RNAI CONSTRUCTS FOR INHIBITING PNPLA3 EXPRESSION

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/597,841, filed December 12, 2017, the contents of which are incorporated herein by reference.

SEQUENCE LISTING

[0002] The present application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 12, 2018, is named A-2219-WO-PCT_SL.txt and is 708,961 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to compositions and methods for modulating liver expression of patatin-like phospholipase domain-containing 3 (PNPLA3), In particular, the present invention relates to nucleic acid-based therapeutics for reducing PNPLA3 expression via RNA interference and methods of using such nucleic acid-based therapeutics to treat or prevent liver disease, such as nonalcoholic fatty liver disease (NAFLD).

[0004] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 12, 2018, is named A-2219-WO-PCT_SL.txt and is 708,961 bytes in size.

BACKGROUND OF THE INVENTION

[0005] Comprising a spectrum of hepatic pathologies, nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world, the prevalence of which doubled in the last 20 years and now is estimated to affect approximately 20% of the world population (Sattar et al. (2014) BMJ 349:g4596; Loomba and Sanyal (2013) Nature Reviews

Gastroenterology & hepatology 10(11):686-690; Kim and Kim (2017) Clin Gastroenterol Hepatol 15(4):474-485; Petta et al. (2016) Dig Liver Dis 48(3):333-342). NAFLD begins with the accumulation of triglyceride in the liver and is defined by the presence of cytoplasmic lipid droplets in more than 5% of hepatocytes in an individual 1) without a history of significant alcohol consumption and 2) in which the diagnosis of other types of liver disease have been excluded (Zhu et al (2016) World J Gastroenterol 22(36):8226-33; Rinella (2015) JAMA 313(22):2263-73; Yki-Jarvinen (2016) Diabetologia 59(6):1104-11). In some individuals the accumulation of ectopic fat in the liver, called steatosis, triggers inflammation and hepatocellular injury leading to a more advanced stage of disease called, nonalcoholic steatohepatitis (NASH) (Rinella, supra). As of 2015, 75-100 million Americans are predicted to have NAFLD; NASH accounting for approximately 10-30% of NAFLD diagnoses (Rinella, supra; Younossi et al (2016) Hepatology 64(5):1577-1586).

[0006] Patatin-like phospholipase domain-containing 3 (PNPLA3), formerly known as adiponutrin (ADPN) and calcium-independent phospholipase A2-epsilon (iPLA(2)ε), is a type II transmembrane protein (Wilson et al (2006) J Lipid Res 47(9):1940-9; Jenkins et al (2004) J Biol Chem 279(47):48968-75). Initially identified in adipose cells as a membrane-associated, adipose-enriched protein induced during adipogenesis in mice, it is now well characterized to be expressed in other tissues, including the liver (Wilson et al, supra; Baulande et al (2001) J Biol Chem 276(36):33336-44; Moldes et al. (2006) Eur J Endocrinol 155(3):461-8; Faraj et al. (2006) J Endocrinol 191(2):427-35; Liu et al (2004) J Clin Endocrinol Metab 89(6):2684-9; Lake et al (2005) J Lipid Res 46(11) :2477-87). In cell-free biochemical systems, recombinant PNPLA3 protein can exhibit either triacylglycerol lipase or transacylation activity (Jenkins et al., supra; Kumari et al (2012) Cell Metab 15(5):691-702; He et al (2010) J Biol Chem 285(9):6706-15). In hepatocytes, PNPLA3 is expressed on the endoplasmic reticulum and lipid membranes and predominantly exhibits triacylglycerol hydrolase activity (He et al., supra; Huang et al (2010) Proc Natl Acad Sci USA 107(17):7892-7; Ruhanen et al (2014) J Lipid Res 55(4):739-46; Pingitore et al. (2014) Biochim Biophys Acta 1841(4):574-80). Although lacking a secretory signal, data indicates PNPLA3 is secreted and can be found in human plasma as disulfide-bond dependent multimers (Winberg et al. (2014) Biochem Biophys Res Commun 446(4):1114-9). Accordingly,

novel therapeutics targeting PNPLA3 function represents a novel approach to reducing PNPLA3 levels and treating hepatologic diseases, such as nonalcoholic fatty liver disease.

SUMMARY OF THE INVENTION

[0007] The present invention is based, in part, on the design and generation of RNAi constructs that target the PNPLA3 gene and reduce expression of PNPLA3 in liver cells. The sequence specific inhibition of PNPLA3 expression is useful for treating or preventing conditions associated with PNPLA3 expression, such as liver-related diseases, such as, for example, simple fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or PNPLA3 related obesity. Accordingly, in one embodiment, the present invention provides an RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is complementary to a PNPLA3 mRNA sequence. In certain embodiments, the antisense strand comprises a region having at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2. In some embodiments, the RNAi of the present invention selectively inhibits PNPLA3-rs738409, PNPLA3-rs738408, and/or PNPLA3-rs738409-rs738408 minor alleles over the reference allele which does not contain these changes.

[0008] In some embodiments, the sense strand of the RNAi constructs described herein comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length. In these and other embodiments, the sense and antisense strands each are about 15 to about 30 nucleotides in length. In some embodiments, the RNAi constructs comprise at least one blunt end. In other embodiments, the RNAi constructs comprise at least one nucleotide overhang. Such nucleotide overhangs may comprise at least 1 to 6 unpaired nucleotides and can be located at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense and antisense strand. In certain embodiments, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the sense strand and the 3' end of the antisense strand. In other embodiments, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the antisense strand and a blunt end of the 3' end of the sense strand/5' end of the antisense strand.

[0009] The RNAi constructs of the invention may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some embodiments, the RNAi constructs comprise one or more 2'-modified nucleotides. Such 2'-modified nucleotides can include 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNA), glycol nucleic acids (GNAs), inverted bases (e.g. inverted adenosine) or combinations thereof In one particular embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some embodiments, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides.

[0010] In some embodiments, the RNAi constructs comprise at least one backbone modification, such as a modified internucleotide or internucleoside linkage. In certain embodiments, the RNAi constructs described herein comprise at least one phosphorothioate internucleotide linkage. In particular embodiments, the phosphorothioate internucleotide linkages may be positioned at the3' or 5' ends of the sense and/or antisense strands.

[0011] In some embodiments, the antisense strand and/or the sense strand of the RNAi constructs of the invention may comprise or consist of a sequence from the antisense and sense sequences listed in Tables 1 or 2. In certain embodiments, the RNAi construct may be any one of the duplex compounds listed in any one of Tables 1 to 2.

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1A-D show screening of five siRNA molecules for both dose-dependent mRNA knockdown and functional durability in vivo.

[0013] Figure 2A-G shows effect of PNPLA3 siRNA molecules in vivo in mice, liver weight, confirmation of human PNPLA3 expression, hepatic triglyceride content, serum TIMP1 levels, and histological indication of steatosis or inflammation.

[0014] Figure 3A-G shows effect of PNPLA3 siRNA molecules in vivo, liver weight, confirmation of human PNPLA3 expression, hepatic triglyceride content, serum TIMP1 levels, and histological indication of steatosis or inflammation.

[0015] Figure 4A-D shows the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule to rescue disease-associated phenotypes due to overexpression of PNPLA3$^{rs738409\text{-}rs738408}$, hepatic triglyceride content, serum TIMP1 levels, and histological indication of steatosis or inflammation.

[0016] Figure 5A-L shows the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule to prevent the development of early fibrosis.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The present invention is directed to compositions and methods for regulating the expression of the Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene. In some embodiments, the gene may be within a cell or subject, such as a mammal (e.g. a human). In some embodiments, compositions of the invention comprise RNAi constructs that target a PNPLA3 mRNA and reduce PNPLA3 expression in a cell or mammal. Such RNAi constructs are useful for treating or preventing various forms of liver-related diseases, such as, for example, simple fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or PNPLA3 related obesity.

[0018] In 2008, a genome wide association study (GWAS) exploring nonsynomonous sequence variations, or single nucleotide polymorphisms (SNPs), associated with NAFLD identified a variant in PNPLA3, (rs738409[G], encoding I148M; which can be referred to as PNPLA3-rs738409, PNPLA3-ma or PNPLA3-minor allele), as significantly associated with hepatic fat content. Since this initial report, subsequent GWAS confirm PNPLA3 rs738409 as the major genetic determinant of NAFLD, significantly associated with 1) increased levels of the serum biomarker for liver damage, alanine transaminase (ALT), 2) NAFLD incidence, progression and severity, 3) both obese and lean individuals, and 4) the only known SNP shown to be significantly associated with all stages of NAFLD: steatosis, NASH, cirrhosis and hepatic cell carcinoma. The consensus among numerous GWAS indicate the association of PNPLA3 rs738409 with NAFLD is independent of age, gender, ethnicity, metabolic syndrome, body mass index, insulin resistance, and serum lipids. Furthermore, statistical analyses from multiple sources estimate approximately 50% of NAFLD patients carry the PNPLA3 rs738409 mutation. Patients can be homozygous or heterozygous for the PNPLA3 rs738409 mutation. Additionally, it has

been discovered that patients having the PNPLA3 rs738409 mutation often also carry an rs738408 mutation 3 base pairs away (Tian et al (2010) Nature Genetics 42:21-23). Thus, a patient can have a PNPLA3-rs738409 minor allele, PNPLA3-rs738408 minor allele or PNPLA3-rs738409-rs738408 double minor allele mutation (PNPLA3-dma).

[0019] Investigators have developed mouse models for exploring PNPLA3 function in vivo. To date, no detectable metabolic phenotype has been identified as the result of *Pnpla3*-deficiency or *Pnpla3* over-expression. In contrast, expression of $Pnpla3^{I148M}$ in both transgenic mice and knock-in mice, led to increased hepatic triglyceride levels akin to NAFLD Thus, combined, the in vivo mouse model data points to expression of the mutant $Pnpla3^{I148M}$ protein, and not over-expression of the wild type protein, as the driver of the disease phenotype. These findings, in addition to the high frequency of the minor allele in NAFLD-affected individuals and prevailing association with the disease, underline PNPLA3 rs738409 as a prime therapeutic target for NAFLD.

[0020] RNA interference (RNAi) is the process of introducing exogeneous RNA into a cell leading to specific degradation of the mRNA encoding the targeted protein with a resultant decrease in protein expression. Advances in both the RNAi technology and hepatic delivery and growing positive outcomes with other RNAi-based therapies, suggest RNAi as a compelling means to therapeutically treat NAFLD by directly targeting PNPLA3I148M . Numerous GWAS indicate a dose-dependent effect of PNPLA3 rs738409 on NAFLD incidence, progression and severity (GWAS); the odds ratio tending to be double, if not more, for homozygote carriers versus heterozygote carriers, yet still at least two-fold more for heterozygotes versus wild type individuals. Thus, silencing PNPLA3 employing allelic discrimination specificity could be both a potential means to reduce hepatic triglyceride in PNPLA3I148M carriers, but also present a scenario in which heterozygotes may gain benefit without silencing of the wild type allele. Along these lines, we identified SNP-specific short interfering RNAs (siRNA) to PNPLA3I148M and demonstrate proof of concept in vitro. Using both hepatoma cell lines, Hep3B (homozygous for the reference allele, PNPLA3I148I) and HEPG2 (homozygous for the minor allele, PNPLA3I148M), we identified siRNA sequences capable of inhibiting specifically PNPLA3I148M gene expression. The inhibitory effect of these sequences were confirmed by

screening on Chinese hamster ovary (CHO) cells over-expressing either PNPLA3I148I or PNPLA3I148M. Using adeno-associated virus (AAV) to overexpress human PNPLA3I148M in vivo, we then demonstrated treatment with minor allele-specific SNPs not only specifically reduced human PNPLA3I148M expression in mice, but also significantly reversed hepatic triglyceride accumulation induced by over-expression of human PNPLA3I148M.

[0021] As used herein, the term "RNAi construct" refers to an agent comprising a RNA molecule that is capable of downregulating expression of a target gene (e.g. PNPLA3) via a RNA interference mechanism when introduced into a cell. RNA interference is the process by which a nucleic acid molecule induces the cleavage and degradation of a target RNA molecule (e.g. messenger RNA or mRNA molecule) in a sequence-specific manner, e.g. through a RNA induced silencing complex (RISC) pathway. In some embodiments, the RNAi construct comprises a double-stranded RNA molecule comprising two antiparallel strands of contiguous nucleotides that are sufficiently complementary to each other to hybridize to form a duplex region. "Hybridize" or "hybridization" refers to the pairing of complementary polynucleotides, typically via hydrogen bonding (e.g. Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary bases in the two polynucleotides. The strand comprising a region having a sequence that is substantially complementary to a target sequence (e.g. target mRNA) is referred to as the "antisense strand." The "sense strand" refers to the strand that includes a region that is substantially complementary to a region of the antisense strand. In some embodiments, the sense strand may comprise a region that has a sequence that is substantially identical to the target sequence.

[0022] In some embodiments, the invention is an RNAi directed to PNPLA3. In some embodiments, the invention is an RNAi that binds at the PNPLA3 rs738409 site. In some embodiments, the invention is an RNAi that binds at the PNPLA3 rs738408 site. In some embodiments, the invention is an RNAi that binds at both the PNPLA3 rs738409 rs738408 sites. In some embodiments, the invention is an RNAi that preferentially binds PNPLA3 rs738409 over the native PNPLA3 sequence (PNPLA3-ref). In some embodiments, the invention is an RNAi that preferentially binds PNPLA3 rs738408 over the PNPLA3-ref sequence. In some embodiments, the invention is an RNAi that preferentially binds PNPLA3-dma over PNPLA3-ma. In some

embodiments, the invention in an RNAi molecule that contains any of the sequences found in Table 1 or 2.

[0023] A double-stranded RNA molecule may include chemical modifications to ribonucleotides, including modifications to the ribose sugar, base, or backbone components of the ribonucleotides, such as those described herein or known in the art. Any such modifications, as used in a double-stranded RNA molecule (e.g. siRNA, shRNA, or the like), are encompassed by the term "double-stranded RNA" for the purposes of this disclosure.

[0024] As used herein, a first sequence is "complementary" to a second sequence if a polynucleotide comprising the first sequence can hybridize to a polynucleotide comprising the second sequence to form a duplex region under certain conditions, such as physiological conditions. Other such conditions can include moderate or stringent hybridization conditions, which are known to those of skill in the art. A first sequence is considered to be fully complementary (100% complementary) to a second sequence if a polynucleotide comprising the first sequence base pairs with a polynucleotide comprising the second sequence over the entire length of one or both nucleotide sequences without any mismatches. A sequence is "substantially complementary" to a target sequence if the sequence is at least about 80%, 85%, 90%, 95%,96%, 97%, 98%, 99% or 100% complementary to a target sequence. Percent complementarity can be calculated by dividing the number of bases in a first sequence that are complementary to bases at corresponding positions in a second or target sequence by the total length of the first sequence. A sequence may also be said to be substantially complementary to another sequence if there are no more than 5, 4, 3, 2, or 1 mismatches over a 30 base pair duplex region when the two sequences are hybridized. Generally, if any nucleotide overhangs, as defined herein, are present, the sequence of such overhangs is not considered in determining the degree of complementarity between two sequences. By way of example, a sense strand of 21 nucleotides in length and an antisense strand of 21 nucleotides in length that hybridize to form a 19 base pair duplex region with a 2 nucleotide overhang at the 3' end of each strand would be considered to be fully complementary as the term is used herein.

[0025] In some embodiments, a region of the antisense strand comprises a sequence that is fully complementary to a region of the target RNA sequence (e.g. PNPLA3 mRNA). In such

embodiments, the sense strand may comprise a sequence that is fully complementary to the sequence of the antisense strand. In other such embodiments, the sense strand may comprise a sequence that is substantially complementary to the sequence of the antisense strand, e.g. having 1, 2, 3, 4, or 5 mismatches in the duplex region formed by the sense and antisense strands. In certain embodiments, it is preferred that any mismatches occur within the terminal regions (e.g. within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' and/or 3' ends of the strands). In one embodiment, any mismatches in the duplex region formed from the sense and antisense strands occur within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' end of the antisense strand.

[0026] In certain embodiments, the sense strand and antisense strand of the double-stranded RNA may be two separate molecules that hybridize to form a duplex region, but are otherwise unconnected. Such double-stranded RNA molecules formed from two separate strands are referred to as "small interfering RNAs" or "short interfering RNAs" (siRNAs). Thus, in some embodiments, the RNAi constructs of the invention comprise a siRNA.

[0027] Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. 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. The maximum number of base pairs in the duplex is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

[0028] In other embodiments, the sense strand and the antisense strand that hybridize to form a duplex region may be part of a single RNA molecule, i.e. the sense and antisense strands are part of a self-complementary region of a single RNA molecule. In such cases, a single RNA molecule comprises a duplex region (also referred to as a stem region) and a loop region. The 3' end of the sense strand is connected to the 5' end of the antisense strand by a contiguous sequence of unpaired nucleotides, which will form the loop region. The loop region is typically of a sufficient length to allow the RNA molecule to fold back on itself such that the antisense strand can base pair with the

sense strand to form the duplex or stem region. The loop region can comprise from about 3 to about 25, from about 5 to about 15, or from about 8 to about 12 unpaired nucleotides. Such RNA molecules with at least partially self-complementary regions are referred to as "short hairpin RNAs" (shRNAs). In some embodiments, the loop region can comprise at least 1, 2, 3, 4, 5, 10, 20, or 25 unpaired nucleotides. In some embodiments, the loop region can have 10, 9, 8, 7, 6, 5, 4, 3, 2, or fewer unpaired nucleotides. In certain embodiments, the RNAi constructs of the invention comprise a shRNA. The length of a single, at least partially self-complementary RNA molecule can be from about 35 nucleotides to about 100 nucleotides, from about 45nucleotides to about 85 nucleotides, or from about 50 to about 60 nucleotides and comprise a duplex region and loop region each having the lengths recited herein.

[0029] In some embodiments, the RNAi constructs of the invention comprise a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is substantially or fully complementary to a PNPLA3 messenger RNA (mRNA) sequence. As used herein, a "PNPLA3 mRNA sequence" refers to any messenger RNA sequence, including splice variants, encoding a PNPLA3 protein, including PNPLA3 protein variants or isoforms from any species (e.g. mouse, rat, non-human primate, human). PNPLA3 protein is also known as adiponutrin (ADPN) and calcium-independent phospholipase A2-epsilon (iPLA(2)□)).

[0030] A PNPLA3 mRNA sequence also includes the transcript sequence expressed as its complementary DNA (cDNA) sequence. A cDNA sequence refers to the sequence of an mRNA transcript expressed as DNA bases (e.g. guanine, adenine, thymine, and cytosine) rather than RNA bases (e.g. guanine, adenine, uracil, and cytosine). Thus, the antisense strand of the RNAi constructs of the invention may comprise a region having a sequence that is substantially or fully complementary to a target PNPLA3 mRNA sequence or PNPLA3 cDNA sequence. A PNPLA3 mRNA or cDNA sequence can include, but is not limited to, any PNPLA3 mRNA or cDNA sequence such as can be derived from the NCBI Reference sequence NM_025225.2.

[0031] A region of the antisense strand can be substantially complementary or fully complementary to at least 15 consecutive nucleotides of the PNPLA3 mRNA sequence. In some embodiments, the target region of the PNPLA3 mRNA sequence to which the antisense strand comprises a region of complementarity can range from about 15 to about 30 consecutive

nucleotides, from about 16 to about 28 consecutive nucleotides, from about 18 to about 26 consecutive nucleotides, from about 17 to about 24 consecutive nucleotides, from about 19 to about 25 consecutive nucleotides, from about 19 to about 23 consecutive nucleotides, or from about 19 to about 21 consecutive nucleotides. In certain embodiments, the region of the antisense strand comprising a sequence that is substantially or fully complementary to a PNPLA3 mRNA sequence may, in some embodiments, comprise at least 15 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2. In other embodiments, the antisense sequence comprises at least 16, at least 17, at least 18, or at least 19 contiguous nucleotides from an antisense sequence listed in Table 1 or Table 2. In some embodiments, the sense and/or antisense sequence comprises at least 15 nucleotides from a sequence listed in Table 1 or 2 with no more than 1, 2, or 3 nucleotide mismatches.

[0032] The sense strand of the RNAi construct typically comprises a sequence that is sufficiently complementary to the sequence of the antisense strand such that the two strands hybridize under physiological conditions to form a duplex region. A "duplex region" refers to the region in two complementary or substantially complementary polynucleotides that form base pairs with one another, either by Watson-Crick base pairing or other hydrogen bonding interaction, to create a duplex between the two polynucleotides. The duplex region of the RNAi construct should be of sufficient length to allow the RNAi construct to enter the RNA interference pathway, e.g. by engaging the Dicer enzyme and/or the RISC complex. For instance, in some embodiments, the duplex region is about 15 to about 30 base pairs in length. Other lengths for the duplex region within this range are also suitable, such as about 15 to about 28 base pairs, about 15 to about 26 base pairs, about 15 to about 24 base pairs, about 15 to about 22 base pairs, about 17 to about 28 base pairs, about 17 to about 26 base pairs, about 17 to about 24 base pairs, about 17 to about 23 base pairs, about 17 to about 21 base pairs, about 19 to about 25 base pairs, about 19 to about 23 base pairs, or about 19 to about 21 base pairs. In one embodiment, the duplex region is about 17 to about 24 base pairs in length. In another embodiment, the duplex region is about 19 to about 21 base pairs in length.

[0033] In some embodiments, an RNAi agent of the invention contains a duplex region of about 24 to about 30 nucleotides that interacts with a target RNA sequence, e.g., an PNPLA3 target

mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells can be broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) Genes Dev. 15:485). Dicer, a ribonuclease-III-like enzyme, 15 processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) Nature 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) Cell107:309). Upon binding to the appropriate target 20 mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15: 188).

[0034] For embodiments in which the sense strand and antisense strand are two separate molecules (e.g. RNAi construct comprises a siRNA), the sense strand and antisense strand need not be the same length as the length of the duplex region. For instance, one or both strands maybe longer than the duplex region and have one or more unpaired nucleotides or mismatches flanking the duplex region. Thus, in some embodiments, the RNAi construct comprises at least one nucleotide overhang. As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that extend beyond the duplex region at the terminal ends of the strands. Nucleotide overhangs are typically created when the 3' end of one strand extends beyond the 5' end of the other strand or when the 5' end of one strand extends beyond the 3' end of the other strand. The length of a nucleotide overhang is generally between 1 and 6 nucleotides, 1and 5 nucleotides, 1 and 4 nucleotides, 1 and 3 nucleotides, 2 and 6 nucleotides, 2 and 5 nucleotides, or 2 and 4 nucleotides. In some embodiments, the nucleotide overhang comprises 1, 2, 3, 4, 5, or 6 nucleotides. In one particular embodiment, the nucleotide overhang comprises 1 to 4 nucleotides. In certain embodiments, the nucleotide overhang comprises 2 nucleotides. The nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified nucleotides as described herein. In some embodiments, the overhang comprises a 5'-uridineuridine-3' (5'-UU-3') dinucleotide. In such embodiments, the UU dinucleotide may comprise ribonucleotides or modified nucleotides, e.g. 2'-modified nucleotides. In other embodiments, the overhang comprises a 5'-deoxythymidine-deoxythymidine-3' (5'-dTdT-3') dinucleotide.

[0035] The nucleotide overhang can be at the 5' end or 3' end of one or both strands. For example, in one embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the sense strand. In some embodiments, the RNAi construct comprises a nucleotide overhang at the 5' end of the sense strand and the 5' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand and the 3' end of the antisense strand.

[0036] The RNAi constructs may comprise a single nucleotide overhang at one end of the double-stranded RNA molecule and a blunt end at the other. A "blunt end" means that the sense strand and antisense strand are fully base-paired at the end of the molecule and there are no unpaired nucleotides that extend beyond the duplex region. In some embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand and a blunt end at the 5' end of the sense strand and 3' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the antisense strand and a blunt end at the 5' end of the antisense strand and the 3' end of the sense strand. In certain embodiments, the RNAi construct comprises a blunt end at both ends of the double-stranded RNA molecule. In such embodiments, the sense strand and antisense strand have the same length and the duplex region is the same length as the sense and antisense strands (i.e. the molecule is double-stranded over its entire length).

[0037] The sense strand and antisense strand can each independently be about 15 to about 30 nucleotides in length, about 18 to about 28 nucleotides in length, about 19 to about 27 nucleotides in length, about 19 to about 25 nucleotides in length, about 19 to about 23 nucleotides in length, about 21 to about 25 nucleotides in length, or about 21 to about 23 nucleotides in length. In certain embodiments, the sense strand and antisense strand are each about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 nucleotides in length. In some embodiments, the sense strand and antisense strand have the same length but form a duplex region that is shorter than the strands such that the RNAi construct has two nucleotide overhangs. For instance, in one embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 21 nucleotides in length, (ii) a duplex region that is 19 base pairs in length, and (iii) nucleotide

overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 23 nucleotides in length, (ii) a duplex region that is 21 base pairs in length, and (iii) nucleotide overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In other embodiments, the sense strand and antisense strand have the same length and form a duplex region over their entire length such that there are no nucleotide overhangs on either end of the double-stranded molecule. In one such embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 21 nucleotides in length, and (ii) a duplex region that is 21 base pairs in length. In another such embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 23 nucleotides in length, and (ii) a duplex region that is 23 base pairs in length.

[0038] In other embodiments, the sense strand or the antisense strand is longer than the other strand and the two strands form a duplex region having a length equal to that of the shorter strand such that the RNAi construct comprises at least one nucleotide overhang. For example, in one embodiment, the RNAi construct comprises (i) a sense strand that is 19 nucleotides in length, (ii)an antisense strand that is 21 nucleotides in length, (iii) a duplex region of 19 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand that is 21 nucleotides in length, (ii) an antisense strand that is 23 nucleotides in length, (iii) a duplex region of 21 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand.

[0039] The antisense strand of the RNAi constructs of the invention can comprise the sequence of any one of the antisense sequences listed in Table 1 or Table 2 or the sequence of nucleotides 1-19 of any of these antisense sequences. Each of the antisense sequences listed in Tables 1 and 6 comprises a sequence of 19 consecutive nucleotides (first 19 nucleotides counting from the 5' end) that is complementary to a PNPLA3 mRNA sequence plus a two nucleotide overhang sequence. Thus, in some embodiments, the antisense strand comprises a sequence of nucleotides 1-19 of any one of SEQ ID NOs: 1-166 or 167-332.

Modified nucleotides

[0040] The RNAi constructs of the invention may comprise one or more modified nucleotides. A "modified nucleotide" refers to a nucleotide that has one or more chemical modifications to the nucleoside, nucleobase, pentose ring, or phosphate group. As used herein, modified nucleotides do not encompass ribonucleotides containing adenosine monophosphate, guanosine monophosphate, uridine monophosphate, and cytidine monophosphate, and deoxyribonucleotides containing deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxythymidine monophosphate, and deoxycytidine monophosphate. However, the RNAi constructs may comprise combinations of modified nucleotides, ribonucleotides, and deoxyribonucleotides. Incorporation of modified nucleotides into one or both strands of double-stranded RNA molecules can improve the in vivo stability of the RNA molecules, e.g., by reducing the molecules' susceptibility to nucleases and other degradation processes. The potency of RNAi constructs for reducing expression of the target gene can also be enhanced by incorporation of modified nucleotides.

[0041] In certain embodiments, the modified nucleotides have a modification of the ribose sugar. These sugar modifications can include modifications at the 2' and/or 5' position of the pentose ring as well as bicyclic sugar modifications. A 2'-modified nucleotide refers to a nucleotide having a pentose ring with a substituent at the 2' position other than H or OH. Such 2' modifications include, but are not limited to, 2'-O-alkyl (e.g. O-C1-C10 or O-C1-C10 substituted alkyl), 2'-O-allyl (O-CH2CH=CH2), 2'-C-allyl, 2'-fluoro, 2'-O-methyl (OCH3), 2'-O-methoxyethyl (O-(CH2)2OCH3), 2'-OCF3, 2'-O(CH2)2SCH3, 2'-O-aminoalkyl, 2'-amino (e.g.NH2), 2'-O-ethylamine, and 2'-azido. Modifications at the 5' position of the pentose ring include, but are not limited to, 5'-methyl (R or S); 5'-vinyl, and 5'-methoxy.

[0042] A "bicyclic sugar modification" refers to a modification of the pentose ring where a bridge connects two atoms of the ring to form a second ring resulting in a bicyclic sugar structure. In some embodiments the bicyclic sugar modification comprises a bridge between the 4' and 2' carbons of the pentose ring. Nucleotides comprising a sugar moiety with a bicyclic sugar modification are referred to herein as bicyclic nucleic acids or BNAs. Exemplary bicyclic sugar modifications include, but are not limited to, □-L-Methyleneoxy (4'-CH2-O-2') bicyclicnucleic

acid (BNA); □-D-Methyleneoxy (4'-CH2-O-2') BNA (also referred to as a locked nucleic acid or LNA); Ethyleneoxy (4'-(CH2)2-O-2') BNA; Aminooxy (4'-CH2-O-N(R)- 2')BNA; Oxyamino (4'-CH2-N(R)-O-2') BNA; Methyl(methyleneoxy) (4'-CH(CH3)-O-2') BNA (also referred to as constrained ethyl or cEt); methylene-thio (4'-CH2-S-2') BNA; methylene-amino (4'-CH2-N(R)-2') BNA; methyl carbocyclic (4'-CH2-CH(CH3)-2') BNA; propylene carbocyclic (4'-(CH2)3-2') BNA; and Methoxy(ethyleneoxy) (4'-CH(CH2OMe)-O-2')BNA (also referred to as constrained MOE or cMOE). These and other sugar-modified nucleotides that can be incorporated into the RNAi constructs of the invention are described in U.S. Patent No. 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, Chemistry and Biology, Vol. 19: 937-954, 2012, all of which are hereby incorporated by reference in their entireties.

[0043] In some embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNAs), or combinations thereof. In certain embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, or combinations thereof. In one particular embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides or combinations thereof.

[0044] Both the sense and antisense strands of the RNAi constructs can comprise one or multiple modified nucleotides. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In certain embodiments, all nucleotides in the sense strand are modified nucleotides. In some embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In other embodiments, all nucleotides in the antisense strand are modified nucleotides. In certain other embodiments, all nucleotides in the sense strand and all nucleotides in the antisense strand are modified nucleotides. In these and other embodiments, the modified nucleotides can be 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof.

[0045] In some embodiments, all pyrimidine nucleotides preceding an adenosine nucleotide in the sense strand, antisense strand, or both strands are modified nucleotides. For example, where the sequence 5'-CA-3' or 5'-UA-3' appears in either strand, the cytidine and uridine nucleotides are

modified nucleotides, preferably 2'-O-methyl modified nucleotides. In certain embodiments, all pyrimidine nucleotides in the sense strand are modified nucleotides (e.g. 2'-O-methyl modified nucleotides), and the 5' nucleotide in all occurrences of the sequence 5'-CA-3' or 5'-UA-3' in the antisense strand are modified nucleotides (e.g. 2'-O-methyl modified nucleotides). In other embodiments, all nucleotides in the duplex region are modified nucleotides. In such embodiments, the modified nucleotides are preferably 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides or combinations thereof.

[0046] In embodiments in which the RNAi construct comprises a nucleotide overhang, the nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified nucleotides. In one embodiment, the nucleotides in the overhang are deoxyribonucleotides, e.g., deoxythymidine. In another embodiment, the nucleotides in the overhang are modified nucleotides. For instance, in some embodiments, the nucleotides in the overhang are 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, 2'-methoxyethyl modified nucleotides, or combinations thereof.

[0047] The RNAi constructs of the invention may also comprise one or more modified internucleotide linkages. As used herein, the term "modified internucleotide linkage" refers to an internucleotide linkage other than the natural 3' to 5' phosphodiester linkage. In some embodiments, the modified internucleotide linkage is a phosphorous-containing internucleotide linkage, such as a phosphotriester, aminoalkyl phosphotriester, an alkylphosphonate (e.g.methylphosphonate, 3' -alkylene phosphonate), a phosphinate, a phosphoramidate (e.g. 3'-aminophosphoramidate and aminoalkylphosphoramidate), a phosphorothioate (P=S), a chiralphosphorothioate, a phosphorodithioate, a thionophosphoramidate, a thionoalkylphosphonate, athionoalkylphosphotriester, and a boranophosphate. In one embodiment, a modified internucleotide linkage is a 2' to 5' phosphodiester linkage. In other embodiments, the modified internucleotide linkage is a non-phosphorous-containing internucleotide linkage and thus can be referred to as a modified internucleoside linkage. Such non-phosphorous-containing linkages include, but are not limited to, morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane linkages (-O-Si(H)2-O-); sulfide, sulfoxide and sulfone linkages; formacetyl and thioformacetyl linkages; alkene containing backbones;

sulfamate backbones; methylenemethylimino (-CH2-N(CH3)-O-CH2-) and methylenehydrazino linkages; sulfonate and sulfonamide linkages; amide linkages; and others having mixed N, O, S and CH2 component parts. In one embodiment, the modified internucleoside linkage is a peptide-based linkage (e.g. aminoethylglycine) to create a peptide nucleic acid or PNA, such as those described in U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262. Other suitable modified internucleotide and internucleoside linkages that may be employed in the RNAi constructs of the invention are described in U.S. Patent No. 6,693,187, U.S. Patent No. 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, Chemistry and Biology, Vol. 19: 937-954, 2012, all of which are hereby incorporated by reference in their entireties.

[0048] In certain embodiments, the RNAi constructs comprise one or more phosphorothioate internucleotide linkages. The phosphorothioate internucleotide linkages may be present in the sense strand, antisense strand, or both strands of the RNAi constructs. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. In other embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7,8, or more phosphorothioate internucleotide linkages. In still other embodiments, both strands comprise 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. The RNAi constructs can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For instance, in certain embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 3'-end of the sense strand, the antisense strand, or both strands. In other embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In one embodiment, the RNAi construct comprises a single phosphorothioate internucleotide linkage at the 3' end of the sense strand and a single phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand (i.e. a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at the 3' end of the antisense strand). In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and

5' ends of the antisense strand. In yet another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at the 5' end of the sense strand. In still another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the sense strand (i.e. a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both the 5' and 3' ends of the antisense strand and a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both the 5' and 3' ends of the sense strand). In any of the embodiments in which one or both strands comprises one or more phosphorothioate internucleotide linkages, the remaining internucleotide linkages within the strands can be the natural 3' to 5' phosphodiester linkages. For instance, in some embodiments, each internucleotide linkage of the sense and antisense strands is selected from phosphodiester and phosphorothioate, wherein at least one internucleotide linkage is a phosphorothioate.

[0049] In embodiments in which the RNAi construct comprises a nucleotide overhang, two or more of the unpaired nucleotides in the overhang can be connected by a phosphorothioate internucleotide linkage. In certain embodiments, all the unpaired nucleotides in a nucleotide overhang at the 3' end of the antisense strand and/or the sense strand are connected by phosphorothioate internucleotide linkages. In other embodiments, all the unpaired nucleotides in a nucleotide overhang at the 5' end of the antisense strand and/or the sense strand are connected by phosphorothioate internucleotide linkages. In still other embodiments, all the unpaired nucleotides in any nucleotide overhang are connected by phosphorothioate internucleotide linkages.

[0050] In certain embodiments, the modified nucleotides incorporated into one or both of the strands of the RNAi constructs of the invention have a modification of the nucleobase (also referred to herein as "base"). A "modified nucleobase" or "modified base" refers to a base other than the naturally occurring purine bases adenine (A) and guanine (G) and pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases can be synthetic or naturally occurring modifications and include, but are not limited to, universal bases, 5-methylcytosine (5-

me-C), 5-hydroxymethyl cytosine, xanthine (X), hypoxanthine (I), 2-aminoadenine, 6-methyladenine, 6-methylguanine, and other alkyl derivatives of adenine and guanine, 2-propyland 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-daazaadenine and 3-deazaguanine and 3-deazaadenine.

[0051] In some embodiments, the modified base is a universal base. A "universal base" refers to a base analog that indiscriminately forms base pairs with all of the natural bases in RNA and DNA without altering the double helical structure of the resulting duplex region. Universal bases are known to those of skill in the art and include, but are not limited to, inosine, C-phenyl, C-naphthyl and other aromatic derivatives, azole carboxamides, and nitroazole derivatives, such as3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole.

[0052] Other suitable modified bases that can be incorporated into the RNAi constructs of the invention include those described in Herdewijn, Antisense Nucleic Acid Drug Dev., Vol. 10:297-310, 2000 and Peacock et al., J. Org. Chern., Vol. 76: 7295-7300, 2011, both of which are hereby incorporated by reference in their entireties. The skilled person is well aware that guanine, cytosine, adenine, thymine, and uracil may be replaced by other nucleobases, such as the modified nucleobases described above, without substantially altering the base pairing properties of a polynucleotide comprising a nucleotide bearing such replacement nucleobase.

[0053] In some embodiments of the RNAi constructs of the invention, the 5' end of the sense strand, antisense strand, or both the antisense and sense strands comprises a phosphate moiety. As used herein, the term "phosphate moiety" refers to a terminal phosphate group that includes unmodified phosphates (-O-P=O)(OH)OH) as well as modified phosphates. Modified phosphates include phosphates in which one or more of the O and OH groups is replaced with H, O, S, N(R) or alkyl where R is H, an amino protecting group or unsubstituted or substituted alkyl. Exemplary phosphate moieties include, but are not limited to, 5'-monophosphate; 5'diphosphate; 5'-triphosphate; 5'-guanosine cap (7-methylated or non-methylated); 5'-adenosinecap or any other

modified or unmodified nucleotide cap structure; 5'-monothiophosphate (phosphorothioate); 5'-monodithiophosphate (phosphorodithioate); 5'-alpha-thiotriphosphate; 5'-gamma-thiotriphosphate, 5'-phosphoramidates; 5'-vinylphosphates; 5'-alkylphosphonates (e.g.,alkyl= methyl, ethyl, isopropyl, propyl, etc.); and 5'-alkyletherphosphonates (e.g., alkylether =methoxymethyl, ethoxymethyl, etc.).

[0054] The modified nucleotides that can be incorporated into the RNAi constructs of the invention may have more than one chemical modification described herein. For instance, the modified nucleotide may have a modification to the ribose sugar as well as a modification to the nucleobase. By way of example, a modified nucleotide may comprise a 2' sugar modification(e.g. 2'-fluoro or 2'-methyl) and comprise a modified base (e.g. 5-methyl cytosine or pseudouracil). In other embodiments, the modified nucleotide may comprise a sugar modification in combination with a modification to the 5' phosphate that would create a modified internucleotide or internucleoside linkage when the modified nucleotide was incorporated into a polynucleotide. For instance, in some embodiments, the modified nucleotide may comprise a sugar modification, such as a 2'-fluoro modification, a 2'-O-methyl modification, or a bicyclic sugar modification, as well as a 5' phosphorothioate group. Accordingly, in some embodiments, one or both strands of the RNAi constructs of the invention comprise a combination of 2' modified nucleotides or BNAs and phosphorothioate internucleotide linkages. In certain embodiments, both the sense and antisense strands of the RNAi constructs of the invention comprise a combination of 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, and phosphorothioate internucleotide linkages. Exemplary RNAi constructs comprising modified nucleotides and internucleotide linkages are shown in Table 2.

Function of RNAi constructs

[0055] Preferably, the RNAi constructs of the invention reduce or inhibit the expression of PNPLA3 in cells, particularly liver cells. Accordingly, in one embodiment, the present invention provides a method of reducing PNPLA3 expression in a cell by contacting the cell with any RNAi construct described herein. The cell may be in vitro or in vivo. PNPLA3 expression can be assessed by measuring the amount or level of PNPLA3 mRNA, PNPLA3 protein, or another biomarker linked to PNPLA3 expression. The reduction of PNPLA3 expression in cells or animals treated

with an RNAi construct of the invention can be determined relative to the PNPLA3 expression in cells or animals not treated with the RNAi construct or treated with a control RNAi construct. For instance, in some embodiments, reduction of PNPLA3 expression is assessed by (a) measuring the amount or level of PNPLA3 mRNA in liver cells treated with a RNAi construct of the invention, (b) measuring the amount or level of PNPLA3 mRNA in liver cells treated with a control RNAi construct (e.g., RNAi agent directed to a RNA molecule not expressed in liver cells or a RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured PNPLA3 mRNA levels from treated cells in (a) to the measured PNPLA3 mRNA levels from control cells in (b). The PNPLA3 mRNA levels in the treated cells and controls cells can be normalized to RNA levels for a control gene (e.g. 18S ribosomal RNA) prior to comparison. PNPLA3 mRNA levels can be measured by a variety of methods, including Northern blot analysis, nuclease protection assays, fluorescence in situ hybridization (FISH), reverse-transcriptase (RT)-PCR, real-time RT-PCR, quantitative PCR, and the like.

[0056] In other embodiments, reduction of PNPLA3 expression is assessed by (a) measuring the amount or level of PNPLA3 protein in liver cells treated with a RNAi construct of the invention,(b) measuring the amount or level of PNPLA3 protein in liver cells treated with a control RNAi construct (e.g. RNAi agent directed to a RNA molecule not expressed in liver cells or a RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured PNPLA3 protein levels from treated cells in (a) to the measured PNPLA3 protein levels from control cells in (b). Methods of measuring PNPLA3 protein levels are known to those of skill in the art, and include Western Blots, immunoassays (e.g. ELISA), and flow cytometry. An exemplary immunoassay-based method for assessing PNPLA3 protein expression is described in Example 2. Example 3 describes an exemplary method for measuring PNPLA3 mRNA using RNA FISH. Any method capable of measuring PNPLA3 mRNA or protein can be used to assess the efficacy of the RNAi constructs of the invention.

[0057] In some embodiments, the methods to assess PNPLA3 expression levels are performed in vitro in cells that natively express PNPLA3 (e.g. liver cells) or cells that have been engineered to express PNPLA3. In certain embodiments, the methods are performed in vitro in liver cells. Suitable liver cells include, but are not limited to, primary hepatocytes (e.g. human, non-human

primate, or rodent hepatocytes), HepAD38 cells, HuH-6 cells, HuH-7 cells, HuH-5-2 cells,BNLCL2 cells, Hep3B cells, or HepG2 cells. In one embodiment, the liver cells are Hep3B cells. In another embodiment, the liver cells are HepG2 cells.

[0058] In other embodiments, the methods to assess PNPLA3 expression levels are performed in vivo. The RNAi constructs and any control RNAi constructs can be administered to an animal (e.g. rodent or non-human primate) and PNPLA3 mRNA or protein levels assessed in liver tissue harvested from the animal following treatment. Alternatively or additionally, a biomarker or functional phenotype associated with PNPLA3 expression can be assessed in the treated animals.

[0059] In certain embodiments, expression of PNPLA3 is reduced in liver cells by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% by an RNAi construct of the invention. In some embodiments, expression of PNPLA3 is reduced in liver cells by at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% by an RNAi construct of the invention. In other embodiments, the expression of PNPLA3 is reduced in liver cells by about 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%,97%, 98%, 99%, or more by an RNAi construct of the invention. The percent reduction of PNPLA3 expression can be measured by any of the methods described herein as well as others known in the art. For instance, in certain embodiments, the RNAi constructs of the invention inhibit at least 45% of PNPLA3 expression at 5 nM in Hep3B cells (contains wild type PNPLA3) in vitro. In related embodiments, the RNAi constructs of the invention inhibit at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of PNPLA3 expression at 5 nM in Hep3B cells in vitro. In other embodiments, the RNAi constructs of the invention inhibit at least 80%, at least85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of PNPLA3 expression at 5 nM in Hep3B cells in vitro. In certain embodiments, the RNAi constructs of the invention inhibit at least 45% of PNPLA3 expression at 5 nM in HepG2 cells (contains the PNPLA3-rs738409-rs738408 double minor allele) in vitro. In related embodiments, the RNAi constructs of the invention inhibit at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of PNPLA3 expression at 5 nM in HepG2 cells in vitro. In other embodiments, the RNAi constructs of the invention inhibit at least 80%, at least85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of PNPLA3 expression at 5 nM in HepG2 cells in vitro. In certain embodiments, the RNAi constructs of the invention inhibit at least 45%

of PNPLA3 expression at 5 nM in CHO transfectedcells expressing human PNPLA3 I148I or I148M cells in vitro. In related embodiments, the RNAi constructs of the invention inhibit at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of PNPLA3 expression at 5 nM in CHO transfectedcells expressing human PNPLA3 I148I or I148M in vitro. In other embodiments, the RNAi constructs of the invention inhibit at least 80%, at least85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of PNPLA3 expression at 5 nM in CHO transfectedcells expressing human PNPLA3 I148I or I148M in vitro. Reduction of PNPLA3 can be measured using a variety of techniques including RNA FISH or droplet digital PCR, as described in Examples 2 and 3.

