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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF GLUCOCORTICOID RECEPTOR (GCR) GENES

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



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COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF GLUCOCORTICOID RECEPTOR (GCR) GENES

This invention relates to double-stranded ribonucleic acids (dsRNAs), and their use in mediating RNA interference to inhibit the expression of the GCR gene. Furthermore, the use of said dsRNAs to treat/prevent a wide range of diseases/disorders which are associated with the expression of the GCR gene, like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression is part of the invention.

Glucocorticoids are responsible for several physiological functions including answer to stress, immune and inflammatory responses as well as stimulation of hepatic gluconeogenesis and glucose utilization at the periphery. Glucocorticoids act via an intracellular glucocorticoid receptor (GCR) belonging to the family of the nuclear steroidal receptors. The non-activated GCR is located in the cellular cytoplasm and is associated with several chaperone proteins. When a ligand activates the receptor, the complex is translocated in the cell nucleus and interacts with the glucocorticoid response element which is located in several gene promoters. The receptor could act in the cell nucleus as an homodimer or an heterodimer. Moreover several associated co-activators or co-repressors could also interact with the complex. This large range of possible combinations leads to several GCR conformations and several possible physiological answers making difficult to identify a small chemical entity which can act as a full and specific GCR inhibitor.

Pathologies like diabetes, Cushing's syndrome or depression have been associated with moderate to severe hypercortisolism (Chiodini et al, Eur. J. Endocrinol. 2005, Vol. 153, pp 837-844; Young, Stress 2004, Vol. 7 (4), pp 205-208). GCR antagonist administration has been proven to be clinically active in depression (Flores et al, Neuropsychopharmacology 2006, Vol. 31, pp 628-636) or in Cushing's syndrome (Chu et al, J. Clin. Endocrinol. Metab. 2001, Vol. 86, pp 3568-3573). These clinical evidences illustrate the potential clinical value of a potent and selective GCR antagonist in many indications like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression (Von Geldern et al, J. Med. Chem. 2004, Vol 47 (17), pp 4213-4230; Hu et al, Drug Develop. Res. 2006, Vol. 67, pp 871-883; Andrews, Handbook of the

stress and the brain 2005, Vol. 15, pp 437-450). This approach might also improve peripheral insulin sensitivity (Zinker et al, Meta. Clin. Exp. 2007, Vol. 57, pp 380-387) and protect pancreatic beta cells (Delauney et al, J. Clin. Invest. 1997, Vol. (100, pp 2094-2098).

Diabetic patients have an increased level of fasting blood glucose which has been
5 correlated in clinic with an impaired control of gluconeogenesis (DeFronzo, Med. Clin. N. Am. 2004, Vol. 88 pp 787-835). The hepatic gluconeogenesis process is under the control of glucocorticoids. Clinical administration of a non-specific GCR antagonist (RU486 / mifepristone) leads acutely to a decrease of fasting plasma glucose in normal volunteers (Garrel et al, J. Clin. Endocrinol. Metab. 1995, Vol. 80 (2), pp 379-385) and chronically to a decrease of
10 plasmatic HbA1c in Cushing's patients (Nieman et al, J. Clin. Endocrinol. Metab. 1985, Vol. 61 (3), pp 536-540). Moreover, this drug given to leptin deficient animals normalizes fasting plasma glucose (ob/ob mice, Gettys et al, Int. J. Obes. 1997, Vol. 21, pp 865-873) as well as the activity of gluconeogenic enzymes (db/db mice, Friedman et al, J. Biol. Chem. 1997, Vol. 272 (50) pp 31475-31481). Liver-specific knockout mice have been produced and these animals display a
15 moderate hypoglycemia when they are fasted for 48h minimizing the risk of severe hypoglycemia (Opherk et al, Mol. Endocrinol. 2004, Vol. 18 (6), pp 1346-1353). Moreover, hepatic and adipose tissue GCR silencing in diabetic mice (db/db mice) with an antisense approach leads to significant reduction of blood glucose (Watts et al, Diabetes, 2005, Vol 54, pp 1846-1853).

20 Endogenous corticosteroid secretion at the level of the adrenal gland can be modulated by the Hypothalamus-Pituitary gland- Adrenal gland axis (HPA). Low plasma level of endogenous corticosteroids can activate this axis via a feed-back mechanism which leads to an increase of endogenous corticosteroids circulating in the blood. Mifepristone which crosses the blood brain barrier is known to stimulate the HPA axis which ultimately leads to an increase of endogenous
25 corticosteroids circulating in the blood (Gaillard et al, Pro. Natl. Acad. Sci. 1984, Vol. 81, pp 3879-3882). Mifepristone also induces some adrenal insufficiency symptoms after long term treatment (up to 1 year, for review see: Sitruk-Ware et al, 2003, Contraception, Vol. 68, pp 409-420). Moreover because of its lack of tissue selectivity Mifepristone inhibits the effect of glucocorticoids at the periphery in preclinical models as well as in human (Jacobson et al, 2005
30 J. Pharm. Exp. Ther. Vol 314 (1) pp 191-200; Gaillard et al, 1985 J. Clin. Endo. Met., Vol. 61 (6), pp 1009-1011)

For GCR modulator to be used in indications such as diabetes, dyslipidemia, obesity, hypertension and cardiovascular diseases it is necessary to limit the risk to activate or inhibit the HPA axis and to inhibit GCR at the periphery in other organs than liver. Silencing directly GCR in hepatocytes can be an approach to modulate/normalize hepatic gluconeogenesis as demonstrated recently. However this effect has been seen only at rather high concentrations (in vitro IC₅₀ in the range of 25nM / Watts et al, Diabetes, 2005, Vol 54,pp 1846-1853). To minimize the risk of off target effect as well as to limit pharmacological activity at the periphery in other organs than liver it would be necessary to get more potent GCR silencing agent.

Double-stranded ribonucleic acid (dsRNA) molecules have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). The invention provides double-stranded ribonucleic acid (dsRNA) molecules able to selectively and efficiently decrease the expression of GCR. The use of GCR RNAi provides a method for the therapeutic and/or prophylactic treatment of diseases/disorders which are associated with any dysregulation of the glucocorticoid pathway. These diseases/disorders can occur due to systemic or local overproduction of endogenous glucocorticoids or due to treatment with synthetic glucocorticoids (e.g. diabetic-like syndrome in patients treated with high doses of glucocorticoids). Particular disease/disorder states include the therapeutic and/or prophylactic treatment of type 2 diabetes, obesity, dyslipidemia, diabetic atherosclerosis, hypertension and depression, which method comprises administration of dsRNA targeting GCR to a human being or animal. Further, the invention provides a method for the therapeutic and/or prophylactic treatment of Metabolic Syndrome X, Cushing's Syndrome, Addison's disease, inflammatory diseases such as asthma, rhinitis, and arthritis, allergy, autoimmune disease, immunodeficiency, anorexia, cachexia, bone loss or bone frailty, and wound healing. Metabolic Syndrome X refers to a cluster of risk factors that include obesity, dyslipidemia, particularly high triglycerides, glucose intolerance, high blood sugar and high blood pressure.

In one preferred embodiment the described dsRNA molecule is capable of inhibiting the expression of a GCR gene by at least 70 %, preferably by at least 80%, most preferably by at least 90%. The invention also provides compositions and methods for specifically targeting the liver with GCR dsRNA, for treating pathological conditions and diseases caused by the expression of the GCR gene including those described above. In other embodiments the invention provides compositions and methods for specifically targeting other tissues or organs affected, including, but not limited to adipose tissue, the hypothalamus, kidneys or the pancreas.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a GCR gene, in particular the expression of the mammalian or human GCR gene. The dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence
5 and an antisense strand may comprise a second sequence, see sequences provided in the sequence listing and also provision of specific dsRNA pairs in the appended tables 1 and 4. In one embodiment the sense strand comprises a sequence which has an identity of at least 90% to at least a portion of an mRNA encoding GCR. Said sequence is located in a region of complementarity of the sense strand to the antisense strand, preferably within nucleotides 2-7 of
10 the 5' terminus of the antisense strand. In one preferred embodiment the dsRNA targets particularly the human GCR gene, in yet another preferred embodiment the dsRNA targets the mouse (*Mus musculus*) and rat (*Rattus norvegicus*) GCR gene.

In one embodiment, the antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding said GCR gene, and the
15 region of complementarity is most preferably less than 30 nucleotides in length. Furthermore, it is preferred that the length of the herein described inventive dsRNA molecules (duplex length) is in the range of about 16 to 30 nucleotides, in particular in the range of about 18 to 28 nucleotides. Particularly useful in context of this invention are duplex lengths of about 19, 20, 21, 22, 23 or 24 nucleotides. Most preferred are duplex stretches of 19, 21 or 23 nucleotides. The
20 dsRNA, upon contacting with a cell expressing a GCR gene, inhibits the expression of a GCR gene in vitro by at least 70%, preferably by at least 80%, most preferred by 90%.

Appended Table 13 relates to preferred molecules to be used as dsRNA in accordance with this invention. Also modified dsRNA molecules are provided herein and are in particular disclosed in appended tables 1 and 4, providing illustrative examples of modified dsRNA
25 molecules of the present invention. As pointed out herein above, Table 1 provides for illustrative examples of modified dsRNAs of this invention (whereby the corresponding sense strand and antisense strand is provided in this table). The relation of the unmodified preferred molecules shown in Table 13 to the modified dsRNAs of Table 1 is illustrated in Table 14. Yet, the illustrative modifications of these constituents of the inventive dsRNAs are provided herein as
30 examples of modifications.

Tables 2 and 3 provide for selective biological, clinically and pharmaceutical relevant parameters of certain dsRNA molecules of this invention.

Most preferred dsRNA molecules are provided in the appended table 13 and, inter alia and preferably, wherein the sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos 873, 929, 1021, 1023, 967 and 905 and the antisense strand is selected from the from the group consting of the nucleic acid sequences depicted in SEQ ID Nos
5 874, 930, 1022, 1024, 968 and 906. Accordingly, the inventive dsRNA molecule may, inter alia, comprise the sequence pairs selected from the group consisting of SEQ ID NOs: 873/874, 929/930, 1021/1022, 1023/1024, 967/968 and 905/906. In context of specific dsRNA molecules provided herein, pairs of SEQ ID NOs relate to corresponding sense and antisense strands sequences (5' to 3') as also shown in appended and included tables.

10 In one embodiment said dsRNA molecules comprise an antisense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the antisense strand comprises uracil or nucleotides which are complementary to the mRNA encoding GCR.

15 In another preferred embodiment, said dsRNA molecules comprise a sense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are identical to the mRNA encoding GCR.

20 In another preferred embodiment, said dsRNA molecules comprise a sense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length, and an antisense strand with a 3' overhang of 1-5 nucleotides length, preferably of 1-2 nucleotides length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are at least 90% identical to the mRNA encoding GCR and said overhang of the antisense strand comprises
25 uracil or nucleotides which are at least 90% complementary to the mRNA encoding GCR

Most preferred dsRNA molecules are provided in the tables 1 and 4 below and, inter alia and preferably, wherein the sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 7, 31, 3, 25, 33, 55, 83, 747 and 764 the antisense strand is
30 selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 8, 32, 4, 26, 34, 56, 84, 753 and 772. Accordingly, the inventive dsRNA molecule may, inter alia, comprise the sequence pairs selected from the group consisting of SEQ ID NOs: 7/8, 31/32, 3/4,

25/26, 33/34, 55/56, 83/84, 747/753 and 764/772. In context of specific dsRNA molecules provided herein, pairs of SEQ ID NOs relate to corresponding sense and antisense strands sequences (5' to 3') as also shown in appended and included tables.

The dsRNA molecules of the invention may be comprised of naturally occurring
5 nucleotides or may be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, inverted deoxythymidine and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. 2' modified nucleotides may have the additional advantage that certain immunostimulatory factors or cytokines are suppressed when the inventive dsRNA molecules
10 are employed in vivo, for example in a medical setting. Alternatively and non-limiting, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. In one preferred embodiment the dsRNA molecules
15 comprises at least one of the following modified nucleotides: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group and a deoxythymidine. In another preferred embodiment all pyrimidines of the sense strand are 2'-O-methyl modified nucleotides, and all pyrimidines of the antisense strand are 2'-deoxy-2'-fluoro modified nucleotides. In one preferred embodiment two deoxythymidine nucleotides are found at the 3' of both strands of the dsRNA
20 molecule. In another embodiment at least one of these deoxythymidine nucleotides at the 3' end of both strands of the dsRNA molecule comprises a 5'-phosphorothioate group. In another embodiment all cytosines followed by adenine, and all uracils followed by either adenine, guanine or uracil in the sense strand are 2'-O-methyl modified nucleotides, and all cytosines and uracils followed by adenine of the antisense strand are 2'-O-methyl modified nucleotides.
25 Preferred dsRNA molecules comprising modified nucleotides are given in tables 1 and 4.

In a preferred embodiment the inventive dsRNA molecules comprise modified nucleotides as detailed in the sequences given in tables 1 and 4. In one preferred embodiment the inventive dsRNA molecule comprises sequence pairs selected from the group consisting of SEQ ID NOs: 7/8, 31/32, 3/4, 25/26, 33/34, 55/56 and 83/84, and comprise modifications as detailed
30 in table 1.

In another embodiment the inventive dsRNAs comprise modified nucleotides on positions different from those disclosed in tables 1 and 4. In one preferred embodiment two

deoxythymidine nucleotides are found at the 3' of both strands of the dsRNA molecule. In another preferred embodiment one of those deoxythymidine nucleotides at the 3' of both strand is a inverted deoxythymidine.

In one embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 9 hours. In one preferred embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 9 hours in human serum. In another embodiment the dsRNA molecules of the invention comprise of a sense and an antisense strand wherein both strands have a half-life of at least 24 hours in human serum.

In another embodiment the dsRNA molecules of the invention are non-immunostimulatory, e.g. do not stimulate INF-alpha and TNF-alpha in vitro.

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

Furthermore, the invention relates to a method for inhibiting the expression of a GCR gene, in particular a mammalian or human GCR gene, in a cell, tissue or organism comprising the following steps:

- (a) introducing into the cell, tissue or organism a double-stranded ribonucleic acid (dsRNA) as defined herein;
- (b) maintaining said cell, tissue or organism produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GCR gene, thereby inhibiting expression of a GCR gene in a given cell.

The invention also relates to pharmaceutical compositions comprising the inventive dsRNAs of this invention. These pharmaceutical compositions are particularly useful in the inhibition of the expression of a GCR gene in a cell, a tissue or an organism. The pharmaceutical composition comprising one or more of the dsRNA of the invention may also comprise (a) pharmaceutically acceptable carrier(s), diluent(s) and/or excipient(s).

In another embodiment, the invention provides methods for treating, preventing or managing disorders which are associated type 2 diabetes, obesity, dislipidemia, diabetic atherosclerosis, hypertension and depression, said method comprising administering to a subject in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention. Preferably, said subject is a mammal, most preferably a human patient.

In one embodiment, the invention provides a method for treating a subject having a pathological condition mediated by the expression of a GCR gene. Such conditions comprise disorders associated with diabetes and obesity, as described above. In this embodiment, the dsRNA acts as a therapeutic agent for controlling the expression of a GCR gene. The method comprises administering a pharmaceutical composition of the invention to the patient (e.g., human), such that expression of a GCR gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target mRNAs of a GCR gene. In one preferred embodiment the described dsRNAs specifically decrease GCR mRNA levels and do not directly affect the expression and / or mRNA levels of off-target genes in the cell. In another preferred embodiment the described dsRNAs specifically decrease GCR mRNA levels as well as mRNA levels of genes that are normally activated by GCR. In another embodiment the inventive dsRNAs decrease glucose levels in vivo.

In one preferred embodiment the described dsRNA decrease GCR mRNA levels in the liver by at least 70%, preferably by at least 80%, most preferably by at least 90% in vivo. Preferably the dsRNAs of the invention decrease glycemia without change in liver transaminases. In another embodiment the described dsRNAs decrease GCR mRNA levels in vivo for at least 4 days. In another embodiment the described dsRNAs decrease GCR mRNA levels in vivo by at least 60% for at least 4 days.

Particularly useful with respect to therapeutic dsRNAs is the set of dsRNAs targeting mouse and rat GCR which can be used to estimate toxicity, therapeutic efficacy and effective dosages and in vivo half-lives for the individual dsRNAs in an animal or cell culture model.

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

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

In one embodiment, the method comprises administering a composition comprising a
10 dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of a GCR gene of the mammal to be treated. As pointed out above, also vectors and cells comprising nucleic acid molecules that encode for at least one strand of the herein defined dsRNA molecules can be used as pharmaceutical compositions and may, therefore, also be employed in the herein disclosed methods of treating a subject in need of
15 medical intervention. It is also of note that these embodiments relating to pharmaceutical compositions and to corresponding methods of treating a (human) subject also relate to approaches like gene therapy approaches. GCR specific dsRNA molecules as provided herein or nucleic acid molecules encoding individual strands of these inventive dsRNA molecules may also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy
20 vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery
25 vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

In another aspect of the invention, GCR specific dsRNA molecules that modulate GCR gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Skillern, A., et al., International PCT Publication No. WO 00/22113). These
30 transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can

also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

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

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

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

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

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

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

DEFINITIONS

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

"G," "C," "A," "U" and "T" or "dT" respectively, each generally stand for a nucleotide that contains guanine, cytosine, adenine, uracil and deoxythymidine as a base, respectively. However, the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. Sequences comprising such replacement moieties are embodiments of the invention. As detailed below, the herein described dsRNA molecules may also comprise "overhangs", i.e. unpaired, overhanging nucleotides which are not directly involved in the RNA double helical structure normally formed by the herein defined pair of "sense strand" and "anti sense strand". Often, such an overhanging stretch comprises the deoxythymidine nucleotide, in most embodiments, 2 deoxythymidines in the 3' end. Such overhangs will be described and illustrated below.

The term „GCR“ as used herein relates in particular to the intracellular glucocorticoid receptor (GCR) and said term relates to the corresponding gene, also known as NR3C1 gene, encoded mRNA, encoded protein/polypeptide as well as functional fragments of the same. Preferred is the human GCR gene. In other preferred embodiments the dsRNAs of the invention target the GCR gene of rat (*Rattus norvegicus*) and mouse (*Mus musculus*), in yet another preferred embodiment the dsRNAs of the invention target the human (*H.sapiens*) and cynomolgous monkey (*Macaca fascicularis*) GCR gene. The term "GCR gene/sequence" does not only relate to (the) wild-type sequence(s) but also to mutations and alterations which may be comprised in said gene/sequence. Accordingly, the present invention is not limited to the specific dsRNA molecules provided herein. The invention also relates to dsRNA molecules that comprise an antisense strand that is at least 85% complementary to the corresponding nucleotide stretch of an RNA transcript of a GCR gene that comprises such mutations/alterations.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a GCR gene, including mRNA that is a product of RNA processing of a primary transcription product.

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

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

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

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

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

The term “double-stranded RNA”, “dsRNA molecule”, or “dsRNA”, as used herein,
25 refers to a ribonucleic acid molecule, or complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one
30 strand and the 5' end of the respective other strand forming the duplex structure, the connecting

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

The term “antisense strand” refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term “region of complementarity” refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated outside nucleotides 2-7 of the 5' terminus of the antisense strand

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

"Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells in vitro; a dsRNA may also be

5 "introduced into a cell", wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for in vivo delivery, dsRNA can be injected into a tissue site or administered systemically. It is, for example envisaged that the dsRNA molecules of this invention be administered to a subject in need of medical intervention. Such an administration may comprise the injection of the dsRNA, the

10 vector or an cell of this invention into a diseased side in said subject, for example into liver tissue/cells or into cancerous tissues/cells, like liver cancer tissue. However, also the injection in close proximity of the diseased tissue is envisaged. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence", "inhibit the expression of" and "knock down", in as far as they refer

15 to a GCR gene, herein refer to the at least partial suppression of the expression of a GCR gene, as manifested by a reduction of the amount of mRNA transcribed from a GCR gene which may be isolated from a first cell or group of cells in which a GCR gene is transcribed and which has or have been treated such that the expression of a GCR gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which

20 has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

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

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to the GCR gene transcription, e.g. the amount of protein

25 encoded by a GCR gene which is secreted by a cell, or the number of cells displaying a certain phenotype.

As illustrated in the appended examples and in the appended tables provided herein, the inventive dsRNA molecules are capable of inhibiting the expression of a human GCR by at least about 70%, preferably by at least 80%, most preferably by at least 90% in vitro assays, i.e. in

30 vitro. The term "in vitro" as used herein includes but is not limited to cell culture assays. In

another embodiment the inventive dsRNA molecules are capable of inhibiting the expression of a mouse or rat GCR by at least 70 %,preferably by at least 80%, most preferably by at least 90%. The person skilled in the art can readily determine such an inhibition rate and related effects, in particular in light of the assays provided herein.

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

10 Particular preferred dsRNAs are provided, for example in appended Table 1 and 2 (sense strand and antisense strand sequences provided therein in 5' to 3' orientation), with the most preferred dsRNAs in table 2.

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

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

20 The terms "treat", "treatment", and the like, mean in context of this invention to relief from or alleviation of a disorder related to GCR expression, like diabetes, dyslipidemia, obesity, hypertension, cardiovascular diseases or depression.

25 As used herein, a “pharmaceutical composition” comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. However, such a “pharmaceutical composition” may also comprise individual strands of such a dsRNA molecule or the herein described vector(s) comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a sense or an antisense strand comprised in the dsRNAs of this invention. It is also envisaged that cells, tissues or isolated organs that express or comprise the herein defined dsRNAs may be used as “pharmaceutical compositions”. As used herein, “pharmacologically effective amount,” “therapeutically effective amount” or

simply “effective amount” refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result.

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

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

For intramuscular, subcutaneous and intravenous use, the pharmaceutical compositions of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. In a preferred embodiment, the carrier consists exclusively of an aqueous buffer. In this context, “exclusively” means no auxiliary agents or encapsulating substances are present which might affect or mediate uptake of dsRNA in the cells that express a GCR gene. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate. The pharmaceutical compositions useful according to the invention also include encapsulated formulations to protect the dsRNA against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid.

Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in PCT publication WO 91/06309 which is incorporated by reference herein.

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

10 It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Table 1 and 4 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. As pointed out above, in most embodiments of this invention, the dsRNA molecules provided herein comprise a duplex length (i.e. without “overhangs”) of about 16 to about 30 nucleotides. Particular useful dsRNA duplex lengths are
15 about 19 to about 25 nucleotides. Most preferred are duplex structures with a length of 19 nucleotides. In the inventive dsRNA molecules, the antisense strand is at least partially complementary to the sense strand.

In one preferred embodiment the inventive dsRNA molecules comprise nucleotides 1-19 of the sequences given in Table 13.

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

As mentioned above, at least one end/strand of the dsRNA may have a single-stranded nucleotide overhang of 1 to 5, preferably 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one
30 nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its

overall stability. dsRNA having only one overhang has proven particularly stable and effective in vivo, as well as in a variety of cells, cell culture mediums, blood, and serum. Preferably, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA may also have a blunt end, preferably located at the 5'-end of the antisense strand. Preferably, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

The dsRNA of the present invention may also be chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Eds.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Chemical modifications may include, but are not limited to 2' modifications, introduction of non-natural bases, covalent attachment to a ligand, and replacement of phosphate linkages with thiophosphate linkages. In this embodiment, the integrity of the duplex structure is strengthened by at least one, and preferably two, chemical linkages. Chemical linking may be achieved by any of a variety of well-known techniques, for example by introducing covalent, ionic or hydrogen bonds; hydrophobic interactions, van der Waals or stacking interactions; by means of metal-ion coordination, or through use of purine analogues. Preferably, the chemical groups that can be used to modify the dsRNA include, without limitation, methylene blue; bifunctional groups, preferably bis-(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxybenzoyl)cystamine; 4-thiouracil; and psoralen. In one preferred embodiment, the linker is a hexa-ethylene glycol linker. In this case, the dsRNA are produced by solid phase synthesis and the hexa-ethylene glycol linker is incorporated according to standard methods (e.g., Williams, D.J., and K.B. Hall, *Biochem.* (1996) 35:14665-14670). In a particular embodiment, the 5'-end of the antisense strand and the 3'-end of the sense strand are chemically linked via a hexaethylene glycol linker. In another embodiment, at least one nucleotide of the dsRNA comprises a phosphorothioate or phosphorodithioate groups. The chemical bond at the ends of the dsRNA is preferably formed by triple-helix bonds.

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

purines. In further embodiments, a chemical bond may be formed by azabenzene units introduced into the double-stranded structure. In still further embodiments, a chemical bond may be formed by branched nucleotide analogs instead of nucleotides introduced into the double-stranded structure. In certain embodiments, a chemical bond may be induced by ultraviolet light.

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

The compounds of the present invention can be synthesized using one or more inverted nucleotides, for example inverted thymidine or inverted adenine (see, for example, Takei, et al., 2002, *JBC* 277(26):23800-06).

20 Modifications of dsRNA molecules provided herein may positively influence their stability in vivo as well as in vitro and also improve their delivery to the (diseased) target side. Furthermore, such structural and chemical modifications may positively influence physiological reactions towards the dsRNA molecules upon administration, e.g. the cytokine release which is preferably suppressed. Such chemical and structural modifications are known in the art and are,
25 inter alia, illustrated in Nawrot (2006) *Current Topics in Med Chem*, 6, 913-925.

Conjugating a ligand to a dsRNA can enhance its cellular absorption as well as targeting to a particular tissue. In certain instances, a hydrophobic ligand is conjugated to the dsRNA to facilitate direct permeation of the cellular membrane. Alternatively, the ligand conjugated to the dsRNA is a substrate for receptor-mediated endocytosis. These approaches have been used to
30 facilitate cell permeation of antisense oligonucleotides. For example, cholesterol has been conjugated to various antisense oligonucleotides resulting in compounds that are substantially

more active compared to their non-conjugated analogs. See M. Manoharan *Antisense & Nucleic Acid Drug Development* 2002, 12, 103. Other lipophilic compounds that have been conjugated to oligonucleotides include 1-pyrene butyric acid, 1,3-bis-O-(hexadecyl)glycerol, and menthol. One example of a ligand for receptor-mediated endocytosis is folic acid. Folic acid enters the cell by
5 folate-receptor-mediated endocytosis. dsRNA compounds bearing folic acid would be efficiently transported into the cell via the folate-receptor-mediated endocytosis. Attachment of folic acid to the 3'-terminus of an oligonucleotide results in increased cellular uptake of the oligonucleotide (Li, S.; Deshmukh, H. M.; Huang, L. *Pharm. Res.* 1998, 15, 1540). Other ligands that have been conjugated to oligonucleotides include polyethylene glycols, carbohydrate clusters, cross-linking
10 agents, porphyrin conjugates, and delivery peptides.

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

The ligand-conjugated dsRNA of the invention may be synthesized by the use of a dsRNA that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the dsRNA. This reactive oligonucleotide may be reacted directly with
20 commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the invention facilitate the synthesis of ligand-conjugated dsRNA by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates,
25 optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the invention via reaction of a selected serum-binding ligand with a linking moiety located on the 5' position of a nucleoside or oligonucleotide. In certain instances, an dsRNA bearing an alkyl ligand attached to the 3'-terminus of the dsRNA is prepared by first covalently attaching a monomer building block to a controlled-pore-glass
30 support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The

monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

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

Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. No. 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. Nos. 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β -lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. No. 5,587,361 drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. No. 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. No. 6,262,241 drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

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

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand

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

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

In some preferred embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5'-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5'-position through a linking group. The amino group at the 5'-terminus can be prepared utilizing a 5'-Amino-Modifier C6 reagent. In a preferred embodiment, ligand molecules may be conjugated to oligonucleotides at the 5'-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5'-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5'-terminus.

In one preferred embodiment of the methods of the invention, the preparation of ligand conjugated oligonucleotides commences with the selection of appropriate precursor molecules upon which to construct the ligand molecule. Typically, the precursor is an appropriately-protected derivative of the commonly-used nucleosides. For example, the synthetic precursors for the synthesis of the ligand-conjugated oligonucleotides of the invention include, but are not limited to, 2'-aminoalkoxy-5'-ODMT-nucleosides, 2'-6-aminoalkylamino-5'-ODMT-nucleosides, 5'-6-aminoalkoxy-2'-deoxy-nucleosides, 5'-6-aminoalkoxy-2'-protected-nucleosides, 3'-6-

aminoalkoxy-5'-ODMT-nucleosides, and 3'-aminoalkylamino-5'-ODMT-nucleosides that may be protected in the nucleobase portion of the molecule. Methods for the synthesis of such amino-linked protected nucleoside precursors are known to those of ordinary skill in the art.

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

Representative hydroxyl protecting groups, as well as other representative protecting groups, are disclosed in Greene and Wuts, *Protective Groups in Organic Synthesis*, Chapter 2, 2d ed., John Wiley & Sons, New York, 1991, and *Oligonucleotides And Analogues A Practical Approach*, Ekstein, F. Ed., IRL Press, N.Y, 1991.

Amino-protecting groups stable to acid treatment are selectively removed with base treatment, and are used to make reactive amino groups selectively available for substitution. Examples of such groups are the Fmoc (E. Atherton and R. C. Sheppard in *The Peptides*, S. Udenfriend, J. Meienhofer, Eds., Academic Press, Orlando, 1987, volume 9, p.1) and various substituted sulfonyl ethyl carbamates exemplified by the Nsc group (Samukov et al., *Tetrahedron Lett.*, 1994, 35:7821).

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

Many solid supports are commercially available and one of ordinary skill in the art can readily select a solid support to be used in the solid-phase synthesis steps. In certain

embodiments, a universal support is used. A universal support allows for preparation of oligonucleotides having unusual or modified nucleotides located at the 3'-terminus of the oligonucleotide. For further details about universal supports see Scott et al., *Innovations and Perspectives in solid-phase Synthesis, 3rd International Symposium*, 1994, Ed. Roger Epton, Mayflower Worldwide, 115-124]. In addition, it has been reported that the oligonucleotide can be cleaved from the universal support under milder reaction conditions when oligonucleotide is bonded to the solid support via a *syn*-1,2-acetoxyposphate group which more readily undergoes basic hydrolysis. See Guzaev, A. I.; Manoharan, M. *J. Am. Chem. Soc.* 2003, 125, 2380.

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

The aralkyl-ligand-conjugated oligonucleotides of the invention also include conjugates of oligonucleotides and linked nucleosides wherein the ligand is attached directly to the nucleoside or nucleotide without the intermediacy of a linker group. The ligand may preferably be attached, via linking groups, at a carboxyl, amino or oxo group of the ligand. Typical linking groups may be ester, amide or carbamate groups.

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

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

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

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

Preferred modified internucleoside linkages or backbones that do not include a
15 phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and
20 thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

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

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleoside units are replaced with novel groups. The
30 nucleobase units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligonucleotide, an oligonucleotide mimetic, that has been shown to have

excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to atoms of the amide portion of the backbone. Teaching of PNA compounds can be found for example in U.S. Pat. No. 5,539,082.

Some preferred embodiments of the invention employ oligonucleotides with phosphorothioate linkages and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--O--CH₂--, --CH₂--N(CH₃)--O--CH₂-- [known as a methylene (methylinino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂--, and --O--N(CH₃)--CH₂-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH₂--] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

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

Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in the *Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC

Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotides of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-Methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C. (Id., pages 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-methoxyethyl sugar modifications.

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

In certain embodiments, the oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-, S-, or N-alkenyl, or O, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly preferred are O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂ CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. a preferred modification includes 2'-methoxyethoxy [2'-O--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE], i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in U.S. Pat. No. 6,127,533, filed on Jan. 30, 1998, the contents of which are incorporated by reference.

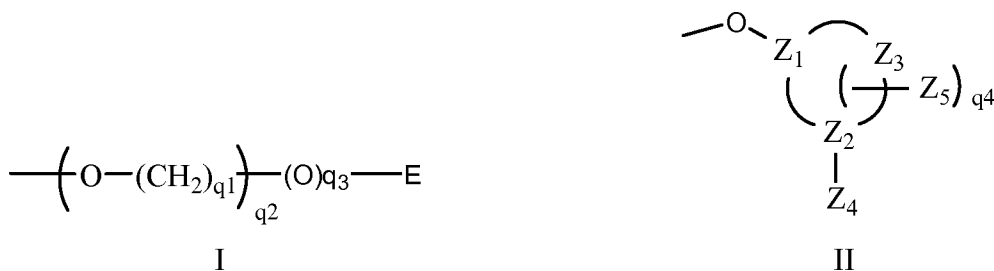
Other preferred modifications include 2'-methoxy (2'-O--CH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications may also be made at other

positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides.

As used herein, the term "sugar substituent group" or "2'-substituent group" includes groups attached to the 2'-position of the ribofuranosyl moiety with or without an oxygen atom.

5 Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole and polyethers of the formula (O-alkyl)_m, wherein m is 1 to about 10. Preferred among these polyethers are linear and cyclic polyethylene glycols (PEGs), and (PEG)-containing groups, such as crown ethers and, inter alia, those which are disclosed by Delgado et. al. (*Critical Reviews in Therapeutic Drug*
10 *Carrier Systems* 1992, 9:249), which is hereby incorporated by reference in its entirety. Further sugar modifications are disclosed by Cook (*Anti-fibrosis Drug Design*, 1991, 6:585-607). Fluoro, O-alkyl, O-alkylamino, O-alkyl imidazole, O-alkylaminoalkyl, and alkyl amino substitution is described in U.S. Patent 6,166,197, entitled "Oligomeric Compounds having Pyrimidine Nucleotide(s) with 2' and 5' Substitutions," hereby incorporated by reference in its entirety.

Additional sugar substituent groups amenable to the invention include 2'-SR and 2'-NR₂ groups, wherein each R is, independently, hydrogen, a protecting group or substituted or unsubstituted alkyl, alkenyl, or alkynyl. 2'-SR Nucleosides are disclosed in U.S. Pat. No. 5,670,633, hereby incorporated by reference in its entirety. The incorporation of 2'-SR monomer synthons is disclosed by Hamm et al. (*J. Org. Chem.*, 1997, 62:3415-3420). 2'-NR nucleosides are disclosed by Goettingen, M., *J. Org. Chem.*, 1996, 61, 6273-6281; and Polushin et al., *Tetrahedron Lett.*, 1996, 37, 3227-3230. Further representative 2'-substituent groups amenable to the invention include those having one of formula I or II:



wherein,

25 E is C₁ -C₁₀ alkyl, N(Q₃)(Q₄) or N=C (Q₃)(Q₄); each Q₃ and Q₄ is, independently, H, C₁-C₁₀ alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate

group, a linker to a solid support; or Q₃ and Q₄, together, form a nitrogen protecting group or a ring structure optionally including at least one additional heteroatom selected from N and O;

q₁ is an integer from 1 to 10;

q₂ is an integer from 1 to 10;

5 q₃ is 0 or 1;

q₄ is 0, 1 or 2;

each Z₁, Z₂ and Z₃ is, independently, C₄-C₇ cycloalkyl, C₅-C₁₄ aryl or C₃-C₁₅ heterocyclyl, wherein the heteroatom in said heterocyclyl group is selected from oxygen, nitrogen and sulfur;

10 Z₄ is OM₁, SM₁, or N(M₁)₂; each M₁ is, independently, H, C₁-C₈ alkyl, C₁-C₈ haloalkyl, C(=NH)N(H)M₂, C(=O)N(H)M₂ or OC(=O)N(H)M₂; M₂ is H or C₁-C₈ alkyl; and

Z₅ is C₁-C₁₀ alkyl, C₁-C₁₀ haloalkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₆-C₁₄ aryl, N(Q₃)(Q₄), OQ₃, halo, SQ₃ or CN.

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

Sugars having O-substitutions on the ribosyl ring are also amenable to the invention. Representative substitutions for ring O include, but are not limited to, S, CH₂, CHF, and CF₂.

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

Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide. For example, one additional

modification of the ligand-conjugated oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more additional non-ligand moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties, such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Lett.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923).

