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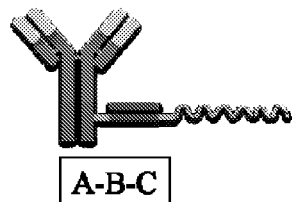
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(54) Title: COMPOSITIONS AND METHODS OF TREATING MUSCLE ATROPHY AND MYOTONIC DYSTROPHY

FIG. 19A



(57) Abstract: This application disclose a polynucleic acid molecule conjugate comprising an antibody or binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of an atrogene; wherein the polynucleic acid molecule comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein the polynucleic acid molecule conjugate mediates RNA interference against the atrogene, thereby treating muscle atrophy or myotonic dystrophy in a subject. In a certain embodiment, atrogene comprises atrogin-1 gene (FBXO32), MuRF1 gene (TRIA63), FOXO1, FOXO3, or MSTN.

COMPOSITIONS AND METHODS OF TREATING MUSCLE ATROPHY AND MYOTONIC DYSTROPHY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/595,545, filed December 6, 2017, and U.S. Provisional Application No. 62/725,883, filed August 31, 2018, which each of the applications is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] 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 6, 2018, is named 45532-722_601_SL.txt and is 3,143,382 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0003] Gene suppression by RNA-induced gene silencing provides several levels of control: transcription inactivation, small interfering RNA (siRNA)-induced mRNA degradation, and siRNA-induced transcriptional attenuation. In some instances, RNA interference (RNAi) provides long lasting effect over multiple cell divisions. As such, RNAi represents a viable method useful for drug target validation, gene function analysis, pathway analysis, and disease therapeutics.

SUMMARY OF THE DISCLOSURE

[0004] Disclosed herein, in certain embodiments, are polynucleic acid molecules and pharmaceutical compositions for modulating a gene associated with muscle atrophy (or an atrogene). In some embodiments, also described herein are methods of treating muscle atrophy with a polynucleic acid molecule or a polynucleic acid molecule conjugate disclosed herein.

[0005] Disclosed herein, in certain embodiments, is a molecule of Formula (I): A-X₁-B-X₂-C (Formula I) wherein, A is a binding moiety; B is a polynucleotide that hybridizes to a target sequence of an atrogene; C is a polymer; and X₁ and X₂ are each independently selected from a bond or a non-polymeric linker; wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein A and C are not attached to B at the same terminus. In some embodiments, the atrogene comprises a differentially regulated (e.g., an upregulated or downregulated) gene within the IGF1-Akt-FoxO pathway, the glucocorticoids-GR pathway, the PGC1 α -FoxO pathway, the TNF α -NF κ B pathway, or the myostatin-ActRIIb-Smad2/3 pathway. In some embodiments, the atrogene encodes an E3 ligase. In some embodiments, the atrogene encodes a Forkhead box transcription factor. In some embodiments, the atrogene comprises atrogin-1 gene (*FBXO32*), MuRF1 gene (*TRIM63*), *FOXO1*, *FOXO3*, or *MSTN*. In some embodiments, the atrogene comprises *DMPK*. In some embodiments, B consists of a polynucleotide that hybridizes to a target sequence of an atrogene. In some embodiments, C consists of a

polymer. In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the at least one inverted abasic moiety is at at least one terminus. In some embodiments, the polynucleotide comprises a single strand which hybridizes to the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule, wherein either the first polynucleotide or the second polynucleotide also hybridizes to the target sequence of an atrogene. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the polynucleotide hybridizes to at least 8 contiguous bases of the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a sequence that is at least 60%, 70%, 80%, 85%, 90%, 95%, or 99% complementary to a sequence as set forth in SEQ ID NOs: 28-141, 370-480, and 703-3406. In some embodiments, the polynucleotide is between about 8 and about 50 nucleotides in length. In some embodiments, the polynucleotide is between about 10 and about 30 nucleotides in length. In some embodiments, the first polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, the second polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, X_1 and X_2 are independently a C_1 - C_6 alkyl group. In some embodiments, X_1 and X_2 are independently a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C_1 - C_6 alkyl group. In some embodiments, A is an antibody or binding fragment thereof. In some embodiments, A comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, A is an anti-transferrin receptor antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, A- X_1 is conjugated to the 5' end of B and X_2 -C is conjugated to the 3' end of B. In some embodiments, X_2 -C is conjugated to the 5' end of B and A- X_1 is conjugated to the 3' end of B. In some embodiments, A is directly conjugated to X_1 . In some embodiments, C is directly conjugated to X_2 . In some embodiments, B is directly conjugated to X_1 and X_2 . In some embodiments, the molecule further comprises D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is an endosomolytic polymer.

[0006] Disclosed herein, in certain embodiments, is a polynucleic acid molecule conjugate comprising a binding moiety conjugated to a polynucleotide that hybridizes to a target sequence of an atrogene; wherein the polynucleotide optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein the polynucleic acid molecule conjugate mediates RNA interference against the atrogene, thereby treating muscle atrophy in a subject. In some embodiments, the atrogene comprises a differentially regulated (e.g., an upregulated or downregulated) gene within the IGF1-Akt-FoxO pathway, the glucocorticoids-GR pathway, the PGC1 α -FoxO pathway, the TNF α -NF κ B pathway, or the myostatin-ActRIIb-Smad2/3 pathway. In some embodiments, the atrogene encodes an E3 ligase. In some embodiments, the atrogene encodes a Forkhead box transcription factor. In some embodiments, the atrogene comprises ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. In some embodiments, the atrogene comprises *DMPK*. In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, the binding moiety comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, the binding moiety is an anti-transferrin receptor antibody or binding fragment thereof. In some embodiments, the binding moiety is cholesterol. In some embodiments, the polynucleotide comprises a single strand which hybridizes to the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule, wherein either the first polynucleotide or the second polynucleotide also hybridizes to the target sequence of an atrogene. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the polynucleotide hybridizes to at least 8 contiguous bases of the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a sequence that is at least 60%, 70%, 80%, 85%, 90%, 95%, or 99% complementary to a sequence as set forth in SEQ ID NOs: 28-141, 370-480, and 703-3406. In some embodiments, the polynucleotide is between about 8 and about 50 nucleotides in length. In some embodiments, the polynucleotide is between about 10 and about 30 nucleotides in length. In some embodiments, the first polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, the second polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, the polynucleic acid molecule conjugate optionally comprises a linker connecting the binding moiety to the polynucleotide. In some embodiments, the polynucleic acid molecule conjugate further comprises a polymer, optionally indirectly conjugated to the polynucleotide by an additional linker. In some embodiments, the linker and the additional linker are each independently a bond or a non-polymeric

linker. In some embodiments, the polynucleic acid molecule conjugate comprises a molecule of Formula (I): A-X₁-B-X₂-C (Formula I) wherein, A is a binding moiety; B is a polynucleotide that hybridizes to a target sequence of an atrogene; C is a polymer; and X₁ and X₂ are each independently selected from a bond or a non-polymeric linker; wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein A and C are not attached to B at the same terminus. In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the at least one inverted abasic moiety is at at least one terminus. In some embodiments, the muscle atrophy is a diabetes-associated muscle atrophy. In some embodiments, the muscle atrophy is a cancer cachexia-associated muscle atrophy. In some embodiments, the muscle atrophy is associated with insulin deficiency. In some embodiments, the muscle atrophy is associated with chronic renal failure. In some embodiments, the muscle atrophy is associated with congestive heart failure. In some embodiments, the muscle atrophy is associated with chronic respiratory disease. In some embodiments, the muscle atrophy is associated with a chronic infection. In some embodiments, the muscle atrophy is associated with fasting. In some embodiments, the muscle atrophy is associated with denervation. In some embodiments, the muscle atrophy is associated with sarcopenia, glucocorticoid treatment, stroke, and/or heart attack. In some cases, myotonic dystrophy type 1 (DM1) is associated with an expansion of CTG repeats in the 3' UTR of the *DMPK* gene.

[0007] Disclosed herein, in certain embodiments, is a pharmaceutical composition comprising: a molecule described above or a polynucleic acid molecule conjugate described above; and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated as a nanoparticle formulation. In some embodiments, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0008] Disclosed herein, in certain embodiments, is a method of treating muscle atrophy or myotonic dystrophy in a subject in need thereof, comprising: administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate comprising a binding moiety conjugated to a polynucleotide that hybridizes to a target sequence of an atrogene; wherein the polynucleotide optionally comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein the polynucleic acid molecule conjugate mediates RNA interference against the atrogene, thereby treating muscle atrophy or myotonic dystrophy in the subject. In some embodiments, the muscle atrophy is a diabetes-associated muscle atrophy. In some embodiments, the muscle atrophy is a cancer cachexia-associated muscle atrophy. In some embodiments, the muscle atrophy is associated with insulin deficiency. In some embodiments, the muscle atrophy is

associated with chronic renal failure. In some embodiments, the muscle atrophy is associated with congestive heart failure. In some embodiments, the muscle atrophy is associated with chronic respiratory disease. In some embodiments, the muscle atrophy is associated with a chronic infection. In some embodiments, the muscle atrophy is associated with fasting. In some embodiments, the muscle atrophy is associated with denervation. In some embodiments, the muscle atrophy is associated with sarcopenia. In some embodiments, the myotonic dystrophy is DM1. In some embodiments, the atrogene comprises a differently regulated (e.g., an upregulated or downregulated) gene within the IGF1-Akt-FoxO pathway, the glucocorticoids-GR pathway, the PGC1 α -FoxO pathway, the TNF α -NF κ B pathway, or the myostatin-ActRIIb-Smad2/3 pathway. In some embodiments, the atrogene encodes an E3 ligase. In some embodiments, the atrogene encodes a Forkhead box transcription factor. In some embodiments, the atrogene comprises atrogen-1 gene (*FBXO32*), MuRF1 gene (*TRIM63*), *FOXO1*, *FOXO3*, or *MSTN*. In some embodiments, the atrogene comprises *DMPK*. In some embodiments, the polynucleic acid molecule conjugate comprises a molecule of Formula (I): A-X₁-B-X₂-C (Formula I) wherein, A is a binding moiety; B is a polynucleotide that hybridizes to the target sequence of an atrogene; C is a polymer; and X₁ and X₂ are each independently selected from a bond or a non-polymeric linker; wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein A and C are not attached to B at the same terminus. In some embodiments, B consists of a polynucleotide that hybridizes to the target sequence of an atrogene. In some embodiments, C consists of a polymer. In some embodiments, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the at least one inverted abasic moiety is at at least one terminus. In some embodiments, the polynucleotide comprises a single strand which hybridizes to the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule, wherein either the first polynucleotide or the second polynucleotide also hybridizes to the target sequence of an atrogene. In some embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the polynucleotide hybridizes to at least 8 contiguous bases of the target sequence of an atrogene. In some embodiments, the polynucleotide comprises a sequence that is at least 60%, 70%, 80%, 85%, 90%, 95%, or 99% complementary to a sequence as set forth in SEQ ID NOs: 28-141, 370-480, and 703-3406. In some embodiments, the polynucleotide is between about 8 and about 50 nucleotides in length. In some embodiments, the polynucleotide is between about 10 and about 30 nucleotides in length. In some embodiments, the first

polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, the second polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some embodiments, X_1 and X_2 are independently a C_1 - C_6 alkyl group. In some embodiments, X_1 and X_2 are independently a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C_1 - C_6 alkyl group. In some embodiments, A is an antibody or binding fragment thereof. In some embodiments, A comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some embodiments, A is an anti-transferrin receptor antibody or binding fragment thereof. In some embodiments, C is polyethylene glycol. In some embodiments, A- X_1 is conjugated to the 5' end of B and X_2 -C is conjugated to the 3' end of B. In some embodiments, X_2 -C is conjugated to the 5' end of B and A- X_1 is conjugated to the 3' end of B. In some embodiments, A is directly conjugated to X_1 . In some embodiments, C is directly conjugated to X_2 . In some embodiments, B is directly conjugated to X_1 and X_2 . In some embodiments, the method further comprises D. In some embodiments, D is conjugated to C or to A. In some embodiments, D is an endosomolytic polymer. In some embodiments, the polynucleic acid molecule conjugate is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration. In some embodiments, the subject is a human.

[0009] Disclosed herein, in certain embodiments, is a kit comprising a molecule described above or a polynucleic acid molecule conjugate described above.

[0009A] In a further aspect, the present invention provides a polynucleic acid molecule conjugate comprising an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of human DMPK mRNA wherein the polynucleic acid molecule comprises a small interfering RNA (siRNA) with a guide strand comprising a nucleic acid sequence selected from a group consisting of SEQ ID NOs: 13463, 14048, 14047, 14049, 14146, 14072, 13478, 14147, 12234, and 14199.

[0009B] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0009C] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Various aspects of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings below. The patent application file contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0011] Fig. 1 illustrates an exemplary structure of cholesterol-myostatin siRNA conjugate.

[0012] Fig. 2 illustrates SAX HPLC chromatogram of TfR mAb-(Cys)-HPRT-PEG5k, DAR1.

[0013] Fig. 3 illustrates SEC HPLC chromatogram of TfR mAb-(Cys)-HPRT-PEG5k, DAR1.

[0014] Fig. 4 illustrates an overlay of DAR1 and DAR2 SAX HPLC chromatograms of TfR1mAb-Cys-BisMal-siRNA conjugates.

[0015] Fig. 5 illustrates an overlay of DAR1 and DAR2 SEC HPLC chromatograms of TfR1mAb-Cys-BisMal-siRNA conjugates.

[0016] Fig. 6 illustrates SEC chromatogram of CD71 Fab-Cys-HPRT-PEG5.

- [0017] Fig. 7 illustrates SAX chromatogram of CD71 Fab-Cys-HPRT-PEG5.
- [0018] Fig. 8 illustrates relative expression levels of Murf1 and atrogen-1 in C2C12 myoblasts and myotubes C2C12 myoblasts and myotubes were generated as described in Example 4. mRNA levels were determined as described in Example 4.
- [0019] Fig. 9A illustrates *in vivo* study design to assess the ability of exemplary conjugates for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle.
- [0020] Fig. 9B shows siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) muscle.
- [0021] Fig. 10A illustrates *in vivo* study design to assess the ability of exemplary conjugates for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle.
- [0022] Fig. 10B shows tissue concentration-time profiles out to 1008 h post-dose of an exemplary molecule of Formula (I).
- [0023] Fig. 10C shows siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) muscle.
- [0024] Fig. 10D shows plasma MSTN protein reduction after siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) muscle.
- [0025] Fig. 10E shows changes in muscle size after siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) muscle.
- [0026] Fig. 10F shows Welch's two-tailed unpaired t-test of Fig. 10E.
- [0027] Fig. 11A illustrates an exemplary *in vivo* study design.
- [0028] Fig. 11B shows tissue accumulation of siRNA in mouse gastrocnemius (gastroc) muscle after a single i.v. administration of an exemplary molecule of Formula (I) at the doses indicated.
- [0029] Fig. 11C shows siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) muscle.
- [0030] Fig. 12A illustrates an exemplary *in vivo* study design.
- [0031] Fig. 12B shows accumulation of siRNA in various muscle tissue.
- [0032] Fig. 12C shows siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc) and heart muscle.
- [0033] Fig. 12D shows RISC loading of the MSTN guide strand in mouse gastrocnemius (gastroc) muscle.
- [0034] Fig. 13A illustrates an exemplary *in vivo* study design.
- [0035] Fig. 13B shows siRNA-mediated mRNA knockdown of mouse MSTN in mouse gastrocnemius (gastroc), quadriceps, triceps, and heart.
- [0036] Fig. 13C illustrates plasma myostatin levels.
- [0037] Fig. 13D illustrates siRNA accumulation in different tissue types: gastrocnemius, triceps, quadriceps, and heart tissues.

- [0038] Fig. 13E shows RISC loading of the MSTN guide strand in mouse gastrocnemius (gastroc) muscle.
- [0039] Fig. 13F shows change in muscle area.
- [0040] Fig. 13G shows Welch's two-tailed unpaired t-test of Fig. 13F.
- [0041] Fig. 14A illustrates an exemplary *in vivo* study design.
- [0042] Fig. 14B shows HPRT mRNA expression of gastrocnemius muscle by exemplary conjugates described herein.
- [0043] Fig. 14C shows SSB mRNA expression of gastrocnemius muscle by exemplary conjugates described herein.
- [0044] Fig. 14D shows HPRT mRNA expression of heart tissue by exemplary conjugates described herein.
- [0045] Fig. 14E shows SSB mRNA expression of heart tissue by exemplary conjugates described herein.
- [0046] Fig. 14F shows accumulation of siRNA in gastrocnemius muscle.
- [0047] Fig. 15A illustrates an exemplary *in vivo* study design.
- [0048] Fig. 15B shows Atrogin-1 downregulation in gastrocnemius (gastroc) muscle.
- [0049] Fig. 15C shows Atrogin-1 downregulation in heart tissue.
- [0050] Fig. 16A illustrates an exemplary *in vivo* study design.
- [0051] Fig. 16B shows MuRF-1 downregulation in gastrocnemius muscle.
- [0052] Fig. 16C shows MuRF-1 downregulation in heart tissue.
- [0053] Fig. 17 illustrates siRNAs that were transfected into mouse C2C12 myoblasts *in vitro*. The four DMPK siRNAs assessed all showed DMPK mRNA knockdown, while the negative control siRNA did not. The dotted lines are three-parameter curves fit by non-linear regression.
- [0054] Fig. 18A-Fig. 18F show *in vivo* results demonstrating robust dose-responses for DMPK mRNA knockdown 7 days after a single i.v. administration of DMPK siRNA-antibody conjugates. Fig. 18A: gastrocnemius; Fig. 18B: Tibialis anterior; Fig. 18C: quadriceps; Fig. 18D: diaphragm; Fig. 18E: heart; and Fig. 18F: liver.
- [0055] Fig. 19A-Fig. 19L show exemplary antibody-nucleic acid conjugates described herein.
- [0056] Fig. 19M presents an antibody cartoon utilized in Fig. 19A-Fig. 19L.
- [0057] Fig. 20A-Fig. 20B illustrate an exemplary 21mer duplex utilized in Example 20. Fig. 20A shows a representative structure of siRNA passenger strand with C6-NH₂ conjugation handle at the 5' end and C6-S-NEM at 3' end. Fig. 20B shows a representative structure of a 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs.
- [0058] Fig. 21A-Fig. 21B illustrate a second exemplary 21 mer duplex utilized in Example 20. Fig. 21A shows a representative structure of siRNA passenger strand with a 5' conjugation handle. Fig. 21B shows a representative structure of a blunt ended duplex with 19 bases of complementarity and one 3' dinucleotide overhang.

- [0059] Fig. 22 shows an illustrative *in vivo* study design.
- [0060] Fig. 23 illustrates a time course of Atrogin-1 mRNA downregulation in gastroc muscle mediated by a TfR1 antibody siRNA conjugate after IV delivery at a dose of a single dose of 3 mg/kg.
- [0061] Fig. 24 illustrates a time course of Atrogin-1 mRNA downregulation in heart muscle mediated by a TfR1 antibody siRNA conjugate after IV delivery at a dose of a single dose of 3 mg/kg.
- [0062] Fig. 25 shows an illustrative *in vivo* study design.
- [0063] Fig. 26 shows MuRF1 mRNA downregulation at 96 hours in gastroc muscle mediated by a TfR1 antibody siRNA conjugate after IV delivery at the doses indicated.
- [0064] Fig. 27 shows MuRF1 mRNA downregulation at 96 hours in heart muscle mediated by a TfR1 antibody siRNA conjugate after IV delivery at the doses indicated.
- [0065] Fig. 28 shows a time course of MuRF1 and Atrogin-1 mRNA downregulation in gastroc muscle mediated by a TfR1 antibody siRNA conjugate (IV delivery at 3 mg/kg siRNA), in the absence and presence of dexamethasone induce muscle atrophy.
- [0066] Fig. 29 shows a time course of MuRF1 and Atrogin-1 mRNA downregulation in heart muscle mediated by a TfR1 antibody siRNA conjugate (IV delivery at 3 mg/kg siRNA), in the absence and presence of dexamethasone induce muscle atrophy.
- [0067] Fig. 30 shows a time course of gastroc weight changes mediated by a TfR1 antibody siRNA conjugate (IV delivery at 3 mg/kg siRNA), in the absence and presence of muscle atrophy.
- [0068] Fig. 31 shows a time course of siRNA tissue concentrations in gastroc and heart muscle mediated by a TfR1 antibody siRNA conjugate (IV delivery at 3 mg/kg siRNA), in the absence and presence of muscle atrophy.
- [0069] Fig. 32 shows an illustrative *in vivo* study design.
- [0070] Fig. 33 shows Atrogin-1 mRNA downregulation in gastroc muscle, 10 days after TfR1 antibody siRNA conjugate, in the absence a presence of dexamethasone induced atrophy (initiated at day 7), relative to the measure concentration of siRNA in the tissue.
- [0071] Fig. 34 shows relative Atrogin-1 mRNA levels in gastroc muscle for the scrambled control groups in the absence (groups 10&13, and groups 11&14)) and presence of dexamethasone induced atrophy (groups 12&15).
- [0072] Fig. 35 shows relative RISC loading of the Atrogin-1 guide strand in mouse gastroc muscle after TfR1-mAb conjugate delivery in the absence and presence of dexamethasone induced atrophy.
- [0073] Fig. 36 shows a time course of MSTN mRNA downregulation in gastroc muscle after TfR1 antibody siRNA conjugate delivery, in the absence (solid lines) and presence (dotted lines) of dexamethasone induced atrophy (initiated at day 7), relative to the PBS control.
- [0074] Fig. 37 shows leg muscle growth rate in gastroc muscle, after TfR1-mAb conjugate delivery in the absence and presence of dexamethasone induced atrophy.
- [0075] Fig. 38 shows an illustrative *in vivo* study design.

[0076] Fig. 39A shows a single treatment of 4.5 mg/kg (siRNA) of either Atrogin-1 siRNA or MuRF1 siRNA or a single dose of both siRNAs combined resulted in up to 75% downregulation of each target in the gastrocnemius.

[0077] Fig. 39B shows mRNA knockdown of both targets in gastrocnemius is maintained at 75% in the intact leg out to 37 days post ASC dose.

[0078] Fig. 39C shows changes in muscle area.

[0079] Fig. 39D shows changes in gastrocnemius weight.

[0080] Fig. 39E shows treatment-induced percentage sparing of muscle wasting in term of leg muscle area. The statistical analysis compared the treatment groups to the scramble siRNA control group using a Welch's TTest.

[0081] Fig. 39F shows the treatment-induced percentage sparing of muscle wasting in term of gastrocnemius weight.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0082] Muscle atrophy is the loss of muscle mass or the progressive weakening and degeneration of muscles, such as skeletal or voluntary muscles that controls movement, cardiac muscles, and smooth muscles. Various pathophysiological conditions including disuse, starvation, cancer, diabetes, and renal failure, or treatment with glucocorticoids result in muscle atrophy and loss of strength. The phenotypical effects of muscle atrophy are induced by various molecular events, including inhibition of muscle protein synthesis, enhanced turnover of muscle proteins, abnormal regulation of satellite cells differentiation, and abnormal conversion of muscle fibers types.

[0083] Extensive research has identified that muscle atrophy is an active process controlled by specific signaling pathways and transcriptional programs. Exemplary pathways involved in this process include, but are not limited to, IGF1-Akt-FoxO, glucocorticoids-GR, PGC1 α -FoxO, TNF α -NF κ B, and myostatin-ActRIIb-Smad2/3.

[0084] In some instances, therapeutic manipulation of mechanisms regulating muscle atrophy has focused on IGF1-Akt, TNF α -NF κ B, and myostatin. While IGF1 analogs were shown to be effective in treating muscle atrophy, the involvement of the IGF1-Akt pathway in promoting tumorigenesis and hypertrophy prevents these therapies. Similar risks are involved in the use of β -adrenergic agonists for the regulation of the Akt-mTOR pathway. Inhibition of myostatin by using soluble ActRIIB or ligand blocking ActRIIB antibodies prevented and reversed skeletal muscle loss, and prolonged the survival of tumor-bearing animals. However the mechanism of the anti-atrophic effects of myostatin blockade remains uncertain as neither expression of a dominant-negative ActRIIB, nor knockdown of Smad2/3 prevented muscle loss following denervation (Satori *et al.*, "Smad2 and 3 transcription factors control muscle mass in adulthood", *Am J Physiol Cell Physiol* **296**: C1248-C1257, 2009).

[0085] Comparing gene expression in different models of muscle atrophy (including diabetes, cancer cachexia, chronic renal failure, fasting and denervation) has led to the identification of atrophy-related genes, named atrogenes (Sacheck *et al.*, "Rapid disuse and denervation atrophy involve transcriptional

changes similar to those of muscle wasting during systemic diseases”, *The FASEB Journal*, **21**(1): 140-155, 2007), that are commonly up- or downregulated in atrophying muscle. Among genes that are strongly upregulated under atrophy conditions are muscle-specific ubiquitin-protein (E3) ligases (e.g. atrogin-1, MuRF1), Forkhead box transcription factors, and proteins mediating stress responses. In some cases, many of these effector proteins are difficult to regulate using traditional drugs.

[0086] Nucleic acid (e.g., RNAi) therapy is a targeted therapy with high selectivity and specificity. However, in some instances, nucleic acid therapy is also hindered by poor intracellular uptake, limited blood stability and non-specific immune stimulation. To address these issues, various modifications of the nucleic acid composition are explored, such as for example, novel linkers for better stabilizing and/or lower toxicity, optimization of binding moiety for increased target specificity and/or target delivery, and nucleic acid polymer modifications for increased stability and/or reduced off-target effect.

[0087] In some embodiments, the arrangement or order of the different components that make-up the nucleic acid composition further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. For example, if the nucleic acid component includes a binding moiety, a polymer, and a polynucleic acid molecule (or polynucleotide), the order or arrangement of the binding moiety, the polymer, and/or the polynucleic acid molecule (or polynucleotide) (e.g., binding moiety-polynucleic acid molecule-polymer, binding moiety-polymer-polynucleic acid molecule, or polymer-binding moiety-polynucleic acid molecule) further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation.

[0088] In some embodiments, described herein include polynucleic acid molecules and polynucleic acid molecule conjugates for the treatment of muscle atrophy or myotonic dystrophy. In some instances, the polynucleic acid molecule conjugates described herein enhance intracellular uptake, stability, and/or efficacy. In some cases, the polynucleic acid molecule conjugates comprise a molecule of Formula (I): A-X₁-B-X₂-C. In some cases, the polynucleic acid molecules that hybridize to target sequences of one or more atrogenes.

[0089] Additional embodiments described herein include methods of treating muscle atrophy or myotonic dystrophy, comprising administering to a subject a polynucleic acid molecule or a polynucleic acid molecule conjugate described herein.

Atrogenes

[0090] Atrogenes, or atrophy-related genes, are genes that are upregulated or downregulated in atrophying muscle. In some instances, upregulated atrogenes include genes that encode ubiquitin ligases, Forkhead box transcription factors, growth factors, deubiquitinating enzymes, or proteins that are involved in glucocorticoid-induced atrophy.

Ubiquitin Ligases

[0091] In some embodiments, an atrogene described herein encodes an E3 ubiquitin ligase. Exemplary E3 ubiquitin ligases include, but are not limited to, Atrogin-1/MAFbx, muscle RING finger 1 (MuRF1),

TNF receptor adaptor protein 6 (TRAF6), F-Box protein 30 (Fbxo30), F-Box protein 40 (Fbxo40), neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4-1), and tripartite motif-containing protein 32 (Trim32). Exemplary mitochondrial ubiquitin ligases include, but are not limited to, Mitochondrial E3 ubiquitin protein ligase 1 (Mul1) and Carboxy terminus of Hsc70 interacting protein (CHIP).

[0092] In some embodiments, an atrogene described herein encodes Atrogin-1, also named Muscle Atrophy F-box (MAFbx), a member of the F-box protein family. Atrogin-1/MAFbx is one of the four subunits of the ubiquitin ligase complex SKP1-cullin-F-box (SCF) that promotes degradation of MyoD, a muscle transcription factor, and eukaryotic translation initiation factor 3 subunit F (eIF3-f). Atrogin-1/MAFbx is encoded by *FBXO32*.

[0093] In some embodiments, an atrogene described herein encodes muscle RING finger 1 (MuRF1). MuRF1 is a member of the muscle-specific RING finger proteins and along with family members MuRF2 and MuRF3 are found at the M-line and Z-line lattices of myofibrils. Further, several studies have shown that MuRF1 interacts with and/or modulates the half-life of muscle structural proteins such as troponin I, myosin heavy chains, actin, myosin binding protein C, and myosin light chains 1 and 2. MuRF1 is encoded by *TRIM63*.

[0094] In some embodiments, an atrogene described herein encodes TNF receptor adaptor protein 6 (TRAF6) (also known as interleukin-1 signal transducer, RING finger protein 85, or RNF85). TRAF6 is a member of the E3 ligase that mediates conjugation of Lys63-linked polyubiquitin chains to target proteins. The Lys63-linked polyubiquitin chains signal autophagy-dependent cargo recognition by scaffold protein p62 (SQSTM1). TRAF6 is encoded by the *TRAF6* gene.

[0095] In some embodiments, an atrogene described herein encodes F-Box protein 30 (Fbxo30) (also known as F-Box only protein, helicase, 18; muscle ubiquitin ligase of SCF complex in atrophy-1; or MUSA1). Fbxo30 is a member of the SCF complex family of E3 ubiquitin ligases. In one study, Fbxo30 is proposed to be inhibited by the bone morphogenetic protein (BMP) pathway and upon atrophy-inducing conditions, are upregulated and subsequently undergoes autoubiquitination. Fbxo30 is encoded by the *FBXO30* gene.

[0096] In some embodiments, an atrogene described herein encodes F-Box protein 40 (Fbxo40) (also known as F-Box only protein 40 or muscle disease-related protein). A second member of the SCF complex family of E3 ubiquitin ligases, Fbxo40 regulates anabolic signals. In some instances, Fbxo40 ubiquitinates and affects the degradation of insulin receptor substrate 1, a downstream effector of insulin receptor-mediated signaling. Fbxo40 is encoded by the *FBXO40* gene.

[0097] In some embodiments, an atrogene described herein encodes neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4-1), a HECT domain E3 ubiquitin ligase which has been shown to be upregulated in muscle cells during disuse. Nedd4-1 is encoded by the *NEDD4* gene.

[0098] In some embodiments, an atrogene described herein encodes tripartite motif-containing protein 32 (Trim32). Trim32 is a member of the E3 ubiquitin ligase that is involved in degradation of thin

filaments such as actin, tropomyosin, and troponins; α -actinin; and desmin. Trim32 is encoded by the *TRIM32* gene.

[0099] In some embodiments, an atrogene described herein encodes Mitochondrial E3 ubiquitin protein ligase 1 (Mul1) (also known as mitochondrial-anchored protein ligase, RING finger protein 218, RNF218, MAPL, MULAN, and GIDE). Mul1 is involved in the mitochondrial network remodeling and is up-regulated by the FoxO family of transcription factors under catabolic conditions, such as for example, denervation or fasting, and subsequently causes mitochondrial fragmentation and removal via autophagy (mitophagy). Furthermore, Mul1 ubiquitinates the mitochondrial pro-fusion protein mitofusin 2, a GTPase that is involved in mitochondrial fusion, leading to the degradation of mitofusin 2. Mul1 is encoded by the *MUL1* gene.

[0100] In some embodiments, an atrogene described herein encodes Carboxy terminus of Hsc70 interacting protein (CHIP) (also known as STIP1 homology and U-Box containing protein 1, STUB1, CLL-associated antigen KW-8, antigen NY-CO-7, SCAR16, SDCCAG7, or UBOX1). CHIP is a mitochondrial ubiquitin ligase that regulates ubiquitination and lysosomal-dependent degradation of filamin C, a muscle protein found in the Z-line. Z-line or Z-disc is the structure formed between adjacent sarcomeres, and sarcomere is the basic unit of muscle. Alterations of filamin structure triggers binding of the co-chaperone BAG3, a complex that comprises chaperones Hsc70 and HspB8 with CHIP. Subsequent ubiquitination of BAG3 and filamin by CHIP activates the autophagy system, leading to degradation of filamin C. CHIP is encoded by the *STUB1* gene.

Forkhead Box Transcription Factors

[0101] In some embodiments, an atrogene described herein encodes a Forkhead box transcription factor. Exemplary Forkhead box transcription factors include, but are not limited to, isoforms Forkhead box protein O1 (FoxO1) and Forkhead box protein O3 (FoxO3).

[0102] In some embodiments, an atrogene described herein encodes Forkhead box protein O1 (FoxO1) (also known as Forkhead homolog in Rhabdomyosarcoma, FKHR, or FKH1). FoxO1 is involved in regulation of gluconeogenesis and glycogenolysis by insulin signaling, and the initiation of adipogenesis by preadipocytes. FoxO1 is encoded by the *FOXO1* gene.

[0103] In some embodiments, an atrogene described herein encodes Forkhead box protein O3 (FoxO3) (also known as Forkhead in Rhabdomyosarcoma-like 1, FKHL1, or FOXO3A). FoxO3 is activated by AMP-activated protein kinase AMPK, which in turn induces expression of atrogin-1 and MuRF1. FoxO3 is encoded by the *FOXO3* gene.

Growth Factors

[0104] In some embodiments, an atrogene described herein encodes a growth factor. An exemplary growth factor includes myostatin.

[0105] In some instances, an atrogene described herein encodes myostatin (Mstn), also known as growth/differentiation factor 8 (GDF-8). Myostatin is intracellularly converted into an activator, and

stimulates muscle degradation and suppresses muscle synthesis by inhibiting Akt through the phosphorylation/activation of Smad (small mothers against decapentaplegic). In some instances, myostatin has been found to be regulated by the Akt-FoxO signaling pathway. In additional instances, myostatin has been shown to suppress differentiation of satellite cells, stimulate muscle degradation through the inhibition of the Akt pathway, and suppress muscle synthesis via the mTOR pathway.

Deubiquitinating Enzymes

[0106] In some embodiments, an atrogene described herein encodes a deubiquitinating enzyme. Exemplary deubiquitinating enzymes include, but are not limited to, Ubiquitin specific peptidase 14 (USP14) and Ubiquitin specific peptidase 19 (USP19). In some instances, an atrogene described herein encodes USP14 (also known as deubiquitinating enzyme 14 or TGT). In other instances, an atrogene described herein encodes USP19 (also known as zinc finger MYND domain-containing protein 9, deubiquitinating enzyme 19, or ZMYND9). USP14 is encoded by the *USP14* gene. USP19 is encoded by the *USP19* gene.

Additional Atrogenes

[0107] In some embodiments, an atrogene described herein encodes regulated in development and DNA damage response 1 (Redd1), also known as DNA-damage-inducible transcript 4 (DDIT4) and HIF-1 responsive protein RTP801. Redd1 represses mTOR function by sequestering 14-3-3 and increases TSC1/2 activity. Furthermore, Redd1 decreases phosphorylation of 4E-BP1 and S6K1, which are involved in muscle protein synthesis. Redd1 is encoded by the *DDIT4* gene.

[0108] In some embodiments, an atrogene described herein encodes cathepsin L2, also known as cathepsin V. Cathepsin L2 is a lysosomal cysteine proteinase. It is encoded by the *CTSL2* gene.

[0109] In some embodiments, an atrogene described herein encodes TG interacting factor, or homeobox protein TGIF1. TG interacting factor is a transcription factor which regulates signaling pathways involved in embryonic development. This protein is encoded by the *TGIF* gene.

[0110] In some embodiments, an atrogene described herein encodes myogenin, also known as myogenic factor 4. Myogenin is a member of the MyoD family of muscle-specific basic-helix-loop-helix (bHLH) transcription factor involved in the coordination of skeletal muscle development and repair. Myogenin is encoded by the *MYOG* gene.

[0111] In some embodiments, an atrogene described herein encodes myotonin-protein kinase (MT-PK), also known as myotonic dystrophy protein kinase (MDPK) or dystrophia myotonica protein kinase (DMK). MT-PK is a Serine/Threonine kinase and further interacts with members of the Rho family of GTPases. In human, MT-PK is encoded by the *DMPK* gene.

[0112] In some embodiments, an atrogene described herein encodes histone deacetylase 2, a member of the histone deacetylase family. Histone deacetylase 2 is encoded by the *HDAC2* gene.

[0113] In some embodiments, an atrogene described herein encodes histone deacetylase 3, another member of the histone deacetylase family. Histone deacetylase 3 is encoded by the *HDAC3* gene.

[0114] In some embodiments, an atro gene described herein encodes metallothionein 1L, a member of the metallothionein family. Metallothioneins (MT) are cysteine-rich, low molecular weight proteins that is capable of binding heavy metals, thereby providing protection against metal toxicity and/or oxidative stress. Metallothionein 1L is encoded by the *MT1L* gene.

[0115] In some embodiments, an atro gene described herein encodes metallothionein 1B, a second member of the metallothionein family. Metallothionein 1B is encoded by the *MT1B* gene.

[0116] In some embodiments, an atro gene described herein is an atro gene listed in Table 14.

Polynucleic Acid Molecules

[0117] In certain embodiments, a polynucleic acid molecule hybridizes to a target sequence of an atrophy-related gene (also referred to as an atro gene). In some instances, a polynucleic acid molecule described herein hybridizes to a target sequence of an ubiquitin ligase (e.g., an E3 ubiquitin ligase or a mitochondrial ubiquitin ligase). In some instances, a polynucleic acid molecule described herein hybridizes to a target sequence of a Forkhead box transcription factor. In some instances, a polynucleic acid molecule described herein hybridizes to a target sequence of a growth factor. In some instances, a polynucleic acid molecule described herein hybridizes to a target sequence of a deubiquitinating enzyme.

[0118] In some embodiments, a polynucleic acid molecule described herein hybridizes to a target sequence of *FBXO32*, *TRIM63*, *TRAF6*, *FBXO30*, *FBXO40*, *NEDD4*, *TRIM32*, *MUL1*, *STUB1*, *FOXO1*, *FOXO3*, *MSTN*, *USP14*, *USP19*, *DDIT4*, *CTSL2*, *TGIF*, *MYOG*, *HDAC2*, *HDAC3*, *MT1L*, *MT1B*, or *DMPK*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FBXO32*, *TRIM63*, *FOXO1*, *FOXO3*, or *MSTN*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FBXO32*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *TRIM63*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *TRAF6*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FBXO30*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FBXO40*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *NEDD4*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *TRIM32*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *MUL1*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *STUB1*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FOXO1*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *FOXO3*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *MSTN*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *USP14*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *USP19*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *DDIT4*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *CTSL2*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *TGIF*. In some cases, a polynucleic acid molecule described

herein hybridizes to a target sequence of *MYOG*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *HDAC2*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *HDAC3*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *MTIL*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *MTIB*. In some cases, a polynucleic acid molecule described herein hybridizes to a target sequence of *DMPK*.

[0119] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480. In some embodiments, the polynucleic acid molecule consists of a target sequence as set forth in SEQ ID NOs: 28-141 and 370-480.

[0120] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to a target sequence as set forth in SEQ ID

NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to a target sequence as set forth in SEQ ID NOs: 703-3406. In some embodiments, the polynucleic acid molecule consists of a target sequence as set forth in SEQ ID NOs: 703-3406.

[0121] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 142-255, 256-369, 481-591, 592-702, and 3407-14222. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 142-255, 481-591, 3407-6110, and 8815-11518, and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 256-369, 592-702, 6111-8814, and 11519-14222.

[0122] In some embodiments, the polynucleic acid molecule comprises a sense strand (e.g., a passenger strand) and an antisense strand (e.g., a guide strand). In some instances, the sense strand (e.g., the passenger strand) comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 142-255, 481-591, 3407-6110, and 8815-11518. In some instances, the antisense strand (e.g., the guide strand) comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,

95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a target sequence as set forth in SEQ ID NOs: 256-369, 592-702, 6111-8814, and 11519-14222.

[0123] In some embodiments, the polynucleic acid molecule described herein comprises RNA or DNA. In some cases, the polynucleic acid molecule comprises RNA. In some instances, RNA comprises short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), double-stranded RNA (dsRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), or heterogeneous nuclear RNA (hnRNA). In some instances, RNA comprises shRNA. In some instances, RNA comprises miRNA. In some instances, RNA comprises dsRNA. In some instances, RNA comprises tRNA. In some instances, RNA comprises rRNA. In some instances, RNA comprises hnRNA. In some instances, the RNA comprises siRNA. In some instances, the polynucleic acid molecule comprises siRNA.

[0124] In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0125] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances, the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 50 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 45 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 40 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 35 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 10 and about 20 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 25 nucleotides in length. In some instances, the polynucleic acid molecule is between about 15 and about 30 nucleotides in length. In some instances, the polynucleic acid molecule is between about 12 and about 30 nucleotides in length.

[0126] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide. In some instances, the polynucleic acid molecule comprises a second polynucleotide. In some instances, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide is a sense strand or passenger strand. In some instances, the second polynucleotide is an antisense strand or guide strand.

[0127] In some embodiments, the polynucleic acid molecule is a first polynucleotide. In some embodiments, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0128] In some instances, the first polynucleotide is about 50 nucleotides in length. In some instances, the first polynucleotide is about 45 nucleotides in length. In some instances, the first polynucleotide is about 40 nucleotides in length. In some instances, the first polynucleotide is about 35 nucleotides in length. In some instances, the first polynucleotide is about 30 nucleotides in length. In some instances, the first polynucleotide is about 25 nucleotides in length. In some instances, the first polynucleotide is about 20 nucleotides in length. In some instances, the first polynucleotide is about 19 nucleotides in length. In some instances, the first polynucleotide is about 18 nucleotides in length. In some instances, the first polynucleotide is about 17 nucleotides in length. In some instances, the first polynucleotide is about 16 nucleotides in length. In some instances, the first polynucleotide is about 15 nucleotides in length. In some instances, the first polynucleotide is about 14 nucleotides in length. In some instances, the first polynucleotide is about 13 nucleotides in length. In some instances, the first polynucleotide is about 12 nucleotides in length. In some instances, the first polynucleotide is about 11 nucleotides in length. In some instances, the first polynucleotide is about 10 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the first polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the first polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the first polynucleotide is between about 12 and about 30 nucleotides in length.

[0129] In some embodiments, the polynucleic acid molecule is a second polynucleotide. In some embodiments, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0130] In some instances, the second polynucleotide is about 50 nucleotides in length. In some instances, the second polynucleotide is about 45 nucleotides in length. In some instances, the second polynucleotide is about 40 nucleotides in length. In some instances, the second polynucleotide is about 35 nucleotides in length. In some instances, the second polynucleotide is about 30 nucleotides in length. In some instances, the second polynucleotide is about 25 nucleotides in length. In some instances, the second polynucleotide is about 20 nucleotides in length. In some instances, the second polynucleotide is about 19 nucleotides in length. In some instances, the second polynucleotide is about 18 nucleotides in length. In some instances, the second polynucleotide is about 17 nucleotides in length. In some instances, the second polynucleotide is about 16 nucleotides in length. In some instances, the second polynucleotide is about 15 nucleotides in length. In some instances, the second polynucleotide is about 14 nucleotides in length. In some instances, the second polynucleotide is about 13 nucleotides in length. In some instances, the second polynucleotide is about 12 nucleotides in length. In some instances, the second polynucleotide is about 11 nucleotides in length. In some instances, the second polynucleotide is about 10 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 50 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 45 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 40 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 35 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 10 and about 20 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 25 nucleotides in length. In some instances, the second polynucleotide is between about 15 and about 30 nucleotides in length. In some instances, the second polynucleotide is between about 12 and about 30 nucleotides in length.

[0131] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the polynucleic acid molecule further comprises a blunt terminus, an overhang, or a combination thereof. In some instances, the blunt terminus is a 5' blunt terminus, a 3' blunt terminus, or both. In some cases, the overhang is a 5' overhang, 3' overhang, or both. In some cases, the overhang comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, 4, 5, or 6 non-base pairing nucleotides. In some cases, the overhang comprises 1 non-base pairing nucleotide. In some cases, the overhang comprises 2 non-base pairing nucleotides. In some cases, the overhang comprises 3 non-base pairing nucleotides. In some cases, the overhang comprises 4 non-base pairing nucleotides.

[0132] In some embodiments, the sequence of the polynucleic acid molecule is at least 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 50% complementary to a target sequence described herein. In some embodiments, the sequence of

the polynucleic acid molecule is at least 60% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 70% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 80% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 90% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 95% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 99% complementary to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule is 100% complementary to a target sequence described herein.

[0133] In some embodiments, the sequence of the polynucleic acid molecule has 5 or less mismatches to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule has 4 or less mismatches to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule has 1 or less mismatches to a target sequence described herein.

[0134] In some embodiments, the specificity of the polynucleic acid molecule that hybridizes to a target sequence described herein is a 95%, 98%, 99%, 99.5% or 100% sequence complementarity of the polynucleic acid molecule to a target sequence. In some instances, the hybridization is a high stringent hybridization condition.

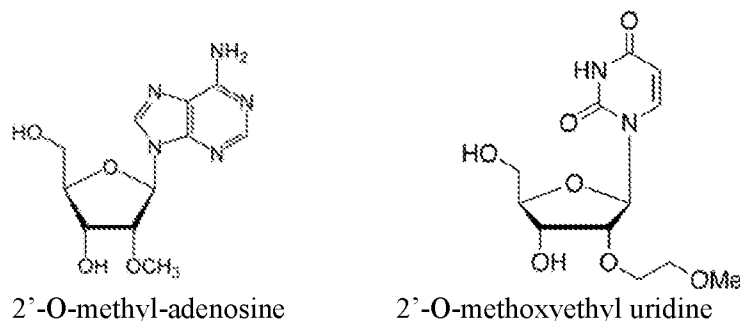
[0135] In some embodiments, the polynucleic acid molecule has reduced off-target effect. In some instances, “off-target” or “off-target effects” refer to any instance in which a polynucleic acid polymer directed against a given target causes an unintended effect by interacting either directly or indirectly with another mRNA sequence, a DNA sequence or a cellular protein or other moiety. In some instances, an “off-target effect” occurs when there is a simultaneous degradation of other transcripts due to partial homology or complementarity between that other transcript and the sense and/or antisense strand of the polynucleic acid molecule.