[0060] In some embodiments, an IC50 value is calculated to assess the potency of an RNAi construct of the invention for inhibiting PNPLA3 expression in liver cells. An "IC50 value" is the dose/concentration required to achieve 50% inhibition of a biological or biochemical function. The IC50 value of any particular substance or antagonist can be determined by constructing a dose-response curve and examining the effect of different concentrations of the substance or antagonist on expression levels or functional activity in any assay. IC50 values can be calculated for a given antagonist or substance by determining the concentration needed to inhibit half of the maximum biological response or native expression levels. Thus, the IC50 value for any RNAi construct can be calculated by determining the concentration of the RNAi construct needed to inhibit half of the native PNPLA3 expression level in liver cells (e.g. PNPLA3 expression level in control liver cells) in any assay, such as the immunoassay or RNA FISH assay or droplet digital PCR assays described in the Examples. The RNAi constructs of the invention may inhibit PNPLA3 expression in liver cells (e.g. Hep3B cells) with an IC50 of less than about 20 nM. For example, the RNAi constructs inhibit PNPLA3 expression in liver cells with an IC50 of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits PNPLA3 expression in liver cells (e.g. Hep3B cells) with an IC50 of about 1 nM to about 10 nM. The RNAi constructs of the invention may inhibit PNPLA3 expression in liver cells (e.g. HepG2 cells) with an IC50 of less than about 20 nM. For example, the RNAi constructs inhibit PNPLA3 expression in liver cells with an IC50 of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about

0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits PNPLA3 expression in liver cells (e.g. HepG2 cells) with an IC50 of about 1 nM to about 10 nM. The RNAi constructs of the invention may inhibit PNPLA3 expression in liver cells (e.g. CHO transfected cells expressing human PNPLA3 I148I or I148M) with an IC50 of less than about 20 nM. For example, the RNAi constructs inhibit PNPLA3 expression in liver cells with an IC50 of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits PNPLA3 expression in liver cells (e.g. CHO transfected cells expressing human PNPLA3 I148I or I148M) with an IC50 of about 1 nM to about 10 nM.

[0061] The RNAi constructs of the invention can readily be made using techniques known in the art, for example, using conventional nucleic acid solid phase synthesis. The polynucleotides of the RNAi constructs can be assembled on a suitable nucleic acid synthesizer utilizing standard nucleotide or nucleoside precursors (e.g. phosphoramidites). Automated nucleic acid synthesizers are sold commercially by several vendors, including DNA/RNA synthesizers from Applied Biosystems (Foster City, CA), MerMade synthesizers from BioAutomation (Irving,TX), and OligoPilot synthesizers from GE Healthcare Life Sciences (Pittsburgh, PA).

[0062] The 2' silyl protecting group can be used in conjunction with acid labile dimethoxytrityl (DMT) at the 5' position of ribonucleosides to synthesize oligonucleotides via phosphoramidite chemistry. Final deprotection conditions are known not to significantly degrade RNA products. All syntheses can be conducted in any automated or manual synthesizer on large, medium, or small scale. The syntheses may also be carried out in multiple well plates, columns, or glass slides.

[0063] The 2'-O-silyl group can be removed via exposure to fluoride ions, which can include any source of fluoride ion, e.g., those salts containing fluoride ion paired with inorganic counterions, e.g., cesium fluoride and potassium fluoride or those salts containing fluoride ion paired with an organic counterion, e.g., a tetraalkylammonium fluoride. A crown ether catalyst can be utilized in combination with the inorganic fluoride in the deprotection reaction. Preferred

fluoride ion source are tetrabutylammonium fluoride or aminohydrofluorides (e.g., combining aqueous HF with triethylamine in a dipolar aprotic solvent, e.g., dimethylformamide).

[0064] The choice of protecting groups for use on the phosphite triesters and phosphotriesters can alter the stability of the triesters towards fluoride. Methyl protection of the phosphotriester or phosphitetriester can stabilize the linkage against fluoride ions and improve process yields.

[0065] Since ribonucleosides have a reactive 2' hydroxyl substituent, it can be desirable to protect the reactive 2' position in RNA with a protecting group that is orthogonal to a 5'-O-dimethoxytrityl protecting group, e.g., one stable to treatment with acid. Silyl protecting groups meet this criterion and can be readily removed in a final fluoride deprotection step that can result in minimal RNA degradation.

[0066] Tetrazole catalysts can be used in the standard phosphoramidite coupling reaction. Preferred catalysts include, e.g., tetrazole, S-ethyl-tetrazole, benzylthiotetrazole, pnitrophenyltetrazole.

[0067] As can be appreciated by the skilled artisan, further methods of synthesizing the RNAi constructs described herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Other synthetic chemistry transformations, protecting groups (e.g., for hydroxyl, amino, etc. present on the bases) and protecting group methodologies (protection and deprotection) useful in synthesizing the RNAi constructs described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof Custom synthesis of RNAi agents is also available from several commercial vendors, including Dharmacon, Inc. (Lafayette, CO), AxoLabs GmbH (Kulmbach, Germany),and Ambion, Inc. (Foster City, CA).

[0068] The RNAi constructs of the invention may comprise a ligand. As used herein, a "ligand" refers to any compound or molecule that is capable of interacting with another compound

or molecule, directly or indirectly. The interaction of a ligand with another compound or molecule may elicit a biological response (e.g. initiate a signal transduction cascade, induce receptor mediated endocytosis) or may just be a physical association. The ligand can modify one or more properties of the double-stranded RNA molecule to which is attached, such as the pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties of the RNA molecule.

[0069] The ligand may comprise a serum protein (e.g., human serum albumin, low-density lipoprotein, globulin), a cholesterol moiety, a vitamin (biotin, vitamin E, vitamin B12), a folate moiety, a steroid, a bile acid (e.g. cholic acid), a fatty acid (e.g., palmitic acid, myristic acid), a carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid), a glycoside, a phospholipid, or antibody or binding fragment thereof (e.g. antibody or binding fragment that targets the RNAi construct to a specific cell type, such as liver). Other examples of ligands include dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons(e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g. EDTA), lipophilic molecules,e.g, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-BisO(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, 03-(oleoyl)lithocholic acid, 03-(oleoyl)cholenic acid,dimethoxytrityl, or phenoxazine), peptides (e.g., antennapedia peptide, Tat peptide, RGDpeptides), alkylating agents, polymers, such as polyethylene glycol (PEG)(e.g., PEG-40K),poly amino acids, and polyamines (e.g. spermine, spermidine).

[0070] In certain embodiments, the ligands have endosomolytic properties. The endosomolytic ligands promote the lysis of the endosome and/or transport of the RNAi construct of the invention, or its components, from the endosome to the cytoplasm of the cell. The endosomolytic ligand may be a polycationic peptide or peptidomimetic which shows pH dependent membrane activity and fusogenicity. In one embodiment, the endosomolytic ligand assumes its active conformation at endosomal pH. The "active" conformation is that conformation in which the endosomolytic ligand promotes lysis of the endosome and/or transport of the RNAi construct of the invention, or its components, from the endosome to the cytoplasm of the cell.

Exemplary endosomolytic ligands include the GALA peptide (Subbarao et al., Biochemistry, Vol. 26: 2964-2972, 1987), the EALA peptide (Vogel et al., J. Am. Chern. Soc.,Vol. 118: 1581-1586, 1996), and their derivatives (Turk et al., Biochem. Biophys. Acta, Vol.1559: 56-68, 2002). In one embodiment, the endosomolytic component may contain a chemical group (e.g., an amino acid) which will undergo a change in charge or protonation in response to a change in pH. The endosomolytic component may be linear or branched.

[0071] In some embodiments, the ligand comprises a lipid or other hydrophobic molecule. In one embodiment, the ligand comprises a cholesterol moiety or other steroid. Cholesterol conjugated oligonucleotides have been reported to be more active than their unconjugated counterparts (Manoharan, Antisense Nucleic Acid Drug Development, Vol. 12: 103-228, 2002).Ligands comprising cholesterol moieties and other lipids for conjugation to nucleic acid molecules have also been described in U.S. Patent Nos. 7,851,615; 7,745,608; and 7,833,992, all of which are hereby incorporated by reference in their entireties. In another embodiment, the ligand comprises a folate moiety. Polynucleotides conjugated to folate moieties can be taken up by cells via a receptor-mediated endocytosis pathway. Such folate-polynucleotide conjugates are described in U.S. Patent No.8, 188,247, which is hereby incorporated by reference in its entirety.

[0072] Given that PNPLA3 is expressed in liver cells (e.g. hepatocytes), in certain embodiments, it is desirable to specifically deliver the RNAi construct to those liver cells. In some embodiments, RNAi constructs can be specifically targeted to the liver by employing ligands that bind to or interact with proteins expressed on the surface of liver cells. For example, in certain embodiments, the ligands may comprise antigen binding proteins (e.g. antibodies or binding fragments thereof (e.g. Fab, scFv)) that specifically bind to a receptor expressed on hepatocytes.

[0073] In certain embodiments, the ligand comprises a carbohydrate. A "carbohydrate" refers to a compound made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Carbohydrates include, but are not limited to, the sugars (e.g., monosaccharides, disaccharides, trisaccharides, tetrasaccharides, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides, such as starches, glycogen, cellulose and polysaccharide gums. In some embodiments, the carbohydrate incorporated into the ligand is a

monosaccharide selected from a pentose, hexose, or heptose and di- and tri-saccharides including such monosaccharide units. In other embodiments, the carbohydrate incorporated into the ligand is an amino sugar, such as galactosamine, glucosamine, N-acetylgalactosamine, and N-acetylglucosamine.

[0074] In some embodiments, the ligand comprises a hexose or hexosamine. The hexose may be selected from glucose, galactose, mannose, fucose, or fructose. The hexosamine may be selected from fructosamine, galactosamine, glucosamine, or mannosamine. In certain embodiments, the ligand comprises glucose, galactose, galactosamine, or glucosamine. In one embodiment, the ligand comprises glucose, glucosamine, or N-acetylglucosamine. In another embodiment, the ligand comprises galactose, galactosamine, or N-acetyl-galactosamine. In particular embodiments, the ligand comprises N-acetyl-galactosamine. Ligands comprising glucose, galactose, and N-acetyl-galactosamine (GalNAc) are particularly effective in targeting compounds to liver cells. See, e.g., D'Souza and Devarajan, J. Control Release, Vol. 203: 126-139, 2015. Examples of GalNAc- or galactose-containing ligands that can be incorporated into the RNAi constructs of the invention are described in U.S. Patent Nos. 7,491,805; 8,106,022; and 8,877,917; U.S. Patent Publication No. 20030130186; and WIPO Publication No. WO2013166155, all of which are hereby incorporated by reference in their entireties.

[0075] In certain embodiments, the ligand comprises a multivalent carbohydrate moiety. As used herein, a "multivalent carbohydrate moiety" refers to a moiety comprising two or more carbohydrate units capable of independently binding or interacting with other molecules. For example, a multivalent carbohydrate moiety comprises two or more binding domains comprised of carbohydrates that can bind to two or more different molecules or two or more different sites on the same molecule. The valency of the carbohydrate moiety denotes the number of individual binding domains within the carbohydrate moiety. For instance, the terms "monovalent," "bivalent," "trivalent," and "tetravalent" with reference to the carbohydrate moiety refer to carbohydrate moieties with one, two, three, and four binding domains, respectively. The multivalent carbohydrate moiety may comprise a multivalent lactose moiety, a multivalent galactose moiety, a multivalent glucose moiety, a multivalent N-acetyl-galactosamine moiety, a multivalent N-acetyl-glucosamine moiety, a multivalent mannose moiety, or a multivalent fucose moiety. In

some embodiments, the ligand comprises a multivalent galactose moiety. In other embodiments, the ligand comprises a multivalent N-acetyl-galactosamine moiety. In these and other embodiments, the multivalent carbohydrate moiety is bivalent, trivalent, or tetravalent. In such embodiments, the multivalent carbohydrate moiety can be bi-antennary or tri-antennary. In one particular embodiment, the multivalent N-acetyl-galactosamine moiety is trivalent or tetravalent. In another particular embodiment, the multivalent galactose moiety is trivalent or tetravalent. Exemplary trivalent and tetravalent GalNAc-containing ligands for incorporation into the RNAi constructs of the invention are described in detail below.

[0076] The ligand can be attached or conjugated to the RNA molecule of the RNAi construct directly or indirectly. For instance, in some embodiments, the ligand is covalently attached directly to the sense or antisense strand of the RNAi construct. In other embodiments, the ligand is covalently attached via a linker to the sense or antisense strand of the RNAi construct. The ligand can be attached to nucleobases, sugar moieties, or internucleotide linkages of polynucleotides (e.g. sense strand or antisense strand) of the RNAi constructs of the invention. Conjugation or attachment to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In certain embodiments, the 2-, 6-, 7-, or 8-positionsof a purine nucleobase are attached to a ligand. Conjugation or attachment to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2-,5-, and 6-positions of a pyrimidine nucleobase can be attached to a ligand. Conjugation or attachment to sugar moieties of nucleotides can occur at any carbon atom. Example carbon atoms of a sugar moiety that can be attached to a ligand include the 2', 3', and 5' carbon atoms. The 1' position can also be attached to a ligand, such as in an a basic residue. Internucleotide linkages can also support ligand attachments. For phosphorus-containing linkages (e.g., phosphodiester, phosphorothioate, phosphorodithiotate, phosphoroamidate, and the like), the ligand can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleoside linkages (e.g., PNA), the ligand can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

[0077] In certain embodiments, the ligand may be attached to the 3' or 5' end of either the sense or antisense strand. In certain embodiments, the ligand is covalently attached to the 5' end of the

sense strand. In other embodiments, the ligand is covalently attached to the 3' end of the sense strand. For example, in some embodiments, the ligand is attached to the 3'-terminal nucleotide of the sense strand. In certain such embodiments, the ligand is attached at the 3'-position of the 3'-terminal nucleotide of the sense strand. In alternative embodiments, the ligand is attached near the 3' end of the sense strand, but before one or more terminal nucleotides (i.e. before 1, 2, 3, or 4 terminal nucleotides). In some embodiments, the ligand is attached at the 2'-position of the sugar of the 3'-terminal nucleotide of the sense strand.

[0078] In certain embodiments, the ligand is attached to the sense or antisense strand via a linker. A "linker" is an atom or group of atoms that covalently joins a ligand to a polynucleotide component of the RNAi construct. The linker may be from about 1 to about 30 atoms in length, from about 2 to about 28 atoms in length, from about 3 to about 26 atoms in length, from about 4to about 24 atoms in length, from about 6 to about 20 atoms in length, from about 7 to about 20atoms in length, from about 8 to about 20 atoms in length, from about 8 to about 18 atoms in length, from about 10 to about 18 atoms in length, and from about 12 to about 18 atoms in length. In some embodiments, the linker may comprise a bifunctional linking moiety, which generally comprises an alkyl moiety with two functional groups. One of the functional groups is selected to bind to the compound of interest (e.g. sense or antisense strand of the RNAi construct) and the other is selected to bind essentially any selected group, such as a ligand as described herein. In certain embodiments, the linker comprises a chain structure or an oligomer of repeating units, such as ethylene glycol or amino acid units. Examples of functional groups that are typically employed in a bifunctional linking moiety include, but are not limited to, electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like.

[0079] Linkers that may be used to attach a ligand to the sense or antisense strand in the RNAi constructs of the invention include, but are not limited to, pyrrolidine, 8-amino-3,6-di oxaoctanoic acid, succinimidy1 4-(N-maleimidomethy1)cyclohexane-1-carboxylate, 6-aminohexanoic acid, substituted C1-C10 alkyl, substituted or unsubstituted C2-C10 alkenyl or substituted or unsubstituted C2-C10 alkynyl. Preferred substituent groups for such linkers include, but are not

limited to, hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

[0080] In certain embodiments, the linkers are cleavable. A cleavable linker is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In some embodiments, the cleavable linker is cleaved at least 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 100 times faster in the target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

[0081] Cleavable linkers are susceptible to cleavage agents, e.g., pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linker by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linker by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

[0082] A cleavable linker may comprise a moiety that is susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable group that is cleaved at a preferred pH, thereby releasing the RNA molecule from the ligand inside the cell, or into the desired compartment of the cell.

[0083] A linker can include a cleavable group that is cleavable by a particular enzyme. The type of cleavable group incorporated into a linker can depend on the cell to be targeted. For example, liver-targeting ligands can be linked to RNA molecules through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more

efficiently in liver cells than in cell types that are not esterase-rich. Other types of cells rich in esterases include cells of the lung, renal cortex, and testis. Linkers that contain peptide bonds can be used when targeting cells rich in peptidases, such as liver cells and synoviocytes.

[0084] In general, the suitability of a candidate cleavable linker can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linker. It will also be desirable to also test the candidate cleavable linker for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In some embodiments, useful candidate linkers are cleaved at least 2, 4, 10, 20, 50, 70, or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

[0085] In other embodiments, redox cleavable linkers are utilized. Redox cleavable linkers are cleaved upon reduction or oxidation. An example of reductively cleavable group is a disulfide linking group (-S-S-). To determine if a candidate cleavable linker is a suitable "reductively cleavable linker," or for example is suitable for use with a particular RNAi construct and particular ligand, one can use one or more methods described herein. For example, a candidate linker can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent known in the art, which mimics the rate of cleavage that would be observed in a cell, e.g., a target cell. The candidate linkers can also be evaluated under conditions which are selected to mimic blood or serum conditions. In a specific embodiment, candidate linkers are cleaved by at most 10% in the blood. In other embodiments, useful candidate linkers are degraded at least 2, 4, 10, 20, 50,70, or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions).

[0086] In yet other embodiments, phosphate-based cleavable linkers are cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that hydrolyzes phosphate groups in cells are enzymes, such as phosphatases in cells. Examples of phosphate-based cleavable groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Specific embodiments include -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -SP(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. Another specific embodiment is -O-P(O)(OH)-O-. These candidate linkers can be evaluated using methods analogous to those described above.

[0087] In other embodiments, the linkers may comprise acid cleavable groups, which are groups that are cleaved under acidic conditions. In some embodiments, acid cleavable groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower), or by agents, such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes, can provide a cleaving environment for acid cleavable groups. Examples of acid cleavable linking groups include, but are not limited to, hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A specific embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiaryalkyl group such as dimethyl, pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

[0088] In other embodiments, the linkers may comprise ester-based cleavable groups, which are cleaved by enzymes, such as esterases and amidases in cells. Examples of ester-based cleavable groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable groups have the general formula -C(O)O-, or -OC(O) -. These candidate linkers can be evaluated using methods analogous to those described above.

[0089] In further embodiments, the linkers may comprise peptide-based cleavable groups, which are cleaved by enzymes, such as peptidases and proteases in cells. Peptide-based cleavable

groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond(i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula -NHCHRAC(O)NHCHRBC(O) -,where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

[0090] Other types of linkers suitable for attaching ligands to the sense or antisense strands in the RNAi constructs of the invention are known in the art and can include the linkers described in U.S. Patent Nos. 7,723,509; 8,017,762; 8,828,956; 8,877,917; and 9,181,551, all of which are hereby incorporated by reference in their entireties.

[0091] In certain embodiments, the ligand covalently attached to the sense or antisense strand of the RNAi constructs of the invention comprises a GalNAc moiety, e.g, a multivalent GalNAc moiety. In some embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 3' end of the sense strand. In other embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 5' end of the sense strand. In yet other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 3' end of the sense strand. In still other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 5' end of the sense strand.

[0092] In some embodiments, the RNAi constructs of the invention may be delivered to a cell or tissue of interest by administering a vector that encodes and controls the intracellular expression of the RNAi construct. A "vector" (also referred to herein as an "expression vector) is a composition of matter which can be used to deliver a nucleic acid of interest to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. Examples of viral vectors

include, but are not limited to, adenoviral vectors, adeno-associated viral vectors, retroviral vectors, and the like. A vector can be replicated in a living cell, or it can be made synthetically.

[0093] Generally, a vector for expressing an RNAi construct of the invention will comprise one or more promoters operably linked to sequences encoding the RNAi construct. The phrase" operably linked" or "under transcriptional control" as used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide sequence to control the initiation of transcription by RNA polymerase and expression of the polynucleotide sequence. A "promoter" refers to a sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene sequence. Suitable promoters include, but are not limited to, RNA pol I, pol II, HI or U6 RNA pol III, and viral promoters (e.g. human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, and the Rous sarcoma virus long terminal repeat). In some embodiments, a HI or U6RNA pol III promoter is preferred. The promoter can be a tissue-specific or inducible promoter. Of particular interest are liver-specific promoters, such as promoter sequences from human alpha!-antitrypsin gene, albumin gene, hemopexin gene, and hepatic lipase gene. Inducible promoters include promoters regulated by ecdysone, estrogen, progesterone, tetracycline, and isopropyl-PD1-thiogalactopyranoside (IPTG).

[0094] In some embodiments in which the RNAi construct comprises a siRNA, the two separate strands (sense and antisense strand) can be expressed from a single vector or two separate vectors. For example, in one embodiment, the sequence encoding the sense strand is operably linked to a promoter on a first vector and the sequence encoding the antisense strand is operably linked to a promoter on a second vector. In such an embodiment, the first and second vectors are co-introduced, e.g., by infection or transfection, into a target cell, such that the sense and antisense strands, once transcribed, will hybridize intracellularly to form the siRNA molecule. In another embodiment, the sense and antisense strands are transcribed from two separate promoters located in a single vector. In some such embodiments, the sequence encoding the sense strand is operably linked to a first promoter and the sequence encoding the antisense strand is operably linked to a second promoter, wherein the first and second promoters are located in a single vector. In one embodiment, the vector comprises a first promoter operably linked to a sequence encoding the

siRNA molecule, and a second promoter operably linked to the same sequence in the opposite direction, such that transcription of the sequence from the first promoter results in the synthesis of the sense strand of the siRNA molecule and transcription of the sequence from the second promoter results in synthesis of the antisense strand of the siRNA molecule.

[0095] In other embodiments in which the RNAi construct comprises a shRNA, a sequence encoding the single, at least partially self-complementary RNA molecule is operably linked to a promoter to produce a single transcript. In some embodiments, the sequence encoding the shRNA comprises an inverted repeat joined by a linker polynucleotide sequence to produce the stem and loop structure of the shRNA following transcription.

[0096] In some embodiments, the vector encoding an RNAi construct of the invention is a viral vector. Various viral vector systems that are suitable to express the RNAi constructs described herein include, but are not limited to, adenoviral vectors, retroviral vectors (e.g., lentiviral vectors, maloney murine leukemia virus), adeno- associated viral vectors; herpes simplex viral vectors; SV 40 vectors; polyoma viral vectors; papilloma viral vectors; picornaviral vectors; and pox viral vectors (e.g. vaccinia virus). In certain embodiments, the viral vector is a retroviral vector (e.g. lentiviral vector).

[0097] Various vectors suitable for use in the invention, methods for inserting nucleic acid sequences encoding siRNA or shRNA molecules into vectors, and methods of delivering the vectors to the cells of interest are within the skill of those in the art. See, e.g., Dornburg , Gene Therap., Vol. 2: 301-310, 1995; Eglitis, Biotechniques, Vol. 6: 608-614, 1988; Miller, HumGene Therap., Vol. 1: 5-14, 1990; Anderson, Nature, Vol. 392: 25-30, 1998; Rubinson D A et al., Nat. Genet., Vol. 33: 401-406, 2003; Brummelkamp et al., Science, Vol. 296: 550-553, 2002;Brummelkamp et al., Cancer Cell, Vol. 2: 243-247, 2002; Lee et al., Nat Biotechnol, Vol. 20:500-505, 2002; Miyagishi et al., Nat Biotechnol, Vol. 20: 497-500, 2002; Paddison et al., GenesDev, Vol. 16: 948-958, 2002; Paul et al., Nat Biotechnol, Vol. 20: 505-508, 2002; Sui et al., ProcNatl Acad Sci USA, Vol. 99: 5515-5520, 2002; and Yu et al., Proc Natl Acad Sci USA, Vol. 99:6047-6052, 2002, all of which are hereby incorporated by reference in their entireties.

[0098] The present invention also includes pharmaceutical compositions and formulations comprising the RNAi constructs described herein and pharmaceutically acceptable carriers,

excipients, or diluents. Such compositions and formulations are useful for reducing expression ofPNPLA3 in a subject in need thereof Where clinical applications are contemplated, pharmaceutical compositions and formulations will be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[0099] The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier, excipient, or diluent" includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the RNAi constructs of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or RNAi constructs of the compositions.

[0100] Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, type and extent of disease or disorder to be treated, or dose to be administered. In some embodiments, the pharmaceutical compositions are formulated based on the intended route of delivery. For instance, in certain embodiments, the pharmaceutical compositions are formulated for parenteral delivery. Parenteral forms of delivery include intravenous, intraarterial, subcutaneous, intrathecal, intraperitoneal or intramuscular injection or infusion. In one embodiment, the pharmaceutical composition is formulated for intravenous delivery. In such an embodiment, the pharmaceutical composition may include a lipid-based delivery vehicle. In another embodiment, the pharmaceutical composition is formulated for subcutaneous delivery. In such an embodiment, the pharmaceutical composition may include a targeting ligand (e.g. GalNAc containing ligands described herein).

[0101] In some embodiments, the pharmaceutical compositions comprise an effective amount of an RNAi construct described herein. An "effective amount" is an amount sufficient to produce a beneficial or desired clinical result. In some embodiments, an effective amount is an amount sufficient to reduce PNPLA3 expression in hepatocytes of a subject. In some embodiments, an effective amount may be an amount sufficient to only partially reduce PNPLA3 expression, for example, to a level comparable to expression of the wild-type PNPLA3 allele in human heterozygotes. Human heterozygous carriers of loss of function PNPLA3 variant alleles were reported to have lower serum levels of non- HDL cholesterol and a lower risk of coronary artery disease and myocardial infarction as compared to non-carriers (Nioi et al., New England Journal of Medicine, Vol. 374(22):2131-2141, 2016). Thus, without being bound by theory, it is believed that partial reduction of PNPLA3 expression may be sufficient to achieve the beneficial reduction of serum non-HDL cholesterol and reduction of risk of coronary artery disease and myocardial infarction.

[0102] An effective amount of an RNAi construct of the invention may be from about 0.01mg/kg body weight to about 100 mg/kg body weight, about 0.05 mg/kg body weight to about 75mg/kg body weight, about 0.1 mg/kg body weight to about 50 mg/kg body weight, about 1mg/kg to about 30 mg/kg body weight, about 2.5 mg/kg of body weight to about 20 mg/kg bodyweight, or about 5 mg/kg body weight to about 15 mg/kg body weight. In certain embodiments, a single effective dose of an RNAi construct of the invention may be about 0.1 mg/kg, about 0.5mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, or about 10 mg/kg. The pharmaceutical composition comprising an effective amount of RNAi construct can be administered weekly, biweekly, monthly, quarterly, or biannually. The precise determination of what would be considered an effective amount and frequency of administration may be based on several factors, including a patient's size, age, and general condition, type of disorder to be treated (e.g. myocardial infarction, heart failure, coronary artery disease, hypercholesterolemia), particular RNAi construct employed, and route of administration. Estimates of effective dosages and in vivo half-lives for any particular RNAi construct of the invention can be ascertained using conventional methods and/or testing in appropriate animal models.

[0103] Administration of the pharmaceutical compositions of the present invention may be via any common route so long as the target tissue is available via that route. Such routes include, but are not limited to, parenteral (e.g., subcutaneous, intramuscular, intraperitoneal or intravenous),oral, nasal, buccal, intradermal, transdermal, and sublingual routes, or by direct injection into liver tissue or delivery through the hepatic portal vein. In some embodiments, the pharmaceutical composition is administered parenterally. For instance, in certain embodiments, the pharmaceutical composition is administered intravenously. In other embodiments, the pharmaceutical composition is administered subcutaneously.

[0104] Colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes, may be used as delivery vehicles for the RNAi constructs of the invention or vectors encoding such constructs. Commercially available fat emulsions that are suitable for delivering the nucleic acids of the invention include Intralipid®, Liposyn®, Liposyn®II, Liposyn®III, Nutrilipid, and other similar lipid emulsions. A preferred colloidal system for use as a delivery vehicle in vivo is a liposome (i.e., an artificial membrane vesicle).The RNAi constructs of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi constructs of the invention may be complexed to lipids, in particular to cationic lipids. Suitable lipids and liposomes include neutral (e.g., dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), and dipalmitoyl phosphatidylcholine (DPPC)), distearolyphosphatidyl choline), negative (e.g., dimyristoylphosphatidyl glycerol (DMPG)), andcationic (e.g., dioleoyltetramethylaminopropyl (DOTAP) and dioleoylphosphatidyl ethanolamine (DOTMA)). The preparation and use of such colloidal dispersion systems is well known in the art. Exemplary formulations are also disclosed in U.S. Pat. No. 5,981,505; U.S. Pat. No.6,217,900; U.S. Pat. No. 6,383,512; U.S. Pat. No. 5,783,565; U.S. Pat. No. 7,202,227; U.S. Pat. No. 6,379,965; U.S. Pat. No. 6,127,170; U.S. Pat. No. 5,837,533; U.S. Pat. No. 6,747,014; andW003/093449.

[0105] In some embodiments, the RNAi constructs of the invention are fully encapsulated in a lipid formulation, e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As

used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a noncationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate).SNALPs and SPLPs are exceptionally useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous injection and accumulate at distal sites (e.g., sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO00/03683. The nucleic acid-lipid particles typically have a mean diameter of about 50 nm to about 150 nm, about 60 nm to about 130 nm, about 70 nm to about 110 nm, or about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO96/40964.

[0106] The pharmaceutical compositions suitable for injectable use include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0107] Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, e.g., as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof

[0108] The compositions of the present invention generally may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with free amino groups) derived from inorganic acids (e.g., hydrochloric or phosphoric acids), or from organic acids (e.g., acetic, oxalic, tartaric, mandelic, and the like). Salts formed with the free carboxyl groups can also be derived from inorganic bases (e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (e.g., isopropylamine, trimethylamine, histidine, procaine and the like).

[0109] For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic for example with sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA standards. In certain embodiments, a pharmaceutical composition of the invention comprises or consists of a sterile saline solution and an RNAi construct described herein. In other embodiments, a pharmaceutical composition of the invention comprises or consists of an RNAi construct described herein and sterile water (e.g. water for

injection, WFI). In still other embodiments, a pharmaceutical composition of the invention comprises or consists of an RNAi construct described herein and phosphate-buffered saline (PBS).

[0110] In some embodiments, the pharmaceutical compositions of the invention are packaged with or stored within a device for administration. Devices for injectable formulations include, but are not limited to, injection ports, pre-filled syringes, auto injectors, injection pumps, on-body injectors, and injection pens. Devices for aerosolized or powder formulations include, but are not limited to, inhalers, insufflators, aspirators, and the like. Thus, the present invention includes administration devices comprising a pharmaceutical composition of the invention for treating or preventing one or more of the disorders described herein.

Methods for inhibiting PNPLA3 expression

[0111] The present invention also provides methods of inhibiting expression of a PNPLA3 gene in a cell. The methods include contacting a cell with an RNAi agent, e.g., double stranded RNAi agent, in an amount effective to inhibit expression of PNPLA3 in the cell, thereby inhibiting expression of PNPLA3 in the cell. Contacting of a cell with an RNAi agent, e.g., a double stranded RNAi agent, may be done in vitro or in vivo. Contacting a cell in vivo with the RNAi agent includes contacting a cell or group of cells within a subject, e.g., a human subject, with the RNAi agent. Combinations of in vitro and in vivo methods of contacting a cell are also possible.

[0112] The present invention provides methods for reducing or inhibiting expression of PNPLA3 in a subject in need thereof as well as methods of treating or preventing conditions, diseases, or disorders associated with PNPLA3 expression or activity. A "condition, disease, or disorder associated with PNPLA3 expression" refers to conditions, diseases, or disorders in which PNPLA3 expression levels are altered or where elevated expression levels of PNPLA3 are associated with an increased risk of developing the condition, disease or disorder.

[0113] Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, e.g., a GalNAc3 ligand, or any other ligand that directs the RNAi agent to a site of interest.

[0114] In one embodiment, contacting a cell with an RNAi includes "introducing" or "delivering the RNAi into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an RNAi can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an RNAi into a cell may be in vitro and/or in vivo. For example, for in vivo introduction, RNAi can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

[0115] The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

[0116] The phrase "inhibiting expression of a PNPLA3" is intended to refer to inhibition of expression of any PNPLA3 gene (such as, e.g., a mouse PNPLA3 gene, a rat PNPLA3 gene, a monkey PNPLA3 gene, or a human PNPLA3 gene) as well as variants or mutants of a PNPLA3 gene. Thus, the PNPLA3 gene may be a wild-type PNPLA3 gene, a mutant PNPLA3 gene (such as a mutant PNPLA3 gene giving rise to amyloid deposition), or a transgenic PNPLA3 gene in the context of a genetically manipulated cell, group of cells, or organism.

[0117] "Inhibiting expression of a PNPLA3 gene" includes any level of inhibition of a PNPLA3 gene, e.g., at least partial suppression of the expression of a PNPLA3 gene. The expression of the PNPLA3 gene may be assessed based on the level, or the change in the level, of any variable associated with PNPLA3 gene expression, e.g., PNPLA3 mRNA level, PNPLA3 protein level, or the number or extent of amyloid deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

[0118] Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with PNPLA3 expression compared with a control level. The control level may be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control). In some embodiments of the methods of the invention, expression of a PNPLA3 gene is inhibited by at least about 5%, at least about

10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%,at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

[0119] Inhibition of the expression of a PNPLA3 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a PNPLA3 gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an RNAi agent of the invention, or by administering an RNAi agent of the invention to a subject in which the cells are or were present) such that the expression of a PNPLA3 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

[0120] Alternatively, inhibition of the expression of a PNPLA3 gene may be assessed in terms of a reduction of a parameter that is functionally linked to PNPLA3 gene expression, e.g. , PNPLA3 protein expression or Hedgehog pathway protein activities. PNPLA3 gene silencing may be determined in any cell expressing PNPLA3, either constitutively or by genomic engineering, and by any assay known in the art.

[0121] Inhibition of the expression of a PNPLA3 protein may be manifested by a reduction in the level of the PNPLA3 protein that is expressed by a cell or group of cells (e.g. , the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of

mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

[0122] A control cell or group of cells that may be used to assess the inhibition of the expression of a PNPLA3 gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an RNAi agent.

[0123] The level of PNPLA3 mRNA that is expressed by a cell or group of cells, or the level of circulating PNPLA3 mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of PNPLA3 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the PNPLA3 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton et al., Nuc. Acids Res. 12:7035), Northern blotting, in situ hybridization, and microarray analysis. Circulating PNPLA3 mRNA may be detected using methods the described in PCT/US2012/043584, the entire contents of which are hereby incorporated herein by reference.

[0124] In one embodiment, the level of expression of PNPLA3 is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific PNPLA3. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

[0125] Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to PNPLA3 mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example

by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of PNPLA3 mRNA.

[0126] An alternative method for determining the level of expression of PNPLA3 in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88: 189-193), self sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87: 1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6: 1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of PNPLA3 is determined by quantitative fluorogenic RT-PCR {i.e., the TaqMan™ System). The expression levels of PNPLA3 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of PNPLA3 expression level may also comprise using nucleic acid probes in solution.

[0127] In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

[0128] The level of PNPLA3 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin

layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), Immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

[0129] In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in a symptom of a PNPLA3 disease, such as reduction in edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; or abdominal pain. These symptoms may be assessed in vitro or in vivo using any method known in the art.

[0130] In some embodiments of the methods of the invention, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of PNPLA3 may be assessed using measurements of the level or change in the level of PNPLA3 mRNA or PNPLA3 protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

Methods of treating or preventing PNPLA3-Associated Diseases

[0131] The present invention provides therapeutic and prophylactic methods which include administering to a subject with a PNPLA3 -associated disease, disorder, and/or condition, or prone to developing, a PNPLA3- associated disease, disorder, and/or condition, compositions comprising an RNAi agent, or pharmaceutical compositions comprising an RNAi agent, or vectors comprising an RNAi of the invention. Non-limiting examples of PNPLA3- associated diseases include, for example, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). In one embodiment, the PNPLA3-associated disease is NAFLD. In another embodiment, the PNPLA3 -associated disease is NASH. In another embodiment, the PNPLA3 -associated disease is fatty liver (steatosis). In another embodiment, the

PNPLA3-associated disease is insulin resistance. In another embodiment, the PNPLA3-associated disease is not insulin resistance.

[0132] In certain embodiments, the present invention provides a method for reducing the expression of PNPLA3 in a patient in need thereof comprising administering to the patient any of the RNAi constructs described herein. The term "patient," as used herein, refers to a mammal, including humans, and can be used interchangeably with the term "subject." Preferably, the expression level of PNPLA3 in hepatocytes in the patient is reduced following administration of the RNAi construct as compared to the PNPLA3 expression level in a patient not receiving the RNAi construct.

[0133] The methods of the invention are useful for treating a subject having a PNPLA3-associated disease, e.g., a subject that would benefit from reduction in PNPLA3 gene expression and/or PNPLA3 protein production. In one aspect, the present invention provides methods of reducing the level of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) gene expression in a subject having nonalcoholic fatty liver disease (NAFLD). In another aspect, the present invention provides methods of reducing the level of PNPLA3 protein in a subject with NAFLD. The present invention also provides methods of reducing the level of activity of the hedgehog pathway in a subject with NAFLD.

[0134] In another aspect, the present invention provides methods of treating a subject having an NAFLD. In one aspect, the present invention provides methods of treating a subject having an PNPLA3-associated disease, e.g., fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, or nonalcoholic fatty liver disease (NAFLD). The treatment methods (and uses) of the invention include administering to the subject, e.g., a human, a therapeutically effective amount of an RNAi agent of the invention targeting a PNPLA3 gene or a pharmaceutical composition comprising an RNAi agent of the invention targeting a PNPLA3 gene or a vector of the invention comprising an RNAi agent targeting an PNPLA3 gene.

[0135] In one aspect, the invention provides methods of preventing at least one symptom in a subject having NAFLD, e.g., the presence of elevated hedgehog signaling pathways, fatigue, weakness, weight loss, loss of apetite, nausea, abdominal pain, spider-like blood vessels, yellowing

of the skin and eyes (jaundice), itching, fluid build up and swelling of the legs (edema), abdomen swelling (ascites), and mental confusion. The methods include administering to the subject a therapeutically effective amount of the RNAi agent, e.g. dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in PNPLA3 gene expression.

[0136] In another aspect, the present invention provides uses of a therapeutically effective amount of an RNAi agent of the invention for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of PNPLA3 gene expression. In a further aspect, the present invention provides uses of an RNAi agent, e.g. , a dsRNA, of the invention targeting an PNPLA3 gene or pharmaceutical composition comprising an RNAi agent targeting an PNPLA3 gene in the manufacture of a medicament for treating a subject, e.g. , a subject that would benefit from a reduction and/or inhibition of PNPLA3 gene expression and/or PNPLA3 protein production, such as a subject having a disorder that would benefit from reduction in PNPLA3 gene expression, e.g., a PNPLA3- associated disease.

[0137] In another aspect, the invention provides uses of an RNAi, e.g., a dsRNA, of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of PNPLA3 gene expression and/or PNPLA3 protein production.

[0138] In a further aspect, the present invention provides uses of an RNAi agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of PNPLA3 gene expression and/or PNPLA3 protein production, such as a PNPLA3-associated disease.

[0139] In one embodiment, an RNAi agent targeting PNPLA3 is administered to a subject having a PNPLA3-associated disease, e.g., nonalcoholic fatty liver disease (NAFLD), such that the expression of a PNPLA3 gene, e.g. , in a cell, tissue, blood or other tissue or fluid of the subject are reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%,

73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more when the dsRNA agent is administered to the subject.

[0140] The methods and uses of the invention include administering a composition described herein such that expression of the target PNPLA3 gene is decreased, such as for about 1, 2, 3, 4 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In one embodiment, expression of the target PNPLA3 gene is decreased for an extended duration, e.g., at least about two, three, four, five, six, seven days or more, e.g., about one week, two weeks, three weeks, or about four weeks or longer.

[0141] Administration of the dsRNA according to the methods and uses of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a PNPLA3- associated disease, e.g., nonalcoholic fatty liver disease (NAFLD). By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%. Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of NAFLD may be assessed, for example, by periodic monitoring of NAFLD symptoms, liver fat levels, or expression of downstream genes. Comparison of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an RNAi targeting PNPLA3 or pharmaceutical composition thereof, "effective against" an PNPLA3 - associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of

symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating NAFLD and/or an PNPLA3 -associated disease and the related causes.

[0142] A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given RNAi drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

[0143] Subjects can be administered a therapeutic amount of RNAi, such as about 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.3 mg/kg, 0.35 mg/kg, 0.4 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.65 mg/kg, 0.7 mg/kg, 0.75 mg/kg, 0.8 mg/kg, 0.85 mg/kg, 0.9 mg/kg, 0.95 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg dsRNA, 2.6 mg/kg dsRNA, 2.7 mg/kg dsRNA, 2.8 mg/kg dsRNA, 2.9 mg/kg dsRNA, 3.0 mg/kg dsRNA, 3.1 mg/kg dsRNA, 3.2 mg/kg dsRNA, 3.3 mg/kg dsRNA, 3.4 mg/kg dsRNA, 3.5 mg/kg dsRNA, 3.6 mg/kg dsRNA, 3.7 mg/kg dsRNA, 3.8 mg/kg dsRNA, 3.9 mg/kg dsRNA, 4.0 mg/kg dsRNA, 4.1 mg/kg dsRNA, 4.2 mg/kg dsRNA, 4.3 mg/kg dsRNA, 4.4 mg/kg dsRNA, 4.5 mg/kg dsRNA, 4.6 mg/kg dsRNA, 4.7 mg/kg dsRNA, 4.8 mg/kg dsRNA, 4.9 mg/kg dsRNA, 5.0 mg/kg dsRNA, 5.1 mg/kg dsRNA, 5.2 mg/kg dsRNA, 5.3 mg/kg dsRNA, 5.4 mg/kg dsRNA, 5.5 mg/kg dsRNA, 5.6 mg/kg dsRNA, 5.7 mg/kg dsRNA, 5.8 mg/kg dsRNA, 5.9 mg/kg dsRNA, 6.0 mg/kg dsRNA, 6.1 mg/kg dsRNA, 6.2 mg/kg dsRNA, 6.3 mg/kg dsRNA, 6.4 mg/kg dsRNA, 6.5 mg/kg dsRNA, 6.6 mg/kg dsRNA, 6.7 mg/kg dsRNA, 6.8 mg/kg dsRNA, 6.9 mg/kg dsRNA, 7.0 mg/kg dsRNA, 7.1 mg/kg dsRNA, 7.2 mg/kg dsRNA, 7.3 mg/kg dsRNA, 7.4 mg/kg dsRNA, 7.5 mg/kg dsRNA, 7.6 mg/kg dsRNA, 7.7 mg/kg dsRNA, 7.8 mg/kg dsRNA, 7.9 mg/kg dsRNA, 8.0 mg/kg dsRNA, 8.1 mg/kg dsRNA, 8.2 mg/kg dsRNA, 8.3 mg/kg dsRNA, 8.4 mg/kg dsRNA, 8.5 mg/kg dsRNA, 8.6 mg/kg dsRNA, 8.7 mg/kg

dsRNA, 8.8 mg/kg dsRNA, 8.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 9.1 mg/kg dsRNA, 9.2 mg/kg dsRNA, 9.3 mg/kg dsRNA, 9.4 mg/kg dsRNA, 9.5 mg/kg dsRNA, 9.6 mg/kg dsRNA, 9.7 mg/kg dsRNA, 9.8 mg/kg dsRNA, 9.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 10 mg/kg dsRNA, 15 mg/kg dsRNA, 20 mg/kg dsRNA, 25 mg/kg dsRNA, 30 mg/kg dsRNA, 35 mg/kg dsRNA, 40 mg/kg dsRNA, 45 mg/kg dsRNA, or about 50 mg/kg dsRNA. In one embodiment, subjects can be administered 0.5 mg/kg of the dsRNA. Values and ranges intermediate to the recited values are also intended to be part of this invention.

[0144] Administration of the RNAi can reduce the presence of PNPLA3 protein levels, e.g. , in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31 %, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

[0145] Before administration of a full dose of the RNAi, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (e.g. , TNF-alpha or INF-alpha) levels.