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

In certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues

(Saison-Behmoaras et al., *EMBO J.*, 1991, 10:111; Kabanov et al., *FEBS Lett.*, 1990, 259:327; Svinarchuk et al., *Biochimie*, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a
5 polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Typical conjugation protocols involve the synthesis of oligonucleotides bearing an aminolinker
10 at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate. The use of a cholesterol conjugate is particularly
15 preferred since such a moiety can increase targeting to tissues in the liver, a site of GCR protein production.

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

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

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be
30 used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including

definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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

5 **Description of figures and appended tables:**

Figure 1- Effect of GCR dsRNA comprising SEQ ID pair 55/56 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA (“on”) or *in silico* predicted off-target sequences (“off 1” to “off 15”; with “off 1” – “off 12” being
10 antisense strand off- targets and “off 13” to “off 15” being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 2 – Effect of GCR dsRNA comprising SEQ ID pair 83/84 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA (“on”) or
15 *in silico* predicted off-target sequences (“off 1” to “off 14”; with “off 1” – “off 11” being antisense strand off- targets and “off 12” and “off 14” being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 3– Effect of GCR dsRNA comprising SEQ ID pair 7/8 on silencing off-target sequences. Expression of renilla luciferase protein after transfection of COS7 cells expressing
20 dual-luciferase constructs, representative for either 19 mer target site of GCR mRNA (“on”) or *in silico* predicted off-target sequences (“off 1” to “off 14”; with “off 1” – “off 11” being antisense strand off- targets and “off 12” to “off 14” being sense strand off -targets), with 50 nM GCR dsRNA. Perfect matching off-target dsRNAs are controls.

Figure 4– mRNA levels, expressed in Quantigene 2.0 units / cell, for GCR (NR3C1)
25 gene, or for housekeeping gene GUSB , in human primary hepatocytes 96h post-transfection with GCR dsRNAs or Luciferase dsRNA control, in comparison to control cells exposed to DharmaFECT-1 transfection reagent alone.

Figure 5– mRNA levels, expressed in Quantigene 2.0 units / cell, for GCR (NR3C1) gene (a), GUSB housekeeping gene (b) and GCR-target genes PCK1 (c) , G6Pc (d) and TAT (e), in human primary hepatocytes exposed for 48h to LNP01-formulated dsRNAs

Figure 6– Glucose output measured in primary human hepatocytes exposed for 48h to LNP01-dsRNAs (a) Luciferase dsRNA control b) GCR dsRNA comprising SEQ ID pair 55/56 c) GCR dsRNA comprising SEQ ID pair 83/84, and starved for 96h before incubation for 5h in the presence of gluconeogenic precursors (lactate and pyruvate).

Figure 7– Cell ATP content measured in primary human hepatocytes exposed for 48h to LNP01-dsRNAs (a) Luciferase dsRNA control b) GCR dsRNA comprising SEQ ID pair 55/56 c) GCR dsRNA comprising SEQ ID pair 83/84, and starved for 96h before incubation for 5h in the presence of gluconeogenic precursors (lactate and pyruvate) .

Figure 8– Liver mRNA levels, relative to GUSB housekeeping mRNA level, obtained for GCR (NR3C1 gene, Figure 8 a) and GCR-upregulated genes TAT (Figure 8a), PCK1 (Figure 8b), G6Pc (Figure 8b), and HES1 (down-regulated by GCR, Figure 8c), 103 h after single iv administration of LPNO1-formulated dsRNAs for GCR comprising SEQ ID pair 517/518 or Luciferase control SEQ ID pair 681/682 in hyperglycemic and diabetic 14 wks-old male db/db mice.

Figure 9– Time-course efficacy on blood glucose levels after single iv administration of LPNO1-dsRNAs in hyperglycemic and diabetic 14 wks-old male db/db mice. (*: $p < 0.05$ versus vehicle). Efficacy of LPNO1-dsRNA for GCR comprising SEQ ID pair 517/518 in decreasing glucose level observed at +55-, +79-, +103h was of -13%, at -31% and -29%, respectively, when compared to the placebo (LNP01-Luciferase dsRNA SEQ ID pair 681/682). $n = 4$, mean values $\pm$ SEM, t-test assuming equal variance for each day.

Figure 10– Time-course plasma levels in ALT and AST in hyperglycemic and diabetic 14 wks-old male db/db mice, 55, 79 and 103h after single iv administration of LPNO1-dsRNAs for GCR comprising SEQ ID pair 517/518 or Luciferase control dsRNA (SEQ ID pair 681/682).

Figure 11 – GCR mRNA levels in liver biopsy of cynomolgus monkeys measured by bDNA assay 3 days post single i.v. bolus injection of Luciferase dsRNA (Seq. ID pair 681/682) or GCR dsRNAs (Seq. ID pair 747/753 or Seq. ID pair 764/772). Dose with respect to dsRNA

given for each group as mg/kg. N=2 female and male cynomolgus monkeys. Values are normalized to mean of GAPDH values of each individual monkey (a), or relative to Luciferase dsRNA (Seq. ID pair 681/682) with error bars indicating variations between monkeys (b).

Table 1 – dsRNA targeting human GCR gene. Letters in capitals represent RNA nucleotides, lower case letters “c”, “g”, “a” and “u” represent 2’ O-methyl-modified nucleotides, “s” represents phosphorothioate and “dT” deoxythymidine, “invdT” inverted deoxythymidine, “f” represents 2’ fluoro modification of the preceding nucleotide.

Table 2 - Characterization of dsRNAs targeting human GCR: Activity testing for dose response in HepG2 and HeLaS3 cells. IC 50: 50 % inhibitory concentration.

Table 3 - Characterization of dsRNAs targeting human GCR: Stability and Cytokine Induction. $t_{1/2}$: half-life of a strand as defined in examples, PBMC: Human peripheral blood mononuclear cells.

Table 4 - dsRNAs targeting mouse and rat GCR genes. Letters in capitals represent RNA nucleotides, lower case letters “c”, “g”, “a” and “u” represent 2’ O-methyl-modified nucleotides, “s” represents phosphorothioate and “dT” deoxythymidine. “f” represents 2’ fluoro modification of the preceding nucleotide.

Table 5 - Characterization of dsRNA targeting mouse and rat GCR genes: Stability and Cytokine Induction. $t_{1/2}$: half-life of a strand as defined in examples, PBMC: Human peripheral blood mononuclear cells.

Table 6 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 55/56.

Table 7 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 83/84.

Table 8 - Selected off-targets of dsRNAs targeting human GCR comprising sequence ID pair 7/8.

Table 9 - Sequences of bDNA probes for determination of human GAPDH; LE= label extender, CE= capture extender, BL= blocking probe.

Table 10 - Sequences of bDNA probes for determination of human GCR; LE= label extender, CE= capture extender, BL= blocking probe.

5 **Table 11** - Sequences of bDNA probes for determination of mouse GCR; LE= label extender, CE= capture extender, BL= blocking probe.

Table 12- Sequences of bDNA probes for determination of mouse GAPDH; LE= label extender, CE= capture extender, BL= blocking probe.

10 **Table 13** – dsRNA targeting human GCR gene. Letters in capitals represent RNA nucleotides.

Table 14 - dsRNA targeting human GCR gene without modifications and their modified counterparts. Letters in capitals represent RNA nucleotides, lower case letters “c”, “g”, “a” and “u” represent 2’ O-methyl-modified nucleotides, “s” represents phosphorothioate and “dT” deoxythymidine, “invdT” inverted deoxythymidine.

15

EXAMPLES

Identification of dsRNAs for therapeutic use

dsRNA design was carried out to identify dsRNAs specifically targeting human GCR for therapeutic use. First, the known mRNA sequences of human (Homo sapiens) GCR
20 (NM_000176.2, NM_001018074.1, NM_001018075.1, NM_001018076.1, NM_001018077.1, NM_001020825.1, NM_001024094.1 listed as SEQ ID NO. 659, SEQ ID NO. 660, SEQ ID NO. 661, SEQ ID NO. 662, SEQ ID NO. 663, SEQ ID NO. 664, and SEQ ID NO. 665) were downloaded from NCBI Genbank.

25 mRNAs of rhesus monkey (Macaca mulatta) GCR (XM_001097015.1, XM_001097126.1, XM_001097238.1, XM_001097341.1, XM_001097444.1, XM_001097542.1, XM_001097640.1, XM_001097749.1, XM_001097846.1 and XM_001097942.1) were downloaded from NCBI Genbank (SEQ ID NO. 666, SEQ ID NO. 667, SEQ ID NO. 668, SEQ

ID NO. 669, SEQ ID NO. 670, SEQ ID NO. 671, SEQ ID NO. 672, SEQ ID NO. 673, SEQ ID NO. 674, and SEQ ID NO. 675).

An EST of cynomolgus monkey (*Macaca fascicularis*) GCR (BB878843.1) was downloaded from NCBI Genbank (SEQ ID NO. 676).

5 The monkey sequences were examined together with the human GCR mRNA sequences (SEQ ID NO. 677) by computer analysis to identify homologous sequences of 19 nucleotides that yield RNA interference (RNAi) agents cross-reactive to human and rhesus monkey or human and cynomolgus monkey sequences.

10 In identifying RNAi agents, the selection was limited to 19mer sequences having at least 2 mismatches in the antisense strand to any other sequence in the human RefSeq database (release 27), which we assumed to represent the comprehensive human transcriptome, by using a proprietary algorithm.

The cynomolgous monkey GCR gene was sequenced (see SEQ ID NO. 678) and examined for target regions of RNAi agents.

15 dsRNAs cross-reactive to human as well as cynomolgous monkey GCR were defined as most preferable for therapeutic use. All sequences containing 4 or more consecutive G's (poly-G sequences) were excluded from the synthesis.

The sequences thus identified formed the basis for the synthesis of the RNAi agents in appended Tables 1, and 14 .

20 **Identification of dsRNAs for *in vivo* proof of concept studies**

dsRNA design was carried out to identify dsRNAs targeting mouse (*Mus musculus*) and rat (*Rattus norvegicus*) for *in vivo* proof-of-concept experiments. First, the transcripts for mouse GCR (NM_008173.3, SEQ ID NO. 679) and rat GCR (NM_012576.2, SEQ ID NO. 680) were examined by computer analysis to identify homologous sequences of 19 nucleotides that yield
25 RNAi agents cross-reactive between these sequences.

In identifying RNAi agents, the selection was limited to 19mer sequences having at least 2 mismatches in the antisense strand to any other sequence in the mouse and rat RefSeq database

(release 27), which we assumed to represent the comprehensive mouse and rat transcriptome, by using a proprietary algorithm.

All sequences containing 4 or more consecutive G's (poly-G sequences) were excluded from the synthesis. The sequences thus identified formed the basis for the synthesis of the RNAi agents in appended Table 4.

dsRNA synthesis

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 μ mole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing 2'-O-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and 2'-O-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at -20 °C until use.

Activity testing

Activity of dsRNAs targeting human GCR

The activity of the GCR-dsRNAs for therapeutic use described above was tested in HeLaS3 cells. Cells in culture were used for quantitation of GCR mRNA by branched DNA in
5 total mRNA derived from cells incubated with GCR-specific dsRNAs.

HeLaS3 cells were obtained from *American Type Culture Collection* (Rockville, Md., cat. No. CCL-2.2) and cultured in Ham's F12 (Biochrom AG, Berlin, Germany, cat. No. FG 0815) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 mg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213) at 37°C in an atmosphere with 5% CO₂ in a humidified incubator (Heraeus HERAAcell, Kendro Laboratory Products, Langenselbold, Germany).

Cell seeding and transfection of dsRNA were performed at the same time. For
15 transfection with dsRNA, HeLaS3 cells were seeded at a density of 2.0.times.10.sup.4 cells/well in 96-well plates. Transfection of dsRNA was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat.No. 11668-019) as described by the manufacturer. In a first single dose experiment dsRNAs were transfected at a concentration of 30 nM. Two independent experiments were performed. Most effective dsRNAs showing a mRNA knockdown of more
20 than 80% from the first single dose screen at 30nM were further characterized by dose response curves. For dose response curves, transfections were performed in HeLaS3 cells as described for the single dose screen above, but with the following concentrations of dsRNA (nM): 24, 6, 1.5, 0.375, 0.0938, 0.0234, 0.0059, 0.0015, 0.0004 and 0.0001 nM . After transfection cells were incubated for 24 h at 37°C and 5 % CO₂ in a humidified incubator (Heraeus GmbH, Hanau,
25 Germany). For measurement of GCR mRNA cells were harvested and lysed at 53°C following procedures recommended by the manufacturer of the QuantiGene 1.0 Assay Kit (Panomics, Fremont, Calif., USA, cat. No. QG-0004) for bDNA quantitation of mRNA. Afterwards, 50 µl of the lysates were incubated with probesets specific to human GCR and human GAPDH (sequence of probesets see table 9 and 10) and processed according to the manufacturer's protocol for
30 QuantiGene. Chemoluminescence was measured in a Victor2-Light (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the human GCR probeset were normalized to the respective human GAPDH values for each well. Unrelated control dsRNAs were used as a negative control.

Inhibition data are given in appended tables 1 and 2.

Activity of dsRNAs targeting rodent GCR

The activity of the GCR-siRNAs for use in rodent models was tested in Hepa1-6 cells. Hepa1-6 cells in culture were used for quantitation of GCR mRNA by branched DNA assay
5 from whole cell lysates derived from cells transfected with GCR-specific siRNAs.

Hepa1-6 cells were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig Germany, cat. No. ACC 175) and cultured in DMEM (Biochrom AG, Berlin, Germany, cat. No. FG 0815) supplemented to contain 10% fetal calf
10 serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 mg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213), L-Glutamine 4 mM (Biochrom AG, Berlin, Germany, cat. No. K0283) at 37°C in an atmosphere with 5% CO₂ in a humidified incubator (Heraeus HERAccl, Kendro Laboratory Products, Langenselbold, Germany).

Cell seeding and transfection of siRNA were performed at the same time. For transfection with siRNA, Hepa1-6 cells were seeded at a density of 15000 cells/well in 96-well plates. Transfection of siRNA was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat.No. 11668-019) as described by the manufacturer. The two chemically
20 different screening sets of siRNAs were transfected at a concentration of 50 nM. For measurement of GCR mRNA cells were harvested 24 h after transfection and lysed at 53°C following procedures recommended by the manufacturer of the Quantigene 1.0 Assay Kit (Panomics, Fremont, Calif., USA, cat. No. QG-0004) for bDNA quantitation of mRNA. Afterwards, 50 µl of the lysates were incubated with probesets specific to mouse GCR and
25 mouse GAPDH (sequence of probesets see below) and processed according to the manufacturer's protocol for QuantiGene. Chemiluminescence was measured in a Victor2-Light (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the mouse GCR probeset were normalized to the respective mouse GAPDH values for each well. Unrelated control siRNAs were used as a negative control.
30 Most efficacious three siRNAs were used for pharmacological prove of concept studies in rodent in vivo experiments.

Inhibition data are given in appended table 4.

Stability of dsRNAs

Stability of dsRNAs was determined in in vitro assays with either human serum or plasma from cynomolgous monkey for dsRNAs targeting human GCR and with mouse serum for dsRNAs targeting mouse/rat PTB1B by measuring the half-life of each single strand.

5 Measurements were carried out in triplicates for each time point, using 3µl 50µM dsRNA sample mixed with 30µl human serum or cynomolgous plasma (Sigma Aldrich). Mixtures were incubated for either 0min, 30min, 1h, 3h, 6h, 24h, or 48h at 37°C. As control for unspecific degradation dsRNA was incubated with 30µl 1x PBS pH 6.8 for 48h. Reactions were stopped by the addition of 4µl proteinase K (20mg/ml), 25µl of “Tissue and Cell Lysis Solution” (Epicentre)
 10 and 38µl Millipore water for 30 min at 65°C. Samples were afterwards spin filtered through a 0.2 µm 96 well filter plate at 1400 rpm for 8 min, washed with 55µl Millipore water twice and spin filtered again.

For separation of single strands and analysis of remaining full length product (FLP), samples were run through an ion exchange Dionex Summit HPLC under denaturing conditions
 15 using as eluent A 20mM Na₃PO₄ in 10% ACN pH=11 and for eluent B 1 M NaBr in eluent A.

The following gradient was applied:

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

20 For every injection, the chromatograms were integrated automatically by the Dionex Chromeleon 6.60 HPLC software, and were adjusted manually if necessary. All peak areas were corrected to the internal standard (IS) peak and normalized to the incubation at t=0 min. The area under the peak and resulting remaining FLP was calculated for each single strand and triplicate
 25 separately. Half-life (t_{1/2}) of a strand was defined by the average time point [h] for triplicates at which half of the FLP was degraded.

Results are given in appended tables 3 and 5.

Cytokine induction

Potential cytokine induction of dsRNAs was determined by measuring the release of INF-a and TNF-a in an *in vitro* PBMC assay.

Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coat blood of two donors by Ficoll centrifugation at the day of transfection. Cells were transfected in quadruplicates with dsRNA and cultured for 24h at 37°C at a final concentration of 130nM in Opti-MEM, using either Gene Porter 2 (GP2) or DOTAP. dsRNA sequences that were known to induce INF-a and TNF-a in this assay, as well as a CpG oligo, were used as positive controls. Chemical conjugated dsRNA or CpG oligonucleotides that did not need a transfection reagent for cytokine induction, were incubated at a concentration of 500nM in culture medium. At the end of incubation, the quadruplicate culture supernatant were pooled.

INF-a and TNF-a was then measured in these pooled supernatants by standard sandwich ELISA with two data points per pool. The degree of cytokine induction was expressed relative to positive controls using a score from 0 to 5, with 5 indicating maximum induction.

Results are given in appended tables 3 and 5.

In vitro off-target analysis of dsRNA targeting human GCR

The psiCHECK™- vector (Promega) contains two reporter genes for monitoring RNAi activity: a synthetic version of the Renilla luciferase (hRluc) gene and a synthetic firefly luciferase gene (hluc+). The firefly luciferase gene permits normalization of changes in Renilla luciferase expression to firefly luciferase expression. Renilla and firefly luciferase activities were measured using the Dual-Glo® Luciferase Assay System (Promega). To use the psiCHECK™ vectors for analyzing off-target effects of the inventive dsRNAs, the predicted off-target sequence was cloned into the multiple cloning region located 3' to the synthetic Renilla luciferase gene and its translational stop codon. After cloning, the vector is transfected into a mammalian cell line, and subsequently cotransfected with dsRNAs targeting GCR. If the dsRNA effectively initiates the RNAi process on the target RNA of the predicted off-target, the fused Renilla target gene mRNA sequence will be degraded, resulting in reduced Renilla luciferase activity.

In silico off-target prediction

The human genome was searched by computer analysis for sequences homologous to the inventive dsRNAs. Homologous sequences that displayed less than 6 mismatches with the inventive dsRNAs were defined as a possible off-targets. Off-targets selected for *in vitro* off-target analysis are given in appended tables 6, 7 and 8.

Generation of psiCHECK vectors containing predicted off-target sequences

The strategy for analyzing off target effects for an dsRNA lead candidate includes the cloning of the predicted off target sites into the psiCHECK2 Vector system (Dual Glo®-system, Promega, Braunschweig, Germany cat. No C8021) via XhoI and NotI restriction sites. Therefore, the off target site is extended with 10 nucleotides upstream and downstream of the dsRNA target site. Additionally, a NheI restriction site is integrated to prove insertion of the fragment by restriction analysis. The single-stranded oligonucleotides were annealed according to a standard protocol (e.g. protocol by Metabion) in a Mastercycler (Eppendorf) and then cloned into psiCHECK (Promega) previously digested with XhoI and NotI. Successful insertion was verified by restriction analysis with NheI and subsequent sequencing of the positive clones. The selected primer (Seq ID No. 677) for sequencing binds at position 1401 of vector psiCHECK. After clonal production the plasmids were analyzed by sequencing and than used in cell culture experiments.

Analysis of dsRNA off-target effects

Cell culture:

Cos7 cells were obtained from Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany, cat. No. ACC-60) and cultured in DMEM (Biochrom AG, Berlin, Germany, cat. No. F0435) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml, and Streptomycin 100 µg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213) and 2 mM L-Glutamine (Biochrom AG, Berlin, Germany, cat. No. K0283) as well as 12 µg/ml Natrium-bicarbonat at 37°C in an atmosphere with 5% CO₂ in a humidified incubator (Heraeus HERAccl, Kendro Laboratory Products, Langenselbold, Germany).

Transfection and Luciferase quantification:

For transfection with plasmids, Cos-7 cells were seeded at a density of 2.25×10^4 cells/well in 96-well plates and transfected directly. Transfection of plasmids was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer at a concentration of 50 ng/well. 4 hours after transfection, the medium was discarded and fresh medium was added. Now the dsRNAs were transfected in a concentration at 50 nM using lipofectamine 2000 as described above. 24h after dsRNA transfection the cells were lysed using Luciferase reagent described by the manufacturer (Dual-Glo™ Luciferase Assay system, Promega, Mannheim, Germany, cat. No. E2980) and Firefly and Renilla Luciferase were quantified according to the manufacturer's protocol. Renilla Luciferase protein levels were normalized to Firefly Luciferase levels. For each dsRNA eight individual data points were collected in two independent experiments. A dsRNA unrelated to all target sites was used as a control to determine the relative Renilla Luciferase protein levels in dsRNA treated cells.

Results are given in figures 1,2 and 3.

Efficacy of dsRNAs targeting GCR in human primary hepatocytes**GCR target gene knockdown after transfection of dsRNAs**

Fresh suspensions of human primary hepatocytes, isolated from surgery resections, were purchased from HepaCult GmbH and were plated in 12 well collagen coated plates, at a density of 325 000 cells / well in William's E media (Sigma-Aldrich Inc, cat. No W1878.) supplemented with 10% Fetal Calf Serum (FCS), 1% GlutaMAX 200 mM (Invitrogen GmbH, cat. No 35050-038.) and antibiotics (penicillin, streptomycin and gentamycin) . After overnight culture (at 37°C in an atmosphere with 5% CO₂ in a humidified incubator), medium was replaced with DMEM medium (Invitrogen GmbH, cat. No 21885) similarly supplemented, and dsRNAs transfections were performed at a final concentration of 15 nM, using DharmaFECT-1 transfection reagent (ThermoFisher Scientific Inc, cat. No T2001). 72h later, medium was replaced with fresh medium supplemented with 2 uM cAMP (Sigma-Aldrich Inc, cat. No S3912) and cells were further cultured overnight to allow for induction of gene expression. Cells were then exposed to Dexamethasone 500nM (Sigma-Aldrich Inc, cat. No D4902) for 6h to trigger activation and translocation of GCR to the nuclei and were recovered for gene expression analysis by branched-DNA technology, according to Panomics/Affymetrix Inc protocols for Quantigene 2.0

technology (http://www.panomics.com/index.php?id=product_1). In these conditions, exposure of human primary hepatocytes to dsRNA for GCR led to up to 90 % KD of GCR gene expression

Results are shown in Figure 4.

5

Effect of LNP01-formulated dsRNAs for GCR on GCR and GCR-regulated genes expression

Human primary hepatocytes were plated and cultivated as described above, except that 450 000 cells were seeded per well. After overnight culture, cells were exposed for 48h to dsRNAs packaged into cationic liposomal formulation LNP01 at doses ranging from 1 to 100 nM. After 32h exposure to dsRNAs, cAMP was added at 2 uM final concentration. Medium was further supplemented with Dexamethasone at 500 nM final concentration 6h before cell recovery for gene expression analysis. In these conditions, cell exposure to LNP01-formulated dsRNA for GCR led to dose response inhibition of GCR gene expression, with 80% KD of GCR gene expression reached at 100 nM exposure without change in the expression of GUSB housekeeping gene. GCR KD translated into strong inhibition of expression of TAT and PCK1 genes, and to a lesser extend, to G6Pc gene inhibition, which expressions are induced by GCR receptor upon activation.

Results are shown in Figure 5.

Effect of LNP01-formulated dsRNAs for GCR on glucose output

Glucose output assays were performed on primary human hepatocytes seeded and exposed to LNP01-formulated dsRNAs as described above, except that 96 well plates format were used with 35 000 cells seeded / well, and that after 48h exposure to LNP01-formulated dsRNAs, cells were cultivated in starvation conditions for 72h in glucose-free RPMI 1640 media (Invitrogen GmbH, cat. No 11879) supplemented with 1% FCS and antibiotics, before medium was refreshed and supplemented with 2 uM cAMP and with 30 nM Dexamethasone for overnight incubation. Control cells treated with cAMP alone, or with cAMP, Dexamethasone and Mifepristone 1 uM (a GCR antagonist), were also performed. Cells were then further incubated in the presence of gluconeogenic precursors (lactate and pyruvate) to induce glucose production for 5h in DPBS (Invitrogen GmbH, cat. No 1404) containing 0.1% free-fatty acid

BSA, 20 mM sodium pyruvate and 2mM lactate. Glucose produced was evaluated with Amplex-Red Glucose/Glucose oxydase assay kit (Invitrogen GmbH, cat. No A22189) in culture supernatants. As an indicator of cell viability, cellular ATP content was also measured using Cell-titer Glo luminescent cell viability assay (Promega Corporation, cat. No G7571). Cell exposure to LNP01-formulated dsRNA for GCR led to dose-response inhibition of glucose production up to the maximum level expected from full antagonism of GCR activity achieved by Mifepristone.

Results are shown in Figure 6 and 7.

In vivo effects of dsRNA targeting mice and rat GCR

RNAi-mediated GCR KD in liver, and efficacy on blood glucose in db/db mice after single i.v. injection.

A group of 30 males db/db mice (Jackson laboratories) were fed a regular chow diet (Kliba 3436). Homogenous groups of 4 mice each were organized according to their BW and blood glucose measured under fed conditions the day of the experiment and 2h after was food removed.

Mice were treated with single iv injection of either LNP01-formulated ds RNA for Luciferase control (SEQ ID pair 681/682) or LNP01-formulated dsRNA for GCR (SEQ ID pair 517/518) at 5.76 mg/kg for up to 103h.

Blood glucose levels were measured with Accu-Chek (Aviva) 2 days, 3 days and 4 days after iv injection (+55h, +79h and +103h post treatment) in the afternoon corresponding to 10h after food was removed. Mice were then sacrificed. Plasma ALT and AST were analyzed by Hitachi. Liver was harvested and snap frozen in liquid nitrogen for mRNA expression analysis of GCR and GCR-regulated genes (TAT, PCK1, G6Pc and HES1 genes) by branched-DNA, processing the largest lobe (left lateral lobe) according to Panomics/Quantigene 2.0 sample processing protocol for animal tissues (Panomics-Affymetrix Inc, cat. No QS0106). Db/db mice treatment with GCR dsRNA resulted in significant KD of GCR gene expression in mice liver and in decreased glycemia without change in liver transaminases.

Results are shown in Figures 8, 9 and 10.

In vivo effects of dsRNA targeting GCR (Macaca fascicularis)

For the following studies a sterile formulation of dsRNA lipid particles in isotonic buffer (e.g. Semple SC et al., Nat Biotechnol. 2010 Feb;28(2):172-6. Epub 2010 Jan 17. Rational
5 design of cationic lipids for siRNA delivery.) were used.

Single Dose Titration Study in Monkeys (Macaca fascicularis)

Monkeys received single i.v. bolus injections of GCR dsRNA (Seq. ID pair 747/753) of either 0.5, 1.5 or 3 mg/kg, or dsRNA (Seq. ID pair 764/772) in a dose of 1.5 mg/kg. Control groups received a 1.5 mg/kg of Luciferase dsRNA (Seq. ID pair 681/682) in order to
10 discriminate between effects caused by the lipid particle and RNAi-mediated effects. All treatment groups were run with one male and one female monkey. Liver biopsy samples were taken on day 3 after injection.

GCR mRNA levels were measured from liver biopsy samples by bDNA assay as described above.

15 GCR dsRNA treated groups showed a dose-dependent decrease in GCR mRNA levels starting with 1.5 mg/kg of GCR dsRNA resulting in a decrease of about 24% by GCR dsRNA (Seq. ID pair 747/753) and 29% decrease by GCR dsRNA (Seq. ID pair 764/772), and reaching a 45% decrease in GCR mRNA with 3 mg/kg of GCR dsRNA (Seq. ID pair 747/753) (Figure 11).

Table 1

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
757	uGucGAAAcAGuuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	93	0
756	uGGucGAAAcAGuuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	93	1
756	uGGucGAAAcAGuuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	93	1
755	uGucGAAAcAGuuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	93	1
748	uGGucGAAAcAGuuuuuuuccdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	93	2
749	uGucGAAAcAGuuuuuuuccdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	93	2
749	uGucGAAAcAGuuuuuuuccdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	93	0
758	uGucGAAAcAGuuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	92	2
754	uGGucGAAAcAGuuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	92	1
755	uGucGAAAcAGuuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	92	0
754	uGGucGAAAcAGuuuuuuuccdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	92	0
758	uGucGAAAcAGuuuuuuucGdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	92	1
748	uGGucGAAAcAGuuuuuuuccdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	92	0
757	uGucGAAAcAGuuuuuuuccdT(abasic)	760	pAGAAAAAACUGUUCGACcAdT(abasic)	92	2
750	uGGucGAAAcAGuuuuuuucGdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	92	0
750	uGGucGAAAcAGuuuuuuucGdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	92	1
751	uGucGAAAcAGuuuuuuucGdT(invdT)	752	pAGAAAAAACUGUUCGACcAdT(invdT)	92	1
759	uGGucGAAAcAGuuuuuuucGdT(abasic)	761	AGAAAAAACUGUUCGACcAdT(abasic)	92	1
751	uGucGAAAcAGuuuuuuucGdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	92	1
746	uGGucGAAAcAGuuuuuuuccdT(invdT)	753	AGAAAAAACUGUUCGACcAdT(invdT)	92	0

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
747	ugGucGAACAGuuuuuuucdT(invdt)	753	AGAAAAAACUGUUCGACCAdT(invdt)	92	1
740	uGGucGAACAGuuuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACCAdTsdT	91	1
742	uGGucGAACAGuuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACCAdTsdT	91	1
740	uGGucGAACAGuuuuuuuccdTsdT	56	AGAAAAAACUGUUCGACCAdTsdT	91	1
743	ugGucGAACAGuuuuuuucGdTsdT	745	pAGAAAAAACUGUUCGACCAdTsdT	91	2
743	ugGucGAACAGuuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACCAdTsdT	91	1
741	ugGucGAACAGuuuuuuuccdTsdT	56	AGAAAAAACUGUUCGACCAdTsdT	91	1
742	uGGucGAACAGuuuuuuucGdTsdT	56	AGAAAAAACUGUUCGACCAdTsdT	91	2
1	cAuGuAcGAccAAuGuAAAdTsdT	2	UfUfACfAUfUfGUfCfGUfACfAUfGdTsdT	91	2
3	uuGcuuAAcuAcAuAuAGAdTsdT	4	UfcfUfAUfAUfGUfAGUfUfAAGCfAAAdTsdT	90	1
5	AAAuAAcuuGcuuAAcuAcdTsdT	6	GUFAGUfUfAAGCfAAAGUfUfAUfUfUfdTsdT	90	2
741	ugGucGAACAGuuuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACCAdTsdT	90	2
739	ugGucGAACAGuuuuuuuccdTsdT	744	pAGAAAAAACUGUUCGACCAdTsdT	90	2
739	ugGucGAACAGuuuuuuuccdTsdT	56	AGAAAAAACUGUUCGACCAdTsdT	90	1
747	ugGucGAACAGuuuuuuuccdT(invdt)	752	pAGAAAAAACUGUUCGACCAdT(invdt)	90	1
746	uGGucGAACAGuuuuuuuccdT(invdt)	752	pAGAAAAAACUGUUCGACCAdT(invdt)	90	2
7	uGcuuAAcuAcAuAuAGAudTsdT	8	AUfCfUfAUfAUfGUfAGUfUfAAGCfAdTsdT	89	2
9	GuAuGAAAACcuuAcuGcudTsdT	10	AGCfAGUfAAGGUfUfUfCfAUfACfAdTsdT	89	1
11	cAGUGAGAGuuGGuuAcucdTsdT	12	GAGUfAACfCAACfUfCfUfCfACfUfGdTsdT	89	2
13	GGGuGGGAGucAuAuAGAcdTsdT	14	GUcUAuAUGAUcUcACCCdTsdT	89	2
762	GuuccAGAcucAAcuuGGcdTsdT	770	pUCcAAGUUUGAGUCUGGAACdTsdT	89	2
762	GuuccAGAcucAAcuuGGcdTsdT	84	UCcAAGUUUGAGUCUGGAACdTsdT	89	2

SEQ ID NO	Sense strand sequence (5' -3')	SEQ ID NO	Antisense strand sequence (5' -3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
55	uGGucGAACAGuuuuuucdTsdT	744	pAGAAAAAACUGUUCGACcAdTsdT	89	3
763	GuuccAGAcucAAcuuGGdTsdT	84	UCcAAGUUGAGUCUGGAACdTsdT	89	1
763	GuuccAGAcucAAcuuGGdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	88	0
765	GuuccAGAcucAAcuuGGdT(invT)	771	pUCcAAGUUGAGUCUGGAACdT(invT)	88	1
768	GuuccAGAcucAAcuuGGdT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	88	1
769	GuuccAGAcucAAcuuGGdT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	88	1
769	GuuccAGAcucAAcuuGGdT(abasic)	773	pUCcAAGUUGAGUCUGGAACdT(abasic)	87	1
765	GuuccAGAcucAAcuuGGdT(invT)	772	UCcAAGUUGAGUCUGGAACdT(invT)	87	1
766	GuuccAGAcucAAcuuGGdT(invT)	771	pUCcAAGUUGAGUCUGGAACdT(invT)	87	1
766	GuuccAGAcucAAcuuGGdT(invT)	772	UCcAAGUUGAGUCUGGAACdT(invT)	87	1
764	GuuccAGAcucAAcuuGGdT(invT)	772	UCcAAGUUGAGUCUGGAACdT(invT)	87	2
767	GuuccAGAcucAAcuuGGdT(abasic)	774	UCcAAGUUGAGUCUGGAACdT(abasic)	87	2
15	GGGuGGAGaucAAuAGAcdTsdT	16	GUfCfUfAUfAUfGUAUfGUAUfCfCfCfCfCfTsdT	87	2
17	cAGuGAGAGuuGGuuAcuedTsdT	18	GAGuAAcCaaCUCUCuACUGdTsdT	87	2
19	cAuAuAGAcAAucAAuGuGcdTsdT	20	GCfACfUfUfGUAUfUfGUfCfUfAUfAUfGdTsdT	87	2
21	ccuAuGuAuGuGuuAucuGdTsdT	22	CfAGAUfAAcCfAcfAUfCfAUfAGGdTsdT	87	1
23	uuAAuGuGucAuuccAccAAudTsdT	24	AUfUfGGUfGGAAUfGACfAUfUfAAAdTsdT	87	2
25	uuGcuuAAcuAAcuAAuAGAdTsdT	26	UCuAuAUGuAGUuAAGcAAAdTsdT	86	3
27	uGGucGAACAGuuuuuucdTsdT	28	AGAAAAAAcCfUfGUfUfCfGACfCfAdTsdT	86	1
29	cAcAcAuuAAuucuGAuuuudTsdT	30	AAAAUfCfAGAUfUfAAUfGUfGUfGdTsdT	86	2
83	GuuccAGAcucAAcuuGGAdTsdT	770	pUCcAAGUUGAGUCUGGAACdTsdT	86	5
768	GuuccAGAcucAAcuuGGdT(abasic)	773	pUCcAAGUUGAGUCUGGAACdT(abasic)	86	2

