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(54) Titre : **MODULATEURS D'EXPRESSION GENETIQUE A BASE D'ACIDE NUCLEIQUE**

(54) Title: **NUCLEIC ACID BASED MODULATORS OF GENE EXPRESSION**

(57) **Abrégé/Abstract:**

Nucleic acid molecules (antisenses or ribozymes) useful as inhibitors of gene expression, especially of HER2, BACE, TERT, PTP-1B, MetAP-2, HBV, phospholamban, presenilin-2 and PKC-alpha. The nucleic acid molecules can be modified in various ways on the sugar and/or base moieties and/or on the phosphate backbone. They are used in pharmaceutical formulations for the treatment of diseases involving increased expression of the target genes. Also disclosed is a method for the synthesis of a modified pyrimidine nucleotide triphosphate and its incorporation into an oligonucleotide.



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(54) Title: NUCLEIC ACID BASED MODULATORS OF GENE EXPRESSION

(57) Abstract: Nucleic acid molecules (antisenses or ribozymes) useful as inhibitors of gene expression, especially of HER2, BACE, TERT, PTP-1B, MetAP-2, HBV, phospholamban, presenilin-2 and PKC-alpha. The nucleic acid molecules can be modified in various ways on the sugar and/or base moieties and/or on the phosphate backbone. They are used in pharmaceutical formulations for the treatment of diseases involving increased expression of the target genes. Also disclosed is a method for the synthesis of a modified pyrimidine nucleotide triphosphate and its incorporation into an oligonucleotide.

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NUCLEIC ACID BASED MODULATORS OF GENE EXPRESSION

Background of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as cancers, diabetes, obesity, Alzheimer's disease, cardiac diseases, age-related diseases, and/or hepatitis B infections and related conditions.

Summary of the Invention

The invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (for example, Cook et al., U.S. Patent 5,359,051)] and methods for their use to modulate the expression of molecular targets impacting the development and progression of cancers, diabetes, obesity, Alzheimer's disease, cardiac diseases, age-related diseases, and/or hepatitis B infections and related conditions

In a preferred embodiment, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (for exaple, Cook et al., U.S. Patent 5,359,051)] and methods for their use for inhibiting the expression of disease related genes, e.g., Protein-Tyrosine-Phosphatase-1b (PTP-1B, Genbank accession No. NM_002827), Methionine Aminopeptidase (MetAP-2, Genbank accession No. U29607), beta-Secretase (BACE, Genbank accession No. AF190725), Presenilin-1 (ps-1, Genbank accession No. L76517), Presenilin-2 (ps-2, Genbank accession No. L43964), Human Epidermal Growth Factor Receptor-2 (HER2/c-erb2/neu, Genbank accession No. X03363), Phospholamban (PLN, Genbank accession No. NM_002667), Telomerase (TERT, Genbank accession No. NM_003219) and Hepatitis B virus genes (HBV, Genbank accession No. AF100308.1). Such ribozymes can be used in a method for treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Thus, in an additional preferred embodiment, the invention features novel nucleic acid-based techniques such as enzymatic nucleic acid molecules and antisense molecules and methods for their use to down regulate or inhibit the expression of genes encoding Protein-Tyrosine-Phosphatase-1b (PTP-1B), Methionine Aminopeptidase (MetAP-2),

beta-Secretase (BACE), Presenilin-1 (ps-1), Presenilin-2 (ps-2), Human Epidermal Growth Factor Receptor-2 (HER2/c-erb2/neu), Phospholamban (PLN), Telomerase (hTERT) PKC alpha. and Hepatitis B (HBV) proteins. In particular, applicant describes the selection and function of nucleic acid molecules capable of cleaving RNAs encoded by these genes and
5 their use to reduce levels of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV proteins in various tissues to treat the diseases discussed herein. Such nucleic acid molecules are also useful for diagnostic uses.

In a preferred embodiment, the invention features the use of one or more of the nucleic acid-based techniques independently or in combination to inhibit the expression of
10 the genes encoding PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV. Specifically, the invention features the use of nucleic acid-based techniques to specifically inhibit the expression of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, PKC alpha, and/or HBV genes.

In yet another preferred embodiment, the invention features the use of an enzymatic
15 nucleic acid molecule, preferably in the hammerhead, NCH (Inozyme), G-cleaver, amberzyme, zinzyme, and/or DNAzyme motif, to inhibit the expression of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, PKC alpha and/or HBV RNA.

Applicant indicates that these nucleic acid molecules are able to inhibit expression of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, PKC alpha, and/or HBV
20 genes. Those of ordinary skill in the art, will find that it is clear from the examples described that other nucleic acid molecules that inhibit target PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV encoding mRNAs may be readily designed and are within the scope of the invention.

By "inhibit" it is meant that the activity of target genes or level of mRNAs or
25 equivalent RNAs encoding target genes is reduced below that observed in the absence of the nucleic acid molecules of the instant invention (*e.g.*, enzymatic nucleic acid molecules), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups). In one embodiment, inhibition with an enzymatic nucleic acid molecule preferably is below that level observed in the
30 presence of an enzymatically attenuated nucleic acid molecule that is able to bind to the same site on the mRNA, but is unable to cleave that RNA. In another embodiment, inhibition with nucleic acid molecules, including enzymatic nucleic acid and antisense

molecules, is preferably greater than that observed in the presence of, for example, an oligonucleotide with scrambled sequence or with mismatches. In another embodiment, inhibition of target genes with the nucleic acid molecule of the instant invention is greater than in the presence of the nucleic acid molecule than in its absence. According to the invention, the activity of telomerase enzyme or the level of RNA encoding one or more protein subunits of the telomerase enzyme is inhibited if it is at least 10% less, 20% less, 50% less, 75% less or even not active or present at all, in the presence of a nucleic acid of the invention relative to the level in the absence of such a nucleic acid.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave target RNA. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. The nucleic acids may be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not meant to be limiting and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it have a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving activity to the molecule (Cech et al., U.S. Patent No. 4,987,071; Cech et al., 1988, JAMA 260:20 3030-4).

By "nucleic acid molecule" as used herein is meant a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and may comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

An example of a nucleic acid molecule according to the invention is a gene which encodes for a macromolecule such as a protein.

By "enzymatic portion" or "catalytic domain" is meant that portion/region of the enzymatic nucleic acid molecule essential for cleavage of a nucleic acid substrate (for example see **Figures 1-5**).

By "substrate binding arm" or "substrate binding domain" is meant that portion/region of a ribozyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in **Figures 1-5**. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme and target RNA together through complementary base-pairing interactions. The ribozyme of the invention may have binding arms that are contiguous or non-contiguous and may be of varying lengths. The length of the binding arm(s) are preferably greater than or equal to four nucleotides and of sufficient length to stably interact with the target RNA; specifically 12-100 nucleotides; more specifically 14-24 nucleotides long. If two binding arms are chosen, the design is such that the length of the binding arms are symmetrical (*i.e.*, each of the binding arms is of the same length; *e.g.*, five and five nucleotides, six and six nucleotides or seven and seven nucleotides long) or asymmetrical (*i.e.*, the binding arms are of different length; *e.g.*, six and three nucleotides; three and six nucleotides long; four and five nucleotides long; four and six nucleotides long; four and seven nucleotides long; and the like). Binding arms can be complementary to the specified substrate, to a portion of the indicated substrate, to the indicated substrate sequence and additional adjacent sequence, or a portion of the indicated sequence and additional adjacent sequence.

By "NCH" or "Inozyme" motif is meant, an enzymatic nucleic acid molecule comprising a motif as described in Ludwig *et al.*, USSN No. 09/406,643, filed September 27, 1999, entitled "COMPOSITIONS HAVING RNA CLEAVING ACTIVITY", and International PCT publication Nos. WO 98/58058 and WO 98/58057, all incorporated by reference herein in their entirety, including the drawings.

By "G-cleaver" motif is meant, an enzymatic nucleic acid molecule comprising a motif as described in Eckstein *et al.*, International PCT publication No. WO 99/16871, incorporated by reference herein in its entirety, including the drawings.

By “zinzyme” motif is meant, a class II enzymatic nucleic acid molecule comprising a motif as described herein and in Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein in its entirety, including the drawings.

5 By “amberzyme” motif is meant, a class I enzymatic nucleic acid molecule comprising a motif as described herein and in Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein in its entirety, including the drawings.

10 By ‘DNAzyme’ is meant, an enzymatic nucleic acid molecule lacking a ribonucleotide (2’-OH) group. In particular embodiments, the enzymatic nucleic acid molecule may have an attached linker(s) or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2’-OH groups. A DNAzyme can be synthesized chemically or can be expressed by means of a single stranded DNA vector or equivalent thereof.

15 By “sufficient length” is meant an oligonucleotide of greater than or equal to 3 nucleotides that is of a length great enough to provide the intended function under the expected condition. For example, for binding arms of enzymatic nucleic acid “sufficient length” means that the binding arm sequence is long enough to provide stable binding to a target site under the expected binding conditions. Preferably, the binding arms are not so long as to prevent useful turnover.

20 By “stably interact” is meant, interaction of the oligonucleotides with target nucleic acid (*e.g.*, by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions).

25 By “equivalent” RNA to PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV is meant to include those naturally occurring RNA molecules having homology (partial or complete) to PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV proteins or encoding for proteins with similar function as PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV in various organisms, including human, rodent, primate, rabbit, pig, protozoans, fungi, plants, and other microorganisms and parasites. The equivalent RNA sequence also includes in addition to
30 the coding region, regions such as 5’-untranslated region, 3’-untranslated region, introns, intron-exon junction and the like in HBV.

By "homology" is meant the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

By "antisense nucleic acid", it is meant a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm *et al.*, 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 *Science* 261, 1004 and Woolf *et al.*, US patent No. 5,849,902). Typically, antisense molecules will be complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule may bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule may bind such that the antisense molecule forms a loop. Thus, the antisense molecule may be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule may be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk *et al.*, 1999, *J. Biol. Chem.*, 274, 21783-21789, Delihias *et al.*, 1997, *Nature*, 15, 751-753, Stein *et al.*, 1997, *Antisense N. A. Drug Dev.*, 7, 151, Crooke, 1998, *Biotech. Genet. Eng. Rev.*, 15, 121-157, Crooke, 1997, *Ad. Pharmacol.*, 40, 1-49. In addition, antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. Antisense DNA can be synthesized chemically or can be expressed via the use of a single stranded DNA expression vector or the equivalent thereof.

By "2-5A antisense chimera" it is meant, an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which, in turn, cleaves the target RNA (Torrence *et al.*, 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" it is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 504).

By "gene" it is meant a nucleic acid that encodes a RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., ribozyme cleavage, antisense or triple helix inhibition. 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 which 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.

At least seven basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. **Table I** summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor of gene expression, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches,

or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme.

The enzymatic nucleic acid molecule that cleave the specified sites in PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV-specific RNAs represent a novel therapeutic approach to treat a variety of pathologic indications, including, HBV infection, hepatitis, hepatocellular carcinoma, tumorigenesis, cirrhosis, liver failure, cancers including breast, ovarian, prostate, and esophageal cancer, tumorigenesis, retinopathy, arthritis, psoriasis, female reproduction, restinosis, certain infectious diseases, transplant rejection and autoimmune disease such as multiple sclerosis, lupus, and AIDS, age related diseases such as macular degeneration and skin ulceration, Alzheimer's disease, dementia, diabetes, obesity and any other condition related to the level of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV in a cell or tissue.

In one of the preferred embodiments of the inventions described herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron, group II intron or RNase P RNA (in association with an RNA guide sequence), *Neurospora* VS RNA, DNazymes, NCH cleaving motifs, or G-cleavers. Examples of such hammerhead motifs are described by Dreyfus, *supra*, Rossi *et al.*, 1992, *AIDS Research and Human Retroviruses* 8, 183. Examples of hairpin motifs are described by Hampel *et al.*, EP0360257, Hampel and Tritz, 1989 *Biochemistry* 28, 4929, Feldstein *et al.*, 1989, *Gene* 82, 53, Haseloff and Gerlach, 1989, *Gene*, 82, 43, Hampel *et al.*, 1990 *Nucleic Acids Res.* 18, 299; and Chowrira & McSwiggen, US. Patent No. 5,631,359. The hepatitis delta virus motif is described by Perrotta and Been, 1992 *Biochemistry* 31, 16. The RNase P motif is described by Guerrier-Takada *et al.*, 1983 *Cell* 35, 849; Forster and Altman, 1990, *Science* 249, 783; and Li and Altman, 1996, *Nucleic Acids Res.* 24, 835. The *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 *Proc. Natl. Acad. Sci. USA* 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799; and Guo and Collins, 1995, *EMBO. J.* 14, 363). Group II introns are described by Griffin *et al.*, 1995, *Chem. Biol.* 2, 761; Michels and Pyle, 1995, *Biochemistry* 34, 2965; and Pyle *et al.*, International PCT Publication No. WO 96/22689. The Group I intron is described by Cech *et al.*, U.S. Patent 4,987,071. DNazymes are described by Usman *et al.*, International PCT Publication No. WO 95/11304; Chartrand *et*

al., 1995, *NAR* 23, 4092; Breaker *et al.*, 1995, *Chem. Bio.* 2, 655; and Santoro *et al.*, 1997, *PNAS* 94, 4262. NCH cleaving motifs are described in Ludwig & Sproat, International PCT Publication No. WO 98/58058; and G-cleavers are described in Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120 and Eckstein *et al.*, International PCT Publication No. WO 99/16871. Additional motifs include the Aptazyme (Breaker *et al.*, WO 98/43993), Amberzyme (Class I motif; **Figure 3**; Beigelman *et al.*, International PCT publication No. WO 99/55857) and Zinzyme (Beigelman *et al.*, International PCT publication No. WO 99/55857), all these references are incorporated by reference herein in their totalities, including drawings and can also be used in the present invention. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule (Cech *et al.*, U.S. Patent No. 4,987,071).

In preferred embodiments of the present invention, a nucleic acid molecule, *e.g.*, an antisense molecule, a triplex DNA, or a ribozyme, is 13 to 100 nucleotides in length, *e.g.*, in specific embodiments 35, 36, 37, or 38 nucleotides in length (*e.g.*, for particular ribozymes or antisense). In particular embodiments, the nucleic acid molecule is 15-100, 17-100, 20-100, 21-100, 23-100, 25-100, 27-100, 30-100, 32-100, 35-100, 40-100, 50-100, 60-100, 70-100, or 80-100 nucleotides in length. Instead of 100 nucleotides being the upper limit on the length ranges specified above, the upper limit of the length range can be, for example, 30, 40, 50, 60, 70, or 80 nucleotides. Thus, for any of the length ranges, the length range for particular embodiments has lower limit as specified, with an upper limit as specified which is greater than the lower limit. For example, in a particular embodiment, the length range can be 35-50 nucleotides in length. All such ranges are expressly included. Also in particular embodiments, a nucleic acid molecule can have a length which is any of the lengths specified above, for example, 21 nucleotides in length.

In a preferred embodiment, the invention provides a method for producing a class of nucleic acid-based gene inhibiting agents which exhibit a high degree of specificity for the RNA of a desired target. For example, the enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of target RNAs encoding PTP-1B, MetAP-

2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV proteins (specifically PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV RNA) such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Such nucleic acid molecules can be delivered exogenously to specific tissue or cellular targets as required. Alternatively, the nucleic acid molecules (e.g., ribozymes and antisense) can be expressed from DNA and/or RNA vectors that are delivered to specific cells.

As used in 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 may be present in an organism which may be a human but is preferably a non-human multicellular organism, e.g., birds, plants and mammals such as cows, sheep, apes, monkeys, swine, dogs, and cats. The cell may be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

By "PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV proteins" is meant, a protein or a mutant protein derivative thereof, comprising sequence expressed and/or encoded by PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, genes and/or the HBV genome respectively.

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.

The enzymatic nucleic acid-based inhibitors of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV expression are useful for the prevention of the diseases and conditions including HBV infection, hepatitis, hepatocellular carcinoma, tumorigenesis, cirrhosis, liver failure, cancers including breast, ovarian, prostate, and esophageal cancer, tumorigenesis, retinopathy, arthritis, psoriasis, female reproduction, restinosis, certain infectious diseases, transplant rejection and autoimmune disease such as multiple sclerosis, lupus, and AIDS, age related diseases such as macular degeneration and skin ulceration, Alzheimer's disease, dementia, diabetes, obesity and any other condition related to the level of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV in a cell or tissue. and any other diseases or conditions that are related to the levels of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV in a cell or tissue.

By “related” is meant that the reduction of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV expression (specifically PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV genes) RNA levels and thus reduction in the level of the respective protein will relieve, to some extent, the symptoms of the disease or condition.

The nucleic acid-based inhibitors 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 preferred embodiments, the enzymatic nucleic acid inhibitors comprise sequences, which are complementary to the substrate sequences in **Tables 3-31, 33, 34, 36-43, 56, 58, 59, 62, 63**. Examples of such enzymatic nucleic acid molecules also are shown in **Tables 3-29, 31, 33, 34, 37-43, 56, 58, 59, 62, 63**. Examples of such enzymatic nucleic acid molecules consist essentially of sequences defined in these tables.

In yet another embodiment, the invention features antisense nucleic acid molecules including sequences complementary to the substrate sequences shown in **Tables 3-31, 33, 34, 36, 37-43, 56, 58, 59, 62, 63**. Such nucleic acid molecules can include sequences as shown for the binding arms of the enzymatic nucleic acid molecules in **Tables 3-29, 31, 33, 34, 37-43, 56, 58, 59, 62, 63**. Similarly, triplex molecules can be provided targeted to the corresponding DNA target regions, and containing the DNA equivalent of a target sequence or a sequence complementary to the specified target (substrate) sequence. Typically, antisense molecules will be complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule may bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule may bind such that the antisense molecule forms a loop. Thus, the antisense molecule may be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule may be complementary to a target sequence or both.

In another aspect, the invention provides mammalian cells containing one or more nucleic acid molecules and/or expression vectors of this invention. The one or more nucleic acid molecules may independently be targeted to the same or different sites.

By “consists essentially of” is meant that the active nucleic acid molecule of the invention, for example, an enzymatic nucleic acid molecule, contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not
5 interfere with such cleavage. Thus, a core region may, for example, include one or more loop or stem-loop structures, which do not prevent enzymatic activity. “X” in the sequences in **Tables 3, 4, 9, 10, 13, 14, 18, 19, 24, 25, 33, 34, 37, 38, 63** can be such a loop. A core sequence for a hammerhead ribozyme can be CUGAUGAG X CGAA where X=GCCGUUAGGC or other stem II region as specifically or generally known in the art.

10 In another aspect of the invention, ribozymes or antisense molecules that interact with target RNA molecules and inhibit PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV (specifically PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV RNA) activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral
15 vectors. Ribozyme or antisense expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes or antisense are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes or antisense. Such vectors
20 might be repeatedly administered as necessary. Once expressed, the ribozymes or antisense bind to the target RNA and inhibit its function or expression. Delivery of ribozyme or antisense expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for
25 introduction into the desired target cell. Antisense DNA can be expressed via the use of a single stranded DNA intracellular expression vector.

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.

30 By “vectors” is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

By "patient" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. Preferably, a patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells.

5 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 above. For example, to treat a disease or condition associated with PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV, the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art, individually or in
10 combination with one or more drugs under conditions suitable for the treatment.

 In a further embodiment, the described molecules, such as antisense or ribozymes, 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 HBV infection, hepatitis, hepatocellular
15 carcinoma, tumorigenesis, cirrhosis, liver failure, cancers including breast, ovarian, prostate, and esophageal cancer, tumorigenesis, retinopathy, arthritis, psoriasis, female reproduction, restinosis, certain infectious diseases, transplant rejection and autoimmune disease such as multiple sclerosis, lupus, and AIDS, age related diseases such as macular degeneration and skin ulceration, Alzheimer's disease, dementia, diabetes, and/or obesity.

20 In another preferred embodiment, the invention features nucleic acid-based inhibitors (*e.g.*, enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups) and methods for their use to down regulate or inhibit the expression of RNA (*e.g.*, PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV) capable of progression
25 and/or maintenance of HBV infection, hepatitis, hepatocellular carcinoma, tumorigenesis, cirrhosis, liver failure, cancers including breast, ovarian, prostate, and esophageal cancer, tumorigenesis, retinopathy, arthritis, psoriasis, female reproduction, restinosis, certain infectious diseases, transplant rejection and autoimmune disease such as multiple sclerosis, lupus, and AIDS, age related diseases such as macular degeneration and skin ulceration,
30 Alzheimer's disease, dementia, diabetes, and/or obesity.

In another preferred embodiment, the invention features nucleic acid-based techniques (*e.g.*, enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups) and methods for their use to down regulate or inhibit the expression of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV RNA expression.

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.

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

The drawings will first briefly be described.

Drawings:

Figure 1 shows the secondary structure model for seven different classes of enzymatic nucleic acid molecules. Arrow indicates the site of cleavage. ----- indicate the target sequence. Lines interspersed with dots are meant to indicate tertiary interactions. - is meant to indicate base-paired interaction. **Group I Intron:** P1-P9.0 represent various stem-loop structures (Cech *et al.*, 1994, *Nature Struc. Bio.*, 1, 273). **RNase P (M1RNA):** EGS represents external guide sequence (Forster *et al.*, 1990, *Science*, 249, 783; Pace *et al.*, 1990, *J. Biol. Chem.*, 265, 3587). **Group II Intron:** 5'SS means 5' splice site; 3'SS means 3'-splice site; IBS means intron binding site; EBS means exon binding site (Pyle *et*

al., 1994, *Biochemistry*, 33, 2716). **VS RNA:** I-VI are meant to indicate six stem-loop structures; shaded regions are meant to indicate tertiary interaction (Collins, International PCT Publication No. WO 96/19577). **HDV Ribozyme:** I-IV are meant to indicate four stem-loop structures (Been *et al.*, US Patent No. 5,625,047). **Hammerhead Ribozyme:** I-III are meant to indicate three stem-loop structures; stems I-III can be of any length and may be symmetrical or asymmetrical (Usman *et al.*, 1996, *Curr. Op. Struct. Bio.*, 1, 527). **Hairpin Ribozyme:** Helix 1, 4 and 5 can be of any length; Helix 2 is between 3 and 8 base-pairs long; Y is a pyrimidine; Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, *i.e.*, m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, o and p is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" $\geq$ is 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond. (Burke *et al.*, 1996, *Nucleic Acids & Mol. Biol.*, 10, 129; Chowrira *et al.*, US Patent No. 5,631,359).

Figure 2 shows examples of chemically stabilized ribozyme motifs. **HH Rz**, represents hammerhead ribozyme motif (Usman *et al.*, 1996, *Curr. Op. Struct. Bio.*, 1, 527); **NCH Rz** represents the NCH ribozyme motif (described herein and in Ludwig & Sproat, International PCT Publication No. WO 98/58058); **G-Cleaver**, represents G-cleaver ribozyme motif (Kore *et al.*, 1998, *Nucleic Acids Research*, 26, 4116-4120). N or

n, represent independently a nucleotide which may be same or different and have complementarity to each other; **rI**, represents ribo-Inosine nucleotide; arrow indicates the site of cleavage within the target. Position 4 of the HH Rz and the NCH Rz is shown as having 2'-C-allyl modification, but those skilled in the art will recognize that this position
5 can be modified with other modifications well known in the art, so long as such modifications do not significantly inhibit the activity of the ribozyme.

Figure 3 shows an example of the Amberzyme ribozyme motif that is chemically stabilized (see, for example, Beigelman *et al.*, International PCT publication No. WO 99/55857; also referred to as Class I Motif). The Amberzyme motif is a class of enzymatic
10 nucleic acid molecules that do not require the presence of a ribonucleotide (2'-OH) group for activity.

Figure 4 shows an example of the Zinzyme A ribozyme motif that is chemically stabilized (see, for example, International PCT publication No. WO 99/55857; also referred to as Class A Motif). The Zinzyme motif is a class of enzymatic nucleic acid
15 molecules that do not require the presence of a ribonucleotide (2'-OH) group for activity.

Figure 5 shows an example of a DNAzyme motif described by Santoro *et al.*, 1997, PNAS, 94, 4262.

Figure 6 is a diagrammatic representation of the hammerhead ribozyme motif known in the art and the NCH motif. Stem II can be 2 base-pair long, preferably, 2, 3,
20 4, 5, 6, 7, 8, and 10 base-pairs long. Each N and N' is independently any base or non-nucleotide as used herein; X is adenosine, cytidine or uridine; Stem I-III are meant to indicate three stem-loop structures; stems I-III can be of any length and may be symmetrical or asymmetrical (Usman *et al.*, 1996, *Curr. Op. Struct. Bio.*, 1, 527); arrow indicates the site of cleavage in the target RNA; Rz refers to ribozyme; Loop II may be
25 present or absent. If Loop II is present it is greater than or equal to three nucleotides, preferably four nucleotides. The Loop II sequence is preferably 5'-GAAA-3' or 5'-GUUA-3'.

Figure 7 shows examples of chemically stabilized ribozyme motifs. **HH Rz**, represents hammerhead ribozyme motif (Usman *et al.*, 1996, *Curr. Op. Struct. Bio.*, 1, 527); **NCH-Inosine Rz** represents the NCH ribozyme motif with riboinosine at 15.1
30 position; **NCH-Xylo Rz** represents the NCH ribozyme with xylo inosine at 15.1 position. **N** or **n**, represent independently a nucleotide which may be same or different and may have

complementarity to each other; **rI**, represents ribo-Inosine nucleotide; **xI** represent xylo-
inosine; arrow indicates the site of cleavage within the target. Position 4 of the HH Rz and
the NCH Rzs is shown as having 2'-C-allyl modification, but those skilled in the art will
recognize that this position can be modified with other modifications well known in the
5 art, so long as such modifications do not significantly inhibit the activity of the ribozyme.

Figure 8 is a graphical representation of data showing inhibition of cell proliferation
mediated by NCH and HH ribozymes targeted against HER2/*neu*/ErbB2 gene. Untreated,
refers to cells not treated with ribozymes; HH RZ refers to hammerhead ribozyme; NCX
RZ refers to the NCH ribozymes of the invention; IA refers to catalytically inactive or
10 attenuated ribozyme used as a control.

Figure 9 is a schematic diagram of the process for the synthesis of beta-D-
xylofuranosyl hypoxanthine 3'-phosphoramidite.

Figure 10 displays a schematic representation of NTP synthesis using nucleoside
substrates.

Figure 11 shows a scheme for an in vitro selection method. A pool of nucleic acid
15 molecules is generated with a random core region and one or more region(s) with a defined
sequence. These nucleic acid molecules are bound to a column containing immobilized
oligonucleotide with a defined sequence, where the defined sequence is complementary to
region(s) of defined sequence of nucleic acid molecules in the pool. Those nucleic acid
20 molecules capable of cleaving the immobilized oligonucleotide (target) in the column are
isolated and converted to complementary DNA (cDNA), followed by transcription using
NTPs to form a new nucleic acid pool.

Figure 12 shows a scheme for a two column in vitro selection method. A pool of
nucleic acid molecules is generated with a random core and two flanking regions (region A
25 and region B) with defined sequences. The pool is passed through a column which has
immobilized oligonucleotides with regions A' and B' that are complementary to regions A
and B of the nucleic acid molecules in the pool, respectively. The column is subjected to
conditions sufficient to facilitate cleavage of the immobilized oligonucleotide target. The
molecules in the pool that cleave the target (active molecules) have A' region of the target
30 bound to their A region, whereas the B region is free. The column is washed to isolate the
active molecules with the bound A' region of the target. This pool of active molecules
may also contain some molecules that are not active to cleave the target (inactive

molecules) but have dissociated from the column. To separate the contaminating inactive molecules from the active molecules, the pool is passed through a second column (column 2) which contains immobilized oligonucleotides with the A' sequence but not the B' sequence. The inactive molecules will bind to column 2 but the active molecules will not
5 bind to column 2 because their A region is occupied by the A' region of the target oligonucleotide from column 1. Column 2 is washed to isolate the active molecules for further processing as described in the scheme shown in **Figure 11**.

Figure 13 is a diagram of a novel 48 nucleotide enzymatic nucleic acid motif which was identified using in vitro methods described in the instant invention. The molecule
10 shown is only exemplary. The 5' and 3' terminal nucleotides (referring to the nucleotides of the substrate binding arms rather than merely the single terminal nucleotide on the 5' and 3' ends) can be varied so long as those portions can base-pair with target substrate sequence. In addition, the guanosine (G) shown at the cleavage site of the substrate can be
15 changed to other nucleotides so long as the change does not eliminate the ability of enzymatic nucleic acid molecules to cleave the target sequence. Substitutions in the nucleic acid molecule and/or in the substrate sequence can be readily tested, for example, as described herein.

Figure 14 is a schematic diagram of HCV luciferase assay used to demonstrate efficacy of class I enzymatic nucleic acid molecule motif.

Figure 15 is a graph indicating the dose curve of an enzymatic nucleic acid molecule
20 targeting site 146 on HCV RNA.

Figure 16 is a bar graph showing enzymatic nucleic acid molecules targeting 4 sites within the HCV RNA are able to reduce RNA levels in cells.

Figure 17 shows secondary structures and cleavage rates for characterized Class II
25 enzymatic nucleic acid motifs.

Figure 18 is a diagram of a novel 35 nucleotide enzymatic nucleic acid motif which was identified using in vitro methods described in the instant invention. The molecule shown is only exemplary. The 5' and 3' terminal nucleotides (referring to the nucleotides of the substrate binding arms rather than merely the single terminal nucleotide on the 5'
30 and 3' ends) can be varied so long as those portions can base-pair with target substrate sequence. In addition, the guanosine (G) shown at the cleavage site of the substrate can be changed to other nucleotides so long as the change does not eliminate the ability of

enzymatic nucleic acid molecules to cleave the target sequence. Substitutions in the nucleic acid molecule and/or in the substrate sequence can be readily tested, for example, as described herein.

Figure 19 is a bar graph showing substrate specificities for Class II (zinzyme) ribozymes.

Figure 20 is a bar graph showing Class II enzymatic nucleic acid molecules targeting 10 representative sites within the HER2 RNA in a cellular proliferation screen.

Figure 21 is a synthetic scheme outlining the synthesis of 5-[3-aminopropynyl(propyl)]uridine 5'-triphosphates and 4-imidazoleaceticacid conjugates.

Figure 22 is a synthetic scheme outlining the synthesis of 5-[3-(N-4-imidazoleacetyl)aminopropynyl(propyl)]uridine 5'-triphosphates.

Figure 23 is a synthetic scheme outlining the synthesis of carboxylate tethered uridine 5'-triphosphates.

Figure 24 is a synthetic scheme outlining the synthesis of 5-(3-aminoalkyl) and 5-[3(N-succinyl)aminopropyl] functionalized cytidines.

Figure 25 is a diagram of a class I ribozyme stem truncation and loop replacement analysis.

Figure 26 is a diagram of class I ribozymes with truncated stem(s) and/or non-nucleotide linkers used in loop structures.

Figure 27 is a diagram of "no-ribo" class II ribozymes.

Figure 28 is a graph showing cleavage reactions with class II ribozymes under differing divalent metal concentrations.

Figure 29 is a diagram of differing class II ribozymes with varying ribo content and their relative rates of catalysis.

Figure 30 is a graph showing class II ribozyme (zinzyme) mediated reduction of HER2 RNA in SKBR3 breast carcinoma cells. Cells were treated with 100 nm, and 200 nm of zinzyme (RPI 18656) targeting site 972 of HER2 RNA and a corresponding scrambled attenuated control complexed with 2.5 µg/ml of lipid. Active zinzymes and scrambled attenuated controls were compared to untreated cells after 24 hours post treatment.

Figure 31 is a graph showing class II ribozyme (zinzyme) mediated dose response anti-proliferation assay in SKBR3 breast carcinoma cells. Cells were treated with 100 nm, and 200 nm of zinzyme (RPI 18656) targeting site 972 of HER2 RNA and a corresponding scrambled attenuated control complexed with 2.0 µg/ml of lipid. Active zinzymes and scrambled attenuated controls were compared to untreated cells after 24 hours post treatment.

Figure 32 is a graph which shows the dose dependent reduction of HER2 RNA in SKOV-3 cells treated with RPI 19293 from 0 to 100 nM with 5.0 µg/ml of cationic lipid.

Figure 33 is a graph which shows the dose dependent reduction of HER2 RNA and inhibition of cellular proliferation in SKBR-3 cells treated with RPI 19293 from 0 to 400 nM with 5.0 µg/ml of cationic lipid.

Figure 34 shows a non-limiting example of the replacement of a 2'-O-methyl 5'-CA-3' with a ribo G in the class II (zinzyme) motif. The representative motif shown for the purpose of the figure is a "seven-ribo" zinzyme motif, however, the interchangeability of a G and a CA in the position shown in **Figure 25** of the class II (zinzyme) motif extends to any combination of 2-O-methyl and ribo residues. For instance, a 2'-O-methyl G can replace the 2'-O-methyl 5'-CA-3' and vice versa.

Figure 35 is a graph which shows a screen of class II ribozymes (zinzymes) targeting site 972 of HER2 RNA which contain ribo-G reductions (RPI 19727 = no ribo, RPI 19728 = one ribo, RPI 19293 = two ribo, RPI 19729 = three ribo, RPI 19730 = four ribo, 19731 = five ribo, and RPI 19292 = seven ribo) for anti-proliferative activity in SKBR3 cells.

Figure 36 summarizes the results of functional group modification studies in which various nucleoside analogs were tested for activity in the NCH ribozyme motif. K_{rel} values describe the cleavage values of a given substituent at position 15.1 relative the Inosine at position 15.1 (I-15.1).

Figure 37 summarizes reported functional group modification studies performed at the A 15.1 residue in the A-15.1 •U-16.1 context of NUH cleaving ribozymes. K_{rel} values describe the cleavage values of a given substituent at position 15.1 relative the adenosine at position 15.1 (A-15.1).

Mechanism of action of Nucleic Acid Molecules of the Invention

Antisense: Antisense molecules may be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides and primarily function by specifically binding to matching sequences resulting in inhibition of peptide synthesis (Wu-Pong, Nov 1994, *BioPharm*, 20-33). The antisense oligonucleotide binds to target RNA by Watson Crick base-pairing and blocks gene expression by preventing ribosomal translation of the bound sequences either by steric blocking or by activating RNase H enzyme. Antisense molecules may also alter protein synthesis by interfering with RNA processing or transport from the nucleus into the cytoplasm (Mukhopadhyay & Roth, 1996, *Crit. Rev. in Oncogenesis* 7, 151-190).

In addition, binding of single stranded DNA to RNA may result in nuclease degradation of the heteroduplex (Wu-Pong, *supra*; Crooke, *supra*). To date, the only backbone modified DNA chemistry which will act as substrates for RNase H are phosphorothioates, phosphorodithioates, and borontrifluoridates. Recently, it has been reported that 2'-arabino and 2'-fluoro arabino- containing oligos can also activate RNase H activity.

A number of antisense molecules have been described that utilize novel configurations of chemically modified nucleotides, secondary structure, and/or RNase H substrate domains (Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, International PCT Publication No. WO 99/54459 ; Hartmann *et al.*, International PCT Publication No. WO 00/17346) all of these are incorporated by reference herein in their entirety.

Antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. Antisense DNA can be chemically synthesized or can be expressed via the use of a single stranded DNA intracellular expression vector or the equivalent thereof.

Triplex Forming Oligonucleotides (TFO): Single stranded DNA may be designed to bind to genomic DNA in a sequence specific manner. TFOs are comprised of pyrimidine-rich oligonucleotides which bind DNA helices through Hoogsteen Base-pairing (Wu-Pong, *supra*). The resulting triple helix composed of the DNA sense, DNA antisense, and TFO disrupts RNA synthesis by RNA polymerase. The TFO mechanism may result in gene expression or cell death since binding may be irreversible (Mukhopadhyay & Roth, *supra*)

2'-5' Oligoadenylates: The 2-5 A system is an interferon-mediated mechanism for RNA degradation found in higher vertebrates (Mitra *et al.*, 1996, *Proc Nat Acad Sci USA* 93, 6780-6785). Two types of enzymes, 2-5A synthetase and RNase L, are required for RNA cleavage. The 2-5A synthetases require double stranded RNA to form 2'-5' oligoadenylates (2-5A). 2-5A then acts as an allosteric effector for utilizing RNase L which has the ability to cleave single stranded RNA. The ability to form 2-5A structures with double stranded RNA makes this system particularly useful for inhibition of viral replication.

(2'-5') oligoadenylate structures may be covalently linked to antisense molecules to form chimeric oligonucleotides capable of RNA cleavage (Torrence, *supra*). These molecules putatively bind and activate a 2-5A dependent RNase, the oligonucleotide/enzyme complex then binds to a target RNA molecule which can then be cleaved by the RNase enzyme. The covalent attachment of 2'-5' oligoadenylate structures is not limited to antisense applications, and can be further elaborated to include attachment to nucleic acid molecules of the instant invention.

Enzymatic Nucleic Acid: Seven basic varieties of naturally-occurring enzymatic RNAs are presently known. In addition, several *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc. London*, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing cleavage and ligation of phosphodiester linkages (Joyce, 1989, *Gene*, 82, 83-87; Beaudry *et al.*, 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.*, 1994, *TIBTECH* 12, 268; Bartel *et al.*, 1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.*, 1995, *FASEB J.*, 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442; Santoro *et al.*, 1997, *Proc. Natl. Acad. Sci.*, 94, 4262; Tang *et al.*, 1997, *RNA* 3, 914; Nakamaye & Eckstein, 1994, *supra*; Long & Uhlenbeck, 1994, *supra*; Ishizaka *et al.*, 1995, *supra*; Vaish *et al.*, 1997, *Biochemistry* 36, 6495; all of these are incorporated by reference herein). Each can catalyze a series of reactions including the hydrolysis of phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions.

In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target

RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target
5 and can repeatedly bind and cleave new targets.

Nucleic acid molecules of this invention will block to some extent PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV protein expression and can be used to treat disease or diagnose disease associated with the levels of PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV.

10 The enzymatic nature of a ribozyme has significant advantages, such as the concentration of ribozyme necessary to affect a therapeutic treatment is low. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not
15 only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a ribozyme.

Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific
20 manner. Such enzymatic nucleic acid molecules can be targeted to virtually any RNA transcript, and achieve efficient cleavage *in vitro* (Zaug *et al.*, 324, *Nature*, 429 1986 ; Uhlenbeck, 1987 *Nature*, 328, 596; Kim *et al.*, 84 *Proc. Natl. Acad. Sci. USA*, 8788, 1987; Dreyfus, 1988, *Einstein Quart. J. Bio. Med.*, 6, 92; Haseloff and Gerlach, 334 *Nature*, 585, 1988; Cech, 260 *JAMA*, 3030, 1988; Jefferies *et al.*, 17 *Nucleic Acids Research*, 1371,
25 1989; and Santoro *et al.*, 1997 *supra*).

Because of their sequence specificity, *trans*-cleaving ribozymes show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Ribozymes can be designed to cleave specific RNA targets within the background of cellular RNA.
30 Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited (Warashina *et al.*, 1999, *Chemistry and Biology*, 6, 237-250.

The nucleic acid molecules of the instant invention are also referred to as GeneBloc™ reagents, which are essentially nucleic acid molecules (e.g.; ribozymes, antisense) capable of down-regulating gene expression.

5 Target sites

Targets for useful ribozymes and antisense nucleic acids can be determined as disclosed in Draper *et al.*, WO 93/23569; Sullivan *et al.*, WO 93/23057; Thompson *et al.*, WO 94/02595; Draper *et al.*, WO 95/04818; McSwiggen *et al.*, US Patent No. 5,525,468, and all hereby incorporated in their entireties by reference herein. Other examples include
10 the following PCT applications, which concern inactivation of expression of disease-related genes: WO 95/23225, WO 95/13380, WO 94/02595, all incorporated by reference herein. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes and antisense to such targets are designed as described in those applications and
15 synthesized to be tested *in vitro* and *in vivo*, as also described. The sequence of human PTP-1B, MetAP-2, BACE, ps-1, ps-2, HER2, PLN, TERT, and/or HBV RNAs (for example, GenBank accession Nos. (PTP-1B, NM_002827), (MetAP-2, U29607), (BACE, AF190725), (ps-1, L76517), (ps-2, L43964), (HER2/c-erb2/neu, X03363), (PLN, NM_002667), (TERT, NM_003219) and (HBV, AF100308.1, HBV strain 2-18;
20 additionally, other HBV strains can be screened by one skilled in the art, see **Table 35** for other possible strains) were screened for optimal enzymatic nucleic acid and antisense target sites using a computer-folding algorithm. Antisense, hammerhead, DNAzyme, NCH (Inozyme), amberzyme, zinzyme or G-Cleaver ribozyme binding/cleavage sites were identified. These sites are shown in **Tables 3-29, 31, 33, 34, 37-43, 56, 58, 59, 62, 63** (all
25 sequences are 5' to 3' in the tables; X can be any base-paired sequence, the actual sequence is not relevant here). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of enzymatic nucleic acid molecule. **Table 36** shows substrate positions selected from Renbo *et al.*, 1987, *Sci. Sin.*, 30, 507, used in Draper, US patent No. 6,017,756 entitled "METHOD AND REAGENT FOR
30 INHIBITING HEPATITIS B VIRUS REPLICATION" and Draper *et al.*, International PCT publication No. WO 93/23569, filed April 29, 1993, entitled "METHOD AND REAGENT FOR INHIBITING VIRAL REPLICATION". While human sequences can be

screened and enzymatic nucleic acid molecule and/or antisense thereafter designed, as discussed in Stinchcomb *et al.*, WO 95/23225, mouse targeted ribozymes may be useful to test efficacy of action of the enzymatic nucleic acid molecule and/or antisense prior to testing in humans.

5 Antisense, hammerhead, DNAzyme, NCH (Inozyme), amberzyme, zinzyme or G-Cleaver ribozyme binding/cleavage sites were identified, as discussed above. The nucleic acid molecules were individually analyzed by computer folding (Jaeger *et al.*, 1989 *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the sequences fold into the appropriate secondary structure. Those nucleic acid molecules with unfavorable intramolecular
10 interactions such as between the binding arms and the catalytic core were eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity.

 Antisense, hammerhead, DNAzyme, NCH, amberzyme, zinzyme or G-Cleaver ribozyme binding/cleavage sites were identified and were designed to anneal to various sites in the RNA target. The binding arms are complementary to the target site sequences
15 described above. The nucleic acid molecules were chemically synthesized. The method of synthesis used follows the procedure for normal DNA/RNA synthesis as described below and in Usman *et al.*, 1987 *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990 *Nucleic Acids Res.*, 18, 5433; Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684; and Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19.

20

Synthesis of Nucleic acid Molecules

 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
25 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, antisense oligonucleotides, hammerhead or the NCH ribozymes) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of RNA structure. Exemplary molecules of the instant invention are
30 chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (e.g.; antisense GeneBlocs) are synthesized using protocols known in the art 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, US patent 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 μ mol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μ mol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μ L of 0.11 M = 4.4 μ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μ L of 0.25 M = 10 μ mol) 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 I₂, 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.

Deprotection of the antisense 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.

The method of synthesis used for normal RNA including certain enzymatic nucleic acid molecules 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, CA) 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 I₂, 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.

5 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,
10 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
15 quenched with 1.5 M NH₄HCO₃.

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
20 min. The sample is cooled at -20 °C and then quenched with 1.5 M NH₄HCO₃.

For purification of the trityl-on oligomers, the quenched NH₄HCO₃ 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,
25 salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

Inactive hammerhead ribozymes or binding attenuated control (BAC) oligonucleotides) are synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252). Similarly, one or more
30 nucleotide substitutions can be introduced in other enzymatic nucleic acid molecules to inactivate the molecule and such molecules can serve as a negative control.

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
5 chemicals used in the reaction.

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).
10

The nucleic acid molecules of the present invention are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 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). Ribozymes are purified by
15 gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and are re-suspended in water.

The sequences of the ribozymes and antisense constructs that are chemically synthesized, useful in this study, are shown in **Tables 3-31, 33, 34, 37-43, 56, 58, 59, 62,**
20 **63.** Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. The ribozyme and antisense construct sequences listed in **Tables 3-31, 33, 34, 37-43, 56, 58, 59, 62, 63** may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes with enzymatic activity are equivalent to
25 the ribozymes described specifically in the Tables.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases may 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
30 93/15187; Rossi *et al.*, International Publication No. WO 91/03162; Sproat, US Patent No.

5,334,711; and Burgin *et al.*, *supra*; all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules herein and are all hereby incorporated by reference herein). Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules (e.g., enzymatic nucleic acid molecules) without significantly effecting catalysis and with significant enhancement in their nuclease stability and efficacy. Enzymatic nucleic acid molecules are modified to enhance stability and/or enhance catalytic activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 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 enzymatic 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, *US Patent* No. 5,334,711 and Beigelman *et al.*, 1995 *J. Biol. Chem.* 270, 25702; 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 enzymatic nucleic acid molecules without inhibiting catalysis, and are incorporated by reference herein. The 2'-position of the sugar in a nucleotide present in the nucleic acid molecules of the instant invention which tolerates substitution is selected from the group comprising -H, -OH, -COOH, -CONH₂, -CONHR¹, -CONR¹R², -NH₂, -NHR¹, -NR¹R², -NHCOR¹, -SH, SR¹, -F, -ONH₂, -ONHR¹, -ONR¹R², -NHOH, -NHOR¹, -NR²OH, -NR²OR¹, substituted or unsubstituted C₁-C₁₀ straight chain or branched alkyl, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkenyl, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkynyl, substituted or unsubstituted C₁-C₁₀ straight chain or branched alkoxy, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkenyloxy, and substituted or unsubstituted C₂-C₁₀ straight chain or branched alkynyloxy. The substituents for sugar 2'

position preferably are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto. R^1 and R^2 can be substituted or unsubstituted alkyl, alkenyl, or alkynyl groups, where the substituents are independently halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto.

5 In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention. Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without inhibiting catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications
10 can be used as described herein to modify the nucleic acid molecules of the instant invention.

Some of the non-limiting examples of base modifications that can be introduced into enzymatic nucleic acids without significantly effecting their catalytic activity include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy
15 benzene, 3-methyluracil, 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) and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or
20 their equivalents; such bases may be used within the catalytic core of the enzyme and/or in the substrate-binding regions.

The nucleic acid bases can be hypoxanthin-9-yl, or a functional equivalent thereof, in position ^{15.1} of the ribozyme; the base at other positions may be guanin-9-yl, hypoxanthin-9-yl or 7-deazaguanin-9-yl in positions 5, 8 and 12 in the ribozyme; adenin-9-yl, 2,6-diaminopurin-9-yl, purin-9-yl or 7-deaza adenin-9-yl in positions 6, 9, 13 and 14; uracil-1-yl, uracil-5-yl, thymine-1-yl or 5-propynyluracil-1-yl in position 4; cytosin-1-yl, 5-methylcytosin-1-yl or 5-propynylcytosin-1-yl in position 3; and adenin-9-yl, cytosin-1-yl, guanin-9-yl, uracil-1-yl, uracil-5-yl, hypoxanthin-9-yl, thymine-1-yl, 5-methylcytosin-1-yl, 2,6-diaminopurin-9-yl, purin-9-yl, 7-deaza adenin-9-yl, 7-deazaguanin-9-yl, 5-propynylcytosin-1-yl, 5-propynyluracil-1-yl, isoguanin-9-yl, 2-aminopurin-9-yl, 6-methyluracil-1-yl, 4-thiouracil-1-yl, 2-pyrimidone-1-yl, quinazoline-2,4-dione-1-yl, xanthin-9-yl, N²-dimethylguanin-9-yl, or a functional equivalent thereof in position 7. The
30

base at position 15.1 is preferably hypoxanthin-9-yl or an analog where no hydrogen bond can form between any group at the 2 position of the base and the 2-oxo group of C^{16.1}. Preferably, B is not guanine-9-yl in position 15.1.

5 In particular, the invention features modified ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.

10 While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, too many of these modifications may cause some toxicity. 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.

15 Nucleic acid molecules having chemical modifications which maintain or enhance activity are provided. Such nucleic acid molecules are also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. Therapeutic nucleic acid molecules delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, nucleic acid molecules must be
20 resistant to nucleases in order to function as effective intracellular therapeutic agents. 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 (all are incorporated by reference herein) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as
25 described above.

Use of these 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 antisense or enzymatic nucleic acid molecules targeted to different genes, nucleic acid molecules coupled with known small molecule inhibitors, or intermittent
30 treatment with combinations of molecules (including different motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules.

Therapeutic nucleic acid molecules (*e.g.*, enzymatic nucleic acid molecules and antisense nucleic acid molecules) delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, these nucleic acid molecules must be 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.

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or *in vivo* where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of these properties is increased or not significantly (less than 10-fold) decreased *in vivo* compared to an all RNA ribozyme or all DNA enzyme.

In yet another preferred embodiment, nucleic acid catalysts having chemical modifications which maintain or enhance enzymatic activity are provided. Such nucleic acid catalysts are also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in a cell and/or *in vivo* even if activity over all is reduced 10 fold (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Such ribozymes herein are said to "maintain" the enzymatic activity of an all RNA ribozyme.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'-cap structure.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Wincott *et al.*, WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may 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-erythrofuransyl) 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 (for more details, see Wincott *et al.*, International PCT publication No. WO 97/26270, incorporated by reference herein).

In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) 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).

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 may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which 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, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which 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, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which 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.

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 interchangeably 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,
5 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,
10 *supra*).

By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule. Such modified
15 nucleotides include dideoxynucleotides which have pharmaceutical utility well known in the art, as well as utility in basic molecular biology methods such as sequencing.

In a preferred embodiment, the invention features modified ribozymes with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amidate carbamate, carboxymethyl,
20 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*,
25 ACS, 24-39. These references are hereby incorporated by reference herein.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, (for more details, see Wincott *et al.*, International PCT publication No. WO 97/26270).

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

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

5 In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH₂ or 2'-O- NH₂, which may be modified or unmodified. Such modified groups are described, for example, in Eckstein et al., U.S. Patent 5,672,695 and Matulic-Adamic et al., WO 98/28317, which are both incorporated by reference in their entireties.

10 Various modifications to nucleic acid (e.g., antisense and ribozyme) 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.

15 Use of these molecules will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes (including different ribozyme motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules.
20 Therapies may be devised which include a mixture of ribozymes (including different ribozyme motifs), antisense and/or 2-5A chimera molecules to one or more targets to alleviate symptoms of a disease.

Administration of Nucleic Acid Molecules

25 Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; and *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995 which are both incorporated herein by reference. Sullivan *et al.*, PCT WO 94/02595, further describes the general methods for delivery of enzymatic RNA molecules. These protocols may be utilized for the delivery of virtually any nucleic
30 acid molecule. Nucleic acid molecules may be administered to cells by a variety of methods known to those familiar to 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. For some indications, nucleic acid molecules may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Many examples in the art describe CNS delivery methods of oligonucleotides by osmotic pump, (see Chun *et al.*, 1998, *Neuroscience Letters*, 257, 135-138, D'Aldin *et al.*, 1998, *Mol. Brain Research*, 55, 151-164, Dryden *et al.*, 1998, *J. Endocrinol.*, 157, 169-175, Ghirnikar *et al.*, 1998, *Neuroscience Letters*, 247, 21-24) or direct infusion (Broadus *et al.*, 1997, *Neurosurg. Focus*, 3, article 4). Other routes of delivery include, but are not limited to oral (tablet or pill form) and/or intrathecal delivery (Gold, 1997, *Neuroscience*, 76, 1153-1158). For a comprehensive review on drug delivery strategies including broad coverage of CNS delivery, see Jain, *Drug Delivery Systems: Technologies and Commercial Opportunities*, Decision Resources, 1998. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of nucleic acid delivery and administration are provided in Sullivan *et al.*, *supra*, Draper *et al.*, PCT WO93/23569; Beigelman *et al.*, PCT WO99/05094, and Klimuk *et al.*, PCT WO99/04819 all of which are incorporated by reference herein.

The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

The negatively charged polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a patient 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 may 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.

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.

5 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 patient, preferably 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
10 charged polymer is desired to be delivered to). 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 which prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation
15 of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged polymers, *e.g.*, nucleic acids, to an accessible diseased tissue. The rate of entry of a drug
20 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 which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also
25 useful. This approach may 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.

By pharmaceutically acceptable formulation is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant
30 invention in the physical location most suitable for their desired activity. Nonlimiting 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, MA; 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.

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, 270, 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; all of which are incorporated herein by reference). 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.

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 may be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents may be used.

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 which 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.

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

Alternatively, certain of the nucleic acid 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; all of these references are hereby incorporated herein, in their totalities, by reference). 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 ribozyme (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; all of these references are hereby incorporated in their totality by reference herein).

In another aspect of the invention, RNA molecules of the present invention are preferably expressed from transcription units (see, for example, Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the nucleic acid molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of nucleic acid molecules. Such vectors might be repeatedly administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA. Delivery of nucleic acid molecule expressing vectors could be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, 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).

In one aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules of the instant invention is disclosed. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operably linked in a manner which allows expression of that nucleic acid molecule.

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); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector may 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 nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences are 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 will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend 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). All of these references are incorporated by reference herein. Several investigators have demonstrated that nucleic acid molecules, such as ribozymes 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. U S A*, 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; Lisiewicz 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 ribozymes in cells (Thompson et al., *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg et al., 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg et al., US Patent No. 5,624,803; Good et al., 1997, *Gene Ther.*, 4, 45; Beigelman et al., International PCT Publication No. WO 96/18736; all of these publications are incorporated by reference herein. The above ribozyme 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*).

In yet another aspect, the invention features an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention, in a manner which allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In another preferred embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; d) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. 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; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

Examples:

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

Example 1: Telomerase

The ribonucleoprotein enzyme telomerase consists of an RNA template subunit and one or more protein subunits including telomerase reverse transcriptase (TERT), which function together to direct the synthesis of telomeres. Telomeres exist as non-nucleosome DNA/protein complexes at the physical ends of eukaryotic chromosomes. These capping structures maintain chromosome stability and replicative potential (Zakian, V. A., 1995, Science, 270, 1601-1607). Telomere structure is characterized by tandem repeats of conserved DNA sequences rich in G-C base pairs. Additional conserved telomere elements include a terminal 3'-overhang in the G-rich strand and non-histone structural proteins that are complexed with telomeric DNA in the nucleus. (Blackburn, "E., 1990, JBC., 265, 5919-5921.). Observed shortening of telomeres coincides with the onset of cellular senescence in most somatic cell lines lacking significant levels of telomerase. This finding has had a profound impact on our views concerning the mechanisms of aging, age related disease, and cancer.

Conventional DNA polymerases are unable to fully replicate the ends of linear chromosomes (Watson, J. D., 1972, Nature, 239, 197-201). This inability stems from the 3' G-rich overhang that is a product of ribonuclease cleavage of the RNA primer used in DNA replication. The overhang prevents DNA polymerase replication since the recessed C-rich parent strand cannot be used as a template. Telomerase overcomes this limitation by extending the 3' end of the chromosome using deoxyribonucleotides as substrates and a sequence within the telomerase RNA subunit as a template. (Lingner, J., 1995, Science, 269, 1533-1534). As such, telomerase is considered a reverse transcriptase that is responsible for telomere maintenance.

Telomerase was first discovered by in *Tetrahymena thermophila* in 1985 (Greider, C. W., 1995, Cell, 43, 405-413). The RNA subunits and their respective genes were later discovered and characterized in protozoa, budding yeast, and mammals. Genetic studies of these genes confirmed the role of telomerase RNA (TR) in determining telomere sequence by mutating genes which encode the telomeric RNA (Yu, G. L., 1990, Nature, 344, 126-132), (Singer, M. S., 1994, Science, 266, 404-409), (Blasco, M. A., 1995, Science, 269, 1267-1270). These studies showed that telomerase activity parallels TR expression in protozoa, yeast and mice. However, the expression of human telomerase RNA (hTR) does not correlate well with telomerase activity in mammalian cells. Many

human tissues express hTR but are devoid of telomerase activity (Feng, J., 1995, Science, 269, 1236-1241). Knockout mice, in which the mTR gene has been deleted from germline cells, have been shown to be viable for at least six generations. Cells from later generations of these mice showed chromosomal abnormalities consistent with telomere degradation, indicating that mTR is necessary for telomere length maintenance, but is not required for embryonic development, oncogenic transformation, or tumor formation in mice (Blasco, M. A., 1997, Cell, 91, 25-34).

The first catalytically active subunit of telomerase (p123) was isolated from *Euplotes aediculatus* along with another subunit (p43) and a 66-kD RNA subunit (Linger, J., 1996, Proc. Natl. Acad. Sci., 93, 10712-10717). Subsequent studies revealed telomerase catalytic subunit homologs from fission yeast (Est2p) and human genes (TRT1). The human homolog, TRT1 encoding hTERT, expressed mRNA with a strong correlation to telomerase activity in human cells (Nakamura, T. M., 1997, Science, 277, 955-959). Reconstitution of telomerase activity with *in vitro* transcribed and translated hTERT and hTR, either co-synthesized or simply mixed, demonstrated that hTERT and hTR represent the minimal components of telomerase. Furthermore, transient expression of hTERT in normal diploid human cells restored telomerase activity, demonstrating that hTERT is the only component necessary to restore telomerase activity in normal human cells (Weinrich, S. L., 1997, Nature Genetics, 17, 498-502). The introduction of telomerase into normal human cells using hTERT expression via transfection has resulted in the extension of life span in these cells. Such findings indicate that telomere loss in the absence of telomerase is the "mitotic clock" that controls the replicative potential of a cell prior to senescence (Bodnar, A. G., 1998, Science, 279, 349-352).

Expression of telomerase is observed in germ cell and most cancer cell lines. These "immortal" cell lines continue to divide without shortening of their telomeres (Kim, N. W., 1994, Science, 266, 2011-2015). A model of tumor progression has evolved from these findings, suggesting a role for telomerase expression in malignant transformation. Successful malignant transformation in human cells was accomplished for the first time by ectopic expression of hTERT in combination with two oncogenes, SV40 large-T and H-ras. Injection of nude mice with cells expressing these oncogenes and hTERT resulted in rapid growth of tumors. These observations indicate that hTERT mediated telomere

maintenance is essential for the formation of human tumor cells (Hahn, W. C., 1999, Nature, 400, 464-468).

Various methods have been developed to assay telomerase activity *in vitro*. The most widely used method to characterize telomerase activity is the telomeric repeat amplification protocol (TRAP). TRAP utilizes RT-PCR of cellular extracts to measure telomerase activity by making the amount of PCR target dependant upon the biochemical activity of the enzyme (Kim, N. W., 1997, Nucleic Acids Research, 25, 2595-2597, which is incorporated by reference herein).

A method based on Kim is as follows. Briefly, for the telomerase assay, 2 µg of protein extract is used. The extract is assayed in 50 µl of reaction mixture containing 0.1 µg TS substrate primer (5'-AATCCGTCGAGCAGAGTT-3', end-labeled using alpha-³²P-ATP and T4 polynucleotide kinase), 0.1 µg ACX return primer (5'-GCGCGG[CTTACC]₃CTAACC-3'), 0.1 µg NT internal control primer (5'-ATCGCTTCTCGGCCTTTT-3'), 0.01 micromol TSNT internal control template (5'-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAACGAT-3'), 50 µM each deoxynucleoside triphosphate, 2 U of Taq DNA polymerase, and 2 µl CHAPS protein extract, all in 1X TRAP buffer (20 mM Tris (pH 8.3), 68 mM KCl, 1.5 mM MgCl₂, 1 mM EGTA, 0.05% Tween 20). Each reaction is placed in a thermocycler block preheated to 30 C and incubated at 30 C for 10 minutes, then cycled for 27 cycles of 94 degrees C for 30 seconds, 60 degrees C for 30 seconds. Reaction products are separated on a denaturing 8% polyacrylamide gel, followed by drying of the gel and autoradiography. The internal control (to control for possible Taq polymerase inhibition) generates a band of 36 nt. Comparison of radioactive signal integrated (*e.g.*, by phosphorimager analysis) for telomerase-extended bands with the radioactive signal from a reaction performed with a known amount of quantification standard template (termed R8; 5'-AATCCGTCGAGCAGAGTTAG [GGTTAG]₇-3') allows expression of telomerase activity as an absolute value. The absolute value = TPG (total product generated) = [(TP - TP_i)/TI]/[(R8-B)/RI] x 100, where TP = telomerase products from test extract, TP_i = telomerase products from a heat-inactivated (75 C, 10 minutes) extract reaction, TI = the signal from the internal control, R8 = the signal from the R8 qualification standard template reaction, B = signal from a lysis buffer-only blank reaction, and RI = the internal control value for the reaction containing R8 template and NT and TSNT control primers.

TPG values of 0-10,000 are possible, with the linear range being from approximately 1 to 1000 TPG. The range of 1 to 1000 TPG encompasses the minimum and maximum levels of telomerase activity in most tumor samples tested, while non-tumor cells most often have no telomerase activity (TPG approximately zero).

5 Telomerase activity may also be assayed as follows. Samples to be assayed for telomerase activity are prepared by extraction into CHAPS lysis buffer (10mM Tris pH 7.5, 1mM MgCl₂, 1mM EGTA, 0.1 mM PMSF, 5mM -mercaptoethanol, 1mM DTT, 0.5% 3-[(3-cholamidopropyl)-dimethyl-amino]-1- propanesulfonate (CHAPS), 10% glycerol and 40 U/ml RNase inhibitor (Promega, Madison, WI, U.S.A.). Cells are
10 suspended in CHAPS lysis buffer and incubated on ice for 30 minutes, which allows lysis of 90-100% of cells. Lysate is then transferred to polyallomer centrifuge tubes and spun at 100,000 x g for 1 hour at 4 degrees C. The supernatant is the protein extract, and concentration ranges of 4-10 µg/µl are suitable for telomerase assay. Extracts may be concentrated if necessary using a Microcon Microfilter 30 (Amicon, Beverly, MA U.S.A.)
15 according to the manufacturer's instructions. Extracts may be stored frozen at -80 degrees C until assayed.

 A variety of animal models have been designed to assay telomerase activity *in vivo*. Inhibition of telomerase activity has been analyzed in rats via cell proliferation studies with MNU (N-methyl-N-nitrosurea) induced mammary carcinomas in response to treatment
20 with 4-(hydroxyphenyl)retinamide (4-HPR), a known inhibitor of mammary carcinogenesis in animal models and premenopausal women (Bednarek, A., 1999, Carcinogenesis, 20, 879-883). Additional studies have focused on the up-regulation of telomerase in transformed cell lines from animal and human model systems (Zhang, P. B., 1998, Leuk. Res., 22, 509-516), (Chadeneau, C., 1995, Oncogene, 11, 893-898),
25 (Greenberg, R., 1999, Oncogene, 18, 1219-1226).

 Human cell culture studies have been established to assay inhibition of telomerase activity in human carcinomas responding to various therapeutics. A human breast cancer model for studying telomerase inhibitors is described (Raymond, E., 1999, Br. J. Cancer, 80, 1332-1341). Human studies of telomerase expression as related to various other
30 cancers are described including cervical cancer (Nakano, K., 1998, Am. J. Pathol, 153, 857-864), endometrial cancer (Kyo, S., 1999, Int. J. Cancer, 80, 60-63), meningeal carcinoma (Kleinschmidt-DeMasters, B. K., 1998, J. Neurol. Sci., 161, 124-134), lung

carcinoma (Yashima, K., 1997, Cancer Reseach, 57, 2372-2377), testicular cancer in response to cisplatin (Burger, A. M., 1997, Eur. J. Cancer, 33, 638-644), and ovarian carcinoma (Counter, C. M., 1994, Proc. Natl. Acad. Sci., 91, 2900-2904).

Particular degenerative and disease states that can be associated with telomerase expression modulation include but are not limited to:

Cancer: Almost all human tumors have detectable telomerase activity (Shay, J. W., 1997, Eur. J. Cancer, 33, 787-791). Treatment with telomerase inhibitors may provide effective cancer therapy with minimal side effects in normal somatic cells that lack telomerase activity. The therapeutic potential exists for the treatment of a wide variety of cancer types.

Restinosis: Telomerase inhibition in vascular smooth muscle cells may inhibit restinosis by limiting proliferation of these cells.

Infectious disease: Telomerase inhibition in infectious cell types that express telomerase activity may provide selective anti-infectious agent activity. Such treatment may prove especially effective in protozoan-based infection such as Giardia and Lesh Meniesis.

Transplant rejection: Telomerase inhibition in endothelial cell types may demonstrate selective immunnosuppressant activity. Activation of telomerase in transplant cells could benefit grafting success through increased proliferative potential.

Autoimmune disease: Telomerase modulation in various immune cells may prove beneficial in treating diseases such as multiple sclerosis, lupus, and AIDS.

Age related disease: Activation of telomerase expression in cells at or nearing senescence as a result of advanced age or premature aging could benefit conditions such as macular degeneration, skin ulceration, and rheumatoid arthritis.

The present body of knowledge in telomerase research indicates the need for methods to assay telomerase activity and for compounds that can regulate telomerase expression for research, diagnostic, trait alteration, animal health and therapeutic use.

Gemcytabine and cyclophosphamide are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drugs such as anti-cancer compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention

(e.g. ribozymes and antisense molecules) and are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example *Cancer: Principles and Practice of Oncology*, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia, USA; incorporated herein by reference) and include, without limitations, antifolates; fluoropyrimidines; cytarabine; purine analogs; adenosine analogs; amsacrine; topoisomerase I inhibitors; anthracyclins; retinoids; antibiotics such as bleomycin, anthracyclins, mitomycin C, dactinomycin, and mithramycin; hexamethylmelamine; dacarbazine; l-asparaginase; platinum analogs; alkylating agents such as nitrogen mustard, melphalan, chlorambucil, busulfan, ifosfamide, 4-hydroperoxycyclophosphamide, nitrosoureas, thiotepa; plant derived compounds such as vinca alkaloids, epipodophyllotoxins, taxol; Tomaxifen; radiation therapy; surgery; nutritional supplements; gene therapy; radiotherapy such as 3D-CRT; immunotoxin therapy such as ricin, monoclonal antibodies herceptin; and the like. For combination therapy, the nucleic acids of the invention are prepared in one of two ways. First, the agents are physically combined in a preparation of nucleic acid and chemotherapeutic agent, such as a mixture of a nucleic acid of the invention encapsulated in liposomes and ifosfamide in a solution for intravenous administration, wherein both agents are present in a therapeutically effective concentration (e.g., ifosfamide in solution to deliver 1000-1250 mg/m²/day and liposome-associated nucleic acid of the invention in the same solution to deliver 0.1-100 mg/kg/day). Alternatively, the agents are administered separately but simultaneously in their respective effective doses (e.g., 1000-1250 mg/m²/d ifosfamide and 0.1 to 100 mg/kg/day nucleic acid of the invention).

Gaeta *et al.*, US patents No. 5,760,062; 5,767,278; 5,770,613 have described small molecule inhibitors of human telomerase RNA (hTR) subunit.

Blasco *et al.*, 1995, Science, 269, 1267-1270 describe the synthesis and testing of antisense oligonucleotides targeted against a specific region of the mouse telomerase RNA (mTR) subunit and reported reduction in telomerase activity in mice.

Bisoffi *et al.*, 1998, Eur. J. Cancer, 34, 1242-1249 have studied the down regulation of human telomerase activity by a retrovirus vector expressing antisense RNA targeted against the hTR RNA.

Norton *et al.*, 1996, Nature Biotechnology, 14, 615-619 have reported the use of a peptide nucleic acid (PNA) molecule targeting hTR RNA to down regulate telomerase activity in human immortal breast epithelial cells.

5 Yokoyama *et al.*, 1998, Cancer Research, 58, 5406-5410 have reported the synthesis and testing of hammerhead ribozyme constructs targeting hTR RNA resulting in a decrease in the telomerase activity in Ishikawa cells.

Henderson, European Patent Application No. 666,313-A2 describes methods of identifying and cloning hTR gene for use in gene therapy approaches for creating aberrant telomeric sequences in transfected human tumor cells. A ribozyme based gene therapy
10 approach to inhibit the expression of hTR gene is described as well. The intended result of such therapies involves incurred genetic instability based on non-native telomeric sequences resulting in rapid cell death of the treated cells.

West *et al.*, US patent No. 5,489,508 describe methods for determining telomere length and telomerase activity in cells. Inhibitors of hTR RNA, including oligonucleotides
15 and/or small molecules are described.

These foregoing approaches of targeting the telomerase RNA subunit (TR) may not be very beneficial, because as demonstrated by Feng *et al.*, (Feng, J., 1995, Science, 269, 1236-1241), telomerase activity in humans does not correlate well to hTR concentration.

Collins *et al.*, International PCT publication No. WO 98/01542 describes assays for
20 the detection of telomerase activity. Four human telomerase subunit proteins are described called p140, p105, p48 and p43. In addition, hybridization probes and primers are described as inhibitors of telomerase gene function. Antibody based inhibitors of telomerase protein subunits are described.

A more attractive approach to telomerase regulation would involve the regulation of
25 human telomerase by modulating the expression of the protein subunits of the enzyme, preferably the reverse transcriptase (hTERT) subunit. Based of reconstitution experiments, hTERT and hTR represent the minimal components of telomerase. Since hTR expression does not correlate well with telomerase activity in human cells and since many human cells express hTR without telomerase activity, targeting hTERT may prove
30 more beneficial than targeting hTR. hTERT is the only component necessary to restore telomerase activity in normal human cells. A study in which the three major subunits of telomerase (hTR, TP1, and hTERT were assayed in normal and malignant endometrial

tissues determined that hTERT is a rate limiting determinant of enzymatic activity of human telomerase (Kyo, S., 1999, Int. J. Cancer, 80, 60-63). Additional protein subunits that have been isolated most likely serve only a structural role in telomerase activity, but may be important in enhancing the activity of the telomerase enzyme. As such, hTERT is one of the better targets for the ectopic regulation of telomerase activity.

Cech *et al.*, International PCT publication No. WO 98/14593 describe compositions and methods related to hTERT for diagnosis, prognosis and treatment of human diseases, for altering proliferative capacity in cells and organisms, and for screening compounds and treatments with potential use as human therapeutics.

Cech *et al.*, International PCT publication No. WO 98/14592 describe nucleic acid and amino acid sequences encoding various telomerase protein subunits and motifs of *Euplotes aediculatus*, and related sequences from *Schizosaccharomyces*, *Saccharomyces* sequences, and human telomerase. The polypeptides comprising telomeric subunits and functional polypeptides and ribonucleoproteins that contain these subunits are described as well. Cech *et al.*, International PCT Publication No. WO 98/14592, mentions in general terms the possibility of using antisense and ribozymes to down regulate the expression of human telomerase reverse transcriptase enzyme.

Identification of Potential Target Sites in Human TERT RNA

The sequence of human TERT was screened for accessible sites using a computer folding algorithm. Regions of the RNA that did not form secondary folding structures and contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables 13-17**.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human TERT RNA

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in TERT RNA, 10 hammerhead ribozyme and three G-Cleaver ribozyme sites were selected for further analysis (**Table 17**). Ribozyme target sites were chosen by analyzing sequences of Human TERT (Nakamura *et al.*, 1997 Science 277, 955-959; Genbank sequence accession number: NM_003219) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*,

1994 *J. Mol. Struct. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes for Efficient Cleavage of TERT RNA

Ribozymes were designed to anneal to various sites in the RNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman *et al.*, (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe *et al.*, (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott *et al.*, *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Wincott *et al.*, *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Table 13-17**.

Ribozyme Cleavage of TERT RNA Target *in vitro*

Ribozymes targeted to the human TERT RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example using the following procedure. The target sequences and the nucleotide location within the TERT RNA are given in **Tables 13-17**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as

substrate RNA without further purification. Alternately, substrates are 5'-³²P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming 15 µl of a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X
5 ribozyme mix to an equal volume (15 µl) of substrate RNA (maximum of 1-5 nM; 5 x 10⁵ to 1 x 10⁷ cpm) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume (30 µl) of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05%
10 xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

15 Example 2: PTP-1B

Protein tyrosine phosphorylation and dephosphorylation are important mechanisms in the regulation of signal transduction pathways that control the processes of cell growth, proliferation, and differentiation (Fantl, W. J., 1993, Annu. Rev. Biochem., 62, 453-481).
20 Cooperative enzyme classes regulate protein tyrosine phosphorylation and dephosphorylation events. These broad classes of enzymes consist of the protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTKs and PTPs can exist as both receptor-type transmembrane proteins and as cytoplasmic protein enzymes. Receptor tyrosine kinases propagate signal transduction events via extracellular receptor-ligand
25 interactions that result in the activation of the tyrosine kinase portion of the PTK in the cytoplasmic domain. Receptor-like transmembrane PTPs function through extracellular ligand binding that modulates dephosphorylation of intracellular phosphotyrosine proteins via cytoplasmic phosphatase domains. Cytoplasmic PTKs and PTPs exert enzymatic activity without receptor-mediated ligand interactions, however, phosphorylation can
30 regulate the activity of these enzymes.

Protein tyrosine phosphatase 1B, a cytoplasmic PTP, was the first PTP to be isolated in homogeneous form (Tonks, N. K., 1988, J. Biol. Chem., 263, 6722-6730), characterized (Tonks, N. K., 1988, J. Biol. Chem., 263, 6731-6737), and sequenced (Charbonneau, H., 1989, Biochemistry, 86, 5252-5256). Cytoplasmic and receptor-like PTPs both share a catalytic domain characterized by eleven conserved amino acids containing cysteine and arginine residues that are critical for phosphatase activity (Streuli, M., 1990, EMBO, 9, 2399-2407). A cysteine residue at position 215 is responsible for the covalent attachment of phosphate to the enzyme (Guan, K., 1991, J. Biol. Chem., 266, 17026-17030). The crystal structure of human PTP1B defined the phosphate binding site of the enzyme as a glycine rich cleft at the surface of the molecule with cysteine 215 positioned at the base of this cleft. The location of cysteine 215 and the shape of the cleft provide specificity of PTPase activity for tyrosine residues but not for serine or threonine residues (Barford, D., 1994, Science, 263, 1397-1404).

Receptor tyrosine kinase and protein tyrosine phosphatase localization plays a key role in the regulation of phosphotyrosine mediated signal transduction. PTP-1B activity and specificity against a panel of receptor tyrosine kinases demonstrated clear differences between substrates, suggesting that cellular compartmentalization is a determinant in defining the activity and function of the enzyme (Lammers, R., 1993, J. Biol. Chem., 268, 22456-22462). Experiments have indicated that PTP-1B is localized predominantly in the endoplasmic reticulum via its 35 amino acid carboxyterminal sequence. PTP-1B is also tightly associated with microsomal membranes with its catalytic phosphatase domain oriented towards the cytoplasm (Frangioni, J. V., 1992, Cell, 68, 545-560).

PTP-1B has been identified as a negative regulator of the insulin response. PTP-1B is widely expressed in insulin sensitive tissues (Goldstein, B. J., 1993, Receptor, 3, 1-15). Isolated PTP-1B dephosphorylates the insulin receptor *in vitro* (Tonks, N. K., 1988, J. Biol. Chem., 263, 6731-6737). PTP-1B dephosphorylation of multiple phosphotyrosine residues of the insulin receptor proceeds sequentially and with specificity for the three tyrosine residues that are critical for receptor autoactivation (Ramachandran, C., 1992, Biochemistry, 31, 4232-4238). In addition to insulin receptor dephosphorylation, PTP-1B also dephosphorylates the insulin related substrate 1 (IRS-1), a principal substrate of the insulin receptor (Lammers, R., 1993, J. Biol. Chem., 268, 22456-22462).

Microinjection of PTP1B into *Xenopus* oocytes results in the inhibition of insulin stimulated tyrosine phosphorylation of endogenous proteins, including the β -subunit of the insulin and insulin-like growth factor receptor proteins. The resulting 3 to 5 fold increase over endogenous PTPase activity also blocks the activation of an S6 peptide kinase (Cicirelli, M. F., 1990, Proc, Natl. Acad. Sci., 87, 5514-5518). Inactivation of recombinant rat PTP-1B with antibody immunoprecipitation results in the dramatic increase in insulin stimulated DNA synthesis and phosphatidylinositol 3'-kinase activity. Insulin stimulated receptor autophosphorylation and insulin receptor substrate 1 tyrosine phosphorylation are increased dramatically as well through PTP-1B inhibition (Ahmad, F., 1995, J. Biol. Chem., 270, 20503-20508).

Increased PTP-1B expression correlates with insulin resistance in hyperglycemic cultured fibroblasts. In this study, desensitized insulin receptor function was observed via impaired insulin-induced autophosphorylation of the receptor. Treatment with insulin sensitivity normalizing thiazolidine derivatives resulted in the amelioration of the hyperglycemic insulin resistance via a normalization in PTP-1B expression (Maegawa, H., 1995, J. Biol. Chem., 270, 7724-7730). A murine model of insulin resistance with a knockout of the heterotrimeric GTP-binding protein subunit $G_{i\alpha 2}$ provides a type 2 diabetes phenotype that correlates with the increased expression of PTP-1B (Moxam, C. M., 1996, Nature, 379, 840-844).

PTP-1B interacts directly with the activated insulin receptor β -subunit. An inactive homolog of PTP-1B was used to precipitate the activated insulin receptor in both purified receptor preparations and whole-cell lysates. Phosphorylation of the insulin receptor's triple tyrosine residues in the kinase domain is necessary for PTP-1B interaction. Furthermore, insulin stimulates tyrosine phosphorylation of PTP-1B (Seely, B. L., 1996, Diabetes, 45, 1379-1385). A similar study confirmed the direct interaction of PTP-1B with the insulin receptor β -subunit as well as the required multiple phosphorylation sites within the receptor and PTP-1B (Bandyopadhyay, D., J. Biol. Chem., 272, 1639-1645).

Knockout mice lacking the PTP-1B gene (both homozygous PTP-1B^{-/-} and heterozygous PTP-1B^{+/-}) have been used to study the specific role of PTP-1B relating to insulin action *in vivo*. The resulting PTP-1B deficient mice were healthy and, in the fed state, had lower blood glucose and circulating insulin levels that were half that of their

PTP-1B^{+/+} expressing littermates. These PTP-1B deficient mice demonstrated enhanced insulin sensitivity in glucose and insulin tolerance tests. At the physiological level, the PTP-1B deficient mice showed increased phosphorylation of the insulin receptor after insulin administration. When fed a high fat diet, the PTP-1B deficient mice were resistant to weight gain and remained insulin sensitive as opposed to normal PTP-1B expressing mice, who rapidly gained weight and become insulin resistant (Elchebly, M., 1999, Science, 283, 1544-1548). As such, modulation of PTP-1B expression could be used to regulate autophosphorylation of the insulin receptor and increase insulin sensitivity *in vivo*. This modulation could prove beneficial in the treatment of insulin related disease states.

In light of the above findings, particular disease states that involve PTP-1B expression include but are not limited to:

Diabetes: Both type 1 and type 2 diabetes may be treated by modulation of PTP-1B expression. Type 2 diabetes correlates to desensitized insulin receptor function (White *et al.*, 1994). Disruption of the PTP-1B dephosphorylation of the insulin receptor *in vivo* manifests in insulin sensitivity and increased insulin receptor autophosphorylation (Elchebly *et al.*, 1999). Insulin dependant diabetes, type 1, may respond to PTP-1B modulation through increased insulin sensitivity.

Obesity: Elchebly *et al.*, 1999, demonstrated that PTP-1B deficient mice were resistant to weight gain when fed a high fat diet compared to normal PTP-1B expressing mice. This finding suggests that PTP-1B modulation may be beneficial in the treatment of obesity. Ahmad *et al.*, 1997, Metab. Clin. Exp., 46, 1140-1145, describe reduced PTPs in adipose tissue and improved insulin sensitivity in obese subjects following weight loss.

Troglitazone is a non-limiting example of a pharmaceutical agent that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drugs such as anti-diabetes and anti-obesity compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. ribozymes and antisense molecules) are hence within the scope of the instant invention.

Methods have been developed to assay PTP-1B activity.

Maegawa *et al.*, 1995, J. Biol. Chem., 270, 7724-7730, describe a tissue culture model in which Rat 1 fibroblasts expressing human insulin receptors can be used to model

hyperglycemia induced insulin resistance. Maegawa *et al.* also describe assays to measure PTPase activity using labeled phosphorylated insulin receptors and by immunoenzymatic techniques.

5 Moxham *et al.*, 1996, Nature, 379, 840-844, describe a murine animal and tissue culture model employing $G\alpha 2$ deficiency to study hyperinsulinaemia, impaired glucose tolerance and resistance to insulin *in vivo*. Assays for PTPase activity and tyrosine phosphorylation of insulin-receptor substrate 1 are described.

10 Khandelwal *et al.*, 1995, Molecular and Cellular Biochemistry, 153, 87-94, describe four different animal models for studying insulin dependent and insulin resistant diabetes mellitus. These models were used to study the effect of vanadate, an insulin mimetic and PTPase inhibitor, on the insulin-stimulated phosphorylation of the insulin receptor and its tyrosine kinase activity.

Wang *et al.*, 1999, Biochim. Biophys. Acta, 1431, 14-23, describe fluorescein monophosphates as fluorogenic substrates for PTPs.

15 Various methods and compounds have been developed to inhibit protein tyrosine phosphatase activity.

Wrobel *et al.*, 1999, J. Med. Chem., 42, 3199-3202, describe PTP-1B inhibition and antihyperglycemic activity in the ob/ob mouse model by 11-arylbenzo[b]naphtho[2,3-d]furans and arylbenzo[b]naphtho[2,3-d]thiophenes.

20 Andersen *et al.*, International PCT publication No. WO 98/DK407 describe the preparation of thienopyridzinones and thienochromenones as modulators of PTPases.

Taing *et al.*, 1999, Biochemistry, 38, 3793-3803, describe potent and highly selective inhibitors of PTP-1B comprising an array of bis(aryldifluorophosphonates).

25 Ham *et al.*, 1999, Bioorg. Med. Chem. Lett., 9, 185-186, describe selective inactivation of PTP-1B by a sulfone analog of naphthoquinone.

Desmarais *et al.*, 1999, Biochem. J., 337, 219-223, describe [Difluoro(phosphono)methyl]phenylalanine-containing peptide inhibitors of PTPs.

Taylor *et al.*, 1998, Bioorg. Med. Chem., 6, 2235, describe potent non-peptidyl inhibitors of PTP-1B.

30 Kotoris *et al.*, 1998, Bioorg. Med. Chem. Lett., 8, 3275-3280, describe novel phosphate mimetics for the design of non-peptidyl inhibitors of PTPs.

Groves *et al.*, 1998, Biochemistry, 37, 17773-17783, describe the structural basis for PTP-1B inhibition by the phosphotyrosine peptide mimetics (difluoronaphthylmethyl)phosphonic acid and the fluoromalonyl tyrosines with complexed crystal structures.

- 5 Yao *et al.*, 1998, Bioorgl Med. Chem., 6, 1799-1810, describe the structure-based design and synthesis of small molecule PTP-1B inhibitors comprising novel naphthyldifluoromethyl phosphonic acids 1 and 2.

Taylor *et al.*, 1998, Bioorg. Med. Chem., 6, 1457-1468, describe potent non-peptidyl inhibitors of PTP-1B.

- 10 Desmarais *et al.*, 1998, Arch. Biochem. Biophys., 354, 225-231, describe inhibition of PTP-1B and CD45 by sulfotyrosyl peptides.

Mjalli *et al.*, application US 96-766114, cont. in part of US patent No. 543,630, describe the preparation of heterocyclic compounds as modulators of proteins with phosphotyrosine recognition units.

- 15 Wang *et al.*, 1998, Bioorg. Med. Chem. Lett., 8, 345-350, describe naphthalenebis[α,α -difluoromethylenephosphonates] as potent inhibitors of PTPs.

Rice *et al.*, 1997, Biochemistry, 36, 15965-15974, describe a targeted library of small molecule tyrosine and dual-specificity phosphatase inhibitors with random side chain variation from a rational core design.

- 20 Olefsky, International PCT publication No. WO 97/US2752 describes a method and phosphopeptides used for the treatment of insulin resistance based on the association of PTP-1B with the activated insulin receptor. Also included is a method for determining whether a compound inhibits PTP-1B binding to the insulin receptor.

- 25 Huyer *et al.*, 1997, J. Biol. Chem., 272, 843-851, describe the mechanism of inhibition of PTPases by vanadate and pervanadate.

Burke *et al.*, 1996, Biochemistry, 35, 15989-15996, describe the structure-based design of PTP-1B inhibitors.

- 30 Tonks *et al.*, International PCT publication No. WO 97/US13016, describe substrate-trapping protein PTPase mutants for identification of tyrosine-phosphorylated protein substrates and their clinical uses.

The human genome is thought to contain up to 100 PTPases, each varying slightly in chemistry but vastly in function. Compounds designed to inhibit PTP-1B activity specifically by covalent binding to or modification of PTP-1B have the potential for multiple side effects. Conventional drug substances that will potently suppress PTP-1B activity with few or no side effects from interaction with other PTPs are difficult to envision. A more attractive approach to PTP-1B modulation would involve the specific regulation of PTP-1B expression with oligonucleotides.

Identification of Potential Target Sites in Human PTP-1B RNA

The sequence of human PTP-1B was screened for accessible sites using a computer folding algorithm. Regions of the RNA that did not form secondary folding structures and contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables 3-8**.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human PTP-1B RNA

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in PTP-1B RNA, 10 hammerhead ribozyme, five NCH and three G-Cleaver ribozyme sites were selected for further analysis (**Table 8**). Ribozyme target sites were chosen by analyzing sequences of Human PTP-1B (Genbank accession number M33689) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, **86**, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes for Efficient Cleavage of PTP-1B RNA

Ribozymes were designed to anneal to various sites in the RNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 J. Am. Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., supra, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; see Wincott et al., supra; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Tables 3-8**.

Ribozyme Cleavage of PTP-1B RNA Target *in vitro*

Ribozymes targeted to the human PTP-1B RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example, using the following procedure. The target sequences and the nucleotide location within the PTP-1B RNA are given in **Tables 3-8**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'- ^{32} P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess.

The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

Example 3: MetAP-2

Methionyl aminopeptidases are metalloproteases that are known to possess post-translational enzymatic activity by hydrolytically cleaving amino-terminal methionine residues from nascent peptide substrates in a non-processive manner (Kendall, R. L., 1992, J. Biol. Chem., 267, 20667-20673). This family of enzymes is divided into two classes (type 1 and type 2) based on differences in sequence, although the overall structure of the two classes are similar (Liu, S., 1998, Science, 282, 1324-1327). Methionine aminopeptidase expression appears to be involved in the control of cellular proliferation. Deletion of the MetAP gene from *E. Coli* is lethal (Chang, S. Y., 1989, J. Bacteriol., 171, 4071-4072). In *Saccharomyces cerevisiae*, deletion of the gene that codes for either MetAP-1 or 2 results in a slow growth phenotype while deletion of both genes is lethal (Li, X., 1995, Proc. Natl. Acad. Sci., 92, 12357-12361). (Human methionine aminopeptidase-1, MetAP-1, accession No. P53582).

The aminopeptidase function of this class of enzymes may serve a regulatory role in activating signal peptides in conjunction with N-myristoyl transferase (NMT) activity. NMT is expressed from a lethal gene in yeast (Duronio, R. J., 1989, Science, 243, 796-800). NMT is responsible for amino-terminal ligation of myristic acid onto nascent peptides and cannot act on peptides with an amino-terminal methionine residue (Resh, M. D., 1996, Cell. Signal., 8, 403-412). Myristoylation of proteins correlates to intracellular localization events that may determine why certain signaling proteins are dependent on NMT for activity (Taunton, J., 1997, Chemistry & Biology, 4, 493-496). Protein tyrosine kinase Src is dependant on myristoylation for activity and has been identified as an upstream regulator of human vascular endothelial growth factor (VEGF) expression

through hypoxic induction in solid tumors (Mukhopadhyay, D., 1995, Nature, 375, 577-581). MetAPs may therefore regulate the activation of signal peptides (such as VEGF) through cotranslational modification of nascent peptides with NMT. Disruption of protein myristoylation by MetAP inhibition could result in the improper localization of signaling proteins resulting in inhibition of cell growth. (Human N-myristoyltransferase, hNMT, accession No. AF043324.)

Fumagillin, a sesquiterpene diepoxide metabolite of the fungus *Aspergillus fumigatus*, and a related compound TNP-470, are strong inhibitors of growth in cultured endothelial cells. The antiproliferative and angiostatic activity of fumagillin was originally discovered by the serendipitous contamination of *Aspergillus fumigatus* in an endothelial cell culture dish in which cells closest to the fungal colony displayed growth inhibition. Synthetic analogs of fumagillin were later synthesized resulting in the discovery of TNP-470, which is 50 times more potent of an inhibitor than fumagillin and is less toxic in mice (Ingber, D., 1990, Nature, 348, 555-557). Treatment of endothelial cells with these compounds results in late G1 phase arrest. TNP-470 inhibits the signaling pathway of retinoblastoma gene product phosphorylation, cyclin dependent kinases cdk2 and cdk4 activation, and cyclins E and A expression (Abe, J., 1994, Cancer Res., 54, 3407-3412). TNP-470 has also been shown to potently inhibit endothelial cell proliferation induced by the growth factors VEGF and bFGF (Toi, M., 1994, Oncology Reports, 1, 423-426).

The bifunctional protein MetAP-2 has been identified as the molecular target for fumagillin and related compounds that demonstrate antiproliferative activity in endothelial cells. The use of affinity chromatography with a fumagillin-biotin conjugate resulted in the isolation of a 67-kDa mammalian protein through covalent interaction with the bound substrate. Analysis of digested peptide fragments from the isolated protein revealed MetAP-2 as the covalently bound substrate. Subsequent growth inhibition studies in yeast utilizing MetAP-1 and MetAP-2 deletion strains determined that MetAP-2 is selectively inhibited by fumagillin *in vivo* (Sin, N., 1997, Proc. Natl. Acad. Sci., 94, 6099-6103). A similar study with TNP-470 and ovalicin, another potent inhibitor of neovascularization, determined that MetAP-2 is the molecular target for these fumagillin-related compounds (Griffith, E. C., 1997, Chemistry & Biology, 4, 461-471).

MetAP-2 expression correlates with cellular growth. Non-dividing cells in culture have no detectable levels of the 67-kDa MetAP-2 protein by immunoassay. MetAP-2 has been shown to affect translational initiation by association with eukaryotic initiation factor 2 α (eIF-2 α) (Ray, M. K., 1992, Proc. Natl. Acad. Sci., 89, 539-543). The binding of MetAP-2 with eIF-2 α inhibits the heme-regulated inhibitor kinase (HRI) phosphorylation of eIF-2 α *in vitro* in reticulocyte lysates (Datta, B., 1988, Proc. Natl. Acad. Sci., 85, 3324-3328). MetAP-2/eIF-2 α binding results in the partial reversal of protein synthesis inhibition by double stranded RNA dependent kinase mediated phosphorylation *in vivo* (Wu, S., 1996, Biochemistry, 35, 8275-8280). Griffith *et al.* also determined that covalent binding of TNP-470 and ovalicin, while potently inhibiting methionine aminopeptidase type 2 activity specifically, did not affect the regulatory activity of MetAP-2 on eIF-2 α . This finding by Griffith *et al.* rules out the possibility that control of eIF-2 α phosphorylation by MetAP-2 is responsible for the inhibition of endothelial cell proliferation by fumagillin related compounds.

Particular angiogenesis related degenerative and disease states that can be associated with MetAP expression modulation include but are not limited to:

Cancer: Solid tumors are unable to grow or metastasize without the formation of new blood vessels (Hanahan, D., 1996, Cell, 86, 353-364). Inhibition of angiogenesis via MetAP modulation can potentially be used to treat a wide variety of cancers.

Diabetic retinopathy and age related macular degeneration: Ocular neovascularization is observed in diabetic retinopathy, which is mediated by up-regulation of VEGF (Adamis, A. P., 1994, Amer. J. Ophthal., 118, 445-450). The requirement of protein kinase Src in hypoxia induced VEGF expression (Mukhopadhyay, D., 1995, Nature, 375, 577-581) indicates that MetAP modulation of aminopeptidase activity can potentially be used to treat conditions involving ocular neovascularization.

Arthritis: The ingrowth of a vascular pannus in arthritis may be mediated by the overexpression of angiogenic factors from infiltrating inflammatory cells, macrophages, and immune cells (Peacock, D. J., 1992, J. exp. Med., 175, 1135-1138). Angiogenesis inhibition through MetAP modulation can potentially be used to treat arthritis.

Psoriasis: Angiogenesis has been implicated in psoriasis due to overexpression of the angiogenic polypeptide interleukin-8 and decreased expression of the angiogenesis inhibitor thrombospondin (Nickoloff, B. J., 1994, Amer. J. Pathol. 44, 820-828).

Angiogenesis inhibition through MetAP modulation can potentially be used to treat psoriasis.

Female reproduction: Angiogenesis in the female reproductive system has been implicated in several disorders of the reproductive tract (Reynolds, L. P., 1992, FASEB, 6, 886-892). Modulation of angiogenesis through control of MetAP may have various applications in the area of female reproduction and fertility.

Various methods have been developed to assay MetAP activity.

Griffith *et al.*, 1998, Proc. Natl. Acad. Sci., 95, 15183-15188, describe an enzymatic assay for MetAP-2 activity *in vitro* and an endothelial cell culture proliferation assay for MetAP-2 activity *in vivo*.

Weber *et al.*, 1999, International PCT publication No. WO 98/US-21231 describe novel fluorescent reporter molecules and an enzymatic assay that can be used for determining the activity of MetAP-2 for drug screening and determining the chemosensitivity of human cancer cells to treatment with chemotherapeutic drugs.

Larrabee, J. A. *et al.*, 1999, Anal. Biochem, 269, 194-198, describe the use of a high-pressure liquid chromatographic (HPLC) method for assaying MetAP-2 activity with application to the study of enzymic inactivation.

Quantitative methods have been developed to assay the efficacy of antiangiogenic therapies.

Wantanabe *et al.*, 1992, Molec. Biol. Cell, 3, 324a, describe the quantitation of angiogenic peptides (bFGF) in human serum as a prognostic test for breast cancer.

Nguyen *et al.*, 1994, J. Natn. Cancer Inst., 86, 356-361, describe the quantitation of angiogenic peptides (bFGF) in the urine of patients with a wide spectrum of cancers.

Li *et al.*, 1994, The Lancet, 344, 82-86, describe the quantitation of angiogenic peptides (bFGF) in the cerebrospinal fluid of children with brain tumors. This work also describes determining the extent of neovascularization in histological sections by utilizing microvessel count.

The present body of knowledge in angiogenesis research indicates the need for compounds that can modulate MetAP activity for research, diagnostic, trait alteration, animal health and therapeutic use.

Griffith *et al.*, International PCT publication No. WO 9856372 describe small molecule inhibitors of MetAP2 and uses thereof.

D'Amato *et al.*, International PCT publication No. WO 9805293 describe the use of AGM-1470 (TNP-470) as an angiogenesis inhibitor for use in regulating the female reproductive system and for treating diseases of the reproductive tissue.

Davidson *et al.*, US patent No. 5,801,146 describe a compound and method for inhibiting angiogenesis using mammalian kringle 5 protein.

Cao *et al.*, US patent No. 5,854,221 describe a protein-based endothelial cell proliferation inhibitor and its method of use.

Chang *et al.*, US patent No. 5,888,796 describe a clone of a nucleotide sequence encoding a protein having two functions comprising methionine aminopeptidase activity and anti eIF-2 phosphorylation activity.

Wang *et al.*, 1998, Proc. Am. Assoc. Cancer Res., 39, 98 (abstr.) describe blocked proliferation of human endothelial cells by human MetAP-2 antisense oligonucleotides.

A rat corneal model has been developed to study ribozyme inhibition of VEGF receptor-mediated angiogenesis (Pavco, P. A., 1999, Nucleic Acids Research, 27, 2569-2577). A similar study employing MetAP-2 inhibition could be used to study ribozyme based inhibition of MetAP-2 induced angiogenesis *in vivo*.

Identification of Potential Target Sites in Human MetAP-2 RNA

The sequence of human MetAP-2 was screened for accessible sites using a computer-folding algorithm. Regions of the RNA that did not form secondary folding structures and contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in Tables 9-12.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human MetAP-2 RNA

To test whether the sites predicted by the computer-based RNA folding algorithm corresponded to accessible sites in MetAP-2 RNA, 11 hammerhead ribozyme, 4 NCH and three G-Cleaver ribozyme sites were selected for further analysis (Table 12). Ribozyme

target sites were chosen by analyzing sequences of Human MetAP-2 (Genbank accession number HSU29607) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, **86**, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes for Efficient Cleavage of MetAP-2 RNA

Ribozymes were designed to anneal to various sites in the RNA message. The binding arms are complementary to the target site sequences described above. The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman *et al.*, (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe *et al.*, (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott *et al.*, *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Table 9-12**.

Ribozyme Cleavage of MetAP-2 RNA Target *in vitro*

Ribozymes targeted to the human MetAP-2 RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for

example, using the following procedure. The target sequences and the nucleotide location within the MetAP-2 RNA are given in **Tables 9-12**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [a-³²P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'-³²P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

Example 4: BACE, ps-1, ps-2

Alzheimer's disease (AD) is a progressive, degenerative disease of the brain which affects approximately 4 million people in the United States alone. An estimated 14 million Americans will have Alzheimer's disease by the middle of the next century if no cure or definitive prevention of the disease is found. Nearly one out of ten people over age 65 and nearly half of those over 85 have Alzheimer's disease. Alzheimer's disease is not confined to the elderly, a small percentage of people in their 30's and 40's are afflicted with early onset AD. Alzheimer's disease is the most common form of dementia, and amounts to the third most expensive disease in the US following heart disease and cancer. An estimated 100 billion dollars are spent annually on Alzheimer's disease (National Alzheimer's Association, 1999).

Alzheimer's disease is characterized by the progressive formation of insoluble plaques and vascular deposits in the brain consisting of the 4 kD amyloid β peptide ($A\beta$). These plaques are characterized by dystrophic neurites that show profound synaptic loss, neurofibrillary tangle formation, and gliosis. $A\beta$ arises from the proteolytic cleavage of the large type I transmembrane protein, β -amyloid precursor protein (APP) (Kang *et al.*, 1987, *Nature*, 325, 733). Processing of APP to generate $A\beta$ requires two sites of cleavage by a β -secretase and a γ -secretase. β -secretase cleavage of APP results in the cytoplasmic release of a 100 kD soluble amino-terminal fragment, APPs β , leaving behind a 12 kD transmembrane carboxy-terminal fragment, C99. Alternately, APP can be cleaved by a α -secretase to generate cytoplasmic APPs α and transmembrane C83 fragments. Both remaining transmembrane fragments, C99 and C83, can be further cleaved by a γ -secretase, leading to the release and secretion of Alzheimer's related $A\beta$ and a non-pathogenic peptide, p3, respectively (Vassar *et al.*, 1999, *Science*, 286, 735-741). Early onset familial Alzheimer's disease is characterized by mutant APP protein with a Met to Leu substitution at position P1, characterized as the "Swedish" familial mutation (Mullan *et al.*, 1992, *Nature Genet.*, 1, 345). This APP mutation is characterized by a dramatic enhancement in β -secretase cleavage (Citron *et al.*, 1992, *Nature*, 360, 672).

The identification of β -secretase, and γ -secretase constituents involved in the release of β -amyloid protein is of primary importance in the development of treatment strategies for Alzheimer's disease. Characterization of α -secretase is also important in this regard since α -secretase cleavage may compete with β -secretase cleavage resulting in non-pathogenic vs. pathogenic protein production. Involvement of the two metalloproteases, ADAM 10, and TACE has been demonstrated in α -cleavage of AAP (Buxbaum *et al.*, 1999, *J. Biol. Chem.*, 273, 27765, and Lammich *et al.*, 1999, *Proc. Natl. Acad. Sci. U.S.A.*, 96, 3922). Studies of γ -secretase activity have demonstrated presenilin dependence (De Strooper *et al.*, 1998, *Nature*, 391, 387, and De Strooper *et al.*, 1999, *Nature*, 398, 518), and as such, presenilins have been proposed as γ -secretase even though presenilin does not present proteolytic activity (Wolfe *et al.*, 1999, *Nature*, 398, 513).

Recently, Vassar *et al.*, 1999, *supra* reported β -secretase cleavage of AAP by the transmembrane aspartic protease beta site APP cleaving enzyme, BACE. While other potential candidates for β -secretase have been proposed (for review see Evin *et al.*, 1999, *Proc. Natl. Acad. Sci. U.S.A.*, 96, 3922), none have demonstrated the full range of characteristics expected from this enzyme. Vassar *et al.*, *supra*, demonstrate that BACE expression and localization are as expected for β -secretase, that BACE overexpression in cells results in increased β -secretase cleavage of APP and Swedish APP, that isolated BACE demonstrates site specific proteolytic activity on APP derived peptide substrates, and that antisense mediated endogenous BACE inhibition results in dramatically reduced β -secretase activity.

Current treatment strategies for Alzheimer's disease rely on either the prevention or the alleviation of symptoms and/or the slowing down of disease progression. Two drugs approved in the treatment of Alzheimer's, donepezil (Aricept®) and tacrine (Cognex®), both cholinomimetics, attempt to slow the loss of cognitive ability by increasing the amount of acetylcholine available to the brain. Antioxidant therapy through the use of antioxidant compounds such as alpha-tocopherol (vitamin E), melatonin, and selegiline (Eldepryl®) attempt to slow disease progression by minimizing free radical damage. Estrogen replacement therapy is thought to incur a possible preventative benefit in the development of Alzheimer's disease based on limited data. The use of anti-inflammatory drugs may be associated with a reduced risk of Alzheimer's as well. Calcium channel blockers such as Nimodipine® are considered to have a potential benefit in treating Alzheimer's disease due to protection of nerve cells from calcium overload, thereby prolonging nerve cell survival. Nootropic compounds, such as acetyl-L-carnitine (Alcar®) and insulin, have been proposed to have some benefit in treating Alzheimer's due to enhancement of cognitive and memory function based on cellular metabolism.

Whereby the above treatment strategies may all improve quality of life in Alzheimer's patients, there exists an unmet need in the comprehensive treatment and prevention of this disease. As such, there exists the need for therapeutics effective in reversing the physiological changes associated with Alzheimer's disease, specifically, therapeutics that can eliminate and/or reverse the deposition of amyloid β peptide. The use of compounds to modulate the expression of proteases that are instrumental in the

release of amyloid β peptide, namely β -secretase (BACE), and γ -secretase (presenilin), is of therapeutic significance.

Tsai *et al.*, 1999, Book of Abstracts, 218th ACS National Meeting, New Orleans, Aug 22-26, describe substrate-based alpha-aminoisobutyric acid derivatives of difluoro ketone peptidomimetic inhibitors of amyloid β peptide through γ -secretase inhibition.

Czech *et al.*, International PCT publication No. WO/9921886, describe peptides capable of inhibiting the interaction between presenilins and the β -amyloid peptide or its precursor for therapeutic use.

Fournier *et al.*, International PCT publication No. WO/9916874, describe human brain proteins capable of interacting with presenilins and cDNAs encoding them toward therapeutic use.

St. George-Hyslop *et al.*, International PCT publication No. WO/9727296, describe genes for proteins that interact with presenilins and their role in Alzheimer's disease toward therapeutic use.

Vassar *et al.*, 1999, *Science*, 286, 735-741, describe specific antisense oligonucleotides targeting BACE, used for inhibition studies of endogenous BACE expression in 101 cells and APPsw cells via lipid mediated transfection.

Vassar *et al.*, 1999, *Science*, 286, 735-741, describe a cell culture model for studying BACE inhibition. Specific antisense nucleic acid molecules targeting BACE mRNA were used for inhibition studies of endogenous BACE expression in 101 cells and APPsw (Swedish type amyloid precursor protein expressing) cells via lipid mediated transfection. Antisense treatment resulted in dramatic reduction of both BACE mRNA by Northern blot analysis, and APPs β sw ("Swedish" type β -secretase cleavage product) by ELISA, with maximum inhibition of both parameters at 75-80%. This model was also used to study the effect of BACE inhibition on amyloid β -peptide production in APPsw cells.

Games *et al.*, 1995, *Nature*, 373, 523-527, describe a transgenic mouse model in which mutant human familial type APP (Phe 717 instead of Val) is overexpressed. This model results in mice that progressively develop many of the pathological hallmarks of Alzheimer's disease, and as such, provides a model for testing therapeutic drugs.

Particular degenerative and disease states that can be associated with BACE expression modulation include but are not limited to Alzheimer's disease and dementia.

Donepezil, tacrine, selegeline, and acetyl-L-carnitine are non-limiting examples of pharmaceutical agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drugs such as diuretic and antihypertensive compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. ribozymes and antisense molecules) are hence within the scope of the instant invention.

Identification of Potential Target Sites in Human BACE RNA

The sequence of human BACE was screened for accessible sites using a computer-folding algorithm. Regions of the RNA that did not form secondary folding structures and contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables 18-23**.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human BACE RNA

Ribozyme target sites were chosen by analyzing sequences of Human BACE (Genbank sequence accession number: AF190725) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of BACE RNA

Ribozymes and antisense constructs were designed to anneal to various sites in the RNA message. The binding arms of the ribozymes are complementary to the target site sequences described above, while the antisense constructs are fully complimentary to the target site sequences described above. The ribozymes and antisense constructs were

chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe et al., (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott et al., *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes and antisense constructs were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes and antisense constructs were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Wincott et al., *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes and antisense constructs used in this study are shown below in **Table 18-23**.

Ribozyme Cleavage of BACE RNA Target *in vitro*

Ribozymes targeted to the human BACE RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example, using the following procedure. The target sequences and the nucleotide location within the BACE RNA are given in **Tables 18-23**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'- ^{32} P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM

EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is
5 determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

Example 5: Phospholamban

Cardiac disease leading to heart failure is the leading cause of combined morbidity
10 and mortality in the developed world. Nearly twenty million people worldwide suffer from heart failure related disease. An estimated five million Americans are afflicted with congestive heart failure (CHF), with 400,000 new cases diagnosed each year. In the US, cardiac disease associated failure results in approximately 40,000 deaths per year, and is associated with an additional 250,000 deaths (Harnish, 1999, *Drug & Market*
15 *Development*, 10, 114-119). Heart failure related disease represents a major public health issue due to an overall increase in prevalence and incidence in aging populations with a greater proportion of survivors of acute myocardial infarction (AMI) (Kannel *et al.*, 1994, *Br. Heart. J.*, 72 (suppl), 3). Heart failure related disease represents the most common reason for hospitalization of elderly patients in the US. The resulting life expectancy of
20 these patients is less than that of many common cancers, with five year survival rates for men and women at only 25% and 38% respectively, and with one year mortality rates for severe heart failure at 50% (Ho *et al.*, 1993, *Circulation*, 88, 107).