[0146] Owing to the inhibitory effects on PNPLA3 expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

[0147] An RNAi of the invention may be administered in "naked" form, where the modified or unmodified RNAi agent is directly suspended in aqueous or suitable buffer solvent, as a "free RNAi." A free RNAi is administered in the absence of a pharmaceutical composition. The free RNAi may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolality of the buffer solution containing the RNAi can be adjusted such that it is suitable for administering to a subject.

[0148] Alternatively, an RNAi of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

[0149] Subjects that would benefit from a reduction and/or inhibition of PNPLA3 gene expression are those having nonalcoholic fatty liver disease (NAFLD) and/or an PNPLA3-associated disease or disorder as described herein.

[0150] Treatment of a subject that would benefit from a reduction and/or inhibition of PNPLA3 gene expression includes therapeutic and prophylactic treatment.

[0151] The invention further provides methods and uses of an RNAi agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of PNPLA3 gene expression, e.g., a subject having a PNPLA3-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, e.g. , with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

[0152] For example, in certain embodiments, an RNAi targeting a PNPLA3 gene is administered in combination with, e.g., an agent useful in treating an PNPLA3-associated disease as described elsewhere herein. For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in PNPLA3 expression, e.g., a subject having a PNPLA3-associated disease, include an RNAi agent targeting a different portion of the PNPLA3 gene, a therapeutic agent, and/or procedures for treating a PNPLA3 -associated disease or a combination of any of the foregoing.

[0153] In certain embodiments, a first RNAi agent targeting a PNPLA3 gene is administered in combination with a second RNAi agent targeting a different portion of the PNPLA3 gene. For example, the first RNAi agent comprises a first sense strand and a first antisense strand forming a double stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'- terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and the second RNAi agent comprises a second sense strand and a second antisense strand forming a

double stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3 '-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

[0154] In one embodiment, all of the nucleotides of the first and second sense strand and/or all of the nucleotides of the first and second antisense strand comprise a modification.

[0155] In one embodiment, the at least one of the modified nucleotides is selected from the group consisting of a 3 '-terminal deoxy-thymine (dT) nucleotide, a 2'-0-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2' -amino-modified nucleotide, a 2' -O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2' -hydroxly- modified nucleotide, a 2'- methoxyethyl modified nucleotide, a 2'-0-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5 '-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

[0156] In certain embodiments, a first RNAi agent targeting a PNPLA3 gene is administered in combination with a second RNAi agent targeting a gene that is different from the PNPLA3 gene. For example, the RNAi agent targeting the PNPLA3 gene may be administered in combination with an RNAi agent targeting the SCAP gene. The first RNAi agent targeting a PNPLA3 gene and the second RNAi agent targeting a gene different from the PNPLA3 gene, e.g. , the SCAP gene, may be administered as parts of the same pharmaceutical composition. Alternatively, the first RNAi agent targeting a PNPLA3 gene and the second RNAi agent targeting a gene different from the PNPLA3 gene, e.g. , the SCAP gene, may be administered as parts of different pharmaceutical compositions.

[0157] The RNAi agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, e.g. , parenterally, or the additional

therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

[0158] The present invention also provides methods of using an RNAi agent of the invention and/or a composition containing an RNAi agent of the invention to reduce and/or inhibit PNPLA3 expression in a cell. In other aspects, the present invention provides an RNAi of the invention and/or a composition comprising an RNAi of the invention for use in reducing and/or inhibiting PNPLA3 gene expression in a cell. In yet other aspects, use of an RNAi of the invention and/or a composition comprising an RNAi of the invention for the manufacture of a medicament for reducing and/or inhibiting PNPLA3 gene expression in a cell are provided. In still other aspects, the the present invention provides an RNAi of the invention and/or a composition comprising an RNAi of the invention for use in reducing and/or inhibiting PNPLA3 protein production in a cell. In yet other aspects, use of an RNAi of the invention and/or a composition comprising an RNAi of the invention for the manufacture of a medicament for reducing and/or inhibiting PNPLA3 protein production in a cell are provided. The methods and uses include contacting the cell with an RNAi, e.g., a dsRNA, of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of an PNPLA3 gene, thereby inhibiting expression of the PNPLA3 gene or inhibiting PNPLA3 protein production in the cell.

[0159] Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of PNPLA3 may be determined by determining the mRNA expression level of PNPLA3 using methods routine to one of ordinary skill in the art, e.g., Northern blotting, qRT-PCR, by determining the protein level of PNPLA3 using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques, flow cytometry methods, ELISA, and/or by determining a biological activity of PNPLA3.

[0160] In the methods and uses of the invention the cell may be contacted in vitro or in vivo, i.e., the cell may be within a subject.

[0161] A cell suitable for treatment using the methods of the invention may be any cell that expresses an PNPLA3 gene, e.g., a cell from a subject having NAFLD or a cell comprising an expression vector comprising a PNPLA3 gene or portion of a PNPLA3 gene. A cell suitable for use in the methods and uses of the invention may be a mammalian cell, e.g., a primate cell (such

as a human cell or a non-human primate cell, e.g., a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (e.g., a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell.

[0162] PNPLA3 gene expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0163] PNPLA3 protein production may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0164] The in vivo methods and uses of the invention may include administering to a subject a composition containing an RNAi, where the RNAi includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the PNPLA3 gene of the mammal to be treated. When the organism to be treated is a human, the composition can be administered by any means known in the art including, but not limited to subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by subcutaneous or intravenous infusion or injection. In one embodiment, the compositions are administered by subcutaneous injection.

[0165] In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired inhibition of PNPLA3, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

[0166] In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi to the subject.

[0167] The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

[0168] In one aspect, the present invention also provides methods for inhibiting the expression of an PNPLA3 gene in a mammal, e.g., a human. The present invention also provides a composition comprising an RNAi, e.g., a dsRNA, that targets an PNPLA3 gene in a cell of a mammal for use in inhibiting expression of the PNPLA3 gene in the mammal. In another aspect, the present invention provides use of an RNAi, e.g., a dsRNA, that targets an PNPLA3 gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the PNPLA3 gene in the mammal.

[0169] The methods and uses include administering to the mammal, e.g., a human, a composition comprising an RNAi, e.g., a dsRNA, that targets an PNPLA3 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the PNPLA3 gene, thereby inhibiting expression of the PNPLA3 gene in the mammal.

[0170] Reduction in gene expression can be assessed in peripheral blood sample of the RNAi-administered subject by any methods known it the art, e.g. qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, e.g., ELISA or Western blotting, described herein. In one embodiment, a tissue sample serves as the tissue material for monitoring the reduction in PNPLA3 gene and/or protein expression. In another embodiment, a blood sample serves as the tissue material for monitoring the reduction in PNPLA3 gene and/or protein expression.

[0171] In one embodiment, verification of RISC medicated cleavage of target in vivo following administration of RNAi agent is done by performing 5 '-RACE or modifications of the protocol as known in the art (Lasham A et al., (2010) Nucleic Acid Res., 38 (3) p-el9) (Zimmermann et al. (2006) Nature 441: 111-4).

[0172] It is understood that all ribonucleic acid sequences disclosed herein can be converted to deoxyribonucleic acid sequences by substituting a thymine base for a uracil base in the sequence. Likewise, all deoxyribonucleic acid sequences disclosed herein can be converted to ribonucleic acid sequences by substituting a uracil base for a thymine base in the sequence. Deoxyribonucleic acid sequences, ribonucleic acid sequences, and sequences containing mixtures of deoxyribonucleotides and ribonucleotides of all sequences disclosed herein are included in the invention.

[0173] Additionally, any nucleic acid sequences disclosed herein may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified polynucleotides is, in certain instances, arbitrary. For example, a polynucleotide comprising a nucleotide having a 2'-OH substituent on the ribose sugar and a thymine base could be described as a DNA molecule having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA molecule having a modified base(thymine (methylated uracil) for natural uracil of RNA).

[0174] Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of a further example and without limitation, a polynucleotide

having the sequence "ATCGATCG" encompasses any polynucleotides having such a sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG" and polynucleotides having other modified bases, such as "ATmeCGAUCG," wherein meC indicates a cytosine base comprising a methyl group at the 5-position.

[0175] The following examples, including the experiments conducted and the results achieved, are provided for illustrative purposes only and are not to be construed as limiting the scope of the appended claims.

INCORPORATION BY REFERENCE

[0176] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

EQUIVALENTS

[0177] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain preferred embodiments of the invention and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

[0178] The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention.

EXAMPLE 1: Selection, Design and Synthesis of Modified PNPLA3 siRNA molecules

[0001] The identification and selection of optimal sequences for therapeutic siRNA molecules targeting patatin-like phospholipase domain-containing 3 (PNPLA3) were identified using bioinformatics analysis of a human PNPLA3 transcript (NM_025225.2). Table 1 shows sequences identified as having therapeutic properties. Throughout the various sequences, {INVAB} is an inverted A basic, {INVDA} is an inverted deoxythymidine, GNA is a glycol nucleic acid, dT is deoxythymidine and dC is deoxycytosine.

Table 1. siRNA sequences directed to PNPLA3

Duplex No.	Sense sequence (5'-3')	SEQ ID NO: (sense)	Antisense sequence (5'-3')	SEQ ID NO: (antisense)
D-1000	GGGCAAUAAAGUACCUGCUUU	1	AGCAGGUACUUUAUUGCCCUU	2
D-1001	CGGCCAAUGUCCACCAGCUUU	3	AGCUGGUGGACAUUGGCCGUU	4
D-1002	GGUCCAGCCUGAACUUCUUUU	5	AAGAAGUUCAGGCUGGACCUU	6
D-1003	GCUUCAUCCCCUUCUACAGUU	7	CUGUAGAAGGGGAUGAAGCUU	8
D-1004	GCGGCUUCCUGGGCUUCUAUU	9	UAGAAGCCCAGGAAGCCGCUU	10
D-1005	GCCUCUGAGCUGAGUUGGUUU	11	ACCAACUCAGCUCAGAGGCUU	12
D-1006	GUGACAACGUACCCUUCAUUU	13	AUGAAGGGUACGUUGUCACUU	14
D-1007	CCCGCCUCCAGGUCCCAAAUU	15	UUUGGGACCUGGAGGCGGGUU	16
D-1008	CUUCAUCCCCUUCUACAGUUU	17	ACUGUAGAAGGGGAUGAAGUU	18
D-1009	GGUAUGUUCCUGCUUCAUGUU	19	CAUGAAGCAGGAACAUACCUU	20
D-1010	GUAUGUUCCUGCUUCAUGCUU	21	GCAUGAAGCAGGAACAUACUU	22
D-1011	UAUGUUCCUGCUUCAUGCCUU	23	GGCAUGAAGCAGGAACAUAUU	24
D-1012	AUGUUCCUGCUUCAUGCCCUU	25	GGGCAUGAAGCAGGAACAUUU	26

D-1013	UGUUCCUGCUUCAUGCCCUUU	27	AGGGCAUGAAGCAGGAACAUU	28
D-1014	GUUCCUGCUUCAUGCCCUUUU	29	AAGGGCAUGAAGCAGGAACUU	30
D-1015	UUCCUGCUUCAUGCCCUUCUU	31	GAAGGGCAUGAAGCAGGAAUU	32
D-1016	UCCUGCUUCAUGCCCUUCUUU	33	AGAAGGGCAUGAAGCAGGAUU	34
D-1017	CCUGCUUCAUGCCCUUCUAUU	35	UAGAAGGGCAUGAAGCAGGUU	36
D-1018	CUGCUUCAUGCCCUUCUACUU	37	GUAGAAGGGCAUGAAGCAGUU	38
D-1019	UGCUUCAUGCCCUUCUACAUU	39	UGUAGAAGGGCAUGAAGCAUU	40
D-1020	GCUUCAUGCCCUUCUACAGUU	41	CUGUAGAAGGGCAUGAAGCUU	42
D-1021	CUUCAUGCCCUUCUACAGUUU	43	ACUGUAGAAGGGCAUGAAGUU	44
D-1022	UUCAUGCCCUUCUACAGUGUU	45	CACUGUAGAAGGGCAUGAAUU	46
D-1023	UCAUGCCCUUCUACAGUGGUU	47	CCACUGUAGAAGGGCAUGAUU	48
D-1024	CAUGCCCUUCUACAGUGGCUU	49	GCCACUGUAGAAGGGCAUGUU	50
D-1025	AUGCCCUUCUACAGUGGCCUU	51	GGCCACUGUAGAAGGGCAUUU	52
D-1026	UGCCCUUCUACAGUGGCCUUU	53	AGGCCACUGUAGAAGGGCAUU	54
D-1027	GCCCUUCUACAGUGGCCUUUU	55	AAGGCCACUGUAGAAGGGCUU	56
D-1028	GGUAUGUUCCUGCUUCAUCUU	57	GAUGAAGCAGGAACAUACCUU	58
D-1029	GUAUGUUCCUGCUUCAUCCUU	59	GGAUGAAGCAGGAACAUACUU	60
D-1030	UAUGUUCCUGCUUCAUCCCUU	61	GGGAUGAAGCAGGAACAUAUU	62
D-1031	AUGUUCCUGCUUCAUCCCCUU	63	GGGGAUGAAGCAGGAACAUUU	64
D-1032	UGUUCCUGCUUCAUCCCCUUU	65	AGGGGAUGAAGCAGGAACAUU	66
D-1033	GUUCCUGCUUCAUCCCCUUUU	67	AAGGGGAUGAAGCAGGAACUU	68
D-1034	UUCCUGCUUCAUCCCCUUCUU	69	GAAGGGGAUGAAGCAGGAAUU	70
D-1035	UCCUGCUUCAUCCCCUUCUUU	71	AGAAGGGGAUGAAGCAGGAUU	72
D-1036	CCUGCUUCAUCCCCUUCUAUU	73	UAGAAGGGGAUGAAGCAGGUU	74
D-1037	CUGCUUCAUCCCCUUCUACUU	75	GUAGAAGGGGAUGAAGCAGUU	76
D-1038	UGCUUCAUCCCCUUCUACAUU	77	UGUAGAAGGGGAUGAAGCAUU	78

D-1039	UUCAUCCCCUUCUACAGUGUU	79	CACUGUAGAAGGGGAUGAAUU	80
D-1040	UCAUCCCCUUCUACAGUGGUU	81	CCACUGUAGAAGGGGAUGAUU	82
D-1041	CAUCCCCUUCUACAGUGGCUU	83	GCCACUGUAGAAGGGGAUGUU	84
D-1042	UCCCCUUCUACAGUGGCCUUU	85	AGGCCACUGUAGAAGGGGAUU	86
D-1043	GAUCAGGACCCGAGCCGAUUU	87	AUCGGCUCGGGUCCUGAUCUU	88
D-1044	UGGGCUUCUACCACGUCGUUU	89	ACGACGUGGUAGAAGCCCAUU	90
D-1045	GAGCGAGCACGCCCCGCAUUU	91	AUGCGGGGCGUGCUCGCUCUU	92
D-1046	UGCACUGCGUCGGCGUCCUUU	93	AGGACGCCGACGCAGUGCAUU	94
D-1047	UGGAGCAGACUCUGCAGGUUU	95	ACCUGCAGAGUCUGCUCCAUU	96
D-1048	UGCAGGUCCUCUCAGAUCUUU	97	AGAUCUGAGAGGACCUGCAUU	98
D-1049	CCCGGCCAAUGUCCACCAUUU	99	AUGGUGGACAUUGGCCGGGUU	100
D-1050	UUCUACAGUGGCCUUAUCUUU	101	AGAUAAGGCCACUGUAGAAUU	102
D-1051	UCUACAGUGGCCUUAUCCUUU	103	AGGAUAAGGCCACUGUAGAUU	104
D-1052	CUUCCUUCAGAGGCGUGCUUU	105	AGCACGCCUCUGAAGGAAGUU	106
D-1053	UUCCUUCAGAGGCGUGCGAUU	107	UCGCACGCCUCUGAAGGAAUU	108
D-1054	GCGUGCGAUAUGUGGAUGUUU	109	ACAUCCACAUAUCGCACGCUU	110
D-1055	CGUGCGAUAUGUGGAUGGAUU	111	UCCAUCCACAUAUCGCACGUU	112
D-1056	UGGAUGGAGGAGUGAGUGAUU	113	UCACUCACUCCUCCAUCCAUU	114
D-1057	ACGUACCCUUCAUUGAUGUUU	115	ACAUCAAUGAAGGGUACGUUU	116
D-1058	UGGACAUCACCAAGCUCAUUU	117	AUGAGCUUGGUGAUGUCCAUU	118
D-1059	CACCUGCGUCUCAGCAUCUUU	119	AGAUGCUGAGACGCAGGUGUU	120
D-1060	ACCUGCGUCUCAGCAUCCUUU	121	AGGAUGCUGAGACGCAGGUUU	122
D-1061	CCAGAGACUGGUGACAUGUUU	123	ACAUGUCACCAGUCUCUGGUU	124
D-1062	AUGGCUUCCAGAUAUGCCUUU	125	AGGCAUAUCUGGAAGCCAUUU	126
D-1063	CCGCCUCCAGGUCCCAAAUUU	127	AUUUGGGACCUGGAGGCGGUU	128
D-1064	UACCUGCUGGUGCUGAGGUUU	129	ACCUCAGCACCAGCAGGUAUU	130

D-1065	ACCUGCUGGUGCUGAGGGUUU	131	ACCCUCAGCACCAGCAGGUUU	132
D-1066	CUCUCCACCUUUCCCAGUUUU	133	AACUGGGAAAGGUGGAGAGUU	134
D-1067	UUUUUCACCUAACUAAAAUUU	135	AUUUUAGUUAGGUGAAAAAUU	136
D-1068	CGGCCAAUGUCCACCAGCUUU	137	AGCUGGUGGACAUUGGCCGUU	138
D-1069	GGUCCAGCCUGAACUUCUUUU	139	AAGAAGUUCAGGCUGGACCUU	140
D-1070	GCGGCUUCCUGGGCUUCUAUU	141	UAGAAGCCCAGGAAGCCGCUU	142
D-1071	GUGACAACGUACCCUUCAUUU	143	AUGAAGGGUACGUUGUCACUU	144
D-1072	GGUAUGUUCCUGCUUCAUGUU	145	CAUGAAGCAGGAACAUACCUU	146
D-1073	GUAUGUUCCUGCUUCAUGCUU	147	GCAUGAAGCAGGAACAUACUU	148
D-1074	UGUUCCUGCUUCAUGCCCUUU	149	AGGGCAUGAAGCAGGAACAUU	150
D-1075	GUUCCUGCUUCAUGCCCUUUU	151	AAGGGCAUGAAGCAGGAACUU	152
D-1076	CCUGCUUCAUGCCCUUCUAUU	153	UAGAAGGGCAUGAAGCAGGUU	154
D-1077	GCUUCAUGCCCUUCUACAGUU	155	CUGUAGAAGGGCAUGAAGCUU	156
D-1078	CUUCAUGCCCUUCUACAGUUU	157	ACUGUAGAAGGGCAUGAAGUU	158
D-1079	UUCAUGCCCUUCUACAGUGUU	159	CACUGUAGAAGGGCAUGAAUU	160
D-1080	AUGGCUUCCAGAUAUGCCUUU	161	AGGCAUAUCUGGAAGCCAUUU	162
D-1081	AUGCCCUUCUACAGUGGCCUU	163	GGCCACUGUAGAAGGGCAUUU	164
D-1082	GCUUCAUGCCCUUCUACAUUU	165	AUGUAGAAGGGCAUGAAGCUU	166
D-1083	GGAAAGACUGUUCCAAAAAUU	333	UUUUUGGAACAGUCUUUCCUU	334
D-1084	GGUAUGUUCCUGCUUCAUGUU	335	CAUGAAGCAGGAACAUACCUU	336
D-1085	GUAUGUUCCUGCUUCAUGCUU	337	GCAUGAAGCAGGAACAUACUU	338
D-1086	UGUUCCUGCUUCAUGCCCUUU	339	AGGGCAUGAAGCAGGAACAUU	340
D-1087	GCUUCAUGCCCUUCUACAGUU	341	CUGUAGAAGGGCAUGAAGCUU	342
D-1088	CUUCAUGCCCUUCUACAGUUU	343	ACUGUAGAAGGGCAUGAAGUU	344
D-1089	GCGGCUUCCUGGGCUUCUAUU	345	UAGAAGCCCAGGAAGCCGCUU	346
D-1090	GUUCCUGCUUCAUGCCCUUUU	347	AAGGGCAUGAAGCAGGAACUU	348

D-1091	AUGGCUUCCAGAUAUGCCUUU	349	AGGCAUAUCUGGAAGCCAUUU	350
D-1092	CCUGCUUCAUGCCCUUCUAUU	351	UAGAAGGGCAUGAAGCAGGUU	352
D-1093	UUCAUGCCCUUCUACAGUUUU	353	AACUGUAGAAGGGCAUGAAUU	354
D-1094	UUCAUGCCCUUCUACAGUGUU	355	CACUGUAGAAGGGCAUGAAUU	356
D-1095	GCUUCAUGCCCUUCUACAUUU	357	AUGUAGAAGGGCAUGAAGCUU	358
D-1096	GGUCCAGCCUGAACUUCUUUU	359	AAGAAGUUCAGGCUGGACCUU	360
D-1097	GCGGCUUCCUGGGCUUCUAUU	361	UAGAAGCCCAGGAAGCCGCUU	362
D-1098	GCGGCUUCCUGGGCUUCUAUU	363	UAGAAGCCCAGGAAGCCGCUU	364
D-1099	GUUCCUGCUUCAUGCCCUUUU	365	AAGGGCAUGAAGCAGGAACUU	366
D-1100	GUUCCUGCUUCAUGCCCUUUU	367	AAGGGCAUGAAGCAGGAACUU	368
D-1101	CCUGCUUCAUGCCCUUCUAUU	369	UAGAAGGGCAUGAAGCAGGUU	370
D-1102	CCUGCUUCAUGCCCUUCUAUU	371	UAGAAGGGCAUGAAGCAGGUU	372
D-1103	AUGCCCUUCUACAGUGGCCUU	373	GGCCACUGUAGAAGGGCAUUU	374
D-1104	AUGGCUUCCAGAUAUGCCUUU	375	AGGCAUAUCUGGAAGCCAUUU	376
D-1105	GUGACAACGUACCCUUCAUUU	377	AUGAAGGGUACGUUGUCACUU	378
D-1106	GUAUGUUCCUGCUUCAUGCUU	379	GCAUGAAGCAGGAACAUACUU	380
D-1107	GUAUGUUCCUGCUUCAUGCUU	381	GCAUGAAGCAGGAACAUACUU	382
D-1108	GUAUGUUCCUGCUUCAUGCUU	383	GCAUGAAGCAGGAACAUACUU	384
D-1109	GUAUGUUCCUGCUUCAUGCCU	385	AGGCAUGAAGCAGGAACAUACUU	386
D-1110	UGGUAUGUUCCUGCUUCAUGU	387	GCAUGAAGCAGGAACAUACCAUU	388
D-1111	GUAUGUUCCUGCUUCAUGU	389	GCAUGAAGCAGGAACAUACUU	390
D-1112	GUAUGUUCCUGCUUCAUGC{INVAB}	391	GCAUGAAGCAGGAACAUACUU	392
D-1113	GCUUCAUGCCCUUCUACAUUU	393	AUGUAGAAGGGCAUGAAGCUU	394
D-1114	GCUUCAUGCCCUUCUACAUUU	395	AUGUAGAAGGGCAUGAAGCUU	396
D-1115	GCUUCAUGCCCUUCUACAUUU	397	AUGUAGAAGGGCAUGAAGCUU	398
D-1116	GCUUCAUGCCCUUCUACAUUU	399	AUGUAGAAGGGCAUGAAGCUU	400

D-1117	GCUUCAUGCCCUUCUACAUUU	401	AUGUAGAAGGGCAUGAAGCUU	402
D-1118	GCUUCAUGCCCUUCUACAUUU	403	AUGUAGAAGGGCAUGAAGCUU	404
D-1119	GCUUCAUGCCCUUCUACAUUU	405	AUGUAGAAGGGCAUGAAGCUU	406
D-1120	GCUUCAUGCCCUUCUACAUUU	407	AUGUAGAAGGGCAUGAAGCUU	408
D-1121	GCUUCAUGCCCUUCUACAUUU	409	AUGUAGAAGGGCAUGAAGCUU	410
D-1122	GCUUCAUGCCCUUCUACAUUU	411	AUGUAGAAGGGCAUGAAGCUU	412
D-1123	GCUUCAUGCCCUUCUACAUUU	413	AUGUAGAAGGGCAUGAAGCUU	414
D-1124	GCUUCAUGCCCUUCUACAUUU	415	AUGUAGAAGGGCAUGAAGCUU	416
D-1125	GCUUCAUGCCCUUCUACAUUU	417	AUGUAGAAGGGCAUGAAGCUU	418
D-1126	GCUUCAUGCCCUUCUACAUUU	419	AUGUAGAAGGGCAUGAAGCUU	420
D-1127	GCUUCAUGCCCUUCUACAUUU	421	AUGUAGAAGGGCAUGAAGCUU	422
D-1128	GCUUCAUGCCCUUCUACAUUU	423	AUGUAGAAGGGCAUGAAGCUU	424
D-1129	GCUUCAUG[DC]CCUUCUACAUUU	425	AUGUAGAAGGGCAUGAAGCUU	426
D-1130	GCUUCAUGCC[DC]UUCUACAUUU	427	AUGUAGAAGGGCAUGAAGCUU	428
D-1131	GCUUCAUGC[DC]CUUCUACAUUU	429	AUGUAGAAGGGCAUGAAGCUU	430
D-1132	GCUUCAUGCCCUUCUACAUUU	431	AUGUAGAAGGGCAUGAAGCUU	432
D-1133	GCUUCAUGCCCUUCUACAUUU	433	AUGUAGAAGGGCAUGAAGCUU	434
D-1134	GCUUCAUGCCCUUCUACAUUU	435	AUGUAGAAGGGCAUGAAGCUU	436
D-1135	GCUUCAUGCCCUUCUACAUUU	437	AUGUAGAAGGGCAUGAAGCUU	438
D-1136	GCUUCAUGCCCUUCUACAUUU	439	AUGUAGAAGGGCAUGAAGCUU	440
D-1137	GCUUCAUGCCCUUCUACAGUU	441	AACUGUAGAAGGGCAUGAAGCUU	442
D-1138	CUGCUUCAUGCCCUUCUACAU	443	AUGUAGAAGGGCAUGAAGCAGUU	444
D-1139	GCUUCAUGCCCUUCUACAU	445	AUGUAGAAGGGCAUGAAGCUU	446
D-1140	GCUUCAUGCCCUUCUACAU{INVAB}	447	AUGUAGAAGGGCAUGAAGCUU	448
D-1141	GCUUCAUGCCCUUCUACAUUU{INVAB}	449	AUGUAGAAGGGCAUGAAGCUU	450
D-1142	GCUUCAUGCCCUUCUACAUUU	451	AUGUAGAAGGGCAUGAAGCUU	452

D-1143	GCUUCAUGCCCUUCUACAUUU	453	AUGUAGAAGGGCAUGAAGCUU	454
D-1144	GCUUCAUGCCCUUCUACAUUU	455	AUGUAGAAGGGCAUGAAGCUU	456
D-1145	GCUUCAUGCCCUUCUACAUUU	457	AUGUAGAAGGGCAUGAAGCUU	458
D-1146	GCUUCAUGCCCUUCUACAUUU	459	AUGUAGAAGGGCAUGAAGCUU	460
D-1147	GCUUCAUGCCCUUCUACAUUU	461	AUGUAGAAGGGCAUGAAGCUU	462
D-1148	GCUUCAUGCCCUUCUACAUUU	463	AUGUAGAAGGGCAUGAAGCUU	464
D-1149	GCUUCAUGCCCUUCUACAUUU	465	AUGUAGAAGGGCAUGAAGCUU	466
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D-1152	GGUAUGUUCCUGCUUCAUUUU	471	AAUGAAGCAGGAACAUACCUU	472
D-1153	GUAUGUUCCUGCUUCAUGUUU	473	ACAUGAAGCAGGAACAUACUU	474
D-1154	CGGCCAAUGUCCACCAGCUUU	475	AGCUGGUGGACAUUGGCCGUU	476
D-1155	UGGAGCAGACUCUGCAGGUUU	477	ACCUGCAGAGUCUGCUCCAUU	478
D-1156	ACGUACCCUUCAUUGAUGUUU	479	ACAUCAAUGAAGGGUACGUUU	480
D-1157	CCAGAGACUGGUGACAUGUUU	481	ACAUGUCACCAGUCUCUGGUU	482
D-1158	[INVAB]GCUUCAUGCCUUUCUACAUUU	483	AUGUAGAAAGGCAUGAAGCUU	484
D-1159	[INVAB]GCUUCAUGCCUUUCUACAUUU	485	AAAUGUAGAAAGGCAUGAAGCUU	486
D-1160	[INVAB]GCUUCAUGCCUUUCUACAUUU	487	AAAUGUAGAAAGGCAUGAAGCUU	488
D-1161	UGCUUCAUGCCUUUCUACAUU	489	UGUAGAAAGGCAUGAAGCAUU	490
D-1162	UAUGUUCCUGCUUCAUGCUUU	491	AGCAUGAAGCAGGAACAUAUU	492
D-1163	UUCCUGCUUCAUGCCUUUUUU	493	AAAAGGCAUGAAGCAGGAAUU	494
D-1164	UCAUGCCUUUCUACAGUGUUU	495	ACACUGUAGAAAGGCAUGAUU	496
D-1165	CAUGCCUUUCUACAGUGGUUU	497	ACCACUGUAGAAAGGCAUGUU	498
D-1166	AUGCCUUUCUACAGUGGCUUU	499	AGCCACUGUAGAAAGGCAUUU	500
D-1167	GGUAUGUUCCUGCUUCAUAUU	501	UAUGAAGCAGGAACAUACCUU	502
D-1168	GUAUGUUCCUGCUUCAUGAUU	503	UCAUGAAGCAGGAACAUACUU	504

D-1169	UAUGUUCCUGCUUCAUGCAUU	505	UGCAUGAAGCAGGAACAUAUU	506
D-1170	UUCCUGCUUCAUGCCUUUAUU	507	UAAAGGCAUGAAGCAGGAAUU	508
D-1171	CUGCUUCAUGCCUUUCUAAUU	509	UUAGAAAGGCAUGAAGCAGUU	510
D-1172	GCUUCAUGCCUUUCUACAAUU	511	UUGUAGAAAGGCAUGAAGCUU	512
D-1173	UUCAUGCCUUUCUACAGUAUU	513	UACUGUAGAAAGGCAUGAAUU	514
D-1174	UCAUGCCUUUCUACAGUGAUU	515	UCACUGUAGAAAGGCAUGAUU	516
D-1175	CAUGCCUUUCUACAGUGGAUU	517	UCCACUGUAGAAAGGCAUGUU	518
D-1176	AUGCCUUUCUACAGUGGCAUU	519	UGCCACUGUAGAAAGGCAUUU	520
D-1177	ACGUACCCUUCAUUGAUGAUU	521	UCAUCAAUGAAGGGUACGUUU	522
D-1178	CCAGAGACUGGUGACAUGAUU	523	UCAUGUCACCAGUCUCUGGUU	524
D-1179	AUGGCUUCCAGAUAUGCCAUU	525	UGGCAUAUCUGGAAGCCAUUU	526
D-1180	GUUCCUGCUUCAUGCCUUUUU	527	AAAGGCAUGAAGCAGGAACUU	528
D-1181	CCUGCUUCAUGCCUUUCUAUU	529	UAGAAAGGCAUGAAGCAGGUU	530
D-1182	GCUUCAUGCCUUUCUACAUUU	531	AUGUAGAAAGGCAUGAAGCUU	532
D-1183	CUUCAUGCCUUUCUACAGUUU	533	ACUGUAGAAAGGCAUGAAGUU	534
D-1184	UUCAUGCCUUUCUACAGUUUU	535	AACUGUAGAAAGGCAUGAAUU	536
D-1185	GCUUCAUCCCUUUCUACAUUU	537	AUGUAGAAAGGGAUGAAGCUU	538
D-1186	[INVAB]GCUUCAUGCCCUUCUACAUUU	539	AUGUAGAAGGGCAUGAAGCUU	540
D-1187	[INVAB]GCUUCAUGCCCUUCUACAUUU	541	AAAUGUAGAAGGGCAUGAAGCUU	542
D-1188	[INVAB]GCUUCAUGCCCUUCUACAUUU	543	AAAUGUAGAAGGGCAUGAAGCUU	544
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D-1190	[INVAB]CUGCGGCUUCCUGGGCUUCUA	547	UAGAAGCCCAGGAAGCCGCAGUU	548
D-1191	CUGCGGCUUCCUGGGCUUCU{INVAB}	549	UAGAAGCCCAGGAAGCCGCAGUU	550
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D-1193	[INVAB]GCGGCUUCCUGGGCUUCUAUU	553	UAGAAGCCCAGGAAGCCGCUU	554
D-1194	CUGCGGCUUCCUGGGCUUCU{INVAB}	555	UAGAAGCCCAGGAAGCCGCAGUU	556

D-1195	[INVAB]CUGCGGCUUCCUGGGCUUCUA	557	UAGAAGCCCAGGAAGCCGCAGUU	558
D-1196	[INVAB]GCGGCUUCCUGGGCUUCUAUU	559	UAGAAGCCCAGGAAGCCGCUU	560
D-1197	CUGCGGCUUCCUGGGCUUCU{INVAB}	561	UAGAAGCCCAGGAAGCCGCAGUU	562
D-1198	[INVAB]CUGCGGCUUCCUGGGCUUCUA	563	UAGAAGCCCAGGAAGCCGCAGUU	564
D-1199	[INVAB]GCGGCUUCCUGGGCUUCUAUU	565	UAGAAGCCCAGGAAGCCGCUU	566
D-1200	[INVAB]CUGCGGCUUCCUGGGCUUCUA	567	UAGAAGCCCAGGAAGCCGCAGUU	568
D-1201	[INVAB]CUGCGGCUUCCUGGGCUUCUA	569	UAGAAGCCCAGGAAGCCGCAGUU	570
D-1202	[INVAB]CUGCGGCUUCCUGGGCUUCUA	571	UAGAAGCCCAGGAAGCCGCAGUU	572
D-1203	[INVAB]AUGGCUUCCAGAUAUGCCUUU	573	AGGCAUAUCUGGAAGCCAUUU	574
D-1204	[INVAB]ACAUGGCUUCCAGAUAUGCCU	575	AGGCAUAUCUGGAAGCCAUGUUU	576
D-1205	ACAUGGCUUCCAGAUAUGCC{INVAB}	577	AGGCAUAUCUGGAAGCCAUGUUU	578
D-1206	[INVAB]ACAUGGCUUCCAGAUAUGCCU	579	AGGCAUAUCUGGAAGCCAUGUUU	580
D-1207	[INVAB]AUGGCUUCCAGAUAUGCCUUU	581	AGGCAUAUCUGGAAGCCAUUU	582
D-1208	ACAUGGCUUCCAGAUAUGCC{INVAB}	583	AGGCAUAUCUGGAAGCCAUGUUU	584
D-1209	[INVAB]ACAUGGCUUCCAGAUAUGCCU	585	AGGCAUAUCUGGAAGCCAUGUUU	586
D-1210	[INVAB]AUGGCUUCCAGAUAUGCCUUU	587	AGGCAUAUCUGGAAGCCAUUU	588
D-1211	ACAUGGCUUCCAGAUAUGCC{INVAB}	589	AGGCAUAUCUGGAAGCCAUGUUU	590
D-1212	[INVAB]ACAUGGCUUCCAGAUAUGCCU	591	AGGCAUAUCUGGAAGCCAUGUUU	592
D-1213	[INVAB]AUGGCUUCCAGAUAUGCCUUU	593	AGGCAUAUCUGGAAGCCAUUU	594
D-1214	ACAUGGCUUCCAGAUAUGCC{INVAB}	595	AGGCAUAUCUGGAAGCCAUGUUU	596
D-1215	[INVAB]ACAUGGCUUCCAGAUAUGCCU	597	AGGCAUAUCUGGAAGCCAUGUUU	598
D-1216	[INVAB]ACAUGGCUUCCAGAUAUGCCU	599	AGGCAUAUCUGGAAGCCAUGUUU	600
D-1217	[INVAB]ACAUGGCUUCCAGAUAUGCCU	601	AGGCAUAUCUGGAAGCCAUGUUU	602
D-1218	[INVAB]ACAUGGCUUCCAGAUAUGCCU	603	AGGCAUAUCUGGAAGCCAUGUUU	604
D-1219	[INVAB]ACGUACCCUUCAUUGAUGUUU	605	ACAUCAAUGAAGGGUACGUUU	606
D-1220	[INVAB]CAACGUACCCUUCAUUGAUGU	607	ACAUCAAUGAAGGGUACGUUGUU	608

D-1221	[INVAB]CAACGUACCCUUCAUUGAUGU	609	ACAUCAAUGAAGGGUACGUUGUU	610
D-1222	[INVAB]ACGUACCCUUCAUUGAUGUUU	611	ACAUCAAUGAAGGGUACGUUU	612
D-1223	CAACGUACCCUUCAUUGAUG{INVAB}	613	ACAUCAAUGAAGGGUACGUUGUU	614
D-1224	[INVAB]ACGUACCCUUCAUUGAUGUUU	615	ACAUCAAUGAAGGGUACGUUU	616
D-1225	CAACGUACCCUUCAUUGAUG{INVAB}	617	ACAUCAAUGAAGGGUACGUUGUU	618
D-1226	[INVAB]CAACGUACCCUUCAUUGAUGU	619	ACAUCAAUGAAGGGUACGUUGUU	620
D-1227	[INVAB]ACGUACCCUUCAUUGAUGUUU	621	ACAUCAAUGAAGGGUACGUUU	622
D-1228	CAACGUACCCUUCAUUGAUG{INVAB}	623	ACAUCAAUGAAGGGUACGUUGUU	624
D-1229	[INVAB]CAACGUACCCUUCAUUGAUGU	625	ACAUCAAUGAAGGGUACGUUGUU	626
D-1230	[INVAB]CAACGUACCCUUCAUUGAUGU	627	ACAUCAAUGAAGGGUACGUUGUU	628
D-1231	[INVAB]CAACGUACCCUUCAUUGAUGU	629	ACAUCAAUGAAGGGUACGUUGUU	630
D-1232	[INVAB]CAACGUACCCUUCAUUGAUGU	631	ACAUCAAUGAAGGGUACGUUGUU	632
D-1233	CUGCUUCAUGCCUUUCUACAU	633	AUGUAGAAAGGCAUGAAGCAGUU	634
D-1234	CUGCUUCAUGCCUUUCUACAU	635	AUGUAGAAAGGCAUGAAGCAGUU	636
D-1235	[INVAB]GCUUCAUGCCUUUCUACAUUU	637	AUGUAGAAAGGCAUGAAGCUU	638
D-1236	[INVAB]GCUUCAUGCCUUUCUACAUUU	639	AUGUAGAAAGGCAUGAAGCUU	640
D-1237	CUGCUUCAUGCCUUUCUACAU	641	AUGUAGAAAGGCAUGAAGCAGUU	642
D-1238	CUGCUUCAUGCCUUUCUACAU	643	AUGUAGAAAGGCAUGAAGCAGUU	644
D-1239	[INVAB]CUGCUUCAUGCCUUUCUACAU	645	AUGUAGAAAGGCAUGAAGCAGUU	646
D-1240	CUGCUUCAUGCCUUUCUACA{INVAB}	647	AUGUAGAAAGGCAUGAAGCAGUU	648
D-1241	[INVAB]CUGCUUCAUGCCUUUCUACAU	649	AUGUAGAAAGGCAUGAAGCAGUU	650
D-1242	[INVAB]CUGCUUCAUGC[DC]UUUCUACAU	651	AUGUAGAAAGGCAUGAAGCAGUU	652
D-1243	CUGCUUCAUGCCUUUCUACAU	653	AUGUAGAAAGGCAUGAAGCAGUU	654
D-1244	[INVAB]CUGCUUCAUGCCUUUCUACAU	655	AUGUAGAAAGGCAUGAAGCAGUU	656
D-1245	CUGCUUCAUGCCUUUCUACAU	657	AUGUAGAAAGGCAUGAAGCAGUU	658
D-1246	CUGCUUCAUGCCUUUCUACA{INVAB}	659	AUGUAGAAAGGCAUGAAGCAGUU	660

D-1247	[INVAB]CUGCUUCAUGCCUUUCUACAU	661	AUGUAGAAAGGCAUGAAGCAGUU	662
D-1248	CUGCUUCAUGCCUUUCUACAU	663	AUGUAGAAAGGCAUGAAGCAGUU	664
D-1249	CUGCUUCAUGCCUUUCUACA{INVAB}	665	AUGUAGAAAGGCAUGAAGCAGUU	666
D-1250	[INVAB]CUGCUUCAUGCCUUUCUACAU	667	AUGUAGAAAGGCAUGAAGCAGUU	668
D-1251	CUGCUUCAUGCCUUUCUACA{INVAB}	669	AUGUAGAAAGGCAUGAAGCAGUU	670
D-1252	[INVAB]CUGCUUCAUGCCUUUCUACAU	671	AUGUAGAAAGGCAUGAAGCAGUU	672
D-1253	[INVAB]GCUUCAUGCCUUUCUACAUUU	673	AUGUAGAAAGGCAUGAAGCUU	674
D-1254	[INVAB]GCUUCAUGCCUUUCUACAUUU	675	AUGUAGAAAGGCAUGAAGCUU	676
D-1255	[INVAB]GCUUCAUGCCUUUCUACAUUU	677	AUGUAGAAAGGCAUGAAGCUU	678
D-1256	[INVAB]GCUUCAUGCCUUUCUACAUUU	679	AUGUAGAAAGGCAUGAAGCUU	680
D-1257	[INVAB]GCUUCAUGCCUUUCUACAUUU	681	AUGUAGAAAGGCAUGAAGCUU	682
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D-1259	[INVAB]GCUUCAUGCCCUUCUACAUUU	685	AUGUAGAAGGGCAUGAAGCUU	686
D-1260	[INVAB]GCUUCAUGCCCUUCUACAUUU	687	AUGUAGAAGGGCAUGAAGCUU	688
D-1261	[INVAB]GCUUCAUGCCCUUCUACAUUU	689	AUGUAGAAGGGCAUGAAGCUU	690
D-1262	CUGCUUCAUGCCCUUCUACA{INVAB}	691	AUGUAGAAGGGCAUGAAGCAGUU	692
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D-1264	[INVAB]GCUUCAUGCCCUUCUACAUUU	695	AUGUAGAAGGGCAUGAAGCUU	696
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D-1266	UGUUCCUGCUUCAUGCCUUUU	699	AAGGCAUGAAGCAGGAACAUU	700
D-1267	UGCCUUUCUACAGUGGCCUUU	701	AGGCCACUGUAGAAAGGCAUU	702
D-1268	CGUACUUCGUCCUUGUAUGUU	703	CAUACAAGGACGAAGUACGUU	704
D-1269	[INVAB]GCUUCAUGC[DC]CUUCUACAUUU	705	AUGUAGAAGGGCAUGAAGCUU	706
D-1270	[INVAB]GCUUCAUGCCCUUCUACAUUU	707	AUGUAGAAGGGCAUGAAGCUU	708
D-1271	[INVAB]GCUUCAUGCCCUUCUACAUUU	709	AUGUAGAAGGGCAUGAAGCUU	710
D-1272	[INVAB]GCUUCAUGCCCUUCUACAUUU	711	AUGUAGAAGGGCAUGAAGCUU	712