[0136] In some embodiments, the polynucleic acid molecule comprises natural or synthetic or artificial nucleotide analogues or bases. In some cases, the polynucleic acid molecule comprises combinations of DNA, RNA and/or nucleotide analogues. In some instances, the synthetic or artificial nucleotide analogues or bases comprise modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

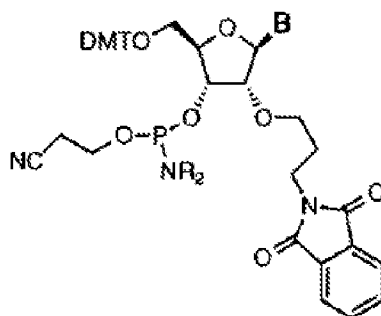
[0137] In some embodiments, nucleotide analogues or artificial nucleotide base comprise a nucleic acid with a modification at a 2' hydroxyl group of the ribose moiety. In some instances, the modification includes an H, OR, R, halo, SH, SR, NH₂, NHR, NR₂, or CN, wherein R is an alkyl moiety. Exemplary alkyl moiety includes, but is not limited to, halogens, sulfurs, thiols, thioethers, thioesters, amines (primary, secondary, or tertiary), amides, ethers, esters, alcohols and oxygen. In some instances, the

alkyl moiety further comprises a modification. In some instances, the modification comprises an azo group, a keto group, an aldehyde group, a carboxyl group, a nitro group, a nitroso group, a nitrile group, a heterocycle (e.g., imidazole, hydrazino or hydroxylamino) group, an isocyanate or cyanate group, or a sulfur containing group (e.g., sulfoxide, sulfone, sulfide, and disulfide). In some instances, the alkyl moiety further comprises a hetero substitution. In some instances, the carbon of the heterocyclic group is substituted by a nitrogen, oxygen or sulfur. In some instances, the heterocyclic substitution includes but is not limited to, morpholino, imidazole, and pyrrolidino.

[0138] In some instances, the modification at the 2' hydroxyl group is a 2'-O-methyl modification or a 2'-O-methoxyethyl (2'-O-MOE) modification. In some cases, the 2'-O-methyl modification adds a methyl group to the 2' hydroxyl group of the ribose moiety whereas the 2'-O-methoxyethyl modification adds a methoxyethyl group to the 2' hydroxyl group of the ribose moiety. Exemplary chemical structures of a 2'-O-methyl modification of an adenosine molecule and 2'-O-methoxyethyl modification of an uridine are illustrated below.



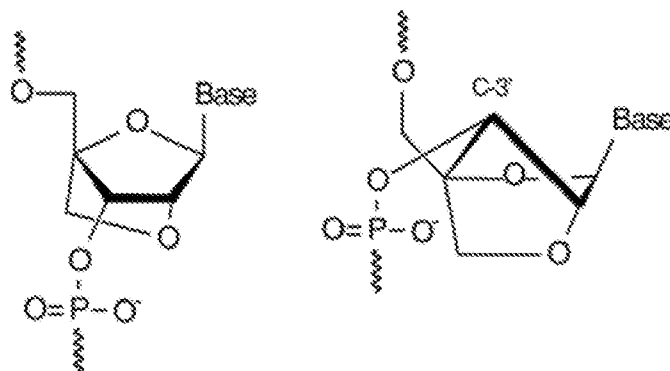
[0139] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties. An exemplary chemical structure of a 2'-O-aminopropyl nucleoside phosphoramidite is illustrated below.



2'-O-aminopropyl nucleoside phosphoramidite

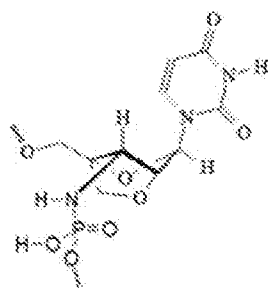
[0140] In some instances, the modification at the 2' hydroxyl group is a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2'-C,4'-C-oxy-methylene-linked bicyclic ribonucleotide monomer. Exemplary representations of the chemical structure of LNA are illustrated

below. The representation shown to the left highlights the chemical connectivities of an LNA monomer. The representation shown to the right highlights the locked 3'-endo (³E) conformation of the furanose ring of an LNA monomer.

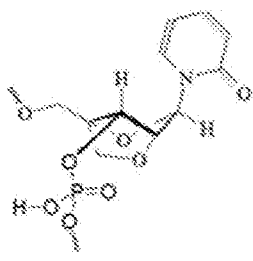


LNA (Locked Nucleic Acids)

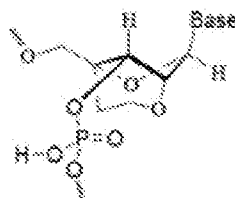
[0141] In some instances, the modification at the 2' hydroxyl group comprises ethylene nucleic acids (ENA) such as for example 2'-4'-ethylene-bridged nucleic acid, which locks the sugar conformation into a C_{3'}'-endo sugar puckering conformation. ENA are part of the bridged nucleic acids class of modified nucleic acids that also comprises LNA. Exemplary chemical structures of the ENA and bridged nucleic acids are illustrated below.



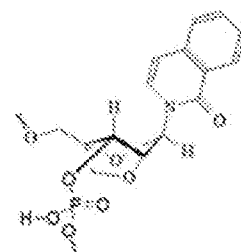
3'-amino-2',4'-BNA



2',4'-BNA-2-pyridone



2',4'-ENA



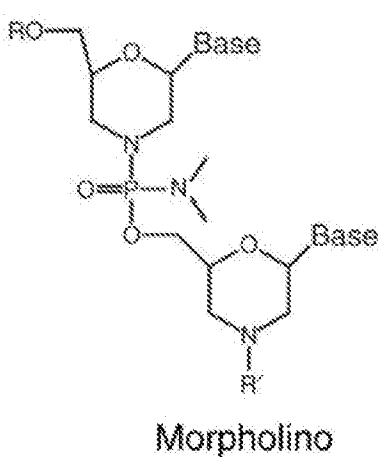
2',4'-BNA-1-isoquinoline

[0142] In some embodiments, additional modifications at the 2' hydroxyl group include 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA).

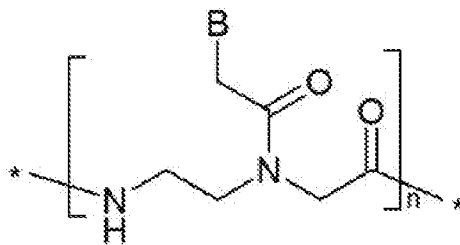
[0143] In some embodiments, nucleotide analogues comprise modified bases such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6-methyladenine, 6-methylguanine, N,N-dimethyladenine, 2-propyladenine, 2-propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5-(2-amino)propyluridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1-methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2-methylguanosine, 7-methylguanosine, 2,2-dimethylguanosine, 5-methylaminoethyluridine, 5-methoxyuridine, deazanucleotides such as 7-deaza-adenosine, 6-azouridine, 6-azocytidine, 6-azothymidine, 5-methyl-2-thiouridine, other thio bases such as 2-thiouridine and 4-thiouridine and 2-thiocytidine, dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O- and N-alkylated purines and pyrimidines such as N6-

methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, pyridine-2-one, phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyl nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or be based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0144] In some embodiments, nucleotide analogues further comprise morpholinos, peptide nucleic acids (PNAs), methylphosphonate nucleotides, thiophosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, 1', 5'- anhydrohexitol nucleic acids (HNAs), or a combination thereof. Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure by deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides.

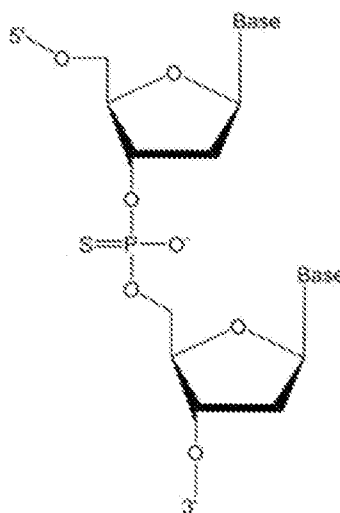


[0145] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.

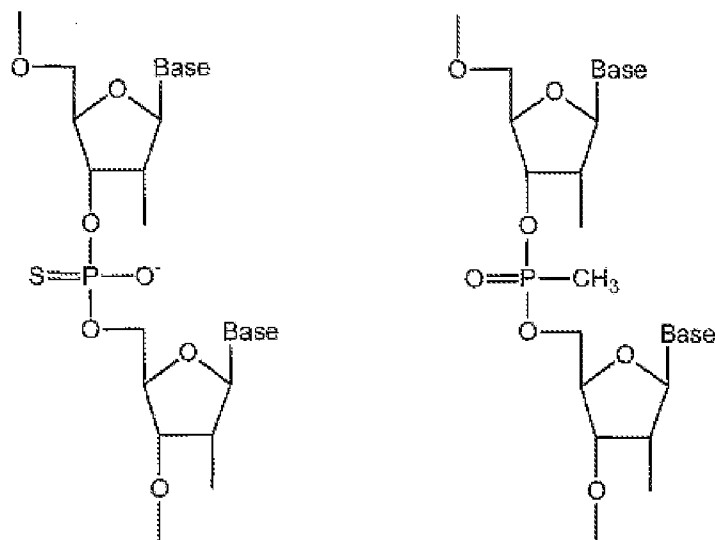


PNA

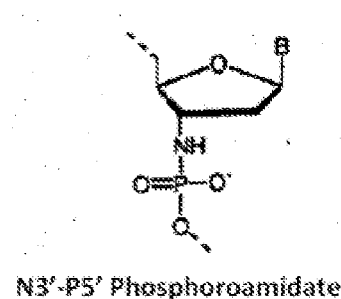
[0146] In some embodiments, one or more modifications optionally occur at the internucleotide linkage. In some instances, modified internucleotide linkage include, but is not limited to, phosphorothioates, phosphorodithioates, methylphosphonates, 5'-alkylenephosphonates, 5'-methylphosphonate, 3'-alkylene phosphonates, borontrifluoridates, borano phosphate esters and selenophosphates of 3'-5' linkage or 2'-5' linkage, phosphotriesters, thionoalkylphosphotriesters, hydrogen phosphonate linkages, alkyl phosphonates, alkylphosphonothioates, arylphosphonothioates, phosphoroselenoates, phosphorodiselenoates, phosphinates, phosphoramidates, 3'-alkylphosphoramidates, aminoalkylphosphoramidates, thionophosphoramidates, phosphoropiperazidates, phosphoroanilothioates, phosphoroanilidates, ketones, sulfones, sulfonamides, carbonates, carbamates, methylenehydrazos, methylenedimethylhydrazos, formacetals, thioformacetals, oximes, methyleneiminos, methylenemethyliminos, thioamides, linkages with riboacetyl groups, aminoethyl glycine, silyl or siloxane linkages, alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms, linkages with morpholino structures, amides, polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly, and combinations thereof. Phosphorothioate antisense oligonucleotides (PS ASO) are antisense oligonucleotides comprising a phosphorothioate linkage. An exemplary PS ASO is illustrated below.



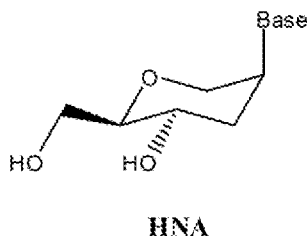
[0147] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left) and methylphosphonate nucleotide (right) are illustrated below.



[0148] In some instances, a modified nucleotide includes, but is not limited to, 2'-fluoro N3-P5'-phosphoramidites illustrated as:



[0149] In some instances, a modified nucleotide includes, but is not limited to, hexitol nucleic acid (or 1', 5'- anhydrohexitol nucleic acids (HNA)) illustrated as:



[0150] In some embodiments, one or more modifications further optionally include modifications of the ribose moiety, phosphate backbone and the nucleoside, or modifications of the nucleotide analogues at the 3' or the 5' terminus. For example, the 3' terminus optionally include a 3' cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus is optionally conjugated with an aminoalkyl group, e.g., a 3' C5-aminoalkyl dT. In an additional alternative, the 3'-terminus is optionally conjugated with an abasic site, e.g., with an apurinic or apyrimidinic site. In some instances, the 5'-terminus is conjugated with an aminoalkyl group, e.g., a 5'-O-alkylamino substituent. In some cases, the 5'-terminus is conjugated with an abasic site, e.g., with an apurinic or apyrimidinic site.

[0151] In some embodiments, the polynucleic acid molecule comprises one or more of the artificial nucleotide analogues described herein. In some instances, the polynucleic acid molecule comprises 1, 2,

3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues selected from 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methyl modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methoxyethyl (2'-O-MOE) modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of thiolphosphonate nucleotides.

[0152] In some instances, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification.

[0153] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 90% modification, from about 20% to about 90% modification, from about 30% to about 90% modification, from about 40% to about 90% modification, from about 50% to about 90% modification, from about 60% to about 90% modification, from about 70% to about 90% modification, and from about 80% to about 100% modification.

[0154] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 80% modification, from about 20% to about 80% modification, from about 30% to about 80% modification, from about 40% to about 80% modification, from about 50% to about 80% modification, from about 60% to about 80% modification, and from about 70% to about 80% modification.

[0155] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 70% modification, from about 20% to about 70% modification, from about 30% to about 70% modification, from about 40% to about 70% modification, from about 50% to about 70% modification, and from about 60% to about 70% modification.

[0156] In some instances, the polynucleic acid molecule comprises at least one of: from about 10% to about 60% modification, from about 20% to about 60% modification, from about 30% to about 60% modification, from about 40% to about 60% modification, and from about 50% to about 60% modification.

[0157] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 50% modification, from about 20% to about 50% modification, from about 30% to about 50% modification, and from about 40% to about 50% modification.

[0158] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 40% modification, from about 20% to about 40% modification, and from about 30% to about 40% modification.

[0159] In some cases, the polynucleic acid molecule comprises at least one of: from about 10% to about 30% modification, and from about 20% to about 30% modification.

[0160] In some cases, the polynucleic acid molecule comprises from about 10% to about 20% modification.

[0161] In some cases, the polynucleic acid molecule comprises from about 15% to about 90%, from about 20% to about 80%, from about 30% to about 70%, or from about 40% to about 60% modifications.

[0162] In additional cases, the polynucleic acid molecule comprises at least about 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% modification.

[0163] In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modifications.

[0164] In some instances, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22 or more modified nucleotides.

[0165] In some instances, from about 5 to about 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 10% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 15% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 20% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 25% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 30% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 35% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 40% of the

polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 45% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 50% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 55% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 60% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 65% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 70% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 75% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 80% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 85% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 90% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 95% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 96% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 97% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 98% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 99% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some instances, about 100% of the polynucleic acid molecule comprises the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof.

[0166] In some embodiments, the polynucleic acid molecule comprises from about 1 to about 25 modifications in which the modification comprises an artificial nucleotide analogues described herein. In some embodiments, the polynucleic acid molecule comprises about 1 modification in which the modification comprises an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 2 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 3 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 4 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 5 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid

molecule comprises about 6 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 7 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 8 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 9 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 10 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 11 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 12 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 13 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 14 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 15 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 16 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 17 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 18 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 19 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 20 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 21 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 22 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 23 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 24 modifications in which the modifications comprise an artificial nucleotide analogue described herein. In some embodiments, the polynucleic acid molecule comprises about 25 modifications in which the modifications comprise an artificial nucleotide analogue described herein.

[0167] In some embodiments, a polynucleic acid molecule is assembled from two separate polynucleotides wherein one polynucleotide comprises the sense strand and the second polynucleotide comprises the antisense strand of the polynucleic acid molecule. In other embodiments, the sense strand

is connected to the antisense strand via a linker molecule, which in some instances is a polynucleotide linker or a non-nucleotide linker.

[0168] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides in the sense strand comprises 2'-O-methylpyrimidine nucleotides and purine nucleotides in the sense strand comprise 2'-deoxy purine nucleotides. In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein pyrimidine nucleotides present in the sense strand comprise 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein purine nucleotides present in the sense strand comprise 2'-deoxy purine nucleotides.

[0169] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides when present in said antisense strand are 2'-O-methyl purine nucleotides.

[0170] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the pyrimidine nucleotides when present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides when present in said antisense strand comprise 2'-deoxy-purine nucleotides.

[0171] In some embodiments, a polynucleic acid molecule comprises a sense strand and antisense strand, wherein the sense strand includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In other embodiments, the terminal cap moiety is an inverted deoxy abasic moiety.

[0172] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a phosphate backbone modification at the 3' end of the antisense strand. In some instances, the phosphate backbone modification is a phosphorothioate.

[0173] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, wherein the antisense strand comprises a glyceryl modification at the 3' end of the antisense strand.

[0174] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and in which the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5,

6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0175] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the sense strand comprises about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and in which the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0176] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or about one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap

molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

[0177] In some embodiments, a polynucleic acid molecule comprises a sense strand and an antisense strand, in which the antisense strand comprises about 1 to about 25 or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 25 or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more phosphorothioate internucleotide linkages, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In other embodiments, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

[0178] In some embodiments, a polynucleic acid molecule described herein is a chemically-modified short interfering nucleic acid molecule having about 1 to about 25, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more phosphorothioate internucleotide linkages in each strand of the polynucleic acid molecule.

[0179] In another embodiment, a polynucleic acid molecule described herein comprises 2'-5' internucleotide linkages. In some instances, the 2'-5' internucleotide linkage(s) is at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both sequence strands. In addition instances, the 2'-5' internucleotide linkage(s) is present at various other positions within one or both sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the polynucleic acid molecule comprise a 2'-5' internucleotide linkage.

[0180] In some embodiments, a polynucleic acid molecule is a single stranded polynucleic acid molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the polynucleic acid molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the polynucleic acid are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro

pyrimidine nucleotides), and wherein any purine nucleotides present in the polynucleic acid are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and a terminal cap modification, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the polynucleic acid molecule optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the polynucleic acid molecule, wherein the terminal nucleotides further comprise one or more (e.g., 1, 2, 3, or 4) phosphorothioate internucleotide linkages, and wherein the polynucleic acid molecule optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

[0181] In some cases, one or more of the artificial nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease when compared to natural polynucleic acid molecules. In some instances, artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or combinations thereof are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribonuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease. In some instances, 2'-O-methyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-aminopropyl modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-deoxy modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, LNA modified polynucleic acid molecule is nuclease

resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, ENA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, HNA modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, morpholinos is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, PNA modified polynucleic acid molecule is resistant to nucleases (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, methylphosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, thiophosphonate nucleotides modified polynucleic acid molecule is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites is nuclease resistance (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistance). In some instances, the 5' conjugates described herein inhibit 5'-3' exonucleolytic cleavage. In some instances, the 3' conjugates described herein inhibit 3'-5' exonucleolytic cleavage.

[0182] In some embodiments, one or more of the artificial nucleotide analogues described herein have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. The one or more of the artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiophosphonate nucleotides, or 2'-fluoro N3-P5'-phosphoramidites have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule has increased binding

affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-O- dimethylaminoethoxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, LNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, ENA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, PNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, HNA modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, morpholino modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, methylphosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, thiophosphonate nucleotides modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some cases, the increased affinity is illustrated with a lower K_d, a higher melt temperature (T_m), or a combination thereof.

[0183] In some embodiments, a polynucleic acid molecule described herein is a chirally pure (or stereo pure) polynucleic acid molecule, or a polynucleic acid molecule comprising a single enantiomer. In some instances, the polynucleic acid molecule comprises L-nucleotide. In some instances, the polynucleic acid molecule comprises D-nucleotides. In some instance, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of its mirror enantiomer. In some cases, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of a racemic mixture. In some instances, the polynucleic acid molecule is a polynucleic acid molecule described in: U.S. Patent Publication Nos: 2014/194610 and 2015/211006; and PCT Publication No.: WO2015107425.

[0184] In some embodiments, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is a DNA aptamer conjugating moiety. In some instances, the aptamer conjugating moiety is Alphamer (Centauri Therapeutics), which comprises an aptamer portion that recognizes a specific cell-surface target and a portion that presents a specific epitopes for attaching to circulating antibodies. In some instance, a polynucleic acid molecule described herein is further modified to include an aptamer conjugating moiety as described in: U.S. Patent Nos: 8,604,184, 8,591,910, and 7,850,975.

[0185] In additional embodiments, a polynucleic acid molecule described herein is modified to increase its stability. In some embodiment, the polynucleic acid molecule is RNA (e.g., siRNA). In some instances, the polynucleic acid molecule is modified by one or more of the modifications described above to increase its stability. In some cases, the polynucleic acid molecule is modified at the 2' hydroxyl position, such as by 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modification or by a locked or bridged ribose conformation (e.g., LNA or ENA). In some cases, the polynucleic acid molecule is modified by 2'-O-methyl and/or 2'-O-methoxyethyl ribose. In some cases, the polynucleic acid molecule also includes morpholinos, PNAs, HNA, methylphosphonate nucleotides, thiophosphonate nucleotides, and/or 2'-fluoro N³-P^{5'}-phosphoramidites to increase its stability. In some instances, the polynucleic acid molecule is a chirally pure (or stereo pure) polynucleic acid molecule. In some instances, the chirally pure (or stereo pure) polynucleic acid molecule is modified to increase its stability. Suitable modifications to the RNA to increase stability for delivery will be apparent to the skilled person.

[0186] In some instances, the polynucleic acid molecule is a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In some instances, the polynucleic acid molecule is assembled from two separate polynucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (e.g., each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19, 20, 21, 22, 23, or more base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the polynucleic acid molecule is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the polynucleic acid molecule are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

[0187] In some cases, the polynucleic acid molecule is a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. In other cases, the polynucleic acid molecule is a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target

nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide is processed either in vivo or in vitro to generate an active polynucleic acid molecule capable of mediating RNAi. In additional cases, the polynucleic acid molecule also comprises a single-stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such polynucleic acid molecule does not require the presence within the polynucleic acid molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide further comprises a terminal phosphate group, such as a 5'-phosphate (see for example Martinez et al., 2002, *Cell*, 110, 563-574 and Schwarz et al., 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate.

[0188] In some instances, an asymmetric hairpin is a linear polynucleic acid molecule comprising an antisense region, a loop portion that comprises nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a loop region comprising about 4 to about 8 nucleotides, and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region. In some cases, the asymmetric hairpin polynucleic acid molecule also comprises a 5'-terminal phosphate group that is chemically modified. In additional cases, the loop portion of the asymmetric hairpin polynucleic acid molecule comprises nucleotides, non-nucleotides, linker molecules, or conjugate molecules.

[0189] In some embodiments, an asymmetric duplex is a polynucleic acid molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complimentary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex polynucleic acid molecule comprises an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22 nucleotides) and a sense region having about 3 to about 18 nucleotides that are complementary to the antisense region.

[0190] In some cases, a universal base refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

Polynucleic Acid Molecule Synthesis

[0191] In some embodiments, a polynucleic acid molecule described herein is constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a polynucleic acid molecule is chemically synthesized using naturally occurring nucleotides or variously

modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the polynucleic acid molecule and target nucleic acids. Exemplary methods include those described in: U.S. Patent Nos. 5,142,047; 5,185,444; 5,889,136; 6,008,400; and 6,111,086; PCT Publication No. WO2009099942; or European Publication No. 1579015. Additional exemplary methods include those described in: Griffey et al., "2'-O-aminopropyl ribonucleotides: a zwitterionic modification that enhances the exonuclease resistance and biological activity of antisense oligonucleotides," *J. Med. Chem.* **39**(26):5100-5109 (1997); Obika, et al. "Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar pucker". *Tetrahedron Letters* **38** (50): 8735 (1997); Koizumi, M. "ENA oligonucleotides as therapeutics". *Current opinion in molecular therapeutics* **8** (2): 144-149 (2006); and Abramova et al., "Novel oligonucleotide analogues based on morpholino nucleoside subunits-antisense technologies: new chemical possibilities," *Indian Journal of Chemistry* **48B**:1721-1726 (2009). Alternatively, the polynucleic acid molecule is produced biologically using an expression vector into which a polynucleic acid molecule has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleic acid molecule will be of an antisense orientation to a target polynucleic acid molecule of interest).

[0192] In some embodiments, a polynucleic acid molecule is synthesized via a tandem synthesis methodology, wherein both strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate fragments or strands that hybridize and permit purification of the duplex.

[0193] In some instances, a polynucleic acid molecule is also assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the molecule.

[0194] Additional modification methods for incorporating, for example, sugar, base and phosphate modifications include: Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. *Nature*, 1990, 344, 565-568; Pieken et al. *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman et al. International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, *J. Biol. Chem.*, 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al., U.S. Pat. No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., U.S. Ser. No. 60/082,404 which was filed on Apr. 20, 1998; Karpeisky et al., 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina et al., 1997, *Bioorg. Med. Chem.*, 5, 1999-2010. Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis.

[0195] In some instances, while chemical modification of the polynucleic acid molecule internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate

linkages improves stability, excessive modifications sometimes cause toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages in some cases is minimized. In such cases, the reduction in the concentration of these linkages lowers toxicity, increases efficacy and higher specificity of these molecules.

Polynucleic Acid Molecule Conjugates

[0196] In some embodiments, a polynucleic acid molecule is further conjugated to a polypeptide A for delivery to a site of interest. In some cases, a polynucleic acid molecule is conjugated to a polypeptide A and optionally a polymeric moiety.

[0197] In some instances, at least one polypeptide A is conjugated to at least one B. In some instances, the at least one polypeptide A is conjugated to the at least one B to form an A-B conjugate. In some embodiments, at least one A is conjugated to the 5' terminus of B, the 3' terminus of B, an internal site on B, or in any combinations thereof. In some instances, the at least one polypeptide A is conjugated to at least two B. In some instances, the at least one polypeptide A is conjugated to at least 2, 3, 4, 5, 6, 7, 8, or more B.

[0198] In some embodiments, at least one polypeptide A is conjugated at one terminus of at least one B while at least one C is conjugated at the opposite terminus of the at least one B to form an A-B-C conjugate. In some instances, at least one polypeptide A is conjugated at one terminus of the at least one B while at least one of C is conjugated at an internal site on the at least one B. In some instances, at least one polypeptide A is conjugated directly to the at least one C. In some instances, the at least one B is conjugated indirectly to the at least one polypeptide A via the at least one C to form an A-C-B conjugate.

[0199] In some instances, at least one B and/or at least one C, and optionally at least one D are conjugated to at least one polypeptide A. In some instances, the at least one B is conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the at least one polypeptide A or are conjugated via an internal site to the at least one polypeptide A. In some cases, the at least one C is conjugated either directly to the at least one polypeptide A or indirectly via the at least one B. If indirectly via the at least one B, the at least one C is conjugated either at the same terminus as the at least one polypeptide A on B, at opposing terminus from the at least one polypeptide A, or independently at an internal site. In some instances, at least one additional polypeptide A is further conjugated to the at least one polypeptide A, to B, or to C. In additional instances, the at least one D is optionally conjugated either directly or indirectly to the at least one polypeptide A, to the at least one B, or to the at least one C. If directly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-D-B conjugate or is optionally conjugated to the at least one B and the at least one C to form an A-D-B-C conjugate. In some instances, the at least one D is directly conjugated to the at least one polypeptide A and indirectly to the at least one B and the at least one C to form a D-A-B-C conjugate. If indirectly to the at least one polypeptide A, the at least one D is also optionally conjugated to the at least one B to form an A-B-D conjugate or is optionally conjugated to the at least one B and the at least one C to form

an A-B-D-C conjugate. In some instances, at least one additional D is further conjugated to the at least one polypeptide A, to B, or to C.

[0200] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19A.

[0201] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19B.

[0202] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19C.

[0203] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19D.

[0204] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19E.

[0205] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19F.

[0206] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19G.

[0207] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19H.

[0208] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19I.

[0209] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19J.

[0210] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19K.

[0211] In some embodiments, a polynucleic acid molecule conjugate comprises a construct as illustrated in Fig. 19L.

[0212] The antibody cartoon as illustrated in Fig. 19M is for representation purposes only and encompasses a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof.

Binding Moiety

[0213] In some embodiments, the binding moiety A is a polypeptide. In some instances, the polypeptide is an antibody or its fragment thereof. In some cases, the fragment is a binding fragment. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment

thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab)₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof.

[0214] In some instances, A is an antibody or binding fragment thereof. In some instances, A is a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab₂, F(ab)₃ fragments, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or binding fragment thereof, or a chemically modified derivative thereof. In some instances, A is a humanized antibody or binding fragment thereof. In some instances, A is a murine antibody or binding fragment thereof. In some instances, A is a chimeric antibody or binding fragment thereof. In some instances, A is a monoclonal antibody or binding fragment thereof. In some instances, A is a monovalent Fab'. In some instances, A is a divalent Fab₂. In some instances, A is a single-chain variable fragment (scFv).

[0215] In some embodiments, the binding moiety A is a bispecific antibody or binding fragment thereof. In some instances, the bispecific antibody is a trifunctional antibody or a bispecific mini-antibody. In some cases, the bispecific antibody is a trifunctional antibody. In some instances, the trifunctional antibody is a full length monoclonal antibody comprising binding sites for two different antigens.

[0216] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab₂, F(ab)₃ fragments, bis-scFv, (scFv)₂, diabody, minibody, triabody, tetrabody or a bi-specific T-cell engager (BiTE). In some embodiments, the bi-specific T-cell engager is a fusion protein that contains two single-chain variable fragments (scFvs) in which the two scFvs target epitopes of two different antigens.

[0217] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab₂. In some instances, A is a bispecific F(ab)₃ fragment. In some cases, A is a bispecific bis-scFv. In some cases, A is a bispecific (scFv)₂. In some embodiments, A is a bispecific diabody. In some embodiments, A is a bispecific minibody. In some embodiments, A is a bispecific triabody. In other embodiments, A is a bispecific tetrabody. In other embodiments, A is a bi-specific T-cell engager (BiTE).

[0218] In some embodiments, the binding moiety A is a trispecific antibody. In some instances, the trispecific antibody comprises F(ab)₃ fragments or a triabody. In some instances, A is a trispecific F(ab)₃ fragment. In some cases, A is a triabody. In some embodiments, A is a trispecific antibody as described in Dimas, *et al.*, "Development of a trispecific antibody designed to simultaneously and efficiently target three different antigens on tumor cells," *Mol. Pharmaceutics*, **12**(9): 3490-3501 (2015).

[0219] In some embodiments, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein on a muscle cell. In some cases, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein on a skeletal muscle cell.

[0220] In some embodiments, exemplary antibodies include, but are not limited to, an anti-myosin antibody, an anti-transferrin antibody, and an antibody that recognizes Muscle-Specific kinase (MuSK). In some instances, the antibody is an anti-transferrin (anti-CD71) antibody.

[0221] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) non-specifically. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue or a cysteine residue, in a non-site specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue in a non-site specific manner. In some cases, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a cysteine residue in a non-site specific manner.

[0222] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) in a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue, a cysteine residue, at the 5'-terminus, at the 3'-terminus, an unnatural amino acid, or an enzyme-modified or enzyme-catalyzed residue, via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a cysteine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 5'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 3'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an unnatural amino acid via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an enzyme-modified or enzyme-catalyzed residue via a site-specific manner.

[0223] In some embodiments, one or more polynucleic acid molecule (B) is conjugated to a binding moiety A. In some instances, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 1 polynucleic acid molecule is conjugated to one binding moiety A. In some instances, about 2 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 3 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 4 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 5 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 6 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 7 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 8 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 9 polynucleic acid molecules are

conjugated to one binding moiety A. In some instances, about 10 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 11 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 12 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 13 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 14 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 15 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 16 polynucleic acid molecules are conjugated to one binding moiety A. In some cases, the one or more polynucleic acid molecules are the same. In other cases, the one or more polynucleic acid molecules are different.

[0224] In some embodiments, the number of polynucleic acid molecule (B) conjugated to a binding moiety A forms a ratio. In some instances, the ratio is referred to as a DAR (drug-to-antibody) ratio, in which the drug as referred to herein is the polynucleic acid molecule (B). In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12 or greater.

[0225] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9. In some instances, the DAR ratio of the

polynucleic acid molecule (B) to binding moiety A is about 10. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 13. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 14. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 15. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 16.

[0226] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 12.

[0227] In some instances, a conjugate comprising polynucleic acid molecule (B) and binding moiety A has improved activity as compared to a conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, improved activity results in enhanced biologically relevant functions, e.g., improved stability, affinity, binding, functional activity, and efficacy in treatment or prevention of a disease state. In some instances, the disease state is a result of one or more mutated exons of a gene. In some instances, the conjugate comprising polynucleic acid molecule (B) and binding moiety A results in increased exon skipping of the one or more mutated exons as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A. In some instances, exon skipping is increased by at least or about 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% in the conjugate comprising polynucleic acid molecule (B) and binding moiety A as compared to the conjugate comprising polynucleic acid molecule (B) without a binding moiety A.

[0228] In some embodiments, an antibody or its binding fragment is further modified using conventional techniques known in the art, for example, by using amino acid deletion, insertion, substitution, addition, and/or by recombination and/or any other modification (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. In some instances, the modification further comprises a modification for modulating interaction with Fc receptors. In some instances, the one or more modifications include those described in, for example, International Publication No. WO97/34631, which discloses amino acid residues involved in the interaction between the Fc domain and the FcRn receptor. Methods for introducing such modifications in the nucleic acid sequence underlying the amino acid sequence of an antibody or its binding fragment is well known to the person skilled in the art.

[0229] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0230] In some instances, the term “single-chain” as used herein means that the first and second domains of a bi-specific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

[0231] In some instances, a bispecific single chain antibody construct relates to a construct comprising two antibody derived binding domains. In such embodiments, bi-specific single chain antibody construct is tandem bi-scFv or diabody. In some instances, a scFv contains a VH and VL domain connected by a linker peptide. In some instances, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities.

[0232] In some embodiments, binding to or interacting with as used herein defines a binding/interaction of at least two antigen-interaction-sites with each other. In some instances, antigen-interaction-site defines a motif of a polypeptide that shows the capacity of specific interaction with a specific antigen or a specific group of antigens. In some cases, the binding/interaction is also understood to define a specific recognition. In such cases, specific recognition refers to that the antibody or its binding fragment is capable of specifically interacting with and/or binding to at least two amino acids of each of a target molecule. For example, specific recognition relates to the specificity of the antibody molecule, or to its ability to discriminate between the specific regions of a target molecule. In additional instances, the specific interaction of the antigen-interaction-site with its specific antigen results in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. In further embodiments, the binding is exemplified by the specificity of a “key-lock-principle”. Thus in some instances, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. In such cases, the specific interaction of the antigen-interaction-site with its specific antigen results as well in a simple binding of the site to the antigen.

[0233] In some instances, specific interaction further refers to a reduced cross-reactivity of the antibody or its binding fragment or a reduced off-target effect. For example, the antibody or its binding fragment that bind to the polypeptide/protein of interest but do not or do not essentially bind to any of the other polypeptides are considered as specific for the polypeptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor, for example, the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

Additional Binding Moieties

[0234] In some embodiments, the binding moiety is a plasma protein. In some instances, the plasma protein comprises albumin. In some instances, the binding moiety A is albumin. In some instances,

albumin is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, albumin is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, albumin is conjugated by lysine conjugation to a polynucleic acid molecule.

[0235] In some instances, the binding moiety is a steroid. Exemplary steroids include cholesterol, phospholipids, di- and triacylglycerols, fatty acids, hydrocarbons that are saturated, unsaturated, comprise substitutions, or combinations thereof. In some instances, the steroid is cholesterol. In some instances, the binding moiety is cholesterol. In some instances, cholesterol is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, cholesterol is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, cholesterol is conjugated by lysine conjugation to a polynucleic acid molecule.

[0236] In some instances, the binding moiety is a polymer, including but not limited to polynucleic acid molecule aptamers that bind to specific surface markers on cells. In this instance the binding moiety is a polynucleic acid that does not hybridize to a target gene or mRNA, but instead is capable of selectively binding to a cell surface marker similarly to an antibody binding to its specific epitope of a cell surface marker.

[0237] In some cases, the binding moiety is a peptide. In some cases, the peptide comprises between about 1 and about 3 kDa. In some cases, the peptide comprises between about 1.2 and about 2.8 kDa, about 1.5 and about 2.5 kDa, or about 1.5 and about 2 kDa. In some instances, the peptide is a bicyclic peptide. In some cases, the bicyclic peptide is a constrained bicyclic peptide. In some instances, the binding moiety is a bicyclic peptide (e.g., bicycles from Bicycle Therapeutics).

[0238] In additional cases, the binding moiety is a small molecule. In some instances, the small molecule is an antibody-recruiting small molecule. In some cases, the antibody-recruiting small molecule comprises a target-binding terminus and an antibody-binding terminus, in which the target-binding terminus is capable of recognizing and interacting with a cell surface receptor. For example, in some instances, the target-binding terminus comprising a glutamate urea compound enables interaction with PSMA, thereby, enhances an antibody interaction with a cell that expresses PSMA. In some instances, a binding moiety is a small molecule described in Zhang et al., "A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules," J Am Chem Soc. 132(36): 12711-12716 (2010); or McEnaney, et al., "Antibody-recruiting molecules: an emerging paradigm for engaging immune function in treating human disease," ACS Chem Biol. 7(7): 1139-1151 (2012).

Production of Antibodies or Binding Fragments Thereof

[0239] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0240] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0241] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0242] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0243] In some embodiments, techniques developed for the production of “chimeric antibodies” (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0244] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0245] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0246] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0247] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0248] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1972, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk⁻, hgp⁺ or apt⁻ cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hyg⁺, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147).

Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0249] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0250] In some instances, any method known in the art for purification or analysis of an antibody or antibody conjugates is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Exemplary chromatography methods included, but are not limited to, strong anion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, and fast protein liquid chromatography.

Conjugation Chemistry

[0251] In some embodiments, a polynucleic acid molecule B is conjugated to a binding moiety. In some instances, the binding moiety comprises amino acids, peptides, polypeptides, proteins, antibodies, antigens, toxins, hormones, lipids, nucleotides, nucleosides, sugars, carbohydrates, polymers such as polyethylene glycol and polypropylene glycol, as well as analogs or derivatives of all of these classes of substances. Additional examples of binding moiety also include steroids, such as cholesterol, phospholipids, di- and triacylglycerols, fatty acids, hydrocarbons (e.g., saturated, unsaturated, or contains substitutions), enzyme substrates, biotin, digoxigenin, and polysaccharides. In some instances, the binding moiety is an antibody or binding fragment thereof. In some instances, the polynucleic acid molecule is further conjugated to a polymer, and optionally an endosomolytic moiety.

[0252] In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety by a chemical ligation process. In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a native ligation. In some instances, the conjugation is as described in: Dawson, et al. "Synthesis of proteins by native chemical ligation," *Science* **1994**, 266, 776–779; Dawson, et al. "Modulation of Reactivity in Native Chemical Ligation through the Use of Thiol Additives," *J. Am. Chem. Soc.* **1997**, 119, 4325–4329; Hackeng, et al. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology.," *Proc. Natl. Acad. Sci. USA* **1999**, 96, 10068–10073; or Wu, et al. "Building complex glycopeptides: Development of a cysteine-free native chemical

ligation protocol,” *Angew. Chem. Int. Ed.* **2006**, 45, 4116–4125. In some instances, the conjugation is as described in U.S. Patent No. 8,936,910. In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety either site-specifically or non-specifically via native ligation chemistry.

[0253] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing a “traceless” coupling technology (Philochem). In some instances, the “traceless” coupling technology utilizes an N-terminal 1,2-aminothiol group on the binding moiety which is then conjugate with a polynucleic acid molecule containing an aldehyde group. (*see* Casi *et al.*, “Site-specific traceless coupling of potent cytotoxic drugs to recombinant antibodies for pharmacodelivery,” *JACS* **134**(13): 5887-5892 (2012))

[0254] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an unnatural amino acid incorporated into the binding moiety. In some instances, the unnatural amino acid comprises *p*-acetylphenylalanine (pAcPhe). In some instances, the keto group of pAcPhe is selectively coupled to an alkoxy-amine derivatived conjugating moiety to form an oxime bond. (*see* Axup *et al.*, “Synthesis of site-specific antibody-drug conjugates using unnatural amino acids,” *PNAS* **109**(40): 16101-16106 (2012)).

[0255] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an enzyme-catalyzed process. In some instances, the site-directed method utilizes SMARTag™ technology (Catalent, Inc.). In some instances, the SMARTag™ technology comprises generation of a formylglycine (FGly) residue from cysteine by formylglycine-generating enzyme (FGE) through an oxidation process under the presence of an aldehyde tag and the subsequent conjugation of FGly to an alkylhydrazine-functionalized polynucleic acid molecule via hydrazino-Pictet-Spengler (HIPS) ligation. (*see* Wu *et al.*, “Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag,” *PNAS* **106**(9): 3000-3005 (2009); Agarwal, *et al.*, “A Pictet-Spengler ligation for protein chemical modification,” *PNAS* **110**(1): 46-51 (2013))

[0256] In some instances, the enzyme-catalyzed process comprises microbial transglutaminase (mTG). In some cases, the polynucleic acid molecule is conjugated to the binding moiety utilizing a microbial transglutaminase-catalyzed process. In some instances, mTG catalyzes the formation of a covalent bond between the amide side chain of a glutamine within the recognition sequence and a primary amine of a functionalized polynucleic acid molecule. In some instances, mTG is produced from *Streptomyces mobarensis*. (*see* Strop *et al.*, “Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates,” *Chemistry and Biology* **20**(2) 161-167 (2013))

[0257] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in PCT Publication No. WO2014/140317, which utilizes a sequence-specific transpeptidase.

[0258] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in U.S. Patent Publication Nos. 2015/0105539 and 2015/0105540.

Polymer Conjugating Moiety

[0259] In some embodiments, a polymer moiety C is further conjugated to a polynucleic acid molecule described herein, a binding moiety described herein, or in combinations thereof. In some instances, a polymer moiety C is conjugated a polynucleic acid molecule. In some cases, a polymer moiety C is conjugated to a binding moiety. In other cases, a polymer moiety C is conjugated to a polynucleic acid molecule-binding moiety molecule. In additional cases, a polymer moiety C is conjugated, as illustrated *supra*.

[0260] In some instances, the polymer moiety C is a natural or synthetic polymer, consisting of long chains of branched or unbranched monomers, and/or cross-linked network of monomers in two or three dimensions. In some instances, the polymer moiety C includes a polysaccharide, lignin, rubber, or polyalkylene oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxypolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethylene terephthalate (also known as poly(ethylene terephthalate), PET, PETG, or PETE), polytetramethylene glycol (PTG), or polyurethane as well as mixtures thereof. As used herein, a mixture refers to the use of different polymers within the same compound as well as in reference to block copolymers. In some cases, block copolymers are polymers wherein at least one section of a polymer is build up from monomers of another polymer. In some instances, the polymer moiety C comprises polyalkylene oxide. In some instances, the polymer moiety C comprises PEG. In some instances, the polymer moiety C comprises polyethylene imide (PEI) or hydroxy ethyl starch (HES).

[0261] In some instances, C is a PEG moiety. In some instances, the PEG moiety is conjugated at the 5' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 3' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated at the 3' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 5' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated to an internal site of the polynucleic acid molecule. In some instances, the PEG moiety, the binding moiety, or a combination thereof, are conjugated to an internal site of the polynucleic acid molecule. In some instances, the conjugation is a direct conjugation. In some instances, the conjugation is via native ligation.

[0262] In some embodiments, the polyalkylene oxide (e.g., PEG) is a polydisperse or monodisperse compound. In some instances, polydisperse material comprises disperse distribution of different molecular weight of the material, characterized by mean weight (weight average) size and dispersity. In some instances, the monodisperse PEG comprises one size of molecules. In some embodiments, C is poly- or monodispersed polyalkylene oxide (e.g., PEG) and the indicated molecular weight represents an average of the molecular weight of the polyalkylene oxide, e.g., PEG, molecules.

[0263] In some embodiments, the molecular weight of the polyalkylene oxide (e.g., PEG) is about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000,

4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da.

[0264] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some embodiments, C is PEG and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some instances, the molecular weight of C is about 200 Da. In some instances, the molecular weight of C is about 300 Da. In some instances, the molecular weight of C is about 400 Da. In some instances, the molecular weight of C is about 500 Da. In some instances, the molecular weight of C is about 600 Da. In some instances, the molecular weight of C is about 700 Da. In some instances, the molecular weight of C is about 800 Da. In some instances, the molecular weight of C is about 900 Da. In some instances, the molecular weight of C is about 1000 Da. In some instances, the molecular weight of C is about 1100 Da. In some instances, the molecular weight of C is about 1200 Da. In some instances, the molecular weight of C is about 1300 Da. In some instances, the molecular weight of C is about 1400 Da. In some instances, the molecular weight of C is about 1450 Da. In some instances, the molecular weight of C is about 1500 Da. In some instances, the molecular weight of C is about 1600 Da. In some instances, the molecular weight of C is about 1700 Da. In some instances, the molecular weight of C is about 1800 Da. In some instances, the molecular weight of C is about 1900 Da. In some instances, the molecular weight of C is about 2000 Da. In some instances, the molecular weight of C is about 2100 Da. In some instances, the molecular weight of C is about 2200 Da. In some instances, the molecular weight of C is about 2300 Da. In some instances, the molecular weight of C is about 2400 Da. In some instances, the molecular weight of C is about 2500 Da. In some instances, the molecular weight of C is about 2600 Da. In some instances, the molecular weight of C is about 2700 Da. In some instances, the molecular weight of C is about 2800 Da. In some instances, the molecular weight of C is about 2900 Da. In some instances, the molecular weight of C is about 3000 Da. In some instances, the molecular weight of C is about 3250 Da. In some instances, the molecular weight of C is about 3350 Da. In some instances, the molecular weight of C is about 3500 Da. In some instances, the molecular weight of C is about 3750 Da. In some instances, the molecular weight of C is about 4000 Da. In some instances, the molecular weight of C is about 4250 Da. In some instances, the molecular weight of C is about 4500 Da. In some instances, the molecular weight of C is about 4600 Da. In some instances, the molecular weight of C is about 4750 Da. In some instances, the molecular weight of C is about 5000 Da. In some instances, the molecular weight of C is about 5500 Da. In some instances, the molecular weight of C is about 6000 Da. In some instances, the molecular weight of C is about 6500 Da. In some instances, the molecular weight of C is about 7000 Da. In some

instances, the molecular weight of C is about 7500 Da. In some instances, the molecular weight of C is about 8000 Da. In some instances, the molecular weight of C is about 10,000 Da. In some instances, the molecular weight of C is about 12,000 Da. In some instances, the molecular weight of C is about 20,000 Da. In some instances, the molecular weight of C is about 35,000 Da. In some instances, the molecular weight of C is about 40,000 Da. In some instances, the molecular weight of C is about 50,000 Da. In some instances, the molecular weight of C is about 60,000 Da. In some instances, the molecular weight of C is about 100,000 Da.

[0265] In some embodiments, the polyalkylene oxide (e.g., PEG) comprises discrete ethylene oxide units (e.g., four to about 48 ethylene oxide units). In some instances, the polyalkylene oxide comprising the discrete ethylene oxide units is a linear chain. In other cases, the polyalkylene oxide comprising the discrete ethylene oxide units is a branched chain.