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
764	GuccAGAcucAAcuuGGAdT(invdT)	771	pUCcAAGUUAGAGUCUGGAACdT(invdT)	86	3
767	GuccAGAcucAAcuuGGAdT(abasic)	773	pUCcAAGUUAGAGUCUGGAACdT(abasic)	83	8
83	GuccAGAcucAAcuuGGAdTsdT	770	pUCcAAGUUAGAGUCUGGAACdTsdT	86	5
31	GuAuGAAAAccuuAcuGcudTsdT	32	AGcAGuAAGGUUUUcAuACdTsdT	85	3
33	cuAcAGGAGucucAcAAGAdTsdT	34	UCUUGAGACUCCUGuAGdTsdT	84	2
35	cuGuAuGAAAAuAccuccdTsdT	36	GGAGGGuAUUUUcAuAcAGdTsdT	85	3
37	uccuAuGuAuGuGuuAucudTsdT	38	AGAuAAcAcAuAcAuAGGAdTsdT	85	5
39	GGuGGAGAucAuAuAGAcAdTsdT	40	UfGUfcfUfAUfAUfGUAUfcfCfCfACfCfdTsdT	84	1
41	AuGuAcGAccAAuGuAAAcdTsdT	42	GUfUfUfACfAUfUfGGUfcfGUfACfAUfdTsdT	84	2
43	AcuGGcAGcGGuuuuAucAdTsdT	44	UfGAUfAAAcCfCfGcUfGcCfCfAGUfdTsdT	84	2
45	AGuGAGAGuuGGuuAcucAdTsdT	46	UfGAGUfAACfCfAACfUfCfUfCfACfUfdTsdT	84	2
47	AAuAAcuuGcuuAAcuAcAdTsdT	48	UfGUfAGUfUfAAAGCfAAAGUfUfAUfUfdTsdT	84	1
49	GuGAGAGuuGGuuAcucAcdTsdT	50	GUfGAGUfAAcCfAACfUfCfUfCfACfCfdTsdT	83	3
51	cAucAucGAuAAAAuucGAdTsdT	52	UfCfGAUfUfUfUfAUfCfGAUfGAUfGdTsdT	83	2
53	cuGuAuGAAAAuAccuccdTsdT	54	GGAGGGUfAUfUfUfCfAUfACfAGdTsdT	83	4
55	uGGucGAACAGuuuuuucdTsdT	56	AGAAAAACUGUUCGACcAdTsdT	82	4
57	AcGAuucAuuccuuuGGAdTsdT	58	UfCfCfAAAAAGGAUfGAAUfCfGUfUfdTsdT	82	2
59	cuGuAuGAAAAccuuAcuGdTsdT	60	cAGuAAGGUUUUcAuAcAGdTsdT	82	3
61	GuGAGAGuuGGuuAcucAcdTsdT	62	GUGAGuAAcAAACUCUcACdTsdT	82	4
63	uGuAcGAccAAuGuAAAcAdTsdT	64	UfGUfUfUfACfAUfUfGGUfCfGUfACfAdTsdT	82	3
65	uAccGGAcAcuAAAcccAAAdTsdT	66	UfUfGGGUfUfUfAGUfGUfCfGGUfAdTsdT	82	2
67	ccGcuAucGAAAAuGucuudTsdT	68	AAGACfAUfUfUfUfCfGAUfAGCfGGdTsdT	81	1
69	AGAuAGAccuGuuGAuAGdTsdT	70	CfUfAUfCfAACfAGGUfCfUfGAUfCfUfdTsdT	81	4

[illegible]

[illegible]

[illegible]

SEQ ID NO	Sense strand sequence (5' -3')	SEQ ID NO	Antisense strand sequence (5' -3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
209	AAAAcAAuAGuuccuGcAAAdTsdT	210	UUGcAGGAACuAUUGUUUUdTsdT	72	3
211	ccGcuAucGAAAAuGuccuudTsdT	212	AAGAcAUUUUCGauAGCGGdTsdT	71	5
213	cAGcAuGccGcuAucGAAAdTsdT	214	UfUfCfGAUfAGCfGGCfAUfGCUfGdTsdT	71	3
215	cuGGuGuGcucuGAuGAAAdTsdT	216	CfUfUfCfAUfCfAGAGCfACfACfGdTsdT	71	3
217	AcGcucAAcAuGuuAGGAGdTsdT	218	CUCcUAacAUUUUGAGCGUdTsdT	71	4
219	ucccAAcAAucuuGGeGcuuTsdT	220	AGCfGCfCfAAGAUfUfGUfUfGGGAdTsdT	71	4
221	AGAcGAAuGAGAGuccuuGdTsdT	222	CfAAAGGACfUfCfUfCfAUfUfCfGUfCfUfGdTsdT	71	6
223	uAccGGAcAcuAAAcceAAAdTsdT	224	UUGGGUuAGUGUCCGGuAdTsdT	70	9
225	cuGcAAcGuuAccAAcAAcudTsdT	226	AGUUGUGGuAACGUUUUGcAGdTsdT	70	4
227	ccAGcAuGccGcuAucGAAdTsdT	228	UUCGAuAGCGGcAUGCUGGdTsdT	70	4
229	AGcAuuGcAAAcceAAuAdTsdT	230	UfAUfUfGAGGUfUfUfGCUfUfGdTsdT	70	5
231	ucccAAcAAucuuGGeGcuuTsdT	232	AGCGCcAAGAUUUGUGGAdTsdT	70	6
233	ccAccAAuucccGuuGGuudTsdT	234	AACcAACGGGAUUUGGUGGdTsdT	70	5
235	ucAGAccuGuuGAuAGAuGdTsdT	236	CfAUfCfUfAUfCfAACfAGGUfCfUfGAdTsdT	70	4
237	uuAccGGAcAcuAAAcceAAAdTsdT	238	UGGGUuAGUGUCCGGuAAAdTsdT	70	8
239	cccAAcAAucuuGGeGcuuTsdT	240	GAGCfGCfCfAAGAUfUfGUfUfGGGdTsdT	70	4
241	uuuuuAAuGGcuAuucAAAdTsdT	242	CUUGAAuAGCcAUuAGAAAdTsdT	70	8
243	uuAAuGucAuuccAccAAAdTsdT	244	AUUGGUGGAAUGAcAUuAAAdTsdT	70	5
245	GGcucAGuAAAGcAAuGcGcdTsdT	246	GCGcAUUUGCUuACUGAGCCdTsdT	69	6
247	GucuuAAcuuGuGGAAAGcuuTsdT	248	AGCUUCCcAAAGUuAAGACdTsdT	69	5
249	ucAuuuuAccGGAcAcuAAAdTsdT	250	UfUfAGUfGUfCfCfGGUfAAAUfGAdTsdT	69	5
251	AGAcGAAuGAGAGuccuuGdTsdT	252	cAAGGACUCUcAUUCGUCUdTsdT	69	6
253	AcuGuAAAAccuuGuGuGGdTsdT	254	CfCfACfACfAAGGUfUfUfUfACfAGUfGdTsdT	68	3

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
255	AAccucAAuAGGucGAccAdTsdT	256	UGGUcGACCuAUUGAGGUUdTsdT	68	4
257	cAuGuGAAuAAuAuuGdTsdT	258	CfAGAUfUAUfUAUfCfAGCfAUfGdTsdT	68	3
259	uGcAAAaccucAAuAGGucGdTsdT	260	CGACCCuAUUGAGGUUUUGcAdTsdT	68	4
261	ccAAcAAuccuuGGcGcucAdTsdT	262	UfGAGCfGCfCfAAgAUfUfGUfUfGGdTsdT	68	4
263	GGuuucAGGAAcuuAcAccdTsdT	264	GGUGuAAGUUCCUGAAACCCdTsdT	68	2
265	GGuuucAGGAAcuuAcAccdTsdT	266	GGUfGUfAAguUfCfCfUfGAAACfCdTsdT	68	2
267	uAGuGAccAGGuuuucAGGdTsdT	268	CCUGAAAAccCUUGGUcACuAdTsdT	68	3
269	cuGcAAcGuuAccAcAAAcudTsdT	270	AGUfUfGUfGGUfAACfGUfUfGCFAGdTsdT	68	3
271	AGcAuGccGeuAucGAAAAAdTsdT	272	UUUUCGAuAGCGGcAUGCUdTsdT	67	4
273	uGcAAcGuuAccAcAAAcudTsdT	274	GAGUUGUGGuAACGUUGcAdTsdT	67	3
275	uGAAccuGAAGuGuuAuAudTsdT	276	AUfAUfAACfAcfUfUfCfAGGUfUfCfAdTsdT	66	3
277	cAccAAAnucccGuuGGuucdTsdT	278	GAACcAACGGGAAUUUGGUdTsdT	66	7
279	ccAGGuAAAGAGAcGA AuGdTsdT	280	CfAUfUfCfGUfCfUfCfUfUfUfACfCfUfGGdTsdT	66	4
281	cucucAAuGGGAcuGuAuAdTsdT	282	UfAUfACfAGUfCfCfCfAUfUfGAGAGdTsdT	66	6
283	uGGeGcucAAAAAAuAGAAdTsdT	284	UfUfCfUfAUfUfUfUfUfUfGAGCfCfCfAdTsdT	66	3
285	AuAccuccucAAAAuAAcudTsdT	286	AGUfUfAUfUfUfGAGGAGGUfAUfdTsdT	65	1
287	GGGcGGcAAAGuGAuuGcAGdTsdT	288	CfUfGcFAUfCfACfUfUfGcfcfcfcCfCdTsdT	65	2
289	uGcuuAAcuAcAuAuAGAAdTsdT	290	AUCuAuAUGuAGUuAAGcAdTsdT	65	4
291	AnuccAcAAAnucccGuuGdTsdT	292	CfAACfGGGAAUfUfGGUfGGAAUfdTsdT	64	5
293	AccucAAuAGGucGAccAGdTsdT	294	CUGGUcGACCCuAUUGAGGUdTsdT	64	4
295	GuucAuGGuGuGAGuAccudTsdT	296	AGGUfACfUfCfACfACfAUfGAACfIdTsdT	63	4
297	ccucucAuuuuAccGGAcAdTsdT	298	UGUCCGGuAAAAUAGAGAGGdTsdT	63	5
299	AGceucucAuuuuAccGGAdTsdT	300	UfCfCfGGUfAAAAUfGAGAGGCCfUfdTsdT	63	5

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
301	ucAAuGGGACuGuAuAuGGdTsdT	302	CfCfAUfAUfACfAGUfCfCfAUfUGAdTsdT	63	6
303	cAGGcuucAGGuAucuuAudTsdT	304	AUfAAGAUfACfCfUfGAAgCfCfUfGdTsdT	63	4
305	AuucAGcAGGccAcuAcAGdTsdT	306	CfUfGUfAGUfGGCfCfUfGcUfGAAUfdTsdT	63	2
307	cccAAcAAucuuGGcGcuCdTsdT	308	GAGCGCcAAGAUUUUUGGGdTsdT	62	4
309	AuGAGAccAGAuGuAAGcudTsdT	310	AGCUuAcAUCUGGUCUCaAUdTsdT	62	5
311	cAuGcuGAaAuAAuAAucuGdTsdT	312	cAGAUuAUuAUUcAGcAUGdTsdT	62	5
313	AcuGGcAGcGGuuuuAucAdTsdT	314	UGAuAAACCCGUCGcAGUdTsdT	62	5
315	AucuGGuuuuGucAAGcccdTsdT	316	GGGCfUfUfGACfAAACfCfAGAUfdTsdT	62	6
317	uGAGAGuuGGuuAcucAcAdTsdT	318	UGUGAGuAACcAACUCUcAdTsdT	61	4
319	ccAccAAuucccGuuGGuudTsdT	320	AACfCfAACfGGGAAUfUGGUfGGdTsdT	61	4
321	AAAcuGGGcAcAGuuuAcudTsdT	322	AGUfAAACfUfGUfGcCfCfAGUfUfUfdTsdT	61	6
323	GuucAuGGGuGuGAGuAccudTsdT	324	AGGuACUcAcAcCcAUGAACdTsdt	60	8
325	AuAccuccucAAAuAAcudTsdT	326	AGUuAUUUAGGAGGGuAUdTsdT	59	5
327	uuAccGGAcAcuAAAcceAdTsdT	328	UfGGGUfUfUfAGUfGUfCfGGUfAAdTsdT	59	5
329	AcuuAcAccuGGAuGAccAdTsdT	330	UfGGUfCfAUfCfCfAGGUfGUfAAAGUfdTsdT	59	3
331	cucAGuAAGcAAuGcGcAGdTsdT	332	CfUfGcGcAUfUfUfGcUfUfUfCfUfGAGdTsdT	59	7
333	uuuGAcAuuuuGcAGGAuudTsdT	334	AAUfCfCfUfGcFAAAAUfGUfCfAAAdTsdT	59	9
335	ucAGAccuGuuGAuAGAuGdTsdT	336	cAUCuAUcAAcAGGUCUGAdTsdT	58	6
337	AuucAGcAGGccAcuAcAGdTsdT	338	CUGuAGUGGCCUGCUGAAUdTsdT	57	5
339	AuAGuuccuGcAAcGuuAcdTsdT	340	GUfAACfGUfUfGcFAGGAACfUfUfdTsdT	56	3
341	uGcAAcGuuAccAcAAAcuCdTsdT	342	GAGUfUfGUfGGUfAACfGUfUfGcFAdTsdT	56	5
343	uAGuuuuuuAuucAuGcuGdTsdT	344	CfAGCfAUfGAUfAAUfAAAAACUfAdTsdT	56	6
345	uGGGAAAuGAccuGGGAuudTsdT	346	AAUfCfCfCfAGGUfCfAUfUfUfCfCfAdTsdT	56	7

SEQ ID NO	Sense strand sequence (5' -3')	SEQ ID NO	Antisense strand sequence (5' -3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
347	uuuGAcAuuuuGcAGGAuudTsdT	348	AAUCCUGcAAAAUGUcAAAdTsdT	56	5
349	AcGcucAAcAuGuuAGGAGdTsdT	350	CfUfCfUfAaCfAUfGUfUfGAGCfGUfAdTsdT	54	6
351	uGcuGuucuuGGuAuuAccAdTsdT	352	UfGGUfAAUfAcfAcfAGAAcfAGCfAdTsdT	54	3
353	cccAGGuAAAGAGAcGAAdTsdT	354	AUUCGUUCUcUuACCUGGGdTsdT	53	11
355	uGcAAAaccucAAuAGGucGdTsdT	356	CfGACfCfUfAUfUfGAGGUfUfGfCfAdTsdT	53	5
357	GccucucAuuuuAccGGAcdTsdT	358	GUCCGGuAAAUAGAGAGGCDTsdT	52	6
359	uGcuGuucuuGGuAuuAccAdTsdT	360	UGGuAAuACcAGAcAGAcAdTsdT	52	4
361	GAAcuGGcAGcGGuuuuAudTsdT	362	AuAAAAccCGCUGCcAGUUCdTsdT	52	4
363	ccuAuGuAuGuGuuAucuGdTsdT	364	cAGAuAAcAcAuAcAuAGGdTsdT	51	5
365	AGAAAGuuucAucGAAcuedTsdT	366	GAGUfUfCfGAUfGAAAUfCfUfUfCfUfAdTsdT	51	6
367	cucuGAAcuuccuGGucGdTsdT	368	CfGACfCfAGGGAAAGUfUfCfAGAGdTsdT	51	3
369	cuGGuGuGcucuGAuGAAgdTsdT	370	CUUcAUcAGAGAcAcAcAGdTsdT	51	6
371	cucAAcuuGGAGGAucAuGdTsdT	372	CfAUfGAUfCfUfCfUfCfAAAGUfUfGAGdTsdT	50	5
373	AGccucucAuuuuAccGGAdTsdT	374	UCCGGuAAAAUAGAGAGGCCUdTsdT	50	7
375	AuAGuuuccuGcAAcGuuAcdTsdT	376	GuAACGUUUGcAGGAACuAUdTsdT	50	6
377	AAcAAuAGuuuccuGcAAcGdTsdT	378	CfGUfUfGcFAGGAACfUfAUfUfGUfUfAdTsdT	50	3
379	AucuGGuuuuGucAAAGccdTsdT	380	GGGCUUGAcAAAAcAcAGAUdTsdT	49	6
381	AcuGuAAAAccuuGuGuGGdTsdT	382	CcAcAcAAGGUUuAcAGUdTsdT	49	6
383	AAcucuuGGAAuuuAuGcAdTsdT	384	UGcAuAGAAUcAcAAGAUUdTsdT	49	7
385	uAGuGAccAGGuuuucAGGdTsdT	386	CfCfUfGAAAcAcCfUfGGUfCfAcUfAdTsdT	49	6
387	AAccucAAuAGGucGAcAdTsdT	388	UfGGUfCfGACfCfUfAUfUfGAGGUfUfAdTsdT	49	5
389	ccucucAuuuuAccGGAcAdTsdT	390	UfGUfCfCfGGUfAAAAUfGAGAGGdTsdT	48	4
391	uGAccAAuGAcccuAcuGdTsdT	392	CfAGUfAGGGUfCfAUfUfUfGGUfCfAdTsdT	48	6

SEQ ID NO	Sense strand sequence (5' -3')	SEQ ID NO	Antisense strand sequence (5' -3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
393	AGAucAGAccuGuuGAuAGdTsdT	394	CuAUcAAcAGGUCUGAUCUdTsdT	48	10
395	cAGGuuucAGGAACuuAcAdTsdT	396	UfGUfAAGUfUfCfCfUfGAAACfCfUfGdTsdT	47	5
397	uAGuuuuuAuucAuGcuGdTsdT	398	cAGcAUGAAuAAAAAAACuAdTsdT	47	7
399	uGuGAuGGAcuuuAuAAAdTsdT	400	UUuAuAGAAAGUCcAUcAcAdTsdT	45	4
401	uGGcGcucAAAAAAuAGAAdTsdT	402	UUCuAUUUUUUUGAGCGCcAdTsdT	45	10
403	AAcAAuAGuuuGcAAcGdTsdT	404	CGUUGcAGGAAACuAUUGUUdTsdT	44	6
405	uGAAccuGAAGuGuuAuAudTsdT	406	AuAuAAcACUUcAGGUUcAdTsdT	44	5
407	cucucAAuGGGAcuGuAuAdTsdT	408	uAuAcAGUCCcAUUGAGAGdTsdT	42	6
409	ucuGuAuGAAAAAccuuAcudTsdT	410	AGuAAGGUUUUcAuAcAGAdTsdT	41	2
411	AuGccGcuAucGAAAAuGudTsdT	412	AcAUUUUCGAuAGCGGcAUdTsdT	41	7
413	uAGuuuGcAAcGuuAccdTsdT	414	GGuAACGUUGcAGGAAACuAdTsdT	40	7
415	AAcAAucuuGGcGcucAAAdTsdT	416	UfUfUfGAGCfGcCfAAAGAUfUfGdTsdT	40	8
417	AAAccucAAuAGGucGAccdTsdT	418	GGUfCfGACfCfUfAUfUfGAGGUfUfGdTsdT	40	4
419	uuuuccAAAGGcucAGuAAAdTsdT	420	UfUfACfUfGAGCfCfUfUfUfGGAAdTsdT	38	7
421	ucucAAuGGGAcuGuAuAdTsdT	422	AuAuAcAGUCCcAUUGAGAdTsdT	38	8
423	AAcAAucuuGGcGcucAAAdTsdT	424	UUUGAGCGCcAAGAUUUGUUdTsdT	38	7
425	AAcucuuGGAAuuuAuGcAdTsdT	426	UfGcFAUfAGAAUfCfCfAAAGAUfUfGdTsdT	38	8
427	AAAAAGAAAGAuucAucGAdTsdT	428	UCGAUGAAAUUCUUCUUUUdTsdT	37	9
429	cAuAuAGAcAAuAcAAAGuGcdTsdT	430	GcACUUUGAUUGUCuAuAUGdTsdT	37	3
431	AcuuAcAccuGGAuGAccAdTsdT	432	UGGUcAUCCcAGGUGuAAGUdTsdT	34	14
433	uuuAccGGAcAcuAAAcccdTsdT	434	GGGUfUfUfAGUfGUCfCfGGUfAAAdTsdT	33	8
435	cAGGuAAAGAGAcGAAuGAdTsdT	436	UcAUUCGUCUCUUAuACCUGdTsdT	32	11
437	cccAGcAuGccGcuAucGAdTsdT	438	UfCfGAUfAGCfGGCfAUfGCUfGCGGdTsdT	31	8

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
439	cccAGcAuGccGcuAucGAdTsdT	440	UCGAuAGCGGcAUGCUGGGdTsdT	31	8
441	GGAGGAcAGAuGuAccAcudTsdT	442	AGUfGGUfAcfAUfCfUfGUfCfCfUfCfAdTsdT	30	5
443	cAuGuAcGAccAAuGuAAAdTsdT	444	UUuAcAUUGGUCCGuAcAUGdTsdT	30	4
445	uAGuuccuGcAAcGuuAccdTsdT	446	GGUfAAcGfGUfUfGcFAGGAACfUfAdTsdT	30	7
447	AAcuuAcAccuGGAuGAcdTsdT	448	GGUfCfAUfCfCfAGGUfGUfAAGUfUfAdTsdT	29	5
449	AAAcAAuAGuuccuGcAAcdTsdT	450	GUUGcAGGAACuAUUGUUdTsdT	29	11
451	uuuuAccGGAcAcuAAAccdTsdT	452	GGUfUfUfAGUfGUfCfCfGGUfAAAdTsdT	28	7
453	uGAGAGuuGGuuAcucAcAdTsdT	454	UfGUfGAGUfAAcCfAAcUfCfUfCfAdTsdT	28	7
455	AcAAuAGuuccuGcAAcGudTsdT	456	ACGUUGcAGGAACuAUUGUdTsdT	27	8
457	GGuccAcccAGGAuuAGuGdTsdT	458	CfAcUfAAUfCfCfUfGGGUfGGAcCfAdTsdT	27	7
459	uGuuAuAuGcAGGAuAuGAdTsdT	460	UcAuAUCCUGcAuAuAAcAdTsdT	27	6
461	AuGAGAccAGAuGuAAGcudTsdT	462	AGCfUfUfAcfAUfCfUfGGUfCfUfCfAUfAdTsdT	26	5
463	AccucAAuAGGucGAceAGdTsdT	464	CfUfGGUfCfGAcCfCfUfAUfUfGAGGUfAdTsdT	26	2
465	AGAAAGuuucAucGAacudTsdT	466	GAGUUCGAUGAAAUUCUdTsdT	26	7
467	AAAccucAAuAGGucGAcdTsdT	468	GGUCGACcAuAUGAGGUUdTsdT	25	7
469	uuuccAccAAuucccGuuGGdTsdT	470	CfCfAAcFggGAAUfUfGGUfGGAAAdTsdT	24	10
471	uGAccAAAuGAcceuAcuGdTsdT	472	cAGuAGGGUcAUUUGGUcAdTsdT	23	6
473	AuuccAccAAuucccGuuGdTsdT	474	cAACGGGAUUUGGUGGAUdTsdT	23	12
475	uGGuccAcccAGGAuuAGudTsdT	476	ACUfAAUfCfUfGGGUfGGAcCfAdTsdT	22	6
477	AGGAuuucAGcAGGccAcudTsdT	478	AGUGCCCUgCUGAAUuCCUdTsdT	22	8
479	AcuucccuGGucGAACaGudTsdT	480	ACUfGUfUfCfGAcCfCfAGGGAAGUfAdTsdT	22	8
481	uuuuAccGGAcAcuAAAccdTsdT	482	GGUUuAGUGUCCCGGuAAAdTsdT	20	12
483	AAAuAAcuuGcuuAAcuAcdTsdT	484	GuAGUuAAcGcAAGUuAUUUDTsdT	20	10

SEQ ID NO	Sense strand sequence (5' -3')	SEQ ID NO	Antisense strand sequence (5' -3')	Activity testing with 30nM dsRNA in HeLaS3 cells	
				mean % knock-down	standard deviation
485	AAGGcucAGuAAGcAAuGcdTsdT	486	GCfAUfUGCfUfUfACfUfGAGCfCfUfUfUfUfTsdT	16	9
487	AGGAuuucAGcAGGccAcudTsdT	488	AGUfGGCfCfUfUGCfUfGAAUfUfCfCfUfUfUfTsdT	16	8
489	cucuGAAcuuccuGGucGdTsdT	490	CGAcCAGGGAAGUUCAGAGdTsdT	15	9
491	ucAAuGGGAcuGuAuAuGGdTsdT	492	CcAuAuAcAGUCCcAUUGAdTsdT	14	8
493	AAAcuGGGcAcAGuuuAcudTsdT	494	AGuAAACUGUGCCcAGUUUdTsdT	13	12
495	AAGccucucAuuuuAccGGdTsdT	496	CfCfGGUfAAAUfGAGAGGcUfUfUfUfTsdT	9	6
497	AcuuuccuGGucGAAcAGudTsdT	498	ACUGUUUCGACcAGGGAAGUdTsdT	8	13
499	AAcuuAcAecuGGAuGAcCdTsdT	500	GGUcAUCCcAGGUGuAAGUUdTsdT	8	6
501	GGAGGAcAGAuGuAAccAcudTsdT	502	AGUGGuAcAUcUGUCCUCCdTsdT	8	8
503	GGuccAcceAGGAuuAGuGdTsdT	504	cACuAAUCCUGGGUGGACCCdTsdT	8	7
505	AAGGcucAGuAAGcAAuGcdTsdT	506	GcAUUGCUuACUGAGCCUUDTsdT	7	7
507	uuuccAccAAuuuccGuuGGdTsdT	508	CcAACGGGAUUUGGUGGAAdTsdT	7	8
509	uuuAccGGAcAcuAAAccdTsdT	510	GGGUUuAGUGUCCCGGuAAAdTsdT	1	13
511	uGGuccAcceAGGAuuAGudTsdT	512	ACuAAUCCUGGGUGGACcAdTsdT	0	15
513	AAGccucucAuuuuAccGGdTsdT	514	CCGGuAAAAUAGAGAGGCUUDTsdT	-1	12
515	AGGcuuuuucAuuAAuGGGdTsdT	516	CCcAUUuAAUGAAAAAGCCUdTsdT	-14	16

Table 2

SEQ ID NO pair	Activity testing for dose response in HeLaS3 cells - transfection 1				Activity testing for dose response in HeLaS3 cells - transfection 2			
	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]
7/8	0.003	0.047	0	87	n.d.	n.d.	n.d.	n.d.
31/32	0.004	0.09	0	89	n.d.	n.d.	n.d.	n.d.
3/4	0.005	0.072	0.001	88	n.d.	n.d.	n.d.	n.d.
25/26	0.006	0.139	0.001	91	n.d.	n.d.	n.d.	n.d.
33/34	0.008	0.114	0.001	86	n.d.	n.d.	n.d.	n.d.
83/84	0.009	0.201	0.002	84	0.0033	0.0739	0.0005	84
55/56	0.009	0.105	0.002	84	0.0055	0.0844	0.001	81
27/28	0.011	0.221	0.001	83	n.d.	n.d.	n.d.	n.d.
9/10	0.012	0.238	0.001	87	n.d.	n.d.	n.d.	n.d.
15/16	0.015	0.131	0.003	86	n.d.	n.d.	n.d.	n.d.
35/36	0.016	0.358	0.002	89	n.d.	n.d.	n.d.	n.d.
17/18	0.025	0.179	0.005	92	n.d.	n.d.	n.d.	n.d.
37/38	0.025	0.563	0.003	82	n.d.	n.d.	n.d.	n.d.
11/12	0.031	0.35	0.005	88	n.d.	n.d.	n.d.	n.d.
13/14	0.036	0.304	0.007	87	n.d.	n.d.	n.d.	n.d.
19/20	0.04	0.446	0.009	86	n.d.	n.d.	n.d.	n.d.
57/58	0.041	1'717	0.006	83	n.d.	n.d.	n.d.	n.d.
59/60	0.044	0.488	0.008	87	n.d.	n.d.	n.d.	n.d.
1/2	0.052	0.397	0.011	90	n.d.	n.d.	n.d.	n.d.
21/22	0.055	0.627	0.009	86	n.d.	n.d.	n.d.	n.d.
5/6	0.056	0.565	0.01	89	n.d.	n.d.	n.d.	n.d.
29/30	0.058	0.824	0.011	85	n.d.	n.d.	n.d.	n.d.

SEQ ID NO pair	Activity testing for dose response in HeLaS3 cells - transfection 1					Activity testing for dose response in HeLaS3 cells - transfection 2			
	mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]		mean IC50 [nM]	mean IC80 [nM]	mean IC20 [nM]	mean maximal inhibition [%]
23/24	0.06	0.798	0.011	85		n.d.	n.d.	n.d.	n.d.
61/62	0.082	0.827	0.016	87		n.d.	n.d.	n.d.	n.d.
85/86	0.083	2'072	0.017	84		n.d.	n.d.	n.d.	n.d.
83/770	n.d.	n.d.	n.d.	n.d.		0.0041	0.0889	0.0006	84
739/744	n.d.	n.d.	n.d.	n.d.		0.0047	0.0549	0.0008	85
755/760	n.d.	n.d.	n.d.	n.d.		0.0051	0.0864	0.0006	87
55/744	n.d.	n.d.	n.d.	n.d.		0.0064	0.1011	0.0009	86
747/753	n.d.	n.d.	n.d.	n.d.		0.0083	0.0895	0.0013	89
764/771	n.d.	n.d.	n.d.	n.d.		0.0087	0.2156	0.0014	83
747/752	n.d.	n.d.	n.d.	n.d.		0.0095	0.1057	0.0016	88
764/772	n.d.	n.d.	n.d.	n.d.		0.0096	0.2988	0.0015	83
767/773	n.d.	n.d.	n.d.	n.d.		0.0105	0.2057	0.0017	85
755/761	n.d.	n.d.	n.d.	n.d.		0.015	0.1494	0.0024	90
767/774	n.d.	n.d.	n.d.	n.d.		0.0268	17'741	0.0033	82

Table 3

SEQ ID NO pair	Stability Human Serum		Stability Cynomolgous Serum		Human PBMC assay	
	Sense strand	Antisense strand	Sense strand	Antisense strand	IFN-a	TNF-a
	t1/2 [hr]	t1/2 [hr]	t1/2 [hr]	t1/2 [hr]		
747/753	>48hrs	>48hrs	>48hrs	>48hrs	0	0
764/772	>48hrs	27.3	>48hrs	24.1	0	0
3/4	>24	>24	5.5	5.3	0	0
7/8	>24	>24	9.3	6.0	0	0
55/56	>24	>24	21.9	8.2	0	0
25/26	>24	13.2	5.3	4.4	0	0
83/84	>24	11.0	4.5	6.4	0	0
31/32	>24	10.7	15.0	10.0	0	0
33/34	>24	9.1	6.7	3.9	0	0

Table 4

Rank	SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
1	517	uGAACuAuGcuuGcucGuudTsdT	518	AACGAGcAAGcAuAGUUCAdTsdT	59	7
2	519	AuGAAuAcAGcAucccunudTsdT	520	AAAGGGAUGCUGuAUUCAdTsdT	58	6
3	521	uucucAGGcAGAuuccAAGdTsdT	522	CUUGGAAUCUGCCUGAGAAAdTsdT	52	6
4	523	AAcAuuAAuuuccGuGuGAdTsdT	524	UcAcACGGAAAUuAAUGUUDTsdT	52	9
5	525	GAAcAuGcuuGcucGuuudTsdT	526	AAACGAGcAAGcAuAGUUCdTsdT	51	9
6	527	uccuAGAcGcuAAcAuuAAAdTsdT	528	UuAAUGUuAGCGUCuAGGAdTsdT	51	6
7	529	uAAuGucAuuccAccAAuudTsdT	530	AAUUGGUGGAAUGAcAUuAdTsdT	50	6
8	531	uuAuuuuAccGGAcAcuAAAdTsdT	532	UuAGUGUCCGGuAAAuAAAdTsdT	50	9
9	533	AAcAuuAAuuuccGuGuGAdTsdT	534	UfcfACfACfGGAUAUfUfAAUfUfUfTsdT	50	5
10	535	AuAucAAAGAGcAuAGGAAAdTsdT	536	UUUCCuAGCUCUUUGAuAUdTsdT	50	14
11	537	GAcGcuAAcAuuAAuuuccdTsdT	538	GGAAAUuAAUGUuAGCGUCdTsdT	50	3
12	539	uuccGuGuGAAAAuGGGucdTsdT	540	GACCCAUUUUcAcACGGAAAdTsdT	49	7
13	541	GuGAAcAuGcuuGcuGudTsdT	542	ACGAGcAAGcAuAGUUCAdTsdT	47	17
14	543	AuAucAAAGAGcAuAGGAAAdTsdT	544	UfUfUfCfUfAGCfUfCfUfUfUfGAUfAUfTsdT	46	10
15	545	uccuAGAcGcuAAcAuuAAAdTsdT	546	UfUfAAUfGUfUfAGCfGUfCfUfAGGAdTsdT	46	11
16	547	uGcAuGuAuGAcAAuGuAdTsdT	548	UfACfAUfUfGGUfCfAUfACfAUfGfCfAdTsdT	45	1
17	549	cccccUGGuAGAGAcGAAAdTsdT	550	CfUfUfGGAAUfCfUfGfCfCfUfGAGAAAdTsdT	45	5
18	551	uuuAucAuGAcAuGuuAuAdTsdT	552	uAuAacAUGUcAUGAuAAAdTsdT	45	19
19	553	AAccucAAuAGGucGAccAdTsdT	554	UGGUCCGACCcAUUUGAGGUUDTsdT	44	6
20	555	uuAuccAAAGccGuuucAcdTsdT	556	GUGAAACGGCUUUGGAuAAAdTsdT	43	21

Rank	SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
21	557	uuuccGuGuGAAAAuGGGucdTsdT	558	GACfCfCAUfUfUfUfCfACfACfGGAAdTsdT	43	9
22	559	AccucAAuAGGucGAccAGdTsdT	560	CUGGUCGACCuAUUGAGGUdTsdT	43	9
23	561	GAAcuAuGcuuGcucGuuudTsdT	562	AAACfGAGCfAAGCfAUfAGUfUfCfAdTsdT	43	6
24	563	AGAcGcuAAcAuuAAuuucdTsdT	564	GAAAUfUfAAUfGUfUfAGCfGUfCfUfAdTsdT	43	8
25	565	uuuAucAuGAcAuGuuAuAdTsdT	566	UfAUfAACfAUfGUfCfAUfGAAAdTsdT	42	18
26	567	GuGAAcuAuGcuuGcucGudTsdT	568	ACfGAGCfAAGCfAUfAGUfUfCfACfAdTsdT	42	19
27	569	AGAcGcuAAcAuuAAuuucdTsdT	570	GAAAUuAUGUuAGCGUCUdTsdT	42	11
28	571	ccGGAcAcuAAAccuAAAAdTsdT	572	UfUfUfUfAGGUfUfUfAGUfGUfCfCGdTsdT	41	8
29	573	uGcAAAccucAAuAGGucGdTsdT	574	CGACCuAUUGAGGUUUGcAdTsdT	41	16
30	575	cuGAAAAcuGGAuAGGuGdTsdT	576	CfACfCfUfAUfUfCfCfAGUfUfUfCfAGdTsdT	40	3
31	577	uGuuAuAuGGuuAAAcccAdTsdT	578	UGGGUUuAACcAuAuAAcAdTsdT	38	13
32	579	uGuuAuAuGGuuAAAcccAdTsdT	580	UfGGGUfUfUfAACfCfAUfAUfAACfAdTsdT	36	2
33	581	uGGuuuAAAuGGucucAAAdTsdT	582	UfUfGAGACfCfAAUfUfUfAAACfCfAdTsdT	35	6
34	583	ccGGAcAcuAAAccuAAAAdTsdT	584	UUUuAGGUUuAGUGUCCGGdTsdT	35	6
35	585	uuAAuGucAuuccAccAAudTsdT	586	AUfUfGGUfGGAAUfGACfAUfUfAAAdTsdT	34	12
36	587	uGuAAuGGuuuAAAuGGudTsdT	588	ACfCfAAUfUfUfAAACfCfAUfUfACfAdTsdT	33	1
37	589	uGGuuuAAAuGGucucAAAdTsdT	590	UUGAGACcAAUuAAACcAdTsdT	33	7
38	591	uuuAAuuAcuGGuAGGAcAdTsdT	592	UGUCCuACcAGuAAUuAAAdTsdT	33	6
39	593	GAcGcuAAcAuuAAuuuccdTsdT	594	GGAAAUfUfAAUfGUfUfAGCfGUfCfAdTsdT	32	6
40	595	uuAuuuuAccGGAcAcuAAAdTsdT	596	UfUfAGUfGUfCfCfGGUfAAAUfAAAdTsdT	32	5
41	597	uuAuccAAAGccGuuucAcdTsdT	598	GUfGAAACfGGCfUfUfUfGGAUfAAAdTsdT	32	24
42	599	uuuAccGGAcAcuAAAccudTsdT	600	AGGUuAGUGUCCGGuAAAdTsdT	31	4
43	601	uuuAAuuAcuGGuAGGAcAdTsdT	602	UfGUfCfCfUfACfCfAGUfAAUfUfAAAdTsdT	30	5