D-1273	[INVAB]GCUUCAUGCCCUUCUACAUUU	713	AUGUAGAAGGGCAUGAAGCUU	714
D-1274	[INVAB]GCUUCAUGCCCUUCUACAUUU	715	AUGUAGAAGGGCAUGAAGCUU	716
D-1275	[INVAB]CCUGCUUCAUGCCUUUCUAUU	717	UAGAAAGGCAUGAAGCAGGUU	718
D-1276	[INVAB]CCUGCUUCAUGCCUUUCUAUU	719	UAGAAAGGCAUGAAGCAGGUU	720
D-1277	UUCCUGCUUCAUGCCUUUCU{INVAB}	721	UAGAAAGGCAUGAAGCAGGAAUU	722
D-1278	[INVAB]CCUGCUUCAUGCCUUUCUAUU	723	UAGAAAGGCAUGAAGCAGGUU	724
D-1279	UUCCUGCUUCAUGCCUUUCU{INVAB}	725	UAGAAAGGCAUGAAGCAGGAAUU	726
D-1280	[INVAB]AUGCCUUUCUACAGUGGCUUU	727	AGCCACUGUAGAAAGGCAUUU	728
D-1281	[INVAB]AUGCCUUUCUACAGUGGCUUU	729	AGCCACUGUAGAAAGGCAUUU	730
D-1282	[INVAB]AUGCCUUUCUACAGUGGCUUU	731	AGCCACUGUAGAAAGGCAUUU	732
D-1283	UCAUGCCUUUCUACAGUGGC{INVAB}	733	AGCCACUGUAGAAAGGCAUGAUU	734
D-1284	[INVAB]AUGCCUUUCUACAGUGGCUUU	735	AGCCACUGUAGAAAGGCAUUU	736
D-1285	[INVAB]UCAUGCCUUUCUACAGUGGCU	737	AGCCACUGUAGAAAGGCAUGAUU	738
D-1286	[INVAB]UGCUUCAUGCCUUUCUACAGU	739	ACUGUAGAAAGGCAUGAAGCAUU	740
D-1287	[INVAB]CUUCAUGCCUUUCUACAGUUU	741	ACUGUAGAAAGGCAUGAAGUU	742
D-1289	UGCUUCAUGCCUUUCUACAG{INVAB}	743	ACUGUAGAAAGGCAUGAAGCAUU	744
D-1290	[INVAB]CUUCAUGCCUUUCUACAGUUU	745	ACUGUAGAAAGGCAUGAAGUU	746
D-1291	UGCUUCAUGCCUUUCUACAG{INVAB}	747	ACUGUAGAAAGGCAUGAAGCAUU	748
D-1292	[INVAB]UGCUUCAUGCCUUUCUACAGU	749	ACUGUAGAAAGGCAUGAAGCAUU	750
D-1293	[INVAB]UGCUUCAUGCCUUUCUACAGU	751	ACUGUAGAAAGGCAUGAAGCAUU	752
D-1294	[INVAB]UGCUUCAUGCCUUUCUACAGU	753	ACUGUAGAAAGGCAUGAAGCAUU	754
D-1295	[INVAB]UGCUUCAUGCCUUUCUACAGU	755	ACUGUAGAAAGGCAUGAAGCAUU	756
D-1296	[INVAB]GUUCCUGCUUCAUGCCUUUUU	757	AAAGGCAUGAAGCAGGAACUU	758
D-1297	[INVAB]CCUGCUUCAUGCCUUUCUAUU	759	UAGAAAGGCAUGAAGCAGGUU	760
D-1298	[INVAB]CUUCAUGCCUUUCUACAGUUU	761	ACUGUAGAAAGGCAUGAAGUU	762
D-1299	[INVAB]UUCCUGCUUCAUGCCUUUCUA	763	UAGAAAGGCAUGAAGCAGGAAUU	764

D-1300	UUCCUGCUUCAUGCCUUUCU{INVAB}	765	UAGAAAGGCAUGAAGCAGGAAUU	766
D-1301	[INVAB]UUCCUGCUUCAUGCCUUUCUA	767	UAGAAAGGCAUGAAGCAGGAAUU	768
D-1302	UUCCUGCUUCAUGCCUUUCU{INVAB}	769	UAGAAAGGCAUGAAGCAGGAAUU	770
D-1303	[INVAB]UUCCUGCUUCAUGCCUUUCUA	771	UAGAAAGGCAUGAAGCAGGAAUU	772
D-1304	[INVAB]UUCCUGCUUCAUGCCUUUCUA	773	UAGAAAGGCAUGAAGCAGGAAUU	774
D-1305	[INVAB]UUCCUGCUUCAUGCCUUUCUA	775	UAGAAAGGCAUGAAGCAGGAAUU	776
D-1306	[INVAB]UUCCUGCUUCAUGCCUUUCUA	777	UAGAAAGGCAUGAAGCAGGAAUU	778
D-1307	[INVAB]UUCCUGCUUCAUGCCUUUCUA	779	UAGAAAGGCAUGAAGCAGGAAUU	780
D-1308	[INVAB]UUCCUGCUUCAUGCCUUUCUA	781	UAGAAAGGCAUGAAGCAGGAAUU	782
D-1309	[INVAB]UCAUGCCUUUCUACAGUGGCU	783	AGCCACUGUAGAAAGGCAUGAUU	784
D-1310	UCAUGCCUUUCUACAGUGGC{INVAB}	785	AGCCACUGUAGAAAGGCAUGAUU	786
D-1311	[INVAB]UCAUGCCUUUCUACAGUGGCU	787	AGCCACUGUAGAAAGGCAUGAUU	788
D-1312	UCAUGCCUUUCUACAGUGGC{INVAB}	789	AGCCACUGUAGAAAGGCAUGAUU	790
D-1313	[INVAB]UCAUGCCUUUCUACAGUGGCU	791	AGCCACUGUAGAAAGGCAUGAUU	792
D-1314	[INVAB]UCAUGCCUUUCUACAGUGGCU	793	AGCCACUGUAGAAAGGCAUGAUU	794
D-1315	UCAUGCCUUUCUACAGUGGC{INVAB}	795	AGCCACUGUAGAAAGGCAUGAUU	796
D-1316	[INVAB]UCAUGCCUUUCUACAGUGGCU	797	AGCCACUGUAGAAAGGCAUGAUU	798
D-1317	[INVAB]UCAUGCCUUUCUACAGUGGCU	799	AGCCACUGUAGAAAGGCAUGAUU	800
D-1318	[INVAB]UCAUGCCUUUCUACAGUGGCU	801	AGCCACUGUAGAAAGGCAUGAUU	802
D-1319	[INVAB]UGCUUCAUGCCUUUCUACAGU	803	ACUGUAGAAAGGCAUGAAGCAUU	804
D-1320	UGCUUCAUGCCUUUCUACAG{INVAB}	805	ACUGUAGAAAGGCAUGAAGCAUU	806
D-1321	UGCUUCAUGCCUUUCUACAG{INVAB}	807	ACUGUAGAAAGGCAUGAAGCAUU	808
D-1322	[INVAB]UGCUUCAUGCCUUUCUACAGU	809	ACUGUAGAAAGGCAUGAAGCAUU	810
D-1323	[INVAB]CUUCAUGCCUUUCUACAGUUU	811	ACUGUAGAAAGGCAUGAAGUU	812
D-1324	[INVAB]UGCUUCAUGCCUUUCUACAGU	813	ACUGUAGAAAGGCAUGAAGCAUU	814
D-1325	[INVAB]GCUUCAUGCCUUUCUACAUUU	815	AUGUAGAAAGGCAUGAAGCUU	816

D-1326	[INVAB]CAUGCCUUUCUACAGUGGUUU	817	ACCACUGUAGAAAGGCAUGUU	818
D-1327	[INVAB]CUGCUUCAUGCCUUUCUAAUU	819	UUAGAAAGGCAUGAAGCAGUU	820
D-1328	[INVAB]GCUUCAUGCCUUUCUACAAUU	821	UUGUAGAAAGGCAUGAAGCUU	822
D-1329	[INVAB]UUCAUGCCUUUCUACAGUAUU	823	UACUGUAGAAAGGCAUGAAUU	824
D-1330	[INVAB]GUUCCUGCUUCAUGCCUUUUU	825	AAAGGCAUGAAGCAGGAACUU	826
D-1331	AUGUUCCUGCUUCAUGCCUU{INVAB}	827	AAAGGCAUGAAGCAGGAACAUUU	828
D-1332	[INVAB]AUGUUCCUGCUUCAUGCCUUU	829	AAAGGCAUGAAGCAGGAACAUUU	830
D-1333	[INVAB]GUUCCUGCUUCAUGCCUUUUU	831	AAAGGCAUGAAGCAGGAACUU	832
D-1334	AUGUUCCUGCUUCAUGCCUU{INVAB}	833	AAAGGCAUGAAGCAGGAACAUUU	834
D-1335	[INVAB]AUGUUCCUGCUUCAUGCCUUU	835	AAAGGCAUGAAGCAGGAACAUUU	836
D-1336	[INVAB]GUUCCUGCUUCAUGCCUUUUU	837	AAAGGCAUGAAGCAGGAACUU	838
D-1337	AUGUUCCUGCUUCAUGCCUU{INVAB}	839	AAAGGCAUGAAGCAGGAACAUUU	840
D-1338	[INVAB]AUGUUCCUGCUUCAUGCCUUU	841	AAAGGCAUGAAGCAGGAACAUUU	842
D-1339	[INVAB]AUGUUCCUGCUUCAUGCCUUU	843	AAAGGCAUGAAGCAGGAACAUUU	844
D-1340	[INVAB]AUGUUCCUGCUUCAUGCCUUU	845	AAAGGCAUGAAGCAGGAACAUUU	846
D-1341	[INVAB]GCUUCAUGCCUUUCUACAGUA	847	UACUGUAGAAAGGCAUGAAGCUU	848
D-1342	[INVAB]GCUUCAUGCCUUUCUACAGUA	849	UACUGUAGAAAGGCAUGAAGCUU	850
D-1343	[INVAB]UUCAUGCCUUUCUACAGUAUU	851	UACUGUAGAAAGGCAUGAAUU	852
D-1344	[INVAB]GCUUCAUGCCUUUCUACAGUA	853	UACUGUAGAAAGGCAUGAAGCUU	854
D-1345	GCUUCAUGCCUUUCUACAGU{INVAB}	855	UACUGUAGAAAGGCAUGAAGCUU	856
D-1346	[INVAB]GCGGCUUCCUGGGCUUCUAUU	857	UAGAAGCCCAGGAAGCCGCUU	858
D-1347	[INVAB]AUGGCUUCCAGAUAUGCCUUU	859	AGGCAUAUCUGGAAGCCAUUU	860
D-1348	[INVAB]ACAUGGCUUCCAGAUAUGCCU	861	AGGCAUAUCUGGAAGCCAUGUUU	862
D-1349	[INVAB]ACAUGGCUUCCAGAUAUGCCU	863	AGGCAUAUCUGGAAGCCAUGUUU	864
D-1350	[INVAB]CAACGUACCCUUCAUUGAUGU	865	ACAUCAAUGAAGGGUACGUUGUU	866
D-1351	[INVAB]CAACGUACCCUUCAUUGAUGU	867	ACAUCAAUGAAGGGUACGUUGUU	868

D-1352	[INVAB]ACGUACCCUUCAUUGAUGUUU	869	ACAUCAAUGAAGGGUACGUUU	870
D-1353	CUGCUUCAUGCCUUUCUACA{INVAB}	871	AUGUAGAAAGGCAUGAAGCAGUU	872
D-1354	[INVAB]CUGCUUCAUGCCUUUCUACAU	873	AUGUAGAAAGGCAUGAAGCAGUU	874
D-1355	[INVAB]GCUUCAUGCCUUUCUACAUUU	875	AUGUAGAAAGGCAUGAAGCUU	876
D-1356	CUGCUUCAUGCCUUUCUACA{INVAB}	877	AUGUAGAAAGGCAUGAAGCAGUU	878
D-1357	[INVAB]CUGCUUCAUGCCUUUCUACAU	879	AUGUAGAAAGGCAUGAAGCAGUU	880
D-1358	CUGCUUCAUGCCUUUCUACA{INVAB}	881	AUGUAGAAAGGCAUGAAGCAGUU	882
D-1359	[INVAB]CUGCUUCAUGCCUUUCUACA{INVAB}	883	AUGUAGAAAGGCAUGAAGCAGUU	884
D-1360	[INVAB]GCUUCAUGCCUUUCUACAUUU	885	AUGUAGAAAGGCAUGAAGCUU	886
D-1361	[INVAB]CUUCAUGCCUUUCUACAGUUU	887	ACUGUAGAAAGGCAUGAAGUU	888
D-1362	UGCUUCAUGCCUUUCUACAG{INVAB}	889	ACUGUAGAAAGGCAUGAAGCAUU	890
D-1363	[INVAB]CUGCUUCAUGCCUUUCUACAU	891	AUGUAGAAAGGCAUGAAGCAGUU	892
D-1364	CUGCUUCAUGCCUUUCUACA{INVAB}	893	AUGUAGAAAGGCAUGAAGCAGUU	894
D-1365	CUGCUUCAUGCCUUUCUACA{INVAB}	895	AUGUAGAAAGGCAUGAAGCAGUU	896
D-1366	[INVAB]CUUCAUCCCUUUCUACAGUUU	897	ACUGUAGAAAGGGAUGAAGUU	898
D-1367	[INVAB]GCUUCAUCCCUUUCUACAUUU	899	AUGUAGAAAGGGAUGAAGCUU	900
D-1368	UGCUUCAUCCCUUUCUACAG{INVAB}	901	ACUGUAGAAAGGGAUGAAGCAUU	902
D-1369	CUGCUUCAUCCCUUUCUACA{INVAB}	903	AUGUAGAAAGGGAUGAAGCAGUU	904
D-1370	[INVAB]ACAUUGCUCUUUCACCUGAUU	905	UCAGGUGAAAGAGCAAUGUUU	906
D-1371	CUGCUUCAUGCCUUUCUACA{INVAB}	907	AUGUAGAAAGGCAUGAAGCAGUU	908
D-1372	CUGCUUCAUGCCUUUCUACA{INVAB}	909	AUGUAGAAAGGCAUGAAGCAGUU	910
D-1373	CUGCUUCAUGCCUUUCUACA{INVAB}	911	AUGUAGAAAGGCAUGAAGCAGUU	912
D-1374	CUGCUUCAUGCCUUUCUACA{INVAB}	913	AUGUAGAAAGGCAUGAAGCAGUU	914
D-1375	CUGCUUCAUGCCUUUCUACA{INVAB}	915	AUGUAGAAAGGCAUGAAGCAGUU	916
D-1376	CUGCUUCAUGCCUUUCUACA{INVAB}	917	AUGUAGAAAGGCAUGAAGCAGUU	918

D-1377	CUGCUUCAUGCCUUUCUACA{INVAB}	919	AUGUAGAAAGGCAUGAAGCAGUU	920
D-1378	CUGCUUCAUGCCUUUCUACA{INVAB}	921	AUGUAGAAAGGCAUGAAGCAGUU	922
D-1379	CUGCUUCAUGCCUUUCUACA{INVAB}	923	AUGUAGAAAGGCAUGAAGCAGUU	924
D-1380	CUGCUUCAUGCCUUUCUACA{INVAB}	925	AUGUAGAAAGGCAUGAAGCAGUU	926
D-1381	CUGCUUCAUGCCUUUCUACA{INVAB}	927	AUGUAGAAAGGCAUGAAGCAGUU	928
D-1381	[INVAB]CUUCAUGCC[DT]UUCUACAGUUU	929	ACUGUAGAAAGGCAUGAAGUU	930
D-1382	[INVAB]CUUCAUGCCUUUCUACAGUUU	931	ACUGUAGAAAGGCAUGAAGUU	932
D-1383	[INVAB]CUUCAUGCCUUUCUACAGUUU	933	ACUGUAGAAAGGCAUGAAGUU	934
D-1384	[INVAB]CUUCAUGCCUUUCUACAGUUU	935	ACUGUAGAAAGGCAUGAAGUU	936
D-1385	[INVAB]CUUCAUGCCUUUCUACAGUUU	937	ACUGUAGAAAGGCAUGAAGUU	938
D-1386	[INVAB]CUUCAUGC[DC]UUUCUACAGUUU	939	ACUGUAGAAAGGCAUGAAGUU	940
D-1387	[INVAB]CUUCAUGCCU[DT]UCUACAGUUU	941	ACUGUAGAAAGGCAUGAAGUU	942
D-1388	[INVAB]GCUUCAUGGGAUUCUACAUUU	943	AUGUAGAAUCCCAUGAAGCUU	944
D-1389	[INVAB]CUUCAUGCGAAUCUACAGUUU	945	ACUGUAGAUUCGCAUGAAGUU	946
D-1390	CUGCUUCAUGCCUUUCUACA{INVAB}	947	UUGUAGAAAGGCAUGAAGCAGUU	948
D-1391	[INVAB]CUGCUUCAUGCCUUUCUACAA	949	UUGUAGAAAGGCAUGAAGCAGUU	950
D-1392	CUGCUUCAUGCCUUUCUACA{INVDA}	951	UUGUAGAAAGGCAUGAAGCAGUU	952
D-1393	CUGCUUCAUGGGAUUCUACA{INVAB}	953	AUGUAGAAUCCCAUGAAGCAGUU	954
D-1394	[INVAB]CUGCUUCAUGGGAUUCUACAU	955	AUGUAGAAUCCCAUGAAGCAGUU	956
D-1395	[INVAB]CUUCAUGCCUUUCUACAGUUU	957	ACUGUAGAAAGGCAUGAAGUU	958
D-1396	[INVAB]CUUCAUGCCUUUCUACAGUUU	959	ACUGUAGAAAGGCAUGAAGUU	960
D-1397	[INVAB]GCUUCAUGCCUUUCUACAUUU	961	AUGUAGAAAGGCAUGAAGCUU	962
D-1398	[INVAB]UGCUUCAUGCCUUUCUACAG{INVAB}	963	ACUGUAGAAAGGCAUGAAGCAUU	964
D-1399	[INVAB]CUUCAUGCCUUUCUACAGUUU	965	ACUGUAGAAAGGCAUGAAGUU	966
D-1400	UGCUUCAUGCCUUUCUACAG{INVAB}	967	ACUGUAGAAAGGCAUGAAGCAUU	968

D-1401	UGCUUCAUGCCUUUCUACAG{INVAB}	969	ACUGUAGAAAGGCAUGAAGCAUU	970
D-1402	UGCUUCAUGCCUUUCUACAG{INVAB}	971	ACUGUAGAAAGGCAUGAAGCAUU	972
D-1403	[INVAB]CUUCAUGCCUUUCUACAGUUU	973	ACUGUAGAAAGGCAUGAAGUU	974
D-1404	UGCUUCAUGCCUUUCUACAG{INVAB}	975	ACUGUAGAAAGGCAUGAAGCAUU	976
D-1405	AUGCCUUUCUACAGUGGCUU{INVAB}	977	AGCCACUGUAGAAAGGCAUGAUU	978
D-1406	UCAUGCCUUUCUACAGUGGC{INVAB}	979	AGCCACUGUAGAAAGGCAUGAUU	980
D-1407	[INVAB]AUGCCUUUCUACAGUGGCUUU	981	AGCCACUGUAGAAAGGCAUUU	982
D-1408	[INVAB]AUGCCUUUCUACAGUGGCUUU	983	AGCCACUGUAGAAAGGCAUUU	984
D-1409	[INVAB]UCAUGCCUUUCUACAGUGGCU	985	AGCCACUGUAGAAAGGCAUGAUU	986
D-1410	UCAUGCCUUUCUACAGUGGC{INVAB}	987	AGCCACUGUAGAAAGGCAUGAUU	988
D-1411	[INVAB]AUGCCUUUCUACAGUGGCUUU	989	AGCCACUGUAGAAAGGCAUUU	990
D-1412	UCAUGCCUUUCUACAGUGGC{INVAB}	991	AGCCACUGUAGAAAGGCAUGAUU	992
D-1413	UCAUGCCUUUCUACAGUGGC{INVAB}	993	AGCCACUGUAGAAAGGCAUGAUU	994
D-1414	[INVAB]UCAUGCCUUUCUACAGUGGC{INVAB}	995	AGCCACUGUAGAAAGGCAUGAUU	996
D-1415	[INVAB]AUGCCUUUCUACAGUGGCAUU	997	UGCCACUGUAGAAAGGCAUUU	998
D-1416	UCAUGCCUUUCUACAGUGGC{INVAB}	999	UGCCACUGUAGAAAGGCAUGAUU	1000
D-1417	[INVAB]UCAUGCCUUUCUACAGUGGCA	1001	UGCCACUGUAGAAAGGCAUGAUU	1002
D-1418	UCAUGCCUUUCUACAGUGGC{INVDA}	1003	UGCCACUGUAGAAAGGCAUGAUU	1004
D-1419	CUGCUUCAUGCCUUUCUACA{INVAB}	1005	AUGUAGAAAGGCAUGAAGCAGUU	1006
D-1420	CUGCUUCAUGCCUUUCUACA{INVAB}	1007	UUGUAGAAAGGCAUGAAGCAGUU	1008
D-1421	CUGCUUCAUGCCUUUCUACA{INVDA}	1009	UUGUAGAAAGGCAUGAAGCAGUU	1010
D-1422	UCAUGCCUUUCUACAGUGGC{INVAB}	1011	UGCCACUGUAGAAAGGCAUGAUU	1012
D-1423	UCAUGCCUUUCUACAGUGGC{INVDA}	1013	UGCCACUGUAGAAAGGCAUGAUU	1014
D-1424	[INVAB]CUUCAUGCCUUUCUACAGUUU	1015	ACUGUAGAAAGGCAUGAAGUU	1016
D-1425	CUGCUUCAUGCCUUUCUACA{INVAB}	1017	AUGUA[AB]AAAGGCAUGAAGCAGUU	1018

D-1426	CUGCUUCAUGCCUUUCUACA{INVAB}	1019	AUGUA[AB]AAAGGCAUGAAGCAGUU	1020
D-1427	UGCUUCAUGCCUUUCUACAG{INVAB}	1021	ACUGU[AB]GAAAGGCAUGAAGCAUU	1022
D-1428	UGCUUCAUGCCUUUCUACAG{INVAB}	1023	ACUGU[AB]GAAAGGCAUGAAGCAUU	1024
D-1429	UCAUGCCUUUCUACAGUGGC{INVAB}	1025	AGCCA[AB]UGUAGAAAGGCAUGAUU	1026
D-1430	UCAUGCCUUUCUACAGUGGC{INVAB}	1027	AGCCA[AB]UGUAGAAAGGCAUGAUU	1028
D-1431	CUGCUUCAUGCCUUUCUACA{INVAB}	1029	AU[GNA-G]UAGAAAGGCAUGAAGCAGUU	1030
D-1432	CUGCUUCAUGCCUUUCUACA{INVAB}	1031	AUG[GNA-U]AGAAAGGCAUGAAGCAGUU	1032
D-1433	CUGCUUCAUGCCUUUCUACA{INVAB}	1033	AUGU[GNA-A]GAAAGGCAUGAAGCAGUU	1034
D-1434	CUGCUUCAUGCCUUUCUACA{INVAB}	1035	AUGUA[GNA-G]AAAGGCAUGAAGCAGUU	1036
D-1435	CUGCUUCAUGCCUUUCUACA{INVAB}	1037	AUGUAG[GNA-A]AAGGCAUGAAGCAGUU	1038
D-1436	CUGCUUCAUGCCUUUCUACA{INVAB}	1039	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1040
D-1437	CUGCUUCAUGCCUUUCUACA{INVAB}	1041	AUGUAGAAAGGCAUGAAGCAGUU	1042
D-1438	[INVAB]CUGCUUCAUGCCUUUCUACAU	1043	AUGUAGAAAGGCAUGAAGCAGUU	1044
D-1439	[INVAB]CUGCUUCAUGCCUUUCUACA{INVAB}	1045	AUGUAGAAAGGCAUGAAGCAGUU	1046
D-1440	CUGCUUCAUGCCUUUCUACA{INVAB}	1047	AUGUAGAAAGGCAUGAAGCAGUU	1048
D-1441	CUGCUUCAUGCCUUUCUACA{INVAB}	1049	AUGUAGAAAGGCAUGAAGCAGUU	1050
D-1442	[INVAB]CUGCUUCAUGCCUUUCUACAU	1051	AUGUAGAAAGGCAUGAAGCAGUU	1052
D-1443	CUGCUUCAUGCCUUUCUACA{INVAB}	1053	AUGUAGAAAGGCAUGAAGCAGUU	1054
D-1444	CUGCUUCAUGCCUUUCUACA{INVAB}	1055	AUGUAGAAAGGCAUGAAGCAGUU	1056
D-1445	[INVAB]CUGCUUCAUGCCUUUCUACAU	1057	AUGUAGAAAGGCAUGAAGCAGUU	1058
D-1446	[INVAB]CUGCUUCAUGCCUUUCUACA{INVAB}	1059	AUGUAGAAAGGCAUGAAGCAGUU	1060
D-1447	CUGCUUCAUGCCUUUCUACA{INVAB}	1061	A[AB]GUAGAAAGGCAUGAAGCAGUU	1062
D-1448	CUGCUUCAUGCCUUUCUACA{INVAB}	1063	AU[AB]UAGAAAGGCAUGAAGCAGUU	1064
D-1449	CUGCUUCAUGCCUUUCUACA{INVAB}	1065	AUG[AB]AGAAAGGCAUGAAGCAGUU	1066
D-1450	CUGCUUCAUGCCUUUCUACA{INVAB}	1067	AUGU[AB]GAAAGGCAUGAAGCAGUU	1068

D-1451	CUGCUUCAUGCCUUUCUACA{INVAB}	1069	AUGUAG[AB]AAGGCAUGAAGCAGUU	1070
D-1452	CUGCUUCAUGCCUUUCUACA{INVAB}	1071	AUGUAGA[AB]AGGCAUGAAGCAGUU	1072
D-1453	CAACGUACCCUUCAUUGAUG{INVAB}	1073	ACAUCAAUGAAGGGUACGUUGUU	1074
D-1454	CAACGUACCCUUCAUUGAUG{INVAB}	1075	ACAUCAAUGAAGGGUACGUUGUU	1076
D-1455	ACAUGGCUUCCAGAUAUGCC{INVAB}	1077	AGGCAUAUCUGGAAGCCAUGUUU	1078
D-1456	ACAUGGCUUCCAGAUAUGCC{INVAB}	1079	AGGCAUAUCUGGAAGCCAUGUUU	1080
D-1457	CUGCUUCAUGCCUUUCUACA{INVAB}	1081	AUGUAGAAAGGCAUGAAGCAGUU	1082
D-1458	CUGCUUCAUGCCUUUCUACA{INVAB}	1083	AUGUAGAAAGGCAUGAAGCAGUU	1084
D-1459	CUGCUUCAUGCCUUUCUACA{INVAB}	1085	AUGUAGAAAGGCAUGAAGCAGUU	1086
D-1460	CUGCGGCUUCCUGGGCUUCU{INVAB}	1087	UAGAAGCCCAGGAAGCCGCAGUU	1088
D-1461	CUGCGGCUUCCUGGGCUUCU{INVAB}	1089	UAGAAGCCCAGGAAGCCGCAGUU	1090
D-1462	UGCUUCAUGCCUUUCUACAG{INVAB}	1091	ACUGUAGAAAGGCAUGAAGCAUU	1092
D-1463	[INVAB]UGCUUCAUGCCUUUCUACAGU	1093	ACUGUAGAAAGGCAUGAAGCAUU	1094
D-1464	[INVAB]UGCUUCAUGCCUUUCUACAG{INVAB}	1095	ACUGUAGAAAGGCAUGAAGCAUU	1096
D-1465	UGCUUCAUGCCUUUCUACAG{INVAB}	1097	ACUGUAGAAAGGCAUGAAGCAUU	1098
D-1466	UGCUUCAUGCCUUUCUACAG{INVAB}	1099	ACUGUAGAAAGGCAUGAAGCAUU	1100
D-1467	UGCUUCAUGCCUUUCUACAG{INVAB}	1101	ACUGUAGAAAGGCAUGAAGCAUU	1102
D-1468	CUGCUUCAUGCCUUUCUACAU	1103	AUGUAGAAAGGCAUGAAGCAGUU	1104
D-1469	CUGCUUCAUGCCUUUCUACAU	1105	AUGUAGAAAGGCAUGAAGCAGUU	1106
D-1470	CUGCUUCAUGCCUUUCUACAU	1107	AUGUAGAAAGGCAUGAAGCAGUU	1108
D-1471	UGCUUCAUGCCUUUCUACAG{INVAB}	1109	UCUGUAGAAAGGCAUGAAGCAUU	1110
D-1472	UGCUUCAUGCCUUUCUACAG{INVDA}	1111	UCUGUAGAAAGGCAUGAAGCAUU	1112
D-1473	CUGCUUCAUGCCUUUCUACA{INVDT}	1113	AUGUAGAAAGGCAUGAAGCAGUU	1114
D-1474	CUGCUUCAUGCCUUUCUACA{INVAB}	1115	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1116
D-1475	CUGCUUCAUGCCUUUCUACA{INVAB}	1117	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1118

D-1476	CUGCUUCAUGCCUUUCUACA{INVAB}	1119	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1120
D-1477	CUGCUUCAUGCCUUUCUACA{INVAB}	1121	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1122
D-1478	CUGCUUCAUGCCUUUCUACA{INVAB}	1123	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1124
D-1479	CUGCUUCAUGCCUUUCUACA{INVAB}	1125	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1126
D-1480	CUGCUUCAUGCCUUUCUACA{INVAB}	1127	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1128
D-1481	CUGCUUCAUGCCUUUCUACA{INVAB}	1129	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1130
D-1482	CUGCUUCAUGCCUUUCUACA{INVAB}	1131	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1132
D-1483	CUGCUUCAUGCCUUUCUACA{INVAB}	1133	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1134
D-1484	CUGCUUCAUGCCUUUCUACA{INVAB}	1135	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1136
D-1485	CUGCUUCAUGCCUUUCUACA{INVAB}	1137	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1138
D-1486	CUGCUUCAUGCCUUUCUACA{INVAB}	1139	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1140
D-1487	CUGCUUCAUGCCUUUCUACA{INVAB}	1141	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1142
D-1488	CUGCUUCAUGCCUUUCUACA{INVAB}	1143	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1144
D-1489	CUGCUUCAUGCCUUUCUACA{INVAB}	1145	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1146
D-1490	CUGCUUCAUGCCUUUCUACA{INVAB}	1147	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1148
D-1491	CUGCUUCAUGCCUUUCUACA{INVAB}	1149	AUGUAGA[GNA-A]A[DG]GCAUGAAGCAGUU	1150
D-1492	CUGCUUCAUGCCUUUCUACA{INVAB}	1151	AUGUAGA[GNA-A][DA]GGCAUGAAGCAGUU	1152
D-1493	CUGCUUCAUGCCUUUCUACA{INVAB}	1153	AUGUAGA[GNA-A]AG[DG]CAUGAAGCAGUU	1154
D-1494	CUGCUUCAUGCCUUUCUACA{INVAB}	1155	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1156
D-1495	CUGCUUCAUGCCUUUCUACA{INVAB}	1157	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1158
D-1496	CUGCUUCAUGCCUUUCUACA{INVAB}	1159	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1160
D-1497	CUGCUUCAUGCCUUUCUACA{INVAB}	1161	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1162
D-1498	CUGCUUCAUGCCUUUCUACA{INVAB}	1163	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1164
D-1499	CUGCUUCAUGCCUUUCUACA{INVAB}	1165	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1166
D-1500	CUGCUUCAUGCCUUUCUACA{INVAB}	1167	AUGUAGAAAGGCAUGAAGCAGUU	1168
D-1501	UGCUUCAUGCCUUUCUACAG{INVDA}	1169	U[GNA-C]UGUAGAAAGGCAUGAAGCAUU	1170

D-1502	UGCUUCAUGCCUUUCUACAG{INVDA}	1171	UC[GNA-U]GUAGAAAGGCAUGAAGCAUU	1172
D-1503	UGCUUCAUGCCUUUCUACAG{INVDA}	1173	UCUG[GNA-U]AGAAAGGCAUGAAGCAUU	1174
D-1504	UGCUUCAUGCCUUUCUACAG{INVDA}	1175	UCUGU[GNA-A]GAAAGGCAUGAAGCAUU	1176
D-1505	UGCUUCAUGCCUUUCUACAG{INVDA}	1177	UCUGUAG[GNA-A]AAGGCAUGAAGCAUU	1178
D-1506	UGCUUCAUGCCUUUCUACAG{INVDA}	1179	U[AB]UGUAGAAAGGCAUGAAGCAUU	1180
D-1507	UGCUUCAUGCCUUUCUACAG{INVDA}	1181	UC[AB]GUAGAAAGGCAUGAAGCAUU	1182
D-1508	UGCUUCAUGCCUUUCUACAG{INVDA}	1183	UCU[AB]UAGAAAGGCAUGAAGCAUU	1184
D-1509	UGCUUCAUGCCUUUCUACAG{INVDA}	1185	UCUG[AB]AGAAAGGCAUGAAGCAUU	1186
D-1510	UGCUUCAUGCCUUUCUACAG{INVDA}	1187	UCUGU[AB]GAAAGGCAUGAAGCAUU	1188
D-1511	UGCUUCAUGCCUUUCUACAG{INVDA}	1189	UCUGUA[AB]AAAGGCAUGAAGCAUU	1190
D-1512	UGCUUCAUGCCUUUCUACAG{INVDA}	1191	UCUGUAG[AB]AAGGCAUGAAGCAUU	1192
D-1513	UCAUGCCUUUCUACAGUGGC{INVDT}	1193	AGCCACUGUAGAAAGGCAUGAUU	1194
D-1514	UCAUGCCUUUCUACAGUGGC{INVAB}	1195	UGCCACUGUAGAAAGGCAUGAUU	1196
D-1515	UCAUGCCUUUCUACAGUGGC{INVDA}	1197	UGCCACUGUAGAAAGGCAUGAUU	1198
D-1516	UCCUGCUUCAUGCCUUUCUA{INVDT}	1199	AUAGAAAGGCAUGAAGCAGGAUU	1200
D-1517	UCCUGCUUCAUGCCUUUCUA{INVAB}	1201	UUAGAAAGGCAUGAAGCAGGAUU	1202
D-1518	UCCUGCUUCAUGCCUUUCUA{INVDA}	1203	UUAGAAAGGCAUGAAGCAGGAUU	1204
D-1519	UAUGUUCCUGCUUCAUGCCU{INVDT}	1205	AAGGCAUGAAGCAGGAACAUAUU	1206
D-1520	UAUGUUCCUGCUUCAUGCCU{INVAB}	1207	UAGGCAUGAAGCAGGAACAUAUU	1208
D-1521	UAUGUUCCUGCUUCAUGCCU{INVDA}	1209	UAGGCAUGAAGCAGGAACAUAUU	1210
D-1522	UCCUGCUUCAUGCCUUUCUA{INVAB}	1211	AUAGAAAGGCAUGAAGCAGGAUU	1212
D-1523	UAUGUUCCUGCUUCAUGCCU{INVAB}	1213	AAGGCAUGAAGCAGGAACAUAUU	1214
D-1524	UCAUGCCUUUCUACAGUGGC{INVDT}	1215	AGCCACUGUAGAAAGGCAUGAUU	1216
D-1525	UCCUGCUUCAUGCCUUUCUA{INVDT}	1217	AUAGAAAGGCAUGAAGCAGGAUU	1218
D-1526	UCCUGCUUCAUGCCUUUCUA{INVDA}	1219	UUAGAAAGGCAUGAAGCAGGAUU	1220
D-1527	UAUGUUCCUGCUUCAUGCCU{INVDA}	1221	UAGGCAUGAAGCAGGAACAUAUU	1222

D-1528	UGCUUCAUGCCUUUCUACAG{INVDA}	1223	UCUGUA[GNA-G]AAAGGCAUGAAGCAUU	1224
D-1529	CUGCUUCAUGCCUUUCUACA{INVAB}	1225	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1226
D-1530	CUGCUUCAUGCCUUUCUACA{INVAB}	1227	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1228
D-1531	CUGCUUCAUGCCUUUCUACA{INVAB}	1229	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1230
D-1532	CUGCUUCAUGCCUUUCUACA{INVAB}	1231	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1232
D-1533	CUGCUUCAUGCCUUUCUACA{INVAB}	1233	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1234
D-1534	CUGCUUCAUGCCUUUCUACA{INVAB}	1235	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1236
D-1535	CUGCUUCAUGCCUUUCUACA{INVAB}	1237	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1238
D-1536	CUGCUUCAUGCCUUUCUACA{INVAB}	1239	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1240
D-1537	CUGCUUCAUGCCUUUCUACA{INVAB}	1241	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1242
D-1538	CUGCUUCAUGCCU[LNA-T]UCUACA{INVAB}	1243	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1244
D-1539	CUGCUUCAUGCC[LNA-T]UUCUACA{INVAB}	1245	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1246
D-1540	CUGCUUCAUGCCUU[LNA-T]CUACA{INVAB}	1247	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1248
D-1541	CUGCUUCAUGCCUUUC[LNA-T]ACA{INVAB}	1249	AUGU[GNA-A]GAAAGGCAUGAAGCAGUU	1250
D-1542	CUGCUUCAUGCCUUUCU[LNA-A]CA{INVAB}	1251	AUG[GNA-U]AGAAAGGCAUGAAGCAGUU	1252
D-1543	CUGCUUCAUGCCUUUCUAC[LNA-A]{INVAB}	1253	A[GNA-U]GUAGAAAGGCAUGAAGCAGUU	1254
D-1544	CUGCUUCAUGCCUU[LNA-T]CUACA{INVAB}	1255	AUGUAG[AB]AAGGCAUGAAGCAGUU	1256
D-1545	CUGCUUCAUGCCUUUC[LNA-T]ACA{INVAB}	1257	AUGU[AB]GAAAGGCAUGAAGCAGUU	1258
D-1546	CUGCUUCAUGCCUUUCU[LNA-A]CA{INVAB}	1259	AUG[AB]AGAAAGGCAUGAAGCAGUU	1260
D-1547	CUGCUUCAUGCCUUUCUACA{INVAB}	1261	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1262
D-1548	CUGCUUCAUGCCUUUCUACA{INVAB}	1263	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1264

D-1549	CUGCUUCAUGCCU[LNA-T]UCUACA{INVAB}	1265	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1266
D-1550	CUGCUUCAUGCCU[LNA-T]UCUACA{INVAB}	1267	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1268
D-1551	CUGCUUCAUGCCU[LNA-T]UCUACA{INVAB}	1269	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1270
D-1552	CUGCUUCAUGCCU[LNA-T]UCUACA{INVAB}	1271	AUGUAGA[GNA-A]AGGCAUGAAGCAGUU	1272
D-1553	UGCUUCAUGCCUUUCU[LNA-A]CAG{INVDA}	1273	UCUG[AB]AGAAAGGCAUGAAGCAUU	1274
D-1554	UGCUUCAUGCCUUUC[LNA-T]ACAG{INVDA}	1275	UCUGU[AB]GAAAGGCAUGAAGCAUU	1276
D-1555	UGCUUCAUGCCUU[LNA-T]CUACAG{INVDA}	1277	UCUGUAG[AB]AAGGCAUGAAGCAUU	1278
D-1556	UGCUUCAUGCCUUUCUAC[LNA-A]G{INVDA}	1279	UC[GNA-U]GUAGAAAGGCAUGAAGCAUU	1280
D-1557	UGCUUCAUGCCUUUCU[LNA-A]CAG{INVDA}	1281	UCUG[GNA-U]AGAAAGGCAUGAAGCAUU	1282
D-1558	UGCUUCAUGCCUUUC[LNA-T]ACAG{INVDA}	1283	UCUGU[GNA-A]GAAAGGCAUGAAGCAUU	1284
D-1559	UGCUUCAUGCCUUUCUACA[LNA-G]{INVDA}	1285	U[AB]UGUAGAAAGGCAUGAAGCAUU	1286
D-1560	UGCUUCAUGCCUUUCUAC[LNA-A]G{INVDA}	1287	UC[AB]GUAGAAAGGCAUGAAGCAUU	1288
D-1561	UCAUGCCUUUCUACA[LNA-G]UGGC{INVAB}	1289	AGCCA[AB]UGUAGAAAGGCAUGAUU	1290
D-1562	UCAUGCCUUUCUACAG[LNA-T]GGC{INVAB}	1291	AGCCA[AB]UGUAGAAAGGCAUGAUU	1292
D-1563	UCAUGCCUUUCUACAGUGGC{INVAB}	1293	AGCCA[AB]UGUAGAAAGGCAUGAUU	1294
D-1564	GGUAUGUUCCUGCUUCAUUUU	2257	AAUGAAGCAGGAACAUACCUU	2258
D-1565	GGUAUGUUCCUGCUUCAUAUU	2259	UAUGAAGCAGGAACAUACCUU	2260
D-1566	GUAUGUUCCUGCUUCAUGUUU	2261	ACAUGAAGCAGGAACAUACUU	2262
D-1567	UAUGUUCCUGCUUCAUGCAUU	2263	UGCAUGAAGCAGGAACAUAUU	2264
D-1568	AUGUUCCUGCUUCAUGCCUUU	2265	AGGCAUGAAGCAGGAACAUUU	2266

D-1569	UGUUCCUGCUUCAUGCCUUUU	2267	AAGGCAUGAAGCAGGAACAUU	2268
D-1570	GUUCCUGCUUCAUGCCUUUUU	2269	AAAGGCAUGAAGCAGGAACUU	2270
D-1571	UUCCUGCUUCAUGCCUUUUUU	2271	AAAAGGCAUGAAGCAGGAAUU	2272
D-1572	UUCCUGCUUCAUGCCUUUAUU	2273	UAAAGGCAUGAAGCAGGAAUU	2274
D-1573	UCCUGCUUCAUGCCUUUCUUU	2275	AGAAAGGCAUGAAGCAGGAUU	2276
D-1574	CCUGCUUCAUGCCUUUCUAUU	2277	UAGAAAGGCAUGAAGCAGGUU	2278
D-1575	CUGCUUCAUGCCUUUCUAUUU	2279	AUAGAAAGGCAUGAAGCAGUU	2280
D-1576	CUGCUUCAUGCCUUUCUAAUU	2281	UUAGAAAGGCAUGAAGCAGUU	2282
D-1577	UGCUUCAUGCCUUUCUACAUU	2283	UGUAGAAAGGCAUGAAGCAUU	2284
D-1578	GCUUCAUGCCUUUCUACAUUU	2285	AUGUAGAAAGGCAUGAAGCUU	2286
D-1579	GCUUCAUGCCUUUCUACAAUU	2287	UUGUAGAAAGGCAUGAAGCUU	2288
D-1580	CUUCAUGCCUUUCUACAGUUU	2289	ACUGUAGAAAGGCAUGAAGUU	2290
D-1581	UUCAUGCCUUUCUACAGUUUU	2291	AACUGUAGAAAGGCAUGAAUU	2292
D-1582	UUCAUGCCUUUCUACAGUAUU	2293	UACUGUAGAAAGGCAUGAAUU	2294
D-1583	UCAUGCCUUUCUACAGUGUUU	2295	ACACUGUAGAAAGGCAUGAUU	2296
D-1584	UCAUGCCUUUCUACAGUGAUU	2297	UCACUGUAGAAAGGCAUGAUU	2298
D-1585	CAUGCCUUUCUACAGUGGUUU	2299	ACCACUGUAGAAAGGCAUGUU	2300
D-1586	CAUGCCUUUCUACAGUGGAUU	2301	UCCACUGUAGAAAGGCAUGUU	2302
D-1587	AUGCCUUUCUACAGUGGCUUU	2303	AGCCACUGUAGAAAGGCAUUU	2304
D-1588	AUGCCUUUCUACAGUGGCAUU	2305	UGCCACUGUAGAAAGGCAUUU	2306
D-1589	UGCCUUUCUACAGUGGCCUUU	2307	AGGCCACUGUAGAAAGGCAUU	2308
D-1590	GCCUUUCUACAGUGGCCUUUU	2309	AAGGCCACUGUAGAAAGGCUU	2310
D-1591	GGUAUGUUCCUGCUUCAUCUU	2311	GAUGAAGCAGGAACAUACCUU	2312
D-1592	GUAUGUUCCUGCUUCAUCCUU	2313	GGAUGAAGCAGGAACAUACUU	2314
D-1593	UAUGUUCCUGCUUCAUCCCUU	2315	GGGAUGAAGCAGGAACAUAUU	2316
D-1594	AUGUUCCUGCUUCAUCCCCUU	2317	GGGGAUGAAGCAGGAACAUUU	2318

D-1595	UGUUCCUGCUUCAUCCCCUUU	2319	AGGGGAUGAAGCAGGAACAUU	2320
D-1596	GUUCCUGCUUCAUCCCCUUUU	2321	AAGGGGAUGAAGCAGGAACUU	2322
D-1597	UUCCUGCUUCAUCCCCUUCUU	2323	GAAGGGGAUGAAGCAGGAAUU	2324
D-1598	UCCUGCUUCAUCCCCUUCUUU	2325	AGAAGGGGAUGAAGCAGGAUU	2326
D-1599	CCUGCUUCAUCCCCUUCUAUU	2327	UAGAAGGGGAUGAAGCAGGUU	2328
D-1600	CUGCUUCAUCCCCUUCUACUU	2329	GUAGAAGGGGAUGAAGCAGUU	2330
D-1601	UGCUUCAUCCCCUUCUACAUU	2331	UGUAGAAGGGGAUGAAGCAUU	2332
D-1602	GCUUCAUCCCCUUCUACAGUU	2333	CUGUAGAAGGGGAUGAAGCUU	2334
D-1603	CUUCAUCCCCUUCUACAGUUU	2335	ACUGUAGAAGGGGAUGAAGUU	2336
D-1604	UUCAUCCCCUUCUACAGUGUU	2337	CACUGUAGAAGGGGAUGAAUU	2338
D-1605	UCAUCCCCUUCUACAGUGGUU	2339	CCACUGUAGAAGGGGAUGAUU	2340
D-1606	CAUCCCCUUCUACAGUGGCUU	2341	GCCACUGUAGAAGGGGAUGUU	2342
D-1607	AUCCCCUUCUACAGUGGCCUU	2343	GGCCACUGUAGAAGGGGAUUU	2344
D-1608	UCCCCUUCUACAGUGGCCUUU	2345	AGGCCACUGUAGAAGGGGAUU	2346
D-1609	CCCCUUCUACAGUGGCCUUUU	2347	AAGGCCACUGUAGAAGGGGUU	2348