Heart disease is characterized by a progressive decrease in cardiac output resulting from insufficient pumping activity of the diseased heart. The resulting venous back-
25 pressure results in peripheral and pulmonary dysfunctional congestion. The heart responds to a variety of mechanical, hemodynamic, hormonal, and pathological stimuli by increasing muscle mass in response to an increased demand for cardiac output. The resulting transformation of heart tissue (myocardial hypertrophy) can arise as a result of genetic, physiologic, and environmental factors, and represents an early indication of
30 clinical heart disease and an important risk factor for subsequent heart failure (Hunter and Chien, 1999, *New England J. of Medicine*, 99, 313-322).

Coronary heart disease is a predominant factor in the development of the cardiac disease state, along with prior AMI, hypertension, diabetes mellitus, and valvular heart disease. Diagnosis of cardiac disease includes determination of coronary heart disease associated left ventricular systolic dysfunction (LVSD) and/or left ventricular diastolic dysfunction (LVDD) by echocardiographic imaging (Cleland, 1997, *Dis Management Health Outcomes*, 1, 169). Promising diagnosis may also rely on assaying atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) concentrations. ANP and BNP levels are indicative of the level of ventricular dysfunction (Davidson *et al.*, 1996, *Am. J. Cardiol.*, 77, 828).

Current treatment strategies for cardiac disease associated failure are varied. Diuretics are often used to reduce pulmonary edema and dyspnea in patients with fluid overload, and are usually used in conjunction with angiotensin converting enzyme (ACE) inhibitors for vasodilation. Digoxin is another popular choice for treating cardiac disease as an inotropic agent, however, doubts remain concerning the long-term efficacy and safety of Digoxin (Harnish, 1999, *Drug & Market Development*, 10, 114-119). Carvedilol, a beta-blocker, has been introduced to complement the above treatments in order to slow down the progression of cardiac disease. Antiarrhythmic agents can be used in order to reduce the risk of sudden death in patients suffering from cardiac disease. Lastly, heart transplants have been effective in the treatment of patients with advanced stages of cardiac disease, however, the limited supply of donor hearts greatly limits the scope of this treatment to the broad population (Harnish, 1999, *Drug & Market Development*, 10, 114-119).

Whereby the above treatment strategies can all improve morbidity and mortality associated with cardiac disease, the only existing definitive approach to curing the diseased heart is replacement by transplant. Even a healthy, transplanted heart can become diseased in response to the various stresses of mechanical, hemodynamic, hormonal, and pathological stimuli associated with extrinsic risk factors. As such there exists the need for therapeutics effective in reversing the physiological changes associated with cardiac disease.

Myocardial hypertrophy and apoptosis are the underlying degenerative process associated with cardiac hypertrophy and failure. A variety of signaling pathways are involved in the progression of myocardial hypertrophy and myocardial apoptosis. Genetic

studies have been instrumental in elucidating these pathways and their involvement in cardiac disease through *in vitro* assays of cardiac muscle cells and *in vivo* studies of genetically engineered animals.

Studies in which the expression of specific genes have been altered in cardiac myocytes have shown that specific peptide hormones, growth factors, and cytokines can activate various features of the hypertrophic response (Hunter and Chien, 1999, *New England J of Medicine*, 99, 313-322). Particular substances that have been characterized from these studies include potential therapeutic and molecular targets involved in heart failure. Hunter *et al.*, in Chien, KR, ed. *Molecular basis of heart disease: a companion to Braunwald's Heart Disease*, Philadelphia: W.B. Saunders, 1999:211-250, describe classes of therapeutic and molecular targets involved in heart failure including:

1. Endothelin 1 and angiotensin II receptor antagonists, and antagonists of ras, p38, and c-jun N-terminal kinase (JNK) for inhibition of pathologic hypertrophy.
2. Insulin like growth factor I and growth hormone receptor stimulation for promotion of physiologic hypertrophy.
3. beta-1-adrenergic receptor blockers for inhibition of neurohumoral over stimulation.
4. Phospholamban and Sarcolipin small molecule inhibitors for relief of sarcoplasmic reticulum calcium ATPase inhibition to provide enhancement of myocardial contractile and relaxation responses.
5. Small molecule inhibitors of β -adrenergic receptor kinase to counteract the desensitization of G protein coupled receptor kinases in order to provide enhancement of myocardial contractile and relaxation responses.
6. Enhancement of angiogenic growth factors (VEGF, FGF-5) for relief of energy deprivation in cardiac tissues.
7. Promoters of myocyte survival including gp 130 ligands (cardiotrophin 1), and Neuregulin for the inhibition of apoptosis of myocytes.
8. Inhibitors of apoptosis such as Caspase inhibitors for the inhibition of apoptosis of myocytes.
9. Inhibitors of cytokines such as TNF-alpha for the inhibition of apoptosis of myocytes.

Congestive heart failure, heart failure, dilated cardiomyopathy and pressure overload hypertrophy are nonlimiting examples of disorders and disease states that can be associated with the above classes of molecular targets.

The failure of cardiac contractile performance leading to cardiac disorders and disease, governed by impairment of cardiac excitation/contraction coupling, points to the importance of the signaling pathways involved in this process. The release and uptake of cytosolic Ca^{2+} by the sarcoplasmic reticulum plays an integral role in each cycle of cardiac contraction and excitation (Minamisawa *et al.*, 1999, *Cell*, 99, 313-322). The process of Ca^{2+} reuptake is mediated by the cardiac sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a). SERCA2a activity is regulated by phospholamban, a p52 muscle specific sarcoplasmic reticulum phosphoprotein (Koss *et al.*, 1996, *Circ. Res.*, 79, 1059-1063, and Simmerman *et al.*, 1998, *Physiol. Rev.*, 78, 921-947). In its active, unphosphorylated state, phospholamban is a potent inhibitor of SERCA2a activity. Phosphorylation of phospholamban at serine 16 by cyclic AMP-dependent protein kinase (PKA) or calmodulin kinase, results in the inhibition of phospholamban interaction with SERCA2a. This phosphorylation event is predominantly responsible for the proportional increase in the rate of Ca^{2+} uptake into the sarcoplasmic reticulum and resultant ventricular relaxation (Tada *et al.*, 1982, *Mol. Cell. Biochem.*, 46, 73-95, and Luo *et al.*, 1998, *J. Biol. Chem.*, 273, 4734-4739).

Since a proportional decrease in Ca^{2+} uptake is a hallmark feature of heart failure (Sordahl *et al.*, 1973, *Am. J. Physiol.*, 224, 497-502) and since an increase in the relative ratio of phospholamban to SERCA2a is an important determinant of sarcoplasmic reticulum dysfunction in heart failure (Hasenfuss, 1998, *Cardiovasc. Res.*, 37, 279-289), the targeting of phospholamban and related regulatory factors as therapeutic targets for heart disorders should prove valuable for cardiac indications.

Pystynen *et al.*, International PCT publication No. WO 99/00132, describe bisethers of 1-oxa, aza and thianaphthalen-2-ones as small molecule inhibitors of phospholamban for increasing coronary flow via direct dilation of the coronary arteries.

Pystynen *et al.*, International PCT publication No. WO 99/15523, describe bisethers of 1-oxa, aza and thianaphthalen-2-ones as small molecule inhibitors of phospholamban that are useful for treating heart failure.

The efficacy of the above mentioned treatment strategies is limited. Small molecule inhibition of a molecular target is often limited by toxicity, which can restrict dosing and overall efficacy.

He *et al.*, 1999, *Circulation*, 100, 974-980, describe endogenous expression of mutant phospholamban and phospholamban antisense RNA to investigate the corresponding effect on SERCA2a activity and cardiac myocyte contractility.

A more attractive approach to the treatment of heart disease would involve the use of
5 ribozymes and/or antisense constructs to modulate the expression of target molecules involved in heart failure. The use of nucleic acid molecules of the instant invention permits highly specific regulation of the molecular targets of interest, including phospholamban (PLN) (GenBank accession No. NM_002667), sarcolipin (SLN) (GenBank accession No. NM_003063), angiotensin II receptor (GenBank accession No.
10 U20860), endothelin 1 receptor (GenBank accession No. NM_001957), K-ras (GenBank accession No. NM_004985), p38 (GenBank accession No. AF092535), c-jun N-terminal kinase (GenBank accession No. NM_002750, L31951, NM_002753), growth hormone receptor (GenBank accession No. NM_000163), insulin-like growth factor I receptor (GenBank accession No. NM_000875), beta-1-adrenergic receptor (GenBank accession
15 No. NM_000024), β 1-adrenergic receptor kinase (GenBank accession No. NM_001619, NM_005160), VEGF receptor (GenBank accession No. U43368, M27281 X15997), fibroblast growth factor 5 (GenBank accession No. NM_004464), cardiotrophin I (GenBank accession No. NM_001330), neuregulin (GenBank accession No. AF009227), TNF-alpha (GenBank accession No. X02910 X02159), PI3 kinase (GenBank accession
20 No. NM_006218, NM_006219, U86453, NM_002649, M61906), and AKT kinase (GenBank accession No. NM_005163, M77198).

Various methods have been developed to assay phospholamban activity *in vitro* and *in vivo*. Holt *et al.*, 1999, *J. Mol. Cell. Cardiol.*, 31, 645-656, describe a cell culture model in which thyroid hormone control of contraction and the Ca^{2+} -ATPase/phospholamban
25 complex is studied in adult rat ventricular myocytes. Slack *et al.* 1997, *J. Biol. Chem.*, 272, 18862-18868, describe studies in which the ectopic expression of phospholamban in mouse fast-twitch skeletal muscle cells alters sarcoplasmic reticulum Ca^{2+} transport and muscle relaxation. MacLennan *et al.*, 1996, *Soc. Gen. Physiol. Ser.*, 51, 89-103, in a review of regulatory interactions between calcium ATPases and phospholamban describe
30 phospholamban/ Ca^{2+} -ATPase interactions in protein expressed in heterologous cell culture experiments. Cornwell *et al.*, 1991, *Mol. Pharmacol.*, 40, 923-931, describe the

regulation of sarcoplasmic reticulum protein phosphorylation by localized cyclic GMP-dependent protein kinase in vascular smooth muscle cells.

Minamisawa *et al.*, 1999, *Cell*, 99, 313-322, describe a phospholamban knockout mouse model which affords protection from induced dilated cardiomyopathy. Dillmann *et al.*, 1999, *Am. J. Cardiol.*, 83, 89H-91H, describe a transgenic rat model for the study of altered expression of calcium regulatory proteins, including phospholamban, and their effect on myocyte contractile response. LekanneDeprez *et al.*, 1998, *J. Mol. Cell.*

Cardiol., 30, 1877-1888, describe a rat pressure-overload model to investigate alterations in gene expression of phospholamban, atrial natriuretic peptide (ANP), sarcoplasmic

endoplasmic reticular calcium ATPase 2 (SERCA2), collagen III α 1, and calsequestrin (CSQ). Jones *et al.*, 1998, *J. Clin. Invest.*, 101, 1385-1393, describe a mouse model for investigating the regulation of calcium signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. In this study, the upregulation and downregulation of calcium uptake and release proteins were determined, including phospholamban. Lorenz

et al., 1997, *Am J. Physiol.*, 273, 6, describe a mouse model for the study of regulatory effects of phospholamban on cardiac function in intact mice. This study makes use of animal models with altered levels of phospholamban to permit *in vivo* evaluation of the physiological role of phospholamban. Arai *et al.*, 1996, *Saishin Igaku*, 51, 1095-1104, presents a review article of gene targeted animal models expressing cardiovascular

abnormalities. The study of phospholamban and other protein expression modification effects in mice is presented. Wankerl *et al.*, 1995, *J. Mol. Med.*, 73, 487-496, presents a review article describing the study of calcium transport proteins in the nonfailing and failing heart. Animal models investigating the major calcium handling myocardial proteins, including phospholamban, are described. These models, as well as others, may

be used to evaluate the effect of treatment with nucleic acid molecules of the instant invention on cardiac function. Endpoints may be, but are not limited to, left ventricular pressure, left ventricular pressure as a function of time (LVdP/dt), and mean arterial blood pressure. Endpoints will be evaluated under basal and stimulated (cardiac load) conditions.

Particular degenerative and disease states that can be associated with phospholamban expression modulation include but are not limited to congestive heart failure, heart failure, dilated cardiomyopathy and pressure overload hypertrophy:

Digoxin, Bendrofluazide, Dofetilide, and Carvedilol are non-limiting examples of pharmaceutical agents that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drugs such as diuretic and antihypertensive compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (*e.g.* ribozymes and antisense molecules) are hence within the scope of the instant invention.

Identification of Potential Target Sites in Human phospholamban RNA

The sequence of human phospholamban was screened for accessible sites using a computer folding algorithm. Regions of the RNA that did not form secondary folding structures and contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables 24-30**.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human phospholamban RNA

Ribozyme target sites were chosen by analyzing sequences of Human phospholamban (Genbank sequence accession number: NM_002667) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struct. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted below, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of phospholamban RNA

Ribozymes and antisense constructs were designed to anneal to various sites in the RNA message. The binding arms of the ribozymes are complementary to the target site sequences described above, while the antisense constructs are fully complimentary to the target site sequences described above. The ribozymes and antisense constructs were

chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 J. Am. Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes and antisense constructs were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51). Ribozymes and antisense constructs were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; see Wincott et al., *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes and antisense constructs used in this study are shown below in **Table 24-30**.

Ribozyme Cleavage of phospholamban RNA Target *in vitro*

Ribozymes targeted to the human phospholamban RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example using the following procedure. The target sequences and the nucleotide location within the phospholamban RNA are given in **Tables 24-30**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'- ^{32} P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM

EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is
5 determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

Tissue distribution of BrdU-labeled antisense in mice

CD1 mice were injected with a single bolus (30 mg/kg) of a BrdU-labeled antisense
10 oligonucleotide or a similar molar amount of BrdU (as a control). At various time points (30 min, 2h and 6 h), mice were sacrificed and major tissues isolated and fixed. Distribution of antisense oligonucleotides was determined by probing with an anti-BrdU antibody and immunohistochemical staining. Tissue slices were probed with an anti-BrdU antibody followed by a reporter enzyme-conjugated second antibody and finally an enzyme
15 substrate. Visualization of the colored product by microscopy indicated nuclear staining, demonstrating effective distribution of antisense oligonucleotide in cardiac tissue.

Tissue distribution of BrdU-labeled ribozymes in monkey

Rhesus monkeys were dosed with BrdU-labeled ribozyme by intravenous bolus
20 injection at 0.1, 1.0, and 10 mg/kg once daily over five days. Saline injection was used in control animals. Animals were sacrificed and major tissues isolated and fixed. Tissue samples were probed with an anti-BrdU antibody followed by a reporter enzyme-conjugated second antibody and finally an enzyme substrate. Significant quantities of chemically modified ribozyme are detected in cardiac tissue following this dosing regimen.

25

Example 6: HBV

Chronic hepatitis B is caused by an enveloped virus, commonly known as the hepatitis B virus or HBV. HBV is transmitted via infected blood or other body fluids, especially saliva and semen, during delivery, sexual activity, or sharing of needles
30 contaminated by infected blood. Individuals may be "carriers" and transmit the infection to others without ever having experienced symptoms of the disease. Persons at highest

risk are those with multiple sex partners, those with a history of sexually transmitted diseases, parenteral drug users, infants born to infected mothers, "close" contacts or sexual partners of infected persons, and healthcare personnel or other service employees who have contact with blood. Transmission is also possible via tattooing, ear or body piercing, and acupuncture; the virus is also stable on razors, toothbrushes, baby bottles, eating utensils, and some hospital equipment such as respirators, scopes and instruments. There is no evidence that HBsAg positive food handlers pose a health risk in an occupational setting, nor should they be excluded from work. Hepatitis B has never been documented as being a food-borne disease. The average incubation period is 60 to 90 days, with a range of 45 to 180; the number of days appears to be related to the amount of virus to which the person was exposed. However, determining the length of incubation is difficult, since onset of symptoms is insidious. Approximately 50% of patients develop symptoms of acute hepatitis that last from 1 to 4 weeks. Two percent or less of these individuals develop fulminant hepatitis resulting in liver failure and death.

The determinants of severity include: (1) The size of the dose to which the person was exposed; (2) the person's age with younger patients experiencing a milder form of the disease; (3) the status of the immune system with those who are immunosuppressed experiencing milder cases; and (4) the presence or absence of co-infection with the Delta virus (hepatitis D), with more severe cases resulting from co-infection. In symptomatic cases, clinical signs include loss of appetite, nausea, vomiting, abdominal pain in the right upper quadrant, arthralgia, and tiredness/loss of energy. Jaundice is not experienced in all cases, however, jaundice is more likely to occur if the infection is due to transfusion or percutaneous serum transfer, and it is accompanied by mild pruritus in some patients. Bilirubin elevations are demonstrated in dark urine and clay-colored stools, and liver enlargement may occur accompanied by right upper-quadrant pain. The acute phase of the disease may be accompanied by severe depression, meningitis, Guillain-Barré syndrome, myelitis, encephalitis, agranulocytosis, and/or thrombocytopenia.

Hepatitis B is generally self-limiting and will resolve in approximately 6 months. Asymptomatic cases can be detected by serologic testing, since the presence of the virus leads to production of large amounts of HBsAg in the blood. This antigen is the first and most useful diagnostic marker for active infections. However, if HBsAg remains positive for 20 weeks or longer, the person is likely to remain positive indefinitely and is now a

carrier. While only 10% of persons over age 6 who contract HBV become carriers, 90% of infants infected during the first year of life do so.

Hepatitis B virus (HBV) infects over 300 million people worldwide (Imperial, 1999, *Gastroenterol. Hepatol.*, 14 (suppl), S1-5). In the United States approximately 1.25
5 million individuals are chronic carriers of HBV as evidenced by the fact that they have measurable hepatitis B virus surface antigen HBsAg in their blood. The risk of becoming a chronic HBsAg carrier is dependent upon the mode of acquisition of infection as well as the age of the individual at the time of infection. For those individuals with high levels of viral replication, chronic active hepatitis with progression to cirrhosis, liver failure and
0 hepatocellular carcinoma (HCC) is common, and liver transplantation is the only treatment option for patients with end-stage liver disease from HBV.

The natural progression of chronic HBV infection over a 10 to 20 year period leads to cirrhosis in 20-to-50% of patients and progression of HBV infection to hepatocellular carcinoma has been well documented. There have been no studies that have determined
5 sub-populations that are most likely to progress to cirrhosis and/or hepatocellular carcinoma, thus all patients have equal risk of progression.

It is important to note that the survival for patients diagnosed with hepatocellular carcinoma is only 0.9 to 12.8 months from initial diagnosis (Takahashi *et al.*, 1993, *American Journal of Gastroenterology*, 88, 240-243). Treatment of hepatocellular
0 carcinoma with chemotherapeutic agents has not proven effective and only 10% of patients will benefit from surgery due to extensive tumor invasion of the liver (Trinchet *et al.*, 1994, *Presse Medicines*, 23, 831-833). Given the aggressive nature of primary hepatocellular carcinoma, the only viable treatment alternative to surgery is liver transplantation (Pichlmayr *et al.*, 1994, *Hepatology.*, 20, 33S-40S).

5 Upon progression to cirrhosis, patients with chronic HCV infection present with clinical features, which are common to clinical cirrhosis regardless of the initial cause (D'Amico *et al.*, 1986, *Digestive Diseases and Sciences*, 31, 468-475). These clinical features may include: bleeding esophageal varices, ascites, jaundice, and encephalopathy (Zakim D, Boyer TD. *Hepatology a textbook of liver disease*, Second Edition Volume 1.
0 1990 W.B. Saunders Company. Philadelphia). In the early stages of cirrhosis, patients are classified as compensated, meaning that although liver tissue damage has occurred, the patient's liver is still able to detoxify metabolites in the blood-stream. In addition, most

patients with compensated liver disease are asymptomatic and the minority with symptoms report only minor symptoms such as dyspepsia and weakness. In the later stages of cirrhosis, patients are classified as decompensated meaning that their ability to detoxify metabolites in the bloodstream is diminished and it is at this stage that the clinical features described above will present.

In 1986, D'Amico *et al.* described the clinical manifestations and survival rates in 1155 patients with both alcoholic and viral associated cirrhosis (D'Amico *supra*). Of the 1155 patients, 435 (37%) had compensated disease although 70% were asymptomatic at the beginning of the study. The remaining 720 patients (63%) had decompensated liver disease with 78% presenting with a history of ascites, 31% with jaundice, 17% had bleeding and 16% had encephalopathy. Hepatocellular carcinoma was observed in six (0.5%) patients with compensated disease and in 30 (2.6%) patients with decompensated disease.

Over the course of six years, the patients with compensated cirrhosis developed clinical features of decompensated disease at a rate of 10% per year. In most cases, ascites was the first presentation of decompensation. In addition, hepatocellular carcinoma developed in 59 patients who initially presented with compensated disease by the end of the six-year study.

With respect to survival, the D'Amico study indicated that the five-year survival rate for all patients on the study was only 40%. The six-year survival rate for the patients who initially had compensated cirrhosis was 54% while the six-year survival rate for patients who initially presented with decompensated disease was only 21%. There were no significant differences in the survival rates between the patients who had alcoholic cirrhosis and the patients with viral related cirrhosis. The major causes of death for the patients in the D'Amico study were liver failure in 49%; hepatocellular carcinoma in 22%; and, bleeding in 13% (D'Amico *supra*).

Hepatitis B virus is a double-stranded circular DNA virus. It is a member of the Hepadnaviridae family. The virus consists of a central core that contains a core antigen (HBcAg) surrounded by an envelope containing a surface protein/surface antigen (HBsAg) and is 42 nm in diameter. It also contains an e antigen (HBeAg) which, along with HBcAg and HBsAg, is helpful in identifying this disease

In HBV virions, the genome is found in an incomplete double-stranded form. HBV uses a reverse transcriptase to transcribe a positive-sense full length RNA version of its genome back into DNA. This reverse transcriptase also contains DNA polymerase activity and thus begins replicating the newly synthesized minus-sense DNA strand. However, it
5 appears that the core protein encapsidates the reverse-transcriptase/polymerase before it completes replication.

From the free-floating form, the virus must first attach itself specifically to a host cell membrane. Viral attachment is one of the crucial steps which determines host and tissue specificity. However, currently there are no in vitro cell-lines that can be infected by
10 HBV. There are some cells lines, such as HepG2, which can support viral replication only upon transient or stable transfection using HBV DNA.

After attachment, fusion of the viral envelope and host membrane must occur to allow the viral core proteins containing the genome and polymerase to enter the cell. Once inside, the genome is translocated to the nucleus where it is repaired and cyclized.

5 The complete closed circular DNA genome of HBV remains in the nucleus and gives rise to four transcripts. These transcripts initiate at unique sites but share the same 3'-ends. The 3.5-kb pregenomic RNA serves as a template for reverse transcription and also encodes the nucleocapsid protein and polymerase. A subclass of this transcript with a 5'-end extension codes for the precore protein that, after processing, is secreted as HBV e
10 antigen. The 2.4-kb RNA encompasses the pre-S1 open reading frame (ORF) that encodes the large surface protein. The 2.1-kb RNA encompasses the pre-S2 and S ORFs that encode the middle and small surface proteins, respectively. The smallest transcript (~0.8-kb) codes for the X protein, a transcriptional activator.

Multiplication of the HBV genome begins within the nucleus of an infected cell.
15 RNA polymerase II transcribes the circular HBV DNA into greater-than-full length mRNA. Since the mRNA is longer than the actual complete circular DNA, redundant ends are formed. Once produced, the pregenomic RNA exits the nucleus and enters the cytoplasm.

The packaging of pregenomic RNA into core particles is triggered by the binding of
20 the HBV polymerase to the 5' epsilon stem-loop. RNA encapsidation is believed to occur as soon as binding occurs. The HBV polymerase also appears to require associated core protein in order to function. The HBV polymerase initiates reverse transcription from the

5' epsilon stem-loop three to four base pairs at which point the polymerase and attached nascent DNA are transferred to the 3' copy of the DR1 region. Once there, the (-)DNA is extended by the HBV polymerase while the RNA template is degraded by the HBV polymerase RNase H activity. When the HBV polymerase reaches the 5' end, a small stretch of RNA is left undigested by the RNase H activity. This segment of RNA is comprised of a small sequence just upstream and including the DR1 region. The RNA oligomer is then translocated and annealed to the DR2 region at the 5' end of the (-)DNA. It is used as a primer for the (+)DNA synthesis which is also generated by the HBV polymerase. It appears that the reverse transcription as well as plus strand synthesis may occur in the completed core particle.

Since the pregenomic RNA is required as a template for DNA synthesis, this RNA is an excellent target for ribozyme cleavage. Nucleoside analogues that have been documented to inhibit HBV replication target the reverse transcriptase activity needed to convert the pregenomic RNA into DNA. Ribozyme cleavage of the pregenomic RNA template would be expected to result in a similar inhibition of HBV replication. Further, targeting the 3'-end of the pregenomic RNA that is common to all HBV transcripts could result in reduction of all HBV gene products and an additional level of inhibition of HBV replication.

As previously mentioned, HBV does not infect cells in culture. However, transfection of HBV DNA (either as a head-to-tail dimer or as an "overlength" genome of >100%) into HuH7 or Hep G2 hepatocytes results in viral gene expression and production of HBV virions released into the media. Thus, HBV replication competent DNA would be co-transfected with ribozymes in cell culture. Such an approach has been used to report intracellular ribozyme activity against HBV (zu Putlitz, *et al.*, 1999, *J. Virol.*, 73, 5381-5387, and Kim *et al.*, 1999, *Biochem. Biophys. Res. Commun.*, 257, 759-765). In addition, stable hepatocyte cell lines have been generated that express HBV. In these cells only ribozyme would need to be delivered; however, a delivery screen would need to be performed. In addition, stable hepatocyte cell lines have been generated that express HBV.

Intracellular HBV gene expression can be assayed by a Taqman® assay for HBV RNA or by ELISA for HBV protein. Extracellular virus can be assayed by PCR for DNA or ELISA for protein. Antibodies are commercially available for HBV surface antigen and

core protein. A secreted alkaline phosphatase expression plasmid can be used to normalize for differences in transfection efficiency and sample recovery.

There are several small animal models to study HBV replication. One is the transplantation of HBV-infected liver tissue into irradiated mice. Viremia (as evidenced
5 by measuring HBV DNA by PCR) is first detected 8 days after transplantation and peaks between 18 – 25 days (Ilan *et al.*, 1999, *Hepatology*, 29, 553-562).

Transgenic mice that express HBV have also been used as a model to evaluate potential anti-virals. HBV DNA is detectable in both liver and serum (Morrey *et al.*, 1999, *Antiviral Res.*, 42, 97-108).

10 An additional model is to establish subcutaneous tumors in nude mice with Hep G2 cells transfected with HBV. Tumors develop in about 2 weeks after inoculation and express HBV surface and core antigens. HBV DNA and surface antigen is also detected in the circulation of tumor-bearing mice (Yao *et al.*, 1996, *J. Viral Hepat.*, 3, 19-22).

Woodchuck hepatitis virus (WHV) is closely related to HBV in its virus structure,
15 genetic organization, and mechanism of replication. As with HBV in humans, persistent WHV infection is common in natural woodchuck populations and is associated with chronic hepatitis and hepatocellular carcinoma (HCC). Experimental studies have established that WHV causes HCC in woodchucks and woodchucks chronically infected with WHV have been used as a model to test a number of anti-viral agents. For example,
20 the nucleoside analogue 3T3 was observed to cause dose dependent reduction in virus (50% reduction after two daily treatments at the highest dose) (Hurwitz *et al.*, 1998. *Antimicrob. Agents Chemother.*, 42, 2804-2809).

Current therapeutic goals of treatment are three-fold: to eliminate infectivity and transmission of HBV to others, to arrest the progression of liver disease and improve the
25 clinical prognosis, and to prevent the development of hepatocellular carcinoma (HCC).

Interferon alpha use is the most common therapy for HBV; however, recently Lamivudine (3TC) has been approved by the FDA. Interferon alpha (IFN-alpha) is one treatment for chronic hepatitis B. The standard duration of IFN-alpha therapy is 16 weeks, however, the optimal treatment length is still poorly defined. A complete response (HBV
30 DNA negative HBeAg negative) occurs in approximately 25% of patients. Several factors have been identified that predict a favorable response to therapy including: High ALT , low HBV DNA , being female, and heterosexual orientation.

There is also a risk of reactivation of the hepatitis B virus even after a successful response, this occurs in around 5% of responders and normally occurs within 1 year.

Side effects resulting from treatment with type 1 interferons can be divided into four general categories including: Influenza-like symptoms, neuropsychiatric, laboratory abnormalities, and other miscellaneous side effects. Examples of influenza-like symptoms include, fatigue, fever; myalgia, malaise, appetite loss, tachycardia, rigors, headache and arthralgias. The influenza-like symptoms are usually short-lived and tend to abate after the first four weeks of dosing (Dusheiko *et al.*, 1994, *Journal of Viral Hepatitis*, 1, 3-5). Neuropsychiatric side effects include irritability, apathy, mood changes, insomnia, cognitive changes, and depression. Laboratory abnormalities include the reduction of myeloid cells, including granulocytes, platelets and to a lesser extent, red blood cells. These changes in blood cell counts rarely lead to any significant clinical sequelae. In addition, increases in triglyceride concentrations and elevations in serum alanine and aspartate aminotransferase concentration have been observed. Finally, thyroid abnormalities have been reported. These thyroid abnormalities are usually reversible after cessation of interferon therapy and can be controlled with appropriate medication while on therapy. Miscellaneous side effects include nausea, diarrhea, abdominal and back pain, pruritus, alopecia, and rhinorrhea. In general, most side effects will abate after 4 to 8 weeks of therapy (Dushieko *et al.*, *supra*).

Lamivudine (3TC) is a nucleoside analogue, which is a very potent and specific inhibitor of HBV DNA synthesis. Lamivudine has recently been approved for the treatment of chronic Hepatitis B. Unlike treatment with interferon, treatment with 3TC does not eliminate the HBV from the patient. Rather, viral replication is controlled and chronic administration results in improvements in liver histology in over 50% of patients. Phase III studies with 3TC, showed that treatment for one year was associated with reduced liver inflammation and a delay in scarring of the liver. In addition, patients treated with Lamivudine (100mg per day) had a 98 percent reduction in hepatitis B DNA and a significantly higher rate of seroconversion, suggesting disease improvements after completion of therapy. However, stopping of therapy resulted in a reactivation of HBV replication in most patients. In addition recent reports have documented 3TC resistance in approximately 30% of patients.

Particular degenerative and disease states that can be associated with HBV expression modulation include but are not limited to, HBV infection, hepatitis, cancer, tumorigenesis, cirrhosis, liver failure and others.

Lamivudine (3TC), L-FMAU, adefovir dipivoxil, type 1 Interferon, therapeutic
5 vaccines, steroids, and 2'-5' Oligoadenylates are non-limiting examples of pharmaceutical agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drugs such as diuretic and antihypertensive compounds or other therapies can similarly and readily be combined with the nucleic acid molecules of the
10 instant invention (e.g. ribozymes and antisense molecules) and are, therefore, within the scope of the instant invention.

Current therapies for treating HBV infection, including interferon and nucleoside analogues, are only partially effective. In addition, drug resistance to nucleoside analogues is now emerging, making treatment of chronic Hepatitis B more difficult. Thus, a need
15 exists for effective treatment of this disease which utilizes antiviral inhibitors which work by mechanisms other than those currently utilized in the treatment of both acute and chronic hepatitis B infections.

Draper, US patent No. 6,017,756, describes the use of ribozymes for the inhibition of Hepatitis B Virus.

20 Passman *et al.*, 2000, *Biochem. Biophys. Res. Commun.*, 268(3), 728-733.; Gan *et al.*, 1998, *J. Med. Coll. PLA*, 13(3), 157-159.; Li *et al.*, 1999, *Jiefangjun Yixue Zazhi*, 24(2), 99-101.; Putlitz *et al.*, 1999, *J. Virol.*, 73(7), 5381-5387.; Kim *et al.*, 1999, *Biochem. Biophys. Res. Commun.*, 257(3), 759-765.; Xu *et al.*, 1998, *Bingdu Xuebao*, 14(4), 365-369.; Welch *et al.*, 1997, *Gene Ther.*, 4(7), 736-743.; Goldenberg *et al.*, 1997,
25 International PCT publication No. WO 97/08309, Wands *et al.*, 1997, *J. of Gastroenterology and Hepatology*, 12(suppl.), S354-S369.; Ruiz *et al.*, 1997, *BioTechniques*, 22(2), 338-345.; Gan *et al.*, 1996, *J. Med. Coll. PLA*, 11(3), 171-175.; Beck and Nassal, 1995, *Nucleic Acids Res.*, 23(24), 4954-62.; Goldenberg, 1995, International PCT publication No. WO 95/22600.; Xu *et al.*, 1993, *Bingdu Xuebao*, 9(4),
30 331-6.; Wang *et al.*, 1993, *Bingdu Xuebao*, 9(3), 278-80, all describe ribozymes that are targeted to cleave a specific HBV target site.

The enzymatic nucleic acid molecules of the instant invention exhibit a high degree of specificity for only the viral mRNA in infected cells. Nucleic acid molecules of the instant invention targeted to highly conserved sequence regions allow the treatment of many strains of human HBV with a single compound. No treatment presently exists which specifically attacks expression of the viral gene(s) that are responsible for transformation of hepatocytes by HBV.

The methods of this invention can be used to treat human hepatitis B virus infections, which include productive virus infection, latent or persistent virus infection, and HBV-induced hepatocyte transformation. The utility can be extended to other species of HBV which infect non-human animals where such infections are of veterinary importance.

Preferred target sites are genes required for viral replication, a non-limiting example includes genes for protein synthesis, such as the 5' most 1500 nucleotides of the HBV pregenomic mRNAs. For sequence references, see Renbao *et al.*, 1987, *Sci. Sin.*, 30, 507.

This region controls the translational expression of the core protein (C), X protein (X) and DNA polymerase (P) genes and plays a role in the replication of the viral DNA by serving as a template for reverse transcriptase. Disruption of this region in the RNA results in deficient protein synthesis as well as incomplete DNA synthesis (and inhibition of transcription from the defective genomes). Target sequences 5' of the encapsidation site can result in the inclusion of the disrupted 3' RNA within the core virion structure and targeting sequences 3' of the encapsidation site can result in the reduction in protein expression from both the 3' and 5' fragments.

Alternative regions outside of the 5' most 1500 nucleotides of the pregenomic mRNA also make suitable targets of enzymatic nucleic acid mediated inhibition of HBV replication. Such targets include the mRNA regions that encode the viral S gene. Selection of particular target regions will depend upon the secondary structure of the pregenomic mRNA. Targets in the minor mRNAs can also be used, especially when folding or accessibility assays in these other RNAs reveal additional target sequences that are unavailable in the pregenomic mRNA species.

A desirable target in the pregenomic RNA is a proposed bipartite stem-loop structure in the 3'-end of the pregenomic RNA which is believed to be critical for viral replication (Kidd and Kidd-Ljunggren, 1996. *Nuc. Acid Res.* 24:3295-3302). The 5'end of the HBV

pregenomic RNA carries a *cis*-acting encapsidation signal, which has inverted repeat sequences that are thought to form a bipartite stem-loop structure. Due to a terminal redundancy in the pregenomic RNA, the putative stem-loop also occurs at the 3'-end. While it is the 5' copy which functions in polymerase binding and encapsidation, reverse transcription actually begins from the 3' stem-loop. To start reverse transcription, a 4 nt primer which is covalently attached to the polymerase is made, using a bulge in the 5' encapsidation signal as template. This primer is then shifted, by an unknown mechanism, to the DR1 primer binding site in the 3' stem-loop structure, and reverse transcription proceeds from that point. The 3' stem-loop, and especially the DR1 primer binding site, appear to be highly effective targets for ribozyme intervention.

Sequences of the pregenomic RNA are shared by the mRNAs for surface, core, polymerase, and X proteins. Due to the overlapping nature of the HBV transcripts, all share a common 3'-end. Ribozyme targeting this common 3'-end will thus cleave the pregenomic RNA as well as all of the mRNAs for surface, core, polymerase and X proteins.

In preferred embodiments, the invention features a method for the analysis of HBV proteins. This method is useful in determining the efficacy of HBV inhibitors. Specifically, the instant invention features an assay for the analysis of HBsAg proteins and secreted alkaline phosphatase (SEAP) control proteins to determine the efficacy of agents used to modulate HBV expression.

The method consists of coating a micro-titer plate with an antibody such as anti-HBsAg Mab (for example, Biostride B88-95-31ad,ay) at 0.1 to 10 µg/ml in a buffer (for example, carbonate buffer, such as Na₂CO₃ 15 mM, NaHCO₃ 35 mM, pH 9.5) at 4°C overnight. The microtiter wells are then washed with PBST or the equivalent thereof, (for example, PBS, 0.05% Tween 20) and blocked for 0.1-24 hr at 37° C with PBST, 1% BSA or the equivalent thereof. Following washing as above, the wells are dried (for example, at 37° C for 30 min). Biotinylated goat anti-HBsAg or an equivalent antibody (for example, Accurate YVS1807) is diluted (for example at 1:1000) in PBST and incubated in the wells (for example, 1 hr. at 37° C). The wells are washed with PBST (for example, 4x). A conjugate, (for example, Streptavidin/Alkaline Phosphatase Conjugate, Pierce 21324) is diluted to 10-10,000 ng/ml in PBST, and incubated in the wells (for example, 1 hr. at 37° C). After washing as above, a substrate (for example, p-nitrophenyl phosphate substrate,

Pierce 37620) is added to the wells, which are then incubated (for example, 1 hr. at 37° C). The optical density is then determined (for example, at 405 nm). SEAP levels are then assayed, for example, using the Great EscAPe® Detection Kit (Clontech K2041-1), as per the manufacturers instructions. In the above example, incubation times and reagent
5 concentrations may be varied to achieve optimum results, a non-limiting example is described in Example 6.

Comparison of this HBsAg ELISA method to a commercially available assay from World Diagnostics, Inc. 15271 NW 60th Ave, #201, Miami Lakes, FL 33014 (305) 827-3304 (Cat. No. EL10018) demonstrates an increase in sensitivity (signal:noise) of 3-20
10 fold.

Identification of Potential Target Sites in Human HBV RNA

The sequence of human HBV was screened for accessible sites using a computer-folding algorithm. Regions of the RNA that did not form secondary folding structures and
15 contained potential ribozyme and/or antisense binding/cleavage sites were identified. The sequences of these cleavage sites are shown in **Tables 36-43**.

Selection of Enzymatic Nucleic Acid Cleavage Sites in Human HBV RNA

Ribozyme target sites were chosen by analyzing sequences of Human HBV
20 (accession number: AF100308.1) and prioritizing the sites on the basis of folding. Ribozymes were designed that could bind each target and were individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem*, 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable
25 intramolecular interactions between the binding arms and the catalytic core were eliminated from consideration. As noted herein, varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of HBV RNA

Ribozymes and antisense constructs were designed to anneal to various sites in the RNA message. The binding arms of the ribozymes are complementary to the target site sequences described above, while the antisense constructs are fully complementary to the target site sequences described above. The ribozymes and antisense constructs were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe et al., (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott et al., *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were typically >98%.

Ribozymes and antisense constructs were also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes and antisense constructs were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; see Wincott et al., *supra*; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Table 43**.

Ribozyme Cleavage of HBV RNA Target *in vitro*

Ribozymes targeted to the human HBV RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example using the following procedure. The target sequences and the nucleotide location within the HBV RNA are given in **Tables 36-43**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex® column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'- ^{32} P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5

at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess.

5 The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is
0 determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

Transfection of HepG2 Cells with psHBV-1 and Ribozymes

The human hepatocellular carcinoma cell line Hep G2 was grown in Dulbecco's
5 modified Eagle media supplemented with 10% fetal calf serum, 2 mM glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 25 mM Hepes, 100 units penicillin, and 100 µg/ml streptomycin. To generate a replication competent cDNA, prior to transfection the HBV genomic sequences are excised from the bacterial plasmid sequence contained in the psHBV-1 vector (Those skilled in the art understand that other methods may be used
10 to generate a replication competent cDNA). This was done with an EcoRI and Hind III restriction digest. Following completion of the digest, a ligation was performed under dilute conditions (20 µg/ml) to favor intermolecular ligation. The total ligation mixture was then concentrated using Qiagen spin columns.

Secreted alkaline phosphatase (SEAP) was used to normalize the HBsAg levels to
15 control for transfection variability. The pSEAP2-TK control vector was constructed by ligating a Bgl II-Hind III fragment of the pRL-TK vector (Promega), containing the herpes simplex virus thymidine kinase promoter region, into *Bgl* II/*Hind* III digested pSEAP2-Basic (Clontech). Hep G2 cells were plated (3×10^4 cells/well) in 96-well microtiter plates and incubated overnight. A lipid/DNA/ribozyme complex was formed containing (at final
20 concentrations) cationic lipid (15 µg/ml), prepared psHBV-1 (4.5 µg/ml), pSEAP2-TK (0.5 µg/ml), and ribozyme (100 µM). Following a 15 min. incubation at 37°C, the

complexes were added to the plated Hep G2 cells. Media was removed from the cells 96 hr. post-transfection for HBsAg and SEAP analysis.

Transfection of the human hepatocellular carcinoma cell line, Hep G2, with replication competent HBV DNA results in the expression of HBV proteins and the production of virions. To investigate the potential use of ribozymes for the treatment of chronic HBV infection, a series of ribozymes that target the 3' terminus of the HBV genome have been synthesized. Ribozymes targeting this region have the potential to cleave all four major HBV RNA transcripts as well as the potential to block the production of HBV DNA by cleavage of the pregenomic RNA. To test the efficacy of these HBV ribozymes, they were co-transfected with HBV genomic DNA into Hep G2 cells, and the subsequent levels of secreted HBV surface antigen (HBsAg) were analyzed by ELISA. To control for variability in transfection efficiency, a control vector which expresses secreted alkaline phosphatase (SEAP), was also co-transfected. The efficacy of the HBV ribozymes was determined by comparing the ratio of HBsAg:SEAP and/or HBeAg:SEAP to that of a scrambled attenuated control (SAC) ribozyme. Twenty-five ribozymes (RPI18341, RPI18356, RPI18363, RPI18364, RPI18365, RPI18366, RPI18367, RPI18368, RPI18369, RPI18370, RPI18371, RPI18372, RPI18373, RPI18374, RPI18303, RPI18405, RPI18406, RPI18407, RPI18408, RPI18409, RPI18410, RPI18411, RPI18418, RPI18419, and RPI18422) have been identified which cause a reduction in the levels of HBsAg and/or HBeAg as compared to the corresponding SAC ribozyme.

Example 6: Analysis of HBsAg and SEAP Levels Following Ribozyme Treatment

Immulon 4 (Dynax) microtiter wells were coated overnight at 4° C with anti-HBsAg Mab (Biostride B88-95-31ad,ay) at 1 µg/ml in Carbonate Buffer (Na₂CO₃ 15 mM, NaHCO₃ 35 mM, pH 9.5). The wells were then washed 4x with PBST (PBS, 0.05% Tween® 20) and blocked for 1 hr at 37° C with PBST, 1% BSA. Following washing as above, the wells were dried at 37° C for 30 min. Biotinylated goat ant-HBsAg (Accurate YVS1807) was diluted 1:1000 in PBST and incubated in the wells for 1 hr. at 37° C. The wells were washed 4x with PBST. Streptavidin/Alkaline Phosphatase Conjugate (Pierce 21324) was diluted to 250 ng/ml in PBST, and incubated in the wells for 1 hr. at 37° C. After washing as above, p-nitrophenyl phosphate substrate (Pierce 37620) was added to the wells, which were then incubated for 1 hr. at 37° C. The optical density at 405 nm was

then determined. SEAP levels were assayed using the Great EscAPe® Detection Kit (Clontech K2041-1), as per the manufacturers instructions.

Example 7: X-gene Reporter Assay

5 The effect of ribozyme treatment on the level of transactivation of a SV40 promoter driven firefly luciferase gene by the HBV X-protein was analyzed in transfected Hep G2 cells. As a control for variability in transfection efficiency, a Renilla luciferase reporter driven by the TK promoter, which is not transactivated by the X protein, was used. Hep G2 cells were plated (3×10^4 cells/well) in 96-well microtiter plates and incubated
10 overnight. A lipid/DNA/ribozyme complex was formed containing (at final concentrations) cationic lipid (2.4 µg/ml), the X-gene vector pSBDR(2.5 µg/ml), the firefly reporter pSV40HCVluc (0.5 µg/ml), the Renilla luciferase control vector pRL-TK (0.5 µg/ml), and ribozyme (100 µM). Following a 15 min. incubation at 37° C, the complexes were added to the plated Hep G2 cells. Levels of firefly and Renilla luciferase were
15 analyzed 48 hr. post transfection, using Promega's Dual-Luciferase Assay System.

 The HBV X protein is a transactivator of a number of viral and cellular genes. Ribozymes which target the X region were tested for their ability to cause a reduction in X protein transactivation of a firefly luciferase gene driven by the SV40 promoter in transfected Hep G2 cells. As a control for transfection variability, a vector containing the
20 Renilla luciferase gene driven by the TK promoter, which is not activated by the X protein, was included in the co-transfections. The efficacy of the HBV ribozymes was determined by comparing the ratio of firefly luciferase: Renilla luciferase to that of a scrambled attenuated control (SAC) ribozyme. Eleven ribozymes (RPI18365, RPI18367, RPI18368, RPI18371, RPI18372, RPI18373, RPI18405, RPI18406, RPI18411, RPI18418, RPI18423)
25 were identified which cause a reduction in the level of transactivation of a reporter gene by the X protein, as compared to the corresponding SAC ribozyme.

Example 8: HBV transgenic mouse study

 A transgenic mouse strain (founder strain 1.3.32 with a C57B1/6 background) that
30 expresses HBV RNA and forms HBV viremia (Morrey *et al.*, 1999, *Antiviral Res.*, 42, 97-108; Guidotti *et al.*, 1995, *J. Virology*, 69, 10, 6158-6169) was utilized to study the *in vivo*

activity of ribozymes of the instant invention. This model is predictive in screening for anti-HBV agents. Ribozyme or the equivalent volume of saline was administered via a continuous s.c. infusion using Alzet® mini-osmotic pumps for 14 days. Alzet® pumps were filled with test material(s) in a sterile fashion according to the manufacturer's instructions. Prior to *in vivo* implantation, pumps were incubated at 37°C overnight (≥ 18 hours) to prime the flow modulators. On the day of surgery, animals were lightly anesthetized with a ketamine/xylazine cocktail (94 mg/kg and 6 mg/kg, respectively; 0.3 ml, IP). Baseline blood samples (200 μ l) were obtained from each animal *via* a retro-orbital bleed. A 2 cm area near the base of the tail was shaved and cleansed with betadine surgical scrub and sequentially with 70% alcohol. A 1 cm incision in the skin was made with a #15 scalpel blade or a blunt pair of scissors near the base of the tail. Forceps were used to open a pocket rostrally (*i.e.*, towards the head) by spreading apart the subcutaneous connective tissue. The pump was inserted with the delivery portal pointing away from the incision. Wounds were closed with sterile 9-mm stainless steel clips or with sterile 4-0 suture. Animals were then allowed to recover from anesthesia on a warm heating pad before being returned to their cage. Wounds were checked daily. Clips or sutures were replaced as needed. Incisions typically healed completely within 7 days post-op. Animals were then deeply anesthetized with the ketamine/xylazine cocktail (150 mg/kg and 10 mg/kg, respectively; 0.5 ml, IP) on day 14 post pump implantation. A midline thoracotomy/ laparotomy was performed to expose the abdominal cavity and the thoracic cavity. The left ventricle was cannulated at the base and animals exsanguinated using a 23G needle and 1 ml syringe. Serum was separated, frozen and analyzed for HBV DNA and antigen levels. Experimental groups were compared to the saline control group in respect to percent change from day 0 to day 14. HBV DNA was assayed by quantitative PCR

Results

Table 44 is a summary of the group designation and dosage levels used in the HBV transgenic mouse study. Baseline blood samples were obtained *via* a retroorbital bleed and animals (N=10/group) received anti-HBV ribozymes (100 mg/kg/day) as a continuous SC infusion. After 14 days, animals treated with a ribozyme targeting site 273 (RPI.18341) of

the HBV RNA showed a significant reduction in serum HBV DNA concentration, compared to the saline treated animals as measured by a quantitative PCR assay. More specifically, the saline treated animals had a 69% increase in serum HBV DNA concentrations over this 2-week period while treatment with the 273 ribozyme (RPI.18341) resulted in a 60% decrease in serum HBV DNA concentrations. Ribozymes directed against sites 1833 (RPI.18371), 1873 (RPI.18418), and 1874 (RPI.18372) decreased serum HBV DNA concentrations by 49%, 15% and 16%, respectively.

Example 7: Activity of NCH Ribozyme to inhibit HER2 gene expression

HER2 (also known as neu, erbB2 and c-erbB2) is an oncogene that encodes a 185-kDa transmembrane tyrosine kinase receptor. HER2 is a member of the epidermal growth factor receptor (EGFR) family and shares partial homology with other family members. In normal adult tissues HER2 expression is low. However, HER2 is overexpressed in at least 25-30% of breast (McGuire & Greene, 1989) and ovarian cancers (Berchuck, *et al.*, 1990). Furthermore, overexpression of HER2 in malignant breast tumors has been correlated with increased metastasis, chemoresistance and poor survival rates (Slamon *et al.*, 1987 *Science* 235: 177-182). Because HER2 expression is high in aggressive human breast and ovarian cancers, but low in normal adult tissues, it is an attractive target for ribozyme-mediated therapy (Thompson *et al.*, *supra*).

The greatest HER2 specific effects have been observed in cancer cell lines that express high levels of HER2 protein (as measured by ELISA). Specifically, in one study that treated five human breast cancer cell lines with the HER2 antibody (anti-erbB2-sFv), the greatest inhibition of cell growth was seen in three cell lines (MDA-MB-361, SKBR-3 and BT-474) that express high levels of HER2 protein. No inhibition of cell growth was observed in two cell lines (MDA-MB-231 and MCF-7) that express low levels of HER2 protein (Wright *et al.*, 1997). Another group successfully used SKBR-3 cells to show HER2 antisense oligonucleotide-mediated inhibition of HER2 protein expression and HER2 RNA knockdown (Vaughn *et al.*, 1995). Other groups have also demonstrated a decrease in the levels of HER2 protein, HER2 mRNA and/or cell proliferation in cultured cells using anti-HER2 ribozymes or antisense molecules (Suzuki, T. *et al.*, 1997; Weichen, *et al.*, 1997; Czubayko, F. *et al.*, 1997; Colomer, *et al.*, 1994; Betram *et al.*, 1994). Because cell lines that express higher levels of HER2 have been more sensitive to anti-

HER2 agents, we are pursuing several medium to high expressing cell lines, including SKBR-3 and T47D, for ribozyme screens in cell culture.

A variety of endpoints have been used in cell culture models to look at HER2-mediated effects after treatment with anti-HER2 agents. Phenotypic endpoints include inhibition of cell proliferation, apoptosis assays and reduction of HER2 protein expression. Because overexpression of HER2 is directly associated with increased proliferation of breast and ovarian tumor cells, a proliferation endpoint for cell culture assays will be our primary screen. There are several methods by which this endpoint can be measured. Following treatment of cells with ribozymes, cells are allowed to grow (typically 5 days) after which either the cell viability, the incorporation of [³H] thymidine into cellular DNA and/or the cell density can be measured. The assay of cell density is very straightforward and can be done in a 96-well format using commercially available fluorescent nucleic acid stains (such as Syto 13 or CyQuant). The assay using CyQuant is in place at RPI and is currently being employed to screen ~100 ribozymes targeting HER2 (details below).

As a secondary, confirmatory endpoint a ribozyme-mediated decrease in the level of HER2 protein expression can be evaluated using a HER2-specific ELISA.

Validation of Cell Lines and Ribozyme Treatment Conditions

Two human breast cancer cell lines (T47D and SKBR-3) that are known to express medium to high levels of HER2 protein, respectively, were considered for ribozyme screening. In order to validate these cell lines for HER2-mediated sensitivity, both cell lines were treated with the HER2 specific antibody, Herceptin® (Genentech) and its effect on cell proliferation was determined. Herceptin was added to cells at concentrations ranging from 0–8 µM in medium containing either no serum (OptiMem), 0.1% or 0.5% FBS and efficacy was determined *via* cell proliferation. Maximal inhibition of proliferation (~50%) in both cell lines was observed after addition of Herceptin at 0.5 nM in medium containing 0.1% or no FBS. The fact that both cell lines are sensitive to an anti-HER2 agent (Herceptin) supports their use in experiments testing anti-HER2 ribozymes.

Prior to ribozyme screening, the choice of the optimal lipid(s) and conditions for ribozyme delivery was determined empirically for each cell line. Applicant has established a panel of proprietary lipids that can be used to deliver ribozymes to cultured cells and are

very useful for cell proliferation assays that are typically 3-5 days in length. Initially, this panel of proprietary lipid delivery vehicles was screened in SKBR-3 and T47D cells using previously established control oligonucleotides. Specific lipids and conditions for optimal delivery were selected for each cell line based on these screens. These conditions were
5 used to deliver HER2 specific ribozymes to cells for primary (inhibition of cell proliferation) and secondary (decrease in HER2 protein) efficacy endpoints.

Primary Screen: Inhibition of Cell Proliferation

Although optimal ribozyme delivery conditions were determined for two cell lines,
10 the SKBR-3 cell line were be used for the initial screen because it has the higher level of HER2 protein, and thus should be most susceptible to a HER2-specific ribozyme. Follow-up studies can be carried out in T47D cells to confirm leads as necessary.

Ribozyme screens were be performed using an automated, high throughput 96-well cell proliferation assay. Cell proliferation were measured over a 5-day treatment period
5 using the nucleic acid stain CyQuant for determining cell density. The growth of cells treated with ribozyme/lipid complexes were compared to both untreated cells and to cells treated with Scrambled-arm Attenuated core Controls (SAC; or IA; **Figure 8**). SACs can no longer bind to the target site due to the scrambled arm sequence and have nucleotide changes in the core that greatly diminish ribozyme cleavage. These SACs are used to
10 determine non-specific inhibition of cell growth caused by ribozyme chemistry (*i.e.* multiple 2' *O*-Me modified nucleotides, a single 2' *C*-allyl uridine, 4 phosphorothioates and a 3' inverted abasic). Lead ribozymes are chosen from the primary screen based on their ability to inhibit cell proliferation in a specific manner. Dose response assays are carried out on these leads and a subset was advanced into a secondary screen using the
15 level of HER2 protein as an endpoint.

Secondary Screen: Decrease in HER2 Protein

A secondary screen that measures the effect of anti-HER2 ribozymes on HER2 protein levels is used to support preliminary findings. A robust HER2 ELISA for both
20 T47D and SKBR-3 cells has been established and is available for use as an additional endpoint.

Ribozyme Mechanism Assays

A Taqman assay for measuring the ribozyme-mediated decrease in HER2 RNA has also been established. This assay is based on PCR technology and can measure in real time the production of HER2 mRNA relative to a standard cellular mRNA such as GAPDH. This RNA assay is used to establish proof that lead ribozymes are working through an RNA cleavage mechanism and result in a decrease in the level of HER2 mRNA, thus leading to a decrease in cell surface HER2 protein receptors and a subsequent decrease in tumor cell proliferation.

Animal Models

Evaluating the efficacy of anti-HER2 agents in animal models is an important prerequisite to human clinical trials. As in cell culture models, the most HER2 sensitive mouse tumor xenografts are those derived from human breast carcinoma cells that express high levels of HER2 protein. In a recent study, nude mice bearing BT-474 xenografts were sensitive to the anti-HER2 humanized monoclonal antibody Herceptin, resulting in an 80% inhibition of tumor growth at a 1 mg kg dose (ip, 2 X week for 4-5 weeks). Tumor eradication was observed in 3 of 8 mice treated in this manner (Baselga *et al.*, 1998). This same study compared the efficacy of Herceptin alone or in combination with the commonly used chemotherapeutics, paclitaxel or doxorubicin. Although, all three anti-HER2 agents caused modest inhibition of tumor growth, the greatest antitumor activity was produced by the combination of Herceptin and paclitaxel (93% inhibition of tumor growth vs 35% with paclitaxel alone). The above studies provide proof that inhibition of HER2 expression by anti-HER2 agents causes inhibition of tumor growth in animals. Lead anti-HER2 ribozymes chosen from *in vitro* assays are further tested in mouse xenograft models. Ribozymes are first tested alone and then in combination with standard chemotherapies.

Animal Model Development

Three human breast tumor cell lines (T47D, SKBR-3 and BT-474) were characterized to establish their growth curves in mice. These three cell lines have been implanted into the mammary papillae of both nude and SCID mice and primary tumor volumes are measured 3 times per week. Growth characteristics of these tumor lines using

a Matrigel implantation format will also be established. In addition, the use of two other breast cell lines that have been engineered to express high levels of HER2 are also being used. The tumor cell line(s) and implantation method that supports the most consistent and reliable tumor growth is used in animal studies testing the lead HER2 ribozyme(s).

- 5 Ribozyme are administered by daily subcutaneous injection or by continuous subcutaneous infusion from Alzet mini osmotic pumps beginning 3 days after tumor implantation and continuing for the duration of the study. Group sizes of at least 10 animals are employed. Efficacy is determined by statistical comparison of tumor volume of ribozyme-treated animals to a control group of animals treated with saline alone. Because the growth of
- 0 these tumors is generally slow (45-60 days), an initial endpoint will be the time in days it takes to establish an easily measurable primary tumor (i.e. 50-100 mm³) in the presence or absence of ribozyme treatment.

Clinical Summary

- 5 Breast cancer is a common cancer in women and also occurs in men to a lesser degree. The incidence of breast cancer in the United States is ~180,000 cases per year and ~46,000 die each year of the disease. In addition, 21,000 new cases of ovarian cancer per year lead to ~13,000 deaths (data from Hung *et al.*, 1995 and the Surveillance, Epidemiology and End Results Program, NCI). Ovarian cancer is a potential secondary
- 0 indication for anti-HER2 ribozyme therapy.

- A full review of breast cancer is given in the NCI PDQ for Breast Cancer. A brief overview is given here. Breast cancer is evaluated or “staged” on the basis of tumor size, and whether it has spread to lymph nodes and/or other parts of the body. In Stage I breast cancer, the cancer is no larger than 2 centimeters and has not spread outside of the breast.
- 5 In Stage II, the patient’s tumor is 2-5 centimeters but cancer may have spread to the axillary lymph nodes. By Stage III, metastasis to the lymph nodes is typical, and tumors are 5 centimeters. Additional tissue involvement (skin, chest wall, ribs, muscles *etc.*) may also be noted. Once cancer has spread to additional organs of the body, it is classed as Stage IV.

- 0 Almost all breast cancers (>90%) are detected at Stage I or II, but 31% of these are already lymph node positive. The 5-year survival rate for node negative patients (with standard surgery/radiation/chemotherapy /hormone regimens) is 97%; however,

involvement of the lymph nodes reduces the 5-year survival to only 77%. Involvement of other organs (Stage III) drastically reduces the overall survival, to 22% at 5 years. Thus, chance of recovery from breast cancer is highly dependent on early detection. Because up to 10% of breast cancers are hereditary, those with a family history are considered to be at high risk for breast cancer and should be monitored very closely.

Breast cancer is highly treatable and often curable when detected in the early stages. (For a complete review of breast cancer treatments, see the NCI PDQ for Breast Cancer.) Common therapies include surgery, radiation therapy, chemotherapy and hormonal therapy. Depending upon many factors, including the tumor size, lymph node involvement and location of the lesion, surgical removal varies from lumpectomy (removal of the tumor and some surrounding tissue) to mastectomy (removal of the breast, lymph nodes and some or all of the underlying chest muscle). Even with successful surgical resection, as many as 21% of the patients may ultimately relapse (10-20 years). Thus, once local disease is controlled by surgery, adjuvant radiation treatments, chemotherapies and/or hormonal therapies are typically used to reduce the rate of recurrence and improve survival. The therapy regimen employed depends not only on the stage of the cancer at its time of removal, but other variables such the type of cancer (ductal or lobular), whether lymph nodes were involved and removed, age and general health of the patient and if other organs are involved.

Common chemotherapies include various combinations cytotoxic drugs to kill the cancer cells. These drugs include paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubin, fluorouracil *etc.* Significant toxicities are associated with these cytotoxic therapies. Well-characterized toxicities include nausea and vomiting, myelosuppression, alopecia and mucosity. Serious cardiac problems are also associated with certain of the combinations, *e.g.* doxorubin and paclitaxel, but are less common.

Testing for estrogen and progesterone receptors helps to determine whether certain anti-hormone therapies might be helpful in inhibiting tumor growth. If either or both receptors are present, therapies to interfere with the action of the hormone ligands, can be given in combination with chemotherapy and are generally continued for several years.

These adjuvant therapies are called SERMs, selective estrogen receptor modulators, and they can give beneficial estrogen-like effects on bone and lipid metabolism while antagonizing estrogen in reproductive tissues. Tamoxifen is one such compound. The

primary toxic effect associated with the use of tamoxifen is a 2 to 7-fold increase in the rate of endometrial cancer. Blood clots in the legs and lung and the possibility of stroke are additional side effects. However, tamoxifen has been determined to reduce breast cancer incidence by 49% in high-risk patients and an extensive, somewhat controversial, clinical study is underway to expand the prophylactic use of tamoxifen. Another SERM, raloxifene, was also shown to reduce the incidence of breast cancer in a large clinical trial where it was being used to treat osteoporosis. In additional studies, removal of the ovaries and/or drugs to keep the ovaries from working are being tested.

Bone marrow transplantation is being studied in clinical trials for breast cancers that have become resistant to traditional chemotherapies or where >3 lymph nodes are involved. Marrow is removed from the patient prior to high-dose chemotherapy to protect it from being destroyed, and then replaced after the chemotherapy. Another type of "transplant" involves the exogenous treatment of peripheral blood stem cells with drugs to kill cancer cells prior to replacing the treated cells in the bloodstream.

One biological treatment, a humanized monoclonal anti-HER2 antibody, Herceptin (Genentech) has been approved by the FDA as an additional treatment for HER2 positive tumors. Herceptin binds with high affinity to the extracellular domain of HER2 and thus blocks its signaling action. Herceptin can be used alone or in combination with chemotherapeutics (*i.e.* paclitaxel, docetaxel, cisplatin, *etc.*) (Pegram, *et al.*, 1998). In Phase III studies, Herceptin significantly improved the response rate to chemotherapy as well as improving the time to progression (Ross & Fletcher, 1998). The most common side effects attributed to Herceptin are fever and chills, pain, asthenia, nausea, vomiting, increased cough, diarrhea, headache, dyspnea, infection, rhinitis, and insomnia. Herceptin in combination with chemotherapy (paclitaxel) can lead to cardiotoxicity (Sparano, 1999), leukopenia, anemia, diarrhea, abdominal pain and infection.

HER2 Protein Levels for Patient Screening and as a Potential Endpoint

Because elevated HER2 levels can be detected in at least 30% of breast cancers, breast cancer patients can be pre-screened for elevated HER2 prior to admission to initial clinical trials testing an anti-HER2 ribozyme. Initial HER2 levels can be determined (by ELISA) from tumor biopsies or resected tumor samples.

During clinical trials, it may be possible to monitor circulating HER2 protein by ELISA (Ross and Fletcher, 1998). Evaluation of serial blood/serum samples over the course of the anti-HER2 ribozyme treatment period could be useful in determining early indications of efficacy. In fact, the clinical course of Stage IV breast cancer was correlated
5 with shed HER2 protein fragment following a dose-intensified paclitaxel monotherapy. In all responders, the HER2 serum level decreased below the detection limit (Luftner *et al.*).

Two cancer-associated antigens, CA27.29 and CA15.3, can also be measured in the serum. Both of these glycoproteins have been used as diagnostic markers for breast cancer. CA27.29 levels are higher than CA15.3 in breast cancer patients; the reverse is
10 true in healthy individuals. Of these two markers, CA27.29 was found to better discriminate primary cancer from healthy subjects. In addition, a statistically significant and direct relationship was shown between CA27.29 and large vs small tumors and node positive vs node negative disease (Gion, *et al.*, 1999). Moreover, both cancer antigens were found to be suitable for the detection of possible metastases during follow-up (Rodriguez
15 de Paterna *et al.*, 1999). Thus, blocking breast tumor growth may be reflected in lower CA27.29 and/or CA15.3 levels compared to a control group. FDA submissions for the use of CA27.29 and CA15.3 for monitoring metastatic breast cancer patients have been filed (reviewed in Beveridge, 1999). Fully automated methods for measurement of either of these markers are commercially available.

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Applicant has designed, synthesized and tested several NCH ribozymes and HH ribozymes targeted against HER2 RNA (see for example **Tables 31 and 34**) in cell proliferation assays.

5

Proliferation assay:

The model proliferation assay used in the study can require a cell plating density of 2000 cells/well in 96-well plates and at least 2 cell doublings over a 5-day treatment period. To calculate cell density for proliferation assays, the FIPS (fluoro-imaging
0 processing system) method well in the art was used. This method allows for cell density measurements after nucleic acids are stained with CyQuant dye, and has the advantage of

accurately measuring cell densities over a very wide range 1,000-100,000 cells/well in 96-well format.

Ribozymes (50-200 nM) were delivered in the presence of cationic lipid at 2.0 µg/mL and inhibition of proliferation was determined on day 5 post-treatment. Two full
5 ribozyme screens were completed and 4 lead HH and 11 lead NCH ribozymes were chosen for further testing. Of the 15 lead Rzs chosen from primary screens, 4 NCH and 1 HH Rzs continued to inhibit cell proliferation in subsequent experiments. NCH Rzs against sites, 2001 (RPI No. 17236), 2783 (RPI No. 17249), 2939 (RPI No. 17251) or 3998 (RPI No. 17262) caused inhibition of proliferation ranging from 25-60% as compared to a
10 scrambled control Rz (IA; RPI No. 17263). Of the five lead Rzs, the most efficacious is the NCH Rz (RPI No. 17251) against site 2939 of HER2 RNA. An example of results from cell culture assay is shown in **Figure 8**. Referring to **Figure 8**, NCH ribozymes and a HH ribozyme targeted against HER2 RNA, are shown to cause significant inhibition of proliferation of cells. This shows that ribozymes, for instance the NCH ribozymes are
5 capable of inhibiting HER2 gene expression in mammalian cells.

Example 8: Activity of Class II (Zinzyme) nucleic acid catalysts to inhibit HER2 gene expression

Applicant has designed, synthesized and tested several class II (zinzyme) ribozymes
10 targeted against HER2 RNA (see, for example, **Tables 58, 59, and 62**) in cell proliferation RNA reduction assays.

Proliferation assay:

The model proliferation assay used in the study requires a cell-plating density of
15 2000-10000 cells/well in 96-well plates and at least 2 cell doublings over a 5-day treatment period. Cells used in proliferation studies were either human breast or ovarian cancer cells (SKBR-3 and SKOV-3 cells respectively). To calculate cell density for proliferation assays, the FIPS (fluoro-imaging processing system) method well known in the art was used. This method allows for cell density measurements after nucleic acids are stained
20 with CyQuant® dye, and has the advantage of accurately measuring cell densities over a very wide range 1,000-100,000 cells/well in 96-well format.

Ribozymes (50-200 nM) were delivered in the presence of cationic lipid at 2.0-5.0 $\mu\text{g/mL}$ and inhibition of proliferation was determined on day 5 post-treatment. Two full ribozyme screens were completed resulting in the selection of 14 ribozymes. Class II (zinzyme) ribozymes against sites, 314 (RPI No. 18653), 443 (RPI No. 18680), 597 (RPI No. 18697), 659 (RPI No. 18682), 878 (RPI Nos. 18683 and 18654), 881 (RPI Nos. 18684 and 18685) 934 (RPI No. 18651), 972 (RPI No. 18656, 19292, 19727, 19728, and 19293), 1292 (RPI No. 18726), 1541 (RPI No. 18687), 2116 (RPI No. 18729), 2932 (RPI No. 18678), 2540 (RPI No. 18715), and 3504 (RPI No. 18710) caused inhibition of proliferation ranging from 25-80% as compared to a scrambled control ribozyme. An example of results from a cell culture assay is shown in **Figure 20**. Referring to **Figure 20**, Class II ribozymes targeted against HER2 RNA are shown to cause significant inhibition of proliferation of cells. This shows that ribozymes, for instance the Class II (zinzyme) ribozymes are capable of inhibiting HER2 gene expression in mammalian cells.

RNA assay:

RNA was harvested 24 hours post-treatment using the Qiagen RNeasy® 96 procedure. Real time RT-PCR (TaqMan® assay) was performed on purified RNA samples using separate primer/probe sets specific for either target HER2 RNA or control actin RNA (to normalize for differences due to cell plating or sample recovery). Results are shown as the average of triplicate determinations of HER2 to actin RNA levels post-treatment. **Figure 30** shows class II ribozyme (zinzyme) mediated reduction in HER2 RNA targeting site 972 vs a scrambled attenuated control.

Dose response assays:

Active ribozyme was mixed with binding arm-attenuated control (BAC) ribozyme to a final oligonucleotide concentration of either 100, 200 or 400 nM and delivered to cells in the presence of cationic lipid at 5.0 $\mu\text{g/mL}$. Mixing active and BAC in this manner maintains the lipid to ribozyme charge ratio throughout the dose response curve. HER2 RNA reduction was measured 24 hours post-treatment and inhibition of proliferation was determined on day 5 post-treatment. The dose response antiproliferation results are summarized in **Figure 31** and the dose-dependent reduction of HER2 RNA results are

summarized in **Figure 32**. **Figure 33** shows a combined dose response plot of both anti-proliferation and RNA reduction data for a class II ribozyme targeting site 972 of HER2 RNA (RPI 19293).

5 Example 9: Compositions having RNA cleaving activity

Hammerhead ribozymes are an example of catalytic RNA molecules which are able to recognize and cleave a given specific RNA substrate (Hutchins *et al.*, 1986, *Nucleic Acids Res.* 14:3627; Keese and Symons, in *Viroids and viroid-like pathogens* (J.J. Semanchik, publ., CRC-Press, Boca Raton, Florida, 1987, pages 1-47). The catalytic center of hammerhead ribozymes is flanked by three stems and can be formed by adjacent sequence regions of the RNA or also by regions, which are separated from one another by many nucleotides. **Figure 6** shows a diagram of such a catalytically active hammerhead structure. The stems have been denoted I, II and III. The nucleotides are numbered according to the standard nomenclature for hammerhead ribozymes (Hertel *et al.*, 1992, *Nucleic Acids Res.* 20:3252). In this nomenclature, bases are denoted by a number, which relates their position relative to the 5' side of the cleavage site. Furthermore, each base that is involved in a stem or loop region has an additional designation (which is denoted by a decimal point and then another number) that defines the position of that base within the stem or loop. A designation of A^{15.1} would indicate that this base is involved in a paired region and that it is the first nucleotide in that stem going away from the core region. This accepted convention for describing hammerhead-derived ribozymes allows for the nucleotides involved in the core of the enzyme to always have the same number relative to all of the other nucleotides. The size of the stems involved in substrate binding or core formation can be any size and of any sequence, and the position of A⁹, for example, will remain the same relative to all of the other core nucleotides. Nucleotides designated, for example, N^{^12} or N^{9^} represent an inserted nucleotide where the position of the caret (^) relative to the number denotes whether the insertion is before or after the indicated nucleotide. Thus, N^{^12} represents a nucleotide inserted before nucleotide position 12, and N^{9^} represents a nucleotide inserted after nucleotide position 9.

0 The consensus sequence of the catalytic core structure is described by Ruffner and Uhlenbeck, 1990, *Nucleic Acids Res.* 18:6025-6029. Perriman *et al.*, 1992, *Gene* 113:157-163, have meanwhile shown that this structure can also contain variations, for example,

naturally occurring nucleotide insertions such as N^9 and N^{12} . Thus, the positive strand of the satellite RNA of the tobacco ring-spot virus does not contain any of the two nucleotide insertions while the +RNA strand of the virusoid of the lucerne transient streak virus (vLTSV) contains a $N^9 = U$ insertion which can be mutated to C or G without loss of activity (Sheldon and Symons, 1989, *Nucleic Acids Res.* 17:5679-5685). Furthermore, in this special case, $N^7 = A$ and $R^{15.1} = A$. On the other hand, the minus strand of the carnation stunt associated viroid (-CarSV) is quite unusual since it contains both nucleotide insertions, that is $N^{12} = A$ and $N^9 = C$ (Hernandez *et al.*, 1992, *Nucleic Acids Res.* 20:6323-6329). In this viroid $N^7 = A$ and $R^{15.1} = A$. In addition, this special hammerhead structure exhibits a very effective self-catalytic cleavage despite the more open central stem.

Possible uses of hammerhead ribozymes include, for example, generation of RNA restriction enzymes and the specific inactivation of the expression of genes in, for example, animal, human or plant cells and prokaryotes, yeasts and plasmodia. A particular biomedical interest is based on the fact that many diseases, including many forms of tumors, are related to the overexpression of specific genes. Inactivating such genes by cleaving the associated mRNA represents a possible way to control and eventually treat such diseases. Moreover there is a great need to develop antiviral, antibacterial, and antifungal pharmaceutical agents. Ribozymes have potential as such anti-infective agents since RNA molecules vital to the survival of the organism can be selectively destroyed.

In addition to needing the correct hybridizing sequences for substrate binding, substrates for hammerhead ribozymes have been shown to strongly prefer the triplet $N^{16.2}U^{16.1}H^{17}$ (NUH) where N can be any nucleotide, U is uridine, and H is either adenosine, cytidine, or uridine (Koizumi *et al.*, 1988, *FEBS Lett.* 228, 228-230; Ruffner *et al.*, 1990, *Biochemistry* 29, 10695-10702 ; Perriman *et al.*, 1992, *Gene* 113, 157-163). NUH is sometimes designated as NUX. The fact that changes to this general rule for substrate specificity result in non-functional substrates implies that there are "non core compatible" structures which are formed when substrates are provided which deviate from the stated requirements. Evidence along these lines was recently reported by Uhlenbeck and co-workers (Uhlenbeck *et al.*, 1997, *Biochemistry* 36:1108-1114) when they demonstrated that the substitution of a G at position 17 caused a functionally catastrophic base pair between G^{17} and C^3 to form, both preventing the correct orientation of the

scissile bond for cleavage and the needed tertiary interactions of C³ (Murray *et al.*, 1995, *Biochem. J.* 311:487-494). The strong preference for a U at position 16.1 may exist for similar reasons. Many experiments have been done in an attempt to isolate ribozymes which are able to efficiently relieve the requirement of a U at position 16.1, however, attempts to find hammerhead type ribozymes which can cleave substrates having a base other than a U at position 16.1 have proven impossible (Perriman *et al.*, 1992, *Gene* 113, 157-163).

Efficient catalytic molecules with reduced or altered requirements in the cleavage region are highly desirable because their isolation would greatly increase the number of available target sequences that molecules of this type could cleave. For example, it would be desirable to have a ribozyme variant that could efficiently cleave substrates containing triplets other than N^{16.2}U^{16.1}H¹⁷ since this would increase the number of potential target cleavage sites.

Chemically modified oligonucleotides which contain a block of deoxyribonucleotides in the middle region of the molecule have potential as pharmaceutical agents for the specific inactivation of the expression of genes (Giles *et al.*, 1992, *Nucleic Acids Res.* 20:763-770). These oligonucleotides can form a hybrid DNA-RNA duplex in which the DNA bound RNA strand is degraded by RNase H. Such oligonucleotides are considered to promote cleavage of the RNA and so cannot be characterized as having an RNA-cleaving activity nor as cleaving an RNA molecule (the RNase H is cleaving). A significant disadvantage of these oligonucleotides for *in vivo* applications is their low specificity, since hybrid formation, and thus cleavage, can also take place at undesired positions on the RNA molecules.

Since, unmodified ribozymes are sensitive to degradation by RNases, chemically modified active substances have to be used in order to administer hammerhead ribozymes exogenously (discussed, for example, by Heidenreich *et al.*, 1994, *J. Biol. Chem.* 269:2131-2138; Kiehntopf *et al.*, 1994, *EMBO J.* 13:4645-4652; Paoletta *et al.*, 1992, *EMBO J.* 11:1913-1919; and Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31:163-164).

Sproat *et al.*, U.S. Pat. No. 5,334,711, describe such chemically modified active substances based on synthetic catalytic oligonucleotide structures with a length of 35 to 40 nucleotides which are suitable for cleaving a nucleic acid target sequence and contain modified nucleotides that contain an optionally substituted alkyl, alkenyl or alkynyl group

with 1 - 10 carbon atoms at the 2'-O atom of the ribose. These oligonucleotides contain modified nucleotide building blocks and form a structure resembling a hammerhead structure. These oligonucleotides are able to cleave specific RNA substrates.

Usman *et al.*, U.S. Patent No. 5,891,684, describe enzymatic nucleic acid molecules
5 with one or more nucleotide base modification(s) in a substrate binding arm.

Thompson *et al.*, US Patent No. 5,599,704 describe enzymatic RNA molecules targeted against ErbB2/*neu*/Her2 RNA.

Sullivan *et al.*, US Patent No. 5,616,490 describe enzymatic RNA molecules targeted against protein kinase C (PKC) RNA.

0 Sioud, International PCT publication No. WO 99/63066 describe hammerhead ribozymes targeted against specific sites within protein kinase C alpha (PKC alpha), VEGF, and TNF alpha RNA.

Jarvis *et al.*, International PCT publication No. WO 98/505030, describe the synthesis of xylo-ribonucleosides and oligonucleotides comprising xylo modifications.

5 This invention relates to novel enzymatic nucleic acid molecules having an RNA-cleavage activity, as well as their use for cleaving RNA substrates *in vitro* and *in vivo*. The compositions contain an active center, the subunits of which are selected from nucleotides and/or nucleotide analogues, as well as flanking regions contributing to the formation of a specific hybridization with an RNA substrate. Preferred compositions form, in
0 combination with an RNA substrate, a structure resembling a hammerhead structure. The active center of the disclosed compositions is characterized by the presence of I^{15.1} which allows cleavage of RNA substrates having C^{16.1}. It is therefore an object of the present invention to provide compositions that cleave RNA, and in particular to provide RNA-cleaving oligomers which at the same time have a high stability, activity, and specificity.

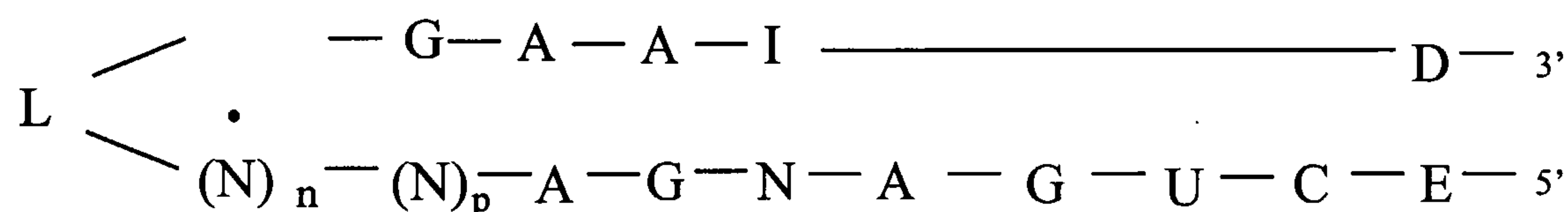
5 This invention relates to novel nucleic acid molecules with catalytic activity, which are particularly useful for cleavage of RNA or DNA or combination thereof. The nucleic acid catalysts of the instant invention are distinct from other nucleic acid catalysts known in the art. Specifically, nucleic acid catalysts of the instant invention are capable of catalyzing an intermolecular or intramolecular endonuclease reaction.

0 It is another object of the present invention to provide compositions that cleave RNA substrates having a cleavage site triplet other than N^{16.2}U^{16.1}H¹⁷ (NUH; **Figure 6**), where N is a nucleotide, U is uridine and H is adenosine, uridine or cytidine. H is used

interchangably with X. Specifically, the enzymatic nucleic acid molecule of the instant invention has an endonuclease activity to cleave RNA substrates having a cleavage triplet $N^{16.2}C^{16.1}H^{17}$ (NCH; **Figure 6**), where N is a nucleotide, C is cytidine and H is adenosine, uridine or cytidine. H is used interchangeably with X. In another aspect the invention

5 features an enzymatic nucleic acid molecule of the instant invention has an endonuclease activity to cleave RNA substrates having a cleavage triplet $N^{16.2}C^{16.1}N^{17}$ (NCN; **Figure 6**), where N is a nucleotide, C is cytidine.

In a preferred embodiment, the invention features an enzymatic nucleic acid molecule having formula 1:



where N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; D and E are independently oligonucleotides of length sufficient to stably interact (*e.g.*, by forming hydrogen bonds with complementary nucleotides in the target) with a target nucleic acid molecule (the target can be an RNA, DNA or mixed

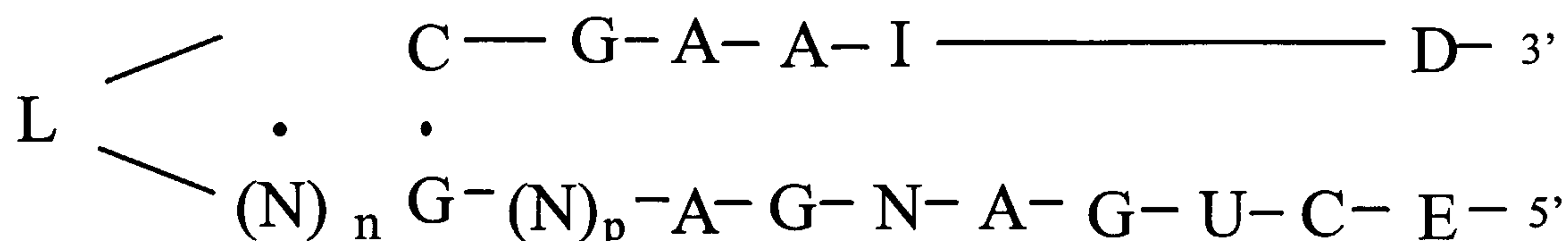
5 polymers), preferably, the length of D and E are independently between 3-20 nucleotides long, specifically, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20; o and n are integers independently greater than or equal to 1 and preferably less than about 100, specifically 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 50, wherein if $(N)_o$ and $(N)_n$ are nucleotides, $(N)_o$ and $(N)_n$ are optionally able to interact by hydrogen bond interaction, in particular if $n=1$

!0 and $o=1$ then $(N)_n$ is preferably a purine (*e.g.*, G, and A) and $(N)_o$ is preferably a pyrimidine (*e.g.*, C and U) and $(N)_n$ preferably forms; • indicates base-paired interaction; L is a linker which may be present or absent (*i.e.*, the molecule may be assembled from two separate oligonucleotides), but when present, is a nucleotide and/or a non-nucleotide linker, which may be a single-stranded and/or double-stranded region; p is an integer 0 or

!5 1, when $p=1$, $(N)_p$ is preferably A or U; and _____ represents a chemical linkage (*e.g.* a phosphate ester linkage, amide linkage, phosphorothioate linkage or others known in the art). A, U, I, C and G represent adenosine, uridine, inosine, cytidine and guanosine nucleotides, respectively. The N in 5'-CUGANGA-3' region of formula 1 is preferably U.

The nucleotides in the formula 1 are unmodified or modified at the sugar, base, and/or phosphate as known in the art.

In a preferred embodiment, the invention features an enzymatic nucleic acid molecule having formula 2:



where N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; D and E are independently oligonucleotides of length sufficient to stably interact (*e.g.*, by forming hydrogen bonds with complementary nucleotides in the target) with a target nucleic acid molecule (the target can be an RNA, DNA or mixed polymers), preferably, the length of D and E are independently between 3-20 nucleotides long, specifically, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20; o and n are integers independently greater than or equal to 0 and preferably less than about 100, specifically 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 50, wherein if (N)_o and (N)_n are nucleotides, (N)_o and (N)_n are optionally able to interact by hydrogen bond interaction; • indicates base-paired interaction; L is a linker which may be present or absent (*i.e.*, the molecule may be assembled from two separate oligonucleotides), but when present, is a nucleotide and/or a non-nucleotide linker, which may be a single-stranded and/or double-stranded region; p is an integer 0 or 1, when p=1, (N)_p is preferably A, C or U; and _____ represents a chemical linkage (*e.g.* a phosphate ester linkage, amide linkage, phosphorothioate linkage or others known in the art). A, U, I, C and G represent adenosine, uridine, inosine, cytidine and guanosine nucleotides, respectively. The N in 5'-CUGANGA-3' region of formula 2 is preferably U. The nucleotides in the formula 2 are unmodified or modified at the sugar, base, and/or phosphate as known in the art.

In a preferred embodiment, the I (inosine) in formula 1 and 2 is preferably a ribo-inosine or a xylo-inosine.

In yet another embodiment, the nucleotide linker (L) is a nucleic acid aptamer, such as an ATP aptamer, HIV Rev aptamer (RRE), HIV Tat aptamer (TAR) and others (for a review see Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; and Szostak & Ellington, 1993, in *The RNA World*, ed. Gesteland and Atkins, pp 511, CSH Laboratory Press). A
5 "nucleic acid aptamer" as used herein is meant to indicate nucleic acid sequence capable of interacting with a ligand. The ligand can be any natural or a synthetic molecule, including but not limited to a resin, metabolites, nucleosides, nucleotides, drugs, toxins, transition state analogs, peptides, lipids, proteins, amino acids, nucleic acid molecules, hormones, carbohydrates, receptors, cells, viruses, bacteria and others. In a preferred embodiment L
0 has the sequence 5'-GAAA-3' or 5'-GUUA-3'.

In yet another embodiment, the non-nucleotide linker (L) is as defined herein.

The term "non-nucleotide", as used herein, includes either abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic*
5 *Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma et al., *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand et al., *Nucleic Acids Res.* 1990, 18:6353; McCurdy et al., *Nucleosides & Nucleotides* 1991, 10:287; Jschke et al., *Tetrahedron Lett.* 1993, 34:301; Ono et al.,
10 *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. Non-nucleotide linkers can be any molecule, which is not an oligomeric sequence, that can be covalently coupled to an
5 oligomeric sequence. Preferred non-nucleotide linkers are oligomeric molecules formed of non-nucleotide subunits. Examples of such non-nucleotide linkers are described by Letsinger and Wu, (*J. Am. Chem. Soc.* 117:7323-7328 (1995)), Benseler *et al.*, (*J. Am. Chem. Soc.* 115:8483-8484 (1993)) and Fu *et al.*, (*J. Am. Chem. Soc.* 116:4591-4598 (1994)). Preferred non-nucleotide linkers, or subunits for non-nucleotide linkers, include
0 substituted or unsubstituted C₁-C₁₀ straight chain or branched alkyl, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkenyl, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkynyl, substituted or unsubstituted C₁-C₁₀ straight chain or

branched alkoxy, substituted or unsubstituted C₂-C₁₀ straight chain or branched alkenyloxy, and substituted or unsubstituted C₂-C₁₀ straight chain or branched alkynyloxy. The substituents for these preferred non-nucleotide linkers (or subunits) can be halogen, cyano, amino, carboxy, ester, ether, carboxamide, hydroxy, or mercapto. Thus, in a preferred embodiment, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule. 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. The terms "abasic" or "abasic nucleotide" as used herein encompass sugar moieties lacking a base or having other chemical groups in place of nucleotide base at the 1' position.

In a preferred embodiment, the invention features modified ribozymes with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, 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.

In a further preferred embodiment of the instant invention, an inverted deoxy abasic moiety is utilized at the 3' end of the enzymatic nucleic acid molecule.

By "pyrimidines" is meant nucleotides comprising modified or unmodified derivatives of a six membered pyrimidine ring. An example of a pyrimidine is modified or unmodified uridine.

In a preferred embodiment, the nucleosides of the instant invention include, 2'-O-methyl-2,6-diaminopurine riboside; 2'-deoxy-2'-amino-2,6-diaminopurine riboside; 2'-(N-alanyl) amino-2'-deoxy-uridine; 2'-(N-phenylalanyl)amino-2'-deoxy-uridine; 2'-deoxy-2'-(N-beta-alanyl) amino ; 2'-deoxy-2'-(lysiyl) amino uridine; 2'-C-allyl uridine; 2'-O-amino-

uridine; 2'-O-methylthiomethyl adenosine; 2'-O-methylthiomethyl cytidine ; 2'-O-methylthiomethyl guanosine; 2'-O-methylthiomethyl-uridine; 2'-Deoxy-2'-(N-histidyl) amino uridine; 2'-deoxy-2'-amino-5-methyl cytidine; 2'-(N- β -carboxamidine-beta-alanyl)amino-2'-deoxy-uridine; 2'-deoxy-2'-(N-beta-alanyl)-guanosine; 2'-O-amino-adenosine; 2'-(N-lysyl)amino -2'-deoxy-cytidine; 2'-Deoxy -2'-(L-histidine) amino Cytidine; and 5-Imidazoleacetic acid 2'-deoxy-5'-triphosphate uridine.

By "oligonucleotide" as used herein is meant a molecule having two or more nucleotides. The polynucleotide can be single, double or multiple stranded and may have modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

In a preferred embodiment, the enzymatic nucleic acid molecule of formula 1 or 2 include at least three ribonucleotide residues, preferably 4, 5, 6, 7, 8, 9, and 10 ribonucleotide residues.

In preferred embodiments, the enzymatic nucleic acid of the instant invention includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety. The necessary RNA components are known in the art (see for *e.g.*, Usman *et al.*, *supra*).

Thus, in one preferred embodiment, the invention features enzymatic nucleic acid molecules that inhibit gene expression and/or cell proliferation *in vitro* or *in vivo* (*e.g.* in patients). These chemically or enzymatically synthesized nucleic acid molecules contain substrate binding domains that bind to accessible regions of specific target nucleic acid molecules. The nucleic acid molecules also contain domains that catalyze the cleavage of target. Upon binding, the enzymatic nucleic acid molecules cleave the target molecules, preventing for example, translation and protein accumulation. In the absence of the expression of the target gene, cell proliferation, for example, is inhibited.

In another preferred embodiment, catalytic activity of the molecules described in the instant invention can be optimized as described by Draper *et al.*, *supra*. The details will not be repeated here, but include altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases and/or enhance their enzymatic activity (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, US Patent No. 5,334,711; and Burgin *et al.*, *supra*; all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of bases from stem loop structures to shorten RNA synthesis times and reduce chemical requirements are desired. (All these publications are hereby incorporated by reference herein.).

By "nucleic acid catalyst" as used herein is meant a nucleic acid molecule (*e.g.*, the molecule of formulae 1 and 2) capable of catalyzing (altering the velocity and/or rate of) a variety of reactions including the ability to repeatedly cleave other separate nucleic acid molecules (endonuclease activity) in a nucleotide base sequence-specific manner. Such a molecule with endonuclease activity may have complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity that specifically cleaves RNA or DNA in that target. That is, the nucleic acid molecule with endonuclease activity is able to intramolecularly or intermolecularly cleave RNA or DNA and thereby inactivate a target RNA or DNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA or DNA to allow the cleavage to occur. 100% complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. The nucleic acids may be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid as used herein is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic oligonucleotides, nucleozyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, oligozyme, finderion or nucleic acid catalyst. All of these terminologies describe nucleic acid molecules of the instant invention with enzymatic activity. The specific examples of enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving activity to the molecule (Cech *et al.*, U.S. Patent No. 4,987,071; Cech *et al.*, 1988, 260 *JAMA* 3030).

The enzymatic nucleic acid molecule of Formula 1 or 2 may independently comprise a cap structure which may independently be present or absent.

By "chimeric nucleic acid molecule" or "mixed polymer" is meant that, the molecule may be comprised of both modified or unmodified nucleotides.

5 In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) 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; 10 modified base nucleotide; phosphorodithioate; *threo*-pentofuransyl 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 15 methylphosphonate and 5'-mercapto moieties (for more details, see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein). 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 20 group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine. The terms "abasic" or "abasic nucleotide" as used herein encompass sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position.

In a preferred embodiment, the invention features 1-(beta-D-xylofuransyl)- 25 xypoxanthine phosphoramidite and a process for the synthesis thereof and incorporation into oligonucleotides, such as enzymatic nucleic acid molecule.

In yet another preferred embodiment, the invention features enzymatic nucleic acid molecules targeted against HER2 RNA, specifically, ribozymes in the hammerhead and NCH motifs.

30 In a preferred embodiment, the invention features enzymatic nucleic acid molecules targeted against PKC alpha RNA, specifically, ribozymes in the hammerhead and NCH motifs.

Targets, for example PKC alpha RNA, for useful ribozymes and antisense nucleic acids can be determined, for example, as described in Draper *et al.*, WO 95/04818; McSwiggen *et al.*, U.S. Patent Nos. 5,525,468 and 5,646,042, all are hereby incorporated by reference herein in their totality. Other examples include the following PCT

5 applications, which concern inactivation of expression of disease-related genes: WO 95/23225, WO 95/13380, WO 94/02595, all incorporated by reference herein.

The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific
0 substrate binding site (*e.g.*, D and E of Formula 1 above) which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving activity to the molecule.

All naturally occurring hammerhead ribozymes have an A^{15.1}-U^{16.1} base pair. In
5 addition, it is known that substrates for ribozymes based on the consensus hammerhead sequence strongly prefer a substrate that contains an N^{16.2}U^{16.1}H¹⁷ triplet in which H¹⁷ is not a guanosine (Koizumi *et al.*, *FEBS Lett.* 228, 228-230 (1988); Ruffner *et al.*, *Biochemistry* 29, 10695-10702 (1990); Perriman *et al.*, *Gene* 113, 157-163 (1992)). Many experiments have been done in an attempt to isolate ribozymes which are able to
10 efficiently relieve the requirement of a U at position 16.1, however, attempts to find ribozymes which can cleave substrates having a base other than a U at position 16.1 have proven largely unsuccessful (Perriman *et al.*, *Gene* 113, 157-163 1992, Singh *et al.*, *Antisense and Nucleic Acid Drug Development* 6:165-168 (1996)).

However, examination of the recently published X-ray crystal structures (Pley *et al.*,
5 *Nature* 372:68-74 (1994), Scott *et al.*, *Cell* 81:991-1002 (1995), and Scott *et al.*, *Science* 274:2065-2069 (1996)) led to the realization that the A^{15.1}-U^{16.1} interaction is a non-standard base pair with a single hydrogen bond between the exocyclic amine (N6) of the adenosine and the 4-oxo group of the uridine. Modeling studies (based on the crystal structure) then led to the discovery that the interaction of the wild-type A^{15.1}-U^{16.1} base pair
0 can be spatially mimicked by replacement with an I^{15.1}-C^{16.1} base pair that adopts an isostructural orientation and which preserves the required contact of the 2-keto group of C^{16.1} with A⁶ of the uridine turn. In the model, the polarity of the stabilizing hydrogen

bond between positions 15.1 and 16.1 is reversed in the $I^{15.1}-C^{16.1}$ interaction, but the correct orientation of the bases around this bond is maintained.

It has been discovered that hammerhead ribozyme analogues containing an inosine at position 15.1 readily cleave RNA substrates containing an $N^{16.2}C^{16.1}H^{17}$ triplet. Based on this, disclosed are compositions, preferably synthetic oligomers, which cleave a nucleic acid target sequence containing the triplet $N^{16.2}C^{16.1}H^{17}$. It is preferred that H^{17} is not guanosine, however, under certain circumstances, NCG triplet containing RNA can be cleaved by the ribozymes of the instant invention. The ability to cleave substrates having $N^{16.2}C^{16.1}X^{17}$ triplets effectively doubles the number of targets available for cleavage by compositions of the type disclosed.

Example 10: Synthesis of 1-(beta-D-xylofuranosyl)-xypoxanthine phosphoramidite

Referring to **Figure 9**, Inosine (1) was 5'-O-monomethoxytritylated and 2'-O-silylated under standard conditions to afford 2 (Charubala, R; Pfeleiderer, W. *Heterocycles* 1990, 30, 1141). Oxidation/reduction procedure afforded 3 in moderate yield (Matulic-Adamic, J.; Daniher, A.T.; Gonzalez, C.; Beigelman, L. *Bioorg. Med. Chem. Lett.* 1999, 9, 157): ^1H NMR ($CDCl_3$) δ 12.80 (br s, 1H, NH), 8.11 (s, 1H, H-8), 8.08 (s, 1H, H-2), 7.45-6.80 (m, 14H, trityl), 5.85 (d, $J_{1',2'}=1.6$, 1H, H-1'), 4.83 (d, $J_{2',3'}=7.2$, 1H, H-2'), 4.46 (br s, 1H, 3'-OH), 4.34 (m, 1H, H-4'), 4.06 (m, 1H, H-3'), 3.77 (s, 6H, 2 x OMe), 3.60 (app d, 2H, H-5', H-5''), 0.89 (s, 9H, *t*-Bu), 0.07 (s, 3H, Me), 0.06 (s, 3H, Me).

Standard phosphitylation of 3 afforded the desired phosphoramidite 4.

More acid stable 5'-O-MMT group is used in this particular case because applicant found that 5'-O-DMT protection is more labile in xylo nucleoside series than in ribo nucleoside series.

The xylo-inosine was incorporated into oligonucleotides using the standard procedures known in the art and as described herein.

Example 11: Activity of the xylo-Inosine-modified NCH Ribozyme

Several NCH ribozymes with xylo-inosine at position 15.1 were designed (**Figure 7**) to cleave RNA containing GCA, ACA, UCA or the CCA triplet. These ribozymes were

synthesized and purified as described herein and tested using standard RNA cleavage reaction conditions (see **Table 31**, for example, and see below).

The ribozymes were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 J. Am. Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., supra, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%.

Ribozymes were purified by gel electrophoresis using general methods or were purified by high pressure liquid chromatography (HPLC; See Wincott et al., supra; the totality of which is hereby incorporated herein by reference) and were resuspended in water. The sequences of the chemically synthesized ribozymes used in this study are shown below in **Table 33**.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [α - ^{32} P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates were 5'- ^{32} P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager[®] quantitation of bands representing the intact substrate and the cleavage products.

The results of the experiments are summarized in **Table 32**, which shows that NCH-xylo ribozymes are catalytically active to cleave target RNA.

Example 12: Activity of NCH Ribozyme variants

5 The nucleic acid molecules of the instant invention allow for the ability to cleave a new set of 12 NCH triplets. Determination of single turnover rate constants at pH 6 of these ribozymes in the all ribo form show that with NCA type triplets, the cleavage rate is higher than at NUA sites. NCC and NUC site rates are similar, and NCU sites are slightly lower than NUU sites. Additional measurements of multiple turnover parameters of the all
0 ribo ribozymes performed under non-saturating conditions using 5nM ribozyme and changing the substrate concentration from 50 to 500 nM at pH 7.4 with 10 mM Mg⁺⁺ at 37 °C gave Km = 100 nM and kcat = 6.5 min⁻¹ for GCA vs Km = 30 nM and kcat = 2.0 min⁻¹ for GUA cleaving all ribo ribozymes. These data verify that the ribozymes with an I•C base pair are efficient catalysts in multiple turnover reactions and the relative order of
5 activity between NCH and NUH cleavers established at pH 6 (Ludwig *et al.*, 1998, *Nucleic Acids Res.*, 26, 2279-2285) remains unchanged.

To gain more insight into the structural requirements of the 15.1- 16.1 base pair of the ribozymes of the instant invention, applicant synthesized several variants of the active I-15.1 •C-16.1 structure and tested these ribozyme analogues with their corresponding
0 substrates. The influence of several core stabilization strategies on the activity of the NCH cleaving ribozymes was also investigated.

Various nucleoside analogs were incorporated at position 15.1 of the ribozyme. Cleavage activity was tested with the complementary Fl* labeled substrates at pH 7.4 in the presence of 10 mM Mg⁺⁺ under conditions of ribozyme excess (i.e. single turnover
5 conditions). The modified oligonucleotides were synthesized by standard oligonucleotide synthesis procedures. Xanthosine was protected using O-2, O-4 pivaloyloxymethyl groups; N,N-dimethylguanosine with 6-O-(2-nitrophenyl)-ethyl and 6-thio-inosine with S-cyanoethyl protecting groups. The cleavage activity of the ribozymes containing the 15.1 analogs is summarized in **Figure 36**. For comparison **Figure 37** summarizes reported
0 functional group modification studies performed at the A 15.1 residue in the A-15.1 •U-16.1 context of NUH cleaving ribozymes.

Modifications at the purine 15.1 N1 and/or C6 positions (Figure 36 A, B, C)

In the 6-thio-inosine (A) (sI) 15.1 substituted ribozyme, the original (I-15.1) position 6 O•H-N (C-16.1) bonds are replaced by weaker (sI-15.1) position 6 S•H-N (C-16.1) hydrogen bonds while all other functional groups remain unchanged. Ribozymes with an adenosine (B) at position 15.1 (A-15.1) are inactive with C-16.1 substrates since the ribozyme geometry requires the [A-15.1] position 6 amino group and the [C-16.1] position 4 amino group hydrogen-bond donor functional groups to be in close proximity. Similarly, low activity is observed with I-15.1 ribozymes and U-16.1 substrates, where the [I-15.1] position 6 keto and [C-16.1] position 4 keto hydrogen-bond acceptor groups are opposed (Figure 37, B). Although inosine can form stable mismatch pairs with uridine in RNA duplexes or in tRNA anticodon-mRNA interactions, these results suggest that the geometry in the I•U mismatches differ from that of the A•U (or I•C) base pair in the active NUH ribozyme. Substitution of N1-Methyl-inosine (C) in place of inosine at position 15.1 leads to complete loss of cleavage activity.

Modifications at the purine 15.1 C2 and/or N3 position (Figure 36 D, E, F)

The extremely low activity observed with the G-15.1 (D) substituted analog may be explained by the formation of a G-C Watson-Crick base pair. The replacement of the I•C pair with a G•C pair can significantly distort the geometry at the 15.1-16.1 position. G-15.1 N2-alkylation (E) gives only minimal recovery of catalytic activity compared to G-15.1, suggesting that the steric problems introduced by the bulky N-methyl groups may interfere with stacking interactions. The activity of this construct is significantly less than that of iso-G-15.1 (Figure 37, E) containing ribozymes in the standard A-U context. Xanthosine 15.1 (F) contains the same functional groups as inosine at the N1 and C6 sites but contains an additional hydrogen-bond donor site at position N3 along with a C2 carbonyl group. The complete lack of activity seen with this construct reinforces the importance of the purine N3 acceptor functionality in transition state formation. Similarly, 3-deaza-adenosine (Figure 37, F) containing ribozymes were also inactive. The C2 carbonyl of the 15.1 purine shows no significant negative interference in iso-guanosine containing 15.1 ribozymes.

Activity of modified core variants

To complete the characterization of the I•C pair containing ribozymes, the acceptance of various core substitution patterns was tested. Short substrates containing GCH and GUH (H= non G) triplets were compared using 3 different modified ribozymes. The acceptance of the U-4 2'-O-alkyl substituent is the greatest with GCA triplets while U-4 = 2'-deoxy-2'-amino uridine and U-4 = ribo uridine substituted ribozymes show a similar level of activity with NCH and NUH triplets. The results of this comparison are summarized in **Table 64**. In addition, a ribozyme construct in which ribo inosine replaces adenosine at positions 14 and 15.1 was tested which demonstrated cleavage activity.

Apart from the A-15.1 •U-16.1 to I-15.1 •C-16.1 change that reverses the polarity of an important H-bond in the ribozyme structure, no other functional group changes at the 15.1 purine residue seem to be compatible with the requirements of efficient catalysis. The I-15.1 and A-15.1 ribozymes are equally suitable for practical applications because there are only minor differences in the acceptance of stabilizing residues.

Example 13: Activity of NCH Ribozyme to inhibit HER2 gene expression

Applicant has designed, synthesized and tested several NCH ribozymes and HH ribozymes targeted against HER2 RNA (see, for example, **Tables 31 and 34**) in cell proliferation assays.

Proliferation assay: The model proliferation assay used in the study can require a cell plating density of 2000 cells/well in 96-well plates and at least 2 cell doublings over a 5-day treatment period. To calculate cell density for proliferation assays, the FIPS (fluoro-imaging processing system) method well in the art was used. This method allows for cell density measurements after nucleic acids are stained with CyQuant® dye, and has the advantage of accurately measuring cell densities over a very wide range 1,000-100,000 cells/well in 96-well format.

Ribozymes (50-200 nM) were delivered in the presence of cationic lipid at 2.0 µg/mL and inhibition of proliferation was determined on day 5 post-treatment. Two full ribozyme screens were completed and 4 lead *HH* and 11 lead *NCH* ribozymes were chosen for further testing. Of the 15 lead Rzs chosen from primary screens, 4 *NCH* and 1

HH Rzs continued to inhibit cell proliferation in subsequent experiments. *NCH* Rzs against sites, 2001 (RPI No. 17236), 2783 (RPI No. 17249), 2939 (RPI No. 17251) or 3998 (RPI No. 17262) caused inhibition of proliferation ranging from 25-60% as compared to a scrambled control Rz (IA; RPI No. 17263). Of the five lead Rzs, the most efficacious is the *NCH* Rz (RPI No. 17251) against site 2939 of HER2 RNA. An example of results from cell culture assay is shown in Figure 3. Referring to Figure 3, *NCH* ribozymes and a *HH* ribozyme targeted against HER2 RNA are shown to cause significant inhibition of proliferation of cells. This shows that ribozymes, for instance, the *NCH* ribozymes are capable of inhibiting HER2 gene expression in mammalian cells.

Example 14: Activity of NCH Ribozyme to inhibit PKC alpha gene expression

The Protein Kinase C family contains twelve currently known isozymes divided into three classes: the classic, Ca^{++} dependent ($\text{PKC}\alpha$, βI , βII , γ), the novel, non- Ca^{++} dependent ($\text{PKC}\delta$, ϵ , μ , η , θ) and the atypical ($\text{PKC}\xi$, i/λ); all of which are serine/threonine kinases. These isozymes show distinct and overlapping tissue, cellular, and subcellular distribution. They aid in the regulation of cell growth and differentiation through their response to second messenger products of lipid metabolism (Blobe, *et al.*, 1996, *Cancer Surveys*, 27, 213-248). These second messengers include diacylglycerol (DAG), inositol-triphosphate (IP3), lysophospholipids, free fatty acids, and phosphatidate which act directly or in addition to changes in the Ca^{++} concentration. A simple model for $\text{PKC}\alpha$ activation follows a two step mechanism. First, membrane association of $\text{PKC}\alpha$ is through Ca^{++} and phospholipid interactions and second, the kinase is activated by interaction with DAG. An example of a signal cascade subsequent to PKC activation is PKC's phosphorylation of c-Raf, which phosphorylates MEK, which phosphorylates MAP, which phosphorylates transcription factors such as Jun and thereby activates a mitogenic program in the nucleus. There are numerous substrates for the various PKC's, one which for $\text{PKC}\alpha$ ultimately stimulates transcription factors that activate P-glycoprotein (P-gp) causing the multi-drug resistant phenotype (MDR) (Blobe, *et al.*, 1994, *Cancer and Metastasis Reviews*, 13, 411-431).