Rank	SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
44	603	uGAACuAuGcuuGcucGuudTsdT	604	AACfGAGCfAAGCfAUfAGUfUfCfAdTsdT	29	10
45	605	GGuuuAAAuuGGucucAAAdTsdT	606	UUUGAGACcAAUuUuAAACCCdTsdT	27	4
46	607	GGuuuAAAuuGGucucAAAdTsdT	608	UfUfUfGAGACfCAUfUfUfAAACfCfdTsdT	26	1
47	609	uGcuGAuAuAAccuGuAGuudTsdT	610	AAcUAcAGGUuAUUcAGcAdTsdT	26	10
48	611	AAuGGGcAAAAGGcGAuAcdTsdT	612	GUfAUfCfGCfCfUfUfUfGcCfCfAUfUfUfTsdT	26	8
49	613	uGuAAuGGuuuAAAuGGudTsdT	614	ACcAAUuUuAAACcAUuAcAdTsdT	25	6
50	615	AuGAAuAcAGcAucccuuudTsdT	616	AAAGGGAUfGcUfUfGUfAUfUfCfAUfUfTsdT	23	4
51	617	uGuuAGucAGcAAuAAcAdTsdT	618	UfGUfAAAUfGGCfUfGACfUfAAcAdTsdT	21	8
52	619	uuAAuGucAuuccAccAAudTsdT	620	AUUGGUGGAUUGAcAUuAAAdTsdT	21	26
53	621	GuGuGGcuucAuAccGuucdTsdT	622	GAACfGUfAUfGAAGCfCfACfCfTsdT	20	4
54	623	GuGuGGcuucAuAccGuucdTsdT	624	GAACGGuAUUGAAGCcAcAcdTsdT	18	5
55	625	uGuuAGucAGcAAuAAcAdTsdT	626	UGuAAAUUGGCUGACuAAcAdTsdT	17	10
56	627	uGuGGcuucAuAccGuucdTsdT	628	GGAAACGGuAUGAAGCcAcAdTsdT	16	5
57	629	uGcuGAuAuAAccuGuAGuudTsdT	630	AACfUfACfAGGUfUfAUfUfCfAGCfAdTsdT	14	20
58	631	uuuAccGGAcAuAAccuudTsdT	632	AGGUfUfUfAGUfUfUfCfCfGGUfAAAdTsdT	14	13
59	633	cuGAAAAcuGGAuAGGuGdTsdT	634	cACCuAUUCcAGUUUcAGdTsdT	13	21
60	635	AAAccucAAuAGGucGAccdTsdT	636	GGUfCfGACfCfUfAUfUfGAGGUfUfUfTsdT	12	8
61	637	AAccucAAuAGGucGAccAdTsdT	638	UfGGUfCfGACfCfUfAUfUfGAGGUfUfUfTsdT	10	1
62	639	AGuAAAUuuAGucAGcAdTsdT	640	UfGGCfUfGACfUfAAcAUfUfUfCfUfTsdT	10	3
63	641	uGcAuGuAuGAccAAuGuAdTsdT	642	uAcAUUUGGUcAuAcAUcAdTsdT	10	26
64	643	uGcAAAccucAAuAGGucGdTsdT	644	CfGACfCfUfAUfUfGAGGUfUfUfGcAdTsdT	2	8
65	645	AAAccucAAuAGGucGAccdTsdT	646	GGUCGACCuAUUGAGGUUUUdTsdT	1	4
66	647	AGuAAAUuuAGucAGcAdTsdT	648	UGGCUGACuAAcAUuACUdTsdT	-2	11

Rank	SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	Activity testing with 50nM dsRNA in Hepa1-6 cells	
					mean % knock-down	standard deviation
67	649	ucuuAuuuuAccGGAcAcudTsdT	650	AGUGUCCGGuAAAAuAAGAdTsdT	-5	5
68	651	AccucAAuAGGucGAccAGdTsdT	652	CfUfGGUfCfGACfCfUfAUfUGAGGUfIdTsdT	-6	12
69	653	AAAuGGGcAAAGGcGAuAcdTsdT	654	GuAUCGCCUUUGCCcAUUUdTsdT	-7	11
70	655	uGuGGcuucAuAccGuuccdTsdT	656	GGAAcFGGUfAUfGAAGCfCfACfAdTsdT	-14	3
71	657	ucuuAuuuuAccGGAcAcudTsdT	658	AGUfGUfCfCfGGUfAAAUfAAGAdTsdT	-19	2

Table 5

Rank	SEQ ID NO pair	Stability Mouse Serum		Stability Rat Serum		Human PBMC assay	
		Sense strand t1/2 [hr]	Antisense strand t1/2 [hr]	Sense strand t1/2 [hr]	Antisense strand t1/2 [hr]	IFN-a	TNF-a
1	517/518	>24	6.3	>24	15.5	0	0
9	533/534	5.1	6	16.4	16.7	0	0
2	519/520	17.5	1.7	23.7	8	0	0

Table 6

	accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
antisense	ON	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		CDS
	OFF-1	Homo sapiens phosphoinositide-3-kinase, catalytic, gamma polypeptide (PIK3CG), mRNA	3.00	4	15 16 17 19	3UTR
	OFF-2	Homo sapiens olfactory receptor, family 7, subfamily A, member 5 (OR7A5), mRNA	3.00	4	14 17 18 19	3UTR
	OFF-3	Homo sapiens ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast) (UBE2G2), transcript variant 1, mRNA	3.00	5	1 13 14 16 19	3UTR
	OFF-4	Homo sapiens zinc finger and BTB domain containing 5 (ZBTB5), mRNA	3.00	3	14 15 17	3UTR
	OFF-5	Homo sapiens Sp4 transcription factor (SP4), mRNA	3.20	3	11 15 17	3UTR
	OFF-6	Homo sapiens ADP-ribosylarginine hydrolase (ADPRH), mRNA	3.20	3	11 14 17	3UTR
	OFF-7	Homo sapiens methyltransferase like 8 (METTL8), mRNA	3.20	4	10 14 17 19	3UTR
	OFF-8	Homo sapiens erythrocyte membrane protein band 4.1 like 4B (EPB41L4B), transcript variant 1, mRNA	3.25	4	9 14 18 19	3UTR
	OFF-9	Homo sapiens attractin-like 1 (ATRNL1), mRNA	3.50	5	1 8 12 14 19	3UTR
	OFF-10	Homo sapiens transforming growth factor beta regulator 1 (TBRG1), transcript variant 1, mRNA	3.70	5	1 8 10 15 19	3UTR
	OFF-11	Homo sapiens chromosome 14 open reading frame 151 (C14orf151), mRNA	11.00	4	1 4 15 19	3UTR
sense	OFF-12	Homo sapiens nucleoporin 133kDa (NUP133), mRNA	11.20	2	5 11	CDS
	OFF-13	Homo sapiens diacylglycerol O-acyltransferase 2-like 3 (DGAT2L3), mRNA	2	3	15 17 19	CDS
	OFF-14	Homo sapiens protocadherin 11 Y-linked (PCDH11Y), transcript variant c, mRNA	11	2	5 14	CDS

		accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
OFF-15		NM_130797.2	Homo sapiens dipeptidyl-peptidase 6 (DPP6), transcript variant 1, mRNA	11.2	3	1 6 11	CDS

Table 7

antisense	accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
ON	NM_001018077.1	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		CDS
OFF-1	NM_213607.1	Homo sapiens coiled-coil domain containing 103 (CCDC103), mRNA	3.00	3	12 13 14	3UTR
OFF-2	NM_001080485.1	Homo sapiens zinc finger protein 275 (ZNF275), mRNA	3.20	4	10 16 17 19	3UTR
OFF-3	NM_002205.2	Homo sapiens integrin, alpha 5 (fibronectin receptor, alpha polypeptide) (ITGA5), mRNA	3.40	4	10 11 14 19	3UTR
OFF-4	XM_001716748.1	PREDICTED: Homo sapiens hypothetical LOC731508 (LOC731508), mRNA	3.45	4	9 10 12 19	3UTR
OFF-5	NM_020476.2	Homo sapiens ankyrin 1, erythrocytic (ANK1), transcript variant 1, mRNA	3.45	5	1 9 11 17 19	3UTR
OFF-6	NM_001025247.1	Homo sapiens TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa (TAF5L), transcript variant 2, mRNA	3.70	4	8 10 17 19	3UTR
OFF-7	NM_001101396.1	Homo sapiens similar to cAMP-regulated phosphoprotein (LOC646227), mRNA	11.00	4	1 3 13 19	3UTR
OFF-8	NM_018667.2	Homo sapiens sphingomyelin phosphodiesterase 3, neutral membrane	11.00	3	1 2 15	3UTR

antisense	accession	description	spec. Score	num. mm	mismatch pos. from 5' end of as	region
OFF-9	NM_001080449.1	(neutral sphingomyelinase II) (SMPD3), mRNA Homo sapiens DNA replication helicase 2 homolog (yeast) (DNA2), mRNA	11.20	4	1 3 11 19	CDS
OFF-10	NM_015039.2	Homo sapiens nicotinamide nucleotide adenylyltransferase 2 (NMNAT2), transcript variant 1, mRNA	12.00	5	1 7 12 14 19	3UTR
OFF-11	NM_000520.4	Homo sapiens hexosaminidase A (alpha polypeptide) (HEXA), mRNA	12.20	4	1 5 10 18	3UTR
sense						
OFF-12	NM_133432.2	Homo sapiens titin (TTN), transcript variant novex-1, mRNA	2	4	1 13 14 19	CDS
OFF-13	NM_033210.3	Homo sapiens zinc finger protein 502 (ZNF502), mRNA	2.25	4	1 9 17 19	3UTR
OFF-14	NM_005076.2	Homo sapiens contactin 2 (axonal) (CNTN2), mRNA	2.5	3	1 8 13	CDS

Table 8

OFF-13	NM_003211.3	Homo sapiens thymine-DNA glycosylase (TDG), mRNA	2.2	4	1 10 17 19	3UTR
OFF-14	NM_002645.2	Homo sapiens phosphoinositide-3-kinase, class 2, alpha polypeptide (PIK3C2A), mRNA	11	4	mis-match	3UTR
			spec.	num.	pos.	region
antisense	accession	description	Score	mm	from 5' end of as	
ON	NM_001018077.1	Homo sapiens nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1), transcript variant 1, mRNA	0.00	0		3UTR
OFF-1	NM_006710.4	Homo sapiens COP9 constitutive photomorphogenic homolog subunit 8 (Arabidopsis) (COPS8), transcript variant 1, mRNA	3.00	4	1 12 15 17	3UTR
OFF-2	NM_194285.2	Homo sapiens SPT2, Suppressor of Ty, domain containing 1 (S. cerevisiae) (SPTY2D1), mRNA	3.00	4	1 16 17 18	3UTR
OFF-3	NM_004929.2	Homo sapiens calbindin 1, 28kDa (CALB1), mRNA	3.20	4	10 17 18 19	3UTR
OFF-4	NM_021101.3	Homo sapiens claudin 1 (CLDN1), mRNA	3.25	3	9 12 18	3UTR
OFF-5	NM_058191.3	Homo sapiens chromosome 21 open reading frame 66 (C21orf66), transcript variant 4, mRNA	3.50	4	1 8 13 18	3UTR
OFF-6	NM_130446.2	Homo sapiens kelch-like 6 (Drosophila) (KLHL6), mRNA	3.75	5	1 8 9 13 19	3UTR
OFF-7	NM_015525.2	Homo sapiens inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), mRNA	11.00	3	1 3 12	3UTR
OFF-8	NM_001080.3	Homo sapiens aldehyde dehydrogenase 5 family, member A1 (succinate-semialdehyde dehydrogenase) (ALDH5A1), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA	12.00	5	1 3 13 18 19	3UTR
OFF-9	NM_018003.2	Homo sapiens uveal autoantigen with coiled-coil domains and ankyrin repeats (UACA), transcript variant 1, mRNA	12.00	3	6 12 15	3UTR
OFF-10	NM_020346.1	Homo sapiens solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 (SLC17A6), mRNA	12.20	4	5 10 17 19	3UTR
OFF-11	NM_004969.2	Homo sapiens insulin-degrading enzyme (IDE), mRNA	12.20	5	1 6 11 14 19	3UTR
sense						
OFF-12	NM_024422.3	Homo sapiens desmocollin 2 (DSC2), transcript variant Dsc2a, mRNA	2	4	1 13 15 19	CDS

Table 9

FPL Name	Function	Sequence	SEQ ID No.
hGAP001	CE	GAATTTGCCATGGGTGGAATTTTCTCTTGAAAGAAAGT	683
hGAP002	CE	GGAGGGATCTCGCTCCTGGATTTTCTCTTGAAAGAAAGT	684
hGAP003	CE	CCCCAGCCTTCTCCATGGTTTCTCTTGAAAGAAAGT	685
hGAP004	CE	GCTCCCCCTGCAATGAGTTTCTCTTGAAAGAAAGT	686
hGAP005	LE	AGCCTTGACGGTGCCATGTTTTAGGCATAGGACCCGTGTCT	687
hGAP006	LE	GATGACAAAGCTTCCCCTCTCTTTTAGGCATAGGACCCGTGTCT	688
hGAP007	LE	AGATGGTGATGGGATTTCCATTTTTAGGCATAGGACCCGTGTCT	689
hGAP008	LE	GCATCGCCCCACTTGATTTTTAGGCATAGGACCCGTGTCT	690
hGAP009	LE	CACGACGTACTCAGCGCCATTTTAGGCATAGGACCCGTGTCT	691
hGAP010	LE	GGCAGAGATGATGACCCCTTTTGTTTTAGGCATAGGACCCGTGTCT	692
hGAP011	BL	GGTGAAGACGCCAGTGGACTC	693

Table 10

FPL Name	Function	Sequence	SEQ ID No.
hGeR3001	CE	TCCCATGCTAATTATCCAGCACCTTTTCTCTTGAAAGAAAGT	694
hGeR3002	CE	TGGCATGCCCAGAGCTCATTTTCTCTTGAAAGAAAGT	695
hGeR3003	CE	GGAGCGTGGCTTCCCTTCATTTTCTCTTGAAAGAAAGT	696
hGeR3004	CE	CCCTGCCCTCTGAATCTGAAGTTTCTCTTGAAAGAAAGT	697
hGeR3005	CE	CCTCCTTACACTTTTATTCCCTTCTTTTCTCTTGAAAGAAAGT	698
hGeR3006	CE	TTTTCTAGAGAGAGCAAAATCCTTTTCTCTTGAAAGAAAGT	699
hGeR3007	CE	GAGGGTATTTTCATACAGCCTTCTTTTCTCTTGAAAGAAAGT	700
hGeR3008	LE	TTTCATAGACACAAATCATGTAGTTTCTTTTAGGCATAGGACCCGTGTCT	701
hGeR3009	LE	TCCATGGTGATGATGTTTTCAGGTTTTCAGGCATAGGACCCGTGTCT	702
hGeR3010	LE	ACAAAAACACATTTCACCTACAGCTACTTTTAGGCATAGGACCCGTGTCT	703

FPL Name	Function	Sequence	SEQ ID No.
hGeR3011	LE	TGACACTAAACCAGACACACACTTTTTAGGCATAGGACCCGTGTCT	704
hGeR3012	LE	AATCTATATGTAGTTAAGCAAGTTATTGAGTTTTAGGCATAGGACCCGTGTCT	705
hGeR3013	BL	GACTTAGGTGAACCTGGAATTGCT	706
hGeR3014	BL	GTTTTTAAAGGGAACATAAAATTATGA	707
hGeR3015	BL	GATCAATGTATTGTATAACAATAATTTTCAT	708

Table 11

FPL Name	Function	Sequence	SEQ ID No.
mmNR3C1 001	CE	ATCTGGTCTCATTCACAGGGCTTTTCTCTTGGAAGAAAGT	709
mmNR3C1 002	CE	CAGGCAGAGTTTGGGAGGTGGTTTTTCTCTTGGAAGAAAGT	710
mmNR3C1 003	CE	TTCCAGGTTCAATCCAGCTTGTTTTCTCTTGGAAGAAAGT	711
mmNR3C1 004	CE	TTTTTTCTTCGTTTTTCGAGCTTTTCTCTTGGAAGAAAGT	712
mmNR3C1 005	CE	AGTGGCTTGCTGAATCCCTTAATTTTCTCTTGGAAGAAAGT	713
mmNR3C1 006	CE	GGAACTATTGTTTTGTAGCGTTTCTTTTCTCTTGGAAGAAAGT	714
mmNR3C1 007	LE	TCCCGTTGCTGTGGAGGATTTTAGGCATAGGACCCGTGTCT	715
mmNR3C1 008	LE	CCGAAGCTTCATCGGAGCACACTTTTAGGCATAGGACCCGTGTCT	716
mmNR3C1 009	LE	CAGCACCCCATATGGCATCTTTTAGGCATAGGACCCGTGTCT	717
mmNR3C1 010	LE	TCCAGCACAAAGGTAATTGTGCTTTTAGGCATAGGACCCGTGTCT	718
mmNR3C1 011	LE	TTTTATCAATGATGCAATCATTTCTTTTAGGCATAGGACCCGTGTCT	719
mmNR3C1 012	LE	AAGACATTTTCGATAGCGGCAATTTTAGGCATAGGACCCGTGTCT	720
mmNR3C1 013	BL	GCTGGACGGAGGAACTCAC	721
mmNR3C1 014	BL	GAAGACTTTACAGCTTCCACACGT	722
mmNR3C1 015	BL	TGTCCTTCCACTGCTCTTTTAA	723
mmNR3C1 016	BL	TGCTGGACAGTTTTTTCTTCGAA	724
mmNR3C1 017	BL	AGAAAGTGCTTGTGAGACTCCTGC	725

Table 12

FPL Name	Function	Sequence	SEQ ID No.
mGAP001	CE	CAATGGCAGCCCTGGTGATTTTCTCTTGGAAGAAAGT	726
mGAP002	CE	CCTTGACTGTGCCGTTGAATTTTCTCTTGGAAGAAAGT	727
mGAP003	CE	GTCTCGCTCCTGGAAGATGGTTTCTCTTGGAAGAAAGT	728
mGAP004	CE	CCCGGCTTCTCCATGGTTTCTCTTGGAAGAAAGT	729
mGAP005	LE	AACAATCTCCACTTTGCCACTGTTTTAGGCATAGGACCCGTGTCT	730
mGAP006	LE	CATGTAGACCATGTAGTTGAGGTCAATTTTAGGCATAGGACCCGTGTCT	731
mGAP007	LE	GACAAGCTTCCCATTCTCGGTTTTAGGCATAGGACCCGTGTCT	732
mGAP008	LE	TGATGGGCTTCCCCTTGATTTTLAGGCATAGGACCCGTGTCT	733
mGAP009	LE	GACATACTCAGCACCGGCCTTTTLAGGCATAGGACCCGTGTCT	734
mGAP010	BL	TGAAGGGTCGTTGATGGC	735
mGAP011	BL	CCGTGAGTGGAGTCATACTGGAA	736
mGAP012	BL	CACCCCATTTGATGTTAGTGGG	737
mGAP013	BL	GGTGAAGACACCAGTAGACTCCAC	738

Table 13

SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')
775	UGCAAACCCUCAAUAGGUCG	776	CGACCUAUUGAGGUUUUGCA
777	AAACCUCAAUAGGUCGACC	778	GGUCGACCUAUUGAGGUUU
779	AACCUCAAUAGGUCGACCA	780	UGGUCGACCUAUUGAGGUU
781	ACCUCAAUAGGUCGACCAG	782	CUGGUCGACCUAUUGAGGU
783	UUAUUGUCAUUCACCAAU	784	AUUGGUGGAUGACAUAUA
785	UGUGAUGGACUUCUAUAAA	786	UUUAUAGAAAGUCCAUACA
787	CCAAGCAGCGAAGACUUUU	788	AAAAGUCUUCGCGCUUGG
789	UUUCCAAAAGGCUCAGUAA	790	UUACUGAGCCUUUUUGGAAA
791	AAGGCUCAGUAAAGCAAUGC	792	GCAUUUGCUUACUGAGCCUU
793	GGCUCAGUAAAGCAAUGCGC	794	GCGCAUUGCUUACUGAGCC
795	CUCAGUAAAGCAAUGCGCAG	796	CUGCGCAUUGCUUACUGAG
797	CUCUCAUUGGACUGUAUA	798	UAUACAGUCCCAUUGAGAG
799	UCUCAUUGGACUGUAUAU	800	AUAUACAGUCCCAUUGAGA
801	UCAUUGGACUGUAUAUGG	802	CCAUUAACAGUCCCAUUGA
803	UGGGAAUAGACCUGGGAUU	804	AAUCCAGGUCAUUUCCCA
805	AGCAUUGCAAACCUCAUA	806	UAUUGAGGUUUGCAAUGCU
807	UUUGACAUUUUGCAGGAUU	808	AAUCCUGCAAUAUUGUCAAA
809	CCCAGGUAAGAGACGAAU	810	AUUCGUCUCUUUACCUGG
811	CCAGGUAAGAGACGAAUG	812	CAUUCGUCUCUUUACCUGG
813	CAGGUAAAGAGACGAAUGA	814	UCAUUCGUCUCUUUACCUG
815	AGACGAAUGAGAGUCCUUG	816	CAAGGACUCUCAUUCGUCU

817	AGAU	CAGACCUGUU	GAUAG			CUAUC	AACAGGUCUG	AUCU
819	UCAG	ACCUGUUG	AUAGAUG			CAUCU	AUCAAACAGGUCUGA	
821	ACGA	UUCAUUCCUUU	UGGA		818	UCCAA	AAGGAUUGAAUUCGU	
823	AAGC	UCUAUUU	ACCGG		820	CCGU	AAAUGAGAGGCUU	
825	AGCC	UCUAUUU	ACCGGA		822	UCCGG	UAAAUGAGAGGCU	
827	GCCU	CUCAUUU	ACCGGAC		824	GUCCG	GUAAAUGAGAGGC	
829	CCUC	CUCAUUU	ACCGGACA		826	UGUCC	GGUAAAUGAGAGG	
831	UCAU	UUUACCGGACACU	AA		828	UUAGU	GUCCGGUAAAUGAGAGGCU	
833	UUU	ACCGGACACU	AAACC		830	GGUUU	AGUCCGGUAAAUGAGAGG	
835	UUU	ACCGGACACU	AAACC		832	GGUUU	AGUCCGGUAAAUGA	
837	UU	ACCGGACACU	AAACCCA		834	UGGGU	UUAGUCCGGUAAAUGA	
839	U	ACCGGACACU	AAACCCAA		836	UUAGU	GUCCGGUAAAUGA	
841	AUC	UGGUUUUGU	CAAGCCC		838	GGUUU	AGUCCGGUAAAUGA	
843	AAAA	AGAAGAUUU	CAUCGA		840	UGGGU	UUAGUCCGGUAAAUGA	
845	AGA	GAUUU	CAUCGAACUC		842	GGCU	UGACAAACCAGAU	
847	AA	ACUGGCACAGU	UUACU		844	UCGA	UGAAAUUCUUCUUUU	
849	UUCU	GUUCAGUGGUGAGU			846	GAGU	UCGAUGAAUUCUUCUUUU	
851	GUUC	AUGGUGAGU	ACCUCU		848	AGU	AAACUGUGCCCAAGUUU	
853	GGAG	GACAGAU	GUACCCACU		850	ACUC	ACACCAUGAACAGAA	
855	CAGC	AUCCCUUUCU	CAACA		852	AGUA	ACUCACACCAUGAAC	
857	AGGA	UCAGAAGCCU	AUUUU		854	AGUG	GUACAUUCUCCUCC	
859	AU	CCACCAAUU	CCCCGUUG		856	UGU	UGAGAAAGGGAUGCUG	
861	U	CCACCAAUU	CCCCGUUGG		858	AAAU	AGGCUUCUGAUCCU	
863	UCC	ACCAAUU	CCCCGUUGU		860	CAAC	GGGAUUGGUGGAAU	
865	CC	ACCAAUU	CCCCGUUGGUU		862	CCAA	CGGGAUUGGUGGAA	
867	CACCA	AUCCCCGUUGGUUC			864	ACCA	ACGGGAUUGGUGGA	
869	CUCUGA	ACUCCCCUGGUCG			866	AACCA	ACGGGAUUGGUGG	
					868	GAACCA	ACGGGAUUGGUG	
					870	CGACCA	AGGAUUCAGAG	

871	ACUUCCUGGUCGAACAGU	872	ACUGUUCGACGAGGAAGU
873	UGGUCGAACAGUUUUUCU	874	AGAAAAACUGUUCGACCA
875	UUUCUAAUGGCUAUUCAAG	876	CUUGAAUAGCCAUUAGAAA
877	AUGAGACCAGAUUAAGCU	878	AGCUUACAUCUGGUCUCAU
879	CCAGAUUAAGCUCUCCUC	880	GAGGAGAGCUUACAUCUGG
881	CUGGUGGUCUCUGAUGAAG	882	CUUCAUCAGAGCACACCAG
883	GUCUUAACUUGUGGAAGCU	884	AGCUUCCACAAGUUAAGAC
885	CAUCAUCGAUAAAUAUCGA	886	UCGAAUUUUUAUCGAUGAUG
887	CCCAGCAUGCCGCUAUCGA	888	UCGAUAGCGGCAUGCUGG
889	CCAGCAUGCCGCUAUCGAA	890	UUCGAUAGCGGCAUGCUGG
891	CAGCAUGCCGCUAUCGAAA	892	UUUCGAUAGCGGCAUGCUG
893	AGCAUGCCGCUAUCGAAAA	894	UUUUCGAUAGCGGCAUGC
895	AUGCCGCUAUCGAAAUAUGU	896	ACAUUUCGAUAGCGGCAU
897	CCGCUAUCGAAAUAUGUCUU	898	AAGACAUUUUUCGAUAGCGG
899	CGCUAUCGAAAUAUGUCUUC	900	GAAGACAUUUUCGAUAGCG
901	AGGAUUUCAGCAGGCCACU	902	AGUGGCCUGCCUGAAUUCU
903	AUUCAGCAGGCCACUACAG	904	CUGUAGUGGCCUGCUGAAU
905	CUACAGGAGUCUCACAAGA	906	UCUUGUGAGACUCCUGUAG
907	AAAACAAUAGUUCUCCUGCAA	908	UUGCAGGAACUAUUUGUUU
909	AAACAAUAGUUCUCCUGCAAC	910	GUUGCAGGAACUAUUUGUU
911	AACAUAUAGUUCUCCUGCAACG	912	CGUUGCAGGAACUAUUUGU
913	ACAAUAGUUCUCCUGCAACGU	914	ACGUUGCAGGAACUAUUUGU
915	AUAGUUCUCCUGCAACGUUAC	916	GUAAACGUUGCAGGAACUAU
917	UAGUUCUCCUGCAACGUUACC	918	GGUAAACGUUGCAGGAACUA
919	CUGCAACGUUACCAACAACU	920	AGUUGUGGUAAACGUUGCAG
921	UGCAACGUUACCAACAACUC	922	GAGUUGUGGUAAACGUUGCA
923	UGAACCUUGAAGUGUUAUAU	924	AUAUAACACUUCAGGUUCA

925	UGUUUAUUGCAGGAUAUGA	926	UCAUAUCCUGCAUAUAACA
927	GCUCUGUUCCAGACUCAAC	928	GUUGAGUCUGGAACAGAGC
929	GUUCCAGACUCAACUUGGA	930	UCCAAGUUUGAGUCUGGAAC
931	CUCAACUUGGAGGAUCAUG	932	CAUGAUCCUCCAAAGUUGAG
933	ACGCUCAACAUGUUAGGAG	934	CUCCUAAACAUGUUGAGGGU
935	GGCGGGCAAGUGAUUGCAG	936	CUGCAUACACUUGCCGCC
937	CAGGUUUCAGGAACUUACA	938	UGUAAGUUCCUGAAACCUG
939	GGUUUCAGGAACUUACACC	940	GGUGUAAGUUCCUGAAACC
941	AACUUACACCUGGAUGACC	942	GGUCAUCCAGGUGUAAGUU
943	ACUACACCUGGAUGACCA	944	UGGUCAUCCAGGUGUAAGU
945	UGACCAAAUGACCCUACUG	946	CAGUAGGGUCAUUUGGUCA
947	GGUGGAGAUCAUAUAGAC	948	GUCUAUAUGAUCUCCACCC
949	GGUGGAGAUCAUAUAGACA	950	UGUCUAUAUGAUCUCCACC
951	GAGAUCAUAUAGACAAUCA	952	UGAUUGUCUAUAUGAUCUC
953	CAUAUAGACAAUCAAGUGC	954	GCACUUGAUUGUCUAUAUG
955	CAUGUACGACCA AUGUAAA	956	UUUACAUAUGGUCGUACAUG
957	AUGUACGACCA AUGUAAAC	958	GUUUACAUAUGGUCGUACA
959	UGUACGACCA AUGUAAACA	960	UGUUUACAUAUGGUCGUACA
961	CAGGCUUCAGGUUAUCUUAU	962	AUAAGAUACCUGAAGCCUG
963	UCUGUAUGAAAACCUUACU	964	AGUAAAGGUUUUACAUAACAG
965	CUGUAUGAAAACCUUACUG	966	CAGUAAGGUUUUACAUAAC
967	GU AUGAAAACCUUACUGCU	968	AGCAGUAAGGUUUUACAUAAC
969	GAAAUUAGAAUGACCUACA	970	UGUAGGUCAUUCUAUUUC
971	GAACUGGCAGCGGUUUUAU	972	AUAAAACCGCUGCCAGUUC
973	ACUGGCAGCGGUUUUAUCA	974	UGAUAAAACCGCUGCCAGU
975	AACUCUUGGAUUCUAUGCA	976	UGCAUAGAAUCCAAGAGUU
977	CACACAUAUAUCUGAUUUU	978	AAAUCAGAUUAUGUGUG

979	UCCCAACAUCUUGGCGCU	980	AGCGCCAAAGAUUUGUUGGA
981	CCCAACAUCUUGGCGCUC	982	GAGCGCCAAGAUUGUUGGG
983	CCAACAUCUUGGCGCUCA	984	UGAGCGCCAAGAUUGUUGG
985	AACAUCUUGGCGCUCAAA	986	UUUGAGCGCCAAAGAUUGUU
987	UUGGCGCUCAAAAAUAGA	988	UCUAUUUUUUUGAGCGCCAA
989	UGGCGCUCAAAAAUAGAA	990	UUCUAUUUUUUUGAGCGCCA
991	AGGCUUUUCAUUAUUGGG	992	CCCAUUUAUGAAAAAGCCU
993	UCCUAUGUAUGUGUUAUCU	994	AGAUAAACACAUAACAUAGGA
995	CCUAUGUAUGUGUUAUCUG	996	CAGAUAAACACAUAACAUAGG
997	CAGUGAGAGUUGGUUACUC	998	GAGUAAACCAACUCUCACUG
999	AGUGAGAGUUGGUUACUCA	1000	UGAGUAAACCAACUCUCACU
1001	GUGAGAGUUGGUUACUCAC	1002	GUGAGUAAACCAACUCUCAC
1003	UGAGAGUUGGUUACUCACA	1004	UGUGAGUAAACCAACUCUCA
1005	UGGUCCACCCAGGAUUAGU	1006	ACUAAUCCUGGGUGGACCA
1007	GGUCCACCCAGGAUUAGUG	1008	CACUAAUCCUGGGUGGACC
1009	UAGUGACCCAGGUUUUCAGG	1010	CCUGAAAAACCUUGGUCACUA
1011	GGCUGUAUGAAAAUACCCU	1012	AGGGUAUUUUUCAUACAGCC
1013	CUGUAUGAAAAUACCCUCC	1014	GGAGGGUAUUUUUCAUACAG
1015	AUACCCUCCUCAAAUAAAU	1016	AGUUUUUUUGAGGAGGGUAU
1017	AAUAACUUGCUUAACUAC	1018	GUAGUUAAGCAAGUUAUUU
1019	AAUAACUUGCUUAACUACA	1020	UGUAGUUAAAGCAAGUUAUU
1021	UUGCUUAACUACAUUAAGA	1022	UCUAUAUGUAGUUAAGCAA
1023	UGCUIAACUACAUUAAGAU	1024	AUCUAUAUGUAGUUAAGCA
1025	UAGUUUUUAUUCUAGCUG	1026	CAGCAUGAAUAAAAACUA
1027	CAUGCUGAAUAAUUAUCUG	1028	CAGAUUAUUAUUCAGCAUG
1029	ACUGUAAACCUUGUGUGG	1030	CCACACAAGGUUUUACAGU
1031	UGCUGUUCUGGUUAUUAACCA	1032	UGGUAAUACCAAGAACAGCA

1033	UGGUCGAACAGUUUUUCC	874	AGAAAAACUGUUCGACCA	
1034	UGGUCGAACAGUUUUUCC	874	AGAAAAACUGUUCGACCA	
1035	GUUCCAGACUCAACUUGC	930	UCCAAGUUUGAGUCUGGAAC	
1036	GUUCCAGACUCAACUUGGU	930	UCCAAGUUUGAGUCUGGAAC	

Table 14

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
955	CAUGUACGACCAUG UAAA	956	UUUACAUUGGUC GUACAUG	1	cAuGuAcGAccAAuGuAA AdTsdT	UfUfUfACfAUfUfGGUfCf GUfACfAUfGdTsdT
1021	UUGC UUAA CUACAUA UAGA	1022	UCUAUAUGUAGU UAAGCAA	3	uuGcuuAAcuAcAuAuAG AdTsdT	UfCfUfAUfAUfGUfAGUfU fAAGCfAAAdTsdT
1017	AAAUAAACUUGCUUAA CUAC	1018	GUAGUUAAAGCAA GUUAUUU	5	AAAuAAcuuGcuuAAcuA cdTsdT	GUfAGUfUfAAAGCfAAAGUf UfAUfUfUfUfTsdT
1023	UGCUUAAACUACAUAU AGAU	1024	AUCUAUAUGUAG UUAAGCA	7	uGcuuAAcuAcAuAuAGA udTsdT	AUfCfUfAUfAUfGUfAGUf UfAAGCfAdTsdT
967	GUAUGAAAACCUUAC UGCU	968	AGCAGUAAAGGUU UUCAUAC	9	GuAuGAAAAccuuAcuGc udTsdT	AGCfAGUfAAAGGUfUfUfU fCfAUfACfUfGdTsdT
997	CAGUGAGAGUUGGUU ACUC	998	GAGUAACCAACU CUCACUG	11	cAGuGAGAGuuGGuuAc ucdTsdT	GAGUfAAACfCfAAACfUfCf UfCfACfUfGdTsdT
947	GGUGGAGAUCAUAU AGAC	948	GUCUAUAUGAUC UCCACCC	13	GGGuGGAGAUcAuAuA GAcdTsdT	GUcCuAuAUGAUcUcAcAC CCdTsdT
947	GGUGGAGAUCAUAU AGAC	948	GUCUAUAUGAUC UCCACCC	15	GGGuGGAGAUcAuAuA GAcdTsdT	GUfCfUfAUfAUfGUfAGUfU fCfCfACfCfCfUfTsdT
997	CAGUGAGAGUUGGUU ACUC	998	GAGUAACCAACU CUCACUG	17	cAGuGAGAGuuGGuuAc ucdTsdT	GAGuAAACcAAACUcUcAc UGdTsdT
953	CAUAUAGACAUAUCAA GUGC	954	GCACUUGAUUUGU CUUAUUG	19	cAuAuAGAcAAucAAAGu GcdTsdT	GCfACfUfUfGUfAUfUfGUfC fUfAUfAUfGdTsdT
995	CCUAUGUAUGUGUUA UCUG	996	CAGAUAAACACAU ACAUAGG	21	ccuAuGuAuGuGuuAucuG dTsdT	CfAGAUfAAACfACfAUfAC fAUfAGGdTsdT
783	UUAAUGUCAUCCAC	784	AUUGGUGGAU	23	uuAAuGucAuuccAccAAu	AUfUfGGUfGGAAUfGACf