[0002] To improve the potency and in vivo stability of PNPLA3 siRNA sequences, chemical modifications were incorporated into PNPLA3 siRNA molecules. Specifically, 2'-O-methyl and 2'-fluoro modifications of the ribose sugar were incorporated at specific positions within the PNPLA3 siRNAs. Phosphorothioate internucleotide linkages were also incorporated at the terminal ends of the antisense and/ or sense sequences. Table 2 below depicts the modifications in the sense and antisense sequences for each of the modified PNPLA3 siRNAs. The nucleotide sequences in Table X are listed according to the following notations: A, U, G, and C =corresponding ribonucleotide; dT = deoxythymidine; a, u, g, and c = corresponding 2'-O-methyl ribonucleotide; Af, Uf, Gf, and Cf = corresponding 2'-deoxy-2'-fluoro ("2'-fluoro") ribonucleotide. Insertion of an "s" in the sequence indicates that the two adjacent nucleotides are connected by a phosphorothiodiester group (e.g. a phosphorothioate internucleotide linkage). Unless indicated otherwise, all other nucleotides are connected by 3'-5' phosphodiester groups. Each of the siRNA

compounds in Table 2 comprises a 19 base pair duplex region with either a 2 nucleotide overhang at the 3' end of both strands or bluntmer at one or both ends. The GalNAc3K2AhxC6 is:

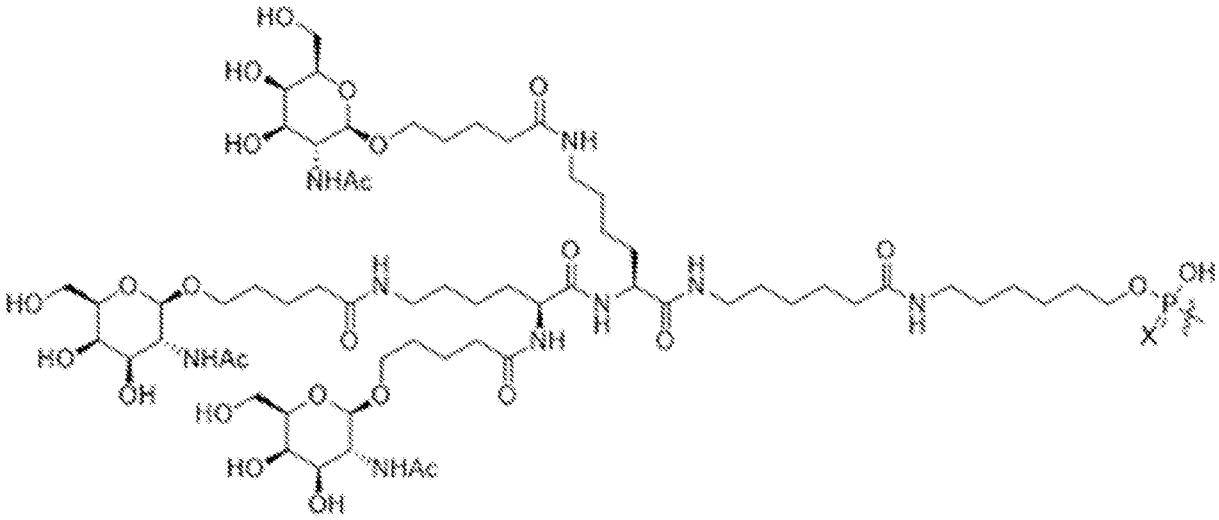


Table 2. siRNA sequences directed to PNPLA3 with modifications

Duplex No.	Sense sequence (5'-3')	SEQ ID NO: (sense)	Antisense sequence (5'-3')	SEQ ID NO: (antisense)
D-2000	GfsgsGfcAfaUfaAfAfGfuAfcCfuGfcUfsusUf	167	asGfscAfgGfuAfcuuUfaUfuGfcCfcsUfsu	168
D-2001	CfsgsGfcCfaAfuGfUfCfcAfcCfaGfcUfsusUf	169	asGfscUfgGfuGfgacAfuUfgGfcCfgsUfsu	170
D-2002	GfsgsUfcCfaGfcCfUfGfaAfcUfuCfuUfsusUf	171	asAfsgAfaGfuUfcagGfcUfgGfaCfcsUfsu	172
D-2003	GfscsUfuCfaUfcCfCfCfuUfcUfaCfaGfsusUf	173	csUfsgUfaGfaAfgggGfaUfgAfaGfcsUfsu	174
D-2004	GfscsGfgCfuUfcCfUfGfgGfcUfuCfuAfsusUf	175	usAfsgAfaGfcCfcagGfaAfgCfcGfcsUfsu	176
D-2005	GfscsCfuCfuGfaGfCfUfgAfgUfuGfgUfsusUf	177	asCfscAfaCfuCfagcUfcAfgAfgGfcsUfsu	178
D-2006	GfsusGfaCfaAfcGfUfAfcCfcUfuCfaUfsusUf	179	asUfsgAfaGfgGfuacGfuUfgUfcAfcsUfsu	180
D-2007	CfscsCfgCfcUfcCfAfGfgUfcCfcAfaAfsusUf	181	usUfsuGfgGfaCfcugGfaGfgCfgGfgsUfsu	182
D-2008	CfsusUfcAfuCfcCfCfUfuCfuAfcAfgUfsusUf	183	asCfsuGfuAfgAfaggGfgAfuGfaAfgsUfsu	184
D-2009	GfsgsUfaUfgUfuCfCfUfgCfuUfcAfuGfsusUf	185	csAfsuGfaAfgCfaggAfaCfaUfaCfcsUfsu	186
D-2010	GfsusAfuGfuUfcCfUfGfcUfuCfaUfgCfsusUf	187	gsCfsaUfgAfaGfcagGfaAfcAfuAfcsUfsu	188
D-2011	UfsasUfgUfuCfcUfGfCfuUfcAfuGfcCfsusUf	189	gsGfscAfuGfaAfgcaGfgAfaCfaUfasUfsu	190
D-2012	AfsusGfuUfcCfuGfCfUfuCfaUfgCfcCfsusUf	191	gsGfsgCfaUfgAfagcAfgGfaAfcAfusUfsu	192
D-2013	UfsgsUfuCfcUfgCfUfUfcAfuGfcCfcUfsusUf	193	asGfsgGfcAfuGfaagCfaGfgAfaCfasUfsu	194

D-2014	GfsusUfcCfuGfcUfUfCfaUfgCfcCfuUfsusUf	195	asAfsgGfgCfaUfgaaGfcAfgGfaAfcsUfsu	196
D-2015	UfsusCfcUfgCfuUfCfAfuGfcCfcUfuCfsusUf	197	gsAfsaGfgGfcAfugaAfgCfaGfgAfasUfsu	198
D-2016	UfscsCfuGfcUfuCfAfUfgCfcCfuUfcUfsusUf	199	asGfsaAfgGfgCfaugAfaGfcAfgGfasUfsu	200
D-2017	CfscsUfgCfuUfcAfUfGfcCfcUfuCfuAfsusUf	201	usAfsgAfaGfgGfcauGfaAfgCfaGfgsUfsu	202
D-2018	CfsusGfcUfuCfaUfGfCfcCfuUfcUfaCfsusUf	203	gsUfsaGfaAfgGfgcaUfgAfaGfcAfgsUfsu	204
D-2019	UfsgsCfuUfcAfuGfCfCfcUfuCfuAfcAfsusUf	205	usGfsuAfgAfaGfggcAfuGfaAfgCfasUfsu	206
D-2020	GfscsUfuCfaUfgCfCfCfuUfcUfaCfaGfsusUf	207	csUfsgUfaGfaAfgggCfaUfgAfaGfcsUfsu	208
D-2021	CfsusUfcAfuGfcCfCfUfuCfuAfcAfgUfsusUf	209	asCfsuGfuAfgAfaggGfcAfuGfaAfgsUfsu	210
D-2022	UfsusCfaUfgCfcCfUfUfcUfaCfaGfuGfsusUf	211	csAfscUfgUfaGfaagGfgCfaUfgAfasUfsu	212
D-2023	UfscsAfuGfcCfcUfUfCfuAfcAfgUfgGfsusUf	213	csCfsaCfuGfuAfgaaGfgGfcAfuGfasUfsu	214
D-2024	CfsasUfgCfcCfuUfCfUfaCfaGfuGfgCfsusUf	215	gsCfscAfcUfgUfagaAfgGfgCfaUfgsUfsu	216
D-2025	AfsusGfcCfcUfuCfUfAfcAfgUfgGfcCfsusUf	217	gsGfscCfaCfuGfuagAfaGfgGfcAfusUfsu	218
D-2026	UfsgsCfcCfuUfcUfAfCfaGfuGfgCfcUfsusUf	219	asGfsgCfcAfcUfguaGfaAfgGfgCfasUfsu	220
D-2027	GfscsCfcUfuCfuAfCfAfgUfgGfcCfuUfsusUf	221	asAfsgGfcCfaCfuguAfgAfaGfgGfcsUfsu	222
D-2028	GfsgsUfaUfgUfuCfCfUfgCfuUfcAfuCfsusUf	223	gsAfsuGfaAfgCfaggAfaCfaUfaCfcsUfsu	224
D-2029	GfsusAfuGfuUfcCfUfGfcUfuCfaUfcCfsusUf	225	gsGfsaUfgAfaGfcagGfaAfcAfuAfcsUfsu	226
D-2030	UfsasUfgUfuCfcUfGfCfuUfcAfuCfcCfsusUf	227	gsGfsgAfuGfaAfgcaGfgAfaCfaUfasUfsu	228
D-2031	AfsusGfuUfcCfuGfCfUfuCfaUfcCfcCfsusUf	229	gsGfsgGfaUfgAfagcAfgGfaAfcAfusUfsu	230
D-2032	UfsgsUfuCfcUfgCfUfUfcAfuCfcCfcUfsusUf	231	asGfsgGfgAfuGfaagCfaGfgAfaCfasUfsu	232
D-2033	GfsusUfcCfuGfcUfUfCfaUfcCfcCfuUfsusUf	233	asAfsgGfgGfaUfgaaGfcAfgGfaAfcsUfsu	234
D-2034	UfsusCfcUfgCfuUfCfAfuCfcCfcUfuCfsusUf	235	gsAfsaGfgGfgAfugaAfgCfaGfgAfasUfsu	236
D-2035	UfscsCfuGfcUfuCfAfUfcCfcCfuUfcUfsusUf	237	asGfsaAfgGfgGfaugAfaGfcAfgGfasUfsu	238
D-2036	CfscsUfgCfuUfcAfUfCfcCfcUfuCfuAfsusUf	239	usAfsgAfaGfgGfgauGfaAfgCfaGfgsUfsu	240
D-2037	CfsusGfcUfuCfaUfCfCfcCfuUfcUfaCfsusUf	241	gsUfsaGfaAfgGfggaUfgAfaGfcAfgsUfsu	242
D-2038	UfsgsCfuUfcAfuCfCfCfcUfuCfuAfcAfsusUf	243	usGfsuAfgAfaGfgggAfuGfaAfgCfasUfsu	244
D-2039	UfsusCfaUfcCfcCfUfUfcUfaCfaGfuGfsusUf	245	csAfscUfgUfaGfaagGfgGfaUfgAfasUfsu	246
D-2040	UfscsAfuCfcCfcUfUfCfuAfcAfgUfgGfsusUf	247	csCfsaCfuGfuAfgaaGfgGfgAfuGfasUfsu	248
D-2041	CfsasUfcCfcCfuUfCfUfaCfaGfuGfgCfsusUf	249	gsCfscAfcUfgUfagaAfgGfgGfaUfgsUfsu	250
D-2042	UfscsCfcCfuUfcUfAfCfaGfuGfgCfcUfsusUf	251	asGfsgCfcAfcUfguaGfaAfgGfgGfasUfsu	252
D-2043	GfsasUfcAfgGfaCfCfCfgAfgCfcGfaUfsusUf	253	asUfscGfgCfuCfgggUfcCfuGfaUfcsUfsu	254
D-2044	UfsgsGfgCfuUfcUfAfCfcAfcGfuCfgUfsusUf	255	asCfsgAfcGfuGfguaGfaAfgCfcCfasUfsu	256
D-2045	GfsasGfcGfaGfcAfCfGfcCfcCfgCfaUfsusUf	257	asUfsgCfgGfgGfcguGfcUfcGfcUfcsUfsu	258
D-2046	UfsgsCfaCfuGfcGfUfCfgGfcGfuCfcUfsusUf	259	asGfsgAfcGfcCfgacGfcAfgUfgCfasUfsu	260

D-2047	UfsgsGfaGfcAfgAfCfUfcUfgCfaGfgUfsusUf	261	asCfscUfgCfaGfaguCfuGfcUfcCfasUfsu	262
D-2048	UfsgsCfaGfgUfcCfUfCfuCfaGfaUfcUfsusUf	263	asGfsaUfcUfgAfgagGfaCfcUfgCfasUfsu	264
D-2049	CfscsCfgGfcCfaAfUfGfuCfcAfcCfaUfsusUf	265	asUfsgGfuGfgAfcauUfgGfcCfgGfgsUfsu	266
D-2050	UfsusCfuAfcAfgUfGfGfcCfuUfaUfcUfsusUf	267	asGfsaUfaAfgGfccaCfuGfuAfgAfasUfsu	268
D-2051	UfscsUfaCfaGfuGfGfCfcUfuAfuCfcUfsusUf	269	asGfsgAfuAfaGfgccAfcUfgUfaGfasUfsu	270
D-2052	CfsusUfcCfuUfcAfGfAfgGfcGfuGfcUfsusUf	271	asGfscAfcGfcCfucuGfaAfgGfaAfgsUfsu	272
D-2053	UfsusCfcUfuCfaGfAfGfgCfgUfgCfgAfsusUf	273	usCfsgCfaCfgCfcucUfgAfaGfgAfasUfsu	274
D-2054	GfscsGfuGfcGfaUfAfUfgUfgGfaUfgUfsusUf	275	asCfsaUfcCfaCfauaUfcGfcAfcGfcsUfsu	276
D-2055	CfsgsUfgCfgAfuAfUfGfuGfgAfuGfgAfsusUf	277	usCfscAfuCfcAfcauAfuCfgCfaCfgsUfsu	278
D-2056	UfsgsGfaUfgGfaGfGfAfgUfgAfgUfgAfsusUf	279	usCfsaCfuCfaCfuccUfcCfaUfcCfasUfsu	280
D-2057	AfscsGfuAfcCfcUfUfCfaUfuGfaUfgUfsusUf	281	asCfsaUfcAfaUfgaaGfgGfuAfcGfusUfsu	282
D-2058	UfsgsGfaCfaUfcAfCfCfaAfgCfuCfaUfsusUf	283	asUfsgAfgCfuUfgguGfaUfgUfcCfasUfsu	284
D-2059	CfsasCfcUfgCfgUfCfUfcAfgCfaUfcUfsusUf	285	asGfsaUfgCfuGfagaCfgCfaGfgUfgsUfsu	286
D-2060	AfscsCfuGfcGfuCfUfCfaGfcAfuCfcUfsusUf	287	asGfsgAfuGfcUfgagAfcGfcAfgGfusUfsu	288
D-2061	CfscsAfgAfgAfcUfGfGfuGfaCfaUfgUfsusUf	289	asCfsaUfgUfcAfccaGfuCfuCfuGfgsUfsu	290
D-2062	AfsusGfgCfuUfcCfAfGfaUfaUfgCfcUfsusUf	291	asGfsgCfaUfaUfcugGfaAfgCfcAfusUfsu	292
D-2063	CfscsGfcCfuCfcAfGfGfuCfcCfaAfaUfsusUf	293	asUfsuUfgGfgAfccuGfgAfgGfcGfgsUfsu	294
D-2064	UfsasCfcUfgCfuGfGfUfgCfuGfaGfgUfsusUf	295	asCfscUfcAfgCfaccAfgCfaGfgUfasUfsu	296
D-2065	AfscsCfuGfcUfgGfUfGfcUfgAfgGfgUfsusUf	297	asCfscCfuCfaGfcacCfaGfcAfgGfusUfsu	298
D-2066	CfsusCfuCfcAfcCfUfUfuCfcCfaGfuUfsusUf	299	asAfscUfgGfgAfaagGfuGfgAfgAfgsUfsu	300
D-2067	UfsusUfuUfcAfcCfUfAfaCfuAfaAfaUfsusUf	301	asUfsuUfuAfgUfuagGfuGfaAfaAfasUfsu	302
D-2068	CfgGfcCfaAfuGfUfCfcAfcCfaGfcUfsusUf	303	asGfscUfgGfuGfgacAfuUfgGfcCfgsUfsu	304
D-2069	GfgUfcCfaGfcCfUfGfaAfcUfuCfuUfsusUf	305	asAfsgAfaGfuUfcagGfcUfgGfaCfcsUfsu	306
D-2070	GfcGfgCfuUfcCfUfGfgGfcUfuCfuAfsusUf	307	usAfsgAfaGfcCfcagGfaAfgCfcGfcsUfsu	308
D-2071	GfuGfaCfaAfcGfUfAfcCfcUfuCfaUfsusUf	309	asUfsgAfaGfgGfuacGfuUfgUfcAfcsUfsu	310
D-2072	GfgUfaUfgUfuCfCfUfgCfuUfcAfuGfsusUf	311	csAfsuGfaAfgCfaggAfaCfaUfaCfcsUfsu	312
D-2073	GfuAfuGfuUfcCfUfGfcUfuCfaUfgCfsusUf	313	gsCfsaUfgAfaGfcagGfaAfcAfuAfcsUfsu	314
D-2074	UfgUfuCfcUfgCfUfUfcAfuGfcCfcUfsusUf	315	asGfsgGfcAfuGfaagCfaGfgAfaCfasUfsu	316
D-2075	GfuUfcCfuGfcUfUfCfaUfgCfcCfuUfsusUf	317	asAfsgGfgCfaUfgaaGfcAfgGfaAfcsUfsu	318
D-2076	CfcUfgCfuUfcAfUfGfcCfcUfuCfuAfsusUf	319	usAfsgAfaGfgGfcauGfaAfgCfaGfgsUfsu	320
D-2077	GfcUfuCfaUfgCfCfCfuUfcUfaCfaGfsusUf	321	csUfsgUfaGfaAfgggCfaUfgAfaGfcsUfsu	322
D-2078	CfuUfcAfuGfcCfCfUfuCfuAfcAfgUfsusUf	323	asCfsuGfuAfgAfaggGfcAfuGfaAfgsUfsu	324
D-2079	UfuCfaUfgCfcCfUfUfcUfaCfaGfuGfsusUf	325	csAfscUfgUfaGfaagGfgCfaUfgAfasUfsu	326

D-2080	AfuGfgCfuUfcCfAfGfaUfaUfgCfcUfsusUf	327	asGfsgCfaUfaUfcugGfaAfgCfcAfusUfsu	328
D-2081	AfuGfcCfcUfuCfUfAfcAfgUfgGfcCfsusUf	329	gsGfscCfaCfuGfuagAfaGfgGfcAfusUfsu	330
D-2082	GfcUfuCfaUfgCfCfCfuUfcUfaCfaUfsusUf	331	asUfsgUfaGfaAfgggCfaUfgAfaGfcsUfsu	332
D-2083	{Phosphate}GfsgsAfaAfgAfcUfGfUfuCfcAfaAfaAfsusUf	1295	{Phosphate}usUfsuUfuGfgAfacaGfuCfuUfuCfcsUfsu	1296
D-2084	{GalNAc3K2AhxC6}ggsuaugUfuCfCfUfGfcuucaugsusu	1297	{Phosphate}csAfsuGfaAfGfcaggAfaCfauaccsusu	1298
D-2085	{GalNAc3K2AhxC6}guauguUfcCfUfGfCfuucaugcsusu	1299	{Phosphate}gsCfsaUfgAfAfgcagGfaAfcauacsusu	1300
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D-2087	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacagsusu	1303	{Phosphate}csUfsgUfaGfAfagggCfaUfgaagcsusu	1304
D-2088	{GalNAc3K2AhxC6}cuucauGfcCfCfUfUfcuacagususu	1305	{Phosphate}asCfsuGfuAfGfaaggGfcAfugaagsusu	1306
D-2089	{GalNAc3K2AhxC6}gcggcuUfcCfUfGfGfgcuucuasusu	1307	{Phosphate}usAfsgAfaGfCfccagGfaAfgccgcsusu	1308
D-2090	{GalNAc3K2AhxC6}guuccuGfcUfUfCfAfugcccuususu	1309	{Phosphate}asAfsgGfgCfAfugaaGfcAfggaacsusu	1310
D-2091	{GalNAc3K2AhxC6}AfuGfgCfuUfcCfAfGfaUfaUfgCfcUfsusUf	1311	{Phosphate}asGfsgCfaUfaUfcugGfaAfgCfcAfusUfsu	1312
D-2092	{GalNAc3K2AhxC6}ccugcuUfcAfUfGfCfccuucuasusu	1313	{Phosphate}usAfsgAfaGfGfgcauGfaAfgcaggsusu	1314
D-2093	{GalNAc3K2AhxC6}uucaugCfcCfUfUfCfuacaguususu	1315	{Phosphate}asAfscUfgUfAfgaagGfgCfaugaasusu	1316
D-2094	{GalNAc3K2AhxC6}uucaugCfcCfUfUfCfuacagugsusu	1317	{Phosphate}csAfscUfgUfAfgaagGfgCfaugaasusu	1318
D-2095	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1319	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1320
D-2096	{GalNAc3K2AhxC6}gguccaGfcCfUfGfAfacuucuususu	1321	{Phosphate}asAfsgAfaGfUfucagGfcUfggaccsusu	1322
D-2097	{GalNAc3K2AhxC6}GfcGfgCfuUfcCfUfGfGfgcUfuCfuAfsusUf	1323	{Phosphate}usAfsgAfaGfCfccagGfaAfgCfcGfcsUfsu	1324
D-2098	{GalNAc3K2AhxC6}GfcGfgCfuUfcCfuGfGfgcUfuCfuAfsusUf	1325	{Phosphate}usAfsgAfaGfCfccAfgGfaAfgCfcGfcsUfsu	1326
D-2099	{GalNAc3K2AhxC6}GfuUfcCfuGfcUfUfCfAfugCfcCfuUfsusUf	1327	{Phosphate}asAfsgGfgCfAfugaaGfcAfgGfaAfcsUfsu	1328
D-2100	{GalNAc3K2AhxC6}GfuUfcCfuGfcUfuCfAfugCfcCfuUfsusUf	1329	{Phosphate}asAfsgGfgCfAfugAfaGfcAfgGfaAfcsUfsu	1330
D-2101	{GalNAc3K2AhxC6}CfcUfgCfuUfcAfUfGfCfccUfuCfuAfsusUf	1331	{Phosphate}usAfsgAfaGfGfgcauGfaAfgCfaGfgsUfsu	1332
D-2102	{GalNAc3K2AhxC6}CfcUfgCfuUfcAfuGfCfccUfuCfuAfsusUf	1333	{Phosphate}usAfsgAfaGfGfgcAfuGfaAfgCfaGfgsUfsu	1334
D-2103	{GalNAc3K2AhxC6}augcccUfuCfUfAfCfaguggccsusu	1335	{Phosphate}gsGfscCfaCfUfguagAfaGfggcaususu	1336
D-2104	{GalNAc3K2AhxC6}auggcuUfcCfAfGfAfuaugccususu	1337	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaususu	1338
D-2105	{GalNAc3K2AhxC6}gugacaAfcGfUfAfCfccuucaususu	1339	{Phosphate}asUfsgAfaGfGfguacGfuUfgucacsusu	1340
D-2106	{GalNAc3K2AhxC6}guauguUfcCfUfGfCfuucaugcsusu	1341	{Phosphate}gsCfsaUfgAfAfgcAfgGfaAfcauacsusu	1342
D-2107	{GalNAc3K2AhxC6}guauguUfcCfuGfCfuucaugcsusu	1343	{Phosphate}gsCfsaUfgAfAfgcagGfaAfcauacsusu	1344
D-2108	{GalNAc3K2AhxC6}guauguUfcCfuGfCfuucaugcsusu	1345	{Phosphate}gsCfsaUfgAfAfgcAfgGfaAfcauacsusu	1346
D-2109	{GalNAc3K2AhxC6}guauguUfcCfUfGfCfuucaugcscsu	1347	{Phosphate}asGfsgCfaUfgAfAfgcagGfaAfcauacsusu	1348
D-2110	{GalNAc3K2AhxC6}ugguauGfuUfCfCfUfgcuucausgsu	1349	{Phosphate}gsCfsaUfgAfaGfCfaggaAfcAfuaccasusu	1350
D-2111	{GalNAc3K2AhxC6}guauguUfcCfUfGfCfuucausgsu	1351	{Phosphate}gsCfsaUfgAfAfgcagGfaAfcauacsusu	1352
D-2112	{GalNAc3K2AhxC6}guauguUfcCfUfGfCfuucaugcs{invAb}	1353	{Phosphate}gsCfsaUfgAfAfgcagGfaAfcauacsusu	1354

D-2113	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucUfaCfaUfsusUf	1355	{Phosphate}asUfsgUfaGfAfagggCfaUfgAfaGfcsUfsu	1356
D-2114	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfcCfUfucUfaCfaUfsusUf	1357	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgAfaGfcsUfsu	1358
D-2115	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1359	{Phosphate}asusguaGfAfagggCfaUfgaagcsusu	1360
D-2116	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucUfaCfaUfsusUf	1361	{Phosphate}asusguaGfAfagggCfaUfgaagcsusu	1362
D-2117	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucuacaususu	1363	{Phosphate}asusguaGfAfagggCfaUfgaagcsusu	1364
D-2118	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1365	{Phosphate}asusguaGfAfagggCfaUfgAfaGfcsUfsu	1366
D-2119	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1367	{Phosphate}asUfsgUfaGfAfagggCfaUfgAfaGfcsUfsu	1368
D-2120	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucUfaCfaUfsusUf	1369	{Phosphate}asusguaGfAfagggCfaUfgaagcsusu	1370
D-2121	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucuacaususu	1371	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1372
D-2122	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucUfaCfaUfsusUf	1373	{Phosphate}asusguaGfAfagggCfaUfgAfaGfcsUfsu	1374
D-2123	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucUfaCfaUfsusUf	1375	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1376
D-2124	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucuacaususu	1377	{Phosphate}asusguaGfAfagggCfaUfgAfaGfcsUfsu	1378
D-2125	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucuacaususu	1379	{Phosphate}asUfsgUfaGfAfagggCfaUfgAfaGfcsUfsu	1380
D-2126	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucUfaCfaUfsusUf	1381	{Phosphate}asUfsgUfaGfAfagggCfaUfgAfaGfcsUfsu	1382
D-2127	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucUfaCfaUfsusUf	1383	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1384
D-2128	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfCfUfucUfaCfaUfsusUf	1385	{Phosphate}asusguaGfAfagggCfaUfgAfaGfcsUfsu	1386
D-2129	{GalNAc3K2AhxC6}gcuucaUfg[dC]CfCfuucuacaususu	1387	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1388
D-2130	{GalNAc3K2AhxC6}gcuucaUfgCfCf[dC]Ufucuacaususu	1389	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1390
D-2131	{GalNAc3K2AhxC6}gcuucaUfgCf[dC]CfUfucuacaususu	1391	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1392
D-2132	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1393	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgaagcsusu	1394
D-2133	{GalNAc3K2AhxC6}gcuucaUfgCfcCfUfucuacaususu	1395	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1396
D-2134	{GalNAc3K2AhxC6}gcuucaUfgCfcCfUfucuacaususu	1397	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgaagcsusu	1398
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D-2136	{GalNAc3K2AhxC6}GfscsUfuCfaUfgCfCfCfUfucUfaCfaUfsusUf	1401	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgAfaGfcsUfsu	1402
D-2137	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacagsusu	1403	{Phosphate}asAfscUfgUfaGfAfagggCfaUfgaagcsusu	1404
D-2138	{GalNAc3K2AhxC6}cugcuuCfaUfGfCfCfcuucuacsasu	1405	{Phosphate}asUfsgUfaGfaAfGfggcaUfgAfagcagsusu	1406
D-2139	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacsasu	1407	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1408
D-2140	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaus{invAb}	1409	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1410
D-2141	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacauuus{invAb}	1411	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1412
D-2142	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1413	{Phosphate}AfsusGfuAfgAfagggCfaUfgaagcsusu	1414
D-2143	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfuCfuacaususu	1415	{Phosphate}AfsusGfuAfgAfagggCfaUfgaagcsusu	1416
D-2144	{GalNAc3K2AhxC6}gcuucaugCfCfCfUfucuacaususu	1417	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1418
D-2145	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfUfucuacaususu	1419	{Phosphate}asUfsgUfaGfAfagggCfaugaagcsusu	1420

D-2146	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfU fucuacaususu	1421	{Phosphate}asusgUfaGfAfagggCfaUfg aagcsusu	1422
D-2147	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfU fucuacaususu	1423	{Phosphate}asUfsgUfagAfagggCfaUfg aagcsusu	1424
D-2148	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfU fucuacaususu	1425	{Phosphate}asusgUfagAfagggCfaUfga agcsusu	1426
D-2149	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfU fucuacaususu	1427	{Phosphate}asUfsguagAfagggCfaUfga agcsusu	1428
D-2150	{GalNAc3K2AhxC6}gcuucaUfgCfCfCfU fucuacaususu	1429	{Phosphate}asusguagAfagggCfaUfgaa gcsusu	1430
D-2151	{GalNAc3K2AhxC6}guauguUfcCfUfGfC fuucaugcuus{invAb}	1431	{Phosphate}gsCfsaUfgAfAfgcagGfaAf cauacsusu	1432
D-2152	{GalNAc3K2AhxC6}gguaugUfuCfCfUfG fcuucauususu	1433	{Phosphate}aAfsuGfaAfGfcaggAfaCfa uaccsusu	1434
D-2153	{GalNAc3K2AhxC6}guauguUfcCfUfGfC fuucaugususu	1435	{Phosphate}asCfsaUfgAfAfgcagGfaAf cauacsusu	1436
D-2154	{GalNAc3K2AhxC6}cggccaAfuGfUfCfC faccagcususu	1437	{Phosphate}asGfscUfgGfUfggacAfuUf ggccgsusu	1438
D-2155	{GalNAc3K2AhxC6}uggagcAfgAfCfUfC fugcaggususu	1439	{Phosphate}asCfscUfgCfAfgaguCfuGf cuccasusu	1440
D-2156	{GalNAc3K2AhxC6}acguacCfcUfUfCfA fuugaugususu	1441	{Phosphate}aCfsaUfcAfAfugaaGfgGfu acgususu	1442
D-2157	{GalNAc3K2AhxC6}ccagagAfcUfGfGfU fgacaugususu	1443	{Phosphate}asCfsaUfgUfCfaccaGfuCf ucuggsusu	1444
D-2158	{GalNAc3K2AhxC6}[invAb]gcuucaUfg CfCfUfUfucuacaususu	1445	{Phosphate}asUfsgUfaGfAfaaggCfaUf gaagcsusu	1446
D-2159	{GalNAc3K2AhxC6}[invAb]gcuucaUfg CfCfUfUfucuacaususu	1447	{Phosphate}asAfsaUfgUfaGfAfaaggCf aUfgaagcsusu	1448
D-2160	{GalNAc3K2AhxC6}[invAb]gcuucaUfg CfcUfUfUfCfuacaususu	1449	{Phosphate}asAfsaUfgUfAfgaaaGfgCf aUfgaagcsusu	1450
D-2161	{GalNAc3K2AhxC6}ugcuucAfuGfCfCfU fuucuacasusu	1451	{Phosphate}usGfsuAfgAfAfaggcAfuGf aagcasusu	1452
D-2162	{GalNAc3K2AhxC6}uauguuCfcUfGfCfU fucaugcususu	1453	{Phosphate}asGfscAfuGfAfagcaGfgAf acauasusu	1454
D-2163	{GalNAc3K2AhxC6}uuccugCfuUfCfAfU fgccuuuususu	1455	{Phosphate}asAfsaAfgGfCfaugaAfgCf aggaasusu	1456
D-2164	{GalNAc3K2AhxC6}ucaugcCfuUfUfCfU facagugususu	1457	{Phosphate}asCfsaCfuGfUfagaaAfgGf caugasusu	1458
D-2165	{GalNAc3K2AhxC6}caugccUfuUfCfUfA fcaguggususu	1459	{Phosphate}asCfscAfcUfGfuagaAfaGf gcaugsusu	1460
D-2166	{GalNAc3K2AhxC6}augccuUfuCfUfAfC faguggcususu	1461	{Phosphate}asGfscCfaCfUfguagAfaAf ggcaususu	1462
D-2167	{GalNAc3K2AhxC6}gguaugUfuCfCfUfG fcuucauasusu	1463	{Phosphate}usAfsuGfaAfGfcaggAfaCf auaccsusu	1464
D-2168	{GalNAc3K2AhxC6}guauguUfcCfUfGfC fuucaugasusu	1465	{Phosphate}usCfsaUfgAfAfgcagGfaAf cauacsusu	1466
D-2169	{GalNAc3K2AhxC6}uauguuCfcUfGfCfU fucaugcasusu	1467	{Phosphate}usGfscAfuGfAfagcaGfgAf acauasusu	1468
D-2170	{GalNAc3K2AhxC6}uuccugCfuUfCfAfU fgccuuuasusu	1469	{Phosphate}usAfsaAfgGfCfaugaAfgCf aggaasusu	1470
D-2171	{GalNAc3K2AhxC6}cugcuuCfaUfGfCfC fuuucuaasusu	1471	{Phosphate}usUfsaGfaAfAfggcaUfgAf agcagsusu	1472
D-2172	{GalNAc3K2AhxC6}gcuucaUfgCfCfUfU fucuacaasusu	1473	{Phosphate}usUfsgUfaGfAfaaggCfaUf gaagcsusu	1474
D-2173	{GalNAc3K2AhxC6}uucaugCfcUfUfUfC fuacaguasusu	1475	{Phosphate}usAfscUfgUfAfgaaaGfgCf augaasusu	1476
D-2174	{GalNAc3K2AhxC6}ucaugcCfuUfUfCfU facagugasusu	1477	{Phosphate}usCfsaCfuGfUfagaaAfgGf caugasusu	1478
D-2175	{GalNAc3K2AhxC6}caugccUfuUfCfUfA fcaguggasusu	1479	{Phosphate}usCfscAfcUfGfuagaAfaGf gcaugsusu	1480
D-2176	{GalNAc3K2AhxC6}augccuUfuCfUfAfC faguggcasusu	1481	{Phosphate}usGfscCfaCfUfguagAfaAf ggcaususu	1482
D-2177	{GalNAc3K2AhxC6}acguacCfcUfUfCfA fuugaugasusu	1483	{Phosphate}usCfsaUfcAfAfugaaGfgGf uacgususu	1484
D-2178	{GalNAc3K2AhxC6}ccagagAfcUfGfGfU fgacaugasusu	1485	{Phosphate}usCfsaUfgUfCfaccaGfuCf ucuggsusu	1486

D-2179	{GalNAc3K2AhxC6}auggcuUfcCfAfGfAfuaugccasusu	1487	{Phosphate}usGfsgCfaUfAfucugGfaAfgccaususu	1488
D-2180	{GalNAc3K2AhxC6}guuccuGfcUfUfCfAfugccuuususu	1489	{Phosphate}asAfsaGfgCfAfugaaGfcAfggaacsusu	1490
D-2181	{GalNAc3K2AhxC6}ccugcuUfcAfUfGfCfcuuucuasusu	1491	{Phosphate}usAfsgAfaAfGfgcauGfaAfgcaggsusu	1492
D-2182	{GalNAc3K2AhxC6}gcuucaUfgCfCfUfUfucuacaususu	1493	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcsusu	1494
D-2183	{GalNAc3K2AhxC6}cuucauGfcCfUfUfUfcuacagususu	1495	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1496
D-2184	{GalNAc3K2AhxC6}uucaugCfcUfUfUfCfuacaguususu	1497	{Phosphate}asAfscUfgUfAfgaaaGfgCfaugaasusu	1498
D-2185	{GalNAc3K2AhxC6}gcuucaUfcCfCfUfUfucuacaususu	1499	{Phosphate}asUfsgUfaGfAfaaggGfaUfgaagcsusu	1500
D-2186	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucuacaususu	1501	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1502
D-2187	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucuacaususu	1503	{Phosphate}asAfsaUfgUfaGfAfagggCfaUfgaagcsusu	1504
D-2188	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfcCfUfUfCfuacaususu	1505	{Phosphate}asAfsaUfgUfAfgaagGfgCfaUfgaagcsusu	1506
D-2189	{GalNAc3K2AhxC6}[invAb]gcggcuUfcCfUfGfGfgcuucuasusu	1507	{Phosphate}usAfsgAfaGfCfccagGfaAfgccgcsusu	1508
D-2190	{GalNAc3K2AhxC6}[invAb]CfuGfcGfgCfuUfcCfuGfGfgcUfuCfsusAf	1509	{Phosphate}usAfsgAfaGfCfccAfgGfaAfgCfcGfcAfgsUfsu	1510
D-2191	{GalNAc3K2AhxC6}cugcggCfuUfCfCfUfgggcuucus{invAb}	1511	{Phosphate}usAfsgAfaGfcCfCfaggaAfgCfcgcagsusu	1512
D-2192	{GalNAc3K2AhxC6}[invAb]cugcggCfuUfCfCfUfgggcuucsusa	1513	{Phosphate}usAfsgAfaGfcCfCfaggaAfgCfcgcagsusu	1514
D-2193	{GalNAc3K2AhxC6}[invAb]gcggcuUfcCfUfGfGfgcUfuCfuAfsusUf	1515	{Phosphate}usAfsgAfaGfCfccagGfaAfgccgcsusu	1516
D-2194	{GalNAc3K2AhxC6}cugcggCfuUfCfCfUfggGfcUfuCfus{invAb}	1517	{Phosphate}usAfsgAfaGfcCfCfaggaAfgCfcgcagsusu	1518
D-2195	{GalNAc3K2AhxC6}[invAb]cugcggCfuUfCfCfUfggGfcUfuCfsusAf	1519	{Phosphate}usAfsgAfaGfcCfCfaggaAfgCfcgcagsusu	1520
D-2196	{GalNAc3K2AhxC6}[invAb]GfcGfgCfuUfcCfuGfGfgcUfuCfuAfsusUf	1521	{Phosphate}usAfsgAfaGfCfccAfgGfaAfgCfcGfcsUfsu	1522
D-2197	{GalNAc3K2AhxC6}CfuGfcGfgCfuUfcCfUfggGfcUfuCfus{invAb}	1523	{Phosphate}usAfsgAfaGfcCfCfagGfaAfgCfcGfcAfgsUfsu	1524
D-2198	{GalNAc3K2AhxC6}[invAb]CfuGfcGfgCfuUfcCfUfggGfcUfuCfsusAf	1525	{Phosphate}usAfsgAfaGfcCfCfagGfaAfgCfcGfcAfgsUfsu	1526
D-2199	{GalNAc3K2AhxC6}[invAb]GfcGfgCfuUfcCfUfGfGfgcuucuasusu	1527	{Phosphate}usAfsgAfaGfCfccagGfaAfgCfcGfcsUfsu	1528
D-2200	{GalNAc3K2AhxC6}[invAb]CfuGfcGfgCfuUfCfCfUfgggcuucsusa	1529	{Phosphate}usAfsgAfaGfcCfCfaggaAfgCfcGfcAfgsUfsu	1530
D-2201	{GalNAc3K2AhxC6}[invAb]cugcggcuUfcCfUfGfGfgcUfuCfusAf	1531	{Phosphate}usAfsgAfaGfCfccagGfaAfgccgcagsusu	1532
D-2202	{GalNAc3K2AhxC6}[invAb]CfuGfcGfgCfuUfcCfUfGfGfgcuucsusa	1533	{Phosphate}usAfsgAfaGfCfccagGfaAfgCfcGfcAfgsUfsu	1534
D-2203	{GalNAc3K2AhxC6}[invAb]auggcuUfcCfAfGfAfuaugccususu	1535	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaususu	1536
D-2204	{GalNAc3K2AhxC6}[invAb]AfcAfuGfgCfuUfcCfaGfAfuaUfgCfscsUf	1537	{Phosphate}asGfsgCfaUfAfucUfgGfaAfgCfcAfuGfusUfsu	1538
D-2205	{GalNAc3K2AhxC6}acauggCfuUfCfCfAfgauaugccs{invAb}	1539	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcaugususu	1540
D-2206	{GalNAc3K2AhxC6}[invAb]acauggCfuUfCfCfAfgauaugcscsu	1541	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcaugususu	1542
D-2207	{GalNAc3K2AhxC6}[invAb]auggcuUfcCfAfGfAfuaUfgCfcUfsusUf	1543	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaususu	1544
D-2208	{GalNAc3K2AhxC6}acauggCfuUfCfCfAfgaUfaUfgCfcs{invAb}	1545	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcaugususu	1546
D-2209	{GalNAc3K2AhxC6}[invAb]acauggCfuUfCfCfAfgaUfaUfgCfscsUf	1547	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcaugususu	1548
D-2210	{GalNAc3K2AhxC6}[invAb]AfuGfgCfuUfcCfaGfAfuaUfgCfcUfsusUf	1549	{Phosphate}asGfsgCfaUfAfucUfgGfaAfgCfcAfusUfsu	1550
D-2211	{GalNAc3K2AhxC6}AfcAfuGfgCfuUfcCfAfgaUfaUfgCfcs{invAb}	1551	{Phosphate}asGfsgCfaUfaUfCfugGfaAfgCfcAfuGfusUfsu	1552