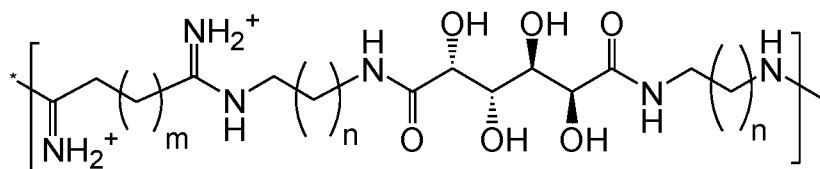
[0266] In some instances, the polymer moiety C is a polyalkylene oxide (e.g., PEG) comprising discrete ethylene oxide units. In some cases, the polymer moiety C comprises between about 4 and about 48 ethylene oxide units. In some cases, the polymer moiety C comprises about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, or about 48 ethylene oxide units.

[0267] In some instances, the polymer moiety C is a discrete PEG comprising, e.g., between about 4 and about 48 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, or about 48 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 4 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 5 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 6 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 7 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 8 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 9 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 10 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 11 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 12 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 13 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 14 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 15 ethylene oxide units. In some cases, the polymer moiety C is a discrete

PEG comprising, e.g., about 16 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 17 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 18 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 19 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 20 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 21 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 22 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 23 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 24 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 25 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 26 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 27 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 28 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 29 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 30 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 31 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 32 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 33 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 34 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 35 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 36 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 37 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 38 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 39 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 40 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 41 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 42 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 43 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 44 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 45 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 46 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 47 ethylene oxide units. In some cases, the polymer moiety C is a discrete PEG comprising, e.g., about 48 ethylene oxide units.

[0268] In some cases, the polymer moiety C is dPEG® (Quanta Biodesign Ltd).

[0269] In some embodiments, the polymer moiety C comprises a cationic mucic acid-based polymer (cMAP). In some instances, cMAP comprises one or more subunit of at least one repeating subunit, and the subunit structure is represented as Formula (V):



Formula V

[0270] wherein m is independently at each occurrence 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 4-6 or 5; and n is independently at each occurrence 1, 2, 3, 4, or 5. In some embodiments, m and n are, for example, about 10.

[0271] In some instances, cMAP is further conjugated to a PEG moiety, generating a cMAP-PEG copolymer, an mPEG-cMAP-PEG_m triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some instances, the PEG moiety is in a range of from about 500 Da to about 50,000 Da. In some instances, the PEG moiety is in a range of from about 500 Da to about 1000 Da, greater than 1000 Da to about 5000 Da, greater than 5000 Da to about 10,000 Da, greater than 10,000 to about 25,000 Da, greater than 25,000 Da to about 50,000 Da, or any combination of two or more of these ranges.

[0272] In some instances, the polymer moiety C is cMAP-PEG copolymer, an mPEG-cMAP-PEG_m triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some cases, the polymer moiety C is cMAP-PEG copolymer. In other cases, the polymer moiety C is an mPEG-cMAP-PEG_m triblock polymer. In additional cases, the polymer moiety C is a cMAP-PEG-cMAP triblock polymer.

[0273] In some embodiments, the polymer moiety C is conjugated to the polynucleic acid molecule, the binding moiety, and optionally to the endosomolytic moiety as illustrated *supra*.

Endosomolytic Moiety

[0274] In some embodiments, a molecule of Formula (I): A-X₁-B-X₂-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic moiety. In some cases, the endosomolytic moiety is a cellular compartmental release component, such as a compound capable of releasing from any of the cellular compartments known in the art, such as the endosome, lysosome, endoplasmic reticulum (ER), golgi apparatus, microtubule, peroxisome, or other vesicular bodies with the cell. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide, an endosomolytic polymer, an endosomolytic lipid, or an endosomolytic small molecule. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide. In other cases, the endosomolytic moiety comprises an endosomolytic polymer.

Endosomolytic Polypeptides

[0275] In some embodiments, a molecule of Formula (I): A-X₁-B-X₂-C, is further conjugated with an endosomolytic polypeptide. In some cases, the endosomolytic polypeptide is a pH-dependent membrane active peptide. In some cases, the endosomolytic polypeptide is an amphipathic polypeptide. In additional cases, the endosomolytic polypeptide is a peptidomimetic. In some instances, the endosomolytic polypeptide comprises INF, melittin, melittin, or their respective derivatives thereof. In some instances, the endosomolytic polypeptide comprises INF or its derivatives thereof. In other cases,

the endosomolytic polypeptide comprises melittin or its derivatives thereof. In additional cases, the endosomolytic polypeptide comprises meucin or its derivatives thereof.

[0276] In some instances, INF7 is a 24 residue polypeptide those sequence comprises CGIFGEIEELIEEGLLENLIDWGNA (SEQ ID NO: 1), or GLFEAIEGFIENGWEGMIDGWYGC (SEQ ID NO: 2). In some instances, INF7 or its derivatives comprise a sequence of: GLFEAIEGFIENGWEGMIWDYGSGSCG (SEQ ID NO: 3), GLFEAIEGFIENGWEGMIDG WYG-(PEG)6-NH₂ (SEQ ID NO: 4), or GLFEAIEGFIENGWEGMIWDYG-SGSC-K(GalNAc)₂ (SEQ ID NO: 5).

[0277] In some cases, melittin is a 26 residue polypeptide those sequence comprises CLIGAILKVLATGLPTLISWIKNKRKQ (SEQ ID NO: 6), or GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 7). In some instances, melittin comprises a polypeptide sequence as described in U.S. Patent No. 8,501,930.

[0278] In some instances, meucin is an antimicrobial peptide (AMP) derived from the venom gland of the scorpion *Mesobuthus eupeus*. In some instances, meucin comprises of meucin-13 those sequence comprises IFGAIAGLLKNIF-NH₂ (SEQ ID NO: 8) and meucin-18 those sequence comprises FFGHLFKLATKIIPSLFQ (SEQ ID NO: 9).

[0279] In some instances, the endosomolytic polypeptide comprises a polypeptide in which its sequence is at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% sequence identity to INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof. In some instances, the endosomolytic moiety comprises INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof.

[0280] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1-5. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2-5. In some cases, the endosomolytic moiety comprises SEQ ID NO: 1. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2-5. In some cases, the endosomolytic moiety consists of SEQ ID NO: 1. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2-5.

[0281] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 6 or 7. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7.

In some cases, the endosomolytic moiety comprises SEQ ID NO: 6. In some cases, the endosomolytic moiety comprises SEQ ID NO: 7. In some cases, the endosomolytic moiety consists of SEQ ID NO: 6. In some cases, the endosomolytic moiety consists of SEQ ID NO: 7.

[0282] In some instances, the endosomolytic moiety is meucin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 8 or 9. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9. In some cases, the endosomolytic moiety comprises SEQ ID NO: 8. In some cases, the endosomolytic moiety comprises SEQ ID NO: 9. In some cases, the endosomolytic moiety consists of SEQ ID NO: 8. In some cases, the endosomolytic moiety consists of SEQ ID NO: 9.

[0283] In some instances, the endosomolytic moiety comprises a sequence as illustrated in **Table 1**.

NAME	ORIGIN	AMINO ACID SEQUENCE	SEQ ID NO:	TYPE
Pep-1	NLS from Simian Virus 40 large antigen and Reverse transcriptase of HIV	KETWWETWWTEWSQPKKKRKV	10	Primary amphipathic
pVEC	VE-cadherin	LLIILRRRRIRKQAHASK	11	Primary amphipathic
VT5	Synthetic peptide	DPKGDPKGVTVTVTVTGKGDP KPD	12	β -sheet amphipathic
C105Y	1-antitrypsin	CSIPPEVKFNKPFVYLI	13	-
Transportan	Galanin and mastoparan	GWTLSAGYLLGKINLKALAALA KKIL	14	Primary amphipathic
TP10	Galanin and mastoparan	AGYLLGKINLKALAALAKKIL	15	Primary amphipathic
MPG	A hydrophobic domain from the fusion sequence of HIV gp41 and NLS of SV40 T antigen	GALFLGFLGAAGSTMGA	16	β -sheet amphipathic
gH625	Glycoprotein gH of HSV type I	HGLASTLTRWAHYNALIRAF	17	Secondary amphipathic α -helical
CADY	PPTG1 peptide	GLWRALWRLLRSLWRLWRA	18	Secondary amphipathic α -helical
GALA	Synthetic peptide	WEAALAEALAEALAEHLAEALAE ALEALAA	19	Secondary amphipathic α -helical
INF	Influenza HA2 fusion peptide	GLFEAIEGFIENGWEGMIDGWYGC	20	Secondary amphipathic α -helical/ pH-dependent

				membrane active peptide
HA2E5-TAT	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDGWYG	21	Secondary amphipathic α -helical/ pH-dependent membrane active peptide
HA2-penetratin	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDGRQIKI WFQNRRMKW KK-amide	22	pH-dependent membrane active peptide
HA-K4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAAGFIENGWEGMIDG-SSKSKK	23	pH-dependent membrane active peptide
HA2E4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFEAIAAGFIENGWEGMIDGGGYC	24	pH-dependent membrane active peptide
H5WYG	HA2 analogue	GLFHAI AHFIHGGWH GLIHGWYG	25	pH-dependent membrane active peptide
GALA-INF3-(PEG)6-NH	INF3 fusion peptide	GLFEAIEGFIENGWEGLAELAEAL EALAA-(PEG)6-NH2	26	pH-dependent membrane active peptide
CM18-TAT11	Cecropin-A-Melittin ₂₋₁₂ (CM ₁₈) fusion peptide	KWKLFKKIGAVLKVLTTG-YGRKKRRQRRR	27	pH-dependent membrane active peptide

[0284] In some cases, the endosomolytic moiety comprises a Bak BH3 polypeptide which induces apoptosis through antagonization of suppressor targets such as Bcl-2 and/or Bcl-x_L. In some instances, the endosomolytic moiety comprises a Bak BH3 polypeptide described in Albarran, *et al.*, “Efficient intracellular delivery of a pro-apoptotic peptide with a pH-responsive carrier,” *Reactive & Functional Polymers* **71**: 261-265 (2011).

[0285] In some instances, the endosomolytic moiety comprises a polypeptide (e.g., a cell-penetrating polypeptide) as described in PCT Publication Nos. WO2013/166155 or WO2015/069587.

Endosomolytic Lipids

[0286] In some embodiments, the endosomolytic moiety is a lipid (e.g., a fusogenic lipid). In some embodiments, a molecule of Formula (I): A-X₁-B-X₂-C, is further conjugated with an endosomolytic lipid (e.g., fusogenic lipid). Exemplary fusogenic lipids include 1,2-dioleoyl-sn-3-phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoyloleoylphosphatidylcholine (POPC), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (Di-Lin), N-methyl(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)methanamine (DLin-k-DMA) and N-methyl-2-(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)ethanamine (XTC).

[0287] In some instances, an endosomolytic moiety is a lipid (e.g., a fusogenic lipid) described in PCT Publication No. WO09/126,933.

Endosomolytic Small Molecules

[0288] In some embodiments, the endosomolytic moiety is a small molecule. In some embodiments, a molecule of Formula (I): A-X₁-B-X₂-C, is further conjugated with an endosomolytic small molecule. Exemplary small molecules suitable as endosomolytic moieties include, but are not limited to, quinine, chloroquine, hydroxychloroquines, amodiaquins (caroquines), amopyroquines, primaquines, mefloquines, nivaquines, halofantrines, quinone imines, or a combination thereof. In some instances, quinoline endosomolytic moieties include, but are not limited to, 7-chloro-4-(4-diethylamino-1-methylbutyl-amino)quinoline (chloroquine); 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutyl-amino)quinoline (hydroxychloroquine); 7-fluoro-4-(4-diethylamino-1-methylbutyl-amino)quinoline; 4-(4-diethylamino-1-methylbutylamino) quinoline; 7-hydroxy-4-(4-diethyl-amino-1-methylbutylamino)quinoline; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-fluoro-4-(4-diethylamino-1-butylamino)quinoline; 4-(4-diethyl-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-butylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-butylamino) quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(4-ethyl-(2-hydroxy-ethyl)-amino-1-methylbutylamino)-quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl-1)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino) quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-

hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino]-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[(4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyl-1,2,3,4-tetrahydroquinoline; 3-chloro-4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethyl-amino)-1-methylbutyl-amino]-6-methoxyquinoline; 3-fluoro-4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 4-(4-hydroxy-alpha, alpha'-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline; 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 3,4-dihydro-1-(2H)-quinolinecarboxyaldehyde; 1,1'-pentamethylene diquinoleinium diiodide; 8-quinolinol sulfate and amino, aldehyde, carboxylic, hydroxyl, halogen, keto, sulfhydryl and vinyl derivatives or analogs thereof. In some instances, an endosomolytic moiety is a small molecule described in Naisbitt et al (1997, J Pharmacol Exp Therapy 280:884-893) and in U.S. Patent No. 5,736,557.

Linkers

[0289] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In other instances, the linker is a non-cleavable linker.

[0290] In some cases, the linker is a non-polymeric linker. A non-polymeric linker refers to a linker that does not contain a repeating unit of monomers generated by a polymerization process. Exemplary non-polymeric linkers include, but are not limited to, C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), homobifunctional cross linkers, heterobifunctional cross linkers, peptide linkers, traceless linkers, self-immolative linkers, maleimide-based linkers, or combinations thereof. In some cases, the non-polymeric linker comprises a C₁-C₆ alkyl group (e.g., a C₅, C₄, C₃, C₂, or C₁ alkyl group), a homobifunctional cross linker, a heterobifunctional cross linker, a peptide linker, a traceless linker, a self-immolative linker, a maleimide-based linker, or a combination thereof. In additional cases, the non-polymeric linker does not comprise more than two of the same type of linkers, e.g., more than two homobifunctional cross linkers, or more than two peptide linkers. In further cases, the non-polymeric linker optionally comprises one or more reactive functional groups.

[0291] In some instances, the non-polymeric linker does not encompass a polymer that is described above. In some instances, the non-polymeric linker does not encompass a polymer encompassed by the polymer moiety C. In some cases, the non-polymeric linker does not encompass a polyalkylene oxide (e.g., PEG). In some cases, the non-polymeric linker does not encompass a PEG.

[0292] In some instances, the linker comprises a homobifunctional linker. Exemplary homobifunctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3'3'-dithiobis(sulfosuccinimidyl propionate) (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N'-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP),

dimethyl suberimidate (DMS), dimethyl-3,3'-dithiobispropionimidate (DTBP), 1,4-di-3'-(2'-pyridyldithio)propionamido)butane (DPDPB), bismaleimido-hexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4'-difluoro-3,3'-dinitrophenylsulfone (DFDNPS), bis- $[\beta$ -(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbonyldiimidazole, o-toluidine, 3,3'-dimethylbenzidine, benzidine, α,α' -p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N'-ethylene-bis(iodoacetamide), or N,N'-hexamethylene-bis(iodoacetamide).

[0293] In some embodiments, the linker comprises a heterobifunctional linker. Exemplary heterobifunctional linker include, but are not limited to, amine-reactive and sulfhydryl cross-linkers such as N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (sulfo-LC-sPDP), succinimidyl-oxycarbonyl- α -methyl- α -(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α -methyl- α -(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacetyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ -maleimidobutyryloxy)succinimide ester (GMBs), N-(γ -maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-(((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (sIAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-(((4-iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-carboxyl-hydrazide-8 (M_2C_2H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), amine-reactive and photoreactive cross-linkers such as N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(p-azidosalicylamido)ethyl-1,3'-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3'-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3'-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(p-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3'-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumarin-3-acetate (sulfo-sAMCA), p-nitrophenyl diazopyruvate (pNPDP), p-nitrophenyl-2-diazo-

3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as 1-(ρ -Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(ρ -azidosalicylamido)butyl]-3'-(2'-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as ρ -azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(ρ -azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as ρ -azidophenyl glyoxal (APG).

[0294] In some instances, the linker comprises a reactive functional group. In some cases, the reactive functional group comprises a nucleophilic group that is reactive to an electrophilic group present on a binding moiety. Exemplary electrophilic groups include carbonyl groups—such as aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide or acid anhydride. In some embodiments, the reactive functional group is aldehyde. Exemplary nucleophilic groups include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0295] In some embodiments, the linker comprises a maleimide group. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC) described above.

[0296] In some embodiments, the maleimide group is a self-stabilizing maleimide. In some instances, the self-stabilizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, *et al.*, “Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates,” *Nat. Biotechnol.* **32**(10):1059-1062 (2014). In some instances, the linker comprises a self-stabilizing maleimide. In some instances, the linker is a self-stabilizing maleimide.

[0297] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, or 6 more amino acid residues. In some instances, the peptide moiety comprises at most 2, 3, 4, 5, 6, 7, or 8 amino acid residues. In some instances, the peptide moiety comprises about 2, about 3, about 4, about 5, or about 6 amino acid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety. In some instances, the peptide moiety comprises Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 14223), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 14224), or Gly-Phe-Leu-Gly (SEQ ID NO: 14225). In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 14223), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit,

Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 14224), or Gly-Phe-Leu-Gly (SEQ ID NO: 14225). In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0298] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[0299] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group. In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[0300] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Patent No. 9,089,614 or PCT Publication No. WO2015038426.

[0301] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of polynucleotide B to the binding moiety A. In some instances, the dendritic type linker comprises PAMAM dendrimers.

[0302] In some embodiments, the linker is a traceless linker or a linker in which after cleavage does not leave behind a linker moiety (e.g., an atom or a linker group) to a binding moiety A, a polynucleotide B, a polymer C, or an endosomolytic moiety D. Exemplary traceless linkers include, but are not limited to, germanium linkers, silicon linkers, sulfur linkers, selenium linkers, nitrogen linkers, phosphorus linkers, boron linkers, chromium linkers, or phenylhydrazide linker. In some cases, the linker is a traceless aryl-triazene linker as described in Hejesen, *et al.*, "A traceless aryl-triazene linker for DNA-directed chemistry," *Org Biomol Chem* **11**(15): 2493-2497 (2013). In some instances, the linker is a traceless linker described in Blaney, *et al.*, "Traceless solid-phase organic synthesis," *Chem. Rev.* **102**: 2607-2024 (2002). In some instances, a linker is a traceless linker as described in U.S. Patent No. 6,821,783.

[0303] In some instances, the linker is a linker described in U.S. Patent Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

[0304] In some embodiments, X₁ and X₂ are each independently a bond or a non-polymeric linker. In some instances, X₁ and X₂ are each independently a bond. In some cases, X₁ and X₂ are each independently a non-polymeric linker.

[0305] In some instances, X_1 is a bond or a non-polymeric linker. In some instances, X_1 is a bond. In some instances, X_1 is a non-polymeric linker. In some instances, the linker is a C_1 - C_6 alkyl group. In some cases, X_1 is a C_1 - C_6 alkyl group, such as for example, a C_5 , C_4 , C_3 , C_2 , or C_1 alkyl group. In some cases, the C_1 - C_6 alkyl group is an unsubstituted C_1 - C_6 alkyl group. As used in the context of a linker, and in particular in the context of X_1 , alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X_1 includes a homobifunctional linker or a heterobifunctional linker described *supra*. In some cases, X_1 includes a heterobifunctional linker. In some cases, X_1 includes sMCC. In other instances, X_1 includes a heterobifunctional linker optionally conjugated to a C_1 - C_6 alkyl group. In other instances, X_1 includes sMCC optionally conjugated to a C_1 - C_6 alkyl group. In additional instances, X_1 does not include a homobifunctional linker or a heterobifunctional linker described *supra*.

[0306] In some instances, X_2 is a bond or a linker. In some instances, X_2 is a bond. In other cases, X_2 is a linker. In additional cases, X_2 is a non-polymeric linker. In some embodiments, X_2 is a C_1 - C_6 alkyl group. In some instances, X_2 is a homobifunctional linker or a heterobifunctional linker described *supra*. In some instances, X_2 is a homobifunctional linker described *supra*. In some instances, X_2 is a heterobifunctional linker described *supra*. In some instances, X_2 comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, X_2 comprises a peptide moiety, such as Val-Cit. In some instances, X_2 comprises a benzoic acid group, such as PABA. In additional instances, X_2 comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, X_2 comprises a mc group. In additional instances, X_2 comprises a mc-val-cit group. In additional instances, X_2 comprises a val-cit-PABA group. In additional instances, X_2 comprises a mc-val-cit-PABA group.

Methods of Use

[0307] Muscle atrophy refers to a loss of muscle mass and/or to a progressive weakening and degeneration of muscles. In some cases, the loss of muscle mass and/or the progressive weakening and degeneration of muscles occurs due to a high rate of protein degradation, a low rate of protein synthesis, or a combination of both. In some cases, a high rate of muscle protein degradation is due to muscle protein catabolism (i.e., the breakdown of muscle protein in order to use amino acids as substrates for gluconeogenesis).

[0308] In one embodiment, muscle atrophy refers to a significant loss in muscle strength. By significant loss in muscle strength is meant a reduction of strength in diseased, injured, or unused muscle tissue in a subject relative to the same muscle tissue in a control subject. In an embodiment, a significant loss in muscle strength is a reduction in strength of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the same muscle tissue in a control subject. In another embodiment, by significant loss in muscle strength is meant a reduction of strength in unused muscle tissue relative to the muscle strength of the same muscle tissue in the same subject prior to a period of nonuse. In an embodiment, a significant loss in muscle strength is a

reduction of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the muscle strength of the same muscle tissue in the same subject prior to a period of nonuse.

[0309] In another embodiment, muscle atrophy refers to a significant loss in muscle mass. By significant loss in muscle mass is meant a reduction of muscle volume in diseased, injured, or unused muscle tissue in a subject relative to the same muscle tissue in a control subject. In an embodiment, a significant loss of muscle volume is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the same muscle tissue in a control subject. In another embodiment, by significant loss in muscle mass is meant a reduction of muscle volume in unused muscle tissue relative to the muscle volume of the same muscle tissue in the same subject prior to a period of nonuse. In an embodiment, a significant loss in muscle tissue is at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, or more relative to the muscle volume of the same muscle tissue in the same subject prior to a period of nonuse. Muscle volume is optionally measured by evaluating the cross-section area of a muscle such as by Magnetic Resonance Imaging (e.g., by a muscle volume/cross-section area (CSA) MRI method).

[0310] Myotonic dystrophy is a multisystemic neuromuscular disease comprising two main types: myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). DM1 is caused by a dominantly inherited “CTG” repeat expansion in the gene DM protein kinase (*DMPK*), which when transcribed into mRNA, forms hairpins that bind with high affinity to the Muscleblind-like (MBNL) family of proteins. MBNL proteins are involved in post-transcriptional splicing and polyadenylation site regulation and loss of the MBNL protein functions lead to downstream accumulation of nuclear foci and increase in mis-splicing events and subsequently to myotonia and other clinical symptoms.

[0311] In some embodiments, described herein is a method of treating muscle atrophy or myotonic dystrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein. In some instances, the muscle atrophy is associated and/or induced by cachexia (e.g., cancer cachexia), denervation, myopathy, motor neuron diseases, diabetes, chronic obstructive pulmonary disease, liver disease, congestive heart failure, chronic renal failure, chronic infection, sepsis, fasting, sarcopenia, glucocorticoid-induced atrophy, disuse, or space flight. In some cases, myotonic dystrophy is DM1.

Cachexia

[0312] Cachexia is an acquired, accelerated loss of muscle caused by an underlying disease. In some instances, cachexia refers to a loss of body mass that cannot be reversed nutritionally, and is generally associated with an underlying disease, such as cancer, COPD, AIDS, heart failure, and the like. When cachexia is seen in a patient with end-stage cancer, it is called “cancer cachexia”. Cancer cachexia affects the majority of patients with advanced cancer and is associated with a reduction in treatment tolerance,

response to therapy, quality of life and duration of survival. In some instances, cancer cachexia is defined as a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass, with or without loss of fat mass, which cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. In some cases, skeletal muscle loss appears to be the most significant event in cancer cachexia. In addition, the classification of cancer cachexia suggests that the diagnostic criteria takes into account not only that weight loss is a signal event of the cachectic process but that the initial reserve of the patient should also be considered, such as low BMI or low level of muscularity.

[0313] In some embodiments, described herein is a method of treating cachexia-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein. In additional embodiments, described herein is a method of treating cancer cachexia-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein.

Denervation

[0314] Denervation is an injury to the peripheral motoneurons with a partial or complete interruption of the nerve fibers between an organ and the central nervous system, resulting in an interruption of nerve conduction and motoneuron firing which, in turn, prevents the contractability of skeletal muscles. This loss of nerve function is either localized or generalized due to the loss of an entire motor neuron unit. The resulting inability of skeletal muscles to contract leads to muscle atrophy. In some instances, denervation is associated with or as a result of degenerative, metabolic, or inflammatory neuropathy (e.g., Guillain-Barre syndrome, peripheral neuropathy, or exposure to environmental toxins or drugs). In additional instances, denervation is associated with a physical injury, e.g., a surgical procedure.

[0315] In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by denervation in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by denervation in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Myopathy

[0316] Myopathy is an umbrella term that describes a disease of the muscle. In some instances, myopathy includes myotonia; congenital myopathy such as nemaline myopathy, multi/minicore myopathy and myotubular (centronuclear) myopathy; mitochondrial myopathy; familial periodic paralysis; inflammatory myopathy; metabolic myopathy, for example, caused by a glycogen or lipid storage disease; dermatomyositis; polymyositis; inclusion body myositis; myositis ossificans; rhabdomyolysis; and myoglobinurias. In some instances, myopathy is caused by a muscular dystrophy syndrome, such as Duchenne, Becker, myotonic, fascioscapulohumeral, Emery-Dreifuss,

oculopharyngeal, scapulohumeral, limb girdle, Fukuyama, a congenital muscular dystrophy, or hereditary distal myopathy. In some instances, myopathy is caused by myotonic dystrophy (e.g., myotonic dystrophy type 1 or DM1). In some instances, myopathy is caused by DM1.

[0317] In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by myopathy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by myopathy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Motor Neuron Diseases

[0318] Motor neuron disease (MND) encompasses a neurological disorder that affects motor neurons, cells that control voluntary muscles of the body. Exemplary motor neuron diseases include, but are not limited to, adult motor neuron diseases, infantile spinal muscular atrophy, amyotrophic lateral sclerosis, juvenile spinal muscular atrophy, autoimmune motor neuropathy with multifocal conductor block, paralysis due to stroke or spinal cord injury, or skeletal immobilization due to trauma.

[0319] In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by a motor neuron disease in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by a motor neuron disease in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Diabetes

[0320] Diabetes (diabetes mellitus, DM) comprises type 1 diabetes, type 2 diabetes, type 3 diabetes, type 4 diabetes, double diabetes, latent autoimmune diabetes (LAD), gestational diabetes, neonatal diabetes mellitus (NDM), maturity onset diabetes of the young (MODY), Wolfram syndrome, Alström syndrome, prediabetes, or diabetes insipidus. Type 2 diabetes, also called non-insulin dependent diabetes, is the most common type of diabetes accounting for 95% of all diabetes cases. In some instances, type 2 diabetes is caused by a combination of factors, including insulin resistance due to pancreatic beta cell dysfunction, which in turn leads to high blood glucose levels. In some cases, increased glucagon levels stimulate the liver to produce an abnormal amount of unneeded glucose, which contributes to high blood glucose levels.

[0321] Type 1 diabetes, also called insulin-dependent diabetes, comprises about 5% to 10% of all diabetes cases. Type 1 diabetes is an autoimmune disease where T cells attack and destroy insulin-producing beta cells in the pancreas. In some embodiments, Type 1 diabetes is caused by genetic and environmental factors.

[0322] Type 4 diabetes is a recently discovered type of diabetes affecting about 20% of diabetic patients age 65 and over. In some embodiments, type 4 diabetes is characterized by age-associated insulin resistance.

[0323] In some embodiments, type 3 diabetes is used as a term for Alzheimer's disease resulting in insulin resistance in the brain.

[0324] In some embodiments, described herein is a method of treating diabetes-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein. In additional embodiments, described herein is a method of treating cancer diabetes-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein.

Chronic Obstructive Pulmonary Disease

[0325] Chronic obstructive pulmonary disease (COPD) is a type of obstructive lung disease characterized by long-term breathing problems and poor airflow. Chronic bronchitis and emphysema are two different types of COPD. In some instances, described herein is a method of treating muscle atrophy associated with or induced by COPD (e.g., chronic bronchitis or emphysema) in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by COPD (e.g., chronic bronchitis or emphysema) in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Liver Diseases

[0326] Liver disease (or hepatic disease) comprises fibrosis, cirrhosis, hepatitis, alcoholic liver disease, hepatic steatosis, a hereditary disease, or primary liver cancer. In some instances, described herein is a method of treating muscle atrophy associated with or induced by a liver disease in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by a liver disease in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Congestive Heart Failure

[0327] Congestive heart failure is a condition in which the heart is unable to pump enough blood and oxygen to the body's tissues. In some instances, described herein is a method of treating muscle atrophy associated with or induced by congestive heart failure in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by

congestive heart failure in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Chronic Renal Failure

[0328] Chronic renal failure or chronic kidney disease is a condition characterized by a gradual loss of kidney function over time. In some instances, described herein is a method of treating muscle atrophy associated with or induced by a chronic renal failure in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by a chronic renal failure in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Chronic Infections

[0329] In some embodiments, chronic infection such as AIDS further leads to muscle atrophy. In some instances, described herein is a method of treating muscle atrophy associated with or induced by a chronic infection (e.g., AIDS) in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by a chronic infection (e.g., AIDS) in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Sepsis

[0330] Sepsis is an immune response to an infection leading to tissue damage, organ failure, and/or death. In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by sepsis in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by sepsis in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Fasting

[0331] Fasting is a willing abstinence or reduction from some or all food, drinks, or both, for a period of time. In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by fasting in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by fasting in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Sarcopenia

[0332] Sarcopenia is the continuous process of muscle atrophy in the course of regular aging that is characterized by a gradual loss of muscle mass and muscle strength over a span of months and years. A regular aging process means herein an aging process that is not influenced or accelerated by the presence of disorders and diseases which promote skeletomuscular neurodegeneration.

[0333] In some embodiments, described herein is a method of treating muscle atrophy associated with or induced by sarcopenia in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating muscle atrophy associated with or induced by sarcopenia in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Glucocorticoid-associated Muscle Atrophy

[0334] In some embodiments, treatment with a glucocorticoid further results in muscle atrophy. Exemplary glucocorticoids include, but are not limited to, cortisol, dexamethasone, betamethasone, prednisone, methylprednisolone, and prednisolone.

[0335] In some embodiments, described herein is a method of treating glucocorticoid-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating glucocorticoid-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Disuse-associated Muscle Atrophy

[0336] Disuse-associated muscle atrophy results when a limb is immobilized (e.g., due to a limb or joint fracture or an orthopedic surgery such as a hip or knee replacement surgery). As used herein, “immobilization” or “immobilized” refers to the partial or complete restriction of movement of limbs, muscles, bones, tendons, joints, or any other body parts for an extended period of time (e.g., for 2 days, 3 days, 4 days, 5 days, 6 days, a week, two weeks, or more). In some instances, a period of immobilization includes short periods or instances of unrestrained movement, such as to bathe, to replace an external device, or to adjust an external device. Limb immobilization is optionally carried out by any variety of external devices including, but are not limited to, braces, slings, casts, bandages, and splints (any of which is optionally composed of hard or soft material including but not limited to cloth, gauze, fiberglass, plastic, plaster, or metal), as well as any variety of internal devices including surgically implanted splints, plates, braces, and the like. In the context of limb immobilization, the restriction of movement involves a single joint or multiple joints (e.g., simple joints such as the shoulder joint or hip joint, compound joints such as the radiocarpal joint, and complex joints such as the knee joint, including

but not limited to one or more of the following: articulations of the hand, shoulder joints, elbow joints, wrist joints, auxiliary articulations, sternoclavicular joints, vertebral articulations, temporomandibular joints, sacroiliac joints, hip joints, knee joints, and articulations of the foot), a single tendon or ligament or multiple tendons or ligaments (e.g., including but not limited to one or more of the following: the anterior cruciate ligament, the posterior cruciate ligament, rotator cuff tendons, medial collateral ligaments of the elbow and knee, flexor tendons of the hand, lateral ligaments of the ankle, and tendons and ligaments of the jaw or temporomandibular joint), a single bone or multiple bones (e.g., including but not limited to one or more of the following: the skull, mandible, clavicle, ribs, radius, ulna, humerus, pelvis, sacrum, femur, patella, phalanges, carpals, metacarpals, tarsals, metatarsals, fibula, tibia, scapula, and vertebrae), a single muscle or multiple muscles (e.g., including but not limited to one or more of the following: latissimus dorsi, trapezius, deltoid, pectorals, biceps, triceps, external obliques, abdominals, gluteus maximus, hamstrings, quadriceps, gastrocnemius, and diaphragm); a single limb or multiple limbs one or more of the arms and legs), or the entire skeletal muscle system or portions thereof (e.g., in the case of a full body cast or spica cast).

[0337] In some embodiments, described herein is a method of treating disuse-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule described herein. In other embodiments, described herein is a method of treating disuse-associated muscle atrophy in a subject, which comprises administering to the subject a therapeutically effective amount of a polynucleic acid molecule conjugate described herein.

Pharmaceutical Formulation

[0338] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular), oral, intranasal, buccal, rectal, or transdermal administration routes. In some instances, the pharmaceutical composition described herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular, intra-arterial, intraperitoneal, intrathecal, intracerebral, intracerebroventricular, or intracranial) administration. In other instances, the pharmaceutical composition described herein is formulated for oral administration. In still other instances, the pharmaceutical composition described herein is formulated for intranasal administration.

[0339] In some embodiments, the pharmaceutical formulations include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0340] In some instances, the pharmaceutical formulation includes multiparticulate formulations. In some instances, the pharmaceutical formulation includes nanoparticle formulations. In some instances, nanoparticles comprise cMAP, cyclodextrin, or lipids. In some cases, nanoparticles comprise solid lipid

[0341] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0343] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0345] In some embodiments, the pharmaceutical formulation comprises a delivery vector, e.g., a recombinant vector, the delivery of the polynucleic acid molecule into cells. In some instances, the recombinant vector is DNA plasmid. In other instances, the recombinant vector is a viral vector. Exemplary viral vectors include vectors derived from adeno-associated virus, retrovirus, adenovirus, or alphavirus. In some instances, the recombinant vectors capable of expressing the polynucleic acid molecules provide stable expression in target cells. In additional instances, viral vectors are used that provide for transient expression of polynucleic acid molecules.

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properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrolidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphatidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999).

[0347] In some instances, the pharmaceutical formulation further includes pH adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0348] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0349] In some instances, the pharmaceutical formulation further includes diluent which are used to stabilize compounds because they provide a more stable environment. Salts dissolved in buffered solutions (which also provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel[®]; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac[®] (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0350] In some cases, the pharmaceutical formulation includes disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term "disintegrate" include both the

dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel[®], or sodium starch glycolate such as Promogel[®] or Explotab[®], a cellulose such as a wood product, methylcrystalline cellulose, e.g., Avicel[®], Avicel[®] PH101, Avicel[®] PH102, Avicel[®] PH105, Elcema[®] P100, Emcocel[®], Vivacel[®], Ming Tia[®], and Solka-Floc[®], methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol[®]), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum[®] HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0351] In some instances, the pharmaceutical formulation includes filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0352] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumarate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex[®]), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet[®], boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax[™], sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid[™], Cab-O-Sil[®], a starch such as corn starch, silicone oil, a surfactant, and the like.

[0353] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers also function as dispersing agents or wetting agents.

[0354] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium docosate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcitol, propylene glycol, and dimethyl isosorbide and the like.

[0355] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0356] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol has a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0357] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic[®] (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0358] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0359] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium docussate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Therapeutic Regimens

[0360] In some embodiments, the pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

[0361] In some embodiments, one or more pharmaceutical compositions are administered simultaneously, sequentially, or at an interval period of time. In some embodiments, one or more

pharmaceutical compositions are administered simultaneously. In some cases, one or more pharmaceutical compositions are administered sequentially. In additional cases, one or more pharmaceutical compositions are administered at an interval period of time (e.g., the first administration of a first pharmaceutical composition is on day one followed by an interval of at least 1, 2, 3, 4, 5, or more days prior to the administration of at least a second pharmaceutical composition).

[0362] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simultaneously. In some cases, the two or more different pharmaceutical compositions are coadministered sequentially without a gap of time between administrations. In other cases, the two or more different pharmaceutical compositions are coadministered sequentially with a gap of about 0.5 hour, 1 hour, 2 hour, 3 hour, 12 hours, 1 day, 2 days, or more between administrations.

[0363] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously; alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0364] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, can be reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

[0365] In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0366] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages is altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[0367] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index and it is expressed as the ratio between LD50 and ED50. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Article of Manufacture

[0368] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more of the compositions and methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0369] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0370] For example, the container(s) include target nucleic acid molecule described herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0371] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[0372] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0373] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a

governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Certain Terminology

[0374] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

[0375] As used herein, ranges and amounts can be expressed as “about” a particular value or range. About also includes the exact amount. Hence “about 5 μ L” means “about 5 μ L” and also “5 μ L.” Generally, the term “about” includes an amount that would be expected to be within experimental error.

[0376] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0377] As used herein, the terms “individual(s),” “subject(s)” and “patient(s)” mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician’s assistant, an orderly or a hospice worker).

[0378] The term “therapeutically effective amount” relates to an amount of a polynucleic acid molecule conjugate that is sufficient to provide a desired therapeutic effect in a mammalian subject. In some cases, the amount is single or multiple dose administration to a patient (such as a human) for treating, preventing, preventing the onset of, curing, delaying, reducing the severity of, ameliorating at least one symptom of a disorder or recurring disorder, or prolonging the survival of the patient beyond that expected in the absence of such treatment. Naturally, dosage levels of the particular polynucleic acid molecule conjugate employed to provide a therapeutically effective amount vary in dependence of the type of injury, the age, the weight, the gender, the medical condition of the subject, the severity of the condition, the route of administration, and the particular inhibitor employed. In some instances, therapeutically effective amounts of polynucleic acid molecule conjugate, as described herein, is estimated initially from cell culture and animal models. For example, IC₅₀ values determined in cell

culture methods optionally serve as a starting point in animal models, while IC₅₀ values determined in animal models are optionally used to find a therapeutically effective dose in humans.

[0379] Skeletal muscle, or voluntary muscle, is generally anchored by tendons to bone and is generally used to effect skeletal movement such as locomotion or in maintaining posture. Although some control of skeletal muscle is generally maintained as an unconscious reflex (e.g., postural muscles or the diaphragm), skeletal muscles react to conscious control. Smooth muscle, or involuntary muscle, is found within the walls of organs and structures such as the esophagus, stomach, intestines, uterus, urethra, and blood vessels.

[0380] Skeletal muscle is further divided into two broad types: Type I (or “slow twitch”) and Type II (or “fast twitch”). Type I muscle fibers are dense with capillaries and are rich in mitochondria and myoglobin, which gives Type I muscle tissue a characteristic red color. In some cases, Type I muscle fibers carries more oxygen and sustain aerobic activity using fats or carbohydrates for fuel. Type I muscle fibers contract for long periods of time but with little force. Type II muscle fibers are further subdivided into three major subtypes (IIa, IIx, and IIb) that vary in both contractile speed and force generated. Type II muscle fibers contract quickly and powerfully but fatigue very rapidly, and therefore produce only short, anaerobic bursts of activity before muscle contraction becomes painful.

[0381] Unlike skeletal muscle, smooth muscle is not under conscious control.

[0382] Cardiac muscle is also an involuntary muscle but more closely resembles skeletal muscle in structure and is found only in the heart. Cardiac and skeletal muscles are striated in that they contain sarcomeres that are packed into highly regular arrangements of bundles. By contrast, the myofibrils of smooth muscle cells are not arranged in sarcomeres and therefore are not striated.

[0383] Muscle cells encompass any cells that contribute to muscle tissue. Exemplary muscle cells include myoblasts, satellite cells, myotubes, and myofibril tissues.

[0384] As used here, muscle force is proportional to the cross-sectional area (CSA), and muscle velocity is proportional to muscle fiber length. Thus, comparing the cross-sectional areas and muscle fibers between various kinds of muscles is capable of providing an indication of muscle atrophy. Various methods are known in the art to measure muscle strength and muscle weight, see, for example, “Musculoskeletal assessment: Joint range of motion and manual muscle strength” by Hazel M. Clarkson, published by Lippincott Williams & Wilkins, 2000. The production of tomographic images from selected muscle tissues by computed axial tomography and sonographic evaluation are additional methods of measuring muscle mass.

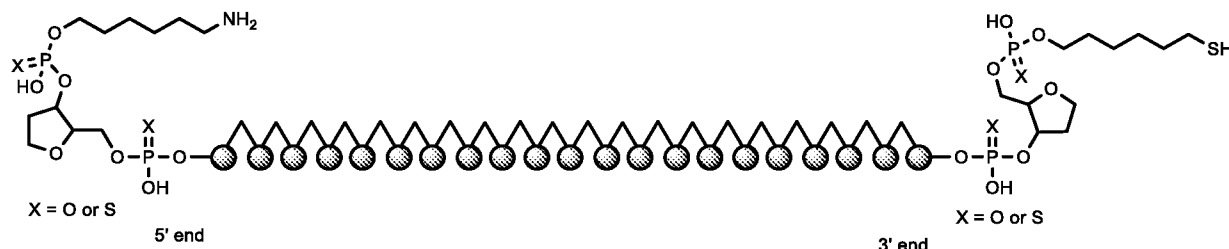
EXAMPLES

[0385] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

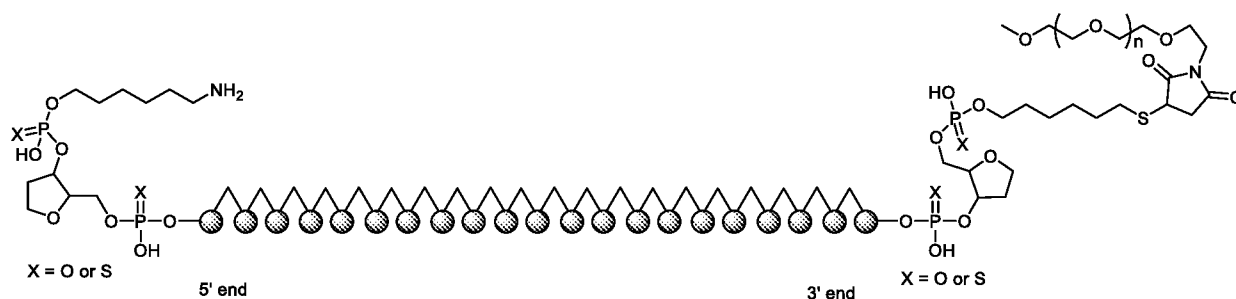
Example 1. siRNA sequences and synthesis

[0386] All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded

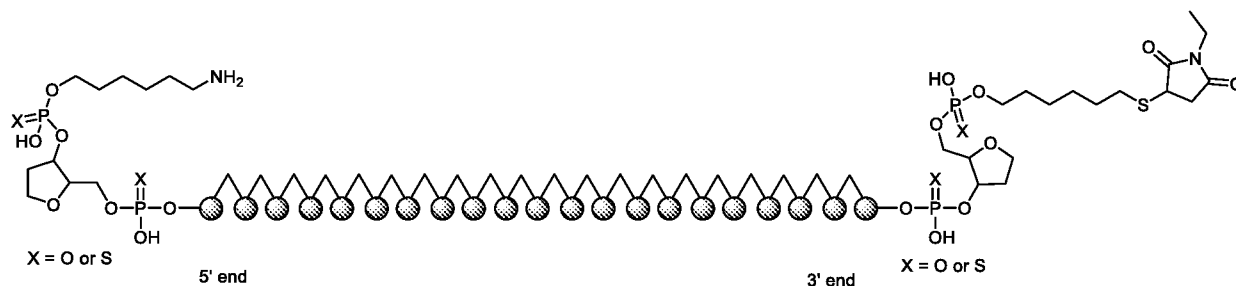
siRNA. All the siRNA passenger strand contains conjugation handles in different formats, C6-NH₂ and/or C6-SH, one at each end of the strand. The conjugation handle or handles were connected to siRNA passenger strand via inverted abasic phosphodiester or phosphorothioate. Below are representative structures of the formats used in the *in vivo* experiments.



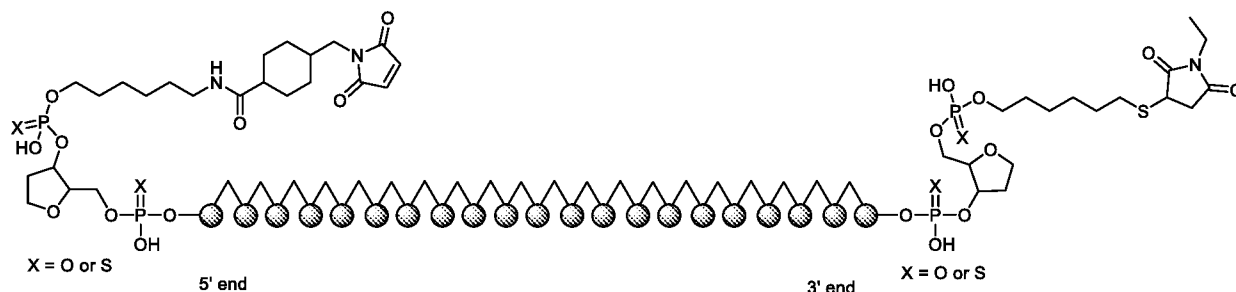
[0387] A representative structure of siRNA with C6-NH₂ conjugation handle at the 5' end and C6-SH at 3' end of the passenger strand.



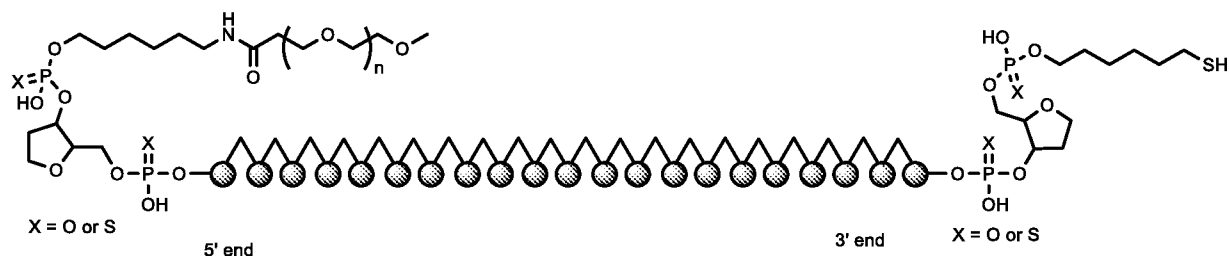
[0388] A representative structure of siRNA passenger strand with C6-NH₂ conjugation handle at the 5' end and C6-S-PEG at 3' end.