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	CAAU		GACAUUAA		dTsdT	AUfUfAAAdTsdT
1021	UUGC UU AACUACAUA UAGA	1022	UCUAUAUGUAGU UAAAGCAA	25	uuGcuuAAcuAcAuAuAG AdTsdT	UCuAuAUGuAGUuAAGcA AdTsdT
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	27	uGGucGAAcAGuuuuuuuu dTsdT	AGAAAAAACfUfGUfUfCf GACfCfAdTsdT
977	CACACAUUAAUCUGA UUUU	978	AAAUCAGAUUA AUGUGUG	29	cAcAcAuAAuAuAuGAAAA dTsdT	AAAAUfCfAGAUfUfAAUf GUfGUfGdTsdT
967	GUAUGAAAACCUUAC UGCU	968	AGCAGUAAAGGU UUCAUAC	31	GuAuGAAAAAccuuAcuGc udTsdT	AGcAGuAAGGUUUUcAu ACdTsdT
905	CUACAGGAGUCUCAC AAGA	906	UCUUGUGAGACU CCUGUAG	33	cuAcAGGAGucucAcAAG AdTsdT	UCUUGUGAGACUCCUGu AGdTsdT
1013	CUGUAUGAAAUAUACC CUCC	1014	GGAGGGUAUUU UCAUACAG	35	cuGuAuGAAAAuAcccucc dTsdT	GGAGGGuAUUUUcAuAc AGdTsdT
993	UCCUAUGUAUGUGUU AUCU	994	AGAUACACAUA CAUAGGA	37	uccuAuGuAuGuGuuAuCu dTsdT	AGAuAAcAcAuAcAuAGG AdTsdT
949	GGUGGAGAUCAUAUA GACA	950	UGUCUAUAUGAU CUCCACC	39	GGuGGAGAcuAuAuAG AcAdTsdT	UfGUfCfUfAUfAUfGAUfC fUfCfCfACfCfAdTsdT
957	AUGUACGACCAAUGU AAAC	958	GUUACAUAUGGU CGUACAU	41	AuGuAcGAcAAuAuGuAA AcdTsdT	GUfUfUfACfAUfUfGGUfC fGUfACfAUfAdTsdT
973	ACUGGCAGCGGUUUU AUCA	974	UGAUAAAACCGC UGCCAGU	43	AcuGGcAGcGGuuuuAuC AdTsdT	UfGAUfAAAAcCfCfGCfUf GCfCfAGUfAdTsdT
999	AGUGAGAGUUGGUUA CUCA	1000	UGAGUAAACCAAC UCUCACU	45	AGuGAGAGuuGGuuAcu cAdTsdT	UfGAGUfAACfCfAACfUf CUfCfACfUfAdTsdT
1019	AAUAAACUUGC UU AAC UACA	1020	UGUAGUUAAAGCA AGUUAUU	47	AAuAAcuuGcuuAAcuAc AdTsdT	UfGUfAGUfUfAAAGCfAAG UfUfAUfUfAdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
1001	GUGAGAGUUGGUUAC UCAC	1002	GUGAGUAACCAA CUCUCAC	49	GuGAGAGuuGGuuAcuc AdTsdT	GUfGAGUfAACfCfAACfU fCfUfCfACfAdTsdT
885	CAUCAUCGAUAAAUAU UCGA	886	UCGAAUUUUUAUC GAUGAUG	51	cAucAucGAuAAAAnuucG AdTsdT	UfCfGAAUfUfUfUfAUfCf GAUfGAUfGdTsdt
1013	CUGUAUGAAAUAUACC CUCC	1014	GGAGGUUAUUU UCAUACAG	53	cuGuAuGAAAAuAccucc dTsdT	GGAGGGUfAUfUfUfUfCf AUfACfAGdTsdt
873	UGGUCGAACAGUUUUU UUCU	874	AGAAAAAACUGU UCGACCA	55	uGGucGAAcAGuuuuuuu dTsdT	AGAAAAAACUGUUCGA CcAdTsdT
821	ACGAUUCAUUCCUUU UGGA	822	UCCAAAAGGAU GAAUCGU	57	AcGAuucAuuccuuuuGGA dTsdT	UfCfCfAAAAAGGAUfGA AUfCfGUfAdTsdT
965	CUGUAUGAAAACCUU ACUG	966	CAGUAAGGUUUU CAUACAG	59	cuGuAuGAAAAccuuAcu GdTsdt	cAGuAAGGUUUUcAuAcA GdTsdt
1001	GUGAGAGUUGGUUAC UCAC	1002	GUGAGUAACCAA CUCUCAC	61	GuGAGAGuuGGuuAcuc AdTsdT	GUGAGuAACcAACUCUc ACdTsdt
959	UGUACGACCA AUGUA AACAA	960	UGUUACA UUGG UCGUACA	63	uGuAcGAccAAuGuAAA c AdTsdT	UfGUfUfUfACfAUfUfGGU fCfGUfACfAdTsdT
839	UACCGGACACUAAAC CCAA	840	UUGGUUUUAGU GUCCGGUA	65	uAccGGAcAcuAAA cccA AdTsdT	UfUfGGGUfUfUfAGUfGUf CfcfGGUfAdTsdT
897	CCGCUAUCGAAAAUG UCUU	898	AAGACAUUUUCG AUAGCGG	67	ccGcuAucGAAAAuGucuu dTsdT	AAGACfAUfUfUfUfCfGA UfAGCfGGdTsdt
817	AGAUCAGACCUGUUG AUAG	818	CUAUC AACAGGU CUGAUCU	69	AGAucAGAccuGuuGAuA GdTsdt	CfUfAUfCfAACfAGGUfCf UfGAUfCfUfAdTsdT
993	UCCUAUGUAUGUGUU AUCU	994	AGAUACACAU CAUAGGA	71	uccuAuGuAuGuGuuAucu dTsdT	AGAUfAACfACfAUfACfA UfAGGAdTsdt
963	UCUGUAUGAAAAACCU	964	AGUAAGGUUUUC	73	ucuGuAuGAAAAccuuAcu	AGUfAAGGUfUfUfUfCfA

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	UACU		AUACAGA		dTsdT	UfACfAGAdTsdt
907	AAAACAAUAGUCCU	908	UUGCAGGAACUA	75	AAAACAAuAGuuccuGcA	UfUfGcFAGGAACfUfAUf
	GCAA		UUGUUUU		AdTsdt	UfGUfUfUfUfTsdt
883	GUCUUAACUUGUGGA	884	AGCUUCCACAAG	77	GucuuAAcuuGuGGAAGc	AGCfUfUfCfCfACfAAGUf
	AGCU		UUAAGAC		udTsdt	UfAAGACfdTsdt
913	ACAAUAGUUCCUGCA	914	ACGUUGCAGGAA	79	AcAAuAGuuccuGcAAcG	ACfGUfUfGcFAGGAACfU
	ACGU		CUAUUGU		udTsdt	fAUfUfGUfUfTsdt
991	AGGCUUUUCAUUA	992	CCCAUUUAAUGA	81	AGGcuuuuAcuuAAuAuGG	CfCfCAUfUfUfAAUfGAA
	UGGG		AAAGCCU		GdTsdt	AAGCfUfUfTsdt
929	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	83	GuuccAGAacAAcuuGG	UCcAAGUUGAGUCUGG
	UGGA		CUGGAAC		AdTsdt	AACdTsdt
957	AUGUACGACCA AUGU	958	GUUUACA UUGGU	85	AuGuAcGAccAAuGuAA	GUUuAcAUUGGUGUGuAc
	AAAC		CGUACAU		AcdTsdT	AUdTsdT
905	CUACAGGAGUCUCAC	906	UCUUGUGAGACU	87	cuAcAGGAGucucAcAAG	UfCfUfUfGUfGAGACfUfC
	AAGA		CCUGUAG		AdTsdt	fCfUfGUfAGdTsdT
959	UGUACGACCA AUGUA	960	UGUUUACA UUGG	89	uGuAcGAccAAuGuAAAc	UGUUuAcAUUGGUGUGuA
	AACA		UCGUACA		AdTsdt	cAdTsdt
857	AGGAUCAGAAGCCUA	858	AAAAUAGGCUUC	91	AGGAucAGAA GccuAuuu	AAAAUfAGGCfUfUfCfUf
	UUUU		UGAUCCU		udTsdt	GAUfCfCfUfUfTsdt
969	GAAAUUAGAAUGACC	970	UGUAGGUCAUUC	93	GAAAUuAGAAuGAccuA	UGuAGGUcAUUUCuAAUU
	UACA		UAAUUUC		cAdTsdt	UCdTsdT
849	UUCUGUUCAUGGUGU	850	ACUCACACCAUG	95	uuuGuucAuGGGuGuGAG	ACfUfCfACfACfCfAUfGA
	GAGU		AACAGAA		udTsdt	ACfAGAAAdTsdt
929	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	97	GuuccAGAcucAAcuuGG	UfCfCAAGUfUfGAGUfCf
	UGGA		CUGGAAC		AdTsdt	UfGGAACfdTsdt

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
879	CCAGAUGAAGCUCU CCUC	880	GAGGAGGCUUA CAUCUGG	99	ccAGAuGuAAGcucucc dTsdT	GAGGAGGCUuAcAUCU GGdTsdT
875	UUUCUAAUGGCUAUU CAAG	876	CUUGAAUAGCCA UUAGAAA	101	uuucuAAuGGcuAuucAA GdTsdT	CfUfGAAUfAGCfCfAUf UfAGAAAdTsdT
895	AUGCCGCUAUCGAAA AUGU	896	ACAUUUUCGAUA GCGGCAU	103	AuGccGcuAucGAAAAuG udTsdT	ACfAUfUfUfCfGAUfAG CfGGCfAUfTdTsdT
889	CCAGCAUGCCGCUAU CGAA	890	UUCGAUAGCGGC AUGCUGG	105	ccAGcAuGccGcuAucGA AdTsdT	UfUfCfGAUfAGCfGGCfA UfGcUfGGdTsdT
987	UUGGCGCUCAAAAAA UAGA	988	UCU AUUUUUUGA GCGCCAA	107	uuGGcGcucAAAAAAuA GAdTsdT	UCuAUUUUUUGAGCGCc AAdTsdT
863	UCCACCAAUUCGCCGU UGGU	864	ACCAACGGGAU UGGUGGA	109	uccAccAAuucccGnuGGud TsdT	ACfCfAAcCfGGGAAUfUfG GUfGGAdTsdT
909	AAACAAUAGUUCUG CAAC	910	GUUGCAGGAACU AUUGUUU	111	AAAcAAuAGuuccuGcAA cdTsdT	GUfUfGcFAGGAAcFfUfAU fUfGUfUfUfTdTsdT
849	UUCUGUUC AUGGUGU GAGU	850	ACUCACACCAUG AACAGAA	113	uuucuGuucAuGGGuGuGAG udTsdT	ACUcAcACcAUGAAAcAGA AdTsdT
805	AGCAUUGCAAACCUC AAUA	806	UAUUGAGGUUU GCAUUGCU	115	AGcAuuGcAAAaccucAAu AdTsdT	uAUUGAGGUUUUGcAAUG CUdTsdT
827	GCCUCUCAUUUUAACC GGAC	828	GUCCGGUAAAAU GAGAGGC	117	GccucucAuuuuAccGGAc TsdT	GUfCfCfGGUfAAAAUfGA GAGGCfTdTsdT
855	CAGCAUCCCUUUCUC AACA	856	UGUUGAGAAAG GGAUGCUG	119	cAGcAucccuuuucAcAd TsdT	UGUUGAGAAAGGGAUG CUGdTsdT
951	GAGAUCAUAUAGACA AUCA	952	UGAUUGUCUAUA UGAUCUC	121	GAGAuCAuAuAGAcAAu cAdTsdT	UGAUUGUCuAuAUGAUC UCdTsdT
1011	GGCUGUAUGAAAAUA	1012	AGGGUAUUUUUCA	123	GGcuGuAuGAAAAAuAc ccc	AGGGuAUUUUcAuAcAG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	CCCU		UACAGCC		udTsdT	CCdTsdT
821	ACGAUUCAUUCCUUU UGGA	822	UCCAAAAGGAU GAAUCGU	125	AcGAuucAuuccuuuuGGA dTsdT	UCcAAAAGGAAUGAAU CGUdTsdT
803	UGGGAAAUGACCUGG GAUU	804	AAUCCCAGGUCA UUUCCCA	127	uGGGAAAuGaccuGGGA uudTsdT	AAUCCCAGGUcAUUUUCC cAdTsdT
809	CCCAGGUAAGAGAC GAUU	810	AUUCGUCUCUUU ACCUGG	129	cccAGGuAAAGAGAcGA AudTsdT	AUfUfCfGUfCfUfUfUf ACfCfUfGGGdTsdT
855	CAGCAUCCCUUUCUC AACA	856	UGUUGAGAAAG GGAUGCUG	131	cAGcAuuccuuuucAAcAd TsdT	UfGUfUfGAGAAAGGGAU fGCfUfGdTsdT
813	CAGGUAAAAGAGACGA AUGA	814	UCAUUCGUCUCU UUACCU	133	cAGGuAAAAGAGAcGAA uGAdTsdT	UfCfAUfUfCfGUfCfUfCfUf UfUfACfCfUfGdTsdT
1019	AAUAAACUUGC UU AAC UACA	1020	UGUAGUU AAGCA AGUU AUU	135	AAuAAcuuGcuuAAcuAc AdTsdT	UGuAGUuAAGcAAGUuA UUdTsdT
965	CUGUAUGAAAACCUU ACUG	966	CAGUAAGGUUUU CAUACAG	137	cuGuAuGAAAAccuuAcu GdTsdT	CfAGUfAAGGUfUfUfUfCf AUfACfAGdTsdT
927	GCUCUGUUC CAGACU CAAC	928	GUUGAGUCUGGA ACAGAGC	139	GcuuGuuccAGAcucAAc dTsdT	GUfUfGAGUfCfUfGGAAC fAGAGCfTdtdT
793	GGCUCAGUAAGCAAU GCGC	794	GCGCAUUGCUUA CUGAGCC	141	GGcucAGuAAGcAAuGc GcdTsdT	GCfGCfAUfUfGCfUfUfACf UfGAGCfCfTdtdT
951	GAGAUCAUAUAGACA AUCA	952	UGAUUGUCUAUA UGAUCUC	143	GAGAuCAuAuAGAcAAu cAdTsdT	UfGAUfUfGUfCfUfAUfAU fGAUfCfUfCfTdtdT
857	AGGAUCAGAAAGCCUA UUUU	858	AAAUAAGGCUUC UGAUCCU	145	AGGAucAGAAAGccuAuuu udTsdT	AAAuAAGGCUUCUGAU CCUdTsdT
891	CAGCAUGCCGCUAUC GAAA	892	UUUCGAUAGCGG CAUGCUG	147	cAGcAuGccGcuAucGAA AdTsdT	UUUCGAuAGCGGcAUGC UGdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
925	UGUUAUAUGCAGGAU AUGA	926	UCAUAUCCUGCA UAUAAACA	149	uGuuAuAuGcAGGAuAu GAdTsdT	UfCfAUfAUfCfCfUfGcCfAU fAUfAACfAdTsdT
899	CGCUAUCGAAAAGU CUUC	900	GAAAGACAUUUUC GAUAGCG	151	cGcuAucGAAAAuGucuuc dTsdT	GAAAGACfAUfUfUfUfCfG AUfAGCfGdTsdT
949	GGUGGAGAUCAUAUA GACA	950	UGUCUAUAUGAU CUCCACC	153	GGuGGAGAuAuAuAG AcAdTsdT	UGUCuAuAuUGAUUCUcA CCdTsdT
987	UUGGCGCUCAAAAA UAGA	988	UCUAUUUUUUGA GCGCCAA	155	uuGGcGcucAAAAAAuA GAdTsdT	UfCfUfAUfUfUfUfUfUfGA GCfGCfCfAAdTsdT
831	UCAUUUUACCGGACA CUAA	832	UUAGUGUCCGGU AAAAUGA	157	ucAuuuuAccGGAcAcuAA dTsdT	UuAGUGUCCGGuAAAAU GAdTsdT
885	CAUCAUCGAUAAAAU UCGA	886	UCGAAUUUAUC GAUGAUG	159	cAucAucGAuAAAAuucG AdTsdT	UCGAAUuUuAUCGAUG AUGdTsdT
811	CCAGGUAAAGAGACG AAUG	812	CAUUCGUCUCU UACCUUG	161	ccAGGuAAAGAGAcGA AuGdTsdT	cAUUCGUCUCUuACC GGdTsdT
961	CAGGCUUCAGGUUAUC UUUAU	962	AUAAGAUACCU AAGCCUG	163	cAGGcuucAGGuAucuuAu dTsdT	AuAAGAuACCUGAAGCC UGdTsdT
789	UUUCCAAAAGGCUCA GUAA	790	UUACUGAGCCU UUGGAAA	165	uuuccAAAAGGcucAGuA AdTsdT	UuACUGAGCCUUUUGG AAAdTsdT
977	CACACAUUAAUCUGA UUUU	978	AAAUCAGAUUA AUGUGUG	167	cAcAcAuAAuAucGAAAA dTsdT	AAAAUcAGAUuAAUGUG UGdTsdT
1011	GGCUGUAUGAAAUA CCCU	1012	AGGUUUUUUCA UACAGCC	169	GGcuGuAuGAAAAuAcce udTsdT	AGGUfAUfUfUfUfCfAUf ACfAGCfCfTsdT
937	CAGGUUUCAGGAACU UACA	938	UGUAAUUUCCUG AAACCUG	171	cAGGuucAGGAACuuAc AdTsdT	UGuAAGUUCUGAAACC UGdTsdT
969	GAAAUUAGAAUGACC	970	UGUAGGUCAUUC	173	GAAAAuuAGAAuGaccuA	UfGUfAGGUfCfAUfUfCfU

unmodified sequence				modified sequence			
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')
	UACA		UAAUUUC		cAdTsdT		fAAUfUfUfCfdTsdT
787	CCAAGCAGCGAAGACUUUU	788	AAAAGUCUUCGCUGCUGG	175	ccAAGcAGcGAAGAcuuuudTsdT	176	AAAAGUfCfUfUfCfGcUfGcUfUfGGdTsdT
863	UCCACCAAUUCGCCGUUGGU	864	ACCAACGGGAUUGGUGA	177	uccAccAAAnucccGnuGGudTsdT	178	ACcAACGGGAUUUGGU GGAdTsdT
983	CCAACAAUCUUGGCGCUCA	984	UGAGCGCCAAAGAUUGUUGG	179	ccAAcAAucuuGGcGeucAdTsdT	180	UGAGCGCcAAGAUAUGU UGGdTsdT
795	CUCAGUAAGCAUAGCGGCAG	796	CUGCACAUAUUGCUUACUGAG	181	cucAGuAAAGcAAuGcGcAGdTsdT	182	CUGCGcAUUGCUuACUG AGdTsdT
799	UCUCAUUGGGACUGUAUAU	800	AUAUACAGUCCC AUUGAGA	183	ucucAAuGGAGAcuGuAuA udTsdT	184	AUfAUfACfAGUfCfCfCfA UfUfGAGAdTsdT
843	AAAAGAAGAUUUCACGCA	844	UCGAUGAAAUUCUUCUUUUU	185	AAAAAGAAAGAuuncAucGAdTsdT	186	UfCfGAUfGAAAUfCfUfUfCfUfUfUfUfdTsdT
971	GAACUGGCAGCGGUUUUAU	972	AUAAAACCGCUG CCAGUUC	187	GAAcuGGcAGcGGuuuuA udTsdT	188	AUfAAAAcfcfcGcUfUfGcCfCfAGUfUfCfdTsdT
927	GCUCUGUUC CAGACUCAAC	928	GUUGAGUCUGGA ACAGAGC	189	GcucuGuuccAGAcucAAc dTsdT	190	GUUGAGUCUGGAAcAG AGCdTsdT
867	CACCAAUUC CCGUUGGUUC	868	GAACCAACGGGA AUUGGUG	191	cAccAAAnucccGnuGGuucdTsdT	192	GAACfcfAACfGGGAAUfUfGGUfGdTsdT
899	CGCUAUCGAAA AUGUCUUC	900	GAA GACA UUUUC GAUAGCG	193	cGcuAucGAAAAuGucuucdTsdT	194	GAAGAcAUUUUUCGAuAG CGdTsdT
893	AGCAUGCCGCUAUCGAAAA	894	UUUUCGAUAGCG GCAUGCU	195	AGcAuGccGcuAucGAAA AdTsdT	196	UfUfUfUfCfGAUfAGCfGGCfAUfGcUfUfdTsdT
931	CUCAACUUGGAGGAUCAUG	932	CAUGAUCCUCCA AGUUGAG	197	cucAAcuuGGAGGAucAuGdTsdT	198	cAUGAUCCUCcAAGUUG AGdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
879	CCAGAUGUAGGCUCU CCUC	880	GAGGAGGCUUA CAUCUGG	199	ccAGAuGuAAGcucuccuc dTsdT	GAGGAGAGCfUfUfACfA UfCfUfGGdTsdT
999	AGUGAGAGUUGGUUA CUA	1000	UGAGUAAACCAAC UCUCACU	201	AGuGAGAGGuuGGuuAcu cAdTsdT	UGAGuAACcAACUCUcA CUdTsdT
935	GGCGGCAAGUGAUU GCAG	936	CUGCAAUCACUU GCCGCC	203	GGGcGGcAAGuGAuuGc AGdTsdT	CUGcAAUcACUUGCCGC CCdTsdT
785	UGUGAUGGACUUCUA UAAA	786	UUUAUAGAAGUC CAUCACA	205	uGuGAuGGAGcuuuAuAA AdTsdT	UfUfUfAUfAGAAAGUfCfCf AUfCfACfAdTsdT
787	CCAAGCAGCGAAGAC UUUU	788	AAAAGUCUUCGC UGCUUGG	207	ccAAGcAGcGAAGAcuuu udTsdT	AAAAGUCUUCGCUGCU UGGdTsdT
907	AAACAAUAGUUCCU GCAA	908	UUGCAGGAACUA UUGUUU	209	AAAcAAuAGuuccuGcA AdTsdT	UUGcAGGAACuAUUGUU UUDTsdT
897	CCGCUAUCGAAAAUG UCUU	898	AAGACAUUUUCG AUAGCGG	211	ccGcuAucGAAAAAuGucuu dTsdT	AAGAcAUUUUCGAuAGC GdTsdT
891	CAGCAUGCCGCUAUC GAAA	892	UUUCGAUAGCGG CAUGCUG	213	cAGcAuGccGcuAucGAA AdTsdT	UfUfUfCfGAUfAGCfGGCf AUfGCfUfGdTsdT
881	CUGGUGUGCUCUGAU GAAG	882	CUUCAUCAGAGC ACACCA	215	cuGGuGuGcucuGAuGAA GdTsdT	CfUfUfCfAUfCfAGAGCfA CfACfCfAGdTsdT
933	ACGCUCAACAUGUUA GGAG	934	CUCCUAACAUGU UGAGCGU	217	AcGcucAAcAuGuuAGGA GdTsdT	CUCCuAAcAUGUUUGAGC GUDTsdT
979	UCCCAACAUCUUUGG CGCU	980	AGCGCCAAGAUU GUUGGGA	219	ucccAAcAAucuuGGcGcu dTsdT	AGCfGCfCfAAGAUfUfGU fUfGGGAdTsdT
815	AGACGAAUGAGAGUC CUUG	816	CAAGGACUCUCA UUCGUCU	221	AGAcGAAuGAGAGuccu uGdTsdT	CfAAGGACfUfCfUfCfAUf UfCfGUfCfUfUfTsdT
839	UACCGGACACUAAAC	840	UUGGGUUUAGU	223	uAccGGAcAcuAAAAcccA	UUGGGUUuAGUGUCCG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	CCAA		GUCCGGUA		AdTsdT	GuAdTsdT
919	CUGCAACGUUACCAC AACU	920	AGUUGUGGUAAC GUUGCAG	225	cuGcAAcGuuAccAcAAcu dTsdT	AGUUGUGGuAACGUUGc AGdTsdT
889	CCAGCAUGCCCGCUAU CGAA	890	UUCGAUAGCGGC AUGCUGG	227	ccAGcAuGccGcuAucGA AdTsdT	UUCGAuAGCGGcAUGCU GGdTsdT
805	AGCAUUGCAAACCUC AAUA	806	UAUUGAGGUUU GCAUUGCU	229	AGcAuuGcAAAaccucAAu AdTsdT	UfAUfUfGAGGUfUfUfGcf AAUfGcfUfdTsdT
979	UCCCAACAUCUUGG CGCU	980	AGGCCAAGAUA GUUGGA	231	ucccAAcAAucuuGGcGcu dTsdT	AGGCcAAAGAUUGUUG GGAdTsdT
865	CCACCAAUUCCCCGUU GGUU	866	AACCAACGGGAA UUGGUGG	233	ccAccAAuucccGuuGGuud TsdT	AACcAACGGGAAUUGG UGGdTsdT
819	UCAGACCUGUUGAUA GAUG	820	CAUCUAUCAACA GGUCUGA	235	ucAGAccuGuuGAuAGAu GdTsdT	CfAUfCfUfAUfCfAACfAG GUfCfUfGAdTsdT
837	UUACCGGACACUAAA CCCA	838	UGGGUUUAGUG UCCGGUAA	237	uuAccGGAcAcuAAAccc AdTsdT	UGGGUuAGUGUCCGGu AAdTsdT
981	CCCAACAACUUGGC GCUC	982	GAGCGCCAAGAU UGUUGGG	239	cccAAcAAucuuGGcGcuc dTsdT	GAGCfGcCfAAAGAUFfG UfUfGGGdTsdT
875	UUUCUAAUGGCUAUU CAAG	876	CUUGAAUAGCCA UUAGAAA	241	uuucuAAuGGcuAuucAA GdTsdT	CUUGAAuAGCcAUuAGA AAdTsdT
783	UUAUGUCAUUCCAC CAAU	784	AUUGGUGGAU GACAUUAA	243	uuAAuGucAuuccAccAAu dTsdT	AUUGGUGGAAUGAcAUu AAdTsdT
793	GGCUCAGUAGCAAU GC GC	794	GCGCAUUGCUUA CUGAGCC	245	GGcucAGuAAGcAAuGc GcdTsdT	GCGcAUUUGCUuACUGAG CCdTsdT
883	GUCUUAACUUGUGGA AGCU	884	AGCUUCCACAAG UUAAGAC	247	GucuuAAcuuGuGGAAgC udTsdT	AGCUUCcAcAAAGUuAAG ACdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
831	UCAUUUUACCGGACA CUAA	832	UUAGUGUCCGGU AAAUGA	249	ucAuuuuAccGGAcAcuAA dTsdT	UfUfAGUfGUfCfCfGGUfA AAUfGAdTsdT
815	AGACGAAUGAGAGUC CUUG	816	CAAGGACUCUCA UUCGUCU	251	AGAcGAAuGAGAGuccu uGdTsdT	cAAGGACUCUcAUUCGU CUdTsdT
1029	ACUGUAAAACCUUGU GUGG	1030	CCACACAAAGGUU UUAACAGU	253	AcuGuAAAAccuuGuGuG GdTsdT	CfCfACfACfAAGGUfUfUf UfACfAGUfdTsdT
779	AACCUCAAUAGGUCG ACCA	780	UGGUCGACCUAU UGAGGUU	255	AAccucAAuAGGucGAcc AdTsdT	UGGUCGACCuAUUGAG GUUdTsdT
1027	CAUGCUGAAUAAUAA UCUG	1028	CAGAUUAUUAU CAGCAUG	257	cAuGcuGAAuAAuAAucu GdTsdT	CfAGAUfUfAUfUfAUfUfC fAGCfAUfGdTsdT
775	UGCAAACCUCAAUAG GUCG	776	CGACCUAUUGAG GUUUGCA	259	uGcAAAccucAAuAGGuc GdTsdT	CGACCuAUUGAGGUUU GcAdTsdT
983	CCAACAUAUCUUGGCG CUCA	984	UGAGCGCCCAAGA UUGUUGG	261	ccAAcAAucuuGGcGcucA dTsdT	UfGAGCfGCfCfAAGAUFU fGUfUfGGdTsdT
939	GGUUUCAGGAACUUA CACC	940	GGUGUAAGUUCC UGAAACC	263	GGuuucAGGAAcuuAcAc cdTsdT	GGUGuAAGUUUCCUGAA ACCDTsdT
939	GGUUUCAGGAACUUA CACC	940	GGUGUAAGUUCC UGAAACC	265	GGuuucAGGAAcuuAcAc cdTsdT	GGUGUfAAAGUfUfCfCfUf GAAACfCfdTsdT
1009	UAGUGACCAGGUUUU CAGG	1010	CCUGAAAACCUG GUCACUA	267	uAGuGAccAGGuuuucAG GdTsdT	CCUGAAAAACCUGGUcAC uAdTsdT
919	CUGCAACGUUACCAC AACU	920	AGUUGUGGUAAAC GUUGCAG	269	cuGcAAcGuuAccAcAAcu dTsdT	AGUfUfGUfGUfUfAACfGU fUfGCfAGdTsdT
893	AGCAUGCCGCUAUCG AAAA	894	UUUUCGAUAGCG GCAUGCU	271	AGcAuGccGcuAucGAAA AdTsdT	UUUUCGAuAGCGGcAUG CUdTsdT
921	UGCAACGUUACCACA	922	GAGUUGUGGUA	273	uGcAAcGuuAccAcAAcuc	GAGUUUGUGGuAACGUU

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	ACUC		ACGUUGCA		dTsdT	GcAdTsdT
923	UGAACCUAGAGUGUU AUU	924	AUAUACACUUC AGGUUCA	275	uGAACcuGAAGuGuuAu AudTsdT	AUfAUfAACfAUfUfCfA GGUfUfCfAdTsdT
867	CACCAAUUCGCCGUUG GUUC	868	GAACCAACGGGA AUUGGUG	277	cAccAAuuucccGuuGGuucd TsdT	GAACcAACCGGGAUUG GUGdTsdT
811	CCAGGUAAAGAGACG AAUG	812	CAUUCGUCUCUU UACCUGG	279	ccAGGuAAAGAGAcGA AuGdTsdT	CfAUfUfCfGUfCfUfCfUf UfACfCfUfGGdTsdT
797	CUCUCAUUGGACUG UAUA	798	UAUACAGUCCCA UUGAGAG	281	cucucAAuGGGAcuGuuAu AdTsdT	UfAUfACfAGUfCfCfCfAUf UfGAGAGdTsdT
989	UGGCGCUCAAAAAAU AGAA	990	UUCUAUUUUUG AGCGCCA	283	uGGcGeucAAAAAAuAG AAdTsdT	UfUfCfUfAUfUfUfUfUf GAGCfGCfCfAdTsdT
1015	AUACCCUCCUCAAAU AACU	1016	AGUUAUUUGAG GAGGGUUAU	285	AuAccuccucAAAuAAcu dTsdT	AGUfUfAUfUfUfGAGGAG GGUfAUfUfUfUfUfUf
935	GGCGGCAAGUGAUU GCAG	936	CUGCAAUCACUU GCCGCC	287	GGCcGGcAAGuAuGc AGdTsdT	CfUfGCfAAUfCfCfUfUfG CfCfGCfCfCfUfUfUf
1023	UGC UUAACUACAUU AGAU	1024	AUCUAUUGUAG UUAAGCA	289	uGcuuAAcuAcAuAuAGA udTsdT	AUCuAuAuGuAGUuAAGc AdTsdT
859	AUCCACCAAUUCCC GUUG	860	CAACGGGAUUUG GUGGAU	291	AuuccAccAAuuucccGuuGd TsdT	CfAACfGGGAUfUfGGUf GGAAUfUfUfUfUfUf
781	ACCUCAAUAGGUCGA CCAG	782	CUGGUCGACCUA UUGAGGU	293	AccucAAuAGGucGAccA GdTsdT	CUGGUCGACCUAUUGAG GUdTsdT
851	GUUCAUGGUGUGAGU ACCU	852	AGGUACUCACAC CAUGAAC	295	GuucAuGGGuGAGuAcc udTsdT	AGGUfACfUfCfACfCfCf AUfGAACfUfUfUfUfUf
829	CCUCUCAUUUUACCG GACA	830	UGUCCGGUAAAA UGAGAGG	297	ccucucAuuuuAccGGAcAd TsdT	UGUCCCGuAAAAUUGAG AGGdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
825	AGCCUCUCAUUUAC CGA	826	UCCGGUAAAAUG AGAGGCU	299	AGccucucAuuuuAccGGA dTsdT	UfcfcfGGUfAAAAUfGAG AGGCfUfdTsdT
801	UCAAUUGGACUGUAU AUGG	802	CCAUUAACAGUC CCAUUGA	301	ucAAuGGGAcuGuAuAu GGdTsdt	CfcfAUfAUfACfAGUfCfCf CfAUfUfGAdTsdt
961	CAGGCUUCAGGUUAUC UUAU	962	AUAGAUAACCUG AAGCCUG	303	cAGGcuucAGGuAucuuAu dTsdt	AUfAAGAUAfACfCfUfGAA GCfCfUfGdTsdt
903	AUUCAGCAGGCCACU ACAG	904	CUGUAGUGGCCU GCUGAAU	305	AuucAGcAGGccAcuAcA GdTsdt	CfUfGUfAGUfGGCfCfUfG CfUfGAAUfdTsdt
981	CCCAACAACUCUUGGC GCUC	982	GAGCGCCAAGAU UGUUGGG	307	cccAAcAAucuuGGcGcuc dTsdt	GAGCGCcAAGAUAUUGUU GGGdTsdt
877	AUGAGACCAGAUUA AGCU	878	AGCUUACAUCUG GUCUCAU	309	AuGAGAccAGAuGuAAG cudTsdt	AGCUuAcAUCUGGUCUc AUdTsdt
1027	CAUGCUGAAUAAUAA UCUG	1028	CAGAUUAUUAUU CAGCAUG	311	cAuGcuGAAuAAuAAucu GdTsdt	cAGAUAuAuAUUcAGcAU GdTsdt
973	ACUGGCAGCGGUUUU AUCA	974	UGAUAAAACCGC UGCCAGU	313	AcuGGcAGcGGuuuuAuc AdTsdt	UGAuAAAACCGCUGCcA GUdTsdt
841	AUCUGGUUUUGUCAA GCCC	842	GGCUUGACAAA ACCAGAU	315	AucuGGuuuuGucAAGccc dTsdt	GGGCfUfUfGACfAAAAcF CfAGAUfdTsdt
1003	UGAGAGUUUGGUUACU CACA	1004	UGUGAGUAAACCA ACUCUCA	317	uGAGAGuuGGuuAcucAc AdTsdt	UGUGAGuAAcCaaCUCU cAdTsdt
865	CCACCAAUUCCCCGUU GGUU	866	AACCAACGGGAA UUGGUGG	319	ccAccAAuucccGuuGGuud Tsdt	AACfCfAACfGGGAUfUf GGUfGGdTsdt
847	AAACUGGGCACAGUU UACU	848	AGUAAACUGUGC CCAGUUU	321	AAAcuGGGcAcAGuuuAc udTsdt	AGUfAAACfUfGUfGCfCf CfAGUfUfUfdTsdt
851	GUUCAUGGUGUGAGU	852	AGGUACUCACAC	323	GuucAuGGGuGuGAGuAcc	AGGuACUcAcAcCaaUGAA