D-2212	{GalNAc3K2AhxC6}[invAb]AfcAfuGfgCfuUfcCfAfgaUfaUfgCfscsUf	1553	{Phosphate}asGfsgCfaUfaUfCfugGfaAfgCfcAfuGfusUfsu	1554
D-2213	{GalNAc3K2AhxC6}[invAb]AfuGfgCfuUfcCfAfGfAfuaugccususu	1555	{Phosphate}asGfsgCfaUfAfucugGfaAfgCfcAfusUfsu	1556
D-2214	{GalNAc3K2AhxC6}AfcAfuGfgCfuUfCfCfAfgauaugccs{invAb}	1557	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcAfuGfusUfsu	1558
D-2215	{GalNAc3K2AhxC6}[invAb]AfcAfuGfgCfuUfCfCfAfgauaugcscsu	1559	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcAfuGfusUfsu	1560
D-2216	{GalNAc3K2AhxC6}[invAb]acauggcuUfcCfAfGfAfuaugcscsu	1561	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaugususu	1562
D-2217	{GalNAc3K2AhxC6}[invAb]acauggcuUfcCfAfGfAfuaUfgCfcsUf	1563	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaugususu	1564
D-2218	{GalNAc3K2AhxC6}[invAb]AfcAfuGfgCfuUfcCfAfGfAfuaugcscsu	1565	{Phosphate}asGfsgCfaUfAfucugGfaAfgCfcAfuGfusUfsu	1566
D-2219	{GalNAc3K2AhxC6}[invAb]acguacCfcUfUfCfAfuugaugususu	1567	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacgususu	1568
D-2220	{GalNAc3K2AhxC6}[invAb]CfaAfcGfuAfcCfcUfuCfAfuuGfaUfsgsUf	1569	{Phosphate}asCfsaUfcAfAfugAfaGfgGfuAfcGfuUfgsUfsu	1570
D-2221	{GalNAc3K2AhxC6}[invAb]caacguAfcCfCfUfUfcauugausgsu	1571	{Phosphate}asCfsaUfcAfaUfGfaaggGfuAfcguugsusu	1572
D-2222	{GalNAc3K2AhxC6}[invAb]acguacCfcUfUfCfAfuuGfaUfgUfsusUf	1573	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacgususu	1574
D-2223	{GalNAc3K2AhxC6}caacguAfcCfCfUfUfcaUfuGfaUfgs{invAb}	1575	{Phosphate}asCfsaUfcAfaUfGfaaggGfuAfcguugsusu	1576
D-2224	{GalNAc3K2AhxC6}[invAb]AfcGfuAfcCfcUfuCfAfuuGfaUfgUfsusUf	1577	{Phosphate}asCfsaUfcAfAfugAfaGfgGfuAfcGfusUfsu	1578
D-2225	{GalNAc3K2AhxC6}CfaAfcGfuAfcCfcUfUfcaUfuGfaUfgs{invAb}	1579	{Phosphate}asCfsaUfcAfaUfGfaaGfgGfuAfcGfuUfgsUfsu	1580
D-2226	{GalNAc3K2AhxC6}[invAb]CfaAfcGfuAfcCfcUfUfcaUfuGfaUfsgsUf	1581	{Phosphate}asCfsaUfcAfaUfGfaaGfgGfuAfcGfuUfgsUfsu	1582
D-2227	{GalNAc3K2AhxC6}[invAb]AfcGfuAfcCfcUfUfCfAfuugaugususu	1583	{Phosphate}asCfsaUfcAfAfugaaGfgGfuAfcGfusUfsu	1584
D-2228	{GalNAc3K2AhxC6}CfaAfcGfuAfcCfCfUfUfcauugaugs{invAb}	1585	{Phosphate}asCfsaUfcAfaUfGfaaggGfuAfcGfuUfgsUfsu	1586
D-2229	{GalNAc3K2AhxC6}[invAb]CfaAfcGfuAfcCfCfUfUfcauugausgsu	1587	{Phosphate}asCfsaUfcAfaUfGfaaggGfuAfcGfuUfgsUfsu	1588
D-2230	{GalNAc3K2AhxC6}[invAb]caacguacCfcUfUfCfAfuugausgsu	1589	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacguugsusu	1590
D-2231	{GalNAc3K2AhxC6}[invAb]caacguacCfcUfUfCfAfuuGfaUfgsUf	1591	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacguugsusu	1592
D-2232	{GalNAc3K2AhxC6}[invAb]CfaAfcGfuAfcCfcUfUfCfAfuugausgsu	1593	{Phosphate}asCfsaUfcAfAfugaaGfgGfuAfcGfuUfgsUfsu	1594
D-2233	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacsasu	1595	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1596
D-2234	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucUfaCfsasUf	1597	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1598
D-2235	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfcUfUfucUfaCfaUfsusUf	1599	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgAfaGfcsUfsu	1600
D-2236	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfCfUfUfucuacaususu	1601	{Phosphate}asUfsgUfaGfAfaaggCfaUfgAfaGfcsUfsu	1602
D-2237	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfgCfcUfUfucUfaCfsasUf	1603	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgAfaGfcAfgsUfsu	1604
D-2238	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfgCfCfUfUfucuacsasu	1605	{Phosphate}asUfsgUfaGfAfaaggCfaUfgAfaGfcAfgsUfsu	1606
D-2239	{GalNAc3K2AhxC6}[invAb]CfuGfcUfuCfaUfgCfCfUfUfucuacsasu	1607	{Phosphate}asUfsgUfaGfAfaaggCfaUfgAfaGfcAfgsUfsu	1608
D-2240	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfgCfCfUfUfucuacas{invAb}	1609	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1610
D-2241	{GalNAc3K2AhxC6}[invAb]CfuGfcUfuCfaUfgCfCfUfUfucuacsasu	1611	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1612
D-2242	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCf[dC]UfUfucuacsasu	1613	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1614
D-2243	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacsasu	1615	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgaagcagsusu	1616
D-2244	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	1617	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgaagcagsusu	1618

D-2245	{GalNAc3K2AhxC6}cugcuucaUfgCfcUfUfucuacsasu	1619	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1620
D-2246	{GalNAc3K2AhxC6}cugcuucaUfgCfcUfUfucuacas{invAb}	1621	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1622
D-2247	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCfcUfUfucuacsasu	1623	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1624
D-2248	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacsasu	1625	{Phosphate}asUfsgUfagAfaaggCfaUfgaagcagsusu	1626
D-2249	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1627	{Phosphate}asUfsgUfagAfaaggCfaUfgaagcagsusu	1628
D-2250	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	1629	{Phosphate}asUfsgUfagAfaaggCfaUfgaagcagsusu	1630
D-2251	{GalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1631	{Phosphate}asUfsguagAfaaggCfaUfgaagcagsusu	1632
D-2252	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	1633	{Phosphate}asUfsguagAfaaggCfaUfgaagcagsusu	1634
D-2253	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfcUfUfucUfaCfaUfsusUf	1635	{Phosphate}asUfsgUfagAfaaGfgCfaUfgAfaGfcsUfsu	1636
D-2254	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfCfUfUfucuacausususu	1637	{Phosphate}asUfsgUfagAfaaggCfaUfgAfaGfcsUfsu	1638
D-2255	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfCfUfUfucuacausususu	1639	{Phosphate}asUfsgUfagAfaaggCfaUfgaagcsusu	1640
D-2256	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacausususu	1641	{Phosphate}asUfsgUfagAfaaGfgCfaUfgaagcsusu	1642
D-2257	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfcUfUfucuacausususu	1643	{Phosphate}asUfsgUfagAfaaGfgCfaUfgaagcsusu	1644
D-2258	{GalNAc3K2AhxC6}cugcuucaUfgCfCfCfUfucuacsasu	1645	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcagsusu	1646
D-2259	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucUfaCfaUfsusUf	1647	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1648
D-2260	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfcCfUfucUfaCfaUfsusUf	1649	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgAfaGfcsUfsu	1650
D-2261	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfCfCfUfucuacausususu	1651	{Phosphate}asUfsgUfaGfAfagggCfaUfgAfaGfcsUfsu	1652
D-2262	{GalNAc3K2AhxC6}cugcuucaUfgCfCfCfUfucuacas{invAb}	1653	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcagsusu	1654
D-2263	{GalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfCfUfucuacsasu	1655	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcagsusu	1656
D-2264	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfCfCfUfucuacausususu	1657	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1658
D-2265	{GalNAc3K2AhxC6}auguucCfuGfCfUfUfcaugccususu	1659	{Phosphate}asGfsgCfaUfGfaagcAfgGfaacausususu	1660
D-2266	{GalNAc3K2AhxC6}uguuccUfgCfUfUfCfaugccuususu	1661	{Phosphate}asAfsgGfcAfUfgaagCfaGfgaacasusu	1662
D-2267	{GalNAc3K2AhxC6}ugccuuUfcUfAfCfAfguggccususu	1663	{Phosphate}asGfsgCfcAfCfuguaGfaAfaggcasusu	1664
D-2268	cguacuUfcGfUfCfCfuuguaugsusu	1665	{Phosphate}csAfsuAfcAfAfggacGfaAfguacgsusu	1666
D-2269	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCf[dC]CfUfucuacausususu	1667	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1668
D-2270	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucuacausususu	1669	{Phosphate}asUfsgUfaGfAfagGfgCfaUfgaagcsusu	1670
D-2271	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfcCfUfucuacausususu	1671	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1672
D-2272	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucuacausususu	1673	{Phosphate}asUfsgUfagAfagggCfaUfgaagcsusu	1674
D-2273	{GalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfCfUfucuacausususu	1675	{Phosphate}asUfsguagAfagggCfaUfgaagcsusu	1676
D-2274	{GalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfcCfUfucuacausususu	1677	{Phosphate}asUfsgUfaGfAfagggCfaUfgaagcsusu	1678
D-2275	{sGalNAc3K2AhxC6}[invAb]ccugcuUfcAfUfGfCfcuUfuCfuAfsusUf	1679	{Phosphate}usAfsgAfaAfGfgcauGfaAfgcaggsusu	1680
D-2276	{sGalNAc3K2AhxC6}[invAb]CfcUfgCfuUfcAfuGfCfcuUfuCfuAfsusUf	1681	{Phosphate}usAfsgAfaAfGfgcAfuGfaAfgCfaGfgsUfsu	1682
D-2277	{sGalNAc3K2AhxC6}UfuCfcUfgCfuUfcAfUfgcCfuUfuCfus{invAb}	1683	{Phosphate}usAfsgAfaAfgGfCfauGfaAfgCfaGfgAfasUfsu	1684

D-2278	{sGalNAc3K2AhxC6}[invAb]CfcUfgCfuUfcAfUfGfCfcuuucuasusu	1685	{Phosphate}usAfsgAfaAfGfgcauGfaAfgCfaGfgsUfsu	1686
D-2279	{sGalNAc3K2AhxC6}UfuCfcUfgCfuUfCfAfUfgccuuucus{invAb}	1687	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaGfgAfasUfsu	1688
D-2280	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfaguggcususu	1689	{Phosphate}asGfscCfaCfUfguagAfaAfggcaususu	1690
D-2281	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfagUfgGfcUfsusUf	1691	{Phosphate}asGfscCfaCfUfguagAfaAfggcaususu	1692
D-2282	{sGalNAc3K2AhxC6}[invAb]AfuGfcCfuUfuCfuAfCfagUfgGfcUfsusUf	1693	{Phosphate}asGfscCfaCfUfguAfgAfaAfgGfcAfusUfsu	1694
D-2283	{sGalNAc3K2AhxC6}UfcAfuGfcCfuUfuCfUfacAfgUfgGfcs{invAb}	1695	{Phosphate}asGfscCfaCfuGfUfagAfaAfgGfcAfuGfasUfsu	1696
D-2284	{sGalNAc3K2AhxC6}[invAb]AfuGfcCfuUfuCfUfAfCfaguggcususu	1697	{Phosphate}asGfscCfaCfUfguagAfaAfgGfcAfusUfsu	1698
D-2285	{sGalNAc3K2AhxC6}[invAb]UfcAfuGfcCfuUfUfCfUfacaguggscsu	1699	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcAfuGfasUfsu	1700
D-2286	{sGalNAc3K2AhxC6}[invAb]ugcuucAfuGfCfCfUfuucuacasgsu	1701	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaagcasusu	1702
D-2287	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuAfcAfgUfsusUf	1703	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1704
D-2288	{sGalNAc3K2AhxC6}UfgCfuUfcAfuGfcCfUfuuCfuAfcAfgs{invAb}	1705	{Phosphate}asCfsuGfuAfgAfAfagGfcAfuGfaAfgCfasUfsu	1706
D-2289	{sGalNAc3K2AhxC6}[invAb]CfuUfcAfuGfcCfUfUfUfcuacagususu	1707	{Phosphate}asCfsuGfuAfGfaaagGfcAfuGfaAfgsUfsu	1708
D-2290	{sGalNAc3K2AhxC6}UfgCfuUfcAfuGfCfCfUfuucuacags{invAb}	1709	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaAfgCfasUfsu	1710
D-2291	{sGalNAc3K2AhxC6}[invAb]UfgCfuUfcAfuGfCfCfUfuucuacasgsu	1711	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaAfgCfasUfsu	1712
D-2292	{sGalNAc3K2AhxC6}[invAb]ugcuucauGfcCfUfUfUfcuacasgsu	1713	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagcasusu	1714
D-2293	{sGalNAc3K2AhxC6}[invAb]ugcuucauGfcCfUfUfUfcuAfcAfgsUf	1715	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagcasusu	1716
D-2294	{sGalNAc3K2AhxC6}[invAb]UfgCfuUfcAfuGfcCfUfUfUfcuacasgsu	1717	{Phosphate}asCfsuGfuAfGfaaagGfcAfuGfaAfgCfasUfsu	1718
D-2295	{sGalNAc3K2AhxC6}[invAb]guuccuGfcUfUfCfAfugccuuususu	1719	{Phosphate}asAfsaGfgCfAfugaaGfcAfggaacsusu	1720
D-2296	{sGalNAc3K2AhxC6}[invAb]ccugcuUfcAfUfGfCfcuuucuasusu	1721	{Phosphate}usAfsgAfaAfGfgcauGfaAfgcaggsusu	1722
D-2297	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1723	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1724
D-2298	{sGalNAc3K2AhxC6}[invAb]UfuCfcUfgCfuUfcAfuGfCfcuUfuCfsusAf	1725	{Phosphate}usAfsgAfaAfGfgcAfuGfaAfgCfaGfgAfasUfsu	1726
D-2299	{sGalNAc3K2AhxC6}uuccugCfuUfCfAfUfgccuuucus{invAb}	1727	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaggaasusu	1728
D-2300	{sGalNAc3K2AhxC6}[invAb]uuccugCfuUfCfAfUfgccuuucsusa	1729	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaggaasusu	1730
D-2301	{sGalNAc3K2AhxC6}uuccugCfuUfCfAfUfgcCfuUfuCfus{invAb}	1731	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaggaasusu	1732
D-2302	{sGalNAc3K2AhxC6}[invAb]uuccugCfuUfCfAfUfgcCfuUfuCfsusAf	1733	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaggaasusu	1734
D-2303	{sGalNAc3K2AhxC6}[invAb]UfuCfcUfgCfuUfcAfUfgcCfuUfuCfsusAf	1735	{Phosphate}usAfsgAfaAfgGfCfauGfaAfgCfaGfgAfasUfsu	1736
D-2304	{sGalNAc3K2AhxC6}[invAb]UfuCfcUfgCfuUfCfAfUfgccuuucsusa	1737	{Phosphate}usAfsgAfaAfgGfCfaugaAfgCfaGfgAfasUfsu	1738
D-2305	{sGalNAc3K2AhxC6}[invAb]uuccugcuUfcAfUfGfCfcuuucsusa	1739	{Phosphate}usAfsgAfaAfGfgcauGfaAfgcaggaasusu	1740
D-2306	{sGalNAc3K2AhxC6}[invAb]uuccugcuUfcAfUfGfCfcuUfuCfsusAf	1741	{Phosphate}usAfsgAfaAfGfgcauGfaAfgcaggaasusu	1742
D-2307	{sGalNAc3K2AhxC6}[invAb]UfuCfcUfgCfuUfcAfUfGfCfcuuucsusa	1743	{Phosphate}usAfsgAfaAfGfgcauGfaAfgCfaGfgAfasUfsu	1744
D-2308	{sGalNAc3K2AhxC6}[invAb]UfcAfuGfcCfuUfuCfuAfCfagUfgGfscsUf	1745	{Phosphate}asGfscCfaCfUfguAfgAfaAfgGfcAfuGfasUfsu	1746
D-2309	{sGalNAc3K2AhxC6}ucaugcCfuUfUfCfUfacaguggcs{invAb}	1747	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcaugasusu	1748
D-2310	{sGalNAc3K2AhxC6}[invAb]ucaugcCfuUfUfCfUfacaguggscsu	1749	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcaugasusu	1750

D-2311	{sGalNAc3K2AhxC6}ucaugcCfuUfUfCfUfacAfgUfgGfcs{invAb}	1751	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcaugasusu	1752
D-2312	{sGalNAc3K2AhxC6}[invAb]ucaugcCfuUfUfCfUfacAfgUfgGfscsUf	1753	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcaugasusu	1754
D-2313	{sGalNAc3K2AhxC6}[invAb]UfcAfuGfcCfuUfuCfUfacAfgUfgGfscsUf	1755	{Phosphate}asGfscCfaCfuGfUfagAfaAfgGfcAfuGfasUfsu	1756
D-2314	{sGalNAc3K2AhxC6}UfcAfuGfcCfuUfUfCfUfacaguggcs{invAb}	1757	{Phosphate}asGfscCfaCfuGfUfagaaAfgGfcAfuGfasUfsu	1758
D-2315	{sGalNAc3K2AhxC6}[invAb]ucaugccuUfuCfUfAfCfaguggscsu	1759	{Phosphate}asGfscCfaCfUfguagAfaAfggcaugasusu	1760
D-2316	{sGalNAc3K2AhxC6}[invAb]ucaugccuUfuCfUfAfCfagUfgGfcsUf	1761	{Phosphate}asGfscCfaCfUfguagAfaAfggcaugasusu	1762
D-2317	{sGalNAc3K2AhxC6}[invAb]UfcAfuGfcCfuUfuCfUfAfCfaguggscsu	1763	{Phosphate}asGfscCfaCfUfguagAfaAfgGfcAfuGfasUfsu	1764
D-2318	{sGalNAc3K2AhxC6}[invAb]UfgCfuUfcAfuGfcCfuUfUfcuAfcAfsgsUf	1765	{Phosphate}asCfsuGfuAfGfaaAfgGfcAfuGfaAfgCfasUfsu	1766
D-2319	{sGalNAc3K2AhxC6}ugcuucAfuGfCfCfUfuucuacags{invAb}	1767	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaagcasusu	1768
D-2320	{sGalNAc3K2AhxC6}ugcuucAfuGfCfCfUfuuCfuAfcAfgs{invAb}	1769	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaagcasusu	1770
D-2321	{sGalNAc3K2AhxC6}[invAb]ugcuucAfuGfCfCfUfuuCfuAfcAfsgsUf	1771	{Phosphate}asCfsuGfuAfgAfAfaggcAfuGfaagcasusu	1772
D-2322	{sGalNAc3K2AhxC6}[invAb]CfuUfcAfuGfcCfuUfUfcuAfcAfgUfsusUf	1773	{Phosphate}asCfsuGfuAfGfaaAfgGfcAfuGfaAfgsUfsu	1774
D-2323	{sGalNAc3K2AhxC6}[invAb]UfgCfuUfcAfuGfcCfUfuuCfuAfcAfsgsUf	1775	{Phosphate}asCfsuGfuAfgAfAfagGfcAfuGfaAfgCfasUfsu	1776
D-2324	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacasusu	1777	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcsusu	1778
D-2325	{sGalNAc3K2AhxC6}[invAb]caugccUfuUfCfUfAfcaguggususu	1779	{Phosphate}asCfscAfcUfGfuagaAfaGfgcaugsusu	1780
D-2326	{sGalNAc3K2AhxC6}[invAb]cugcuuCfaUfGfCfCfuuucuaasusu	1781	{Phosphate}usUfsaGfaAfAfggcaUfgAfagcagsusu	1782
D-2327	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacaasusu	1783	{Phosphate}usUfsgUfaGfAfaaggCfaUfgaagcsusu	1784
D-2328	{sGalNAc3K2AhxC6}[invAb]uucaugCfcUfUfUfCfuacaguasusu	1785	{Phosphate}usAfscUfgUfAfgaaaGfgCfaugaasusu	1786
D-2329	{sGalNAc3K2AhxC6}[invAb]guuccuGfcUfUfCfAfugCfcUfuUfsusUf	1787	{Phosphate}asAfsaGfgCfAfugaaGfcAfggaacsusu	1788
D-2330	{sGalNAc3K2AhxC6}auguucCfuGfCfUfUfcaUfgCfcUfus{invAb}	1789	{Phosphate}asAfsaGfgCfaUfGfaagcAfgGfaacaususu	1790
D-2331	{sGalNAc3K2AhxC6}[invAb]auguucCfuGfCfUfUfcaUfgCfcUfsusUf	1791	{Phosphate}asAfsaGfgCfaUfGfaagcAfgGfaacaususu	1792
D-2332	{sGalNAc3K2AhxC6}[invAb]GfuUfcCfuGfcUfuCfAfugCfcUfuUfsusUf	1793	{Phosphate}asAfsaGfgCfAfugAfaGfcAfgGfaAfcsUfsu	1794
D-2333	{sGalNAc3K2AhxC6}AfuGfuUfcCfuGfcUfUfcaUfgCfcUfus{invAb}	1795	{Phosphate}asAfsaGfgCfaUfGfaaGfcAfgGfaAfcAfusUfsu	1796
D-2334	{sGalNAc3K2AhxC6}[invAb]AfuGfuUfcCfuGfcUfUfcaUfgCfcUfsusUf	1797	{Phosphate}asAfsaGfgCfaUfGfaaGfcAfgGfaAfcAfusUfsu	1798
D-2335	{sGalNAc3K2AhxC6}[invAb]GfuUfcCfuGfcUfUfCfAfugccuuususu	1799	{Phosphate}asAfsaGfgCfAfugaaGfcAfgGfaAfcsUfsu	1800
D-2336	{sGalNAc3K2AhxC6}AfuGfuUfcCfuGfCfUfUfcaugccuus{invAb}	1801	{Phosphate}asAfsaGfgCfaUfGfaagcAfgGfaAfcAfusUfsu	1802
D-2337	{sGalNAc3K2AhxC6}[invAb]AfuGfuUfcCfuGfCfUfUfcaugccususu	1803	{Phosphate}asAfsaGfgCfaUfGfaagcAfgGfaAfcAfusUfsu	1804
D-2338	{sGalNAc3K2AhxC6}[invAb]auguuccuGfcUfUfCfAfugccususu	1805	{Phosphate}asAfsaGfgCfAfugaaGfcAfggaacaususu	1806
D-2339	{sGalNAc3K2AhxC6}[invAb]auguuccuGfcUfUfCfAfugCfcUfusUf	1807	{Phosphate}asAfsaGfgCfAfugaaGfcAfggaacaususu	1808
D-2340	{sGalNAc3K2AhxC6}[invAb]GfcUfuCfaUfgCfcUfuUfCfuaCfaGfsusAf	1809	{Phosphate}usAfscUfgUfAfgaAfaGfgCfaUfgAfaGfcsUfsu	1810
D-2341	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacagsusa	1811	{Phosphate}usAfscUfgUfaGfAfaaggCfaUfgaagcsusu	1812
D-2342	{sGalNAc3K2AhxC6}[invAb]uucaugCfcUfUfUfCfuaCfaGfuAfsusUf	1813	{Phosphate}usAfscUfgUfAfgaaaGfgCfaugaasusu	1814
D-2343	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucUfaCfaGfsusAf	1815	{Phosphate}usAfscUfgUfaGfAfaaggCfaUfgaagcsusu	1816

D-2344	{sGalNAc3K2AhxC6}GfcUfuCfaUfgCfCfUfUfucuacagus{invAb}	1817	{Phosphate}usAfscUfgUfaGfAfaaggCfaUfgAfaGfcsUfsu	1818
D-2345	{sGalNAc3K2AhxC6}[invAb]GfcGfgCfuUfcCfUfGfGfgcuucuasusu	1819	{Phosphate}usAfsgAfaGfCfccagGfaAfgCfcGfcsUfsu	1820
D-2346	{sGalNAc3K2AhxC6}[invAb]auggcuUfcCfAfGfAfuaugccususu	1821	{Phosphate}asGfsgCfaUfAfucugGfaAfgccaususu	1822
D-2347	{sGalNAc3K2AhxC6}[invAb]acauggCfuUfCfCfAfgaUfaUfgCfscsUf	1823	{Phosphate}asGfsgCfaUfaUfCfuggaAfgCfcaugususu	1824
D-2348	{sGalNAc3K2AhxC6}[invAb]AfcAfuGfgCfuUfcCfAfGfAfuaugcscsu	1825	{Phosphate}asGfsgCfaUfAfucugGfaAfgCfcAfuGfusUfsu	1826
D-2349	{sGalNAc3K2AhxC6}[invAb]caacguAfcCfCfUfUfcauugausgsu	1827	{Phosphate}asCfsaUfcAfaUfGfaaggGfuAfcguugsusu	1828
D-2350	{sGalNAc3K2AhxC6}[invAb]caacguacCfcUfUfCfAfuuGfaUfgsUf	1829	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacguugsusu	1830
D-2351	{sGalNAc3K2AhxC6}[invAb]acguacCfcUfUfCfAfuugaugususu	1831	{Phosphate}asCfsaUfcAfAfugaaGfgGfuacgususu	1832
D-2352	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1833	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1834
D-2353	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	1835	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1836
D-2354	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucUfaCfaUfsusUf	1837	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcsusu	1838
D-2355	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucUfaCfas{invAb}	1839	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1840
D-2356	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucUfaCfasUf	1841	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1842
D-2357	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1843	{Phosphate}asUfsguagAfaaggCfaUfgaagcagsusu	1844
D-2358	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacas{invAb}	1845	{Phosphate}asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1846
D-2359	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacaususu	1847	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgaagcsusu	1848
D-2360	{sGalNAc3K2AhxC6}[invAb]CfuUfcAfuGfcCfUfUfUfcuacagususu	1849	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1850
D-2361	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1851	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagcasusu	1852
D-2362	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	1853	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgaagcagsusu	1854
D-2363	{sGalNAc3K2AhxC6}cugcuucaUfgCfcUfUfucuacas{invAb}	1855	{Phosphate}asUfsgUfaGfAfaaggcaUfgaagcagsusu	1856
D-2364	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1857	{Phosphate}asUfsgUfaGfAfaaGfgCfaUfgaagcagsusu	1858
D-2365	{sGalNAc3K2AhxC6}[invAb]cuucauCfcCfUfUfUfcuacagususu	1859	{Phosphate}asCfsuGfuAfGfaaagGfgAfugaagsusu	1860
D-2366	{sGalNAc3K2AhxC6}[invAb]gcuucaUfcCfCfUfUfucuacaususu	1861	{Phosphate}asUfsgUfaGfAfaaggGfaUfgaagcsusu	1862
D-2367	{sGalNAc3K2AhxC6}ugcuucauCfcCfUfUfUfcuacags{invAb}	1863	{Phosphate}asCfsuGfuAfGfaaagGfgAfugaagcasusu	1864
D-2368	{sGalNAc3K2AhxC6}cugcuucaUfcCfCfUfUfucuacas{invAb}	1865	{Phosphate}asUfsgUfaGfAfaaggGfaUfgaagcagsusu	1866
D-2369	{sGalNAc3K2AhxC6}[invAb]acauugCfuCfUfUfUfcaccugasusu	1867	{Phosphate}usCfsaGfgUfGfaaagAfgCfaaugususu	1868
D-2370	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1869	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1870
D-2371	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1871	asUfsgUfaGfAfaaggCfaUfgaagcasgsusu	1872
D-2372	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1873	asUfsgsUfaGfAfaaggCfaUfgaagcagsusu	1874
D-2373	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1875	asUfsgUfaGfAfaaggCfaUfgaagcagusu	1876
D-2374	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1877	asUfsgsUfaGfAfaaggCfaUfgaagcagusu	1878
D-2375	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1879	asUfgUfaGfAfaaggCfaUfgaagcagsusu	1880
D-2376	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1881	asUfgUfaGfAfaaggCfaUfgaagcasgsusu	1882

D-2377	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacsas{invAb}	1883	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1884
D-2378	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuascsas{invAb}	1885	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1886
D-2379	{sGalNAc3K2AhxC6}csugcuucaUfgCfCfUfUfucuacas{invAb}	1887	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1888
D-2380	{sGalNAc3K2AhxC6}csusgcuucaUfgCfCfUfUfucuacas{invAb}	1889	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	1890
D-2381	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCf[dT]UfUfcuacagususu	1891	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1892
D-2382	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1893	{Phosphate}asCfsuGfuAfGfaaAfgGfcAfugaagsusu	1894
D-2383	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfuUfUfcuacagususu	1895	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1896
D-2384	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1897	{Phosphate}asCfsuGfuaGfaaagGfcAfugaagsusu	1898
D-2385	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1899	{Phosphate}asCfsuguaGfaaagGfcAfugaagsusu	1900
D-2386	{sGalNAc3K2AhxC6}[invAb]cuucauGfc[dC]UfUfUfcuacagususu	1901	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1902
D-2387	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUf[dT]Ufcuacagususu	1903	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1904
D-2388	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgGfGfAfUfcuacaususu	1905	{Phosphate}asUfsgUfaGfAfauccCfaUfgaagcsusu	1906
D-2389	{sGalNAc3K2AhxC6}[invAb]cuucauGfcGfAfAfUfcuacagususu	1907	{Phosphate}asCfsuGfuAfGfauucGfcAfugaagsusu	1908
D-2390	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1909	{Phosphate}usUfsgUfaGfAfaaggCfaUfgaagcagsusu	1910
D-2391	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasa	1911	{Phosphate}usUfsgUfaGfAfaaggCfaUfgaagcagsusu	1912
D-2392	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invDA}	1913	{Phosphate}usUfsgUfaGfAfaaggCfaUfgaagcagsusu	1914
D-2393	{sGalNAc3K2AhxC6}cugcuucaUfgGfGfAfUfcuacas{invAb}	1915	{Phosphate}asUfsgUfaGfAfauccCfaUfgaagcagsusu	1916
D-2394	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgGfGfAfUfcuacsasu	1917	{Phosphate}asUfsgUfaGfAfauccCfaUfgaagcagsusu	1918
D-2395	{sGalNAc3K2AhxC6}[invAb]CfuUfcAfuGfcCfUfUfUfcuAfcAfgUfsusUf	1919	{Phosphate}asCfsuGfuAfGfaaagGfcAfugaagsusu	1920
D-2396	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuAfcAfgUfsusUf	1921	{Phosphate}asCfsuGfuAfGfaaagGfcAfuGfaAfgsUfsu	1922
D-2397	{sGalNAc3K2AhxC6}[invAb]gcuucaUfgCfCfUfUfucuacaususu	1923	asUfsgUfaGfAfaaggCfaUfgaagcsusu	1924
D-2398	{sGalNAc3K2AhxC6}[invAb]ugcuucauGfcCfUfUfUfcuacags{invAb}	1925	asCfsuGfuAfGfaaagGfcAfugaagcasusu	1926
D-2399	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1927	{Phosphate}asCfsuGfuAfGfaaagGfcAfuGfaAfgsUfsu	1928
D-2400	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuAfcAfgs{invAb}	1929	asCfsuGfuAfGfaaagGfcAfugaagcasusu	1930
D-2401	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1931	asCfsuGfuAfGfaaAfgGfcAfugaagcasusu	1932
D-2402	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1933	asCfsuguaGfaaagGfcAfugaagcasusu	1934
D-2403	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfUfUfUfcuacagususu	1935	asCfsuGfuAfGfaaagGfcAfugaagsusu	1936
D-2404	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1937	asCfsuGfuAfGfaaagGfcAfugaagcasusu	1938
D-2405	{GalNAc3K2AhxC6}augccuuuCfuAfCfAfGfuggcusus{invAb}	1939	{Phosphate}asGfscCfaCfUfguAfgAfaAfgGfcAfuGfasUfsu	1940
D-2406	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1941	asGfscCfaCfUfguagAfaAfggcaugasusu	1942
D-2407	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfaguggcususu	1943	asGfscCfaCfUfguagAfaAfggcaususu	1944
D-2408	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfagUfgGfcUfsusUf	1945	asGfscCfaCfUfguagAfaAfggcaususu	1946
D-2409	{sGalNAc3K2AhxC6}[invAb]ucaugccuUfuCfUfAfCfaguggscsu	1947	asGfscCfaCfUfguagAfaAfggcaugasusu	1948

D-2410	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfagUfgGfcs{invAb}	1949	asGfscCfaCfUfguagAfaAfggcaugasusu	1950
D-2411	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfaguggcususu	1951	asGfscCfaCfUfguAfgAfaAfggcaususu	1952
D-2412	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1953	asGfscCfaCfUfguAfgAfaAfggcaugasusu	1954
D-2413	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1955	asGfsccacUfguagAfaAfggcaugasusu	1956
D-2414	{sGalNAc3K2AhxC6}[invAb]ucaugccuUfuCfUfAfCfaguggcs{invAb}	1957	asGfscCfaCfUfguagAfaAfggcaugasusu	1958
D-2415	{sGalNAc3K2AhxC6}[invAb]augccuUfuCfUfAfCfaguggcasusu	1959	usGfscCfaCfUfguagAfaAfggcaususu	1960
D-2416	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1961	usGfscCfaCfUfguagAfaAfggcaugasusu	1962
D-2417	{sGalNAc3K2AhxC6}[invAb]ucaugccuUfuCfUfAfCfaguggscsa	1963	usGfscCfaCfUfguagAfaAfggcaugasusu	1964
D-2418	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invDA}	1965	usGfscCfaCfUfguagAfaAfggcaugasusu	1966
D-2419	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1967	asUfsguagAfaaggCfaUfgaagcagsusu	1968
D-2420	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1969	usUfsguagAfaaggCfaUfgaagcagsusu	1970
D-2421	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invDA}	1971	usUfsguagAfaaggCfaUfgaagcagsusu	1972
D-2422	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1973	usGfsccacUfguagAfaAfggcaugasusu	1974
D-2423	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invDA}	1975	usGfsccacUfguagAfaAfggcaugasusu	1976
D-2424	{sGalNAc3K2AhxC6}[invAb]cuucauGfcCfuUfUfcuacagususu	1977	{Phosphate}asCfsuGfuAfGfaaAfgGfcAfugaagsusu	1978
D-2425	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1979	asUfsgUfa[Ab]AfaaggCfaUfgaagcagsusu	1980
D-2426	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1981	asUfsgua[Ab]AfaaggCfaUfgaagcagsusu	1982
D-2427	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1983	asCfsuGfu[Ab]GfaaagGfcAfugaagcasusu	1984
D-2428	{sGalNAc3K2AhxC6}ugcuucauGfcCfUfUfUfcuacags{invAb}	1985	asCfsugu[Ab]GfaaagGfcAfugaagcasusu	1986
D-2429	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1987	asGfscCfa[Ab]UfguagAfaAfggcaugasusu	1988
D-2430	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invAb}	1989	asGfscca[Ab]UfguagAfaAfggcaugasusu	1990
D-2431	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1991	asUfs[GNA-G]uagAfaaggCfaUfgaagcagsusu	1992
D-2432	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1993	asUfsg[GNA-U]agAfaaggCfaUfgaagcagsusu	1994
D-2433	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1995	asUfsgu[GNA-A]gAfaaggCfaUfgaagcagsusu	1996
D-2434	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1997	asUfsgua[GNA-G]AfaaggCfaUfgaagcagsusu	1998
D-2435	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	1999	asUfsguag[GNA-A]aaggCfaUfgaagcagsusu	2000
D-2436	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	2001	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2002
D-2437	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	2003	asUfsgUfagAfaaggCfaUfgaagcagsusu	2004
D-2438	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacsasu	2005	asUfsguagAfaaggCfaUfgaagcagsusu	2006
D-2439	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucuacas{invAb}	2007	asUfsguagAfaaggCfaUfgaagcagsusu	2008
D-2440	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	2009	asUfsguagAfaaGfgCfaUfgaagcagsusu	2010
D-2441	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucUfaCfas{invAb}	2011	asUfsguagAfaaggCfaUfgaagcagsusu	2012
D-2442	{sGalNAc3K2AhxC6}[invAb]cugcuucaUfgCfCfUfUfucUfaCfasUf	2013	asUfsguagAfaaggCfaUfgaagcagsusu	2014

D-2443	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucUfaCfas{invAb}	2015	asUfsguagAfaaGfgCfaUfgaagcagsusu	2016
D-2444	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2017	asUfsgUfaGfAfaaGfgCfaUfgaagcagsus u	2018
D-2445	{sGalNAc3K2AhxC6}[invAb]cugcuuca UfgCfCfUfUfucuacsasu	2019	asUfsgUfaGfAfaaGfgCfaUfgaagcagsus u	2020
D-2446	{sGalNAc3K2AhxC6}[invAb]cugcuuca UfgCfCfUfUfucuacas{invAb}	2021	asUfsgUfaGfAfaaggCfaUfgaagcagsusu	2022
D-2447	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2023	as[Ab]guagAfaaggCfaUfgaagcagsusu	2024
D-2448	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2025	asUfs[Ab]uagAfaaggCfaUfgaagcagsus u	2026
D-2449	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2027	asUfsg[Ab]agAfaaggCfaUfgaagcagsus u	2028
D-2450	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2029	asUfsgu[Ab]gAfaaggCfaUfgaagcagsus u	2030
D-2451	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2031	asUfsguag[Ab]aaggCfaUfgaagcagsusu	2032
D-2452	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2033	asUfsguagAf[Ab]aggCfaUfgaagcagsus u	2034
D-2453	{sGalNAc3K2AhxC6}caacguacCfcUfUf CfAfuugaugs{invAb}	2035	asCfsaucaAfugaaGfgGfuacguugsusu	2036
D-2454	{sGalNAc3K2AhxC6}caacguacCfcUfUf CfAfuugaugs{invAb}	2037	asCfsaUfcAfAfugaaGfgGfuacguugsusu	2038
D-2455	{sGalNAc3K2AhxC6}acauggcuUfcCfAf GfAfuaugccs{invAb}	2039	asGfsgcauAfucugGfaAfgccaugususu	2040
D-2456	{sGalNAc3K2AhxC6}acauggcuUfcCfAf GfAfuaugccs{invAb}	2041	asGfsgCfaUfAfucugGfaAfgccaugususu	2042
D-2457	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2043	asUfsguaGfAfaaggCfaUfgaagcagsusu	2044
D-2458	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucUfacas{invAb}	2045	asUfsguagAfaaggCfaUfgaagcagsusu	2046
D-2459	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuaCfas{invAb}	2047	asUfsguagAfaaggCfaUfgaagcagsusu	2048
D-2460	{sGalNAc3K2AhxC6}cugcggcuUfcCfUf GfGfgcuucus{invAb}	2049	usAfsgAfaGfCfccagGfaAfgccgcagsusu	2050
D-2461	{sGalNAc3K2AhxC6}cugcggcuUfcCfUf GfGfgcuucus{invAb}	2051	usAfsgaagCfccagGfaAfgccgcagsusu	2052
D-2462	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuacags{invAb}	2053	asCfsuGfuaGfaaagGfcAfugaagcasusu	2054
D-2463	{sGalNAc3K2AhxC6}[invAb]ugcuucau GfcCfUfUfUfcuacasgsu	2055	asCfsuGfuaGfaaagGfcAfugaagcasusu	2056
D-2464	{sGalNAc3K2AhxC6}[invAb]ugcuucau GfcCfUfUfUfcuacags{invAb}	2057	asCfsuGfuaGfaaagGfcAfugaagcasusu	2058
D-2465	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuacags{invAb}	2059	asCfsuGfuaGfaaAfgGfcAfugaagcasusu	2060
D-2466	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuAfcAfgs{invAb}	2061	asCfsuGfuaGfaaagGfcAfugaagcasusu	2062
D-2467	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuAfcAfgs{invAb}	2063	asCfsuGfuAfGfaaagGfcAfuGfaAfgCfas Ufsu	2064
D-2468	{sGalNAc3K2AhxC6}cugcuuCfaUfGfCf cuuucuacsasu	2065	asUfsguaGfaaAfggcaUfgAfagcagsusu	2066
D-2469	{sGalNAc3K2AhxC6}cugcuuCfaUfGfCf cuuucuacsasu	2067	asUfsguaGfaaaggcaUfgAfagcagsusu	2068
D-2470	{sGalNAc3K2AhxC6}cugcuuCfaUfGfCf cuuucuacsasu	2069	asUfsguaGfaAfAfggcaUfgAfagcagsusu	2070
D-2471	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuacags{invAb}	2071	usCfsuguaGfaaagGfcAfugaagcasusu	2072
D-2472	{sGalNAc3K2AhxC6}ugcuucauGfcCfUf UfUfcuacags{invDA}	2073	usCfsuguaGfaaagGfcAfugaagcasusu	2074
D-2473	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invDT}	2075	asUfsguagAfaaggCfaUfgaagcagsusu	2076
D-2474	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2077	asUfsguagAf[sGNA-A]aggCfaUfgaagcagsusu	2078
D-2475	{sGalNAc3K2AhxC6}cugcuucaUfgCfCf UfUfucuacas{invAb}	2079	asUfsguagAfs[GNA-A]aggCfaUfgaagcagsusu	2080

D-2476	{sGalNAc3K2AhxC6}cugcuucaUfgCfCfUfUfucuacas{invAb}	2081	asUfsguagAfs[sGNA-A]aggCfaUfgaagcagsusu	2082
D-2477	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2083	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2084
D-2478	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2085	asUfsguagAf[GNA-A]AfggCfaUfgaagcagsusu	2086
D-2479	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2087	asUfsguaga[GNA-A]aggCfaUfgaagcagsusu	2088
D-2480	csusgcuucaUfgCfCfUfuucuacas{invAb}	2089	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2090
D-2481	csusgcuucaUfgCfCfUfuucuacas{invAb}	2091	asUfsguaga[GNA-A]aggCfaUfgaagcagsusu	2092
D-2482	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2093	asUfsguaga[GNA-A]AfggCfaUfgaagcagsusu	2094
D-2483	csusgcuucaUfgCfCfUfuucuacas{invAb}	2095	asUfsguagAf[GNA-A]AfggCfaUfgaagcagsusu	2096
D-2484	csusgcuucaUfgCfCfUfuucuacas{invAb}	2097	asUfsguaga[GNA-A]AfggCfaUfgaagcagsusu	2098
D-2485	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2099	asUfsgUfaGfAf[GNA-A]aggCfaUfgaagcagsusu	2100
D-2486	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2101	asUfsgUfagAf[GNA-A]aGfgCfaUfgaagcagsusu	2102
D-2487	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2103	asUfsgUfagAf[GNA-A]aggCfaUfgaagcagsusu	2104
D-2488	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2105	asUfsgUfaGfAf[GNA-A]aGfgCfaUfgaagcagsusu	2106
D-2489	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2107	asUfsguagAf[GNA-A]aGfgCfaUfgaagcagsusu	2108
D-2490	csusgcuucaUfgCfCfUfUfucUfaCfas{invAb}	2109	asUfsguagAf[GNA-A]aGfgCfaUfgaagcagsusu	2110
D-2491	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2111	asUfsguagAf[GNA-A]a[dG]gCfaUfgaagcagsusu	2112
D-2492	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2113	asUfsguagAf[GNA-A][dA]ggCfaUfgaagcagsusu	2114
D-2493	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2115	asUfsguagAf[GNA-A]ag[dG]CfaUfgaagcagsusu	2116
D-2494	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2117	asUfsguaGfAf[GNA-A]aggCfaUfgaagcagsusu	2118
D-2495	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2119	asUfsguaGfa[GNA-A]aggCfaUfgaagcagsusu	2120
D-2496	csusgcuucaUfgCfCfUfUfucuacas{invAb}	2121	asUfsguaGfa[GNA-A]AfggCfaUfgaagcagsusu	2122
D-2497	csusgcuuCfaUfGfCfcuuucuacas{invAb}	2123	asUfsguaGfa[GNA-A]aggcaUfgAfagcagsusu	2124
D-2498	csusgcuuCfaUfGfCfcuuucuacas{invAb}	2125	asUfsguaGfa[GNA-A]AfggcaUfgAfagcagsusu	2126
D-2499	csusgcuuCfaUfgCfcuuucuacas{invAb}	2127	asUfsguaga[GNA-A]aggCfaUfgAfagcagsusu	2128
D-2500	csusgcuuCfaUfgCfcuuucuacas{invAb}	2129	asUfsguagaaaggCfaUfgAfagcagsusu	2130
D-2501	usgscuucauGfcCfUfUfUfcuacags{invDA}	2131	us[sGNA-C]uguaGfaaagGfcAfugaagcasusu	2132
D-2502	usgscuucauGfcCfUfUfUfcuacags{invDA}	2133	usCfs[GNA-U]guaGfaaagGfcAfugaagcasusu	2134
D-2503	usgscuucauGfcCfUfUfUfcuacags{invDA}	2135	usCfsug[GNA-U]aGfaaagGfcAfugaagcasusu	2136
D-2504	usgscuucauGfcCfUfUfUfcuacags{invDA}	2137	usCfsugu[GNA-A]GfaaagGfcAfugaagcasusu	2138
D-2505	usgscuucauGfcCfUfUfUfcuacags{invDA}	2139	usCfsuguaGf[GNA-A]aagGfcAfugaagcasusu	2140
D-2506	usgscuucauGfcCfUfUfUfcuacags{invDA}	2141	us[Ab]uguaGfaaagGfcAfugaagcasusu	2142
D-2507	usgscuucauGfcCfUfUfUfcuacags{invDA}	2143	usCfs[Ab]guaGfaaagGfcAfugaagcasusu	2144
D-2508	usgscuucauGfcCfUfUfUfcuacags{invDA}	2145	usCfsu[Ab]uaGfaaagGfcAfugaagcasusu	2146