Cell Culture Review

PKC's have been implicated in tumor promotion since the discovery that these molecules can serve as receptors for tumor-promoting phorbol esters. An increase in PKC overexpression in numerous tumor cell lines and tumor tissues has also been

5 demonstrated. PKC overexpression has been shown to be associated with increased invasion and metastasis in mouse Lewis lung carcinoma, mouse B16 melanoma (Lee *et al.*, 1997, *Molecular Carcinogenesis*, 18, 44-53), mouse mammary adenocarcinoma, mouse fibrosarcoma, human lung carcinoma (Wang and Liu, 1998, *Acta Pharmacologica Sinica*, 19, 265-268), human bladder carcinoma, human pancreatic cancer (Denham *et al.*, 1998, 0 *Surgery*, 124, 218-223), and human gastric cancer (Dean *et al.*, 1996, *Cancer Research*, 56, 3499-3507). Mounting evidence suggests PKC α can stimulate adhesion molecule expression and can directly act on these membrane bound species as substrates, thereby modulating cellular adhesion to the extracellular matrix and increasing metastatic potential. Furthermore, human surgical specimens have demonstrated elevated PKC in breast 5 tumors, thyroid carcinomas and melanomas (Becker *et al.*, 1990, *Oncogene*, 5, 1133-1139).

Utz *et al.*, 1994, *Int. J. Cancer*, 57, 104-110, describe a cell proliferation assay in which small molecule inhibitors of PKC demonstrate anti-proliferative activity in CCRF-VCR 1000 and KB-8511 cells with the multidrug resistant (MDR) phenotype. PKC α is 0 overexpressed in tumor tissues that express the MDR phenotype. This phenotype is associated with the expression of a 170 kDa broad specificity drug efflux pump, P-gp. PKC α phosphorylation of P-gp has been shown *in vitro*. In addition, PKC expression correlates with resistance to doxorubicin and high P-gp levels in human renal carcinoma and non-small cell lung carcinoma. Inhibitors of PKC partially reverse the MDR 5 phenotype and decrease phosphorylation of P-gp (Caponigro *et al.*, 1997, *Anti-Cancer Drugs*, 8, 26-33).

Dean *et al.*, 1994, *Journal of Biological Chemistry*, 269, 16416-24, describe cell culture studies in which antisense targeting of PKC α resulted in the potent inhibition of mRNA and protein expression in human lung carcinoma (A549) cells. In this study, PKC 0 α inhibition resulted in the reduced induction of intercellular adhesion molecule 1 (ICAM-1) mRNA by phorbol esters.

Yano *et al.*, 1999, *Endocrinology*, 140, 4622-4632, describe a cell proliferation study in which down regulation of different PKC isoforms, including PKC α , results in the inhibition of insulin like growth factor I induced vascular smooth muscle cell proliferation, migration, and gene expression.

- 5 Wang *et al.*, 1999, *Experimental Cell Research*, 250, 253-263, describe cell culture studies in which antisense inhibition of PKC α results in the reversal of the transformed phenotype in human lung carcinoma (LTEPa-2) cells. In this study, the amounts of PKC α protein and total PKC activity were decreased when compared to control cells.

- Sioud and Sorensen, 1998, *Nature Biotechnology*, 16, 556-561, describe
0 hammerhead ribozyme inhibition of PKC α in rat glioma cell lines (BT4C and BT4Cn). This study demonstrated inhibition of malignant glioma cell proliferation along with the inhibition of regulatory Bcl-x_L protein expression. Bcl-x_L is overexpressed in glioma cells and is an apoptosis inhibitor. The ribozyme mediated inhibition of cell proliferation presumably results from apoptosis induction of transformed glioma cells through
5 suppression of PKC α and Bcl-x_L (Leirdal and Sioud, 1999, *British J. of Cancer*, 80, 1558-1564).

Animal Models

- Evaluating the efficacy of anti-PKC α agents in animal models is an important prerequisite to human clinical trials. A variety of mouse xenograft models using human
0 tumor cell lines have been developed using cell lines which express high levels of PKC α protein. McGraw *et al.*, 1997, *Anti-Cancer Drug Design*, 12, 315-326, describe mouse xenograft models using human breast (MDA MB-321), prostate (Du-145), colon (Colo 205, WiDr), lung (NCI H69, H209, J460, H520, A549), bladder (T-24), and melanoma (SK-mel 1) carcinoma cells. Antisense oligonucleotides targeting PKC α administered
5 intravenously following s.c. transplanted tumor cells resulted in dose dependant decreases in tumor size when compared to controls in most cases. Similar studies using T-24 bladder carcinoma, non-small cell lung carcinoma (A549), and Colo 205 colon carcinoma mouse xenografts are described in Dean *et al.*, 1996, *Biochemical Society Transactions*, 24, 623. Sioud and Sorensen, 1998, *Nature Biotechnology*, 16, 556-561, describe a rat model
0 in which inbred syngeneic BDIX rats were inoculated subcutaneously with BT4Cn glioma cells. After approximately three weeks, rats were treated with a single injection of

ribozyme targeting PKC α resulting in inhibition of tumor growth as determined by tumor size and/or weight when compared to controls. The above studies provide proof that inhibition of PKC α expression by anti-PKC α agents causes inhibition of tumor growth in animals. Lead anti-PKC α ribozymes chosen from *in vitro* assays can be further tested in mouse xenograft models. Ribozymes can be first tested alone and then in combination with standard chemotherapies.

Animal Model Development

Human lung (A549, NCI H520) tumor and breast (MDA-MB 231) cell lines can be characterized to establish their growth curves in mice. These cell lines are been implanted into both nude and SCID mice and primary tumor volumes are measured 3 times per week. Growth characteristics of these tumor lines using a Matrigel implantation format can also be established. In addition, the use of other cell lines that have been engineered to express high levels of PKC α can also be used. The tumor cell line(s) and implantation method that supports the most consistent and reliable tumor growth can be used in animal studies to test promising PKC α ribozyme(s). Ribozymes can be administered by daily subcutaneous injection or by continuous subcutaneous infusion from Alzet mini osmotic pumps beginning 3 days after tumor implantation and continuing for the duration of the study. Group sizes of at least 10 animals are employed. Efficacy is determined by statistical comparison of tumor volume of ribozyme-treated animals to a control group of animals treated with saline alone. Because the growth of these tumors is generally slow (45-60 days), an initial endpoint will be the time in days it takes to establish an easily measurable primary tumor (i.e. 50-100 mm³) in the presence or absence of ribozyme treatment.

Clinical Summary

Overview

Ribozymes targeting PKC α have strong potential to develop into useful therapeutics directed towards numerous cancer types. Lung cancer is the leading cause of cancer deaths for both men and women in the USA. The incidence of lung cancer in the United States is ~172,000 cases per year, accounting for 14% of cancer diagnoses. Approximately 158,000 die each year of lung cancer, accounting for 28% of all cancer deaths. Numerous other

indications exist including cancers of the bladder, colon, breast, prostate, and ovary in addition to melanoma and glioblastoma.

McGraw *et al.*, 1997, *Anti-Cancer Drug Design*, 12, 315-326, describe a Phase I trial for ISIS 3521/CGP 64128A, a PKC alpha antisense construct. In this trial, ISIS 3521/CGP 64128A was administered as either a two-hour i.v. infusion three times per week for three consecutive weeks, or as a continuous i.v. infusion for twenty-one consecutive days. The authors report that patients demonstrated excellent tolerance to the antisense compound when administered at doses of up to 2.5 mg/kg by the two-hour i.v. infusion and at 1.5 mg/kg/day by continuous i.v. infusion. In patients receiving the two-hour i.v. infusion schedule, the post-infusion plasma concentration of the compound increased proportional to the dose, and metabolites were determined to have been cleared rapidly from plasma with a half-life of thirty to forty-five minutes. These metabolites were composed of chain-shortened oligonucleotides, consistent with exonuclease-mediated degradation. No evidence of accumulation, induction, or inhibition of metabolism was found after the administration of repetitive doses.

Therapy

Treatment options for lung cancer are determined by the type and stage of the cancer and include surgery, radiation therapy, and chemotherapy. For many localized cancers, surgery is usually the treatment of choice. Because the disease has usually spread by the time it is discovered, radiation therapy and chemotherapy are often needed in combination with surgery. Chemotherapy alone or combined with radiation has replaced surgery as the treatment of choice for small cell lung cancer; on this regimen, a large percentage of patients experience remission, which in some cases is long-lasting. The 1-year relative survival rates for lung cancer have increased from 32% in 1973 to 41% in 1994, largely due to improvements in surgical techniques. The 5-year relative survival rate for all stages combined is only 14%. The survival rate is 50% for cases detected when the disease is still localized, but only 15% of lung cancers are discovered that early.

Common chemotherapies include various combinations of cytotoxic drugs to kill the cancer cells. These drugs include paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubin, fluorouracil *etc.* Significant toxicities are associated with these cytotoxic therapies. Well-characterized toxicities include nausea and vomiting,

myelosuppression, alopecia and mucosity. Serious cardiac problems are also associated with certain of the combinations, *e.g.* doxorubin and paclitaxel, but are less common.

Applicant has designed several NCH ribozymes targeted against PKC α RNA (Genebank accession No NM_002737) (see, for example, **Table 63**). These ribozymes are
5 used first in a proliferation assay that is used to select ribozyme leads.

Proliferation assay: The model proliferation assay useful in the study can require a cell plating density of 2000 cells/well in 96-well plates and at least 2 cell doublings over a 5-day treatment period. To calculate cell density for proliferation assays, the FIPS (fluoro-imaging processing system) method well known in the art can be used. This method allows
10 for cell density measurements after nucleic acids are stained with CyQuant® dye, and has the advantage of accurately measuring cell densities over a very wide range 1,000-100,000 cells/well in 96-well format.

Ribozymes (50-200 nM) are delivered in the presence of cationic lipid at 2.0 μ g/mL and inhibition of proliferation is determined on day 5 post-treatment. Two full ribozyme
15 screens are usually completed and lead ribozymes are chosen for further testing. Of the lead ribozymes chosen from primary screens, ribozymes which continue to inhibit cell proliferation in subsequent experiments are selected for PKC α RNA and protein inhibition studies.

!0 Example 15: Nucleoside Triphosphates and their incorporation into oligonucleotides

The synthesis of nucleotide triphosphates and their incorporation into nucleic acids using polymerase enzymes has greatly assisted in the advancement of nucleic acid research. The polymerase enzyme utilizes nucleotide triphosphates as precursor molecules to assemble oligonucleotides. Each nucleotide is attached by a phosphodiester bond
!5 formed through nucleophilic attack by the 3' hydroxyl group of the oligonucleotide's last nucleotide onto the 5' triphosphate of the next nucleotide. Nucleotides are incorporated one at a time into the oligonucleotide in a 5' to 3' direction. This process allows RNA to be produced and amplified from virtually any DNA or RNA templates.

Most natural polymerase enzymes incorporate standard nucleotide triphosphates into
!0 nucleic acid. For example, a DNA polymerase incorporates dATP, dTTP, dCTP, and dGTP into DNA and an RNA polymerase generally incorporates ATP, CTP, UTP, and

GTP into RNA. There are however, certain polymerases that are capable of incorporating non-standard nucleotide triphosphates into nucleic acids (Joyce, 1997, *PNAS* 94, 1619-1622, Huang et al., *Biochemistry* 36, 8231-8242).

Before nucleosides can be incorporated into RNA transcripts using polymerase
5 enzymes they must first be converted into nucleotide triphosphates which can be recognized by these enzymes. Phosphorylation of unblocked nucleosides by treatment with POCl₃ and trialkyl phosphates was shown to yield nucleoside 5'-phosphorodichloridates (Yoshikawa *et al.*, 1969, *Bull. Chem. Soc.(Japan)* 42, 3505). Adenosine or 2'-deoxyadenosine 5'-triphosphate was synthesized by adding an additional
10 step consisting of treatment with excess tri-n-butylammonium pyrophosphate in DMF followed by hydrolysis (Ludwig, 1981, *Acta Biochim. et Biophys. Acad. Sci. Hung.* 16, 131-133).

Non-standard nucleotide triphosphates are not readily incorporated into RNA transcripts by traditional RNA polymerases. Mutations have been introduced into RNA
15 polymerase to facilitate incorporation of deoxyribonucleotides into RNA (Sousa & Padilla, 1995, *EMBO J.* 14, 4609-4621, Bonner *et al.*, 1992, *EMBO J.* 11, 3767-3775, Bonner et al., 1994, *J. Biol. Chem.* 42, 25120-25128, Aurup *et al.*, 1992, *Biochemistry* 31, 9636-9641).

McGee *et al.*, International PCT Publication No. WO 95/35102, describes the incorporation of 2'-NH₂-NTP's, 2'-F-NTP's, and 2'-deoxy-2'-benzyloxyamino UTP into
20 RNA using bacteriophage T7 polymerase.

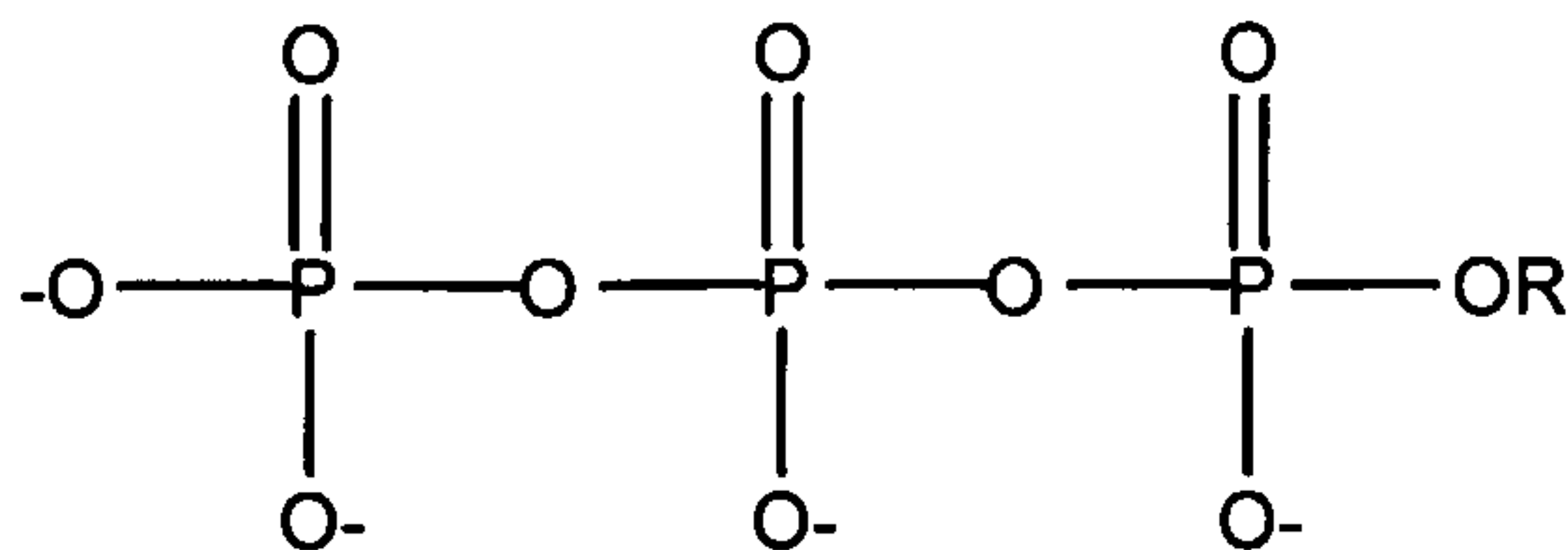
Wieczorek *et al.*, 1994, *Bioorganic & Medicinal Chemistry Letters* 4, 987-994, describes the incorporation of 7-deaza-adenosine triphosphate into an RNA transcript using bacteriophage T7 RNA polymerase.

Lin *et al.*, 1994, *Nucleic Acids Research* 22, 5229-5234, reports the incorporation of
25 2'-NH₂-CTP and 2'-NH₂-UTP into RNA using bacteriophage T7 RNA polymerase and polyethylene glycol containing buffer. The article describes the use of the polymerase synthesized RNA for *in vitro* selection of aptamers to human neutrophil elastase (HNE).

This invention relates to novel nucleotide triphosphate (NTP) molecules, and their incorporation into nucleic acid molecules, including nucleic acid catalysts. The NTPs of
30 the instant invention are distinct from other NTPs known in the art. The invention further relates to incorporation of these nucleotide triphosphates into oligonucleotides using an RNA polymerase; the invention further relates to novel transcription conditions for the

incorporation of modified (non-standard) and unmodified NTP's, into nucleic acid molecules. Further, the invention relates to methods for synthesis of novel NTP's

In a first aspect, the invention features NTP's having the formula triphosphate-OR, for example the following formula 3:



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where R is any nucleoside; specifically the nucleosides 2'-*O*-methyl-2,6-diaminopurine riboside; 2'-deoxy-2'-amino-2,6-diaminopurine riboside; 2'-(*N*-alanyl) amino-2'-deoxy-uridine; 2'-(*N*-phenylalanyl) amino-2'-deoxy-uridine; 2'-deoxy-2'-(*N*-β-alanyl) amino ; 2'-deoxy-2'-(lysiyl) amino uridine; 2'-*C*-allyl uridine; 2'-*O*-amino-uridine; 2'-*O*-methylthiomethyl adenosine; 2'-*O*-methylthiomethyl cytidine ; 2'-*O*-methylthiomethyl guanosine; 2'-*O*-methylthiomethyl-uridine; 2'-deoxy-2'-(*N*-histidyl) amino uridine; 2'-deoxy-2'-amino-5-methyl cytidine; 2'-(*N*-β-carboxamidine-β-alanyl) amino-2'-deoxy-uridine; 2'-deoxy-2'-(*N*-β-alanyl)-guanosine; 2'-*O*-amino-adenosine; 2'-(*N*-lysiyl) amino-2'-deoxy-cytidine; 2'-Deoxy-2'-(*L*-histidine) amino Cytidine; 5-Imidazoleacetic acid 2'-deoxy uridine, 5-[3-(*N*-4-imidazoleacetyl)aminopropynyl]-2'-*O*-methyl uridine, 5-(3-aminopropynyl)-2'-*O*-methyl uridine, 5-(3-aminopropyl)-2'-*O*-methyl uridine, 5-[3-(*N*-4-imidazoleacetyl)aminopropyl]-2'-*O*-methyl uridine, 5-(3-aminopropyl)-2'-deoxy-2-fluoro uridine, 2'-Deoxy-2'-(β-alanyl-*L*-histidyl) amino uridine, 2'-deoxy-2'-β-alaninamido-uridine, 3-(2'-deoxy-2'-fluoro-β-D-ribofuranosyl)piperazino[2,3-*D*]pyrimidine-2-one, 5-[3-(*N*-4-imidazoleacetyl)aminopropyl]-2'-deoxy-2'-fluoro uridine, 5-[3-(*N*-4-imidazoleacetyl)aminopropynyl]-2'-deoxy-2'-fluoro uridine, 5-*E*-(2-carboxyvinyl-2'-deoxy-2'-fluoro uridine, 5-[3-(*N*-4-aspartyl)aminopropynyl]-2'-fluoro uridine, 5-(3-aminopropyl)-2'-deoxy-2-fluoro cytidine, and 5-[3-(*N*-4-succinyl)aminopropyl]-2'-deoxy-2-fluoro cytidine.

In a second aspect, the invention features inorganic and organic salts of the nucleoside triphosphates of the instant invention.

In a third aspect, the invention features a process for the synthesis of pyrimidine nucleotide triphosphate (such as UTP, 2'-*O*-MTM-UTP, dUTP and the like) including the steps of monophosphorylation where the pyrimidine nucleoside is contacted with a mixture having a phosphorylating agent (such as phosphorus oxychloride, phospho-tris-triazolides, phospho-tris-triimidazolides and the like), trialkyl phosphate (such as triethylphosphate or trimethylphosphate or the like) and a hindered base (such as dimethylaminopyridine, DMAP and the like) under conditions suitable for the formation of pyrimidine monophosphate; and pyrophosphorylation where the pyrimidine monophosphate is contacted with a pyrophosphorylating reagent (such as tributylammonium pyrophosphate) under conditions suitable for the formation of pyrimidine triphosphates.

By "nucleotide triphosphate" or "NTP" is meant a nucleoside bound to three inorganic phosphate groups at the 5' hydroxyl group of the modified or unmodified ribose or deoxyribose sugar where the 1' position of the sugar may comprise a nucleic acid base or hydrogen. The triphosphate portion may be modified to include chemical moieties which do not destroy the functionality of the group (*i.e.*, allow incorporation into an RNA molecule).

In another preferred embodiment, nucleotide triphosphates (NTPs) of the instant invention are incorporated into an oligonucleotide using an RNA polymerase enzyme. RNA polymerases include but are not limited to mutated and wild type versions of bacteriophage T7, SP6, or T3 RNA polymerases. Applicant has also found that the NTPs of the present invention can be incorporated into oligonucleotides using certain DNA polymerases, such as Taq polymerase.

In yet another preferred embodiment, the invention features a process for incorporating modified NTP's into an oligonucleotide including the step of incubating a mixture having a DNA template, RNA polymerase, NTP, and an enhancer of modified NTP incorporation under conditions suitable for the incorporation of the modified NTP into the oligonucleotide.

By "enhancer of modified NTP incorporation" is meant a reagent which facilitates the incorporation of modified nucleotides into a nucleic acid transcript by an RNA polymerase. Such reagents include, but are not limited to, methanol, LiCl, polyethylene glycol (PEG), diethyl ether, propanol, methyl amine, ethanol, and the like.

In another preferred embodiment, the modified nucleotide triphosphates can be incorporated by transcription into a nucleic acid molecules including enzymatic nucleic acid, antisense, 2-5A antisense chimera, oligonucleotides, triplex forming oligonucleotide (TFO), aptamers and the like (Stull *et al.*, 1995 *Pharmaceutical Res.* 12, 465).

5 By "triplex forming oligonucleotides (TFO)" it is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89, 504).

0 In yet another preferred embodiment, the modified nucleotide triphosphates of the instant invention can be used for combinatorial chemistry or *in vitro* selection of nucleic acid molecules with novel function. Modified oligonucleotides can be enzymatically synthesized to generate libraries for screening.

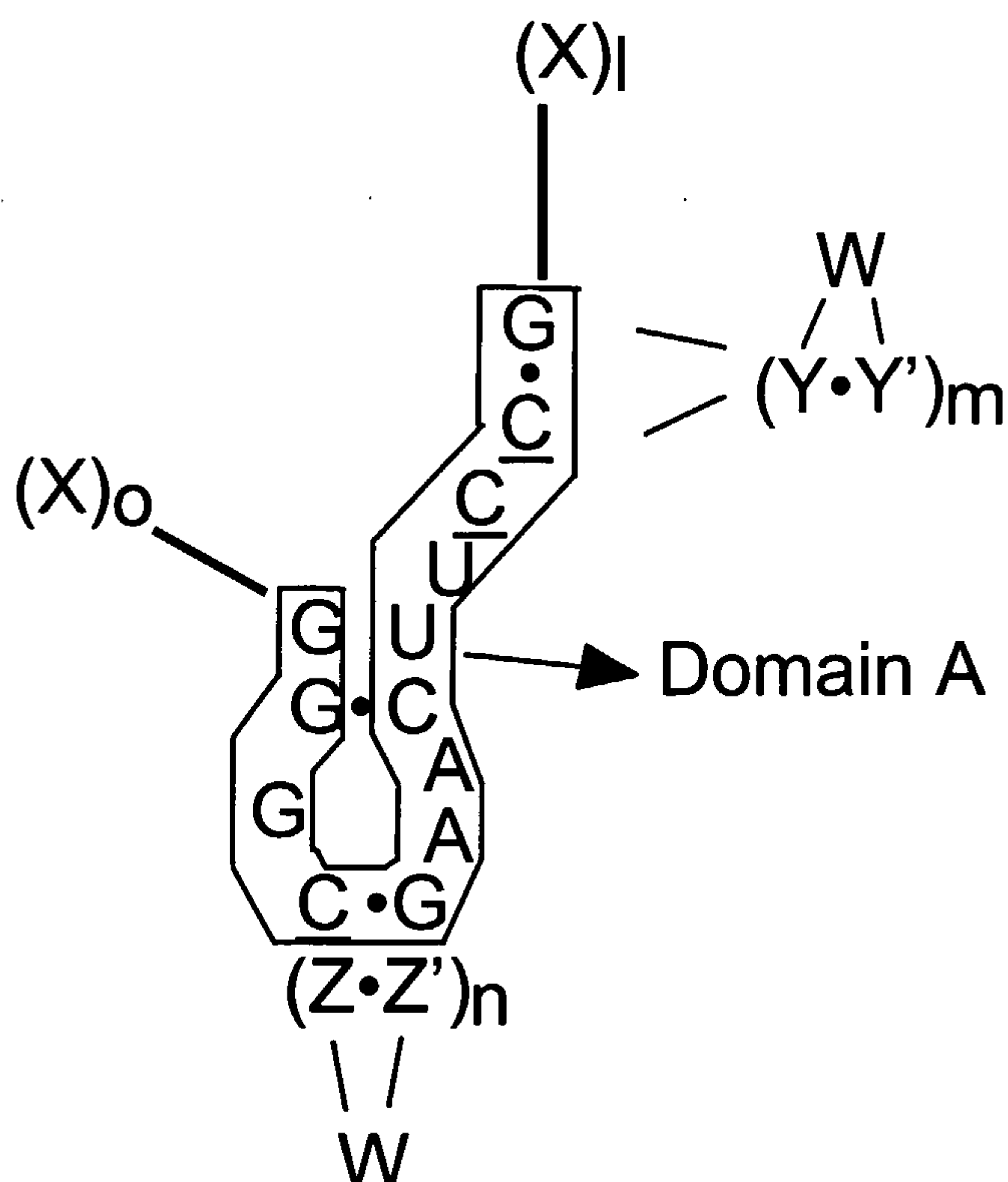
In another preferred embodiment, the invention features nucleic acid based techniques (*e.g.*, enzymatic nucleic acid molecules), antisense nucleic acids, 2-5A
5 antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups) isolated using the methods described in this invention and methods for their use to diagnose, down regulate or inhibit gene expression.

In yet another preferred embodiment, the invention features enzymatic nucleic acid molecules targeted against HER2 RNA, specifically including ribozymes in the class II
10 (zinzyme) motif.

Targets, for example HER2 RNA, for useful ribozymes and antisense nucleic acids can be determined, for example, as described in Draper *et al.*, WO 93/23569; Sullivan *et al.*, WO 93/23057; Thompson *et al.*, WO 94/02595; Draper *et al.*, WO 95/04818; McSwiggen *et al.*, US Patent Nos. 5,525,468 and 5,646,042, all are hereby incorporated by
15 reference herein in their totalities. Other examples include the following PCT applications, which concern inactivation of expression of disease-related genes: WO 95/23225, and WO 95/13380; all of which are incorporated by reference herein.

In yet another preferred embodiment, the invention features a process for incorporating a plurality of compounds of formula 3.

0 In yet another embodiment, the invention features a nucleic acid molecule with catalytic activity having formula 4:

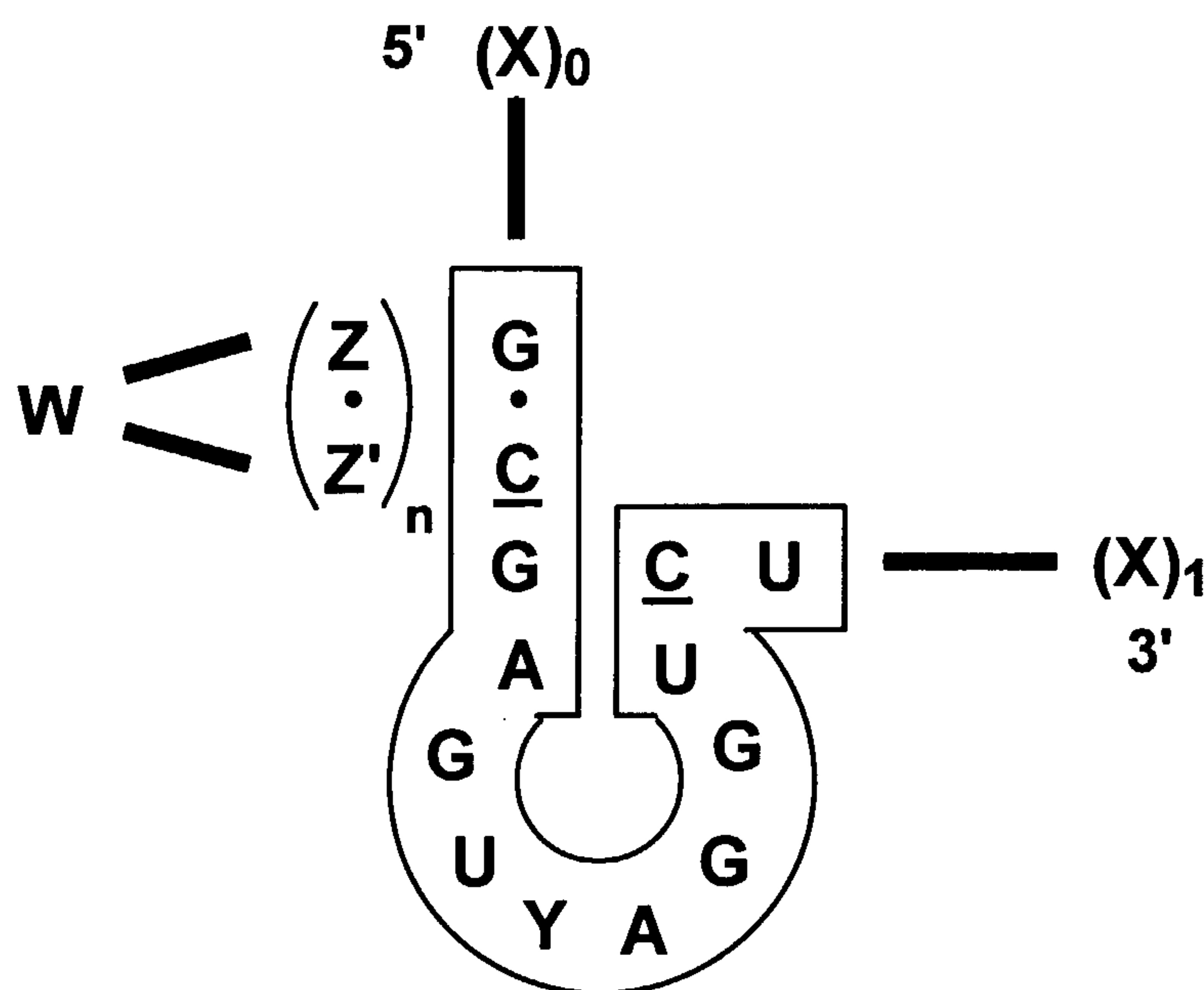


In the formula shown above X, Y, and Z represent independently a nucleotide or a non-nucleotide linker, which may be same or different; • indicates hydrogen bond

- 5 formation between two adjacent nucleotides which may or may not be present; Y' is a nucleotide complementary to Y; Z' is a nucleotide complementary to Z; I is an integer greater than or equal to 3 and preferably less than 20, more specifically 4, 5, 6, 7, 8, 9, 10, 11, 12, or 15; m is an integer greater than 1 and preferably less than 10, more specifically 2, 3, 4, 5, 6, or 7; n is an integer greater than 1 and preferably less than 10, more
- 10 specifically 3, 4, 5, 6, or 7; o is an integer greater than or equal to 3 and preferably less than 20, more specifically 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 15; I and o may be the same length ($I = o$) or different lengths ($I \neq o$); each $X(I)$ and $X(o)$ are oligonucleotides which are of sufficient length to stably interact independently with a target nucleic acid sequence (the target can be an RNA, DNA or RNA/DNA mixed polymers); W is a linker of ≥ 2
- 15 nucleotides in length or may be a non-nucleotide linker; A, U, C, and G represent the nucleotides; G is a nucleotide, preferably 2'-O-methyl or ribo; A is a nucleotide, preferably 2'-O-methyl or ribo; U is a nucleotide, preferably 2'-amino (e.g., 2'-NH₂ or 2'-O-NH₂), 2'-O-methyl or ribo; C represents a nucleotide, preferably 2'-amino (e.g., 2'-NH₂ or 2'-O-

NH₂), and _____ represents a chemical linkage (e.g. a phosphate ester linkage, amide linkage, phosphorothioate, phosphorodithioate or others known in the art).

In yet another embodiment, the invention features a nucleic acid molecule with catalytic activity having formula 5:



- 5 In the formula shown above X, Y, and Z represent independently a nucleotide or a non-nucleotide linker, which may be same or different; • indicates hydrogen bond formation between two adjacent nucleotides which may or may not be present; Z' is a nucleotide complementary to Z; l is an integer greater than or equal to 3 and preferably less than 20, more specifically 4, 5, 6, 7, 8, 9, 10, 11, 12, or 15; n is an integer greater than
- 10 1 and preferably less than 10, more specifically 3, 4, 5, 6, or 7; o is an integer greater than or equal to 3 and preferably less than 20, more specifically 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 15; l and o may be the same length (l = o) or different lengths (l ≠ o); each X_(l) and X_(o) are oligonucleotides which are of sufficient length to stably interact independently with a target nucleic acid sequence (the target can be an RNA, DNA or RNA/DNA mixed
- 15 polymers); X_(o) preferably has a G at the 3'-end, X_(l) preferably has a G at the 5'-end; W is a linker of ≥ 2 nucleotides in length or may be a non-nucleotide linker; Y is a linker of ≥ 1 nucleotides in length, preferably G, 5'-CA-3', or 5'-CAA-3', or may be a non-nucleotide linker; A, U, C, and G represent nucleotides; G is a nucleotide, preferably 2'-O-methyl, 2'-deoxy-2'-fluoro, or 2'-OH; A is a nucleotide, preferably 2'-O-methyl, 2'-deoxy-2'-

fluoro, or 2'-OH; U is a nucleotide, preferably 2'-O-methyl, 2'-deoxy-2'-fluoro, or 2'-OH; C represents a nucleotide, preferably 2'-amino (*e.g.*, 2'-NH₂ or 2'-O- NH₂, and _____ represents a chemical linkage (*e.g.* a phosphate ester linkage, amide linkage, phosphorothioate, phosphorodithioate or others known in the art).

5 The enzymatic nucleic acid molecules of Formula 4 and Formula 5 may independently comprise a cap structure which may independently be present or absent.

 In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide; 4'-thio nucleotide; carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-
10 aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide; 5'-5'-
15 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).

 In another aspect, the invention provides mammalian cells containing one or more
20 nucleic acid molecules and/or expression vectors of this invention. The one or more nucleic acid molecules may independently be targeted to the same or different sites.

Nucleotide Synthesis

 Addition of dimethylaminopyridine (DMAP) to the phosphorylation protocols
25 known in the art can greatly increase the yield of nucleotide monophosphates while decreasing the reaction time. Synthesis of the nucleosides of the invention have been described in several publications and Applicants previous applications (Beigelman *et al.*, International PCT publication No. WO 96/18736; Dudzcy *et al.*, Int. PCT Pub. No. WO 95/11910; Usman *et al.*, Int. PCT Pub. No. WO 95/13378; Matulic-Adamic *et al.*, 1997, *Tetrahedron Lett.* 38, 203; Matulic-Adamic *et al.*, 1997, *Tetrahedron Lett.* 38, 1669; all of
30 which are incorporated herein by reference). These nucleosides are dissolved in triethyl phosphate and chilled in an ice bath. Phosphorus oxychloride (POCl₃) is then added

followed by the introduction of DMAP. The reaction is then warmed to room temperature and allowed to proceed for 5 hours. This reaction allows the formation of nucleotide monophosphates which can then be used in the formation of nucleotide triphosphates.

Tributylamine is added followed by the addition of anhydrous acetonitrile and

5 tributylammonium pyrophosphate. The reaction is then quenched with TEAB and stirred overnight at room temperature (about 20°C). The triphosphate is purified using

Sephadex® column purification or equivalent and/or HPLC and the chemical structure is confirmed using NMR analysis. Those skilled in the art will recognize that the reagents,

temperatures of the reaction, and purification methods can easily be alternated with

10 substitutes and equivalents and still obtain the desired product.

Nucleotide Triphosphates

The invention provides nucleotide triphosphates which can be used for a number of different functions. The nucleotide triphosphates formed from nucleosides found in **Table**
15 **45** are unique and distinct from other nucleotide triphosphates known in the art.

Incorporation of modified nucleotides into DNA or RNA oligonucleotides can alter the properties of the molecule. For example, modified nucleotides can hinder binding of nucleases, thus increasing the chemical half-life of the molecule. This is especially important if the molecule is to be used for cell culture or *in vivo*. It is known in the art that
20 the introduction of modified nucleotides into these molecules can greatly increase the stability and thereby the effectiveness of the molecules (Burgin *et al.*, 1996, *Biochemistry* 35, 14090-14097; Usman *et al.*, 1996, *Curr. Opin. Struct. Biol.* 6, 527-533).

Modified nucleotides are incorporated using either wild type or mutant polymerases.

For example, mutant T7 polymerase is used in the presence of modified nucleotide

25 triphosphate(s), DNA template and suitable buffers. Those skilled in the art will recognize that other polymerases and their respective mutant versions can also be utilized for the

incorporation of NTP's of the invention. Nucleic acid transcripts were detected by incorporating radiolabelled nucleotides (α -³²P NTP). The radiolabeled NTP contained the

same base as the modified triphosphate being tested. The effects of methanol, PEG and

30 LiCl were tested by adding these compounds independently or in combination. Detection

and quantitation of the nucleic acid transcripts was performed using a Molecular Dynamics

PhosphorImager. Efficiency of transcription was assessed by comparing modified nucleotide triphosphate incorporation with all-ribonucleotide incorporation control. Wild-type polymerase was used to incorporate NTP's using the manufacturer's buffers and instructions (Boehringer Mannheim).

5

Transcription Conditions

Incorporation rates of modified nucleotide triphosphates into oligonucleotides can be increased by adding to traditional buffer conditions, several different enhancers of modified NTP incorporation. Applicant has utilized methanol and LiCl in an attempt to increase incorporation rates of dNTP using RNA polymerase. These enhancers of modified NTP incorporation can be used in different combinations and ratios to optimize transcription. Optimal reaction conditions differ between nucleotide triphosphates and can readily be determined by standard experimentation. Overall, however, Applicant has found that inclusion of enhancers of modified NTP incorporation such as methanol or inorganic compound such as lithium chloride increase the mean transcription rates.

10

Applicant synthesized pyrimidine nucleotide triphosphates using DMAP in the reaction. For purines, applicant utilized standard protocols previously described in the art (Yoshikawa *et al supra*; Ludwig, *supra*). Described below is one example of a pyrimidine nucleotide triphosphate and one purine nucleotide triphosphate synthesis.

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Synthesis of purine nucleotide triphosphates: 2'-O-methyl-guanosine-5'-triphosphate

2'-O-methyl guanosine nucleoside (0.25 grams, 0.84 mmol) was dissolved in triethyl phosphate (5.0) ml by heating to 100°C for 5 minutes. The resulting clear, colorless solution was cooled to 0°C using an ice bath under an argon atmosphere. Phosphorous oxychloride (1.8 eq., 0.141 ml) was then added to the reaction mixture with vigorous stirring. The reaction was monitored by HPLC, using a sodium perchlorate gradient. After 5 hours at 0°C, tributylamine (0.65 ml) was added followed by the addition of anhydrous acetonitrile (10.0 ml), and after 5 minutes (reequilibration to 0°C) tributylammonium pyrophosphate (4.0 eq., 1.53 g) was added. The reaction mixture was quenched with 20 ml of 2M TEAB after 15 minutes at 0°C (HPLC analysis with above conditions showed consumption of monophosphate at 10 minutes) then stirred overnight at room temperature, the mixture was evaporated *in vacuo* with methanol co-evaporation

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(4x) then diluted in 50 ml 0.05M TEAB. DEAE sephadex purification was used with a gradient of 0.05 to 0.6 M TEAB to obtain pure triphosphate (0.52 g, 66.0% yield) (elutes around 0.3M TEAB); the purity was confirmed by HPLC and NMR analysis.

5 Synthesis of Pyrimidine nucleotide triphosphates: 2'-O-methylthiomethyl-uridine-5'-triphosphate

2'-O-methylthiomethyl uridine nucleoside (0.27 grams, 1.0 mmol) was dissolved in triethyl phosphate (5.0 ml). The resulting clear, colorless solution was cooled to 0°C with an ice bath under an argon atmosphere. Phosphorus oxychloride (2.0 eq., 0.190 ml) was
10 then added to the reaction mixture with vigorous stirring. Dimethylaminopyridine (DMAP, 0.2eq., 25 mg) was added, the solution warmed to room temperature and the reaction was monitored by HPLC, using a sodium perchlorate gradient. After 5 hours at 20°C, tributylamine (1.0 ml) was added followed by anhydrous acetonitrile (10.0 ml), and after 5 minutes tributylammonium pyrophosphate (4.0 eq., 1.8 g) was added. The reaction
15 mixture was quenched with 20 ml of 2M TEAB after 15 minutes at 20°C (HPLC analysis with above conditions showed consumption of monophosphate at 10 minutes) then stirred overnight at room temperature. The mixture was evaporated *in vacuo* with methanol co-evaporation (4x) then diluted in 50 ml 0.05M TEAB. DEAE fast flow Sepharose purification with a gradient of 0.05 to 1.0 M TEAB was used to obtain pure triphosphate
20 (0.40 g, 44% yield) (elutes around 0.3M TEAB) as determined by HPLC and NMR analysis.

Utilization of DMAP in Uridine 5'-Triphosphate Synthesis

The reactions were performed on 20 mg aliquots of nucleoside dissolved in 1 ml of
25 triethyl phosphate and 19 ul of phosphorus oxychloride. The reactions were monitored at 40 minute intervals automatically by HPLC to generate yield-of-product curves at times up to 18 hours. A reverse phase column and ammonium acetate/ sodium acetate buffer system (50mM & 100mM respectively at pH 4.2) was used to separate the 5', 3', 2' monophosphates (the monophosphates elute in that order) from the 5'-triphosphate and the
30 starting nucleoside. The data is shown in Table 46. These conditions doubled the product yield and resulted in a 10-fold improvement in the reaction time to maximum yield (1200 minutes down to 120 minutes for a 90% yield). Selectivity for 5'-monophosphorylation

was observed for all reactions. Subsequent triphosphorylation occurred in nearly quantitative yield.

Materials Used in Bacteriophage T7 RNA Polymerase Reactions

5 **Buffer 1:** Reagents are mixed together to form a 10X stock solution of buffer 1 (400 mM Tris-Cl [pH 8.1], 200 mM MgCl₂, 100 mM DTT, 50 mM spermidine, and 0.1% triton® X-100). Prior to initiation of the polymerase reaction methanol, LiCl is added and the buffer is diluted such that the final reaction conditions for condition 1 consisted of : 40mM tris (pH 8.1), 20mM MgCl₂, 10 mM DTT, 5 mM spermidine, 0.01% triton® X-
10 100, 10% methanol, and 1 mM LiCl.

BUFFER 2: Reagents are mixed together to form a 10X stock solution of buffer 2 (400 mM Tris-Cl [pH 8.1], 200 mM MgCl₂, 100 mM DTT, 50 mM spermidine, and 0.1% triton® X-100). Prior to initiation of the polymerase reaction PEG, LiCl is added and the buffer is diluted such that the final reaction conditions for buffer 2 consisted of : 40mM
15 tris (pH 8.1), 20mM MgCl₂, 10 mM DTT, 5 mM spermidine, 0.01% triton® X-100, 4% PEG, and 1 mM LiCl.

BUFFER 3: Reagents are mixed together to form a 10X stock solution of buffer 3 (400 mM Tris-Cl [pH 8.0], 120 mM MgCl₂, 50 mM DTT, 10 mM spermidine and 0.02% triton® X-100). Prior to initiation of the polymerase reaction PEG is added and the buffer
20 is diluted such that the final reaction conditions for buffer 3 consisted of : 40mM tris (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% triton® X-100, and 4% PEG.

BUFFER 4: Reagents are mixed together to form a 10X stock solution of buffer 4 (400 mM Tris-Cl [pH 8.0], 120 mM MgCl₂, 50 mM DTT, 10 mM spermidine and 0.02% triton® X-100). Prior to initiation of the polymerase reaction PEG, methanol is added and
25 the buffer is diluted such that the final reaction conditions for buffer 4 consisted of : 40mM tris (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% triton® X-100, 10% methanol, and 4% PEG.

BUFFER 5: Reagents are mixed together to form a 10X stock solution of buffer 5 (400 mM Tris-Cl [pH 8.0], 120 mM MgCl₂, 50 mM DTT, 10 mM spermidine and 0.02% triton® X-100). Prior to initiation of the polymerase reaction PEG, LiCl is added and the
30 buffer is diluted such that the final reaction conditions for buffer 5 consisted of : 40mM

tris (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% triton® X-100, 1 mM LiCl and 4% PEG.

BUFFER 6: Reagents are mixed together to form a 10X stock solution of buffer 6 (400 mM Tris-Cl [pH 8.0], 120 mM MgCl₂, 50 mM DTT, 10 mM spermidine and 0.02% triton® X-100). Prior to initiation of the polymerase reaction PEG, methanol is added and the buffer is diluted such that the final reaction conditions for buffer 6 consisted of : 40mM tris (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% triton® X-100, 10% methanol, and 4% PEG.

BUFFER 7: Reagents are mixed together to form a 10X stock solution of buffer 6 (400 mM Tris-Cl [pH 8.0], 120 mM MgCl₂, 50 mM DTT, 10 mM spermidine and 0.02% triton® X-100). Prior to initiation of the polymerase reaction PEG, methanol and LiCl is added and the buffer is diluted such that the final reaction conditions for buffer 6 consisted of : 40mM tris (pH 8.0), 12 mM MgCl₂, 5 mM DTT, 1 mM spermidine, 0.002% triton® X-100, 10% methanol, 4% PEG, and 1 mM LiCl.

Screening of Modified nucleotide triphosphates with Mutant T7 RNA Polymerase

Modified nucleotide triphosphates were tested in buffers 1 through 6 at two different temperatures (25 and 37°C). Buffers 1-6 tested at 25°C were designated conditions 1-6 and buffers 1-6 tested at 37°C were designated conditions 7-12 (**Table 47**). In each condition, Y639F mutant T7 polymerase (Sousa and Padilla, *supra*) (0.3-2 mg/20 ml reaction), NTP's (2 mM each), DNA template (10 pmol), inorganic pyrophosphatase (5U/ml) and α -³²P NTP (0.8 mCi/pmol template) were combined and heated at the designated temperatures for 1-2 hours. The radiolabeled NTP used was different from the modified triphosphate being testing. The samples were resolved by polyacrylamide gel electrophoresis. Using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), the amount of full-length transcript was quantified and compared with an all-RNA control reaction. The data is presented in **Table 48**; results in each reaction are expressed as a percent compared to the all-ribonucleotide triphosphate (rNTP) control. The control was run with the mutant T7 polymerase using commercially available polymerase buffer (Boehringer Mannheim, Indianapolis, IN).

Incorporation of Modified NTP's using Wild-type T7 RNA polymerase

Bacteriophage T7 RNA polymerase was purchased from Boehringer Mannheim at 0.4 U/ μ L concentration. Applicant used the commercial buffer supplied with the enzyme and 0.2 μ Ci alpha- ^{32} P NTP in a 50 μ L reaction with nucleotides triphosphates at 2 mM each. The template was a double-stranded PCR fragment, which was used in previous screens. Reactions were carried out at 37°C for 1 hour. Ten μ L of the sample was run on a 7.5% analytical PAGE and bands were quantitated using a PhosphorImager. Results are calculated as a comparison to an "all ribo" control (non-modified nucleotide triphosphates) and the results are in **Table 49**.

10 Incorporation of Multiple Modified nucleotide triphosphates Into Oligonucleotides

Combinations of modified nucleotide triphosphates were tested with the transcription protocol described above, to determine the rates of incorporation of two or more of these triphosphates. Incorporation of 2'-Deoxy-2'-(L-histidine) amino uridine (2'-his-NH₂-UTP) was tested with unmodified cytidine nucleotide triphosphates, rATP and rGTP in reaction condition number 9. The data is presented as a percentage of incorporation of modified NTP's compared to the all rNTP control and is shown in **Table 50a**.

Two modified cytidines (2'-NH₂-CTP or 2'dCTP) were incorporated along with 2'-his-NH₂-UTP with identical efficiencies. 2'-his-NH₂-UTP and 2'-NH₂-CTP were then tested with various unmodified and modified adenosine triphosphates in the same buffer (**Table 50b**). The best modified adenosine triphosphate for incorporation with both 2'-his-NH₂-UTP and 2'-NH₂-CTP was 2'-NH₂-DAPTP.

Optimization of Reaction conditions for Incorporation of Modified Nucleotide

25 Triphosphate

The combination of 2'-his-NH₂-UTP, 2'-NH₂-CTP, 2'-NH₂-DAP, and rGTP was tested in several reaction conditions (**Table 51**) using the incorporation protocol described above. The results demonstrate that of the buffer conditions tested, incorporation of these modified nucleotide triphosphates occur in the presence of both methanol and LiCl.

Selection of Novel Enzymatic nucleic acid molecule Motifs using 2'-deoxy-2' amino Modified GTP and CTP

For selection of new enzymatic nucleic acid molecule motifs, pools of enzymatic nucleic acid molecules were designed to have two substrate binding arms (5 and 16 nucleotides long) and a random region in the middle. The substrate has a biotin on the 5' end, 5 nucleotides complementary to the short binding arm of the pool, an unpaired G (the desired cleavage site), and 16 nucleotides complementary to the long binding arm of the pool. The substrate was bound to column resin through an avidin-biotin complex. The general process for selection is shown in **Figure 11**. The protocols described below represent one possible method that may be utilized for selection of enzymatic nucleic acid molecules and are given as a non-limiting example of enzymatic nucleic acid molecule selection with combinatorial libraries.

Construction of Libraries:

The oligonucleotides listed below were synthesized by Operon Technologies (Alameda, CA). Templates were gel purified and then run through a Sep-Pak™ cartridge (Waters, Millford, MA) using the manufacturers protocol. Primers (MST3, MST7c, MST3del) were used without purification.

Primers:

MST3 (30 mer): 5'-CAC TTA GCA TTA ACC CTC ACT AAA GGC CGT-3'
 MST7c (33 mer): 5'-TAA TAC GAC TCA CTA TAG GAA AGG TGT GCA ACC-3'
 MST3del (18 mer): 5'-ACC CTC ACT AAA GGC CGT-3'

Templates:

MSN60c (93 mer): 5'-ACC CTC ACT AAA GGC CGT (N)₆₀ GGT TGC ACA CCT TTG-3'
 MSN40c (73 mer): 5'-ACC CTC ACT AAA GGC CGT (N)₄₀ GGT TGC ACA CCT TTG-3'
 MSN20c (53 mer): 5'-ACC CTC ACT AAA GGC CGT (N)₂₀ GGT TGC ACA CCT TTG-3'

N60 library was constructed using MSN60c as a template and MST3/MST7c as primers. N40 and N20 libraries were constructed using MSN40c (or MSN20c) as template and MST3del/MST7c as primers.

Single-stranded templates were converted into double-stranded DNA by the following protocol: 5 nmol template, 10 nmol each primer, in 10 ml reaction volume using standard PCR buffer, dNTP's, and taq DNA polymerase (all reagents from Boehringer Mannheim). Synthesis cycle conditions were 94°C, 4 minutes; (94°C, 1 minute; 42°C, 1 minute; 72°C, 2 minutes) x 4; 72°C, 10 minutes. Products were checked on agarose gel to confirm the length of each fragment (N60=123 bp, N40=91 bp, N20=71 bp) and then were phenol/chloroform extracted and ethanol precipitated. The concentration of the double-stranded product was 25 µM.

Transcription of the initial pools was performed in a 1 ml volume comprising: 500 pmol double-stranded template (3×10^{14} molecules), 40 mM tris-HCl (pH 8.0), 12 mM MgCl₂, 1 mM spermidine, 5 mM DTT, 0.002% triton X-100, 1 mM LiCl, 4% PEG 8000, 10% methanol, 2 mM ATP (Pharmacia), 2 mM GTP (Pharmacia), 2 mM 2'-deoxy-2'-amino-CTP (USB), 2 mM 2'-deoxy-2'-amino-UTP (USB), 5 U/ml inorganic pyrophosphatase (Sigma), 5 U/µl T7 RNA polymerase (USB; Y639F mutant was used in some cases at 0.1 mg/ml (Sousa and Padilla, *supra*)), 37°C, 2 hours. Transcribed libraries were purified by denaturing PAGE (N60=106 ntds, N40=74, N20=54) and the resulting product was desalted using Sep-PakTM columns and then ethanol precipitated.

Initial column-Selection:

The following biotinylated substrate was synthesized using standard protocols (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):

5'-biotin-C18 spacer-GCC GUG GGU UGC ACA CCU UUC C-C18 spacer-thiol-modifier C6 S-S-inverted abasic-3'

Substrate was purified by denaturing PAGE and ethanol precipitated. 10 nmol of substrate was linked to a NeutrAvidinTM column using the following protocol: 400 µl UltraLink Immobilized NeutrAvidinTM slurry (200 µl beads, Pierce, Rockford, IL) were loaded into a polystyrene column (Pierce). The column was washed twice with 1 ml of binding buffer (20 mM NaPO₄ (pH 7.5), 150 mM NaCl) and then capped off (i.e., a cap was put on the bottom of the column to stop the flow). 200 µl of the substrate suspended in binding buffer was applied and allowed to incubate at room temperature for 30 minutes

with occasional vortexing to ensure even linking and distribution of the solution to the resin. After the incubation, the cap was removed and the column was washed with 1 ml binding buffer followed by 1 ml column buffer (50 mM tris-HCL (pH 8.5), 100 mM NaCl, 50 mM KCl). The column was then ready for use and capped off. 1 nmol of the initial
5 pool RNA was loaded on the column in a volume of 200 µl column buffer. It was allowed to bind the substrate by incubating for 30 minutes at room temperature with occasional vortexing. After the incubation, the cap was removed and the column was washed twice with 1 ml column buffer and capped off. 200 µl of elution buffer (50 mM tris-HCl (pH 8.5), 100 mM NaCl, 50 mM KCl, 25 mM MgCl₂) was applied to the column followed by
10 30 minute incubation at room temperature with occasional vortexing. The cap was removed and four 200 µl fractions were collected using elution buffer.

Second column (counter selection):

A diagram for events in the second column is generally shown in **Figure 12** and
15 substrate oligonucleotide used is shown below:

5'-GGU UGC ACA CCU UUC C-C18 spacer-biotin-inverted abasic-3'

This column substrate was linked to UltraLink NeutrAvidin™ resin as previously described (40 pmol) which was washed twice with elution buffer. The eluent from the first column purification was then run on the second column. The use of this column
20 allowed for binding of RNA that non-specifically diluted from the first column, while RNA that performed a catalytic event and had product bound to it, flowed through the second column. The fractions were ethanol precipitated using glycogen as carrier and rehydrated in sterile water for amplification.

25 Amplification:

RNA and primer MST3 (10-100 pmol) were denatured at 90°C for 3 minutes in water and then snap-cooled on ice for one minute. The following reagents were added to the tube (final concentrations given): 1X PCR buffer (Boehringer Mannheim), 1 mM dNTP's (for PCR, Boehringer Mannheim), 2 U/µl RNase-Inhibitor (Boehringer
30 Mannheim), 10 U/µl Superscript™ II Reverse Transcriptase (BRL). The reaction was incubated for 1 hour at 42°C, then at 95°C for 5 minutes in order to destroy the

Superscript™. The following reagents were then added to the tube to increase the volume five-fold for the PCR step (final concentrations/amounts given): MST7c primer (10-100 pmol, same amount as in RT step), 1X PCR buffer, taq DNA polymerase (0.025-0.05 U/μl, Boehringer Mannheim). The reaction was cycled as follows: 94°C, 4minutes;
5 (94°C, 30s; 42-54°C, 30s; 72°C, 1minute) x 4-30 cycles; 72°C, 5minutes; 30°C, 30 minutes. Cycle number and annealing temperature were decided on a round by round basis. In cases where heteroduplex was observed, the reaction was diluted five-fold with fresh reagents and allowed to progress through 2 more amplification cycles. Resulting products were analyzed for size on an agarose gel (N60=123 bp, N40=103 bp, N20=83 bp)
10 and then ethanol precipitated.

Transcriptions:

Transcription of amplified products was done using the conditions described above with the following modifications: 10-20% of the amplification reaction was used as
15 template, reaction volume was 100-500 μl, and the products sizes varied slightly (N60=106 ntds, N40=86, N20=66). A small amount of ³²P-GTP was added to the reactions for quantitation purposes.

Subsequent rounds:

20 Subsequent rounds of selection used 20 pmols of input RNA and 40 pmol of the 22 nucleotide substrate on the column.

Activity of pools:

Pools were assayed for activity under single turnover conditions every three to four
25 rounds. Activity assay conditions were as follows: 50 mM tris-HCl (pH 8.5), 25 mM MgCl₂, 100 mM NaCl, 50 mM KCl, trace ³²P-labeled substrate, 10 nM RNA pool. 2X pool in buffer and, separately, 2X substrate in buffer were incubated at 90°C for 3 minutes, then at 37°C for 3 minutes. Equal volume 2X substrate was then added the 2X pool tube (t=0). Initial assay time points were taken at 4 and 24 hours: 5 μl was removed and
30 quenched in 8 μl cold Stop buffer (96% formamide, 20 mM EDTA, 0.05% bromphenyl blue/xylene cyanol). Samples were heated 90°C, 3 minutes, and loaded on a 20%

sequencing gel. Quantitation was performed using a Molecular Dynamics Phosphorimager and ImageQuaNT™ software. The data is shown in **Table 52**.

Samples from the pools of oligonucleotide were cloned into vectors and sequenced using standard protocols (Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*,
5 Cold Spring Harbor Laboratory Press). The enzymatic nucleic acid molecules were transcribed from a representative number of these clones using methods described in this application. Individuals from each pool were tested for RNA cleavage from N60 and N40 by incubating the enzymatic nucleic acid molecules from the clones with 5/16 substrate in 2mM MgCl₂, pH 7.5, 10mM KCl at 37°C. The data in **Table 54** shows that the enzymatic
10 nucleic acid molecules isolated from the pool are individually active.

Kinetic Activity:

Kinetic activity of the enzymatic nucleic acid molecule shown in **Table 54**, was determined by incubating enzymatic nucleic acid molecule (10 nM) with substrate in a
15 cleavage buffer (pH 8.5, 25 mM MgCl₂, 100 mM NaCl, 50 mM KCl) at 37°C.

Magnesium Dependence:

Magnesium dependence of round 15 of N20 was tested by varying MgCl₂ while other conditions were held constant (50 mM tris [pH 8.0], 100 mM NaCl, 50 mM KCl,
20 single turnover, 10 nM pool). The data is shown in **Table 55**, which demonstrates increased activity with increased magnesium concentrations.

Selection of Novel Enzymatic nucleic acid molecule Motifs using 2'-Deoxy-2'-(N-histidyl) amino UTP, 2'-Fluoro-ATP, and 2'-deoxy-2'-amino CTP and GTP

The method used for selection of novel enzymatic nucleic acid molecule motifs using 2'-deoxy-2'-amino modified GTP and CTP was repeated using 2'-Deoxy-2'-(N-histidyl) amino UTP, 2'-Fluoro-ATP, and 2'-deoxy-2'-amino CTP and GTP. However, rather than causing cleavage on the initial column with MgCl₂, the initial random modified-RNA pool was loaded onto substrate-resin in the following buffer; 5 mM
25 NaOAc pH 5.2, 1 M NaCl at 4° C. After ample washing, the resin was moved to 22 ° C and the buffer switch 20 mM HEPES pH 7.4, 140 mM KCl, 10 mM NaCl, 1 mM CaCl₂,
30 1 mM MgCl₂. In one selection of N60 oligonucleotides, no divalent cations (MgCl₂,

CaCl₂) was used. The resin was incubated for 10 minutes to allow reaction and the eluant collected.

The enzymatic nucleic acid molecule pools were capable of cleaving 1-3% of the present substrate even in the absence of divalent cations, the background (in the absence of modified pools) was 0.2 - 0.4 %.

Synthesis of 5-substituted 2'-modified nucleosides

When designing monomeric nucleoside triphosphates for selection of therapeutic catalytic RNAs, one has to take into account nuclease stability of such molecules in biological sera. A common approach to increase RNA stability is to replace the sugar 2'-OH group with other groups like 2'-fluoro, 2'-O-methyl or 2'-amino. Fortunately such 2'-modified pyrimidine 5'triphosphates are shown to be substrates for RNA polymerases. (Aurup, H.; Williams, D.M.; Eckstein, F. *Biochemistry* **1992**, *31*, 9637; and Padilla, R.; Sousa, R. *Nucleic Acids Res.* **1999**, *27*, 1561.) On the other hand it was shown that variety of substituents at pyrimidine 5-position is well tolerated by T7 RNA polymerase (Tarasow, T.M.; Eaton, B.E. *Biopolymers* **1998**, *48*, 29), most likely because the natural hydrogen-bonding pattern of these nucleotides is preserved. We have chosen 2'-fluoro and 2'-O-methyl pyrimidine nucleosides as starting materials for attachment of different functionalities to the 5-position of the base. Both rigid (alkynyl) and flexible (alkyl) spacers are used. The choice of imidazole, amino and carboxylate pendant groups is based on their ability to act as general acids, general bases, nucleophiles and metal ligands, all of which can improve the catalytic effectiveness of selected nucleic acids. **Figures 21 – 24** relate to the synthesis of these compounds.

2'-O-methyluridine was 3',5'-bis-acetylated using acetic anhydride in pyridine and then converted to its 5-iodo derivative **1a** using I₂/ceric ammonium nitrate reagent (Asakura, J.; Robins, M.J. *J. Org. Chem.* **1990**, *55*, 4928) (Scheme 1). Both reactions proceeded in a quantitative yield and no chromatographic purifications were needed. Coupling between **1** and *N*-trifluoroacetyl propargylamine using copper(I) iodide and tetrakis(triphenylphosphine)palladium(0) catalyst as described by Hobbs (Hobbs, F.W., Jr. *J. Org. Chem.* **1989**, *54*, 3420) yielded **2a** in 89% yield. Selective *O*-deacylation with aqueous NaOH afforded **3a** which was phosphorylated with POCl₃/triethylphosphate

(TEP) in the presence of 1,8-bis(dimethylamino)naphthalene (Proton-Sponge) (Method A) (Kovácz, T; Ötvös, L. Tetrahedron Lett. 1988, 29, 4525). The intermediate nucleoside phosphorodichloridate was condensed *in situ* with tri-*n*-butylammonium pyrophosphate. At the end, the *N*-TFA group was removed with concentrated ammonia. 5'-Triphosphate
5 was purified on Sephadex® DEAE A-25 ion exchange column using a linear gradient of 0.1-0.8M triethylammonium bicarbonate (TEAB) for elution. Traces of contaminating inorganic pyrophosphate are removed using C-18 RP HPLC to afford analytically pure material. Conversion into Na-salt was achieved by passing the aqueous solution of triphosphate through Dowex 50WX8 ion exchange resin in Na⁺ form to afford **4a** in 45%
10 yield. When Proton-Sponge was omitted in the first phosphorylation step, yields were reduced to 10-20%. Catalytic hydrogenation of **3a** yielded 5-aminopropyl derivative **5a** which was phosphorylated under conditions identical to those described for propynyl derivative **3a** to afford triphosphate **6a** in 50% yield.

For the preparation of imidazole derivatized triphosphates **9a** and **11a**, we
15 developed an efficient synthesis of *N*-diphenylcarbamoyl 4-imidazoleacetic acid (ImAA^{DPC}): Transient protection of carboxyl group as TMS-ester using TMS-Cl/pyridine followed by DPC-Cl allowed for a clean and quantitative conversion of 4-imidazoleacetic acid (ImAA) to its *N*-DPC protected derivative.

Complete deacylation of **2a** afforded 5-(3-aminopropynyl) derivative **8a** which
20 was condensed with 4-imidazoleacetic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) to afford **9a** in 68% yield. Catalytic hydrogenation of **8a** yielded 5-(3-aminopropyl) derivative **10a** which was condensed with ImAA^{DPC} to yield conjugate **11a** in 32% yield. Yields in these couplings were greatly improved when 5'-OH was protected with DMT group (not shown) thus
25 efficiently preventing undesired 5'-*O*-esterification. Both **9a** and **11a** failed to yield triphosphate products in reaction with POCl₃/TEP/Proton-Sponge.

On the contrary, phosphorylation of 3'-*O*-acetylated derivatives **12a** and **13a** using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one followed by pyrophosphate addition and oxidation (Method B, Scheme 2; Ludwig, J., Eckstein, F., *J. Org. Chem.* 1989, 54,
30 631) afforded the desired triphosphates **14a** and **15a** in 57% yield, respectively.

2'-Deoxy-2'-fluoro nucleoside 5'-triphosphates containing amino- (**4b**, **6b**) and imidazole- (**14b**, **15b**) linked groups were synthesized in a manner analogous to that described for the preparation of 2'-O-methyl nucleoside 5'-triphosphates (Schemes 1 and 2). Again, only Ludwig-Eckstein's phosphorylation worked for the preparation of 4-imidazoleacetyl derivatized triphosphates.

It is worth noting that when "one-pot-two-steps" phosphorylation reaction (Kovácz, T; Ötvös, L. *Tetrahedron Lett.* **1988**, 29, 4525) of **5b** was quenched with 40% aqueous methylamine instead of TEAB or H₂O, the γ -amidate **7b** was generated as the only detectable product. Similar reaction was reported recently for the preparation of the γ -amidate of pppA2'p5'A2'p5'A.¹²

Carboxylate group was introduced into 5-position of uridine both on the nucleoside level and post-synthetically (Method C) (Scheme 3). 5-Iodo-2'-deoxy-2'-fluorouridine (**16**) was coupled with methyl acrylate using modified Heck reaction¹³ to yield **17** in 85% yield. 5'-O-Dimethoxytritylation, followed by *in situ* 3'-O-acetylation and subsequent detritylation afforded 3'-protected derivative **18**. Phosphorylation using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one followed by pyrophosphate addition and oxidation (Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, 54, 631) afforded the desired triphosphate in 54% yield. On the other hand, 5-(3-aminopropyl)uridine 5'-triphosphate **6b** was coupled with *N*-hydroxysuccinimide ester of Fmoc-Asp-OFm to afford, after removal of Fmoc and Fm groups with diethylamine, the desired aminoacyl conjugate **20** in 50% yield.

Cytidine derivatives comprising 3-aminopropyl and 3(*N*-succinyl)aminopropyl groups were synthesized according to Scheme 4. Peracylated 5-(3-aminopropynyl)uracil derivative **2b** is reduced using catalytic hydrogenation and then converted in seven steps and 5% overall yield into 3'-acetylated cytidine derivative **25**. This synthesis was plagued by poor solubility of intermediates and formation of the N⁴-cyclized byproduct during ammonia treatment of the 4-triazolyl intermediate. Phosphorylation of **25** as described in reference 11 yielded triphosphate **26** and N⁴-cyclized product **27** in 1:1 ratio. They were easily separated on Sephadex DEAE A-25 ion exchange column using 0.1-0.8M TEAB gradient. It appears that under basic conditions the free primary amine can displace any remaining intact 4-NHBz group leading to the cyclized product. This is similar to displacement of 4-triazolyl group by primary amine as mentioned above.

We reasoned that utilization of *N*⁴-unprotected cytidine will solve this problem. This lead to an improved synthesis of **26**: Iodination of 2'-deoxy-2'-fluorocytidine (**28**) provided the 5-iodo derivative **29** in 58% yield. This compound was then smoothly converted into 5-(3-aminopropynyl) derivative **30**. Hydrogenation afforded 5-(3-aminopropyl) derivative **31** which was phosphorylated directly with POCl₃/ PPI to afford **26** in 37% yield. Coupling of the 5'-triphosphate **26** with succinic anhydride yielded succinylated derivative **32** in 36% yield.

Synthesis of 5-Imidazoleacetic acid 2'-deoxy-5'-triphosphate uridine

5-dintrophenylimidazoleacetic acid 2'-deoxy uridine nucleoside (80 mg) was dissolved in 5 ml of triethylphosphate while stirring under argon, and the reaction mixture was cooled to 0°C. Phosphorous oxychloride (1.8 eq, 22 ml) was added to the reaction mixture at 0°C, three more aliquots were added over the course of 48 hours at room temperature. The reaction mixture was then diluted with anhydrous MeCN (5 ml) and cooled to 0°C, followed by the addition of tributylamine (0.65 ml) and tributylammonium pyrophosphate (4.0 eq, 0.24 g). After 45 minutes, the reaction was quenched with 10 ml aq. methyl amine for four hours. After co-evaporation with MeOH (3x), purified material on DEAE Sephadex followed by RP chromatography to afford 15 mg of triphosphate.

Synthesis of 2'-(*N*-lysyl)-amino-2'-deoxy-cytidine Triphosphate

2'-(*N*-lysyl)-amino-2'-deoxy cytidine (0.180 g, 0.22 mmol) was dissolved in triethyl phosphate (2.00 ml) under Ar. The solution was cooled to 0 °C in an ice bath. Phosphorus oxychloride (99.999%, 3 eq., 0.0672 mL) was added to the solution and the reaction was stirred for two hours at 0 °C. Tributylammonium pyrophosphate (4 eq., 0.400 g) was dissolved in 3.42 mL of acetonitrile and tributylamine (0.165 mL). Acetonitrile (1 mL) was added to the monophosphate solution followed by the pyrophosphate solution which was added dropwise. The resulting solution was clear. The reaction was allowed to warm up to room temperature. After stirring for 45 minutes, methylamine (5 mL) was added and the reaction and stirred at room temperature for 2 hours. A biphasic mixture appeared (little beads at the bottom of the flask). TLC (7:1:2 iPrOH:NH₄OH:H₂O) showed the appearance of triphosphate material. The solution was concentrated, dissolved in water and loaded on a newly prepared DEAE Sephadex A-25 column. The column was washed with a gradient up to 0.6 M TEAB buffer and the product eluted off in fractions

90-95. The fractions were analyzed by ion exchange HPLC. Each fraction showed one triphosphate peak that eluted at ~4.000 minutes. The fractions were combined and pumped down from methanol to remove buffer salt to yield 15.7 mg of product.

Synthesis of 2'-deoxy-2'-(L-histidine)amino Cytidine Triphosphate

5 2'-[N-Fmoc, *Nimid*-dinitrophenyl-histidyl]amino-2'-cytidine (0.310 g, 4.04 mmol) was dissolved in triethyl phosphate (3 ml) under Ar. The solution was cooled to 0 °C. Phosphorus oxychloride (1.8 eq., 0.068 mL) was added to the solution and stored overnight in the freezer. The next morning TLC (10% MeOH in CH₂Cl₂) showed significant starting material, one more equivalent of POCl₃ was added. After two hours, 10 TLC still showed starting material. Tributylamine (0.303 mL) and Tributylammonium pyrophosphate (4 eq., 0.734 g) dissolved in 6.3 mL of acetonitrile (added dropwise) were added to the monophosphate solution. The reaction was allowed to warm up to room temperature. After stirring for 15 min, methylamine (10 mL) was added at room temperature and stirring continued for 2 hours. TLC (7:1:2 iPrOH:NH₄OH:H₂O) showed 15 the appearance of triphosphate material. The solution was concentrated, dissolved in water and loaded on a DEAE Sephadex A-25 column. The column was washed with a gradient up to 0.6 M TEAB buffer and the product eluted off in fractions 170-179. The fractions were analyzed by ion exchange HPLC. Each fraction showed one triphosphate peak that eluted at ~6.77 minutes. The fractions were combined and pumped down from methanol 20 to remove buffer salt to afford 17 mg of product.

Screening for Novel Enzymatic nucleic acid molecule Motifs Using Modified NTPs (Class I Motif)

Our initial pool contained 3×10^{14} individual sequences of 2'-amino-dCTP/2'-amino-dUTP RNA. We optimized transcription conditions in order to increase the amount 25 of RNA product by inclusion of methanol and lithium chloride. 2'-amino-2'-deoxynucleotides do not interfere with the reverse transcription and amplification steps of selection and confer nuclease resistance. We designed the pool to have two binding arms complementary to the substrate, separated by the random 40 nucleotide region. The 16-mer substrate had two domains, 5 and 10 nucleotides long, that bind the pool, separated by 30 an unpaired guanosine. On the 5' end of the substrate was a biotin attached by a C18 linker. This enabled us to link the substrate to a NeutrAvidin™ resin in a column format.

The desired reaction would be cleavage at the unpaired G upon addition of magnesium cofactor followed by dissociation from the column due to instability of the 5 base pair helix. A detailed protocol follows:

Enzymatic nucleic acid molecule Pool Prep: The initial pool DNA was prepared by converting the following template oligonucleotides into double-stranded DNA by filling in with taq polymerase. (template=5'-ACC CTC ACT AAA GGC CGT (N)₄₀ GGT TGC ACA CCT TTC-3'; primer 1=5'-CAC TTA GCA TTA ACC CTC ACT AAA GGC CGT-3'; primer 2=5'-TAA TAC GAC TCA CTA TAG GAA AGG TGT GCA ACC-3'.) All DNA oligonucleotides were synthesized by Operon technologies. Template oligos were purified by denaturing PAGE and Sep-pak chromatography columns (Waters). RNA substrate oligos were using standard solid phase chemistry and purified by denaturing PAGE followed by ethanol precipitation. Substrates for *in vitro* cleavage assays were 5'-end labeled with gamma-³²P-ATP and T4 polynucleotide kinase followed by denaturing PAGE purification and ethanol precipitation.

5 nmole of template, 10 nmole of each primer and 250 U taq polymerase were incubated in a 10 ml volume with 1X PCR buffer (10 mM tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl) and 0.2 mM each dNTP as follows: 94°C, 4 minutes; (94°C, 1 min; 42°C, 1 min; 72°C, 2 min) through four cycles; and then 72°C, for 10 minutes. The product was analyzed on 2% Separide™ agarose gel for size and then was extracted twice with buffered phenol, then chloroform-isoamyl alcohol, and ethanol precipitated. The initial RNA pool was made by transcription of 500 pmole (3 x 10¹⁴ molecules) of this DNA as follows. Template DNA was added to 40 mM tris-HCl (pH 8.0), 12 mM MgCl₂, 5 mM dithiothreitol (DTT), 1 mM spermidine, 0.002% triton X-100, 1 mM LiCl, 4% PEG-8000, 10% methanol, 2 mM ATP, 2 mM GTP, 2 mM 2'-amino-dCTP, 2 mM 2'-amino-dUTP, 5 U/ml inorganic pyrophosphatase, and 5 U/μl T7 RNA polymerase at room temperature for a total volume of 1 ml. A separate reaction contained a trace amount of alpha-³²P-GTP for detection. Transcriptions were incubated at 37°C for 2 hours followed by addition of equal volume STOP buffer (94% formamide, 20 mM EDTA, 0.05% bromophenol blue). The resulting RNA was purified by 6% denaturing PAGE gel, Sep-pak™ chromatography, and ethanol precipitated.

INITIAL SELECTION: 2 nmole of 16 mer 5'-biotinylated substrate (5'-biotin-C18 linker-GCC GUG GGU UGC ACA C-3') was linked to 200 μl UltraLink Immobilized

NeutrAvidin™ resin (400 µl slurry, Pierce) in binding buffer (20 mM NaPO₄ (pH 7.5), 150 mM NaCl) for 30 minutes at room temperature. The resulting substrate column was washed with 2 ml binding buffer followed by 2 ml column buffer (50 mM tris-HCl (pH 8.5), 100 mM NaCl, 50 mM KCl). The flow was capped off and 1000 pmole of initial
5 pool RNA in 200 µl column buffer was added to the column and incubated 30 minutes at room temperature. The column was uncapped and washed with 2 ml column buffer, then capped off. 200 µl elution buffer (=column buffer + 25 mM MgCl₂) was added to the column and allowed to incubate 30 minutes at room temperature. The column was uncapped and eluent collected followed by three 200 µl elution buffer washes. The
10 eluent/washes were ethanol precipitated using glycogen as carrier and rehydrated in 50 µl sterile H₂O. The eluted RNA was amplified by standard reverse transcription/PCR amplification techniques. 5-31 µl RNA was incubated with 20 pmol of primer 1 in 14 µl volume 90° for 3 min then placed on ice for 1 minute. The following reagent were added (final concentrations noted): 1X PCR buffer, 1 mM each dNTP, 2 U/µl RNase Inhibitor,
15 10 U/µl SuperScript™ II reverse transcriptase. The reaction was incubated 42° for 1 hour followed by 95° for 5 min in order to inactivate the reverse transcriptase. The volume was then increased to 100 µl by adding water and reagents for PCR: 1X PCR buffer, 20 pmol primer 2, and 2.5 U taq DNA polymerase. The reaction was cycled in a Hybaid thermocycler: 94°, 4 min; (94°C, 30 sec; 54°C, 30 sec; 72°C, 1 min) X 25; 72°C, 5 min.
20 Products were analyzed on agarose gel for size and ethanol precipitated. One-third to one-fifth of the PCR DNA was used to transcribe the next generation, in 100 µl volume, as described above. Subsequent rounds used 20 pmol RNA for the column with 40 pmol substrate.

TWO COLUMN SELECTION: At generation 8 (G8), the column selection was
25 changed to the two column format. 200 pmoles of 22 mer 5'-biotinylated substrate (5'-biotin-C18 linker-GCC GUG GGU UGC ACA CCU UUC C-C18 linker-thiol modifier C6 S-S-inverted abasic-3') was used in the selection column as described above. Elution was in 200 µl elution buffer followed by a 1 ml elution buffer wash. The 1200 µl eluent was passed through a product trap column by gravity. The product trap column was prepared
30 as follows: 200 pmol 16 mer 5'-biotinylated "product" (5'-GGU UGC ACA CCU UUC C-C18 linker-biotin-3') was linked to the column as described above and the column was equilibrated in elution buffer. Eluent from the product column was precipitated as

previously described. The products were amplified as above only with 2.5-fold more volume and 100 pmol each primer. 100 μ l of the PCR reaction was used to do a cycle course; the remaining fraction was amplified the minimal number of cycles needed for product. After 3 rounds (G11), there was visible activity in a single turnover cleavage
5 assay. By generation 13, 45% of the substrate was cleaved at 4 hours; k_{obs} of the pool was 0.037 min^{-1} in 25 mM MgCl_2 . We subcloned and sequenced generation 13; the pool was still very diverse. Since our goal was a enzymatic nucleic acid molecule that would work in a physiological environment, we decided to change selection pressure rather than exhaustively catalog G13.

10 Reselection of the N40 pool was started from G12 DNA. Part of the G12 DNA was subjected to hypermutagenic PCR (Vartanian *et al.*, 1996, *Nucleic Acids Research* 24, 2627-2631) to introduce a 10% per position mutation frequency and was designated N40H. At round 19, part of the DNA was hypermutagenized again, giving N40M and N40HM (a total of 4 parallel pools). The column substrates remained the same; buffers
15 were changed and temperature of binding and elution was raised to 37°C. Column buffer was replaced by physiological buffer (50 mM tris-HCl (pH 7.5), 140 mM KCl, 10 mM NaCl) and elution buffer was replaced by 1 mM Mg buffer (physiological buffer + 1 mM MgCl_2). Amount of time allowed for the pool to bind the column was eventually reduced to 10 min and elution time was gradually reduced from 30 min to 20 sec. Between rounds
20 18 and 23, k_{obs} for the N40 pool stayed relatively constant at $0.035\text{-}0.04 \text{ min}^{-1}$. Generation 22 from each of the 4 pools was cloned and sequenced.

CLONING AND SEQUENCING: Generations 13 and 22 were cloned using Novagen's Perfectly Blunt™ Cloning kit (pT7Blue-3 vector) following the kit protocol. Clones were screened for insert by PCR amplification using vector-specific primers.
25 Positive clones were sequenced using ABI Prism 7700 sequence detection system and vector-specific primer. Sequences were aligned using MacVector software; two-dimensional folding was performed using Mulfold software (Zuker,, 1989, *Science* 244, 48-52; Jaeger *et al.*, 1989, *Biochemistry* 86, 7706-7710; Jaeger *et al.*, 1989, R. F. Doolittle ed., *Methods in Enzymology*, 183, 281-306). Individual clone transcription units were
30 constructed by PCR amplification with 50 pmol each primer 1 and primer 2 in 1X PCR buffer, 0.2 mM each dNTP, and 2.5 U of taq polymerase in 100 μ l volume cycled as follows: 94°C, 4 min; (94°C, 30 sec; 54°C, 30 sec; 72°C, 1 min) X 20; 72°C, 5 min.

Transcription units were ethanol precipitated, rehydrated in 30 μ l H₂O, and 10 μ l was transcribed in 100 μ l volume and purified as previously described.

Thirty-six clones from each pool were sequenced and were found to be variations of the same consensus motif. Unique clones were assayed for activity in 1 mM MgCl₂ and physiological conditions; nine clones represented the consensus sequence and were used in subsequent experiments. There were no mutations that significantly increased activity; most of the mutations were in regions believed to be duplex, based on the proposed secondary structure. In order to make the motif shorter, we deleted the 3'-terminal 25 nucleotides necessary to bind the primer for amplification. The measured rates of the full length and truncated molecules were both 0.04 min⁻¹; thus we were able reduce the size of the motif from 86 to 61 nucleotides. The molecule was shortened even further by truncating base pairs in the stem loop structures as well as the substrate recognition arms to yield a 48 nucleotide molecule. In addition, many of the ribonucleotides were replaced with 2-*O*-methyl modified nucleotides to stabilize the molecule. An example of the new motif is given in **Figure 13**. Those of ordinary skill in the art will recognize that the molecule is not limited to the chemical modifications shown in the figure and that it represents only one possible chemically modified molecule.

Kinetic Analysis:

Single turnover kinetics were performed with trace amounts of 5'-³²P-labeled substrate and 10-1000 nM pool of enzymatic nucleic acid molecule. 2X substrate in 1X buffer and 2X pool/enzymatic nucleic acid molecule in 1X buffer were incubated separately 90° for 3 min followed by equilibration to 37° for 3 min. Equal volume of 2X substrate was added to pool/enzymatic nucleic acid molecule at t₀ and the reaction was incubated at 37°C. Time points were quenched in 1.2 vol STOP buffer on ice. Samples were heated to 90°C for 3 min prior to separation on 15% sequencing gels. Gels were imaged using a PhosphorImager and quantitated using ImageQuant™ software (Molecular Dynamics). Curves were fit to double-exponential decay in most cases, although some of the curves required linear fits.

STABILITY: Serum stability assays were performed as previously described (Beigelman *et al.*, 1995, *J. Biol. Chem.* 270, 25702-25708). 1 μ g of 5'-³²P-labeled

synthetic enzymatic nucleic acid molecule was added to 13 μ l cold and assayed for decay in human serum. Gels and quantitation were as described in kinetics section.

SUBSTRATE REQUIREMENTS: **Table 60** outlines the substrate requirements for Class I motif. Substrates maintained Watson-Crick or wobble base pairing with mutant Class I constructs. Activity in single turnover kinetic assay is shown relative to wild type Class I and 22 mer substrate (50 mM Tris-HCL (pH 7.5), 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 100 nM ribozyme, 5 nM substrate, 37°C).

RANDOM REGION MUTATION ALIGNMENT: **Table 61** outlines the random region alignment of 134 clones from generation 22 (1.x = N40, 2.x = N40M, 3.x = N40H, 4.x = N40HM). The number of copies of each mutant is in parenthesis in the table, deviations from consensus are shown. Mutations that maintain base pair U19:A34 are shown in italic. Activity in single turnover kinetic assay is shown relative to the G22 pool rate (50 mM Tris-HCL pH 7.5, 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 100 nM ribozyme, trace substrate, 37°C).

STEM TRUNCATION AND LOOP REPLACEMENT ANALYSIS: **Figure 25** shows a representation of Class I ribozyme stem truncation and loop replacement analysis. The K_{rel} is compared to a 61 mer Class I ribozyme measured as described above. **Figure 26** shows examples of Class I ribozymes with truncated stem(s) and/or non-nucleotide linker replaced loop structures.

Inhibition of HCV Using Class I (Amberzyme) Motif

During HCV infection, viral RNA is present as a potential target for enzymatic nucleic acid molecule cleavage at several processes: uncoating, translation, RNA replication and packaging. Target RNA may be more or less accessible to enzymatic nucleic acid molecule cleavage at any one of these steps. Although the association between the HCV initial ribosome entry site (IRES) and the translation apparatus is mimicked in the HCV 5'UTR/luciferase reporter system, these other viral processes are not represented in the OST7 system. The resulting RNA/protein complexes associated with the target viral RNA are also absent. Moreover, these processes may be coupled in an HCV-infected cell which could further impact target RNA accessibility. Therefore, we

tested whether enzymatic nucleic acid molecules designed to cleave the HCV 5'UTR could effect a replicating viral system.

Recently, Lu and Wimmer characterized an HCV-poliovirus chimera in which the poliovirus IRES was replaced by the IRES from HCV (Lu & Wimmer, 1996, *Proc. Natl. Acad. Sci. USA.* 93, 1412-1417). Poliovirus (PV) is a positive strand RNA virus like HCV, but unlike HCV is non-enveloped and replicates efficiently in cell culture. The HCV-PV chimera expresses a stable, small plaque phenotype relative to wild type PV.

The capability of the new enzymatic nucleic acid molecule motifs to inhibit HCV RNA intracellularly was tested using a dual reporter system that utilizes both firefly and Renilla luciferase (**Figure 14**). A number of enzymatic nucleic acid molecules having the new class I motif (Amberzyme) were designed and tested (**Table 56**). The Amberzyme ribozymes were targeted to the 5' HCV UTR region, which when cleaved, would prevent the translation of the transcript into luciferase. OST-7 cells were plated at 12,500 cells per well in black walled 96-well plates (Packard) in medium DMEM containing 10% fetal bovine serum, 1% pen/strep, and 1% L-glutamine and incubated at 37°C overnight. A plasmid containing T7 promoter expressing 5' HCV UTR and firefly luciferase (T7C1-341 (Wang *et al.*, 1993, *J. of Virol.* 67, 3338-3344)) was mixed with a pRLSV40 Renilla control plasmid (Promega Corporation) followed by enzymatic nucleic acid molecule, and cationic lipid to make a 5X concentration of the reagents (T7C1-341 (4 µg/ml), pRLSV40 renilla luciferase control (6 µg/ml), enzymatic nucleic acid molecule (250 nM), transfection reagent (28.5 µg/ml).

The complex mixture was incubated at 37°C for 20 minutes. The media was removed from the cells and 120 µl of Opti-mem media was added to the well followed by 30 µl of the 5X complex mixture. 150 µl of Opti-mem was added to the wells holding the untreated cells. The complex mixture was incubated on OST-7 cells for 4 hours, lysed with passive lysis buffer (Promega Corporation) and luminescent signals were quantified using the Dual Luciferase Assay Kit using the manufacturer's protocol (Promega Corporation). The data shown in **Figure 15** is a dose curve of enzymatic nucleic acid molecule targeting site 146 of the HCV RNA and is presented as a ratio between the firefly and Renilla luciferase fluorescence. The enzymatic nucleic acid molecule was able to reduce the quantity of HCV RNA at all enzymatic nucleic acid molecule concentrations

yielding an IC_{50} of approximately 5 nM. Other sites were also efficacious (**Figure 16**), in particular enzymatic nucleic acid molecules targeting sites 133, 209, and 273 were also able to reduce HCV RNA compared to the irrelevant (IRR) controls.

5 Cleavage of Substrates Using Completely Modified class I (Amberzyme) enzymatic nucleic acid molecule

10 The ability of an enzymatic nucleic acid, which is modified at every 2' position to cleave a target RNA was tested to determine if any ribonucleotide positions are necessary in the Amberzyme motif. Enzymatic nucleic acid molecules were constructed with 2'-*O*-methyl, and 2'-amino (NH_2) nucleotides and included no ribonucleotides (**Table 56**; gene name: no ribo) and kinetic analysis was performed as described in example 13. 100 nM enzymatic nucleic acid was mixed with trace amounts of substrate in the presence of 1 mM $MgCl_2$ at physiological conditions (37°C). The Amberzyme with no ribonucleotide present in it has a K_{rel} of 0.13 compared to the enzymatic nucleic acid with a few
15 ribonucleotides present in the molecule shown in **Table 56** (ribo). This shows that Amberzyme enzymatic nucleic acid molecule may not require the presence of 2'-OH groups within the molecule for activity.

Substrate Recognition Rules for Class II (zinzyme) enzymatic nucleic acid molecules

Class II (zinzyme) ribozymes were tested for their ability to cleave base-paired substrates with all sixteen possible combinations of bases immediately 5' and 3' proximal to the bulged cleavage site G. Ribozymes were identical in all remaining positions of their 7 base pair binding arms. Activity was assessed at two and twenty-four hour time points under standard reaction conditions [20 mM HEPES pH 7.4, 140 mM KCl, 10 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂ – 37⁰ C]. **Figure 19** shows the results of this study. Base paired substrate UGG (not shown in the figure) cleaved as poorly as CGG shown in the figure. The figure shows the cleavage site substrate triplet in the 5'- 3' direction and 2 and 24 hour time points are shown top to bottom respectively. The results indicate the cleavage site triplet is most active with a 5'- Y-G-H –3' (where Y is C or U and H is A, C or U with cleavage between G and H); however, activity is detected particularly with the 24 hour time point for most paired substrates. All positions outside of the cleavage triplet were found to tolerate any base pairings (data not shown).

All possible mispairs immediately 5' and 3' proximal to the bulged cleavage site G were tested to a class II ribozyme designed to cleave a 5'-C-G-C –3'. It was observed the 5' and 3' proximal sites are as active with G:U wobble pairs, in addition, the 5' proximal site will tolerate a mismatch with only a slight reduction in activity [data not shown].

Screening for Novel Enzymatic nucleic acid molecule Motifs (Class II Motifs)

The selections were initiated with pools of > 10¹⁴ modified RNA's of the following sequence: 5'-GGGAGGAGGAAGUGCCU (N)₃₅ UGCCGCGCUCGCUCCCAGUCC-3'. The RNA was enzymatically generated using the mutant T7 Y639F RNA polymerase prepared by Rui Souza. The following modified NTP's were incorporated: 2'-deoxy-2'-fluoro-adenine triphosphate, 2'-deoxy-2'-fluoro-uridine triphosphate or 2'-deoxy-2'-fluoro-5-[(N-imidazole-4acetyl)propyl amine] uridine triphosphate, and 2'-deoxy-2'-amino-cytidine triphosphate; natural guanine triphosphate was used in all selections so that alpha –³²P-GTP could be used to label pool RNA's. RNA pools were purified by denaturing gel electrophoresis 8% polyacrilamide 7 M Urea.

The following target RNA (resin A) was synthesized and coupled to Iodoacetyl Ultralink™ resin (Pierce) by the supplier's procedure: 5' –b-L-GGACUGGGAGCGAGCGCGGCAGGCACU GAAG-L-S-B-3'; where b is biotin (Glenn

Research cat# 10-1953-nn), L is polyethylene glycol spacer (Glenn Research cat# 10-1918-nn), S is thiol-modifier C6 S-S (Glenn Research cat# 10-1936-nn), B is a standard inverted deoxy abasic.

RNA pools were added to 100 μ l of 5 μ M Resin A in the buffer A (20 mM HEPES pH 7.4, 140 mM KCL, 10 mM NaCl) and incubated at 22⁰C for 5 minutes. The temperature was then raised to 37⁰C for 10 minutes. The resin was washed with 5 ml buffer A. Reaction was triggered by the addition of buffer B(20 mM HEPES pH 7.4, 140 mM KCL, 10 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂). Incubation proceeded for 20 minutes in the first generation and was reduced progressively to 1 minute in the final generations; with 13 total generations. The reaction eluent was collected in 5 M NaCl to give a final concentration of 2 M NaCl. To this was added 100 μ l of 50% slurry Ultralink NeutraAvidinTM (Pierce). Binding of cleaved biotin product to the avidin resin was allowed by 20 minute incubation at 22⁰ C. The resin was subsequently washed with 5 ml of 20 mM HEPES pH 7.4, 2 M NaCl. Desired RNA's were removed by a 1.2 ml denaturing wash 1M NaCl, 10 M Urea at 94⁰ C over 10 minutes. RNA's were double precipitated in 0.3 M sodium acetate to remove Cl⁻ ions inhibitory to reverse transcription. Standard protocols of reverse transcription and PCR amplification were performed. RNA's were again transcribed with the modified NTP's described above. After 13 generations cloning and sequencing provided 14 sequences which were able to cleave the target substrate. Six sequences were characterized to determine secondary structure and kinetic cleavage rates. The structures and kinetic data are given in **Figure 17**. The sequences of eight other enzymatic nucleic acid molecule sequences are given in **Table 57**. The size, sequence, and chemical compositions of these molecules can be modified as described below or using other techniques well known in the art.

Nucleic Acid Catalyst Engineering

Sequence, chemical and structural variants of Class I and Class II enzymatic nucleic acid molecule can be engineered and re-engineered using the techniques shown in this application and known in the art. For example, the size of class I and class II enzymatic nucleic acid molecules can, be reduced or increased using the techniques known in the art (Zaug *et al.*, 1986, *Nature*, 324, 429; Ruffner *et al.*, 1990, *Biochem.*, 29, 10695; Beaudry *et*

al., 1990, *Biochem.*, 29, 6534; McCall *et al.*, 1992, *Proc. Natl. Acad. Sci., USA.*, 89, 5710; Long *et al.*, 1994, *supra*; Hendry *et al.*, 1994, *BBA* 1219, 405; Benseler *et al.*, 1993, *JACS*, 115, 8483; Thompson *et al.*, 1996, *Nucl. Acids Res.*, 24, 4401; Michels *et al.*, 1995, *Biochem.*, 34, 2965; Been *et al.*, 1992, *Biochem.*, 31, 11843; Guo *et al.*, 1995, *EMBO. J.*, 14, 368; Pan *et al.*, 1994, *Biochem.*, 33, 9561; Cech, 1992, *Curr. Op. Struc. Bio.*, 2, 605; Sugiyama *et al.*, 1996, *FEBS Lett.*, 392, 215; Beigelman *et al.*, 1994, *Bioorg. Med. Chem.*, 4, 1715; Santoro *et al.*, 1997, *PNAS* 94, 4262; all are incorporated in their totality by reference herein), to the extent that the overall catalytic activity of the ribozyme is not significantly decreased.

Further rounds of *in vitro* selection strategies described herein and variations thereof can be readily used by a person skilled in the art to evolve additional nucleic acid catalysts and such new catalysts are within the scope of the instant invention.

Example 16: Activity of Class II (zinzyme) nucleic acid catalysts to inhibit HER2 gene expression

Applicant has designed, synthesized and tested several class II (zinzyme) ribozymes targeted against HER2 RNA (see, for example, **Tables 58, 59, and 62**) in cell proliferation RNA reduction assays.

Proliferation assay:

The model proliferation assay used in the study can require a cell-plating density of 2000-10000 cells/well in 96-well plates and at least 2 cell doublings over a 5-day treatment period. Cells used in proliferation studies were either human breast or ovarian cancer cells (SKBR-3 and SKOV-3 cells respectively). To calculate cell density for proliferation assays, the FIPS (fluoro-imaging processing system) method well known in the art was used. This method allows for cell density measurements after nucleic acids are stained with CyQuant® dye, and has the advantage of accurately measuring cell densities over a very wide range 1,000-100,000 cells/well in 96-well format.

Ribozymes (50-200 nM) were delivered in the presence of cationic lipid at 2.0-5.0 µg/mL and inhibition of proliferation was determined on day 5 post-treatment. Two full ribozyme screens were completed resulting in the selection of 14 ribozymes. Class II (zinzyme) ribozymes against sites, 314 (RPI No. 18653), 443 (RPI No. 18680), 597 (RPI

No. 18697), 659 (RPI No. 18682), 878 (RPI Nos. 18683 and 18654), 881 (RPI Nos. 18684 and 18685) 934 (RPI No. 18651), 972 (RPI No. 18656, 19292, 19727, 19728, and 19293), 1292 (RPI No. 18726), 1541 (RPI No. 18687), 2116 (RPI No. 18729), 2932 (RPI No. 18678), 2540 (RPI No. 18715), and 3504 (RPI No. 18710) caused inhibition of proliferation ranging from 25-80% as compared to a scrambled control ribozyme. An example of results from a cell culture assay is shown in **Figure 20**. Referring to **Figure 20**, Class II ribozymes targeted against HER2 RNA are shown to cause significant inhibition of proliferation of cells. This shows that ribozymes, for instance the Class II (zinzyme) ribozymes are capable of inhibiting HER2 gene expression in mammalian cells.

RNA assay:

RNA was harvested 24 hours post-treatment using the Qiagen RNeasy® 96 procedure. Real time RT-PCR (TaqMan® assay) was performed on purified RNA samples using separate primer/probe sets specific for either target HER2 RNA or control actin RNA (to normalize for differences due to cell plating or sample recovery). Results are shown as the average of triplicate determinations of HER2 to actin RNA levels post-treatment. **Figure 30** shows class II ribozyme (zinzyme) mediated reduction in HER2 RNA targeting site 972 vs a scrambled attenuated control.

Dose response assays:

Active ribozyme was mixed with binding arm-attenuated control (BAC) ribozyme to a final oligonucleotide concentration of either 100, 200 or 400 nM and delivered to cells in the presence of cationic lipid at 5.0 µg/mL. Mixing active and BAC in this manner maintains the lipid to ribozyme charge ratio throughout the dose response curve. HER2 RNA reduction was measured 24 hours post-treatment and inhibition of proliferation was determined on day 5 post-treatment. The dose response antiproliferation results are summarized in **Figure 31** and the dose-dependent reduction of HER2 RNA results are summarized in **Figure 32**. **Figure 33** shows a combined dose response plot of both anti-proliferation and RNA reduction data for a class II ribozyme targeting site 972 of HER2 RNA (RPI 19293).

Example 17: Reduction of ribose residues in Class II (zinzyme) nucleic acid catalysts

Class II (zinzyme) nucleic acid catalysts were tested for their activity as a function of ribonucleotide content. A Zinzyme having no ribonucleotide residue (*ie.*, no 2'-OH group at the 2' position of the nucleotide sugar) against the K-Ras site 521 was designed. This molecules were tested utilizing the chemistry shown in **Figure 27a**. The *in vitro* catalytic activity of the zinzyme construct was not significantly effected (the cleavage rate reduced only 10 fold).

The Kras zinzyme shown in **Figure 27a** was tested in physiological buffer with the divalent concentrations as indicated in the legend (high NaCl is an altered monovalent condition shown) of **Figure 28**. The 1 mM Ca^{++} condition yielded a rate of 0.005 min^{-1} while the 1 mM Mg^{++} condition yielded a rate of 0.002 min^{-1} . The ribose containing wild type yields a rate of 0.05 min^{-1} while substrate in the absence of zinzyme demonstrates less than 2% degradation at the longest time point under reaction conditions shown. This illustrates a well-behaved cleavage reaction done by a non-ribose containing catalyst with only a 10-fold reduced cleavage as compared to ribonucleotide-containing zinzyme and vastly above non-catalyzed degradation.

A more detailed investigation into the role of ribose positions in the Class II (zinzyme) motif was carried out in the context of the HER2 site 972 (Applicant has further designed a fully modified Zinzyme as shown in **Figure 27b** targeting the HER2 RNA site 972). **Figure 29** is a diagram of the alternate formats tested and their relative rates of catalysis. The effect of substitution of ribose G for the 2'-O-methyl C-2'-O-methyl A in the loop of Zinzyme (see **Figure 34**) was insignificant when assayed with the Kras target but showed a modest rate enhancement in the HER2 assays. The activity of all Zinzyme motifs, including the fully stabilized "0 ribose" (RPI 19727) are well above background noise level degradation. Zinzyme with only two ribose positions (RPI 19293) are sufficient to restore "wild-type" activity. Motifs containing 3 (RPI 19729), 4 (RPI 19730) or 5 ribose (RPI 19731) positions demonstrated a greater extent of cleavage and profiles almost identical to the 2 ribose motif. Applicant has thus demonstrated that a Zinzyme with no ribonucleotides present at any position can catalyze efficient RNA cleavage activity. Thus, Zinzyme enzymatic nucleic acid molecules do not require the presence of 2'-OH group within the molecule for catalytic activity.

Example 18: Activity of reduced ribose containing Class II (zinzyme) nucleic acid catalysts to inhibit HER2 gene expression

A cell proliferation assay for testing reduced ribo class II (zinzyme) nucleic acid catalysts (50-400 nM) targeting HER2 site 972 was performed as described in example 19.

- 5 The results of this study are summarized in **Figure 35**. These results indicate significant inhibition of HER2 gene expression using stabilized Class II (zinzyme) motifs, including two ribo (RPI 19293), one ribo (RPI 19728), and non-ribo (RPI 19727) containing nucleic acid catalysts.

10 Applications

- The use of NTP's described in this invention have several research and commercial applications. These modified nucleotide triphosphates can be used for *in vitro* selection (evolution) of oligonucleotides with novel functions. Examples of *in vitro* selection protocols are incorporated herein by reference (Joyce, 1989, *Gene*, 82, 83-87; Beaudry *et al.*, 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.*, 1994, *TIBTECH* 12, 268; Bartel *et al.*, 1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.*, 1995, *FASEB J.*, 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442).

- 20 Additionally, these modified nucleotide triphosphates can be employed to generate modified oligonucleotide combinatorial chemistry libraries. Several references for this technology exist (Brenner *et al.*, 1992, *PNAS* 89, 5381-5383, Eaton, 1997, *Curr. Opin. Chem. Biol.* 1, 10-16), which are all incorporated herein by reference.

Diagnostic uses

- 25 Enzymatic nucleic acid molecules of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of specific RNA in a cell. The close relationship between enzymatic nucleic acid molecule 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
- 30 RNA. By using multiple enzymatic nucleic acid molecules described in this invention, one may 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 enzymatic nucleic acid

molecules may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with known small molecule inhibitors, radiation or intermittent treatment with combinations of enzymatic nucleic acid molecules and/or other chemical or biological molecules). Other *in vitro* uses of enzymatic nucleic acid molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a enzymatic nucleic acid molecule using standard methodology.

In a specific example, enzymatic nucleic acid molecules which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first enzymatic nucleic acid molecule is used to identify wild-type RNA present in the sample and the second enzymatic nucleic acid molecule 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 enzymatic nucleic acid molecules to demonstrate the relative enzymatic nucleic acid molecule 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 can involve two enzymatic nucleic acid molecules, two substrates and one unknown sample which can be combined into six reactions. The presence of cleavage products can 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 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.

Additional Uses

5 Potential usefulness of sequence-specific enzymatic nucleic acid molecules of the instant invention can have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans *et al.*, 1975 *Ann. Rev. Biochem.* 44:273). For example, the pattern of restriction fragments can be used to establish sequence relationships between two related RNAs, and large RNAs could be
10 specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the enzymatic nucleic acid molecule is ideal for cleavage of RNAs of unknown sequence. Applicant describes the use of nucleic acid molecules to down-regulate gene expression of target genes in bacterial, microbial, fungal, viral, and eukaryotic systems including plant, or mammalian cells.

15 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.

 One skilled in the art would readily appreciate that the present invention is well
20 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
25 of the claims.

 It will be readily apparent to one skilled in the art that varying substitutions and modifications may 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.

30 The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is 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.

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.

Thus, additional embodiments are within the scope of the invention and within the following claims

Table 1

TABLE 1

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-OH of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintenance of the active structure.
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [i,ii].
- Complete kinetic framework established for one ribozyme [iii,iv,v,vi].
- Studies of ribozyme folding and substrate docking underway [vii,viii,ix].
- Chemical modification investigation of important residues well established [x,xi].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the *Tetrahymena* group I intron has been used to repair a "defective" β -galactosidase message by the ligation of new β -galactosidase sequences onto the defective message [xii].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [xiii].
- Reaction mechanism: possible attack by M^{2+} -OH to generate cleavage products with 3'-OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [xiv,xv].
- Important phosphate and 2' OH contacts recently identified [xvi,xvii].

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [xviii,xix].

Table 1

- Sequence requirements not fully determined.
- Reaction mechanism: 2'-OH of an internal adenosine generates cleavage products with 3'-OH and a "lariat" RNA containing a 3'-5' and a 2'-5' branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [xx,xxi] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [xxii].
- Important 2' OH contacts beginning to be identified [xxiii]
- Kinetic framework under development [xxiv]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [xxv].
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme

(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures [xxvi,xxvii]
- Minimal ligation activity demonstrated (for engineering through *in vitro* selection) [xxviii]
- Complete kinetic framework established for two or more ribozymes [xxix].
- Chemical modification investigation of important residues well established [xxx].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [xxxi,xxxii,xxxiii,xxxiv]

Table 1

- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through *in vitro* selection [xxxv]
- Complete kinetic framework established for one ribozyme [xxxvi].
- Chemical modification investigation of important residues begun [xxxvii, xxxviii].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [xxxix].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [xl].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [xli]

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Table 2

Table 2:

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 min	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	

Table 2

Acetonitrile	NA	2.64 mL	NA	NA	NA
C. 0.2 µmol Synthesis Cycle 96 well Instrument					
Reagent	Equivalents	Amount	Wait Time*	Wait Time* 2'-O-methyl	Wait Time* Ribo
	DNA/2'-O-methyl/Ribo	DNA/2'-O-methyl/Ribo	DNA		
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
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.