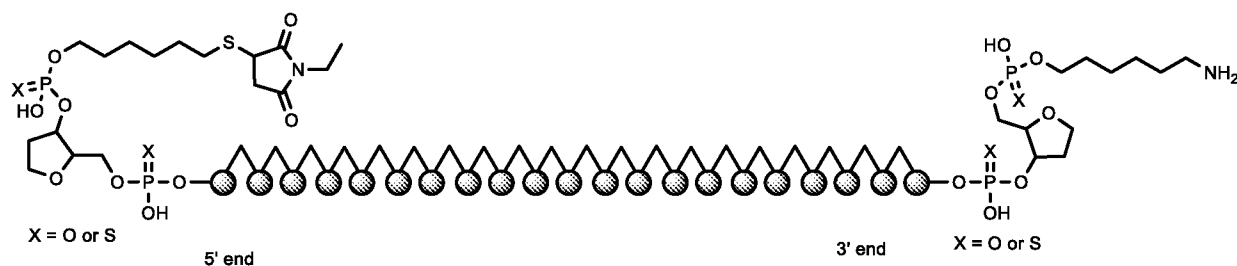
[0389] A representative structure of siRNA passenger strand with C6-NH₂ conjugation handle at the 5' end and C6-S-NEM at 3' end.



[0390] A representative structure of siRNA passenger strand with C6-N-SMCC conjugation handle at the 5' end and C6-S-NEM at 3' end.



[0391] A representative structure of siRNA passenger strand with PEG at the 5' end and C6-SH at 3' end.



[0392] A representative structure of siRNA passenger strand with C6-S-NEM at the 5' end and C6-NH₂ conjugation handle at 3' end.

Cholesterol-myostatin siRNA conjugate

[0393] The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 1169 for the mouse mRNA transcript for MSTN (UUAUUAUUUGUUCUUUGCCUU; SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained a 5' cholesterol which was conjugated as described below in Fig. 1.

Example 2. General Experimental Protocol and Materials

[0394] Animals

[0395] All animal studies were conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the "Guide for the Care and Use of Laboratory Animals" (National Research Council publication, 8th Ed., revised in 2011). All mice were obtained from either Charles River Laboratories or Harlan Laboratories.

[0396] Wild type CD-1 mice (4-6 week old) were dosed via intravenous (iv) injection with the indicated ASCs (or antibody-nucleic acid conjugate) and doses.

[0397] Anti-transferrin receptor antibody

[0398] Anti-mouse transferrin receptor antibody or CD71 mAb is a rat IgG2a subclass monoclonal antibody that binds mouse CD71 or mouse transferrin receptor 1 (mTfR1). The antibody was produced by BioXcell and it is commercially available (Catalog # BE0175).

[0399] **IgG2a isotype control antibody**

[0400] Rat IgG2a isotype control antibody was purchased from BioXcell (Clone 2A3, Catalog # BE0089) and this antibody is specific to trinitrophenol and does not have any known antigens in mouse.

[0401] **Anti-EGFR antibody**

[0402] Anti-EGFR antibody is a fully human IgG1 κ monoclonal antibody directed against the human epidermal growth factor receptor (EGFR). It is produced in the Chinese Hamster Ovary cell line DJT33, which has been derived from the CHO cell line CHO-K1SV by transfection with a GS vector carrying the antibody genes derived from a human anti-EGFR antibody producing hybridoma cell line (2F8). Standard mammalian cell culture and purification technologies are employed in the manufacturing of anti-EGFR antibody.

[0403] The theoretical molecular weight (MW) of anti-EGFR antibody without glycans is 146.6 kDa. The experimental MW of the major glycosylated isoform of the antibody is 149 kDa as determined by mass spectrometry. Using SDS-PAGE under reducing conditions the MW of the light chain was found to be approximately 25 kDa and the MW of the heavy chain to be approximately 50 kDa. The heavy chains are connected to each other by two inter-chain disulfide bonds, and one light chain is attached to each heavy chain by a single inter-chain disulfide bond. The light chain has two intra-chain disulfide bonds and the heavy chain has four intra-chain disulfide bonds. The antibody is N-linked glycosylated at Asn305 of the heavy chain with glycans composed of N-acetyl-glucosamine, mannose, fucose and galactose. The predominant glycans present are fucosylated bi-antennary structures containing zero or one terminal galactose residue.

[0404] The charged isoform pattern of the IgG1 κ antibody has been investigated using imaged capillary IEF, agarose IEF and analytical cation exchange HPLC. Multiple charged isoforms are found, with the main isoform having an isoelectric point of approximately 8.7.

[0405] The major mechanism of action of anti-EGFR antibody is a concentration dependent inhibition of EGF-induced EGFR phosphorylation in A431 cancer cells. Additionally, induction of antibody-dependent cell-mediated cytotoxicity (ADCC) at low antibody concentrations has been observed in pre-clinical cellular in vitro studies.

[0406] **In vitro evaluation of siRNA potency and efficacy**

[0407] C2C12 myoblasts (ATCC) were grown in DMEM supplemented with 10% v/v FBS. For transfection, cells were plated at a density of 10,000 cells/well in 24-well plates, and transfected within 24 hours. C2C12 myotubes were generated by incubating confluent C2C12 myoblast cultures in DMEM supplemented with 2% v/v horse serum for 3-4 days. During and after differentiation the medium was changed daily. Pre-differentiated primary human skeletal muscle cells were obtained from ThermoFisher and plated in DMEM with 2% v/v horse serum according to recommendations by the manufacturer. Human SJCRH30 rhabdomyosarcoma myoblasts (ATCC) were grown in DMEM supplemented with

10% v/v heat-inactivated fetal calf serum, 4.5 mg/mL glucose, 4 mM L-glutamine, 10 mM HEPES, and 1 mM sodium pyruvate. For transfections cells were plated in a density of 10,000-20,000 cells/well in 24-well plates and transfected within 24 hours. All cells were transfected with various concentrations of the siRNAs (0.0001-100 nM; 10-fold dilutions) using RNAiMax (ThermoFisher) according to the recommendation by the manufacturer. Transfected cells were incubated in 5% CO₂ at 37°C for 2 days, then washed with PBS, and harvested in 300 µl TRIzol (ThermoFisher) and stored at -80°C. RNA was prepared using a ZYMO 96-well RNA kit (ThermoFisher) and relative RNA expression levels quantified by RT-qPCR using commercially available TaqMan probes (LifeTechnology). Expression data were analyzed using the $\Delta\Delta$ CT method normalized to Ppib expression, and are presented as % KD relative to mock-transfected cells. Data were analyzed by nonlinear regression using a 3 parameter dose response inhibition function (GraphPad Prism 7.02). All knock down results present the maximal observed KD under these experimental conditions.

[0408] Myostatin ELISA

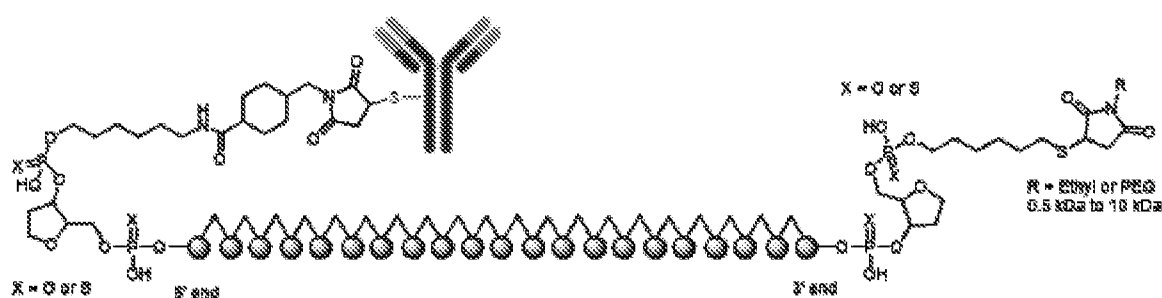
[0409] Myostatin protein in plasma was quantified using the GDF-8 (Myostatin) Quantikine ELISA Immunoassay (part# DGDF80) from R&D Systems according to the manufacturer's instructions.

[0410] RISC loading assay

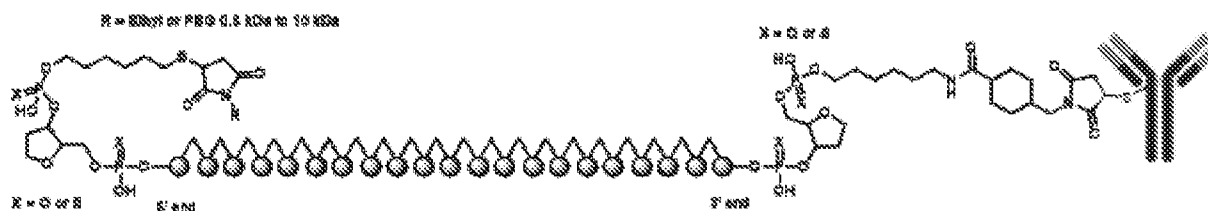
[0411] Specific immunoprecipitation of the RISC from tissue lysates and quantification of small RNAs in the immunoprecipitates were determined by stem-loop PCR, using an adaptation of the assay described by Pei *et al.* Quantitative evaluation of siRNA delivery in vivo. RNA (2010), 16:2553–2563.

Example 3. Conjugate synthesis

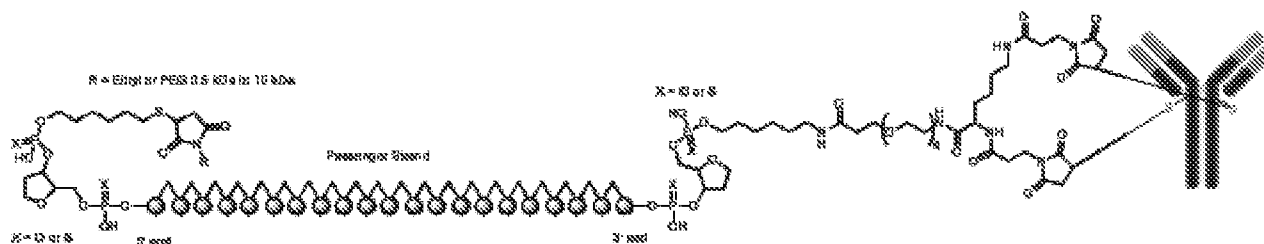
[0412] The following structures illustrate exemplary A-X₁-B-X₂-Y (Formula I) architectures described herein.



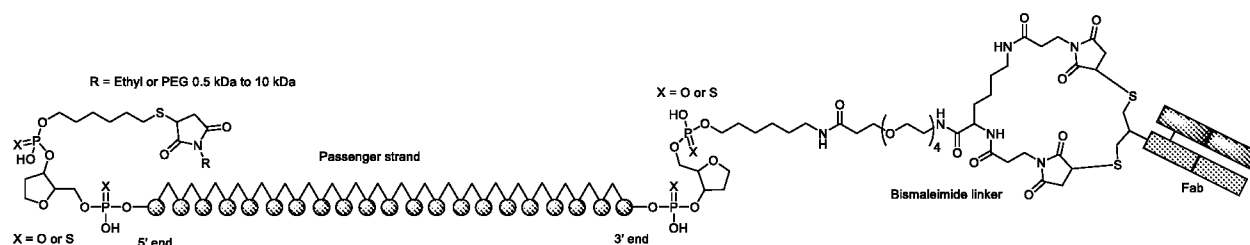
[0413] **Architecture-1:** Antibody-Cys-SMCC-5'-passenger strand. This conjugate was generated by antibody inter-chain cysteine conjugation to maleimide (SMCC) at the 5' end of passenger strand.



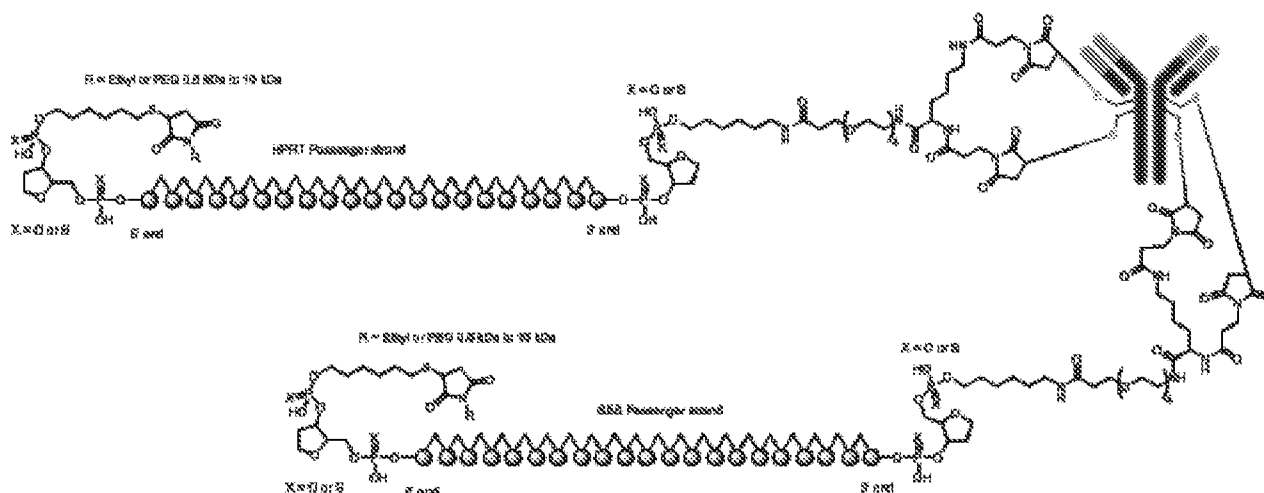
[0414] **Architecture-2:** Antibody-Cys-SMCC-3'-Passenger strand. This conjugate was generated by antibody inter-chain cysteine conjugation to maleimide (SMCC) at the 3' end of passenger strand.



[0415] **ASC Architecture-3:** Antibody-Cys-bisMal-3'-Passenger strand. This conjugate was generated by antibody inter-chain cysteine conjugation to bismaleimide (bisMal) linker at the 3' end of passenger strand.

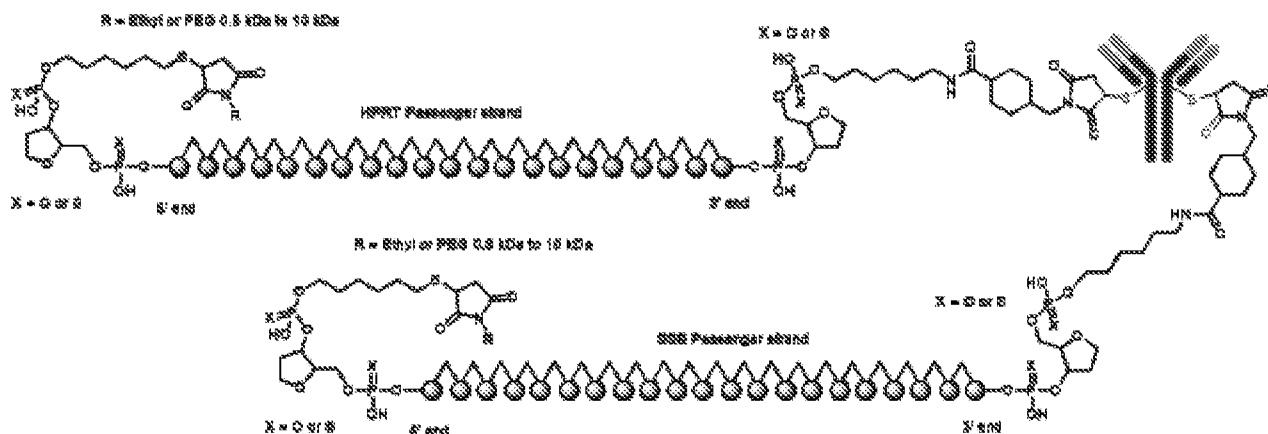


[0416] **ASC Architecture-4:** A model structure of the Fab-Cys-bisMal-3'-Passenger strand. This conjugate was generated by Fab inter-chain cysteine conjugation to bismaleimide (bisMal) linker at the 3' end of passenger strand.



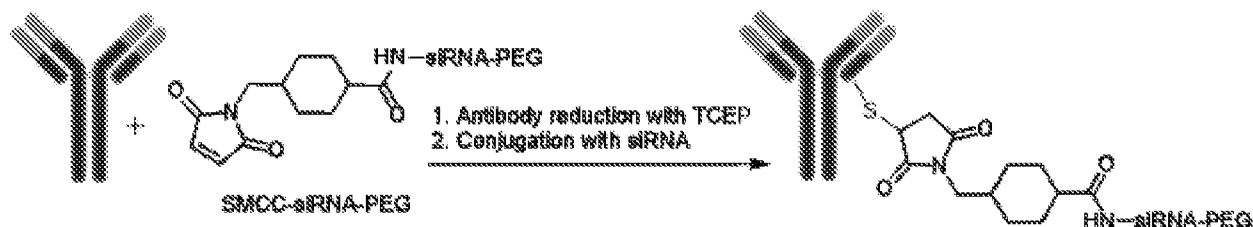
[0417] **ASC Architecture-5:** A model structure of the antibody siRNA conjugate with two different siRNAs attached to one antibody molecule. This conjugate was generated by conjugating a mixture of

SSB and HPRT siRNAs to the reduced mAb inter-chain cysteines to bismaleimide (bisMal) linker at the 3' end of passenger strand of each siRNA.



[0418] **ASC Architecture-6:** A model structure of the antibody siRNA conjugate with two different siRNAs attached. This conjugate was generated by conjugating a mixture of SSB and HPRT siRNAs to the reduced mAb inter-chain cysteines to maleimide (SMCC) linker at the 3' end of passenger strand of each siRNA.

Example 3.1 Antibody siRNA Conjugate Synthesis using SMCC linker



Synthesis scheme-1: Antibody-Cys-SMCC-siRNA-PEG conjugates via antibody cysteine conjugation

[0419] Step 1: Antibody interchain disulfide reduction with TCEP

[0420] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 10 mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was buffer exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of SMCC-C6-siRNA or SMCC-C6-siRNA-C6-NHCO-PEG-XkDa (2 equivalents) (X= 0.5 kDa to 10 kDa) in pH 7.4 PBS containing 5 mM EDTA at RT and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0421] Step 2: Purification

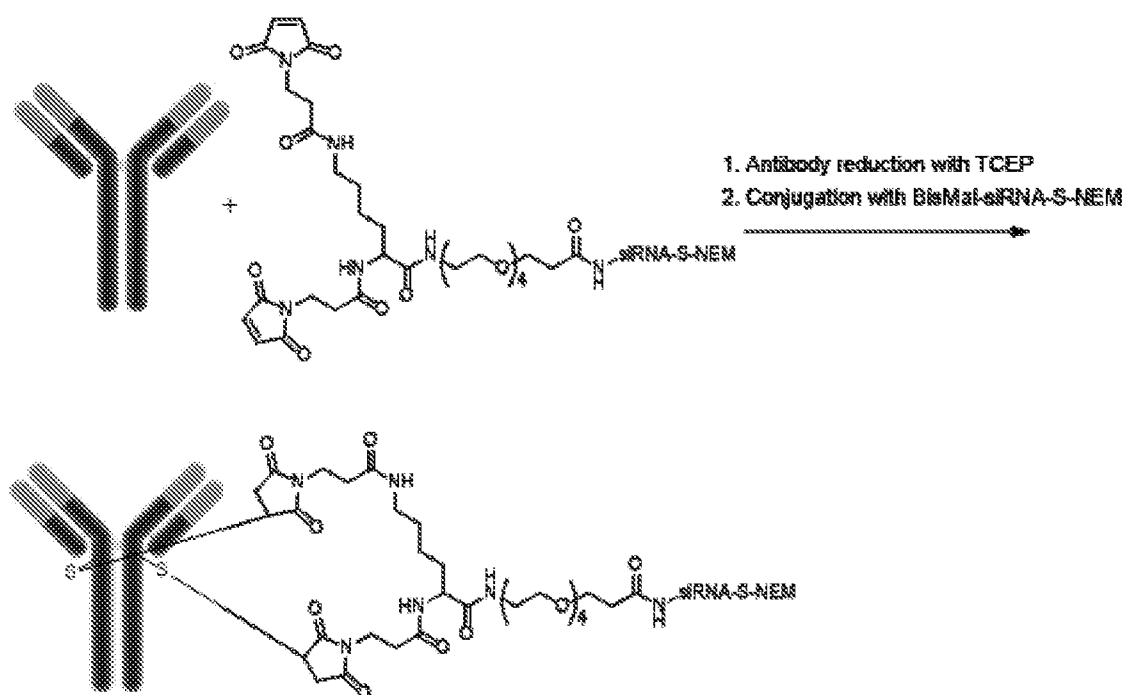
[0422] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1 as described in Example 3.4. Fractions containing DAR1 and DAR>2 antibody-siRNA-PEG conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0423] Step-3: Analysis of the purified conjugate

[0424] The isolated conjugates were characterized by SEC, SAX chromatography and SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3. Both methods are described in Example 3.4. Isolated DAR1 conjugates are typically eluted at 9.0 ± 0.3 min on analytical SAX method and are greater than 90% pure. The typical DAR>2 cysteine conjugate contains more than 85% DAR2 and less than 15% DAR3.

[0425] Fig. 2 illustrates SAX HPLC chromatogram of TfR mAb-(Cys)-HPRT-PEG5k, DAR1.

[0426] Fig. 3 illustrates SEC HPLC chromatogram of TfR mAb-(Cys)-HPRT-PEG5k, DAR1.

[0427] Example 3.2. Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

Synthesis scheme-2: Antibody-Cys-BisMal-siRNA-PEG conjugates

[0428] Step 1: Antibody reduction with TCEP

[0429] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 5mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of BisMal-C6-siRNA-C6-S-NEM (2 equivalents) in pH 7.4 PBS containing 5 mM EDTA at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0430] Step 2: Purification

[0431] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

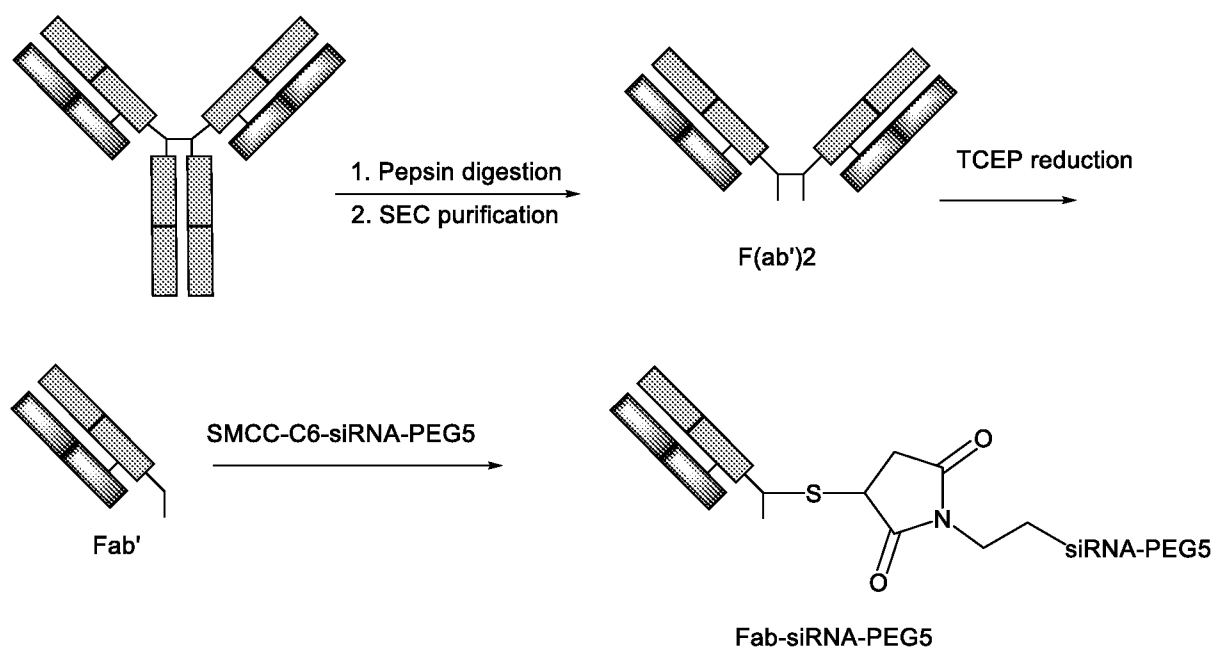
[0432] **Step-3: Analysis of the purified conjugate**

[0433] The isolated conjugates were characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or 3 as well as size exclusion chromatography method-1.

[0434] Fig. 4 illustrates an overlay of DAR1 and DAR2 SAX HPLC chromatograms of TfR1mAb-Cys-BisMal-siRNA conjugates.

[0435] Fig. 5 illustrates an overlay of DAR1 and DAR2 SEC HPLC chromatograms of TfR1mAb-Cys-BisMal-siRNA conjugates.

[0436] **Example 3.3. Fab' generation from mAb and conjugation to siRNA**



Scheme-3: Fab-siRNA conjugate generation

[0437] **Step 1: Antibody digestion with pepsin**

[0438] Antibody was buffer exchanged with pH 4.0, 20 mM sodium acetate/acetic acid buffer and made up to 5mg/ml concentration. Immobilized pepsin (Thermo Scientific, Prod#20343) was added and incubated for 3 hours at 37 °C. The reaction mixture was filtered using 30 kDa MWCO Amicon spin filters and pH 7.4 PBS. The retentate was collected and purified using size exclusion chromatography to isolate $F(ab')_2$. The collected $F(ab')_2$ was then reduced by 10 equivalents of TCEP and conjugated with SMCC-C6-siRNA-PEG5 at room temperature in pH 7.4 PBS. Analysis of reaction mixture on SAX chromatography showed Fab-siRNA conjugate along with unreacted Fab and siRNA-PEG.

[0439] **Step 2: Purification**

[0440] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR2 Fab-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0441] **Step-3: Analysis of the purified conjugate**

[0442] The characterization and purity of the isolated conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 or 3 as well as by SEC method-1.

[0443] Fig. 6 illustrates SEC chromatogram of CD71 Fab-Cys-HPRT-PEG5.

[0444] Fig. 7 illustrates SAX chromatogram of CD71 Fab-Cys-HPRT-PEG5.

[0445] **Example 3.4. Purification and analytical Methods**

[0446] **Anion exchange chromatography method (SAX)-1.**

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 μ m
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:

a.	%A	%B	Column Volume
b.	100	0	1.00
c.	60	40	18.00
d.	40	60	2.00
e.	40	60	5.00
f.	0	100	2.00
g.	100	0	2.00

[0447] **Anion exchange chromatography (SAX) method-2**

1. Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min
3. Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00		90
d.	11.00	40	60
e.	13.00	40	60
f.	15.00	90	10
g.	20.00	90	10

[0448] **Anion exchange chromatography (SAX) method-3**

1. Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl
3. Flow Rate: 0.75 ml/min
4. Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	23.00	40	60
f.	25.00	90	10
g.	30.00	90	10

[0449] Size exclusion chromatography (SEC) method-1

1. Column: TOSOH Biosciences, TSKgelG3000SW XL, 7.8 X 300 mm, 5 μ M
2. Mobile phase: 150 mM phosphate buffer
3. Flow Rate: 1.0 ml/min for 15 mins

Example 4. In vitro screen: Atrogin-1**[0450] Identification of siRNAs for the regulation of mouse and human/NHP atrogin-1**

[0451] A bioinformatics screen conducted identified 56 siRNAs (19mers) that bind specifically to mouse atrogin-1 (Fbxo32; NM_026346.3). In addition, 6 siRNAs were identified that target mouse atrogin-1 and human atrogin-1 (FBXO32; NM_058229.3). A screen for siRNAs (19mers) targeting specifically human/NHP atrogin-1 (FBXO32; NM_058229.3) yielded 52 candidates (Table 2A-Table 2B). All selected siRNA target sites do not harbor SNPs (pos. 2-18).

[0452] Tables 2A and 2B illustrate identified siRNA candidates for the regulation of mouse and human/NHP atrogin-1.

Table 2A.

19mer pos. in NM_0263 46.3	exon #	mouse	mouse x human	sequence of total 23mer target site in NM_026346.3	SEQ ID NO:
7	1	X		GGGCAGCGGCCCGGGAUAAAUAC	28
8	1	X		GGCAGCGGCCCGGGAUAAAUACU	29
499	3-Feb	X		AACCAAAACUCAGUACUCCAUC	30
553	4-Mar	X		UACGAAGGAGCGCCAUGGAUACU	31
590	4	X	X	GCUUUCAACAGACUGGACUUCUC	32
631	4	X		CAGAAGAUUCAACUACGUAGUAA	33
694	5	X		GAGUGGCAUCGCCCAAAGAACU	34
772	6	X		AAGACUUAUACGGGAACUUCUCC	35
1178	8	X		AAGCUUGUACGAUGUUACCCAAG	36
1179	8	X		AGCUUGUACGAUGUUACCCAAGA	37
1256	9-Aug	X	X	UGGAAGGGCACUGACCAUCCGUG	38
1258	9-Aug	X	X	GAAGGGCACUGACCAUCCGUGCA	39
1260	9	X	X	AGGGCACUGACCAUCCGUGCACG	40
1323	9	X		AAGACUUUAUCAUUUGUUAAG	41
1401	9	X		GGGAGUCGGGACACUUAUUUGU	42
1459	9	X		CGGGGGAUACGUCAUUGAGGAGA	43
1504	9	X		UUGCCGAUGGAAAUUACAAAUUG	44
1880	9	X	X	CCACACAAUGGUCUACCUCUAAA	45
1884	9	X	X	ACAAUGGUCUACCUCUAAAAGCA	46
2455	9	X		CUGAUAGAUGUGUUCGUCUAAAA	47
2570	9	X		AUCUCAGGGCUUAAGGAGUUAUU	48
2572	9	X		CUCAGGGCUUAAGGAGUUAUUUC	49

2936	9	X		CUGAUUUGCAGGGUCUUACAUCU	50
3006	9	X		CUGGUGGCCAAAUAAGUUGAAU	51
3007	9	X		UGGUGGCCAAAUAAGUUGAAU	52
3115	9	X		GAGAUUACAAACAUUGUAAACAGA	53
3668	9	X		CAGCGCAAAACUAGUUAGCCAGU	54
3676	9	X		AACUAGUUAGCCAGUCUUACAGA	55
3715	9	X		AAGUCAUAUAGCAUCCAUAACACC	56
3800	9	X		UAGUAGGUGCUUGCAGGUUCUCC	57
3845	9	X		AUGGUAUGUGACACAACCGAAGA	58
3856	9	X		CACAACCGAAGAAUCGUUUGACG	59
4026	9	X		GGCAAGCAAGAUACCCAUAUUAG	60
4095	9	X		AGCUCUUAGGACAUUAAUAGUCU	61
4139	9	X		UGCAGGACUCCCAGACUUA AAAAC	62
4183	9	X		CCCAGAACUGCUAGUACAAAAGC	63
4203	9	X		AGCAAGAGGGGUGUGGCUAUAGA	64
4208	9	X		GAGGGGUGUGGCUAUAGAAGUUG	65
4548	9	X		GACCAUGUCGCUACUACCAUUGC	66
4554	9	X		GUCGCUACUACCAUUGCUUCAAG	67
4563	9	X		ACCAUUGCUUCAAGUGGGUAUCU	68
4567	9	X		UUGCUUCAAGUGGGUAUCUCAGU	69
4673	9	X		CUGGUUAGUGAUGAUCAACUUCA	70
4858	9	X		UGCCGCUUCAUACGGGAGAAAAA	71
4970	9	X		UCGGCUUCAACGCAUUGUUUAUU	72
5022	9	X		CUGCCUGGUUAUAAAGCAAUAAC	73
5235	9	X		ACCUGUUAGUGCUUAAACAGACU	74
5237	9	X		CUGUUAGUGCUUAAACAGACUCA	75
5279	9	X		GGGGCAAACGCAGGGGUGUUACU	76
5292	9	X		GGGUGUUACUCUUUGAUUAUACA	77
5443	9	X		AUCCAGACUUUAGACCAAAGG	78
5640	9	X		UUGUGGACGUGUGUAAAUUCAUG	79
6000	9	X		UUCAUUGACCAACCAGUCUUAAG	80
6105	9	X		UGCCGCAACCUCCCAAGUCAUAU	81
6530	9	X		GAGUAUAGACAUGCGUGUUAACU	82
6537	9	X		GACAUGCGUGUUAACUAUGCACA	83
6608	9	X		UUGGUUCCAUCUUUAUACCAAU	84
6668	9	X		GUGUCUAAGCUUAGAAGCUUUA	85
6720	9	X		UGGGUUGAACACUUUAACUAAAC	86
6797	9	X		AUCUGAAUCCUGUAUAACUUAUU	87
6799	9	X		CUGAAUCCUGUAUAACUUAUUUG	88
6803	9	X		AUCCUGUAUAACUUAUUUGCACA	89
19mer pos. in NM_058229.3		human + NHP		sequence of total 23mer target site in NM_058229.3	

586		x		UUCCAGAAGAUUUAACUACGUGG	90
589		x		CAGAAGAUUUAACUACGUGGUCC	91
1068		x		AGCGGCAGAUCCGCAAACGAUUA	92
1071		x		GGCAGAUCCGCAAACGAUUAUUU	93
1073		x		CAGAUCCGCAAACGAUUAUUUCU	94
1075		x		GAUCCGCAAACGAUUAUUUCUGU	95
1076		x		AUCCGCAAACGAUUAUUUCUGUC	96
1077		x		UCCGCAAACGAUUAUUUCUGUCA	97
1079		x		CGCAAACGAUUAUUUCUGUCAGA	98
1083		x		AACGAUUAUUUCUGUCAGACAAA	99
1127		x		AUGUAUUUCAAAACUUGUCCGAUG	100
1142		x		GUCCGAUGUUAACCAAGGAAAGA	101
1164		x		AGCAGUAUGGAGAUACCCUUCAG	102
1228		x		CCAUCCGUGCACUGCCAAUAACC	103
1254		x		AGAGCUGCUCGCUUUCACUUA	104
1361		x		UGGGAUAUUGGCAUUUGGACACU	105
1492		x		UGUGAACUUCUCACUAGAAUUGG	106
1500		x		UCUCACUAGAAUUGGUAUGGAAA	107
1563		x		CAGCAAGACUAUAAGGGCAAUAA	108
1566		x		CAAGACUAUAAGGGCAAUAAUUC	109
1635		x		UCUUAUAGUUCCCUAGGAAGAAA	110
1679		x		AUAGGACGCUUUGUUUACAAUGU	111
2487		x		UUUUCUUUAGGUCCAACAUCAAA	112
2488		x		UUUCUUUAGGUCCAACAUCAAAA	113
2582		x		AGGAGAGGUACCACAAGUUCAUC	114
2661		x		GAGGCAAUAUCAGCAGGUAACU	115
2663		x		GGCAAUAUCAGCAGGUAACUGU	116
2790		x		UUUCCUACAACAAUGUACAUAUA	117
2999		x		AAGAGACAAGCUAUGAUACAACA	118
3875		x		GAAAUCAACCUUUAUGGUUCUCU	119
4036		x		GUGCCACGUGGUAUCUGUUAAGU	120
4039		x		CCACGUGGUAUCUGUUAAGUAUG	121
4059		x		AUGGCCAGAGCCUCACAUAUAAG	122
4062		x		GCCAGAGCCUCACAUAUAAGUGA	123
4065		x		AGAGCCUCACAUAUAAGUGAAGA	124
4117		x		AUAAUAGUCUAUAGAAUUUCUAU	125
4444		x		GCCUAGAGUCUCUUGAGAGUAAA	126
4653		x		GAAAGCAUCCCCAAUGUAUCAGU	127
4665		x		AAUGUAUCAGUUGUGAGAUGAUU	128
4787		x		CUACUAGCACUUGGGCAGUAAGG	129
5162		x		UUAACUAAACUCUAUCAUCAUUU	130
5261		x		CUGGCCUAAAAUCCUAUUAGUGC	131
5270		x		AAUCCUAUUAGUGCUUAAACAGA	132

5272		x		UCCUAUUAGUGCUUAAACAGACC	133
5338		x		UUUGAUUAUUCUUGGGUCCUUGA	134
5737		x		UGGCUGUUAACGUUUCCAUUUCA	135
5739		x		GCUGUUAACGUUUCCAUUUCAAG	136
6019		x		CUCAGAGGUACAUUUAAUCCAUC	137
6059		x		CAGGACCAGCUAUGAGAUUCAGU	138
6140		x		GGGGGAUUAUCCAUGAGGCAGC	139
6431		x		GGCUCCAAGCUGUAUUCUAUACU	140
6720		x		UUUGUACCAGACGGUGGCAUAUU	141

Table 2B.

19mer pos. in NM_0263 46.3	exon #	sense strand sequence (5'-3')	SEQ ID NO:	antisense strand sequence (5'-3')	SEQ ID NO:
7	1	GCAGCGGCCCGGGAUAA AU	142	AUUUAUCCCGGGCCGCU GC	256
8	1	CAGCGGCCCGGGAUAAA UA	143	UAUUUAUCCCGGGCCGC UG	257
499	3-Feb	CCAAAACUCAGUACUUC CA	144	UGGAAGUACUGAGUUUU GG	258
553	4-Mar	CGAAGGAGCGCCAUGGA UA	145	UAUCCAUGGCGCUCCUU CG	259
590	4	UUUCAACAGACUGGACU UC	146	GAAGUCCAGUCUGUUGA AA	260
631	4	GAAGAUUCAACUACGUA GU	147	ACUACGUAGUUGAAUCU UC	261
694	5	GUGGCAUCGCCCAAAG AA	148	UUCUUUUGGGCGAUGCC AC	262
772	6	GACUUAUACGGGAACUU CU	149	AGAAGUCCCGUAUAAG UC	263
1178	8	GCUUGUACGAUGUUACC CA	150	UGGGUAACAUCGUACAA GC	264
1179	8	CUUGUACGAUGUUACCC AA	151	UUGGGUAACAUCGUACA AG	265
1256	9-Aug	GAAGGGCACUGACCAUC CG	152	CGGAUGGUCAGUGCCCU UC	266
1258	9-Aug	AGGGCACUGACCAUCCG UG	153	CACGGAUGGUCAGUGCC CU	267
1260	9	GGCACUGACCAUCCGUG CA	154	UGCACGGAUGGUCAGUG CC	268
1323	9	GACUUUAUCAAUUUGUU CA	155	UGAACAAAUUGAUAAAG UC	269
1401	9	GAGUCGGGACACUUCAU UU	156	AAAUGAAGUGUCCCGAC UC	270
1459	9	GGGGAUACGUCAUUGAG GA	157	UCCUCAUGACGUAUCC CC	271
1504	9	GCCGAUGGAAAUUUACA AA	158	UUUGUAAAUUUCCAUCG GC	272
1880	9	ACACAAUGGUCUACCUC UA	159	UAGAGGUAGACCAUUGU GU	273

1884	9	AAUGGUCUACCUCUAAA AG	160	CUUUUAGAGGUAGACCA UU	274
2455	9	GAUAGAUGUGUUCGUCU UA	161	UAAGACGAACACAUCUA UC	275
2570	9	CUCAGGGCUUAAGGAGU UA	162	UAAUCUCCUUAAGCCCUG AG	276
2572	9	CAGGGCUUAAGGAGUUA AU	163	AUUAACUCCUUAAGCCC UG	277
2936	9	GAUUUGCAGGGUCUUAAC AU	164	AUGUAAGACCCUGCAAA UC	278
3006	9	GGUGGCCAAAUAAGUU GA	165	UCAACUUAUUUUGGCCA CC	279
3007	9	GUGGCCAAAUAAGUUG AA	166	UUCAACUUAUUUUGGCC AC	280
3115	9	GAUUACAAACAUUGUAA CA	167	UGUUACAAUGUUUGUAA UC	281
3668	9	GCGCAAAACUAGUUAGC CA	168	UGGCUAACUAGUUUUGC GC	282
3676	9	CUAGUUAGCCAGUCUUA CA	169	UGUAAGACUGGCUAACU AG	283
3715	9	GUCAUAUAGCAUCCAUA CA	170	UGUAUGGAUGCUAUAUG AC	284
3800	9	GUAGGUGCUUGCAGGUU CU	171	AGAACCUGCAAGCACCU AC	285
3845	9	GGUAUGUGACACAACCG AA	172	UUCGGUUGUGUCACAU CC	286
3856	9	CAACCGAAGAAUCGUUU GA	173	UCAAACGAUUCUUCGGU UG	287
4026	9	CAAGCAAGAUACCCAUA UU	174	AAUAUGGGUAUCUUGCU UG	288
4095	9	CUCUUAGGACAUUAAUA GU	175	ACUAUUAUGUCCUAAG AG	289
4139	9	CAGGACUCCCAGACUUA AA	176	UUUAAGUCUGGGAGUCC UG	290
4183	9	CAGAACUGCUAGUACAA AA	177	UUUUGUACUAGCAGUUC UG	291
4203	9	CAAGAGGGGUGUGGCUA UA	178	UAUAGCCACACCCCUCU UG	292
4208	9	GGGGUGUGGCUAUAGAA GU	179	ACUUCUAUAGCCACACC CC	293
4548	9	CCAUGUCGCUACUACCA UU	180	AAUGGUAGUAGCGACAU GG	294
4554	9	CGCUACUACCAUUGCUU CA	181	UGAAGCAAUGGUAGUAG CG	295
4563	9	CAUUGCUUCAAGUGGGU AU	182	AUACCCACUUGAAGCAA UG	296
4567	9	GCUUCAAGUGGGUAUCU CA	183	UGAGAUACCCACUUGAA GC	297
4673	9	GGUUAGUGAUGAUCAAC UU	184	AAGUUGAUCAUACUAA CC	298
4858	9	CCGCUUCAUACGGGAGA AA	185	UUUCUCCCGUAUGAAGC GG	299
4970	9	GGCUUCAACGCAUUGUU UA	186	UAAACAAUGCGUUGAAG CC	300

5022	9	GCCUGGUUAUAAAGCAA UA	187	UAUUGCUUUAUAACCAG GC	301
5235	9	CUGUUAGUGCUUAAACA GA	188	UCUGUUUAAGCACUAAC AG	302
5237	9	GUUAGUGCUUAAACAGA CU	189	AGUCUGUUUAAGCACUA AC	303
5279	9	GGCAAACGCAGGGGUGU UA	190	UACACCCCUGCGUUUG CC	304
5292	9	GUGUUACUCUUUGAUAU AU	191	AUAUAUCAAAGAGUAAC AC	305
5443	9	CCCAGACUUUAGACCAA AA	192	UUUUGGUCUAAAGUCUG GG	306
5640	9	GUGGACGUGUGUAAAUU CA	193	UGAAUUUACACACGUCC AC	307
6000	9	CAUUGACCAACCAGUCU UA	194	UAAGACUGGUUGGUCAA UG	308
6105	9	CCGCAACCUCCCAAGUC AU	195	AUGACUUGGGAGGUUGC GG	309
6530	9	GUUAGACAUGCGUGUU AA	196	UUAACACGCAUGUCUAU AC	310
6537	9	CAUGCGUGUUAACUAUG CA	197	UGCAUAGUUAACACGCA UG	311
6608	9	GGUCCAUCUUUAUACC AA	198	UUGGUUAUAAAGAUGGAA CC	312
6668	9	GUCUAAGCUUAGAAGCU UU	199	AAAGCUUCUAAGCUUAG AC	313
6720	9	GGUUGAACACUUUAACU AA	200	UUAGUUAAGUGUUCAA CC	314
6797	9	CUGAAUCCUGUAUAACU UA	201	UAAGUUAUACAGGAUUC AG	315
6799	9	GAAUCCUGUAUAACUUA UU	202	AAUAAGUUAUACAGGAU UC	316
6803	9	CCUGUAUAACUUAUUUG CA	203	UGCAAUAAGUUAUACA GG	317
19mer pos. in NM_05822 9.3		sense strand sequence (5'-3')		antisense strand sequence (5'-3')	
586		CCAGAAGAUUUAACUAC GU	204	ACGUAGUAAAUCUUCU GG	318
589		GAAGAUUUAACUACGUG GU	205	ACCACGUAGUAAAUCU UC	319
1068		CGGCAGAUCCGCAAACG AU	206	AUCGUUUGCGGAUCUGC CG	320
1071		CAGAUCCGCAAACGAUU AA	207	UUAUUCGUUUGCGGAUC UG	321
1073		GAUCCGCAAACGAUUAA UU	208	AAUUAUUCGUUUGCGGA UC	322
1075		UCCGCAAACGAUUAAUU CU	209	AGAAUUAUUCGUUUGCG GA	323
1076		CCGCAAACGAUUAAUUC UG	210	CAGAAUUAUUCGUUUGC GG	324
1077		CGCAAACGAUUAAUUCU GU	211	ACAGAAUUAUUCGUUUG CG	325
1079		CAAACGAUUAAUUCUGU	212	UGACAGAAUUAUUCGUU	326

		CA		UG	
1083		CGAUUAAUUCUGUCAGA CA	213	UGUCUGACAGAAUUAU CG	327
1127		GUAUUUCAAACUUGUCC GA	214	UCGGACAAGUUUGAAU AC	328
1142		CCGAUGUUACCCAAGGA AA	215	UUUCCUUGGGUAACAUC GG	329
1164		CAGUAUGGAGAUACCCU UC	216	GAAGGGUAUCUCCAUA UG	330
1228		AUCCGUGCACUGCCAAU AA	217	UUAUUGGCAGUGCACGG AU	331
1254		AGCUGCUCCGUUUCACU UU	218	AAAGUGAAACGGAGCAG CU	332
1361		GGAAUAUGGCAUUUGGA CA	219	UGUCCAAAUGCCAUAU CC	333
1492		UGAACUUCUCACUAGAA UU	220	AAUUCUAGUGAGAAGUU CA	334
1500		UCACUAGAAUUGGUAUG GA	221	UCCAUACCAAUUCUAGU GA	335
1563		GCAAGACUAUAAGGGCA AU	222	AUUGCCCUUAUAGUCUU GC	336
1566		AGACUAUAAGGGCAAUA AU	223	AUUAUUGCCCUUAUAGU CU	337
1635		UUAUAGUUCCCUAGGAA GA	224	UCUCCUAGGGAACUAU AA	338
1679		AGGACGCUUUGUUUACA AU	225	AUUGUAAACAAAGCGUC CU	339
2487		UUCUUUAGGUCCAACA CA	226	UGAUGUUGGACCUA AA	340
2488		UCUUUAGGUCCAACAUC AA	227	UUGAUGUUGGACCUA GA	341
2582		GAGAGGUACCACAAGUU CA	228	UGAACUUGUGGUACCUC UC	342
2661		GGCAAAUAUCAGCAGGU AA	229	UUACCUGCUGAUUUUG CC	343
2663		CAAAUAUCAGCAGGUAA CU	230	AGUUACCUGCUGAUUU UG	344
2790		UCCUACAACAAUGUACA UA	231	UAUGUACAUUGUUGUAG GA	345
2999		GAGACAAGCUAUGAUAC AA	232	UUGUAUCAUAGCUUGUC UC	346
3875		AAUCAACCUUUAUGGUU CU	233	AGAACCAUAAAGGUUGA UU	347
4036		GCCACGUGGUUAUCUGUU AA	234	UUAACAGAUACCACGUG GC	348
4039		ACGUGGUUAUCUGUUAAG UA	235	UACUUAACAGAUACCAC GU	349
4059		GGCCAGAGCCUCACAUA UA	236	UAUAUGUGAGGCUCUGG CC	350
4062		CAGAGCCUCACAUAUAA GU	237	ACUUAUAUGUGAGGCUC UG	351
4065		AGCCUCACAUAUAAGUG AA	238	UUCACUUAUAUGUGAGG CU	352
4117		AAUAGUCUAUAGAAUUU	239	AGAAAUUCUAUAGACUA	353

		CU		UU	
4444		CUAGAGUCUCUUGAGAG UA	240	UACUCUCAAGAGACUCU AG	354
4653		AAGCAUCCCCAAUGUAU CA	241	UGAUACAUUGGGGAUGC UU	355
4665		UGUAUCAGUUGUGAGAU GA	242	UCAUCUCACAACUGAUA CA	356
4787		ACUAGCACUUGGGCAGU AA	243	UUACUGCCCAAGUGCUA GU	357
5162		AACUAAACUCUAUCAUC AU	244	AUGAUGAUAGAGUUUAG UU	358
5261		GGCCUAAAAUCCUAUUA GU	245	ACUAAUAGGAUUUUAGG CC	359
5270		UCCUAUUAGUGCUUAAA CA	246	UGUUUAAGCACUAUAG GA	360
5272		CUAUUAGUGCUUAAACA GA	247	UCUGUUUAAGCACUAAU AG	361
5338		UGAUUAUCUUGGGUCC UU	248	AAGGACCCAAGAUUAU CA	362
5737		GCUGUUAACGUUCCAU UU	249	AAAUGGAAACGUUAA GC	363
5739		UGUUAACGUUCCAUUU CA	250	UGAAAUGGAAACGUUAA CA	364
6019		CAGAGGUACAUUUAAUC CA	251	UGGAUUAAAUGUACCUC UG	365
6059		GGACCAGCUAUGAGAUU CA	252	UGAAUCUCAUAGCUGGU CC	366
6140		GGGAUUUUAUCCAUAGAG CA	253	UGCCUCAUGGAAUAAUC CC	367
6431		CUCCAAGCUGUAUUCUA UA	254	UAUAGAAUACAGCUUGG AG	368
6720		UGUACCAGACGGUGGCA UA	255	UAUGCCACCGUCUGGUA CA	369

[0453] Evaluation of selected atrogin-1 siRNAs in transfected mouse C2C12 myoblasts, mouse C2C12 myotubes, pre-differentiated myotubes of primary human skeletal muscle cells, and human SJCRH30 rhabdomyosarcoma myoblasts.