D-2509	usgscuucauGfcCfUfUfUfcuacags{invDA}	2147	usCfsug[Ab]aGfaaagGfcAfugaagcasusu	2148
D-2510	usgscuucauGfcCfUfUfUfcuacags{invDA}	2149	usCfsugu[Ab]GfaaagGfcAfugaagcasusu	2150
D-2511	usgscuucauGfcCfUfUfUfcuacags{invDA}	2151	usCfsugua[Ab]aaagGfcAfugaagcasusu	2152
D-2512	usgscuucauGfcCfUfUfUfcuacags{invDA}	2153	usCfsuguaGf[Ab]aagGfcAfugaagcasusu	2154
D-2513	uscsaugccuUfuCfUfAfCfaguggcs{invDT}	2155	asGfsccacUfguagAfaAfggcaugasusu	2156
D-2514	uscsaugccuUfuCfUfAfCfaguggcs{invAb}	2157	usGfsccacUfguagAfaAfggcaugasusu	2158
D-2515	uscsaugccuUfuCfUfAfCfaguggcs{invDA}	2159	usGfsccacUfguagAfaAfggcaugasusu	2160
D-2516	uscscugcuuCfaUfGfCfCfuuucuas{invDT}	2161	asUfsagaaAfggcaUfgAfagcaggasusu	2162
D-2517	uscscugcuuCfaUfGfCfCfuuucuas{invAb}	2163	usUfsagaaAfggcaUfgAfagcaggasusu	2164
D-2518	uscscugcuuCfaUfGfCfCfuuucuas{invDA}	2165	usUfsagaaAfggcaUfgAfagcaggasusu	2166
D-2519	usasuguuccUfgCfUfUfCfaugccus{invDT}	2167	asAfsggcaUfgaagCfaGfgaacauasusu	2168
D-2520	usasuguuccUfgCfUfUfCfaugccus{invAb}	2169	usAfsggcaUfgaagCfaGfgaacauasusu	2170
D-2521	usasuguuccUfgCfUfUfCfaugccus{invDA}	2171	usAfsggcaUfgaagCfaGfgaacauasusu	2172
D-2522	{sGalNAc3K2AhxC6}uccugcuuCfaUfGfCfCfuuucuas{invAb}	2173	asUfsagaaAfggcaUfgAfagcaggasusu	2174
D-2523	{sGalNAc3K2AhxC6}uauguuccUfgCfUfUfCfaugccus{invAb}	2175	asAfsggcaUfgaagCfaGfgaacauasusu	2176
D-2524	{sGalNAc3K2AhxC6}ucaugccuUfuCfUfAfCfaguggcs{invDT}	2177	asGfsccacUfguagAfaAfggcaugasusu	2178
D-2525	{sGalNAc3K2AhxC6}uccugcuuCfaUfGfCfCfuuucuas{invDT}	2179	asUfsagaaAfggcaUfgAfagcaggasusu	2180
D-2526	{sGalNAc3K2AhxC6}uccugcuuCfaUfGfCfCfuuucuas{invDA}	2181	usUfsagaaAfggcaUfgAfagcaggasusu	2182
D-2527	{sGalNAc3K2AhxC6}uauguuccUfgCfUfUfCfaugccus{invDA}	2183	usAfsggcaUfgaagCfaGfgaacauasusu	2184
D-2528	usgscuucauGfcCfUfUfUfcuacags{invDA}	2185	usCfsugua[GNA-G]aaagGfcAfugaagcasusu	2186
D-2529	cugcuucaUfgCfCfUfUfsucuacas{invAb}	2187	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2188
D-2530	cugcuucaUfgCfCfUfsUfucuacas{invAb}	2189	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2190
D-2531	cugcuucaUfgCfCfUfsUfsucuacas{invAb}	2191	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2192
D-2532	cugcuucaUfgCfCfUfsUfucuacas{invAb}	2193	asUfsguagAfs[GNA-A]aggCfaUfgaagcagsusu	2194
D-2533	cugcuucaUfgCfCfUfsUfsucuacas{invAb}	2195	asUfsguagAfs[GNA-A]aggCfaUfgaagcagsusu	2196
D-2534	cugcuucaUfgCfCfUfUfsucuacas{invAb}	2197	asUfsguagAf[sGNA-A]aggCfaUfgaagcagsusu	2198
D-2535	cugcuucaUfgCfCfUfsUfsucuacas{invAb}	2199	asUfsguagAf[sGNA-A]aggCfaUfgaagcagsusu	2200
D-2536	cugcuucaUfgCfCfUfUfsucuacas{invAb}	2201	asUfsguagAfs[sGNA-A]aggCfaUfgaagcagsusu	2202
D-2537	cugcuucaUfgCfCfUfsUfucuacas{invAb}	2203	asUfsguagAfs[sGNA-A]aggCfaUfgaagcagsusu	2204
D-2538	cugcuucaUfgCfCfUf[LNA-T]ucuacas{invAb}	2205	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2206
D-2539	cugcuucaUfgCfCf[LNA-T]Ufucuacas{invAb}	2207	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2208
D-2540	cugcuucaUfgCfCfUfUf[LNA-T]cuacas{invAb}	2209	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2210
D-2541	cugcuucaUfgCfCfUfUfuc[LNA-T]acas{invAb}	2211	asUfsgu[GNA-A]gAfaaggCfaUfgaagcagsusu	2212

D-2542	cugcuucaUfgCfCfUfUfucu[LNA-A]cas{invAb}	2213	asUfsg[GNA-U]agAfaaggCfaUfgaagcagsusu	2214
D-2543	cugcuucaUfgCfCfUfUfucuac[sLNA-A]{invAb}	2215	as[sGNA-U]guagAfaaggCfaUfgaagcagsusu	2216
D-2544	cugcuucaUfgCfCfUfUf[LNA-T]cuacas{invAb}	2217	asUfsguag[Ab]aaggCfaUfgaagcagsusu	2218
D-2545	cugcuucaUfgCfCfUfUfuc[LNA-T]acas{invAb}	2219	asUfsgu[Ab]gAfaaggCfaUfgaagcagsusu	2220
D-2546	cugcuucaUfgCfCfUfUfucu[LNA-A]cas{invAb}	2221	asUfsg[Ab]agAfaaggCfaUfgaagcagsusu	2222
D-2547	cugcuucaUfgCfCfUfUfucUfaCfas{invAb}	2223	asUfsgUfagAf[GNA-A]aggCfaUfgaagcagsusu	2224
D-2548	cugcuucaUfgCfCfUfUfucUfaCfas{invAb}	2225	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2226
D-2549	cugcuucaUfgCfCfUfs[LNA-T]ucuacas{invAb}	2227	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2228
D-2550	cugcuucaUfgCfCfUfs[sLNA-T]ucuacas{invAb}	2229	asUfsguagAf[GNA-A]aggCfaUfgaagcagsusu	2230
D-2551	cugcuucaUfgCfCfUf[LNA-T]ucuacas{invAb}	2231	asUfsguagAf[sGNA-A]aggCfaUfgaagcagsusu	2232
D-2552	cugcuucaUfgCfCfUf[LNA-T]ucuacas{invAb}	2233	asUfsguagAfs[GNA-A]aggCfaUfgaagcagsusu	2234
D-2553	usgscuucauGfcCfUfUfUfcu[LNA-A]cags{invDA}	2235	usCfsug[Ab]aGfaaagGfcAfugaagcasusu	2236
D-2554	usgscuucauGfcCfUfUfUfc[LNA-T]acags{invDA}	2237	usCfsugu[Ab]GfaaagGfcAfugaagcasusu	2238
D-2555	usgscuucauGfcCfUfUf[LNA-T]cuacags{invDA}	2239	usCfsuguaGf[Ab]aagGfcAfugaagcasusu	2240
D-2556	usgscuucauGfcCfUfUfUfcuac[LNA-A]gs{invDA}	2241	usCfs[GNA-U]guaGfaaagGfcAfugaagcasusu	2242
D-2557	usgscuucauGfcCfUfUfUfcu[LNA-A]cags{invDA}	2243	usCfsug[GNA-U]aGfaaagGfcAfugaagcasusu	2244
D-2558	usgscuucauGfcCfUfUfUfc[LNA-T]acags{invDA}	2245	usCfsugu[GNA-A]GfaaagGfcAfugaagcasusu	2246
D-2559	usgscuucauGfcCfUfUfUfcuaca[sLNA-G]{invDA}	2247	us[Ab]uguaGfaaagGfcAfugaagcasusu	2248
D-2560	usgscuucauGfcCfUfUfUfcuac[LNA-A]gs{invDA}	2249	usCfs[Ab]guaGfaaagGfcAfugaagcasusu	2250
D-2561	ucaugccuUfuCfUfAfCfa[LNA-G]uggcs{invAb}	2251	asGfscca[Ab]UfguagAfaAfggcaugasusu	2252
D-2562	ucaugccuUfuCfUfAfCfag[LNA-T]ggcs{invAb}	2253	asGfscca[Ab]UfguagAfaAfggcaugasusu	2254
D-2563	ucaugccuUfuCfUfAfCfasgsuggcs{invAb}	2255	asGfscca[Ab]UfguagAfaAfggcaugasusu	2256
D-2564	{GalNAc3K2AhxC6}GfgUfaUfgUfuCfCfUfGfcuUfcAfuUfsusUf	2349	{Phosphate}asAfsuGfaAfGfcaggAfaCfaUfaCfcsUfsu	2350
D-2565	{GalNAc3K2AhxC6}GfgUfaUfgUfuCfCfUfGfcuUfcAfuAfsusUf	2351	{Phosphate}usAfsuGfaAfGfcaggAfaCfaUfaCfcsUfsu	2352
D-2566	{GalNAc3K2AhxC6}GfuAfuGfuUfcCfUfGfCfuuCfaUfgUfsusUf	2353	{Phosphate}asCfsaUfgAfAfgcagGfaAfcAfuAfcsUfsu	2354
D-2567	{GalNAc3K2AhxC6}UfaUfgUfuCfcUfGfCfUfucAfuGfcAfsusUf	2355	{Phosphate}usGfscAfuGfAfagcaGfgAfaCfaUfasUfsu	2356
D-2568	{GalNAc3K2AhxC6}AfuGfuUfcCfuGfCfUfUfUfcaUfgCfcUfsusUf	2357	{Phosphate}asGfsgCfaUfGfaagcAfgGfaAfcAfusUfsu	2358
D-2569	{GalNAc3K2AhxC6}UfgUfuCfcUfgCfUfUfCfauGfcCfuUfsusUf	2359	{Phosphate}asAfsgGfcAfUfgaagCfaGfgAfaCfasUfsu	2360
D-2570	{GalNAc3K2AhxC6}GfuUfcCfuGfcUfUfCfAfugCfcUfuUfsusUf	2361	{Phosphate}asAfsaGfgCfAfugaaGfcAfgGfaAfcsUfsu	2362
D-2571	{GalNAc3K2AhxC6}UfuCfcUfgCfuUfCfAfUfgcCfuUfuUfsusUf	2363	{Phosphate}asAfsaAfgGfCfaugaAfgCfaGfgAfasUfsu	2364
D-2572	{GalNAc3K2AhxC6}UfuCfcUfgCfuUfCfAfUfgcCfuUfuAfsusUf	2365	{Phosphate}usAfsaAfgGfCfaugaAfgCfaGfgAfasUfsu	2366
D-2573	{GalNAc3K2AhxC6}UfcCfuGfcUfuCfAfUfGfccUfuUfcUfsusUf	2367	{Phosphate}asGfsaAfaGfGfcaugAfaGfcAfgGfasUfsu	2368
D-2574	{GalNAc3K2AhxC6}CfcUfgCfuUfcAfUfGfCfcuUfuCfuAfsusUf	2369	{Phosphate}usAfsgAfaAfGfgcauGfaAfgCfaGfgsUfsu	2370

D-2575	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfGfCfCfuuUfcUfaUfsusUf	2371	{Phosphate}asUfsaGfaAfAfggcaUfgAfaGfcAfgsUfsu	2372
D-2576	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfGfCfCfuuUfcUfaAfsusUf	2373	{Phosphate}usUfsaGfaAfAfggcaUfgAfaGfcAfgsUfsu	2374
D-2577	{GalNAc3K2AhxC6}UfgCfuUfcAfuGfCfCfUfuuCfuAfcAfsusUf	2375	{Phosphate}usGfsuAfgAfAfaggcAfuGfaAfgCfasUfsu	2376
D-2578	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfUfUfucUfaCfaUfsusUf	2377	{Phosphate}asUfsgUfaGfAfaaggCfaUfgAfaGfcsUfsu	2378
D-2579	{GalNAc3K2AhxC6}GfcUfuCfaUfgCfCfUfUfucUfaCfaAfsusUf	2379	{Phosphate}usUfsgUfaGfAfaaggCfaUfgAfaGfcsUfsu	2380
D-2580	{GalNAc3K2AhxC6}CfuUfcAfuGfcCfUfUfUfcuAfcAfgUfsusUf	2381	{Phosphate}asCfsuGfuAfGfaaagGfcAfuGfaAfgsUfsu	2382
D-2581	{GalNAc3K2AhxC6}UfuCfaUfgCfcUfUfUfCfuaCfaGfuUfsusUf	2383	{Phosphate}asAfscUfgUfAfgaaaGfgCfaUfgAfasUfsu	2384
D-2582	{GalNAc3K2AhxC6}UfuCfaUfgCfcUfUfUfCfuaCfaGfuAfsusUf	2385	{Phosphate}usAfscUfgUfAfgaaaGfgCfaUfgAfasUfsu	2386
D-2583	{GalNAc3K2AhxC6}UfcAfuGfcCfuUfUfCfUfacAfgUfgUfsusUf	2387	{Phosphate}asCfsaCfuGfUfagaaAfgGfcAfuGfasUfsu	2388
D-2584	{GalNAc3K2AhxC6}UfcAfuGfcCfuUfUfCfUfacAfgUfgAfsusUf	2389	{Phosphate}usCfsaCfuGfUfagaaAfgGfcAfuGfasUfsu	2390
D-2585	{GalNAc3K2AhxC6}CfaUfgCfcUfuUfCfUfAfcaGfuGfgUfsusUf	2391	{Phosphate}asCfscAfcUfGfuagaAfaGfgCfaUfgsUfsu	2392
D-2586	{GalNAc3K2AhxC6}CfaUfgCfcUfuUfCfUfAfcaGfuGfgAfsusUf	2393	{Phosphate}usCfscAfcUfGfuagaAfaGfgCfaUfgsUfsu	2394
D-2587	{GalNAc3K2AhxC6}AfuGfcCfuUfuCfUfAfCfagUfgGfcUfsusUf	2395	{Phosphate}asGfscCfaCfUfguagAfaAfgGfcAfusUfsu	2396
D-2588	{GalNAc3K2AhxC6}AfuGfcCfuUfuCfUfAfCfagUfgGfcAfsusUf	2397	{Phosphate}usGfscCfaCfUfguagAfaAfgGfcAfusUfsu	2398
D-2589	{GalNAc3K2AhxC6}UfgCfcUfuUfcUfAfCfAfguGfgCfcUfsusUf	2399	{Phosphate}asGfsgCfcAfCfuguaGfaAfaGfgCfasUfsu	2400
D-2590	{GalNAc3K2AhxC6}GfcCfuUfuCfuAfCfAfGfugGfcCfuUfsusUf	2401	{Phosphate}asAfsgGfcCfAfcuguAfgAfaAfgGfcsUfsu	2402
D-2591	{GalNAc3K2AhxC6}GfgUfaUfgUfuCfCfUfGfcuUfcAfuCfsusUf	2403	{Phosphate}gsAfsuGfaAfGfcaggAfaCfaUfaCfcsUfsu	2404
D-2592	{GalNAc3K2AhxC6}GfuAfuGfuUfcCfUfGfCfuuCfaUfcCfsusUf	2405	{Phosphate}gsGfsaUfgAfAfgcagGfaAfcAfuAfcsUfsu	2406
D-2593	{GalNAc3K2AhxC6}UfaUfgUfuCfcUfGfCfUfucAfuCfcCfsusUf	2407	{Phosphate}gsGfsgAfuGfAfagcaGfgAfaCfaUfasUfsu	2408
D-2594	{GalNAc3K2AhxC6}AfuGfuUfcCfuGfCfUfUfcaUfcCfcCfsusUf	2409	{Phosphate}gsGfsgGfaUfGfaagcAfgGfaAfcAfusUfsu	2410
D-2595	{GalNAc3K2AhxC6}UfgUfuCfcUfgCfUfUfCfauCfcCfcUfsusUf	2411	{Phosphate}asGfsgGfgAfUfgaagCfaGfgAfaCfasUfsu	2412
D-2596	{GalNAc3K2AhxC6}GfuUfcCfuGfcUfUfCfAfucCfcCfuUfsusUf	2413	{Phosphate}asAfsgGfgGfAfugaaGfcAfgGfaAfcsUfsu	2414
D-2597	{GalNAc3K2AhxC6}UfuCfcUfgCfuUfCfAfUfccCfcUfuCfsusUf	2415	{Phosphate}gsAfsaGfgGfGfaugaAfgCfaGfgAfasUfsu	2416
D-2598	{GalNAc3K2AhxC6}UfcCfuGfcUfuCfAfUfCfccCfuUfcUfsusUf	2417	{Phosphate}asGfsaAfgGfGfgaugAfaGfcAfgGfasUfsu	2418
D-2599	{GalNAc3K2AhxC6}CfcUfgCfuUfcAfUfCfCfccUfuCfuAfsusUf	2419	{Phosphate}usAfsgAfaGfGfggauGfaAfgCfaGfgsUfsu	2420
D-2600	{GalNAc3K2AhxC6}CfuGfcUfuCfaUfCfCfCfcuUfcUfaCfsusUf	2421	{Phosphate}gsUfsaGfaAfGfgggaUfgAfaGfcAfgsUfsu	2422
D-2601	{GalNAc3K2AhxC6}UfgCfuUfcAfuCfCfCfuuCfuAfcAfsusUf	2423	{Phosphate}usGfsuAfgAfAfggggAfuGfaAfgCfasUfsu	2424
D-2602	{GalNAc3K2AhxC6}GfcUfuCfaUfcCfCfCfUfucUfaCfaGfsusUf	2425	{Phosphate}csUfsgUfaGfAfagggGfaUfgAfaGfcsUfsu	2426
D-2603	{GalNAc3K2AhxC6}CfuUfcAfuCfcCfCfUfUfcuAfcAfgUfsusUf	2427	{Phosphate}asCfsuGfuAfGfaaggGfgAfuGfaAfgsUfsu	2428
D-2604	{GalNAc3K2AhxC6}UfuCfaUfcCfcCfUfUfCfuaCfaGfuGfsusUf	2429	{Phosphate}csAfscUfgUfAfgaagGfgGfaUfgAfasUfsu	2430
D-2605	{GalNAc3K2AhxC6}UfcAfuCfcCfcUfUfCfUfacAfgUfgGfsusUf	2431	{Phosphate}csCfsaCfuGfUfagaaGfgGfgAfuGfasUfsu	2432
D-2606	{GalNAc3K2AhxC6}CfaUfcCfcCfuUfCfUfAfcaGfuGfgCfsusUf	2433	{Phosphate}gsCfscAfcUfGfuagaAfgGfgGfaUfgsUfsu	2434
D-2607	{GalNAc3K2AhxC6}AfuCfcCfcUfuCfUfAfCfagUfgGfcCfsusUf	2435	{Phosphate}gsGfscCfaCfUfguagAfaGfgGfgAfusUfsu	2436

D-2608	{GalNAc3K2AhxC6}UfcCfcCfuUfcUfAf CfAfguGfgCfcUfsusUf	2437	{Phosphate}asGfsgCfcAfCfuguaGfaAf gGfgGfasUfsu	2438
D-2609	{GalNAc3K2AhxC6}CfcCfcUfuCfuAfCf AfGfugGfcCfuUfsusUf	2439	{Phosphate}asAfsgGfcCfAfcuguAfgAf aGfgGfgsUfsu	2440

EXAMPLE 2: Efficacy of select PNPLA3 siRNA molecules in RNA FISH assay

[0003] A panel of fully chemically modified siRNA, including siRNA spanning the rs738409 and/or the rs738408 SNP in PNPLA3, were prepared and tested for potency and selectivity of mRNA knockdown in vitro. Each siRNA duplex consisted of two strands, the sense or 'passenger' strand and the antisense or 'guide' strand. The strands are 21 or 23 nucleotides in length with 19 complementary base pairs. In some instances, there are two base pair 3' overhangs. The siRNA were prepared with substitution of the natural 2'-OH in the ribose of each nucleotide with either a 2'-OMe or 2'-F group. Optionally, phosphosdiester internucleotide linkages at one or both strands were replaced with phosphorothioates to reduce degradation by exonucleases.

[0004] The efficacy of each of the siRNA molecules in reducing PNPLA3 expression was assessed using a 384-well format in vitro siRNA transfection assay followed by a fluorescent in situ hybridization targeting ribonucleic acid molecules (RNA FISH) assay to determine IC50 and maximum activity values. This assay was performed on human hepatocellular carcinoma cell line Hep3B cells (ATCC HB-8064) and Chinese hamster ovary (CHO) cells expressing human PNPLA3 I148I. Human hepatocellular carcinoma HepB3 was maintained in EMEM media (ATCC 30-2003) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic at 37°C and 5% CO2. CHO cells expressing human PNPLA3 I148I was maintained in media containing 50% CD-CHO (Life Technologies), 50% Ex-Cell CHO 5 Medium (Sigma), 8mM L-Glutamine, 1 x HT, 1% antibiotic/antimycotic, and 10μg/mL Puromycin at 37°C and 5% CO2.

[0005] For Hep3B cell assays, transfection complexes of the siRNA molecules and the Lipofectamine RNAiMAX transfection reagent (Life Technologies) in EMEM media (ATCC 30-2003) were prepared in 384-well plates (PerkinElmer), at 10μg per well, in accordance with manufacturer's recommendations. For CHO human cell assays, transfection complexes of the siRNA molecules and the Lipofectamine RNAiMAX transfection reagent in F12K media (Mediatech) were prepared in 384-well plates, at 10μg per well, in accordance with manufacturer's recommendations. Cells were diluted to 67,000 cells/ml in antibiotic/antimycotic-free media and

30µl added to each well, a final density of 2000 cells/well in 40µl media. After a 20 minute incubation at room temperature, plates were transferred to a 37°C and 5% CO2 incubator. Hep3B cell transfection assays were incubated for 72 hours and CHO human PNPLA3 I148I transfection assays were incubated for 48 hours.

[0184] At harvest, the cells were fixed in an 8% formaldehyde fixative solution (Thermo Scientific) for 15 minutes at room temperature. The plates were then subjected to dehydration with sequential 50%, 70%, and 100% ethanol washes. Plates were then sealed and stored at -20°C.

[0185] The RNA FISH assay was performed using the Affymetrix QuantiGene® View RNA HC Screening Assay kit (QVP0011), the Affymetrix View HC Signal Amplification Kit 3-plex (QVP0213), and Affymetrix gene specific probes: PNPLA3 Human 0.33mL View RNA Type 6 (650 label) VA6-20279-01 and PPIB Human 0.44mL View RNA Type 1 (488 label) VA1-10148-01.

[0186] Plates were first rehydrated with sequential 100%, 70%, and 50% ethanol washes. Cells were then washed with PBS, and then permeabilized and protease-digested according to the kit instructions. The target Working Probe Sets were prepared according to the manufacturer's protocol, added to the wells, and incubated for 3 hours at 40°C. The manufacturer's protocol was followed for the sequential hybridizations with the Working Probe Sets, the Working PreAmps, the Working Amps, and the Working LPs. Last, nuclei counterstains were applied (Hoechst 33342 and Cell Mask Blue; Molecular Probes). Plates were incubated for 30 minutes at room temperature, washed with PBS, overlaid with 80µl of PBS, and then the plates were sealed for imaging.

[0187] All plates were imaged on an Opera Phenix High Content Screening System (PerkinElmer), using the UV Channel for Hoechst 33342 and Cell Mask Blue, the 488 Channel for Type1 probes, and the 647 Channel for Type6 probes.

[0188] RNA FISH data was analyzed using Columbus software and images were generated using Genedata Screener. The results of the assay for CHO transfected PNPLA3 I148I are shown in Table 3. The results of the assay for CHO transfected PNPLA3 I148M are shown in Table 4.

PNPLA3 knockdown provides a percentage of knockdown compared to control. Negative values indicate a decrease in PNPLA3 levels.

Table 3. RNA FISH assay on CHO transfected PNPLA3 I148I

Duplex No.	IC50 (μM)	PNPLA3 knockdown (%)
D-2001	.0589	-33.9
D-2002	.0158	-67.2
D-2003	.0427	-43.4
D-2004	.00835	-63.5
D-2006	.0177	-77.8
D-2008	.125	-10.7
D-2009	.00769	-45.5
D-2010	.00558	-80
D-2011	.035	-3
D-2012	> 0.5	-3.7
D-2013	.036	-6.4
D-2014	.0122	-58.2
D-2015	> 0.5	8
D-2016	> 0.5	8
D-2017	.0153	-73.2
D-2018	.00386	-31.5
D-2019	.125	
D-2020	> 0.5	
D-2021	> 0.5	8
D-2022	> 0.167	-6.8
D-2023	.0257	-36.9
D-2024	> 0.5	4
D-2025	> 0.5	-2.5
D-2026	.022	-35.1
D-2027	.00172	-16.5
D-2028	> 0.5	10
D-2029	.0106	-56.2
D-2032	.00205	-52.9
D-2033	.0107	-61.7
D-2034	> 0.5	6
D-2035	> 0.5	-3.8
D-2036	.00665	-55.9
D-2037	> 0.5	4

D-2038	> 0.5	10
D-2039	.0116	-23.9
D-2040	> 0.5	-25.4
D-2041	> 0.5	9
D-2042	.00959	-25.5
D-2043	> 0.5	9
D-2044	.00552	-29
D-2045	> 0.5	9
D-2046	> 0.5	9
D-2047	> 0.5	-5.9
D-2048	.00618	-56.3
D-2049	> 0.5	12
D-2050	> 0.5	10
D-2051	> 0.5	-17.2
D-2052	> 0.5	-8.3
D-2053	> 0.5	11
D-2054	> 0.5	-14.9
D-2055	> 0.5	-10.6
D-2056	> 0.5	10
D-2057	.00485	-59.2
D-2058	.014	-53
D-2059	> 0.5	-4.9
D-2060	> 0.5	18
D-2061	.00795	-44.8
D-2062	.000668	-74.6
D-2063	> 0.5	-21.8
D-2064	> 0.5	9
D-2065	> 0.5	-10.5
D-2066	.0412	-42.2
D-2067	> 0.5	10

Table 4. RNA FISH assay on CHO transfected PNPLA3 I148M

Duplex No.	IC50 (μM)	PNPLA3 knockdown (%)
D-2000	.0316	-29.2
D-2001	.0131	-81.8
D-2002	.00216	-90.5
D-2003	.022	-50.4

D-2004	.00429	-88
D-2005	> 0.5	15
D-2006	.00301	-89.2
D-2007	> 0.5	6
D-2009	.00274	-86.9
D-2010	.00203	-93.3
D-2011	.000694	-11.9
D-2012	> 0.5	-18
D-2013	.011	-66.4
D-2014	.0057	-84.3
D-2015	> 0.5	-13.5
D-2016	> 0.5	-12.4
D-2017	.00448	-89.4
D-2018	.0104	-36.2
D-2019	.0302	-7.7
D-2020	.01	-78.9
D-2021	.00435	-83.5
D-2022	.00628	-88.6
D-2023	.0143	-44.3
D-2024	> 0.5	11
D-2025	.00355	-58.2
D-2026	.000867	-39.4
D-2027	> 0.5	32
D-2028	.00205	-89.9
D-2029	.0019	-94
D-2030	> 0.5	-9.4
D-2031	> 0.5	4

RNA FISH was also run on a hepatic cell line containing the double mutant PNPLA3-rs738408-rs738409, as well as a control wild-type cell line, Hep3B. Hep3B and HepG2 cells (purchased from ATCC) were cultured in minimal essential medium (MEM from Corning for Hep3B and EMEM from ATCC for HepG2) supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% penicillin-streptomycin (P-S, Corning). The siRNA transfection was performed as follows: 1 μL of test siRNAs and 4 μL of plain MEM or EMEM, depending on the cell line, were added to PDL-coated CellCarrier-384 Ultra assay plates (PerkinElmer) by BioMek FX (Beckman Coulter). 5 μL of Lipofectamine RNAiMAX (Thermo Fisher Scientific), pre-diluted in plain MEM or

EMEM (specifically 0.035 μL RNAiMAX in 5 μL MEM for Hep3B and 0.06 μL of RNAiMAX in 5 μL EMEM for HepG2), was then dispensed into the assay plates by Multidrop Combi Reagent Dispenser (Thermo Fisher Scientific). After 20 mins incubation of the siRNA/RNAiMAX mixture at room temperature (RT), 30 μL of either Hep3B or HepG2 cells (2000 cells per well) in MEM or EMEM supplemented with 10% FBS and 1% P-S were added to the transfection complex using Multidrop Combi Reagent Dispenser. The assay plates were incubated for 20 mins at RT prior to being placed in an incubator. Cells were then incubated for 72 hrs at 37 °C and 5% CO2. ViewRNA ISH Cell Assay was performed following manufacture's protocol (Thermo Fisher Scientific) using an in-house assembled automated FISH assay platform for liquid handling. In brief, cells were fixed in 4% formaldehyde (Thermo Fisher Scientific) for 15 mins at RT, permeabilized with detergent for 3 mins at RT and then treated with protease solution for 10 mins at RT. Incubation of target-specific probe pairs (Thermo Fisher Scientific) was done for 3 hrs, while for Preamplifiers, Amplifiers and Label Probes (Thermo Fisher Scientific) were for 1 hr each. All hybridization steps were carried out at 40 °C in Cytomat 2 C-LIN automated incubator (Thermo Fisher Scientific). After hybridization reactions, cells were stained for 30 mins with Hoechst and CellMask Blue (Thermo Fisher Scientific) and then imaged on Opera Phenix (PerkinElmer). The images were analyzed using Columbus Image Data Storage and Analysis System (PerkinElmer) to obtain mean spot counts per cell. The spot counts were normalized using the high (containing phosphate-buffered saline, Corning) and low (without target probe pairs) control wells. The normalized values against the total siRNA concentrations were plotted and the data were fit to a four-parameter sigmoidal model in Genedata Screener (Genedata) to obtain IC50 and maximum activity. The results of the assay for HepG2 cells is shown in Table 5 and the results of the assay for Hep3B cells is shown in Table 6. PNPLA3 knockdown provides a percentage of knockdown compared to control. Negative values indicate a decrease in PNPLA3 levels. In instances where a duplex was run more than once, the average IC50 is shown, with a standard deviation.

Table 5. RNA FISH assay on hepatic HepG2 cells

Duplex No.	IC50 (μM)	PNPLA3 knockdown (%)
D-2001	.0132	-70.5
D-2003	.0458	-46.9
D-2004	.0049	-74.2
D-2006	.00283	-69.6
D-2009	.00448	-62.2
D-2010	.00206	-52
D-2013	.00319	-71.7
D-2014	.00164	-67.4
D-2017	.00222	-60.9
D-2018	.00717	-52.7
D-2020	.00664	-68.3
D-2021	.00559	-61.5
D-2022	.004	-30.5
D-2023	> 0.5	-18
D-2025	.0041	-61.9
D-2026	.00937	-34.6
D-2564	0.009	-64.021
D-2565	0.00609	-70.965
D-2566	0.00255	-52.65
D-2567	0.00584	-56.603
D-2568	0.0157	-63.002
D-2569	0.00327	-64.898
D-2570	0.00144	-55.424
D-2571	> 0.1	-18.661
D-2572	0.00557	-25.668
D-2573	0.0115	-55.329
D-2574	0.00289	-69.84
D-2575	0.00378	-69.491
D-2576	0.00527	-64.841
D-2577	0.00511	-46.449
D-2578	0.0026	-67.821
D-2579	0.00402	-67.057
D-2580	0.00119	-64.422
D-2581	0.00915	-73.008
D-2582	0.000823	-77.053
D-2583	0.00851	-66.555
D-2584	0.00513	-54.442

D-2585	0.0154	-67.707
D-2586	0.00701	-69.624
D-2587	0.00732	-66.627
D-2588	0.00226	-70.854
D-2589	0.00837	-31.221
D-2590	0.0249	-41.857
D-2591	0.009	-64.021
D-2592	0.00609	-70.965
D-2593	0.00255	-52.65
D-2594	0.00584	-56.603
D-2595	0.0157	-63.002
D-2596	0.00327	-64.898
D-2597	0.00144	-55.424
D-2598	> 0.1	-18.661
D-2599	0.00557	-25.668
D-2600	0.0115	-55.329
D-2601	0.00289	-69.84
D-2602	0.00378	-69.491
D-2603	0.00527	-64.841
D-2604	0.00511	-46.449
D-2605	0.0026	-67.821
D-2606	0.00402	-67.057
D-2607	0.00119	-64.422
D-2608	0.00915	-73.008
D-2609	0.000823	-77.053
D-2591	0.00851	-66.555
D-2592	0.00513	-54.442
D-2593	0.0154	-67.707
D-2594	0.00701	-69.624
D-2595	0.00732	-66.627
D-2596	0.00226	-70.854
D-2597	0.00837	-31.221
D-2598	0.0249	-41.857
D-2426	85.098	-14.902
D-2444	31.575 +/- 6.79	-68.425 +/- 6.79
D-2454	53.215	-46.785
D-2473	29.35	-70.65

Table 6. RNA FISH assay on hepatic Hep3B cells

Duplex No.	IC50 (μM)	PNPLA3 knockdown (%)
D-2001	.00842	-37
D-2003	.0158	-32.1
D-2004	.00266	-32.4
D-2006	.00948	-54.1
D-2009	.00228	-29.5
D-2010	.00219	-37.2
D-2013	.00524	-31.5
D-2014	.00148	-37.6
D-2017	.00333	-37.6
D-2018	.00315	-21.3
D-2020	> 0.5	6
D-2021	> 0.5	-1.6
D-2022	.00272	-30.9
D-2023	> 0.5	24
D-2025	.0101	-30.3
D-2026	.00551	-23
D-2564	0.01	-63.199
D-2565	0.00938	-58.392
D-2566	0.00343	-61.484
D-2567	0.0175	-53.489
D-2568	> 0.1	-18.367
D-2569	0.0195	-62.568
D-2570	0.0127	-77.141
D-2571	> 0.1	-15.922
D-2572	> 0.1	-12.434
D-2573	> 0.1	-14.649
D-2574	0.0215	-52.515
D-2575	0.0203	-53.175
D-2576	0.018	-48.137
D-2577	> 0.1	-16.105
D-2578	> 0.1	-21.309
D-2579	> 0.1	-17.510
D-2580	> 0.1	-24.616
D-2581	> 0.1	-13.987
D-2582	0.0574	-30.543

D-2583	> 0.1	-23.990
D-2584	> 0.1	-6.715
D-2585	> 0.1	-17.518
D-2586	> 0.1	-24.518
D-2587	0.0391	-58.478
D-2588	0.0218	-56.609
D-2589	> 0.1	-17.418
D-2590	> 0.1	-21.161
D-2591	0.0167	-59.366
D-2592	0.0104	-61.548
D-2593	Undefined	-61.879
D-2594	0.0211	-43.856
D-2595	0.0272	-63.020
D-2596	> 0.1	-10.278
D-2597	0.0546	-31.743
D-2598	Undefined	-47.517
D-2599	0.00489	-70.825
D-2600	> 0.1	-8.522
D-2601	0.0364	-31.836
D-2602	0.00577	-65.062
D-2603	0.01	-58.287
D-2604	0.00353	-40.649
D-2605	0.0113	-50.691
D-2606	> 0.1	-5.097
D-2607	0.0261	-49.898
D-2608	> 0.1	-23.747
D-2609	> 0.1	-23.804
D-2426	> 0.1	-15.207
D-2454	0.00187	-51.735
D-2473	> 0.1	-21.333

EXAMPLE 3: Droplet digital PCR assay of siRNA for PNPLA3-rs738409 and PNPLA3-rs738409-rs738408

[0189] Following the manufacturers protocol, thawed human primary hepatocyte cells (Xenotech/Sekisui donor lot#HC3-38) in OptiThaw media (Xenotech cat#K8000), cells were centrifuged and post media aspiration, resuspended in OptiPlate hepatocyte media (Xenotech cat#K8200) and plated into 96 well collagen coated plates (Greiner cat#655950). Following a 2-

4 hour incubation period, media was removed and replaced with OptiCulture hepatocyte media (Xenotech cat#K8300). 2-4 hours post addition of OptiCulture media, delivered GalNAc conjugated siRNAs to cells via free uptake (no transfection reagent). Cells were incubated 24-72 hours at 37°C and 5% CO2. Cells were then lysed with Qiagen RLT buffer (79216) +1% 2-mercaptoethanol (Sigma, M-3148), and lysates were stored at -20°C. RNA was purified using a Qiagen QIACube HT instrument (9001793) and a Qiagen RNeasy 96 QIACube HT Kit (74171) according to manufacturer's instructions. Samples were analyzed using a QIAxpert system (9002340). cDNA was synthesized from RNA samples using the Applied Biosystems High Capacity cDNA Reverse Transcription kit (4368813), reactions were assembled according to manufacturer's instructions, input RNA concentration varied by sample. Reverse transcription was carried out on a BioRad tetrad thermal cycler (model# PTC-0240G) under the following conditions: 25°C 10 minutes, 37°C 120 minutes, 85°C 5 minutes followed by (an optional) 4°C infinite hold.

[0190] Droplet digital PCR (ddPCR) was performed using BioRad's QX200 AutoDG droplet digital PCR system according to manufacturer's instructions. Reactions were assembled into an Eppendorf clear 96 well PCR plate (951020303) using BioRad ddPCR Supermix for Probes (1863010), and fluorescently labeled qPCR assays for PNPLA3 (IDT Hs.PT.58.21464637, primer to probe ratio 3.6:1 and TBP (IDT Hs.PT.53a.20105486, primer to probe ratio 3.6:1) and RNase free water (Ambion, AM9937). Final primer/probe concentration is 900nM/250nM respectively, input cDNA concentration varied among wells. Droplets were formed using a BioRad Auto DG droplet generator (1864101) set up with manufacturer recommended consumables (BioRad DG32 cartridges 1864108, BioRad tips 1864121, Eppendorf blue 96 well PCR plate 951020362, BioRad droplet generation oil for probes 1864110 and a BioRad droplet plate assembly). Droplets were amplified on a BioRad C1000 touch thermal cycler (1851197) using the following conditions: enzyme activation 95°C 10 minutes, denaturation 94°C 30 seconds followed by annealing/extension 60°C for one minute, 40 cycles using a 2°C/second ramp rate, enzyme deactivation 98°C 10 minutes followed by (an optional) 4°C infinite hold. Samples were then read on a BioRad QX200 Droplet Reader measuring FAM/HEX signal that correlates to PNPLA3 or TBP concentration. Data was analyzed using BioRad's QuantaSoft software package. Samples are gated by channel (fluorescent label) to determine the concentration per sample. Each sample

is then expressed as the ratio of the concentration of the gene of interest (PNPLA3)/concentration of the housekeeping gene (TBP) to control for differences in sample loading. Data is then imported into Genedata Screener, where each test siRNA is normalized to the median of the neutral control wells (buffer only). IC50 values are reported in Table 7.

Table 7. ddPCR assay on primary hepatocyte cells

Duplex No.	IC50 (μM)	% PNPLA3 knockdown
D-2068	.0339	-49.628
D-2069	.00408	-52.997
D-2070	.00433	-42.193
D-2072	.00884	-53.16
D-2073	> 2.0	-7.435
D-2078	.0044	-43.123
D-2084	0.00499	-38.791
D-2085	0.00539	-64.312
D-2086	> 2.0	-14.938
D-2087	> 2.0	-25.465
D-2088	0.207	-34.944
D-2089	0.0107	-38.791
D-2090	0.0218	-38.977
D-2091	0.0508	-41.209
D-2092	0.00192	-44
D-2093	0.00634	-30.233
D-2094	> 2.0	4.93
D-2095	0.00181	-59.814
D-2096	0.0181	-52.807
D-2099	0.00549	-39.296
D-2100	0.0142	-55.281
D-2158	0.0681	-48.649
D-2159	0.0325	-36.036
D-2160	> 0.667	-13.514
D-2161	> 2.0	-24.229
D-2162	0.0726	-28.634
D-2163	> 0.667	-16.3
D-2164	> 2.0	-15.418
D-2165	0.00644	-26.872
D-2166	0.00192	-30.045

D-2167	> 0.667	-6.726
D-2168	> 2.0	-15.418
D-2169	> 2.0	-13.004
D-2170	> 2.0	-9.417
D-2171	0.00505	-44.395
D-2172	0.003	-55.336
D-2173	0.00598	-46.188
D-2174	> 2.0	-9.009
D-2175	0.017	-27.928
D-2176	0.00452	-35.426
D-2177	> 2.0	4.5
D-2178	> 2.0	1.8
D-2179	> 2.0	-6.306
D-2180	0.00546	-40.969
D-2181	0.00152	-43.119
D-2182	0.00317	-54.128
D-2183	0.00948	-58.079
D-2184	0.0109	-50.459
D-2185	> 2.0	-7.339
D-2186	0.0021	-48.624
D-2187	> 2.0	-11.009
D-2188	> 2.0	1.32
D-2191	0.0984	-66.923
D-2192	0.124	-64.231
D-2193	0.138	-60.606
D-2194	0.0478	-54.182
D-2195	0.0801	-47.515
D-2199	0.0517	-62.973
D-2201	0.0517	-62.973
D-2202	0.0165	-72.404
D-2203	0.00946	-49.459
D-2204	0.0241	-58.545
D-2205	0.0382	-45.576
D-2206	0.0222	-50.946
D-2209	0.0867	-46.622
D-2212	0.358	-60
D-2216	0.0826	-58.942
D-2218	0.0242	-63.462
D-2220	0.0113	-69.333
D-2221	0.138	-55.541

D-2224	0.0198	-66.486
D-2225	0.245	-68.077
D-2228	0.155	-44.606
D-2229	0.0651	-38.909
D-2231	0.0512	-56.892
D-2232	0.0678	-67.981
D-2233	0.00802	-57.182
D-2234	0.00473	-55.947
D-2235	0.00816	-62.115
D-2236	0.00245	-51.542
D-2237	0.00495	-60
D-2238	0.00561	-63.017
D-2239	0.00453	-55.537
D-2240	0.00584	-56.116
D-2241	0.00755	-54.76
D-2242	0.0137	-56.332
D-2243	0.00329	-57.118
D-2244	0.0127	-56.909
D-2245	0.00697	-58.364
D-2246	0.00713	-56.828
D-2247	0.00875	-57.797
D-2248	0.0098	-58.59
D-2249	0.00603	-57.759
D-2250	0.0105	-62.155
D-2251	0.00521	-59.914
D-2252	0.00988	-58.678
D-2253	0.00481	-57.118
D-2254	0.00721	-56.332
D-2255	0.00788	-52.838
D-2256	0.00831	-55.455
D-2257	0.00503	-54.545
D-2258	0.00626	-54.545
D-2259	0.00401	-55.947
D-2260	0.00379	-52.423
D-2261	0.00151	-54.31
D-2262	0.00292	-53.448
D-2263	0.00607	-59.483
D-2264	0.00703	-59.504
D-2265	> 4.0	-20.524
D-2266	0.0129	-32.727

D-2267	0.107	-27.273
D-2275	0.00359	-45.701
D-2276	0.00416	-43.891
D-2277	0.00218	-49.14
D-2278	0.00743	-42.986
D-2279	0.0116	-53.846
D-2280	0.00347	-36.652
D-2281	0.00449	-43.891
D-2282	0.0134	-37.557
D-2283	0.00864	-34.842
D-2284	0.00738	-49.558
D-2285	0.0202	-38.053
D-2286	0.00543	-45.487
D-2287	0.00934	-47.611
D-2288	0.00652	-55.575
D-2289	0.0259	-61.593
D-2290	0.00549	-53.805
D-2291	0.00476	-51.062
D-2292	0.0105	-42.584
D-2293	0.0059	-45.455
D-2294	0.0117	-45.646
D-2295	0.0109	-52.823
D-2296	0.01246 +/- 0.015	-59.847 +/- 15.2
D-2297	0.00828	-44.444
D-2298	0.0279	-41.346
D-2299	0.00529	-55.926
D-2300	0.0195	-50.423
D-2301	0.00838	-35.577
D-2302	0.00832	-40.865
D-2303	0.00371	-40.096
D-2304	0.00563	-38.365
D-2305	0.00639	-40.385
D-2306	0.00669	-41.25
D-2307	0.00212	-38.462
D-2308	0.00573	-37.736
D-2309	0.0645	-33.962
D-2310	0.00232	-33.019
D-2311	0.00181	-31.132
D-2312	0.0447	-45.283
D-2313	0.00655	-42.453

D-2314	0.00613	-41.509
D-2315	0.00941	-50
D-2316	0.0218	-41.784
D-2317	0.0142	-42.723
D-2318	0.0182	-31.455
D-2319	> 4.0	-23.005
D-2320	0.0228	-37.089
D-2321	0.00809	-45.352
D-2322	0.0165	-44.601
D-2323	0.0184	-38.373
D-2324	0.00766	-41.627
D-2325	0.00815	-46.507
D-2326	0.0168	-48.325
D-2327	0.00663	-62.254
D-2328	0.00716	-39.367
D-2329	0.044	-52.036
D-2330	0.00282	-65.701
D-2331	0.00411	-53.991
D-2332	0.0127	-51.222
D-2333	0.012	-48.357
D-2334	0.019	-42.593
D-2335	0.00448	-47.418
D-2336	0.00944	-37.327
D-2337	0.00514	-37.327
D-2338	0.154	-38.249
D-2339	0.0089	-40.092
D-2340	0.0169	-48.148
D-2341	0.00274	-46.296
D-2342	0.0225	-42.723
D-2343	0.00222 +/- 0.00136	-43.218 +/- 4.81
D-2344	0.0136	-56.561
D-2345	0.0222	-50.226
D-2346	0.0273	-55.385
D-2347	0.0164	-41.784
D-2348	0.0314	-60.282
D-2349	0.0103	-65.1
D-2350	0.0427	-63.9

Example 4: Efficacy screening of select PNPLA3 siRNA molecules in a humanized mouse model

[0191] Associated adenovirus (AAV; serotype AAV8 or AAV7; endotoxin-free, prepared internally by Amgen) diluted in phosphate buffered saline (Thermo Fisher Scientific,14190-136) to 4e11 up to 1e12 viral particles per animal, was injected intravenously into the tail vein of C57BL/6NCrl male mice (Charles River Laboratories Inc.) to drive expression of either human *PNPLA3*^{WT} *(PNPLA3-WT)*, *PNPLA3*^{rs738409} *(PNPLA3-I148M), or PNPLA3*$^{rs738409\text{-}rs738408}$ *(PNPLA3-I148M DM)* in the liver. Mice were generally 10-12 weeks of age and an n=4-6 animals were included per group. Every round of screening included at least two vehicle-treated control groups: AAV-empty vector and AAV-*PNPLA3*^{WT} or *PNPLA3*^{rs738409} and *PNPLA3*$^{rs738409\text{-}rs738408}$ treated with vehicle. All siRNAs were tested against AAV-*PNPLA3*^{WT}, *PNPLA3*^{rs738409}, *and/or PNPLA3*$^{rs738409\text{-}rs738408}$. Two weeks after AAV injection, mice were treated with a single dose of siRNA D-2324 (0.5mM), via subcutaneous injection, at 0.5, 1.0, 3.0 or 5.0 milligrams per kilogram of animal, diluted in phosphate buffered saline (Thermo Fisher Scientific,14190-136). At 8, 15, 22, 28 or 42 days post-siRNA injection, livers were collected from the animals, snap frozen in liquid nitrogen, processed for purified RNA using a QIACube HT instrument (Qiagen, 9001793) and RNeasy 96 QIACube HT kits (Qiagen, 74171) according to manufacturer's instructions. Samples were analyzed using a QIAxpert system (Qiagen, 9002340). RNA was treated with RQ1 RNase-Free DNase (Promega, M6101) and prepared for Real-Time qPCR using the TaqMan™ RNA-to-C_T™ 1-Step kit (Applied Biosystems, 4392653). Real-Time qPCR was run on a QuantStudio Real-Time PCR machine. Results are based on gene expression of human *PNPLA3* as normalized to mouse *Gapdh* (TaqMan™ assays from Invitrogen, hs00228747_m1 and 4352932E, respectively), and presented as the relative knockdown of human *PNPLA3* mRNA expression compared to vehicle-treated control animals. Endogenous mouse *Pnpla3* expression was determined for comparison (Invitrogen, Mm00504420_m1).