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	ACCU		CAUGAAC		udTsdT	CdTsdT
1015	AUACCCUCCUCAAAU AACU	1016	AGUUAUUUGAG GAGGUUAU	325	AuAccuccucAAAUAAcu dTsdT	AGUuAUUUGAGGGGu AUdTsdT
837	UUACCCGGACACUAAA CCCA	838	UGGGUUUAGUG UCCGGUAA	327	uuAccGGAcAcuAAAcccc AdTsdT	UfGGGUfUfAGUfGUfCf CfGGUfAAAdTsdT
943	ACUUAACACCGGAUG ACCA	944	UGGUCAUCCAGG UGUAAAGU	329	AcuuAcAccuGGAUAGacc AdTsdT	UfGGUfCfAUfCfCfAGGUf GUfAAAGUfTsdT
795	CUCAGUAAGCAAUGC GCAG	796	CUGCGCAUUGCU UACUGAG	331	cucAGuAAGcAAuGcGcA GdTsdT	CfUfGCfGCfAUfUfGCfUfU fACfUfGAGdTsdT
807	UUUGACAUUUUGCAG GAUU	808	AAUCCUGCAAAA UGUCAAA	333	uuuGAcAuuuuGcAGGAu udTsdT	AAUfCfCfUfGCfAAAAUf GUfCfAAAdTsdT
819	UCAGACCUGUUUGAUA GAUG	820	CAUCUAUCAACA GGUCUGA	335	ucAGAccuGuuGAuAGAu GdTsdT	cAUCuAUcAAcAGGUCUG AdTsdT
903	AUUCAGCAGGCCACU ACAG	904	CUGUAGUGGCCU GCUGAAU	337	AuucAGcAGGccAcuAcA GdTsdT	CUGuAGUGGCCUUGCUGA AUdTsdT
915	AUAGUUCCUGCAACG UUAC	916	GUAACGUUGCAG GAACUUAU	339	AuAGuuuccuGcAAcGuuAc dTsdT	GUfAAACfGUfUfGCfAGGA ACfUfAUfTsdT
921	UGCAACGUUAACCACA ACUC	922	GAGUUUGUGUA ACGUUGCA	341	uGcAAcGuuAccAcAAAcuc dTsdT	GAGUfUfGUfGGUfAAACfG UfUfGCfAdTsdT
1025	UAGUUUUUUUUUCAU GCUG	1026	CAGCAUGAAUAA AAAACUA	343	uAGuuuuuuAuucAuGeuG dTsdT	CfAGCfAUfGAUfAAAA AACfUfAdTsdT
803	UGGGAAAUGACCCUGG GAUU	804	AAUCCCAGGUCA UUUCCCA	345	uGGGAAAUAGaccuGGGA uudTsdT	AAUfCfCfCfAGGUfCfAUf UfUfCfCfCfAdTsdT
807	UUUGACAUUUUGCAG GAUU	808	AAUCCUGCAAAA UGUCAAA	347	uuuGAcAuuuuGcAGGAu udTsdT	AAUCCUGcAAAAUUGUcA AAdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
933	ACGCUCAACAUGUUA GGAG	934	CUCCUAACAUGU UGAGCGU	349	AcGcucAAcAuGuuAGGA GdTsdT	CfUfCfCfUfAACfAUfGUfU fGAGCfGUfdTsdT
1031	UGCUGUUCUGGUAAU ACCA	1032	UGGUAUACCAG AACAGCA	351	uGcuGuucuuGGuAuAuuAccA dTsdT	UfGGUfAAUfACfCfAGAA CfAGCfAdTsdT
809	CCCAGGUAAAGAGAC GAU	810	AUUCGUCUCUUU ACCUUGG	353	cccAGGuAAAGAGAcGA AudTsdT	AUUCGUCUCUUuACCUG GGdTsdT
775	UGCAAACCCUCAAUAG GUCG	776	CGACCUAUUGAG GUUUGCA	355	uGcAAAAccucAAuAGGuc GdTsdT	CfGACfCfUfAUfUfGAGG UfUfUfGCfAdTsdT
827	GCCUCUCAUUUUUACC GGAC	828	GUCCGGUAAAAU GAGAGGC	357	GccucucAuuuuAccGGAc dTsdT	GUCCGGuAAAAUUGAGA GGCdTsdT
1031	UGCUGUUCUGGUAAU ACCA	1032	UGGUAUACCAG AACAGCA	359	uGcuGuucuuGGuAuAuuAccA dTsdT	UGGuAAuACcAGAAcAGc AdTsdT
971	GAACUGGCAGCGGUU UUU	972	AUAAAACCGCUG CCAGUUC	361	GAAcuGGcAGcGGGuuuuA udTsdT	AuAAAAACCGCUGCcAGU UCdTsdT
995	CCUAUGUAUGUGUUA UCUG	996	CAGUAACACAU ACAUAGG	363	ccuAuGuAuGuGuuAucuG dTsdT	cAGAuAAcAcAuAcAuAG GdTsdT
845	AGAGAUUUUCAUCGA ACUC	846	GAGUUCGAUGAA AUCUUCU	365	AGAAGAuuuAcuGAAAcu cdTsdT	GAGUfUfCfGAUfGAAAUf CfUfUfCfUfAdTsdT
869	CUCUGAACUUCCCCUG GUCG	870	CGACCAGGGAAG UUCAGAG	367	cucuGAAAcuucccuGGucGd TsdT	CfGACfCfAGGGAAAGUfUf CfAGAGdTsdT
881	CUGGUGGUCUCUGAU GAAG	882	CUUCAUCAGAGC ACACCA	369	cuGGuGuGcuuGGuGAA GdTsdT	CUUcAUcAGAGAcAcAcA GdTsdT
931	CUCAACUUUGGAGGAU CAUG	932	CAUGAUCCUCCA AGUUGAG	371	cucAAcuuGGAGGAucAu GdTsdT	CfAUfGAUfCfUfCfCfAA GUfUfGAGdTsdT
825	AGCCUCUCAUUUUUAC	826	UCCGGUAAAAAUG	373	AGccucucAuuuuAccGGA	UCCGGuAAAAUUGAGAG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	CGGA		AGAGGCU		dTsdT	GCUdTsdT
915	AUAGUUCCUGCAACG UUAC	916	GUAACGUUGCAG GAACUUAU	375	AuAGuuccuGcAAcGuuAc dTsdT	GuAACGUUGcAGGAACu AUdTsdT
911	AACAAUAGUUCUCCG AACG	912	CGUUGCAGGAAC UAUUGUU	377	AAcAAuAGuuccuGcAAc GdTsdT	CfGUfUGCfAGGAACfUf AUfUGUfUfTsdT
841	AUCUGGUUUUGUCA GCCC	842	GGCUUGACAAA ACCAGAU	379	AucuGGuuuuGucAAGccc dTsdT	GGGCUUGAcAAAAcAcAG AUdTsdT
1029	ACUGUAAAACCUUGU GUGG	1030	CCACACAAGGUU UUACAGU	381	AcuGuAAAAccuuGuGuG GdTsdT	CcAcAcAAGGUUUuAcAG UdTsdT
975	AACUCUUGGAUUCUA UGCA	976	UGCAUAGAAUCC AAGAGUU	383	AAcucuuGGAuucuuAuGcA dTsdT	UGcAuAGAAUCCcAAGAG UUDTsdT
1009	UAGUGACCAGGUUUU CAGG	1010	CCUGAAAACCUG GUCACUA	385	uAGuGAccAGGuuuuAcG GdTsdT	CfCfUfGAAAACfCfUfGG UfCfACfUfAdTsdT
779	AACCUCAAUAGGUUG ACCA	780	UGGUCGACCUAU UGAGGUU	387	AAccucAAuAGGucGAcc AdTsdT	UfGGUfCfGACfCfUfAUfU fGAGGUfUfTsdT
829	CCUCUCAUUUUACCG GACA	830	UGUCCGGUAAAA UGAGAGG	389	ccucucAuuuuAccGGAcAd TsdT	UfGUfCfGfGGUfAAAAUf GAGAGGdTsdT
945	UGACCAAUUGACCCU ACUG	946	CAGUAGGGUCAU UUGGUCA	391	uGAccAAAuGAcccuAcu GdTsdT	CfAGUfAGGGUfCfAUfUf UfGGUfCfAdTsdT
817	AGAUACAGACCUUGU AUAG	818	CUAUCACAGGU CUGAUCU	393	AGAuCAGAccuGuuGAuA GdTsdT	CuAUcAAcAGGUCUGAU CUdTsdT
937	CAGGUUUCAGGAACU UACA	938	UGUAAGUUCCUG AAACCUG	395	cAGGuuucAGGAACuuAc AdTsdT	UfGUfAAGUfUfCfCfUfGA AACfCfUfGdTsdT
1025	UAGUUUUUUUUAUUCAU GCUG	1026	CAGCAUGAAUAA AAAACUA	397	uAGGuuuuuuAuucAuGcuG dTsdT	cAGcAUUGAAuAAAAAAC uAdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
785	UGUGAUGGACUUCUA UAAA	786	UUUAUAGAAAGUC CAUCACA	399	uGuGAuGGAcuucuAuAA AdTsdT	UUuAuAGAAAGUCcAUcAc AdTsdT
989	UGGCGCUCAAAAAU AGAA	990	UUCUAUUUUUUG AGCGCCA	401	uGGcGcucAAAAAAuAG AAAdTsdT	UUCuAUUUUUUGAGCG CcAdTsdT
911	AACAAUAGUUCUGC AACG	912	CGUUGCAGGAAC UAUUGUU	403	AAcAAuAGuuucuGcAAc GdTsdT	CGUUGcAGGAACuAUUG UUDTsdT
923	UGAACCCUGAAGUGUU AUAU	924	AUAUACACUUC AGGUUCA	405	uGAAccuGAAGuGuuAu AudTsdT	AuAuAAcACUUcAGGUUc AdTsdT
797	CUCUCAUUGGACUG UAUA	798	UAUACAGUCCCA UUGAGAG	407	cucucAAuGGGAcuGuAu AdTsdT	uAuAcAGUCCcAUUGAG AGdTsdT
963	UCUGUAUGAAACCU UACU	964	AGUAGGUUUUC AUACAGA	409	ucuGuAuGAAAAAccuuAcu dTsdT	AGuAAGGUUUUcAuAcA GAdTsdT
895	AUGCCGCUAUCGAAA AUGU	896	ACAUUUUCGAUA GCGGCAU	411	AuGccGcuAucGAAAAuG udTsdT	AcAUUUUCGAuAGCGGc AUdTsdT
917	UAGUUCUGCAACGU UACC	918	GGUAAACGUUGCA GGAACUA	413	uAGuuucuGcAAcGuuAcc dTsdT	GGuAACGUUGcAGGAAC uAdTsdT
985	AACAACUCUUGGCGCU CAAA	986	UUUGAGCGCCAA GAUUGUU	415	AAcAAucuuGGcGcucAA AdTsdT	UfUfUGAGCfGcfcfAAG AUfUGUfUfTsdT
777	AAACCUCAAUAGGUC GACC	778	GGUCGACCUAUU GAGGUUU	417	AAAccucAAuAGGucGAc cdTsdT	GGUfCfGACfCfUfAUfUG AGGUfUfUfTsdT
789	UUUCCAAAAGGCUCA GUAA	790	UUACUGAGCCUU UUGGAAA	419	uuuccAAAAGGcucAGuA AdTsdT	UfUfACfUfGAGCfCfUfUf UfUfGGAAAdTsdT
799	UCUCAUUGGACUGU AUAU	800	AUAACAGUCCC AUUGAGA	421	ucucAAuGGGAcuGuAuA udTsdT	AuAuAcAGUCCcAUUGA GAdTsdT
985	AACAACUCUUGGCGCU	986	UUUGAGCGCCAA	423	AAcAAucuuGGcGcucAA	UUUGAGCGCcAAGAUAU

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	CAAA		GAUUGUU		AdTsdT	GUUdTsdT
975	AACUCUUGGAUUCUA UGCA	976	UGCAUAGAAUCC AAGAGUU	425	AAcuuuGGAnuucAuGcA dTsdT	UfGcFAUfAGAAUfCfCfA AGAGUfUfdTsdT
843	AAAAAGAGAUUUCUA UCGA	844	UCGAUGAAAUUCU UCUUUUU	427	AAAAAGAGAAuuucAuc GAdTsdT	UCGAUGAAAUUCUUCUU UUUdTsdT
953	CAUAUAGACAAUCAA GUGC	954	GCACUUGAUUUGU CUAU AUG	429	cAuAuAGAcAAuAcAAGu GcdTsdT	GcACUUGAUUUGUCuAuA UGdTsdT
943	ACUACACCUUGGAUG ACCA	944	UGGUCAUCCAGG UGUAAUG	431	AcuuAcAccuGGAuGAcc AdTsdT	UGGUcAUcCAGGUGuAA GUdTsdT
835	UUUACCGGACACUAA ACCC	836	GGUUUAGUGUC CGGUAAA	433	uuuAccGGAcAcuAAAacc dTsdT	GGGUfUfAGUfGUfCfCf GGUfAAAdTsdT
813	CAGGUAAAAGAGACGA AUGA	814	UCAUUCGUCUCU UUACCU	435	cAGGuAAAGAGAcGAA uGAdTsdT	UcAUUCGUCUCUUuACC UGdTsdT
887	CCCAGCAUGCCGCUA UCGA	888	UCGAUAGCGGCA UGCUGG	437	cccAGcAuGccGcuAucGA dTsdT	UfCfGAUfAGCfGGCfAUf GCfUfGGGdTsdT
887	CCCAGCAUGCCGCUA UCGA	888	UCGAUAGCGGCA UGCUGG	439	cccAGcAuGccGcuAucGA dTsdT	UCGAuAGCGGcAUGCUG GGdTsdT
853	GGAGGACAGAUGUAC CACU	854	AGUGGUACAUCU GUCCUCC	441	GGAGGAcAGAuGuAaccA cudTsdT	AGUfGGUfACfAUfCfUfG UfCfCfUfCfCfTsdT
955	CAUGUACGACCA AUG UAAA	956	UUUACA UUGGUC GUACAUG	443	cAuGuAcGAccAAuGuAA AdTsdT	UUuAcAUUGGUCGuAcA UGdTsdT
917	UAGUUCCUGCAACGU UACC	918	GGUAAACGUUGCA GGAACUA	445	uAGuuucuGcAAcGuuAcc dTsdT	GGUfAACfGUfUfGcFAGG AACfUfAdTsdT
941	AACUUACACCUUGGAU GACC	942	GGUCAUCCAGGU GUAAGUU	447	AAcuuAcAccuGGAuGAc cdTsdT	GGUfCfAUfCfCfAGGUfG UfAAGUfUfdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
909	AAACAAUAGUUCCUG CAAC	910	GUUGCAGGAACU AUUGUUU	449	AAcAAuAGuuccuGcAA cdTsdT	GUUGcAGGAACuAUUGU UUDTsdT
833	UUUACCGGACACUA AACC	834	GGUUUAGUGUCC GGUAAA	451	uuuuAccGGAcAcuAAAcc dTsdT	GGUfUfUfAGUfGUfCfG GUfAAAAdTsdT
1003	UGAGAGUUGGUUACU CACA	1004	UGUGAGUAAACCA ACUCUCA	453	uGAGAGuuGGuuAcucAc AdTsdT	UfGUfGAGUfAACfCfAAC fUfCfUfCfAdTsdT
913	ACAAUAGUUCCUGCA ACGU	914	ACGUUGCAGGAA CUAUUGU	455	AcAAuAGuuccuGcAAcG udTsdT	ACGUUGcAGGAACuAUU GUDTsdT
1007	GGUCCACCCAGGAUU AGUG	1008	CACUAAUCCUGG GUGGACC	457	GGuccAcccAGGAuuAGu GdTsdT	CfACfUfAAUfCfCfUfGGG UfGGACfCfTsdT
925	UGUUAUAUGCAGGAU AUGA	926	UCAUAUCCUGCA UAUAAAC	459	uGuuAuAuGcAGGAuAu GAdTsdT	UcAuAUCCUGcAuAuAAc AdTsdT
877	AUGAGACCAGAUGUA AGCU	878	AGCUUACAUCUG GUCUCAU	461	AuGAGAccAGAuGuAAG cudTsdT	AGCfUfUfACfAUfCfUfGG UfCfUfCfAUfTsdT
781	ACCUCAAUAGGUCGA CCAG	782	CUGGUCGACCUA UUGAGGU	463	AccucAAuAGGucGAccA GdTsdT	CfUfGGUfCfGACfCfUfAUf UfGAGGUfTsdT
845	AGAGAUAUUAUCGA ACUC	846	GAGUUCGAUGAA AUCUUCU	465	AGAAAGuuuucAucGAACu cdTsdT	GAGUUCGAUGAAAUCU UCUdTsdT
777	AAACCCUCAAUAGGUC GACC	778	GGUCGACCUAU GAGGUUU	467	AAAccucAAuAGGucGAc cdTsdT	GGUCGACCuAUUUGAGG UUUdTsdT
861	UCCACCAAUUCCCG UUGG	862	CCAACGGGAUU GGUGGAA	469	uuuccAccAAuuuccGuuGGd TsdT	CfCfAACfGGGAUuUfGG UfGGAAAdTsdT
945	UGACCAAAUGACCCU ACUG	946	CAGUAGGGUCAU UUGGUCA	471	uGAccAAAuGAcccuAcu GdTsdT	cAGuAGGGUcAUUUGGU cAdTsdT
859	AUUCACCAAUUCCC	860	CAACGGGAUUUG	473	AuuuccAccAAuuuccGuuGd	cAACGGGAUUGGUGG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	GUUG		GUGGAU		TsdT	AAUdTsdT
1005	UGGUCCACCCAGGAU UAGU	1006	ACUAAUCCUGGG UGGACCA	475	uGGuccAcccAGGAuuuAG udTsdT	ACfUfAAUfCfCfUfGGGUf GGACfCfAdTsdT
901	AGGAAUUCAGCAGGC CACU	902	AGUGGCCUGCUG AAUUCU	477	AGGAAuucAGcAGGccA cudTsdT	AGUGGCCUUGCUGAAUU CCUdTsdT
871	ACUUCCCUUGGUCGAA CAGU	872	ACUGUUCGACCA GGGAAGU	479	AcuucccuGGcGAAcAGu dTsdT	ACfUfGUfUfCfGACfCfAG GGAAGUfdTsdT
833	UUUAACCGGACACUA AACC	834	GGUUAGUGUCC GGUAAA	481	uuuuAccGGAcAcuAAAcc dTsdT	GGUUuAGUGUCCGGuAA AAdTsdT
1017	AAAUAAUUGCUUAA CUAC	1018	GUAGUUAAAGCAA GUUAUUU	483	AAAuAAcuuGcuuAAcuA cdTsdT	GuAGUuAAGcAAGUuAU UUDTsdT
791	AAGGCUCAGUAAAGCA AUGC	792	GCAUUGCUUACU GAGCCUU	485	AAGGcucAGuAAGcAAu GcdTsdT	GCfAUfUfGcUfUfACfUf GAGCfCfUfUfdTsdT
901	AGGAAUUCAGCAGGC CACU	902	AGUGGCCUGCUG AAUUCU	487	AGGAAuucAGcAGGccA cudTsdT	AGUfGGCfCfUfGcUfGAG AUfUfCfUfUfdTsdT
869	CUCUGAACUUUCCCUUG GUCG	870	CGACCCAGGGAAG UUCAGAG	489	cucuGAAcuuucccuGGucGd TsdT	CGACcAGGGAAAGUUCAG AGdTsdT
801	UCAAUGGGACUGUAU AUGG	802	CCAUAUACAGUC CCAUGA	491	ucAAuGGGAcuGuAuAu GGdTsdT	CcAuAuAcAGUCCcAUUG AdTsdT
847	AAACUGGGCACAGUU UACU	848	AGUAAACUGUGC CCAGUUU	493	AAAcuGGGcAcAGuuuAc udTsdT	AGuAAACUGUGCCcAGU UUDTsdT
823	AAGCCUCUCAUUUUA CCGG	824	CCGGUAAAUAUGA GAGGCUU	495	AAGccucucAuuuuAccGG dTsdT	CfCfGGUfAAAAUfGAGA GGCfUfUfdTsdT
871	ACUUCCCUUGGUCGAA CAGU	872	ACUGUUCGACCA GGGAAGU	497	AcuucccuGGcGAAcAGu dTsdT	ACUGUUUCGACcAGGGAA GUdTsdT

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
941	AACUUACACCUGGAU GACC	942	GGUCAUCCAGGU GUAAGUU	499	AAcuuAcAccuGGAuGAc cdTsdT	GGUcAUCcAGGUGuAAG UUDTsdT
853	GGAGGACAGAUGUAC CACU	854	AGUGGUACAUCU GUCCUCC	501	GGAGGAcAGAuGuAccA cudTsdT	AGUGGuAcAUCUGUCCU CCdTsdT
1007	GGUCCACCCAGGAUU AGUG	1008	CACUAAUCCUGG GUGGACC	503	GGuccAcccAGGAuuAGu GdTsdT	cACuAAUCCUGGGUGGA CCdTsdT
791	AAGGCUCAGUAAAGCA AUGC	792	GCAUUGCUUACU GAGCCUU	505	AAGGcucAGuAAGcAAu GcdTsdT	GcAUUGCUuACUGAGCC UUDTsdT
861	UUCACCAAUUCCCCG UUGG	862	CCAACGGGAAUU GGUGGAA	507	uuccAccAAuucccGuuGGd TsdT	CcAACGGGAUUUGGUG GAAdTsdT
835	UUUACCGGACACUAA ACCC	836	GGUUUAGUGUC CGGUAAA	509	uuuAccGGAcAcuAAAccc dTsdT	GGGUuAGUGUCCCGGuA AAdTsdT
1005	UGGUCCACCCAGGAU UAGU	1006	ACUAAUCCUGGG UGGACCA	511	uGGuccAcccAGGAuuAG udTsdT	ACuAAUCCUGGGUGGAC cAdTsdT
823	AAGCCUCUCAUUUUA CCGG	824	CCGUAAAUAUGA GAGGCUU	513	AAGccuucAuuuuAccGG dTsdT	CCGGuAAAAUUGAGAGG CUUDTsdT
991	AGGCUUUUCAUUAAA UGGG	992	CCCAUUUAUUGA AAAGCCU	515	AGGcuuuuAuAAuAGG GdTsdT	CCcAUUuAAUGAAAAAGC CUdTsdT
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	55	uGGucGAAcAGuuuuuuuu dTsdT	pAGAAAAAACUGUUUCG ACcAdTsdT
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	739	ugGucGAAcAGuuuuuuuu dTsdT	pAGAAAAAACUGUUUCG ACcAdTsdT
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	739	ugGucGAAcAGuuuuuuuu dTsdT	AGAAAAAACUGUUUCGA CcAdTsdT
1033	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	740	uGGucGAAcAGuuuuuuuu	pAGAAAAAACUGUUUCG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	UUC		UCGACCA		dTsdT	ACcAdTsdT
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	741	ugGucGAACAGuuuuuuu dTsdT	pAGAAAAAACUGUUUCG ACcAdTsdT
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	740	uGGucGAACAGuuuuuuu dTsdT	AGAAAAAACUGUUUCG CcAdTsdT
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	741	ugGucGAACAGuuuuuuu dTsdT	AGAAAAAACUGUUUCG CcAdTsdT
1034	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	742	uGGucGAACAGuuuuuuu GdTsdT	pAGAAAAAACUGUUUCG ACcAdTsdT
1034	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	743	ugGucGAACAGuuuuuuu dTsdT	pAGAAAAAACUGUUUCG ACcAdTsdT
1034	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	742	uGGucGAACAGuuuuuuu GdTsdT	AGAAAAAACUGUUUCG CcAdTsdT
1034	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	743	ugGucGAACAGuuuuuuu dTsdT	AGAAAAAACUGUUUCG CcAdTsdT
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	746	uGGucGAACAGuuuuuuu dT(inv dT)	pAGAAAAAACUGUUUCG ACcAdT(inv dT)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	747	ugGucGAACAGuuuuuuu dT(inv dT)	pAGAAAAAACUGUUUCG ACcAdT(inv dT)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	746	uGGucGAACAGuuuuuuu dT(inv dT)	AGAAAAAACUGUUUCG CcAdT(inv dT)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	747	ugGucGAACAGuuuuuuu dT(inv dT)	AGAAAAAACUGUUUCG CcAdT(inv dT)
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	748	uGGucGAACAGuuuuuuu dT(inv dT)	pAGAAAAAACUGUUUCG ACcAdT(inv dT)

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	749	ugGucGAAAcAGuuuuuuucc dT(invdT)	pAGAAAAAACUGUUUCG ACcAdT(invdT)
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	748	uGGucGAAAcAGuuuuuuucc dT(invdT)	AGAAAAAACUGUUUCG CcAdT(invdT)
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	749	ugGucGAAAcAGuuuuuuucc dT(invdT)	AGAAAAAACUGUUUCG CcAdT(invdT)
1034	UGGUCGAACAGUUUU UUCG	874	AGAAAAAACUGU UCGACCA	750	uGGucGAAAcAGuuuuuuucc GdT(invdT)	pAGAAAAAACUGUUUCG ACcAdT(invdT)
1034	UGGUCGAACAGUUUU UUCG	874	AGAAAAAACUGU UCGACCA	751	ugGucGAAAcAGuuuuuuuccG dT(invdT)	pAGAAAAAACUGUUUCG ACcAdT(invdT)
1034	UGGUCGAACAGUUUU UUCG	874	AGAAAAAACUGU UCGACCA	750	uGGucGAAAcAGuuuuuuucc GdT(invdT)	AGAAAAAACUGUUUCG CcAdT(invdT)
1034	UGGUCGAACAGUUUU UUCG	874	AGAAAAAACUGU UCGACCA	751	ugGucGAAAcAGuuuuuuuccG dT(invdT)	AGAAAAAACUGUUUCG CcAdT(invdT)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	754	uGGucGAAAcAGuuuuuuuccu dT(abasic)	pAGAAAAAACUGUUUCG ACcAdT(abasic)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	755	ugGucGAAAcAGuuuuuuuccu dT(abasic)	pAGAAAAAACUGUUUCG ACcAdT(abasic)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	754	uGGucGAAAcAGuuuuuuuccu dT(abasic)	AGAAAAAACUGUUUCG CcAdT(abasic)
873	UGGUCGAACAGUUUU UUCU	874	AGAAAAAACUGU UCGACCA	755	ugGucGAAAcAGuuuuuuuccu dT(abasic)	AGAAAAAACUGUUUCG CcAdT(abasic)
1033	UGGUCGAACAGUUUU UUC	874	AGAAAAAACUGU UCGACCA	756	uGGucGAAAcAGuuuuuuucc dT(abasic)	pAGAAAAAACUGUUUCG ACcAdT(abasic)
1033	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	757	ugGucGAAAcAGuuuuuuucc	pAGAAAAAACUGUUUCG

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
	UUC		UCGACCA		dT(abasic)	ACcAdT(abasic)
1033	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	756	uGGucGAACAcAGuuuuuucc	AGAAAAAACUGUUCGA
1033	UUC		UCGACCA		dT(abasic)	CcAdT(abasic)
1033	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	757	ugGucGAACAcAGuuuuuucc	AGAAAAAACUGUUCGA
1033	UUC		UCGACCA		dT(abasic)	CcAdT(abasic)
1034	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	758	ugGucGAACAcAGuuuuuucc	pAGAAAAAACUGUUCG
1034	UUC		UCGACCA		dT(abasic)	ACcAdT(abasic)
1034	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	759	uGGucGAACAcAGuuuuuucc	AGAAAAAACUGUUCGA
1034	UUC		UCGACCA		GdT(abasic)	CcAdT(abasic)
1034	UGGUCGAACAGUUUU	874	AGAAAAAACUGU	758	ugGucGAACAcAGuuuuuucc	AGAAAAAACUGUUCGA
1034	UUC		UCGACCA		dT(abasic)	CcAdT(abasic)
929	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	83	GuuccAGAcucAAcuuGG	pUCcAAGUUUGAGUCUGG
929	UGG		CUGGAAC		AdTsdT	AACdTsdT
1035	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	762	GuuccAGAcucAAcuuGGc	pUCcAAGUUUGAGUCUGG
1035	UGG		CUGGAAC		dTsdT	AACdTsdT
1035	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	762	GuuccAGAcucAAcuuGGc	UCcAAGUUUGAGUCUGG
1035	UGG		CUGGAAC		dTsdT	AACdTsdT
1036	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	763	GuuccAGAcucAAcuuGGu	pUCcAAGUUUGAGUCUGG
1036	UGG		CUGGAAC		dTsdT	AACdTsdT
1036	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	763	GuuccAGAcucAAcuuGGu	UCcAAGUUUGAGUCUGG
1036	UGG		CUGGAAC		dTsdT	AACdTsdT
929	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	764	GuuccAGAcucAAcuuGG	pUCcAAGUUUGAGUCUGG
929	UGG		CUGGAAC		AdT(invdt)	AACdT(invdt)
929	GUUCCAGACUCAACU	930	UCCAAGUUUGAGU	764	GuuccAGAcucAAcuuGG	UCcAAGUUUGAGUCUGG
929	UGG		CUGGAAC		AdT(invdt)	AACdT(invdt)

unmodified sequence				modified sequence		
SEQ ID NO	Sense strand sequence (5'-3')	SEQ ID NO	Antisense strand sequence (5'-3')	SEQ ID NO	Sense strand sequence (5'-3')	Antisense strand sequence (5'-3')
1035	GUUCCAGACUCUAAACU UGGC	930	UCCAAGUUUGAGU CUGGAAC	765	GuuccAGAcucAAcuuGGc dT(invvdT)	pUCcAAGUUGAGUCUGG AACdT(invvdT)
1035	GUUCCAGACUCUAAACU UGGC	930	UCCAAGUUUGAGU CUGGAAC	765	GuuccAGAcucAAcuuGGc dT(invvdT)	UCcAAGUUGAGUCUGG AACdT(invvdT)
1036	GUUCCAGACUCUAAACU UGGU	930	UCCAAGUUUGAGU CUGGAAC	766	GuuccAGAcucAAcuuGGu dT(invvdT)	pUCcAAGUUGAGUCUGG AACdT(invvdT)
1036	GUUCCAGACUCUAAACU UGGU	930	UCCAAGUUUGAGU CUGGAAC	766	GuuccAGAcucAAcuuGGu dT(invvdT)	UCcAAGUUGAGUCUGG AACdT(invvdT)
929	GUUCCAGACUCUAAACU UGGA	930	UCCAAGUUUGAGU CUGGAAC	767	GuuccAGAcucAAcuuGG AdT(abasic)	pUCcAAGUUGAGUCUGG AACdT(abasic)
929	GUUCCAGACUCUAAACU UGGA	930	UCCAAGUUUGAGU CUGGAAC	767	GuuccAGAcucAAcuuGG AdT(abasic)	UCcAAGUUGAGUCUGG AACdT(abasic)
1035	GUUCCAGACUCUAAACU UGGC	930	UCCAAGUUUGAGU CUGGAAC	768	GuuccAGAcucAAcuuGGc dT(abasic)	pUCcAAGUUGAGUCUGG AACdT(abasic)
1035	GUUCCAGACUCUAAACU UGGC	930	UCCAAGUUUGAGU CUGGAAC	768	GuuccAGAcucAAcuuGGc dT(abasic)	UCcAAGUUGAGUCUGG AACdT(abasic)
1036	GUUCCAGACUCUAAACU UGGU	930	UCCAAGUUUGAGU CUGGAAC	769	GuuccAGAcucAAcuuGGu dT(abasic)	pUCcAAGUUGAGUCUGG AACdT(abasic)
1036	GUUCCAGACUCUAAACU UGGU	930	UCCAAGUUUGAGU CUGGAAC	769	GuuccAGAcucAAcuuGGu dT(abasic)	UCcAAGUUGAGUCUGG AACdT(abasic)

Claims

1. A double-stranded ribonucleic acid molecule capable of inhibiting the expression of GCR gene in vitro by at least 70 %, preferably by at least 80% and most preferably by at least 90%.
5
2. The double-stranded ribonucleic acid molecule of claim 1, wherein said double-stranded ribonucleic acid molecule comprises a sense strand and an antisense strand, the antisense strand being at least partially complementary to the sense strand, whereby the sense strand comprises a sequence, which has an identity of at least 90 % to at least a portion of
10 an mRNA encoding GCR, wherein said sequence is (i) located in the region of complementarity of said sense strand to said antisense strand; and (ii) wherein said sequence is less than 30 nucleotides in length.
3. The double-stranded ribonucleic acid molecule of claims 1 to 2, wherein said sense
15 strand comprises nucleotide acid sequences depicted in SEQ ID Nos: 873, 929, 1021, 1023, 967 and 905, and said antisense strand comprises nucleic acid sequences depicted in SEQ ID Nos: 874, 930, 1022, 1024, 968 and 906, wherein said double-stranded ribonucleic acid molecule comprises the sequence pairs selected from the group consisting of SEQ ID NOs: 873/874, 929/930, 1021/1022, 1023/1024, 967/968 and
20 905/906.
4. The double-stranded ribonucleic acid molecule of claim 3, wherein the antisense strand further comprises a 3' overhang of 1-5 nucleotides in length, preferably of 1-2 nucleotides in length.
25
5. The double-stranded ribonucleic acid molecule of claim 4, wherein the overhang of the antisense strand comprises uracil or nucleotides which are complementary to the mRNA encoding GCR.
- 30 6. The double-stranded ribonucleic acid molecule of any of claims 3 to 5, wherein the sense strand further comprises a 3' overhang of 1-5 nucleotides in length, preferably of 1-2 nucleotides in length.

7. The double-stranded ribonucleic acid molecule of claim 6 wherein the overhang of the sense strand comprises uracil or nucleotides which are identical to the mRNA encoding GCR.
- 5 8. The double-stranded ribonucleic acid molecule of any one of claims 1 to 7, wherein said double-stranded ribonucleic acid molecule comprises at least one modified nucleotide.
9. The double-stranded ribonucleic acid molecule of claim 8, wherein said modified nucleotide is selected from the group consisting of a 2'-O-methyl modified nucleotide, a
10 nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, an inverted deoxythymidine, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural
15 base comprising nucleotide.
10. The double-stranded ribonucleic acid molecule of any one of claims 8 and 9, wherein said modified nucleotide is a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, an inverted deoxythymidine and a deoxythymidine.
20
11. The double-stranded ribonucleic acid molecule of claims 3 to 10, wherein said sense strand and / or said antisense strand comprise an overhang of 1-2 deoxythymidines and/or
inverted deoxythymidine.
- 25 12. The double-stranded ribonucleic acid molecule of any one of claims 1 to 11, wherein said sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos: 3, 7, 55, 25, 83, 31, 33, 747 and 764 and said antisense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID Nos: 4, 8, 56, 26, 84, 32, 34, 753 and 772 wherein said double-stranded ribonucleic acid
30 molecule comprises the sequence pairs selected from the group consisting of SEQ ID NOs: 3/4, 7/8, 55/56, 25/26, 83/84, 31/32, 33/34, 747/753 and 764/772.