Table 3

Table 3: Human PTP-1B Hammerhead Ribozyme and Target Sequence

Nt. Position	Ribozyme Sequence	Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
15	UGCCGCUC CUGAUGAG X CGAA AGGCCGCG	1	CGCGGCCT A GAGCGGCA	529
72	AUCUCCAU CUGAUGAG X CGAA ACGGGCCA	2	TGGCCCGT C ATGGAGAT	530
92	UCUGCUCG CUGAUGAG X CGAA ACUCCUUU	3	AAAGGAGT T CGAGCAGA	531
93	AUCUGCUC CUGAUGAG X CGAA AACUCCUU	4	AAGGAGTT C GAGCAGAT	532
102	GACUUGUC CUGAUGAG X CGAA AUCUGCUC	5	GAGCAGAT C GACAAGTC	533
110	AGCUCCCG CUGAUGAG X CGAA ACUUGUCG	6	CGACAAGT C CGGGAGCT	534
129	UCCUGGUA CUGAUGAG X CGAA AUGGCCGC	7	GCGGCCAT T TACCAGGA	535
130	AUCCUGGU CUGAUGAG X CGAA AAUGGCCG	8	CGGCCATT T ACCAGGAT	536
131	UAUCCUGG CUGAUGAG X CGAA AAAUGGCC	9	GGCCATTT A CCAGGATA	537
139	AUGUCGGA CUGAUGAG X CGAA AUCCUGGU	10	ACCAGGAT A TCCGACAT	538
141	UCAUGUCG CUGAUGAG X CGAA AUAUCCUG	11	CAGGATAT C CGACATGA	539
161	UACAUGGG CUGAUGAG X CGAA AGUCACUG	12	CAGTGACT T CCCATGTA	540
162	CUACAUGG CUGAUGAG X CGAA AAGUCACU	13	AGTGACTT C CCATGTAG	541
169	GGCCACUC CUGAUGAG X CGAA ACAUGGGA	14	TCCCATGT A GAGTGGCC	542
183	UUCUAGG CUGAUGAG X CGAA AGCUUGGC	15	GCCAAGCT T CCTAAGAA	543
184	GUUCUAG CUGAUGAG X CGAA AAGCUUGG	16	CCAAGCTT C CTAAGAAC	544
187	UUUGUUCU CUGAUGAG X CGAA AGGAAGCU	17	AGCTTCCT A AGAACAAA	545
205	UCUGUACC CUGAUGAG X CGAA AUUUCGGU	18	ACCGAAAT A GGTACAGA	546
209	CGUCUCUG CUGAUGAG X CGAA ACCUAUUU	19	AAATAGGT A CAGAGACG	547
219	AAGGGACU CUGAUGAG X CGAA ACGUCUCU	20	AGAGACGT C AGTCCCTT	548
223	GUCAAAGG CUGAUGAG X CGAA ACUGACGU	21	ACGTCAGT C CCTTTGAC	549
227	UAUGGUCA CUGAUGAG X CGAA AGGGACUG	22	CAGTCCCT T TGACCATA	550
228	CUAUGGUC CUGAUGAG X CGAA AAGGGACU	23	AGTCCCTT T GACCATAG	551
235	AAUCCGAC CUGAUGAG X CGAA AUGGUCAA	24	TTGACCAT A GTCGGATT	552
238	UUUAAUCC CUGAUGAG X CGAA ACUAUGGU	25	ACCATAGT C GGATTAAA	553
243	UGUAGUUU CUGAUGAG X CGAA AUCCGACU	26	AGTCGGAT T AAATACA	554
244	AUGUAGUU CUGAUGAG X CGAA AAUCCGAC	27	GTCGGATT A AACTACAT	555
249	UCUUGAUG CUGAUGAG X CGAA AGUUUAAU	28	ATTAAACT A CATCAAGA	556
253	AUCUUCUU CUGAUGAG X CGAA AUGUAGUU	29	AACTACAT C AAGAAGAT	557
262	AUAGUCAU CUGAUGAG X CGAA AUCUUCUU	30	AAGAAGAT A ATGACTAT	558
269	CGUUGAUA CUGAUGAG X CGAA AGUCAUUA	31	TAATGACT A TATCAACG	559
271	AGCGUUGA CUGAUGAG X CGAA AUAGUCAU	32	ATGACTAT A TCAACGCT	560
273	CUAGCGUU CUGAUGAG X CGAA AUAUAGUC	33	GACTATAT C AACGCTAG	561
280	UAUCAAAC CUGAUGAG X CGAA AGCGUUGA	34	TCAACGCT A GTTTGATA	562
283	UUUUUAUCA CUGAUGAG X CGAA ACUAGCGU	35	ACGCTAGT T TGATAAAA	563
284	UUUUUAUC CUGAUGAG X CGAA AACUAGCG	36	CGCTAGTT T GATAAAAA	564
288	UCCAUUUU CUGAUGAG X CGAA AUCAAACU	37	AGTTTGAT A AAAATGGA	565
313	AAGAAUGU CUGAUGAG X CGAA ACUCCUUU	38	AAAGGAGT T ACATTCTT	566
314	UAAGAAUG CUGAUGAG X CGAA AACUCCUU	39	AAGGAGTT A CATTCTTA	567
318	UGGGUAAG CUGAUGAG X CGAA AUGUAACU	40	AGTTACAT T CTTACCCA	568
319	CUGGGUAA CUGAUGAG X CGAA AAUGUAAC	41	GTTACATT C TTACCCAG	569
321	CCCUGGGU CUGAUGAG X CGAA AGAAUGUA	42	TACATTCT T ACCCAGGG	570
322	GCCCUGGG CUGAUGAG X CGAA AAGAAUGU	43	ACATTCTT A CCCAGGGC	571
334	GUUAGGCA CUGAUGAG X CGAA AGGGCCCU	44	AGGGCCCT T TGCCTAAC	572

Table 3

335	UGUUAGGC CUGAUGAG X CGAA AAGGGCCC	45	GGGCCCTT T GCCTAACA	573
340	GCAUGUGU CUGAUGAG X CGAA AGGCAAAG	46	CTTTGCCT A ACACATGC	574
352	CCAAAAGU CUGAUGAG X CGAA ACCGCAUG	47	CATGCGGT C ACTTTTGG	575
356	UCUCCCAA CUGAUGAG X CGAA AGUGACCG	48	CGGTCACT T TTGGGAGA	576
357	AUCUCCCA CUGAUGAG X CGAA AAGUGACC	49	GGTCACTT T TGGGAGAT	577
358	CAUCUCCC CUGAUGAG X CGAA AAAGUGAC	50	GTCACCTT T GGGAGATG	578
393	AGCAUGAC CUGAUGAG X CGAA ACACCCCU	51	AGGGGTGT C GTCATGCT	579
396	UUGAGCAU CUGAUGAG X CGAA ACGACACC	52	GGTGTCTG C ATGCTCAA	580
402	ACUCUGUU CUGAUGAG X CGAA AGCAUGAC	53	GTCATGCT C AACAGAGT	581
424	UUUUAACG CUGAUGAG X CGAA ACCUUUCU	54	AGAAAGGT T CGTTAAAA	582
425	AUUUUAAC CUGAUGAG X CGAA AACCUUUC	55	GAAAGGTT C GTTAAAT	583
428	CGCAUUUU CUGAUGAG X CGAA ACGAACCU	56	AGGTTCTG T AAAATGCG	584
429	GCGCAUUU CUGAUGAG X CGAA AACGAACC	57	GGTTCGTT A AAATGCGC	585
443	GUGGCCAG CUGAUGAG X CGAA AUUGUGCG	58	CGCACAAT A CTGGCCAC	586
474	UCUUCAAA CUGAUGAG X CGAA AUCAUCUC	59	GAGATGAT C TTTGAAGA	587
476	UGUCUUCA CUGAUGAG X CGAA AGAUCAUC	60	GATGATCT T TGAAGACA	588
477	GUGUCUUC CUGAUGAG X CGAA AAGAUCAU	61	ATGATCTT T GAAGACAC	589
490	UAAUUUCA CUGAUGAG X CGAA AUUUGUGU	62	ACACAAAT T TGAAATTA	590
491	UUAAUUUC CUGAUGAG X CGAA AAUUUGUG	63	CACAAATT T GAAATTAA	591
497	UCAAUGUU CUGAUGAG X CGAA AUUUCAAA	64	TTTGAAAT T AACATTGA	592
498	AUCAAUGU CUGAUGAG X CGAA AAUUUCAA	65	TTGAAATT A ACATTGAT	593
503	CAGAGAUC CUGAUGAG X CGAA AUGUUAAU	66	ATTAACAT T GATCTCTG	594
507	UCUUCAGA CUGAUGAG X CGAA AUCAAUGU	67	ACATTGAT C TCTGAAGA	595
509	UAUCUUCA CUGAUGAG X CGAA AGAUCAAU	68	ATTGATCT C TGAAGATA	596
517	UGACUUGA CUGAUGAG X CGAA AUCUUCAG	69	CTGAAGAT A TCAAGTCA	597
519	UAUGACUU CUGAUGAG X CGAA AUAUCUUC	70	GAAGATAT C AAGTCATA	598
524	UAUAAUAU CUGAUGAG X CGAA ACUUGAUA	71	TATCAAGT C ATATTATA	599
527	CUGUAUAA CUGAUGAG X CGAA AUGACUUG	72	CAAGTCAT A TTATACAG	600
529	CACUGUAU CUGAUGAG X CGAA AUAUGACU	73	AGTCATAT T ATACAGTG	601
530	GCACUGUA CUGAUGAG X CGAA AAUAUGAC	74	GTCATATT A TACAGTGC	602
532	UCGCACUG CUGAUGAG X CGAA AUAAUAUG	75	CATATTAT A CAGTGCGA	603
546	UCCAAUUC CUGAUGAG X CGAA AGCUGUCG	76	CGACAGCT A GAATTGGA	604
551	GGUUUUC CUGAUGAG X CGAA AUUCUAGC	77	GCTAGAAT T GGAAAACC	605
561	UGGGUUGU CUGAUGAG X CGAA AGGUUUUC	78	GAAAACCT T ACAACCCA	606
562	UUGGGUUG CUGAUGAG X CGAA AAGGUUUU	79	AAAACCTT A CAACCCAA	607
577	GAUCUCUC CUGAUGAG X CGAA AGUUUCUU	80	AAGAACT C GAGAGATC	608
585	AAAUGUAA CUGAUGAG X CGAA AUCUCUCG	81	CGAGAGAT C TTACATTT	609
587	GGAAAUUG CUGAUGAG X CGAA AGAUCUCU	82	AGAGATCT T ACATTTCC	610
588	UGGAAAUUG CUGAUGAG X CGAA AAGAUCUC	83	GAGATCTT A CATTTCCA	611
592	AUAGUGGA CUGAUGAG X CGAA AUGUAAGA	84	TCTTACAT T TCCACTAT	612
593	UAUAGUGG CUGAUGAG X CGAA AAUGUAAG	85	CTTACATT T CCACTATA	613
594	GUUAGUG CUGAUGAG X CGAA AAAUGUAA	86	TTACATTT C CACTATAC	614
599	AUGUGGUA CUGAUGAG X CGAA AGUGGAAA	87	TTTCCACT A TACCACAT	615
601	CCAUGUGG CUGAUGAG X CGAA AUAGUGGA	88	TCCACTAT A CCACATGG	616
617	GGACUCCA CUGAUGAG X CGAA AGUCAGGC	89	GCCTGACT T TGGAGTCC	617
618	GGGACUCC CUGAUGAG X CGAA AAGUCAGG	90	CCTGACTT T GGAGTCCC	618
624	GAUUCAGG CUGAUGAG X CGAA ACUCCAAA	91	TTTGAGT C CCTGAATC	619

Table 3

632	AGGCUGGU CUGAUGAG X CGAA AUUCAGGG	92	CCCTGAAT C ACCAGCCT	620
641	UCAAGAAU CUGAUGAG X CGAA AGGCUGGU	93	ACCAGCCT C ATTCTTGA	621
644	AGUUCAAG CUGAUGAG X CGAA AUGAGGCU	94	AGCCTCAT T CTTGAACT	622
645	AAGUUCAA CUGAUGAG X CGAA AAUGAGGC	95	GCCTCATT C TTGAACTT	623
647	GAAAGUUC CUGAUGAG X CGAA AGAAUGAG	96	CTCATTCT T GAACTTTC	624
653	UGAAAAGA CUGAUGAG X CGAA AGUUCAAG	97	CTTGAACT T TCTTTTCA	625
654	UUGAAAAG CUGAUGAG X CGAA AAGUUCAA	98	TTGAACTT T CTTTTCOA	626
655	UUUGAAAA CUGAUGAG X CGAA AAAGUUCA	99	TGAACTTT C TTTTCAAA	627
657	ACUUUGAA CUGAUGAG X CGAA AGAAAGUU	100	AACTTTCT T TTCAAAGT	628
658	GACUUUGA CUGAUGAG X CGAA AAGAAAGU	101	ACTTTCTT T TCAAAGTC	629
659	GGACUUUG CUGAUGAG X CGAA AAAGAAAG	102	CTTTCTTT T CAAAGTCC	630
660	CGGACUUU CUGAUGAG X CGAA AAAAGAAA	103	TTTCTTTT C AAAGTCCG	631
666	GACUCUCG CUGAUGAG X CGAA ACUUUGAA	104	TTCAAAGT C CGAGAGTC	632
674	GUGACCCU CUGAUGAG X CGAA ACUCUCGG	105	CCGAGAGT C AGGGTCAC	633
680	GGCUGAGU CUGAUGAG X CGAA ACCCUGAC	106	GTCAGGGT C ACTCAGCC	634
684	UCCGGGCU CUGAUGAG X CGAA AGUGACCC	107	GGGTCACT C AGCCCGGA	635
705	UGCACCAC CUGAUGAG X CGAA ACGGGCCC	108	GGGCCCCG T GTGGTGCA	636
729	GACCUGCC CUGAUGAG X CGAA AUGCCUGC	109	GCAGGCAT C GGCAGGTC	637
737	AGGUUCCA CUGAUGAG X CGAA ACCUGCCG	110	CGGCAGGT C TGGAACCT	638
746	CCAGACAG CUGAUGAG X CGAA AGGUUCCA	111	TGGAACCT T CTGTCTGG	639
747	GCCAGACA CUGAUGAG X CGAA AAGGUUCC	112	GGAACCTT C TGTCTGGC	640
751	AUCAGCCA CUGAUGAG X CGAA ACAGAAGG	113	CCTTCTGT C TGGCTGAT	641
760	GAGGCAGG CUGAUGAG X CGAA AUCAGCCA	114	TGGCTGAT A CCTGCCTC	642
768	AUCAGCAA CUGAUGAG X CGAA AGGCAGGU	115	ACCTGCCT C TTGCTGAT	643
770	CCAUCAGC CUGAUGAG X CGAA AGAGGCAG	116	CTGCCTCT T GCTGATGG	644
796	AACGGAAG CUGAUGAG X CGAA AGGGUCUU	117	AAGACCCT T CTTCCGTT	645
797	CAACGGAA CUGAUGAG X CGAA AAGGGUCU	118	AGACCCTT C TTCCGTTG	646
799	AUCAACGG CUGAUGAG X CGAA AGAAGGGU	119	ACCCTTCT T CCGTTGAT	647
800	UAUCAACG CUGAUGAG X CGAA AAGAAGGG	120	CCCTTCTT C CGTTGATA	648
804	UUGAUUUC CUGAUGAG X CGAA ACGGAAGA	121	TCTTCCGT T GATATCAA	649
808	UUUCUUGA CUGAUGAG X CGAA AUCAACGG	122	CCGTTGAT A TCAAGAAA	650
810	ACUUUCUU CUGAUGAG X CGAA AUAUCAAC	123	GTTGATAT C AAGAAAGT	651
824	UCAUUUCU CUGAUGAG X CGAA ACAGCACU	124	AGTGCTGT T AGAAATGA	652
825	CUCAUUUC CUGAUGAG X CGAA AACAGCAC	125	GTGCTGTT A GAAATGAG	653
839	CCAUCCGA CUGAUGAG X CGAA ACUUCCUC	126	GAGGAAGT T TCGGATGG	654
840	CCCAUCCG CUGAUGAG X CGAA AACUCCCU	127	AGGAAGTT T CGGATGGG	655
841	CCCCAUCC CUGAUGAG X CGAA AAACUUCC	128	GGAAGTTT C GGATGGGG	656
855	GCUGUCUG CUGAUGAG X CGAA AUCAGCCC	129	GGGCTGAT C CAGACAGC	657
878	GGUAGGAG CUGAUGAG X CGAA AGCGCAGC	130	GCTGCGCT T CTCCTACC	658
879	AGGUAGGA CUGAUGAG X CGAA AAGCGCAG	131	CTGCGCTT C TCCTACCT	659
881	CCAGGUAG CUGAUGAG X CGAA AGAAGCGC	132	GCGCTTCT C CTACCTGG	660
884	CAGCCAGG CUGAUGAG X CGAA AGGAGAAG	133	CTTCTCCT A CCTGGCTG	661
897	GCACCUUC CUGAUGAG X CGAA AUCACAGC	134	GCTGTGAT C GAAGGTGC	662
911	CCAUGAUG CUGAUGAG X CGAA AUUUGGCA	135	TGCCAAAT T CATCATGG	663
912	CCCAUGAU CUGAUGAG X CGAA AAUUGGCG	136	GCCAAATT C ATCATGGG	664
915	UCCCCCAU CUGAUGAG X CGAA AUGAAUUU	137	AAATTCAT C ATGGGGGA	665
926	GCACGGAA CUGAUGAG X CGAA AGUCCCCC	138	GGGGGACT C TTCCGTGC	666

Table 3

928	CUGCACGG CUGAUGAG X CGAA AGAGUCCC	139	GGGACTCT T CCGTGCAG	667
929	CCUGCACG CUGAUGAG X CGAA AAGAGUCC	140	GGACTCTT C CGTGCAGG	668
940	CUUCCACU CUGAUGAG X CGAA AUCCUGCA	141	TGCAGGAT C AGTGGAAG	669
954	UCGUGGGA CUGAUGAG X CGAA AGCUCCUU	142	AAGGAGCT T TCCCACGA	670
955	CUCGUGGG CUGAUGAG X CGAA AAGCUCCU	143	AGGAGCTT T CCCACGAG	671
956	CCUCGUGG CUGAUGAG X CGAA AAAGCUCC	144	GGAGCTTT C CCACGAGG	672
988	UGGGGGGA CUGAUGAG X CGAA AUGCUCGG	145	CCGAGCAT A TCCCCCA	673
990	GGUGGGGG CUGAUGAG X CGAA AUAUGCUC	146	GAGCATAT C CCCCCACC	674
1000	UGGCCGGG CUGAUGAG X CGAA AGGUGGGG	147	CCCCACCT C CCCGGCCA	675
1020	GGCUCCAG CUGAUGAG X CGAA AUUCGUUU	148	AAACGAAT C CTGGAGCC	676
1052	UUGGGAAG CUGAUGAG X CGAA ACUCCCUG	149	CAGGGAGT T CTTCCCAA	677
1053	UUUGGGA CUGAUGAG X CGAA AACUCCCU	150	AGGGAGTT C TTCCCAA	678
1055	GAUJUGGG CUGAUGAG X CGAA AGAACUCC	151	GGAGTTCT T CCCAAATC	679
1056	UGAUJUGG CUGAUGAG X CGAA AAGAACUC	152	GAGTTCTT C CCAAATCA	680
1063	CCACUGGU CUGAUGAG X CGAA AUJUGGGA	153	TCCCAAAT C ACCAGTGG	681
1096	GCAGUCUU CUGAUGAG X CGAA AUCCUCCU	154	AGGAGGAT A AAGACTGC	682
1110	UCUUCUU CUGAUGAG X CGAA AUGGGGCA	155	TGCCCCAT C AAGGAAGA	683
1133	CGGCAUUU CUGAUGAG X CGAA AGGGGCUU	156	AAGCCCCT T AAATGCCG	684
1134	GCGGCAUU CUGAUGAG X CGAA AAGGGGCU	157	AGCCCCTT A AATGCCGC	685
1148	CGAUGCCG CUGAUGAG X CGAA AGGGUGCG	158	CGCACCTT A CGGCATCG	686
1155	AUGCUUUC CUGAUGAG X CGAA AUGCCGUA	159	TACGGCAT C GAAAGCAT	687
1168	AGUGUCUU CUGAUGAG X CGAA ACUCAUGC	160	GCATGAGT C AAGACACT	688
1182	CGACUUCU CUGAUGAG X CGAA ACUUCAGU	161	ACTGAAGT T AGAAGTCG	689
1183	CCGACUUC CUGAUGAG X CGAA AACUUCAG	162	CTGAAGTT A GAAGTCGG	690
1189	CACGACCC CUGAUGAG X CGAA ACUUCUAA	163	TTAGAAGT C GGGTCGTG	691
1194	CCCCCCAC CUGAUGAG X CGAA ACCCGACU	164	AGTCGGGT C GTGGGGGG	692
1207	ACCUCGAA CUGAUGAG X CGAA ACUUCCCC	165	GGGGAAGT C TTCGAGGT	693
1209	GCACCUCG CUGAUGAG X CGAA AGACUUC	166	GGAAGTCT T CGAGGTGC	694
1210	GGCACCUC CUGAUGAG X CGAA AAGACUUC	167	GAAGTCTT C GAGGTGCC	695
1229	UGGCUGGG CUGAUGAG X CGAA AGGCAGCC	168	GGCTGCCT C CCCAGCCA	696
1250	CGGGCAGU CUGAUGAG X CGAA ACGGCUCC	169	GGAGCCGT C ACTGCCCCG	697
1285	CUUCCAGU CUGAUGAG X CGAA ACUCAGUG	170	CACTGAGT T ACTGGAAG	698
1286	GCUUCCAG CUGAUGAG X CGAA AACUCAGU	171	ACTGAGTT A CTGGAAGC	699
1298	UGACCAGG CUGAUGAG X CGAA AGGGCUUC	172	GAAGCCCT T CCTGGTCA	700
1299	UUGACCAG CUGAUGAG X CGAA AAGGGCUU	173	AAGCCCTT C CTGGTCAA	701
1305	CACAUGUU CUGAUGAG X CGAA ACCAGGAA	174	TTCCTGGT C AACATGTG	702
1321	GAGGACCG CUGAUGAG X CGAA AGCCACGC	175	GCGTGGCT A CGGTCCTC	703
1326	GCCGUGAG CUGAUGAG X CGAA ACCGUAGC	176	GCTACGGT C CTCACGGC	704
1329	CCGGCCGU CUGAUGAG X CGAA AGGACCGU	177	ACGGTCCT C ACGGCCGG	705
1342	GCAGAGGU CUGAUGAG X CGAA AGCGCCGG	178	CCGGCGCT T ACCTCTGC	706
1343	AGCAGAGG CUGAUGAG X CGAA AAGCGCCG	179	CGGCGCTT A CCTCTGCT	707
1347	CUGUAGCA CUGAUGAG X CGAA AGGUAAGC	180	GCTTACCT C TGCTACAG	708
1352	GGAACCUG CUGAUGAG X CGAA AGCAGAGG	181	CCTCTGCT A CAGGTTCC	709
1358	UGAACAGG CUGAUGAG X CGAA ACCUGUAG	182	CTACAGGT T CCTGTTCA	710
1359	UUGAACAG CUGAUGAG X CGAA AACCUGUA	183	TACAGGTT C CTGTTCAA	711
1364	UGCUGUUG CUGAUGAG X CGAA ACAGGAAC	184	GTTCTGT T CAACAGCA	712
1365	UUGCUGUU CUGAUGAG X CGAA AACAGGAA	185	TTCCTGTT C AACAGCAA	713

Table 3

1379	GGUCAGGC CUGAUGAG X CGAA AUGUGUUG	186	CAACACAT A GCCTGACC	714
1390	GAGUGGAG CUGAUGAG X CGAA AGGGUCAG	187	CTGACCCT C CTCCACTC	715
1393	GUGGAGUG CUGAUGAG X CGAA AGGAGGGU	188	ACCCTCCT C CACTCCAC	716
1398	UGGAGGUG CUGAUGAG X CGAA AGUGGAGG	189	CCTCCACT C CACCTCCA	717
1404	AGUGGGUG CUGAUGAG X CGAA AGGUGGAG	190	CTCCACCT C CACCCACT	718
1415	CAGAGGCG CUGAUGAG X CGAA ACAGUGGG	191	CCCACTGT C CGCCTCTG	719
1421	UGCGGGCA CUGAUGAG X CGAA AGGCGGAC	192	GTCCGCCT C TGCCCGCA	720
1446	AUGCCUGC CUGAUGAG X CGAA AGUCGGGC	193	GCCCGACT A GCAGGCAT	721
1463	CCCUJACC CUGAUGAG X CGAA ACCGCGGC	194	GCCGCGGT A GGTAAGGG	722
1467	GCGGCCCCU CUGAUGAG X CGAA ACCUACCG	195	CGGTAGGT A AGGGCCGC	723
1486	CGGCUCUC CUGAUGAG X CGAA ACGCGGUC	196	GACCGCGT A GAGAGCCG	724
1511	GCAGAACC CUGAUGAG X CGAA ACGUCCGU	197	ACGGACGT T GGTTCTGC	725
1515	UAGUGCAG CUGAUGAG X CGAA ACCAACGU	198	ACGTTGGT T CTGACTA	726
1516	UUAGUGCA CUGAUGAG X CGAA AACCAACG	199	CGTTGGTT C TGACTAA	727
1523	AUGGGUUU CUGAUGAG X CGAA AGUGCAGA	200	TCTGCACT A AAACCCAT	728
1532	CCGGGGAA CUGAUGAG X CGAA AUGGGUUU	201	AAACCCAT C TTCCCCGG	729
1534	AUCCGGGG CUGAUGAG X CGAA AGAUGGGU	202	ACCCATCT T CCCC GGAT	730
1535	CAUCCGGG CUGAUGAG X CGAA AAGAUGGG	203	CCCATCTT C CCCGGATG	731
1549	AGGGGUGA CUGAUGAG X CGAA ACACACAU	204	ATGTGTGT C TCACCCCT	732
1551	UGAGGGGU CUGAUGAG X CGAA AGACACAC	205	GTGTGTCT C ACCCCTCA	733
1558	AAAAGGAU CUGAUGAG X CGAA AGGGGUGA	206	TCACCCCT C ATCCTTTT	734
1561	AGUAAAAG CUGAUGAG X CGAA AUGAGGGG	207	CCCCTCAT C CTTTTACT	735
1564	AAAAGUAA CUGAUGAG X CGAA AGGAUGAG	208	CTCATCCT T TTACTTTT	736
1565	AAAAAGUA CUGAUGAG X CGAA AAGGAUGA	209	TCATCCTT T TACTTTTT	737
1566	CAAAAAGU CUGAUGAG X CGAA AAAGGAUG	210	CATCCTTT T ACTTTTTG	738
1567	GCAAAAAG CUGAUGAG X CGAA AAAAGGAU	211	ATCCTTTT A CTTTTTGC	739
1570	GGGGCAAA CUGAUGAG X CGAA AGUAAAAG	212	CTTTTACT T TTTGCCCC	740
1571	AGGGGCAA CUGAUGAG X CGAA AAGUAAAA	213	TTTTACTT T TTGCCCCT	741
1572	AAGGGGCA CUGAUGAG X CGAA AAAGUAAA	214	TTTACTTT T TGCCCCTT	742
1573	GAAGGGGC CUGAUGAG X CGAA AAAAGUAA	215	TTACTTTT T GCCCCTTC	743
1580	CAAAGUGG CUGAUGAG X CGAA AGGGGCAA	216	TTGCCCCT T CCACTTTG	744
1581	UCAAGUG CUGAUGAG X CGAA AAGGGGCA	217	TGCCCCTT C CACTTTGA	745
1586	GGUACUCA CUGAUGAG X CGAA AGUGGAAG	218	CTTCCACT T TGAGTACC	746
1587	UGGUACUC CUGAUGAG X CGAA AAGUGGAA	219	TTCCACTT T GAGTACCA	747
1592	GGAUUUGG CUGAUGAG X CGAA ACUCAAAG	220	CTTTGAGT A CCAAATCC	748
1599	GGCUUGUG CUGAUGAG X CGAA AUUUGGUA	221	TACCAAAT C CACAAGCC	749
1610	CCUCAAAA CUGAUGAG X CGAA AUGGCUUG	222	CAAGCCAT T TTTTGAGG	750
1611	UCCUCAAA CUGAUGAG X CGAA AAUGGCUU	223	AAGCCATT T TTTGAGGA	751
1612	CUCCUCAA CUGAUGAG X CGAA AAAUGGCU	224	AGCCATT T TTTGAGGAG	752
1613	UCUCCUCA CUGAUGAG X CGAA AAAAUGGC	225	GCCATTTT T TGAGGAGA	753
1614	CUCUCCUC CUGAUGAG X CGAA AAAAAUGG	226	CCATTTTT T GAGGAGAG	754
1634	CAGCAUGG CUGAUGAG X CGAA ACUCUCUU	227	AAGAGAGT A CCATGCTG	755
1665	GACGGGUG CUGAUGAG X CGAA AGGCCCCU	228	AGGGGCCT A CACCCGTC	756
1673	AGCCCCAA CUGAUGAG X CGAA ACGGGUGU	229	ACACCCGT C TTGGGGCT	757
1675	CGAGCCCC CUGAUGAG X CGAA AGACGGGU	230	ACCCGTCT T GGGGCTCG	758
1682	GGUGGGGC CUGAUGAG X CGAA AGCCCCAA	231	TTGGGGCT C GCCCCACC	759
1698	CCAGGAGG CUGAUGAG X CGAA AGCCCUGG	232	CCAGGGCT C CCTCCTGG	760

Table 3

1702	UGCUC CAG CUGAUGAG X CGAA AGGGAGCC	233	GGCTCCCT C CTGGAGCA	761
1712	CCGCCUGG CUGAUGAG X CGAA AUGCUCCA	234	TGGAGCAT C CCAGGCGG	762
1746	GCAGAUUC CUGAUGAG X CGAA AGGGGGGG	235	CCCCCCT T GAATCTGC	763
1751	UCCCUGCA CUGAUGAG X CGAA AUUCAAGG	236	CCTTGAAT C TGCAGGGA	764
1766	GGAGUGGA CUGAUGAG X CGAA AGUUGCUC	237	GAGCAACT C TCCACTCC	765
1768	AUGGAGUG CUGAUGAG X CGAA AGAGUUGC	238	GCAACTCT C CACTCCAT	766
1773	UAAAU AUG CUGAUGAG X CGAA AGUGGAGA	239	TCTCCACT C CATATTTA	767
1777	UAAAUAAA CUGAUGAG X CGAA AUGGAGUG	240	CACTCCAT A TTTATTTA	768
1779	UUUAAUA CUGAUGAG X CGAA AUAUGGAG	241	CTCCATAT T TATTTAAA	769
1780	GUUUAAAU CUGAUGAG X CGAA AAUAUGGA	242	TCCATATT T ATTTAAAC	770
1781	UGUUUAAA CUGAUGAG X CGAA AAAUAUGG	243	CCATATTT A TTAAACA	771
1783	AUUGUUUA CUGAUGAG X CGAA AUAAAUAU	244	ATATTTAT T TAAACAAT	772
1784	AAUUGUUU CUGAUGAG X CGAA AAUAAAUA	245	TATTTATT T AAACAATT	773
1785	AAAUUGUU CUGAUGAG X CGAA AAUAAAUA	246	ATTTATTT A AACAATTT	774
1792	GGGGAAAA CUGAUGAG X CGAA AUUGUUUA	247	TAAACAAT T TTTTCCCC	775
1793	UGGGGAAA CUGAUGAG X CGAA AAUUGUUU	248	AAACAATT T TTTCCCCA	776
1794	UUGGGGAA CUGAUGAG X CGAA AAUUGUUU	249	AACAATTT T TTCCCCAA	777
1795	UUUGGGGA CUGAUGAG X CGAA AAAAUUGU	250	ACAATTTT T TCCCCAAA	778
1796	CUUUGGGG CUGAUGAG X CGAA AAAAAUUG	251	CAATTTTT T CCCCAAAG	779
1797	CCUUGGGG CUGAUGAG X CGAA AAAAAAUU	252	AATTTTTT C CCCAAAGG	780
1809	GCACUAUG CUGAUGAG X CGAA AUGCCUUU	253	AAAGGCAT C CATAGTGC	781
1813	UAGUGCAC CUGAUGAG X CGAA AUGGAUGC	254	GCATCCAT A GTGCACTA	782
1821	GAAAAUGC CUGAUGAG X CGAA AGUGCACU	255	AGTGCACT A GCATTTTC	783
1826	UUCAAGAA CUGAUGAG X CGAA AUGCUAGU	256	ACTAGCAT T TTCTTGAA	784
1827	GUUCAAGA CUGAUGAG X CGAA AAUGCUAG	257	CTAGCATT T TCTTGAAC	785
1828	GGUUCAAG CUGAUGAG X CGAA AAAUGCUA	258	TAGCATT T CTTGAACC	786
1829	UGGUUCAA CUGAUGAG X CGAA AAAAUGCU	259	AGCATTTT C TTGAACCA	787
1831	AUUGGUUC CUGAUGAG X CGAA AGAAAAUG	260	CATTTTCT T GAACCAAT	788
1840	UAAUACAU CUGAUGAG X CGAA AUUGGUUC	261	GAACCAAT A ATGTATTA	789
1845	AAUUUUA CUGAUGAG X CGAA ACAUUUAU	262	AATAATGT A TTAAAATT	790
1847	AAAAUUU CUGAUGAG X CGAA AUACAUUA	263	TAATGTAT T AAAATTTT	791
1848	AAAAUUU CUGAUGAG X CGAA AAUACAUU	264	AATGTATT A AAATTTT	792
1853	CAUCAAAA CUGAUGAG X CGAA AUUUUAAU	265	ATTAAAAT T TTTTGATG	793
1854	ACAUCAAA CUGAUGAG X CGAA AAUUUUA	266	TTAAAATT T TTTGATGT	794
1855	GACAUCAA CUGAUGAG X CGAA AAAUUUUA	267	TAAAATTT T TTGATGTC	795
1856	UGACAUCA CUGAUGAG X CGAA AAAAAUUU	268	AAAATTTT T TGATGTCA	796
1857	CUGACAU CUGAUGAG X CGAA AAAAAUUU	269	AAATTTTT T GATGTCAG	797
1863	GCAAGGCU CUGAUGAG X CGAA ACAUCAAA	270	TTTGATGT C AGCCTTGC	798
1869	CUUGAUGC CUGAUGAG X CGAA AGGCUGAC	271	GTCAGCCT T GCATCAAG	799
1874	AAGCCCUU CUGAUGAG X CGAA AUGCAAGG	272	CCTTGCAT C AAGGGCTT	800
1882	UUUUGAUA CUGAUGAG X CGAA AGCCCUUG	273	CAAGGGCT T TATCAAAA	801
1883	UUUUUGAU CUGAUGAG X CGAA AAGCCCUU	274	AAGGGCTT T ATCAAAAA	802
1884	CUUUUGA CUGAUGAG X CGAA AAAGCCCU	275	AGGGCTTT A TCAAAAAG	803
1886	UACUUUUU CUGAUGAG X CGAA AUAAAGCC	276	GGCTTTAT C AAAAAGTA	804
1894	UAUUAUUG CUGAUGAG X CGAA ACUUUUUG	277	CAAAAAGT A CAATAATA	805
1899	GGAUUUAU CUGAUGAG X CGAA AUUGUACU	278	AGTACAAT A ATAAATCC	806
1902	UGAGGAUU CUGAUGAG X CGAA AUUAUUGU	279	ACAATAAT A AATCCTCA	807

Table 3

1906	UACCUGAG CUGAUGAG X CGAA AUUUAUUA	280	TAATAAAT C CTCAGGTA	808
1909	UACUACCU CUGAUGAG X CGAA AGGAUUUA	281	TAAATCCT C AGGTAGTA	809
1914	CCCAGUAC CUGAUGAG X CGAA ACCUGAGG	282	CCTCAGGT A GTACTGGG	810
1917	AUUCCCAG CUGAUGAG X CGAA ACUACCUG	283	CAGGTAGT A CTGGGAAT	811
1934	CCAUGGCA CUGAUGAG X CGAA AGCCUUC	284	GGAAGGCT T TGCCATGG	812
1935	CCCAUGGC CUGAUGAG X CGAA AAGCCUUC	285	GAAGGCTT T GCCATGGG	813
1954	ACUGGUCU CUGAUGAG X CGAA ACGCAGCA	286	TGCTGCGT C AGACCAGT	814
1963	CUUCCCAG CUGAUGAG X CGAA ACUGGUCU	287	AGACCAGT A CTGGGAAG	815
1981	CUGCUUAC CUGAUGAG X CGAA ACCGUCCU	288	AGGACGGT T GTAAGCAG	816
1984	CAACUGCU CUGAUGAG X CGAA ACAACCGU	289	ACGGTTGT A AGCAGTTG	817
1991	UAAAUAA CUGAUGAG X CGAA ACUGCUIA	290	TAAGCAGT T GTTATTTA	818
1994	CACUAAAU CUGAUGAG X CGAA ACAACUGC	291	GCAGTTGT T ATTTAGTG	819
1995	UCACUAAA CUGAUGAG X CGAA AACCAACUG	292	CAGTTGTT A TTTAGTGA	820
1997	UAUCACUA CUGAUGAG X CGAA AUAACAAC	293	GTTGTTAT T TAGTGATA	821
1998	AUAUCACU CUGAUGAG X CGAA AAUAACAA	294	TTGTTATT T AGTGATAT	822
1999	AAUAUCAC CUGAUGAG X CGAA AAAUAACA	295	TGTTATTT A GTGATATT	823
2005	ACCCACAA CUGAUGAG X CGAA AUCACUAA	296	TTAGTGAT A TTGTGGGT	824
2007	UUACCCAC CUGAUGAG X CGAA AUAUCACU	297	AGTGATAT T GTGGGTAA	825
2014	UCUCACGU CUGAUGAG X CGAA ACCCACAA	298	TTGTGGGT A ACGTGAGA	826
2027	CAUUGUUC CUGAUGAG X CGAA AUCUUCUC	299	GAGAAGAT A GAACAATG	827
2038	AUAUAUUA CUGAUGAG X CGAA AGCAUUGU	300	ACAATGCT A TAATATAT	828
2040	UUAUAUUA CUGAUGAG X CGAA AUAGCAUU	301	AATGCTAT A ATATATAA	829
2043	UCAUUAUA CUGAUGAG X CGAA AUUAUAGC	302	GCTATAAT A TATAATGA	830
2045	GUUCAUUA CUGAUGAG X CGAA AUAUUAUA	303	TATAATAT A TAATGAAC	831
2047	GUGUUCAU CUGAUGAG X CGAA AUAUAUUA	304	TAATATAT A ATGAACAC	832
2062	UUAUUAAA CUGAUGAG X CGAA ACCCACGU	305	ACGTGGGT A TTTAATAA	833
2064	UCUUAUUA CUGAUGAG X CGAA AUACCCAC	306	GTGGGTAT T TAATAAGA	834
2065	UUCUUAUU CUGAUGAG X CGAA AAUACCCA	307	TGGGTATT T AATAAGAA	835
2066	UUUCUUAU CUGAUGAG X CGAA AAAUACCC	308	GGGTATTT A ATAAGAAA	836
2069	AUGUUUCU CUGAUGAG X CGAA AUUAAUUA	309	TATTTAAT A AGAAACAT	837
2088	GACAAAGU CUGAUGAG X CGAA AUCUCACA	310	TGTGAGAT T ACTTTGTC	838
2089	GGACAAAG CUGAUGAG X CGAA AAUCUCAC	311	GTGAGATT A CTTTGTCC	839
2092	GCGGGACA CUGAUGAG X CGAA AGUAAUCU	312	AGATTACT T TGTCCCGC	840
2093	AGCGGGAC CUGAUGAG X CGAA AAGUAAUC	313	GATTACTT T GTCCCGCT	841
2096	AUAAGCGG CUGAUGAG X CGAA ACAAAGUA	314	TACTTTGT C CCGCTTAT	842
2102	AGCAGAAU CUGAUGAG X CGAA AGCGGGAC	315	GTCCCGCT T ATTCTGCT	843
2103	GAGCAGAA CUGAUGAG X CGAA AAGCGGGA	316	TCCCGCTT A TTCTGCTC	844
2105	GGGAGCAG CUGAUGAG X CGAA AUAAGCGG	317	CCGCTTAT T CTGCTCCC	845
2106	AGGGAGCA CUGAUGAG X CGAA AAUAAGCG	318	CGCTTATT C TGCTCCCT	846
2111	AUAACAGG CUGAUGAG X CGAA AGCAGAAU	319	ATTCTGCT C CCTGTTAT	847
2117	UAGCAGAU CUGAUGAG X CGAA ACAGGGAG	320	CTCCCTGT T ATCTGCTA	848
2118	CUAGCAGA CUGAUGAG X CGAA AACAGGGA	321	TCCCTGTT A TCTGCTAG	849
2120	AUCUAGCA CUGAUGAG X CGAA AUAACAGG	322	CCTGTTAT C TGCTAGAT	850
2125	ACUAGAUC CUGAUGAG X CGAA AGCAGAUU	323	TATCTGCT A GATCTAGT	851
2129	GAGAACUA CUGAUGAG X CGAA AUCUAGCA	324	TGCTAGAT C TAGTTCTC	852
2131	UUGAGAAC CUGAUGAG X CGAA AGAUCUAG	325	CTAGATCT A GTTCTCAA	853
2134	UGAUUGAG CUGAUGAG X CGAA ACUAGAUC	326	GATCTAGT T CTCAATCA	854

Table 3

2135	GUGAUUGA CUGAUGAG X CGAA AACUAGAU	327	ATCTAGTT C TCAATCAC	855
2137	CAGUGAUU CUGAUGAG X CGAA AGAACUAG	328	CTAGTTCT C AATCACTG	856
2141	GGAGCAGU CUGAUGAG X CGAA AUUGAGAA	329	TTCTCAAT C ACTGCTCC	857
2148	ACACGGGG CUGAUGAG X CGAA AGCAGUGA	330	TCACTGCT C CCCC GTGT	858
2159	CAUUCUAA CUGAUGAG X CGAA ACACACGG	331	CCGTGTGT A TTAGAATG	859
2161	UGCAUUCU CUGAUGAG X CGAA AUACACAC	332	GTGTGTAT T AGAATGCA	860
2162	AUGCAUUC CUGAUGAG X CGAA AAUACACA	333	TGTGTATT A GAATGCAT	861
2173	GAAGACCU CUGAUGAG X CGAA ACAUGCAU	334	ATGCATGT A AGGTCTTC	862
2178	CACAAGAA CUGAUGAG X CGAA ACCUUA CA	335	TGTAAGGT C TTCTTG TG	863
2180	GACACAAG CUGAUGAG X CGAA AGACCUUA	336	TAAGGTCT T CTTGTG TC	864
2181	GGACACAA CUGAUGAG X CGAA AAGACCUU	337	AAGGTCTT C TTGTGT CC	865
2183	CAGGACAC CUGAUGAG X CGAA AGAAGACC	338	GGTCTTCT T GTGTCCTG	866
2188	UUCAUCAG CUGAUGAG X CGAA ACACAAGA	339	TCTTGTGT C CTGATGAA	867
2201	CAAGCACA CUGAUGAG X CGAA AUUUUUA	340	TGAAAAAT A TGTGCTTG	868
2208	CUCAUUUC CUGAUGAG X CGAA AGCACAU A	341	TATGTGCT T GAAATGAG	869
2222	AGAGAUCA CUGAUGAG X CGAA AGUUUCUC	342	GAGAAACT T TGATCTCT	870
2223	CAGAGAUC CUGAUGAG X CGAA AAGUUUCU	343	AGAAACTT T GATCTCTG	871
2227	UAAGCAGA CUGAUGAG X CGAA AUCAAAGU	344	ACTTTGAT C TCTGCTTA	872
2229	AGUAAGCA CUGAUGAG X CGAA AGAUCAAA	345	TTTGATCT C TGCTTACT	873
2234	ACAUUAGU CUGAUGAG X CGAA AGCAGAGA	346	TCTCTGCT T ACTAATGT	874
2235	CACAUUAG CUGAUGAG X CGAA AAGCAGAG	347	CTCTGCTT A CTAATGTG	875
2238	GGGCACAU CUGAUGAG X CGAA AGUAAGCA	348	TGCTTACT A ATGTGCCC	876
2252	UGGACUUG CUGAUGAG X CGAA ACAUGGGG	349	CCCCATGT C CAAGTCCA	877
2258	GCAGGUUG CUGAUGAG X CGAA ACUUGGAC	350	GTCCAAGT C CAACCTGC	878
2283	CAUGUAAU CUGAUGAG X CGAA AUCAGGUC	351	GACCTGAT C ATTACATG	879
2286	AGCCAUGU CUGAUGAG X CGAA AUGAUCAG	352	CTGATCAT T ACATGGCT	880
2287	CAGCCAUG CUGAUGAG X CGAA AAUGAUCA	353	TGATCATT A CATGGCTG	881
2300	GGCUUAGG CUGAUGAG X CGAA ACCACAGC	354	GCTGTGGT T CCTAAGCC	882
2301	AGGCUUAG CUGAUGAG X CGAA AACCACAG	355	CTGTGGTT C CTAAGCCT	883
2304	AACAGGCU CUGAUGAG X CGAA AGGAACCA	356	TGGTTCCT A AGCCTGTT	884
2312	ACUUCAGC CUGAUGAG X CGAA ACAGGCUU	357	AAGCCTGT T GCTGAAGT	885
2321	GCGACAAU CUGAUGAG X CGAA ACUUCAGC	358	GCTGAAGT C ATTGTCGC	886
2324	UGAGCGAC CUGAUGAG X CGAA AUGACUUC	359	GAAGTCAT T GTCGCTCA	887
2327	UGCUGAGC CUGAUGAG X CGAA ACAAUAGAC	360	GTCATTGT C GCTCAGCA	888
2331	CUAUUGCU CUGAUGAG X CGAA AGCGACAA	361	TTGTGCGT C AGCAATAG	889
2338	CUGCACCC CUGAUGAG X CGAA AUUGCUGA	362	TCAGCAAT A GGGTGCAG	890
2348	UCCUGGAA CUGAUGAG X CGAA ACUGCACC	363	GGTGCAGT T TTCCAGGA	891
2349	UUCCUGGA CUGAUGAG X CGAA AACUGCAC	364	GTGCAGTT T TCCAGGAA	892
2350	AUCCUGG CUGAUGAG X CGAA AAACUGCA	365	TGCAGTTT T CCAGGAAT	893
2351	UAUCCUG CUGAUGAG X CGAA AAAACUGC	366	GCAGTTT C CAGGAATA	894
2359	CAAAUGCC CUGAUGAG X CGAA AUCCUGG	367	CCAGGAAT A GGCATTTG	895
2365	AUUAGGCA CUGAUGAG X CGAA AUGCCUAU	368	ATAGGCAT T TGCCTAAT	896
2366	AAUAGGC CUGAUGAG X CGAA AAUGCCUA	369	TAGGCATT T GCCTAATT	897
2371	CCAGGAAU CUGAUGAG X CGAA AGGCAAAU	370	ATTTGCCT A ATTCCTGG	898
2374	AUGCCAGG CUGAUGAG X CGAA AUUAGGCA	371	TGCCTAAT T CCTGGCAT	899
2375	CAUGCCAG CUGAUGAG X CGAA AAUAGGCC	372	GCCTAATT C CTGGCATG	900
2389	AGUCACUA CUGAUGAG X CGAA AGUGUCAU	373	ATGACACT C TAGTGACT	901

Table 3

2391	GAAGUCAC CUGAUGAG X CGAA AGAGUGUC	374	GACACTCT A GTGACTTC	902
2398	UCACCAGG CUGAUGAG X CGAA AGUCACUA	375	TAGTGACT T CCTGGTGA	903
2399	CUCACCAG CUGAUGAG X CGAA AAGUCACU	376	AGTGACTT C CTGGTGAG	904
2419	UGUACCAG CUGAUGAG X CGAA ACAGGCUG	377	CAGCCTGT C CTGGTACA	905
2425	CCCUGCUG CUGAUGAG X CGAA ACCAGGAC	378	GTCCTGGT A CAGCAGGG	906
2435	UACAGCAA CUGAUGAG X CGAA ACCCUGCU	379	AGCAGGGT C TTGCTGTA	907
2437	GUUACAGC CUGAUGAG X CGAA AGACCCUG	380	CAGGGTCT T GCTGTAAC	908
2443	GUCUGAGU CUGAUGAG X CGAA ACAGCAAG	381	CTTGCTGT A ACTCAGAC	909
2447	GAAUGUCU CUGAUGAG X CGAA AGUACAG	382	CTGTAAC T C AGACATTC	910
2454	ACCCUUGG CUGAUGAG X CGAA AUGUCUGA	383	TCAGACAT T CCAAGGGT	911
2455	UACCCUUG CUGAUGAG X CGAA AAUGUCUG	384	CAGACATT C CAAGGGTA	912
2463	GCUUCCCA CUGAUGAG X CGAA ACCCUUGG	385	CCAAGGGT A TGGGAAGC	913
2475	GGUGUGAA CUGAUGAG X CGAA AUGGCUUC	386	GAAGCCAT A TTCACACC	914
2477	GAGGUGUG CUGAUGAG X CGAA AUAUGGCU	387	AGCCATAT T CACACCTC	915
2478	UGAGGUGU CUGAUGAG X CGAA AAUAUGGC	388	GCCATATT C ACACCTCA	916
2485	CAGAGCGU CUGAUGAG X CGAA AGGUGUGA	389	TCACACCT C ACGCTCTG	917
2491	CAUGUCCA CUGAUGAG X CGAA AGCGUGAG	390	CTCACGCT C TGGACATG	918
2502	CUUCCCUA CUGAUGAG X CGAA AUCAUGUC	391	GACATGAT T TAGGGAAG	919
2503	GCUUCCCU CUGAUGAG X CGAA AAUCAUGU	392	ACATGATT T AGGGAAGC	920
2504	UGC UCCC CUGAUGAG X CGAA AAAUCAUG	393	CATGATT T A GGGAAGCA	921
2536	UGAUCCCA CUGAUGAG X CGAA AGGUGGGG	394	CCCCACCT T TGGGATCA	922
2537	CUGAUCCC CUGAUGAG X CGAA AAGGUGGG	395	CCCACCTT T GGGATCAG	923
2543	CGGAGGCU CUGAUGAG X CGAA AUCCCAA	396	TTTGGGAT C AGCCTCCG	924
2549	GAAUGGCG CUGAUGAG X CGAA AGGCUGAU	397	ATCAGCCT C CGCCATTC	925
2556	CGACUUGG CUGAUGAG X CGAA AUGGCGGA	398	TCCGCCAT T CCAAGTCG	926
2557	UCGACUUG CUGAUGAG X CGAA AAUGGCGG	399	CCGCCATT C CAAGTCGA	927
2563	AGAGUGUC CUGAUGAG X CGAA ACUUGGAA	400	TTCCAAGT C GACACTCT	928
2570	CUCAAGAA CUGAUGAG X CGAA AGUGUCGA	401	TCGACACT C TTCTTGAG	929
2572	UGCUC AAG CUGAUGAG X CGAA AGAGUGUC	402	GACACTCT T CTTGAGCA	930
2573	CUGCUC AA CUGAUGAG X CGAA AAGAGUGU	403	AACTCTT C TTGAGCAG	931
2575	GUCUGCUC CUGAUGAG X CGAA AGAAGAGU	404	ACTCTTCT T GAGCAGAC	932
2590	CUCUCCA CUGAUGAG X CGAA AUCACGGU	405	ACCGTGAT T TGGAAGAG	933
2591	UCUCUCC CUGAUGAG X CGAA AAUCACGG	406	CCGTGATT T GGAAGAGA	934
2622	GUUUC AAG CUGAUGAG X CGAA AGUGUGGU	407	ACCACACT T CTTGAAAC	935
2623	UGUUC AA CUGAUGAG X CGAA AAGUGUGG	408	CCACACTT C TTGAAACA	936
2625	GCUGUUC CUGAUGAG X CGAA AGAAGUGU	409	AACTTCT T GAAACAGC	937
2646	GCCUAAAG CUGAUGAG X CGAA ACCGUCAC	410	GTGACGGT C CTTTAGGC	938
2649	GCUGCCUA CUGAUGAG X CGAA AGGACCGU	411	ACGGTCCT T TAGGCAGC	939
2650	GGCUGCCU CUGAUGAG X CGAA AAGGACCG	412	CGGTCCTT T AGGCAGCC	940
2651	AGGCUGCC CUGAUGAG X CGAA AAAGGACC	413	GGTCCTTT A GGCAGCCT	941
2668	GGGACAGA CUGAUGAG X CGAA ACGGCGGC	414	GCCGCCGT C TCTGTCCC	942
2670	CCGGGACA CUGAUGAG X CGAA AGACGGCG	415	CGCCGTCT C TGTCCCGG	943
2674	UGAACCGG CUGAUGAG X CGAA ACAGAGAC	416	GTCTCTGT C CCGGTTCA	944
2680	GCAAGGUG CUGAUGAG X CGAA ACCGGGAC	417	GTCCCGGT T CACCTTGC	945
2681	GGCAAGGU CUGAUGAG X CGAA AACCGGGA	418	TCCCGGTT C ACCTTGCC	946
2686	CUCUCGGC CUGAUGAG X CGAA AGGUGAAC	419	GTTCACCT T GCCGAGAG	947
2703	GUGGGGCA CUGAUGAG X CGAA ACGCGCCU	420	AGGCGCGT C TGCCCCAC	948

Table 3

2715	CAGGGUUU CUGAUGAG X CGAA AGGGUGGG	421	CCCACCCT C AAACCCTG	949
2741	AGAGUCGU CUGAUGAG X CGAA AGCACCAU	422	ATGGTGCT C ACGACTCT	950
2748	UGCAGGAA CUGAUGAG X CGAA AGUCGUGA	423	TCACGACT C TTCCTGCA	951
2750	UUUGCAGG CUGAUGAG X CGAA AGAGUCGU	424	ACGACTCT T CCTGCAAA	952
2751	CUUUGCAG CUGAUGAG X CGAA AAGAGUCG	425	CGACTCTT C CTGCAAAG	953
2774	UUAAUGUG CUGAUGAG X CGAA AGGUCUUC	426	GAAGACCT C CACATTAA	954
2780	AGCCACUU CUGAUGAG X CGAA AUGUGGAG	427	CTCCACAT T AAGTGGCT	955
2781	AAGCCACU CUGAUGAG X CGAA AAUGUGGA	428	TCCACATT A AGTGGCTT	956
2789	AUGUAAA CUGAUGAG X CGAA AGCCACUU	429	AAGTGGCT T TTTAACAT	957
2790	CAUGUAAA CUGAUGAG X CGAA AAGCCACU	430	AGTGGCTT T TTAACATG	958
2791	UCAUGUUA CUGAUGAG X CGAA AAAGCCAC	431	GTGGCTTT T TAACATGA	959
2792	UUCAUGUU CUGAUGAG X CGAA AAAAGCCA	432	TGGCTTTT T AACATGAA	960
2793	UUUCAUGU CUGAUGAG X CGAA AAAAAGCC	433	GGCTTTTT A ACATGAAA	961
2816	UCGGGAGC CUGAUGAG X CGAA ACAGCUGC	434	GCAGCTGT A GCTCCCGA	962
2820	UAGCUCGG CUGAUGAG X CGAA AGCUACAG	435	CTGTAGCT C CCGAGCTA	963
2828	CAAGAGAG CUGAUGAG X CGAA AGCUCGGG	436	CCCGAGCT A CTCTCTTG	964
2831	UGGCAAGA CUGAUGAG X CGAA AGUAGCUC	437	GAGCTACT C TCTTGCCA	965
2833	GCUGGCAA CUGAUGAG X CGAA AGAGUAGC	438	GCTACTCT C TTGCCAGC	966
2835	AUGCUGGC CUGAUGAG X CGAA AGAGAGUA	439	TACTCTCT T GCCAGCAT	967
2844	AAUGUGAA CUGAUGAG X CGAA AUGCUGGC	440	GCCAGCAT T TTCACATT	968
2845	AAAUGUGA CUGAUGAG X CGAA AAUGCUGG	441	CCAGCATT T TCACATTT	969
2846	AAAAUGUG CUGAUGAG X CGAA AAAUGCUG	442	CAGCATTT T CACATTTT	970
2847	CAAAAUGU CUGAUGAG X CGAA AAAAUGCU	443	AGCATTTT C ACATTTTG	971
2852	AAAGGCAA CUGAUGAG X CGAA AUGUGAAA	444	TTTCACAT T TTGCCTTT	972
2853	GAAAGGCA CUGAUGAG X CGAA AAUGUGAA	445	TTCACATT T TGCCTTTC	973
2854	AGAAAGGC CUGAUGAG X CGAA AAAUGUGA	446	TCACATTT T GCCTTTCT	974
2859	CCACGAGA CUGAUGAG X CGAA AGGCAAAA	447	TTTTGCCT T TCTCGTGG	975
2860	ACCACGAG CUGAUGAG X CGAA AAGGCAAA	448	TTTGCCTT T CTCGTGGT	976
2861	UACCACGA CUGAUGAG X CGAA AAAGGCAA	449	TTGCCTTT C TCGTGGTA	977
2863	UCUACCAC CUGAUGAG X CGAA AGAAAGGC	450	GCCTTTCT C GTGGTAGA	978
2869	CUGGCUUC CUGAUGAG X CGAA ACCACGAG	451	CTCGTGGT A GAAGCCAG	979
2879	UUUCUCUG CUGAUGAG X CGAA ACUGGCUU	452	AAGCCAGT A CAGAGAAA	980
2889	CACCACAG CUGAUGAG X CGAA AUUUCUCU	453	AGAGAAAT T CTGTGGTG	981
2890	CCACCACA CUGAUGAG X CGAA AAUUCUCU	454	GAGAAATT C TGTGGTGG	982
2905	ACACCUCG CUGAUGAG X CGAA AUGUCCCC	455	GGGAACAT T CGAGGTGT	983
2906	GACACCUC CUGAUGAG X CGAA AAUGUCCC	456	GGAACATT C GAGGTGTC	984
2914	UGCAGGGU CUGAUGAG X CGAA ACACCUCG	457	CGAGGTGT C ACCCTGCA	985
2928	CCUCACCA CUGAUGAG X CGAA AGCUCUGC	458	GCAGAGCT A TGGTGAGG	986
2944	CUAAGCCU CUGAUGAG X CGAA AUCCACAC	459	GTGTGGAT A AGGCTTAG	987
2950	UGGCACCU CUGAUGAG X CGAA AGCCUUAU	460	ATAAGGCT T AGGTGCCA	988
2951	CUGGCACC CUGAUGAG X CGAA AAGCCUUA	461	TAAGGCTT A GGTGCCAG	989
2965	AGAAUGCU CUGAUGAG X CGAA ACAGCCUG	462	CAGGCTGT A AGCATTCT	990
2971	CAGCUCAG CUGAUGAG X CGAA AUGCUUAC	463	GTAAGCAT T CTGAGCTG	991
2972	CCAGCUCA CUGAUGAG X CGAA AAUGCUUA	464	TAAGCATT C TGAGCTGG	992
2983	AAAACAAC CUGAUGAG X CGAA AGCCAGCU	465	AGCTGGCT T GTTGTTTT	993
2986	UUAAAAAC CUGAUGAG X CGAA ACAAGCCA	466	TGGCTTGT T GTTTTAA	994
2989	GACUAAA CUGAUGAG X CGAA ACAACAAG	467	CTTGTTGT T TTTAAGTC	995

Table 3

2990	GGACUUA CUGAUGAG X CGAA AACAACAA	468	TTGTTGTT T TTAAGTCC	996
2991	AGGACUUA CUGAUGAG X CGAA AAACAACA	469	TGTTGTTT T TAAGTCCT	997
2992	CAGGACUU CUGAUGAG X CGAA AAAACAAC	470	GTTGTTTT T AAGTCCTG	998
2993	ACAGGACU CUGAUGAG X CGAA AAAAACA	471	TTGTTTTT A AGTCCTGT	999
2997	AUAUACAG CUGAUGAG X CGAA ACUUA AAA	472	TTTTAAGT C CTGTATAT	1000
3002	CAUACAUA CUGAUGAG X CGAA ACAGGACU	473	AGTCCTGT A TATGTATG	1001
3004	UACAUACA CUGAUGAG X CGAA AUACAGGA	474	TCCTGTAT A TGTATGTA	1002
3008	CUACUACA CUGAUGAG X CGAA ACAUAUAC	475	GTATATGT A TGTAGTAG	1003
3012	CAAACUAC CUGAUGAG X CGAA ACAUACAU	476	ATGTATGT A GTAGTTTG	1004
3015	ACCCAAAC CUGAUGAG X CGAA ACUACAUA	477	TATGTAGT A GTTTGGGT	1005
3018	CACACCCA CUGAUGAG X CGAA ACUACUAC	478	GTAGTAGT T TGGGTGTG	1006
3019	ACACACCC CUGAUGAG X CGAA AACUACUA	479	TAGTAGTT T GGGTGTGT	1007
3028	ACUAUAUA CUGAUGAG X CGAA ACACACCC	480	GGGTGTGT A TATATAGT	1008
3030	CUACUAUA CUGAUGAG X CGAA AUACACAC	481	GTGTGTAT A TATAGTAG	1009
3032	UGCUCUA CUGAUGAG X CGAA AUAUACAC	482	GTGTATAT A TAGTAGCA	1010
3034	AAUGCUAC CUGAUGAG X CGAA AUAUAUAC	483	GTATATAT A GTAGCATT	1011
3037	UGAAAUGC CUGAUGAG X CGAA ACUAUAUA	484	TATATAGT A GCATTTCA	1012
3042	CAUUUUGA CUGAUGAG X CGAA AUGCUACU	485	AGTAGCAT T TCAAAATG	1013
3043	CCAUUUUG CUGAUGAG X CGAA AAUGCUAC	486	GTAGCATT T CAAAATGG	1014
3044	UCCAUUUU CUGAUGAG X CGAA AAAUGCUA	487	TAGCATTT C AAAATGGA	1015
3056	UAAACCAG CUGAUGAG X CGAA ACGUCCAU	488	ATGGACGT A CTGGTTTA	1016
3062	GGAGGUUA CUGAUGAG X CGAA ACCAGUAC	489	GTACTGGT T TAACCTCC	1017
3063	AGGAGGUU CUGAUGAG X CGAA AACCAGUA	490	TACTGGTT T AACCTCCT	1018
3064	UAGGAGGU CUGAUGAG X CGAA AAACCAGU	491	ACTGGTTT A ACCTCCTA	1019
3069	AAGGAUAG CUGAUGAG X CGAA AGGUUAAA	492	TTTAACCT C CTATCCTT	1020
3072	UCCAAGGA CUGAUGAG X CGAA AGGAGGUU	493	AACCTCCT A TCCTTGGA	1021
3074	UCUCCAAG CUGAUGAG X CGAA AUAGGAGG	494	CCTCCTAT C CTTGGAGA	1022
3077	UGCUCUCC CUGAUGAG X CGAA AGGAUAGG	495	CCTATCCT T GGAGAGCA	1023
3093	AAGGUGGA CUGAUGAG X CGAA AGCCAGCU	496	AGCTGGCT C TCCACCTT	1024
3095	ACAAGGUG CUGAUGAG X CGAA AGAGCCAG	497	CTGGCTCT C CACCTTGT	1025
3101	UGUGUAAC CUGAUGAG X CGAA AGGUGGAG	498	CTCCACCT T GTTACACA	1026
3104	UAAUGUGU CUGAUGAG X CGAA ACAAGGUG	499	CACCTTGT T ACACATTA	1027
3105	AUAAUGUG CUGAUGAG X CGAA AACAAGGU	500	ACCTTGTT A CACATTAT	1028
3111	UCUAACAU CUGAUGAG X CGAA AUGUGUAA	501	TTACACAT T ATGTTAGA	1029
3112	CUCUAACA CUGAUGAG X CGAA AAUGUGUA	502	TACACATT A TGTTAGAG	1030
3116	ACCUCUCU CUGAUGAG X CGAA ACAUAAUG	503	CATTATGT T AGAGAGGT	1031
3117	UACCUCUC CUGAUGAG X CGAA AACAUA AU	504	ATTATGTT A GAGAGGTA	1032
3125	CAGCUCGC CUGAUGAG X CGAA ACCUCUCU	505	AGAGAGGT A GCGAGCTG	1033
3136	ACAUAGCA CUGAUGAG X CGAA AGCAGCUC	506	GAGCTGCT C TGCTATGT	1034
3141	UAAGGACA CUGAUGAG X CGAA AGCAGAGC	507	GCTCTGCT A TGTCTTA	1035
3145	GGCUAAG CUGAUGAG X CGAA ACAUAGCA	508	TGCTATGT C CTTAAGCC	1036
3148	AUUGGCUU CUGAUGAG X CGAA AGGACAU	509	TATGTCCT T AAGCCAAT	1037
3149	UAUUGGCU CUGAUGAG X CGAA AAGGACAU	510	ATGTCCTT A AGCCAATA	1038
3157	UGAGUAAA CUGAUGAG X CGAA AUUGGCUU	511	AAGCCAAT A TTTACTCA	1039
3159	GAUGAGUA CUGAUGAG X CGAA AUAUUGGC	512	GCCAATAT T TACTCATC	1040
3160	UGAUGAGU CUGAUGAG X CGAA AAUAUUGG	513	CCAATATT T ACTCATCA	1041
3161	CUGAUGAG CUGAUGAG X CGAA AAUAUUG	514	CAATATTT A CTCATCAG	1042

Table 3

3164	GACCUGAU CUGAUGAG X CGAA AGUAAAUA	515	TATTTACT C ATCAGGTC	1043
3167	AAUGACCU CUGAUGAG X CGAA AUGAGUAA	516	TTACTCAT C AGGTCATT	1044
3172	AAAAUAAU CUGAUGAG X CGAA ACCUGAUG	517	CATCAGGT C ATTATTTT	1045
3175	UAAAAAAU CUGAUGAG X CGAA AUGACCUG	518	CAGGTCAT T ATTTTTTA	1046
3176	GUAAAAAA CUGAUGAG X CGAA AAUGACCU	519	AGGTCATT A TTTTTTAC	1047
3178	UUGUAAAA CUGAUGAG X CGAA AUAAUGAC	520	GTCATTAT T TTTTACAA	1048
3179	AUUGUAAA CUGAUGAG X CGAA AAUAAUGA	521	TCATTATT T TTTACAAT	1049
3180	CAUUGUAA CUGAUGAG X CGAA AAAUAAUG	522	CATTATTT T TTACAATG	1050
3181	CCAUUGUA CUGAUGAG X CGAA AAAAUAAU	523	ATTATTTT T TACAATGG	1051
3182	GCCAUUGU CUGAUGAG X CGAA AAAAAUAA	524	TTATTTTT T ACAATGGC	1052
3183	GGCCAUUG CUGAUGAG X CGAA AAAAAAUA	525	TATTTTTT A CAATGGCC	1053
3199	AAAUGGUU CUGAUGAG X CGAA AUUCCAUG	526	CATGGAAT A AACCATTT	1054
3206	UUUGUAAA CUGAUGAG X CGAA AUGGUUUA	527	TAAACCAT T TTTACAAA	1055
3207	UUUUGUAA CUGAUGAG X CGAA AAUGGUUU	528	AAACCATT T TTACAAA	1056

Input Sequence = PTPN1 (Homo sapiens protein tyrosine phosphatase, non-receptor type 1 (PTPN1)
3215 bp)

Cut Site = UH.

Table 4

Table 4: Human PTP-1B NCH Ribozyme and Target Sequence

Nt. Position	Ribozyme Sequence	Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
13	CCGCUCUA CUGAUGAG X CGAA ICCGCGUC	1057	GACGCGGC C TAGAGCGG	1781
14	GCCGCUCU CUGAUGAG X CGAA IGCCGCGU	1058	ACGCGGCC T AGAGCGGC	1782
23	GCGCCGUC CUGAUGAG X CGAA ICCGUCUCU	1059	AGAGCGGC A GACGGCGC	1783
32	CGGCCCAC CUGAUGAG X CGAA ICGCCGUC	1060	GACGGCGC A GTGGGCCG	1784
39	UCCUUCUC CUGAUGAG X CGAA ICCCACUG	1061	CAGTGGGC C GAGAAGGA	1785
53	GCGGCUGC CUGAUGAG X CGAA ICGCCUCC	1062	GGAGGCGC A GCAGCCGC	1786
56	AGGGCGGC CUGAUGAG X CGAA ICUGCGCC	1063	GGCGCAGC A GCCGCCCT	1787
59	GCCAGGGC CUGAUGAG X CGAA ICUGCUGC	1064	GCAGCAGC C GCCCTGGC	1788
62	CGGGCCAG CUGAUGAG X CGAA ICGGCUGC	1065	GCAGCCGC C CTGGCCCG	1789
63	ACGGGCCA CUGAUGAG X CGAA ICGGGCUG	1066	CAGCCGCC C TGGCCCGT	1790
64	GACGGGCC CUGAUGAG X CGAA IGGCGGCU	1067	AGCCGCCC T GGCCCGTC	1791
68	CCAUGACG CUGAUGAG X CGAA ICCAGGGC	1068	GCCCTGGC C CGTCATGG	1792
69	UCCAUGAC CUGAUGAG X CGAA IGCCAGGG	1069	CCCTGGCC C GTCATGGA	1793
73	CAUCUCCA CUGAUGAG X CGAA IACGGGCC	1070	GGCCCGTC A TGGAGATG	1794
98	UGUCGAUC CUGAUGAG X CGAA ICUCGAAC	1071	GTTCGAGC A GATCGACA	1795
106	CCCGGACU CUGAUGAG X CGAA IUCGAUCU	1072	AGATCGAC A AGTCCGGG	1796
111	CAGCUCCC CUGAUGAG X CGAA IACUUGUC	1073	GACAAGTC C GGGAGCTG	1797
118	GGCCGCCC CUGAUGAG X CGAA ICUCCCGG	1074	CCGGGAGC T GGGCGGCC	1798
126	UGGUAAAU CUGAUGAG X CGAA ICCGCCCA	1075	TGGGCGGC C ATTTACCA	1799
127	CUGGUAAA CUGAUGAG X CGAA IGCCGCCC	1076	GGGCGGCC A TTTACCAG	1800
133	GAUAUCCU CUGAUGAG X CGAA IUAAAUGG	1077	CCATTTAC C AGGATATC	1801
134	GGUAUACC CUGAUGAG X CGAA IGUAAAUG	1078	CATTTACC A GGATATCC	1802
142	UUCAUGUC CUGAUGAG X CGAA IAUAUCCU	1079	AGGATATC C GACATGAA	1803
146	UGGCUUCA CUGAUGAG X CGAA IUCGGAUA	1080	TATCCGAC A TGAAGCCA	1804
153	AAGUCACU CUGAUGAG X CGAA ICUUCAUG	1081	CATGAAGC C AGTGAATT	1805
154	GAAGUCAC CUGAUGAG X CGAA IGCUUCAU	1082	ATGAAGCC A GTGACTTC	1806
160	ACAUGGGA CUGAUGAG X CGAA IUCACUGG	1083	CCAGTGAC T TCCCATGT	1807
163	UCUACAUG CUGAUGAG X CGAA IAAGUCAC	1084	GTGACTTC C CATGTAGA	1808
164	CUCUACAU CUGAUGAG X CGAA IGAAGUCA	1085	TGACTTCC C ATGTAGAG	1809
165	ACUCUACA CUGAUGAG X CGAA IGGAAGUC	1086	GACTTCCC A TGTAAGT	1810
177	GGAAGCUU CUGAUGAG X CGAA ICCACUCU	1087	AGAGTGGC C AAGCTTCC	1811
178	AGGAAGCU CUGAUGAG X CGAA IGCCACUC	1088	GAGTGGCC A AGCTTCCT	1812
182	UCUUAGGA CUGAUGAG X CGAA ICUUGGCC	1089	GGCCAAGC T TCCTAAGA	1813
185	UGUUCUUA CUGAUGAG X CGAA IAAGCUUG	1090	CAAGCTTC C TAAGAACA	1814
186	UUGUUCUU CUGAUGAG X CGAA IGAAGCUU	1091	AAGCTTCC T AAGAACAA	1815
193	UCGGUUUU CUGAUGAG X CGAA IUUCUUAG	1092	CTAAGAAC A AAAACCGA	1816
199	CCUAUUUC CUGAUGAG X CGAA IUUUUUGU	1093	ACAAAAAC C GAAATAGG	1817
211	GACGUCUC CUGAUGAG X CGAA IUACCUAU	1094	ATAGGTAC A GAGACGTC	1818
220	AAAGGGAC CUGAUGAG X CGAA IACGUCUC	1095	GAGACGTC A GTCCCTTT	1819
224	GGUCAAA CUGAUGAG X CGAA IACUGACG	1096	CGTCAGTC C CTTTGACC	1820
225	UGGUCAA CUGAUGAG X CGAA IGACUGAC	1097	GTCAGTCC C TTTGACCA	1821
226	AUGGUCAA CUGAUGAG X CGAA IGGACUGA	1098	TCAGTCCC T TTGACCAT	1822
232	CCGACUAU CUGAUGAG X CGAA IUCAAAGG	1099	CCTTTGAC C ATAGTCGG	1823

Table 4

233	UCCGACUA CUGAUGAG X CGAA IGUCAAAG	1100	CTTTGACC A TAGTCGGA	1824
248	CUUGAUGU CUGAUGAG X CGAA IUUUAAUC	1101	GATTAAAC T ACATCAAG	1825
251	CUUCUUGA CUGAUGAG X CGAA IUAGUUUA	1102	TAAACTAC A TCAAGAAG	1826
254	UAUCUUCU CUGAUGAG X CGAA IAUGUAGU	1103	ACTACATC A AGAAGATA	1827
268	GUUGAUUAU CUGAUGAG X CGAA IUCAUUUAU	1104	ATAATGAC T ATATCAAC	1828
274	ACUAGCGU CUGAUGAG X CGAA IAUUAUAGU	1105	ACTATATC A ACGCTAGT	1829
279	AUCAAACU CUGAUGAG X CGAA ICGUUGAU	1106	ATCAACGC T AGTTTGAT	1830
303	CUCCUUUG CUGAUGAG X CGAA ICUUCUUC	1107	GAAGAAGC C CAAAGGAG	1831
304	ACUCCUUU CUGAUGAG X CGAA IGCUUCUU	1108	AAGAAGCC C AAAGGAGT	1832
305	AACUCCUU CUGAUGAG X CGAA IGGCUUCU	1109	AGAAGCCC A AAGGAGTT	1833
316	GGUAAGAA CUGAUGAG X CGAA IUAACUCC	1110	GGAGTTAC A TTCTTACC	1834
320	CCUGGGUA CUGAUGAG X CGAA IAAUGUAA	1111	TTACATTC T TACCCAGG	1835
324	GGGCCCUG CUGAUGAG X CGAA IUAAGAAU	1112	ATTCTTAC C CAGGGCCC	1836
325	AGGGCCCU CUGAUGAG X CGAA IGUAAGAA	1113	TTCTTACC C AGGGCCCT	1837
326	AAGGGCCC CUGAUGAG X CGAA IGGUAAGA	1114	TCTTACCC A GGGCCCTT	1838
331	AGGCAAAG CUGAUGAG X CGAA ICCUGGG	1115	CCCAGGGC C CTTTGCCT	1839
332	UAGGCAA CUGAUGAG X CGAA IGCCUGG	1116	CCAGGGCC C TTTGCCTA	1840
333	UUAGGCAA CUGAUGAG X CGAA IGGCCCUG	1117	CAGGGCCC T TTGCCTAA	1841
338	AUGUGUUA CUGAUGAG X CGAA ICAAAGGG	1118	CCCTTTGC C TAACACAT	1842
339	CAUGUGUU CUGAUGAG X CGAA IGCAAAGG	1119	CCTTTGCC T AACACATG	1843
343	ACCGCAUG CUGAUGAG X CGAA IUUAGGCA	1120	TGCCTAAC A CATGCGGT	1844
345	UGACCGCA CUGAUGAG X CGAA IUGUAGG	1121	CCTAACAC A TGCGGTCA	1845
353	CCCAAAG CUGAUGAG X CGAA IACCGCAU	1122	ATGCGGTC A CTTTTGGG	1846
355	CUCCCAA CUGAUGAG X CGAA IUGACCGC	1123	GCGGTCAC T TTTGGGAG	1847
377	UGCUIIUC CUGAUGAG X CGAA ICUCCAC	1124	GTGGGAGC A GAAAAGCA	1848
385	GACACCCC CUGAUGAG X CGAA ICUUIUCU	1125	AGAAAAGC A GGGGTGTC	1849
397	GUUGAGCA CUGAUGAG X CGAA IACGACAC	1126	GTGTCGTC A TGCTCAAC	1850
401	CUCUGUUG CUGAUGAG X CGAA ICAUGACG	1127	CGTCATGC T CAACAGAG	1851
403	CACUCUGU CUGAUGAG X CGAA IAGCAUGA	1128	TCATGCTC A ACAGAGTG	1852
406	CAUCACUC CUGAUGAG X CGAA IUUGAGCA	1129	TGCTCAAC A GAGTGATG	1853
438	CAGUAUUG CUGAUGAG X CGAA ICGCAUUU	1130	AAATGCGC A CAATACTG	1854
440	GCCAGUAU CUGAUGAG X CGAA IUGCGCAU	1131	ATGCGCAC A ATACTGGC	1855
445	UUGUGGCC CUGAUGAG X CGAA IUAUUGUG	1132	CACAATAC T GGCCACAA	1856
449	CUUUUUGU CUGAUGAG X CGAA ICCAGUAU	1133	ATACTGGC C ACAAAAAG	1857
450	UCUUUUUG CUGAUGAG X CGAA IGCCAGUA	1134	TACTGGCC A CAAAAGA	1858
452	CUUCUUUU CUGAUGAG X CGAA IUGGCCAG	1135	CTGGCCAC A AAAAGAAG	1859
475	GUCUUCUA CUGAUGAG X CGAA IAUCAUCU	1136	AGATGATC T TTGAAGAC	1860
484	CAAAUUG CUGAUGAG X CGAA IUCUUCUA	1137	TTGAAGAC A CAAATTTG	1861
486	UUCAAUU CUGAUGAG X CGAA IUGUCUUC	1138	GAAGACAC A AATTTGAA	1862
501	GAGAUCAA CUGAUGAG X CGAA IUUAAUUU	1139	AAATTAAC A TTGATCTC	1863
508	AUCUUCAG CUGAUGAG X CGAA IAUCAAUG	1140	CATTGATC T CTGAAGAT	1864
510	AUAUCUUC CUGAUGAG X CGAA IAGAUCAA	1141	TTGATCTC T GAAGATAT	1865
520	AUAUGACU CUGAUGAG X CGAA IAUAUUU	1142	AAGATATC A AGTCATAT	1866
525	GUAAUAUA CUGAUGAG X CGAA IACUUGAU	1143	ATCAAGTC A TATTATAC	1867
534	UGUCGCAC CUGAUGAG X CGAA IUAUAAUA	1144	TATTATAC A GTGCGACA	1868
542	AUUCUAGC CUGAUGAG X CGAA IUCGCACU	1145	AGTGCGAC A GCTAGAAT	1869
545	CCAAUUCU CUGAUGAG X CGAA ICUGUCGC	1146	GCGACAGC T AGAATTGG	1870

Table 4

559	GGUUGUAA CUGAUGAG X CGAA IUUUUCCA	1147	TGGAAAAC C TTACAACC	1871
560	GGGUUGUA CUGAUGAG X CGAA IGUUUUCC	1148	GGAAAACC T TACAACCC	1872
564	UCUUGGGU CUGAUGAG X CGAA IUAAGGUU	1149	AACCTTAC A ACCCAAGA	1873
567	GUUUCUUG CUGAUGAG X CGAA IUUGUAAG	1150	CTTACAAC C CAAGAAAC	1874
568	AGUUUCUU CUGAUGAG X CGAA IGUUGUAA	1151	TTACAACC C AAGAAACT	1875
569	GAGUUUCU CUGAUGAG X CGAA IGGUUGUA	1152	TACAACCC A AGAAACTC	1876
576	AUCUCUCG CUGAUGAG X CGAA IUUUCUUG	1153	CAAGAAAC T CGAGAGAT	1877
586	GAAAUGUA CUGAUGAG X CGAA IAUCUCUC	1154	GAGAGATC T TACATTTC	1878
590	AGUGGAAA CUGAUGAG X CGAA IUAAGAUC	1155	GATCTTAC A TTTCCACT	1879
595	GGUAUAGU CUGAUGAG X CGAA IAAAUGUA	1156	TACATTTC C ACTATACC	1880
596	UGGUAUAG CUGAUGAG X CGAA IGAAAUGU	1157	ACATTTCC A CTATACCA	1881
598	UGUGGUAU CUGAUGAG X CGAA IUGGAAAU	1158	ATTTCCAC T ATACCACA	1882
603	GGCCAUGU CUGAUGAG X CGAA IUUAUAGU	1159	CACTATAC C ACATGGCC	1883
604	AGGCCAUG CUGAUGAG X CGAA IGUAUAGU	1160	ACTATACC A CATGGCCT	1884
606	UCAGGCCA CUGAUGAG X CGAA IUGGUUAU	1161	TATACCAC A TGGCCTGA	1885
611	CAAAGUCA CUGAUGAG X CGAA ICCAUGUG	1162	CACATGGC C TGACTTTG	1886
612	CCAAAGUC CUGAUGAG X CGAA IGCCAUGU	1163	ACATGGCC T GACTTTGG	1887
616	GACUCCAA CUGAUGAG X CGAA IUCAGGCC	1164	GGCCTGAC T TTGGAGTC	1888
625	UGAUUCAG CUGAUGAG X CGAA IACUCCAA	1165	TTGGAGTC C CTGAATCA	1889
626	GUGAUUCA CUGAUGAG X CGAA IGACUCCA	1166	TGGAGTCC C TGAATCAC	1890
627	GGUGAUUC CUGAUGAG X CGAA IGGACUCC	1167	GGAGTCCC T GAATCACC	1891
633	GAGGCUGG CUGAUGAG X CGAA IAUUCAGG	1168	CCTGAATC A CCAGCCTC	1892
635	AUGAGGCU CUGAUGAG X CGAA IUGAUUCA	1169	TGAATCAC C AGCCTCAT	1893
636	AAUGAGGC CUGAUGAG X CGAA IGUGAUUC	1170	GAATCACC A GCCTCATT	1894
639	AAGAAUGA CUGAUGAG X CGAA ICUGGUGA	1171	TCACCAGC C TCATTCTT	1895
640	CAAGAAUG CUGAUGAG X CGAA IGCUGGUG	1172	CACCAGCC T CATTCCTG	1896
642	UUCAAGAA CUGAUGAG X CGAA IAGGCUGG	1173	CCAGCCTC A TTCTTGAA	1897
646	AAAGUUCA CUGAUGAG X CGAA IAAUGAGG	1174	CCTCATTC T TGAACCTT	1898
652	GAAAAGAA CUGAUGAG X CGAA IUUCAAGA	1175	TCTTGAAC T TTCTTTTC	1899
656	CUUUGAAA CUGAUGAG X CGAA IAAAGUUC	1176	GAACCTTC T TTTCAAAG	1900
661	UCGGACUU CUGAUGAG X CGAA IAAAAGAA	1177	TTCTTTTC A AAGTCCGA	1901
667	UGACUCUC CUGAUGAG X CGAA IACUJUGA	1178	TCAAAGTC C GAGAGTCA	1902
675	AGUGACCC CUGAUGAG X CGAA IACUCUCG	1179	CGAGAGTC A GGGTCACT	1903
681	GGGCUGAG CUGAUGAG X CGAA IACCCUGA	1180	TCAGGGTC A CTCAGCCC	1904
683	CCGGGCUG CUGAUGAG X CGAA IUGACCCU	1181	AGGGTCAC T CAGCCCGG	1905
685	CUCCGGGC CUGAUGAG X CGAA IAGUGACC	1182	GGTCACTC A GCCCGGAG	1906
688	GUGCUCCG CUGAUGAG X CGAA ICUGAGUG	1183	CACTCAGC C CGGAGCAC	1907
689	CGUGCUC CUGAUGAG X CGAA IGCUGAGU	1184	ACTCAGCC C GGAGCAGC	1908
695	CGGGCCCG CUGAUGAG X CGAA ICUCCGGG	1185	CCCGGAGC A CGGGCCCG	1909
701	CCACAACG CUGAUGAG X CGAA ICCCGUGC	1186	GCACGGGC C CGTTGTGG	1910
702	ACCACAAC CUGAUGAG X CGAA IGCCCGUG	1187	CACGGGCC C GTTGTGGT	1911
713	CACUGCAG CUGAUGAG X CGAA ICACCACA	1188	TGTGGTGC A CTGCAGTG	1912
715	UGCACUGC CUGAUGAG X CGAA IUGCACCA	1189	TGGTGCAC T GCAGTGCA	1913
718	GCCUGCAC CUGAUGAG X CGAA ICAGUGCA	1190	TGCACTGC A GTGCAGGC	1914
723	CCGAUGCC CUGAUGAG X CGAA ICACUGCA	1191	TGCAGTGC A GGCATCGG	1915
727	CCUGCCGA CUGAUGAG X CGAA ICCUGCAC	1192	GTGCAGGC A TCGGCAGG	1916
733	UCCAGACC CUGAUGAG X CGAA ICCGAUGC	1193	GCATCGGC A GGTCTGGA	1917

Table 4

738	AAGGUUCC CUGAUGAG X CGAA IACCUGCC	1194	GGCAGGTC T GGAACCTT	1918
744	AGACAGAA CUGAUGAG X CGAA IUUCCAGA	1195	TCTGGAAC C TTCTGTCT	1919
745	CAGACAGA CUGAUGAG X CGAA IGUUCAG	1196	CTGGAACC T TCTGTCTG	1920
748	AGCCAGAC CUGAUGAG X CGAA IAAGGUUC	1197	GAACCTTC T GTCTGGCT	1921
752	UAUCAGCC CUGAUGAG X CGAA IACAGAAG	1198	CTTCTGTC T GGCTGATA	1922
756	CAGGUAUC CUGAUGAG X CGAA ICCAGACA	1199	TGTCTGGC T GATACCTG	1923
762	AAGAGGCA CUGAUGAG X CGAA IUAUCAGC	1200	GCTGATAC C TGCCTCTT	1924
763	CAAGAGGC CUGAUGAG X CGAA IGUAUCAG	1201	CTGATACC T GCCTCTTG	1925
766	CAGCAAGA CUGAUGAG X CGAA ICAGGUAU	1202	ATACCTGC C TCTTGCTG	1926
767	UCAGCAAG CUGAUGAG X CGAA IGCAGGUA	1203	TACCTGCC T CTTGCTGA	1927
769	CAUCAGCA CUGAUGAG X CGAA IAGGCAGG	1204	CCTGCCTC T TGCTGATG	1928
773	UGUCCAUC CUGAUGAG X CGAA ICAAGAGG	1205	CCTCTTGC T GATGGACA	1929
781	UUUCCUCU CUGAUGAG X CGAA IUCCAUA	1206	TGATGGAC A AGAGGAAA	1930
793	GGAAGAAG CUGAUGAG X CGAA IUCUUUCC	1207	GGAAAGAC C CTTCTTCC	1931
794	CGGAAGAA CUGAUGAG X CGAA IGUCUUUC	1208	GAAAGACC C TTCTTCCG	1932
795	ACGGAAGA CUGAUGAG X CGAA IGGUCUUU	1209	AAAGACCC T TCTTCCGT	1933
798	UCAACGGA CUGAUGAG X CGAA IAAGGGUC	1210	GACCCTTC T TCCGTTGA	1934
801	AUAUCAAC CUGAUGAG X CGAA IAAGAAGG	1211	CCTTCTTC C GTTGATAT	1935
811	CACUUUCU CUGAUGAG X CGAA IAUAUCAA	1212	TTGATATC A AGAAAGTG	1936
821	UUUCUAAC CUGAUGAG X CGAA ICACUUUC	1213	GAAAGTGC T GTTAGAAA	1937
851	UCUGGAUC CUGAUGAG X CGAA ICCCAUC	1214	GATGGGGC T GATCCAGA	1938
856	GGCUGUCU CUGAUGAG X CGAA IAUCAGCC	1215	GGCTGATC C AGACAGCC	1939
857	CGGCUGUC CUGAUGAG X CGAA IGAUCAGC	1216	GCTGATCC A GACAGCCG	1940
861	UGGUCGGC CUGAUGAG X CGAA IUCUGGAU	1217	ATCCAGAC A GCCGACCA	1941
864	AGCUGGUC CUGAUGAG X CGAA ICUGUCUG	1218	CAGACAGC C GACCAGCT	1942
868	GCGCAGCU CUGAUGAG X CGAA IUCGGCUG	1219	CAGCCGAC C AGCTGCGC	1943
869	AGCGCAGC CUGAUGAG X CGAA IGUCGGCU	1220	AGCCGACC A GCTGCGCT	1944
872	AGAAGCGC CUGAUGAG X CGAA ICUGGUCG	1221	CGACCAGC T GCGCTTCT	1945
877	GUAGGAGA CUGAUGAG X CGAA ICGCAGCU	1222	AGCTGCGC T TCTCCTAC	1946
880	CAGGUAGG CUGAUGAG X CGAA IAAGCGCA	1223	TGCGCTTC T CCTACCTG	1947
882	GCCAGGUA CUGAUGAG X CGAA IAGAAGCG	1224	CGCTTCTC C TACCTGGC	1948
883	AGCCAGGU CUGAUGAG X CGAA IGAGAAGC	1225	GCTTCTCC T ACCTGGCT	1949
886	CACAGCCA CUGAUGAG X CGAA IUAGGAGA	1226	TCTCCTAC C TGGCTGTG	1950
887	UCACAGCC CUGAUGAG X CGAA IGUAGGAG	1227	CTCCTACC T GGCTGTGA	1951
891	UCGAUCAC CUGAUGAG X CGAA ICCAGGUA	1228	TACCTGGC T GTGATCGA	1952
906	AUGAAUUU CUGAUGAG X CGAA ICACCUUC	1229	GAAGGTGC C AAATTCAT	1953
907	GAUGAAUU CUGAUGAG X CGAA IGCACCUU	1230	AAGGTGCC A AATTCATC	1954
913	CCCAUGA CUGAUGAG X CGAA IAAUUUGG	1231	CCAAATTC A TCATGGGG	1955
916	GUCCCCCA CUGAUGAG X CGAA IAUGAAUU	1232	AATTCATC A TGGGGGAC	1956
925	CACGGAAG CUGAUGAG X CGAA IUCCCCCA	1233	TGGGGGAC T CTTCCGTG	1957
927	UGCACGGA CUGAUGAG X CGAA IAGUCCCC	1234	GGGGACTC T TCCGTGCA	1958
930	UCCUGCAC CUGAUGAG X CGAA IAAGAGUC	1235	GACTCTTC C GTGCAGGA	1959
935	ACUGAUCC CUGAUGAG X CGAA ICACGGAA	1236	TTCCGTGC A GGATCAGT	1960
941	CCUCCAC CUGAUGAG X CGAA IAUCCUGC	1237	GCAGGATC A GTGGAAGG	1961
953	CGUGGGAA CUGAUGAG X CGAA ICUCCUUC	1238	GAAGGAGC T TTCCCACG	1962
957	UCCUCGUG CUGAUGAG X CGAA IAAAGCUC	1239	GAGCTTTC C CACGAGGA	1963
958	GUCCUCGU CUGAUGAG X CGAA IGAAAGCU	1240	AGCTTTCC C ACGAGGAC	1964

Table 4

959	GGUCCUCG CUGAUGAG X CGAA IGGAAAGC	1241	GCTTTCCC A CGAGGACC	1965
967	GGGCUCCA CUGAUGAG X CGAA IUCCUCGU	1242	ACGAGGAC C TGGAGCCC	1966
968	GGGGCUCC CUGAUGAG X CGAA IGUCCUCG	1243	CGAGGACC T GGAGCCCC	1967
974	CGGGUGGG CUGAUGAG X CGAA ICUCCAGG	1244	CCTGGAGC C CCCACCCG	1968
975	UCGGGUGG CUGAUGAG X CGAA IGCUCCAG	1245	CTGGAGCC C CCACCCGA	1969
976	CUCGGGUG CUGAUGAG X CGAA IGGCUCCA	1246	TGGAGCCC C CACCCGAG	1970
977	GCUCGGGU CUGAUGAG X CGAA IGGGCUCC	1247	GGAGCCCC C ACCCGAGC	1971
978	UGCUCGGG CUGAUGAG X CGAA IGGGGCUC	1248	GAGCCCCC A CCCGAGCA	1972
980	UAUGCUCG CUGAUGAG X CGAA IUGGGGGC	1249	GCCCCCAC C CGAGCATA	1973
981	AUAUGCUC CUGAUGAG X CGAA IGUGGGGG	1250	CCCCCACC C GAGCATAT	1974
986	GGGGGAUA CUGAUGAG X CGAA ICUCGGGU	1251	ACCCGAGC A TATCCCCC	1975
991	AGGUGGGG CUGAUGAG X CGAA IAUAUGCU	1252	AGCATATC C CCCCACCT	1976
992	GAGGUGGG CUGAUGAG X CGAA IGAUAUGC	1253	GCATATCC C CCCACCTC	1977
993	GGAGGUGG CUGAUGAG X CGAA IGGAUAUG	1254	CATATCCC C CCACCTCC	1978
994	GGGAGGUG CUGAUGAG X CGAA IGGGAUAU	1255	ATATCCCC C CACCTCCC	1979
995	GGGGAGGU CUGAUGAG X CGAA IGGGGAUA	1256	TATCCCCC C ACCTCCCC	1980
996	CGGGGAGG CUGAUGAG X CGAA IGGGGGAU	1257	ATCCCCCC A CCTCCCCG	1981
998	GCCGGGGA CUGAUGAG X CGAA IUGGGGGG	1258	CCCCCCAC C TCCCCGGC	1982
999	GGCCGGGG CUGAUGAG X CGAA IGUGGGGG	1259	CCCCCACC T CCCC GGCC	1983
1001	GUGGCCGG CUGAUGAG X CGAA IAGGUGGG	1260	CCCACCTC C CCGGCCAC	1984
1002	GGUGGCCG CUGAUGAG X CGAA IGAGGUGG	1261	CCACCTCC C CGGCCACC	1985
1003	GGGUGGCC CUGAUGAG X CGAA IGGAGGUG	1262	CACCTCCC C GGCCACCC	1986
1007	GUUUGGGU CUGAUGAG X CGAA ICCGGGGA	1263	TCCCCGGC C ACCCAAAC	1987
1008	CGUUUGGG CUGAUGAG X CGAA IGCCGGGG	1264	CCCCGGCC A CCCAAACG	1988
1010	UUCGUUUG CUGAUGAG X CGAA IUUGCCGG	1265	CCGGCCAC C CAAACGAA	1989
1011	AUUCGUUU CUGAUGAG X CGAA IGUGGCCG	1266	CGGCCACC C AAACGAAT	1990
1012	GAUUCGUU CUGAUGAG X CGAA IGGUGGCC	1267	GGCCACCC A AACGAATC	1991
1021	UGGCUCCA CUGAUGAG X CGAA IAUUCGUU	1268	AACGAATC C TGGAGCCA	1992
1022	GUGGCUCC CUGAUGAG X CGAA IGAUUCGU	1269	ACGAATCC T GGAGCCAC	1993
1028	CAUUGUGU CUGAUGAG X CGAA ICUCCAGG	1270	CCTGGAGC C ACACAATG	1994
1029	CCAUUGUG CUGAUGAG X CGAA IGCUCCAG	1271	CTGGAGCC A CACAATGG	1995
1031	UCCCAUUG CUGAUGAG X CGAA IUGGCUCC	1272	GGAGCCAC A CAATGGGA	1996
1033	UUUCCCAU CUGAUGAG X CGAA IUUGGCCU	1273	AGCCACAC A ATGGGAAA	1997
1045	GAACUCCC CUGAUGAG X CGAA ICAUUUCC	1274	GGAAATGC A GGGAGTTC	1998
1054	AUUUGGGA CUGAUGAG X CGAA IAACUCCC	1275	GGGAGTTC T TCCCAAAT	1999
1057	GUGAUUUG CUGAUGAG X CGAA IAAGAACU	1276	AGTTCTTC C CAAATCAC	2000
1058	GGUGAUUU CUGAUGAG X CGAA IGAAGAAC	1277	GTTCTTCC C AAATCACC	2001
1059	UGGUGAUU CUGAUGAG X CGAA IGGAAGAA	1278	TTCTTCCC A AATCACCA	2002
1064	CCCACUGG CUGAUGAG X CGAA IAUUUGGG	1279	CCCAAATC A CCAGTGGG	2003
1066	CACCCACU CUGAUGAG X CGAA IUGAUUUG	1280	CAAATCAC C AGTGGGTG	2004
1067	UCACCCAC CUGAUGAG X CGAA IGUGAUUU	1281	AAATCACC A GTGGGTGA	2005
1086	UCCUCCUG CUGAUGAG X CGAA IUCUCUUC	1282	GAAGAGAC C CAGGAGGA	2006
1087	AUCCUCCU CUGAUGAG X CGAA IGUCUCUU	1283	AAGAGACC C AGGAGGAT	2007
1088	UAUCCUCC CUGAUGAG X CGAA IGGUCUCU	1284	AGAGACCC A GGAGGATA	2008
1102	GAUGGGGC CUGAUGAG X CGAA IUCUUUUAU	1285	ATAAAGAC T GCCCCATC	2009
1105	CUUGAUGG CUGAUGAG X CGAA ICAGUCUU	1286	AAGACTGC C CCATCAAG	2010
1106	CCUUGAUG CUGAUGAG X CGAA IGCAGUCU	1287	AGACTGCC C CATCAAGG	2011

Table 4

1107	UCCUUGAU CUGAUGAG X CGAA IGGCAGUC	1288	GACTGCCC C ATCAAGGA	2012
1108	UUCCUUGA CUGAUGAG X CGAA IGGGCAGU	1289	ACTGCCCC A TCAAGGAA	2013
1111	UUCUUCU CUGAUGAG X CGAA IAUGGGGC	1290	GCCCCATC A AGGAAGAA	2014
1129	AUUUAAGG CUGAUGAG X CGAA ICUUCCU	1291	AAGGAAGC C CCTTAAAT	2015
1130	CAUUUAAG CUGAUGAG X CGAA IGCUCCU	1292	AGGAAGCC C CTTAAATG	2016
1131	GCAUUUAA CUGAUGAG X CGAA IGGCUUCC	1293	GGAAGCCC C TTAAATGC	2017
1132	GGCAUUUA CUGAUGAG X CGAA IGGGCUUC	1294	GAAGCCCC T TAAATGCC	2018
1140	UAGGGUGC CUGAUGAG X CGAA ICAUUUAA	1295	TTAAATGC C GCACCCTA	2019
1143	CCGUAGGG CUGAUGAG X CGAA ICGGCAU	1296	AATGCCGC A CCCTACGG	2020
1145	UGCCGUAG CUGAUGAG X CGAA IUGCGGCA	1297	TGCCGCAC C CTACGGCA	2021
1146	AUGCCGUA CUGAUGAG X CGAA IGUGCGGC	1298	GCCGCACC C TACGGCAT	2022
1147	GAUGCCGU CUGAUGAG X CGAA IGGUGCGG	1299	CCGCACCC T ACGGCATC	2023
1153	GCUUUCGA CUGAUGAG X CGAA ICCGUAGG	1300	CCTACGGC A TCGAAAGC	2024
1162	UUGACUCA CUGAUGAG X CGAA ICUUUCGA	1301	TCGAAAGC A TGAGTCAA	2025
1169	CAGUGUCU CUGAUGAG X CGAA IACUCAUG	1302	CATGAGTC A AGACACTG	2026
1174	AACUUCAG CUGAUGAG X CGAA IUCUUGAC	1303	GTCAAGAC A CTGAAGTT	2027
1176	CUAACUUC CUGAUGAG X CGAA IUGUCUUG	1304	CAAGACAC T GAAGTTAG	2028
1208	CACCUCGA CUGAUGAG X CGAA IACUUCCC	1305	GGGAAGTC T TCGAGGTG	2029
1218	GCAGCCUG CUGAUGAG X CGAA ICACCUCG	1306	CGAGGTGC C CAGGCTGC	2030
1219	GGCAGCCU CUGAUGAG X CGAA IGCACCUC	1307	GAGGTGCC C AGGCTGCC	2031
1220	AGGCAGCC CUGAUGAG X CGAA IGGCACCUC	1308	AGGTGCCC A GGCTGCCT	2032
1224	GGGGAGGC CUGAUGAG X CGAA ICCUGGGC	1309	GCCCAGGC T GCCTCCCC	2033
1227	GCUGGGGA CUGAUGAG X CGAA ICAGCCUG	1310	CAGGCTGC C TCCCCAGC	2034
1228	GGCUGGGG CUGAUGAG X CGAA IGCAGCCU	1311	AGGCTGCC T CCCCAGCC	2035
1230	UUGGCUGG CUGAUGAG X CGAA IAGGCAGC	1312	GCTGCCTC C CCAGCCAA	2036
1231	UUUGGCUG CUGAUGAG X CGAA IGAGGCAG	1313	CTGCCTCC C CAGCCAAA	2037
1232	CUUUGGCU CUGAUGAG X CGAA IGGAGGCA	1314	TGCCTCCC C AGCCAAAG	2038
1233	CCUUGGCU CUGAUGAG X CGAA IGGGAGGC	1315	GCCTCCCC A GCCAAAGG	2039
1236	UCCCCUJU CUGAUGAG X CGAA ICUGGGGA	1316	TCCCCAGC C AAAGGGGA	2040
1237	CUCCCCUJ CUGAUGAG X CGAA IGCUGGGG	1317	CCCCAGCC A AAGGGGAG	2041
1247	GCAGUGAC CUGAUGAG X CGAA ICUCCCCU	1318	AGGGGAGC C GTCAGTGC	2042
1251	UCGGGCAG CUGAUGAG X CGAA IACGGCUC	1319	GAGCCGTC A CTGCCCCA	2043
1253	UCUCGGGC CUGAUGAG X CGAA IUGACGGC	1320	GCCGTCAC T GCCCGAGA	2044
1256	CCUUCUCG CUGAUGAG X CGAA ICAGUGAC	1321	GTCAGTGC C CGAGAAGG	2045
1257	UCCUUCUC CUGAUGAG X CGAA IGCAGUGA	1322	TCACTGCC C GAGAAGGA	2046
1273	CAGUGCAU CUGAUGAG X CGAA IUCCUCGU	1323	ACGAGGAC C ATGCACTG	2047
1274	UCAGUGCA CUGAUGAG X CGAA IGUCCUCG	1324	CGAGGACC A TGCAGTGA	2048
1278	UAACUCAG CUGAUGAG X CGAA ICAUGGUC	1325	GACCATGC A CTGAGTTA	2049
1280	AGUAACUC CUGAUGAG X CGAA IUGCAUGG	1326	CCATGCAC T GAGTTACT	2050
1288	GGGCUUCC CUGAUGAG X CGAA IUAACUCA	1327	TGAGTTAC T GGAAGCCC	2051
1295	CCAGGAAG CUGAUGAG X CGAA ICUUCCAG	1328	CTGGAAGC C TTCCTGGG	2052
1296	ACCAGGAA CUGAUGAG X CGAA IGCUUCCA	1329	TGGAAGCC C TTCCTGGT	2053
1297	GACCAGGA CUGAUGAG X CGAA IGGCUUCC	1330	GGAAGCCC T TCCTGGTC	2054
1300	GUUGACCA CUGAUGAG X CGAA IAAGGGCU	1331	AGCCCTTC C TGGTCAAC	2055
1301	UGUUGACC CUGAUGAG X CGAA IGAAGGGC	1332	GCCCTTCC T GGTCAACA	2056
1306	GCACAUGU CUGAUGAG X CGAA IACCAGGA	1333	TCCTGGTC A ACATGTGC	2057
1309	CACGCACA CUGAUGAG X CGAA IUUGACCA	1334	TGGTCAAC A TGTGCGTG	2058