[0454] From the 62 identified siRNAs targeting mouse atrogin-1 and 52 targeting human atrogin-1, 30 and 20 siRNAs were selected for synthesis and functional analysis, respectively. The activity of these siRNAs was analyzed in transfected mouse C2C12 myoblasts, mouse C2C12 myotubes, pre-differentiated myotubes of primary human skeletal muscle cells, and human SJCRH30 rhabdomyosarcoma myoblasts.

[0455] None of the tested siRNAs targeting mouse atrogin-1 showed significant activity in mouse C2C12 myotubes (at 10 nM), however 3 siRNAs downregulated mouse atrogin-1 mRNA by >75% in C2C12 myoblasts (Table 3). In contrast, siRNAs targeting Murf1, which is exclusively expressed in C2C12 myotubes (Fig. 8), were active in C2C12 myotubes, demonstrating that siRNAs can be transfected into C2C12 myotubes. To determine whether atrogin-1 might be alternatively spliced in C2C12 myoblasts and myotubes, various positions in the atrogin-1 mRNA were probed by RT-qPCR, but

yielded similar results. Among the 20 tested siRNAs targeting human atrogin-1 only four yielded >75% KD. For both, mouse and human atrogin-1, active siRNAs localized either within or close to the coding region. One of the siRNAs targeting mouse atrogin-1 (1179) was strongly cross-reactive with human atrogin-1. While this siRNA failed to show significant activity in mouse C2C12 myotubes, it effectively downregulated human atrogin-1 in myotubes of primary human skeletal muscle cells. All efficacious siRNAs downregulated their respective targets with subnanomolar potency.

[0456] Table 3 illustrates activity of selected atrogin-1 siRNAs in transfected mouse C2C12 myoblasts, mouse C2C12 myotubes, pre-differentiated myotubes of primary human skeletal muscle cells, and human SJCRH30 rhabdomyosarcoma myoblasts. For experimental procedures see Example 2.

mu atrogin-1 NM_026346. 3 ID#	muC2C12 myotubes %KD (10 nM)	muC2C12 myoblasts %KD	muC2C12 myoblasts IC50(nM)	huSkMC myotubes %KD	huSkMC myotubes IC50(nM)	huSJCRH30 myoblasts %KD	huSJCRH30 myoblasts IC50 (nM)
8	no KD	no KD					
499	8.5	34.4					
553	9.7	10.6					
590	10.1	30.5	0.628	62.8			
631	14.4	83.7	0.159	81.5	0.129	54.5	0.614
694	10.2	50.5	1.228	72.4	0.011	64.6	0.004
772	8.8	64.7	0.872				0.084
1179	7.2	76.6	0.160	88.5	0.010	86.0	0.015
1256	2.7	32.5					
1260	no KD	13.6					
1459	16.1	60.8	0.258	24.4	1.714		
1504	12.8	76.8	0.092	29.1	0.433	17.3	0.423
1880	14.3	58.6	0.192	66.5			
1884	7.7	54.6	0.135	54.8			0.002
2572	13.7	61.5	0.928	16.0	2.664	18.4	0.027
3007	14.4	32.7					
3668	1.4	6.9					
3715	0.7	17.2					
3856	no KD	5.9					
4139	2.7	10.5					
4567	12.8	56.1	1.589	0.0		37.2	1.028
4673	11.6	34.9					
4970	15.6	35.7					
5292	20.3	49.6	0.106	19.3	0.441	11.2	0.02
5640	13.6	40.4					
6000	19.7	21.2					
6530	3.5	no KD					
6608	7.4	no KD					
6720	17.5	no KD					
6799	15.4	no KD					

hu atrogin-1 NM_058229. 3 ID#	muC2C12 myotubes %KD(10 nM)	muC2C12 myoblasts %KD	muC2C12 myoblasts IC50(nM)	huSkMC myotubes %KD	huSkMC myotubes IC50(nM)	huSICRH30 myoblasts %KD	huSICRH30 myoblasts IC50 (nM)
586		66.1	0.326	89.9	0.008	90.6	0.011
1071		0.0	no KD	55.9			
1077		14.4	1.774	93.9	0.009	93.9	0.016
1083		no KD	no KD	92.8	0.047	92.2	0.056
1361		no KD		80.1	0.003	81.4	0.118
1566				49.9			
1679		15.1	1.471	55.8			
2582				46.2			
2663		no KD	no KD	64.2			
2999		no KD	no KD	55.0			
4036		no KD	0.200	64.7			
4059				3.2			
4117		24.2	1.541	68.0			
5162				15.7			
5261				44.4			
5272				47.4			
5737		no KD	no KD	60.8			
6019				44.4			
6059		no KD	no KD	57.6			
6431		no KD	no KD	65.0			

Example 5. In vitro screen: MuRF-1

[0457] Identification of siRNAs targeting mouse Murf1 (Trim63) and/ or human/NHP MuRF1 (TRIM63)

[0458] A bioinformatics screen was conducted and identified 51 siRNAs (19mers) that bind specifically to mouse Murf1 sequences that show >3 sequence derivations from mouse Murf2 (Trim55; NM_001039048.2) or Murf3 (Trim54). In addition, 9 siRNAs were identified that target mouse Murf1 and human MuRF1 (TRIM63; NM_032588.3). A screen for siRNAs (19mers) targeting specifically human and NHP MuRF1 (NM_032588.3) yielded 52 candidates (Table 4A-Table 4B). All selected siRNA target sites do not harbor SNPs (pos. 2-18).

[0459] Tables 4A and 4B illustrate identified siRNA candidates for the regulation of mouse and human/NHP MuRF1.

Table 4A

19mer pos. in NM_0010 39048.2	exon #	mouse	mouse X human	sequence of total 23mer target site in NM_001039048.2	SEQ ID NO:
33	1	x		GAGGAUCCGAGUGGGUUUGGAGA	370
82	1	x		CGAGACAGUCGCAUUUCAAGCA	371

109	1	x		GGAUUUAUAAAUCUAGCCUGAUUC	372
130	1	x		UCCUGAUGGAAACGCUAUGGAGA	373
264	2	x		AGGCUGCGAAUCCCUACUGGACC	374
318	2	x		GUCGUUUCGUGGCCCCUCGUGC	375
328	2	x		UUGCCCCUCGUGCCGCCAUGAAG	376
329	2	x		UGCCCCUCGUGCCGCCAUGAAGU	377
330	2	x		GCCCCUCGUGCCGCCAUGAAGUG	378
337	2	x		GUGCCGCCAUGAAGUGAUGG	379
346	2	x		UGAAGUGAUGAUGGACCGGCACG	380
423	3-Feb	x	X	AGCAGGAGUGCUCAGUCGGCCC	381
457	3	x		CAGCCACCCGAUGUGCAAGGAAC	382
460	3	x		CCACCCGAUGUGCAAGGAACACG	383
495	3	x	X	UCAACAUCUACUGUCUCACGUGU	384
497	3	x		AACAUCUACUGUCUCACGUGUGA	385
499	3	x	X	CAUCUACUGUCUCACGUGUGAGG	386
500	3	x	X	AUCUACUGUCUCACGUGUGAGGU	387
502	3	x	X	CUACUGUCUCACGUGUGAGGUGC	388
505	3	x	X	CUGUCUCACGUGUGAGGUGCCUA	389
507	3	x		GUCUCACGUGUGAGGUGCCUACU	390
511	3	x		CACGUGUGAGGUGCCUACUUGCU	391
538	3	x		GUGCAAGGUGUUUGGGGCUCACC	392
609	4	x		CUGAGCUGAGUAAACUGCAUCUCC	393
616	4	x		GAGUAAACUGCAUCUCCAUGCUGG	394
646	4	x		CAACGACCGAGUGCAGACGAUCA	395
651	4	x		ACCGAGUGCAGACGAUCAUCUCU	396
787	5	x	X	GCUGCAGCGGAUCACGCAGGAGC	397
790	5	x		GCAGCGGAUCACGCAGGAGCAGG	398
911	5	x		GAGCCCGGAGGGGCUACCUUCCU	399
1012	7	x		UGAGAACAUGGACUACUUUACUC	400
1016	7	x		AACAUGGACUACUUUACUCUGGA	401
1018	7	x		CAUGGACUACUUUACUCUGGACU	402
1022	7	x		GACUACUUUACUCUGGACUUAGA	403
1130	8	x		GAGGAAGAGGGCGUGACCACAGA	404
1266	9	x		UACAAUAGGGAAGUGUGUCUUCU	405
1351	9	x		ACACAAUUGGAAAUGUAUCCAAA	406
1364	9	x		UGUAUCCAAAACGUCACAGGACA	407
1366	9	x		UAUCCAAAACGUCACAGGACACU	408
1369	9	x		CCAAAACGUCACAGGACACUUUU	409
1380	9	x		CAGGACACUUUUUCUACGUUGGUG	410
1386	9	x		ACUUUUCUACGUUGGUGCGAAAU	411
1387	9	x		CUUUUCUACGUUGGUGCGAAAUG	412
1390	9	x		UUCUACGUUGGUGCGAAAUGAAA	413
1391	9	x		UCUACGUUGGUGCGAAAUGAAAU	414

1393	9	x		UACGUUGGUGCGAAAUGAAAUAU	415
1397	9	x		UUGGUGCGAAAUGAAAUAUUUUG	416
1454	9	x	X	UAUAUGUAUGCCAAUUUGGUGCU	417
1458	9	x	X	UGUAUGCCAAUUUGGUGCUUUUU	418
1460	9	x		UAUGCCAAUUUGGUGCUUUUUGU	419
1462	9	x		UGCCAAUUUGGUGCUUUUUGUAC	420
1466	9	x		AAUUUGGUGCUUUUUGUACGAGA	421
1478	9	x		UUUGUACGAGAACUUUUGUAUGA	422
1480	9	x		UGUACGAGAACUUUUGUAUGAUC	423
1481	9	x		GUACGAGAACUUUUGUAUGAUC	424
1483	9	x		ACGAGAACUUUUGUAUGAUCACG	425
1520	9	x		GACUGGCGAUUGUCACAAAGUGG	426
1658	9	x		GGAUAGGACUGAAUUUGUGUUAU	427
1660	9	x		AUAGGACUGAAUUUGUGUUAUAU	428
19mer pos. in NM_032588.3		+ human NHP		sequence of total 23mer target site in NM_032588.3	
28		X		GGAAGCCAACAGGAUCCGACCCG	429
75		X		CCCAGGUCUACUUAGAGCAAAGU	430
77		X		CAGGUCUACUUAGAGCAAAGUUA	431
153		X		AGUCGAGCCUGAUCCAGGAUGGG	432
239		X		CCAGUGGUCAUCUUGCCGUGCCA	433
245		X		GUCAUCUUGCCGUGCCAGCACAA	434
248		X		AUCUUGCCGUGCCAGCACAAACCU	435
249		X		UCUUGCCGUGCCAGCACAAACCUG	436
259		X		CCAGCACAAACCUGUGCCGGAAGU	437
339		X		UGUCCAUGUCUGGAGGCCGUUUC	438
367		X		CCCCACCUGCCGCCACGAGGUGA	439
368		X		CCCACCUGCCGCCACGAGGUGAU	440
370		X		CACCUGCCGCCACGAGGUGAUCA	441
371		X		ACCUGCCGCCACGAGGUGAUCAU	442
372		X		CCUGCCGCCACGAGGUGAUCAUG	443
373		X		CUGCCGCCACGAGGUGAUCAUGG	444
374		X		UGCCGCCACGAGGUGAUCAUGGA	445
375		X		GCCGCCACGAGGUGAUCAUGGAU	446
379		X		CCACGAGGUGAUCAUGGAUCGUC	447
380		X		CACGAGGUGAUCAUGGAUCGUCA	448
381		X		ACGAGGUGAUCAUGGAUCGUCAC	449
384		X		AGGUGAUCAUGGAUCGUCACGGA	450
385		X		GGUGAUCAUGGAUCGUCACGGAG	451
386		X		GUGAUCAUGGAUCGUCACGGAGU	452
387		X		UGAUCAUGGAUCGUCACGGAGUG	453
451		X		CAUCUACAAACAGGAGUGCUCCA	454

458		X		AAACAGGAGUGCUCAGUCGGCC	455
459		X		AACAGGAGUGCUCAGUCGGCCG	456
461		X		CAGGAGUGCUCAGUCGGCCGCU	457
491		X		GGCAGUCACCCCAUGUGCAAGGA	458
499		X		CCCAUGUGCAAGGAGCACGAAG	459
503		X		AUGUGCAAGGAGCACGAAGAUGA	460
531		X		UCAACAUCUACUGUCUCACGUGU	461
535		X		CAUCUACUGUCUCACGUGUGAGG	462
539		X		UACUGUCUCACGUGUGAGGUGCC	463
564		X		CCUGCUCCAUGUGCAAGGUGUUU	464
568		X		CUCCAUGUGCAAGGUGUUUGGA	465
610		X		GGCCCAUUGCAGAGUGUCUCC	466
612		X		CCCAUUGCAGAGUGUCUCCAG	467
645		X		CUGAACUGAAUAACUGUAUCUCC	468
647		X		GAACUGAAUAACUGUAUCUCCA	469
670		X		GCUGGUGGCGGGGAAUGACCGUG	470
671		X		CUGGUGGCGGGGAAUGACCGUGU	471
672		X		UGGUGGCGGGGAAUGACCGUGUG	472
673		X		GGUGGCGGGGAAUGACCGUGUGC	473
812		X		AAAAGUGAGUUGCUGCAGCGGAU	474
860		X		AGCUUCAUCGAGGCCCUCAUCCA	475
968		X		CUCUUGACUGCCAAGCAACUCAU	476
970		X		CUUGACUGCCAAGCAACUCAUCA	477
977		X		GCCAAGCAACUCAUCAAAGCAU	478
979		X		CAAGCAACUCAUCAAAGCAUUG	479
980		X		AAGCAACUCAUCAAAGCAUUGU	480

Table 4B

19mer pos. in NM_0010 39048.2	exon #	sense strand sequence (5'-3')	SEQ ID NO:	antisense strand sequence (5'-3')	SEQ ID NO:
33	1	GGAUCCGAGUGGGUUUG GA	481	UCCAAACCCACUCGGAUC C	592
82	1	AGACAGUCGCAUUUCAA G	482	CUUUGAAAUGCGACUGUC U	593
109	1	AUUAUAAAUCUAGCCUGA U	483	AUCAGGCUAGAUUUUAUA AU	594
130	1	CUGAUGGAAACGCUAUGG A	484	UCCAUAGCGUUUCCAUCA G	595
264	2	GCUGCGAAUCCCUACUGG A	485	UCCAGUAGGGAUUCGCAG C	596
318	2	CGUUUCCGUUGCCCCUCG U	486	ACGAGGGGCAACGGAAAC G	597
328	2	GCCCCUCGUGCCGCCAUG A	487	UCAUGGCGGCACGAGGGG C	598
329	2	CCCCUCGUGCCGCCAUGA	488	UUCAUGGCGGCACGAGGG	599

		A		G	
330	2	CCCUCGUGCCGCCAUGAA G	489	CUUCAUGGCGGCACGAGG G	600
337	2	GCCGCCAUGAAGUGAUC U	490	AUGAUCACUUCAUGGCGG C	601
346	2	AAGUGAUCAUGGACCGGC A	491	UGCCGGUCCAUGAUCACU U	602
423	3- Fe b	CAGGAGUGCUCCAGUCGG C	492	GCCGACUGGAGCACUCCU G	603
457	3	GCCACCCGAUGUGCAAGG A	493	UCCUUGCACAUCGGGUGG C	604
460	3	ACCCGAUGUGCAAGGAAC A	494	UGUCCUUGCACAUCGGG U	605
495	3	AACAUCUACUGUCUCACG U	495	ACGUGAGACAGUAGAUG UU	606
497	3	CAUCUACUGUCUCACGUG U	496	ACACGUGAGACAGUAGAU G	607
499	3	UCUACUGUCUCACGUGUG A	497	UCACACGUGAGACAGUAG A	608
500	3	CUACUGUCUCACGUGUGA G	498	CUCACACGUGAGACAGUA G	609
502	3	ACUGUCUCACGUGUGAGG U	499	ACCUCACACGUGAGACAG U	610
505	3	GUCUCACGUGUGAGGUGC C	500	GGCACCUCACACGUGAGA C	611
507	3	CUCACGUGUGAGGUGCCU A	501	UAGGCACCUCACACGUGA G	612
511	3	CGUGUGAGGUGCCUACUU G	502	CAAGUAGGCACCUCACAC G	613
538	3	GCAAGGUGUUUGGGGCUC A	503	UGAGCCCCAAACACCUUG C	614
609	4	GAGCUGAGUAAACUGCAUC U	504	AGAUGCAGUUACUCAGCU C	615
616	4	GUAACUGCAUCUCCAUGC U	505	AGCAUGGAGAUGCAGUU AC	616
646	4	ACGACCGAGUGCAGACGA U	506	AUCGUCUGCACUCGGUCG U	617
651	4	CGAGUGCAGACGAUCAUC U	507	AGAUGAUCGUCUGCACUC G	618
787	5	UGCAGCGGAUCACGCAGG A	508	UCCUGCGUGAUCCGCUGC A	619
790	5	AGCGGAUCACGCAGGAGC A	509	UGCUCUGCGUGAUCCGC U	620
911	5	GCCCGGAGGGGCUACCUU C	510	GAAGGUAGCCCCUCCGGG C	621
1012	7	AGAACAUGGACUACUUUA C	511	GUAAAGUAGUCCAUGUUC U	622
1016	7	CAUGGACUACUUUACUCU G	512	CAGAGUAAAGUAGUCCA G	623
1018	7	UGGACUACUUUACUCUGG A	513	UCCAGAGUAAAGUAGUCC A	624
1022	7	CUACUUUACUCUGGACUU A	514	UAAGUCCAGAGUAAAGU AG	625

1130	8	GGAAGAGGGCGUGACCAC A	515	UGUGGUCACGCCCUCUUC C	626
1266	9	CAAUAGGGAAGUGUGUC UU	516	AAGACACACUCCCCAUU G	627
1351	9	ACAAUUGGAAAUGUAUCC A	517	UGGAUACAUUUCCAAUUG U	628
1364	9	UAUCCAAAACGUCACAGG A	518	UCCUGUGACGUUUUGGAU A	629
1366	9	UCCAAAACGUCACAGGAC A	519	UGUCCUGUGACGUUUUGG A	630
1369	9	AAAACGUCACAGGACACU U	520	AAGUGUCCUGUGACGUUU U	631
1380	9	GGACACUUUUCUACGUUG G	521	CCAACGUAGAAAAGUGUC C	632
1386	9	UUUUCUACGUUGGUGCGA A	522	UUCGCACCAACGUAGAAA A	633
1387	9	UUUCUACGUUGGUGCGAA A	523	UUUCGCACCAACGUAGAA A	634
1390	9	CUACGUUGGUGCGAAAUG A	524	UCAUUUCGCACCAACGUA G	635
1391	9	UACGUUGGUGCGAAAUG AA	525	UUCAUUUCGCACCAACGU A	636
1393	9	CGUUGGUGCGAAAUGAA AU	526	AUUUCAUUUCGCACCAAC G	637
1397	9	GGUGCGAAAUGAAAUAU UU	527	AAAUAUUUCAUUUCGCAC C	638
1454	9	UAUGUAUGCCAAUUUGG UG	528	CACCAAAUUGGCAUACAU A	639
1458	9	UAUGCCAAUUUGGUGCUU U	529	AAAGCACCAAAUUGGCAU A	640
1460	9	UGCCAAUUUGGUGCUUUU U	530	AAAAAGCACCAAAUUGGC A	641
1462	9	CCAAUUUGGUGCUUUUUG U	531	ACAAAAGCACCAAAUUG G	642
1466	9	UUUGGUGCUUUUUGUAC GA	532	UCGUACAAAAAGCACCAA A	643
1478	9	UGUACGAGAACUUUUGU AU	533	AUACAAAAGUUCUCGUAC A	644
1480	9	UACGAGAACUUUUGUAU GA	534	UCAUACAAAAGUUCUCGU A	645
1481	9	ACGAGAACUUUUGUAUG AU	535	AUCAUACAAAAGUUCUCG U	646
1483	9	GAGAACUUUUGUAUGAU CA	536	UGAUCAUACAAAAGUUCU C	647
1520	9	CUGGCGAUUGUCACAAAG U	537	ACUUUGUGACAAUCGCCA G	648
1658	9	AUAGGACUGAAUUUGUG UU	538	AACACAAAUUCAGUCCUA U	649
1660	9	AGGACUGAAUUUGUGUU AU	539	AUAACACAAAUUCAGUCC U	650
19mer pos. in NM_032588 .3		sense strand sequence (5'-3')		antisense strand sequence (5'- 3')	
28		AAGCCAACAGGAUCCGAC	540	GGUCGGAUCCUGUUGGCU	651

		C		U	
75		CAGGUCUACUUAGAGCAA A	541	UUUGCUCUAAGUAGACCU G	652
77		GGUCUACUUAGAGCAAAG U	542	ACUUUGCUCUAAGUAGAC C	653
153		UCGAGCCUGAUCCAGGAU G	543	CAUCCUGGAUCAGGCUCG A	654
239		AGUGGUCAUCUUGCCGUG C	544	GCACGGCAAGAUGACCAC U	655
245		CAUCUUGCCGUGCCAGCA C	545	GUGCUGGCACGGCAAGAU G	656
248		CUUGCCGUGCCAGCACAA C	546	GUUGUGCUGGCACGGCAA G	657
249		UUGCCGUGCCAGCACAA C	547	GGUUGUGCUGGCACGGCA A	658
259		AGCACAACCUGUGCCGGA A	548	UUCCGGCACAGGUUGUGC U	659
339		UCCAUGUCUGGAGGCCGU U	549	AACGGCCUCCAGACAUGG A	660
367		CCACCUGCCGCCACGAGG U	550	ACCUCGUGGCGGCAGGUG G	661
368		CACCUGCCGCCACGAGGU G	551	CACCUCGUGGCGGCAGGU G	662
370		CCUGCCGCCACGAGGUGA U	552	AUCACCUCGUGGCGGCAG G	663
371		CUGCCGCCACGAGGUGAU C	553	GAUACCUCGUGGCGGCA G	664
372		UGCCGCCACGAGGUGAUC A	554	UGAUCACCUCGUGGCGGC A	665
373		GCCGCCACGAGGUGAUCA U	555	AUGAUCACCUCGUGGCGG C	666
374		CCGCCACGAGGUGAUCAU G	556	CAUGAUCACCUCGUGGCG G	667
375		CGCCACGAGGUGAUCAUG G	557	CCAUGAUCACCUCGUGGC G	668
379		ACGAGGUGAUCAUGGAUC G	558	CGAUCCAUGAUCACCUCG U	669
380		CGAGGUGAUCAUGGAUCG U	559	ACGAUCCAUGAUCACCUC G	670
381		GAGGUGAUCAUGGAUCG UC	560	GACGAUCCAUGAUCACCU C	671
384		GUGAUCAUGGAUCGUCAC G	561	CGUGACGAUCCAUGAUCA C	672
385		UGAUCAUGGAUCGUCACG G	562	CCGUGACGAUCCAUGAUC A	673
386		GAUCAUGGAUCGUCACGG A	563	UCCGUGACGAUCCAUGAU C	674
387		AUCAUGGAUCGUCACGGA G	564	CUCCGUGACGAUCCAUGA U	675
451		UCUACAAACAGGAGUGCU C	565	GAGCACUCCUGUUUGUAG A	676
458		ACAGGAGUGCUCCAGUCG G	566	CCGACUGGAGCACUCCUG U	677
459		CAGGAGUGCUCCAGUCGG	567	GCCGACUGGAGCACUCCU	678

		C		G	
461		GGAGUGCUCCAGUCGGCC G	568	CGGCCGACUGGAGCACUC C	679
491		CAGUCACCCCAUGUGCAA G	569	CUUGCACAUGGGGUGACU G	680
499		CCAUGUGCAAGGAGCACG A	570	UCGUGCUCCUUGCACAUG G	681
503		GUGCAAGGAGCACGAAGA U	571	AUCUUCGUGCUCCUUGCA C	682
531		AACAUCUACUGUCUCACG U	572	ACGUGAGACAGUAGAUG UU	683
535		UCUACUGUCUCACGUGUG A	573	UCACACGUGAGACAGUAG A	684
539		CUGUCUCACGUGUGAGGU G	574	CACCUCACACGUGAGACA G	685
564		UGCUCCAUGUGCAAGGUG U	575	ACACCUUGCACAUGGAGC A	686
568		CCAUGUGCAAGGUGUUUG G	576	CCAAACACCUUGCACAUG G	687
610		CCCCAUUGCAGAGUGUCU U	577	AAGACACUCUGCAAUGGG G	688
612		CCAUUGCAGAGUGUCUUC C	578	GGAAGACACUCUGCAAUG G	689
645		GAACUGAAUAACUGUAUC U	579	AGAUACAGUUAUUCAGU UC	690
647		ACUGAAUAACUGUAUCUC C	580	GGAGAUACAGUUAUUCA GU	691
670		UGGUGGCGGGGAAUGACC G	581	CGGUCAUUCCCCGCCACC A	692
671		GGUGGCGGGGAAUGACCG U	582	ACGGUCAUUCCCCGCCAC C	693
672		GUGGCGGGGAAUGACCGU G	583	CACGGUCAUUCCCCGCCA C	694
673		UGGCGGGGAAUGACCGUG U	584	ACACGGUCAUUCCCCGCC A	695
812		AAGUGAGUUGCUGCAGCG G	585	CCGCUGCAGCAACUCACU U	696
860		CUUCAUCGAGGCCCUCAU C	586	GAUGAGGGCCUCGAUGAA G	697
968		CUUGACUGCCAAGCAACU C	587	GAGUUGCUUGGCAGUCAA G	698
970		UGACUGCCAAGCAACUCA U	588	AUGAGUUGCUUGGCAGUC A	699
977		CAAGCAACUCAUCAAAG C	589	GCUUUUGAUGAGUUGCU UG	700
979		AGCAACUCAUCAAAGCA U	590	AUGCUUUUGAUGAGUUG CU	701
980		GCAACUCAUCAAAGCAU U	591	AAUGCUUUUGAUGAGUU GC	702

[0460] Activity of selected MuRF1 siRNAs in transfected mouse C2C12 myotubes and pre-differentiated myotubes of primary human skeletal muscle cells

[0461] From the 60 identified siRNAs targeting mouse Murf1 and 25 siRNAs targeting human MuRF1, 35 and 25 siRNAs were selected for synthesis, respectively. The activity of these siRNAs was analyzed in transfected mouse C2C12 myotubes and pre-differentiated primary human skeletal muscle cells (Table 5). Among the siRNAs targeting mouse MurF1, 14 displayed >70% knock down of Murf1, but <20% knock down of Murf2 and Murf3 in C2C12 myotubes. At least 6 of these 14 siRNAs were cross reactive with human MuRF1. Among the tested siRNAs targeting human MuRF1, 8 displayed >70% knock down of MuRF1, but <20% knock down of MuRF2 and MuRF3 in pre-differentiated myotubes of primary human skeletal muscle cells. Only 1 of these 8 siRNAs showed significant cross-reactivity with mouse Muf1. All efficacious siRNAs downregulated their respective targets with subnanomolar potency.

[0462] Table 5 illustrates activity of selected MuRF1 siRNAs in transfected mouse C2C12 myotubes and pre-differentiated myotubes of primary human skeletal muscle cells. Cells were grown and transfected and RNAs isolated and analyzed as described in Example 5.

19mer position in NM_02634 6.3	muC2C12 myotubes mMuRF1 KD(%)	muC2C12 myotubes mMuRF2 KD(%)	muC2C12 myotubes mMuRF3 KD(%)	muC2C12 myotubes mMuRF1 IC50(nM)	huSkMC myotubes hMuRF1 KD(%)	huSkMC myotubes hMuRF2 KD(%)	huSkMC myotubes hMuRF3 KD(%)	huSkMC myotubes hMuRF1 IC50(nM)
33	24.3	0.0	5.5					
109	73.4	0.0	6.0	0.073				
130	45.8	10.4	16.0					
264	79.6	3.7	22.0	0.172	80.9	5.6	0.0	0.018
337	42.9	10.4	7.9					
423	65.9	15.5	8.9	0.288	64.5			
460	56.4	16.6	17.8					
495	70.7	9.6	31.9	0.495	76.1	49.0	4.4	0.105
499	73.8	12.7	7.0	0.116	76.0	11.3	24.0	0.150
500	69.0	20.7	11.5	0.167	92.5	23.3	50.0	
538	48.6	10.7	16.5					
651	78.3	5.8	0.0	0.082	53.9	0.0	4.2	0.150
787	26.0	13.5	7.9					
911	43.5	0.0	10.0					
1012	74.1	0.0	27.5	0.014	83.1	6.6	0.0	0.019
1018	72.6	12.3	6.3	0.121				
1022	70.9	26.5	22.9	0.042				
1130	60.6	22.0	16.3	0.206				
1266	73.5	27.1	19.6	0.007	83.8	7.5	42.3	0.184
1351	77.5	44.2	0.0	0.008	79.0	6.6	52.4	0.509
1364	71.6	0.8	2.8	0.012	27.9			
1387	79.1	9.5	14.9	0.007	66.7	15.0	13.3	0.059
1390	73.6	33.1	10.0	0.012				
1393	75.2	28.4	4.5	0.044	73.3	34.0	12.1	0.141
1397	78.8	5.2	16.4	0.008	74.9	23.0	12.3	0.009
1454	73.8	6.5	8.7	0.019	68.2	7.0	1.7	0.004

1458	73.1	0.0	6.4	0.016	85.8	13.7	42.7	0.008
1462	69.9	17.2	19.8	0.017	63.8	3.8	0.0	0.005
1466	75.0	17.8	19.1	0.012	68.4	6.2	0.0	0.045
1480	71.1	11.9	6.9	0.022				
1481	65.8	12.2	0.4	0.266				
1483	74.6	1.1	4.0	0.030				
1520	72.8	1.6	8.8	0.012	30.6			
1658	73.7	21.6	9.8	0.028	26.0	15.6	6.6	0.005
1660	76.2	0.0	0.0	0.017				
19mer position in NM_02634 _6.3	muC2C12 myotubes mMuRF1 KD(%)	muC2C12 myotubes mMuRF2 KD(%)	muC2C12 myotubes mMuRF3 KD(%)	muC2C12 myotubes mMuRF1 IC50(nM)	huSkMC myotubes hMuRF1 KD(%)	huSkMC myotubes hMuRF2 KD(%)	huSkMC myotubes hMuRF3 KD(%)	huSkMC myotubes hMuRF1 IC50(nM)
75	25.8				69.6	0.0	0.0	
77	14.5	17.4	13.8		83.7	7.1	13.9	0.152
245	60.3			0.905	70.9	33.1	14.3	
259	49.7			2.759	75.1	84.5	71.1	0.053
339					52.7	1.2	14.3	
367					0.0	0.0	0.0	
370	8.8			0.033	21.2	25.0	4.1	
373					64.6	21.8	14.1	
374	33.7	7.0	14.8	0.659	89.7	10.0	19.7	0.110
380	19.5			0.013	70.6	8.5	0.4	
386					69.9	19.9	16.4	0.002
459	66.7			0.148	78.1	25.2	7.1	
491					57.1	16.3	1.3	
503	56.4			0.101	52.0	89.9	86.1	
535	70.8	32.4	13.4	0.074	82.1	24.0	9.5	0.008
564	8.2			0.002	72.4	26.1	0.6	
610	6.5	18.7	17.0		89.6	9.4	15.2	0.107
645	46.2	13.9	2.3	3.475	85.2	0.0	0.0	0.007
647	77.5	20.1	0.0	0.211	94.6	4.4	19.4	0.006
673					35.5	13.4	4.5	
860					77.1	22.5	0.0	
970	8.8	29.3	10.6		84.9	12.9	7.3	0.056
977	19.9	5.9	0.0	2.838	93.6	51.0	12.6	0.117
979					87.4	29.6	17.4	0.058
980	0.0	36.0	2.1		93.6	4.7	20.5	0.118

Example 6. 2017-PK-279-WT - CD71 vs IgG2A isotype, HPRT vs MSTN siRNA design and synthesis

[0463] MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands

were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0464] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 14227). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 3' end and a C6-SH at the 5' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0465] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker.

[0466] ASC synthesis and characterization

[0467] The CD71 mAb-siRNA DAR1 conjugates were made, purified and characterized as described in Example 3. All conjugates were made through cysteine conjugation, a SMCC linker and the PEG was attached at the thiol using architecture 1 for MSTN and the scrambled siRNA and architecture 2 for the HPRT siRNA, see Example 3. Conjugates were characterized chromatographically as described in Table 6.

Table 6. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
1-4	TfR-mAb-HPRT-PEG5k; DAR1	8.8	7.1
5-8	IgG2a-mAb-HPRT-PEG5k; DAR1	8.9	7.7
9-12	TfR-mAb-MSTN-PEG5k; DAR1	8.7	7.2
13-16	IgG2a-mAb-MSTN-PEG5k; DAR1	8.9	7.7
17-20	TfR-mAb-scrambled-PEG5k; DAR1	8.4	7.2

[0468] In vivo study design

[0469] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv)

injection with PBS vehicle control and the indicated ASCs and doses, see Fig. 9A. After 96 hours, gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0470] Results

[0471] For gastrocnemius muscle harvested 96 hours post-dose, maximum MSTN mRNA downregulation of greater than 90% was observed after a single intravenous dose of 3 mg/kg of siRNA, see Fig. 9B. In addition, a dose response was also observed (dose range: 0.3 to 3.0 mg/kg siRNA) and no significant mRNA downregulation was observed for the control groups.

[0472] Conclusions

[0473] In gastrocnemius muscle, it was demonstrated that an ASC is able to downregulate a muscle specific gene. The ASC was made with an anti-transferrin antibody conjugated to an siRNA designed to down regulate MSTN mRNA. Mouse gastroc muscle expresses the transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in accumulation of the conjugates in gastroc muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the MSTN mRNA.

Example 7. 2017-PK-289-WT - CD71 mAb MSTN time course for phenotype siRNA design and synthesis

[0474] MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0475] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified

single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0476] ASC synthesis and characterization

[0477] The CD71 mAb-siRNA DAR1 conjugates were made and characterized as described in Example 3. All conjugates were made through cysteine conjugation, a SMCC linker and the thiol was end capped with NEM using architecture 1. Conjugates were characterized chromatographically as described in Table 7.

Table 7. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
1-4, 13 & 14	TfR-mAb-MSTN-NEM ; DAR1	8.7	10.0
5-8	TfR-mAb-scrambled-NEM; DAR1	8.9	10.0

[0478] In vivo study design

[0479] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 10A. Plasma and tissue samples were also taken as indicated in Fig. 10A. Muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (Δ Ct) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$ Ct). Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0480] Plasma myostatin levels were determined using an ELISA, see Example 2 for full experimental details. Changes in leg muscle area were determined: The leg-to-be-measured were shaved and a line was drawn using indelible ink to mark region of measurement. Mice were restrained in a cone restraint and the right leg was held by hand. Digital calipers were used to take one measurement on the sagittal plane and another on the coronal plane. The procedure was repeated twice per week.

[0481] Results

[0482] Quantifiable levels of siRNA accumulated in muscle tissue after a single intravenous dose of the antibody siRNA conjugates, see Fig. 10B. Robust MSTN mRNA downregulation was observed in gastrocnemius muscle, which resulted in a reduction in the levels of MSTN protein in the plasma, after a single intravenous dose of 3 mg/kg of siRNA, see Fig. 10C and Fig. 10D. Maximum mRNA downregulation of ~90% was observed between 7-14 days post-dose. At 6 weeks post-dose gastroc muscle had approximately 75% mRNA downregulation, which corresponded to about a 50% reduction in plasma protein levels relative to the PBS or anti-transferrin antibody conjugated scrambled controls. Downregulation of MSTN resulted in statistically significant increases in muscle size, see Fig. 10E and Fig. 10F.

[0483] Conclusions

[0484] In this example it was demonstrated that accumulation of siRNA in various muscle tissues after a single dose of an anti-transferrin antibody targeted siRNA conjugate. In Gastroc muscle, significant and long-lasting siRNA mediated MSTN mRNA downregulation was observed. Mouse gastroc muscle expresses transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in accumulation of the conjugates in gastroc muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the MSTN gene.

Example 8: 2017-PK-299-WT - MSTN Zalu vs TfR, mAb vs Fab, DAR1 vs DAR2 siRNA design and synthesis

[0485] MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0486] MSTN*: MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained one conjugation handle, a C6-NH₂ at the 5' end, which was connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0487] ASC synthesis and characterization

[0488] The CD71 mAb-siRNA DAR1 and DAR2 conjugates were made and characterized as described in Example 3. Groups 1-8 and 17-20 were made through cysteine conjugation and a BisMal linker using architecture 3. Groups 9-16 were made through cysteine conjugation, a SMCC linker and the free thiol was end capped with NEM PEG using architecture 1. Conjugates were characterized chromatographically as described in Table 8.

Table 8. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
1-4	TfR-Fab-MSTN; DAR1	8.7	10.0
5-8	EGFR-Fab-MSTN; DAR1	8.9	10.0
9-12	TfR-mAb-MSTN-NEM; DAR1	9.5	7.9
13-16	TfR-mAb-MSTN-nEM; DAR2	10.3	8.1
17-18	EGFR-mAb-MSTN; DAR1	9.3	NT
19-20	EGFR-mAb-MSTN; DAR2	10.2	NT

[0489] In vivo study design

[0490] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 11A. Plasma and tissue samples were also taken as indicated in Fig. 11A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta \Delta Ct$). Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0491] Results

[0492] Quantifiable levels of siRNA accumulated in muscle tissue after a single intravenous dose of the antibody and Fab siRNA conjugates, see Fig. 11B. Robust MSTN mRNA downregulation was observed in gastroc muscle, when the anti-transferrin antibody conjugate was administered as a DAR1 or DAR2, or as a Fab DAR1 conjugate, see Fig. 11C.

[0493] Conclusions

[0494] In this example it was demonstrated that accumulation of siRNA in gastroc muscle tissue after a single dose of an anti-transferrin antibody and Fab targeted siRNA conjugates. In Gastroc muscle, siRNA mediated MSTN mRNA downregulation with DAR1 and DAR2 antibody conjugates were observed, in addition to the DAR1 Fab conjugate. Mouse gastroc muscle expresses transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody or Fab to target the siRNA, resulting in accumulation of the conjugates in gastroc muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the MSTN gene.

Example 9: 2017-PK-303-WT - Dose response MSTN mAb vs Fab vs Chol siRNA design and synthesis

[0495] MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0496] MSTN*: MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained one conjugation handle, a C6-NH₂ at the 5' end, which was connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0497] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0498] ASC synthesis and characterization

[0499] The CD71 mAb-siRNA DAR1 and DAR2 conjugates were made and characterized as described in Example 3. Groups 5-12 made through cysteine conjugation, a BisMal linker using architecture 3. Groups 13-16 were made through cysteine conjugation, a SMCC linker, the free thiol was end capped with NEM using architecture 1. Groups 17-20 were made through cysteine conjugation, a BisMal linker, the free thiol was end capped with NEM using architecture 3. Conjugates were characterized chromatographically as described Table 9.

Table 9. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
5-8	TfR-Fab-MSTN*; DAR1	8.7	10.0
9-12	TfR-mAb-MSTN*; DAR1	9.3	7.8
13-16	TfR-mAb-MSTN; DAR1	9.5	7.9
17-20	TfR-mAb-scramble; DAR1	9.1	7.3

[0500] In vivo study design

[0501] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 12A. Tissue samples were also taken as indicated in Fig. 12A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$). Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0502] Intracellular RISC loading was determined as described in Example 2.

[0503] Results

[0504] Quantifiable levels of siRNA accumulated in gastroc and heart tissue, see Fig. 12B, after a single intravenous dose of the antibody and Fab siRNA conjugates. Robust MSTN mRNA downregulation was observed in gastroc muscle, when the ASC was targeted with either the anti-transferrin receptor antibody or Fab see Fig. 12B and Fig. 12C. Much higher concentrations of siRNA were delivered to heart tissue, but this did not result in robust myostatin mRNA downregulation, see Fig. 12B. Compared to the cholesterol siRNA conjugate, much lower doses of the ASCs were required to

achieve equivalent mRNA downregulation. The amount of RISC loading of the MSTN siRNA guide strand correlated with downregulation of the mRNA, see Fig. 12D.

[0505] Conclusions

[0506] In this example it was demonstrated that accumulation of siRNA in gastrocnemius muscle tissue after a single dose of an anti-transferrin antibody and Fab targeted siRNA conjugates. In Gastroc muscle, siRNA mediated MSTN mRNA downregulation with the DAR1 anti-transferrin antibody or Fab conjugates was observed. Mouse gastroc muscle expresses transferrin receptor and the conjugate have a mouse specific anti-transferrin antibody or Fab to target the payload, resulting in accumulation of the conjugates in gastroc muscle and loading into the RISC complex. Receptor mediate uptake resulted in siRNA mediated MSTN mRNA downregulation.

Example 10: 2017-PK-304-WT - PK with MSTN phenotype mAb vs Chol siRNA design and synthesis

[0507] MSTN: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAUUAUUUGUUCUUUGCCUU (SEQ ID NO: 14226). Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0508] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0509] ASC synthesis and characterization

[0510] The CD71 mAb-siRNA DAR1 and DAR2 conjugates were made and characterized as described in example 3. Groups 5-12 were made through cysteine conjugation, a SMCC linker, the free thiol was end capped with NEM using architecture 1. Groups 13-16 were made through cysteine

conjugation, a BisMal linker, the free thiol was end capped with NEM using architecture 3. Conjugates were characterized chromatographically as described in Table 10.

Table 10. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
5-8	TfR-mAb-MSTN; DAR1	9.5	7.9
9-12	TfR-mAb-MSTN; DAR2	10.3	7.6
13-16	TfR-mAb-scramble; DAR1	9.1	7.3

[0511] In vivo study design

[0512] The conjugates were assessed for their ability to mediate mRNA downregulation of myostatin (MSTN) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 13A. Tissue samples were also taken as indicated in Fig. 13A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$). Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0513] Intracellular RISC loading was determined as described in Example 2. Plasma MSTN protein levels were measured by ELISA as described in Example 2.

[0514] Changes in leg muscle area were determined: the leg-to-be measured were shaved and a line was drawn using indelible ink to mark region of measurement. Mice were restrained in a small decapicone bag. Digital calipers were used to take one measurement on the sagittal plane and another on the coronal plane. The procedure was repeated twice per week.

[0515] Results

[0516] Quantifiable levels of siRNA accumulated in gastrocnemius, triceps, quadriceps (Quad), and heart tissues, see Fig. 13D, after a single intravenous dose of the antibody siRNA conjugates at 3 mg/kg. MSTN mRNA downregulation was observed in gastrocnemius, quadriceps, and triceps with the DAR1 and DAR2 conjugates but not in heart tissue, see Fig. 13B. MSTN mRNA downregulation resulted in a reduction in the plasma concentration of MSTN protein, as measured by ELISA, see Fig. 13C. The

amount of RISC loading of the MSTN siRNA guide strand correlated with downregulation of the mRNA, see Fig. 13E. Downregulation of MSTN resulted in statistically significant increases in muscle size, see Fig. 13F and Fig. 13G.

[0517] Conclusions

[0518] In this example it was demonstrated that accumulation of siRNA in gastrocnemius, quadriceps, and triceps muscle tissues after a single dose of anti-transferrin antibody siRNA conjugates, DAR1 and DAR2. In all three tissues, measurable siRNA mediated MSTN mRNA downregulation with the DAR1 and DAR2 anti-transferrin antibody conjugates was observed. mRNA downregulation correlated with a reduced level of plasma MSTN protein and RISC loading of the siRNA guide strand. All three muscle tissues expressed transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in accumulation of the conjugates in muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the MSTN gene.