13. A nucleic acid sequence encoding a sense strand and/or an antisense strand comprised in the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12.
14. A vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one of a sense strand or an antisense strand comprised in the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12 or comprising the nucleic acid sequence of claim 13.
15. A cell, tissue or non-human organism comprising the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13 or the vector of claim 14.
16. A pharmaceutical composition comprising the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13, the vector of claim 14 or the cell or tissue of claim 15.
17. The pharmaceutical composition of claim 16, further comprising a pharmaceutically acceptable carrier, stabilizer and/or diluent.
18. A method for inhibiting the expression of GCR gene in a cell, a tissue or an organism comprising the following steps:
- (a) introducing into the cell, tissue or organism the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, the nucleic acid molecule of claim 13, the vector of claim 14; and
 - (b) maintaining the cell, tissue or organism produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a GCR gene, thereby inhibiting expression of a GCR gene in the cell.
19. A method of treating, preventing or managing pathological conditions and diseases caused by the expression of the GCR gene disease comprising administering to a subject in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a the double-stranded ribonucleic acid molecule as

defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a pharmaceutical composition as defined in claims 16 or 17.

20. The method of claim 19, wherein said subject is a human.

5

21. A double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a pharmaceutical composition as defined in claims 16 or 17 for use in treating type 2 diabetes, obesity, dislipidemia, diabetic atherosclerosis, hypertension or depression.

10

22. Use of a the double-stranded ribonucleic acid molecule as defined in any one of claims 1 to 12, a nucleic acid molecule of claim 13, a vector of claim 14 and/or a cell or tissue of claim 15 for the preparation of a pharmaceutical composition for the treatment of type 2 diabetes, obesity, dislipidemia, diabetic atherosclerosis, hypertension or depression.

15

Fig 1

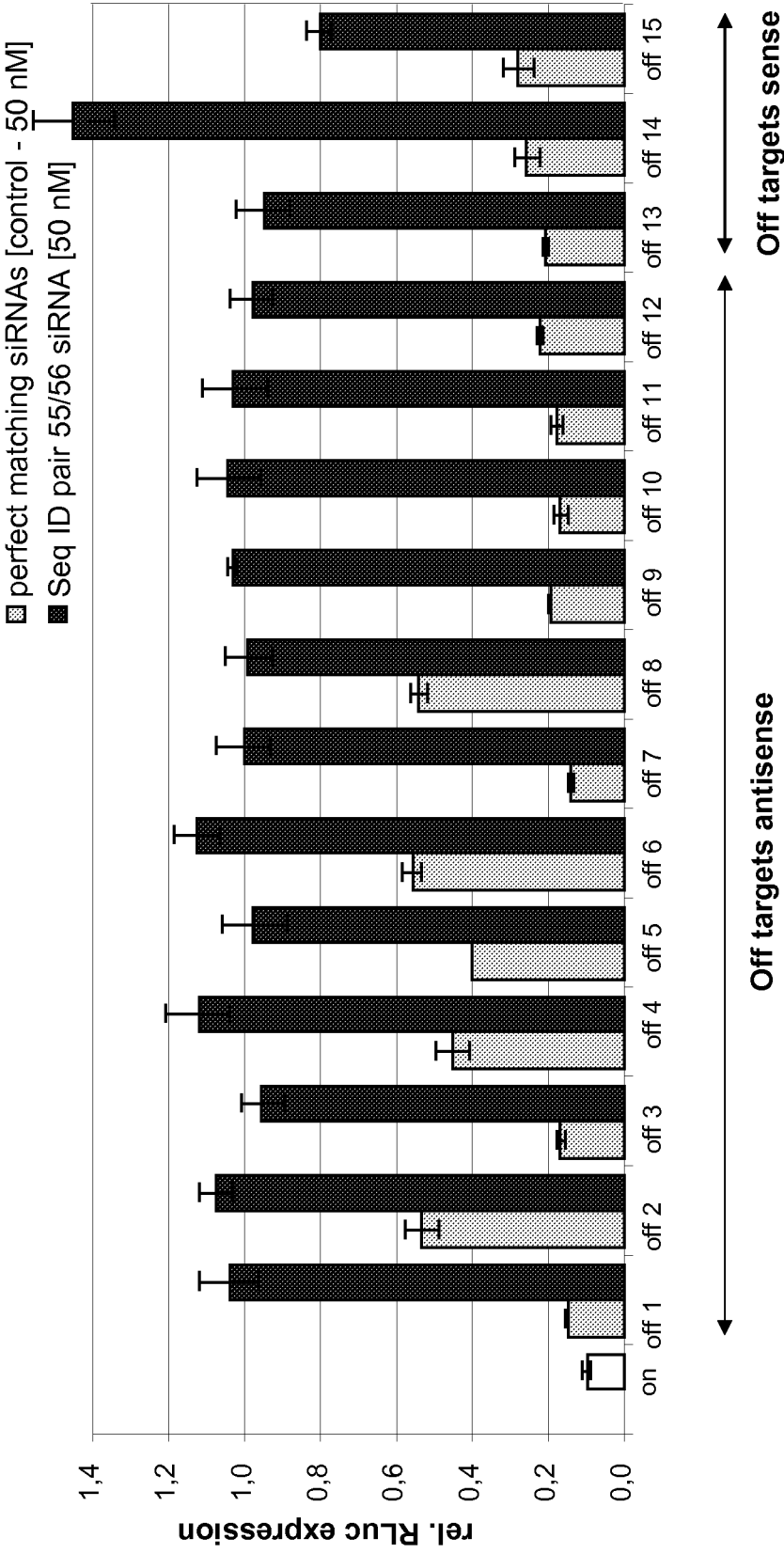


Fig 2

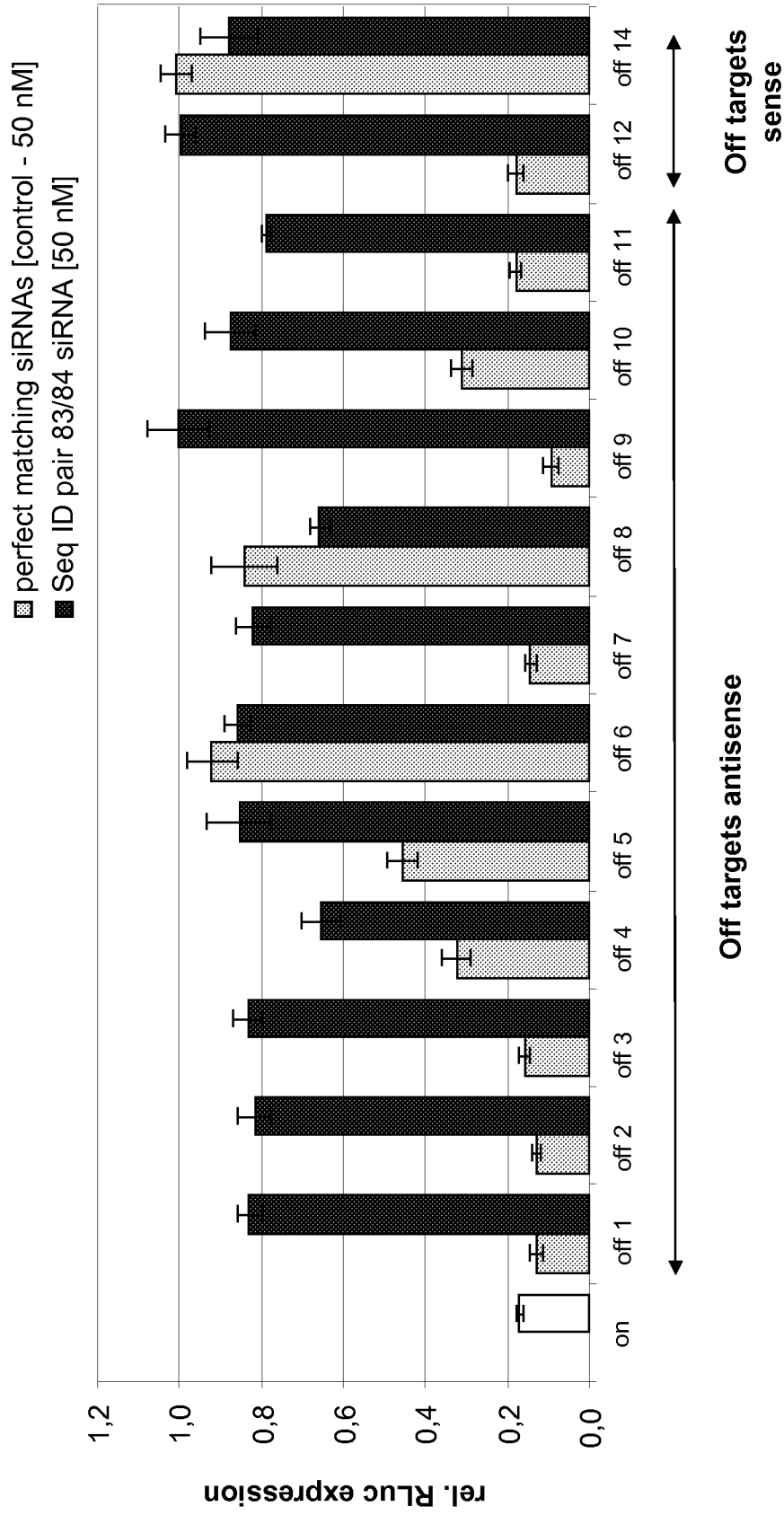


Fig 3

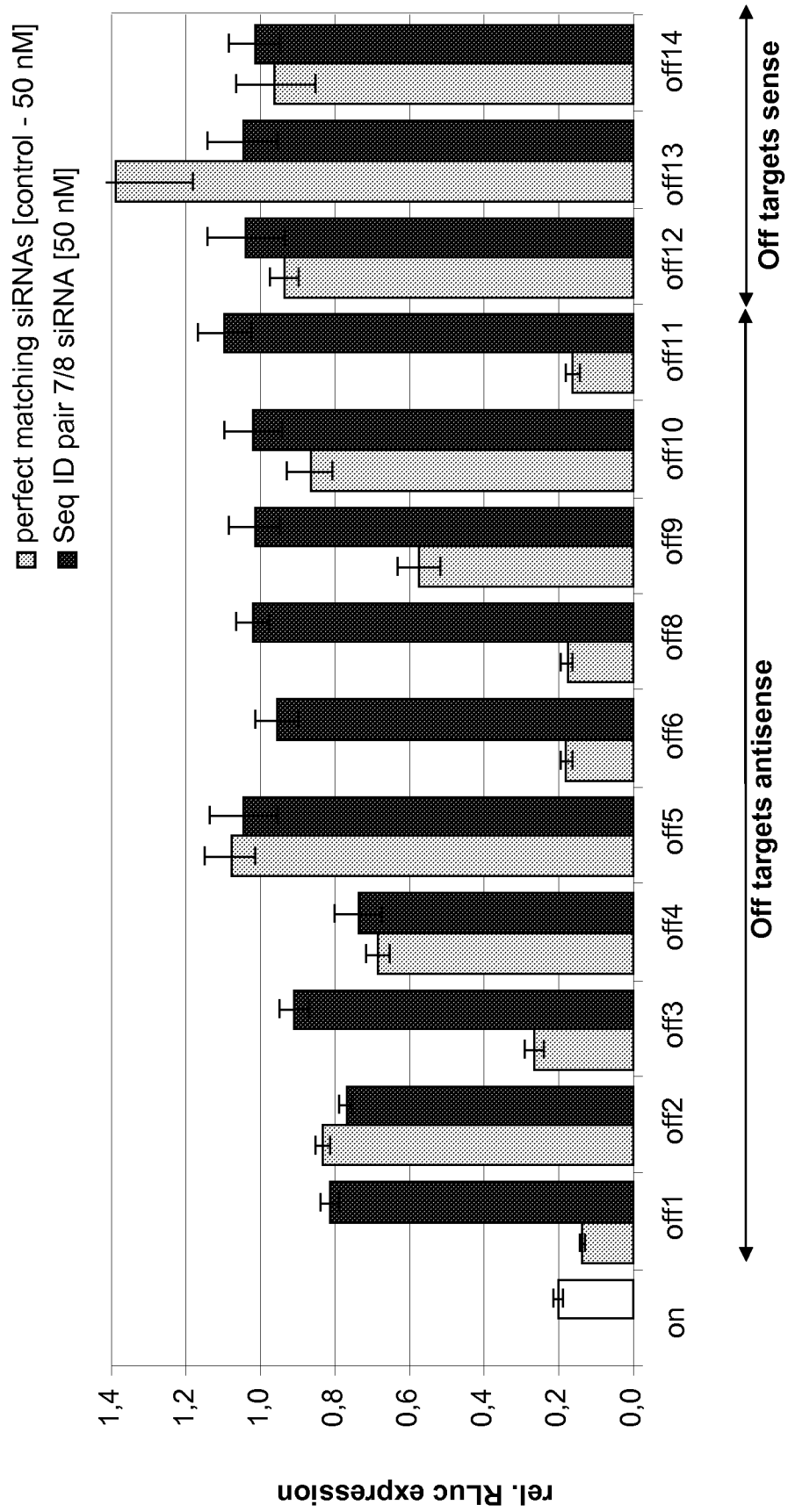


Fig 4

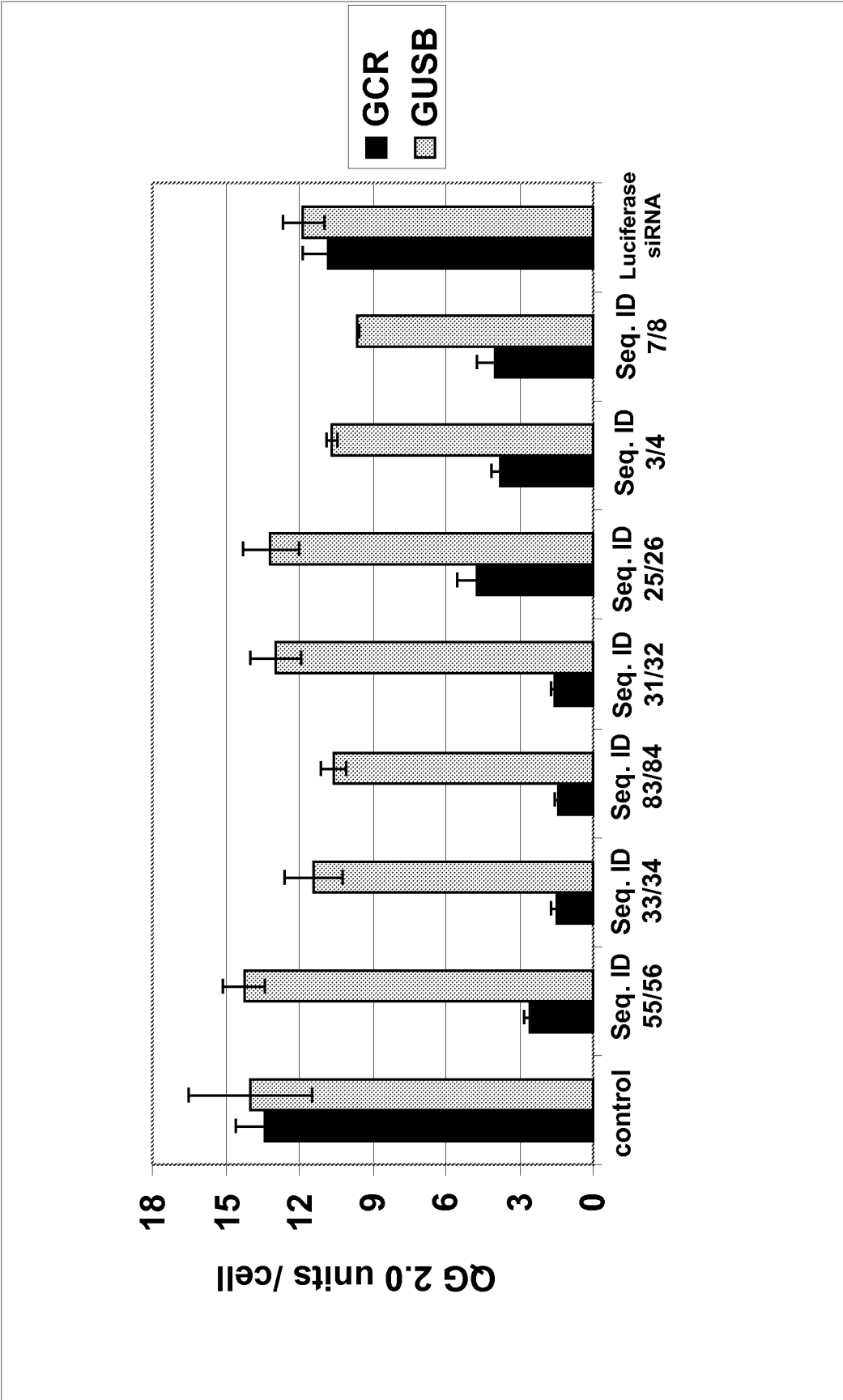


Fig 5 a)

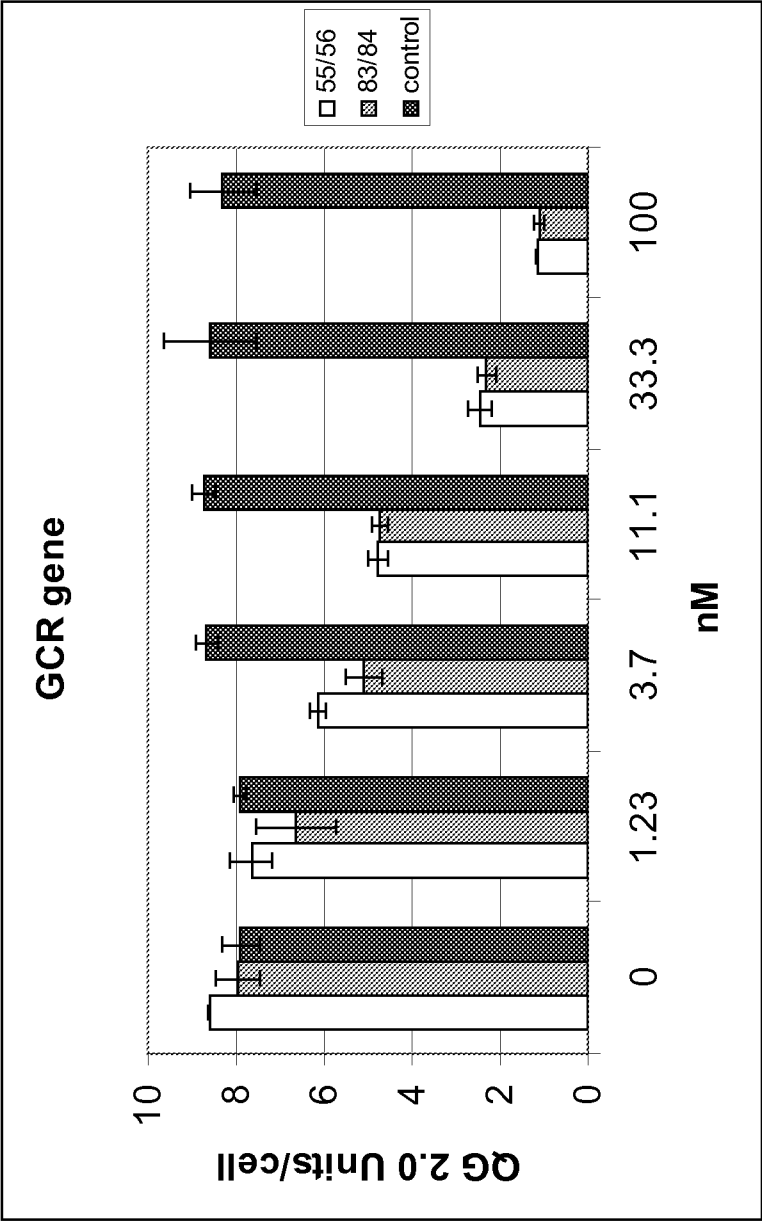


Fig 5b)

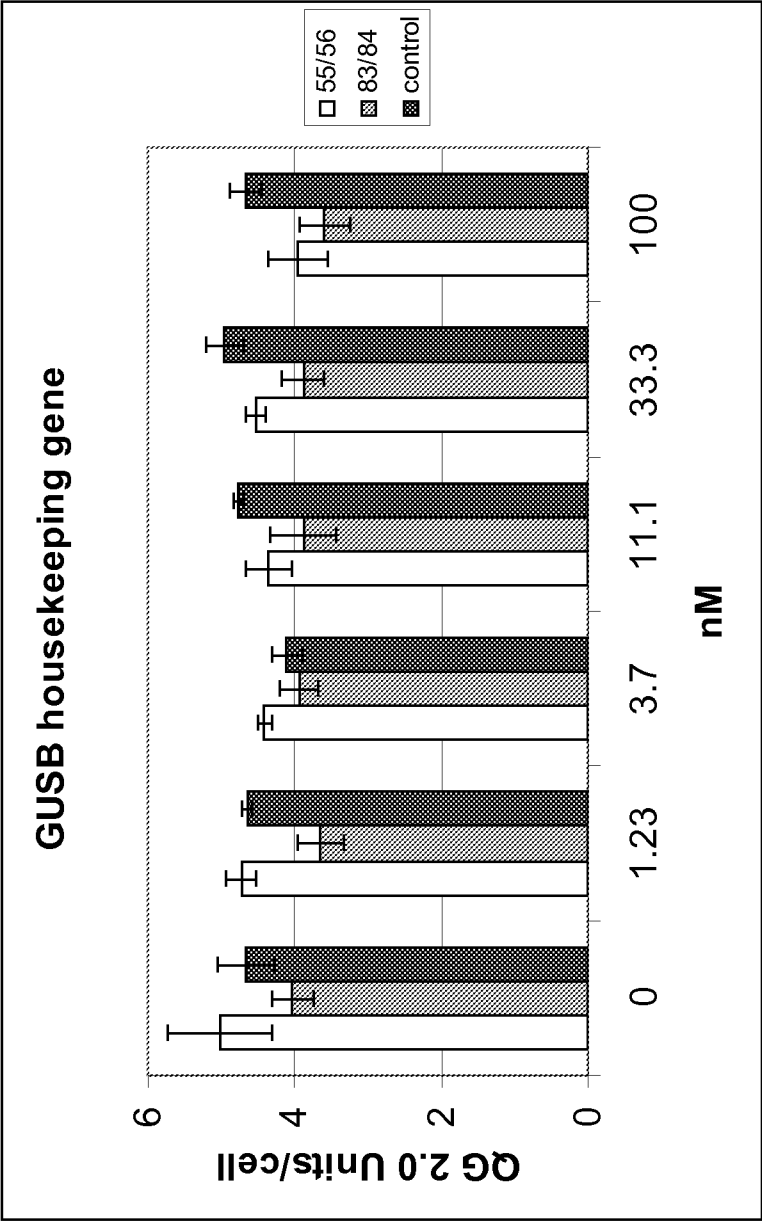


Fig 5c)

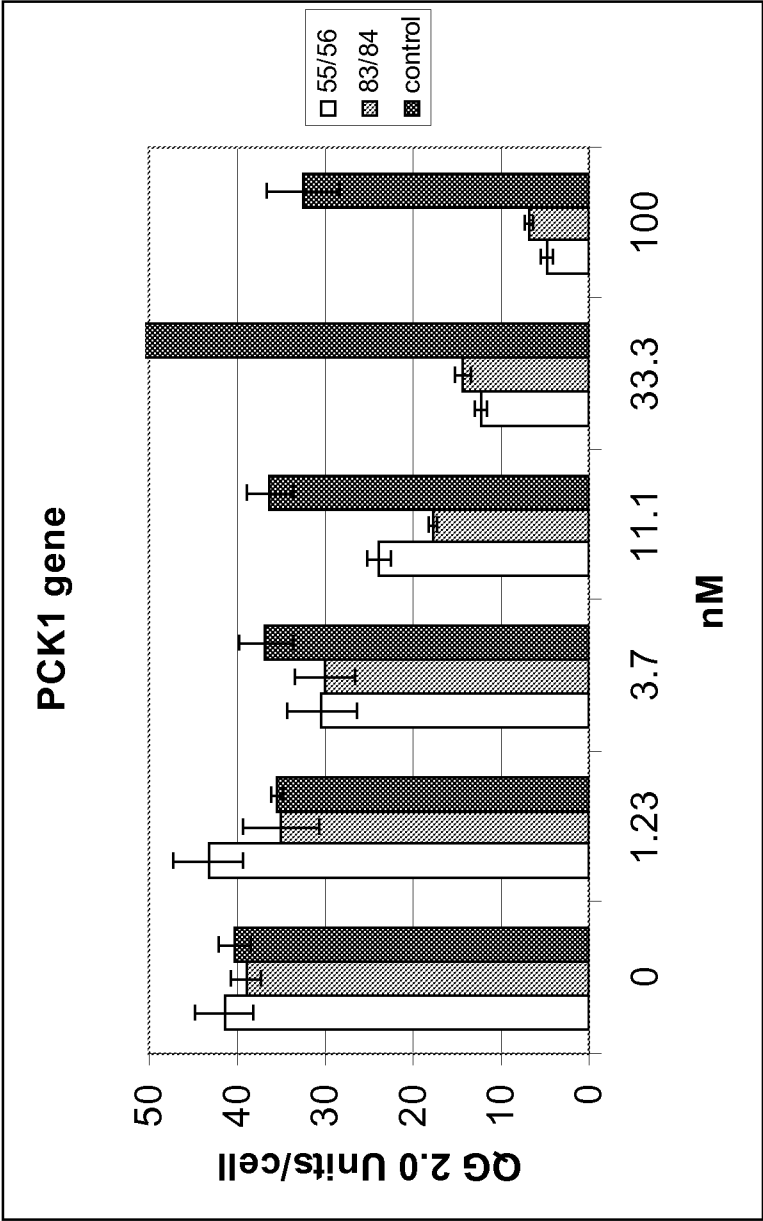


Fig 5d)

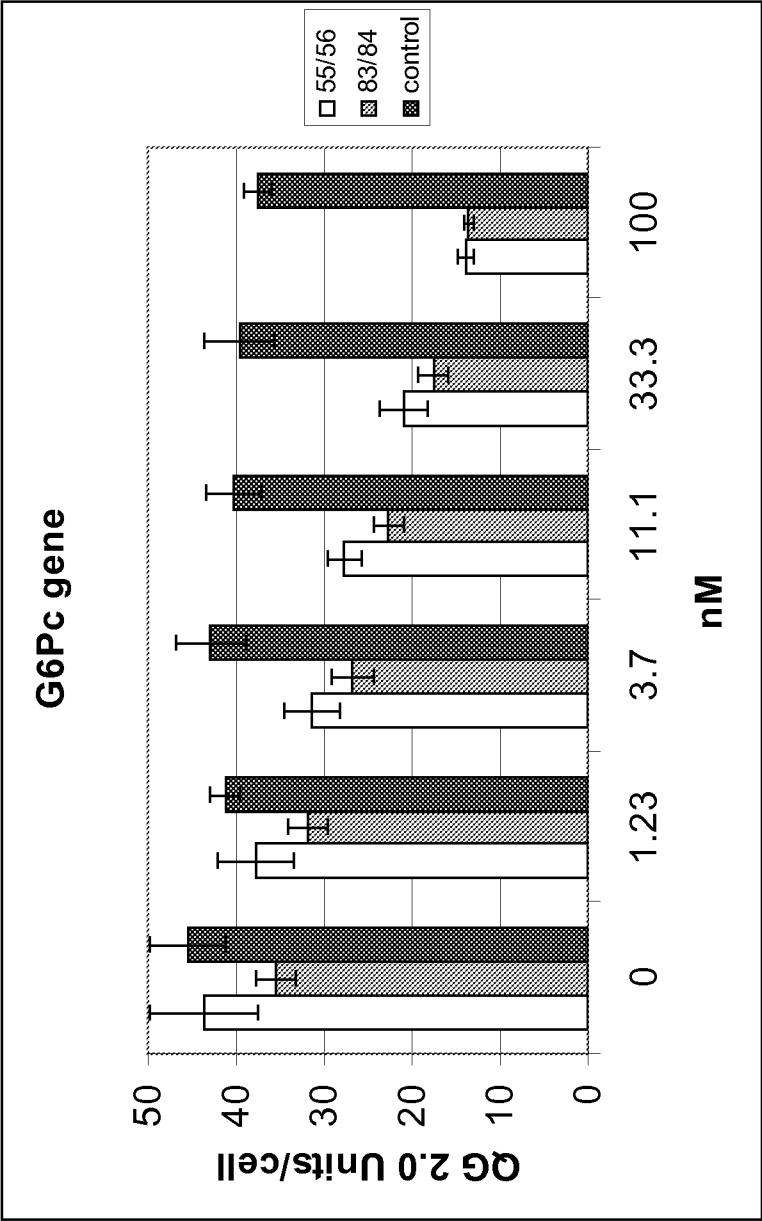


Fig 5e)

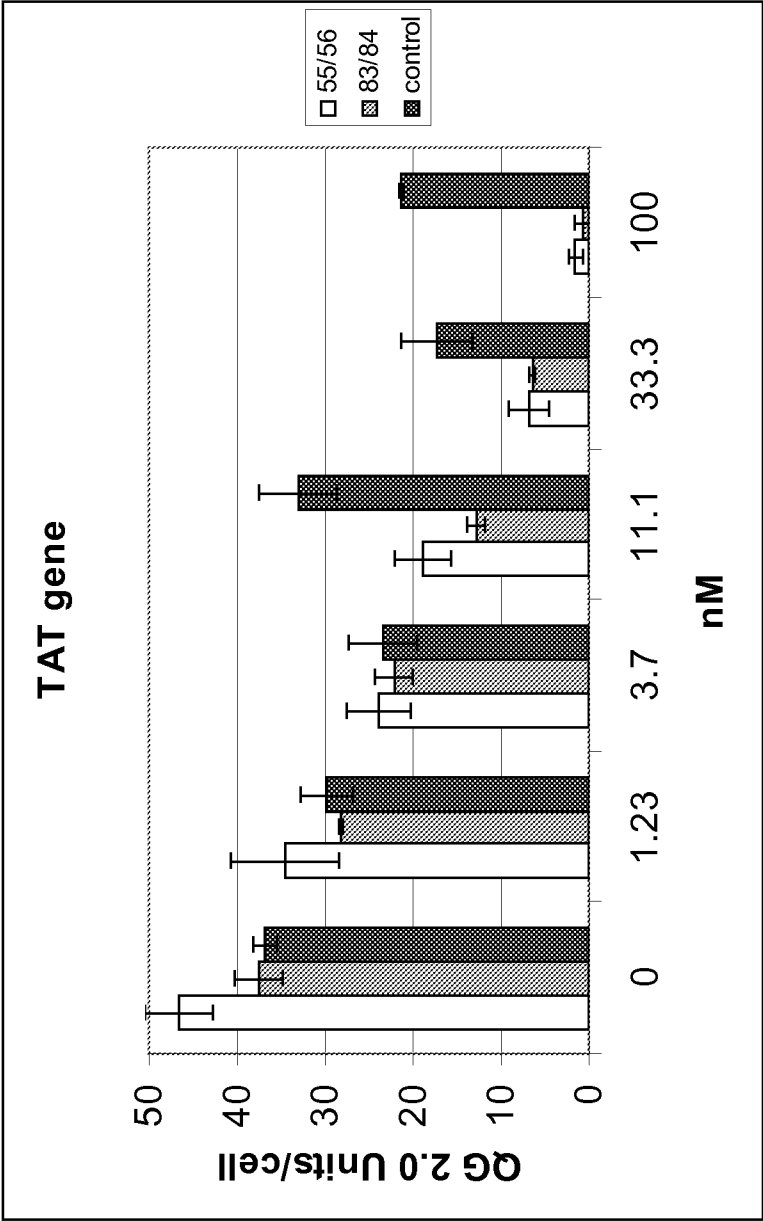


Fig 6 a)

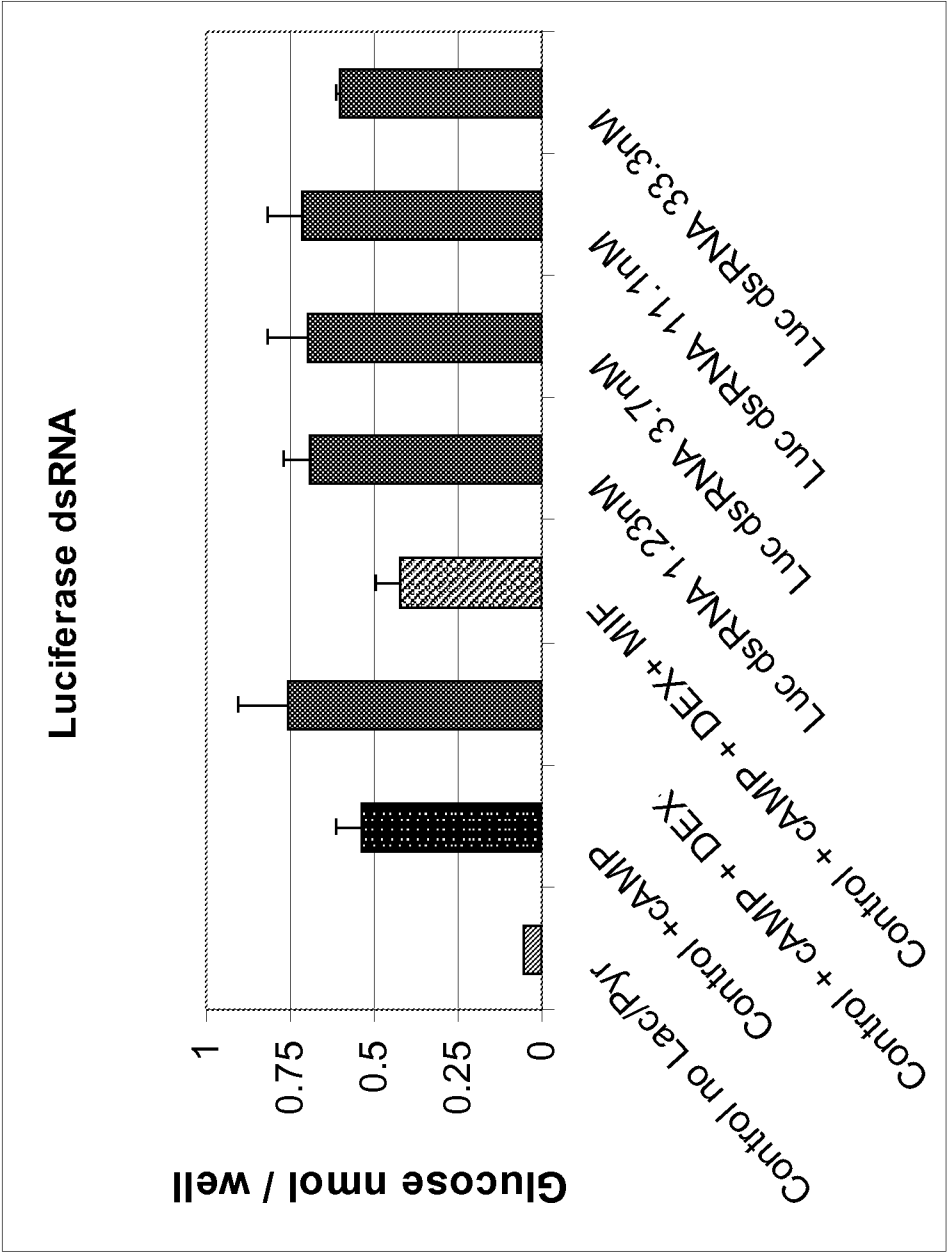


Fig 6b)