Table 4

1320	AGGACCGU CUGAUGAG X CGAA ICCACGCA	1335	TGCGTGGC T ACGGTCCT	2059
1327	GGCCGUGA CUGAUGAG X CGAA IACCGUAG	1336	CTACGGTC C TCACGGCC	2060
1328	CGGCCGUG CUGAUGAG X CGAA IGACCGUA	1337	TACGGTCC T CACGGCCG	2061
1330	GCCGGCCG CUGAUGAG X CGAA IAGGACCG	1338	CGGTCCTC A CGGCCGGC	2062
1335	UAAGCGCC CUGAUGAG X CGAA ICCGUGAG	1339	CTCACGGC C GGCGCTTA	2063
1341	CAGAGGUA CUGAUGAG X CGAA ICGCCGGC	1340	GCCGGCGC T TACCTCTG	2064
1345	GUAGCAGA CUGAUGAG X CGAA IUAAGCGC	1341	GCGCTTAC C TCTGCTAC	2065
1346	UGUAGCAG CUGAUGAG X CGAA IGUAAGCG	1342	CGCTTACC T CTGCTACA	2066
1348	CCUGUAGC CUGAUGAG X CGAA IAGGUAAG	1343	CTTACCTC T GCTACAGG	2067
1351	GAACCUGU CUGAUGAG X CGAA ICAGAGGU	1344	ACCTCTGC T ACAGGTTC	2068
1354	CAGGAACC CUGAUGAG X CGAA IUAGCAGA	1345	TCTGCTAC A GGTTCCCTG	2069
1360	GUUGAACA CUGAUGAG X CGAA IAACCUGU	1346	ACAGGTTC C TGTTCAAC	2070
1361	UGUUGAAC CUGAUGAG X CGAA IGAACCUG	1347	CAGGTTC T GTTCAACA	2071
1366	GUUGCUGU CUGAUGAG X CGAA IAACAGGA	1348	TCCTGTTC A ACAGCAAC	2072
1369	UGUGUUGC CUGAUGAG X CGAA IUUGAACA	1349	TGTTCAAC A GCAACACA	2073
1372	CUAUGUGU CUGAUGAG X CGAA ICUGUUGA	1350	TCAACAGC A ACACATAG	2074
1375	AGGCUAUG CUGAUGAG X CGAA IUUGCUGU	1351	ACAGCAAC A CATAGCCT	2075
1377	UCAGGCUA CUGAUGAG X CGAA IUGUUGCU	1352	AGCAACAC A TAGCCTGA	2076
1382	GAGGGUCA CUGAUGAG X CGAA ICUAUGUG	1353	CACATAGC C TGACCCTC	2077
1383	GGAGGGUC CUGAUGAG X CGAA IGCUAUGU	1354	ACATAGCC T GACCCTCC	2078
1387	UGGAGGAG CUGAUGAG X CGAA IUCAGGCU	1355	AGCCTGAC C CTCCTCCA	2079
1388	GUGGAGGA CUGAUGAG X CGAA IGUCAGGC	1356	GCCTGACC C TCCTCCAC	2080
1389	AGUGGAGG CUGAUGAG X CGAA IGGUCAGG	1357	CCTGACCC T CCTCCACT	2081
1391	GGAGUGGA CUGAUGAG X CGAA IAGGGUCA	1358	TGACCCTC C TCCACTCC	2082
1392	UGGAGUGG CUGAUGAG X CGAA IGAGGGUC	1359	GACCCTCC T CCACTCCA	2083
1394	GGUGGAGU CUGAUGAG X CGAA IAGGAGGG	1360	CCCTCCTC C ACTCCACC	2084
1395	AGGUGGAG CUGAUGAG X CGAA IGAGGAGG	1361	CCTCCTCC A CTCCACCT	2085
1397	GGAGGUGG CUGAUGAG X CGAA IUGGAGGA	1362	TCCTCCAC T CCACCTCC	2086
1399	GUGGAGGU CUGAUGAG X CGAA IAGUGGAG	1363	CTCCACTC C ACCTCCAC	2087
1400	GGUGGAGG CUGAUGAG X CGAA IGAGUGGA	1364	TCCACTCC A CCTCCACC	2088
1402	UGGGUGGA CUGAUGAG X CGAA IUGGAGUG	1365	CACTCCAC C TCCACCCA	2089
1403	GUGGGUGG CUGAUGAG X CGAA IGUGGAGU	1366	ACTCCACC T CCACCCAC	2090
1405	CAGUGGGU CUGAUGAG X CGAA IAGGUGGA	1367	TCCACCTC C ACCCACTG	2091
1406	ACAGUGGG CUGAUGAG X CGAA IGAGGUGG	1368	CCACCTCC A CCCACTGT	2092
1408	GGACAGUG CUGAUGAG X CGAA IUGGAGGU	1369	ACCTCCAC C CACTGTCC	2093
1409	CGGACAGU CUGAUGAG X CGAA IGUGGAGG	1370	CCTCCACC C ACTGTCCG	2094
1410	GCGGACAG CUGAUGAG X CGAA IGGUGGAG	1371	CTCCACCC A CTGTCCGC	2095
1412	AGGCGGAC CUGAUGAG X CGAA IUGGGUGG	1372	CCACCCAC T GTCCGCCT	2096
1416	GCAGAGGC CUGAUGAG X CGAA IACAGUGG	1373	CCACTGTC C GCCTCTGC	2097
1419	CGGGCAGA CUGAUGAG X CGAA ICGGACAG	1374	CTGTCCGC C TCTGCCCCG	2098
1420	GCGGGCAG CUGAUGAG X CGAA ICGGACA	1375	TGTCCGCC T CTGCCCGC	2099
1422	CUGCGGGC CUGAUGAG X CGAA IAGGCGGA	1376	TCCGCCTC T GCCCGCAG	2100
1425	GCUCUGCG CUGAUGAG X CGAA ICAGAGGC	1377	GCCTCTGC C CGCAGAGC	2101
1426	GGCUCUGC CUGAUGAG X CGAA IGCAGAGG	1378	CCTCTGCC C GCAGAGCC	2102
1429	GUGGGCUC CUGAUGAG X CGAA ICGGGCAG	1379	CTGCCCCG A GAGCCCAC	2103
1434	CGGGCGUG CUGAUGAG X CGAA ICUCUGCG	1380	CGCAGAGC C CACGCCCG	2104
1435	UCGGGCGU CUGAUGAG X CGAA IGCUCUGC	1381	GCAGAGCC C ACGCCCGA	2105

Table 4

1436	GUCGGGCG CUGAUGAG X CGAA IGGCUCUG	1382	CAGAGCCC A CGCCCGAC	2106
1440	GCUAGUCG CUGAUGAG X CGAA ICGUGGGC	1383	GCCCACGC C CGACTAGC	2107
1441	UGCUAGUC CUGAUGAG X CGAA ICGUGGGG	1384	CCCACGCC C GACTAGCA	2108
1445	UGCCUGCU CUGAUGAG X CGAA IUCGGGCG	1385	CGCCCGAC T AGCAGGCA	2109
1449	GGCAUGCC CUGAUGAG X CGAA ICUAGUCG	1386	CGACTAGC A GGCATGCC	2110
1453	CCGCGGCA CUGAUGAG X CGAA ICCUGCUA	1387	TAGCAGGC A TGCCGCGG	2111
1457	CCUACCGC CUGAUGAG X CGAA ICAUGCCU	1388	AGGCATGC C GCGGTAGG	2112
1473	GGUCCGGC CUGAUGAG X CGAA ICCCUUAC	1389	GTAAGGGC C GCCGGACC	2113
1476	CGCGGUCC CUGAUGAG X CGAA ICGGCCC	1390	AGGGCCGC C GGACCGCG	2114
1481	CUCUACGC CUGAUGAG X CGAA IUCCGGCG	1391	CGCCGGAC C GCGTAGAG	2115
1493	CGGGGCCC CUGAUGAG X CGAA ICUCUCUA	1392	TAGAGAGC C GGGCCCCG	2116
1498	CCGUCCGG CUGAUGAG X CGAA ICCCGGCU	1393	AGCCGGGC C CCGGACGG	2117
1499	UCCGUCCG CUGAUGAG X CGAA IGCCCGGC	1394	GCCGGGCC C CGGACGGA	2118
1500	GUCCGUCC CUGAUGAG X CGAA IGGCCCGG	1395	CCGGGCCC C GGACGGAC	2119
1517	UUUAGUGC CUGAUGAG X CGAA IAACCAAC	1396	GTTGGTTC T GCACTAAA	2120
1520	GGUUUAG CUGAUGAG X CGAA ICAGAACC	1397	GGTTCTGC A CTAAAACC	2121
1522	UGGUUUU CUGAUGAG X CGAA IUGCAGAA	1398	TTCTGCAC T AAAACCCA	2122
1528	GGAAGAUG CUGAUGAG X CGAA IUUUUAGU	1399	ACTAAAAC C CATCTTCC	2123
1529	GGGAAGAU CUGAUGAG X CGAA IGUUUAG	1400	CTAAAACC C ATCTTCCC	2124
1530	GGGAAGA CUGAUGAG X CGAA IGGUUUA	1401	TAAAACCC A TCTTCCCC	2125
1533	UCCGGGGA CUGAUGAG X CGAA IAUGGGUU	1402	AACCCATC T TCCCCGGA	2126
1536	ACAUCCGG CUGAUGAG X CGAA IAAGAUGG	1403	CCATCTTC C CCGGATGT	2127
1537	CACAUCCG CUGAUGAG X CGAA IGAAGAUG	1404	CATCTTCC C CGGATGTG	2128
1538	ACACAUCC CUGAUGAG X CGAA IGGAAGAU	1405	ATCTTCCC C GGATGTGT	2129
1550	GAGGGGUG CUGAUGAG X CGAA IACACACA	1406	TGTGTGTC T CACCCCTC	2130
1552	AUGAGGGG CUGAUGAG X CGAA IAGACACA	1407	TGTGTCTC A CCCCTCAT	2131
1554	GGAUGAGG CUGAUGAG X CGAA IUGAGACA	1408	TGTCTCAC C CCTCATCC	2132
1555	AGGAUGAG CUGAUGAG X CGAA IGUGAGAC	1409	GTCTCACC C CTCATCCT	2133
1556	AAGGAUGA CUGAUGAG X CGAA IGGUGAGA	1410	TCTCACCC C TCATCCTT	2134
1557	AAAGGAUG CUGAUGAG X CGAA IGGGUGAG	1411	CTCACCCC T CATCCTTT	2135
1559	UAAAAGGA CUGAUGAG X CGAA IAGGGGUG	1412	CACCCCTC A TCCTTTTA	2136
1562	AAGUAAAA CUGAUGAG X CGAA IAUGAGGG	1413	CCCTCATC C TTTTACTT	2137
1563	AAAGUAAA CUGAUGAG X CGAA IGAUGAGG	1414	CCTCATCC T TTTACTTT	2138
1569	GGGCAAAA CUGAUGAG X CGAA IUAAAAGG	1415	CCTTTTAC T TTTTGCCC	2139
1576	GUGGAAGG CUGAUGAG X CGAA ICAAAAAG	1416	CTTTTTCG C CCTTCCAC	2140
1577	AGUGGAAG CUGAUGAG X CGAA IGCAAAAA	1417	TTTTTGCC C CTTCCACT	2141
1578	AAGUGGAA CUGAUGAG X CGAA IGGCAAAA	1418	TTTTGCCC C TTCCACTT	2142
1579	AAAGUGGA CUGAUGAG X CGAA IGGGCAAA	1419	TTTGCCCC T TCCACTTT	2143
1582	CUCAAAGU CUGAUGAG X CGAA IAAGGGGC	1420	GCCCCTTC C ACTTTGAG	2144
1583	ACUCAAAG CUGAUGAG X CGAA IGAAGGGG	1421	CCCCTTCC A CTTTGAGT	2145
1585	GUACUCAA CUGAUGAG X CGAA IUGGAAGG	1422	CCTTCCAC T TTGAGTAC	2146
1594	GUGGAUUU CUGAUGAG X CGAA IUACUCAA	1423	TTGAGTAC C AAATCCAC	2147
1595	UGUGGAUU CUGAUGAG X CGAA IGUACUCA	1424	TGAGTACC A AATCCACA	2148
1600	UGGCUUGU CUGAUGAG X CGAA IAUUUGGU	1425	ACCAAATC C ACAAGCCA	2149
1601	AUGGCUUG CUGAUGAG X CGAA IGAUUUGG	1426	CCAAATCC A CAAGCCAT	2150
1603	AAAUUGCU CUGAUGAG X CGAA IUGGAUUU	1427	AAATCCAC A AGCCATTT	2151
1607	CAAAAAAU CUGAUGAG X CGAA ICUUGUGG	1428	CCACAAGC C ATTTTGTG	2152

Table 4

1608	UCAAAAAA CUGAUGAG X CGAA IGCUGUG	1429	CACAAGCC A TTTTGTGA	2153
1636	GCCAGCAU CUGAUGAG X CGAA IUACUCUC	1430	GAGAGTAC C ATGCTGGC	2154
1637	CGCCAGCA CUGAUGAG X CGAA IGUACUCU	1431	AGAGTACC A TGCTGGCG	2155
1641	GCGCCGCC CUGAUGAG X CGAA ICAUGGUA	1432	TACCATGC T GGCGGCGC	2156
1650	CUUCCCUC CUGAUGAG X CGAA ICGCCGCC	1433	GGCGGCGC A GAGGGAAG	2157
1663	CGGGUGUA CUGAUGAG X CGAA ICCCCUUC	1434	GAAGGGGC C TACACCCG	2158
1664	ACGGGUGU CUGAUGAG X CGAA IGCCCCU	1435	AAGGGGCC T ACACCCGT	2159
1667	AAGACGGG CUGAUGAG X CGAA IUAGGCC	1436	GGGCTAC A CCCGTCTT	2160
1669	CCAAGACG CUGAUGAG X CGAA IUGUAGGC	1437	GCCTACAC C CGTCTTGG	2161
1670	CCAAGAC CUGAUGAG X CGAA IGUGUAGG	1438	CCTACACC C GTCTTGGG	2162
1674	GAGCCCCA CUGAUGAG X CGAA IACGGGUG	1439	CACCCGTC T TGGGGCTC	2163
1681	GUGGGGCG CUGAUGAG X CGAA ICCCAAG	1440	CTTGGGGC T CGCCCCAC	2164
1685	CUGGGUGG CUGAUGAG X CGAA ICGAGCCC	1441	GGGCTCGC C CCACCCAG	2165
1686	CCUGGGUG CUGAUGAG X CGAA ICGAGGCC	1442	GGCTCGCC C CACCCAGG	2166
1687	CCCUGGGU CUGAUGAG X CGAA IGGCGAGC	1443	GCTCGCCC C ACCCAGGG	2167
1688	GCCCUGGG CUGAUGAG X CGAA IGGGCGAG	1444	CTCGCCCC A CCCAGGGC	2168
1690	GAGCCCUG CUGAUGAG X CGAA IUGGGGCG	1445	CGCCCCAC C CAGGGCTC	2169
1691	GGAGCCCU CUGAUGAG X CGAA IGUGGGGC	1446	GCCCCACC C AGGGCTCC	2170
1692	GGGAGCCC CUGAUGAG X CGAA IGGUGGGG	1447	CCCCACCC A GGGCTCCC	2171
1697	CAGGAGGG CUGAUGAG X CGAA ICCUGGG	1448	CCCAGGGC T CCCTCCTG	2172
1699	UCCAGGAG CUGAUGAG X CGAA IAGCCUG	1449	CAGGGCTC C CTCCTGGA	2173
1700	CUCCAGGA CUGAUGAG X CGAA IGAGCCCU	1450	AGGGCTCC C TCCTGGAG	2174
1701	GCUCCAGG CUGAUGAG X CGAA IGGAGCCC	1451	GGGCTCCC T CCTGGAGC	2175
1703	AUGCUGCA CUGAUGAG X CGAA IAGGGAGC	1452	GCTCCCTC C TGGAGCAT	2176
1704	GAUGCUGC CUGAUGAG X CGAA IGAGGGAG	1453	CTCCCTCC T GGAGCATC	2177
1710	GCCUGGGA CUGAUGAG X CGAA ICUCCAGG	1454	CCTGGAGC A TCCCAGGC	2178
1713	CCCGCCUG CUGAUGAG X CGAA IAUGCUGC	1455	GGAGCATC C CAGGCGGG	2179
1714	GCCCGCCU CUGAUGAG X CGAA IGAUGCUC	1456	GAGCATCC C AGGCGGGC	2180
1715	CGCCCGCC CUGAUGAG X CGAA IGGAUGCU	1457	AGCATCCC A GGCGGGCG	2181
1726	GUCUGGCG CUGAUGAG X CGAA ICCGCCCC	1458	CGGGCGGC A CGCCAGAC	2182
1730	GGCUGUCU CUGAUGAG X CGAA ICGUGCCG	1459	CGGCACGC C AGACAGCC	2183
1731	GGGCUGUC CUGAUGAG X CGAA ICGUGGCC	1460	GGCACGCC A GACAGCCC	2184
1735	GGGGGGGC CUGAUGAG X CGAA IUCUGGCG	1461	CGCCAGAC A GCCCCCCC	2185
1738	AAGGGGGG CUGAUGAG X CGAA ICUGUCUG	1462	CAGACAGC C CCCCCCTT	2186
1739	CAAGGGGG CUGAUGAG X CGAA IGCUGUCU	1463	AGACAGCC C CCCCCCTG	2187
1740	UCAAGGGG CUGAUGAG X CGAA IGGCUGUC	1464	GACAGCCC C CCCCTTGA	2188
1741	UUCAAGGG CUGAUGAG X CGAA IGGGCUGU	1465	ACAGCCCC C CCCTTGAA	2189
1742	AUUCAAGG CUGAUGAG X CGAA IGGGGCUG	1466	CAGCCCCC C CCTTGAAT	2190
1743	GAUUCAAG CUGAUGAG X CGAA IGGGGGCU	1467	AGCCCCCC C CTTGAATC	2191
1744	AGAUUCA CUGAUGAG X CGAA IGGGGGGC	1468	GCCCCCCC C TTGAATCT	2192
1745	CAGAUUCA CUGAUGAG X CGAA IGGGGGGG	1469	CCCCCCCC T TGAATCTG	2193
1752	CUCCCUGC CUGAUGAG X CGAA IAUUCAAG	1470	CTTGAATC T GCAGGGAG	2194
1755	UUGCUCUU CUGAUGAG X CGAA ICAGAUUC	1471	GAATCTGC A GGGAGCAA	2195
1762	UGGAGAGU CUGAUGAG X CGAA ICUCCCUG	1472	CAGGGAGC A ACTCTCCA	2196
1765	GAGUGGAG CUGAUGAG X CGAA IUUGCUCU	1473	GGAGCAAC T CTCCACTC	2197
1767	UGGAGUGG CUGAUGAG X CGAA IAGUUGCU	1474	AGCAACTC T CCACTCCA	2198
1769	UAUGGAGU CUGAUGAG X CGAA IAGAGUUG	1475	CAACTCTC C ACTCCATA	2199

Table 4

1770	AUAUGGAG CUGAUGAG X CGAA IGAGAGUU	1476	AACTCTCC A CTCCATAT	2200
1772	AAAU AUGG CUGAUGAG X CGAA IUGGAGAG	1477	CTCTCCAC T CCATATTT	2201
1774	AUAAAUAU CUGAUGAG X CGAA IAGUGGAG	1478	CTCCACTC C ATATTTAT	2202
1775	AAUAAAUA CUGAUGAG X CGAA IGAGUGGA	1479	TCCACTCC A TATTTATT	2203
1789	GAAAAAAU CUGAUGAG X CGAA IUUUAAAU	1480	ATTTAAAC A ATTTTTC	2204
1798	GCCUUUGG CUGAUGAG X CGAA IAAAAAU	1481	ATTTTTC C CCAAAGGC	2205
1799	UGCCUUUG CUGAUGAG X CGAA IGAAAAA	1482	TTTTTTC C CAAAGGCA	2206
1800	AUGCCUUU CUGAUGAG X CGAA IGGAAAA	1483	TTTTTCC C AAAGGCAT	2207
1801	GAUGCCUU CUGAUGAG X CGAA IGGGAAAA	1484	TTTTCCC A AAGGCATC	2208
1807	ACUAUGGA CUGAUGAG X CGAA ICCUUUGG	1485	CCAAAGGC A TCCATAGT	2209
1810	UGCACUAU CUGAUGAG X CGAA IAUGCCUU	1486	AAGGCATC C ATAGTGCA	2210
1811	GUGCACUA CUGAUGAG X CGAA IGAUGCCU	1487	AGGCATCC A TAGTGCAC	2211
1818	AAUGCUAG CUGAUGAG X CGAA ICACUAUG	1488	CATAGTGC A CTAGCATT	2212
1820	AAA AUGCU CUGAUGAG X CGAA IUGCACUA	1489	TAGTGCAC T AGCATTTT	2213
1824	CAAGAAAA CUGAUGAG X CGAA ICUAGUGC	1490	GCACTAGC A TTTTCTTG	2214
1830	UUGGUUCA CUGAUGAG X CGAA IAAA AUGC	1491	GCATTTTC T TGAACCAA	2215
1836	ACAUUAU CUGAUGAG X CGAA IUUCAAGA	1492	TCTTGAAC C AATAATGT	2216
1837	UACAUUAU CUGAUGAG X CGAA IGUUCAAG	1493	CTTGAACC A ATAATGTA	2217
1864	UGCAAGGC CUGAUGAG X CGAA IACAUCAA	1494	TTGATGTC A GCCTTGCA	2218
1867	UGAUGCAA CUGAUGAG X CGAA ICUGACAU	1495	ATGTCAGC C TTGCATCA	2219
1868	UUGAUGCA CUGAUGAG X CGAA IGCUGACA	1496	TGTCAGCC T TGCATCAA	2220
1872	GCCCUUGA CUGAUGAG X CGAA ICAAGGCU	1497	AGCCTTGC A TCAAGGGC	2221
1875	AAAGCCCU CUGAUGAG X CGAA IAUGCAAG	1498	CTTGCATC A AGGGCTTT	2222
1881	UUUGAUAA CUGAUGAG X CGAA ICCCUUGA	1499	TCAAGGGC T TTATCAA	2223
1887	GUACUUUU CUGAUGAG X CGAA IAUAAAGC	1500	GCTTTATC A AAAAGTAC	2224
1896	UUUAUUUAU CUGAUGAG X CGAA IUACUUUU	1501	AAAAGTAC A ATAATAAA	2225
1907	CUACCUGA CUGAUGAG X CGAA IAUUUUAU	1502	AATAAATC C TCAGGTAG	2226
1908	ACUACCUG CUGAUGAG X CGAA IGAUUUAU	1503	ATAAATCC T CAGGTAGT	2227
1910	GUACUACC CUGAUGAG X CGAA IAGGAUUU	1504	AAATCCTC A GG TAGTAC	2228
1919	CCAUUCCC CUGAUGAG X CGAA IUACUACC	1505	GGTAGTAC T GGGAATGG	2229
1933	CAUGGCAA CUGAUGAG X CGAA ICCUCCA	1506	TGGAAGGC T TTGCCATG	2230
1938	AGGCCCAU CUGAUGAG X CGAA ICAAAGCC	1507	GGCTTTGC C ATGGGCCT	2231
1939	CAGGCCCA CUGAUGAG X CGAA IGCAAAGC	1508	GCTTTGCC A TGGGCCTG	2232
1945	ACGCAGCA CUGAUGAG X CGAA ICCCAUGG	1509	CCATGGGC C TGCTGCGT	2233
1946	GACGCAGC CUGAUGAG X CGAA IGCCCAUG	1510	CATGGGCC T GCTGCGTC	2234
1949	UCUGACGC CUGAUGAG X CGAA ICAGGCC	1511	GGGCCTGC T GCGTCAGA	2235
1955	UACUGGUC CUGAUGAG X CGAA IACGCAGC	1512	GCTGCGTC A GACCAGTA	2236
1959	CCAGUACU CUGAUGAG X CGAA IUCUGACG	1513	CGTCAGAC C AGTACTGG	2237
1960	CCCAGUAC CUGAUGAG X CGAA IGUCUGAC	1514	GTCAGACC A GTACTGGG	2238
1965	UCCUCCCC CUGAUGAG X CGAA IUACUGGU	1515	ACCAGTAC T GGGAAGGA	2239
1988	AUAACAAC CUGAUGAG X CGAA ICUUACAA	1516	TTGTAAGC A GTTGTTAT	2240
2032	UAUAGCAU CUGAUGAG X CGAA IUUCUAUC	1517	GATAGAAC A ATGCTATA	2241
2037	UAUAUUUAU CUGAUGAG X CGAA ICAUUGUU	1518	AACAATGC T ATAATATA	2242
2054	UACCCACG CUGAUGAG X CGAA IUUCAUUA	1519	TAATGAAC A CGTGGGTA	2243
2076	UCACAUCA CUGAUGAG X CGAA IUUUCUUA	1520	TAAGAAAC A TGATGTGA	2244
2091	CGGGACAA CUGAUGAG X CGAA IUAAUCUC	1521	GAGATTAC T TTGTCCCG	2245
2097	AAUAAGCG CUGAUGAG X CGAA IACAAAGU	1522	ACTTTGTC C CGCTTATT	2246

Table 4

2098	GAAUAAGC CUGAUGAG X CGAA IGACAAAG	1523	CTTTGTCC C GCTTATTC	2247
2101	GCAGAAUA CUGAUGAG X CGAA ICGGGACA	1524	TGTCCCGC T TATTCTGC	2248
2107	CAGGGAGC CUGAUGAG X CGAA IAAUAAGC	1525	GCTTATTC T GCTCCCTG	2249
2110	UAACAGGG CUGAUGAG X CGAA ICAGAAUA	1526	TATTCTGC T CCCTGTTA	2250
2112	GAUAACAG CUGAUGAG X CGAA IAGCAGAA	1527	TTCTGCTC C CTGTTATC	2251
2113	AGAUACA CUGAUGAG X CGAA IGAGCAGA	1528	TCTGCTCC C TGTTATCT	2252
2114	CAGAUAA CUGAUGAG X CGAA IGGAGCAG	1529	CTGCTCCC T GTTATCTG	2253
2121	GAUCUAGC CUGAUGAG X CGAA IAUAACAG	1530	CTGTTATC T GCTAGATC	2254
2124	CUAGAUCU CUGAUGAG X CGAA ICAGAUAA	1531	TTATCTGC T AGATCTAG	2255
2130	UGAGAACU CUGAUGAG X CGAA IAUCUAGC	1532	GCTAGATC T AGTTCTCA	2256
2136	AGUGAUUG CUGAUGAG X CGAA IAACUAGA	1533	TCTAGTTC T CAATCACT	2257
2138	GCAGUGAU CUGAUGAG X CGAA IAGAACUA	1534	TAGTTCTC A ATCACTGC	2258
2142	GGGAGCAG CUGAUGAG X CGAA IAUUGAGA	1535	TCTCAATC A CTGCTCCC	2259
2144	GGGGGAGC CUGAUGAG X CGAA IUGAUUGA	1536	TCAATCAC T GCTCCCCC	2260
2147	CACGGGGG CUGAUGAG X CGAA ICAGUGAU	1537	ATCACTGC T CCCCCGTG	2261
2149	CACACGGG CUGAUGAG X CGAA IAGCAGUG	1538	CACTGCTC C CCCGTGTG	2262
2150	ACACACGG CUGAUGAG X CGAA IGAGCAGU	1539	ACTGCTCC C CCGTGTGT	2263
2151	UACACACG CUGAUGAG X CGAA IGGAGCAG	1540	CTGCTCCC C CGTGTGTA	2264
2152	AUACACAC CUGAUGAG X CGAA IGGGAGCA	1541	TGCTCCCC C GTGTGTAT	2265
2169	ACCUUACA CUGAUGAG X CGAA ICAUUCUA	1542	TAGAATGC A TGTAAGGT	2266
2179	ACACAAGA CUGAUGAG X CGAA IACCUUAC	1543	GTAAGGTC T TCTTGTGT	2267
2182	AGGACACA CUGAUGAG X CGAA IAAGACCU	1544	AGGTCTTC T TGTGTCCT	2268
2189	UUUCAUCA CUGAUGAG X CGAA IACACAAG	1545	CTTGTGTC C TGATGAAA	2269
2190	UUUUCAUC CUGAUGAG X CGAA IGACACAA	1546	TTGTGTCC T GATGAAA	2270
2207	UCAUUUCA CUGAUGAG X CGAA ICACAUAU	1547	ATATGTGC T TGAAATGA	2271
2221	GAGAUCAA CUGAUGAG X CGAA IUUUCUCA	1548	TGAGAAAC T TTGATCTC	2272
2228	GUAAGCAG CUGAUGAG X CGAA IAUCAAAG	1549	CTTTGATC T CTGCTTAC	2273
2230	UAGUAAGC CUGAUGAG X CGAA IAGAUCAA	1550	TTGATCTC T GCTTACTA	2274
2233	CAUUAGUA CUGAUGAG X CGAA ICAGAGAU	1551	ATCTCTGC T TACTAATG	2275
2237	GGCACAUC CUGAUGAG X CGAA IUAAGCAG	1552	CTGCTTAC T AATGTGCC	2276
2245	GGACAUGG CUGAUGAG X CGAA ICACAUUA	1553	TAATGTGC C CCATGTCC	2277
2246	UGGACAUG CUGAUGAG X CGAA IGCACAUC	1554	AATGTGCC C CATGTCCA	2278
2247	UUGGACAU CUGAUGAG X CGAA IGGCACAU	1555	ATGTGCC C ATGTCCAA	2279
2248	CUUGGACA CUGAUGAG X CGAA IGGGCACA	1556	TGTGCCCC A TGTCCAAG	2280
2253	UUGGACUU CUGAUGAG X CGAA IACAUGGG	1557	CCCATGTC C AAGTCCAA	2281
2254	GUUGGACU CUGAUGAG X CGAA IGACAUGG	1558	CCATGTCC A AGTCCAAC	2282
2259	GGCAGGUU CUGAUGAG X CGAA IACUUGGA	1559	TCCAAGTC C AACCTGCC	2283
2260	AGGCAGGU CUGAUGAG X CGAA IGACUUGG	1560	CCAAGTCC A ACCTGCCT	2284
2263	CACAGGCA CUGAUGAG X CGAA IUUGGACU	1561	AGTCCAAC C TGCCTGTG	2285
2264	GCACAGGC CUGAUGAG X CGAA IGUUGGAC	1562	GTCCAACC T GCCTGTGC	2286
2267	CAUGCACA CUGAUGAG X CGAA ICAGGUUG	1563	CAACCTGC C TGTGCATG	2287
2268	UCAUGCAC CUGAUGAG X CGAA IGCAGGUU	1564	AACCTGCC T GTGCATGA	2288
2273	UCAGGUCA CUGAUGAG X CGAA ICACAGGC	1565	GCCTGTGC A TGACCTGA	2289
2278	AAUGAUCA CUGAUGAG X CGAA IUCAUGCA	1566	TGCATGAC C TGATCATT	2290
2279	UAAUGAUC CUGAUGAG X CGAA IGUCAUGC	1567	GCATGACC T GATCATT	2291
2284	CCAUGUAA CUGAUGAG X CGAA IAUCAGGU	1568	ACCTGATC A TTACATGG	2292
2289	CACAGCCA CUGAUGAG X CGAA IUAAUGAU	1569	ATCATTAC A TGGCTGTG	2293

Table 4

2294	GGAACCAC CUGAUGAG X CGAA ICCAUGUA	1570	TACATGGC T GTGGTTCC	2294
2302	CAGGCUUA CUGAUGAG X CGAA IAACCACA	1571	TGTGGTTC C TAAGCCTG	2295
2303	ACAGGCUU CUGAUGAG X CGAA IGAACCAC	1572	GTGGTTCC T AAGCCTGT	2296
2308	CAGCAACA CUGAUGAG X CGAA ICUUAGGA	1573	TCCTAAGC C TGTTGCTG	2297
2309	UCAGCAAC CUGAUGAG X CGAA IGCUUAGG	1574	CCTAAGCC T GTTGCTGA	2298
2315	AUGACUUC CUGAUGAG X CGAA ICAACAGG	1575	CCTGTTGC T GAAGTCAT	2299
2322	AGCGACAA CUGAUGAG X CGAA IACUUCAG	1576	CTGAAGTC A TTGTCGCT	2300
2330	UAUUGCUG CUGAUGAG X CGAA ICGACAAU	1577	ATTGTCGC T CAGCAATA	2301
2332	CCUAUUGC CUGAUGAG X CGAA IAGCGACA	1578	TGTCGCTC A GCAATAGG	2302
2335	CACCCUUA CUGAUGAG X CGAA ICUGAGCG	1579	CGCTCAGC A ATAGGGTG	2303
2345	UGGAAAAC CUGAUGAG X CGAA ICACCCUA	1580	TAGGGTGC A GTTTTCCA	2304
2352	CUAUUCCU CUGAUGAG X CGAA IAAAACUG	1581	CAGTTTTC C AGGAATAG	2305
2353	CCUAUUCC CUGAUGAG X CGAA IGAAAACU	1582	AGTTTTC C A GGAATAGG	2306
2363	UAGGCAAA CUGAUGAG X CGAA ICCUAUUC	1583	GAATAGGC A TTTGCCTA	2307
2369	AGGAAUUA CUGAUGAG X CGAA ICAAAUGC	1584	GCATTTGC C TAATTCCT	2308
2370	CAGGAAUU CUGAUGAG X CGAA IGCAAUG	1585	CATTTGCC T AATTCCTG	2309
2376	UCAUGCCA CUGAUGAG X CGAA IAAUUAGG	1586	CCTAATTC C TGGCATGA	2310
2377	GUCAUGCC CUGAUGAG X CGAA IGAAUUAG	1587	CTAATTC T GGCATGAC	2311
2381	GAGUGUCA CUGAUGAG X CGAA ICCAGGAA	1588	TTCCTGGC A TGACACTC	2312
2386	CACUAGAG CUGAUGAG X CGAA IUCAUGCC	1589	GGCATGAC A CTCTAGTG	2313
2388	GUCACUAG CUGAUGAG X CGAA IUGUCAUG	1590	CATGACAC T CTAGTGAC	2314
2390	AAGUCACU CUGAUGAG X CGAA IAGUGUCA	1591	TGACACTC T AGTGACTT	2315
2397	CACCAGGA CUGAUGAG X CGAA IUCACUAG	1592	CTAGTGAC T TCCTGGTG	2316
2400	CCUCACCA CUGAUGAG X CGAA IAAGUCAC	1593	GTGACTTC C TGGTGAGG	2317
2401	GCCUCACC CUGAUGAG X CGAA IGAAGUCA	1594	TGACTTCC T GGTGAGGC	2318
2410	ACAGGCUG CUGAUGAG X CGAA ICCUCACC	1595	GGTGAGGC C CAGCCTGT	2319
2411	GACAGGCU CUGAUGAG X CGAA IGCCUCAC	1596	GTGAGGCC C AGCCTGTC	2320
2412	GGACAGGC CUGAUGAG X CGAA IGGCCUCA	1597	TGAGGCCC A GCCTGTCC	2321
2415	CCAGGACA CUGAUGAG X CGAA ICUGGGCC	1598	GGCCCAGC C TGTCCTGG	2322
2416	ACCAGGAC CUGAUGAG X CGAA IGCUGGGC	1599	GCCCAGCC T GTCCTGGT	2323
2420	CUGUACCA CUGAUGAG X CGAA IACAGGCU	1600	AGCCTGTC C TGGTACAG	2324
2421	GCUGUACC CUGAUGAG X CGAA IGACAGGC	1601	GCCTGTCC T GGTACAGC	2325
2427	GACCCUGC CUGAUGAG X CGAA IUACCAGG	1602	CCTGGTAC A GCAGGGTC	2326
2430	CAAGACCC CUGAUGAG X CGAA ICUGUACC	1603	GGTACAGC A GGGTCTTG	2327
2436	UUACAGCA CUGAUGAG X CGAA IACCCUGC	1604	GCAGGGTC T TGCTGTAA	2328
2440	UGAGUUAC CUGAUGAG X CGAA ICAAGACC	1605	GGTCTTGC T GTAACCTA	2329
2446	AAUGUCUG CUGAUGAG X CGAA IUUACAGC	1606	GCTGTAAC T CAGACATT	2330
2448	GGAAUGUC CUGAUGAG X CGAA IAGUUACA	1607	TGTAACCTC A GACATTCC	2331
2452	CCUUGGAA CUGAUGAG X CGAA IUCUGAGU	1608	ACTCAGAC A TTCCAAGG	2332
2456	AUACCCUU CUGAUGAG X CGAA IAAUGUCU	1609	AGACATTC C AAGGGTAT	2333
2457	CAUACCCU CUGAUGAG X CGAA IGAAUGUC	1610	GACATTCC A AGGGTATG	2334
2472	GUGAAUAU CUGAUGAG X CGAA ICUUCCCA	1611	TGGGAAGC C ATATTCAC	2335
2473	UGUGAAUA CUGAUGAG X CGAA IGCUUCCC	1612	GGGAAGCC A TATTCACA	2336
2479	GUGAGGUG CUGAUGAG X CGAA IAAUAUGG	1613	CCATATTC A CACCTCAC	2337
2481	GCGUGAGG CUGAUGAG X CGAA IUGAAUAU	1614	ATATTCAC A CCTCACGC	2338
2483	GAGCGUGA CUGAUGAG X CGAA IUGUGAAU	1615	ATTCACAC C TCACGCTC	2339
2484	AGAGCGUG CUGAUGAG X CGAA IGUGUGAA	1616	TTCACACC T CACGCTCT	2340

Table 4

2486	CCAGAGCG CUGAUGAG X CGAA IAGGUGUG	1617	CACACCTC A CGCTCTGG	2341
2490	AUGUCCAG CUGAUGAG X CGAA ICGUGAGG	1618	CCTCACGC T CTGGACAT	2342
2492	UCAUGUCC CUGAUGAG X CGAA IAGCGUGA	1619	TCACGCTC T GGACATGA	2343
2497	CUAAAUCA CUGAUGAG X CGAA IUCCAGAG	1620	CTCTGGAC A TGATTTAG	2344
2512	GGUGUCCC CUGAUGAG X CGAA ICUUCCCU	1621	AGGGAAGC A GGGACACC	2345
2518	GCGGGGGG CUGAUGAG X CGAA IUCCCUGC	1622	GCAGGGAC A CCCCCCGC	2346
2520	GGGCGGGG CUGAUGAG X CGAA IUGUCCCU	1623	AGGGACAC C CCCC GCCC	2347
2521	GGGGCGGG CUGAUGAG X CGAA IGUGUCCC	1624	GGGACACC C CCGCCCCC	2348
2522	GGGGGCGG CUGAUGAG X CGAA IGGUGUCC	1625	GGACACCC C CCGCCCCC	2349
2523	GGGGGGCG CUGAUGAG X CGAA IGGGUGUC	1626	GACACCCC C CGCCCCCC	2350
2524	UGGGGGGC CUGAUGAG X CGAA IGGGGUGU	1627	ACACCCCC C GCCCCCA	2351
2527	AGGUGGGG CUGAUGAG X CGAA ICGGGGGG	1628	CCCCCGC C CCCCACCT	2352
2528	AAGGUGGG CUGAUGAG X CGAA ICGGGGGG	1629	CCCCCGCC C CCCACCTT	2353
2529	AAAGGUGG CUGAUGAG X CGAA IGGCGGGG	1630	CCCCGCC C CCACCTTT	2354
2530	CAAAGGUG CUGAUGAG X CGAA IGGGCGGG	1631	CCCGCCCC C CACCTTTG	2355
2531	CCAAAGGU CUGAUGAG X CGAA IGGGGCGG	1632	CCGCCCCC C ACCTTTGG	2356
2532	CCCAAAGG CUGAUGAG X CGAA IGGGGGCG	1633	CGCCCCCC A CCTTTGGG	2357
2534	AUCCCAA CUGAUGAG X CGAA IUGGGGGG	1634	CCCCCAC C TTTGGGAT	2358
2535	GAUCCCAA CUGAUGAG X CGAA IGUGGGGG	1635	CCCCACC T TTGGGATC	2359
2544	GCGGAGGC CUGAUGAG X CGAA IAUCCCAA	1636	TTGGGATC A GCCTCCGC	2360
2547	AUGGCGGA CUGAUGAG X CGAA ICUGAUCC	1637	GGATCAGC C TCCGCCAT	2361
2548	AAUGGCGG CUGAUGAG X CGAA IGCUGAUC	1638	GATCAGCC T CCGCCATT	2362
2550	GGAAUGGC CUGAUGAG X CGAA IAGGCUGA	1639	TCAGCCTC C GCCATTCC	2363
2553	CUUGGAAU CUGAUGAG X CGAA ICGGAGGC	1640	GCCTCCGC C ATTCCAAG	2364
2554	ACUUGGAA CUGAUGAG X CGAA ICGGAGGC	1641	CCTCCGCC A TTCCAAGT	2365
2558	GUCGACUU CUGAUGAG X CGAA IAAUGGCG	1642	CGCCATTC C AAGTCGAC	2366
2559	UGUCGACU CUGAUGAG X CGAA IGAAUGGC	1643	GCCATTCC A AGTCGACA	2367
2567	AAGAAGAG CUGAUGAG X CGAA IUCCGACUU	1644	AAGTCGAC A CTCTTCTT	2368
2569	UCAAGAAG CUGAUGAG X CGAA IUGUCGAC	1645	GTCGACAC T CTTCTTGA	2369
2571	GCUCAAGA CUGAUGAG X CGAA IAGUGUCG	1646	CGACACTC T TCTTGAGC	2370
2574	UCUGCUCA CUGAUGAG X CGAA IAAGAGUG	1647	CACTCTTC T TGAGCAGA	2371
2580	UCACGGUC CUGAUGAG X CGAA ICUCAAGA	1648	TCTTGAGC A GACCGTGA	2372
2584	CAAUCCAC CUGAUGAG X CGAA IUCUGCUC	1649	GAGCAGAC C GTGATTTG	2373
2603	CCAGCAGG CUGAUGAG X CGAA ICCUCUCU	1650	AGAGAGGC A CCTGCTGG	2374
2605	UJCCAGCA CUGAUGAG X CGAA IUGCCUCU	1651	AGAGGCAC C TGCTGGAA	2375
2606	UUJCCAGC CUGAUGAG X CGAA IGUGCCUC	1652	GAGGCACC T GCTGGAAA	2376
2609	UGGUUJCC CUGAUGAG X CGAA ICAGGUGC	1653	GCACCTGC T GGAAACCA	2377
2616	AGAAGUGU CUGAUGAG X CGAA IUUJCCAG	1654	CTGGAAAC C ACACTTCT	2378
2617	AAGAAGUG CUGAUGAG X CGAA IGUUJCCA	1655	TGGAAACC A CACTTCTT	2379
2619	UCAAGAAG CUGAUGAG X CGAA IUGGUJUC	1656	GAAACCAC A CTTCTTGA	2380
2621	UUUCAAGA CUGAUGAG X CGAA IUGUGGUU	1657	AACCACAC T TCTTGAAA	2381
2624	CUGUUUCA CUGAUGAG X CGAA IAAGUGUG	1658	CACACTTC T TGAAACAG	2382
2631	ACCCAGGC CUGAUGAG X CGAA IUUJCAAG	1659	CTTGAAAC A GCCTGGGT	2383
2634	GUCACCCA CUGAUGAG X CGAA ICUGUUUC	1660	GAAACAGC C TGGGTGAC	2384
2635	CGUCACCC CUGAUGAG X CGAA IGCUGUUU	1661	AAACAGCC T GGGTGACG	2385
2647	UGCCUAAA CUGAUGAG X CGAA IACCGUCA	1662	TGACGGTC C TTTAGGCA	2386
2648	CUGCCUAA CUGAUGAG X CGAA IGACCGUC	1663	GACGGTCC T TTAGGCAG	2387

Table 4

2655	CGGCAGGC CUGAUGAG X CGAA ICCUAAAG	1664	CTTTAGGC A GCCTGCCG	2388
2658	CGGCGGCA CUGAUGAG X CGAA ICUGCCUA	1665	TAGGCAGC C TGCCGCCG	2389
2659	ACGGCGGC CUGAUGAG X CGAA IGCUGCCU	1666	AGGCAGCC T GCCGCCGT	2390
2662	GAGACGGC CUGAUGAG X CGAA ICAGGCUG	1667	CAGCCTGC C GCCGTCTC	2391
2665	ACAGAGAC CUGAUGAG X CGAA ICGGCAGG	1668	CCTGCCGC C GTCTCTGT	2392
2669	CGGGACAG CUGAUGAG X CGAA IACGGCGG	1669	CCGCCGTC T CTGTCCCG	2393
2671	ACCGGGAC CUGAUGAG X CGAA IAGACGGC	1670	GCCGTCTC T GTCCCGGT	2394
2675	GUGAACCG CUGAUGAG X CGAA IACAGAGA	1671	TCTCTGTC C CGGTTACAC	2395
2676	GGUGAACC CUGAUGAG X CGAA IGACAGAG	1672	CTCTGTCC C GGTTCACC	2396
2682	CGGCAAGG CUGAUGAG X CGAA IAACCGGG	1673	CCCGGTTC A CCTTGCCG	2397
2684	CUCGGCAA CUGAUGAG X CGAA IUGAACCG	1674	CGGTTACAC C TTGCCGAG	2398
2685	UCUCGGCA CUGAUGAG X CGAA IGUGAACCG	1675	GGTTCACC T TGCCGAGA	2399
2689	CCUCUCUC CUGAUGAG X CGAA ICAAGGUG	1676	CACCTTGC C GAGAGAGG	2400
2704	GGUGGGGC CUGAUGAG X CGAA IACGCGCC	1677	GGCGCGTC T GCCCCACC	2401
2707	GAGGGUGG CUGAUGAG X CGAA ICAGACGC	1678	GCGTCTGC C CCACCCTC	2402
2708	UGAGGGUG CUGAUGAG X CGAA IGCAGACG	1679	CGTCTGCC C CACCCTCA	2403
2709	UUGAGGGU CUGAUGAG X CGAA IGGCAGAC	1680	GTCTGCCC C ACCCTCAA	2404
2710	UUUGAGGG CUGAUGAG X CGAA IGGGCAGA	1681	TCTGCCCC A CCCTCAAA	2405
2712	GGUUUGAG CUGAUGAG X CGAA IUGGGGCA	1682	TGCCCCAC C CTCAAACC	2406
2713	GGGUUUGA CUGAUGAG X CGAA IGUGGGGC	1683	GCCCCACC C TCAAACCC	2407
2714	AGGGUUUG CUGAUGAG X CGAA IGGUGGGG	1684	CCCCACCC T CAAACCCT	2408
2716	ACAGGGUU CUGAUGAG X CGAA IAGGGUGG	1685	CCACCCTC A AACCCTGT	2409
2720	CCCCACAG CUGAUGAG X CGAA IUUUGAGG	1686	CCTCAAAC C CTGTGGGG	2410
2721	GCCCCACA CUGAUGAG X CGAA IGUUUGAG	1687	CTCAAACC C TGTGGGGC	2411
2722	GGCCCCAC CUGAUGAG X CGAA IGGUUUGA	1688	TCAAACCC T GTGGGGCC	2412
2730	CACCAUCA CUGAUGAG X CGAA ICCCCACA	1689	TGTGGGGC C TGATGGTG	2413
2731	GCACCAUC CUGAUGAG X CGAA IGCCCCAC	1690	GTGGGGCC T GATGGTGC	2414
2740	GAGUCGUG CUGAUGAG X CGAA ICACCAUC	1691	GATGGTGC T CACGACTC	2415
2742	AAGAGUCG CUGAUGAG X CGAA IAGCACCA	1692	TGGTGCTC A CGACTCTT	2416
2747	GCAGGAAG CUGAUGAG X CGAA IUCGUGAG	1693	CTCACGAC T CTTCTGTC	2417
2749	UUGCAGGA CUGAUGAG X CGAA IAGUCGUG	1694	CACGACTC T TCCTGCAA	2418
2752	CCUUUGCA CUGAUGAG X CGAA IAAGAGUC	1695	GACTCTTC C TGCAAAGG	2419
2753	CCCUUUGC CUGAUGAG X CGAA IGAAGAGU	1696	ACTCTTCC T GCAAAGGG	2420
2756	GUUCCCUU CUGAUGAG X CGAA ICAGGAAG	1697	CTTCCTGC A AAGGGAAC	2421
2765	AGGUCUUC CUGAUGAG X CGAA IUUCCCUU	1698	AAGGGAAC T GAAGACCT	2422
2772	AAUGUGGA CUGAUGAG X CGAA IUCUUCAG	1699	CTGAAGAC C TCCACATT	2423
2773	UAAUGUGG CUGAUGAG X CGAA IGUCUUCA	1700	TGAAGACC T CCACATTA	2424
2775	CUUAAUGU CUGAUGAG X CGAA IAGGUCUU	1701	AAGACCTC C ACATTAAG	2425
2776	ACUUA AUG CUGAUGAG X CGAA IGAGGUCU	1702	AGACCTCC A CATTAAGT	2426
2778	CCACUUA CUGAUGAG X CGAA IUGGAGGU	1703	ACCTCCAC A TTAAGTGG	2427
2788	UGUUAAAA CUGAUGAG X CGAA ICCACUUA	1704	TAAGTGGC T TTTTAACA	2428
2796	GUUUUUA CUGAUGAG X CGAA IUUAAAAA	1705	TTTTTAAC A TGAAAAAC	2429
2805	AGCUGCCG CUGAUGAG X CGAA IUUUUUA	1706	TGAAAAAC A CGGCAGCT	2430
2810	GCUACAGC CUGAUGAG X CGAA ICCGUGUU	1707	AACACGGC A GCTGTAGC	2431
2813	GGAGCUAC CUGAUGAG X CGAA ICUGCCGU	1708	ACGGCAGC T GTAGCTCC	2432
2819	AGCUCGGG CUGAUGAG X CGAA ICUACAGC	1709	GCTGTAGC T CCCGAGCT	2433
2821	GUAGCUCG CUGAUGAG X CGAA IAGCUACA	1710	TGTAGCTC C CGAGCTAC	2434

Table 4

2822	AGUAGCUC CUGAUGAG X CGAA IGAGCUAC	1711	GTAGCTCC C GAGCTACT	2435
2827	AAGAGAGU CUGAUGAG X CGAA ICUCGGGA	1712	TCCCGAGC T ACTCTCTT	2436
2830	GGCAAGAG CUGAUGAG X CGAA IUAGCUCG	1713	CGAGCTAC T CTCTTGCC	2437
2832	CUGGCAAG CUGAUGAG X CGAA IAGUAGCU	1714	AGCTACTC T CTTGCCAG	2438
2834	UGCUGGCA CUGAUGAG X CGAA IAGAGUAG	1715	CTACTCTC T TGCCAGCA	2439
2838	AAAAUGCU CUGAUGAG X CGAA ICAAGAGA	1716	TCTCTTGC C AGCATTTT	2440
2839	GAAAAUGC CUGAUGAG X CGAA IGCAAGAG	1717	CTCTTGCC A GCATTTTC	2441
2842	UGUGAAAA CUGAUGAG X CGAA ICUGGCAA	1718	TTGCCAGC A TTTTCACA	2442
2848	GCAAAAUG CUGAUGAG X CGAA IAAAAUGC	1719	GCATTTTC A CATTTTGC	2443
2850	AGGCAAAA CUGAUGAG X CGAA IUGAAAAU	1720	ATTTTCAC A TTTTGCCT	2444
2857	ACGAGAAA CUGAUGAG X CGAA ICAAAAUG	1721	CATTTTGC C TTTCTCGT	2445
2858	CACGAGAA CUGAUGAG X CGAA IGCAAAAU	1722	ATTTTGCC T TTCTCGTG	2446
2862	CUACCACG CUGAUGAG X CGAA IAAAGGCA	1723	TGCCTTTC T CGTGGTAG	2447
2875	UCUGUACU CUGAUGAG X CGAA ICUUCUAC	1724	GTAGAAGC C AGTACAGA	2448
2876	CUCUGUAC CUGAUGAG X CGAA IGCUUCUA	1725	TAGAAGCC A GTACAGAG	2449
2881	AAUUUCUC CUGAUGAG X CGAA IUACUGGC	1726	GCCAGTAC A GAGAAATT	2450
2891	CCCACCAC CUGAUGAG X CGAA IAAUUUCU	1727	AGAAATTC T GTGGTGGG	2451
2903	ACCUCGAA CUGAUGAG X CGAA IUUCCAC	1728	GTGGGAAC A TTCGAGGT	2452
2915	CUGCAGGG CUGAUGAG X CGAA IACACCUC	1729	GAGGTGTC A CCCTGCAG	2453
2917	CUCUGCAG CUGAUGAG X CGAA IUGACACC	1730	GGTGTAC C CTGCAGAG	2454
2918	GCUCUGCA CUGAUGAG X CGAA IGUGACAC	1731	GTGTCACC C TGCAGAGC	2455
2919	AGCUCUGC CUGAUGAG X CGAA IGGUGACA	1732	TGTCACCC T GCAGAGCT	2456
2922	CAUAGCUC CUGAUGAG X CGAA ICAGGGUG	1733	CACCCTGC A GAGCTATG	2457
2927	CUCACCAU CUGAUGAG X CGAA ICUCUGCA	1734	TGCAGAGC T ATGGTGAG	2458
2949	GGCACCUA CUGAUGAG X CGAA ICCUUAUC	1735	GATAAGGC T TAGGTGCC	2459
2957	UACAGCCU CUGAUGAG X CGAA ICACCUA	1736	TTAGGTGC C AGGCTGTA	2460
2958	UUACAGCC CUGAUGAG X CGAA IGCACCUA	1737	TAGGTGCC A GGCTGTAA	2461
2962	AUGCUUAC CUGAUGAG X CGAA ICCUGGCA	1738	TGCCAGGC T GTAAGCAT	2462
2969	GCUCAGAA CUGAUGAG X CGAA ICUUACAG	1739	CTGTAAGC A TTCTGAGC	2463
2973	GCCAGCUC CUGAUGAG X CGAA IAAUGCUU	1740	AAGCATTC T GAGCTGGC	2464
2978	AACAAGCC CUGAUGAG X CGAA ICUCAGAA	1741	TTCTGAGC T GGCTTGTT	2465
2982	AAACAACA CUGAUGAG X CGAA ICCAGCUC	1742	GAGCTGGC T TGTTGTTT	2466
2998	CAUAUACA CUGAUGAG X CGAA IACUUA	1743	TTTAAGTC C TGTATATG	2467
2999	ACAUUAC CUGAUGAG X CGAA IGACUUA	1744	TTAAGTCC T GTATATGT	2468
3040	UUUUGAAA CUGAUGAG X CGAA ICUACU	1745	ATAGTAGC A TTTCAAAA	2469
3045	GUCCAUUU CUGAUGAG X CGAA IAAAUGCU	1746	AGCATTC A AAATGGAC	2470
3058	GUUAAACC CUGAUGAG X CGAA IUACGUCC	1747	GGACGTAC T GGTTTAAC	2471
3067	GGAUAGGA CUGAUGAG X CGAA IUUAAACC	1748	GGTTTAAC C TCCTATCC	2472
3068	AGGAUAGG CUGAUGAG X CGAA IGUUAAC	1749	GTTTAACC T CCTATCCT	2473
3070	CAAGGAUA CUGAUGAG X CGAA IAGGUUA	1750	TTAACCTC C TATCCTTG	2474
3071	CCAAGGAU CUGAUGAG X CGAA IGAGGUUA	1751	TAACCTCC T ATCCTTGG	2475
3075	CUCUCCAA CUGAUGAG X CGAA IAUAGGAG	1752	CTCCTATC C TTGGAGAG	2476
3076	GCUCUCCA CUGAUGAG X CGAA IGAUAGGA	1753	TCCTATCC T TGGAGAGC	2477
3085	GAGCCAGC CUGAUGAG X CGAA ICUCUCCA	1754	TGGAGAGC A GCTGGCTC	2478
3088	GGAGAGCC CUGAUGAG X CGAA ICUGCUCU	1755	AGAGCAGC T GGCTCTCC	2479
3092	AGGUGGAG CUGAUGAG X CGAA ICCAGCUG	1756	CAGCTGGC T CTCCACCT	2480
3094	CAAGGUGG CUGAUGAG X CGAA IAGCCAGC	1757	GCTGGCTC T CCACCTTG	2481

Table 4

3096	AACAAGGU CUGAUGAG X CGAA IAGAGCCA	1758	TGGCTCTC C ACCTTGTT	2482
3097	UAACAAGG CUGAUGAG X CGAA IGAGAGCC	1759	GGCTCTCC A CCTTGTTA	2483
3099	UGUAACAA CUGAUGAG X CGAA IUGGAGAG	1760	CTCTCCAC C TTGTTACA	2484
3100	GUGUAACA CUGAUGAG X CGAA IGUGGAGA	1761	TCTCCACC T TGTTACAC	2485
3107	ACAUAAUG CUGAUGAG X CGAA IUAACAAG	1762	CTTGTTAC A CATTATGT	2486
3109	UAACAUAU CUGAUGAG X CGAA IUGUAACA	1763	TGTTACAC A TTATGTTA	2487
3132	AGCAGAGC CUGAUGAG X CGAA ICUCGCUA	1764	TAGCGAGC T GCTCTGCT	2488
3135	CAUAGCAG CUGAUGAG X CGAA ICAGCUCG	1765	CGAGCTGC T CTGCTATG	2489
3137	GACAUAGC CUGAUGAG X CGAA IAGCAGCU	1766	AGCTGCTC T GCTATGTC	2490
3140	AAGGACAU CUGAUGAG X CGAA ICAGAGCA	1767	TGCTCTGC T ATGTCCTT	2491
3146	UGGCUUAA CUGAUGAG X CGAA IACAUAGC	1768	GCTATGTC C TTAAGCCA	2492
3147	UUGGCUUA CUGAUGAG X CGAA IGACAUAG	1769	CTATGTCC T TAAGCCAA	2493
3153	UAAAUUUU CUGAUGAG X CGAA ICUUAAGG	1770	CCTTAAGC C AATATTTA	2494
3154	GUAAAUUU CUGAUGAG X CGAA IGCUUAAG	1771	CTTAAGCC A ATATTTAC	2495
3163	ACCUGAUG CUGAUGAG X CGAA IUAAAUUU	1772	ATATTTAC T CATCAGGT	2496
3165	UGACCUGA CUGAUGAG X CGAA IAGUAAAU	1773	ATTTACTC A TCAGGTCA	2497
3168	UAAUGACC CUGAUGAG X CGAA IAUGAGUA	1774	TACTCATC A GGTCATTA	2498
3173	AAAAAUAA CUGAUGAG X CGAA IACCUGAU	1775	ATCAGGTC A TTATTTT	2499
3185	AUGGCCAU CUGAUGAG X CGAA IUAAAAAA	1776	TTTTTTAC A ATGGCCAT	2500
3191	UAUUCCAU CUGAUGAG X CGAA ICCAUUGU	1777	ACAATGGC C ATGGAATA	2501
3192	UUUUCCAU CUGAUGAG X CGAA IGCCAUUG	1778	CAATGGCC A TGGAATAA	2502
3203	GUAAAAAU CUGAUGAG X CGAA IUUUUUUC	1779	GAATAAAC C ATTTTAC	2503
3204	UGUAAAAA CUGAUGAG X CGAA IGUUUUUU	1780	AATAAACC A TTTTACA	2504

Table 5

Table 5: Human PTP-1B G-Cleaver Ribozyme and Target Sequence

Nt. position	Ribozyme Sequence		Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
25	CACUG	UGAUG GCAUGCACUAUGC GCG GCCGUCUGCC	2505	GGCAGACGGC G CAGTG	2670
35	CUUCU	UGAUG GCAUGCACUAUGC GCG GGCCACACUGC	2506	GCAGTGGGC G AGAAG	2671
46	UGCUG	UGAUG GCAUGCACUAUGC GCG GCCUCCUUCU	2507	AGAAGGAGGC G CAGCA	2672
55	CAGGG	UGAUG GCAUGCACUAUGC GCG GGCUGCUGCG	2508	CGCAGCAGCC G CCCTG	2673
89	CUGCU	UGAUG GCAUGCACUAUGC GCG GAACUCCUUU	2509	AAAGGAGTTC G AGCAG	2674
98	CUUGU	UGAUG GCAUGCACUAUGC GCG GAUCUGCUCG	2510	CGAGCAGATC G ACAAG	2675
138	CAUGU	UGAUG GCAUGCACUAUGC GCG GGAUAUCCUG	2511	CAGGATATCC G ACATG	2676
143	GGCUU	UGAUG GCAUGCACUAUGC GCG AUGUCGGAUA	2512	TATCCGACAT G AAGCC	2677
152	GAAGU	UGAUG GCAUGCACUAUGC GCG ACUGGCUUCA	2513	TGAAGCCAGT G ACTTC	2678
195	UAUUU	UGAUG GCAUGCACUAUGC GCG GGUUUUUGUU	2514	AACAAAAACC G AAATA	2679
224	AUGGU	UGAUG GCAUGCACUAUGC GCG AAAGGGACUG	2515	CAGTCCCTTT G ACCAT	2680
260	AUAGU	UGAUG GCAUGCACUAUGC GCG AUUAUCUUCU	2516	AGAAGATAAT G ACTAT	2681
272	ACUAG	UGAUG GCAUGCACUAUGC GCG GUUGAUUAG	2517	CTATATCAAC G CTAGT	2682
280	UUUAU	UGAUG GCAUGCACUAUGC GCG AAACUAGCGU	2518	ACGCTAGTTT G ATAAA	2683
331	UUAGG	UGAUG GCAUGCACUAUGC GCG AAAGGGCCCU	2519	AGGGCCCTTT G CCTAA	2684
342	GACCG	UGAUG GCAUGCACUAUGC GCG AUGUGUUAGG	2520	CCTAACACAT G CGGTC	2685
394	UUGAG	UGAUG GCAUGCACUAUGC GCG AUGACGACAC	2521	GTGTCGTCAT G CTCAA	2686
406	UCCAU	UGAUG GCAUGCACUAUGC GCG ACUCUGUUGA	2522	TCAACAGAGT G ATGGA	2687
429	GUGCG	UGAUG GCAUGCACUAUGC GCG AUUUUAAACGA	2523	TCGTTAAAAAT G CGCAC	2688
431	UUGUG	UGAUG GCAUGCACUAUGC GCG GCAUUUUAAC	2524	GTTAAAAATGC G CACAA	2689
466	AAGAU	UGAUG GCAUGCACUAUGC GCG AUCUCUUUUU	2525	AAAAAGAGAT G ATCTT	2690
473	GUCUU	UGAUG GCAUGCACUAUGC GCG AAAGAUCAUC	2526	GATGATCTTT G AAGAC	2691
487	AAUUU	UGAUG GCAUGCACUAUGC GCG AAUUUUGUGU	2527	ACACAAATTT G AAATT	2692
499	GAGAU	UGAUG GCAUGCACUAUGC GCG AAUGUUAAUU	2528	AATTAAACATT G ATCTC	2693
506	AUCUU	UGAUG GCAUGCACUAUGC GCG AGAGAUCAAU	2529	ATTGATCTCT G AAGAT	2694
532	UGUCG	UGAUG GCAUGCACUAUGC GCG ACUGUAUAAU	2530	ATTATACAGT G CGACA	2695
534	GCUGU	UGAUG GCAUGCACUAUGC GCG GCACUGUAUA	2531	TATACAGTGC G ACAGC	2696
573	UCUCU	UGAUG GCAUGCACUAUGC GCG GAGUUUCUUG	2532	CAAGAAACTC G AGAGA	2697

Table 5

608	AAAGU UGAUG GCAUGCACUAUGC GCG AGGCCAUGUG	2533	CACATGGCCT G ACTTT	2698
623	UGAUU UGAUG GCAUGCACUAUGC GCG AGGACUCCA	2534	TGGAGTCCCT G AATCA	2699
643	AAGUU UGAUG GCAUGCACUAUGC GCG AAGAAUGAGG	2535	CCTCATTTCTT G AACTT	2700
663	ACUCU UGAUG GCAUGCACUAUGC GCG GGACUUUGAA	2536	TTCAAAGTCC G AGAGT	2701
706	CAGUG UGAUG GCAUGCACUAUGC GCG ACCACAACGG	2537	CCGTTGTGGT G CACTG	2702
711	CACUG UGAUG GCAUGCACUAUGC GCG AGUGCACCAC	2538	GTGGTGCACT G CAGTG	2703
716	GCCUG UGAUG GCAUGCACUAUGC GCG ACUGCAGUGC	2539	GCACTGCAGT G CAGGC	2704
752	GGUAU UGAUG GCAUGCACUAUGC GCG AGCCAGACAG	2540	CTGTCTGGCT G ATACC	2705
759	AGAGG UGAUG GCAUGCACUAUGC GCG AGGUAUCAGC	2541	GCTGATACCT G CCTCT	2706
766	AUCAG UGAUG GCAUGCACUAUGC GCG AAGAGGCAGG	2542	CCTGCCTCTT G CTGAT	2707
769	UCCAU UGAUG GCAUGCACUAUGC GCG AGCAAAGAGGC	2543	GCCTCTTGCT G ATGGA	2708
800	GAUUA UGAUG GCAUGCACUAUGC GCG AACGGAAGAA	2544	TTCTTCCGTT G ATATC	2709
814	AACAG UGAUG GCAUGCACUAUGC GCG ACUUCUUGA	2545	TCAAGAAAGT G CTGTT	2710
826	UUCCU UGAUG GCAUGCACUAUGC GCG AUUUCUAACA	2546	TGTTAGAAAT G AGGAA	2711
847	UGGAU UGAUG GCAUGCACUAUGC GCG AGCCCCAUCC	2547	GGATGGGCT G ATCCA	2712
860	CUGGU UGAUG GCAUGCACUAUGC GCG GCGUGUCUGG	2548	CCAGACAGCC G ACCAG	2713
868	AAGCG UGAUG GCAUGCACUAUGC GCG AGCUGGUCGG	2549	CCGACCAGCT G CGCTT	2714
870	AGAAG UGAUG GCAUGCACUAUGC GCG GCAGCUGGUC	2550	GACCAGCTGC G CTTCT	2715
889	UCGAU UGAUG GCAUGCACUAUGC GCG ACAGCCAGGU	2551	ACCTGGCTGT G ATCGA	2716
893	ACCUU UGAUG GCAUGCACUAUGC GCG GAUCACAGCC	2552	GGCTGTGATC G AAGGT	2717
899	UUUGG UGAUG GCAUGCACUAUGC GCG ACCUUCGAUC	2553	GATCGAAGGT G CCAAA	2718
928	UCCUG UGAUG GCAUGCACUAUGC GCG ACGGAAGAGU	2554	ACTCTTCCGT G CAGGA	2719
956	GUCCU UGAUG GCAUGCACUAUGC GCG GUGGAAAGC	2555	GCTTTCCAC G AGGAC	2720
977	AUGCU UGAUG GCAUGCACUAUGC GCG GGGUGGGGC	2556	GCCCCACCC G AGCAT	2721
1011	GGAUU UGAUG GCAUGCACUAUGC GCG GUUUGGGUGG	2557	CCACCCTAAC G AATCC	2722
1038	CCCUG UGAUG GCAUGCACUAUGC GCG AUUUCCTCAU	2558	AATGGGAAAT G CAGGG	2723
1069	UCCUU UGAUG GCAUGCACUAUGC GCG ACCCACUGGU	2559	ACCAGTGGGT G AAGGA	2724
1098	UGGGG UGAUG GCAUGCACUAUGC GCG AGUCUUUAUC	2560	GATAAAGACT G CCCC	2725
1133	UGCGG UGAUG GCAUGCACUAUGC GCG AUUUAAGGGG	2561	CCCCTTAAAT G CCGCA	2726
1136	GGGUG UGAUG GCAUGCACUAUGC GCG GGCAUUUAAG	2562	CTTAAATGCC G CACCC	2727
1151	GCUUU UGAUG GCAUGCACUAUGC GCG GAUGCCGUAG	2563	CTACGGCATC G AAAGC	2728

Table 5

1159	UGACU UGAUG GCAUGCACUAUGC GCG AUGCUUUUGA	2564	TCGAAAGCAT G AGTCA	2729
1172	AACUU UGAUG GCAUGCACUAUGC GCG AGUGUCUUGA	2565	TCAAGACACT G AAGTT	2730
1206	CACCU UGAUG GCAUGCACUAUGC GCG GAAGACUUC	2566	GGAAGTCTTC G AGGTG	2731
1211	CUGGG UGAUG GCAUGCACUAUGC GCG ACCUCGAAGA	2567	TCTTCGAGGT G CCCAG	2732
1220	GGAGG UGAUG GCAUGCACUAUGC GCG AGCCUGGGCA	2568	TGCCCAGGCT G CCTCC	2733
1249	UCGGG UGAUG GCAUGCACUAUGC GCG AGUGACGGCU	2569	AGCCGTCAC T G CCCGA	2734
1253	CUUCU UGAUG GCAUGCACUAUGC GCG GGCAGUGAC	2570	GTCAC TGCCC G AGAAG	2735
1262	GUCCU UGAUG GCAUGCACUAUGC GCG GUCCUUCUCG	2571	CGAGAAGGAC G AGGAC	2736
1271	CAGUG UGAUG GCAUGCACUAUGC GCG AUGGUCCUCG	2572	CGAGGACCAT G CACTG	2737
1276	UAACU UGAUG GCAUGCACUAUGC GCG AGUGCAUGGU	2573	ACCATGCACT G AGTTA	2738
1308	CCACG UGAUG GCAUGCACUAUGC GCG ACAUGUUGAC	2574	GTCACACATGT G CGTGG	2739
1334	GUAAG UGAUG GCAUGCACUAUGC GCG GCCGGCCGUG	2575	CACGGCCGGC G CTTAC	2740
1344	UGUAG UGAUG GCAUGCACUAUGC GCG AGAGGUAAGC	2576	GCTTACCTCT G CTACA	2741
1379	AGGGU UGAUG GCAUGCACUAUGC GCG AGGCUAUGUG	2577	CACATAGCCT G ACCCT	2742
1412	AGAGG UGAUG GCAUGCACUAUGC GCG GGACAGUGGG	2578	CCCAC TGTC G CCTCT	2743
1418	GCGGG UGAUG GCAUGCACUAUGC GCG AGAGGCGGAC	2579	GTCGCGCTCT G CCCGC	2744
1422	CUCUG UGAUG GCAUGCACUAUGC GCG GGCAGAGGC	2580	GCCTCTGCCC G CAGAG	2745
1433	UCGGG UGAUG GCAUGCACUAUGC GCG GUGGGCUCUG	2581	CAGAGCCCAC G CCCGA	2746
1437	CUAGU UGAUG GCAUGCACUAUGC GCG GGGCGUGGGC	2582	GCCCACGCCC G ACTAG	2747
1450	CGCGG UGAUG GCAUGCACUAUGC GCG AUGCCUGCUA	2583	TAGCAGGCAT G CCGCG	2748
1453	UACCG UGAUG GCAUGCACUAUGC GCG GGCAUGCCUG	2584	CAGGCATGCC G CGGTA	2749
1469	UCCGG UGAUG GCAUGCACUAUGC GCG GGCCCUUACC	2585	GGTAAGGGCC G CCGGA	2750
1477	CUACG UGAUG GCAUGCACUAUGC GCG GGUCCGGCGG	2586	CCGCCGGACC G CGTAG	2751
1513	UAGUG UGAUG GCAUGCACUAUGC GCG AGAACCAACG	2587	CGTTGGTTCT G CACTA	2752
1569	AGGGG UGAUG GCAUGCACUAUGC GCG AAAAAGUAAA	2588	TTTACTTTTT G CCCCT	2753
1583	GUACU UGAUG GCAUGCACUAUGC GCG AAAGUGGAAG	2589	CTTCCACTTT G AGTAC	2754
1610	CUCCU UGAUG GCAUGCACUAUGC GCG AAAAAAUGGC	2590	GCCATTTTTT G AGGAG	2755
1619	UCUUU UGAUG GCAUGCACUAUGC GCG ACUCUCCUCA	2591	TGAGGAGAGT G AAAGA	2756
1634	GCCAG UGAUG GCAUGCACUAUGC GCG AUGGUACUCU	2592	AGAGTACCAT G CTGGC	2757
1643	CUCUG UGAUG GCAUGCACUAUGC GCG GCCGCCAGCA	2593	TGCTGGCGGC G CAGAG	2758
1678	UGGGG UGAUG GCAUGCACUAUGC GCG GAGCCCCAAG	2594	CTTGGGGCTC G CCCCCA	2759

Table 5

1723	UCUGG UGAUG GCAUGCACUAUGC GCG GUGCCGCCCG	2595	CGGGGGGCAC G CCAGA	2760
1742	AGAUU UGAUG GCAUGCACUAUGC GCG AAGGGGGGG	2596	CCCCCCCCCTT G AATCT	2761
1748	CCCUG UGAUG GCAUGCACUAUGC GCG AGAUUCAAGG	2597	CCTTGAATCT G CAGGG	2762
1811	UAGUG UGAUG GCAUGCACUAUGC GCG ACUAUGGAUG	2598	CATCCATAGT G CACTA	2763
1827	UGGUU UGAUG GCAUGCACUAUGC GCG AAGAAAAUUC	2599	GCATTTTCTT G AACCA	2764
1853	GACAU UGAUG GCAUGCACUAUGC GCG AAAAAAUUUU	2600	AAAAATTTT G ATGTC	2765
1865	UGAUG UGAUG GCAUGCACUAUGC GCG AAGGCUGACA	2601	TGTCAGCCTT G CATCA	2766
1931	CAUGG UGAUG GCAUGCACUAUGC GCG AAAGCCUUC	2602	GGAAGGCTT G CCATG	2767
1942	CGCAG UGAUG GCAUGCACUAUGC GCG AGGCCCAUGG	2603	CCATGGGCTT G CTGCG	2768
1945	UGACG UGAUG GCAUGCACUAUGC GCG AGCAGGCCCA	2604	TGGGCTGCT G CGTCA	2769
1997	AAUUA UGAUG GCAUGCACUAUGC GCG ACUAAAUAA	2605	GTTATTTAGT G ATATT	2770
2014	CUUCU UGAUG GCAUGCACUAUGC GCG ACGUUACCCA	2606	TGGGTAACGT G AGAAG	2771
2030	UAUAG UGAUG GCAUGCACUAUGC GCG AUUGUUCUAU	2607	ATAGAACAAT G CTATA	2772
2045	GUGUU UGAUG GCAUGCACUAUGC GCG AUUAUAUAUU	2608	AATATATAAT G AACAC	2773
2073	CACAU UGAUG GCAUGCACUAUGC GCG AUGUUUCUUA	2609	TAAGAAAACAT G ATGTG	2774
2078	AAUCU UGAUG GCAUGCACUAUGC GCG ACAUCAUGUU	2610	AACATGATGT G AGATT	2775
2094	AUAAG UGAUG GCAUGCACUAUGC GCG GGGACAAAGU	2611	ACTTTGTCCC G CTTAT	2776
2103	GGGAG UGAUG GCAUGCACUAUGC GCG AGAAUAAAGC	2612	CGCTTATTCT G CTCCC	2777
2117	UCUAG UGAUG GCAUGCACUAUGC GCG AGAUAAACAGG	2613	CCTGTTATCT G CTAGA	2778
2140	GGGAG UGAUG GCAUGCACUAUGC GCG AGUGAUUGAG	2614	CTCAATCACT G CTCCC	2779
2162	ACAUG UGAUG GCAUGCACUAUGC GCG AUUCUAAUAC	2615	GTATTAGAAT G CATGT	2780
2186	UUCAU UGAUG GCAUGCACUAUGC GCG AGGACACAA	2616	CTTGTGTCCT G ATGAA	2781
2189	UUUUU UGAUG GCAUGCACUAUGC GCG AUCAGGACAC	2617	GTGTCCTGAT G AAAAA	2782
2200	UCAAG UGAUG GCAUGCACUAUGC GCG ACAUAUUUUU	2618	AAAAATATGT G CTTGA	2783
2204	CAUUU UGAUG GCAUGCACUAUGC GCG AAGCACAUUA	2619	ATATGTGCTT G AAATG	2784
2209	UUUCU UGAUG GCAUGCACUAUGC GCG AUUUCAAAGCA	2620	TGCTTGAAAT G AGAAA	2785
2219	GAGAU UGAUG GCAUGCACUAUGC GCG AAAGUUUUCUC	2621	GAGAAACTTT G ATCTC	2786
2226	GUAAG UGAUG GCAUGCACUAUGC GCG AGAGAUCAAA	2622	TTTGATCTCT G CTTAC	2787
2238	UGGGG UGAUG GCAUGCACUAUGC GCG ACAUUAGUAA	2623	TTACTAATGT G CCCCC	2788
2260	ACAGG UGAUG GCAUGCACUAUGC GCG AGGUUGGACU	2624	AGTCCAACCT G CCTGT	2789
2266	UCAUG UGAUG GCAUGCACUAUGC GCG ACAGGCAGGU	2625	ACCTGCCTGT G CATGA	2790

Table 5

2270	CAGGU UGAUG GCAUGCACUAUGC GCG AUGCACAGGC	2626	GCCTGTGCAT G ACCTG	2791
2275	AUGAU UGAUG GCAUGCACUAUGC GCG AGGUCAUGCA	2627	TGCATGACCT G ATCAT	2792
2308	UUCAG UGAUG GCAUGCACUAUGC GCG AACAGGCUUA	2628	TAAGCCTGTT G CTGAA	2793
2311	GACUU UGAUG GCAUGCACUAUGC GCG AGCAACAGGC	2629	GCCTGTTGCT G AAGTC	2794
2323	CUGAG UGAUG GCAUGCACUAUGC GCG GACAAUGACU	2630	AGTCATTGTC G CTCAG	2795
2338	AACUG UGAUG GCAUGCACUAUGC GCG ACCCUAUUGC	2631	GCAATAGGGT G CAGTT	2796
2362	UUAGG UGAUG GCAUGCACUAUGC GCG AAUAGCCUUAU	2632	ATAGGCATTT G CCTAA	2797
2378	AGUGU UGAUG GCAUGCACUAUGC GCG AUGCCAGGAA	2633	TTCCTGGCAT G ACACT	2798
2389	GAAGU UGAUG GCAUGCACUAUGC GCG ACUAGAGUGU	2634	ACACTCTAGT G ACTTC	2799
2400	GGCCU UGAUG GCAUGCACUAUGC GCG ACCAGGAAGU	2635	ACTTCCTGGT G AGGCC	2800
2433	UACAG UGAUG GCAUGCACUAUGC GCG AAGACCCUGC	2636	GCAGGGTCTT G CTGTA	2801
2483	CAGAG UGAUG GCAUGCACUAUGC GCG GUGAGGUGUG	2637	CACACCTCAC G CTCTG	2802
2494	UAAAU UGAUG GCAUGCACUAUGC GCG AUGUCCAGAG	2638	CTCTGGACAT G ATTTA	2803
2520	GGGGG UGAUG GCAUGCACUAUGC GCG GGGGGGUGUC	2639	GACACCCCCC G CCCCC	2804
2546	AAUGG UGAUG GCAUGCACUAUGC GCG GGAGGCUGAU	2640	ATCAGCCTCC G CCATT	2805
2559	AGUGU UGAUG GCAUGCACUAUGC GCG GACUUGGAU	2641	ATTCCAAGTC G ACACT	2806
2571	CUGCU UGAUG GCAUGCACUAUGC GCG AAGAAGAGUG	2642	CACTCTTCTT G AGCAG	2807
2582	CAAAU UGAUG GCAUGCACUAUGC GCG ACGGUCUGCU	2643	AGCAGACCGT G ATTTG	2808
2602	UCCAG UGAUG GCAUGCACUAUGC GCG AGGUGCCUCU	2644	AGAGGCACCT G CTGGA	2809
2621	UGUUU UGAUG GCAUGCACUAUGC GCG AAGAAGUGUG	2645	CACACTTCTT G AAACA	2810
2635	ACCGU UGAUG GCAUGCACUAUGC GCG ACCCAGGCUG	2646	CAGCCTGGGT G ACGGT	2811
2655	GGCGG UGAUG GCAUGCACUAUGC GCG AGGUGCCCUA	2647	TAGGCAGCCT G CCGCC	2812
2658	GACGG UGAUG GCAUGCACUAUGC GCG GGCAGGCUGC	2648	GCAGCCTGCC G CCGTC	2813
2682	CUCGG UGAUG GCAUGCACUAUGC GCG AAGGUGAACC	2649	GGTTCACCTT G CCGAG	2814
2685	UCUCU UGAUG GCAUGCACUAUGC GCG GGCAAGGUGA	2650	TCACCTTGCC G AGAGA	2815
2694	AGACG UGAUG GCAUGCACUAUGC GCG GCCUCUCUCG	2651	CGAGAGAGGC G CGTCT	2816
2700	UGGGG UGAUG GCAUGCACUAUGC GCG AGACGCGCCU	2652	AGGCGCGTCT G CCCCCA	2817
2727	ACCAU UGAUG GCAUGCACUAUGC GCG AGGCCCCACA	2653	TGTGGGGCCT G ATGGT	2818
2733	GUGAG UGAUG GCAUGCACUAUGC GCG ACCAUCAGGC	2654	GCCTGATGGT G CTCAC	2819
2739	AGAGU UGAUG GCAUGCACUAUGC GCG GUGAGCACCA	2655	TGGTGCTCAC G ACTCT	2820
2749	CUUUG UGAUG GCAUGCACUAUGC GCG AGGAAGAGUC	2656	GACTCTTCCT G CAAAG	2821

Table 5

2761	GUCUU UGAUG GCAUGCACUAUGC GCG AGUUCUCCUUU	2657	AAAGGGAAC T G AAGAC	2822
2793	UUUUU UGAUG GCAUGCACUAUGC GCG AUGUUA AAAA	2658	TTTTTAACAT G AAAAA	2823
2818	UAGCU UGAUG GCAUGCACUAUGC GCG GGGAGCUACA	2659	TGTAGCTCCC G AGCTA	2824
2831	GCUGG UGAUG GCAUGCACUAUGC GCG AAGAGAGUAG	2660	CTACTCTCTT G CCAGC	2825
2850	AAAGG UGAUG GCAUGCACUAUGC GCG AAAAUGUGAA	2661	TTCACATTTT G CCTTT	2826
2902	CACCU UGAUG GCAUGCACUAUGC GCG GAAUGUUCCC	2662	GGGAACATTC G AGGTG	2827
2915	CUCUG UGAUG GCAUGCACUAUGC GCG AGGGUGACAC	2663	GTGTCACCC T G CAGAG	2828
2928	CACCU UGAUG GCAUGCACUAUGC GCG ACCAUAGCUC	2664	GAGCTATGGT G AGGTG	2829
2950	CCUGG UGAUG GCAUGCACUAUGC GCG ACCUAAGCCU	2665	AGGCTTAGGT G CCAGG	2830
2969	CAGCU UGAUG GCAUGCACUAUGC GCG AGAAUGCUUA	2666	TAAGCATTTCT G AGCTG	2831
3123	CAGCU UGAUG GCAUGCACUAUGC GCG GCUACCUCUC	2667	GAGAGGTAGC G AGCTG	2832
3128	CAGAG UGAUG GCAUGCACUAUGC GCG AGCUCGCUAC	2668	GTAGCGAGCT G CTCTG	2833
3133	CAUAG UGAUG GCAUGCACUAUGC GCG AGAGCAGCUC	2669	GAGCTGCTCT G CTATG	2834

Table 6

Table 6: Human PTP-1B DNAzyme and Target Sequence

Nt. Position	DNAzyme Sequence	Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
11	GCTCTAGG GGCTAGCTACAACGA GCGTGGC	2835	GCGACGCG G CCUAGAGC	3545
18	GTCTGCCG GGCTAGCTACAACGA TCTAGGCC	2836	GGCCUAGA G CGGCAGAC	3546
21	GCCGTCTG GGCTAGCTACAACGA CGCTCTAG	2837	CUAGAGCG G CAGACGGC	3547
25	CTGCGCCG GGCTAGCTACAACGA CTGCCGCT	2838	AGCGGCAG A CGGCGCAG	3548
28	CCACTGCG GGCTAGCTACAACGA CGTCTGCC	2839	GGCAGACG G CGCAGUGG	3549
30	GCCCACTG GGCTAGCTACAACGA GCCGTCTG	2840	CAGACGGC G CAGUGGGC	3550
33	TCGGGCCA GGCTAGCTACAACGA TGCGCCGT	2841	ACGGCGCA G UGGGCCGA	3551
37	CTTCTCGG GGCTAGCTACAACGA CCACTGCG	2842	CGCAGUGG G CCGAGAAG	3552
49	CTGCTGCG GGCTAGCTACAACGA CTCCTTCT	2843	AGAAGGAG G CGCAGCAG	3553
51	GGCTGCTG GGCTAGCTACAACGA GCCTCCTT	2844	AAGGAGGC G CAGCAGCC	3554
54	GGCGGCTG GGCTAGCTACAACGA TGCGCCTC	2845	GAGGCGCA G CAGCCGCC	3555
57	CAGGGCGG GGCTAGCTACAACGA TGCTGCGC	2846	GCGCAGCA G CCGCCCG	3556
60	GGCCAGGG GGCTAGCTACAACGA GGCTGCTG	2847	CAGCAGCC G CCCUGGCC	3557
66	ATGACGGG GGCTAGCTACAACGA CAGGGCGG	2848	CCGCCCCUG G CCGGUCAU	3558
70	CTCCATGA GGCTAGCTACAACGA GGGCCAGG	2849	CCUGGCCG G UCAUGGAG	3559
73	CACTCCA GGCTAGCTACAACGA GACGGGCC	2850	GGCCCGUC A UGGAGAUG	3560
79	CTTTTCCA GGCTAGCTACAACGA CTCCATGA	2851	UCAUGGAG A UGGAAAAG	3561
90	TGCTCGAA GGCTAGCTACAACGA TCCTTTTC	2852	GAAAAGGA G UUCGAGCA	3562
96	TCGATCTG GGCTAGCTACAACGA TCGAACTC	2853	GAGUUCGA G CAGAUCGA	3563
100	CTTGTCGA GGCTAGCTACAACGA CTGCTCGA	2854	UCGAGCAG A UCGACAAG	3564
104	CGGACTTG GGCTAGCTACAACGA CGATCTGC	2855	GCAGAUCC A CAAGUCCG	3565
108	CTCCCGGA GGCTAGCTACAACGA TTGTCGAT	2856	AUCGACAA G UCCGGGAG	3566
116	CCGCCCCAG GGCTAGCTACAACGA TCCCGGAC	2857	GUCCGGGA G CUGGGCGG	3567
121	AATGGCCG GGCTAGCTACAACGA CCAGCTCC	2858	GGAGCUGG G CGGCCAUU	3568
124	GTAAATGG GGCTAGCTACAACGA CGCCCAGC	2859	GCUGGGCG G CCAUUUAC	3569
127	CTGGTAAA GGCTAGCTACAACGA GGCCGCC	2860	GGCGGGCC A UUUACCAG	3570
131	TATCCTGG GGCTAGCTACAACGA AAATGGCC	2861	GGCCAUUU A CCAGGAUA	3571
137	GTCGGATA GGCTAGCTACAACGA CCTGGTAA	2862	UUACCAGG A UAUCCGAC	3572

Table 6

139	ATGTCGGA GGCTAGCTACAACGA ATCCTGGT	2863	ACCAGGAU A UCCGACAU	3573
144	GCTTCATG GGCTAGCTACAACGA CGGATATC	2864	GAUAUCCG A CAUGAAGC	3574
146	TGGCTTCA GGCTAGCTACAACGA GTCGGATA	2865	UAUCCGAC A UGAAGCCA	3575
151	GTCACCTGG GGCTAGCTACAACGA TTCAATGTC	2866	GACAUGAA G CCAGUGAC	3576
155	GGAAGTCA GGCTAGCTACAACGA TGGCTTCA	2867	UGAAGCCA G UGACUUC	3577
158	ATGGGAAG GGCTAGCTACAACGA CACTGGCT	2868	AGCCAGUG A CUUCCCAU	3578
165	ACTCTACA GGCTAGCTACAACGA GGAAGTCT	2869	GACUUCCT A UGUAGAGU	3579
167	CCACTCTA GGCTAGCTACAACGA ATGGGAAG	2870	CUUCCCAU G UAGAGUGG	3580
172	CTTGGCCA GGCTAGCTACAACGA TCTACATG	2871	CAUGUAGA G UGGCCAAG	3581
175	AAGCTTGG GGCTAGCTACAACGA CACTCTAC	2872	GUAGAGUG G CCAAGCUU	3582
180	TTAGGAAG GGCTAGCTACAACGA TTGGCCAC	2873	GUGGCCAA G CUUCCUAA	3583
191	GGTTTTTG GGCTAGCTACAACGA TCTTAGGA	2874	UCCUAGA A CAAAAACC	3584
197	TATTTTCGG GGCTAGCTACAACGA TTTTGTTC	2875	GAACAAAA A CCGAAUAU	3585
203	TGTACCTA GGCTAGCTACAACGA TTCGGTTT	2876	AAACCGAA A UAGGUACA	3586
207	TCTCTGTA GGCTAGCTACAACGA CTATTTCG	2877	CGAAAUAG G UACAGAGA	3587
209	CGTCTCTG GGCTAGCTACAACGA ACCTATT	2878	AAAUAGGU A CAGAGACG	3588
215	GACTGACG GGCTAGCTACAACGA CTCTGTAC	2879	GUACAGAG A CGUCAGUC	3589
217	GGGACTGA GGCTAGCTACAACGA GTCTCTGT	2880	ACAGAGAC G UCAGUCCC	3590
221	CAAAAGGA GGCTAGCTACAACGA TGACGTCT	2881	AGACGUCA G UCCCUIUG	3591
230	GACTATGG GGCTAGCTACAACGA CAAAGGGA	2882	UCCCUUUG A CCAUAGUC	3592
233	TCCGACTA GGCTAGCTACAACGA GGTCAAAG	2883	CUUUGACC A UAGUCGGA	3593
236	TAATCCGA GGCTAGCTACAACGA TATGTTCA	2884	UGACCAUA G UCGGAUUA	3594
241	TAGTTTAA GGCTAGCTACAACGA CCGACTAT	2885	AUAGUCGG A UUAACUAU	3595
246	TGATGTAG GGCTAGCTACAACGA TTAATCCG	2886	CGGAUUA A CUACAUA	3596
249	TCTTGATG GGCTAGCTACAACGA AGTTTAAT	2887	AUUAACU A CAUCAAGA	3597
251	CTTCTTGA GGCTAGCTACAACGA GTAGTTTA	2888	UAAACUAC A UCAAGAAG	3598
260	AGTCATTA GGCTAGCTACAACGA CTTCTTGA	2889	UCAAGAAG A UAAUGACU	3599
263	TATAGTCA GGCTAGCTACAACGA TATCTTCT	2890	AGAAGUA A UGACUAUA	3600
266	TGATATAG GGCTAGCTACAACGA CATTATCT	2891	AGAUAUG A CUAUAUCA	3601
269	CGTTGATA GGCTAGCTACAACGA AGTCATTA	2892	UAAUGACU A UAUCAACG	3602
271	AGCGTTGA GGCTAGCTACAACGA ATAGTCAT	2893	AUGACUAU A UCAACGCU	3603

Table 6

275	AACTAGCG	GGCTAGCTACAACGA	TGATATAG	2894	CUAUAUCA A	CGCUAGUU	3604
277	CAAACTAG	GGCTAGCTACAACGA	GTTGATAT	2895	AUAUCAAC G	CUAGUUUG	3605
281	TTATCAAA	GGCTAGCTACAACGA	TAGCGTTG	2896	CAACGCUA G	UUUGAUAA	3606
286	CATTTTTA	GGCTAGCTACAACGA	CAAACTAG	2897	CUAGUUUG A	UAAAAAUG	3607
292	TTCTTTCCA	GGCTAGCTACAACGA	TTTTATCA	2898	UGAUAAAA A	UGGAAGAA	3608
301	CCTTTGGG	GGCTAGCTACAACGA	TTCTTTCCA	2899	UGGAAGAA G	CCCAAAGG	3609
311	GAATGTAA	GGCTAGCTACAACGA	TCCTTTGG	2900	CCAAAGGA G	UUACAUUC	3610
314	TAAGAATG	GGCTAGCTACAACGA	AACTCCTT	2901	AAGGAGUU A	CAUUCUUA	3611
316	GGTAAGAA	GGCTAGCTACAACGA	GTAACCTC	2902	GGAGUUAC A	UUCUUACC	3612
322	GCCCTGGG	GGCTAGCTACAACGA	AAGAATGT	2903	ACAUUCUU A	CCCAGGGC	3613
329	GCAAAGGG	GGCTAGCTACAACGA	CCTGGGTA	2904	UACCCAGG G	CCCUUUGC	3614
336	GTGTTAGG	GGCTAGCTACAACGA	AAAGGGCC	2905	GGCCUUU G	CCUAACAC	3615
341	CGCATGTG	GGCTAGCTACAACGA	TAGGCAAA	2906	UUUGCCUA A	CACAUGCG	3616
343	ACCGCATG	GGCTAGCTACAACGA	GTTAGGCA	2907	UGCCUAAC A	CAUGCGGU	3617
345	TGACCGCA	GGCTAGCTACAACGA	GTGTTAGG	2908	CCUAACAC A	UGCGGUCA	3618
347	AGTGACCG	GGCTAGCTACAACGA	ATGTGTTA	2909	UAACACAU G	CGGUCACU	3619
350	AAAAGTGA	GGCTAGCTACAACGA	CGCATGTG	2910	CACAUGCG G	UCACUUUU	3620
353	CCCAAAAG	GGCTAGCTACAACGA	GACCGCAT	2911	AUGCGGUC A	CUUUUGGG	3621
364	CCACACCA	GGCTAGCTACAACGA	CTCCCAAA	2912	UUUGGGAG A	UGGUGUGG	3622
367	CTCCCCCA	GGCTAGCTACAACGA	CATCTCCC	2913	GGGAGAU G	UGUGGGAG	3623
369	TGCTCCCC	GGCTAGCTACAACGA	ACCATCTC	2914	GAGAUGGU G	UGGGAGCA	3624
375	CTTTTCTG	GGCTAGCTACAACGA	TCCCACAC	2915	GUGUGGGA G	CAGAAAAG	3625
383	CACCCCTG	GGCTAGCTACAACGA	TTTTCTGC	2916	GCAGAAAA G	CAGGGGUG	3626
389	TGACGACA	GGCTAGCTACAACGA	CCCTGCTT	2917	AAGCAGGG G	UGUCGUCA	3627
391	CATGACGA	GGCTAGCTACAACGA	ACCCCTGC	2918	GCAGGGGU G	UCGUCAU G	3628
394	GAGCATGA	GGCTAGCTACAACGA	GACACCCC	2919	GGGGUGUC G	UCAUGCUC	3629
397	GTTGAGCA	GGCTAGCTACAACGA	GACGACAC	2920	GUGUCGUC A	UGCUCAAC	3630
399	CTGTTGAG	GGCTAGCTACAACGA	ATGACGAC	2921	GUCGUCAU G	CUCAACAG	3631
404	TCACCTCTG	GGCTAGCTACAACGA	TGAGCATG	2922	CAUGCUCA A	CAGAGUGA	3632
409	CTCCATCA	GGCTAGCTACAACGA	TCTGTTGA	2923	UCAACAGA G	UGAUGGAG	3633
412	TTTCTCCA	GGCTAGCTACAACGA	CACCTCTG	2924	ACAGAGUG A	UGGAGAAA	3634

Table 6

422	TTAACGAA GGCTAGCTACAACGA CTTTCTCC	2925	GGAGAAAG G UUCGUUAA	3635
426	CATTTTAA GGCTAGCTACAACGA GAACCTTT	2926	AAAGGUUC G UUA AAAUG	3636
432	TGTGCGCA GGCTAGCTACAACGA TTTAACGA	2927	UCGUUAAA A UGCGCACA	3637
434	ATTGTGCG GGCTAGCTACAACGA ATTTTAAC	2928	GUUAAAAU G CGCACAAU	3638
436	GTATTGTG GGCTAGCTACAACGA GCATTTTA	2929	UAAAAUGC G CACAAUAC	3639
438	CAGTATTG GGCTAGCTACAACGA GCGCATTT	2930	AAUUGCGC A CAUACUG	3640
441	GGCCAGTA GGCTAGCTACAACGA TGTGCGCA	2931	UGCGCACA A UACUGGCC	3641
443	GTGGCCAG GGCTAGCTACAACGA ATTGTGCG	2932	CGCACAAU A CUGGCCAC	3642
447	TTTTGTGG GGCTAGCTACAACGA CAGTATTG	2933	CAUACUG G CCACAAA	3643
450	TCTTTTTG GGCTAGCTACAACGA GGCCAGTA	2934	UACUGGCC A CAAAAAGA	3644
469	AAAGATCA GGCTAGCTACAACGA CTCCTTTT	2935	AAAAAGAG A UGAUCUUU	3645
472	TTCAAAGA GGCTAGCTACAACGA CATCTCTT	2936	AAGAGAUG A UCUUUGAA	3646
482	AATTGTG GGCTAGCTACAACGA CTTCAAAG	2937	CUUUGAAG A CACAAAUU	3647
484	CAAATTG GGCTAGCTACAACGA GTCTTCAA	2938	UUGAAGAC A CAAAUJUG	3648
488	ATTTCAAA GGCTAGCTACAACGA TTGTGTCT	2939	AGACACAA A UUUGAAAU	3649
495	AATGTTAA GGCTAGCTACAACGA TTCAAATT	2940	AAUUUGAA A UUAACA AU	3650
499	GATCAATG GGCTAGCTACAACGA TAATTTC A	2941	UGAAAUA A CAUUGAUC	3651
501	GAGATCAA GGCTAGCTACAACGA GTTAATTT	2942	AAAUUAAC A UUGAUCUC	3652
505	TTCAGAGA GGCTAGCTACAACGA CAATGTTA	2943	UAACAUG A UCUCUGAA	3653
515	ACTTGATA GGCTAGCTACAACGA CTTCAGAG	2944	CUCUGAAG A UAUCAAGU	3654
517	TGACTTGA GGCTAGCTACAACGA ATCTTCAG	2945	CUGAAGAU A UCAAGUCA	3655
522	TAATATGA GGCTAGCTACAACGA TTGATATC	2946	GAUAUCAA G UCAUAUUA	3656
525	GTATAATA GGCTAGCTACAACGA GACTTGAT	2947	AUCAAGUC A UAUAUAUC	3657
527	CTGTATAA GGCTAGCTACAACGA ATGACTTG	2948	CAAGUCAU A UUAUACAG	3658
530	GCACTGTA GGCTAGCTACAACGA AATATGAC	2949	GUCAUAU A UACAGUGC	3659
532	TCGCACTG GGCTAGCTACAACGA ATAATATG	2950	CAUAUAU A CAGUGCGA	3660
535	CTGTGCGA GGCTAGCTACAACGA TGTATAAT	2951	AUAUAACA G UGCGACAG	3661
537	AGCTGTGCG GGCTAGCTACAACGA ACTGTATA	2952	UAUACAGU G CGACAGCU	3662
540	TCTAGCTG GGCTAGCTACAACGA CGCACTGT	2953	ACAGUGCG A CAGCUAGA	3663
543	AATTCTAG GGCTAGCTACAACGA TGTGCGAC	2954	GUGCGACA G CUAGAAU	3664
549	TTTTTCCAA GGCTAGCTACAACGA TCTAGCTG	2955	CAGCUAGA A UUGGAAAA	3665

Table 6

557	TTGTAAGG	GGCTAGCTACAACGA	TTTCCAAT	2956	AUUGGAAA A CCUUACAA	3666
562	TTGGGTTG	GGCTAGCTACAACGA	AAGGTTT	2957	AAAACCUU A CAACCCAA	3667
565	TTCTTTGG	GGCTAGCTACAACGA	TGTAAGGT	2958	ACCUUACA A CCAAGAA	3668
574	CTCTCGAG	GGCTAGCTACAACGA	TTCTTGGG	2959	CCCAAGAA A CUCGAGAG	3669
583	ATGTAAGA	GGCTAGCTACAACGA	CTCTCGAG	2960	CUCGAGAG A UCUUACAU	3670
588	TGGAAATG	GGCTAGCTACAACGA	AAGATCTC	2961	GAGAUUU A CAUUUCCA	3671
590	AGTGGAAA	GGCTAGCTACAACGA	GTAAGATC	2962	GAUCUUAC A UUUCCACU	3672
596	TGGTATAG	GGCTAGCTACAACGA	GGAAATGT	2963	ACAUUUCC A CUAUACCA	3673
599	ATGTGGTA	GGCTAGCTACAACGA	AGTGGA	2964	UUUCCACU A UACCACAU	3674
601	CCATGTGG	GGCTAGCTACAACGA	ATAGTGGA	2965	UCCACUAA A CCACAUGG	3675
604	AGGCCATG	GGCTAGCTACAACGA	GGTATAGT	2966	ACUUAUCC A CAUGGCCU	3676
606	TCAGGCCA	GGCTAGCTACAACGA	GTGGTATA	2967	UAUACCCAC A UGGCCUGA	3677
609	AAGTCAGG	GGCTAGCTACAACGA	CATGTGGT	2968	ACCACAUG G CCUGACUU	3678
614	CTCCAAAG	GGCTAGCTACAACGA	CAGGCCAT	2969	AUGGCCUG A CUUUGGAG	3679
622	TTCAGGGA	GGCTAGCTACAACGA	TCCAAAGT	2970	ACUUUGGA G UCCUGAA	3680
630	GCTGGTGA	GGCTAGCTACAACGA	TCAGGGAC	2971	GUCCUGA A UCACCAGC	3681
633	GAGGCTGG	GGCTAGCTACAACGA	GATTCAGG	2972	CCUGAAUC A CCAGCCUC	3682
637	GAATGAGG	GGCTAGCTACAACGA	TGGTGATT	2973	AAUCACCA G CCUCAUUC	3683
642	TTCAAGAA	GGCTAGCTACAACGA	GAGGCTGG	2974	CCAGCCUC A UUCUUGAA	3684
650	AAAGAAAG	GGCTAGCTACAACGA	TCAAGAAT	2975	AUUCUUGA A CUUUCUUU	3685
664	CTCTCGGA	GGCTAGCTACAACGA	TTTGAAAA	2976	UUUUCAAA G UCCGAGAG	3686
672	GACCCTGA	GGCTAGCTACAACGA	TCTCGGAC	2977	GUCCGAGA G UCAGGGUC	3687
678	CTGAGTGA	GGCTAGCTACAACGA	CCTGACTC	2978	GAGUCAGG G UCACUCAG	3688
681	GGGCTGAG	GGCTAGCTACAACGA	GACCCTGA	2979	UCAGGGUC A CUCAGCCC	3689
686	GCTCCGGG	GGCTAGCTACAACGA	TGAGTGAC	2980	GUACACUA G CCCGGAGC	3690
693	GGCCCGTG	GGCTAGCTACAACGA	TCCGGGCT	2981	AGCCCGGA G CACGGGCC	3691
695	CGGGCCCG	GGCTAGCTACAACGA	GCTCCGGG	2982	CCCGGAGC A CGGGCCCG	3692
699	ACAACGGG	GGCTAGCTACAACGA	CCGTGCTC	2983	GAGCACGG G CCCGUUGU	3693
703	CACCACAA	GGCTAGCTACAACGA	GGGCCCGT	2984	ACGGGCCG G UUGUGGUG	3694
706	GTGCACCA	GGCTAGCTACAACGA	AACGGGCC	2985	GGCCCGUU G UGGUGCAC	3695
709	GCAGTGCA	GGCTAGCTACAACGA	CACAACGG	2986	CCGUUGUG G UGCACUGC	3696

Table 6

711	CTGCAGTG	GGCTAGCTACAACGA	ACCACAAC	2987	GUUGUGGU G CACUGCAG	3697
713	CACTGCAG	GGCTAGCTACAACGA	GCACCACA	2988	UGUGGUGC A CUGCAGUG	3698
716	CTGCACTG	GGCTAGCTACAACGA	AGTGCACC	2989	GGUGCACU G CAGUGCAG	3699
719	TGCCTGCA	GGCTAGCTACAACGA	TGCAGTGC	2990	GCACUGCA G UGCAGGCA	3700
721	GATGCCCTG	GGCTAGCTACAACGA	ACTGCAGT	2991	ACUGCAGU G CAGGCAUC	3701
725	TGCCGATG	GGCTAGCTACAACGA	CTGCACTG	2992	CAGUGCAG G CAUCGGCA	3702
727	CCTGCCCGA	GGCTAGCTACAACGA	GCCTGCAC	2993	GUGCAGGC A UCGGCAGG	3703
731	CAGACCTG	GGCTAGCTACAACGA	CGATGCCT	2994	AGGCAUCG G CAGGUCUG	3704
735	GTTCCAGA	GGCTAGCTACAACGA	CTGCCGAT	2995	AUCGGCAG G UCUGGAAC	3705
742	ACAGAAAG	GGCTAGCTACAACGA	TCCAGACC	2996	GGUCUGGA A CCUUCUGU	3706
749	CAGCCAGA	GGCTAGCTACAACGA	AGAAGGTT	2997	AACCUUCU G UCUGGCUG	3707
754	GGTATCAG	GGCTAGCTACAACGA	CAGACAGA	2998	UCUGUCUG G CUGAUACC	3708
758	GGCAGGTA	GGCTAGCTACAACGA	CAGCCAGA	2999	UCUGGCUG A UACCGGCC	3709
760	GAGGCAGG	GGCTAGCTACAACGA	ATCAGCCA	3000	UGGCUGAU A CCUGCCUC	3710
764	GCAAGAGG	GGCTAGCTACAACGA	AGGTATCA	3001	UGAUACCU G CCUCUUGC	3711
771	TCCATCAG	GGCTAGCTACAACGA	AAGAGGCA	3002	UGCCUCUU G CUGAUGGA	3712
775	CTTGTCCA	GGCTAGCTACAACGA	CAGCAAGA	3003	UCUUGCUG A UGGACAAG	3713
779	TCCTCTTG	GGCTAGCTACAACGA	CCATCAGC	3004	GCUGAUGG A CAAGAGGA	3714
791	AAGAAAGG	GGCTAGCTACAACGA	CTTTCCTC	3005	GAGGAAAG A CCCUUCUU	3715
802	GATATCAA	GGCTAGCTACAACGA	GGAAGAAG	3006	CUUCUUCG G UUGAUUUC	3716
806	TCTTGATA	GGCTAGCTACAACGA	CAACGGAA	3007	UUCCGUUG A UAUCAAGA	3717
808	TTTCTTTGA	GGCTAGCTACAACGA	ATCAACGG	3008	CCGUUGAU A UCAAGAAA	3718
817	TAACAGCA	GGCTAGCTACAACGA	TTTCTTGA	3009	UCAAGAAA G UGCUGUUA	3719
819	TCTAACAG	GGCTAGCTACAACGA	ACTTCTTT	3010	AAGAAAGU G CUGUUAGA	3720
822	ATTTCTAA	GGCTAGCTACAACGA	AGCACTTT	3011	AAAGUGCU G UUAGAAAU	3721
829	CTTCCCTCA	GGCTAGCTACAACGA	TTCTAACA	3012	UGUUAGAA A UGAGGAAG	3722
837	ATCCGAAA	GGCTAGCTACAACGA	TTCTCTCAT	3013	AUGAGGAA G UUUCGGAU	3723
844	CAGCCCCA	GGCTAGCTACAACGA	CCGAAACT	3014	AGUUUCGG A UGGGCUG	3724
849	TGGATCAG	GGCTAGCTACAACGA	CCCATCCG	3015	CGGAUGGG G CUGAUCCA	3725
853	TGTCTGGA	GGCTAGCTACAACGA	CAGCCCCA	3016	UGGGGCUG A UCCAGACA	3726
859	GTCGGCTG	GGCTAGCTACAACGA	CTGGATCA	3017	UGAUCCAG A CAGCCGAC	3727