Example 11: 2017-PK-355-WT Multiple siRNA dosing siRNA design and synthesis

[0519] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 14227). Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained a single conjugation handles, a C6-NH₂ at the 5', which was connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0520] SSB: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against mouse MSTN. The sequence (5' to 3') of the guide/antisense strand was UUACAUUAAAGUCUGUUGUUU (SEQ ID NO: 14229). Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained a single conjugation handles, a C6-NH₂ at the 5', which was connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker.

[0521] ASC synthesis and characterization

[0522] The CD71 mAb-siRNA conjugates were made and characterized as described in Example 3.

[0523] Groups 1-4 and 5-8 were made through cysteine conjugation, a BisMal linker, no 3' conjugation handle on the passenger strand using architecture 3. Groups 13-16 were made through cysteine conjugation, a BisMal linker, no 3' conjugation handle on the passenger strand, but were DAR2

conjugates made with a mixture of HPRT and SSB siRNAs using architecture 4. Conjugates were characterized chromatographically as described in Table 11.

Table 11. HPLC retention time (RT) in minutes

Conjugate	RT, SAX Method-2	RT, SEC Method-1
TfR-mAb-HPRT; DAR1	9.0	12.5 (0.5 ml flow rate, 25 min run)
TfR-mAb-SSB; DAR1	9.4	No Data
TfR-mAb-HPRT/SSB (1:1) DAR2	10.09	No Data

[0524] In vivo study design

[0525] The conjugates were assessed for their ability to mediate mRNA downregulation of two house keeper genes (HPRT and SSB) in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 14A. Tissue samples were also taken as indicated in Fig. 14A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta \Delta Ct$). Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0526] The RISC loading assay was conducted as described in Example 2.

[0527] Results

[0528] After a single intravenous dose of the antibody siRNA conjugates at the indicated doses, mRNA downregulation was observed in gastroc and heart tissue, see Fig. 14B - Fig. 14D. Co-administration of a mixture of two ASC's, targeting two different genes (HPRT and SSB) resulted in efficient mRNA downregulation of both targets in gastroc and heart tissue. In addition, administration of a DAR2 conjugate synthesized using a 1:1 mixture of the two different siRNAs (HPRT and SSB) also resulted in efficient mRNA down regulation of both targets in gastroc and heart tissue. All approaches to delivery resulted in measurable amounts of siRNA accumulating in gastroc tissue, see Fig. 14F.

[0529] Conclusions

[0530] In this example, it was demonstrated that accumulation of siRNA in gastroc and heart tissue after a single dose of anti-transferrin antibody siRNA conjugates. Two genes were downregulated by co-administration of two ASC produced with the same anti-transferrin antibody but conjugated to two

different siRNAs (HPRT and SSB). In addition, two genes were downregulated by an anti-transferrin mAb DAR2 conjugate synthesized using a 1:1 mixture of two different siRNAs (HPRT and SSB). In some instances, simultaneous downregulation of more than one gene is useful in muscle atrophy.

Example 12: 2017-PK-380-WT Activity of Atrogin-1 siRNAs in vivo (dose response) siRNA design and synthesis

[0531] Atrogin-1 siRNAs: 4 different 21mer duplexes with 19 bases of complementarity and 3' dinucleotide overhangs were designed against Atrogin-1, see Example 4 for details of the sequence. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. The same design was used for all four siRNAs. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0532] ASC synthesis and characterization

[0533] The CD71 mAb-siRNA conjugates were made and characterized as described in Example 3.

[0534] Groups 1-16 were made through cysteine conjugation, a BisMal linker, the free thiol was end capped with NEM using architecture 3. Conjugates were characterized chromatographically as described Table 12.

Table 12. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
1-4	mTfR1(Cys)-BisMal-N-mAtrogin#1179; DAR1	9.2	7.7
5-8	mTfR1(Cys)-BisMal-N-mAtrogin#1504; DAR1	9.3	7.8
9-12	mTfR1(Cys)-BisMal-N-mAtrogin#631; DAR1	9.3	7.8
13-16	mTfR1(Cys)-BisMal-N-mAtrogin#586; DAR1	9.2	7.8

[0535] In vivo study design

[0536] The conjugates were assessed for their ability to mediate mRNA downregulation of Atrogin-1 in skeletal muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 15A. Tissue samples were taken as indicated in Fig. 15A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct

value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0537] Results

[0538] After a single intravenous dose of the antibody siRNA conjugates at the indicated doses, up to 80% atrogin-1 mRNA downregulation was observed in gastroc muscle and up to 50% in heart tissue, see Fig. 15B and Fig. 15C.

[0539] Conclusions

[0540] As illustrated in this example, antibody siRNA conjugates differentially downregulate Atrogin-1 in muscle and heart.

Example 13: 2017-PK-383-WT Activity of MuRF1 siRNA in vivo (dose response) siRNA design and synthesis

[0541] MuRF1 siRNAs: 4 different 21mer duplexes with 19 bases of complementarity and 3' dinucleotide overhangs were designed against Atrogin-1, see Example 5 for details of the sequence. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. The same design was used for all four siRNAs. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0542] ASC synthesis and characterization

[0543] The CD71 mAb-siRNA conjugates were made and characterized as described in Example 3.

[0544] Groups 1-16 were made through cysteine conjugation, a BisMal linker, the free thiol was end capped with NEM using architecture 3. Conjugates were characterized chromatographically as described Table 13.

Table 13. HPLC retention time (RT) in minutes

Groups	Conjugate	RT, SAX Method-2	RT, SEC Method-1
1-4	mTfR1(Cys)-BisMal-N-MuRF#651-S-NEM; DAR1	9.2	7.8
5-8	mTfR1(Cys)-BisMal-N-MuRF#1387-S-NEM; DAR1	9.3	7.8
9-13	mTfR1(Cys)-BisMal-N-MuRF#1454-S-NEM; DAR1	9.1	7.8
14-18	mTfR1(Cys)-BisMal-N-MuRF#1660-S-NEM; DAR1	9.1	7.8

[0545] In vivo study design

[0546] The conjugates were assessed for their ability to mediate mRNA downregulation of MuRF-1 in skeletal and heart muscle *in vivo* in wild type CD-1 mice. Mice were dosed via intravenous (iv) injection

with PBS vehicle control and the indicated ASCs at the doses indicated in Fig. 16A. Tissue samples were taken as indicated in Fig. 16A. Gastrocnemius (gastroc) muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0547] Results

[0548] After a single intravenous dose of the antibody siRNA conjugates at the indicated doses, MuRF1 mRNA in gastroc muscle was downregulated to up to 70% and up to 50% in heart tissue, see Fig. 16B and Fig. 16C.

[0549] Conclusions

[0550] As illustrated in this example, antibody siRNA conjugates differentially downregulate MuRF1 in muscle and heart.

Example 14.

[0551] Table 14 illustrates exemplary siRNA (or atrogene) targets to regulate muscle atrophy. In some instances, a polynucleic acid molecule hybridizes to a target region of an atrogene described in Table 14.

Function	Gene	Name
Protein Degradation	FBXO32	Atrogin-1
	Trim63	MuRF1
	TRAF6	TNF receptor- associated factor 6
	USP14	Ubiquitin specific protease 14
	CTSL2	Cathepsin L2
Transcription	Foxo1	Forkhead box O1
	Foxo3	Forkhead box O3
	TGIF	TG interacting factor
	MYOG	myogenin
	HDAC2	Histone deacetylase 2
	HDAC3	Histone deacetylase 3
Stress Response	MT1L	Metallothionein 1L
	MT1B	Metallothionein 1B

Example 15: Sequences

[0552] 23-mer target sequences within one DMPK transcript variant (NM_001288766) are assigned with SEQ ID NOs: 703-3406. The set of 23-mer target sequences for this transcript variant was generated by walking down the length of the transcript one base at a time, and a similar set of target sequences could be generated for the other DMPK transcript variants using the same procedure. One

common siRNA structure that can be used to target these sites in the DMPK transcript is a 19-mer fully complementary duplex with 2 overhanging (not base-paired) nucleotides on the 3' end of each strand. Thus, adding the 19-mer with both of the 2 nucleotide overhangs results in a total of 23 bases for the target site. Since the overhangs can be comprised of a sequence reflecting that of the target transcript or other nucleotides (for example a non-related dinucleotide sequence such as "UU"), the 19-mer fully complementary sequence can be used to describe the siRNA for each 23-mer target site.

[0553] 19-mer sense and antisense sequences for siRNA duplexes targeting each site within the DMPK transcript are assigned with SEQ ID NOs: 3407-8814 (with the first sense and antisense pairing as SEQ ID NO: 3407 and SEQ ID NO: 6111). SEQ ID NOs: 3407-6110 illustrate the sense strand. SEQ ID NOs: 6111-8814 illustrate the antisense strand. The DMPK transcript variant NM_001288766 has been used for illustration but a similar set of siRNA duplexes can be generated by walking through the other DMPK transcript variants. When the antisense strand of the siRNA loads into Ago2, the first base associates within the Ago2 binding pocket while the other bases (starting at position 2 of the antisense strand) are displayed for complementary mRNA binding. Since "U" is the thermodynamically preferred first base for binding to Ago2 and does not bind the target mRNA, all of the antisense sequences can have "U" substituted into the first base without affecting the target complementarity and specificity. Correspondingly, the last base of the sense strand 19-mer (position 19) is switched to "A" to ensure base pairing with the "U" at the first position of the antisense strand.

[0554] SEQ ID NOs: 8815-11518 are similar to SEQ ID NOs: 3407-6110 except the last position of the 19-mer sense strand substituted with base "A".

[0555] SEQ ID NOs: 11519-14222 are similar to SEQ ID NOs: 6111-8814 except the first position of the 19-mer antisense strand substituted with base "U".

[0556] SEQ ID NO: 8815 and SEQ ID NO: 11519 for the first respective sense and antisense pairing.

Example 16: Initial screening of a selected set of DMPK siRNAs for *in vitro* activity

[0557] The initial set of DMPK siRNAs from SEQ ID NOs: 8815-14222 was narrowed down to a list of 81 siRNA sequences using a bioinformatic analysis aimed at selecting the sequences with the highest probability of on-target activity and the lowest probability of off-target activity. The bioinformatic methods for selecting active and specific siRNAs are well described in the field of RNAi and a person skilled in the arts would be able to generate a similar list of DMPK siRNA sequences against any of the other DMPK transcript variants. The DMPK siRNAs in the set of 81 sequences were synthesized on small scale using standard solid phase synthesis methods that are described in the oligonucleotide synthesis literature. Both unmodified and chemically modified siRNAs are known to produce effective knockdown following *in vitro* transfection. The DMPK siRNA sequences were synthesized using base, sugar and phosphate modifications that are described in the field of RNAi to optimize the potency of the duplex and reduce immunogenicity. Two human cell lines were used to assess the *in vitro* activity of the DMPK siRNAs: first, SJCRH30 human rhabdomyosarcoma cell line (ATCC® CRL-2061™); and second, Myotonic Dystrophy Type 1 (DM1) patient-derived immortalized human skeletal myoblasts. For

the initial screening of the DMPK siRNA library, each DMPK siRNA was transfected into SJCRH30 cells at 1 nM and 0.01 nM final concentration, as well as into DM1 myoblasts at 10 nM and 1 nM final concentration. The siRNAs were formulated with transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's "forward transfection" instructions. Cells were plated 24 h prior to transfection in triplicate on 96-well tissue culture plates, with 8500 cells per well for SJCRH30 and 4000 cells per well for DM1 myoblasts. At 48 h (SJCRH30) or 72 h (DM1 myoblasts) post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with DMPK-specific and PPIB-specific TaqMan human gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). DMPK values were normalized within each sample to PPIB gene expression. The quantification of DMPK downregulation was performed using the standard $2^{-\Delta\Delta C_t}$ method. All experiments were performed in triplicate, with Table 15A and Table 15B) presenting the mean values of the triplicates.

Table 15A

ID # ¹	sense strand sequence (5'-3') Passenger Strand (PS)	SEQ ID NO:	antisense strand sequence (5'-3') Guide Strand (GS)	SEQ ID NO:
385	GCUUAAGGAGGUCCGACUA	9199	UAGUCGGACCUCCUUAAGC	11903
443	GGGGCGUUCAGCGAGGUAA	9257	UUACCUCGCUGAACGCCCC	11961
444	GGGCGUUCAGCGAGGUAGA	9258	UCUACCUCGCUGAACGCCC	11962
445	GGCGUUCAGCGAGGUAGCA	9259	UGC UACCUCGCUGAACGCC	11963
533	AGGGGCGAGGUGUCGUGCA	9347	UGCACGACACCUCGCCCCU	12051
534	GGGGCGAGGUGUCGUGCUA	9348	UAGCACGACACCUCGCCCC	12052
535	GGGCGAGGUGUCGUGCUUA	9349	UAAGCACGACACCUCGCCC	12053
539	GAGGUGUCGUGCUUCCGUA	9353	UACGGAAGCACGACACCUC	12057
540	AGGUGUCGUGCUUCCGUGA	9354	UCACGGAAGCACGACACCU	12058
541	GGUGUCGUGCUUCCGUGAA	9355	UUCACGGAAGCACGACACC	12059
543	UGUCGUGCUUCCGUGAGGA	9357	UCCUCACGGAAGCACGACA	12061
544	GUCGUGCUUCCGUGAGGAA	9358	UUCUCACGGAAGCACGAC	12062
576	UGAAUGGGGACCGGCGGUA	9390	UACCGCCGGUCCCCAUUCA	12094
577	GAAUGGGGACCGGCGGUGA	9391	UCACCGCCGGUCCCCAUUC	12095
581	GGGGACCGGCGGUGGAUCA	9395	UGAUCCACCGCCGGUCCCC	12099
583	GGACCGGCGGUGGAUCACA	9397	UGUGAUCCACCGCCGGUCC	12101
584	GACCGGCGGUGGAUCACGA	9398	UCGUGAUCCACCGCCGGUC	12102
690	AGUUUGGGGAGCGGAUUCA	9504	UGAAUCCGCUCCCCAAACU	12208
716	AUGGCGCGCUUCUACCUGA	9530	UCAGGUAGAAGCGCGCCAU	12234
717	UGGCGCGCUUCUACCUGGA	9531	UCCAGGUAGAAGCGCGCCA	12235
785	AGGGACAUCAAACCCGACA	9599	UGUCGGGUUUGAUGUCCCU	12303
786	GGGACAUCAAACCCGACAA	9600	UUGUCGGGUUUGAUGUCCC	12304
789	ACAUCAAACCCGACAACAA	9603	UUGUUGUCGGGUUUGAUGU	12307
1026	GGCAGACGCCCUUCUACGA	9840	UCGUAGAAGGGCGUCUGCC	12544

1027	GCAGACGCCCUCUACGCA	9841	UGCGUAGAAGGGCGUCUGC	12545
1028	CAGACGCCCUCUACGCGA	9842	UCGCGUAGAAGGGCGUCUG	12546
1029	AGACGCCCUCUACGCGGA	9843	UCCGCGUAGAAGGGCGUCU	12547
1037	UUCUACGCGGAUUCCACGA	9851	UCGUGGAAUCCGCGUAGAA	12555
1039	CUACGCGGAUUCCACGGCA	9853	UGCCGUGGAAUCCGCGUAG	12557
1041	ACGCGGAUUCCACGGCGGA	9855	UCCGCCGUGGAAUCCGCGU	12559
1043	GCGGAUUCCACGGCGGAGA	9857	UCUCCGCCGUGGAAUCCGC	12561
1044	CGGAUUCCACGGCGGAGAA	9858	UUCUCCGCCGUGGAAUCCG	12562
1047	AUUCCACGGCGGAGACCUA	9861	UAGGUCUCCGCCGUGGAAU	12565
1071	AGAUCGUCCACUACAAGGA	9885	UCCUUGUAGUGGACGAUCU	12589
1073	AUCGUCCACUACAAGGAGA	9887	UCUCCUUGUAGUGGACGAU	12591
1262	CCCUUUACACCGGAUUUCA	10076	UGAAAUCCGGUGUAAAGGG	12780
1263	CCUUUACACCGGAUUUCGA	10077	UCGAAAUCCGGUGUAAAGG	12781
1264	CUUUACACCGGAUUUCGAA	10078	UUCGAAAUCCGGUGUAAAG	12782
1265	UUUACACCGGAUUUCGAAA	10079	UUUCGAAAUCCGGUGUAAA	12783
1267	UACACCGGAUUUCGAAGGA	10081	UCCUUCGAAAUCCGGUGUA	12785
1268	ACACCGGAUUUCGAAGGUA	10082	UACCUUCGAAAUCCGGUGU	12786
1269	CACCGGAUUUCGAAGGUGA	10083	UCACCUUCGAAAUCCGGUG	12787
1274	GAUUUCGAAGGUGCCACCA	10088	UGGUGGCACCUUCGAAAUC	12792
1276	UUUCGAAGGUGCCACCGAA	10090	UUCGGUGGCACCUUCGAAA	12794
1283	GGUGCCACCGACACAUGCA	10097	UGCAUGUGUCGGUGGCACC	12801
1297	AUGCAACUUCGACUUGGUA	10111	UACCAAGUCGAAGUUGCAU	12815
1342	ACUGUCGGACAUUCGGGAA	10156	UUCCCGAAUGUCCGACAGU	12860
1343	CUGUCGGACAUUCGGGAAA	10157	UUUCCCGAAUGUCCGACAG	12861
1344	UGUCGGACAUUCGGGAAGA	10158	UCUUCCCGAAUGUCCGACA	12862
1346	UCGGACAUUCGGGAAGGUA	10160	UACCUUCCCGAAUGUCCGA	12864
1825	UGCUCUGUUCGCCGUUGA	10639	UCAACGGCGAACAGGAGCA	13343
1886	CCACGCCGGCCAACUCACA	10700	UGUGAGUUGGCCGGCGUGG	13404
1890	GCCGGCCAACUCACCGCAA	10704	UUGCGGUGAGUUGGCCGGC	13408
1898	ACUCACCGCAGUCUGGCGA	10712	UCGCCAGACUGCGGUGAGU	13416
1945	CCCUAGAACUGUCUUCGAA	10759	UUCGAAGACAGUUCUAGGG	13463
1960	CGACUCCGGGGCCCCGUUA	10774	UAACGGGGCCCCGGAGUCG	13478
2126	GCCGGCGAACGGGGCUCGA	10940	UCGAGCCCCGUUCGCCGGC	13644
2127	CCGGCGAACGGGGCUCGAA	10941	UUCGAGCCCCGUUCGCCGG	13645
2149	UCCUUGUAGCCGGGAAUGA	10963	UCAUUCCCGGCUACAAGGA	13667
2150	CCUUGUAGCCGGGAAUGCA	10964	UGCAUUCCCGGCUACAAGG	13668
2268	CCCUGACGUGGAUGGGCAA	11082	UUGCCCAUCCACGUCAGGG	13786
2272	GACGUGGAUGGGCAAACUA	11086	UAGUUUGCCCAUCCACGUC	13790
2528	GCUUCGGCGGUUUGGAUAA	11342	UUAUCCAAACCGCCGAAGC	14046
2529	CUUCGGCGGUUUGGAUAUA	11343	UAUAUCCAAACCGCCGAAG	14047
2530	UUCGGCGGUUUGGAUAUUA	11344	UAAUAUCCAAACCGCCGAA	14048
2531	UCGGCGGUUUGGAUAUUUA	11345	UAAUAUCCAAACCGCCGA	14049
2532	CGGCGGUUUGGAUAUUUAA	11346	UUAAUAUCCAAACCGCCG	14050
2554	CCUCGUCCUCCGACUCGCA	11368	UGCGAGUCGGAGGACGAGG	14072
2558	GUCCUCCGACUCGCUGACA	11372	UGUCAGCGAGUCGGAGGAC	14076
2600	CAAUCCACGUUUUGGAUGA	11414	UCAUCCAAAACGUGGAUUG	14118

2628	CCGACAUUCCUCGGUAUUA	11442	UAAUACCGAGGAAUGUCGG	14146
2629	CGACAUUCCUCGGUAUUUA	11443	UAAAUACCGAGGAAUGUCG	14147
2631	ACAUUCCUCGGUAUUUAUA	11445	UAUAAAUACCGAGGAAUGU	14149
2636	CCUCGGUAUUUAUUGUCUA	11450	UAGACAAUAAAUAACCGAGG	14154
2639	CGGUAAUUUAUUGUCUGUA	11453	UGACAGACAAUAAAUAACCG	14157
2675	CCCCGACCCUCGCGAAUAA	11489	UUAUUCGCGAGGGUCGGGG	14193
2676	CCCGACCCUCGCGAAUAAA	11490	UUUAUUCGCGAGGGUCGGG	14194
2679	GACCCUCGCGAAUAAAAGA	11493	UCUUUUUAUUCGCGAGGGUC	14197
2680	ACCCUCGCGAAUAAAAGGA	11494	UCCUUUUUAUUCGCGAGGGU	14198
2681	CCCUCGCGAAUAAAAGGCA	11495	UGCCUUUUUAUUCGCGAGGG	14199
2682	CCUCGCGAAUAAAAGGCCA	11496	UGGCCUUUUUAUUCGCGAGG	14200
Neg. Control	n/a	n/a	n/a	n/a

¹19mer position in NM_001288766.1

Table 15B

ID # ¹	qPCR ²	qPCR ³	qPCR ⁴	qPCR ⁵	ID # ¹	qPCR ²	qPCR ³	qPCR ⁴	qPCR ⁵
385	150.8	153.8	64.1	142.5	1269	42.6	49.1	42.4	96.7
443	112.7	95.8	56.7	127.8	1274	63.6	55.4	78.0	98.5
444	76.5	66.2	36.7	113.6	1276	52.2	36.9	35.2	82.5
445	61.4	107.7	29.4	110.8	1283	35.2	62.8	56.6	95.9
533	168.8	119.8	85.7	118.1	1297	20.3	55.7	32.2	91.0
534	91.4	44.8	26.7	94.2	1342	44.6	46.7	41.5	94.5
535	101.0	65.9	33.1	109.9	1343	65.8	80.0	56.1	119.2
539	81.7	70.2	34.1	102.4	1344	30.9	63.7	51.7	116.7
540	68.3	56.8	40.0	114.6	1346	133.8	102.9	98.0	104.0
541	112.1	107.3	73.8	120.6	1825	54.1	69.2	28.6	86.7
543	42.6	59.9	41.9	117.8	1886	786.9	282.0	130.5	98.4
544	42.4	107.5	66.9	154.7	1890	28.8	30.3	51.5	94.4
576	107.4	119.0	85.0	127.5	1898	125.5	57.5	67.7	97.6
577	101.6	90.1	72.1	106.6	1945	23.5	22.6	21.8	57.6
581	199.3	97.7	69.9	103.5	1960	28.4	33.7	35.7	87.9
583	66.6	77.5	66.4	100.3	2126	147.9	87.2	86.8	98.1
584	26.3	37.3	31.0	88.3	2127	46.5	51.9	52.7	96.2
690	163.6	84.1	58.0	92.7	2149	44.7	41.5	62.0	99.6
716	29.0	39.6	29.4	86.0	2150	110.4	89.1	63.4	114.1
717	44.4	45.7	52.8	102.5	2268	53.5	48.6	60.8	113.1
785	79.9	93.2	71.3	101.0	2272	56.5	54.7	46.9	92.5
786	85.5	63.8	54.3	92.2	2528	32.5	32.8	32.7	76.9
789	45.4	51.3	43.8	96.9	2529	19.6	25.8	21.4	59.5
1026	55.6	77.3	32.0	110.4	2530	29.5	25.9	32.8	68.1
1027	98.9	94.7	35.2	108.3	2531	22.2	31.6	25.4	64.3
1028	132.1	104.9	27.3	87.3	2532	44.4	35.6	29.2	74.0
1029	62.2	95.5	45.9	94.2	2554	13.7	22.6	26.8	60.9
1037	68.2	80.2	65.3	97.0	2558	54.6	47.4	28.0	72.0
1039	42.3	79.3	53.6	97.0	2600	205.4	209.6	n.d.	n.d.

1041	67.2	64.4	73.2	98.6	2628	12.6	28.5	20.1	56.2
1043	342.8	86.6	61.5	96.5	2629	12.8	39.5	20.6	63.8
1044	109.5	84.8	42.7	94.0	2631	97.4	68.6	39.7	104.4
1047	101.3	72.1	35.2	90.7	2636	62.0	68.6	16.8	58.7
1071	88.5	99.6	91.6	101.3	2639	33.2	46.1	22.2	81.0
1073	134.3	63.0	36.3	93.6	2675	57.7	82.5	n.d.	n.d.
1262	36.5	59.6	27.3	117.5	2676	31.1	53.0	n.d.	n.d.
1263	47.6	79.7	33.9	104.3	2679	44.7	75.7	n.d.	n.d.
1264	64.2	54.5	43.7	95.4	2680	89.2	61.5	n.d.	n.d.
1265	19.8	57.6	30.9	91.6	2681	19.0	28.6	n.d.	n.d.
1267	61.3	85.9	73.4	97.1	2682	98.2	61.8	n.d.	n.d.
1268	32.0	28.3	38.0	92.3	Neg. Control	101.2	100.6	101.1	106.4

²DM1 myoblasts; 10 nM; % DMPK mRNA

³DM1 myoblasts; 1 nM; % DMPK mRNA

⁴SJCRH30; 1 nM; % DMPK mRNA

⁵SJCRH30; 0.01 nM; % DMPK mRNA

Example 17: *In vitro* dose response curves for a selected set of DMPK siRNAs

[0558] To further validate the activity of the DMPK siRNAs, many of the sequences that showed the best activity in the initial screen were selected for a follow-up evaluation in dose response format. Once again, two human cell lines were used to assess the *in vitro* activity of the DMPK siRNAs: first, SJCRH30 human rhabdomyosarcoma cell line; and second, Myotonic Dystrophy Type 1 (DM1) patient-derived immortalized human skeletal myoblasts. The selected siRNAs were transfected in a 10-fold dose response at 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 nM final concentrations or in a 9-fold dose response at 50, 5.55556, 0.617284, 0.068587, 0.007621, 0.000847, and 0.000094 nM final concentrations. The siRNAs were formulated with transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's "forward transfection" instructions. Cells were plated 24 h prior to transfection in triplicate on 96-well tissue culture plates, with 8500 cells per well for SJCRH30 and 4000 cells per well for DM1 myoblasts. At 48 h (SJCRH30) or 72 h (DM1 myoblasts) post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with DMPK-specific and PPIB-specific TaqMan human gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). DMPK values were normalized within each sample to PPIB gene expression. The quantification of DMPK downregulation was performed using the standard $2^{-\Delta\Delta Ct}$ method. All experiments were performed in triplicate, with Tables 16A-B, 17A-B, and 18A-B presenting the mean values of the triplicates as well as the calculated IC₅₀ values determined from fitting curves to the dose-response data by non-linear regression.

Table 16A

ID # ¹	sense strand sequence (5'-3') Passenger Strand (PS)	SEQ ID NO:	antisense strand sequence (5'-3') Guide Strand (GS)	SEQ ID NO:
535	GGGCGAGGUGUCGUGCUUA	9349	UAAGCACGACACCUCGCCC	12053
584	GACCGGCGGUGGAUCACGA	9398	UCGUGAUCCACCGCCGGUC	12102
716	AUGGCGCGCUUCUACCUGA	9530	UCAGGUAGAAGCGCGCCAU	12234
1028	CAGACGCCCUCUACGCGA	9842	UCGCGUAGAAGGGCGUCUG	12546
1276	UUUCGAAGGUGCCACCGAA	10090	UUCGGUGGCACCUUCGAAA	12794
1825	UGCUCUGUUCGCCGUUGA	10639	UCAACGGCGAACAGGAGCA	13343
1945	CCCUAGAACUGUCUUCGAA	10759	UUCGAAGACAGUUCUAGGG	13463
2529	CUUCGGCGGUUUGGAUUA	11343	UAUAUCCAAACCGCCGAAG	14047
2558	GUCCUCCGACUCGCUGACA	11372	UGUCAGCGAGUCGGAGGAC	14076
2628	CCGACAUUCCUCGGUAUUA	11442	UAAUACCGAGGAAUGUCGG	14146
2636	CCUCGGUAUUUAUUGUCUA	11450	UAGACAAUAAAUACCGAGG	14154

¹19mer position in NM_001288766.1

Table 16B

ID # ¹	qPCR ²	qPCR ³	qPCR ⁴	qPCR ⁵	qPCR ⁶	qPCR ⁷	qPCR ⁸	IC50 (nM)
535	111.9	105.4	106.3	82.4	36.7	29.5	35.7	0.165
584	90.5	90.2	84.7	67.8	38.0	25.8	28.3	0.190
716	88.9	85.2	81.9	62.0	32.6	19.3	20.3	0.181
1028	88.5	81.8	83.0	61.3	32.7	27.3	31.5	0.127
1276	87.0	85.0	84.0	66.1	40.5	34.0	36.4	0.150
1825	85.1	85.9	83.7	69.1	36.2	25.2	25.0	0.259
1945	85.0	81.7	74.4	44.9	22.9	17.7	17.2	0.070
2529	83.3	81.8	75.3	50.6	24.6	17.5	17.7	0.103
2558	84.3	81.1	74.3	45.4	23.4	13.3	11.8	0.088
2628	85.3	84.0	79.5	59.8	30.3	23.5	25.1	0.140
2636	86.3	86.9	74.3	44.0	19.8	12.4	13.0	0.070

²SJCRH30; 0.0001 nM; % DMPK mRNA

³SJCRH30; 0.001 nM; % DMPK mRNA

⁴SJCRH30; 0.01 nM; % DMPK mRNA

⁵SJCRH30; 0.1 nM; % DMPK mRNA

⁶SJCRH30; 1 nM; % DMPK mRNA

⁷SJCRH30; 10 nM; % DMPK mRNA

⁸SJCRH30; 100 nM; % DMPK mRNA

Table 17A

ID # ¹	sense strand sequence (5'-3') Passenger Strand (PS)	SEQ ID NO:	antisense strand sequence (5'-3') Guide Strand (GS)	SEQ ID NO:
2600	CAAUCCACGUUUUGGAUGA	11414	UCAUCCAAAACGUGGAUUG	14118
2636	CCUCGGUAUUUAUUGUCUA	11450	UAGACAAUAAAUACCGAGG	14154
2675	CCCCGACCCUCGCGAAUAA	11489	UUAUUCGCGAGGGUCGGGG	14193
2676	CCCGACCCUCGCGAAUAAA	11490	UUUAUUCGCGAGGGUCGGG	14194

2679	GACCCUCGCGAAUAAAAGA	11493	UCUUUUUAUUCGCGAGGGUC	14197
2680	ACCCUCGCGAAUAAAAGGA	11494	UCCUUUUUAUUCGCGAGGGU	14198
2681	CCCUCGCGAAUAAAAGGCA	11495	UGCCUUUUUAUUCGCGAGGG	14199
2682	CCUCGCGAAUAAAAGGCCA	11496	UGGCCUUUUUAUUCGCGAGG	14200

¹19mer position in NM_001288766.1

Table 17B

ID # ¹	qPCR ²	qPCR ³	qPCR ⁴	qPCR ⁵	qPCR ⁶	qPCR ⁷	IC50 (nM)
2600	107.5	107.6	108.1	106.3	103.1	72.7	31.31
2636	81.1	81.1	74.0	47.2	25.7	11.5	0.073
2675	88.1	88.3	84.3	64.6	38.1	20.7	0.151
2676	88.9	78.9	84.4	72.7	44.9	35.6	0.204
2679	84.0	87.3	82.7	53.3	31.4	13.5	0.091
2680	87.4	85.3	85.1	68.5	44.5	39.6	0.110
2681	87.0	85.4	77.6	49.6	26.5	16.0	0.061
2682	82.4	83.9	77.1	50.8	27.3	31.1	0.047

²SJCRH30; 0.000094 nM; % DMPK mRNA

³SJCRH30; 0.000847 nM; % DMPK mRNA

⁴SJCRH30; 0.007621 nM; % DMPK mRNA

⁵SJCRH30; 0.068587 nM; % DMPK mRNA

⁶SJCRH30; 0.617284 nM; % DMPK mRNA

⁷SJCRH30; 5.55556 nM; % DMPK mRNA

Table 18A

ID # ¹	sense strand sequence (5'-3') Passenger Strand (PS)	SEQ ID NO:	antisense strand sequence (5'-3') Guide Strand (GS)	SEQ ID NO:
584	GACCGGCGGUGGAUCACGA	9398	UCGUGAUCCACCGCCGGUC	12102
716	AUGGCGCGCUUCUACCUGA	9530	UCAGGUAGAAGCGCGCCAU	12234
1265	UUUACACCGGAUUUCGAAA	10079	UUUCGAAAUCCGGUGUAAA	12783
1297	AUGCAACUUCGACUUGGUA	10111	UACCAAGUCGAAGUUGCAU	12815
1945	CCCUAGAACUGUCUUCGAA	10759	UUCGAAGACAGUUCUAGGG	13463
1960	CGACUCCGGGGCCCCGUUA	10774	UAACGGGGCCCCGGAGUCG	13478
2529	CUUCGGCGGUUUGGAUAUA	11343	UAUAUCCAAACCGCCGAAG	14047
2530	UUCGGCGGUUUGGAUAUUA	11344	UAAUAUCCAAACCGCCGAA	14048
2531	UCGGCGGUUUGGAUAUUA	11345	UAAUAUCCAAACCGCCGA	14049
2554	CCUCGUCCUCCGACUCGCA	11368	UGCGAGUCGGAGGACGAGG	14072
2628	CCGACAUUCCUCGGUAUUA	11442	UAAUACCGAGGAAUGUCGG	14146
2629	CGACAUUCCUCGGUAUUA	11443	UAAAUACCGAGGAAUGUCG	14147
2681	CCCUCGCGAAUAAAAGGCA	11495	UGCCUUUUUAUUCGCGAGGG	14199

¹19mer position in NM_001288766.1

Table 18B

ID # ¹	qPCR ²	qPCR ³	qPCR ⁴	qPCR ⁵	qPCR ⁶	qPCR ⁷	IC50 (nM)
584	90.8	77.0	97.7	71.9	45.0	29.7	0.228
716	96.5	82.5	77.0	64.6	43.3	33.9	0.080
1265	68.5	80.9	68.0	57.1	37.5	25.7	0.146
1297	71.4	67.2	69.4	53.5	40.5	25.4	0.171
1945	71.8	62.3	41.7	29.8	22.4	15.3	0.006
1960	63.0	65.4	62.1	45.8	31.1	28.3	0.068
2529	63.5	58.7	49.2	31.1	22.9	21.9	0.017
2530	69.3	66.7	53.1	43.2	38.8	24.5	0.016
2531	69.9	72.4	57.3	40.2	35.4	25.6	0.018
2554	68.2	70.1	51.2	43.0	32.1	17.3	0.043
2628	69.7	67.9	62.5	38.4	31.6	17.1	0.042
2629	72.1	65.6	69.0	42.1	34.4	13.7	0.078
2681	82.4	91.5	87.6	55.5	29.3	19.6	0.084

²DM1 myoblasts; 0.000094 nM; % DMPK mRNA

³DM1 myoblasts; 0.000847 nM; % DMPK mRNA

⁴DM1 myoblasts; 0.007621 nM; % DMPK mRNA

⁵DM1 myoblasts; 0.068587 nM; % DMPK mRNA

⁶DM1 myoblasts; 0.617284 nM; % DMPK mRNA

⁷DM1 myoblasts; 5.55556 nM; % DMPK mRNA

Example 18: *In vitro* experiments to determine species cross-reactivity in mouse

[0559] The selected siRNAs were transfected at 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 nM final concentrations into C2C12 mouse muscle myoblasts (ATCC® CRL-1772™). The siRNAs were formulated with transfection reagent Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's "forward transfection" instructions. Cells were plated 24 h prior to transfection in triplicate on 96-well tissue culture plates, with 4000 cells per well for C2C12 seeding. At 48 h post-transfection cells were washed with PBS and harvested with TRIzol® reagent (Life Technologies). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with DMPK-specific and PPIB-specific TaqMan mouse gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). DMPK values were normalized within each sample to PPIB gene expression. The quantification of DMPK downregulation was performed using the standard $2^{-\Delta\Delta C_t}$ method. All experiments were performed in triplicate, with the results shown in Fig. 17. Four DMPK siRNAs (the numbers indicated in the Fig. 17 legend correspond to the ID # that is listed in Table 19 (Tables 19A-19B)) were shown to effectively cross-react with mouse DMPK mRNA, producing robust mRNA knockdown in the mouse C2C12 myoblast cell line. Two of the siRNAs (ID #s 535 and 1028) were slightly less effective and only produced approximately 70% maximum mRNA knockdown. Two of the siRNAs (ID #s 2628 and 2636) were more effective and produced approximately 90% maximum mRNA knockdown.

Example 19: *In vivo* experiments to determine species cross-reactivity in mouse**[0560] Animals**

[0561] All animal studies were conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the “Guide for the Care and Use of Laboratory Animals” (National Research Council publication, 8th Ed., revised in 2011). All mice were obtained from either Charles River Laboratories or Harlan Laboratories.

[0562] Conjugate preparation

[0563] The *in vivo* studies used a total of five siRNAs: four DMPK siRNAs that were shown to be cross-reactive with mouse *in vitro* (Fig. 17) and one siRNA with a scrambled sequence that does not produce DMPK knockdown and can be used as a negative control. All siRNAs were synthesized using standard solid phase synthesis methods that are described in the oligonucleotide synthesis literature. The single strands were purified by HPLC using standard methods and then pure single strands were mixed at equimolar ratios to generate pure duplexes. All siRNAs were synthesized with a hexylamine linker on the 5' end of the passenger (sense) strand that can act as a conjugation handle for linkage to the antibody. The siRNAs were synthesized using optimal base, sugar, and phosphate modifications that are well described in the field of RNAi to maximize the potency, maximize the metabolic stability, and minimize the immunogenicity of the duplex.

[0564] The anti-mouse transferrin receptor (TfR1, also known as CD71) monoclonal antibody (mAb) is a rat IgG2a subclass monoclonal antibody that binds mouse CD71 protein with high affinity. This CD71 antibody was produced by BioXcell and it is commercially available (Catalog # BE0175). The antibody-siRNA conjugates were synthesized using the CD71 mAb from BioXcell and the respective DMPK or scramble siRNAs. All conjugates were synthesized through cysteine conjugation to the antibody and amine conjugation to the siRNA (through the hexylamine) utilizing a bismaleimide-TFP ester linker as previously described. All conjugates were purified by strong cation exchange (SAX) to isolate only the conjugate with a drug-antibody ratio (DAR) equal to 1 (i.e. a molar ratio of 1 siRNA per mAb), as previously described. All antibody-siRNA conjugates were formulated by dilution in PBS for *in vivo* dosing.

[0565] *In vivo* dosing and analysis

[0566] Purified DAR1 antibody-siRNA conjugates were dosed into groups (n=4) of female wild-type CD-1 mice (4-6 weeks old) at 0.1, 0.3, 1, and 3 mg/kg (based on the weight of siRNA) by a single i.v. bolus injection into the tail vein at a dosing volume of 5 mL/kg. A single sham dose of PBS vehicle was injected at matched dose volumes into a control group (n=5) of female wild-type CD-1 mice (also 4-6 weeks old). The mice were sacrificed by CO₂ asphyxiation 7 days post-dose and 20-30 mg pieces of multiple tissues (gastrocnemius, tibialis anterior, quadriceps, diaphragm, heart, and liver) were harvested from each mouse and snap-frozen in liquid nitrogen. TRIzol® reagent (Life Technologies) was added and then each tissue piece was homogenized using a TissueLyser II (Qiagen). RNA was isolated using the Direct-zol-96 RNA Kit (Zymo Research) according to the manufacturer's instructions. 10 µl of RNA

was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. cDNA samples were evaluated by qPCR with DMPK-specific and PPIB-specific TaqMan mouse gene expression probes (Thermo Fisher) using TaqMan® Fast Advanced Master Mix (Applied Biosystems). DMPK values were normalized within each sample to PPIB gene expression. The quantification of DMPK downregulation was performed using the standard $2^{-\Delta\Delta C_t}$ method by comparing the treated animals to the PBS control group. The *in vivo* DMPK mRNA knockdown results are presented in Fig. 18A-Fig. 18F. All four DMPK siRNAs (the numbers indicated in the Fig. 18A-Fig. 18F legends correspond to the ID # that is listed in Table 19 (Tables 19A-19B)) were shown to effectively reduce levels of DMPK mRNA in all skeletal muscles that were analyzed (gastrocnemius, tibialis anterior, quadriceps, and diaphragm) in a dose-dependent manner. The most active siRNA achieved greater than 75% DMPK mRNA knockdown in all skeletal muscles at the highest dose (3 mg/kg). The *in vivo* DMPK knockdown observed in skeletal muscles of mice (Fig. 18A-Fig. 18F) correlated well with the *in vitro* DMPK knockdown observed in the mouse C2C12 myoblast cell line (Fig. 17), with siRNA ID #s 2628 and 2636 demonstrating higher mRNA knockdown than siRNA ID #s 535 and 1028. In addition to DMPK mRNA knockdown in skeletal muscle, strong activity (greater than 50% mRNA knockdown) was observed in mouse cardiac muscle (heart) as well. Finally, poor activity (less than 50% mRNA knockdown) was observed in mouse liver. These results demonstrate that it is possible to achieve robust DMPK mRNA knockdown in multiple mouse muscle groups (including both skeletal and cardiac), while minimizing the knockdown in off-target tissues such as the liver.

Example 20: siRNA synthesis

[0567] All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified using HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All the siRNA passenger strands contained a C6-NH₂ conjugation handle on the 5' end, see Fig. 20A-Fig. 21B. For the 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs, the conjugation handle was connected to siRNA passenger strand via an inverted abasic phosphodiester, see Fig. 20A-Fig. 20B for the structures. For the blunt ended duplex with 19 bases of complementarity and one 3' dinucleotide overhang the conjugation handle was connected to siRNA passenger strand via a phosphodiester on the terminal base, see Fig. 21A-Fig. 21B for the structures.

[0568] Purified single strands were duplexed to get the double stranded siRNA.

Example 21: 2017-PK-401-C57BL6: In vivo transferrin mAb conjugate delivery of various Atrogin-1 siRNAs

[0569] For groups 1-4, see study design in Fig. 22, the 21mer Atrogin-1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCUACGUAGUUGAAUCUUCUU (SEQ ID NO: 14230). The guide and fully complementary RNA passenger strands were assembled on solid phase

using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0570] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

[0571] Step 1: Antibody reduction with TCEP

[0572] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37 °C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0573] Step 2: Purification

[0574] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated, and buffer exchanged with pH 7.4 PBS.

[0575] Anion exchange chromatography method (SAX)-1.

[0576] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 µm

[0577] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0578] Gradient:

a.	%A%B	Column Volume	
b.	100 0	1	
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100 0	2	

[0579] Anion exchange chromatography (SAX) method-2

[0580] Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm

[0581] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min

[0582] Gradient:

a.	Time	%A	%B

b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0583] Step-3: Analysis of the purified conjugate

[0584] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2. For conjugate mTfR1-mAb-Atrogin-1 (DAR1), the SAX retention time was 9.1 min and % purity (by chromatographic peak area) was 99.

[0585] In vivo study design

[0586] The conjugates were assessed for their ability to mediate mRNA downregulation of Atrogin-1 in skeletal muscle, in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Fig. 22. After the indicated time points, gastrocnemius (gastroc) and heart muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0587] Results

[0588] The Atrogin-1 siRNA guide strands was able to mediate downregulation of the target gene in gastroc and heart muscle when conjugated to an anti-TfR mAb targeting the transferrin receptor, see Fig. 23 and Fig. 24.

[0589] Conclusions

[0590] In this example, it was demonstrated that a TfR1-siAtrogin-1 conjugate, after *in vivo* delivery, mediated specific down regulation of the target gene in gastroc and heart muscle. The ASC was made with an anti-transferrin antibody, mouse gastroc and heart muscle expresses the transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in accumulation of the conjugates in gastroc and heart muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the target mRNA.

Example 22: 2017-PK-413-C57BL6: In vivo transferrin mAb conjugate delivery of various MuRF1 sequence

[0591] For groups 1-2, see study design in Fig. 25, the 21mer MuRF1 (2089) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UUUCGCACCAACGUAGAAAUU (SEQ ID NO: 14231). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0592] For groups 3-6, see study design in figure G, the 21mer MuRF1 (2265) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCGUGAGACAGUAGAUGUUUU (SEQ ID NO: 14232). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained a single conjugation handle, a C6-NH₂ at the 5' end connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker.

[0593] For groups 7-10, see study design in figure G, the 21mer MuRF1 (2266) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCACACGUGAGACAGUAGAUU (SEQ ID NO: 14233). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained a single conjugation handle, a C6-NH₂ at the 5' end connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker.

[0594] For groups 11-14, see study design in figure G, the 21mer MuRF1 (2267) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UUCACACGUGAGACAGUAGUU (SEQ ID NO: 14234). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained a single conjugation handle, a C6-NH₂ at the 5' end connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker.

[0595] For groups 15-18, see study design in figure G, the 21mer MuRF1 (2268) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UAAUAUUUCAUUUCGCACCUU (SEQ ID NO: 14235). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained a single conjugation handle, a C6-NH₂ at the 5' end connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker.

[0596] For groups 19-22, see study design in figure G, the 21mer MuRF1 (2269) guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UAAGCACCAAUUGGCAUAUU (SEQ ID NO: 14236). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained a single conjugation handle, a C6-NH₂ at the 5' end connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker.

[0597] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

[0598] Step 1: Antibody reduction with TCEP

[0599] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37°C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0600] Step 2: Purification

[0601] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

[0602] Anion exchange chromatography method (SAX)-1.

[0603] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 μm

[0604] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0605] Gradient:

a.	%A	%B	Column Volume
b.	100	0	1

c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

[0606] Anion exchange chromatography (SAX) method-2**[0607]** Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm**[0608]** Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min**[0609]** Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0610] Step-3: Analysis of the purified conjugate**[0611]** The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 (Table 19).

Table 19.

Conjugate	SAX retention time (min)	% purity (by peak area)
mTfR1-mAb-MuRF1(R2089) (DAR1)	9.3	99
mTfR1-mAb-MuRF1(R2265) (DAR1)	9.1	95
mTfR1-mAb-MuRF1(R2266) (DAR1)	9.1	98
mTfR1-mAb-MuRF1(R2267) (DAR1)	9.1	98
mTfR1-mAb-MuRF1(R2268) (DAR1)	9.1	97
mTfR1-mAb-MuRF1(R2269) (DAR1)	9.2	97

[0612] In vivo study design**[0613]** The conjugates were assessed for their ability to mediate mRNA downregulation of MuRF1 in muscle (gastroc and heart), in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Fig. 25. After 96 hours, gastrocnemius (gastroc) and heart muscle tissues were harvested and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the

comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0614] Results

[0615] The MuRF1 siRNA guide strands was able to mediate downregulation of the target gene in gastroc and heart muscle when conjugated to an anti-TfR1 mAb targeting the transferrin receptor 1, see Fig. 26 and Fig. 27.