[0192] For hepatic triglyceride content analysis, approximately 0.05-0.1 milligrams of frozen liver from an animal was homogenized in one milliliter of isopropanol. After one hour of incubation on ice, samples were spun at 10,000 rpm in a microfuge, and supernatants transferred to a clean deep-well 96-well plate. Triglyceride content was determined using the colorimetric Infinity Triglyceride Reagent (Thermo Fisher Scientific, TR22421) and Triglyceride Standard (Pointe Scientific, T7531-STD) according to manufacturer's instructions. Results are presented as milligrams of triglyceride per milligrams of tissue.

[0193] All animal experiments described herein were approved by the Institutional Animal Care and Use Committee (IACUC) of Amgen and cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8th* Edition (National Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of Laboratory Animals., Institute for Laboratory Animal Research (U.S.), and National Academies Press (U.S.) (2011) Guide for the care and use of laboratory animals. 8th Ed., National Academies Press, Washington, D.C. Mice were single-housed in an air-conditioned room at 22±2°C with a twelve-hour light; twelve-hour darkness cycle (0600-1800 hours). Animals had *ad libitum* access to a regular chow diet (Envigo, 2920X) and to water (reverse osmosis-purified) via automatic watering system, unless otherwise indicated. At termination, blood was collected by cardiac puncture under deep anesthesia, and then, following Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines, euthanized by a secondary physical method. **Figure 1A-D.** An example of five siRNA molecules screened for both dose-dependent mRNA knockdown and functional durability in vivo. Mice expressing human *PNPLA3*$^{rs738409\text{-}rs738408}$ were treated with siRNA two-weeks after intravenous AAV injections. N=6 mice per group; data represented as the average and the standard error of the mean. (A) siRNAs were injected at 0.5, 1.0, 3.0, or 5.0 milligrams per kilogram of body weight subcutaneously into the abdomen of the mouse. Four weeks after siRNA treatment, mice were sacrificed, and the livers were collected and processed for gene expression analysis. The data represents the average relative knockdown of human *PNPLA3*$^{rs738409\text{-}rs738408}$ in each group set to the vehicle-treated control group. (B) Livers from the same four-week treatment group were also processed for triglyceride content to access functional efficacy. The data represents the average milligrams of triglyceride per gram of tissue processed. (C) siRNAs were injected at 1.0 and 3.0 milligrams per kilogram of body weight subcutaneously into the abdomen of a parallel cohort of animals. Mice were harvested six weeks after siRNA treatment to compare durability of the siRNA molecules in vivo. The livers were collected and processed for gene expression analysis. The data represents the average relative knockdown of human *PNPLA3*$^{rs738409\text{-}rs738408}$ in each group set to the vehicle-treated control group. (D) Livers from the same six-week treatment group were also processed for triglyceride content to access functional efficacy over time. The data represents the average milligrams of triglyceride per gram of tissue processed.

[0194] Data for relative knockdown is shown in Tables 8-12, showing relative knockdown at day 8, 15, 22, 28, and 42, respectively and the various doses. PNPLA3 knockdown is a percentage, with negative values indicating a decrease in PNPLA3 levels.

Table 8. Day 8 PNPLA3 knockdown assay

Duplex number	AAV vector	AAV particles/ animal	Dose administered (mg/kg)	PNPLA3 knockdown (%)
D-2092	PNPLA3-I148M	1.00E+12	3	-91.51
D-2092	PNPLA3-I148M	1.00E+12	5	-92.64
D-2095	PNPLA3-I148M	1.00E+12	3	-79.21
D-2095	PNPLA3-I148M	1.00E+12	5	-86.38
D-2068	PNPLA3-I148M	4.00E+11	5	-34.15
D-2069	PNPLA3-I148M	4.00E+11	5	-82.74
D-2070	PNPLA3-I148M	4.00E+11	5	-79.64
D-2071	PNPLA3-I148M	4.00E+11	5	-48.80
D-2071	PNPLA3-WT	4.00E+11	5	-59.43
D-2075	PNPLA3-I148M	4.00E+11	5	-79.10
D-2075	PNPLA3-WT	4.00E+11	5	-60.60
D-2079	PNPLA3-I148M	4.00E+11	5	-50.08
D-2072	PNPLA3-I148M	4.00E+11	5	-44.40
D-2072	PNPLA3-WT	4.00E+11	5	-43.75
D-2077	PNPLA3-I148M	4.00E+11	5	-28.46
D-2076	PNPLA3-I148M	4.00E+11	5	-83.25
D-2078	PNPLA3-I148M	4.00E+11	5	-49.10
D-2078	PNPLA3-WT	4.00E+11	5	-3.45
D-2073	PNPLA3-I148M	4.00E+11	5	-39.42
D-2074	PNPLA3-I148M	4.00E+11	5	-46.68
D-2074	PNPLA3-WT	4.00E+11	5	-5.11
D-2084	PNPLA3-I148M	4.00E+11	5	-87.49
D-2084	PNPLA3-I148M	8.00E+11	5	-88.75
D-2084	PNPLA3-I148M	8.00E+11	1	-16.49
D-2084	PNPLA3-WT	8.00E+11	1	-15.42
D-2085	PNPLA3-I148M	4.00E+11	5	-77.14
D-2085	PNPLA3-I148M	8.00E+11	5	-84.42
D-2085	PNPLA3-I148M	8.00E+11	1	-25.19
D-2085	PNPLA3-WT	8.00E+11	1	-21.14

D-2086	PNPLA3-I148M	4.00E+11	5	-64.54
D-2086	PNPLA3-I148M	4.00E+11	5	-52.95
D-2087	PNPLA3-I148M	4.00E+11	5	-20.15
D-2088	PNPLA3-I148M	4.00E+11	5	-47.18
D-2088	PNPLA3-I148M	8.00E+11	5	-66.96
D-2089	PNPLA3-I148M	8.00E+11	5	-85.47
D-2089	PNPLA3-WT	8.00E+11	1	-21.01
D-2089	PNPLA3-I148M	8.00E+11	1	-34.21
D-2089	PNPLA3-I148M	1.00E+12	5	-90.55
D-2090	PNPLA3-I148M	8.00E+11	5	-89.58
D-2090	PNPLA3-I148M	8.00E+11	1	-50.13
D-2090	PNPLA3-WT	8.00E+11	1	8.76
D-2091	PNPLA3-WT	8.00E+11	5	-35.70
D-2092	PNPLA3-I148M	8.00E+11	5	-92.34
D-2092	PNPLA3-I148M	8.00E+11	1	-51.35
D-2092	PNPLA3-WT	8.00E+11	1	-42.88
D-2093	PNPLA3-I148M	8.00E+11	5	-83.87
D-2094	PNPLA3-I148M	8.00E+11	5	-70.12
D-2095	PNPLA3-I148M	8.00E+11	1	-29.95
D-2095	PNPLA3-WT	8.00E+11	1	67.40
D-2095	PNPLA3-I148M	8.00E+11	5	-85.42
D-2096	PNPLA3-WT	8.00E+11	5	-90.44
D-2081	PNPLA3-I148M	8.00E+11	5	6.25
D-2081	PNPLA3-I148M	8.00E+11	5	-11.81
D-2097	PNPLA3-I148M	8.00E+11	5	-87.61
D-2098	PNPLA3-I148M	8.00E+11	5	-79.84
D-2099	PNPLA3-I148M	8.00E+11	5	-84.36
D-2100	PNPLA3-I148M	8.00E+11	5	-79.67
D-2101	PNPLA3-I148M	8.00E+11	5	-89.64
D-2102	PNPLA3-I148M	8.00E+11	5	-61.49
D-2103	PNPLA3-I148M	8.00E+11	5	-19.65
D-2104	PNPLA3-I148M	8.00E+11	5	-79.70
D-2104	PNPLA3-I148M	1.00E+12	5	-82.87
D-2105	PNPLA3-I148M	8.00E+11	5	-84.49
D-2105	PNPLA3-I148M	1.00E+12	5	-87.71
D-2152	PNPLA3-I148M	1.00E+12	5	-39.35
D-2153	PNPLA3-I148M	1.00E+12	5	-79.04
D-2154	PNPLA3-I148M	1.00E+12	5	-66.72
D-2155	PNPLA3-I148M	1.00E+12	5	-44.68

D-2156	PNPLA3-I148M	1.00E+12	5	-84.72
D-2157	PNPLA3-I148M	1.00E+12	5	-17.25
D-2280	PNPLA3-I148M DM	1.00E+12	5	-99.70
D-2280	PNPLA3 WT	1.00E+12	5	-51.13
D-2295	PNPLA3-WT	1.00E+12	5	-35.90
D-2295	PNPLA3-I148M DM	1.00E+12	5	-94.68
D-2296	PNPLA3-WT	1.00E+12	5	-23.24
D-2296	PNPLA3-I148M DM	1.00E+12	5	-92.78
D-2297	PNPLA3-WT	1.00E+12	5	43.71
D-2297	PNPLA3-I148M DM	1.00E+12	5	-94.59
D-2324	PNPLA3-WT	1.00E+12	5	6.53
D-2324	PNPLA3-I148M DM	1.00E+12	5	-97.39
D-2326	PNPLA3-WT	1.00E+12	5	-8.25
D-2326	PNPLA3-I148M DM	1.00E+12	5	-77.82
D-2328	PNPLA3-WT	1.00E+12	5	-2.12
D-2328	PNPLA3-I148M DM	1.00E+12	5	-92.49

Table 9. Day 15 PNPLA3 knockdown assay

Duplex number	AAV vector	AAV particles/ animal	Dose administered (mg/kg)	PNPLA3 knockdown (%)
D-2092	PNPLA3-I148M	1.00E+12	3	-87.58
D-2092	PNPLA3-I148M	1.00E+12	5	-93.96
D-2095	PNPLA3-I148M	1.00E+12	3	-81.73
D-2095	PNPLA3-I148M	1.00E+12	5	-72.99
D-2131	PNPLA3-I148M	1.00E+12	5	-66.02
D-2186	PNPLA3-I148M	1.00E+12	5	-87.67
D-2089	PNPLA3-I148M	1.00E+12	5	-95.01
D-2104	PNPLA3-I148M	1.00E+12	5	-76.01
D-2105	PNPLA3-I148M	1.00E+12	5	-72.67
D-2123	PNPLA3-I148M	1.00E+12	5	-56.93
D-2128	PNPLA3-I148M	1.00E+12	5	-37.26
D-2138	PNPLA3-I148M	1.00E+12	5	-62.16
D-2149	PNPLA3-I148M	1.00E+12	5	-72.34
D-2156	PNPLA3-I148M	1.00E+12	5	-75.81
D-2259	PNPLA3-I148M	1.00E+12	5	-79.15
D-2260	PNPLA3-I148M	1.00E+12	5	-69.97
D-2261	PNPLA3-I148M	1.00E+12	5	-50.40

D-2262	PNPLA3-I148M	1.00E+12	5	-84.36
D-2263	PNPLA3-I148M	1.00E+12	5	-77.08
D-2264	PNPLA3-I148M	1.00E+12	5	-40.86
D-2269	PNPLA3-I148M	1.00E+12	5	-37.55
D-2270	PNPLA3-I148M	1.00E+12	5	-77.42
D-2271	PNPLA3-I148M	1.00E+12	5	-26.87
D-2272	PNPLA3-I148M	1.00E+12	5	-56.05
D-2280	PNPLA3-I148M DM	1.00E+12	3	-86.30
D-2287	PNPLA3-I148M DM	1.00E+12	3	-94.73
D-2289	PNPLA3-I148M DM	1.00E+12	3	-93.48
D-2292	PNPLA3-I148M DM	1.00E+12	3	-82.48
D-2297	PNPLA3-I148M DM	1.00E+12	3	-72.61
D-2322	PNPLA3-I148M DM	1.00E+12	3	-32.92
D-2324	PNPLA3-I148M DM	1.00E+12	3	-87.56
D-2327	PNPLA3-I148M DM	1.00E+12	3	-86.70
D-2345	PNPLA3-WT	1.00E+12	5	-83.48
D-2346	PNPLA3-WT	1.00E+12	5	-75.93
D-2347	PNPLA3-WT	1.00E+12	5	-22.83
D-2348	PNPLA3-WT	1.00E+12	5	-20.09
D-2349	PNPLA3-WT	1.00E+12	5	-67.70
D-2350	PNPLA3-WT	1.00E+12	5	-57.51
D-2351	PNPLA3-WT	1.00E+12	5	-56.11
D-2352	PNPLA3-I148M DM	1.00E+12	3	-92.21
D-2353	PNPLA3-I148M DM	1.00E+12	3	-89.55
D-2354	PNPLA3-I148M DM	1.00E+12	3	-90.21
D-2358	PNPLA3-I148M DM	1.00E+12	3	-95.10
D-2359	PNPLA3-I148M DM	1.00E+12	3	-91.17
D-2360	PNPLA3-I148M DM	1.00E+12	3	-63.98
D-2361	PNPLA3-I148M DM	1.00E+12	3	-92.47
D-2362	PNPLA3-I148M DM	1.00E+12	3	-95.10
D-2364	PNPLA3-I148M DM	1.00E+12	3	-95.31
D-2370	PNPLA3-I148M DM	1.00E+12	3	-95.99
D-2370	PNPLA3 WT	1.00E+12	3	11.88
D-2371	PNPLA3-I148M DM	1.00E+12	3	-91.14
D-2372	PNPLA3-I148M DM	1.00E+12	3	-93.71
D-2373	PNPLA3-I148M DM	1.00E+12	3	-73.80
D-2374	PNPLA3-I148M DM	1.00E+12	3	-75.98
D-2375	PNPLA3-I148M DM	1.00E+12	3	-96.68
D-2376	PNPLA3-I148M DM	1.00E+12	3	-96.78
D-2377	PNPLA3-I148M DM	1.00E+12	3	-96.88

D-2378	PNPLA3-I148M DM	1.00E+12	3	-97.60
D-2379	PNPLA3-I148M DM	1.00E+12	3	-94.73
D-2380	PNPLA3-I148M DM	1.00E+12	3	-94.73
D-2381	PNPLA3-I148M DM	1.00E+12	3	-76.24
D-2382	PNPLA3-I148M DM	1.00E+12	3	-80.33
D-2383	PNPLA3-I148M DM	1.00E+12	3	-71.98
D-2384	PNPLA3-I148M DM	1.00E+12	3	-87.24
D-2385	PNPLA3-I148M DM	1.00E+12	3	-78.77
D-2386	PNPLA3-I148M DM	1.00E+12	3	-70.58
D-2387	PNPLA3-I148M DM	1.00E+12	3	-67.09
D-2390	PNPLA3-I148M DM	1.00E+12	3	-92.97
D-2391	PNPLA3-I148M DM	1.00E+12	3	-94.10
D-2392	PNPLA3-I148M DM	1.00E+12	3	-92.14
D-2395	PNPLA3-I148M DM	1.00E+12	3	-85.63
D-2396	PNPLA3-I148M DM	1.00E+12	3	-94.63
D-2397	PNPLA3-I148M DM	1.00E+12	3	-92.90
D-2398	PNPLA3-I148M DM	1.00E+12	3	-95.48
D-2399	PNPLA3-I148M DM	1.00E+12	3	-93.40
D-2400	PNPLA3-I148M DM	1.00E+12	3	-97.55
D-2401	PNPLA3-I148M DM	1.00E+12	3	-96.98
D-2402	PNPLA3-I148M DM	1.00E+12	3	-97.25
D-2403	PNPLA3 WT	1.00E+12	3	36.90
D-2404	PNPLA3-I148M DM	1.00E+12	3	-95.08
D-2405	PNPLA3-I148M DM	1.00E+12	3	-96.93
D-2406	PNPLA3 WT	1.00E+12	3	28.80
D-2395	PNPLA3-I148M DM	1.00E+12	3	-17.25
D-2396	PNPLA3-I148M DM	1.00E+12	3	-93.70
D-2413	PNPLA3-I148M DM	1.00E+12	3	-94.80
D-2415	PNPLA3-I148M DM	1.00E+12	3	-90.95
D-2418	PNPLA3-I148M DM	1.00E+12	3	-88.45
D-2419	PNPLA3 WT	1.00E+12	3	43.72
D-2453	PNPLA3 WT	1.00E+12	3	-81.33
D-2454	PNPLA3 WT	1.00E+12	3	-83.38
D-2455	PNPLA3 WT	1.00E+12	3	-68.85
D-2456	PNPLA3 WT	1.00E+12	3	-89.03
D-2460	PNPLA3 WT	1.00E+12	3	-68.65
D-2461	PNPLA3 WT	1.00E+12	3	-47.85

Table 10. Day 22 PNPLA3 knockdown assay

Duplex number	AAV vector	AAV particles/ animal	Dose administered (mg/kg)	PNPLA3 knockdown (%)
D-2092	PNPLA3-I148M	1.00E+12	3	-74.61
D-2092	PNPLA3-I148M	1.00E+12	5	-85.78
D-2095	PNPLA3-I148M	1.00E+12	3	-27.78
D-2095	PNPLA3-I148M	1.00E+12	5	-33.60
D-2324	PNPLA3-I148M DM	1.00E+12	3	-56.68
D-2324	PNPLA3-I148M DM	1.00E+12	5	-88.02
D-2089	PNPLA3-I148M	1.00E+12	5	-80.86
D-2104	PNPLA3-I148M	1.00E+12	5	-65.61
D-2105	PNPLA3-I148M	1.00E+12	5	-39.67
D-2280	PNPLA3-I148M DM	1.00E+12	5	-84.12
D-2297	PNPLA3-I148M DM	1.00E+12	3	-58.01
D-2297	PNPLA3-I148M DM	1.00E+12	5	-76.43
D-2352	PNPLA3-I148M DM	1.00E+12	5	-92.74
D-2353	PNPLA3-I148M DM	1.00E+12	5	-84.54
D-2354	PNPLA3-I148M DM	1.00E+12	5	-89.06
D-2355	PNPLA3-I148M DM	1.00E+12	5	-95.53
D-2356	PNPLA3-I148M DM	1.00E+12	5	-94.99
D-2357	PNPLA3-I148M DM	1.00E+12	5	-97.37

Table 11. Day 28 PNPLA3 knockdown assay

Duplex number	AAV vector	AAV particles/ animal	Dose administered (mg/kg)	PNPLA3 knockdown (%)
D-2092	PNPLA3-I148M	1.00E+12	3	-72.57
D-2092	PNPLA3-I148M	1.00E+12	5	-82.74
D-2095	PNPLA3-I148M	1.00E+12	3	-32.93
D-2095	PNPLA3-I148M	1.00E+12	5	-55.31
D-2089	PNPLA3-I148M	1.00E+12	5	-63.49
D-2104	PNPLA3-I148M	1.00E+12	5	-44.39
D-2105	PNPLA3-I148M	1.00E+12	5	-39.80
D-2370	PNPLA3-I148M DM	1.00E+12	3	-89.28
D-2400	PNPLA3-I148M DM	1.00E+12	3	-88.23

D-2401	PNPLA3-I148M DM	1.00E+12	3	-91.95
D-2402	PNPLA3-I148M DM	1.00E+12	0.5	-50.65
D-2402	PNPLA3-I148M DM	1.00E+12	1	-69.37
D-2402	PNPLA3-I148M DM	1.00E+12	3	-96.43
D-2402	PNPLA3-I148M DM	1.00E+12	3	-85.44
D-2402	PNPLA3-I148M DM	1.00E+12	3	-90.52
D-2404	PNPLA3-I148M DM	1.00E+12	3	-95.67
D-2419	PNPLA3-I148M DM	1.00E+12	0.5	-68.83
D-2419	PNPLA3-I148M DM	1.00E+12	1	-76.88
D-2419	PNPLA3-I148M DM	1.00E+12	3	-97.53
D-2419	PNPLA3-I148M DM	1.00E+12	3	-93.95
D-2419	PNPLA3-I148M DM	1.00E+12	3	-89.66
D-2419	PNPLA3-I148M DM	1.00E+12	3	-93.25
D-2420	PNPLA3-I148M DM	1.00E+12	3	-95.93
D-2421	PNPLA3-I148M DM	1.00E+12	0.5	-50.01
D-2421	PNPLA3-I148M DM	1.00E+12	1	-66.30
D-2421	PNPLA3-I148M DM	1.00E+12	3	-94.99
D-2421	PNPLA3-I148M DM	1.00E+12	3	-80.05
D-2421	PNPLA3 WT	1.00E+12	3	-36.65
D-2421	PNPLA3-I148M DM	1.00E+12	3	-91.08
D-2425	PNPLA3-I148M DM	1.00E+12	3	-16.07
D-2426	PNPLA3-I148M DM	1.00E+12	3	-37.54
D-2427	PNPLA3-I148M DM	1.00E+12	3	-25.19

D-2428	PNPLA3-I148M DM	1.00E+12	3	-16.71
D-2437	PNPLA3-I148M DM	1.00E+12	3	-93.78
D-2438	PNPLA3-I148M DM	1.00E+12	3	-90.63
D-2439	PNPLA3-I148M DM	1.00E+12	3	-88.10
D-2440	PNPLA3-I148M DM	1.00E+12	3	-95.25
D-2441	PNPLA3-I148M DM	1.00E+12	3	-90.13
D-2442	PNPLA3-I148M DM	1.00E+12	3	-57.24
D-2443	PNPLA3-I148M DM	1.00E+12	3	-95.43
D-2444	PNPLA3-I148M DM	1.00E+12	3	-90.58
D-2445	PNPLA3-I148M DM	1.00E+12	3	-84.55
D-2446	PNPLA3-I148M DM	1.00E+12	3	-81.50
D-2427	PNPLA3-I148M DM	1.00E+12	3	-90.09
D-2462	PNPLA3-I148M DM	1.00E+12	3	-89.20
D-2463	PNPLA3-I148M DM	1.00E+12	3	-41.25
D-2464	PNPLA3-I148M DM	1.00E+12	3	-60.53
D-2465	PNPLA3-I148M DM	1.00E+12	3	-91.35
D-2466	PNPLA3-I148M DM	1.00E+12	3	-93.68
D-2467	PNPLA3-I148M DM	1.00E+12	3	-85.15
D-2472	PNPLA3-I148M DM	1.00E+12	0.5	-46.58
D-2472	PNPLA3-I148M DM	1.00E+12	1	-74.47
D-2472	PNPLA3-I148M DM	1.00E+12	3	-88.79
D-2472	PNPLA3 WT	1.00E+12	3	-23.16
D-2472	PNPLA3-I148M DM	1.00E+12	3	-90.54

D-2473	PNPLA3-I148M DM	1.00E+12	0.5	-57.95
D-2473	PNPLA3-I148M DM	1.00E+12	1	-71.96
D-2473	PNPLA3-I148M DM	1.00E+12	3	-91.70
D-2473	PNPLA3-I148M DM	1.00E+12	3	-85.94
D-2473	PNPLA3 WT	1.00E+12	3	-18.70

Table 12. Day 42 PNPLA3 knockdown assay

Duplex number	AAV vector	AAV particles/ animal	Dose administered (mg/kg)	PNPLA3 knockdown (%)
D-2402	PNPLA3-I148M DM	1.00E+12	1	-57.74
D-2402	PNPLA3-I148M DM	1.00E+12	3	-83.75
D-2419	PNPLA3-I148M DM	1.00E+12	1	-71.07
D-2419	PNPLA3-I148M DM	1.00E+12	3	-70.8
D-2421	PNPLA3-I148M DM	1.00E+12	1	-62.21
D-2421	PNPLA3-I148M DM	1.00E+12	3	-80.12
D-2472	PNPLA3-I148M DM	1.00E+12	1	-60.54
D-2472	PNPLA3-I148M DM	1.00E+12	3	-74.77
D-2473	PNPLA3-I148M DM	1.00E+12	1	-54.55
D-2473	PNPLA3-I148M DM	1.00E+12	3	-81.13

Example 5: Prevention and rescue of NAFLD by siRNA molecules in a humanized *PNPLA3*$^{rs738409\text{-}rs738408}$ mouse model

[0195] The 'American Lifestyle-Induced Obesity Syndrome', or ALIOS mouse model for NAFLD/NASH is developed by feeding mice with a diet high in trans-fats (45% of the total

amount of fat) and sugar (Tetri 2008). For these studies, eight to ten-week old C57BL/6NCrl male mice (Charles River Laboratories Inc.) were injected with AAV-empty vector or AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$, as described previously. At the time of AAV injection, mice were either maintained on a normal chow diet or placed on the ALIOS diet (Envigo, TD.06303) complete with drinking water composed of 55% fructose and 45% glucose (Sigma, F0127 and G7021, respectively) until harvest. In previous experiments, it was established that over-expression of PNPLA3$^{rs738409\text{-}rs738408}$, in this context, both accelerates and worsens NAFLD phenotypes (data not shown).

[0196] Two weeks after AAV injection and diet initiation, mice were treated with a single dose of siRNA D-2324 (0.5mM), via subcutaneous injection, at 5.0 milligrams per kilogram of animal, diluted in phosphate buffered saline (Thermo Fisher Scientific,14190-136) or a vehicle control. Dosing was repeated biweekly until harvest. At the time of harvest, body weights were collected, followed by serum via cardiac puncture under isoflurane anesthesia, followed by liver weights. The median lobe was fixed via 10% neutral buffered formalin, followed by paraffin processing and embedding. The remainder of the liver was snap frozen for content and gene expression analysis, as described previously.

[0197] Snap frozen liver tissue was processed for RNA and gene expression analysis, as described previously. Results are shown as both the raw Ct value and relative mRNA expression of the indicated gene normalized to mouse *Gapdh*. (TaqMan™ assays from Invitrogen: human *PNPLA3*, hs00228747_m1; mouse *Pnpla3*, Mm00504420_m1; mouse *Gapdh*, 4352932E).

[0198] Formalin-fixed tissues were processed for Hemotoxylin and Eosin staining (Dako, CS70030-2, CS70130-2, respectively) according to the manufacturer's instructions. Scoring for steatosis and inflammation was performed by a board-certified pathologist.

[0199] Serum analysis included TIMP1, a biomarker associated with NASH and NASH-related fibrosis (Youssani 2011). The TIMP1 ELISA (R&D Systems, MTM100) was performed according to the manufacturer's instructions.

[0200] **Figure 2A-G.** To evaluate the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule D-2324 to prevent the development of phenotypes associated with NAFLD and overexpression of *PNPLA3*$^{rs738409\text{-}rs738408}$, mice received AAV8-empty vector (EV), or AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$, or vehicle, and were maintained on a regular chow diet or transitioned to

the ALIOS diet. Two weeks after AAV injections, mice were treated with siRNA or vehicle, every other week for six weeks; a total of three injection rounds. Mice were harvested at the eight-week time point. Results are presented as the group average and standard error, N=8 per group. Asterisks represent statistical significance to the AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ cohort, generated by One-way ANOVA employing Dunnett's multiple comparisons test. (A) The ratio of liver weight (grams) to body weight (grams) at the time of harvest. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, **=0.0018, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (B) Confirmation of human *PNPLA3* mRNA expression and silencing in the liver by qPCR. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the ALIOS-fed *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (C) Analysis of mouse *Pnpla3* mRNA expression in liver by qPCR indicates endogenous Pnpla3 is not significantly altered by AAV-mediated over-expression or siRNA silencing. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the chow-fed no-AAV group to the ALIOS-fed *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (D) Hepatic triglyceride content presented as milligrams of triglyceride per gram of liver tissue. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, *=0.0393, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.0063. (E) Serum TIMP1 presented as picograms per milliliter of serum. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (F) Histological indication of steatosis based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, no significance, AAV-*PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.0012. (G) Histological indication of inflammation based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001.

[0201] **Figure 3A-G.** To evaluate the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule to prevent further progression of PNPLA3$^{rs738409\text{-}rs738408}$-mediated disease after disease onset, mice received AAV8-empty vector (EV), or AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$, or vehicle, and were maintained on a regular chow diet or transitioned to the ALIOS diet. Eight weeks after AAV

injections and diet changes, mice were treated with siRNA or vehicle, every other week for eight more weeks; a total of four injection rounds. Mice were harvested at the sixteen-week time point. Although no change was observed in steatosis, if siRNA treatment began after disease induction, several other disease-associated end points were significantly reduced. Results are presented as averages and standard error, N=8 per group. Asterisks represent statistical significance to the AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ cohort, generated by One-way ANOVA employing Dunnett's multiple comparisons test. (A) The ratio of liver weight (grams) to body weight (grams) at the time of harvest. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ***=0.0006. (B) Confirmation of human *PNPLA3* mRNA expression and silencing in the liver by qPCR. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the ALIOS-fed *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (C) Analysis of mouse *Pnpla3* mRNA expression in liver by qPCR indicates endogenous Pnpla3 is not significantly altered by AAV-mediated over-expression or siRNA silencing. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the chow-fed no-AAV group to the ALIOS-fed *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (D) Hepatic triglyceride content presented as milligrams of triglyceride per gram of liver tissue. Adjusted P values: AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, *=0.0403. (E) Serum TIMP1 presented as picograms per milliliter of serum. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, **=0.0027, AAV-*PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.002. (F) Histological indication of steatosis based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, not significant. (G) Histological indication of inflammation based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.0068.

[0202] **Figure 4A-D.** To evaluate the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule to rescue disease-associated phenotypes due to overexpression of PNPLA3$^{rs738409\text{-}rs738408}$, liver and serum from ALIOS-fed eight-week AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ -vehicle treated mice

were compared to livers and serum from ALIOS-fed sixteen-week vehicle- or siRNA-treated AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ mice. Although no change was observed in steatosis at this time point with siRNA treatment, hepatic triglyceride, serum TIMP1, and inflammation were all statistically lower at sixteen weeks compared to vehicle controls at eight weeks. Results are presented as averages and standard error, N=8 per group. Asterisks represent statistical significance to the eight-week AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ vehicle-treated cohort, generated by One-way ANOVA employing Dunnett's multiple comparisons test. (A) Hepatic triglyceride content presented as milligrams of triglyceride per gram of liver tissue. Adjusted P values: 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle, not significant; 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.0011. (B) Serum Timp1 presented as picograms per milliliter of serum. Adjusted P values: 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle, not significant; 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, *=0.0134. (C) Histological indication of steatosis based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle, not significant; 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, not significant. (D) Histological indication of inflammation based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle, not significant; 16WK AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, *=0.0112.

Example 6: Prevention of liver fibrosis by siRNA molecules in a humanized *PNPLA3*$^{rs738409\text{-}rs738408}$ mouse model

[0203] The "AMLN" diet, developed by Amylin Pharmaceuticals (Clapper 2013), is a modified version of the ALIOS diet. The feed includes a ten-fold increase in cholesterol (2%) and additional sucrose. Mice placed on the "AMLN" diet develop mild to moderate fibrosis after 20-30 weeks (Clapper, Mells and Kristiansen papers). For this study, eight to ten-week old C57BL/6NCrl male mice (Charles River Laboratories Inc.) were injected with AAV-empty vector or AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$, as described above, to accelerate disease onset. At the time of AAV injection, mice were continued on a normal chow diet or placed on the Envigo diet, TD.170748, complete with drinking water composed of 55% fructose and 45% glucose (Sigma, F0127 and G7021, respectively) until harvest.

[0204] Two weeks post-AAV injection and diet initiation, mice were treated with a single dose of siRNA D-2324 (0.5mM), via subcutaneous injection, at 5.0 milligrams per kilogram of animal, diluted in phosphate buffered saline (Thermo Fisher Scientific,14190-136) or a vehicle control. Dosing was repeated biweekly until harvest. At the time of harvest, body weights were collected, followed by serum via cardiac puncture under isoflurane anesthesia, followed by liver weights. The median lobe was fixed via 10% neutral buffered formalin, followed by paraffin processing and embedding. The remainder of the liver was snap frozen for gene expression analysis.

[0205] Snap frozen liver tissue was processed for RNA and gene expression analysis, as described previously. Results are shown as both the raw Ct value and relative mRNA expression of the indicated gene normalized to mouse *Gapdh*. (TaqMan™ assays from Invitrogen: human *PNPLA3*, hs00228747_m1; mouse *Pnpla3*, Mm00504420_m1; mouse *Col1a1*, Mm00801666_g1; mouse *Col3a1*, Mm01254471_g1; *Col4a1*, Mm01210125_m1; mouse *Gapdh*, 4352932E). Col1a1, Col3a1 and Col4a1 are extracellular matrix markers associated with hepatic stellate cell activation and liver fibrosis (Baiocchini 2016).

[0206] Formalin-fixed tissues were processed for Hemotoxylin, Eosin, and Masson's Trichrome staining (Dako, CS70030-2, CS70130-2, AR17311-2, respectively) according to the manufacturer's instructions. Anti-Smooth Muscle Actin staining was performed without antigen retrieval and using a DAKO autostainer. Slides were processed using Peroxidazed 1 and Sniper (Biocare, PX968 and BS966, respectively) and stained with monoclonal anti-Actin, alpha-Smooth Muscle antibody (Sigma, F3777), followed by Rabbit anti-FITC (Invitrogen, 711900), Envision-Rabbit HRP polymer (Dako, K4003), DAB+ (Dako, K3468), and Hemotoxylin. Scoring for the amount of steatosis, inflammation, oval cell/ bile duct hyperplasia, and aSMA-positive cells was performed by a board-certified pathologist.

[0207] Serum was analyzed for mouse TIMP1 (R&D Systems, MTM100) and Mouse Cytokeratin 18-M30 (Cusabio, CSB-E14265m) according to manufacturer's instructions. In addition to TIMP1, Cytokine 18-M30 has been identified as a potential biomarker for NAFLD/NASH, including early fibrosis (Neuman 2014 and Yang 2015). **Figure 5A-L.** To evaluate the ability of a PNPLA3$^{rs738409\text{-}rs738408}$ – specific siRNA molecule to prevent the development of early fibrosis, mice received AAV8-empty vector (EV), or AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$, or vehicle, and were maintained on a regular chow diet or transitioned to the AMLN diet.

Two weeks after AAV injections, mice were treated with siRNA, D-2324, or vehicle, every other week for ten more weeks; a total of six injection rounds. Mice were harvested at the ten-week time point. Results are presented as averages and standard error, Chow-fed no AAV + vehicle and AMLN-fed AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle, N=8 per group; AMLN-fed AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, N=12 per group. Asterisks represent statistical significance to the AAV8-*PNPLA3*$^{rs738409\text{-}rs738408}$ – vehicle-treated cohort, generated by One-way ANOVA employing Dunnett's multiple comparisons test. (A) The ratio of liver weight (grams) to body weight (grams) at the time of harvest. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (B) Confirmation of human *PNPLA3* mRNA expression and silencing in the liver by qPCR. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (C) Analysis of mouse *Pnpla3* mRNA expression in liver by qPCR indicates endogenous Pnpla3 is not significantly altered by AAV-mediated over-expression or siRNA silencing. (left) Raw Ct value and (right) relative fold mRNA expression normalized to mouse *Gapdh*; comparing the chow-fed no-AAV group to the AMLN-fed *PNPLA3*$^{rs738409\text{-}rs738408}$ + vehicle and *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA groups. (D) Serum Timp1 presented as picograms per milliliter of serum. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (E) Serum CK18m30 presented as picograms per milliliter of serum. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (F) Histological indication of inflammation based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, *=0.0108, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (G) Histological indication of oval cell/bile duct hyperplasia based on H&E staining, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **=0.0081. (H) Immunohistochemical staining for anti-Smooth Muscle Actin, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle,

*=0.0101, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ***=0.0002. (I) Masson's Trichrome staining for fibrosis, scored as: Within normal limits (0), minimal (1), mild (2), moderate (3), and severe (4). Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, not significant, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, not significant. (J) Mouse *Col1a1* mRNA expression in liver by qPCR. Relative fold mRNA expression normalized to mouse *Gapdh*. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (K) Mouse *Col3a1* mRNA expression in liver by qPCR. Relative fold mRNA expression normalized to mouse *Gapdh*. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ****<0.0001, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, ****<0.0001. (L) Mouse *Col4a1* mRNA expression in liver by qPCR. Relative fold mRNA expression normalized to mouse *Gapdh*. Adjusted P values: no AAV + vehicle group, ****<0.0001, AAV-EV + vehicle, ***<0.0005, AAV- *PNPLA3*$^{rs738409\text{-}rs738408}$ + siRNA, **<0.0041.

What is Claimed

1. An RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 1 or 2, and wherein the RNAi construct inhibits the expression of Patatin-Like Phospholipase Domain Containing 3 (PNPLA3).
2. The RNAi construct of claim 1, wherein the antisense strand comprises a region that is complementary to a PNPLA3 mRNA sequence.
3. The RNAi construct any of the above claims, wherein the sense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 1 or 2.
4. The RNAi construct any of the above claims, wherein the construct preferentially inhibits a PNPLA3-rs738409 minor allele.
5. The RNAi construct of claim 4, wherein the construct has at least 10% greater inhibition of a PNPLA3-rs738409 minor allele as compared to the major allele.
6. The RNAi construct of any of the above claims, wherein the construct preferentially inhibits a PNPLA3-rs738408 minor allele.
7. The RNAi construct of claim 6, wherein the construct has at least 10% greater inhibition of a PNPLA3-rs738409-rs738408 double minor allele as compared to the major allele.
8. The RNAi construct any of the above claims, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length.
9. The RNAi construct of claim 8, wherein the duplex region is about 17 to about 24 base pairs in length.
10. The RNAi construct of claim 8, wherein the duplex region is about 19 to about 21 base pairs in length.
11. The RNAi construct of claim 10, wherein the duplex region is 19 base pairs in length.

12. The RNAi construct of any one of claims 8 to 11, wherein the sense strand and the antisense strand are each about 15 to about 30 nucleotides in length.

13. The RNAi construct of claim 12, wherein the sense strand and the antisense strand are each about 19 to about 27 nucleotides in length.

14. The RNAi construct of claim 12, wherein the sense strand and the antisense strand are each about 21 to about 25 nucleotides in length.

15. The RNAi construct of claim 12, wherein the sense strand and the antisense strand are each about 21 to about 23 nucleotides in length.

16. The RNAi construct of any one of claims 1 to 15, wherein the RNAi construct comprises at least one blunt end.

17. The RNAi construct of any one of claims 1 to 15, wherein the RNAi construct comprises at least one nucleotide overhang of 1 to 4 unpaired nucleotides.

18. The RNAi construct of claim 17, wherein the nucleotide overhang has 2 unpaired nucleotides.

19. The RNAi construct of claim 17 or 18, wherein the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense strand and the antisense strand.

20. The RNAi construct of any one of claims 17 to 19**,** wherein the nucleotide overhang comprises a 5'-UU-3' dinucleotide or a 5'-dTdT-3' dinucleotide.

21. The RNAi construct of any one of claims 1 to 20, wherein the RNAi construct comprises at least one modified nucleotide.

22. The RNAi construct of claim 21, wherein the modified nucleotide is a 2'-modified nucleotide.

23. The RNAi construct of claim 21, wherein the modified nucleotide is a 2'-fluoro modified nucleotide, a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-O-allyl modified nucleotide, a bicyclic nucleic acid (BNA), a glycol nucleic acid, an inverted base or combinations thereof.

24. The RNAi construct of claim 23, wherein the modified nucleotide is a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-fluoro modified nucleotide, or combinations thereof.

25. The RNAi construct of claim 21, wherein all of the nucleotides in the sense and antisense strands are modified nucleotides.

26. The RNAi construct of claim 25, wherein the modified nucleotides are 2'-O-methylmodified nucleotides, 2'-fluoro modified nucleotides, or combinations thereof.

27. The RNAi construct of any one of claims 1 to 26, wherein the RNAi construct comprises at least one phosphorothioate internucleotide linkage.

28. The RNAi construct of claim 27, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand.

29. The RNAi construct of claim 27, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at the 5' end of the sense strand.

30. The RNAi construct of any one of claims 1 to 29, wherein the antisense strand comprises a sequence selected from the antisense sequences listed in Table 1 or Table 2.

31. The RNAi construct of claim 30, wherein the sense strand comprises a sequence selected from the sense sequences listed in Table 1 or Table 2.

32. The RNAi construct of any one of claims 1 to 31, wherein the RNAi construct is any one of the duplex compounds listed in any one of Tables 1 to 2.

33. The RNAi construct of any one of claims 1 to 32, wherein the RNAi construct reduces the expression level of PNPLA3 in liver cells following incubation with the RNAi construct as compared to the PNPLA3 expression level in liver cells that have been incubated with a control RNAi construct.

34. The RNAi construct of claim 27, wherein the liver cells are Hep3B or HepG2 cells.

35. The RNAi construct of any one of claims 1 to 26, wherein the RNAi construct inhibits at least 10% of PNPLA3 expression at 5 nM in Hep3B cells *in vitro*.

36. The RNAi construct of any one of claims 1 to 26, wherein the RNAi construct inhibits at least 10% of PNPLA3 expression at 5 nM in HepG2 cells *in vitro*.

37. The RNAi construct of any one of claims 1 to 26, wherein the RNAi construct inhibits PNPLA3 expression in Hep3B cells with an IC50 of less than about 1 nM.

38. The RNAi construct of any one of claims 1 to 26, wherein the RNAi construct inhibits PNPLA3 expression in HepG2 cells with an IC50 of less than about 1 nM.

39. A pharmaceutical composition comprising the RNAi construct of any one of claims 1 to 38 and a pharmaceutically acceptable carrier, excipient, or diluent.

40. A method for reducing the expression of PNPLA3 in a patient in need thereof comprising administering to the patient the RNAi construct of any one of claims 1 to 38.

41. The method of claim 40, wherein the expression level of PNPLA3 in hepatocytes is reduced in the patient following administration of the RNAi construct as compared to the PNPLA3 expression level in a patient not receiving the RNAi construct.