Table 6

862	CTGGTCGG	GGCTAGCTACAACGA	TGTCTGGA	3018	UCCAGACA G	CCGACCAG	3728
866	GCAGCTGG	GGCTAGCTACAACGA	CGGCTGTC	3019	GACAGCCG A	CCAGCUGC	3729
870	AAGCGCAG	GGCTAGCTACAACGA	TGGTCGGC	3020	GCCGACCA G	CUGGCGCU	3730
873	GAGAAGCG	GGCTAGCTACAACGA	AGCTGGTC	3021	GACCAGCU G	CGCUUCUC	3731
875	AGGAGAAG	GGCTAGCTACAACGA	GCAGCTGG	3022	CCAGCUGC G	CUUCUCCU	3732
884	CAGCCAGG	GGCTAGCTACAACGA	AGGAGAAG	3023	CUUCUCCU A	CCUGGCGU	3733
889	GATCACAG	GGCTAGCTACAACGA	CAGGTAGG	3024	CCUACCCU G	CUGUGAUC	3734
892	TTCGATCA	GGCTAGCTACAACGA	AGCCAGGT	3025	ACCUGGCU G	UGAUCGAA	3735
895	ACCTTCGA	GGCTAGCTACAACGA	CACAGCCA	3026	UGGCUGUG A	UCGAAGGU	3736
902	ATTGGCA	GGCTAGCTACAACGA	CTTCGATC	3027	GAUCGAAG G	UGCCAAAU	3737
904	GAATTGG	GGCTAGCTACAACGA	ACCTTCGA	3028	UCGAAGGU G	CCTAAAUUC	3738
909	ATGATGAA	GGCTAGCTACAACGA	TTGGCACC	3029	GGUGCCAA A	UUCAUCAU	3739
913	CCCCATGA	GGCTAGCTACAACGA	GAATTGG	3030	CCTAAAUUC A	UCAUGGGG	3740
916	GTCCCCCA	GGCTAGCTACAACGA	GATGAATT	3031	AAUUCauc A	UGGGGGAC	3741
923	CGGAAGAG	GGCTAGCTACAACGA	CCCCCATG	3032	CAUGGGGG A	CUCUUCGG	3742
931	ATCCTGCA	GGCTAGCTACAACGA	GGAAGAGT	3033	ACUCUUCG G	UGCAGGAU	3743
933	TGATCCTG	GGCTAGCTACAACGA	ACGGAAGA	3034	UCUUCCGU G	CAGGAUCA	3744
938	TCCACTGA	GGCTAGCTACAACGA	CCTGCACG	3035	CGUGCAGG A	UCAGUGGA	3745
942	TCCTTCCA	GGCTAGCTACAACGA	TGATCCTG	3036	CAGGAUCA G	UGGAAGGA	3746
951	TGGGAAAG	GGCTAGCTACAACGA	TCCTTCCA	3037	UGGAAGGA G	CUUUCCCA	3747
959	GGTCCTCG	GGCTAGCTACAACGA	GGGAAAGC	3038	GCUUUCCC A	CGAGGACC	3748
965	GCTCCAGG	GGCTAGCTACAACGA	CCTCGTGG	3039	CCACGAGG A	CCUGGAGC	3749
972	GGTGGGGG	GGCTAGCTACAACGA	TCCAGGTC	3040	GACCUGGA G	CCCCCACC	3750
978	TGCTCGGG	GGCTAGCTACAACGA	GGGGGCTC	3041	GAGCCCCC A	CCCGAGCA	3751
984	GGGATATG	GGCTAGCTACAACGA	TCGGGTGG	3042	CCACCCGA G	CAUAUCCC	3752
986	GGGGGATA	GGCTAGCTACAACGA	GCTCGGGT	3043	ACCCGAGC A	UAUCCCCC	3753
988	TGGGGGGA	GGCTAGCTACAACGA	ATGCTCGG	3044	CCGAGCAU A	UCCCCCCA	3754
996	CGGGGAGG	GGCTAGCTACAACGA	GGGGGGAT	3045	AUCCCCCC A	CCUCCCCG	3755
1005	TTGGGTGG	GGCTAGCTACAACGA	CGGGGAGG	3046	CCUCCCCG G	CCACCCAA	3756
1008	CGTTTGGG	GGCTAGCTACAACGA	GGCCGGGG	3047	CCCCGGCC A	CCCAAACG	3757
1014	AGGATTCC	GGCTAGCTACAACGA	TTGGGTGG	3048	CCACCCAA A	CGAAUCCU	3758

Table 6

1018	CTCCAGGA GGCTAGCTACAACGA TCGTTTGG	3049	CCAAACGA A UCCUGGAG	3759
1026	TTGTGTGG GGCTAGCTACAACGA TCCAGGAT	3050	AUCCUGGA G CCACACAA	3760
1029	CCATTGTG GGCTAGCTACAACGA GGCTCCAG	3051	CUGGAGCC A CACAAUGG	3761
1031	TCCCATTG GGCTAGCTACAACGA GTGGCTCC	3052	GGAGCCAC A CAAUGGGA	3762
1034	ATTTCCCA GGCTAGCTACAACGA TGTGTGGC	3053	GCCACACA A UGGGAAAU	3763
1041	TCCCTGCA GGCTAGCTACAACGA TTCCCAT	3054	AAUGGGAA A UGCAGGGA	3764
1043	ACTCCCTG GGCTAGCTACAACGA ATTTCCCA	3055	UGGGAUU G CAGGGAGU	3765
1050	GGGAAGAA GGCTAGCTACAACGA TCCCTGCA	3056	UGCAGGGA G UUCUCCCC	3766
1061	ACTGGTGA GGCTAGCTACAACGA TTGGGAAG	3057	CUUCCCAA A UCACCAGU	3767
1064	CCCACCTG GGCTAGCTACAACGA GATTGGG	3058	CCCAAUC A CCAGUGGG	3768
1068	TTCAACCA GGCTAGCTACAACGA TGGTGATT	3059	AAUCACCA G UGGGUGAA	3769
1072	TTCTTTCA GGCTAGCTACAACGA CCACTGGT	3060	ACCAGUGG G UGAAGGAA	3770
1084	CTCCTGGG GGCTAGCTACAACGA CTCCTTCT	3061	AGGAAGAG A CCCAGGAG	3771
1094	AGTCTTTA GGCTAGCTACAACGA CCTCCTGG	3062	CCAGGAGG A UAAAGACU	3772
1100	TGGGGCAG GGCTAGCTACAACGA CTTTATCC	3063	GGAUAAAG A CUGCCCCA	3773
1103	TGATGGGG GGCTAGCTACAACGA AGTCTTTA	3064	UAAAGACU G CCCCAUCA	3774
1108	TTCCCTTA GGCTAGCTACAACGA GGGGCAGT	3065	ACUGCCCC A UCAAGGAA	3775
1127	TTAAGGGG GGCTAGCTACAACGA TTCCTTTT	3066	AAAAGGAA G CCCCUUAA	3776
1136	GTGCGGCA GGCTAGCTACAACGA TTAAGGGG	3067	CCCCUUA A UGCCGCAC	3777
1138	GGGTGCGG GGCTAGCTACAACGA ATTTAAGG	3068	CCUUAAAU G CCGCACCC	3778
1141	GTAGGGTG GGCTAGCTACAACGA GGCATTTA	3069	UAAUUGCC G CACCCUAC	3779
1143	CCGTAGGG GGCTAGCTACAACGA GCGGCATT	3070	AAUGCCGC A CCCUACGG	3780
1148	CGATGCCG GGCTAGCTACAACGA AGGGTGCG	3071	CGCACCCU A CGGCAUCG	3781
1151	TTTCGATG GGCTAGCTACAACGA CGTAGGGT	3072	ACCCUACG G CAUCGAAA	3782
1153	GCTTTTCGA GGCTAGCTACAACGA GCCGTAGG	3073	CCUACGGC A UCGAAAAGC	3783
1160	GACTCATG GGCTAGCTACAACGA TTTCGATG	3074	CAUCGAAA G CAUGAGUC	3784
1162	TTGACTCA GGCTAGCTACAACGA GCTTTCGA	3075	UCGAAAAGC A UGAGUCA	3785
1166	TGTCCTTA GGCTAGCTACAACGA TCATGCTT	3076	AAGCAUGA G UCAAGACA	3786
1172	CTTCAGTG GGCTAGCTACAACGA CTTGACTC	3077	GAGUCAAG A CACUGAAG	3787
1174	AACTTCAG GGCTAGCTACAACGA GTCTTGAC	3078	GUCAAGAC A CUGAAAGUU	3788
1180	ACTTCTAA GGCTAGCTACAACGA TTCAGTGT	3079	ACACUGAA G UUAGAAGU	3789

Table 6

1187	CGACCCGA GGCTAGCTACAACGA TTCTAACT	3080	AGUUAGAA G UCGGGUCG	3790
1192	CCCCACGA GGCTAGCTACAACGA CCGACTTC	3081	GAAGUCGG G UCGUGGGG	3791
1195	TCCCCCCA GGCTAGCTACAACGA GACCCGAC	3082	GUCGGGUC G UGGGGGGA	3792
1205	CTCGAAGA GGCTAGCTACAACGA TTCCCCCC	3083	GGGGGGA G UCUCGAG	3793
1214	CCTGGGCA GGCTAGCTACAACGA CTCGAAGA	3084	UCUUCGAG G UGCCCAGG	3794
1216	AGCCTGGG GGCTAGCTACAACGA ACCTCGAA	3085	UUCGAGGU G CCCAGGCU	3795
1222	GGAGGCAG GGCTAGCTACAACGA CTGGGCAC	3086	GUGCCCAG G CUGCCUCC	3796
1225	TGGGGAGG GGCTAGCTACAACGA AGCCTGGG	3087	CCCAGGCU G CCUCCCCA	3797
1234	CCCTTTGG GGCTAGCTACAACGA TGGGGAGG	3088	CCUCCCCA G CCAAAGGG	3798
1245	AGTGACGG GGCTAGCTACAACGA TCCCCTTT	3089	AAAGGGGA G CCGUCACU	3799
1248	GGCAGTGA GGCTAGCTACAACGA GGCTCCCC	3090	GGGGAGCC G UCACUGCC	3800
1251	TCGGGCAG GGCTAGCTACAACGA GACGGCTC	3091	GAGCCGUC A CUGCCCCG	3801
1254	TTCTCGGG GGCTAGCTACAACGA AGTGACGG	3092	CCGUCACU G CCGGAGAA	3802
1265	GGTCCTCG GGCTAGCTACAACGA CCTTCTCG	3093	CGAGAAGG A CGAGGACC	3803
1271	GTGCATGG GGCTAGCTACAACGA CCTCGTCC	3094	GGACGAGG A CCAUGCAC	3804
1274	TCAGTGCA GGCTAGCTACAACGA GGTCCCTCG	3095	CGAGGACC A UGCACUGA	3805
1276	ACTCAGTG GGCTAGCTACAACGA ATGGTCCT	3096	AGGACCAU G CACUGAGU	3806
1278	TAACTCAG GGCTAGCTACAACGA GCATGGTC	3097	GACCAUGC A CUGAGUUA	3807
1283	TCCAGTAA GGCTAGCTACAACGA TCAGTGCA	3098	UGCACUGA G UUACUGGA	3808
1286	GCTTCCAG GGCTAGCTACAACGA AACTCAGT	3099	ACUGAGUU A CUGGAAGC	3809
1293	AGGAAGGG GGCTAGCTACAACGA TTCCAGTA	3100	UACUGGAA G CCCUCCU	3810
1303	CATGTTGA GGCTAGCTACAACGA CAGGAAGG	3101	CCUCCUG G UCAACAUG	3811
1307	CGCACATG GGCTAGCTACAACGA TGACCAGG	3102	CCUGGUCA A CAUGUGCG	3812
1309	CACGCACA GGCTAGCTACAACGA GTTGACCA	3103	UGGUCAAC A UGUGCGUG	3813
1311	GCCACGCA GGCTAGCTACAACGA ATGTTGAC	3104	GUCAACAU G UGCGUGGC	3814
1313	TAGCCACG GGCTAGCTACAACGA ACATGTTG	3105	CAACAUGU G CGUGGCUA	3815
1315	CGTAGCCA GGCTAGCTACAACGA GCACATGT	3106	ACAUGUGC G UGGCUACG	3816
1318	GACCGTAG GGCTAGCTACAACGA CACGCACA	3107	UGUGCGUG G CUACGGUC	3817
1321	GAGGACCG GGCTAGCTACAACGA AGCCACGC	3108	GCGUGGCU A CGGUCCUC	3818
1324	CGTGAGGA GGCTAGCTACAACGA CGTAGCCA	3109	UGGCUACG G UCCUCACG	3819
1330	GCCGGCCG GGCTAGCTACAACGA GAGGACCG	3110	CGGUCCUC A CGGCCGGC	3820

Table 6

1333	AGCGCCGG	GGCTAGCTACAACGA	CGTGAGGA	3111	UCCUCACG G	CGGCGGCU	3821
1337	GGTAAGCG	GGCTAGCTACAACGA	CGGCCGTG	3112	CACGGCCG G	CGCUUACC	3822
1339	GAGGTAAG	GGCTAGCTACAACGA	GCCGGCCG	3113	CGGCCGGC G	CUUACCUC	3823
1343	AGCAGAGG	GGCTAGCTACAACGA	AAGCGCCG	3114	CGGCGCUU A	CCUCUGCU	3824
1349	ACCTGTAG	GGCTAGCTACAACGA	AGAGGTAA	3115	UUACCUCU G	CUACAGGU	3825
1352	GGAACCTG	GGCTAGCTACAACGA	AGCAGAGG	3116	CCUCUGCU A	CAGGUUCC	3826
1356	AACAGGAA	GGCTAGCTACAACGA	CTGTAGCA	3117	UGCUCACG G	UUCUUGUU	3827
1362	CTGTTGAA	GGCTAGCTACAACGA	AGGAACCT	3118	AGGUUCCU G	UUCAACAG	3828
1367	TGTTGCTG	GGCTAGCTACAACGA	TGAACAGG	3119	CCUGUUCA A	CAGCAACA	3829
1370	ATGTGTTG	GGCTAGCTACAACGA	TGTTGAAC	3120	GUUCAACA G	CAACACAU	3830
1373	GCTATGTG	GGCTAGCTACAACGA	TGCTGTTG	3121	CAACAGCA A	CACAUAGC	3831
1375	AGGCTATG	GGCTAGCTACAACGA	GTTGCTGT	3122	ACAGCAAC A	CAUAGCCU	3832
1377	TCAGGCTA	GGCTAGCTACAACGA	GTGTTGCT	3123	AGCAACAC A	UAGCCUGA	3833
1380	GGGTCAGG	GGCTAGCTACAACGA	TATGTGTT	3124	AACACAUA G	CCUGACCC	3834
1385	GAGGAGGG	GGCTAGCTACAACGA	CAGGCTAT	3125	AUAGCCUG A	CCCUCUCC	3835
1395	AGGTGGAG	GGCTAGCTACAACGA	GGAGGAGG	3126	CCUCCUCC A	CUCCACCU	3836
1400	GGTGGAGG	GGCTAGCTACAACGA	GGAGTGGA	3127	UCCACUCC A	CCUCCACC	3837
1406	ACAGTGGG	GGCTAGCTACAACGA	GGAGGTGG	3128	CCACCUCU A	CCCACUGU	3838
1410	GCGGACAG	GGCTAGCTACAACGA	GGGTGGAG	3129	CUCCACCC A	CUGUCCGC	3839
1413	GAGGCGGA	GGCTAGCTACAACGA	AGTGGGTG	3130	CACCCACU G	UCCGCCUC	3840
1417	GGCAGAGG	GGCTAGCTACAACGA	GGACAGTG	3131	CACUGUCC G	CCUCUGCC	3841
1423	TCTGCGGG	GGCTAGCTACAACGA	AGAGGCGG	3132	CCGCCUCU G	CCCGCAGA	3842
1427	GGGCTCTG	GGCTAGCTACAACGA	GGGCAGAG	3133	CUCUGCCC G	CAGAGCCC	3843
1432	GGCGTGGG	GGCTAGCTACAACGA	TCTGCGGG	3134	CCCGCAGA G	CCCACGCC	3844
1436	GTCGGGCG	GGCTAGCTACAACGA	GGGCTCTG	3135	CAGAGCCC A	CGCCCGAC	3845
1438	TAGTCGGG	GGCTAGCTACAACGA	GTGGGCTC	3136	GAGCCAC G	CCCGACUA	3846
1443	CCTGCTAG	GGCTAGCTACAACGA	CGGGCGTG	3137	CACGCCCG A	CUAGCAGG	3847
1447	CATGCCCTG	GGCTAGCTACAACGA	TAGTCGGG	3138	CCCGACUA G	CAGGCAUG	3848
1451	GCGGCATG	GGCTAGCTACAACGA	CTGCTAGT	3139	ACUAGCAG G	CAUGCCGC	3849
1453	CCGCGGCA	GGCTAGCTACAACGA	GCCTGCTA	3140	UAGCAGGC A	UGCCGCGG	3850
1455	TACCGCGG	GGCTAGCTACAACGA	ATGCCTGC	3141	GCAGGCAU G	CCGCGGUA	3851

Table 6

1458	ACCTACCG	GGCTAGCTACAACGA	GGCATGCC	3142	GGCAUGCC G	CGGUAGGU	3852
1461	CTTACCTA	GGCTAGCTACAACGA	CGCGGCAT	3143	AUGCCCGG G	UAGGUAAG	3853
1465	GGCCCTTA	GGCTAGCTACAACGA	CTACCGCG	3144	CGCGGUAG G	UAAGGGCC	3854
1471	TCCGGCGG	GGCTAGCTACAACGA	CCTTACCT	3145	AGGUAAGG G	CCGCCGGA	3855
1474	CGGTCCGG	GGCTAGCTACAACGA	GGCCCTTA	3146	UAAGGGCC G	CCGGACCG	3856
1479	CTACGCGG	GGCTAGCTACAACGA	CCGGCGGC	3147	GCCGCCGG A	CCGCGUAG	3857
1482	TCTCTACG	GGCTAGCTACAACGA	GGTCCGGC	3148	GCCGGACC G	CGUAGAGA	3858
1484	GCTCTCTA	GGCTAGCTACAACGA	GCGGTCCG	3149	CGGACCGC G	UAGAGAGC	3859
1491	GGGCCCCG	GGCTAGCTACAACGA	TCTCTACG	3150	CGUAGAGA G	CCGGGGCC	3860
1496	GTCCGGGG	GGCTAGCTACAACGA	CCGGCTCT	3151	AGAGCCGG G	CCCCGGAC	3861
1503	AACGTCCG	GGCTAGCTACAACGA	CCGGGGCC	3152	GGCCCCGG A	CGGACGUU	3862
1507	AACCAACG	GGCTAGCTACAACGA	CCGTCCGG	3153	CCGGACGG A	CGUUGGUU	3863
1509	AGAAACCA	GGCTAGCTACAACGA	GTCCGTCC	3154	GGACGGAC G	UUGGUUCU	3864
1513	GTGCAGAA	GGCTAGCTACAACGA	CAACGTCC	3155	GGACGUUG G	UUCUGCAC	3865
1518	TTTTAGTG	GGCTAGCTACAACGA	AGAACCAA	3156	UUGGUUCU G	CACUAAAA	3866
1520	GGTTTTAG	GGCTAGCTACAACGA	GCAGAACC	3157	GGUUCUGC A	CUAAAAACC	3867
1526	AAGATGGG	GGCTAGCTACAACGA	TTTAGTGC	3158	GCACUAAA A	CCCAUCUU	3868
1530	GGGGAAGA	GGCTAGCTACAACGA	GGGTTTTA	3159	UAAAAACC A	UCUUCCCC	3869
1541	GACACACA	GGCTAGCTACAACGA	CCGGGGAA	3160	UUCCCCCG A	UGUGUGUC	3870
1543	GAGACACA	GGCTAGCTACAACGA	ATCCGGGG	3161	CCCCGGAU G	UGUGUCUC	3871
1545	GTGAGACA	GGCTAGCTACAACGA	ACATCCGG	3162	CCGGAUGU G	UGUCUCAC	3872
1547	GGGTGAGA	GGCTAGCTACAACGA	ACACATCC	3163	GGAUGUGU G	UCUCACCC	3873
1552	ATGAGGGG	GGCTAGCTACAACGA	GAGACACA	3164	UGUGUCUC A	CCCCUCAU	3874
1559	TAAAAGGA	GGCTAGCTACAACGA	GAGGGGTG	3165	CACCCUC A	UCCUUJUA	3875
1567	GCAAAAAG	GGCTAGCTACAACGA	AAAAGGAT	3166	AUCCUUUU A	CUUUUUGC	3876
1574	GGAAGGGG	GGCTAGCTACAACGA	AAAAAGTA	3167	UACUUUUU G	CCCCUUC	3877
1583	ACTCAAAG	GGCTAGCTACAACGA	GGAAGGGG	3168	CCCCUUC A	CUUUGAGU	3878
1590	ATTTGGTA	GGCTAGCTACAACGA	TCAAAGTG	3169	CACUUUGA G	UACCAAAU	3879
1592	GGATTTGG	GGCTAGCTACAACGA	ACTCAAAG	3170	CUUUGAGU A	CCAAAUCC	3880
1597	CTTGTGGA	GGCTAGCTACAACGA	TTGGTACT	3171	AGUACCAA A	UCCACAAG	3881
1601	ATGGCTTG	GGCTAGCTACAACGA	GGATTTGG	3172	CCAAAUCC A	CAAGCCAU	3882

Table 6

1605	AAAAATGG	GGCTAGCTACAACGA	TTGTGGAT	3173	AUCCACAA G CCAUUUUU	3883
1608	TCAAAAAA	GGCTAGCTACAACGA	GGCTTGTG	3174	CACAAGCC A UUUUUUGA	3884
1622	CTCTTTCA	GGCTAGCTACAACGA	TCTCCTCA	3175	UGAGGAGA G UGAAAGAG	3885
1632	GCATGGTA	GGCTAGCTACAACGA	TCTCTTTC	3176	GAAAGAGA G UACCAUGC	3886
1634	CAGCATGG	GGCTAGCTACAACGA	ACTCTCTT	3177	AAGAGAGU A CCAUGCUG	3887
1637	CGCCAGCA	GGCTAGCTACAACGA	GGTACTCT	3178	AGAGUACC A UGCUGGCG	3888
1639	GCCGCCAG	GGCTAGCTACAACGA	ATGGTACT	3179	AGUACCAU G CUGGCGGC	3889
1643	CTGCGCCG	GGCTAGCTACAACGA	CAGCATGG	3180	CCAUGCUG G CGGCGCAG	3890
1646	CCTCTGCG	GGCTAGCTACAACGA	CGCCAGCA	3181	UGCUGGCG G CGCAGAGG	3891
1648	TCCCTCTG	GGCTAGCTACAACGA	GCCGCCAG	3182	CUGGCGGC G CAGAGGGA	3892
1661	GGTGTAGG	GGCTAGCTACAACGA	CCCTTCCC	3183	GGGAAGGG G CCUACACC	3893
1665	GACGGGTG	GGCTAGCTACAACGA	AGGCCCTT	3184	AGGGGCCU A CACCCGUC	3894
1667	AAGACGGG	GGCTAGCTACAACGA	GTAGGCCC	3185	GGGCUUAC A CCCGUCUU	3895
1671	CCCCAAGA	GGCTAGCTACAACGA	GGTGTTAG	3186	CUACACCC G UCUGGGG	3896
1679	GGGCGGAG	GGCTAGCTACAACGA	CCCAAGAC	3187	GUCUUGGG G CUCGCCCC	3897
1683	GGGTGGGG	GGCTAGCTACAACGA	GAGCCCCA	3188	UGGGGCUC G CCCCACCC	3898
1688	GCCCTGGG	GGCTAGCTACAACGA	GGGGCGAG	3189	CUCGCCCC A CCCAGGGC	3899
1695	GGAGGGAG	GGCTAGCTACAACGA	CCTGGGTG	3190	CACCCAGG G CUCCCUCC	3900
1708	CTGGGATG	GGCTAGCTACAACGA	TCCAGGAG	3191	CUCCUGGA G CAUCCCCAG	3901
1710	GCCTGGGA	GGCTAGCTACAACGA	GCTCCAGG	3192	CCUGGAGC A UCCCAGGC	3902
1717	GCCGCCCG	GGCTAGCTACAACGA	CTGGGATG	3193	CAUCCCCAG G CGGGCGGC	3903
1721	GCGTGCCG	GGCTAGCTACAACGA	CCGCCTGG	3194	CCAGGCGG G CGGCACGC	3904
1724	CTGGCGTG	GGCTAGCTACAACGA	CGCCCGCC	3195	GGCGGGCG G CACGCCAG	3905
1726	GTCTGGCG	GGCTAGCTACAACGA	GCCGCCCG	3196	CGGGCGGC A CGCCAGAC	3906
1728	CTGTCTGG	GGCTAGCTACAACGA	GTGCCGCC	3197	GGCGGCAC G CCAGACAG	3907
1733	GGGGGCTG	GGCTAGCTACAACGA	CTGGCGTG	3198	CACGCCAG A CAGCCCCC	3908
1736	GGGGGGGG	GGCTAGCTACAACGA	TGTCTGGC	3199	GCCAGACA G CCCCCCCC	3909
1749	CCTGCAGA	GGCTAGCTACAACGA	TCAAGGGG	3200	CCCCUUGA A UCUGCAGG	3910
1753	GCTCCCTG	GGCTAGCTACAACGA	AGATTCAA	3201	UUGAAUCU G CAGGGAGC	3911
1760	GAGAGTTG	GGCTAGCTACAACGA	TCCCTGCA	3202	UGCAGGGA G CAACUCUC	3912
1763	GTGGAGAG	GGCTAGCTACAACGA	TGCTCCCT	3203	AGGGAGCA A CUCUCCAC	3913

Table 6

1770	ATATGGAG	GGCTAGCTACAACGA	GGAGAGTT	3204	AACUCUCC A CUCCAUAU	3914
1775	AATAAATA	GGCTAGCTACAACGA	GGAGTGGA	3205	UCCACUCC A UAUUUUUU	3915
1777	TAAATAAA	GGCTAGCTACAACGA	ATGGAGTG	3206	CACUCCAU A UUAUUUUA	3916
1781	TGTTTAAA	GGCTAGCTACAACGA	AAATATGG	3207	CCAUAUUU A UUA AAAACA	3917
1787	AAAAATTG	GGCTAGCTACAACGA	T TAAATAA	3208	UUAUUUUA A CAAUUUUU	3918
1790	GGAAAAAA	GGCTAGCTACAACGA	TGTTTAAA	3209	UUUAAAACA A UUUUUUCC	3919
1805	TATGGATG	GGCTAGCTACAACGA	CTTTGGGG	3210	CCCCAAAG G CAUCCAUA	3920
1807	ACTATGGA	GGCTAGCTACAACGA	GCCTTTGG	3211	CCAAAGGC A UCCAUAUG	3921
1811	GTGCACTA	GGCTAGCTACAACGA	GGATGCCT	3212	AGGCAUCC A UAGUGCAC	3922
1814	CTAGTGCA	GGCTAGCTACAACGA	TATGGATG	3213	CAUCCAUA G UGCACUAG	3923
1816	TGCTAGTG	GGCTAGCTACAACGA	ACTATGGA	3214	UCCAUAUG G CACUAGCA	3924
1818	AATGCTAG	GGCTAGCTACAACGA	GCACTATG	3215	CAUAGUGC A CUAGCAUU	3925
1822	AGAAAAATG	GGCTAGCTACAACGA	TAGTGCAC	3216	GUGCACUA G CAUUUUUCU	3926
1824	CAAGAAAA	GGCTAGCTACAACGA	GCTAGTGC	3217	GCACUAGC A UUUUCUUG	3927
1834	ATTATTGG	GGCTAGCTACAACGA	TCAAGAAA	3218	UUUCUUGA A CCAAUAAU	3928
1838	ATACATTA	GGCTAGCTACAACGA	TGGTTCAA	3219	UUGAACCA A UAAUGUAU	3929
1841	T TAAATACA	GGCTAGCTACAACGA	TATTGGTT	3220	AACCAAUA A UGUUUUAA	3930
1843	TTTTTAATA	GGCTAGCTACAACGA	ATTATTGG	3221	CCAUAUAU G UAUUAAAA	3931
1845	AATTTTAA	GGCTAGCTACAACGA	ACATTATT	3222	AAUAAUGU A UUA AAAAUU	3932
1851	TCAAAAAA	GGCTAGCTACAACGA	TTTAATAC	3223	GUUUUAAA A UUUUUUGA	3933
1859	GGCTGACA	GGCTAGCTACAACGA	CAAAAAAT	3224	AUUUUUUG A UGUCAGCC	3934
1861	AAGGCTGA	GGCTAGCTACAACGA	ATCAAAAA	3225	UUUUUUGAU G UCAGCCUU	3935
1865	ATGCAAGG	GGCTAGCTACAACGA	TGACATCA	3226	UGAUGUCA G CCUUGCAU	3936
1870	CCTTGATG	GGCTAGCTACAACGA	AAGGCTGA	3227	UCAGCCUU G CAUCAAGG	3937
1872	GCCCTTGA	GGCTAGCTACAACGA	GCAAGGCT	3228	AGCCUUGC A UCAAGGGC	3938
1879	TGATAAAG	GGCTAGCTACAACGA	CCTTGATG	3229	CAUCAAGG G CUUUAUCA	3939
1884	CTTTT TGA	GGCTAGCTACAACGA	AAAGCCCT	3230	AGGGCUUU A UCAAAAAG	3940
1892	TTATT TGA	GGCTAGCTACAACGA	TTTTTTGAT	3231	AUCAAAAA G UACAAUAA	3941
1894	TATTATTG	GGCTAGCTACAACGA	ACTTTTTG	3232	CAAAAAGU A CAAUAAUA	3942
1897	ATTTATTA	GGCTAGCTACAACGA	TGTACTTT	3233	AAAGUACA A UAAUAAAU	3943
1900	AGGATTTA	GGCTAGCTACAACGA	TATTGTAC	3234	GUACAAUA A UAAAUCCU	3944

Table 6

1904	CCTGAGGA	GGCTAGCTACAACGA	TTATTATT	3235	AAUAAUAA A UCCUCAGG	3945
1912	CAGTACTA	GGCTAGCTACAACGA	CTGAGGAT	3236	AUCCUCAG G UAGUACUG	3946
1915	TCCCAGTA	GGCTAGCTACAACGA	TACCTGAG	3237	CUCAGGUA G UACUGGGA	3947
1917	ATTCCCAG	GGCTAGCTACAACGA	ACTACCTG	3238	CAGGUAGU A CUGGGAU	3948
1924	GCCTTCCA	GGCTAGCTACAACGA	TCCCAGTA	3239	UACUGGGA A UGGAAGGC	3949
1931	TGGCAAAG	GGCTAGCTACAACGA	CTTCCATT	3240	AAUGGAAG G CUUUGCCA	3950
1936	GCCCATGG	GGCTAGCTACAACGA	AAAGCCTT	3241	AAGGCUUU G CCAUGGGC	3951
1939	CAGGCCCA	GGCTAGCTACAACGA	GGCAAAGC	3242	GCUUUGCC A UGGGCCUG	3952
1943	GCAGCAGG	GGCTAGCTACAACGA	CCATGGCA	3243	UGCCAUGG G CCUGCUGC	3953
1947	TGACGCAG	GGCTAGCTACAACGA	AGGCCCAT	3244	AUGGGCCU G CUGCGUCA	3954
1950	GTCTGACG	GGCTAGCTACAACGA	AGCAGGCC	3245	GGCCUGCU G CGUCAGAC	3955
1952	TGGTCTGA	GGCTAGCTACAACGA	GCAGCAGG	3246	CCUGCUGC G UCAGACCA	3956
1957	AGTACTGG	GGCTAGCTACAACGA	CTGACGCA	3247	UGCUCAG A CCAGUACU	3957
1961	TCCCAGTA	GGCTAGCTACAACGA	TGGTCTGA	3248	UCAGACCA G UACUGGGA	3958
1963	CTTCCCAG	GGCTAGCTACAACGA	ACTGGTCT	3249	AGACCAGU A CUGGGAAG	3959
1976	TACAACCG	GGCTAGCTACAACGA	CCTCCTTC	3250	GAAGGAGG A CGGUUGUA	3960
1979	GCTTACAA	GGCTAGCTACAACGA	CGTCCTCC	3251	GGAGGACG G UUGUAAGC	3961
1982	ACTGCTTA	GGCTAGCTACAACGA	AACCGTCC	3252	GGACGGUU G UAAGCAGU	3962
1986	AACAACCTG	GGCTAGCTACAACGA	TTACAACC	3253	GGUUGUAA G CAGUUGUU	3963
1989	AATAACAA	GGCTAGCTACAACGA	TGCTTACA	3254	UGUAAGCA G UUGUUAUU	3964
1992	CTAAATAA	GGCTAGCTACAACGA	AACTGCTT	3255	AAGCAGUU G UUAUUUAG	3965
1995	TCACTAAA	GGCTAGCTACAACGA	AACAAC TG	3256	CAGUUGUU A UUUAGUGA	3966
2000	CAATATCA	GGCTAGCTACAACGA	TAAATAAC	3257	GUUAUUUA G UGAUAUUG	3967
2003	CCACAATA	GGCTAGCTACAACGA	CACTAAAT	3258	AUUUAGUG A UAUUGUGG	3968
2005	ACCCACAA	GGCTAGCTACAACGA	ATCACTAA	3259	UUAGUGAU A UUGUGGGU	3969
2008	GTTACCCA	GGCTAGCTACAACGA	AATATCAC	3260	GUGAUUUU G UGGGUAAC	3970
2012	TCACGTTA	GGCTAGCTACAACGA	CCACAATA	3261	UAUUGUGG G UAACGUGA	3971
2015	TTCTCACG	GGCTAGCTACAACGA	TACCCACA	3262	UGUGGGUA A CGUGAGAA	3972
2017	TCCTTCTCA	GGCTAGCTACAACGA	GTTACCCA	3263	UGGGUAAC G UGAGAAGA	3973
2025	TTGTTCTA	GGCTAGCTACAACGA	CTTCTCAC	3264	GUGAGAAG A UAGAACAA	3974
2030	TAGCATTG	GGCTAGCTACAACGA	TCTATCTT	3265	AAGAUAGA A CAAUGCUA	3975

Table 6

2033	TTATAGCA	GGCTAGCTACAACGA	TGTTCTAT	3266	AUAGAACA A UGCUAUAA	3976
2035	TATTATAG	GGCTAGCTACAACGA	ATTGTTCT	3267	AGAACAAU G CUUAUAAU	3977
2038	ATATATTA	GGCTAGCTACAACGA	AGCATTTG	3268	ACAAUGCU A UAAUUAU	3978
2041	ATTATATA	GGCTAGCTACAACGA	TATAGCAT	3269	AUGCUAUA A UAUUAU	3979
2043	TCATTATA	GGCTAGCTACAACGA	ATTATAGC	3270	GCUUAUAA A UAUAAUGA	3980
2045	GTTTCATTA	GGCTAGCTACAACGA	ATATTATA	3271	UAUAAUAA A UAAUGAAC	3981
2048	CGTGTTC	GGCTAGCTACAACGA	TATATATT	3272	AAUUAUA A UGAACACG	3982
2052	CCCACGTG	GGCTAGCTACAACGA	TCATTATA	3273	UAUAAUGA A CACGUGGG	3983
2054	TACCCACG	GGCTAGCTACAACGA	GTTTCATTA	3274	UAAUGAAC A CGUGGGUA	3984
2056	AATACCCA	GGCTAGCTACAACGA	GTGTTTCAT	3275	AUGAACAC G UGGGUUU	3985
2060	ATTAAATA	GGCTAGCTACAACGA	CCACGTGT	3276	ACACGUGG G UAUUUAAU	3986
2062	TTATTAAA	GGCTAGCTACAACGA	ACCCACGT	3277	ACGUGGGU A UUUAAUAA	3987
2067	GTTTCTTA	GGCTAGCTACAACGA	TAAATACC	3278	GGUAUUUA A UAAGAAAC	3988
2074	ACATCATG	GGCTAGCTACAACGA	TTCTTATT	3279	AAUAAAGAA A CAUGAUGU	3989
2076	TCACATCA	GGCTAGCTACAACGA	GTTTCTTA	3280	UAAGAAAC A UGAUGUGA	3990
2079	ATCTCACA	GGCTAGCTACAACGA	CATGTTTC	3281	GAAACAUG A UGUGAGAU	3991
2081	TAATCTCA	GGCTAGCTACAACGA	ATCATGTT	3282	AACAUGAU G UGAGAUUA	3992
2086	CAAAGTAA	GGCTAGCTACAACGA	CTCACATC	3283	GAUGUGAG A UUACUUUG	3993
2089	GGACAAAG	GGCTAGCTACAACGA	AATCTCAC	3284	GUGAGAUU A CUUUGUCC	3994
2094	AAGCGGGA	GGCTAGCTACAACGA	AAAGTAAT	3285	AUUACUUU G UCCCGCUU	3995
2099	AGAAATAAG	GGCTAGCTACAACGA	GGACAAA	3286	UUUGUCCC G CUUAUUCU	3996
2103	GAGCAGAA	GGCTAGCTACAACGA	AAGCGGGA	3287	UCCCGCUU A UUCUGCUC	3997
2108	ACAGGGAG	GGCTAGCTACAACGA	AGAATAAG	3288	CUUAUUCU G CUCCCUUG	3998
2115	GCAGATAA	GGCTAGCTACAACGA	AGGAGCA	3289	UGCUCUUU G UUAUCUGC	3999
2118	CTAGCAGA	GGCTAGCTACAACGA	AACAGGGA	3290	UCCUGUUU A UCUGCUAG	4000
2122	AGATCTAG	GGCTAGCTACAACGA	AGATAACA	3291	UGUUUAUCU G CUAGAUCU	4001
2127	GAACCTAGA	GGCTAGCTACAACGA	CTAGCAGA	3292	UCUGCUAG A UCUAGUUC	4002
2132	ATTGAGAA	GGCTAGCTACAACGA	TAGATCTA	3293	UAGAUCUA G UUCUCAAU	4003
2139	AGCAGTGA	GGCTAGCTACAACGA	TGAGAACT	3294	AGUUCUCA A UCACUGCU	4004
2142	GGGAGCAG	GGCTAGCTACAACGA	GATTGAGA	3295	UCUCAAU A CUGCUCCC	4005
2145	CGGGGGAG	GGCTAGCTACAACGA	AGTGATTG	3296	CAUACACU G CUCCCCCG	4006

Table 6

2153	AATACACA	GGCTAGCTACAACGA	GGGGGAGC	3297	GCUCCCCC G UGUGUAUU	4007
2155	CTAATACA	GGCTAGCTACAACGA	ACGGGGGA	3298	UCCCCCGU G UGUUUUAG	4008
2157	TTCTAATA	GGCTAGCTACAACGA	ACACGGGG	3299	CCCCGUGU G UAUUAGAA	4009
2159	CATTCTAA	GGCTAGCTACAACGA	ACACACGG	3300	CCGUGUGU A UUAGAAUG	4010
2165	TACATGCA	GGCTAGCTACAACGA	TCTAATAC	3301	GUUUUAGA A UGCAUGUA	4011
2167	CTTACATG	GGCTAGCTACAACGA	ATTCTAAT	3302	AUUAGAAU G CAUGUAAG	4012
2169	ACCTTACA	GGCTAGCTACAACGA	GCATTCTA	3303	UAGAAUGC A UGUAAGGU	4013
2171	AGACCTTA	GGCTAGCTACAACGA	ATGCATTC	3304	GAAUGCAU G UAAGGUCU	4014
2176	CAAGAAGA	GGCTAGCTACAACGA	CTTACATG	3305	CAUGUAAG G UCUUCUUG	4015
2184	TCAGGACA	GGCTAGCTACAACGA	AAGAAGAC	3306	GUCUUUUU G UGUCCUGA	4016
2186	CATCAGGA	GGCTAGCTACAACGA	ACAAGAAG	3307	CUUCUUGU G UCCUGAUG	4017
2192	ATTTTTC	GGCTAGCTACAACGA	CAGGACAC	3308	GUGUCCUG A UGAAAAAU	4018
2199	AGCACATA	GGCTAGCTACAACGA	TTTTTCATC	3309	GAUGAAAA A UAUGUGCU	4019
2201	CAAGCACA	GGCTAGCTACAACGA	ATTTTTC	3310	UGAAAAAU A UGUGCUUG	4020
2203	TTCAAGCA	GGCTAGCTACAACGA	ATATTTT	3311	AAAAUAU G UGCUUGAA	4021
2205	ATTTCAAG	GGCTAGCTACAACGA	ACATATTT	3312	AAUAUGU G CUUGAAAA	4022
2212	GTTTCTCA	GGCTAGCTACAACGA	TTCAAGCA	3313	UGCUUGAA A UGAGAAAC	4023
2219	GATCAAAG	GGCTAGCTACAACGA	TTCTCAT	3314	AAUGAGAA A CUUUGAUC	4024
2225	AGCAGAGA	GGCTAGCTACAACGA	CAAAGTTT	3315	AAACUUUG A UCUCUGCU	4025
2231	TTAGTAAG	GGCTAGCTACAACGA	AGAGATCA	3316	UGAUCUCU G CUUACUAA	4026
2235	CACATTAG	GGCTAGCTACAACGA	AAGCAGAG	3317	CUCUGCUU A CUAUUGUG	4027
2239	GGGCACA	GGCTAGCTACAACGA	TAGTAAGC	3318	GCUUACUA A UGUGCCCC	4028
2241	ATGGGGCA	GGCTAGCTACAACGA	ATTAGTAA	3319	UUACUAAU G UGCCCCAU	4029
2243	ACATGGGG	GGCTAGCTACAACGA	ACATTAGT	3320	ACUAAUGU G CCCCAGU	4030
2248	CTTGGACA	GGCTAGCTACAACGA	GGGGCACA	3321	UGUGCCCC A UGUCCAAG	4031
2250	GACTTGGA	GGCTAGCTACAACGA	ATGGGGCA	3322	UGCCCCAU G UCCAAGUC	4032
2256	AGGTTGGA	GGCTAGCTACAACGA	TTGGACAT	3323	AUGUCCAA G UCCAACCU	4033
2261	CAGGCAGG	GGCTAGCTACAACGA	TGGACTTG	3324	CAAGUCCA A CCUGCCUG	4034
2265	TGCACAGG	GGCTAGCTACAACGA	AGGTTGGA	3325	UCCAACCU G CCUGUGCA	4035
2269	GTCAATGA	GGCTAGCTACAACGA	AGGCAGGT	3326	ACCUGCCU G UGCAUGAC	4036
2271	AGGTCATG	GGCTAGCTACAACGA	ACAGGCAG	3327	CUGCCUGU G CAUGACCU	4037

Table 6

2273	TCAGGTCA GGCTAGCTACAACGA GCACAGGC	3328	GCCUGUGC A UGACCUGA	4038
2276	TGATCAGG GGCTAGCTACAACGA CATGCACA	3329	UGUGCAUG A CCUGAUGA	4039
2281	TGTAATGA GGCTAGCTACAACGA CAGGTCAT	3330	AUGACCUG A UCAUUAUA	4040
2284	CCATGTAA GGCTAGCTACAACGA GATCAGGT	3331	ACCUGAUC A UUACAUGG	4041
2287	CAGCCATG GGCTAGCTACAACGA AATGATCA	3332	UGAUCAUU A CAUGGCUG	4042
2289	CACAGCCA GGCTAGCTACAACGA GTAATGAT	3333	AUCAUUAC A UGGCUGUG	4043
2292	AACCACAG GGCTAGCTACAACGA CATGTAAT	3334	AUUACAUG G CUGUGGUU	4044
2295	AGGAACCA GGCTAGCTACAACGA AGCCATGT	3335	ACAUGGCU G UGGUUCUU	4045
2298	CTTAGGAA GGCTAGCTACAACGA CACAGCCA	3336	UGGCUGUG G UUCCUAAG	4046
2306	GCAACAGG GGCTAGCTACAACGA TTAGGAAC	3337	GUUCCUAA G CCUGUJUG	4047
2310	TTCAGCAA GGCTAGCTACAACGA AGGCTTAG	3338	CUAAGCCU G UUGCUGAA	4048
2313	GACTTCAG GGCTAGCTACAACGA AACAGGCT	3339	AGCCUGUU G CUGAAGUC	4049
2319	GACAAATGA GGCTAGCTACAACGA TTCAGCAA	3340	UUGCUGAA G UCAUUGUC	4050
2322	AGCGACAA GGCTAGCTACAACGA GACTTCAG	3341	CUGAAGUC A UUGUGGCU	4051
2325	CTGAGCGA GGCTAGCTACAACGA AATGACTT	3342	AAGUCAUU G UCGCUCAG	4052
2328	TTGCTGAG GGCTAGCTACAACGA GACAATGA	3343	UCAUUGUC G CUCAGCAA	4053
2333	CCCTATTG GGCTAGCTACAACGA TGAGCGAC	3344	GUCGCUCA G CAAUAGGG	4054
2336	GCACCCCTA GGCTAGCTACAACGA TGCTGAGC	3345	GCUCAGCA A UAGGGUGC	4055
2341	AAACTGCA GGCTAGCTACAACGA CCTATTGC	3346	GCAAUAGG G UGCAGUUU	4056
2343	GAAAACTG GGCTAGCTACAACGA ACCCTATT	3347	AAUAGGGU G CAGUUUUC	4057
2346	CTGGAAAA GGCTAGCTACAACGA TGCACCCCT	3348	AGGGUGCA G UUUUCCAG	4058
2357	AATGCCCTA GGCTAGCTACAACGA TCCTGGAA	3349	UUCCAGGA A UAGGCAUU	4059
2361	GGCAAATG GGCTAGCTACAACGA CTATTTCCT	3350	AGGAAUAG G CAUUUGCC	4060
2363	TAGGCAAA GGCTAGCTACAACGA GCCTATTTC	3351	GAAUAGGC A UUUGCCUA	4061
2367	GAATTAGG GGCTAGCTACAACGA AAATGCCT	3352	AGGCAUUU G CCUAAUUC	4062
2372	GCCAGGAA GGCTAGCTACAACGA TAGGCAAA	3353	UUUGCCUA A UUCCUGGC	4063
2379	GTGTCATG GGCTAGCTACAACGA CAGGAATT	3354	AAUUCUG G CAUGACAC	4064
2381	GAGTGTCA GGCTAGCTACAACGA GCCAGGAA	3355	UUCUUGGC A UGACACUC	4065
2384	CTAGAGTG GGCTAGCTACAACGA CATGCCAG	3356	CUGGCAUG A CACUCUAG	4066
2386	CACTAGAG GGCTAGCTACAACGA GTCATGCC	3357	GGCAUGAC A CUCUAGUG	4067
2392	GGAAGTCA GGCTAGCTACAACGA TAGAGTGT	3358	ACACUCUA G UGACUUCC	4068

Table 6

2395	CCAGGAAG GGCTAGCTACAACGA CACTAGAG	3359	CUCUAGUG A CUUCCUGG	4069
2403	GGGCCCTCA GGCTAGCTACAACGA CAGGAAGT	3360	ACUUCUCUG G UGAGGCC	4070
2408	AGGCTGGG GGCTAGCTACAACGA CTCACCAG	3361	CUGGUGAG G CCCAGCCU	4071
2413	AGGACAGG GGCTAGCTACAACGA TGGGCCTC	3362	GAGGCCCA G CCUGUCCU	4072
2417	TACCAGGA GGCTAGCTACAACGA AGGCTGGG	3363	CCCAGCCU G UCCUGGUA	4073
2423	CTGCTGTA GGCTAGCTACAACGA CAGGACAG	3364	CUGUCCUG G UACAGCAG	4074
2425	CCCTGCTG GGCTAGCTACAACGA ACCAGGAC	3365	GUCCUGGU A CAGCAGGG	4075
2428	AGACCCCTG GGCTAGCTACAACGA TGTACCAG	3366	CUGGUACA G CAGGGUCU	4076
2433	CAGCAAGA GGCTAGCTACAACGA CCTGCTGT	3367	ACAGCAGG G UCUUGCUG	4077
2438	AGTTACAG GGCTAGCTACAACGA AAGACCCT	3368	AGGGUCUU G CUGUAACU	4078
2441	CTGAGTTA GGCTAGCTACAACGA AGCAAGAC	3369	GUCUUGCU G UAACUCAG	4079
2444	TGTCTGAG GGCTAGCTACAACGA TACAGCAA	3370	UUGCUGUA A CUCAGACA	4080
2450	TTGGAATG GGCTAGCTACAACGA CTGAGTTA	3371	UAACUCAG A CAUCCCAA	4081
2452	CCTTGGAA GGCTAGCTACAACGA GTCTGAGT	3372	ACUCAGAC A UUCAAGG	4082
2461	TTCCCATTA GGCTAGCTACAACGA CCTTGGAA	3373	UUCCAAGG G UAUGGGAA	4083
2463	GCTTCCCA GGCTAGCTACAACGA ACCCTTGG	3374	CCAAGGGU A UGGGAAGC	4084
2470	GAATATGG GGCTAGCTACAACGA TTCCCATTA	3375	UAUGGGAA G CCAUAUUC	4085
2473	TGTGAATA GGCTAGCTACAACGA GGCTTCCC	3376	GGGAAGCC A UAUUCACA	4086
2475	GGTGTGAA GGCTAGCTACAACGA ATGGCTTC	3377	GAAGCCAU A UUCACACC	4087
2479	GTGAGGTG GGCTAGCTACAACGA GAATATGG	3378	CCAUAUUC A CACCUCAC	4088
2481	GCGTGAGG GGCTAGCTACAACGA GTGAATAT	3379	AUAUUCAC A CCUCACGC	4089
2486	CCAGAGCG GGCTAGCTACAACGA GAGGTGTG	3380	CACACCUC A CGCUCUGG	4090
2488	GTCCAGAG GGCTAGCTACAACGA GTGAGGTG	3381	CACCUCAC G CUCUGGAC	4091
2495	AAATCATG GGCTAGCTACAACGA CCAGAGCG	3382	CGCUCUGG A CAUGAUUU	4092
2497	CTAAATCA GGCTAGCTACAACGA GTCCAGAG	3383	CUCUGGAC A UGAUUUAG	4093
2500	TCCCTAAA GGCTAGCTACAACGA CATGTCCA	3384	UGGACAUG A UUUAGGGA	4094
2510	TGTCCCTG GGCTAGCTACAACGA TTCCCTAA	3385	UUAGGGAA G CAGGGACA	4095
2516	GGGGGGTG GGCTAGCTACAACGA CCCTGCTT	3386	AAGCAGGG A CACCCCCC	4096
2518	GCGGGGGG GGCTAGCTACAACGA GTCCCTGC	3387	GCAGGGAC A CCCCCCGC	4097
2525	GTGGGGGG GGCTAGCTACAACGA GGGGGGTG	3388	CACCCCCC G CCCCCCAC	4098
2532	CCCAAAGG GGCTAGCTACAACGA GGGGGGCG	3389	CGCCCCCC A CCUUUGGG	4099

Table 6

2541	GAGGCTGA	GGCTAGCTACAACGA	CCCAAAGG	3390	CCUUUGG A UCAGCCUC	4100
2545	GGCGGAGG	GGCTAGCTACAACGA	TGATCCCA	3391	UGGGAUCA G CCUCCGCC	4101
2551	TGGAATGG	GGCTAGCTACAACGA	GGAGGCTG	3392	CAGCCUCC G CCAUUCCA	4102
2554	ACTTGGAA	GGCTAGCTACAACGA	GGCGGAGG	3393	CCUCCGCC A UUCAAGU	4103
2561	AGTGTCGA	GGCTAGCTACAACGA	TTGGAATG	3394	CAUCCCAA G UCGACACU	4104
2565	GAAGAGTG	GGCTAGCTACAACGA	CGACTTGG	3395	CCAAGUCG A CACUCUUC	4105
2567	AAGAAGAG	GGCTAGCTACAACGA	GTCGACTT	3396	AAGUCGAC A CUCUUCUU	4106
2578	ACGGTCTG	GGCTAGCTACAACGA	TCAAGAAG	3397	CUUCUUGA G CAGACCGU	4107
2582	AATCACGG	GGCTAGCTACAACGA	CTGCTCAA	3398	UUGAGCAG A CCGUGAUU	4108
2585	CCAAATCA	GGCTAGCTACAACGA	GGTCTGCT	3399	AGCAGACC G UGAUUUGG	4109
2588	CTTCCAAA	GGCTAGCTACAACGA	CACGGTCT	3400	AGACCGUG A UUUGGAAG	4110
2601	AGCAGGTG	GGCTAGCTACAACGA	CTCTCTTC	3401	GAAGAGAG G CACCUGCU	4111
2603	CCAGCAGG	GGCTAGCTACAACGA	GCCTCTCT	3402	AGAGAGGC A CCUGCUGG	4112
2607	GTTTCCAG	GGCTAGCTACAACGA	AGGTGCCT	3403	AGGCACCU G CUGGAAAC	4113
2614	AAGTGTGG	GGCTAGCTACAACGA	TTCAGCA	3404	UGCUGGAA A CCACACUU	4114
2617	AAGAAAGT	GGCTAGCTACAACGA	GGTTTCCA	3405	UGGAAACC A CACUUCUU	4115
2619	TCAAGAAG	GGCTAGCTACAACGA	GTGGTTTC	3406	GAAACCAC A CUUCUUGA	4116
2629	CCAGGCTG	GGCTAGCTACAACGA	TTCAGAA	3407	UUCUUGAA A CAGCCUGG	4117
2632	CACCCAGG	GGCTAGCTACAACGA	TGTTTCAA	3408	UUGAAACA G CCUGGGUG	4118
2638	GACCGTCA	GGCTAGCTACAACGA	CCAGGCTG	3409	CAGCCUGG G UGACGGUC	4119
2641	AAGGACCG	GGCTAGCTACAACGA	CACCCAGG	3410	CCUGGGUG A CGGUCCUU	4120
2644	CTAAAGGA	GGCTAGCTACAACGA	CGTCACCC	3411	GGGUGACG G UCCUUUAG	4121
2653	GCAGGCTG	GGCTAGCTACAACGA	CTAAAGGA	3412	UCCUUUAG G CAGCCUGC	4122
2656	GCGGCAGG	GGCTAGCTACAACGA	TGCCTAAA	3413	UUUAGGCA G CCUGCCGC	4123
2660	GACGGCGG	GGCTAGCTACAACGA	AGGCTGCC	3414	GGCAGCCU G CCGCCGUC	4124
2663	AGAGACGG	GGCTAGCTACAACGA	GGCAGGCT	3415	AGCCUGCC G CCGUCUCU	4125
2666	GACAGAGA	GGCTAGCTACAACGA	GGCGGCAG	3416	CUGCCGCC G UCUCUGUC	4126
2672	AACCGGGA	GGCTAGCTACAACGA	AGAGACGG	3417	CCGUCUCU G UCCCGGUU	4127
2678	AAGGTGAA	GGCTAGCTACAACGA	CGGGACAG	3418	CUGUCCCG G UUCACCUU	4128
2682	CGGCAAGG	GGCTAGCTACAACGA	GAACCGGG	3419	CCCGGUUC A CCUUGCCG	4129
2687	TCTCTCGG	GGCTAGCTACAACGA	AAGGTGAA	3420	UUCACCUU G CCGAGAGA	4130

Table 6

2697	CAGACGGG	GGCTAGCTACAACGA	CTCTCTCG	3421	CGAGAGAG	G CGGUCUG	4131
2699	GGCAGACG	GGCTAGCTACAACGA	GCCTCTCT	3422	AGAGAGGC	G CGUCUGCC	4132
2701	GGGGCAGA	GGCTAGCTACAACGA	GCGCCTCT	3423	AGAGGCGC	G UCUGCCCC	4133
2705	GGGTGGGG	GGCTAGCTACAACGA	AGACGGCG	3424	GCGCGUCU	G CCCCACCC	4134
2710	TTTGAGGG	GGCTAGCTACAACGA	GGGGCAGA	3425	UCUGCCCC	A CCCUCAAA	4135
2718	CCACAGGG	GGCTAGCTACAACGA	TTGAGGGT	3426	ACCCUCA	A CCCUGUGG	4136
2723	AGGCCCCA	GGCTAGCTACAACGA	AGGGTTTG	3427	CAAACCCU	G UGGGGCCU	4137
2728	CCATCAGG	GGCTAGCTACAACGA	CCCACAGG	3428	CCUGUGGG	G CCUGAUGG	4138
2733	GAGCACCA	GGCTAGCTACAACGA	CAGGCCCC	3429	GGGGCCUG	A UGGUGCUC	4139
2736	CGTGAGCA	GGCTAGCTACAACGA	CATCAGGC	3430	GCCUGAUG	G UGCUCACG	4140
2738	GTCGTGAG	GGCTAGCTACAACGA	ACCATCAG	3431	CUGAUGGU	G CUCACGAC	4141
2742	AAGAGTCG	GGCTAGCTACAACGA	GAGCACCA	3432	UGGUGCUC	A CGACUCUU	4142
2745	AGGAAGAG	GGCTAGCTACAACGA	CGTGAGCA	3433	UGCUCACG	A CUCUUCCU	4143
2754	TCCCTTTG	GGCTAGCTACAACGA	AGGAAGAG	3434	CUCUUCCU	G CAAAAGGA	4144
2763	GTCCTTCAG	GGCTAGCTACAACGA	TCCCTTTG	3435	CAAAGGGA	A CUGAAGAC	4145
2770	TGTGGAGG	GGCTAGCTACAACGA	CTTCAGTT	3436	AACUGAAG	A CCUCCACA	4146
2776	ACTTAATG	GGCTAGCTACAACGA	GGAGTCT	3437	AGACCUC	A CAUUAAGU	4147
2778	CCACTTAA	GGCTAGCTACAACGA	GTGGAGGT	3438	ACCUCCAC	A UUAAGUGG	4148
2783	AAAAGCCA	GGCTAGCTACAACGA	TTAATGTG	3439	CACAUUAA	G UGGCUUUU	4149
2786	TTAAAAG	GGCTAGCTACAACGA	CACCTAAT	3440	AUUAAGUG	G CUUUUUAA	4150
2794	TTTTTCATG	GGCTAGCTACAACGA	TAAAAGC	3441	GCUUUUUA	A CAUGAAAA	4151
2796	GTTTTTCA	GGCTAGCTACAACGA	GTAAAAA	3442	UUUUUAAC	A UGAAAAAC	4152
2803	CTGCCGTG	GGCTAGCTACAACGA	TTTTTCATG	3443	CAUGAAAA	A CACGGCAG	4153
2805	AGCTGCCG	GGCTAGCTACAACGA	GTTTTTCA	3444	UGAAAAAC	A CGGCAGCU	4154
2808	TACAGCTG	GGCTAGCTACAACGA	CGTGTTTT	3445	AAAACACG	G CAGCUGUA	4155
2811	AGCTACAG	GGCTAGCTACAACGA	TGCCGTGT	3446	ACACGGCA	G CUGUAGCU	4156
2814	GGGAGCTA	GGCTAGCTACAACGA	AGCTGCCG	3447	CGGCAGCU	G UAGCUCCC	4157
2817	CTCGGGAG	GGCTAGCTACAACGA	TACAGCTG	3448	CAGCUGUA	G CUCCCCGAG	4158
2825	GAGAGTAG	GGCTAGCTACAACGA	TCGGGAGC	3449	GCUCCCGA	G CUACUCUC	4159
2828	CAAGAGAG	GGCTAGCTACAACGA	AGCTCGGG	3450	CCCGAGCU	A CUCUCUUG	4160
2836	AATGCTGG	GGCTAGCTACAACGA	AAGAGAGT	3451	ACUCUCUU	G CCAGCAUU	4161

Table 6

2840	TGAAAAATG	GGCTAGCTACAACGA	TGGCAAGA	3452	UCUUGCCA G CAUUUUA	4162
2842	TGTGAAA	GGCTAGCTACAACGA	GCTGGCAA	3453	UUGCCAGC A UUUUCACA	4163
2848	GCAAAATG	GGCTAGCTACAACGA	GAAAATGC	3454	GCAUUUC A CAUUUUGC	4164
2850	AGGCAAAA	GGCTAGCTACAACGA	GTGAAAAT	3455	AUUUUCAC A UUUUGCCU	4165
2855	GAGAAAGG	GGCTAGCTACAACGA	AAAATGTG	3456	CACAUUUU G CCUUUCUC	4166
2864	TTCTACCA	GGCTAGCTACAACGA	GAGAAAGG	3457	CCUUUCUC G UGGUAGAA	4167
2867	GGCTTCTA	GGCTAGCTACAACGA	CACGAGAA	3458	UUCUCGUG G UAGAAGCC	4168
2873	TGTACTGG	GGCTAGCTACAACGA	TTCTACCA	3459	UGGUAGAA G CCAGUACA	4169
2877	TCTCTGTA	GGCTAGCTACAACGA	TGGCTTCT	3460	AGAAGCCA G UACAGAGA	4170
2879	TTTCTCTG	GGCTAGCTACAACGA	ACTGGCTT	3461	AAGCCAGU A CAGAGAAA	4171
2887	CCACAGAA	GGCTAGCTACAACGA	TTCTCTGT	3462	ACAGAGAA A UUCUGUGG	4172
2892	TCCCACCA	GGCTAGCTACAACGA	AGAATTTC	3463	GAAAUUCU G UGGUGGGA	4173
2895	TGTTCCCA	GGCTAGCTACAACGA	CACAGAA	3464	AUUCUGUG G UGGGAACA	4174
2901	CTCGAATG	GGCTAGCTACAACGA	TCCCACCA	3465	UGGUGGGA A CAUUCGAG	4175
2903	ACCTCGAA	GGCTAGCTACAACGA	GTTCCAC	3466	GUGGGAAC A UUCGAGGU	4176
2910	GGGTGACA	GGCTAGCTACAACGA	CTCGAATG	3467	CAUUCGAG G UGUCACCC	4177
2912	CAGGGTGA	GGCTAGCTACAACGA	ACCTCGAA	3468	UUCGAGGU G UCACCCUG	4178
2915	CTGCAGGG	GGCTAGCTACAACGA	GACACCTC	3469	GAGGUGUC A CCCUGCAG	4179
2920	TAGCTCTG	GGCTAGCTACAACGA	AGGGTGAC	3470	GUCACCCU G CAGAGCUA	4180
2925	CACCATAG	GGCTAGCTACAACGA	TCTGCAGG	3471	CCUGCAGA G CUAUGGUG	4181
2928	CCTCACCA	GGCTAGCTACAACGA	AGCTCTGC	3472	GCAGAGCU A UGGUGAGG	4182
2931	ACACCTCA	GGCTAGCTACAACGA	CATAGCTC	3473	GAGCUAUG G UGAGGUGU	4183
2936	TATCCACA	GGCTAGCTACAACGA	CTCACCAT	3474	AUGGUGAG G UGUGGAUA	4184
2938	CTTATCCA	GGCTAGCTACAACGA	ACCTCAC	3475	GGUGAGGU G UGGAUAAG	4185
2942	AAGCCTTA	GGCTAGCTACAACGA	CCACACCT	3476	AGGUGUGG A UAAAGGCUU	4186
2947	CACCTAAG	GGCTAGCTACAACGA	CTTATCCA	3477	UGGAUAAG G CUUAGGUG	4187
2953	GCCTGGCA	GGCTAGCTACAACGA	CTAAGCCT	3478	AGGCUUAG G UGCCAGGC	4188
2955	CAGCCTGG	GGCTAGCTACAACGA	ACCTAAGC	3479	GCUUAGGU G CCAGGCUG	4189
2960	GCTTACAG	GGCTAGCTACAACGA	CTGGCACC	3480	GGUGCCAG G CUGUAAGC	4190
2963	AATGCTTA	GGCTAGCTACAACGA	AGCCTGGC	3481	GCCAGGCU G UAAGCAUU	4191
2967	TCAGAATG	GGCTAGCTACAACGA	TTACAGCC	3482	GGCUGUAA G CAUUCUGA	4192

Table 6

2969	GCTCAGAA	GGCTAGCTACAACGA	GCTTACAG	3483	CUGUAAGC A UUCUGAGC	4193
2976	CAAGCCAG	GGCTAGCTACAACGA	TCAGAATG	3484	CAUUCUGA G CUGGCUUG	4194
2980	ACAACAAG	GGCTAGCTACAACGA	CAGCTCAG	3485	CUGAGCUG G CUUGUUUG	4195
2984	AAAAACAA	GGCTAGCTACAACGA	AAGCCAGC	3486	GCUGGCUU G UUGUUUUU	4196
2987	CTTAAAAA	GGCTAGCTACAACGA	AACAAGCC	3487	GGCUUGUU G UUUUUAAG	4197
2995	ATACAGGA	GGCTAGCTACAACGA	TAAAAAAC	3488	GUUUUUAA G UCCUGUAA	4198
3000	TACATATA	GGCTAGCTACAACGA	AGGACTTA	3489	UAAGUCCU G UAUUAUGA	4199
3002	CATACATA	GGCTAGCTACAACGA	ACAGGACT	3490	AGUCCUGU A UAUGUAUG	4200
3004	TACATACA	GGCTAGCTACAACGA	ATACAGGA	3491	UCCUGUAA A UGUAUGUA	4201
3006	ACTACATA	GGCTAGCTACAACGA	ATATACAG	3492	CUGUAUAA G UAUGUAUG	4202
3008	CTACTACA	GGCTAGCTACAACGA	ACATATAC	3493	GUUAUUGU A UGUAGUAG	4203
3010	AACATACTA	GGCTAGCTACAACGA	ATACATAT	3494	AUAUGUAA G UAGUAGUU	4204
3013	CCAAACTA	GGCTAGCTACAACGA	TACATACA	3495	UGUAUGUA G UAGUUUGG	4205
3016	CACCCAAA	GGCTAGCTACAACGA	TACTACAT	3496	AUGUAUGA G UUUGGGUG	4206
3022	TATACACA	GGCTAGCTACAACGA	CCAAACTA	3497	UAGUUUGG G UGUGUAUA	4207
3024	TATATACA	GGCTAGCTACAACGA	ACCCAAAC	3498	GUUUGGGU G UGUAAUUA	4208
3026	TATATATA	GGCTAGCTACAACGA	ACACCCAA	3499	UUGGGUGU G UAUUAUUA	4209
3028	ACTATATA	GGCTAGCTACAACGA	ACACACCC	3500	GGGUGUGU A UAUUAUAG	4210
3030	CTACTATA	GGCTAGCTACAACGA	ATACACAC	3501	GUGUGUAA A UAUAGUAG	4211
3032	TGCTACTA	GGCTAGCTACAACGA	ATATACAC	3502	GUGUAUAA A UAGUAGCA	4212
3035	AAATGCTA	GGCTAGCTACAACGA	TATATATA	3503	UAUAUUA G UAGCAUUU	4213
3038	TTGAAATG	GGCTAGCTACAACGA	TACTATAT	3504	AUAUAGUA G CAUUUCAAA	4214
3040	TTTTTGAA	GGCTAGCTACAACGA	GCTACTAT	3505	AUAGUAGC A UUUCAAAA	4215
3048	TACGTCCA	GGCTAGCTACAACGA	TTTGAAAT	3506	AUUUCAAA A UGGACGUA	4216
3052	CCAGTACG	GGCTAGCTACAACGA	CCATTTTG	3507	CAAAUUGG A CGUACUGG	4217
3054	AACCAAGTA	GGCTAGCTACAACGA	GTCCATTT	3508	AAAUUGGAC G UACUGGUU	4218
3056	TAAACCAG	GGCTAGCTACAACGA	ACGTCCAT	3509	AUGGACGU A CUGGUUUA	4219
3060	AGGTTAAA	GGCTAGCTACAACGA	CAGTACGT	3510	ACGUACUG G UUUAAACCU	4220
3065	ATAGGAGG	GGCTAGCTACAACGA	TAAACCAG	3511	CUGGUUUA A CCUCCUAA	4221
3072	TCCAAGGA	GGCTAGCTACAACGA	AGGAGGTT	3512	AACCUCCU A UCCUUGGA	4222
3083	GCCAGCTG	GGCTAGCTACAACGA	TCTCCAAG	3513	CUUGGAGA G CAGCUGGC	4223

Table 6

3086	AGAGCCAG	GGCTAGCTACAACGA	TGCTCTCC	3514	GGAGAGCA	G	CUGGCUCU	4224
3090	GTGGAGAG	GGCTAGCTACAACGA	CAGCTGCT	3515	AGCAGCUG	G	CUCUCCAC	4225
3097	TAAACAAGG	GGCTAGCTACAACGA	GGAGAGCC	3516	GGCUCUCC	A	CCUUGUUA	4226
3102	ATGTGTAA	GGCTAGCTACAACGA	AAGGTGGA	3517	UCCACCCU	G	UUACACAU	4227
3105	ATAATGTG	GGCTAGCTACAACGA	AACAAGGT	3518	ACCUUGUU	A	CACAUUAU	4228
3107	ACATAATG	GGCTAGCTACAACGA	GTAACAAG	3519	CUUGUUAC	A	CAUUAUGU	4229
3109	TAAACATAA	GGCTAGCTACAACGA	GTGTAACA	3520	UGUUACAC	A	UUAUGUUA	4230
3112	CTCTAACA	GGCTAGCTACAACGA	AATGTGTA	3521	UACACAUU	A	UGUUAGAG	4231
3114	CTCTCTAA	GGCTAGCTACAACGA	ATAATGTG	3522	CACAUUAU	G	UUAGAGAG	4232
3123	GCTCGCTA	GGCTAGCTACAACGA	CTCTCTAA	3523	UUAGAGAG	G	UAGCGAGC	4233
3126	GCAGCTCG	GGCTAGCTACAACGA	TACCTCTC	3524	GAGAGGUA	G	CGAGCUGC	4234
3130	CAGAGCAG	GGCTAGCTACAACGA	TCGCTACC	3525	GGUAGCGA	G	CUGCUCUG	4235
3133	TAGCAGAG	GGCTAGCTACAACGA	AGCTCGCT	3526	AGCGAGCU	G	CUCUGCUA	4236
3138	GGACATAG	GGCTAGCTACAACGA	AGAGCAGC	3527	GCUGCUCU	G	CUAUGUCC	4237
3141	TAAGGACA	GGCTAGCTACAACGA	AGCAGAGC	3528	GCUCUGCU	A	UGUCCUUA	4238
3143	CTTAAGGA	GGCTAGCTACAACGA	ATAGCAGA	3529	UCUGCUAU	G	UCCUUAAG	4239
3151	AATATTGG	GGCTAGCTACAACGA	TTAAGGAC	3530	GUCCUUAU	G	CCAUAUUA	4240
3155	AGTAAATA	GGCTAGCTACAACGA	TGGCTTAA	3531	UUAAGCCA	A	UAUUUACU	4241
3157	TGAGTAAA	GGCTAGCTACAACGA	ATTGGCTT	3532	AAGCCAAU	A	UUUACUCA	4242
3161	CTGATGAG	GGCTAGCTACAACGA	AAATATTG	3533	CAAUAUUU	A	CUCAUCAG	4243
3165	TGACCTGA	GGCTAGCTACAACGA	GAGTAAAT	3534	AUUUACUC	A	UCAGGUCA	4244
3170	AATAATGA	GGCTAGCTACAACGA	CTGATGAG	3535	CUCAUCAG	G	UCAUUUAU	4245
3173	AAAAATAA	GGCTAGCTACAACGA	GACCTGAT	3536	AUCAGGUC	A	UUUUUUUU	4246
3176	GTAATAAA	GGCTAGCTACAACGA	AATGACCT	3537	AGGUCAUU	A	UUUUUUUAC	4247
3183	GGCCATTG	GGCTAGCTACAACGA	AAAAAATA	3538	UAUUUUUU	A	CAAUGGCC	4248
3186	CATGGCCA	GGCTAGCTACAACGA	TGTAAAAA	3539	UUUUUACA	A	UGGCCAUG	4249
3189	TTCCATGG	GGCTAGCTACAACGA	CATTGTAA	3540	UUACAAUG	G	CCAUGGAA	4250
3192	TTATTCCA	GGCTAGCTACAACGA	GGCCATTG	3541	CAAUGGCC	A	UGGAAUAA	4251
3197	ATGGTTTA	GGCTAGCTACAACGA	TCCATGGC	3542	GCCAUGGA	A	UAAACCAU	4252
3201	AAAAATGG	GGCTAGCTACAACGA	TTATTCCA	3543	UGGAAUAA	A	CCAUUUUU	4253
3204	TGTAAAAA	GGCTAGCTACAACGA	GGTTTATT	3544	AAUAAACC	A	UUUUUACA	4254

Table 7

Table 7: Human PTP-1B Hairpin Ribozyme and Target Sequence

Nt. Position	Ribozyme sequence		Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
10	GCUCUA	AGAA GCGU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4255	ACGC GGCC TAGAGC	4331
23	UGCGCC	AGAA GCCG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4256	CGGC AGAC GCGGCA	4332
56	CAGGGC	AGAA GCUG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4257	CAGC AGCC GCCCTG	4333
59	GGCCAG	AGAA GCUG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4258	CAGC CGCC CTGGCC	4334
98	UUGUCG	AGAA GCUC ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4259	GAGC AGAT CGACAA	4335
220	CAAAGG	AGAA GACG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4260	CGTC AGTC CCTTTG	4336
239	AGUUUA	AGAA GACU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4261	AGTC GGAT TAAACT	4337
612	UCCAAA	AGAA GGCC ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4262	GGCC TGAC TTTTGA	4338
636	GAAUGA	AGAA GGUG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4263	CACC AGCC TCATTG	4339
685	GCUCCG	AGAA GAGU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4264	ACTC AGCC CGGAGC	4340
702	CACCAC	AGAA GGCC ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4265	GGCC CGTT GTGGTG	4341
748	CAGCCA	AGAA GAAG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4266	CTTC TGTC TGGCTG	4342
763	GCAAGA	AGAA GGUA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4267	TACC TGCC TCTTGC	4343
773	UUGUCC	AGAA GCAA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4268	TTGC TGAT GGACAA	4344
801	GAUAUC	AGAA GAAG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4269	CTTC CGTT GATATC	4345
842	AGCCCC	AGAA GAAA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4270	TTTC GGAT GGGGCT	4346
851	GUCUGG	AGAA GCCC ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4271	GGGC TGAT CCAGAC	4347
861	CUGGUC	AGAA GUCU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4272	AGAC AGCC GACCAG	4348
864	CAGCUG	AGAA GCUG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4273	CAGC CGAC CAGCTG	4349
869	AAGCGC	AGAA GGUC ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4274	GACC AGCT GCGCTT	4350
1102	UGAUGG	AGAA GUCU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4275	AGAC TGCC CCATCA	4351
1224	UGGGGA	AGAA GCCU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4276	AGGC TGCC TCCCCA	4352
1253	UUCUCG	AGAA GUGA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4277	TCAC TGCC CGAGAA	4353
1323	CGUGAG	AGAA GUAG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4278	CTAC GGTC CTCACG	4354
1332	AGCGCC	AGAA GUGA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4279	TCAC GGCC GCGGCT	4355
1361	CUGUUG	AGAA GGAA ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4280	TTCC TGTT CAACAG	4356
1383	AGGAGG	AGAA GGCU ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4281	AGCC TGAC CCTCCT	4357
1412	GAGGCG	AGAA GUGG ACCAGAGAAAACA X GUACAUAUACCUUGGUA	4282	CCAC TGTC CGCCTC	4358

Table 7

1416	GGCAGA AGAA GACA ACCAGAGAAAACA X GUACAUUACCUGGUA	4283	TGTC CGCC TCTGCC	4359
1422	UCUGCG AGAA GAGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4284	CCTC TGCC CGCAGA	4360
1441	CUGCUA AGAA GCGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4285	CGCC CGAC TAGCAG	4361
1460	CUUACC AGAA GCGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4286	CCGC GGTA GGTAAAG	4362
1473	CGGUCC AGAA GCCC ACCAGAGAAAACA X GUACAUUACCUGGUA	4287	GGGC CGCC GGACCCG	4363
1477	UACGCG AGAA GCGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4288	CGCC GGAC CGCGTA	4364
1501	ACGUCC AGAA GGGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4289	CCCC GGAC GGACGT	4365
1505	ACCAAC AGAA GUCC ACCAGAGAAAACA X GUACAUUACCUGGUA	4290	GGAC GGAC GTTGGT	4366
1539	ACACAC AGAA GGGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4291	CCCC GGAT GTGTGT	4367
1670	CCCCAA AGAA GGUG ACCAGAGAAAACA X GUACAUUACCUGGUA	4292	CACC CGTC TTGGGG	4368
1735	GGGGGG AGAA GUCU ACCAGAGAAAACA X GUACAUUACCUGGUA	4293	AGAC AGCC CCCCCC	4369
1864	AUGCAA AGAA GACA ACCAGAGAAAACA X GUACAUUACCUGGUA	4294	TGTC AGCC TTGCAT	4370
1946	UGACGC AGAA GGCC ACCAGAGAAAACA X GUACAUUACCUGGUA	4295	GGCC TGCT GCGTCA	4371
1955	GUACUG AGAA GACG ACCAGAGAAAACA X GUACAUUACCUGGUA	4296	CGTC AGAC CAGTAC	4372
1960	UCCCG AGAA GGUC ACCAGAGAAAACA X GUACAUUACCUGGUA	4297	GACC AGTA CTGGGA	4373
1978	GCUUAC AGAA GUCC ACCAGAGAAAACA X GUACAUUACCUGGUA	4298	GGAC GGTT GTAAGC	4374
1988	AAUAAAC AGAA GCUU ACCAGAGAAAACA X GUACAUUACCUGGUA	4299	AAGC AGTT GTTATT	4375
2098	AGAAUA AGAA GGAC ACCAGAGAAAACA X GUACAUUACCUGGUA	4300	GTCC CGCT TATTCT	4376
2107	ACAGGG AGAA GAAU ACCAGAGAAAACA X GUACAUUACCUGGUA	4301	ATTG TGCT CCCTGT	4377
2144	CGGGGG AGAA GUGA ACCAGAGAAAACA X GUACAUUACCUGGUA	4302	TCAC TGCT CCCCCG	4378
2190	UUUUUC AGAA GGAC ACCAGAGAAAACA X GUACAUUACCUGGUA	4303	GTCC TGAT GAAAAA	4379
2230	UUAGUA AGAA GAGA ACCAGAGAAAACA X GUACAUUACCUGGUA	4304	TCTC TGCT TACTAA	4380
2264	UGCACA AGAA GGUU ACCAGAGAAAACA X GUACAUUACCUGGUA	4305	AACC TGCC TGTGCA	4381
2279	GUAAUG AGAA GGUC ACCAGAGAAAACA X GUACAUUACCUGGUA	4306	GACC TGAT CATTAC	4382
2309	UUCAGC AGAA GGCU ACCAGAGAAAACA X GUACAUUACCUGGUA	4307	AGCC TGTG GCTGAA	4383
2345	CUGGAA AGAA GCAC ACCAGAGAAAACA X GUACAUUACCUGGUA	4308	GTGC AGTT TTCCAG	4384
2412	AGGACA AGAA GGGC ACCAGAGAAAACA X GUACAUUACCUGGUA	4309	GCCC AGCC TGTCCT	4385
2416	UACCAG AGAA GGCU ACCAGAGAAAACA X GUACAUUACCUGGUA	4310	AGCC TGTC CTGGTA	4386
2524	GUGGGG AGAA GGGG ACCAGAGAAAACA X GUACAUUACCUGGUA	4311	CCCC CGCC CCCCAC	4387
2544	GGCGGA AGAA GAUC ACCAGAGAAAACA X GUACAUUACCUGGUA	4312	GATC AGCC TCCGCC	4388
2580	AUCACG AGAA GCUC ACCAGAGAAAACA X GUACAUUACCUGGUA	4313	GAGC AGAC CGTGAT	4389

Table 7

2606	GUUUC AGAA GGUG ACCAGAGAAACA X GUACAUUACCUUGUA	4314	CACC TGCT GGAAAC	4390
2631	CACCCA AGAA GUUU ACCAGAGAAACA X GUACAUUACCUUGUA	4315	AAAC AGCC TGGGTG	4391
2643	CUAAAG AGAA GUCA ACCAGAGAAACA X GUACAUUACCUUGUA	4316	TGAC GGTC CTTTAG	4392
2655	GCGGCA AGAA GCCU ACCAGAGAAACA X GUACAUUACCUUGUA	4317	AGGC AGCC TGCCGC	4393
2659	GACGGC AGAA GGCU ACCAGAGAAACA X GUACAUUACCUUGUA	4318	AGCC TGCC GCCGTC	4394
2662	AGAGAC AGAA GCAG ACCAGAGAAACA X GUACAUUACCUUGUA	4319	CTGC CGCC GTCCTC	4395
2665	GACAGA AGAA GCGG ACCAGAGAAACA X GUACAUUACCUUGUA	4320	CCGC CGTC TCTGTC	4396
2671	AACCGG AGAA GAGA ACCAGAGAAACA X GUACAUUACCUUGUA	4321	TCTC TGTC CCGGTT	4397
2677	AAGGUG AGAA GGGG ACCAGAGAAACA X GUACAUUACCUUGUA	4322	TCCC GGTT CACCTT	4398
2704	GGGUGG AGAA GACG ACCAGAGAAACA X GUACAUUACCUUGUA	4323	CGTC TGCC CCACCC	4399
2731	AGCACC AGAA GGCC ACCAGAGAAACA X GUACAUUACCUUGUA	4324	GGCC TGAT GGTGCT	4400
2810	AGCUAC AGAA GCCG ACCAGAGAAACA X GUACAUUACCUUGUA	4325	CGGC AGCT GTAGCT	4401
2813	GGGAGC AGAA GCUG ACCAGAGAAACA X GUACAUUACCUUGUA	4326	CAGC TGTA GCTCCC	4402
2876	UCUCUG AGAA GGCU ACCAGAGAAACA X GUACAUUACCUUGUA	4327	AGCC AGTA CAGAGA	4403
2999	UACAUU AGAA GGAC ACCAGAGAAACA X GUACAUUACCUUGUA	4328	GTCC TGTA TATGTA	4404
3085	AGAGCC AGAA GCUC ACCAGAGAAACA X GUACAUUACCUUGUA	4329	GAGC AGCT GGCTCT	4405
3132	UAGCAG AGAA GCUC ACCAGAGAAACA X GUACAUUACCUUGUA	4330	GAGC TGCT CTGCTA	4406

Table 8

Table 8: Anti Human PTP-1B HH, NCH, and G Cleaver Ribozymes

Alias	Ribozyme Sequence	Seq. ID Nos	Substrate Seq.	Seq. ID Nos.
HH				
PTP1B-599	UGUGGUA CUGAUGAGGCCCGUAGGCCGAA AGUGGAA	4407	UUCCACU A UACCACA	4425
PTP1B-879	GGUAGGA CUGAUGAGGCCCGUAGGCCGAA AAGCGCA	4408	UGCGCUU C UCCUACC	4426
PTP1B-1393	UGGAGUG CUGAUGAGGCCCGUAGGCCGAA AGGAGGG	4409	CCCUCUU C CACUCCA	4427
PTP1B-1398	GGAGGUG CUGAUGAGGCCCGUAGGCCGAA AGUGGAG	4410	CUCCACU C CACCUCU	4428
PTP1B-1404	GUGGGUG CUGAUGAGGCCCGUAGGCCGAA AGGUGGA	4411	UCCACCU C CACCCAC	4429
PTP1B-2118	UAGCAGA CUGAUGAGGCCCGUAGGCCGAA AACAGGG	4412	CCCUGUU A UCUGCUA	4430
PTP1B-2181	GACACAA CUGAUGAGGCCCGUAGGCCGAA AAGACCU	4413	AGGUCUU C UUGUGUC	4431
PTP1B-2183	AGGACAC CUGAUGAGGCCCGUAGGCCGAA AGAAGAC	4414	GUCUUCU U GUGUCCU	4432
PTP1B-2238	GGCACAU CUGAUGAGGCCCGUAGGCCGAA AGUAAGC	4415	GCUUACU A AUGUGCC	4433
PTP1B-2252	GGACUUG CUGAUGAGGCCCGUAGGCCGAA ACAUGGG	4416	CCCAUGU C CAAGUCC	4434
NCH				
PTP1B-1395	GGUGGAG CUGAUGAGGCCCGUAGGCCGAA IGAGGAG	4417	CUCCUCC A CUCCACC	4435
PTP1B-1408	GACAGUG CUGAUGAGGCCCGUAGGCCGAA IUGGAGG	4418	CCUCCAC C CACUGUC	4436
PTP1B-1555	GGAUGAG CUGAUGAGGCCCGUAGGCCGAA IGUGAGA	4419	UCUCACC C CUCAUCC	4437
PTP1B-1578	AGUGGAA CUGAUGAGGCCCGUAGGCCGAA IGGCAAA	4420	UUUGCCC C UUCCACU	4438
PTP1B-2113	GAUAAAC CUGAUGAGGCCCGUAGGCCGAA IGAGCAG	4421	CUGCUCC C UGUUAUC	4439
G-Cleaver				
PTP1B-1267	GUCCU UGAUGGCAUGCACUAUGCGG GUCCUUCUCG	4422	CGAGAAAGGAC G AGGAC	4440
PTP1B-2184	GGACA UGAUGGCAUGCACUAUGCGG AAGAAGACCU	4423	AGGUUUCUU G UGUCC	4441
PTP1B-2241	GGGCA UGAUGGCAUGCACUAUGCGG AUUAGUAAGC	4424	GCUUACUAAU G UGCCC	4442

Table 9

Table 9: Human methionine aminopeptidase type 2 (Met AP-2) Hammerhead Ribozyme and Target Sequence

Nt. position	Ribozyme Sequence	Seq ID nos.	Substrate Sequence	Seq ID nos.
9	CCGAGAGA CUGAUGAG X CGAA ACGAGGGA	1	TCCCTCGT C TCTCTCGG	413
11	GCCCGAGA CUGAUGAG X CGAA AGACGAGG	2	CCTCGTCT C TCTCGGGC	414
13	UUGCCCGA CUGAUGAG X CGAA AGAGACGA	3	TCGTCTCT C TCGGGCAA	415
15	UGUUGCCC CUGAUGAG X CGAA AGAGAGAC	4	GTCTCTCT C GGGCAACA	416
43	GAGGCCGC CUGAUGAG X CGAA ACCUCCUC	5	GAGGAGGT A GCGGCCTC	417
51	GGCUCCCG CUGAUGAG X CGAA AGGCCGCU	6	AGCGGCCT C CGGGAGCC	418
80	GUCGUCUG CUGAUGAG X CGAA AUCCAGGU	7	ACCTGGAT C CAGACGAC	419
108	CAGCCGUA CUGAUGAG X CGAA AGGCAGCU	8	AGCTGCCT C TACGGCTG	420
110	CUCAGCCG CUGAUGAG X CGAA AGAGGCAG	9	CTGCCTCT A CGGCTGAG	421
167	UGCUGCAG CUGAUGAG X CGAA AGGCCCUU	10	AAGGGCCT T CTGCAGCA	422
168	CUGCUGCA CUGAUGAG X CGAA AAGGCCCU	11	AGGGCCTT C TGCAGCAG	423
194	UGAUUCUU CUGAUGAG X CGAA AUCAGGUU	12	AACCTGAT A AAGAATCA	424
201	AGGCUCCU CUGAUGAG X CGAA AUUCUUUA	13	TAAAGAAT C AGGAGCCT	425
210	CAUCCACU CUGAUGAG X CGAA AGGCUCCU	14	AGGAGCCT C AGTGGATG	426
223	UGUCUUGC CUGAUGAG X CGAA ACUUCAUC	15	GATGAAGT A GCAAGACA	427
234	AUCUUUCC CUGAUGAG X CGAA ACUGUCUU	16	AAGACAGT T GGAAAGAT	428
243	CCAAUGCU CUGAUGAG X CGAA AUCUUUCC	17	GGAAAGAT C AGCATTGG	429
249	UAUCUUC CUGAUGAG X CGAA AUGCUGAU	18	ATCAGCAT T GGAAGATA	430
257	UCUUUCUU CUGAUGAG X CGAA AUCUCCA	19	TGGAAGAT A AAGAAAGA	431
355	UCUGUUUG CUGAUGAG X CGAA ACUUUUGG	20	CCAAAAGT T CAAACAGA	432
356	GUCUGUUU CUGAUGAG X CGAA AACUUUUG	21	CAAAAGTT C AAACAGAC	433
368	AACUGAGG CUGAUGAG X CGAA AGGGUCUG	22	CAGACCCT C CCTCAGTT	434
372	UUGGAACU CUGAUGAG X CGAA AGGGAGGG	23	CCCTCCCT C AGTTCCAA	435
376	CAUAUUGG CUGAUGAG X CGAA ACUGAGGG	24	CCCTCAGT T CCAATATG	436
377	ACAUAUUG CUGAUGAG X CGAA AACUGAGG	25	CCTCAGTT C CAATATGT	437
382	AGGUCACA CUGAUGAG X CGAA AUUGGAAC	26	GTTCCAAT A TGTGACCT	438
393	CAUVAGGA CUGAUGAG X CGAA ACAGGUCA	27	TGACCTGT A TCCTAATG	439
395	ACCAUUAG CUGAUGAG X CGAA AUACAGGU	28	ACCTGTAT C CTAATGGT	440
398	UACACCAU CUGAUGAG X CGAA AGGAUACA	29	TGTATCCT A ATGGTGTA	441
406	UUGGGAAA CUGAUGAG X CGAA ACACCAUU	30	AATGGTGT A TTTCCCAA	442
408	CUUUGGGA CUGAUGAG X CGAA AUACACCA	31	TGGTGTAT T TCCCAAAG	443
409	CCUUUGGG CUGAUGAG X CGAA AAUACACC	32	GGTGTATT T CCCAAAGG	444
410	UCCUUUGG CUGAUGAG X CGAA AAUACAC	33	GTGTATTT C CCAAAGGA	445
432	UGGGUGGG CUGAUGAG X CGAA AUUCGCAU	34	ATGCGAAT A CCCACCCA	446
464	AGUUCUCC CUGAUGAG X CGAA AGCAGCUG	35	CAGCTGCT T GGAGAACT	447
473	UUCACUUG CUGAUGAG X CGAA AGUUCUCC	36	GGAGAACT A CAAGTGAA	448
495	CCUGAUCU CUGAUGAG X CGAA AUGCUUUC	37	GAAAGCAT T AGATCAGG	449
496	GCCUGAUC CUGAUGAG X CGAA AAUGCUUU	38	AAAGCATT A GATCAGGC	450
500	ACUUGCCU CUGAUGAG X CGAA AUCUAAUG	39	CATTAGAT C AGGCAAGT	451
517	UCAUUCCA CUGAUGAG X CGAA AUCUCUUC	40	GAAGAGAT T TGGAATGA	452
518	AUCAUUC CUGAUGAG X CGAA AAUCUCUU	41	AAGAGATT T GGAATGAT	453
527	UUCUCGAA CUGAUGAG X CGAA AUCAUUC	42	GGAATGAT T TTCGAGAA	454

Table 9

528	CUUCUCGA CUGAUGAG X CGAA AAUCAUUC	43	GAATGATT T TCGAGAAG	455
529	GCUUCUCG CUGAUGAG X CGAA AAAUCAUU	44	AATGATTT T CGAGAAGC	456
530	AGCUUCUC CUGAUGAG X CGAA AAAAUCAU	45	ATGATTTT C GAGAAGCT	457
551	AACUUGUC CUGAUGAG X CGAA AUGUGCUU	46	AAGCACAT C GACAAGTT	458
559	UAUUUUCU CUGAUGAG X CGAA ACUUGUCG	47	CGACAAGT T AGAAAATA	459
560	GUAUUUUC CUGAUGAG X CGAA AACUUGUC	48	GACAAGTT A GAAAATAC	460
567	UCAUUACG CUGAUGAG X CGAA AUUUUCUA	49	TAGAAAAT A CGTAATGA	461
571	CAGCUCAU CUGAUGAG X CGAA ACGUAUUU	50	AAATACGT A ATGAGCTG	462
583	CCAGGCUU CUGAUGAG X CGAA AUCCAGCU	51	AGCTGGAT C AAGCCTGG	463
604	CAGAUUUC CUGAUGAG X CGAA AUCAUUGU	52	ACAATGAT A GAAATCTG	464
610	UUUUCACA CUGAUGAG X CGAA AUUUCUAU	53	ATAGAAAT C TGTGAAAA	465
621	AGUCUUC CUGAUGAG X CGAA ACUUUUCU	54	TGAAAAGT T GGAAGACT	466
632	CUUGCGUG CUGAUGAG X CGAA ACAGUCUU	55	AAGACTGT T CACGCAAG	467
633	ACUUGCGU CUGAUGAG X CGAA AACAGUCU	56	AGACTGTT C ACGCAAGT	468
642	CUUUUAUU CUGAUGAG X CGAA ACUUGCGU	57	ACGCAAGT T AATAAAG	469
643	UCUUUUUAU CUGAUGAG X CGAA AACUUGCG	58	CGCAAGTT A ATAAAAGA	470
646	UUCUCUUU CUGAUGAG X CGAA AUUAACUU	59	AAGTTAAT A AAAGAGAA	471
660	CUGCAUUU CUGAUGAG X CGAA AUCCAUUC	60	GAATGGAT T AAATGCAG	472
661	CCUGCAUU CUGAUGAG X CGAA AAUCCAUU	61	AATGGATT A AATGCAGG	473
678	CAGUAGGA CUGAUGAG X CGAA AUGCCAGG	62	CCTGGCAT T TCCTACTG	474
679	CCAGUAGG CUGAUGAG X CGAA AAUGCCAG	63	CTGGCATT T CCTACTGG	475
680	UCCAGUAG CUGAUGAG X CGAA AAAUGCCA	64	TGGCATTT C CTACTGGA	476
683	ACAUCCAG CUGAUGAG X CGAA AGGAAAUG	65	CATTTCTT A CTGGATGT	477
692	AUUGAGAG CUGAUGAG X CGAA ACAUCCAG	66	CTGGATGT T CTCTCAAT	478
693	UAUUGAGA CUGAUGAG X CGAA ACAUCCA	67	TGGATGTT C TCTCAATA	479
695	AUUAUUGA CUGAUGAG X CGAA AGAACAUC	68	GATGTTCT C TCAATAAT	480
697	CAAUUUUU CUGAUGAG X CGAA AGAGAACA	69	TGTTCTCT C AATAATTG	481
701	AGCACAAU CUGAUGAG X CGAA AUUGAGAG	70	CTCTCAAT A ATTGTGCT	482
704	GGCAGCAC CUGAUGAG X CGAA AUUAUUGA	71	TCAATAAT T GTGCTGCC	483
716	GGGAGUAU CUGAUGAG X CGAA AUGGGCAG	72	CTGCCCAT T ATACTCCC	484
717	UGGGAGUA CUGAUGAG X CGAA AAUGGGCA	73	TGCCCATT A TACTCCCA	485
719	AUUGGGAG CUGAUGAG X CGAA AUAAUGGG	74	CCCATTAT A CTCCCAAT	486
722	GGCAUUGG CUGAUGAG X CGAA AGUAUAAU	75	ATTATACT C CCAATGCC	487
745	UACUGUAA CUGAUGAG X CGAA ACUGUUGU	76	ACAACAGT A TTACAGTA	488
747	CAUACUGU CUGAUGAG X CGAA AUACUGUU	77	AACAGTAT T ACAGTATG	489
748	UCAUACUG CUGAUGAG X CGAA AAUACUGU	78	ACAGTATT A CAGTATGA	490
753	UGUCAUCA CUGAUGAG X CGAA ACUGUAAU	79	ATTACAGT A TGATGACA	491
763	AUUUUACA CUGAUGAG X CGAA AUGUCAUC	80	GATGACAT C TGTAATAAT	492
767	GUCUAUUU CUGAUGAG X CGAA ACAGAUGU	81	ACATCTGT A AAATAGAC	493
772	CCAAAGUC CUGAUGAG X CGAA AUUUUACA	82	TGTAATAAT A GACTTTGG	494
777	GUGUUGCA CUGAUGAG X CGAA AGUCUAUU	83	AATAGACT T TGGAACAC	495
778	UGUGUUC CUGAUGAG X CGAA AAGUCUAU	84	ATAGACTT T GGAACACA	496
788	ACCACUUA CUGAUGAG X CGAA AUGUGUUC	85	GAACACAT A TAAGTGGT	497
790	CUACCACU CUGAUGAG X CGAA AUAUGUGU	86	ACACATAT A AGTGGTAG	498
797	AAUAAUCC CUGAUGAG X CGAA ACCACUUA	87	TAAGTGGT A GGATTATT	499
802	CAGUCAAU CUGAUGAG X CGAA AUCCUACC	88	GGTAGGAT T ATTGACTG	500
803	ACAGUCAA CUGAUGAG X CGAA AAUCCUAC	89	GTAGGATT A TTGACTGT	501

Table 9

805	GCACAGUC CUGAUGAG X CGAA AUAUCCU	90	AGGATTAT T GACTGTGC	502
815	GACAGUAA CUGAUGAG X CGAA AGCACAGU	91	ACTGTGCT T TTACTGTC	503
816	UGACAGUA CUGAUGAG X CGAA AAGCACAG	92	CTGTGCTT T TACTGTCA	504
817	GUGACAGU CUGAUGAG X CGAA AAAGCACA	93	TGTGCTTT T ACTGTCAC	505
818	AGUGACAG CUGAUGAG X CGAA AAAAGCAC	94	GTGCTTTT A CTGTCACT	506
823	UUAAAAGU CUGAUGAG X CGAA ACAGUAAA	95	TTTACTGT C ACTTTTAA	507
827	GGGAUUA CUGAUGAG X CGAA AGUGACAG	96	CTGTCACT T TTAATCCC	508
828	UGGGAUUA CUGAUGAG X CGAA AAGUGACA	97	TGTCACTT T TAATCCCA	509
829	UUGGGAU CUGAUGAG X CGAA AAAGUGAC	98	GTCACCTT T AATCCCAA	510
830	UUUGGGAU CUGAUGAG X CGAA AAAAGUGA	99	TCACTTTT A ATCCCAA	511
833	AUAUUUGG CUGAUGAG X CGAA AUUAAAAG	100	CTTTTAAT C CCAAATAT	512
840	ACGUAUCA CUGAUGAG X CGAA AUUUGGGA	101	TCCCAAAT A TGATACGT	513
845	UAAUAACG CUGAUGAG X CGAA AUCAUAU	102	AATATGAT A CGTTATTA	514
849	CUUUUAAU CUGAUGAG X CGAA ACGUAUCA	103	TGATACGT T ATTAAAAG	515
850	GCUUUUAA CUGAUGAG X CGAA AACGUAUC	104	GATACGTT A TTAAAAGC	516
852	CAGCUUUU CUGAUGAG X CGAA AUAACGUA	105	TACGTTAT T AAAAGCTG	517
853	ACAGCUUU CUGAUGAG X CGAA AAUAACGU	106	ACGTTATT A AAAGCTGT	518
862	GCAUCUUU CUGAUGAG X CGAA ACAGCUUU	107	AAAGCTGT A AAAGATGC	519
872	AGUGUUAG CUGAUGAG X CGAA AGCAUCUU	108	AAGATGCT A CTAACACT	520
875	UCCAGUGU CUGAUGAG X CGAA AGUAGCAU	109	ATGCTACT A AACTGGA	521
886	GCACACUU CUGAUGAG X CGAA AUUCCAGU	110	ACTGGAAT A AAGTGTGC	522
901	CGAACAU CUGAUGAG X CGAA AUUCCAGC	111	GCTGGAAT T GATGTTCC	523
907	CACAGACG CUGAUGAG X CGAA ACAUCAU	112	ATTGATGT T CGTCTGTG	524
908	ACACAGAC CUGAUGAG X CGAA AACAUCAA	113	TTGATGTT C GTCTGTGT	525
911	AUCACACA CUGAUGAG X CGAA ACGAACAU	114	ATGTTTCGT C TGTGTGAT	526
922	GCCUCACC CUGAUGAG X CGAA ACAUCACA	115	TGTGATGT T GGTGAGGC	527
934	ACUUCUUG CUGAUGAG X CGAA AUGGCCUC	116	GAGGCCAT C CAAGAAGT	528
943	GACUCCA CUGAUGAG X CGAA ACUUCUUG	117	CAAGAAGT T ATGGAGTC	529
944	GGACUCCA CUGAUGAG X CGAA AACUUCUU	118	AAGAAGTT A TGGAGTCC	530
951	CUUCAUAG CUGAUGAG X CGAA ACUCCAUA	119	TATGGAGT C CTATGAAG	531
954	CAACUUCA CUGAUGAG X CGAA AGGACUCC	120	GGAGTCCT A TGAAGTTG	532
961	UCUAUUUC CUGAUGAG X CGAA ACUUCAUA	121	TATGAAGT T GAAATAGA	533
967	UUCCCAUC CUGAUGAG X CGAA AUUUCAAC	122	GTTGAAAT A GATGGGAA	534
981	UCACUUGA CUGAUGAG X CGAA AUGUCUUC	123	GAAGACAT A TCAAGTGA	535
983	UUUCACUU CUGAUGAG X CGAA AUAUGUCU	124	AGACATAT C AAGTGAAA	536
997	AGAUUACG CUGAUGAG X CGAA AUUGGUUU	125	AAACCAAT C CGTAATCT	537
1001	AUUUAGAU CUGAUGAG X CGAA ACGGAUUG	126	CAATCCGT A ATCTAAAT	538
1004	UCCAUUUA CUGAUGAG X CGAA AUUACGGA	127	TCCGTAAT C TAAATGGA	539
1006	UGUCCAUA CUGAUGAG X CGAA AGAUUACG	128	CGTAATCT A AATGGACA	540
1016	CCCAAUUG CUGAUGAG X CGAA AUGUCCA	129	ATGGACAT T CAATTGGG	541
1017	GCCCAAU CUGAUGAG X CGAA AAUGUCCA	130	TGGACATT C AATTGGGC	542
1021	UAUUGCCC CUGAUGAG X CGAA AUUGAAUG	131	CATTCAAT T GGGCAATA	543
1029	GUUUUUA CUGAUGAG X CGAA AUUGCCCA	132	TGGGCAAT A TAGAATAC	544
1031	AUGUAUUC CUGAUGAG X CGAA AUAUUGCC	133	GGCAATAT A GAATACAT	545
1036	CCAGCAUG CUGAUGAG X CGAA AUUCUAUA	134	TATAGAAT A CATGCTGG	546
1060	CCUUUCAC CUGAUGAG X CGAA AUCGGCAC	135	GTGCCGAT T GTGAAAGG	547
1102	AUUGCAUA CUGAUGAG X CGAA ACUUCUCC	136	GGAGAAGT A TATGCAAT	548

Table 9

1104	CAAUUGCA CUGAUGAG X CGAA AUACUUCU	137	AGAAGTAT A TGCAATTG	549
1111	AAGGUUUC CUGAUGAG X CGAA AUUGCAUA	138	TATGCAAT T GAAACCTT	550
1119	UACUACCA CUGAUGAG X CGAA AGGUJUUA	139	TGAAACCT T TGGTAGTA	551
1120	GUACUACC CUGAUGAG X CGAA AAGGUUUC	140	GAAACCTT T GGTAGTAC	552
1124	UCCUGUAC CUGAUGAG X CGAA ACCAAAGG	141	CCTTTGGT A GTACAGGA	553
1127	UUUUCUG CUGAUGAG X CGAA ACUACCAA	142	TTGGTAGT A CAGGAAAA	554
1141	UCAUGAAC CUGAUGAG X CGAA ACACCUUU	143	AAAGGTGT T GTTCATGA	555
1144	UCAUCAUG CUGAUGAG X CGAA ACAACACC	144	GGTGTGT T CATGATGA	556
1145	AUCAUCAU CUGAUGAG X CGAA AACACAC	145	GTGTTGTT C ATGATGAT	557
1154	ACAUUCCA CUGAUGAG X CGAA AUCAUCAU	146	ATGATGAT A TGAATGT	558
1163	GUAUUGUG CUGAUGAG X CGAA ACAUUCCA	147	TGAATGT T CACATTAC	559
1164	UGUAAUGU CUGAUGAG X CGAA AACAUUCC	148	GGAATGTT C ACATTACA	560
1169	UUUCAUGU CUGAUGAG X CGAA AUGUGAAC	149	GTTACAT T ACATGAAA	561
1170	UUUCAUG CUGAUGAG X CGAA AAUGUGAA	150	TTACATT A CATGAAA	562
1181	AACAUCAA CUGAUGAG X CGAA AUUUUUA	151	TGAAAAT T TTGATGTT	563
1182	CAACAUCA CUGAUGAG X CGAA AAUUUUUC	152	GAAAATT T TGATGTTG	564
1183	CCAACAUC CUGAUGAG X CGAA AAUUUUU	153	AAAAATT T GATGTTGG	565
1189	ACAUGUCC CUGAUGAG X CGAA ACAUCAA	154	TTTGATGT T GGACATGT	566
1204	GGAAGCCU CUGAUGAG X CGAA AUUGGCAC	155	GTGCCAAT A AGGCTTCC	567
1210	GUUCUUG CUGAUGAG X CGAA AGCCUUAU	156	ATAAGGCT T CCAAGAAC	568
1211	UGUUCUUG CUGAUGAG X CGAA AAGCCUUA	157	TAAGGCTT C CAAGAACA	569
1227	CAUUUAA CUGAUGAG X CGAA AGUGUUUU	158	AAAACACT T GTTAAATG	570
1230	UGACAUUU CUGAUGAG X CGAA ACAAGUGU	159	ACACTTGT T AAATGTCA	571
1231	AUGACAUU CUGAUGAG X CGAA AACAAGUG	160	CACTTGT A AATGTCAT	572
1237	UCAUUGAU CUGAUGAG X CGAA ACAUUUAA	161	TTAAATGT C ATCAATGA	573
1240	UUUUCAUU CUGAUGAG X CGAA AUGACAUU	162	AATGTCAT C AATGAAAA	574
1251	GGGUUCCA CUGAUGAG X CGAA AGUUUUA	163	TGAAACT T TGGAACCC	575
1252	AGGUUCC CUGAUGAG X CGAA AAGUUUUC	164	GAAACTT T GGAACCCT	576
1261	CAGAAGGC CUGAUGAG X CGAA AGGUUCC	165	GGAACCCT T GCCTTCTG	577
1266	UGCGGCAG CUGAUGAG X CGAA AGGCAAGG	166	CCTTGCCT T CTGCCGCA	578
1267	CUGCGGCA CUGAUGAG X CGAA AAGGCAAG	167	CTTGCCTT C TGCCGCA	579
1286	UCCCAAGC CUGAUGAG X CGAA AUCCAGCC	168	GGCTGGAT C GCTTGGA	580
1290	UUUCUCCC CUGAUGAG X CGAA AGCGAUCC	169	GGATCGCT T GGGAGAAA	581
1301	CAAGUAUU CUGAUGAG X CGAA ACUUUCUC	170	GAGAAAGT A AATACTTG	582
1305	CCAUCAAG CUGAUGAG X CGAA AUUUACUU	171	AAGTAAAT A CTTGATGG	583
1308	GAGCCAUC CUGAUGAG X CGAA AGUAUUUA	172	TAAATACT T GATGGCTC	584
1316	AUUCUUA CUGAUGAG X CGAA AGCCAUA	173	TGATGGCT C TGAAGAAT	585
1325	GUCACACA CUGAUGAG X CGAA AUUCUUA	174	TGAAGAAT C TGTGTGAC	586
1335	CAAUGCCC CUGAUGAG X CGAA AGUCACAC	175	GTGTGACT T GGGCATTG	587
1342	GGAUCUAC CUGAUGAG X CGAA AUGCCCAA	176	TTGGGCAT T GTAGATCC	588
1345	UAUGGAUC CUGAUGAG X CGAA ACAUAGCC	177	GGCATTGT A GATCCATA	589
1349	UGGAUAUG CUGAUGAG X CGAA AUCUACAA	178	TTGTAGAT C CATATCCA	590
1353	AUGGUGGA CUGAUGAG X CGAA AUGGAUCU	179	AGATCCAT A TCCACCAT	591
1355	UAAUGGUG CUGAUGAG X CGAA AUAUGGAU	180	ATCCATAT C CACCATTA	592
1362	UGUCACAU CUGAUGAG X CGAA AUGGUGGA	181	TCCACCAT T ATGTGACA	593
1363	AUGUCACA CUGAUGAG X CGAA AAUGGUGG	182	CCACCATT A TGTGACAT	594
1372	GAUCCUUU CUGAUGAG X CGAA AUGUCACA	183	TGTGACAT T AAAGGATC	595