[0616] Conclusions

[0617] In this example, it was demonstrated that TfR1-MuRF1 conjugates, after *in vivo* delivery, mediated specific down regulation of the target gene in gastroc and heart muscle. The ASC was made with an anti-transferrin1 antibody, mouse gastroc and heart muscle expresses the transferrin receptor1 and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in accumulation of the conjugates in gastroc muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the target mRNA.

Example 23: 2017-PK-412-C57BL6: Prevention of dexamethasone induce muscle atrophy with Atrogin-1 and MuRF1 TfR1-mAb conjugates

[0618] For this experiment three different siRNAs were used:

[0619] (1): A 21mer Atrogin-1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCUACGUAGUUGAAUCUUCUU (SEQ ID NO: 14230). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0620] (2): A 21mer MuRF1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UUUCGCACCAACGUAGAAAUU (SEQ ID NO: 14231). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0621] (3): Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0622] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

[0623] Step 1: Antibody reduction with TCEP

[0624] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37°C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0625] Step 2: Purification

[0626] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

[0627] Anion exchange chromatography method (SAX)-1.

[0628] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 µm

[0629] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0630] Gradient:

a.	%A	%B	Column Volume
b.	100	0	1
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

[0631] Anion exchange chromatography (SAX) method-2

[0632] Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm

[0633] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min

[0634] Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0635] Step-3: Analysis of the purified conjugate

[0636] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 (Table 20).

Table 20.

Conjugate	SAX retention time (min)	% purity (by peak area)
mTfR1-Atrogin-1 (DAR1)	9.3	97
mTfR1-MuRF1 (DAR1)	9.5	98
mTfR1-SC (DAR1)	9.0	99

[0637] **In vivo study design**

[0638] The conjugates were assessed for their ability to mediate mRNA downregulation of MuRF1 and Atrogin-1 in muscle (gastroc) in the presence and absence of muscle atrophy, in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Table 21. Seven days post conjugate delivery, for groups 2-4, 9-11, and 16-18, muscle atrophy was induced by the daily administration, via intraperitoneal injection (10 mg/kg) of dexamethasone for 21 days. For the control groups 5-7, 12-14 and 19-21 (no induction of muscle atrophy) PBS was administered by the daily intraperitoneal injection. Groups 1, 8, 15 and 22 were harvested at day 7 to establish the baseline measurements of mRNA expression and muscle weighted, prior to induction of muscle atrophy. At the time points indicated, gastrocnemius (gastroc) and heart muscle tissues were harvested, weighed and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta \Delta Ct$).

[0639] Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 21.

Animal and Group Info			Dex/PBS Dosing				Compound Info		
Group	Test Article	N	ROA	Dose Volume (mL/kg)	# of Doses	Dose Schedule	siRNA Dose (mg/kg)	# of Doses	Harvest Time (d)
1	mTfR1-Atrogin-1 (DAR1)	5	-	-	-	-	3	1	7
2	mTfR1-Atrogin-1 (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	10
3	mTfR1-Atrogin-1 (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	17
4	mTfR1-Atrogin-1 (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	28
5	mTfR1-Atrogin-1 (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	10
6	mTfR1-Atrogin-1 (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	17
7	mTfR1-Atrogin-1 (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	28
8	mTfR1-Atrogin-1 (DAR1) + mTfR1-MuRF1 (DAR1)	5	-	-	-	-	3+3	1	7
9	mTfR1-Atrogin-1 (DAR1) + mTfR1-MuRF1 (DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3+3	1	10
10	mTfR1-Atrogin-1 (DAR1) + mTfR1-MuRF1 (DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3+3	1	17
11	mTfR1-Atrogin-1 (DAR1) + mTfR1-MuRF1 (DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3+3	1	28
12	mTfR1-Atrogin-1 (DAR1) + mTfR1-MuRF1 (DAR1), +PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3+3	1	10
13	mTfR1-Atrogin-1 (DAR1) + mTfR1-	5	IP	6.25	Daily; 21	192h Post	3+3	1	17

	MuRF1 (DAR1), +PBS				Days	ASC			
14	mTfR1-Atrogin-1 (DAR1) + mTfR1- MuRF1 (DAR1), +PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3+3	1	28
15	mTfR1-SC (DAR1)	5	-	-	-	-	3	1	7
16	mTfR1-SC DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	10
17	mTfR1-SC (DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	17
18	mTfR1-SC (DAR1), +DEX (10 mg/kg)	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	28
19	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	10
20	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	17
21	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 21 Days	192h Post ASC	3	1	28
22	PBS Control	5	-	-	-	-	-	1	7

[0640] Results

[0641] The data are summarized in Fig. 28-Fig. 31. Co-delivery of Atrogin-1 and MuRF1 siRNAs efficiently downregulated Atrogin-1 and MuRF1 mRNA expression in normal and atrophic muscles, when delivered using a TfR1 mAb conjugate. Induction of atrophy transiently induces Atrogin-1 and MuRF1 expression about 4-fold. A single dose of mTfR1-Atrogin-1 + TfR1.mAb-siMuRF1 (3 mg/kg, each and dose as a mixture) reduced Atrogin-1 and MuRF1 mRNA levels by >70% in normal and atrophic gastrocnemius muscle. Downregulation of MuRF1 and Atrogin-1 mRNA increases gastrocnemius weight by 5-10% and reduces DEX-induced gastrocnemius weight loss by 50%. Downregulation of Atrogin-1 alone has no significant effect on gastrocnemius weight changes. In the absence of muscle atrophy treatment with Atrogin-1/MuRF1 siRNAs induces muscle hypertrophy.

[0642] Conclusions

[0643] In this example, it was demonstrated that co-delivery of Atrogin-1 and MuRF1 siRNAs efficiently downregulated Atrogin-1 and MuRF1 mRNA expression in normal and atrophic gastroc muscles, when delivered using a TfR1 mAb conjugate. The conjugates had little effect on heart muscle, where downregulation of Atrogin-1 could be detrimental. Downregulation of MuRF1 and Atrogin-1 mRNA increased gastroc muscle weight by 5-10% and reduced DEX-induced gastroc muscle weight loss by 50%. Downregulation of Atrogin-1 alone has no significant effect on gastrocnemius weight changes. The ASC were made with an anti-transferrin antibody, mouse gastroc muscle expresses the transferrin receptor and the conjugate has a mouse specific anti-transferrin antibody to target the siRNA, resulting in

accumulation of the conjugates in gastroc muscle. Receptor mediate uptake resulted in siRNA mediated knockdown of the target mRNA.

Example 24: 2017-PK-435-C57BL6: *In vivo* dose response experiment for transferrin mAb conjugate delivery of Atrogin-1

[0644] For groups 1-12, see study design in Fig. 32, the 21mer Atrogin-1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCGUAGUAAAUCUUCUGGUU (SEQ ID NO: 14237). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in figure A. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0645] For groups 13-18 see study design in Fig. 32, a 21mer negative control siRNA sequence (scramble) (published by Burke et al. (2014) Pharm. Res., 31(12):3445-60) with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0646] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

[0647] Step 1: Antibody reduction with TCEP

[0648] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37 °C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0649] Step 2: Purification

[0650] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

[0651] Anion exchange chromatography method (SAX)-1.

[0652] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 μ m

[0653] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0654] Gradient:

a.	%A	%B	Column Volume
b.	100	0	1
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

[0655] Anion exchange chromatography (SAX) method-2

[0656] Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm

[0657] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min

[0658] Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0659] Step-3: Analysis of the purified conjugate

[0660] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 (Table 22).

Table 22.

Conjugate	SAX retention time (min)	% purity (by peak area)
TfR1-Atrogin-1 DAR1	9.2	99
TfR1-Scramble DAR1	8.9	93

[0661] In vivo study design

[0662] The conjugates were assessed for their ability to mediate mRNA downregulation of Atrogin-1 in muscle (gastroc) in the presence and absence of muscle atrophy, in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Fig. 32. Seven days post conjugate delivery, for groups 3, 6, 9, 12, and 15, muscle atrophy was induced by the daily administration via intraperitoneal injection (10 mg/kg) of dexamethasone for 3 days. For the control groups 2, 5, 8, 11, and 14 (no induction of muscle atrophy) PBS was administered by the daily intraperitoneal injection. Groups 1, 4, 7, 10, and 13 were harvested at day 7 to establish the baseline measurements of mRNA expression and muscle weighted, prior to induction of muscle atrophy. At three days post-atrophy induction (or 10 days post conjugate delivery), gastrocnemius (gastroc) muscle tissues were harvested, weighed and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0663] Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0664] Results

[0665] The data are summarized in Fig. 33-Fig. 35. The Atrogin-1 siRNA guide strands were able to mediate downregulation of the target gene in gastroc muscle when conjugated to an anti-TfR mAb targeting the transferrin receptor, see Fig. 33. Increasing the dose from 3 to 9 mg/kg reduced atrophy-induced Atrogin-1 mRNA levels 2-3 fold. The maximal KD achievable with this siRNA was 80% and a tissue concentration of 40 nM was needed to achieve maximal KD in atrophic muscles. This highlights the conjugate delivery approach is able to change disease induce mRNA expression levels of Atrogin-1 (see Fig. 34), by increasing the increasing the dose. Fig. 35 highlights that mRNA down regulation is mediated by RISC loading of the Atrogin-1 guide strands and is concentration dependent.

[0666] Conclusions

[0667] In this example, it was demonstrated that a TfR1-Atrogin-1 conjugates, after *in vivo* delivery, mediated specific down regulation of the target gene in gastroc muscle in a dose dependent manner. After induction of atrophy the conjugate was able to mediate disease induce mRNA expression levels of Atrogin-1 at the higher doses. Higher RISC loading of the Atrogin-1 guide strand correlated with increased mRNA downregulation.

Example 25: 2017-PK-381-C57BL6: Myostatin (MSTN) downregulation reduces muscle loss in dexamethasone-treated Mice

[0668] For groups 1-12, see study design in Table 24, the 21mer Atrogin-1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UCGUAGUAAAUCUUCUGGUU (SEQ ID NO: 14237). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0669] For groups 13-18 see study design in Table 24, a 21mer negative control siRNA sequence (scramble) (published by Burke et al. (2014) Pharm. Res., 31(12):3445-60) with 19 bases of complementarity and 3' dinucleotide overhangs were used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0670] Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker

[0671] Step 1: Antibody reduction with TCEP

[0672] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37 °C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0673] Step 2: Purification

[0674] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

[0675] Anion exchange chromatography method (SAX)-1.

[0676] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 μm

[0677] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0678] Gradient:

a.	%A	%B	Column Volume
b.	100	0	1
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

[0679] Anion exchange chromatography (SAX) method-2

[0680] Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm

[0681] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min

[0682] Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0683] Step-3: Analysis of the purified conjugate

[0684] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 (Table 23).

Table 23.

Conjugate	SAX retention time (min)	% purity (by peak area)
mTfR1-MSTN (DAR1)	9.2	98
mTfR1-SC (DAR1)	8.9	98

[0685] **In vivo study design**

[0686] The conjugates were assessed for their ability to mediate mRNA downregulation of MSTN in muscle (gastroc) in the presence and absence of muscle atrophy, in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Table 24. Seven days post conjugate delivery, for groups 2, 3, 4, 9, 10 and 11, muscle atrophy was induced by the daily administration via intraperitoneal injection (10 mg/kg) of

dexamethasone for 13 days. For the control groups 5, 6, 7, 12, 13 and 14 (no induction of muscle atrophy), PBS was administered by the daily intraperitoneal injection. Groups 1 and 8 were harvested at day 7 to establish the baseline measurements of mRNA expression and muscle weighted, prior to induction of muscle atrophy. At 3, 7, and 14 days post-atrophy induction (or 10, 14, and 21 days post conjugate delivery), gastrocnemius (gastroc) muscle tissues were harvested, weighed and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (Δ Ct) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$ Ct).

[0687] Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 24

Animal and Group Info			Dex/PBS Dosing				Compound Info		
Group	Test Article	N	ROA	Dose Volume (mL/kg)	# of Doses	Dose Schedule	siRNA Dose (mg/kg)	ROA	Harvest Time (d)
1	mTfR1-MSTN (DAR1)	5	-	-	-	-	3	IV	7
2	mTfR1-MSTN (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	10
3	mTfR1-MSTN (DAR=1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	14
4	mTfR1-MSTN (DAR=1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	21
5	mTfR1-MSTN (DAR1), PBS	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	10
6	mTfR1-MSTN	5	IP	6.25	Daily; 13	192h Post ASC	3	IV	14

	(DAR1), PBS				Days				
7	mTfR1-MSTN (DAR1), PBS	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	21
8	mTfR1-SC (DAR1)	5	-	-	-	-	3	IV	7
9	mTfR1-SC (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	10
10	mTfR1-SC (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	14
11	mTfR1-SC (DAR1), +DEX (10mg/kg)	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	21
12	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	10
13	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	14
14	mTfR1-SC (DAR1), PBS	5	IP	6.25	Daily; 13 Days	192h Post ASC	3	IV	21
15	PBS Control	5	-	-	-	-	-	IV	7

[0688] Results

[0689] The data are summarized in Fig. 36 and Fig. 37. The MSTN siRNA guide strands were able to mediate downregulation of the target gene in gastroc muscle when conjugated to an anti-TfR mAb targeting the transferrin receptor, see Fig. 36, in the presence and absence of dexamethasone induced atrophy. A single of 3 mg/kg siRNA downregulated MSTN mRNA levels by >75%. In the presence of dexamethasone induced atrophy, MSTN downregulation increased muscle mass and attenuates Dex-induced muscle loss, see Fig. 37.

[0690] Conclusions

[0691] In this example, it was demonstrated that a TfR1-MSTN conjugate, after *in vivo* delivery, mediated specific down regulation of the target gene in gastroc muscle. After induction of atrophy the conjugate was able to increase muscle mass and attenuate Dex-induced muscle loss.

Example 26: 2017-PK-496-C57BL6: Atrogin-1 and MuRF1 downregulation reduces leg muscle loss upon sciatic nerve denervation in Mice

[0692] For groups 1-4, see study design in Fig. 38, the 21mer Atrogin-1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UUGGGUAAACAUCGUACAAGUU (SEQ ID

NO: 14238). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0693] For groups 5-6, see study design in figure V, the 21mer MuRF1 guide strand was designed. The sequence (5' to 3') of the guide/antisense strand was UUUCGCACCAACGUAGAAUU (SEQ ID NO: 14231). The guide and fully complementary RNA passenger strands were assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. Purified single strands were duplexed to get the double stranded siRNA described in Fig. 20B. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linkers. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0694] For groups 7-12, the Atrogin-1 and MuRF1 were design as above. After conjugation to the TfR1 mAb and after purification and isolation of the individual DAR1 species, were mixed and co-administered.

[0695] For group 13, see study design in Fig. 38, a 21mer negative control siRNA sequence (scramble) (published by Burke *et al.* (2014) Pharm. Res., 31(12):3445-60) with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 14228). The same base, sugar and phosphate modifications that were used for the active MSTN siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phosphoramidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH₂ at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphodiester linker. Because the free thiol was not being used for conjugation, it was end capped with N-ethylmaleimide.

[0696] **Antibody siRNA Conjugate Synthesis using bis-maleimide (BisMal) linker**

[0697] **Step 1: Antibody reduction with TCEP**

[0698] Antibody was buffer exchanged with 25mM borate buffer (pH 8) with 1mM DTPA and made up to 10mg/ml concentration. To this solution, 4 equivalents of TCEP in the same borate buffer were added and incubated for 2 hours at 37 °C. The resultant reaction mixture was combined with a solution of BisMal-siRNA (1.25 equivalents) in pH 6.0 10 mM acetate buffer at RT and kept at 4 °C overnight.

Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA. The reaction mixture was treated with 10 EQ of N-ethylmaleimide (in DMSO at 10 mg/mL) to cap any remaining free cysteine residues.

[0699] Step 2: Purification

[0700] The crude reaction mixture was purified by AKTA Pure FPLC using anion exchange chromatography (SAX) method-1. Fractions containing DAR1 and DAR2 antibody-siRNA conjugates were isolated, concentrated and buffer exchanged with pH 7.4 PBS.

[0701] Anion exchange chromatography method (SAX)-1.

[0702] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 μ m

[0703] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0704] Gradient:

a.	%A	%B	Column Volume
b.	100	0	1
c.	81	19	0.5
d.	50	50	13
e.	40	60	0.5
f.	0	100	0.5
g.	100	0	2

[0705] Anion exchange chromatography (SAX) method-2

[0706] Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm

[0707] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 0.75 ml/min

[0708] Gradient:

a.	Time	%A	%B
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	14.00	40	60
f.	15.00	20	80
g.	16.00	90	10
h.	20.00	90	10

[0709] Step-3: Analysis of the purified conjugate

[0710] The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2 (Table 25).

Table 25

Conjugate	SAX retention time (min)	% purity (by peak area)
TfR1-Atrogin-1 DAR1	9.2	95
TfR1-MuRF1 DAR1	9.3	92
mTfR1-SC (DAR1)	8.9	76

[0711] In vivo study design

[0712] The conjugates were assessed for their ability to mediate mRNA downregulation of MuRF1 and Atrogin-1 in muscle (gastroc) in the presence and absence of sciatic nerve denervation, in an *in vivo* experiment (C57BL6 mice). Mice were dosed via intravenous (iv) injection with PBS vehicle control and the indicated ASCs and doses, see Fig. 38. Seven days post conjugate delivery, for groups 2-4, 6-8, 10-12 and 14-16, leg muscle atrophy was induced by sciatic nerve denervation. Denervation was not induced for the control groups 1, 5, 9, 13, and 17.

[0713] For the Denervation procedure at day 7, mice were anesthetized (5% isoflurane) and administer a subcutaneous dose of 0.1 mg/kg Buprenorphine. The right dorsal pelvic region was shaved from the sciatic notch to the knee. The area was disinfected with alternating alcohol and povidone-iodine. The sciatic notch was identified by palpation and an incision made from the sciatic notch towards the knee, approximately 1cm. The bicep femoris muscle was split to expose the sciatic nerve and about a 1cm fragment was removed by cauterizing both ends. The muscle and skin were then sutured to close the incision. The operative limb was then inspected daily to observe the condition of the surgical wound and observe the animal for overall health.

[0714] For groups 4, 8, 12, and 16 changes in leg muscle area were determined: The leg-to-be-measured were shaved and a line was drawn using indelible ink to mark region of measurement. Mice were restrained in a cone restraint and the right leg was held by hand. Digital calipers were used to take one measurement on the sagittal plane and another on the coronal plane. The procedure was repeated twice per week. For all groups at the time points indicated, gastrocnemius (gastroc) and heart muscle tissues were harvested, weighed and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta Ct$).

[0715] Quantitation of tissue siRNA concentrations was determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

[0716] Fig. 39A shows a single treatment of 4.5 mg/kg (siRNA) of either Atrogin-1 siRNA or MuRF1 siRNA or a single dose of both siRNAs combined resulted in up to 75% downregulation of each target in the gastrocnemius.

[0717] Fig. 39B shows mRNA knockdown of both targets in gastrocnemius is maintained at 75% in the intact leg out to 37 days post ASC dose.

[0718] In the denerved leg, Atrogin1 mRNA knockdown is maintained 3 days post denervation, but is reduced to 20% by 10 days post denervation and to 0% by 30 days post denervation. MuRF1 mRNA knockdown in the denerved leg is enhanced to 80-85% 3 days post denervation, but is reduced to 50% by 10 days post denervation and to 40% by 30 days post denervation (Fig. 39C).

[0719] The mRNA knockdown of each target was not impacted by the knockdown of the other target when treated with the combination of both siRNAs (Fig. 39D).

[0720] Based on leg muscle area measurements, siRNA-mediated downregulation of MuRF1 and the combination of MuRF1 and Atrogin-1 reduced denervation-induced muscle wasting by up to 30%. Treatment with MuRF1 siRNA alone showed similar responses than treatment with the combination of MuRF1 and Atrogin-1. Downregulation of Atrogin-1 alone had no significant effect on leg muscle area. The statistical analysis compared the treatment groups to the scramble siRNA control group using a Welch's TTest. *See Fig. 39E.*

[0721] Based on the Gastrocnemius weight only MuRF1 showed statistically significant differences from the scramble siRNA control group. Similar to the results obtained by measuring leg muscle area, downregulation of MuRF1 showed an up to 35% reduction in denervation-induced muscle wasting. These results agree with effects of MuRF1 knock out in mice (Bodine et al., Science 291, 2001). *See Fig. 39F.*

[0722] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleic acid molecule conjugate comprising an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of human DMPK mRNA wherein the polynucleic acid molecule comprises a small interfering RNA (siRNA) with a guide strand comprising a nucleic acid sequence selected from a group consisting of SEQ ID NOs: 13463, 14048, 14047, 14049, 14146, 14072, 13478, 14147, 12234, and 14199.
2. The polynucleic acid molecule conjugate of claim 1, wherein the polynucleic acid molecule comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, and/or at least one inverted abasic moiety.
3. The polynucleic acid molecule conjugate according to claim 1 or 2, wherein the anti-transferrin receptor antibody binds to a transferrin receptor on a cell surface of a muscle cell.
4. The polynucleic acid molecule conjugate according to any one of claims 1 to 3, wherein the polynucleic acid molecule conjugate comprises a linker connecting the anti-transferrin receptor antibody to the polynucleic acid molecule.
5. The polynucleic acid molecule conjugate according to any one of claims 2 to 4, wherein the at least one 2' modified nucleotide: comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-

O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide; comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA); or comprises a combination thereof.

6. The polynucleic acid molecule conjugate according to any one of claims 2 to 5, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage.
7. The polynucleic acid molecule conjugate according to any one of claims 2 to 6, wherein the polynucleic acid molecule comprises 3 or more 2' modified nucleotides selected from 2'-O-methyl and 2'-deoxy-2'-fluoro.
8. The polynucleic acid molecule conjugate according to any one of claims 1 to 7, wherein the polynucleic acid molecule conjugate has a drug to antibody ratio of from about 1 to about 4.
9. The polynucleic acid molecule conjugate according to any one of claims 1 to 8, wherein the polynucleic acid molecule comprises a 5'-terminal vinylphosphonate modified nucleotide.
10. The polynucleic acid molecule conjugate according to any one of claims 1 to 9, wherein the anti-transferrin receptor antibody comprises two light chain variable domains and two heavy chain variable domains.

11. The polynucleic acid molecule conjugate according to any one of claims 1 to 10, wherein the anti-transferrin receptor antibody or antigen-binding fragment thereof comprises a humanized antibody or antigen-binding fragment thereof, chimeric antibody or antigen-binding fragment thereof, monoclonal antibody or antigen-binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, single-domain antibody (sdAb), or camelid antibody or antigen-binding fragment thereof.
12. A method for treating muscle atrophy or myotonic dystrophy in a subject in need thereof, comprising: administering to the subject a therapeutically effective amount of the polynucleic acid molecule conjugate of any one of claims 1 to 11 to the subject, thereby treating myotonic dystrophy in the subject.
13. Use of a therapeutically effective amount the polynucleic acid molecule conjugate of any one of claims 1 to 11 in the manufacture of a medicament for treating muscle atrophy or myotonic dystrophy.
14. The method of claim 12 or the use of claim 13, wherein the muscle atrophy is associated with myotonic dystrophy type 1 (DM1).
15. The method of claim 12 or the use of claim 13, wherein the myotonic dystrophy is DM1.
16. The method of claim 12 or the use of claim 13, the polynucleic acid molecule conjugate is formulated for parenteral administration.

FIG. 1

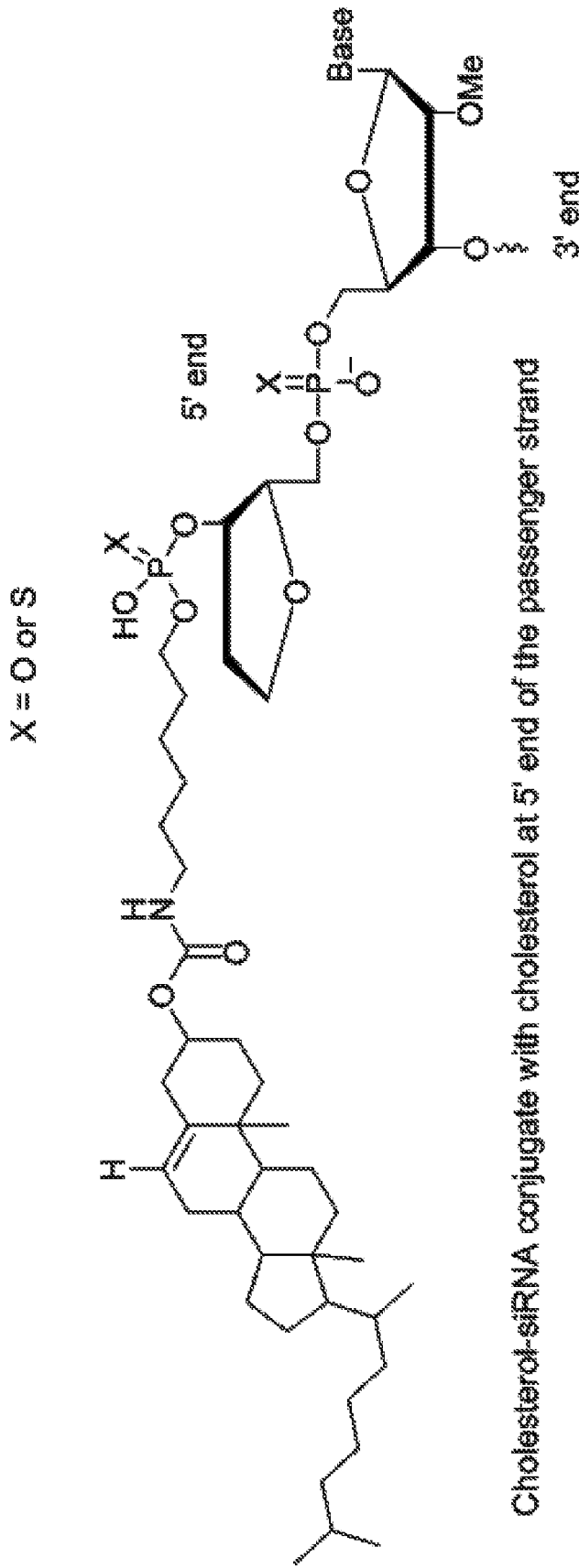


FIG. 2

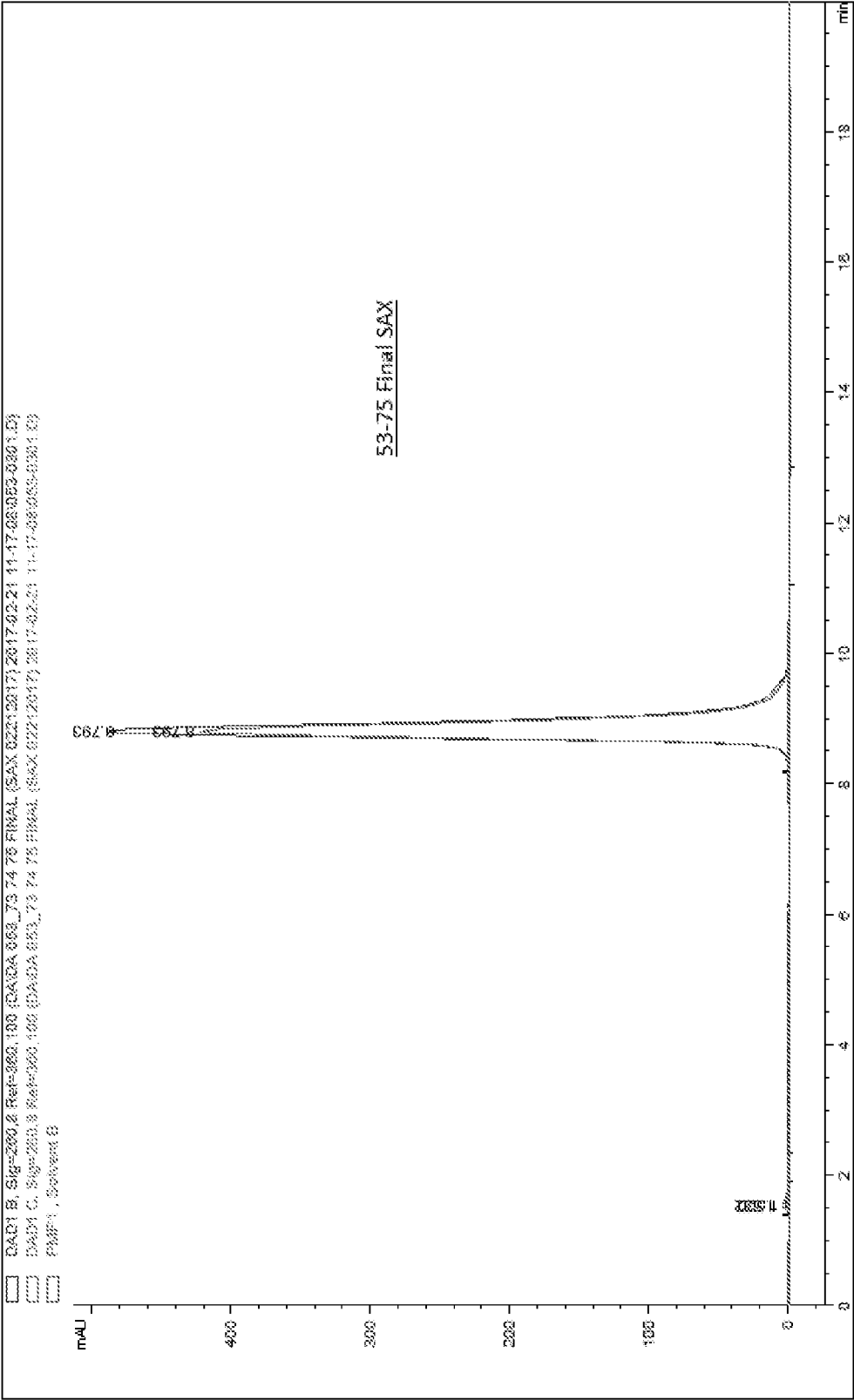


FIG. 3

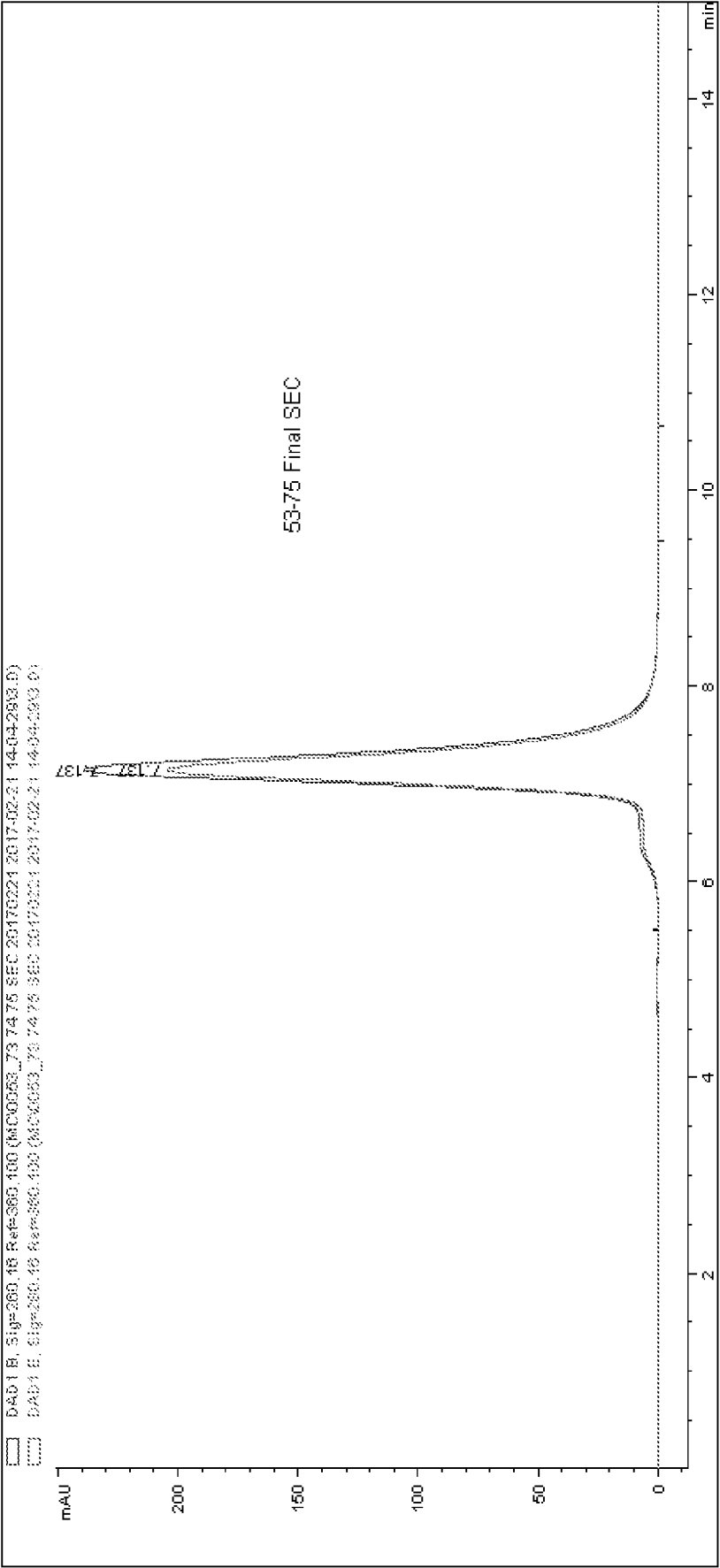


FIG. 4

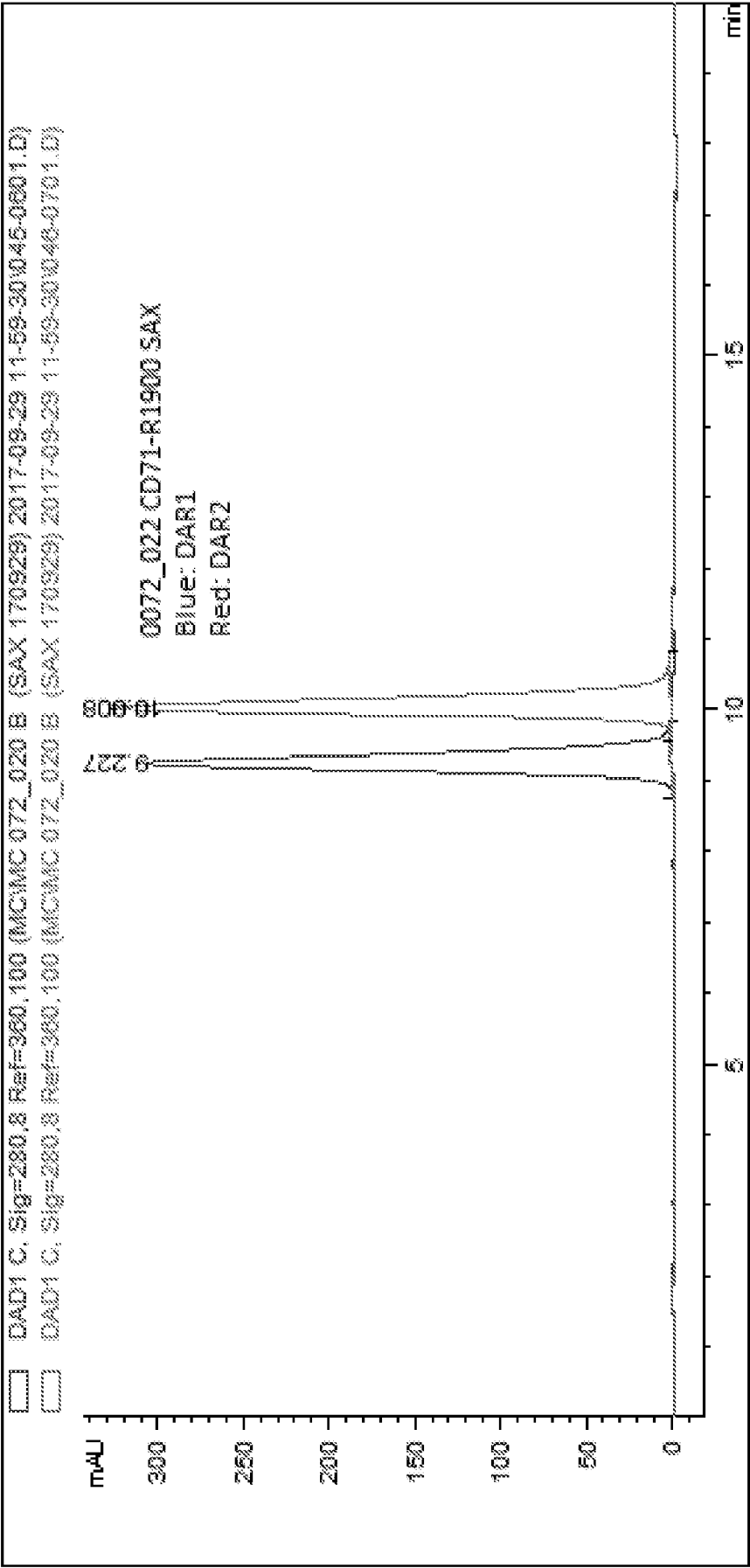


FIG. 5

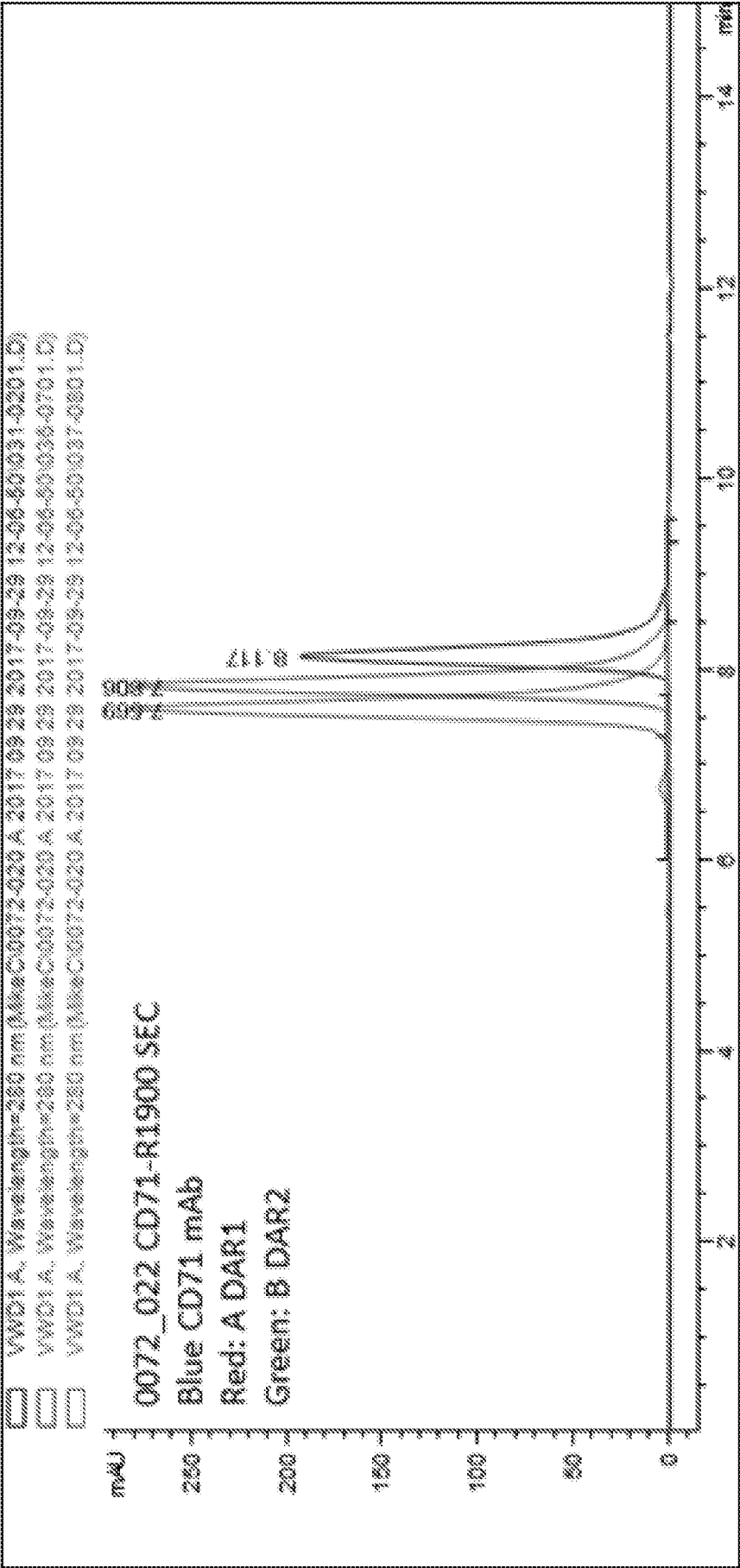


FIG. 6

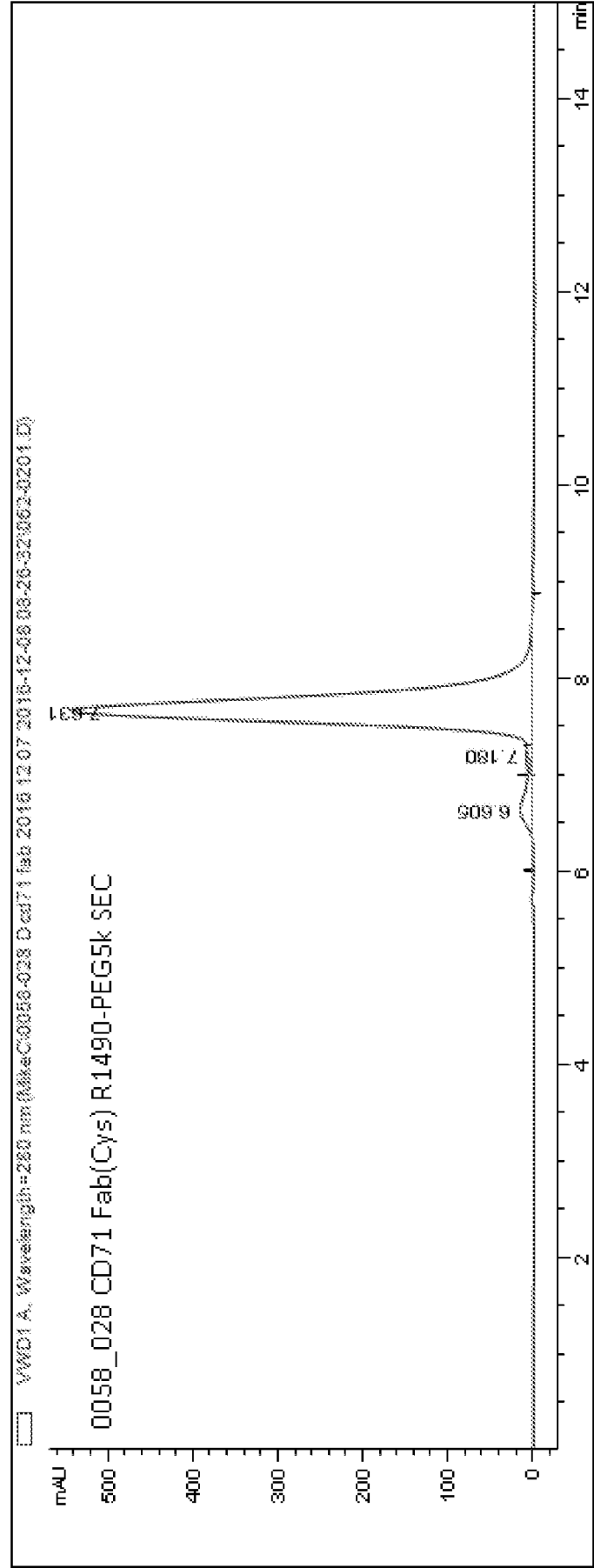


FIG. 7

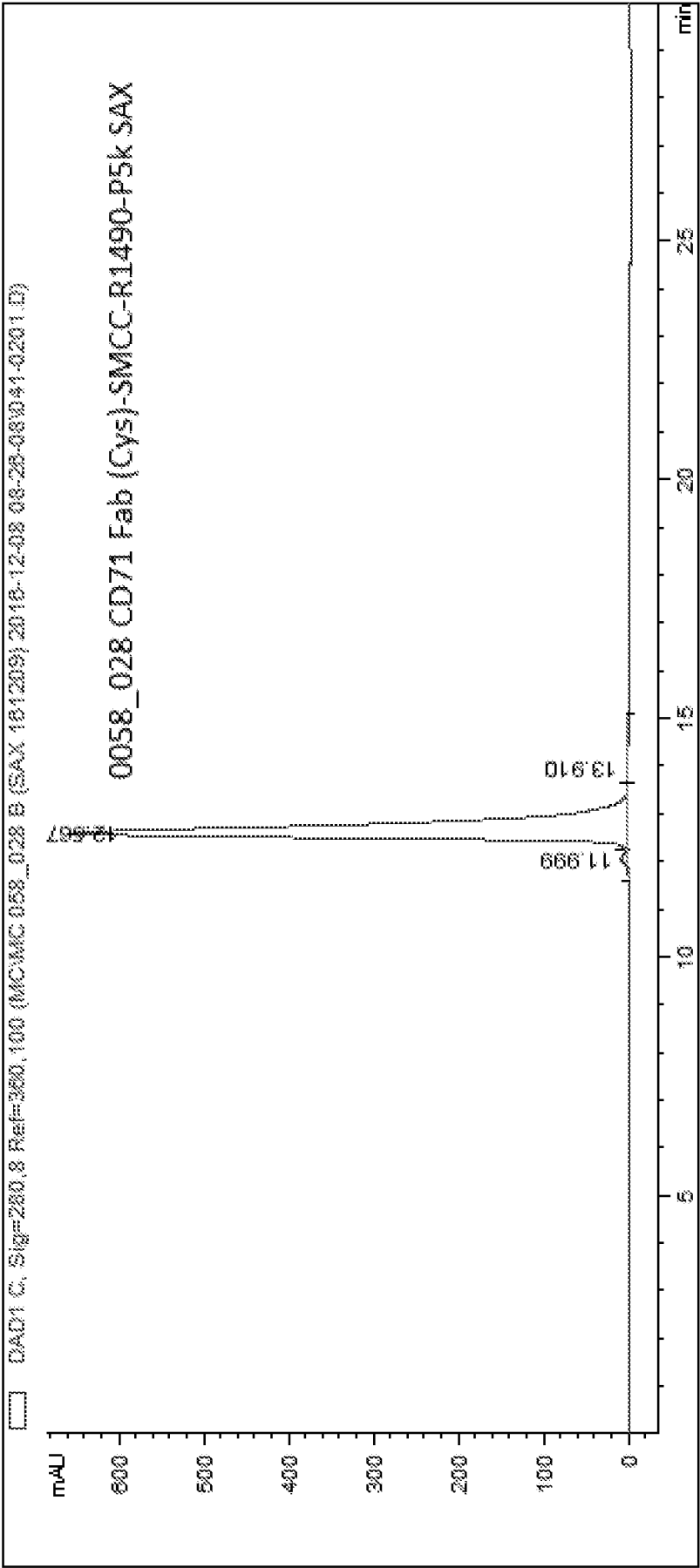


FIG. 8

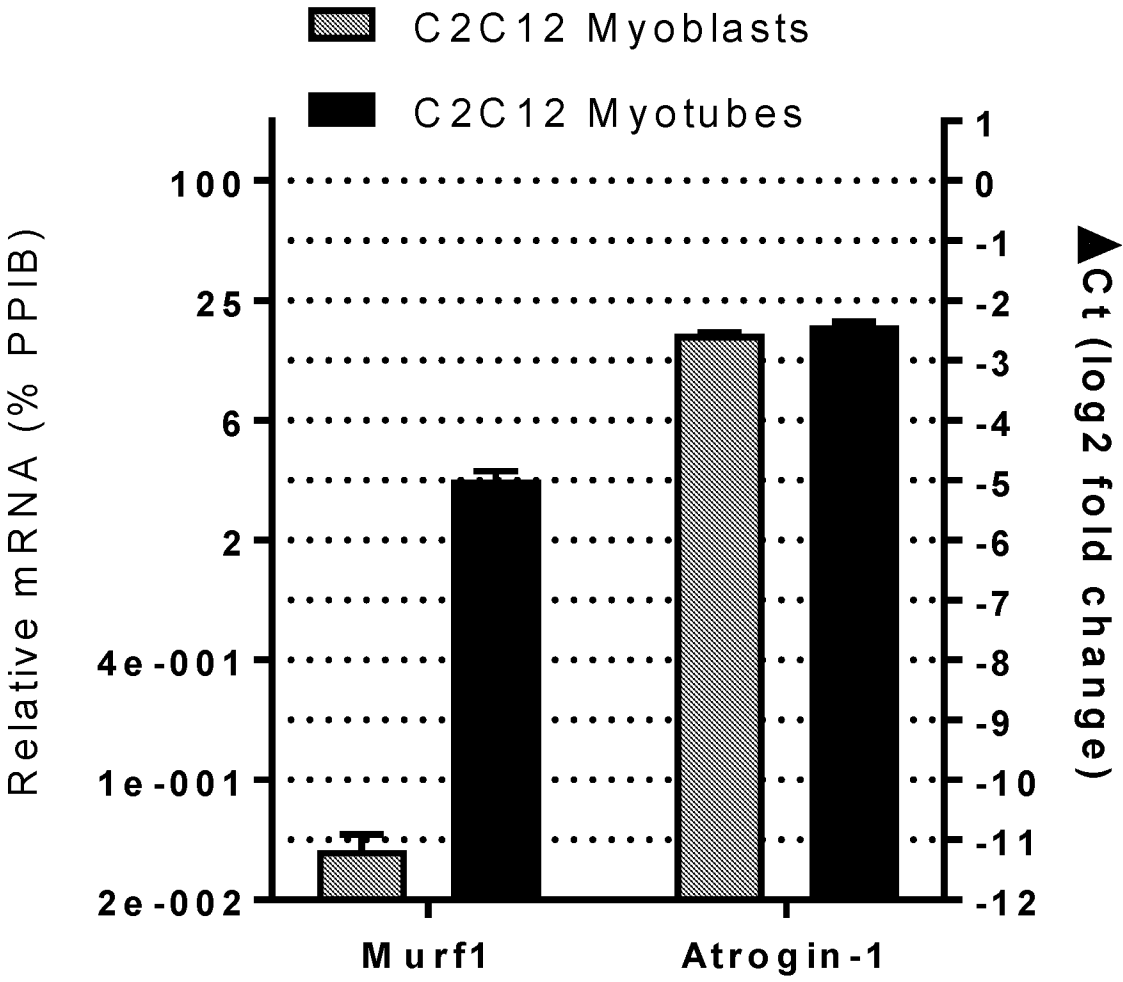


FIG. 9A

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	TfR-mAb-HPRT-PEG5k (n=1)	4	3	5.0	1	96
2	TfR-mAb-HPRT-PEG5k (n=1)	4	1	5.0	1	96
3	TfR-mAb-HPRT-PEG5k (n=1)	4	0.3	5.0	1	96
4	TfR-mAb-HPRT-PEG5k (n=1)	4	0.1	5.0	1	96
5	IgG2a-mAb-HPRT-PEG5k (n=1)	4	3	5.0	1	96
6	IgG2a-mAb-HPRT-PEG5k (n=1)	4	1	5.0	1	96
7	IgG2a-mAb-HPRT-PEG5k (n=1)	4	0.3	5.0	1	96
8	IgG2a-mAb-HPRT-PEG5k (n=1)	4	0.1	5.0	1	96
9	TfR-mAb-MSTN-PEG5k (n=1)	4	3	5.0	1	96
10	TfR-mAb-MSTN-PEG5k (n=1)	4	1	5.0	1	96
11	TfR-mAb-MSTN-PEG5k (n=1)	4	0.3	5.0	1	96
12	TfR-mAb-MSTN-PEG5k (n=1)	4	0.1	5.0	1	96
13	IgG2a-mAb-MSTN-PEG5k (n=1)	4	3	5.0	1	96
14	IgG2a-mAb-MSTN-PEG5k (n=1)	4	1	5.0	1	96
15	IgG2a-mAb-MSTN-PEG5k (n=1)	4	0.3	5.0	1	96
16	IgG2a-mAb-MSTN-PEG5k (n=1)	4	0.1	5.0	1	96
17	TfR-mAb-scramble-PEG5k (n=1)	4	3	5.0	1	96
18	TfR-mAb-scramble-PEG5k (n=1)	4	1	5.0	1	96
19	TfR-mAb-scramble-PEG5k (n=1)	4	0.3	5.0	1	96
20	TfR-mAb-scramble-PEG5k (n=1)=1)	4	0.1	5.0	1	96
21	PBS Control	5	-	5.0	1	96