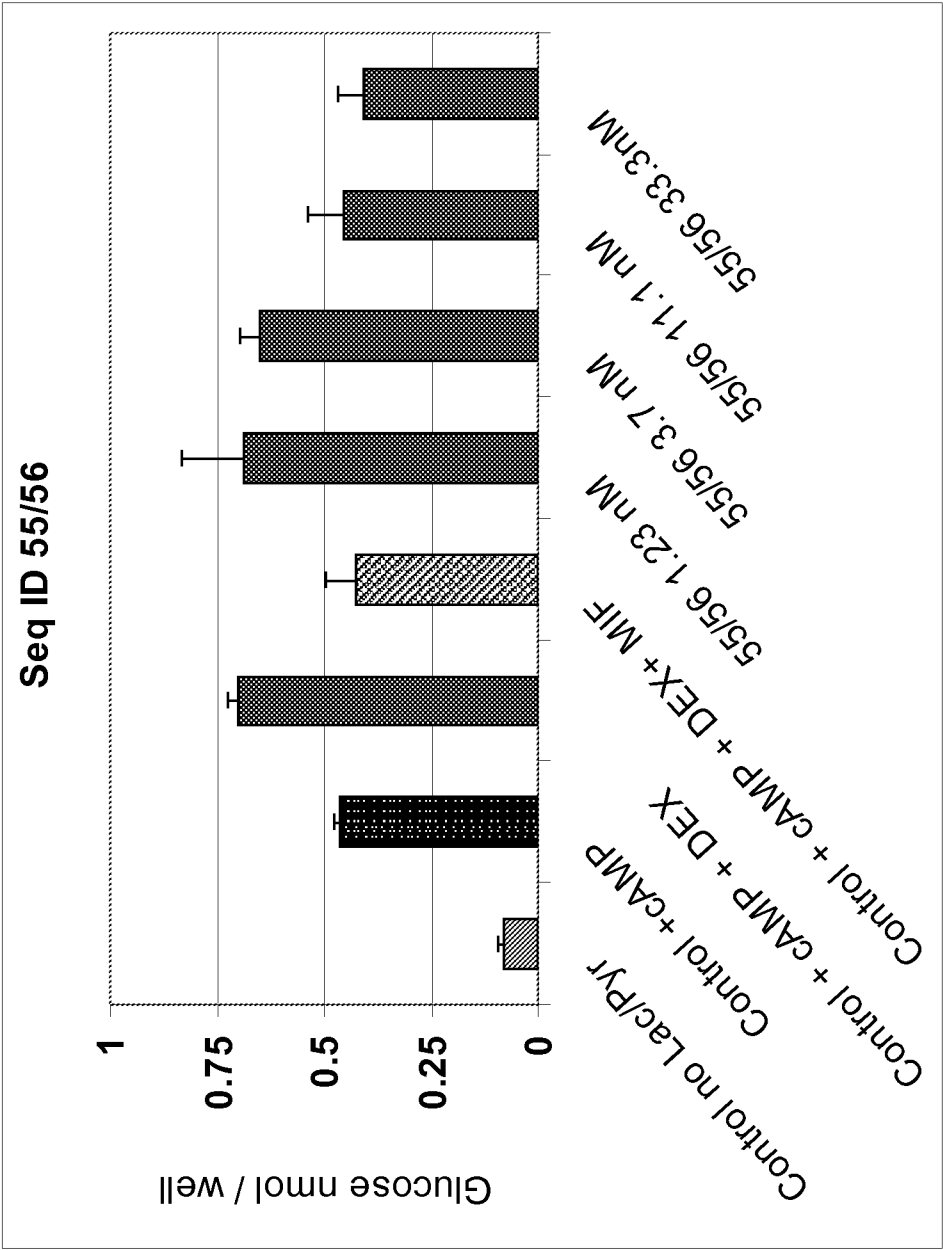


Fig. 6c)

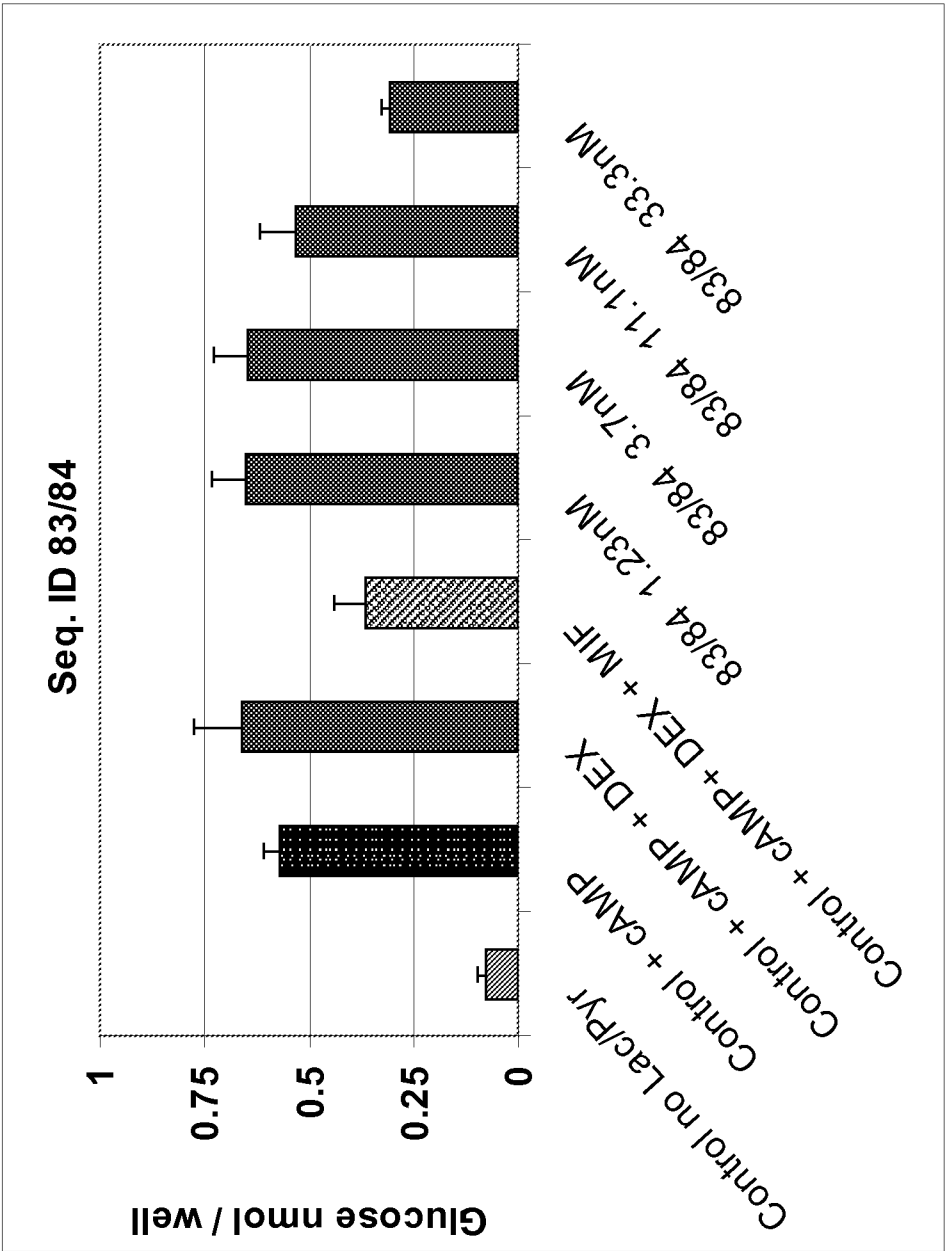


Fig. 7a)

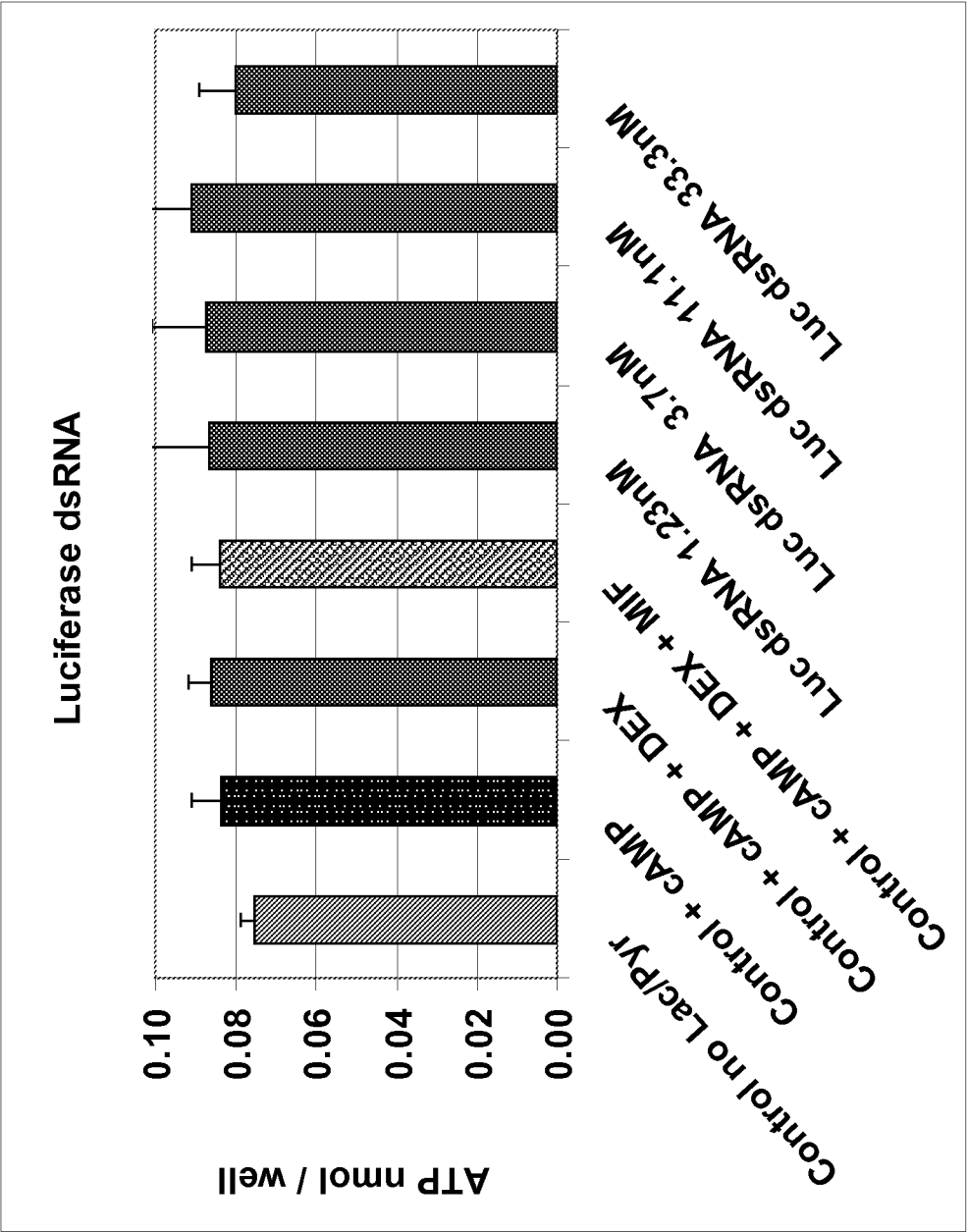


Fig 7b)

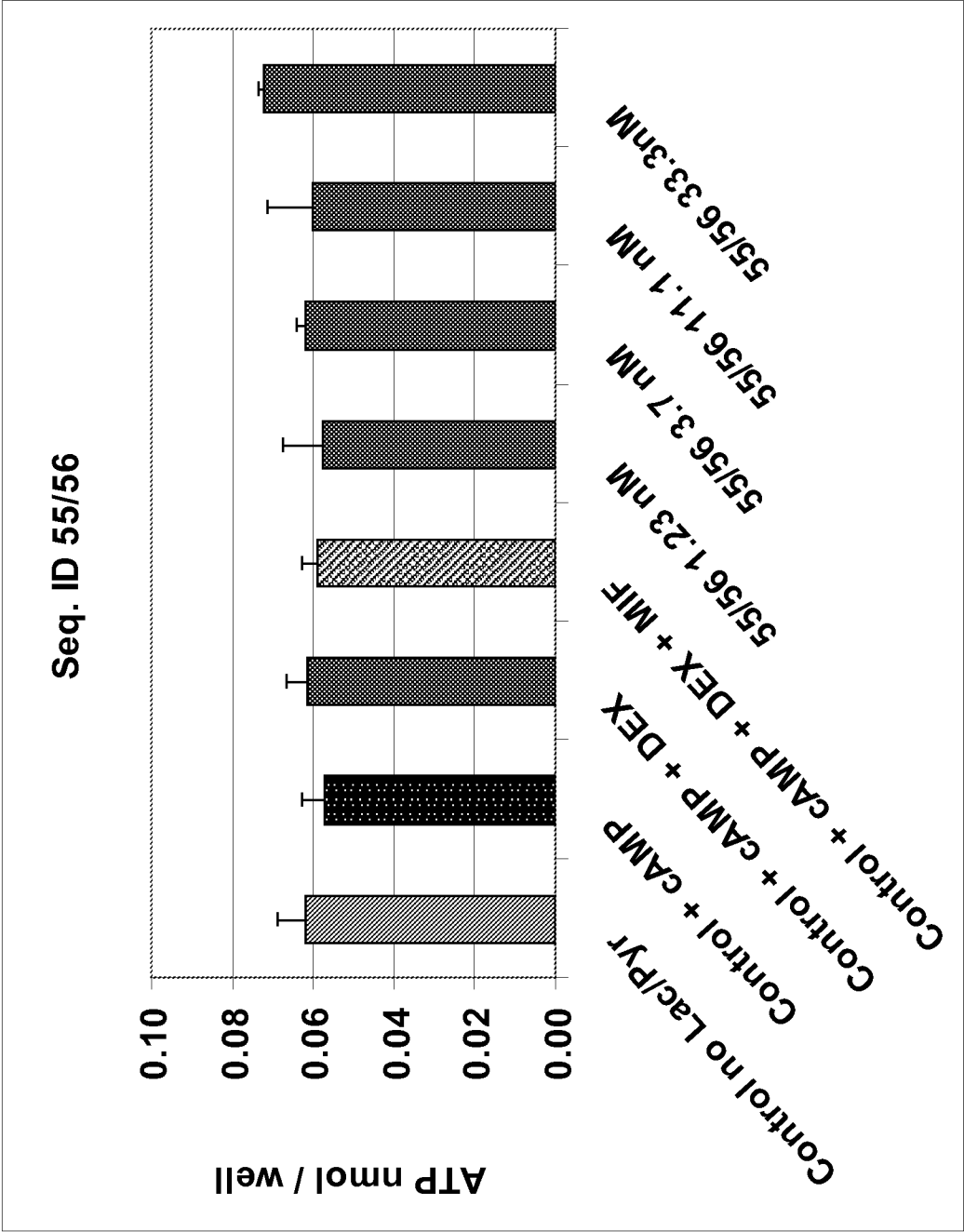


Fig 7c)

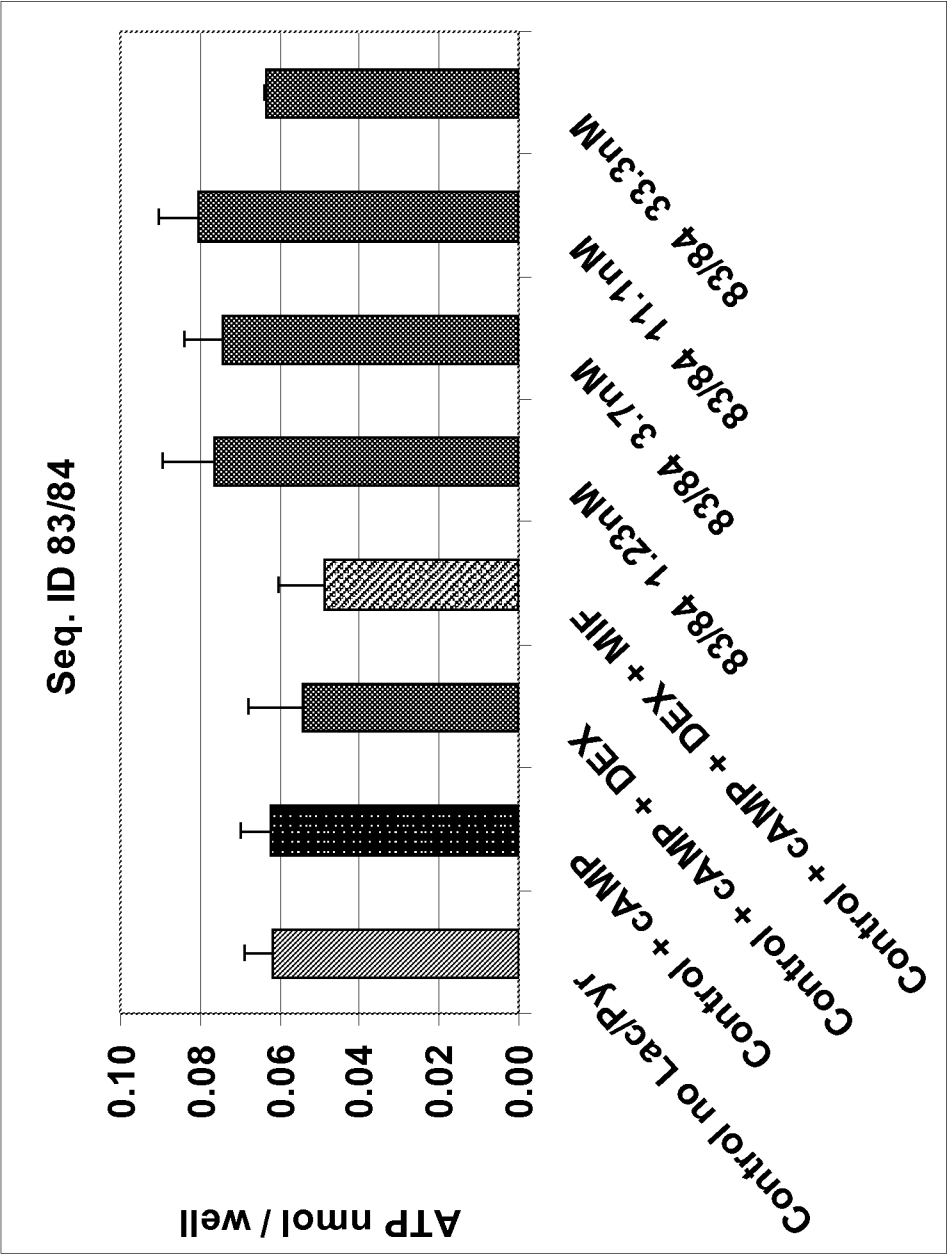


Fig. 8 a)

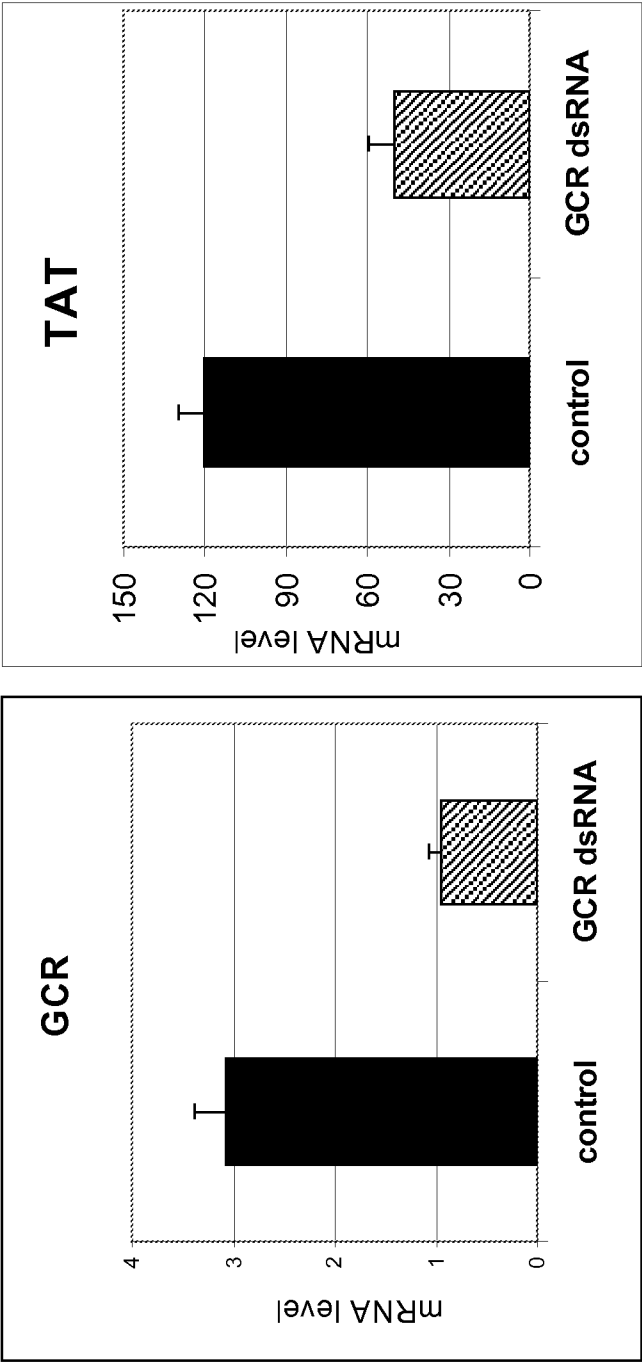
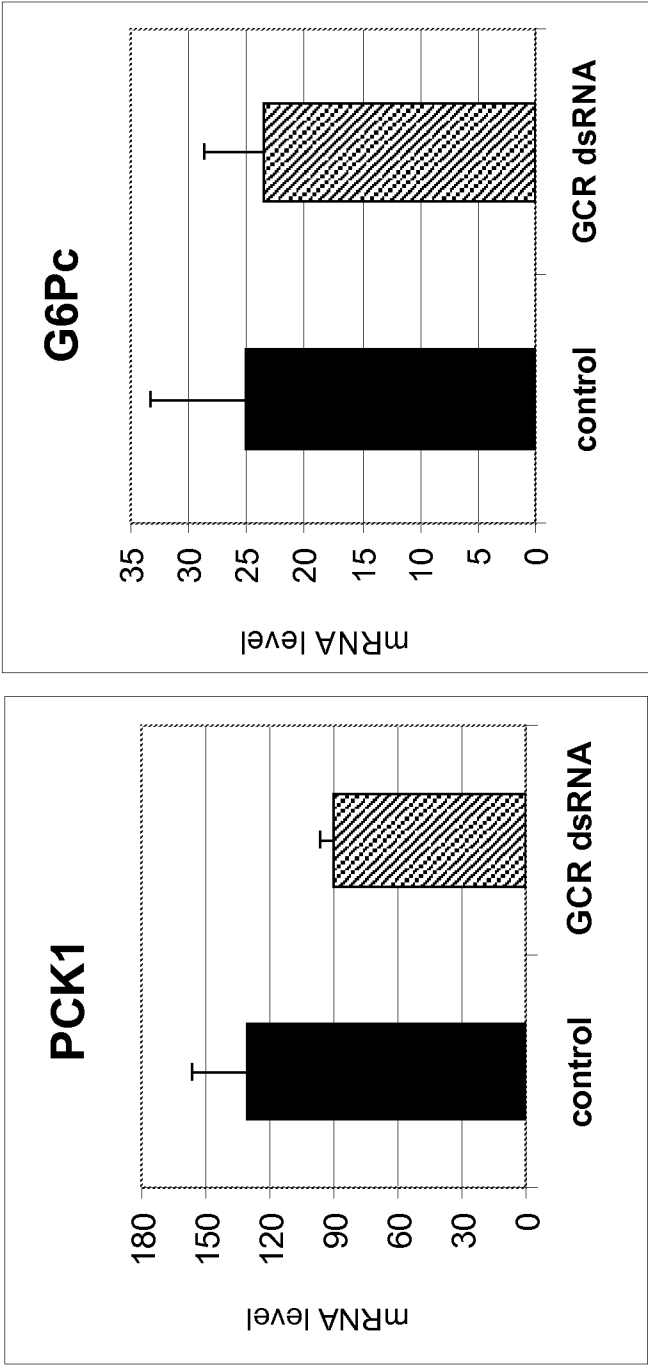


Fig 8 b)



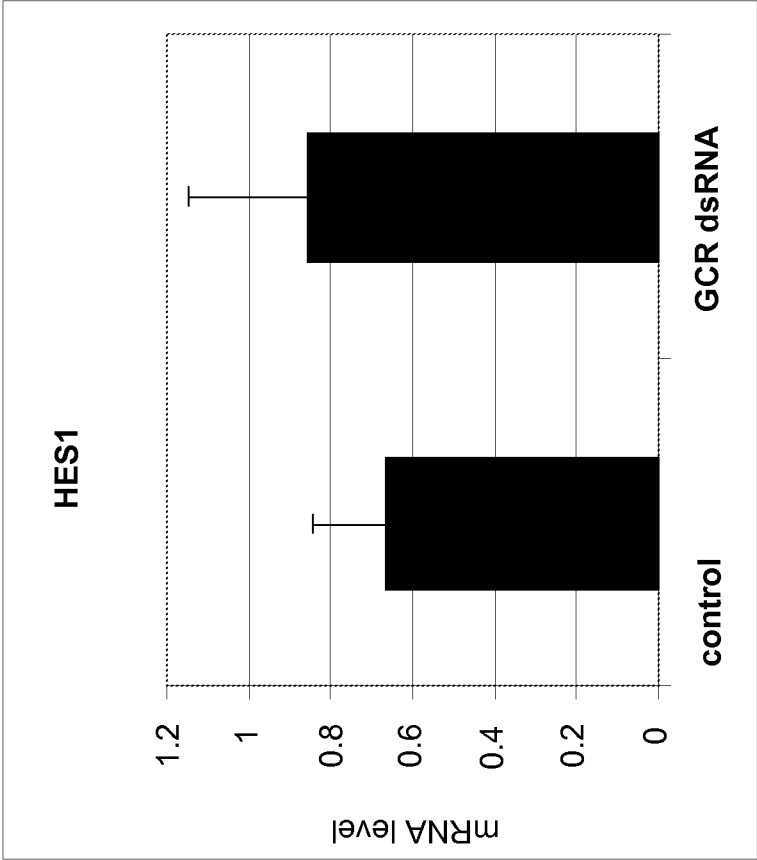
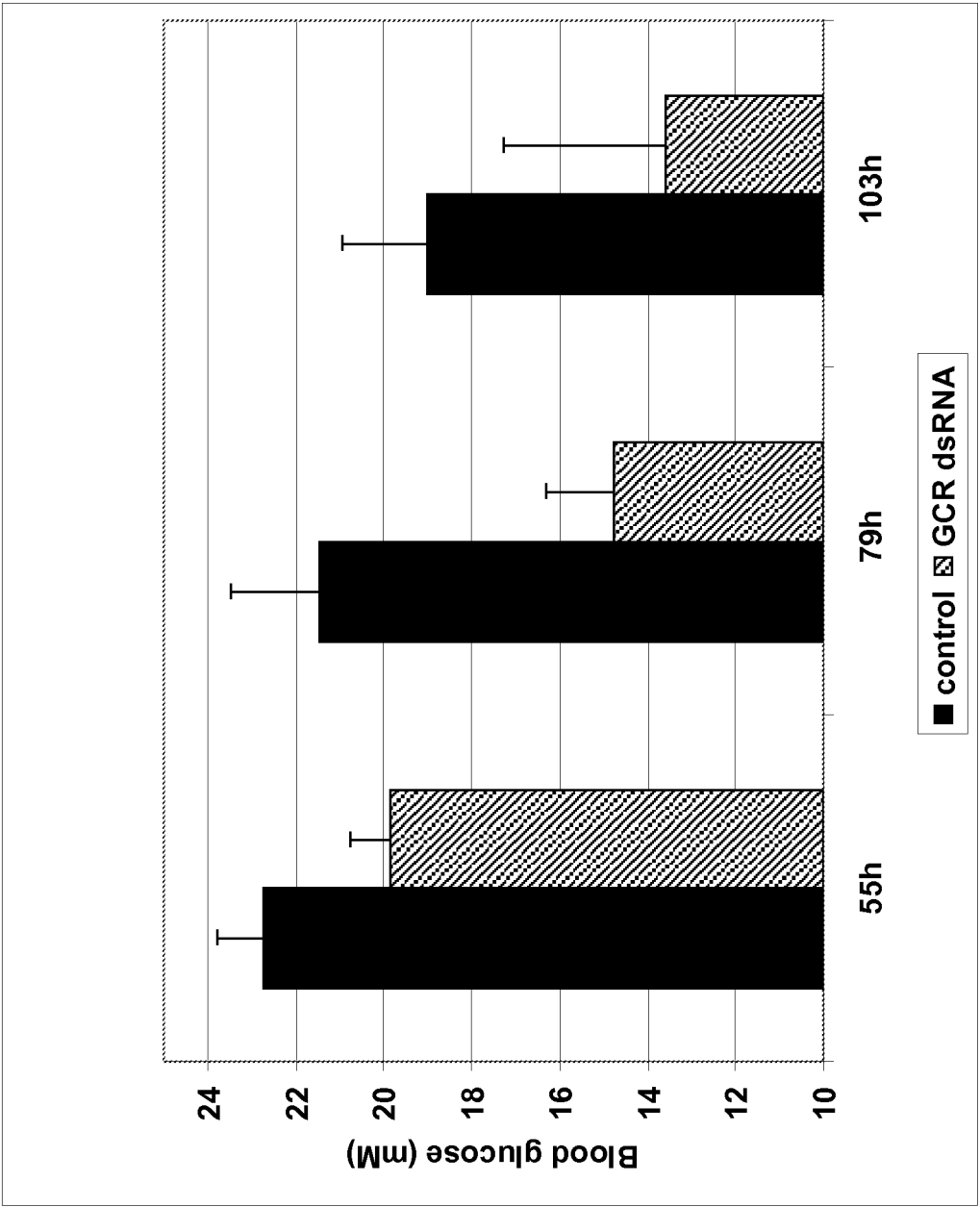


Fig. 8c)

Fig 9



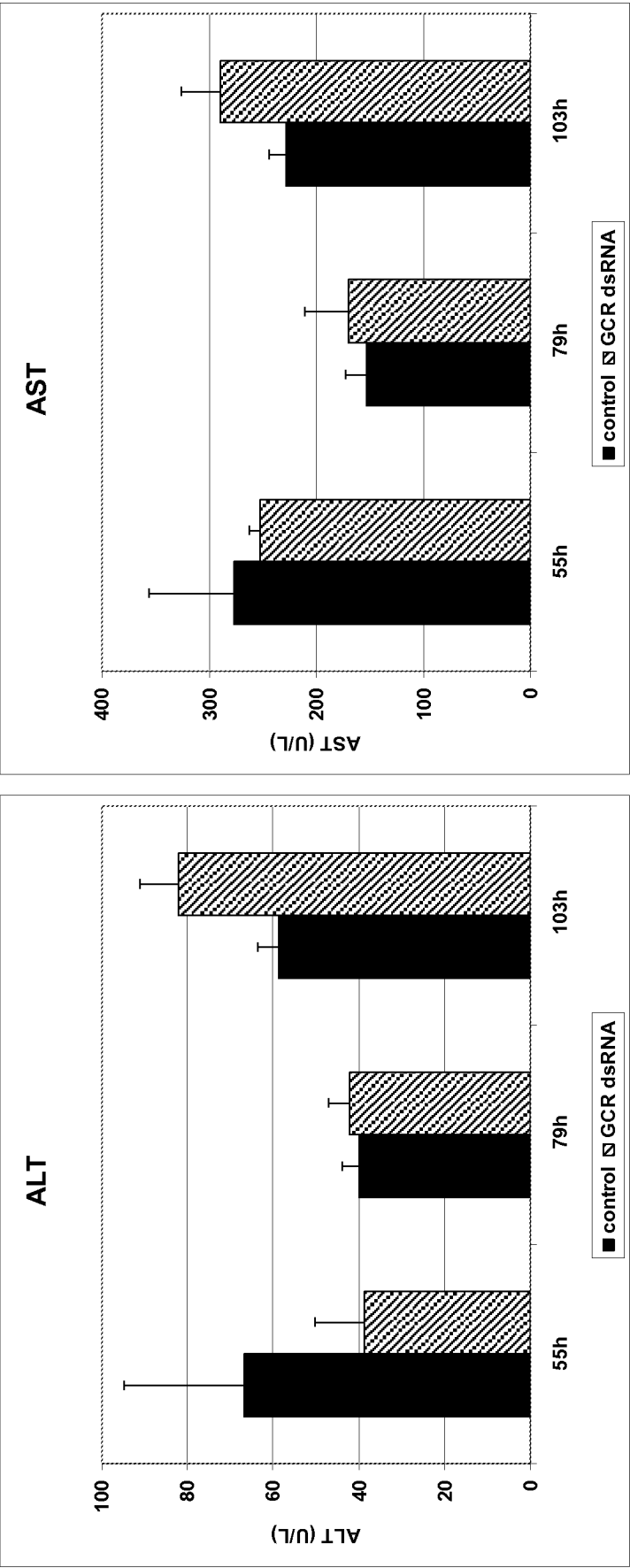


Fig 10

Fig.11a)

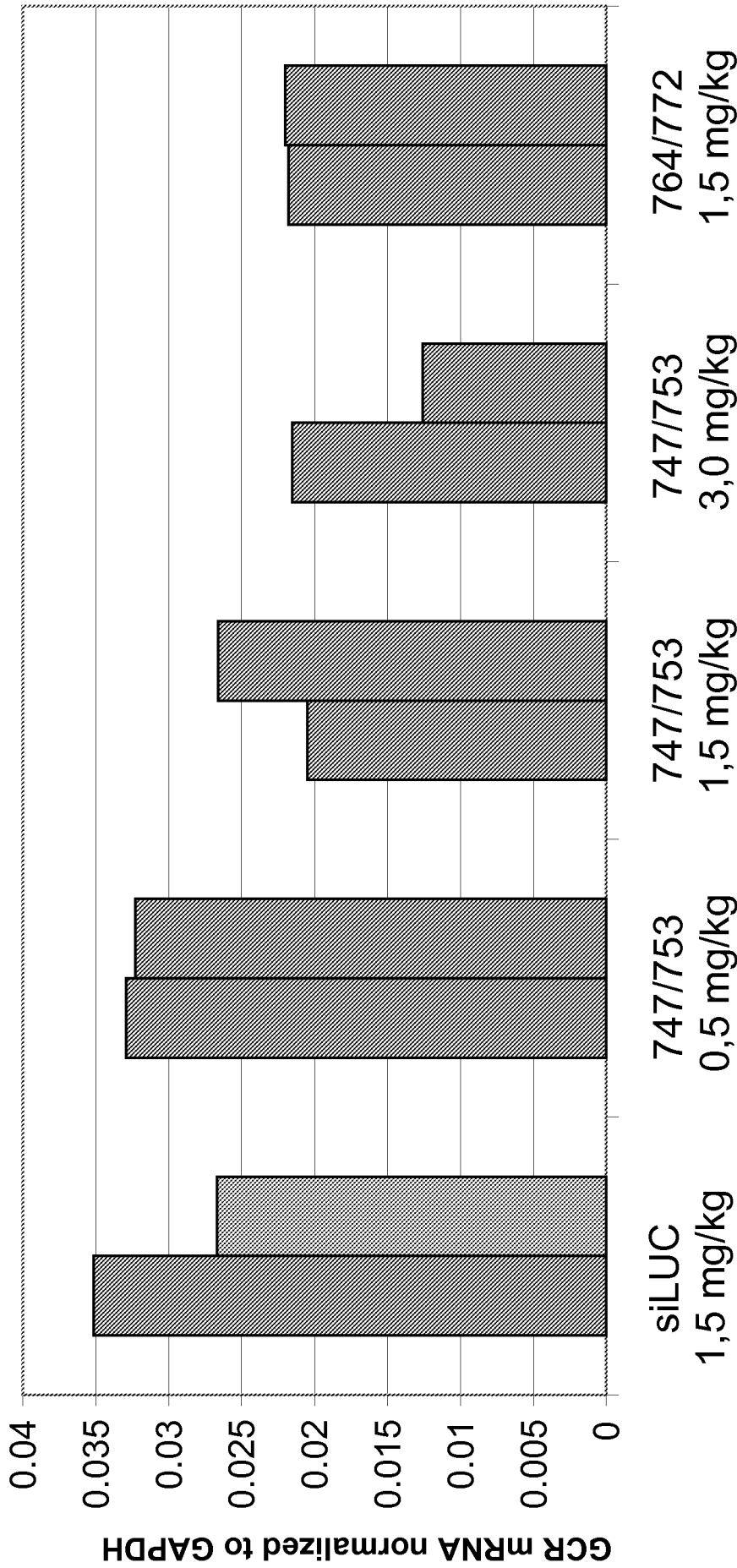


Fig.11b)