Table 9

1373	UGAUCCUU CUGAUGAG X CGAA AAUGUCAC	184	GTGACATT A AAGGATCA	596
1380	CUGUAUUAU CUGAUGAG X CGAA AUCCUUUA	185	TAAAGGAT C ATATACAG	597
1383	GCGCUGUA CUGAUGAG X CGAA AUGAUCCU	186	AGGATCAT A TACAGCGC	598
1385	UUGCGCUG CUGAUGAG X CGAA AUAUGAUC	187	GATCATAT A CAGCGCAA	599
1395	UAUGUUA CUGAUGAG X CGAA AUUGCGCU	188	AGCGCAAT T TGAACATA	600
1396	GUAUGUUC CUGAUGAG X CGAA AAUUGCGC	189	GCGCAATT T GAACATAC	601
1403	CAGGAUGG CUGAUGAG X CGAA AUGUUCUA	190	TTGAACAT A CCATCCTG	602
1408	CGCAACAG CUGAUGAG X CGAA AUGGUAUG	191	CATACCAT C CTGTTGCG	603
1413	UUGGACGC CUGAUGAG X CGAA ACAGGAUG	192	CATCCTGT T GCGTCCAA	604
1418	ACAUGUUG CUGAUGAG X CGAA ACGCAACA	193	TGTTGCGT C CAACATGT	605
1427	AACUUCUU CUGAUGAG X CGAA ACAUGUUG	194	CAACATGT A AAGAAGTT	606
1435	CUGCUGAC CUGAUGAG X CGAA ACUUCUUU	195	AAAGAAGT T GTCAGCAG	607
1438	CCUCUGCU CUGAUGAG X CGAA ACAACUUC	196	GAAGTTGT C AGCAGAGG	608
1455	AAGUUUAA CUGAUGAG X CGAA AGUCAUCU	197	AGATGACT A TTAACTT	609
1457	CUAAGUUU CUGAUGAG X CGAA AUAGUCAU	198	ATGACTAT T AAACCTAG	610
1458	ACUAAGUU CUGAUGAG X CGAA AAUAGUCA	199	TGACTATT A AACTTAGT	611
1463	UUUGGACU CUGAUGAG X CGAA AGUUUAAU	200	ATTAACT T AGTCCAAA	612
1464	CUUUGGAC CUGAUGAG X CGAA AAGUUUAA	201	TTAACTT A GTCCAAAG	613
1467	UGGCUUUG CUGAUGAG X CGAA ACUAAGUU	202	AACTTAGT C CAAAGCCA	614
1479	AAGGUGUU CUGAUGAG X CGAA AGGUGGCU	203	AGCCACCT C AACACCTT	615
1487	AGAAAAUA CUGAUGAG X CGAA AGGUGUUG	204	CAACACCT T TATTTTCT	616
1488	CAGAAAAU CUGAUGAG X CGAA AAGGUGUU	205	AACACCTT T ATTTTCTG	617
1489	UCAGAAAA CUGAUGAG X CGAA AAAGGUGU	206	ACACCTTT A TTTTCTGA	618
1491	GCUCAGAA CUGAUGAG X CGAA AUAAAGGU	207	ACCTTTAT T TTCTGAGC	619
1492	AGCUCAGA CUGAUGAG X CGAA AAUAAAGG	208	CCTTTATT T TCTGAGCT	620
1493	AAGCUCAG CUGAUGAG X CGAA AAUAAAG	209	CTTTATTT T CTGAGCTT	621
1494	AAAGCUCA CUGAUGAG X CGAA AAAAUAAA	210	TTTATTTT C TGAGCTTT	622
1501	UUCCAACA CUGAUGAG X CGAA AGCUCAGA	211	TCTGAGCT T TGTTGGAA	623
1502	UUCCAAC CUGAUGAG X CGAA AAGCUCAG	212	CTGAGCTT T GTTGGAAA	624
1505	UGUUUCC CUGAUGAG X CGAA ACAAAGCU	213	AGCTTTGT T GGAAAACA	625
1518	AAUUCUGG CUGAUGAG X CGAA AUCAUGUU	214	AACATGAT A CCAGAATT	626
1526	GGCAAAU CUGAUGAG X CGAA AUUCUGGU	215	ACCAGAAT T AATTTGCC	627
1527	UGGCAAAU CUGAUGAG X CGAA AAUUCUGG	216	CCAGAATT A ATTTGCCA	628
1530	AUGUGGCA CUGAUGAG X CGAA AUUAAUUC	217	GAATTAAT T TGCCACAT	629
1531	CAUGUGGC CUGAUGAG X CGAA AAUUAUU	218	AATTAATT T GCCACATG	630
1541	AAACAGAC CUGAUGAG X CGAA ACAUGUGG	219	CCACATGT T GTCTGTTT	631
1544	UUAACA CUGAUGAG X CGAA ACAACAUG	220	CATGTTGT C TGTTTTAA	632
1548	ACUGUUA CUGAUGAG X CGAA ACAGACAA	221	TTGTCTGT T TTAACAGT	633
1549	CACUGUA CUGAUGAG X CGAA AACAGACA	222	TGTCTGTT T TAACAGTG	634
1550	CCACUGUU CUGAUGAG X CGAA AAACAGAC	223	GTCTGTTT T AACAGTGG	635
1551	UCCACUGU CUGAUGAG X CGAA AAAACAGA	224	TCTGTTTT A ACAGTGGA	636
1567	AAAAGUAU CUGAUGAG X CGAA ACAUGGGU	225	ACCCATGT A ATACTTTT	637
1570	GAUAAAAG CUGAUGAG X CGAA AUUACAUG	226	CATGTAAT A CTTTTATC	638
1573	AUGGAUAA CUGAUGAG X CGAA AGUAUUAC	227	GTAATACT T TTATCCAT	639
1574	CAUGGAUA CUGAUGAG X CGAA AAGUAUUA	228	TAATACTT T TATCCATG	640
1575	ACAUGGAU CUGAUGAG X CGAA AAAGUAUU	229	AATACTTT T ATCCATGT	641
1576	AACAUGGA CUGAUGAG X CGAA AAAAGUAU	230	ATACTTTT A TCCATGTT	642

Table 9

1578	UAAACAUG CUGAUGAG X CGAA AUAAAAGU	231	ACTTTTAT C CATGTTTA	643
1584	CUUUUUUA CUGAUGAG X CGAA ACAUGGAU	232	ATCCATGT T TAAAAAAG	644
1585	UCUUUUUU CUGAUGAG X CGAA AACAUUGA	233	TCCATGTT T AAAAAAGA	645
1586	UUCUUUUU CUGAUGAG X CGAA AAACAUGG	234	CCATGTTT A AAAAAAGAA	646
1600	UUUGUCCA CUGAUGAG X CGAA AUUCCUUC	235	GAAGGAAT T TGGACAAA	647
1601	CUUUGUCC CUGAUGAG X CGAA AAUCCUUC	236	AAGGAATT T GGACAAAG	648
1619	UUACAUUA CUGAUGAG X CGAA ACGGUUUG	237	CAAACCGT C TAATGTAA	649
1621	AAUUAUUA CUGAUGAG X CGAA AGACGGUU	238	AACCGTCT A ATGTAATT	650
1626	UGGUUAAU CUGAUGAG X CGAA ACAUUAGA	239	TCTAATGT A ATTAACCA	651
1629	CGUUGGUU CUGAUGAG X CGAA AUUACAUU	240	AATGTAAT T AACCAACG	652
1630	UCGUUGGU CUGAUGAG X CGAA AAUUAUUA	241	ATGTAATT A ACCAACGA	653
1646	AGUCCGGA CUGAUGAG X CGAA AGCUUUUU	242	AAAAAGCT T TCCGGACT	654
1647	AAGUCCGG CUGAUGAG X CGAA AAGCUUUU	243	AAAAGCTT T CCGGACTT	655
1648	AAAGUCCG CUGAUGAG X CGAA AAAGCUUU	244	AAAGCTTT C CGGACTTT	656
1655	GCAUUUAA CUGAUGAG X CGAA AGUCCGGA	245	TCCGGACT T TTAAATGC	657
1656	AGCAUUUA CUGAUGAG X CGAA AAGUCCGG	246	CCGGACTT T TAAATGCT	658
1657	UAGCAUUU CUGAUGAG X CGAA AAAGUCCG	247	CGGACTTT T AAATGCTA	659
1658	UUAGCAUU CUGAUGAG X CGAA AAAAGUCC	248	GGACTTTT A AATGCTAA	660
1665	AAAACAGU CUGAUGAG X CGAA AGCAUUUA	249	TAAATGCT A ACTGTTTT	661
1671	AGGGGAAA CUGAUGAG X CGAA ACAGUUAG	250	CTAACTGT T TTTCCCCT	662
1672	AAGGGGAA CUGAUGAG X CGAA AACAGUUA	251	TAAGTGT T TTCCCCTT	663
1673	GAAGGGGA CUGAUGAG X CGAA AAACAGUU	252	AACTGTTT T TCCCCTTC	664
1674	GGAAGGGG CUGAUGAG X CGAA AAAACAGU	253	ACTGTTTT T CCCCTTCC	665
1675	AGGAAGGG CUGAUGAG X CGAA AAAAACAG	254	CTGTTTTT C CCCTTCCT	666
1680	UAGACAGG CUGAUGAG X CGAA AGGGGAAA	255	TTTCCCCT T CCTGTCTA	667
1681	CUAGACAG CUGAUGAG X CGAA AAGGGGAA	256	TTCCCCTT C CTGTCTAG	668
1686	UUUUCUA CUGAUGAG X CGAA ACAGGAAG	257	CTTCCTGT C TAGGAAAA	669
1688	CAUUUCC CUGAUGAG X CGAA AGACAGGA	258	TCCTGTCT A GGAAAATG	670
1699	GAGCUUUA CUGAUGAG X CGAA AGCAUUUU	259	AAAATGCT A TAAAGCTC	671
1701	UUGAGCUU CUGAUGAG X CGAA AUAGCAUU	260	AATGCTAT A AAGCTCAA	672
1707	ACUAAUUU CUGAUGAG X CGAA AGCUUUUU	261	ATAAAGCT C AAATTAGT	673
1712	UCCUAACU CUGAUGAG X CGAA AUUUGAGC	262	GCTCAAAT T AGTTAGGA	674
1713	UUCCUAAC CUGAUGAG X CGAA AAUUGAG	263	CTCAAAT A GTTAGGAA	675
1716	UCAUCCU CUGAUGAG X CGAA ACUAAUUU	264	AAATTAGT T AGGAATGA	676
1717	GUCAUCC CUGAUGAG X CGAA AACUAAUU	265	AATTAGTT A GGAATGAC	677
1727	AAACGUUU CUGAUGAG X CGAA AGUCAUUC	266	GAATGACT T ATACGTTT	678
1728	AAAACGUA CUGAUGAG X CGAA AAGUCAUU	267	AATGACTT A TACGTTTT	679
1730	ACAAAACG CUGAUGAG X CGAA AUAAGUCA	268	TGACTTAT A CGTTTTGT	680
1734	CAAAACAA CUGAUGAG X CGAA ACGUAUUA	269	TTATACGT T TTGTTTTG	681
1735	UCAAAACA CUGAUGAG X CGAA AACGUUAU	270	TATACGTT T TGTTTTGA	682
1736	UUCAAAAC CUGAUGAG X CGAA AAACGUUU	271	ATACGTTT T GTTTTGAA	683
1739	GUUUUCAA CUGAUGAG X CGAA ACAAACG	272	CGTTTTGT T TTGAATAC	684
1740	GGUAUUUA CUGAUGAG X CGAA AACAAAAC	273	GTTTTGTT T TGAATACC	685
1741	AGGUUUUC CUGAUGAG X CGAA AAACAAAA	274	TTTTGTTT T GAATACCT	686
1746	CUCUUAGG CUGAUGAG X CGAA AUUCAAAA	275	TTTTGAAT A CCTAAGAG	687
1750	GUAUCUCU CUGAUGAG X CGAA AGGUUUUC	276	GAATACCT A AGAGATAC	688
1757	CCAAAAG CUGAUGAG X CGAA AUCUCUUA	277	TAAGAGAT A CTTTTTGG	689

Table 9

1760	UAUCCAAA CUGAUGAG X CGAA AGUAUCUC	278	GAGATACT T TTTGGATA	690
1761	AUAUCCAA CUGAUGAG X CGAA AAGUAUCU	279	AGATACTT T TTGGATAT	691
1762	AAUAUCCA CUGAUGAG X CGAA AAAGUAUC	280	GATACTTT T TGGATATT	692
1763	AAAUAUCC CUGAUGAG X CGAA AAAAGUAU	281	ATACTTTT T GGATATTT	693
1768	AAUAUAAA CUGAUGAG X CGAA AUCCAAAA	282	TTTTGGAT A TTTATATT	694
1770	GCAAUUAU CUGAUGAG X CGAA AUAUCCAA	283	TTGGATAT T TATATTGC	695
1771	GGCAUAU CUGAUGAG X CGAA AAUAUCCA	284	TGGATATT T ATATTGCC	696
1772	UGGCAUAU CUGAUGAG X CGAA AAAUAUCC	285	GGATATTT A TATTGCCA	697
1774	UAUGGCAA CUGAUGAG X CGAA AUAAAUAU	286	ATATTTAT A TTGCCATA	698
1776	AAUAUGGC CUGAUGAG X CGAA AUUAUAAU	287	ATTTATAT T GCCATATT	699
1782	AGUAAGAA CUGAUGAG X CGAA AUGGCAAU	288	ATTGCCAT A TTCTTACT	700
1784	CAAGUAAG CUGAUGAG X CGAA AUAUGGCA	289	TGCCATAT T CTTACTTG	701
1785	UCAAGUAA CUGAUGAG X CGAA AAUAUGGC	290	GCCATATT C TTACTIONTGA	702
1787	AUUCAAGU CUGAUGAG X CGAA AGAAUAUG	291	CATATTCT T ACTTGAAT	703
1788	CAUUCAAG CUGAUGAG X CGAA AAGAAUAU	292	ATATTCTT A CTTGAATG	704
1791	AAGCAUUC CUGAUGAG X CGAA AGUAAGAA	293	TTCTTACT T GAATGCTT	705
1799	GUCAUUCA CUGAUGAG X CGAA AGCAUUCA	294	TGAATGCT T TGAATGAC	706
1800	AGUCAUUC CUGAUGAG X CGAA AAGCAUUC	295	GAATGCTT T GAATGACT	707
1809	ACUGGAUG CUGAUGAG X CGAA AGUCAUUC	296	GAATGACT A CATCCAGT	708
1813	CAGAACUG CUGAUGAG X CGAA AUGUAGUC	297	GACTACAT C CAGTTCTG	709
1818	AGGUGCAG CUGAUGAG X CGAA ACUGGAUG	298	CATCCAGT T CTGCACCT	710
1819	UAGGUGCA CUGAUGAG X CGAA AACUGGAU	299	ATCCAGTT C TGCACCTA	711
1827	AGAGGGUA CUGAUGAG X CGAA AGGUGCAG	300	CTGCACCT A TACCCTCT	712
1829	CCAGAGGG CUGAUGAG X CGAA AUAGGUGC	301	GCACCTAT A CCCTCTGG	713
1834	CAACACCA CUGAUGAG X CGAA AGGGUUAU	302	TATACCCT C TGGTGTG	714
1841	UAAAAAGC CUGAUGAG X CGAA ACACCAGA	303	TCTGGTGT T GCTTTTTA	715
1845	AGGUUAAA CUGAUGAG X CGAA AGCAACAC	304	GTGTTGCT T TTAAACCT	716
1846	AAGGUUAA CUGAUGAG X CGAA AAGCAACA	305	TGTTGCTT T TTAACCTT	717
1847	GAAGGUUA CUGAUGAG X CGAA AAAGCAAC	306	GTTGCTTT T TAACCTTC	718
1848	GGAAGGUU CUGAUGAG X CGAA AAAAGCAA	307	TTGCTTTT T AACCTTCC	719
1849	AGGAAGGU CUGAUGAG X CGAA AAAAAGCA	308	TGCTTTTT A ACCTTCCT	720
1854	AUCCAGG CUGAUGAG X CGAA AGGUUAAA	309	TTAAACCT T CCTGGAAT	721
1855	GAUCCAG CUGAUGAG X CGAA AAGGUUAA	310	TTAACCTT C CTGGAATC	722
1863	AGAAAUG CUGAUGAG X CGAA AUCCAGG	311	CCTGGAAT C CATTTTCT	723
1867	UUUUAGAA CUGAUGAG X CGAA AUGGAUUC	312	GAATCCAT T TTCTAAAA	724
1868	UUUUUAGA CUGAUGAG X CGAA AAUGGAUU	313	AATCCATT T TCTAAAAA	725
1869	UUUUUUAG CUGAUGAG X CGAA AAAUGGAU	314	ATCCATTT T CTAAAAAA	726
1870	AUUUUUUA CUGAUGAG X CGAA AAAAUGGA	315	TCCATTTT C TAAAAAAT	727
1872	UUAUUUUU CUGAUGAG X CGAA AGAAAAUG	316	CATTTTCT A AAAAATAA	728
1879	UGUGUCUU CUGAUGAG X CGAA AUUUUUUA	317	TAAAAAAT A AAGACACA	729
1889	CUGAGAAG CUGAUGAG X CGAA AUGUGUCU	318	AGACACAT T CTTCTCAG	730
1890	GCUGAGAA CUGAUGAG X CGAA AAUGUGUC	319	GACACATT C TTCTCAGC	731
1892	GUGCUGAG CUGAUGAG X CGAA AGAAUGUG	320	CACATTCT T CTCAGCAC	732
1893	GGUGCUGA CUGAUGAG X CGAA AAGAAUGU	321	ACATTCTT C TCAGCACC	733
1895	GUGGUGCU CUGAUGAG X CGAA AGAAGAAU	322	ATTCTTCT C AGCACCAC	734
1913	UUUUGGAA CUGAUGAG X CGAA AGGUGUUG	323	CAACACCT A TTCCAAAA	735
1915	GAUUUUGG CUGAUGAG X CGAA AUAGGUGU	324	ACACCTAT T CCAAATC	736

Table 9

1916	CGAUUUUG CUGAUGAG X CGAA AAUAGGUG	325	CACCTATT C CAAAATCG	737
1923	AUGUGGUC CUGAUGAG X CGAA AUUUUGGA	326	TCCAAAAT C GACCACAT	738
1932	CUUCCAAA CUGAUGAG X CGAA AUGUGGUC	327	GACCACAT A TTTGGAAG	739
1934	UACUCCA CUGAUGAG X CGAA AUAUGUGG	328	CCACATAT T TGGAAGTA	740
1935	UUACUCC CUGAUGAG X CGAA AAUAUGUG	329	CACATATT T GGAAGTAA	741
1942	GAGAGCUU CUGAUGAG X CGAA ACUUCCAA	330	TTGGAAGT A AAGCTCTC	742
1948	GCUGAGGA CUGAUGAG X CGAA AGCUUUAC	331	GTAAAGCT C TCCTCAGC	743
1950	UUGCUGAG CUGAUGAG X CGAA AGAGCUUU	332	AAAGCTCT C CTCAGCAA	744
1953	CAUUUGCU CUGAUGAG X CGAA AGGAGAGC	333	GCTCTCCT C AGCAAATG	745
1963	UGUUCUUU CUGAUGAG X CGAA ACAUUUGC	334	GCAAATGT A AAAGAACA	746
1977	UUUGUUAU CUGAUGAG X CGAA AUUUCUGU	335	ACAGAAAT T ATAACAAA	747
1978	GUUUGUUA CUGAUGAG X CGAA AAUUUCUG	336	CAGAAATT A TAACAAAC	748
1980	CAGUUUGU CUGAUGAG X CGAA AUAAUUUC	337	GAAATTAT A ACAAACTG	749
1990	GUCUGAGA CUGAUGAG X CGAA ACAGUUUG	338	CAAACCTGT C TCTCAGAC	750
1992	UGGUCUGA CUGAUGAG X CGAA AGACAGUU	339	AACTGTCT C TCAGACCA	751
1994	UGUGGUCU CUGAUGAG X CGAA AGAGACAG	340	CTGTCTCT C AGACCACA	752
2005	UUUGGUUA CUGAUGAG X CGAA ACUGUGGU	341	ACCACAGT A TAACCAAA	753
2007	AGUUUGGU CUGAUGAG X CGAA AUACUGUG	342	CACAGTAT A ACCAACT	754
2016	CUGAGUUC CUGAUGAG X CGAA AGUUUGGU	343	ACCAAACT A GAACTCAG	755
2022	UJAAUCCU CUGAUGAG X CGAA AGUUCUAG	344	CTAGAACT C AGGATTAA	756
2028	AGUUUCUU CUGAUGAG X CGAA AUCCUGAG	345	CTCAGGAT T AAGAACT	757
2029	GAGUUUCU CUGAUGAG X CGAA AAUCCUGA	346	TCAGGATT A AGAACTC	758
2037	UUUUGAGU CUGAUGAG X CGAA AGUUUCUU	347	AAGAACT C ACTCAAAA	759
2041	GUGGUUUU CUGAUGAG X CGAA AGUGAGUU	348	AACTCACT C AAAACCAC	760
2056	UUUCCAUG CUGAUGAG X CGAA AGUUGUGU	349	ACACAAC T CATGGAAA	761
2079	UCAUUCAG CUGAUGAG X CGAA AGCAGGUU	350	AACCTGCT C CTGAATGA	762
2090	GUAUCCAG CUGAUGAG X CGAA AGUCAUUC	351	GAATGACT A CTGGATAC	763
2097	UUGUUAUG CUGAUGAG X CGAA AUCCAGUA	352	TACTGGAT A CATAACAA	764
2101	CAUUUUGU CUGAUGAG X CGAA AUGUAUCC	353	GGATACAT A ACAAATG	765
2121	AACAUCUU CUGAUGAG X CGAA AUUUCUGC	354	GCAGAAAT A AAGATGTT	766
2129	UUUUAAAG CUGAUGAG X CGAA ACAUCUUU	355	AAAGATGT T CTTTAAAA	767
2130	GUUUUAAA CUGAUGAG X CGAA AACAUCUU	356	AAGATGTT C TTTAAAC	768
2132	UGGUUUUA CUGAUGAG X CGAA AGAACAUC	357	GATGTTCT T TAAACCA	769
2133	UUGGUUUU CUGAUGAG X CGAA AAGAACAU	358	ATGTTCTT T AAAACCAA	770
2134	AUUGGUUU CUGAUGAG X CGAA AAAGAACA	359	TGTTCTTT A AAACCAAT	771
2162	GAUUCUGG CUGAUGAG X CGAA AUGUUGUG	360	CACAACAT A CCAGAATC	772
2170	GUCCCAGA CUGAUGAG X CGAA AUUCUGGU	361	ACCAGAAT C TCTGGGAC	773
2172	GUGUCCCA CUGAUGAG X CGAA AGAUUCUG	362	CAGAATCT C TGGGACAC	774
2183	CUGCUUUG CUGAUGAG X CGAA AUGUGUCC	363	GGACACAT T CAAAGCAG	775
2184	ACUGCUUU CUGAUGAG X CGAA AAUGUGUC	364	GACACATT C AAAGCAGT	776
2197	UUUCCUC CUGAUGAG X CGAA ACACACUG	365	CAGTGTGT A GAGGGAAA	777
2207	GUGCUAUA CUGAUGAG X CGAA AUUUCUU	366	AGGGAAAT T TATAGCAC	778
2208	AGUGCUAU CUGAUGAG X CGAA AAUUCUU	367	GGGAAATT T ATAGCACT	779
2209	UAGUGCUA CUGAUGAG X CGAA AAAUUCUU	368	GGAAATTT A TAGCACTA	780
2211	UUUAGUGC CUGAUGAG X CGAA AUAAAUUU	369	AAATTTAT A GCACTAAA	781
2217	UGGGCAUU CUGAUGAG X CGAA AGUGCUAU	370	ATAGCACT A AATGCCCA	782
2244	AUUUAGA CUGAUGAG X CGAA AUUUCUG	371	CAGGAAAT A TCTAAAAT	783

Table 9

2246	CAAUUUUA CUGAUGAG X CGAA AUAUUUCC	372	GGAAATAT C TAAAATTG	784
2248	GUCAAUUU CUGAUGAG X CGAA AGAUUUUU	373	AAATATCT A AAATTGAC	785
2253	AGGGUGUC CUGAUGAG X CGAA AUUUUAGA	374	TCTAAAAT T GACACCCT	786
2262	UGUGAUGU CUGAUGAG X CGAA AGGGUGUC	375	GACACCCT A ACATCACA	787
2267	UUAUUUGU CUGAUGAG X CGAA AUGUUAGG	376	CCTAACAT C ACAATTAA	788
2273	GUUCUUUU CUGAUGAG X CGAA AUUGUGAU	377	ATCACAAT T AAAAGAAC	789
2274	AGUUCUUU CUGAUGAG X CGAA AAUUGUGA	378	TCACAATT A AAAGAACT	790
2283	UGCUCUC CUGAUGAG X CGAA AGUUCUUU	379	AAAGAACT A GAGAAGCA	791
2305	AGCUUUUC CUGAUGAG X CGAA AUGUGUUU	380	AAACACAT T GAAAAGCT	792
2314	CCUUCUCU CUGAUGAG X CGAA AGCUUUUC	381	GAAAAGCT A AGAGAAGG	793
2331	AUCUUAGU CUGAUGAG X CGAA AUUUCUUG	382	CAAGAAAT A ACTAAGAT	794
2335	UCUGAUCU CUGAUGAG X CGAA AGUUAUUU	383	AAATAACT A AGATCAGA	795
2340	UCUGCUCU CUGAUGAG X CGAA AUCUUAGU	384	ACTAAGAT C AGAGCAGA	796
2361	UGUGUCUC CUGAUGAG X CGAA AUUUCUUC	385	AAGGAAAT A GAGACACA	797
2377	UUUUUGAA CUGAUGAG X CGAA AGUUUUUU	386	AAAAAACT C TTCAAAAA	798
2379	AUUUUUUG CUGAUGAG X CGAA AGAGUUUU	387	AAAACCTCT T CAAAAAAT	799
2380	GAUUUUUU CUGAUGAG X CGAA AAGAGUUU	388	AAACTCTT C AAAAAATC	800
2388	GAUUCAUU CUGAUGAG X CGAA AUUUUUUG	389	CAAAAAAT C AATGAATC	801
2396	AGCUCCUG CUGAUGAG X CGAA AUUCAUUG	390	CAATGAAT C CAGGAGCT	802
2408	UUUCAAAA CUGAUGAG X CGAA ACCAGCUC	391	GAGCTGGT T TTTTGAAA	803
2409	GUUUCAAA CUGAUGAG X CGAA AACCAGCU	392	AGCTGGTT T TTTGAAAC	804
2410	CGUUUCAA CUGAUGAG X CGAA AAACCAGC	393	GCTGGTTT T TTGAAACG	805
2411	UCGUUUCA CUGAUGAG X CGAA AAAACCAG	394	CTGGTTTT T TGAAACGA	806
2412	AUCGUUUC CUGAUGAG X CGAA AAAAACCA	395	TGGTTTTT T GAAACGAT	807
2421	AUUUUGUU CUGAUGAG X CGAA AUCGUUUC	396	GAAACGAT C AACAAAAT	808
2430	UGUCUAUC CUGAUGAG X CGAA AUUUUGUU	397	AACAAAAT T GATAGACA	809
2434	CUAGUGUC CUGAUGAG X CGAA AUCAAUUU	398	AAATTGAT A GACACTAG	810
2441	AGUCUUGC CUGAUGAG X CGAA AGUGUCUA	399	TAGACACT A GCAAGACT	811
2450	UUCUUUAU CUGAUGAG X CGAA AGUCUUGC	400	GCAAGACT A ATAAAGAA	812
2453	UUCUUCUU CUGAUGAG X CGAA AUUAGUCU	401	AGACTAAT A AAGAAGAA	813
2475	UUCUAUUU CUGAUGAG X CGAA AUUCUUCU	402	AGAAGAAT C AAATAGAA	814
2480	AUUGCUUC CUGAUGAG X CGAA AUUUGAUU	403	AATCAAAT A GAAGCAAT	815
2489	UCAUUUUU CUGAUGAG X CGAA AUUGCUUC	404	GAAGCAAT A AAAAATGA	816
2499	AUCCCCUU CUGAUGAG X CGAA AUCAUUUU	405	AAAATGAT A AAGGGGAT	817
2508	GGUGGUGA CUGAUGAG X CGAA AUCCCCUU	406	AAGGGGAT A TCACCACC	818
2510	UUGGUGGU CUGAUGAG X CGAA AUAUCCCC	407	GGGGATAT C ACCACCAA	819
2520	UUCUGUGG CUGAUGAG X CGAA AUUGGUGG	408	CCACCAAT C CCACAGAA	820
2531	UGGUGGUU CUGAUGAG X CGAA AUUUCUGU	409	ACAGAAAT A AACCACCA	821
2541	UAUUCUCU CUGAUGAG X CGAA AUGGUGGU	410	ACCACCAT C AGAGAATA	822
2549	GUUUGUAG CUGAUGAG X CGAA AUUCUCUG	411	CAGAGAAT A CTACAAAC	823
2552	GGUGUUUG CUGAUGAG X CGAA AGUAUUCU	412	AGAATACT A CAAACACC	824

Input Sequence = HSU29607. Cut Site = UH/.

Stem Length = 8. Core Sequence = CUGAUGAG X CGAA (X = GCCGUUAGGC or other stem II)

Seq1 = HSU29607 (Human methionine aminopeptidase mRNA, complete cds., 2569 bp)

Table 10

Table 10: Human methionine aminopeptidase type 2 (MetAP-2) NCH Ribozyme and Target Sequence

Nt. position	Ribozyme Sequence	Seq. ID Nos.	Substrate Sequence	Seq. ID Nos.
10	CCCGAGAG CUGAUGAG X CGAA IACGAGGG	825	CCCTCGTC T CTCTCGGG	1255
12	UGCCCGAG CUGAUGAG X CGAA IAGACGAG	826	CTCGTCTC T CTCGGGCA	1256
14	GUUGCCCG CUGAUGAG X CGAA IAGAGACG	827	CGTCTCTC T CGGGCAAC	1257
20	CGCCAUGU CUGAUGAG X CGAA ICCCGAGA	828	TCTCGGGC A ACATGGCG	1258
23	GCCCGCCA CUGAUGAG X CGAA IUUGCCCG	829	CGGGCAAC A TGGCGGGC	1259
49	CUCCCGGA CUGAUGAG X CGAA ICCGCUAC	830	GTAGCGGC C TCCGGGAG	1260
50	GCUCCCGG CUGAUGAG X CGAA IGCCGCUA	831	TAGCGGCC T CCGGGAGC	1261
52	UGGCUCCC CUGAUGAG X CGAA IAGGCCGC	832	GCGGCCTC C GGGAGCCA	1262
59	AUUCAGGU CUGAUGAG X CGAA ICUCCCGG	833	CCGGGAGC C ACCTGAAT	1263
60	CAUUCAGG CUGAUGAG X CGAA IGCUCCCG	834	CGGGAGCC A CCTGAATG	1264
62	GCCAUUCA CUGAUGAG X CGAA IUGGCUCC	835	GGAGCCAC C TGAATGGC	1265
63	CGCCAUUC CUGAUGAG X CGAA IGUGGCUC	836	GAGCCACC T GAATGGCG	1266
74	UGGAUCCA CUGAUGAG X CGAA IUCGCCAU	837	ATGGCGAC C TGGATCCA	1267
75	CUGGAUCC CUGAUGAG X CGAA IGUCGCCA	838	TGGCGACC T GGATCCAG	1268
81	UGUCGUCU CUGAUGAG X CGAA IAUCCAGG	839	CCTGGATC C AGACGACA	1269
82	CUGUCGUC CUGAUGAG X CGAA IGAUCCAG	840	CTGGATCC A GACGACAG	1270
89	UUCUUCUU CUGAUGAG X CGAA IUCGUCUG	841	CAGACGAC A GGGAAGAA	1271
103	GUAGAGGC CUGAUGAG X CGAA ICUCCUUC	842	GAAGGAGC T GCCTCTAC	1272
106	GCCGUAGA CUGAUGAG X CGAA ICAGCUCC	843	GGAGCTGC C TCTACGGC	1273
107	AGCCGUAG CUGAUGAG X CGAA IGCAGCUC	844	GAGCTGCC T CTACGGCT	1274
109	UCAGCCGU CUGAUGAG X CGAA IAGGCAGC	845	GCTGCCTC T ACGGCTGA	1275
115	GCUUCCUC CUGAUGAG X CGAA ICCGUAGA	846	TCTACGGC T GAGGAAGC	1276
124	UUCUUGGC CUGAUGAG X CGAA ICUUCCUC	847	GAGGAAGC A GCCAAGAA	1277
127	UUUUUCUU CUGAUGAG X CGAA ICUGCUUC	848	GAAGCAGC C AAGAAAAA	1278
128	UUUUUUCU CUGAUGAG X CGAA IGCUGCUU	849	AAGCAGCC A AGAAAAAA	1279
158	AGGCCCUU CUGAUGAG X CGAA ICUCUUCU	850	AGAAGAGC A AAGGGCCT	1280
165	CUGCAGAA CUGAUGAG X CGAA ICCCUUUG	851	CAAAGGGC C TTCTGCAG	1281
166	GCUGCAGA CUGAUGAG X CGAA IGCCCUUU	852	AAAGGGCC T TCTGCAGC	1282
169	CCUGCUGC CUGAUGAG X CGAA IAAGGCCC	853	GGGCCTTC T GCAGCAGG	1283
172	UCCCCUGC CUGAUGAG X CGAA ICAGAAGG	854	CCTTCTGC A GCAGGGGA	1284
175	UGUCCCCC CUGAUGAG X CGAA ICUGCAGA	855	TCTGCAGC A GGGGAACA	1285
183	CAGGUUCC CUGAUGAG X CGAA IUUCCCCU	856	AGGGGAAC A GGAACCTG	1286
189	CUUUAUCA CUGAUGAG X CGAA IUUCCUGU	857	ACAGGAAC C TGATAAAG	1287
190	UCUUUAUC CUGAUGAG X CGAA IGUUCUG	858	CAGGAACC T GATAAAGA	1288
202	GAGGCUCC CUGAUGAG X CGAA IAUUCUUU	859	AAAGAATC A GGAGCCTC	1289
208	UCCACUGA CUGAUGAG X CGAA ICUCCUGA	860	TCAGGAGC C TCAGTGGA	1290
209	AUCCACUG CUGAUGAG X CGAA IGCUCCUG	861	CAGGAGCC T CAGTGGAT	1291
211	UCAUCCAC CUGAUGAG X CGAA IAGGCUCC	862	GGAGCCTC A GTGGATGA	1292
226	AACUGUCU CUGAUGAG X CGAA ICUACUUC	863	GAAGTAGC A AGACAGTT	1293
231	UUUCCAAC CUGAUGAG X CGAA IUCUUGCU	864	AGCAAGAC A GTTGGAAG	1294
244	UCCAAUGC CUGAUGAG X CGAA IAUUUUUC	865	GAAAGATC A GCATTGGA	1295
247	UCUCCAA CUGAUGAG X CGAA ICUGAUCU	866	AGATCAGC A TTGGAAGA	1296

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D’UN TOME.

CECI EST LE TOME 1 DE 3
CONTENANT LES PAGES 1 À 250

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 3
CONTAINING PAGES 1 TO 250

NOTE: For additional volumes, please contact the Canadian Patent Office

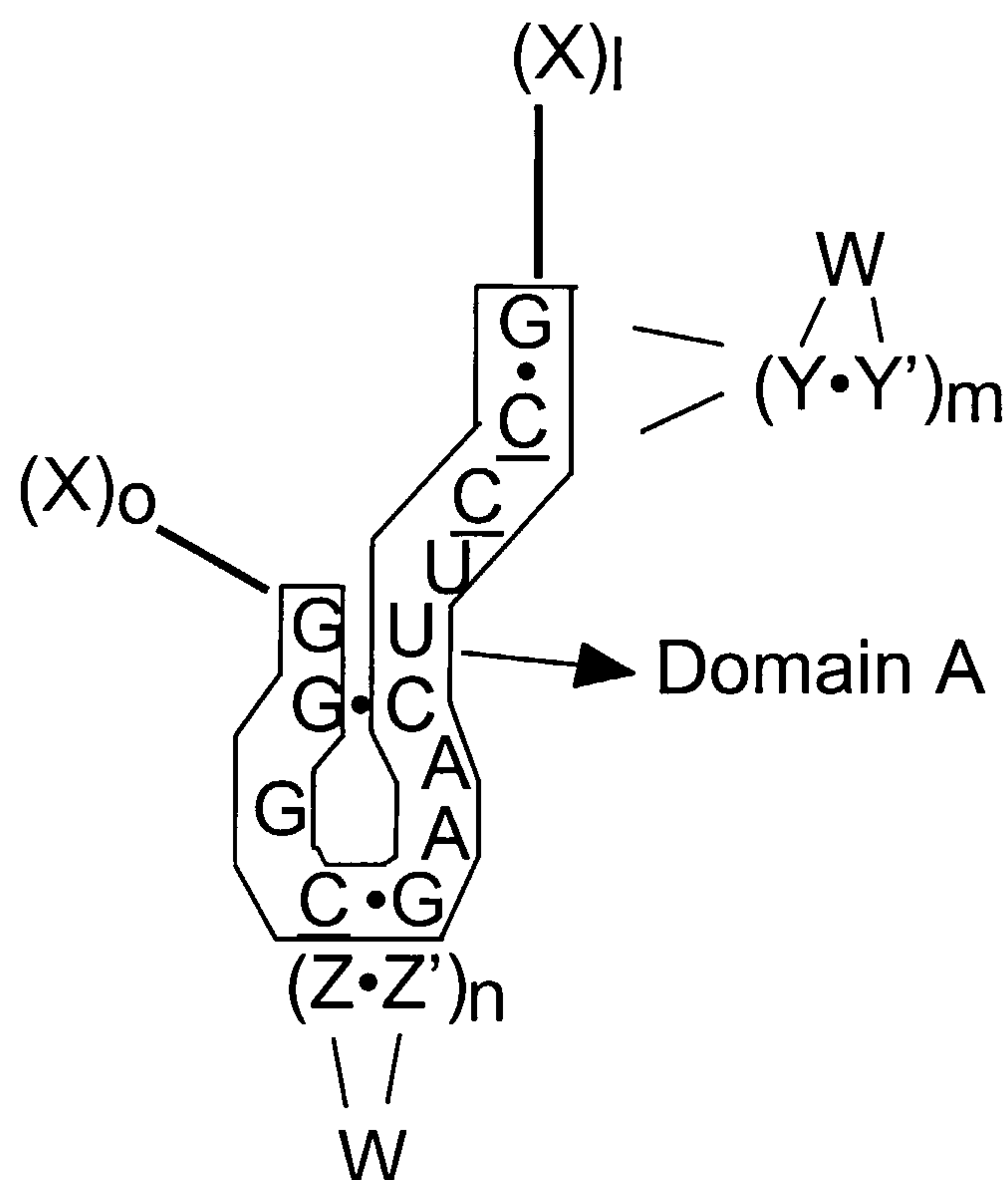
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NOTE POUR LE TOME / VOLUME NOTE:

Claims:

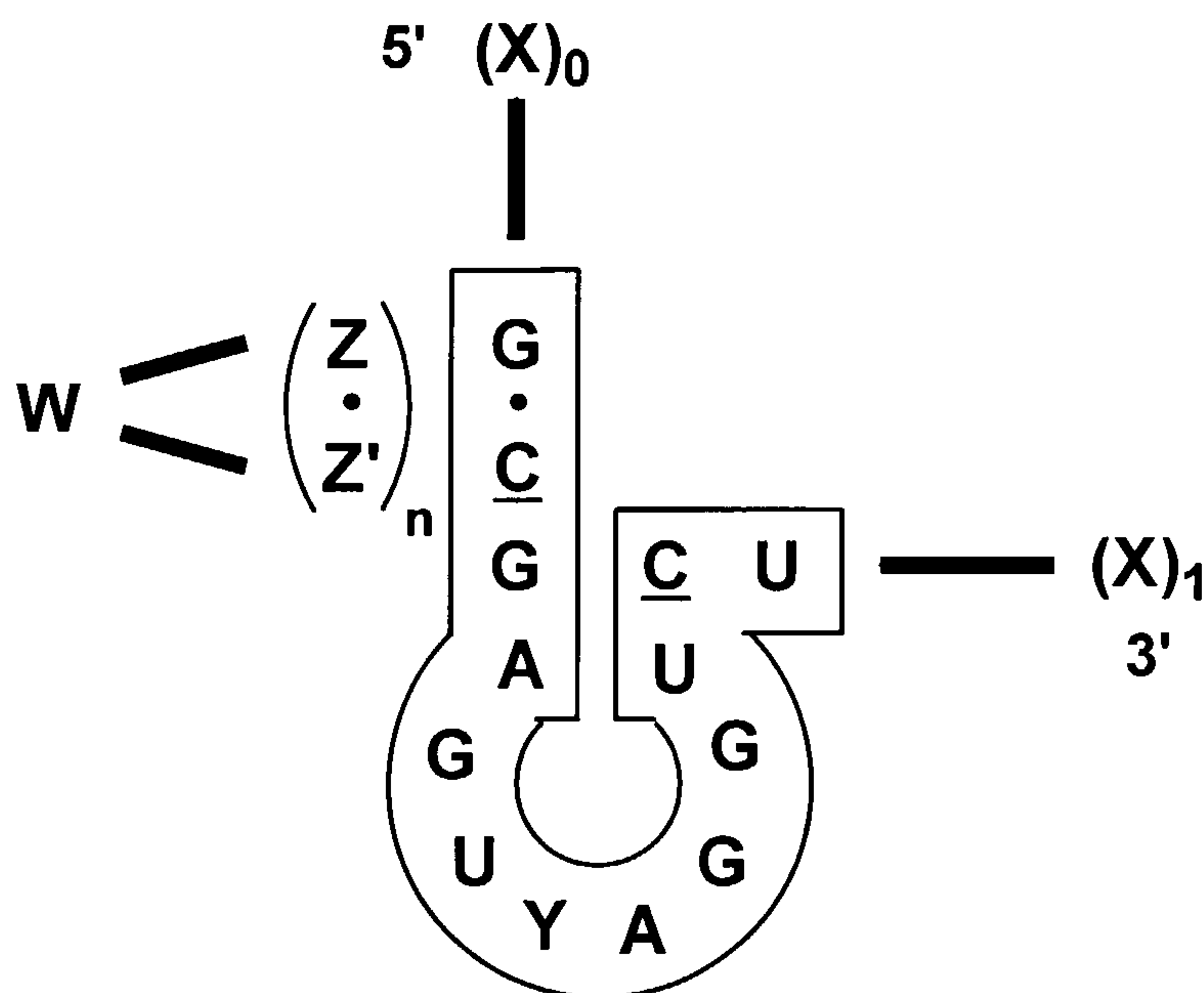
We claim:

1. An enzymatic nucleic acid molecule having formula 4 namely:



wherein each X, Y, and Z represents independently a nucleotide which may be the same or different; l is an integer greater than or equal to 3; m is an integer greater than 1 ; n is an integer greater than 1; 0 is an integer greater than or equal to 3; Z' is a nucleotide complementary to Z; Y' is a nucleotide complementary to Y; each X(l) and X(o) are oligonucleotides which are of sufficient length to stably interact independently with a target nucleic acid sequence; W is a linker of ≥ 2 nucleotides; A, U, G, and C represent nucleotides; C is 2'-amino; and ____ represents a chemical linkage.

2. An enzymatic nucleic acid molecule having formula 5 namely:



5 wherein each X, Y, and Z represents independently a nucleotide which may be the same or different; l is an integer greater than or equal to 3; n is an integer greater than 1; 0 is an integer greater than or equal to 3; Z' is a nucleotide complementary to Z; each X_(l) and X_(o) are oligonucleotides which are of sufficient length to stably interact independently with a target nucleic acid sequence; W is a linker of ≥ 2 nucleotides in
 10 length or may be a non-nucleotide linker; A, U, G, and C represent nucleotides; C is 2'-amino; and ___ represents a chemical linkage.

3. The enzymatic nucleic acid molecule of claims 1 or 2, wherein l is selected from the group consisting of 4, 5, 6, 7, 8, 9, 10, 11, 12, and 15.
4. The enzymatic nucleic acid molecule of claim 1, wherein m is selected from the
 15 group consisting of 2, 3, 4, 5, 6, and 7.
5. The enzymatic nucleic acid molecule of claims 1 or 2, wherein n is selected from the group consisting of 2, 3, 4, 5, 6, and 7.
6. The enzymatic nucleic acid molecule of claims 1 or 2, wherein o is selected from the group consisting of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 15.

7. The enzymatic nucleic acid molecule of claims 1 or 2, wherein 1 and o are of the same length.
8. The enzymatic nucleic acid molecule of claims 1 or 2, wherein 1 and o are of different length.
- 5 9. The enzymatic nucleic acid molecule of claims 1 or 2, wherein the target nucleic acid sequence is selected from the group consisting of an RNA, DNA and RNA/DNA mixed polymer.
10. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said chemical linkage is selected from the group consisting of phosphate ester linkage, amide
10 linkage, phosphorothioate, and phosphorodithioate.
11. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said C is selected from the group consisting of 2'-deoxy-2'-NH₂ and 2'-deoxy-2'-O-NH₂.
12. A method for inhibiting expression of a gene in a cell, comprising the step of
15 administering to said cell the enzymatic nucleic acid molecule of claims 1 or 2 under conditions suitable for said inhibition.
13. A method of cleaving a separate RNA molecule comprising, contacting the enzymatic nucleic acid molecule of claims 1 or 2 with said separate RNA molecule under conditions suitable for the cleavage of said separate RNA molecule.
14. The method of claim 13, wherein said cleavage is carried out in the presence of a
20 divalent cation.
15. The method of claim 14, wherein said divalent cation is Mg²⁺.
16. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic nucleic acid molecule is chemically synthesized.
17. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic
25 nucleic acid molecule comprises at least one ribonucleotide.
18. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic nucleic acid molecule comprises no ribonucleotide residues.
19. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic nucleic acid molecule comprises at least one 2-amino modification.

20. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic nucleic acid molecule comprises at least three phosphorothioate modifications.

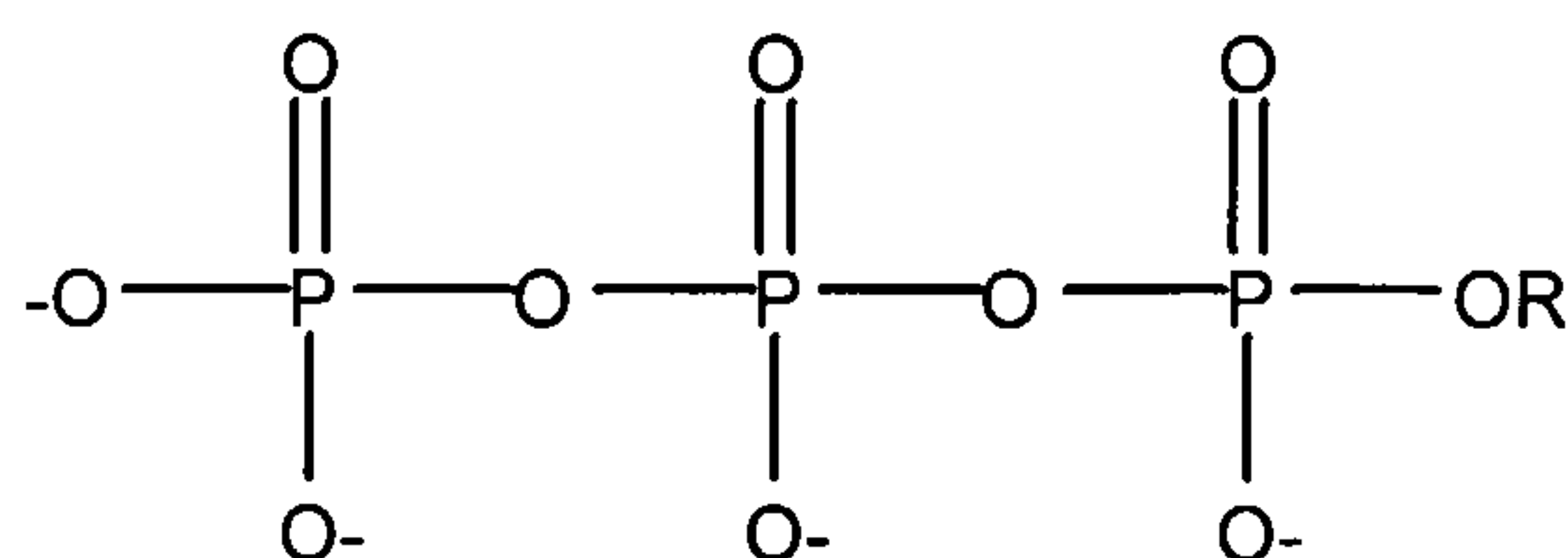
21. The enzymatic nucleic acid molecule of claim 20, wherein said phosphorothioate modification is at the 5'-end of said enzymatic nucleic acid molecule.

5 22. The enzymatic nucleic acid molecule of claims 1 or 2, wherein said enzymatic nucleic acid molecule comprises a 5'-cap or a 3'-cap or both a 5'-cap and a 3'-cap.

23. The enzymatic nucleic acid molecule of claim 22, wherein said 5-cap is phosphorothioate modification.

10 24. The enzymatic nucleic acid molecule of claim 22, wherein said 3'-cap is an inverted abasic moiety.

25. A compound having the formula 3:



wherein R is independently any nucleoside selected from the group consisting of 2'-*O*-methyl-2,6-diaminopurine riboside; 2'-deoxy-2'-amino-2,6-diaminopurine riboside; 2'-
 15 (N-alanyl) amino-2'-deoxy-uridine; 2'-(N-phenylalanyl)amino-2'-deoxy-uridine; 2'-deoxy-2'-(N-β-alanyl) amino ; 2'-deoxy-2'-(lysiyl) amino uridine; 2'-*C*-allyl uridine; 2'-*O*-amino-uridine; 2'-*O*-methylthiomethyl adenosine; 2'-*O*-methylthiomethyl cytidine ; 2'-*O*-methylthiomethyl guanosine; 2'-*O*-methylthiomethyl-uridine; 2'-deoxy-2'-(N-histidyl) amino uridine; 2'-deoxy-2'-amino-5-methyl cytidine; 2'-(N-β-carboxamidine-β-alanyl)amino-2'-deoxy-uridine; 2'-deoxy-2'-(N-β-alanyl)-guanosine;
 20 2'-*O*-amino-adenosine; 2'-(N-lysiyl)amino-2'-deoxy-cytidine; 2'-Deoxy-2'-(L-histidine) amino Cytidine; 5-Imidazoleacetic acid 2'-deoxy uridine, 5-[3-(N-4-imidazoleacetyl)aminopropynyl]-2'-*O*-methyl uridine, 5-(3-aminopropynyl)-2'-*O*-methyl uridine, 5-(3-aminopropyl)-2'-*O*-methyl uridine, 5-[3-(N-4-imidazoleacetyl)aminopropyl]-2'-*O*-methyl uridine, 5-(3-aminopropyl)-2'-deoxy-2-fluoro uridine, 2'-Deoxy-2'-(β-alanyl-L-histidyl)amino uridine, 2'-deoxy-2'-β-alaninamido-uridine, 3-(2'-deoxy-2'-fluoro-β-D-ribofuranosyl)piperazino[2,3-D]pyrimidine-2-one, 5-[3-(N-4-imidazoleacetyl)aminopropyl]-2'-deoxy-2'-fluoro uridine, 5-[3-(N-4-imidazoleacetyl)aminopropynyl]-2'-deoxy-2'-fluoro uridine, 5-E-

(2-carboxyvinyl-2'-deoxy-2'-fluoro uridine, 5-[3-(N-4-aspartyl)aminopropynyl-2'-fluoro uridine, 5-(3-aminopropyl)-2'-deoxy-2-fluoro cytidine, and 5-[3-(N-4-succynyl)aminopropyl-2'-deoxy-2-fluoro cytidine.

26. A process for incorporation of the compounds of claim 25 into an oligonucleotide
5 comprising the step of contacting said compound with a mixture comprising a nucleic acid template, an RNA polymerase enzyme, and an enhancer of modified nucleotide triphosphate incorporation, under conditions suitable for the incorporation of said compound into said oligonucleotide.

27. The process of claim 26, wherein said RNA polymerase is a T7 RNA polymerase.

10 28. The process of claim 26, wherein said RNA polymerase is a mutant T7 RNA polymerase.

29. The process of claim 26, wherein said RNA polymerase is a SP6 RNA polymerase.

30. The process of claim 26, wherein said RNA polymerase is a mutant SP6 RNA polymerase.

15 31. The process of claim 26, wherein said RNA polymerase is a T3 RNA polymerase.

32. The process of claim 26, wherein said RNA polymerase is a mutant T3 RNA polymerase.

33. The process of claim 26, wherein said enhancer of modified nucleotide triphosphate incorporation is selected from the group consisting of LiCl, methanol,
20 polyethylene glycol, diethyl ether, propanol, methylamine, and ethanol.

34. A process for the synthesis of a pyrimidine nucleotide triphosphate comprising the steps of:

a. monophosphorylation, wherein a pyrimidine nucleoside is contacted with a mixture comprising a phosphorylating reagent, a trialkyl phosphate and
25 dimethylaminopyridine, under conditions suitable for the formation of a pyrimidine nucleotide monophosphate; and

b. pyrophosphorylation, wherein said pyrimidine monophosphate from step (a) is contacted with a pyrophosphorylating reagent under conditions suitable for the formation of said pyrimidine nucleotide triphosphate.

35. The process of claim 34, wherein said pyrimidine nucleoside triphosphate is uridine triphosphate.

36. The process of claim 34, wherein said uridine triphosphate has a 2'-sugar modification.

5 37. The process of claim 36, wherein said uridine triphosphate is 2'-*O*-methylthiomethyl uridine triphosphate.

38. The process of claim 34, wherein said phosphorylating agent is selected from the group consisting of phosphorus oxychloride, phospho-tris-triazolides and phospho-tris-triimidazolides.

10 39. The process of claim 34, wherein said trialkylphosphate is triethyl phosphate.

40. The process of claim 34, wherein said pyrophosphorylating reagent is tributyl ammonium pyrophosphate.

41. The process of claim 26, wherein said oligonucleotide is RNA.

15 42. The process of claim 26, wherein said oligonucleotide is an enzymatic nucleic acid molecule.

43. The process of claim 26, wherein said oligonucleotide is an aptamer.

44. A kit for synthesis of an oligonucleotide comprising an RNA polymerase, an enhancer of modified nucleotide triphosphate incorporation and at least one compound of claim 25.

20 45. A kit for synthesis of an oligonucleotide comprising a DNA polymerase, an enhancer of modified nucleotide triphosphate incorporation and at least one compound of claim 25.

46. The kit of claim 44, wherein said RNA polymerase is a bacteriophage T7 RNA polymerase.

25 47. The kit of claim 44, wherein said RNA polymerase is a bacteriophage SP6 RNA polymerase.

48. The kit of claim 44, wherein said RNA polymerase is a bacteriophage T3 RNA polymerase.

49. The kit of claim 44, wherein said RNA polymerase is a mutant T7 RNA polymerase.
50. The kit of claim 44 or 45, wherein said kit comprises at least two different compounds of claim 25.
- 5 51. A nucleic acid catalyst comprising a histidyl modification, wherein said nucleic acid catalyst is able to catalyze an endonuclease reaction in the absence of a metal ion co-factor.
52. The nucleic acid catalyst of claim 51, wherein said catalyst is able to cleave a separate nucleic acid molecule.
- 10 53. The nucleic acid catalyst of claim 52, wherein said separate nucleic acid molecule is an RNA molecule.
54. The nucleic acid catalyst of claim 52, wherein said separate nucleic acid molecule is a DNA molecule.
- 15 55. The nucleic acid catalyst of claim 51, wherein said nucleic acid catalyst comprises at least one ribonucleotide.
56. The enzymatic nucleic acid molecule of claim 2, wherein said nucleic acid molecule has an endonuclease activity to cleave RNA of HER2 gene.
57. The enzymatic nucleic acid molecule of claim 56, wherein said nucleic acid molecule comprises sequences complementary to any of substrate sequences defined as Target sequence in Tables 58, 59 and 62.
- 20 58. The enzymatic nucleic acid molecule of claim 56, wherein said nucleic acid molecule comprises any of ribozyme sequences defined as Ribozyme sequence in Tables 58, 59 and 62.
59. A method for treating cancer using the enzymatic nucleic acid molecule of claim 25 56.
60. The method of claim 59, wherein said cancer is breast cancer.
61. A method for treating conditions associated with the level of HER2 gene using the enzymatic nucleic acid molecule of claim 56.

62. The enzymatic nucleic acid molecule of claim 56, wherein said enzymatic nucleic acid molecule comprises a substrate binding region which has between 5 and 30 nucleotides complementary to the RNA.
63. The enzymatic nucleic acid molecule of claim 56, wherein said enzymatic nucleic acid molecule comprises a substrate binding region which has between 7 and 12 nucleotides complementary to the RNA.
64. A mammalian cell including the enzymatic nucleic acid molecule of claim 56.
65. The mammalian cell of claim 64, wherein said mammalian cell is a human cell.
66. A mammalian cell including the enzymatic nucleic acid molecule of claims 1 or 2.
67. The mammalian cell of claim 66, wherein said mammalian cell is a human cell.
68. A method for inhibiting expression of HER2 gene in a cell, comprising the step of administering to said cell the enzymatic nucleic acid molecule of claim 56 under conditions suitable for said inhibition.
69. A method of cleaving RNA derived from HER2 gene comprising, contacting the enzymatic nucleic acid molecule of claim 56 with said RNA molecule under conditions suitable for the cleavage of said RNA molecule.
70. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of any of claims 1 or 2.
71. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of claim 56.
72. A method of treatment of a patient having a condition associated with the level of HER2, wherein said patient is administered the enzymatic nucleic acid molecule of claim 56 under conditions suitable for said treatment.
73. The method of claim 72, wherein said method is performed in conjunction with one or more other therapies.
74. The method of claim 59, wherein said enzymatic nucleic acid molecule is used in conjunction with one or more other therapies.
75. The enzymatic nucleic acid molecule of claim 56, wherein said enzymatic nucleic acid molecule comprises at least one sugar modification.

76. The enzymatic nucleic acid molecule of claim 56, wherein said enzymatic nucleic acid molecule comprises at least one nucleic acid base modification.
77. The enzymatic nucleic acid molecule of claim 56, wherein said enzymatic nucleic acid molecule comprises at least one phosphate backbone modification.
- 5 78. The enzymatic nucleic acid molecule of claim 56, wherein said phosphate backbone modification is selected from the group consisting of phosphorothioate, phosphorodithioate and amide.
79. An enzymatic nucleic acid molecule which down regulates expression of genes selected from the group consisting of beta site APP-cleaving enzyme (BACE) and
10 telomerase reverse transcriptase (TERT) genes.
80. The enzymatic nucleic acid molecule of claim 79, wherein said gene is the beta site APP-cleaving enzyme (BACE).
81. The enzymatic nucleic acid molecule of claim 79, wherein said gene is the telomerase reverse transcriptase (TERT).
- 15 82. A nucleic acid molecule which down regulates expression of genes selected from the group consisting of protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.
83. The nucleic acid molecule of claim 82, wherein said nucleic acid molecule is an
20 enzymatic nucleic acid molecule.
84. The nucleic acid molecule of claim 82, wherein said nucleic acid molecule is an antisense nucleic acid molecule.
85. The nucleic acid molecule of any of claims 82-84, wherein said gene is the protein-tyrosine phosphatase-1B (PTP-1B).
- 25 86. The nucleic acid molecule of any of claims 82-84, wherein said gene is the methionine aminopeptidase (MetAP-2).
87. The nucleic acid molecule of any of claims 82-84, wherein said gene is the hepatitis B virus (HBV).

88. The nucleic acid molecule of any of claims 82-84, wherein said gene is the phospholamban (PLN).

89. The nucleic acid molecule of any of claims 82-84, wherein said gene is the presenilin (ps-2).

5 90. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is adapted for use to treat diseases and conditions related to the expression of genes selected from the group consisting of beta site APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2),
10 hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.

91. The nucleic acid molecule of claim 82, wherein said nucleic acid molecule is adapted for use to treat diseases and conditions related to the expression of genes selected from the group consisting of protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban
15 (PLN), and presenilin (ps-2) genes.

92. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule has an endonuclease activity to cleave RNA encoded by said beta site APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine phosphatase-1B (PTP-1B), methionine
20 aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.

93. The enzymatic nucleic acid of any of claims 79 or 83, wherein a binding arm of said enzymatic nucleic acid molecule comprise sequences complementary to any of the sequences defined as Target or Substrate sequence in Tables 3-30, and 36-
25 43.

94. The enzymatic nucleic acid molecule of any of claims 79 or 83 wherein said enzymatic nucleic acid molecule comprises any of the sequences defined as Ribozyme or DNzyme sequence in Tables 3-29, and 37-43.

95. The nucleic acid molecule of claim 84, wherein said antisense nucleic acid
30 molecule comprises sequence complementary to any of the sequences defined as Target or Substrate sequence in Tables 3-12, 24-30, and 36-43.

96. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a hammerhead (HH) motif.
97. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a zinzyme (Class II) motif.
- 5 98. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a amberzyme (Class 1) motif.
99. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid, or RNase P nucleic acid motif.
- 10 100. The enzymatic nucleic acid molecule of claim 97, wherein said zinzyme motif comprises sequences complementary to any of the substrate sequences shown in Tables 21, 27 and 40.
101. The enzymatic nucleic acid molecule of claim 98, wherein said amberzyme motif comprises sequences complementary to any of the substrate sequences
15 shown in Tables 23, 29, and 42.
102. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a NCH motif.
103. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is in a G-cleaver motif.
- 20 104. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule is a DNAzyme.
105. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule comprises between 12 and 100 bases complementary to the RNA of genes selected from the group consisting of beta site
25 APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.
106. The enzymatic nucleic acid of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule comprises between 14 and 24 bases complementary to the
30 RNA of genes selected from the group consisting of beta site APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine

phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.

107. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid is chemically synthesized.

5 108. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid comprises at least one 2'-sugar modification.

109. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid comprises at least one nucleic acid base modification.

10 110. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid comprises at least one phosphate backbone modification.

111. A mammalian cell including the enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said mammalian cell is not a living human.

112. The mammalian cell of claim 111, wherein said mammalian cell is a human cell.

15 113. The antisense nucleic acid molecule of claim 84, wherein said antisense nucleic acid is chemically synthesized.

114. The antisense nucleic acid molecule of claim 84, wherein said antisense nucleic acid comprises at least one 2'-sugar modification.

20 115. The antisense nucleic acid molecule of claim 84, wherein said antisense nucleic acid comprises at least one nucleic acid base modification.

116. The antisense nucleic acid molecule of claim 84, wherein said antisense nucleic acid comprises at least one phosphate backbone modification.

117. A mammalian cell including the antisense nucleic acid molecule of claim 84, wherein said mammalian cell is not a living human.

25 118. The mammalian cell of claim 117, wherein said mammalian cell is a human cell.

119. A method of reducing BACE activity in a cell, comprising the step of contacting said cell with the enzymatic nucleic acid molecule of claim 80, under conditions suitable for said inhibition.

120. A method of reducing TERT activity in a cell, comprising the step of contacting said cell with the enzymatic nucleic acid molecule of claim 81, under conditions suitable for said inhibition.

121. A method of reducing PTP-1B activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 85, under conditions suitable for said inhibition.

122. A method of reducing MetAP-2 activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 86, under conditions suitable for said inhibition.

123. A method of reducing HBV activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 87, under conditions suitable for said inhibition.

124. A method of reducing phospholamban (PLN) activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 88, under conditions suitable for said inhibition.

125. A method of reducing presenilin-2 (ps-2) activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 89, under conditions suitable for said inhibition.

126. A method of treatment of a patient having a condition associated with the level of BACE, comprising contacting cells of said patient with the enzymatic nucleic acid molecule of claim 80, under conditions suitable for said treatment.

127. A method of treatment of a patient having a condition associated with the level of TERT, comprising contacting cells of said patient with the enzymatic nucleic acid molecule of claim 81, under conditions suitable for said treatment.

128. A method of treatment of a patient having a condition associated with the level of PTP-1B, comprising contacting cells of said patient with the nucleic acid molecule of claim 85, under conditions suitable for said treatment.

129. A method of treatment of a patient having a condition associated with the level of MetAP-2, comprising contacting cells of said patient with the nucleic acid molecule of claim 86, under conditions suitable for said treatment.

130. A method of treatment of a patient having a condition associated with the level of HBV, comprising contacting cells of said patient with the nucleic acid molecule of claim 87, under conditions suitable for said treatment.

131. A method of treatment of a patient having a condition associated with the level of phospholamban (PLN), comprising contacting cells of said patient with the nucleic acid molecule of claim 88, under conditions suitable for said treatment.

132. A method of treatment of a patient having a condition associated with the level of presenilin-2 (ps-2), comprising contacting cells of said patient with the nucleic acid molecule of claim 89, under conditions suitable for said treatment.

133. The method of any of claims 126-132 further comprising the use of one or more drug therapies under conditions suitable for said treatment.

134. A method of cleaving RNA of BACE gene, comprising, contacting the enzymatic nucleic acid molecule of claim 80, with said RNA under conditions suitable for the cleavage of said RNA.

135. A method of cleaving RNA of TERT gene, comprising, contacting the enzymatic nucleic acid molecule of claim 81, with said RNA under conditions suitable for the cleavage of said RNA.

136. A method of cleaving RNA of PTP-1B gene, comprising, contacting the enzymatic nucleic acid molecule of claim 85, with said RNA under conditions suitable for the cleavage of said RNA.

137. A method of cleaving RNA of MetAP-2 gene, comprising, contacting the enzymatic nucleic acid molecule of claim 86, with said RNA under conditions suitable for the cleavage of said RNA.

138. A method of cleaving RNA of HBV gene, comprising, contacting the enzymatic nucleic acid molecule of claim 87, with said RNA under conditions suitable for the cleavage of said RNA.

139. A method of cleaving RNA of phospholamban (PLN) gene, comprising, contacting the enzymatic nucleic acid molecule of claim 88, with said RNA under conditions suitable for the cleavage of said RNA.

140. A method of cleaving RNA of presenilin-2 (ps-2) gene, comprising, contacting the enzymatic nucleic acid molecule of claim 89, with said RNA under conditions suitable for the cleavage of said RNA.
141. The method of any of claims 134-140, wherein said cleavage is carried out in the presence of a divalent cation.
142. The method of claim 141, wherein said divalent cation is Mg^{2+} .
143. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid comprises a cap structure, wherein the cap structure is at the 5'-end or 3'-end or both the 5'-end and the 3'-end.
144. The antisense nucleic acid molecule of claim 84, wherein said antisense nucleic acid comprises a cap structure, wherein the cap structure is at the 5'-end or 3'-end or both the 5'-end and the 3'-end.
145. The enzymatic nucleic acid molecule of claim 96, wherein said hammerhead motif comprises sequences complementary to any of sequences defined as Target or Substrate sequences in Tables 3, 9, 13, 18, 24, and 37.
146. The enzymatic nucleic acid molecule of claim 102, wherein said NCH motif comprises sequences complementary to any of sequences defined as Target or Substrate sequences in Tables 4, 10, 14, 19, 25, and 38.
147. The enzymatic nucleic acid molecule of claim 103, wherein said G-cleaver motif comprises sequences complementary to any of sequences defined as Target or Substrate sequences in Tables 5, 11, 15, 20, 26, and 39.
148. The enzymatic nucleic acid molecule of claim 104, wherein said DNAzyme comprises sequences complementary to any of sequences defined as Target or Substrate sequences in Tables 6, 16, 22, 28, and 41.
149. The method of any of claims 119-125 or 133, wherein said enzymatic nucleic acid molecule is in a hammerhead motif.
150. The method of any of claims 119-125 or 133, wherein said nucleic acid molecule is a DNAzyme.

151. An expression vector comprising nucleic acid sequence encoding at least one enzymatic nucleic acid molecule of any of claims 79 or 83, in a manner which allows expression of that enzymatic nucleic acid molecule.
152. An expression vector comprising nucleic acid sequence encoding at least one antisense nucleic acid molecule of claim 84, in a manner which allows expression of that antisense nucleic acid molecule.
153. A mammalian cell including an expression vector of any of claims 151 or 152, wherein said mammalian cell is not a living human.
154. The mammalian cell of claim 153, wherein said mammalian cell is a human cell.
155. The expression vector of claim 151, wherein said enzymatic nucleic acid molecule is in a hammerhead motif.
156. The expression vector of claim 151, wherein said expression vector further comprises a sequence for an antisense nucleic acid molecule complementary to the RNA of genes selected from the group consisting of beta site APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.
157. The expression vector of claim 151, wherein said expression vector comprises sequence encoding at least two said enzymatic nucleic acid molecules, which may be same or different.
158. The expression vector of claim 157, wherein one said expression vector further comprises sequence encoding antisense nucleic acid molecule complementary to the RNA of genes selected from the group consisting of beta site APP-cleaving enzyme (BACE), telomerase reverse transcriptase (TERT), protein-tyrosine phosphatase-1B (PTP-1B), methionine aminopeptidase (MetAP-2), hepatitis B virus (HBV), phospholamban (PLN), and presenilin (ps-2) genes.
159. A method for treatment of Alzheimer's disease comprising the step of administering to a patient the enzymatic nucleic acid molecule of claim 80 under conditions suitable for said treatment.

160. The method of claim 159, wherein said treatment of Alzheimer's disease is treatment of dementia.

161. A method for treatment of Alzheimer's disease comprising the step of administering to a patient the antisense nucleic acid molecule of claim 89 under
5 conditions suitable for said treatment.

162. A method for treatment of diabetes comprising the step of administering to a patient the nucleic acid molecule of claim 85 under conditions suitable for said treatment.

163. The method of claim 162, wherein said diabetes is type I diabetes.

10 164. The method of claim 162, wherein said diabetes is type II diabetes.

165. A method for treatment of diabetes comprising the step of administering to a patient the antisense nucleic acid molecule of claim 85 under conditions suitable for said treatment.

15 166. A method for treatment of obesity comprising the step of administering to a patient the nucleic acid molecule of claim 85 under conditions suitable for said treatment.

167. A method for treatment of obesity comprising the step of administering to a patient the antisense nucleic acid molecule of claim 85 under conditions suitable for said treatment.

20 168. A method for treatment of heart disease comprising the step of administering to a patient the nucleic acid molecule of claim 88 under conditions suitable for said treatment.

169. The method of claim 168, wherein said heart disease is heart failure.

170. The method of claim 168, wherein said heart disease is congestive heart failure.

25 171. A method for treatment of pressure overload hypertrophy, or dilated cardiomyopathy, or both, comprising the step of administering to a patient the nucleic acid molecule of claim 88 under conditions suitable for said treatment.

172. A method for treatment of cancer comprising the step of administering to a patient the nucleic acid molecule of claim 86 under conditions suitable for said treatment.

173. A method for treatment of hepatitis comprising the step of administering to a patient the nucleic acid molecule of claim 87 under conditions suitable for said treatment.

174. A method for treatment of hepatocellular carcinoma comprising the step of administering to a patient the nucleic acid molecule of claim 87 under conditions suitable for said treatment.

175. The method of claim 159, wherein said enzymatic nucleic acid molecule is in a hammerhead motif.

176. The method of claim 159, wherein said method further comprises administering to said patient the enzymatic nucleic acid molecule in conjunction with one or more of other therapies.

177. The method of any of claims 162, 165-168, or 171-174, wherein said nucleic acid molecule is an enzymatic nucleic acid molecule.

178. The method of any of claims 162, 166-168, or 171-174, wherein said nucleic acid molecule is an antisense nucleic acid molecule.

179. The method of any of claims 162, 165-168, or 171-174, wherein said method further comprises administering to said patient the nucleic acid molecule in conjunction with one or more of other therapies.

180. The enzymatic nucleic acid molecule of any of claims 79 or 83, wherein said enzymatic nucleic acid molecule comprises at least five ribose residues; at least ten 2'-O-methyl modifications, and a 3'- end modification.

181. The enzymatic nucleic acid molecule of claim 180, wherein said enzymatic nucleic acid molecule further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

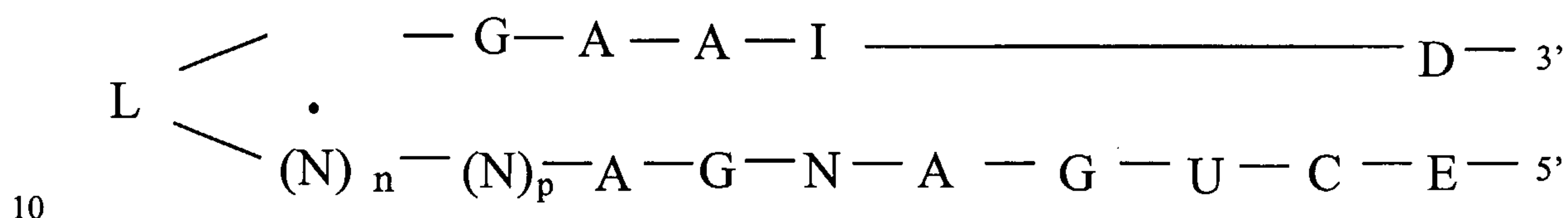
182. The enzymatic nucleic acid molecule of claim 180, wherein said 3'- end modification is 3'-3' inverted abasic moiety.

183. The enzymatic nucleic acid molecule of claim 104, wherein said DNAzyme comprises at least ten 2'-O-methyl modifications and a 3'-end modification.

184. The enzymatic nucleic acid molecule of claim 183, wherein said DNAzyme further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

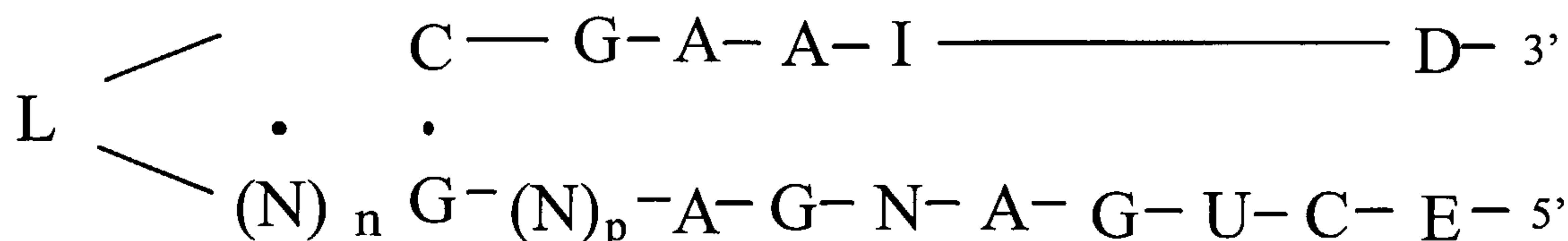
185. The enzymatic nucleic acid molecule of claim 183, wherein said 3'- end modification is 3'-3' inverted abasic moiety.

186. An enzymatic nucleic acid molecule having formula 1:



wherein N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; D and E are independently oligonucleotides of length sufficient to stably interact with a target RNA molecule; o and n are integers independently greater than or equal to 1, wherein if (N)o and (N)n are nucleotides, (N)o and (N)n are optionally able to interact by hydrogen bond interaction; • indicates base-paired interaction; L is a linker which may be present or absent, but when present, is a nucleotide linker, a non-nucleotide linker, or a combination of nucleotide and a non-nucleotide linker; p is an integer 0 or 1; — represents a chemical linkage; and A, U, I, C and G represent adenosine, uridine, inosine, cytidine and guanosine nucleotides, respectively.

187. An enzymatic nucleic acid molecule having formula 2:



wherein N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; D and E are independently oligonucleotides of length sufficient to stably interact with a target RNA molecule; o and n are integers independently greater than or equal to 0, wherein if (N)o and (N)n are nucleotides,

(N)o and (N)n are optionally able to interact by hydrogen bond interaction; • indicates base-paired interaction; L is a linker which may be present or absent, but when present, is a nucleotide linker, a non-nucleotide linker, or a combination of nucleotide and a non-nucleotide linker; p is an integer 0 or 1; represents a chemical linkage; and A, U, I, C and G represent adenosine, uridine, inosine, cytidine and guanosine nucleotides, respectively.

188. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said D and E are independently of length selected from the group consisting of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20 nucleotides.
- 10 189. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said D and E are of the same length.
190. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said D and E are of different length.
191. The enzymatic nucleic acid molecule of claim 186, wherein said o and n are
15 independently integers selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, and 50.
192. The enzymatic nucleic acid molecule of claim 187, wherein said o and n are independently integers selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, and 50.
- 20 193. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said (N)o and (N)n comprise nucleotides that are complementary to each other.
194. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said (N)o and (N)n are of the same length.
195. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said (N)o
25 and (N)n are of different length.
196. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said L is a nucleotide linker.
197. The enzymatic nucleic acid molecule of claim 196, wherein said nucleotide linker is of length between 3-50 nucleotides.

198. The enzymatic nucleic acid molecule of claim 196, wherein said nucleotide linker is an aptamer.

199. The enzymatic nucleic acid molecule of claim 196 wherein said nucleotide linker is selected from the group consisting of 5'-GAAA-3' and 5'-GUUA-3'.

5 200. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said L is a non-nucleotide linker.

201. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said chemical linkage is independently or in combination selected from the group consisting of phosphate ester linkage, amide linkage, phosphorothioate, arabino,
10 arabinofluoro, and phosphorodithioate.

202. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said p is 1.

203. The enzymatic nucleic acid molecule of claim 202, wherein said N of (N)_p is independently selected from the group consisting of adenosine, uridine, and cytidine.

15 204. The enzymatic nucleic acid molecule of claims 186 or 187 wherein said enzymatic nucleic acid molecule is chemically synthesized.

205. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least three ribonucleotide residues.

20 206. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least four ribonucleotide residues.

207. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least five ribonucleotide residues.

208. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said I is selected from the group consisting of ribo-inosine and xylo-inosine.

25 209. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least one sugar modification.

210. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least nucleic acid base modification.

211. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule comprises at least one phosphate backbone modification.

212. The enzymatic nucleic acid molecule of claim 209, wherein said sugar modification is selected from the group consisting of 2'-H, 2'-O-methyl, 2'-O-allyl, and 2'-deoxy-2'-amino.

213. The enzymatic nucleic acid molecule of claim 211, wherein said phosphate backbone modification is selected from the group consisting of phosphorothioate, phosphorodithioate and amide.

214. The enzymatic nucleic acid molecule of claims 186 or 187 wherein said enzymatic nucleic acid molecule comprises a 5'-cap or a 3'-cap or both a 5'-cap and a 3'-cap.

215. The enzymatic nucleic acid molecule of claim 214, wherein said 5'-cap is a phosphorothioate modification of at least one 5'-terminal nucleotide in said enzymatic nucleic acid molecule.

216. The enzymatic nucleic acid molecule of claim 214, wherein said 5'-cap is a phosphorothioate modification of at least two 5'-terminal nucleotide in said enzymatic nucleic acid molecule.

217. The enzymatic nucleic acid molecule of claim 214, wherein said 5'-cap is a phosphorothioate modification of at least three 5'-terminal nucleotide in said enzymatic nucleic acid molecule.

218. The enzymatic nucleic acid molecule of claim 214, wherein said 3'-cap is a 3'-3' inverted abasic moiety.

219. The enzymatic nucleic acid molecule of claim 214, wherein said 3'-cap is a 3'-3' inverted nucleotide moiety.

220. A method for inhibiting expression of a gene in a cell, comprising the step of administering to said cell the enzymatic nucleic acid molecule of claims 186 or 187 under conditions suitable for said inhibition.

221. A method of cleaving a separate RNA molecule comprising, contacting the enzymatic nucleic acid molecule of claims 186 or 187 with said separate RNA

molecule under conditions suitable for the cleavage of said separate RNA molecule.

222. The method of claim 221, wherein said cleavage is carried out in the presence of a divalent cation.

5 223. The method of claim 222, wherein said divalent cation is Mg^{2+} .

224. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said enzymatic nucleic acid molecule has an endonuclease activity to cleave RNA derived from HER2 gene.

10 225. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises sequences complementary to any of NCH substrate sequence of Table 34.

226. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises any of the NCH ribozyme sequences shown in Table 34.

15 227. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule is used to treat cancer.

228. The enzymatic nucleic acid molecule of claim 224, wherein said cancer is breast cancer.

20 229. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule is used to treat conditions associated with the level of HER2 gene.

230. An enzymatic nucleic acid molecule, wherein said enzymatic nucleic acid molecule comprises any of sequence shown as NCH ribozyme sequence in Table 31.

25 231. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises a substrate binding region which has between 5 and 30 nucleotides complementary to the RNA.

30 232. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises a substrate binding region which has between 7 and 12 nucleotides complementary to the RNA.

233. A mammalian cell including the enzymatic nucleic acid molecule of claim 224, wherein said mammalian cell is not a living human.

234. The mammalian cell of claim 233, wherein said mammalian cell is a human cell.

5 235. A mammalian cell including the enzymatic nucleic acid molecule of claims 186 or 187, wherein said mammalian cell is not a living human.

236. The mammalian cell of claim 235, wherein said mammalian cell is a human cell.

10 237. A method for inhibiting expression of HER2 gene in a cell, comprising the step of administering to said cell the enzymatic nucleic acid molecule of claim 224 under conditions suitable for said inhibition.

238. A method of cleaving RNA derived from HER2 gene comprising, contacting the enzymatic nucleic acid molecule of claim 224 with said RNA molecule under conditions suitable for the cleavage of said RNA molecule.

15 239. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of any of claims 186 or 187.

240. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of claim 224.

20 241. A method of treatment of a patient having a condition associated with the level of HER2, wherein said patient is administered the enzymatic nucleic acid molecule of claim 224 under conditions suitable for said treatment.

242. The method of claim 241, wherein said method is performed in conjunction with one or more other therapies.

25 243. The enzymatic nucleic acid molecule of claim 227, wherein said enzymatic nucleic acid molecule is used in conjunction with one or more other therapies.

244. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said nucleic acid molecule comprises at least five ribose residues; a 2'-C-allyl modification at position No. 4 of said enzymatic nucleic acid; at least ten 2'-O-alkyl modifications, and a 3'-cap structure.

245. The enzymatic nucleic acid molecule of claim 244, wherein said 2'-O-alkyl modifications is selected from the group consisting of 2'-O-methyl and 2'-O-allyl.

246. The enzymatic nucleic acid molecule of claim 244, wherein said 3'-cap is 3'-3' inverted abasic moiety.

5 247. The enzymatic nucleic acid molecule of claim 244, wherein said 3'-cap is 3'-3' inverted nucleotide.

248. The enzymatic nucleic acid molecule of claim 244, wherein said enzymatic nucleic acid comprises phosphorothioate linkages in at least three of the 5' terminal nucleotides.

10 249. The enzymatic nucleic acid molecule of claims 186 or 187, wherein said nucleic acid molecule comprises at least five ribose residues; a 2'-deoxy-2'-amino modification at position Nos. 4 and 7 of said enzymatic nucleic acid; at least ten 2'-O-alkyl modifications, and a 3'-cap structure.

15 250. The enzymatic nucleic acid molecule of claim 249, wherein said 2'-O-alkyl modifications is selected from the group consisting of 2'-O-methyl and 2'-O-allyl.

251. The enzymatic nucleic acid molecule of claim 249, wherein said 3'-cap is 3'-3' inverted abasic moiety.

252. The enzymatic nucleic acid molecule of claim 249, wherein said 3'-cap is 3'-3' inverted nucleotide.

20 253. The enzymatic nucleic acid molecule of claim 249, wherein said enzymatic nucleic acid comprises phosphorothioate linkages in at least three of the 5' terminal nucleotides.

254. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises at least one sugar modification.

25 255. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises at least one nucleic acid base modification.

256. The enzymatic nucleic acid molecule of claim 224, wherein said enzymatic nucleic acid molecule comprises at least one phosphate backbone modification.

257. The enzymatic nucleic acid molecule of claim 224, wherein said phosphate backbone modification is selected from the group consisting of phosphorothioate, phosphorodithioate and amide.

258. The enzymatic nucleic acid molecule of claim 224, wherein said nucleic acid molecule comprises at least five ribose residues; a 2'-C-allyl modification at position No. 4 of said enzymatic nucleic acid; at least ten 2'-O-alkyl modifications, and a 3'-cap structure.

259. The enzymatic nucleic acid molecule of claim 258, wherein said 2'-O-alkyl modifications is selected from the group consisting of 2'-O-methyl and 2'-O-allyl.

260. The enzymatic nucleic acid molecule of claim 258, wherein said 3'-cap is 3'-3' inverted abasic moiety.

261. The enzymatic nucleic acid molecule of claim 258, wherein said 3'-cap is 3'-3' inverted nucleotide.

262. The enzymatic nucleic acid molecule of claim 258, wherein said enzymatic nucleic acid comprises phosphorothioate linkages in at least three of the 5' terminal nucleotides.

263. The enzymatic nucleic acid molecule of claim 224, wherein said nucleic acid molecule comprises at least five ribose residues; a 2'-deoxy-2'-amino modification at position Nos. 4 and 7 of said enzymatic nucleic acid; at least ten 2'-O-alkyl modifications, and a 3'-cap structure.

264. The enzymatic nucleic acid molecule of claim 263, wherein said 2'-O-alkyl modifications is selected from the group consisting of 2'-O-methyl and 2'-O-allyl.

265. The enzymatic nucleic acid molecule of claim 263, wherein said 3'-cap is 3'-3' inverted abasic moiety.

266. The enzymatic nucleic acid molecule of claim 263, wherein said 3'-cap is 3'-3' inverted nucleotide.

267. The enzymatic nucleic acid molecule of claim 263, wherein said enzymatic nucleic acid comprises phosphorothioate linkages in at least three of the 5' terminal nucleotides.

268. The enzymatic nucleic acid molecule of claim 186, wherein said enzymatic nucleic acid molecule is capable of down-regulating the expression of protein kinase C alpha (PKC alpha) gene.

269. A method for inhibiting expression of a PKC alpha gene in a cell, comprising the step of administering to said cell the enzymatic nucleic acid molecule of claim 268 under conditions suitable for said inhibition.

270. A method of cleaving a PKC alpha RNA molecule comprising, contacting the enzymatic nucleic acid molecule of claim 268 with said separate PKC alpha RNA molecule under conditions suitable for the cleavage of said PKC alpha RNA molecule.

271. The method of claim 270, wherein said cleavage is carried out in the presence of a divalent cation.

272. The method of claim 271, wherein said divalent cation is Mg^{2+} .

273. The enzymatic nucleic acid molecule of claim 268, wherein said enzymatic nucleic acid molecule has an endonuclease activity to cleave RNA derived from PKC alpha gene.

274. The enzymatic nucleic acid molecule of claim 273, wherein said enzymatic nucleic acid molecule comprises sequences complementary to any of NCH substrate sequence of Table 63.

275. The enzymatic nucleic acid molecule of claim 273 wherein said enzymatic nucleic acid molecule comprises any of the NCH ribozyme sequences shown in Table 63.

276. The enzymatic nucleic acid molecule of claim 268, wherein said enzymatic nucleic acid molecule is used to treat cancer.

277. The enzymatic nucleic acid molecule of claim 276, wherein said cancer is selected from the group consisting of lung, breast, colon, prostate, bladder, ovary, melanoma, and glioblastoma cancer.

278. The enzymatic nucleic acid molecule of claim 268, wherein said enzymatic nucleic acid molecule is used to treat conditions associated with the level of PKC alpha gene.

279. The enzymatic nucleic acid molecule of claim 268, wherein said D and E independently has between 5 and 30 nucleotides complementary to the RNA.

280. The enzymatic nucleic acid molecule of claim 268, wherein said D and E independently has between 7 and 12 nucleotides complementary to the RNA.

5 281. A mammalian cell including the enzymatic nucleic acid molecule of claim 268, wherein said mammalian cell is not a living human.

282. The mammalian cell of claim 281, wherein said mammalian cell is a human cell.

10 283. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of claim 238.

284. A pharmaceutical composition comprising the enzymatic nucleic acid molecule of claim 273.

15 285. A method of treatment of a patient having a condition associated with the level of PKC alpha, wherein said patient is administered the enzymatic nucleic acid molecule of claim 268 under conditions suitable for said treatment.

286. The method of claim 285, wherein said method is performed in conjunction with one or more other therapies.

287. The enzymatic nucleic acid molecule of claim 286, wherein said enzymatic nucleic acid molecule is used in conjunction with one or more other therapies.

20 288. An antisense nucleic acid molecule comprising sequence complementary to any of substrate sequence in Tables 13-23.

289. The antisense nucleic acid molecule of claim 288, wherein said enzymatic nucleic acid is chemically synthesized.

25 290. The antisense nucleic acid molecule of claim 288, wherein said antisense nucleic acid comprises at least one 2'-sugar modification.

291. The antisense nucleic acid molecule of claim 288, wherein said antisense nucleic acid comprises at least one nucleic acid base modification.

292. The antisense nucleic acid molecule of claim 288, wherein said antisense nucleic acid comprises at least one phosphate backbone modification.

293. A mammalian cell including the antisense nucleic acid molecule of claim 288, wherein said mammalian cell is not a living human.

294. The mammalian cell of claim 293, wherein said mammalian cell is a human cell.

Figure 1: Ribozyme Motifs

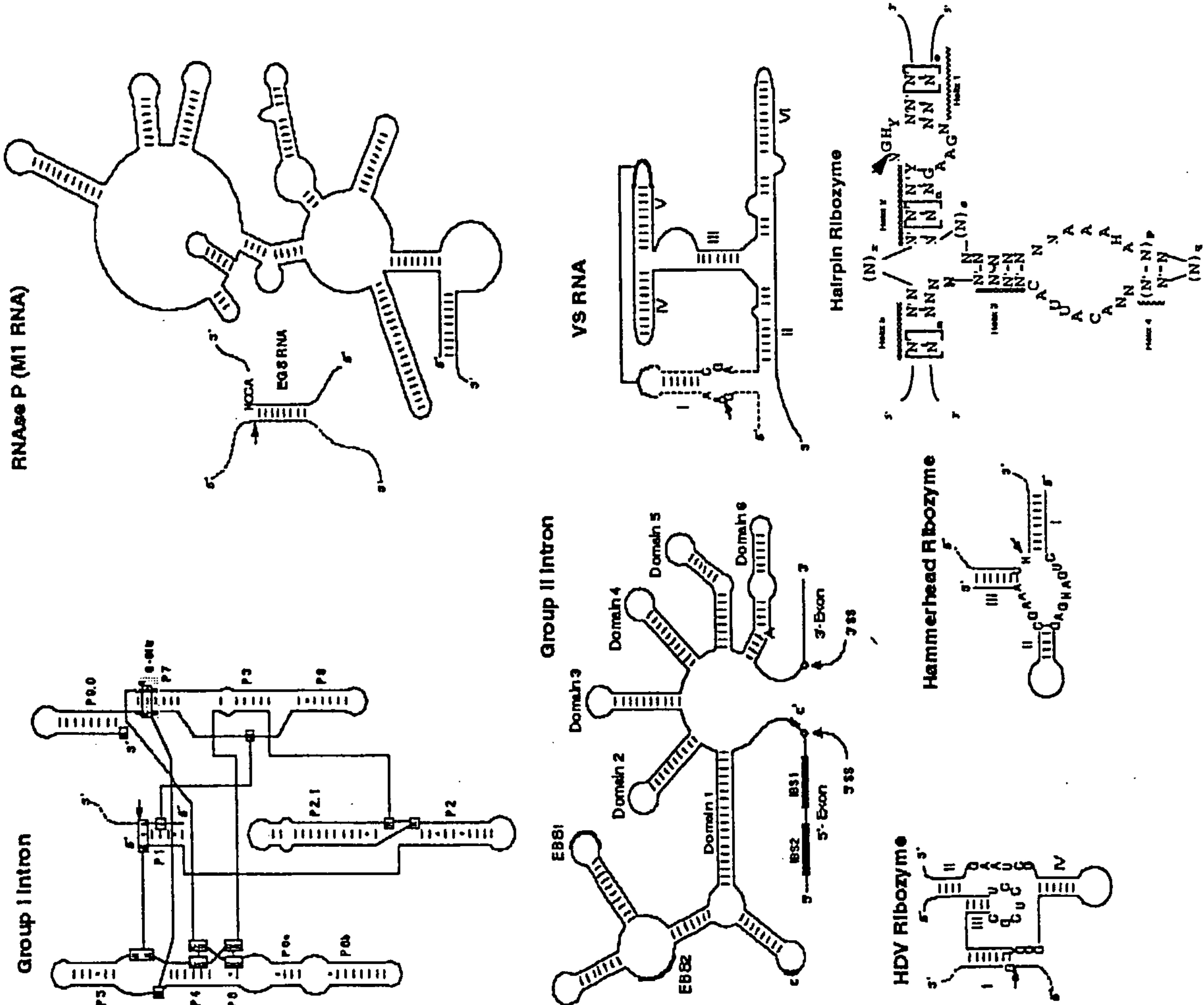
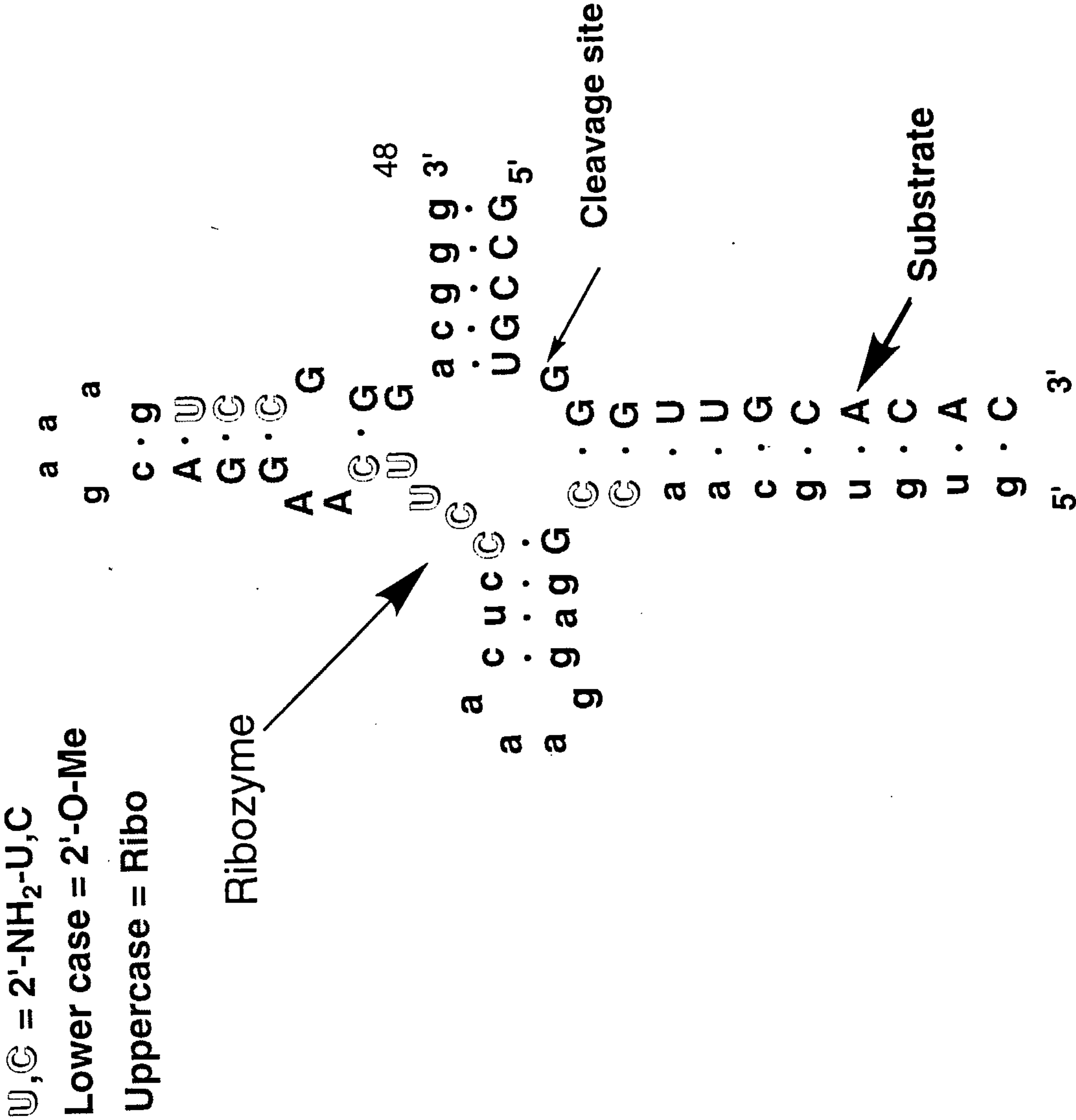
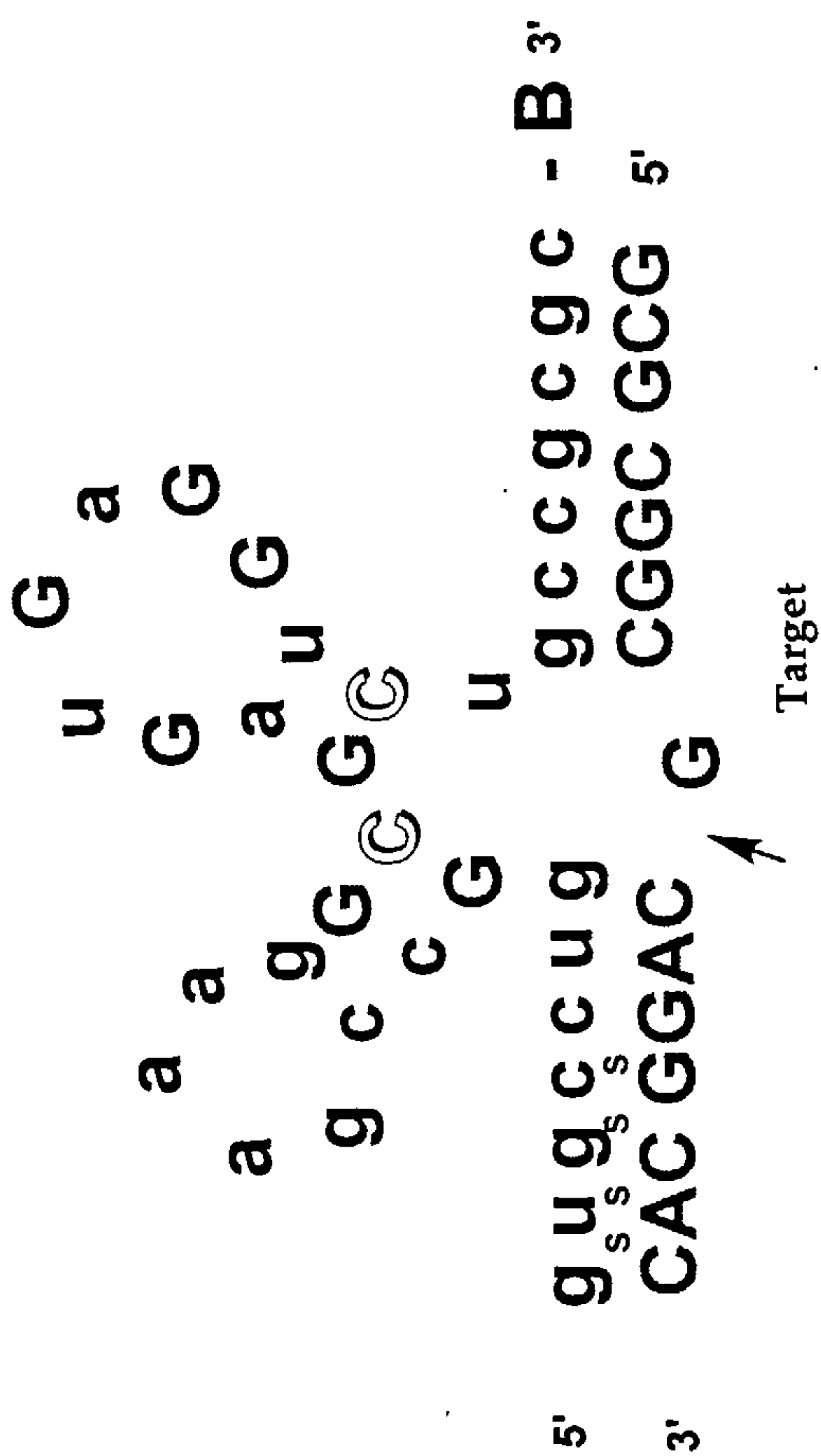


Figure 3: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif



Zinzyme A-motif RZ



Legend

Uppercase indicates natural ribo residues

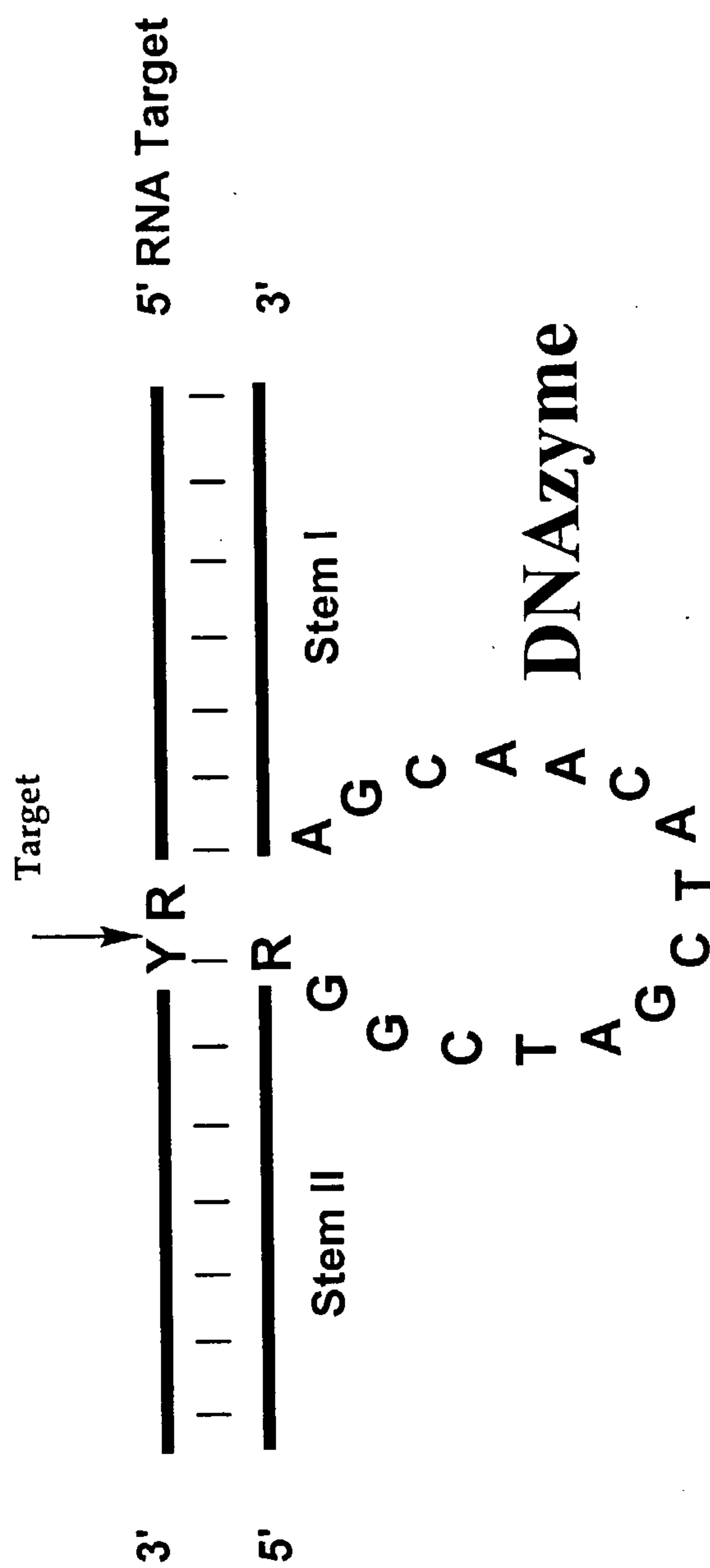
© indicates 2' - d-NH₂-C

Lowercase: 2'-O-Me

Subscript s indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 5: DNAzyme Motif



Legend

$$Y = U \text{ or } C$$

R = A or G

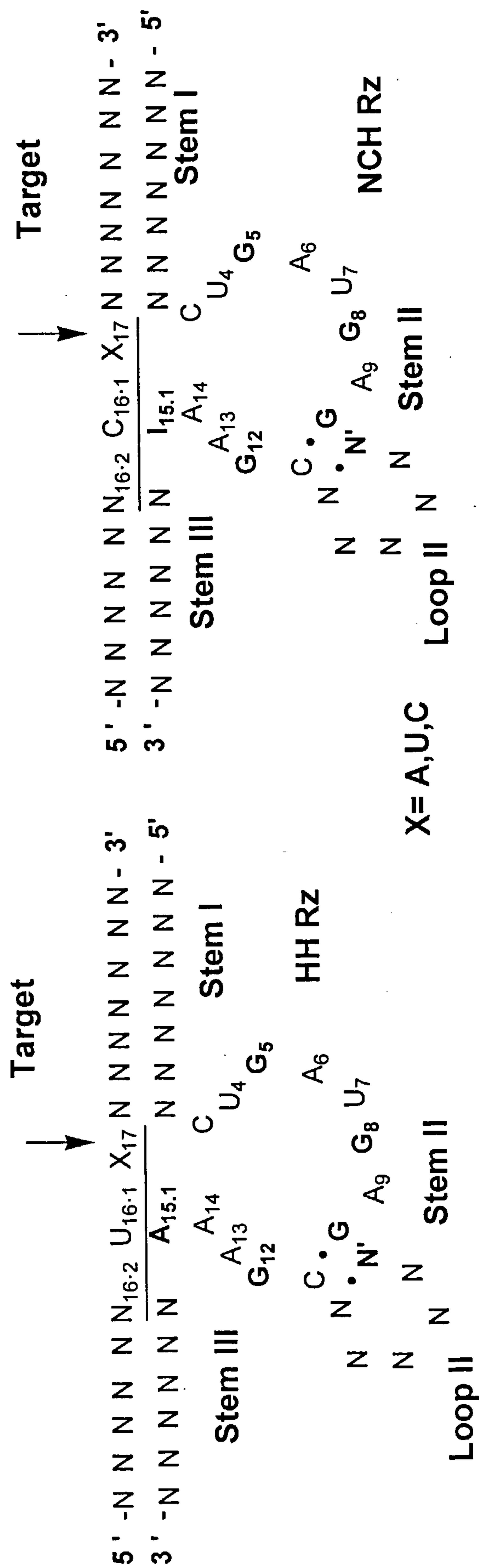


Figure 7: Examples of Nuclease Stable Ribozyme Motifs

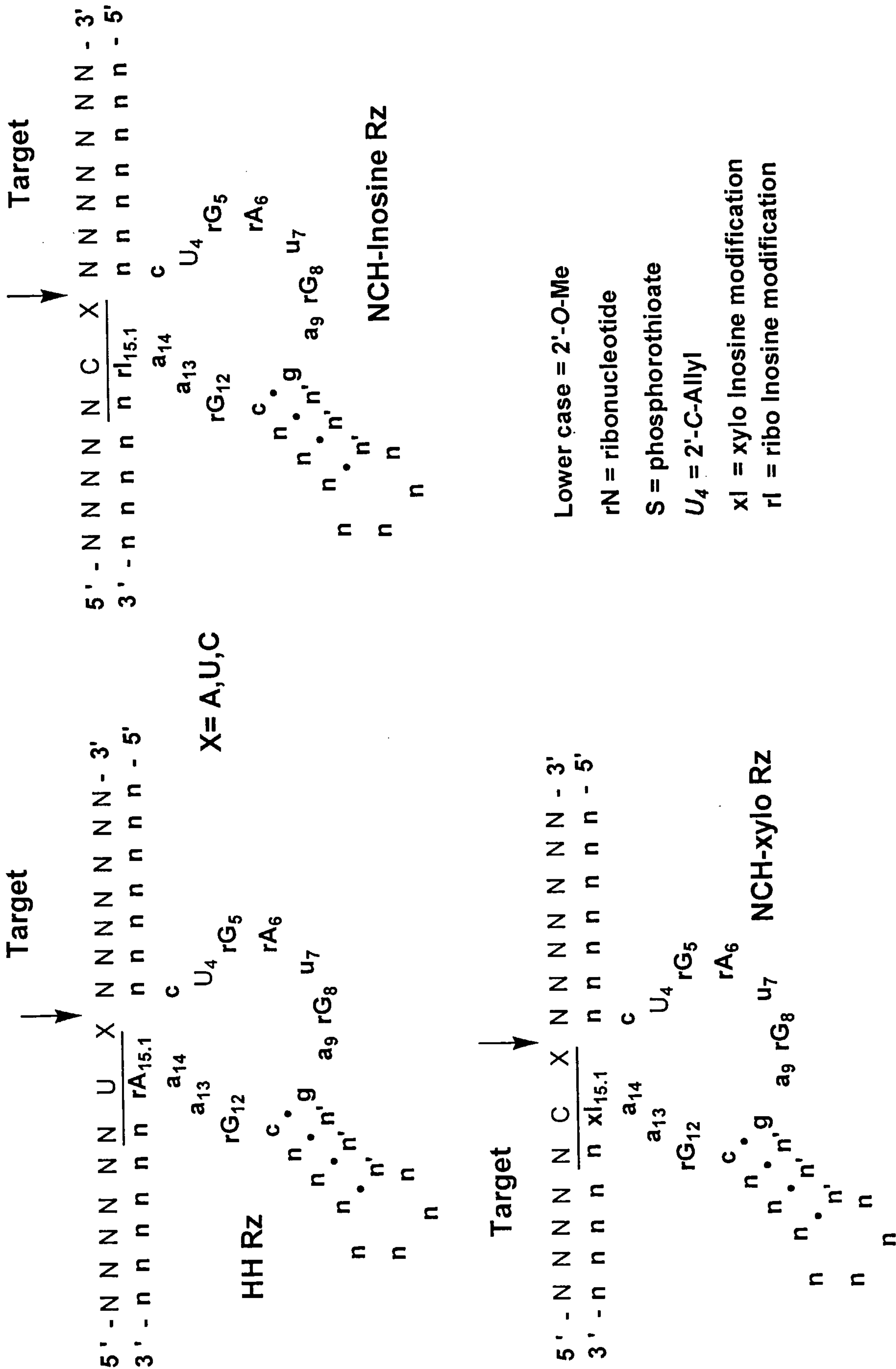


Figure 8: Inhibition of Cell Proliferation by Anti-Her2 Ribozymes

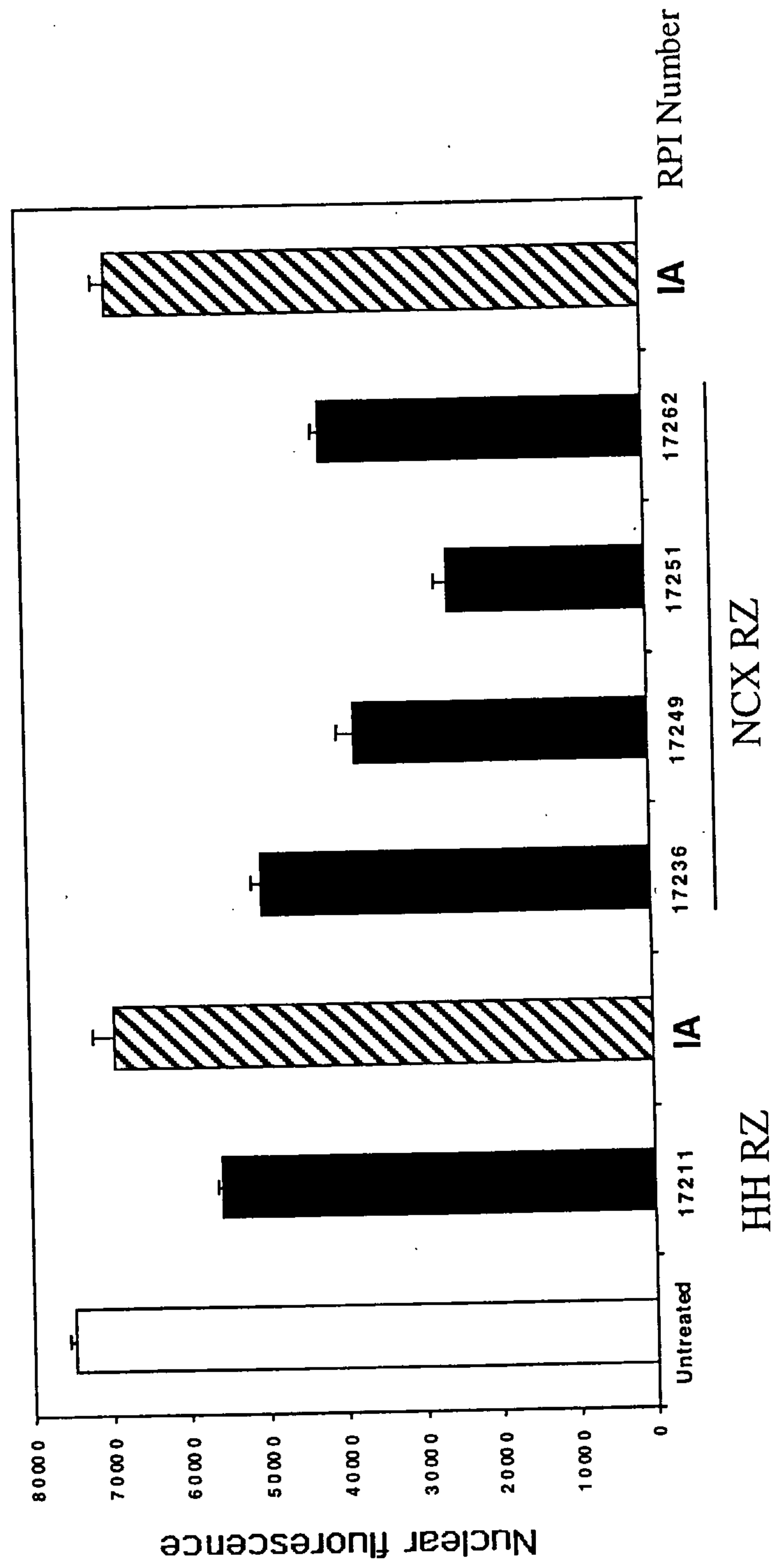
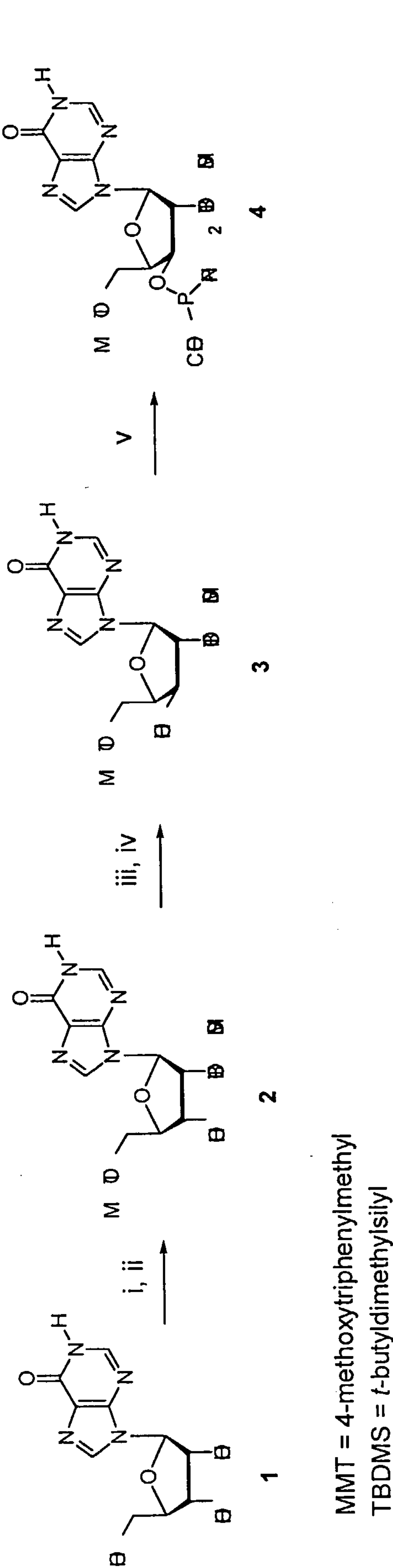
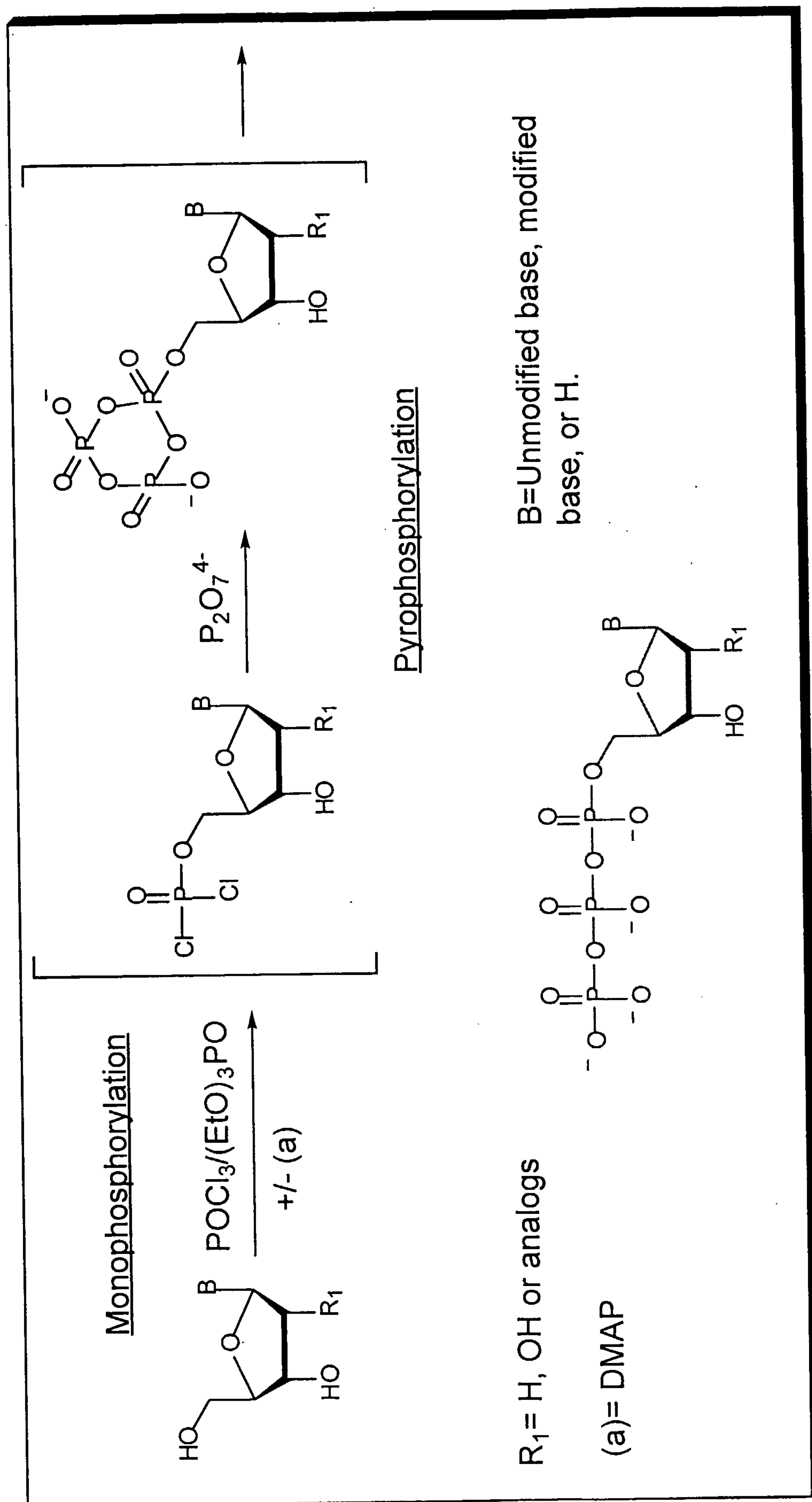


Figure 9: Synthesis of b-D-xylofuranosyl hypoxanthine 3'-phosphoramidite





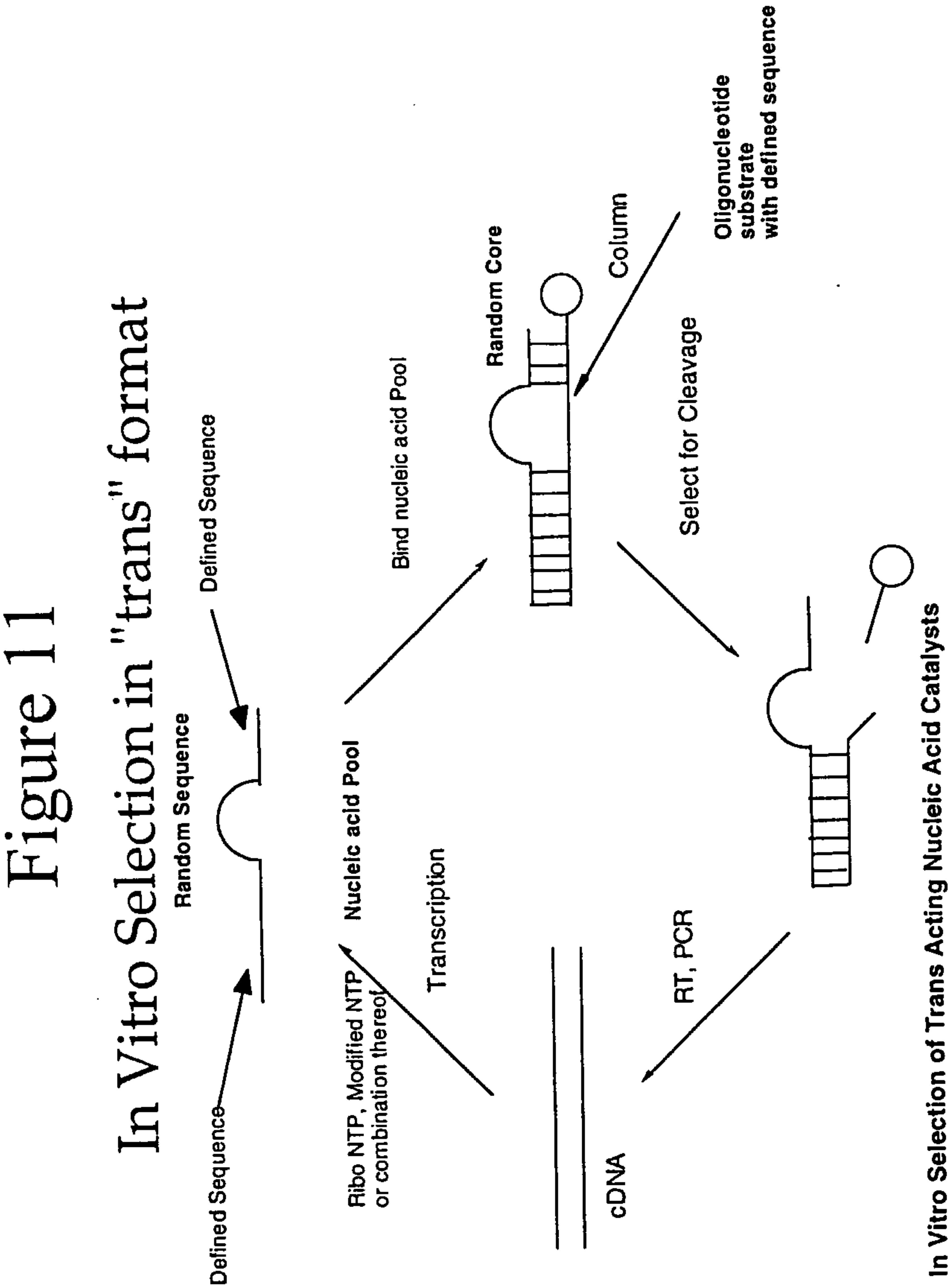


Figure 12. Removal of “parasitic RNA” using a Second Selection column

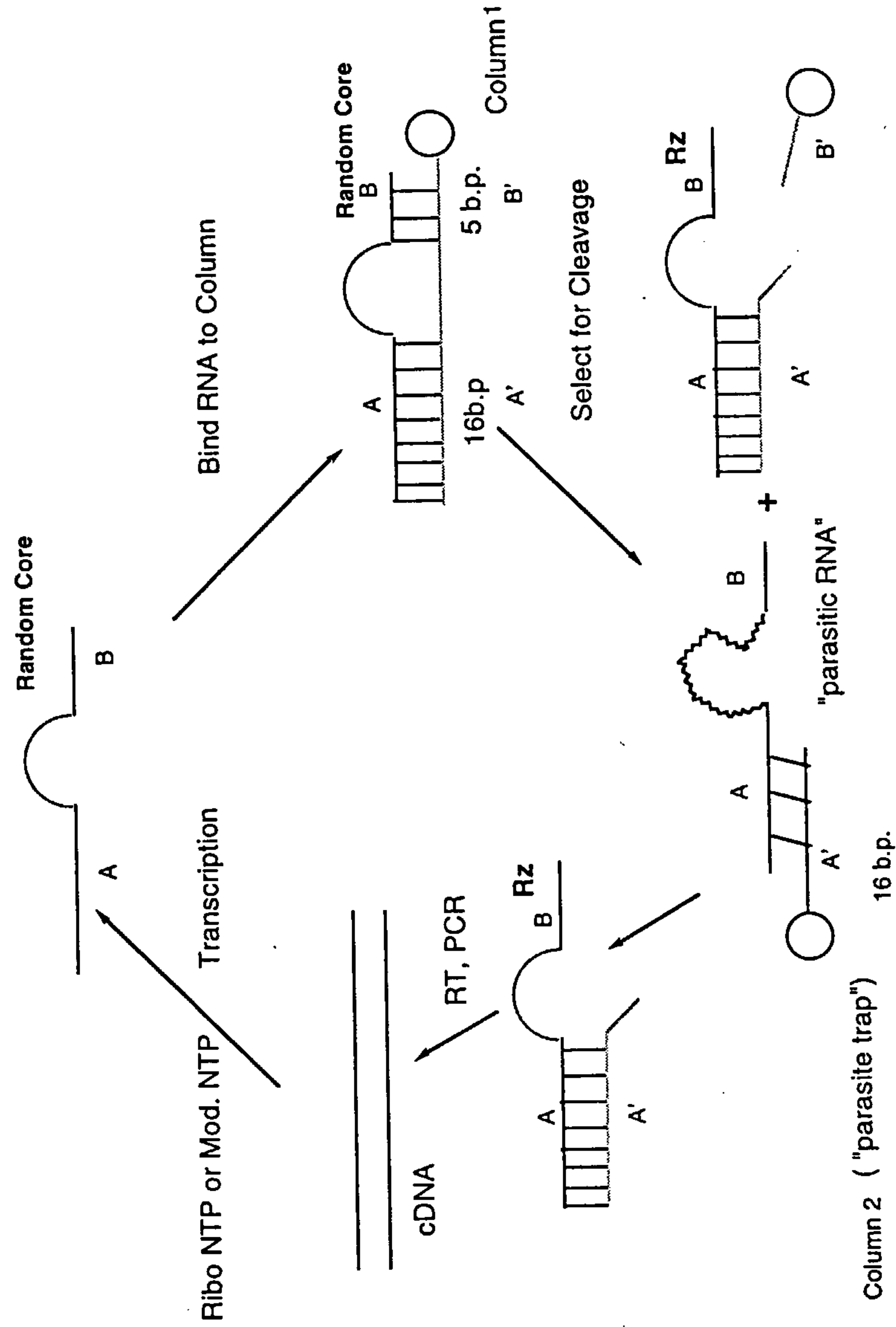


FIGURE 14. Dual Reporter System for Cytoplasmic HCV Target

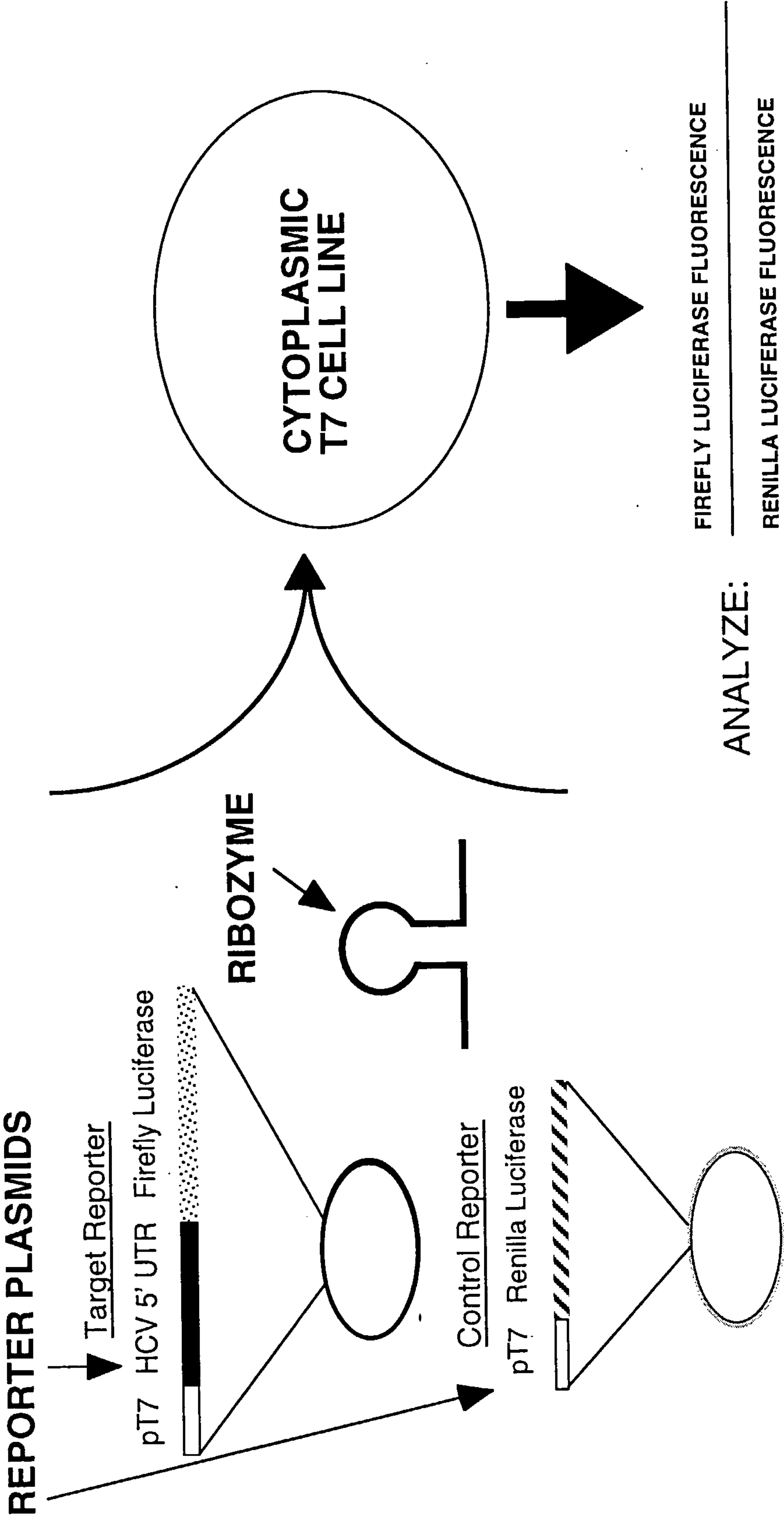


Figure 15. Dose-dependent inhibition of HCV-IRES mediated luciferase activity

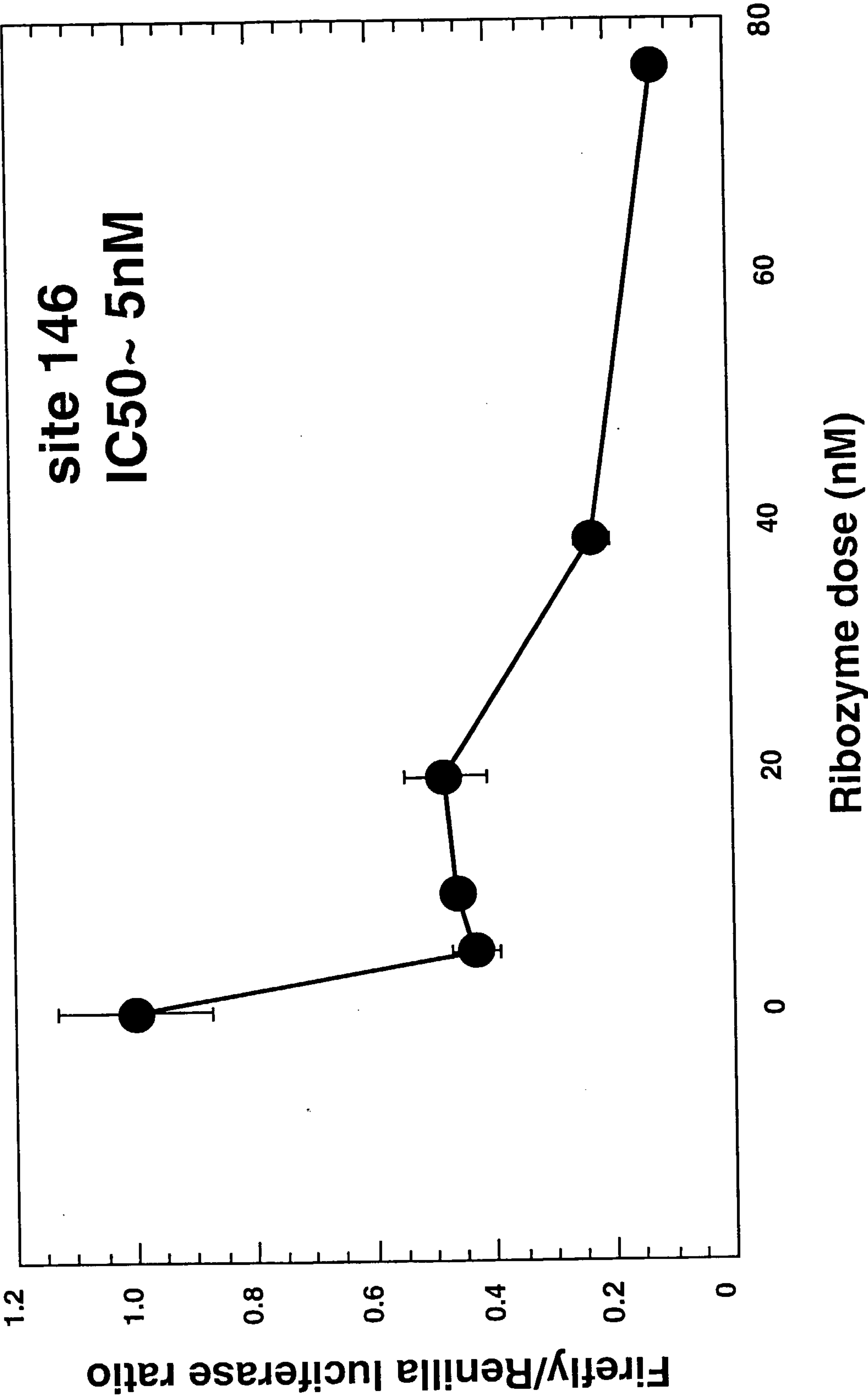
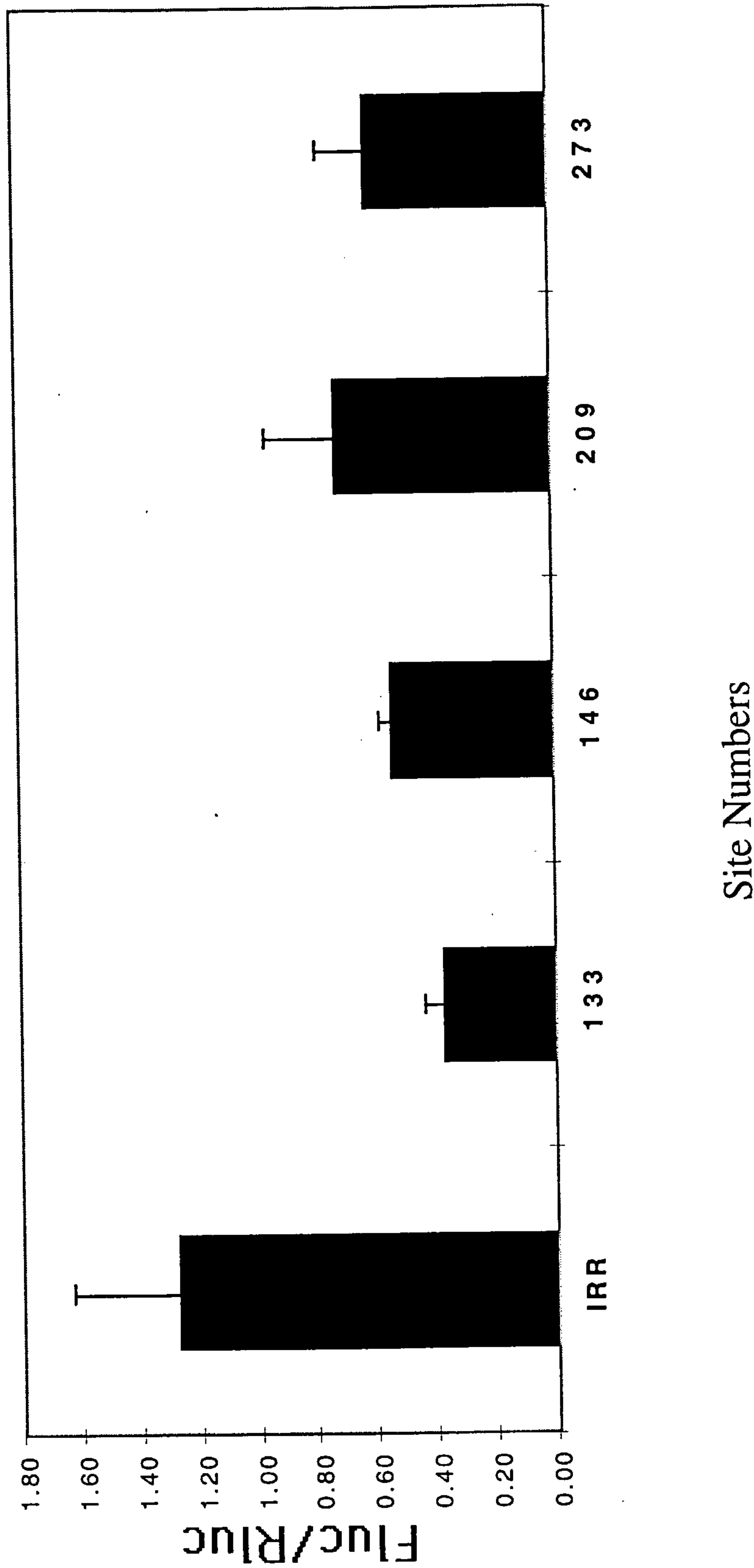


Figure 16. Efficacious Ribozymes Targeting 5'UTR HCV RNA



Sequence and chemical compositions for site numbers are given in table XII

Figure 17. Characterized Class II Enzymatic Nucleic Acid Motifs

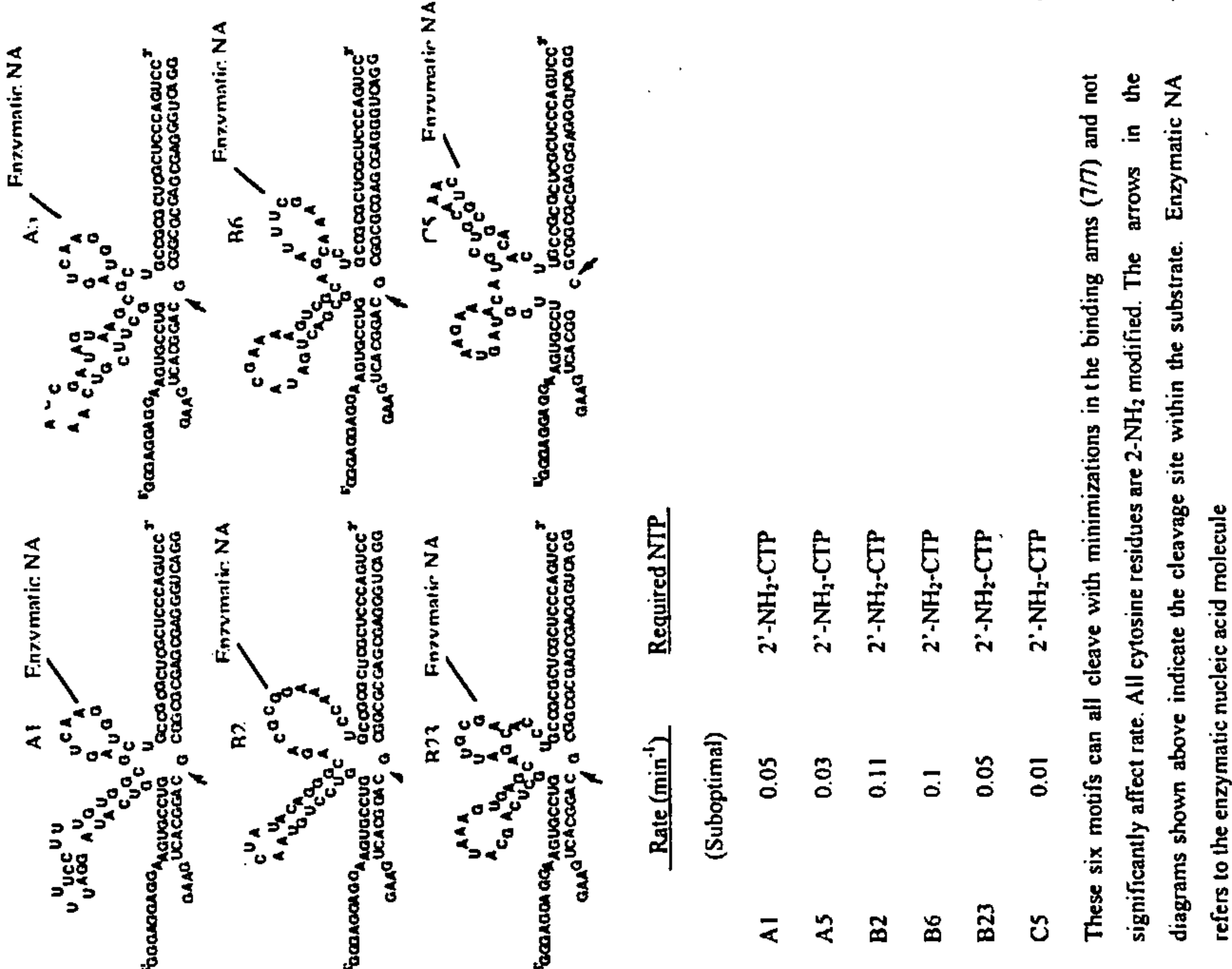
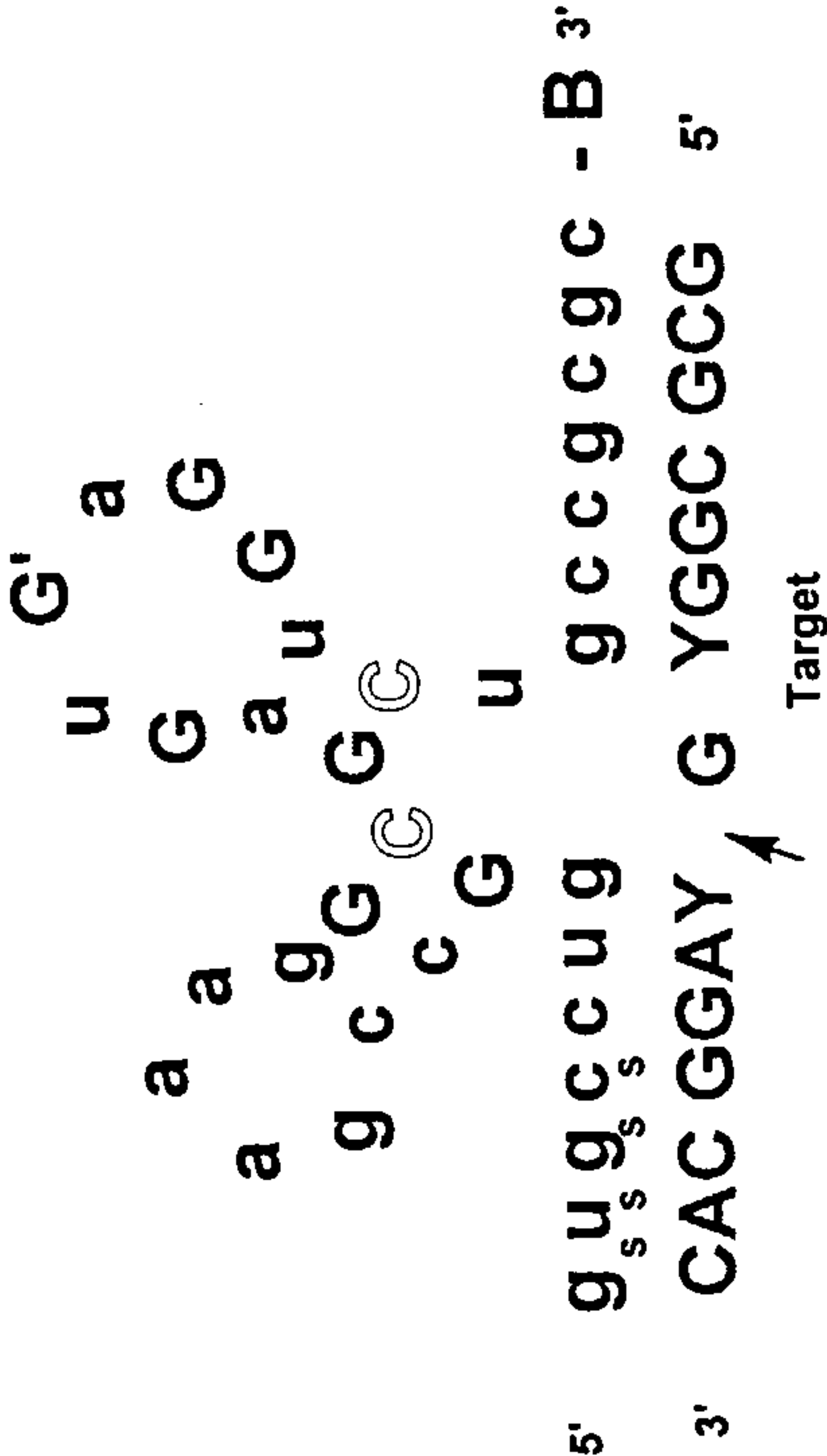


Figure 18: Chemically Stabilized Class II Motif



Legend

Uppercase indicates natural ribo residues

Ⓒ indicates 2' - d-NH₂-C

Lowercase: 2'-O- Me

Subscript _s indicates phosphothioate linkage

B: 3'-3' abasic moiety

Y = U or C

G' can be G, ca, or caa

The gaaa tetraloop can be replaced by 18 atom polyethylene glycol (Spacer)

All ribo G's can be replaced with 2'-O-methyl/ G

Figure 19: Substrate specificities of Class II (zinzyme) ribozymes

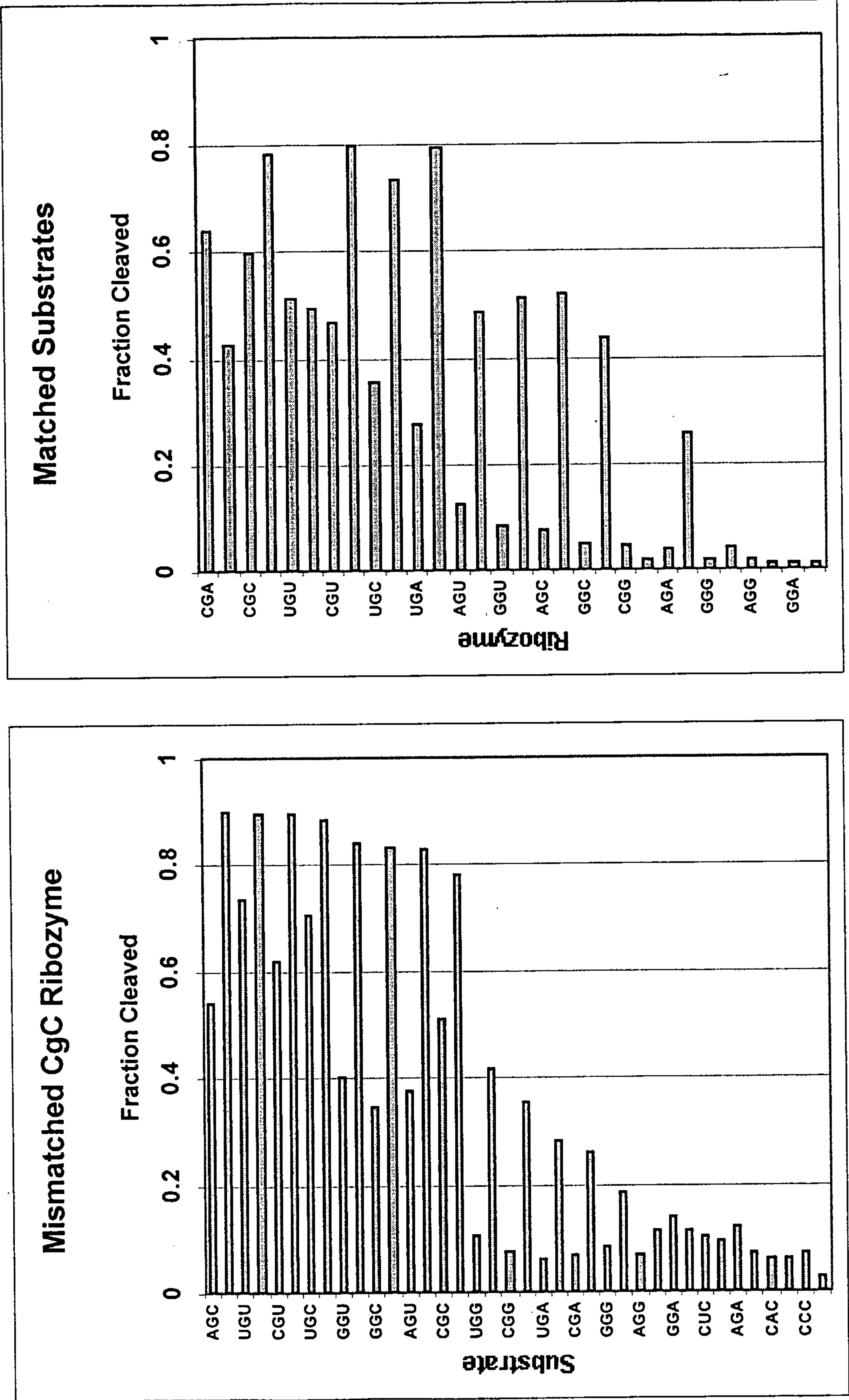
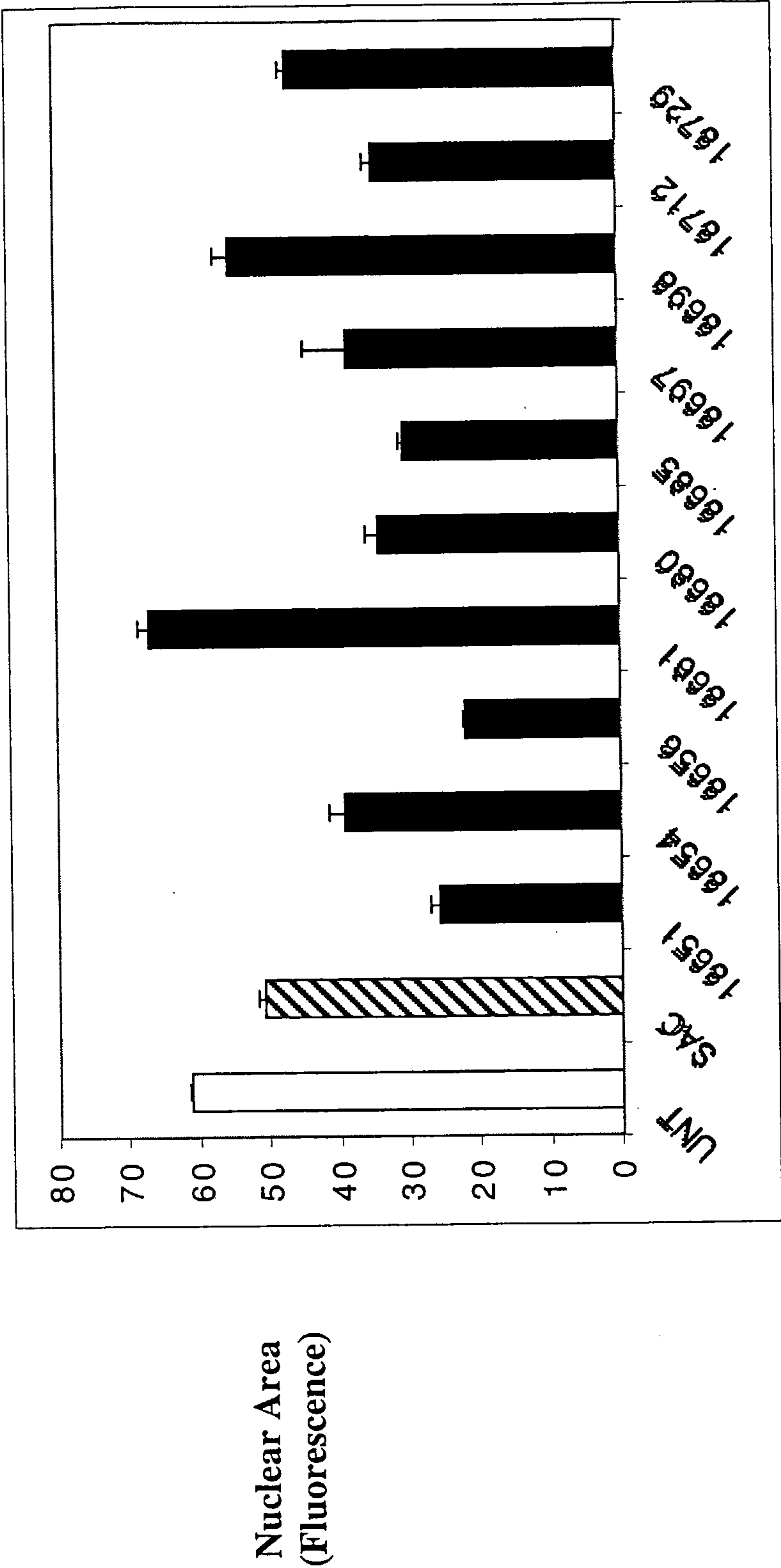
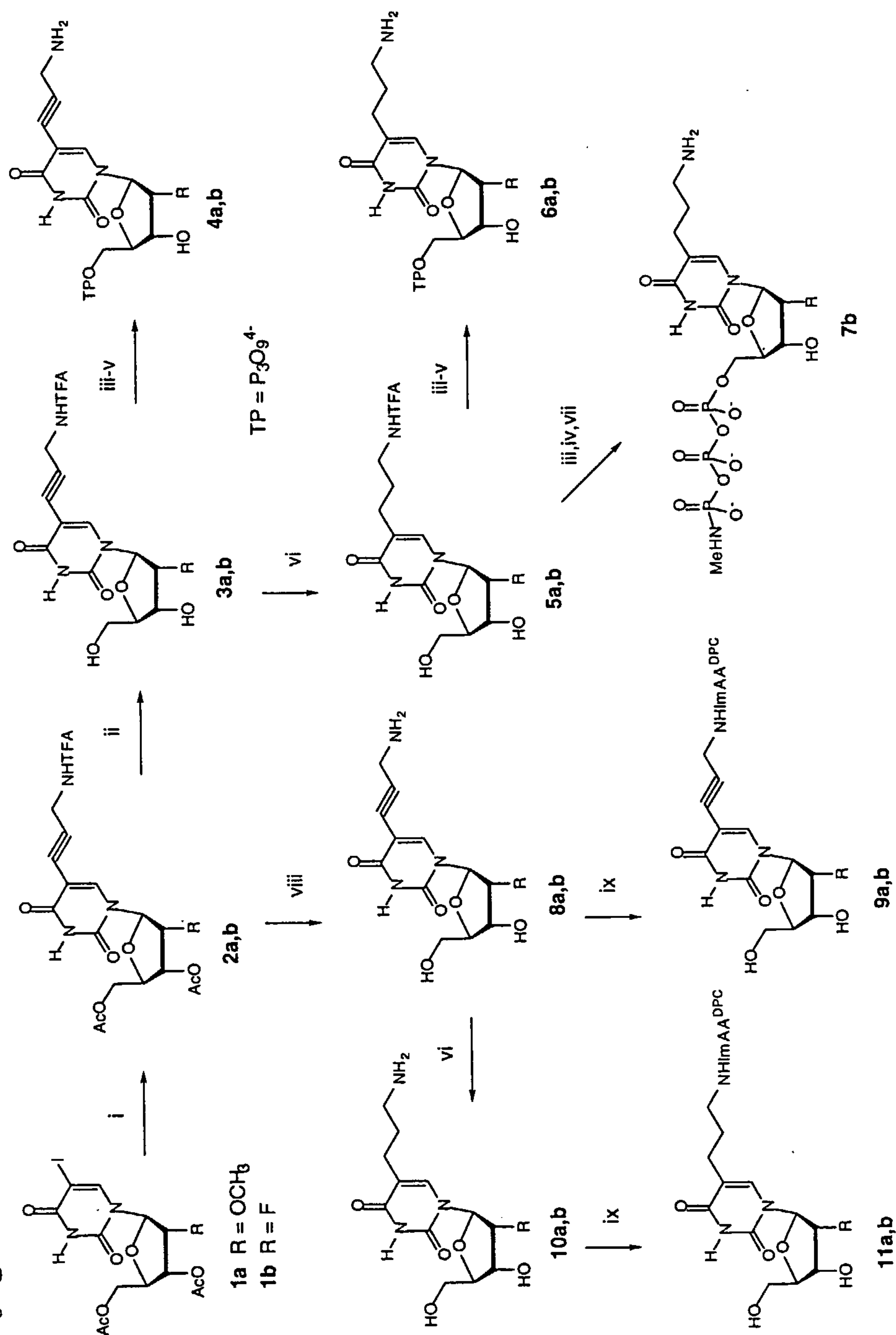


Figure 20: Representative data of HER2 cell proliferation primary screen of Class II (zinczyme) Ribozymes



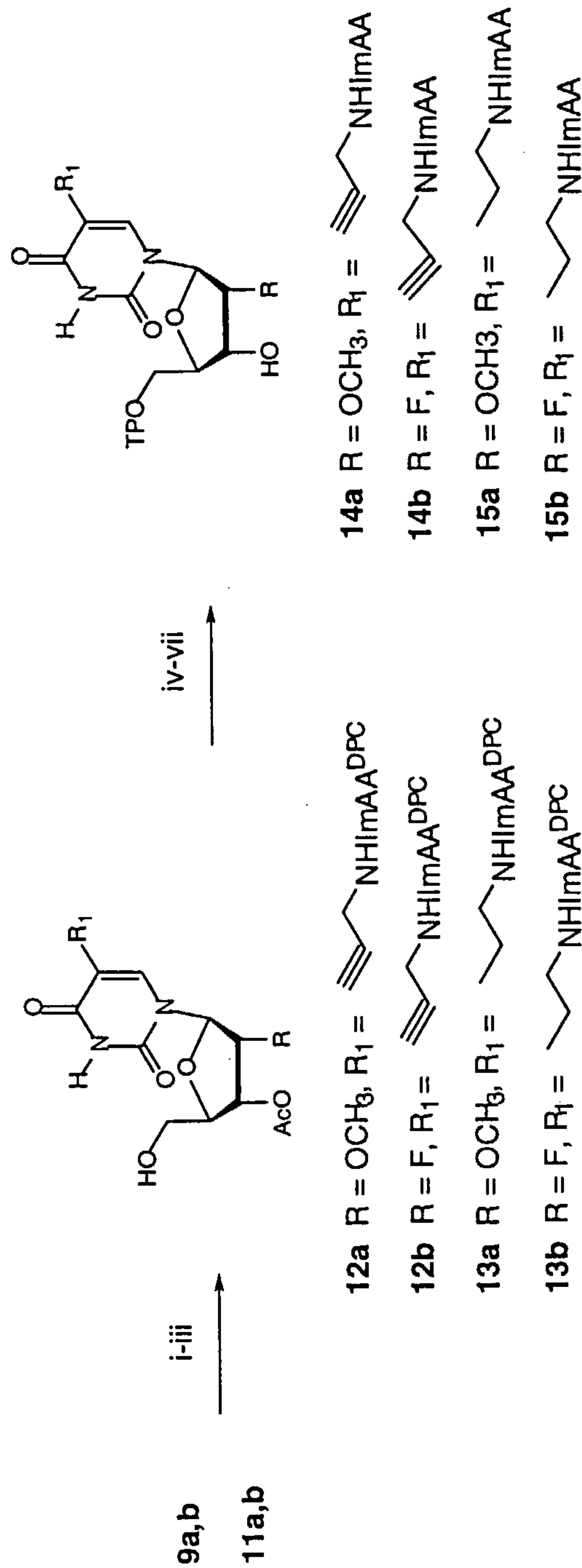
SKBR3 breast carcinoma cells
2 µg/mL RPI.9649 (lipid)
200 nM ribozymes
120 hour timepoint
UNT = untreated
SAC = scrambled attenuated control

Figure 21: Synthesis of 5-[3-aminopropynyl(propyl)]uridine 5'-triphosphates and 4-imidazoleacetic acid conjugates



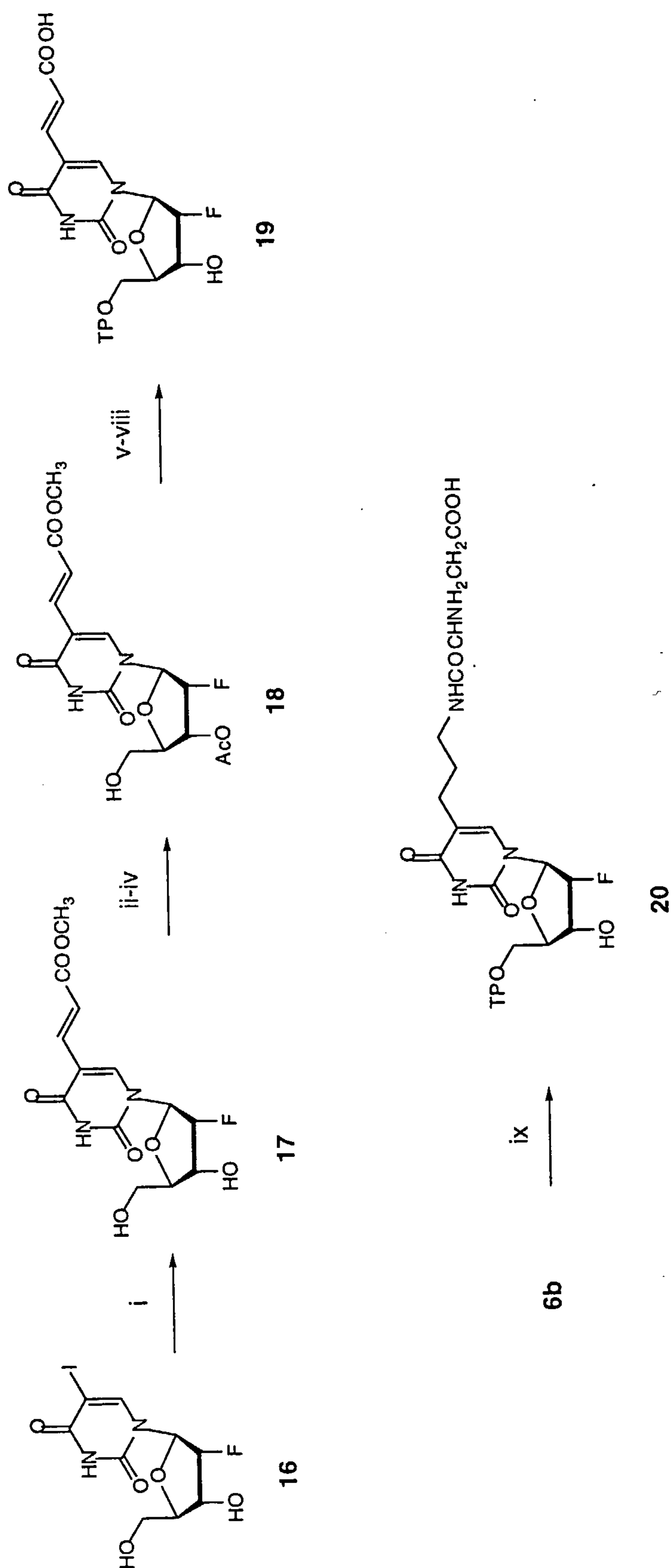
Reagents and Conditions: (i) *N*-TFA propargylamine, CuI, tetrakis(Ph₃P)Pd(0), Et₃N, DMF, 16 h, (ii) aq NaOH, pyr, MeOH, 0 °C, 1 h, (iii) POCl₃, Proton-Sponge, (EtO)₃PO, 2 h, (iv) *n*-Bu₃N PPI, MeCN, 15 min., (v) 1MEt₃NH⁺HCO₃⁻, then NH₄OH, 16 h, (vi) H₂, 5% Pd-C, 24 h, 40 psi, (vii) 40% MeNH₂, 3 h, (viii) NH₄OH, 4 °C, 16 h, (ix) ImAA^{DPC}, EDCHCl, DMF, 16 h.

Figure 22: Synthesis of 5-[3-(N-4-imidazoleacetyl0aminopropynyl(propyl)]uridine 5'-triphosphates



Reagents and Conditions: (I) DMT-Cl, pyr, 16 h, (ii) Ac₂O, pyr, 2 h, (iii) 3%TCA, CH₂Cl₂, 2 h, (iv) 2-Cl-4*H*-1,3,2-benzo-dioxaphosphorin-4-one, pyr, dioxane, 30 min., (v) *n*-Bu₃N PPI, DMF, 30 min., (vi) I₂, pyr-H₂O, 20 min., (vii) NH₄OH, 2 h.

Figure 23: Synthesis of Carboxylate tethered uridine 5'-triphosphates



Reagents and Conditions: (1) methyl acrylate, Ph_3P , $\text{Pd}(\text{II})\text{acetate}$, Et_3N , dioxane, 30 min., reflux,

(ii) DMT-Cl , pyr, 16 h. (iii) Ac_2O , pyr, 3 h. (iv) 3% TCA , CH_2Cl_2 , 1 h,

(v) 2-Cl-4H-1,3,2-benzodioxaphosphorin-4-one, pyr, dioxane, 30 min.,

(vi) $n\text{-Bu}_3\text{N}$ PPI , DMF , 30 min., (vii) I_2 , pyr- H_2O , 20 min., (viii) 1N NaOH , 5 h,

(ix) Fmoc-Asp-OFm NHS-ester, DMF -0.1M $\text{Na}_2\text{B}_4\text{O}_7$, 16 h, then Et_2NH , 3 h.

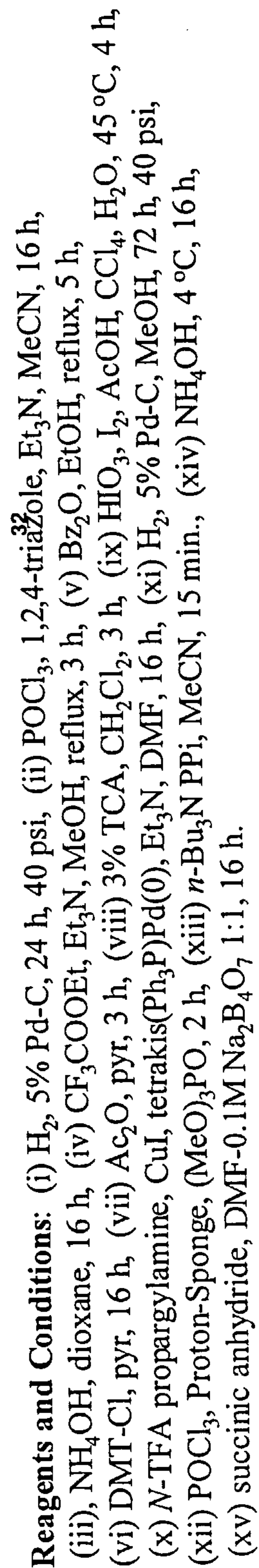
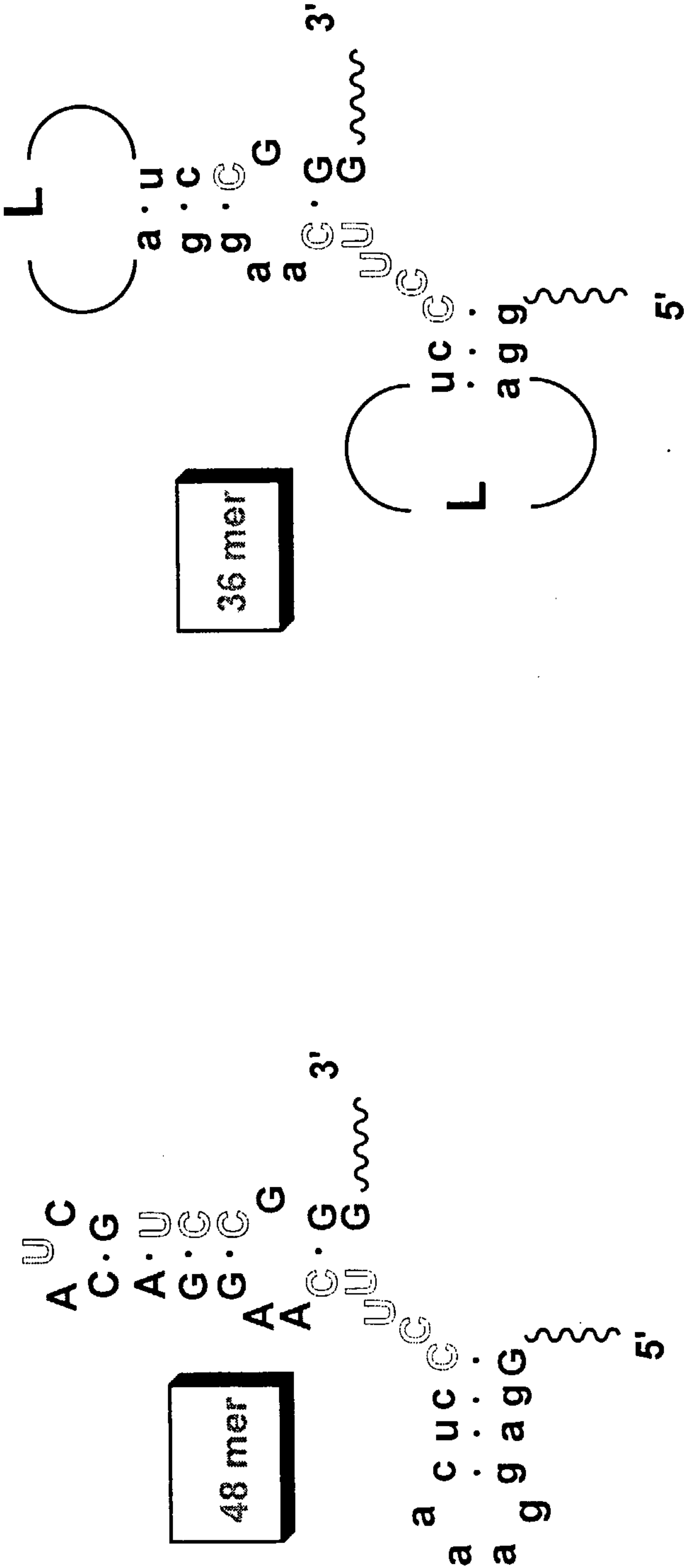


Figure 26: Class I ribozyme Stem truncation and Loop replacement



C and U = 2'-deoxy-2'-amino C, U

Upper case = ribo

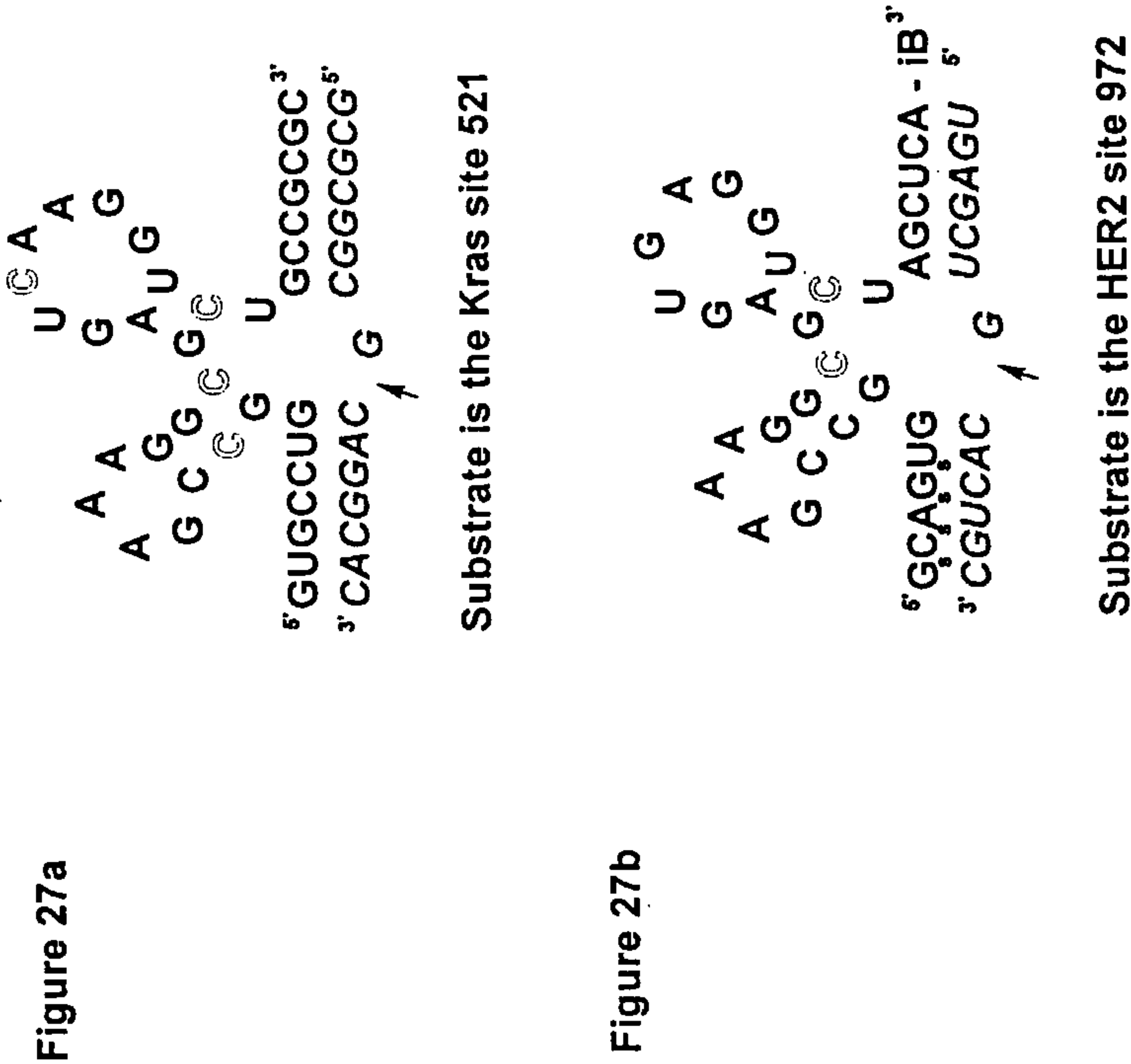
lower case = 2'-O-methyl

L = hexaethylene glycol linker

~~~~~ = binding arms



Figure 27: Non ribo Class II (zinzyme) motifs



Legend  
*Italic* Indicates natural ribo residues  
G Indicates 2' - NH<sub>2</sub>-C  
A G C U Indicates 2'-OMe residues  
Subscript <sub>s</sub> indicates phosphothioate linkage  
IB Indicates inverted deoxy abasic residue



Figure 28: Non ribo Class II (zinzyme) cleavage reactions

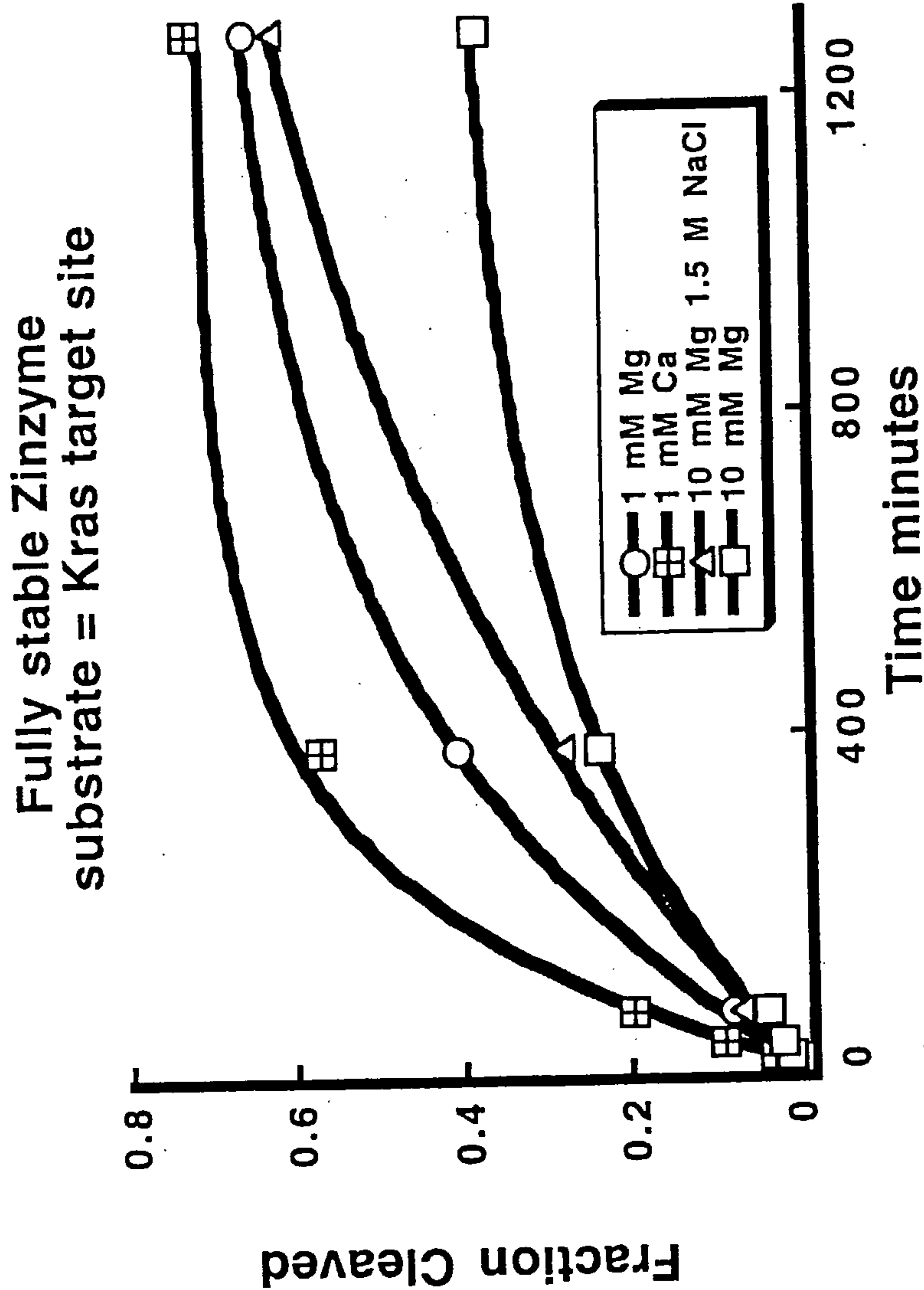
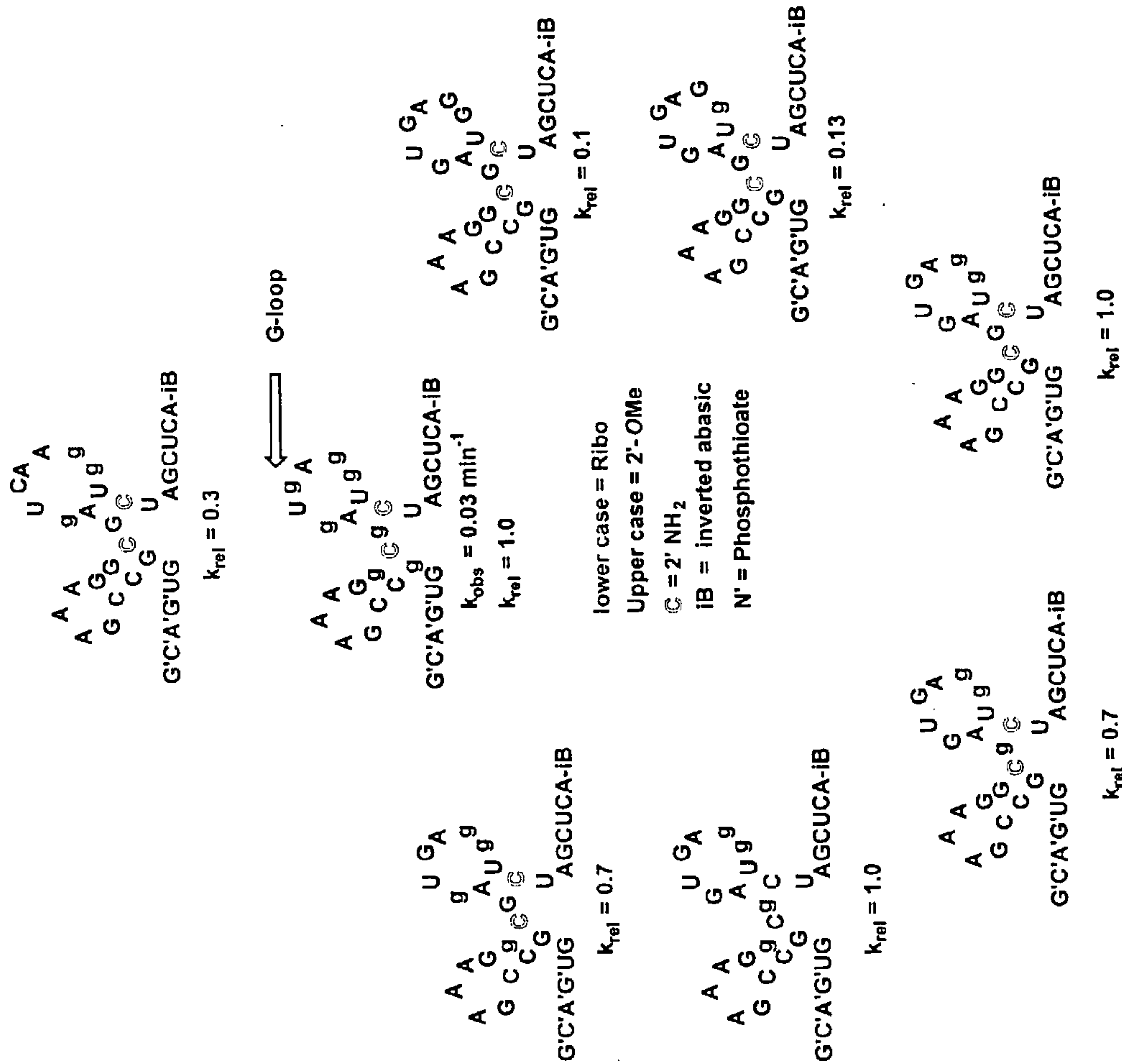




Figure 29: Positional testing of ribose positions in Class II (zinzyme) nucleic acid catalysts targeting HER2 site 972



All assays done under selection conditions - physiological buffer  
1 mM Mg, 1 mM Ca 37° C  
Substrate 15-mer HER2 site 972



Figure 30: RPI 18656 Mediated Decrease in HER2 RNA  
site 972 vs SAC

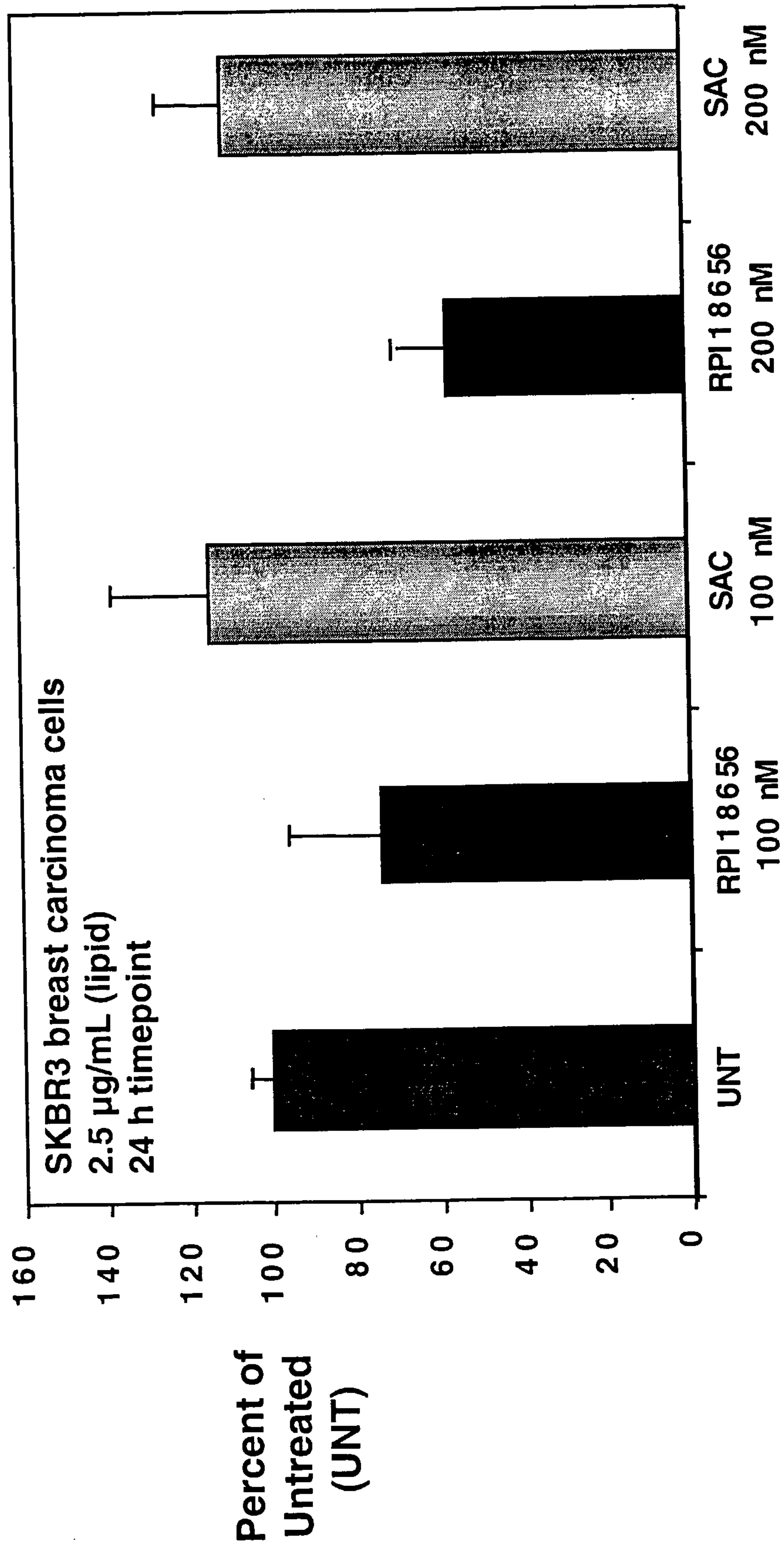




Figure 31: Dose Response of RPI 18656 Against Site 972 in Antiproliferation Assay

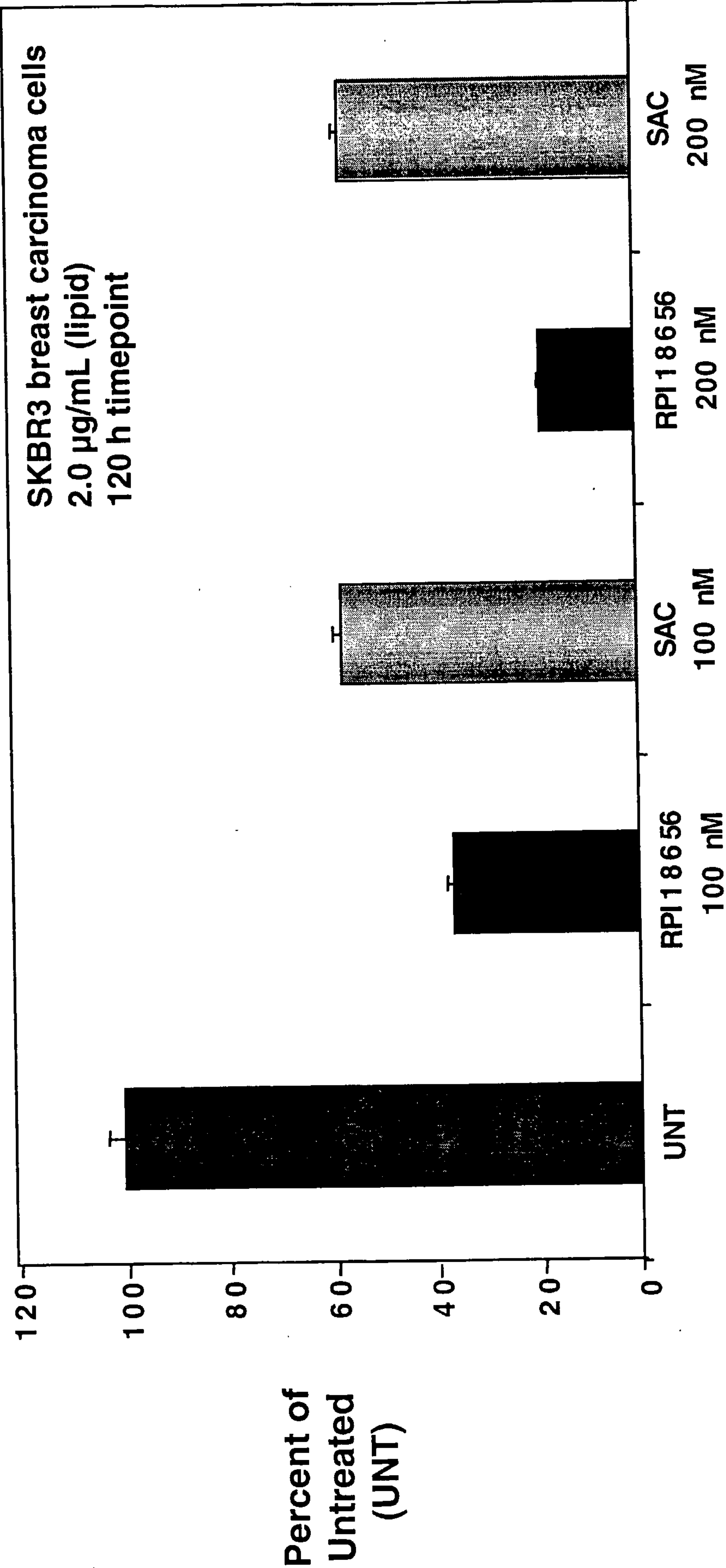




Figure 32: Dose-Dependent HER2 RNA Reduction after Treatment with RPI 19293

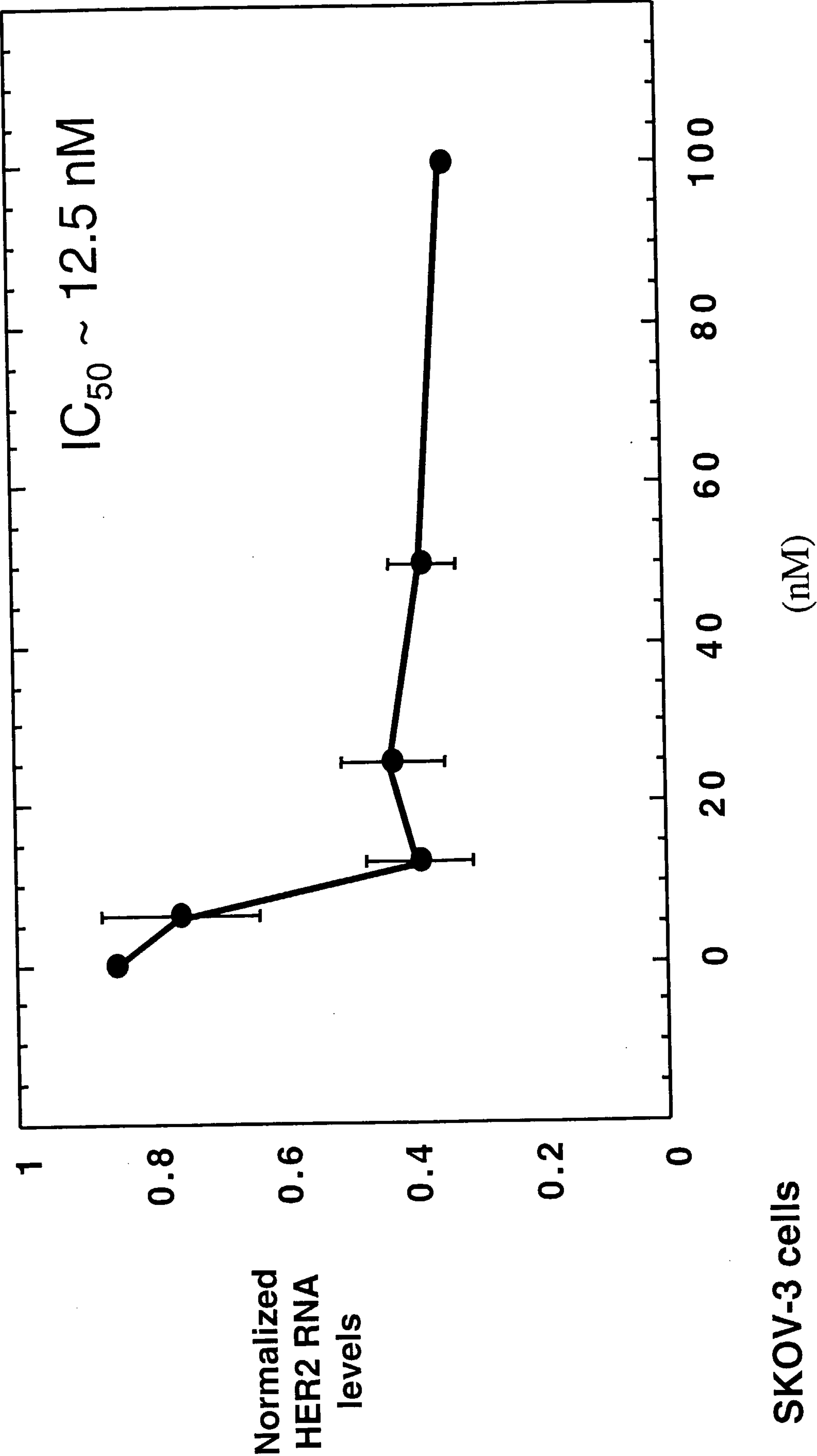




Figure 33: Dose-Dependent HER2 RNA Reduction & Inhibition of Cell Proliferation (RPI.19293)

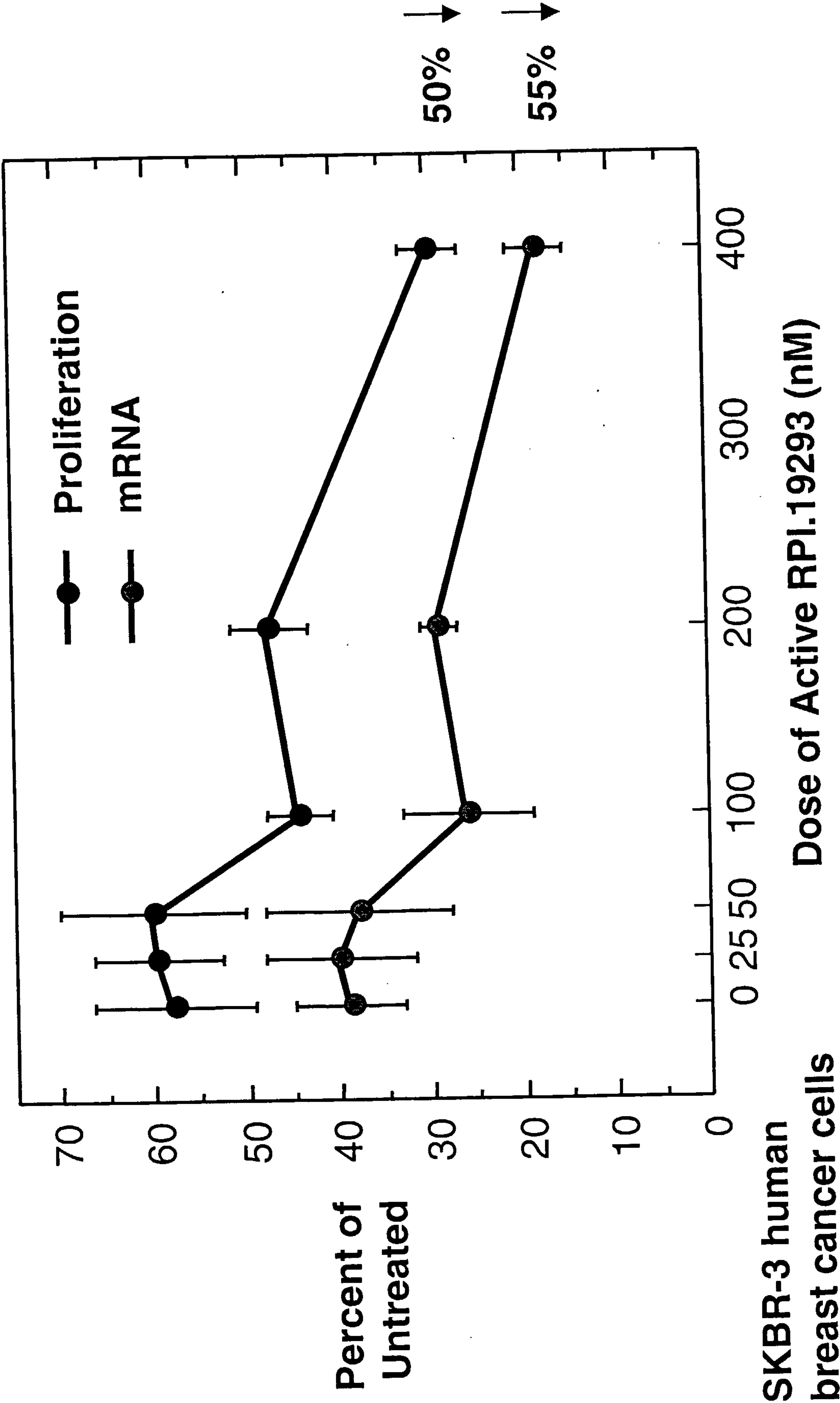
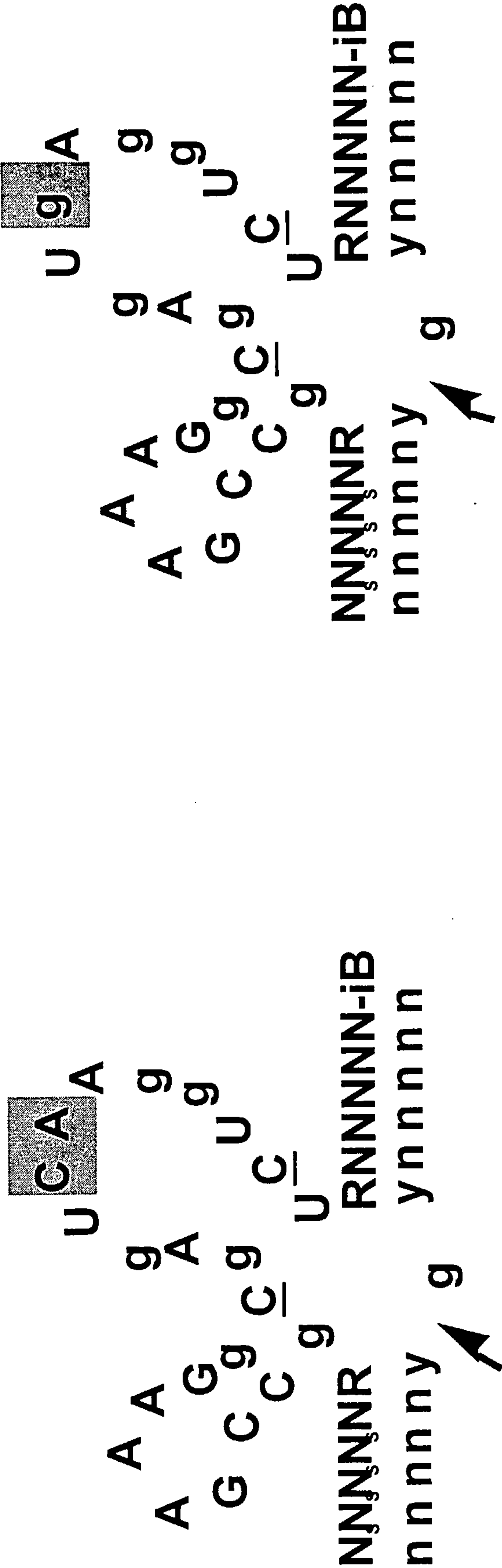




Figure 34: Zinzyme CA →G loop (7-ribo)



Lower case = 2'-OH  
Underline = 2'-NH<sub>2</sub>  
UPPER CASE = 2'-O-Me  
N<sub>s</sub> = Phosphothioate linkage



Figure 35: Screen of Zinzymes (containing ribose-G reductions) for Anti-proliferative Activity

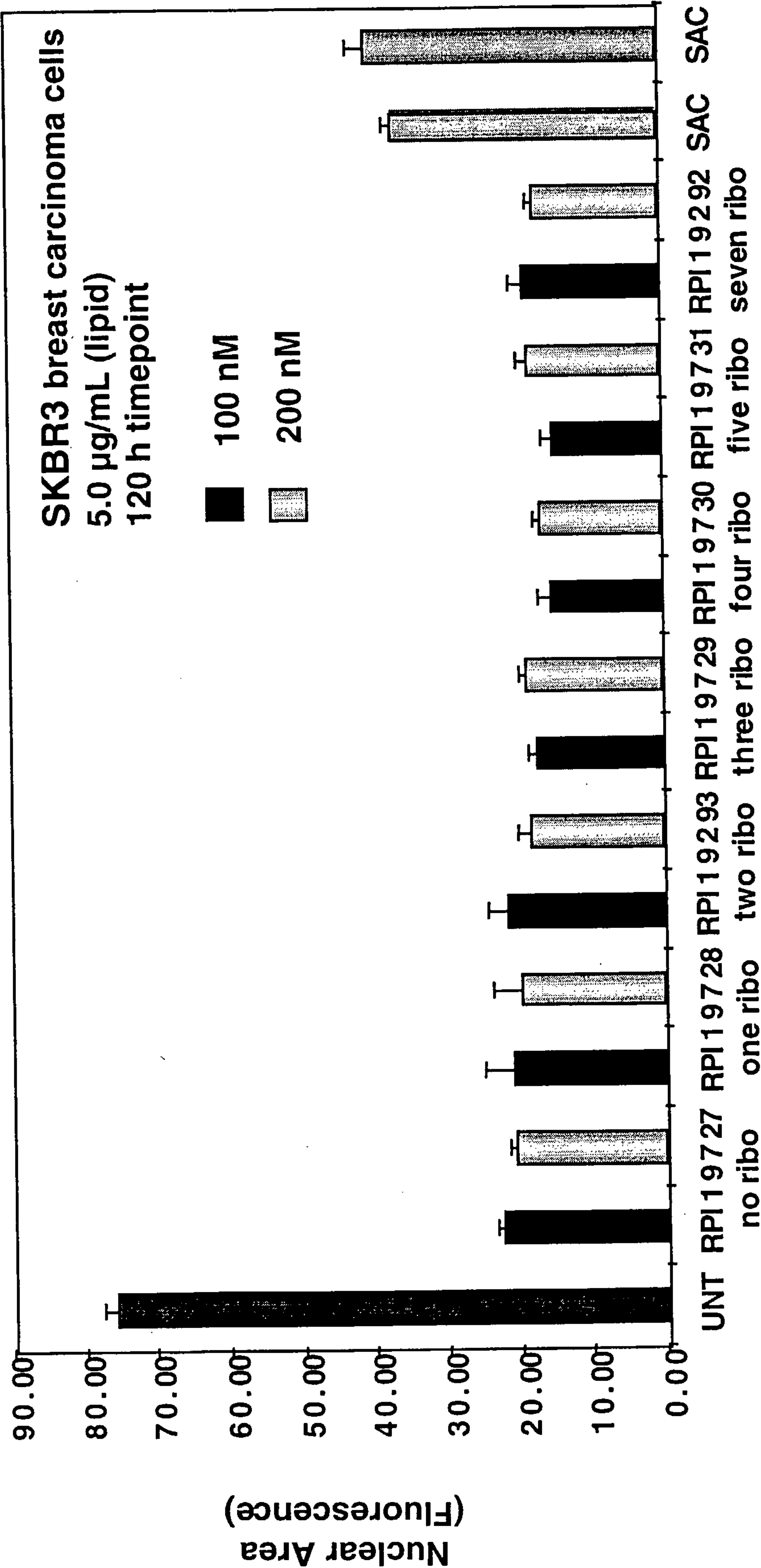
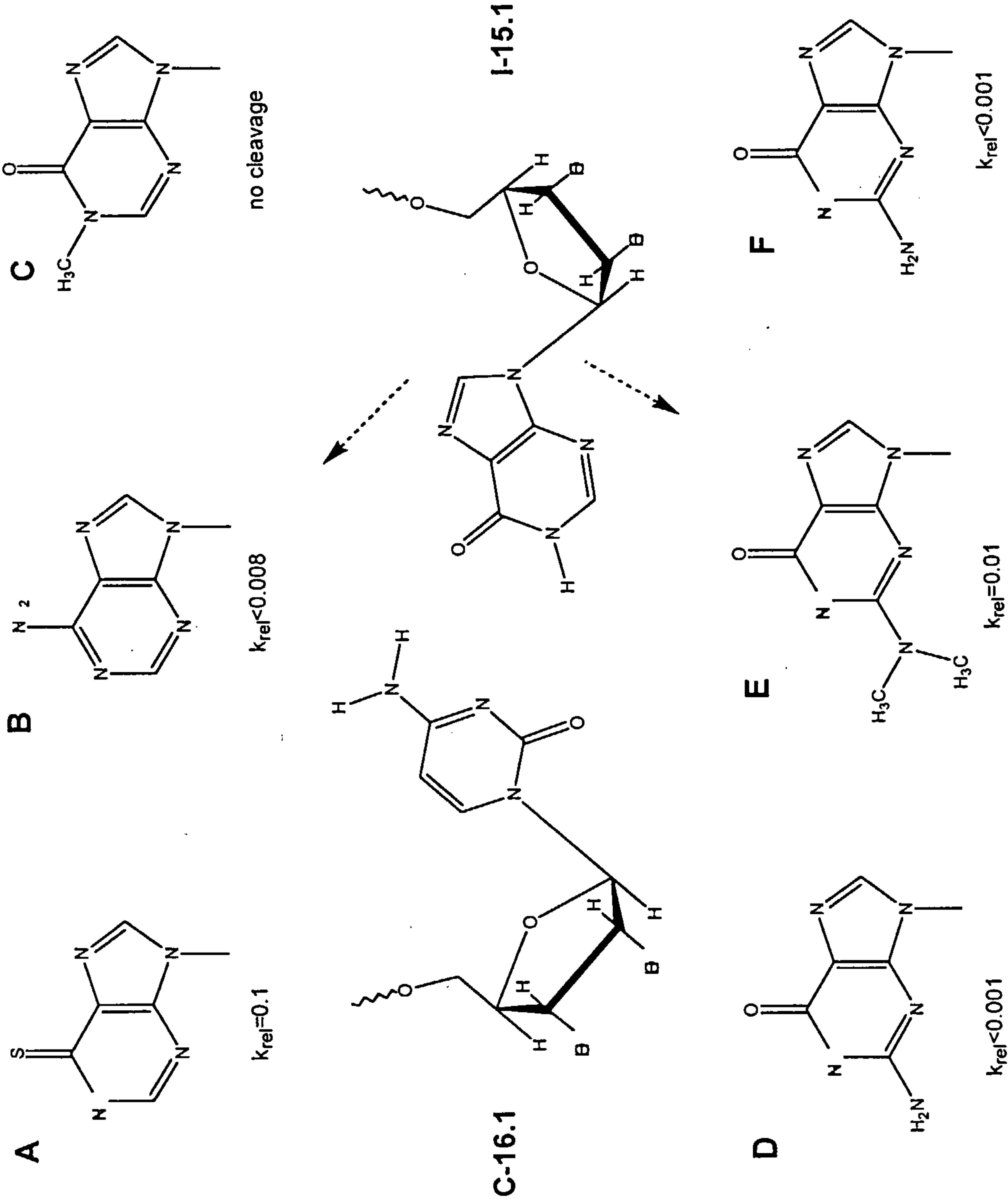




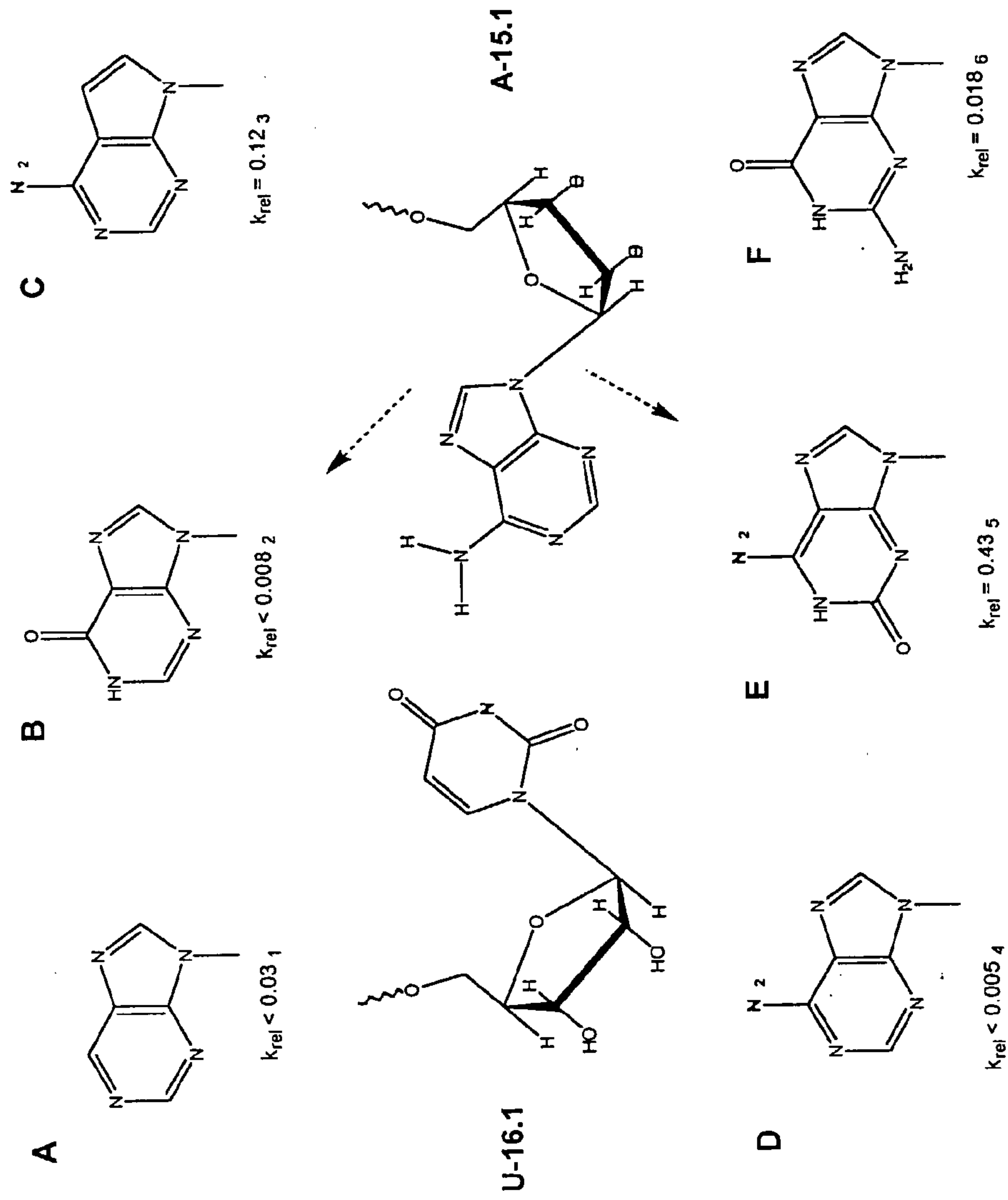
Figure 36: Effect of substitutions at NCH ribozyme position 15.1



$k_{rel}$  values describe the cleavage rate relative to I-15.1 activity



**Figure 37: Effect of substitutions at Hammerhead Ribozyme position 15.1**



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$k_{rel}$  values describe the cleavage rate relative to A-15.1 activity