85

WT mice (CD-1)

FIG. 9B

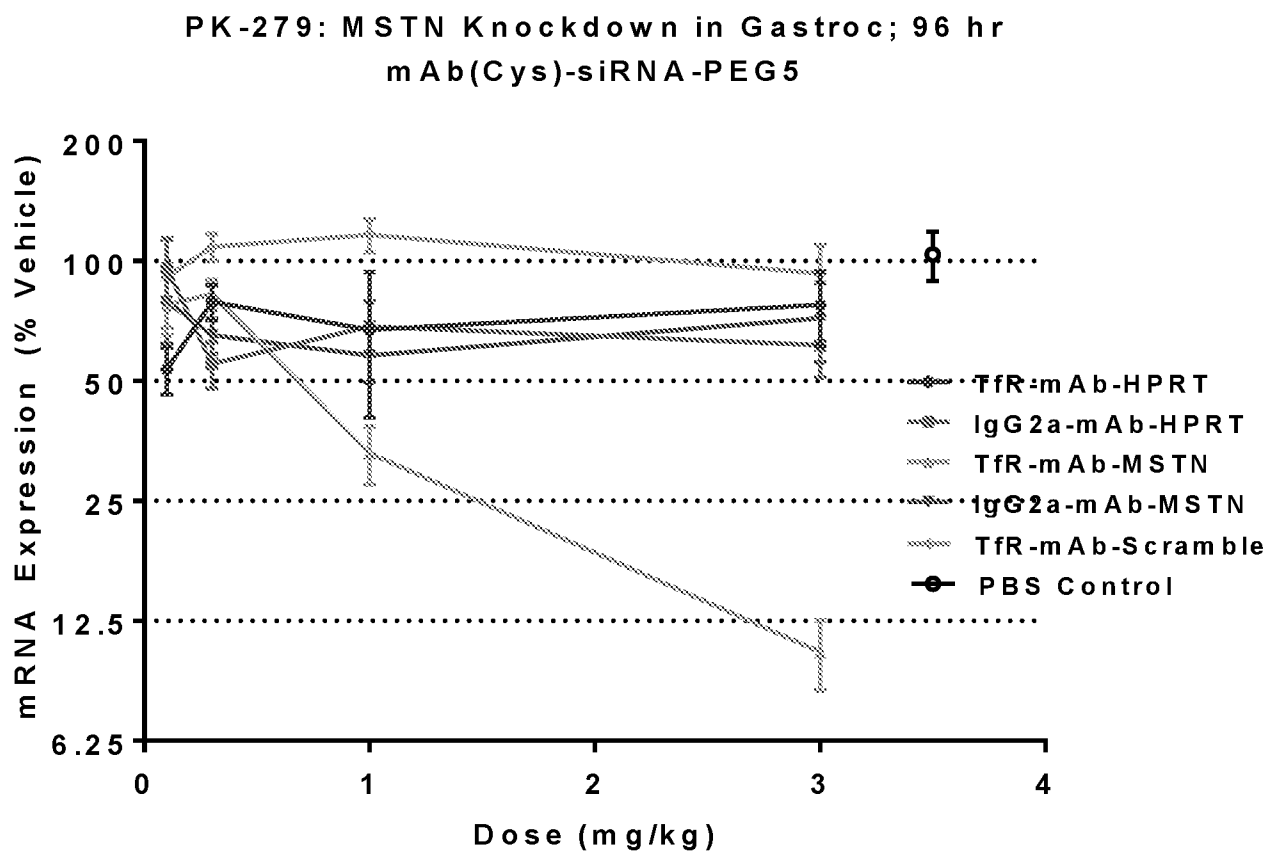


FIG. 10A

Group	Test Article	N	siRNA Dose (mg/kg)	# of Doses	Survival Bleed (h)	Terminal Bleed (h)	Harvest Time (h)
1	TfR-mAb-MSTN (n=1)	4	3	1	0.25	24	24
2	TfR-mAb-MSTN (n=1)	4	3	1	1	96	96
3	TfR-mAb-MSTN (n=1)	4	3	1	2	168	168
4	TfR-mAb-MSTN (n=1)	4	3	1	4	336	336
5	TfR-mAb-scramble (n=1)	4	3	1	0.25	24	24
6	TfR-mAb-scramble (n=1)	4	3	1	1	96	96
7	TfR-mAb-scramble (n=1)	4	3	1	2	168	168
8	TfR-mAb-scramble (n=1)	4	3	1	4	336	336
9	PBS Control	4	-	1	-	-	24
10	PBS Control	4	-	1	-	-	96
11	PBS Control	4	-	1	-	-	168
12	PBS Control	4	-	1	-	-	336
13	TfR-mAb-MSTN (n=1)	5	3	1	504	840	840
14	TfR-mAb-MSTN (n=1)	5	3	1	672	1,008	1,008
15	PBS Control	5	-	1	504	840	840
16	PBS control	5	-	1	672	1,008	1,008

FIG. 10B

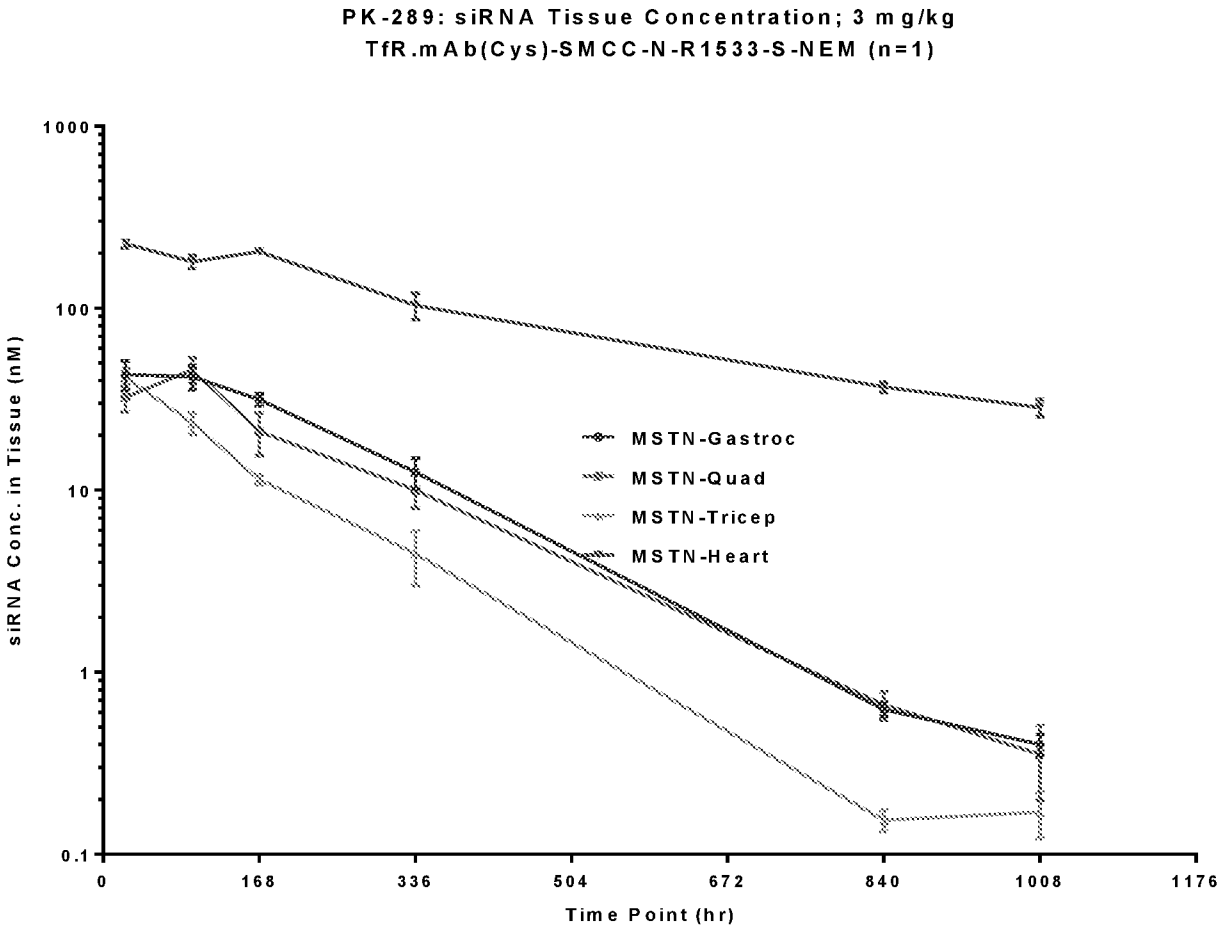


FIG. 10C

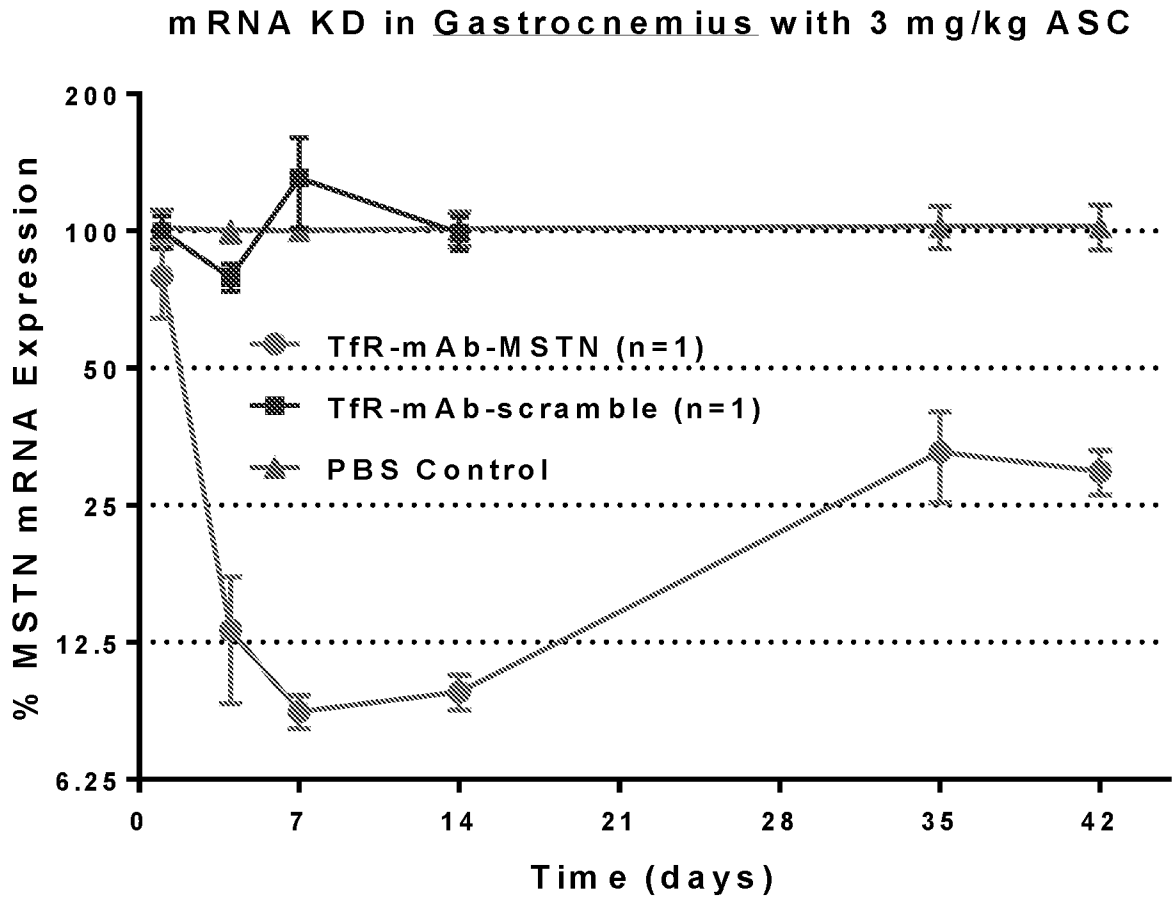


FIG. 10D

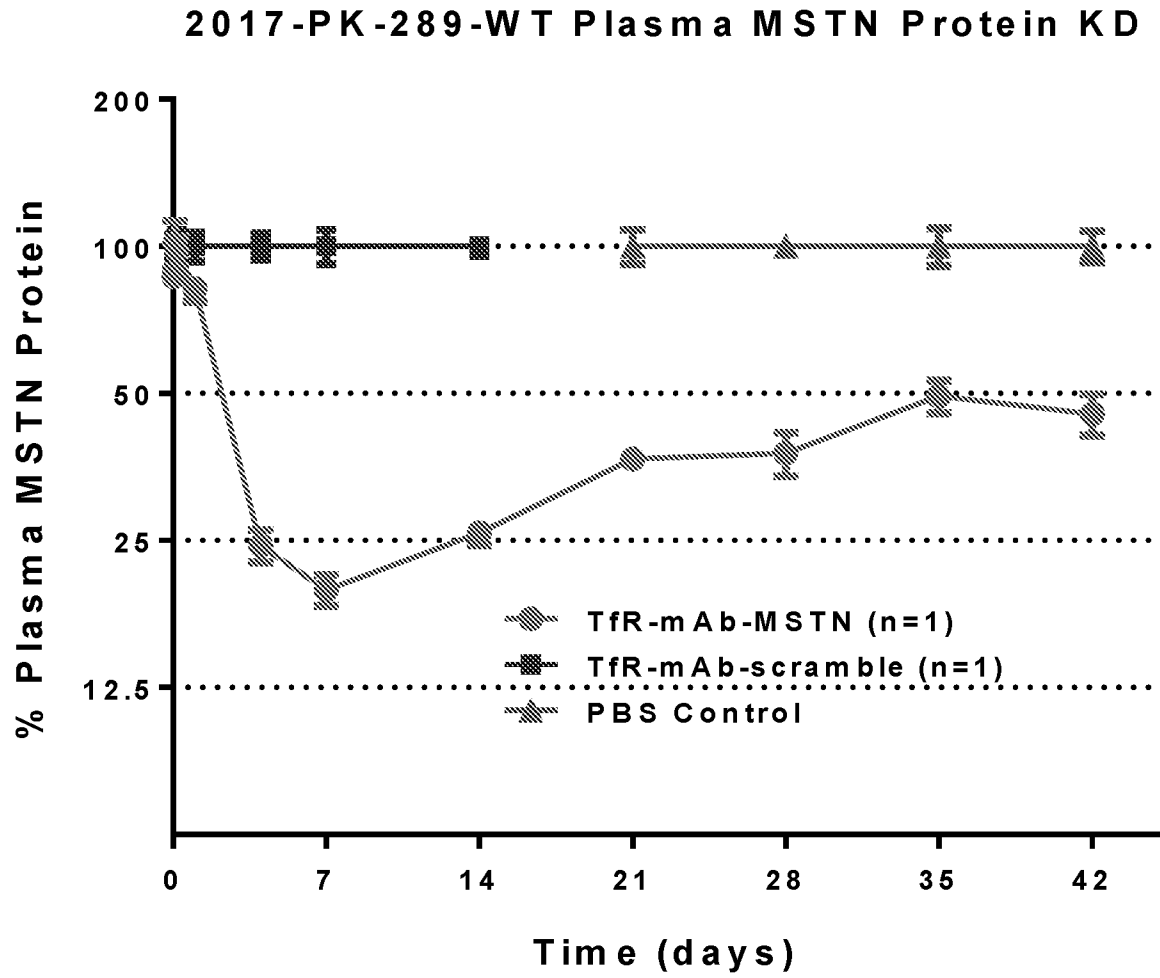


FIG. 10E

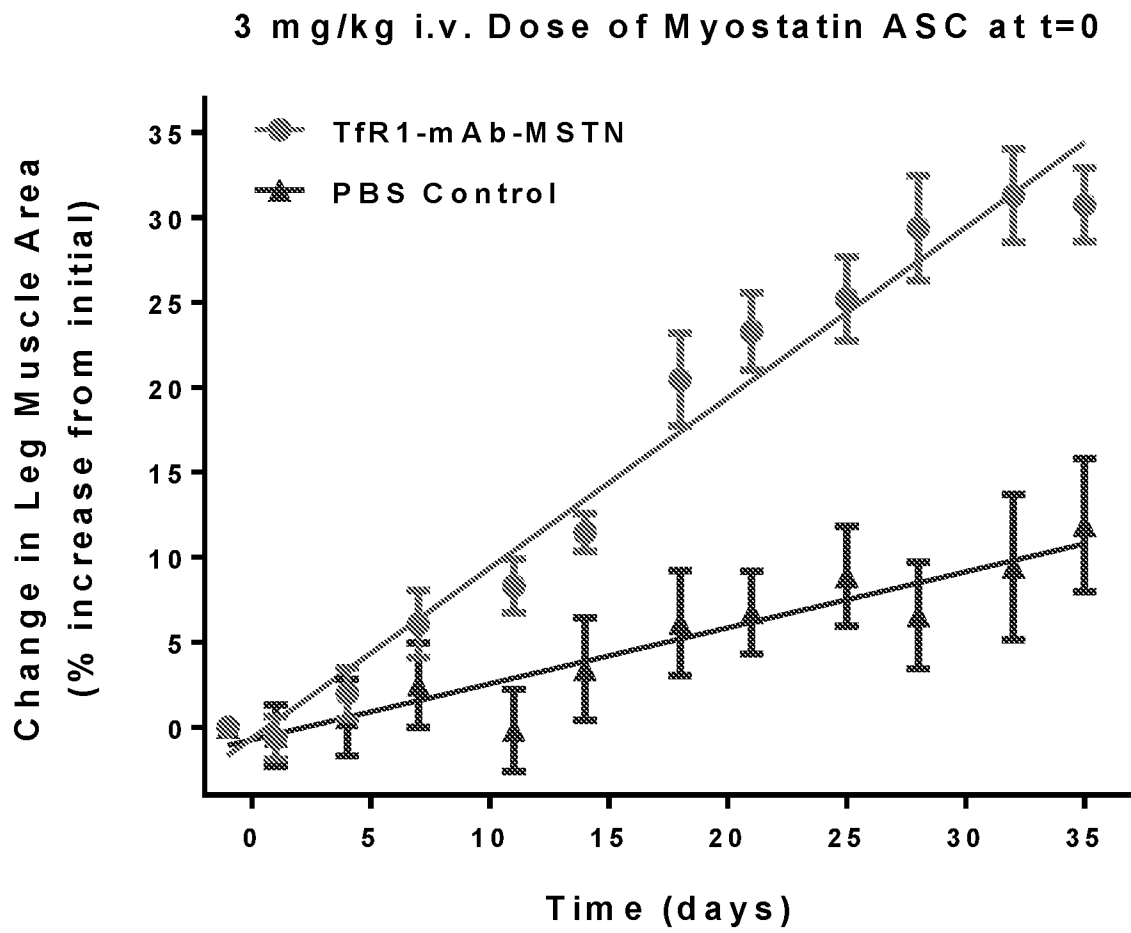


FIG. 10F

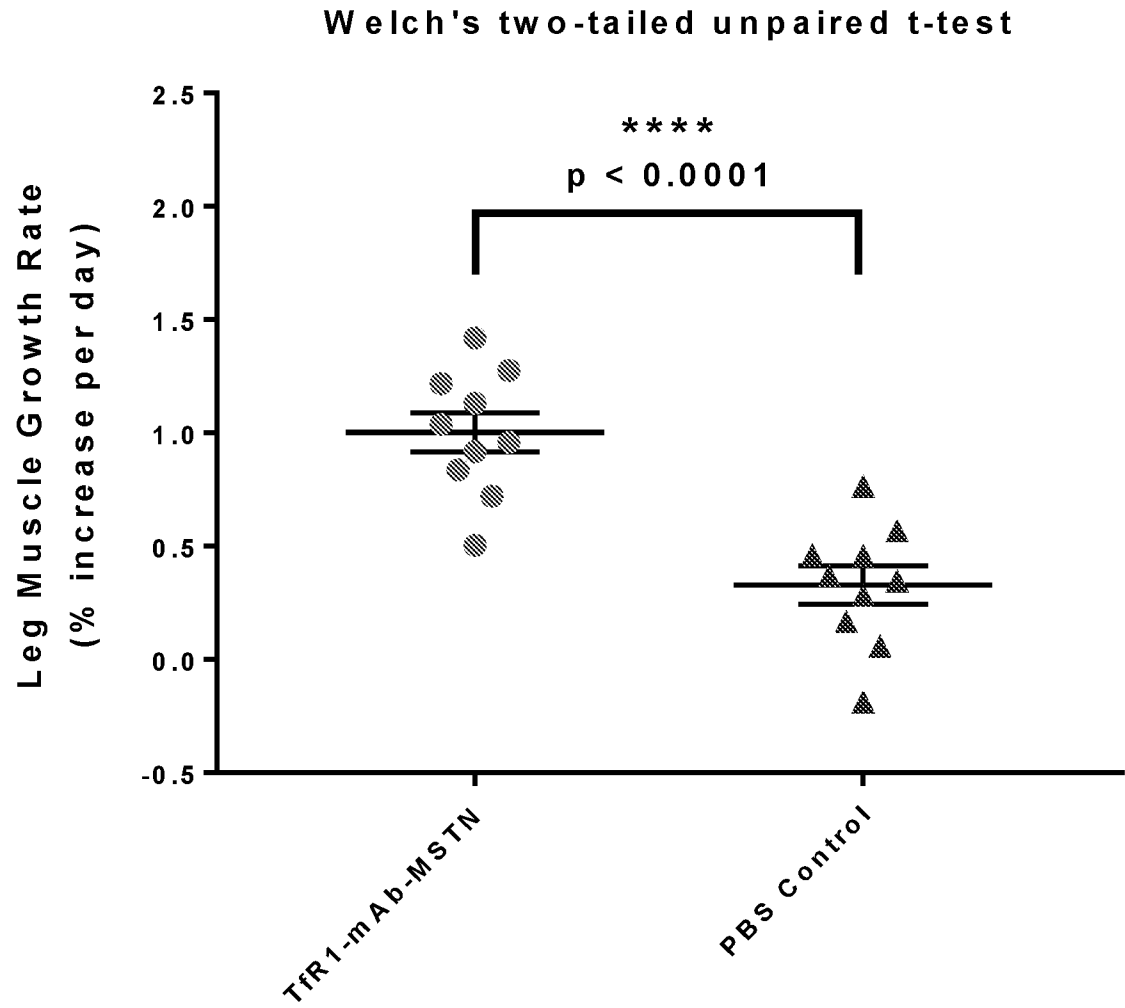


FIG. 11A

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	# of Doses	Dose Schedule	Harvest Time (h)
1	TfR-Fab-MSTN* (n=1)	4	6	5.0	1	t=0	96
2	TfR-Fab-MSTN* (n=1)	4	2	5.0	1	t=0	96
3	TfR-Fab-MSTN* (n=1)	4	0.6	5.0	1	t=0	96
4	TfR-Fab-MSTN* (n=1)	4	0.2	5.0	1	t=0	96
5	EGFR-Fab-MSTN* (n=1)	4	6	5.0	1	t=0	96
6	EGFR-Fab-MSTN* (n=1)	4	2	5.0	1	t=0	96
7	EGFR-Fab-MSTN* (n=1)	4	0.6	5.0	1	t=0	96
8	EGFR-Fab-MSTN* (n=1)	4	0.2	5.0	1	t=0	96
9	TfR-mAb-MSTN (n=1)	4	3	5.0	1	t=0	96
10	TfR-mAb-MSTN (n=1)	4	1	5.0	1	t=0	96
11	TfR-mAb-MSTN (n=1)	4	0.3	5.0	1	t=0	96
12	TfR-mAb-MSTN (n=1)	4	0.1	5.0	1	t=0	96
13	TfR-mAb-MSTN (n=2)	4	6	5.0	1	t=0	96
14	TfR-mAb-MSTN (n=2)	4	2	5.0	1	t=0	96
15	TfR-mAb-MSTN (n=2)	4	0.6	5.0	1	t=0	96
16	TfR-mAb-MSTN (n=2)	4	0.2	5.0	1	t=0	96
17	EGFR-mAb-MSTN* (n=1)	4	2.6	5.0	1	t=0	96
18	EGFR-mAb-MSTN* (n=1)	4	1	5.0	1	t=0	96
19	EGFR-mAb-MSTN* (n=2)	4	3	5.0	1	t=0	96
20	EGFR-mAb-MSTN* (n=2)	4	1	5.0	1	t=0	96
21	PBS Control	5	-	5.0	1	t=0	96

85

WT mice (CD-1)

FIG. 11B

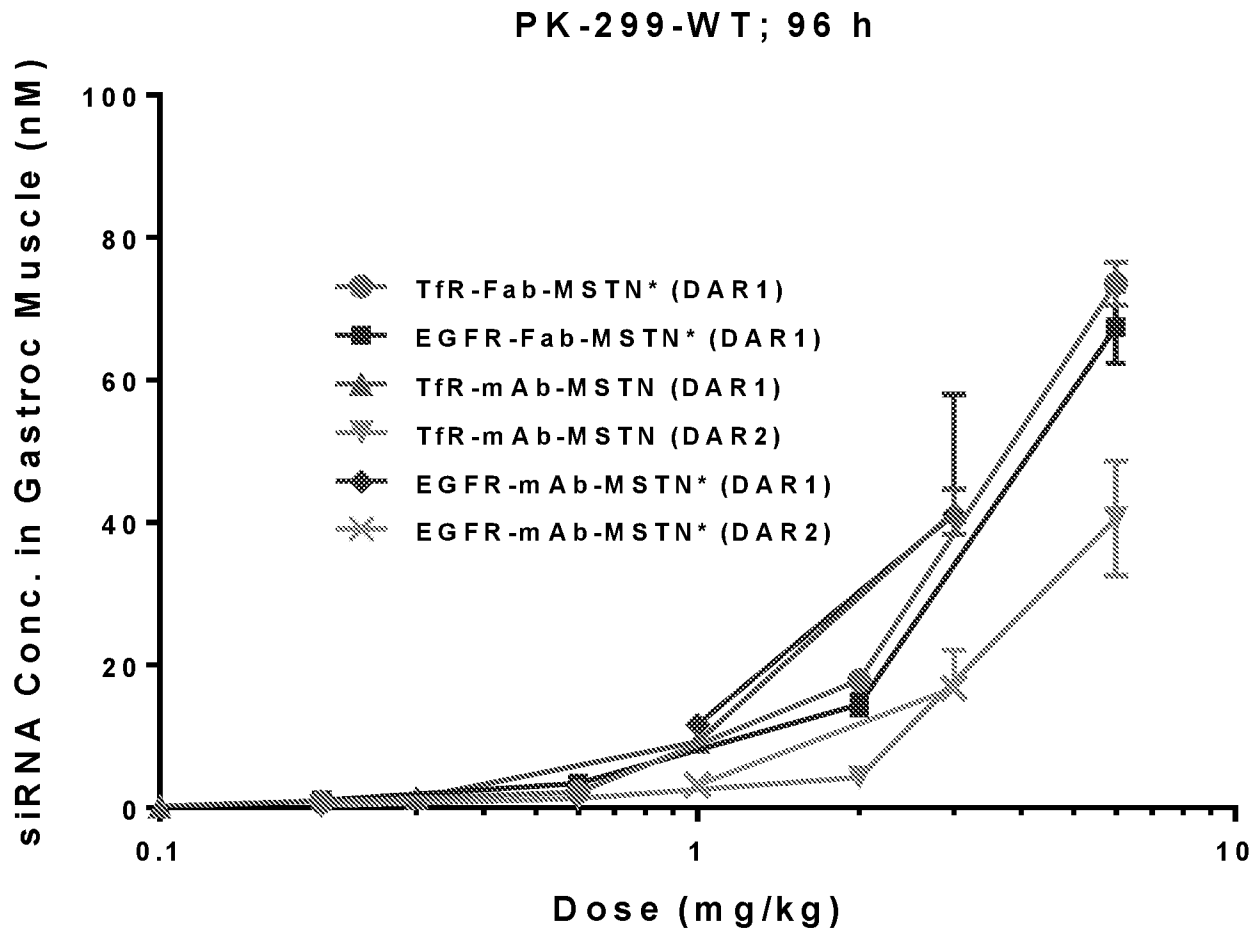


FIG. 11C

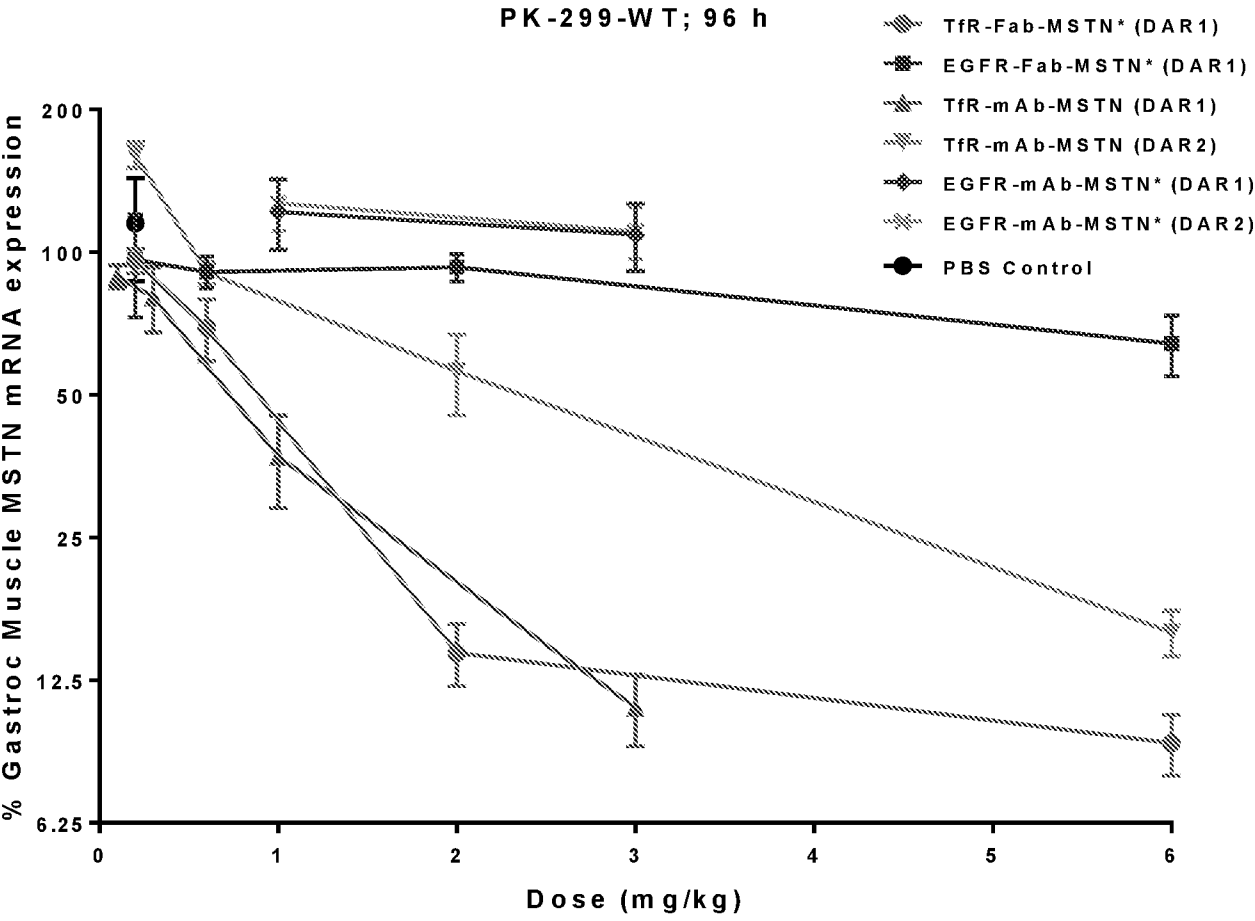


FIG. 12A

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	Dose Schedule	Harvest Time (h)
1	Chol-MSTN Control	4	60	5.0	t=0	96
2	Chol-MSTN Control	4	20	5.0	t=0	96
3	Chol-MSTN Control	4	6	5.0	t=0	96
4	Chol-MSTN Control	4	2	5.0	t=0	96
5	TfR-Fab-MSTN* (n=1)	4	6	5.0	t=0	96
6	TfR-Fab-MSTN* (n=1)	4	2	5.0	t=0	96
7	TfR-Fab-MSTN* (n=1)	4	0.6	5.0	t=0	96
8	TfR-Fab-MSTN* (n=1)	4	0.2	5.0	t=0	96
9	TfR-mAb-MSTN* (n=1)	4	3	5.0	t=0	96
10	TfR-mAb-MSTN* (n=1)	4	1	5.0	t=0	96
11	TfR-mAb-MSTN* (n=1)	4	0.3	5.0	t=0	96
12	TfR-mAb-MSTN* (n=1)	4	0.1	5.0	t=0	96
13	TfR-mAb-MSTN (n=1)	4	3	5.0	t=0	96
14	TfR-mAb-MSTN (n=1)	4	1	5.0	t=0	96
15	TfR-mAb-MSTN (n=1)	4	0.3	5.0	t=0	96
16	TfR-mAb-MSTN (n=1)	4	0.1	5.0	t=0	96
17	TfR-mAb-scramble (n=1)	4	3	5.0	t=0	96
18	TfR-mAb-scramble (n=1)	4	1	5.0	t=0	96
19	TfR-mAb-scramble (n=1)	4	0.3	5.0	t=0	96
20	TfR-mAb-scramble (n=1)	4	0.1	5.0	t=0	96
21	PBS Control	5	-	5.0	t=0	96

85 WT mice (CD-1)

FIG. 12B

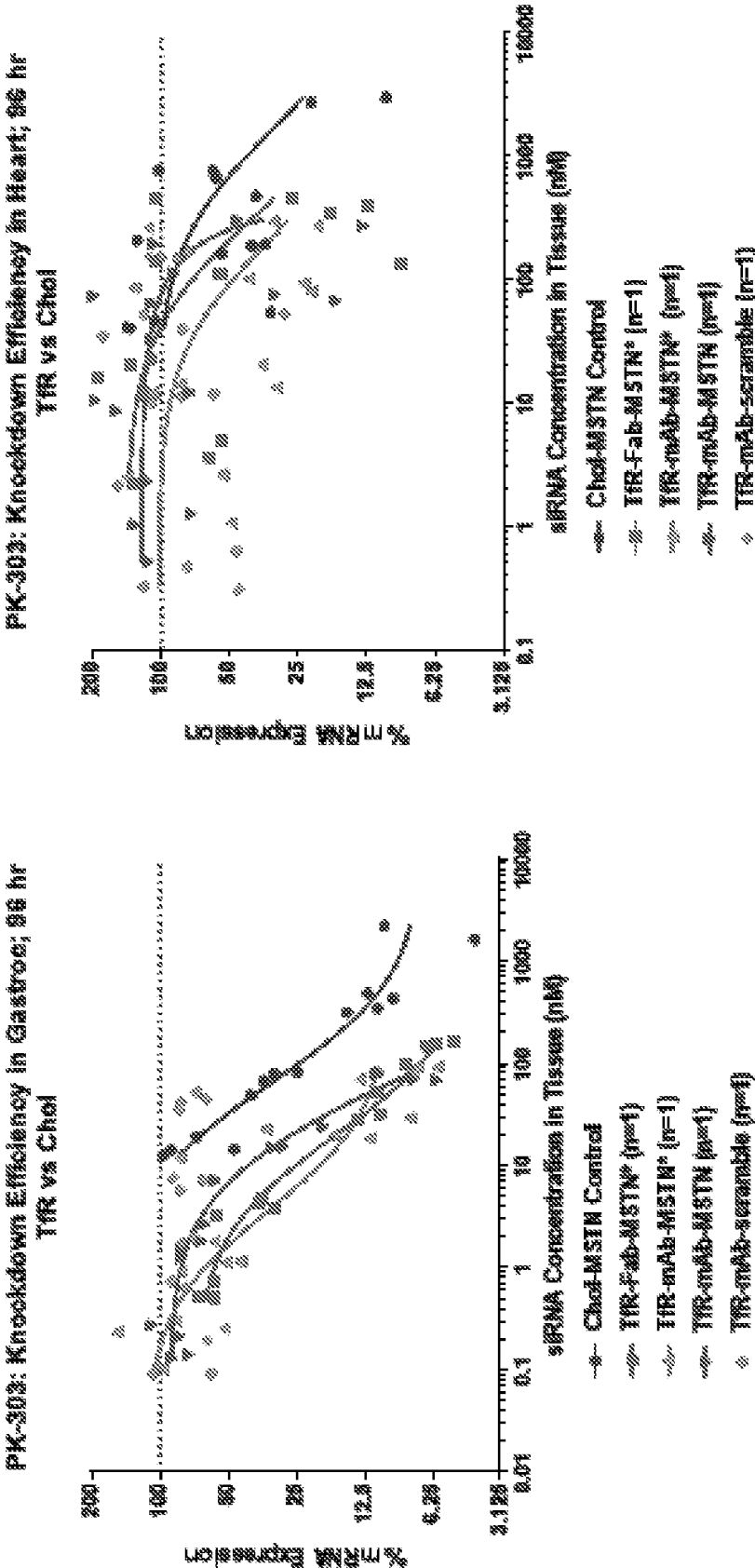


FIG. 12C

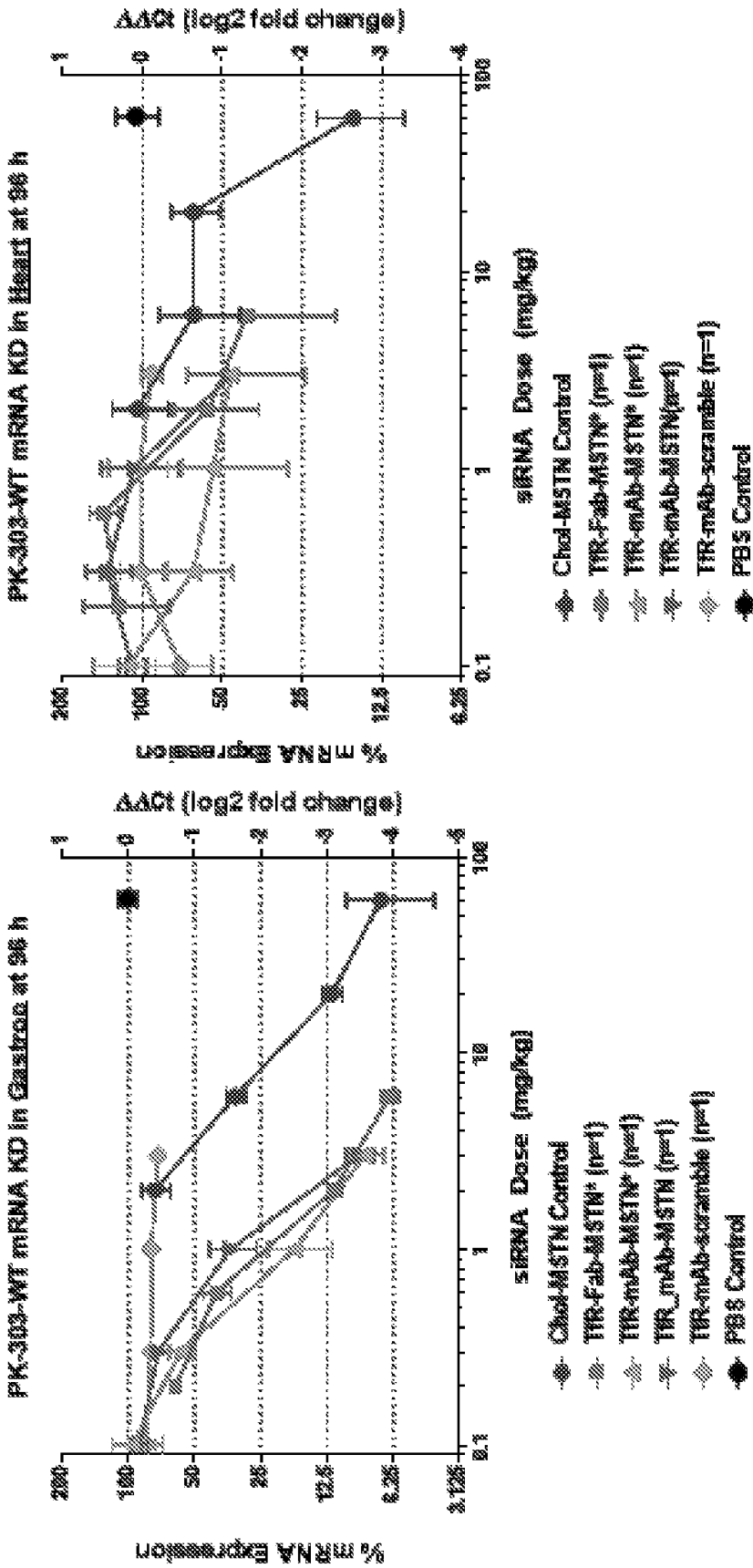


FIG. 12D

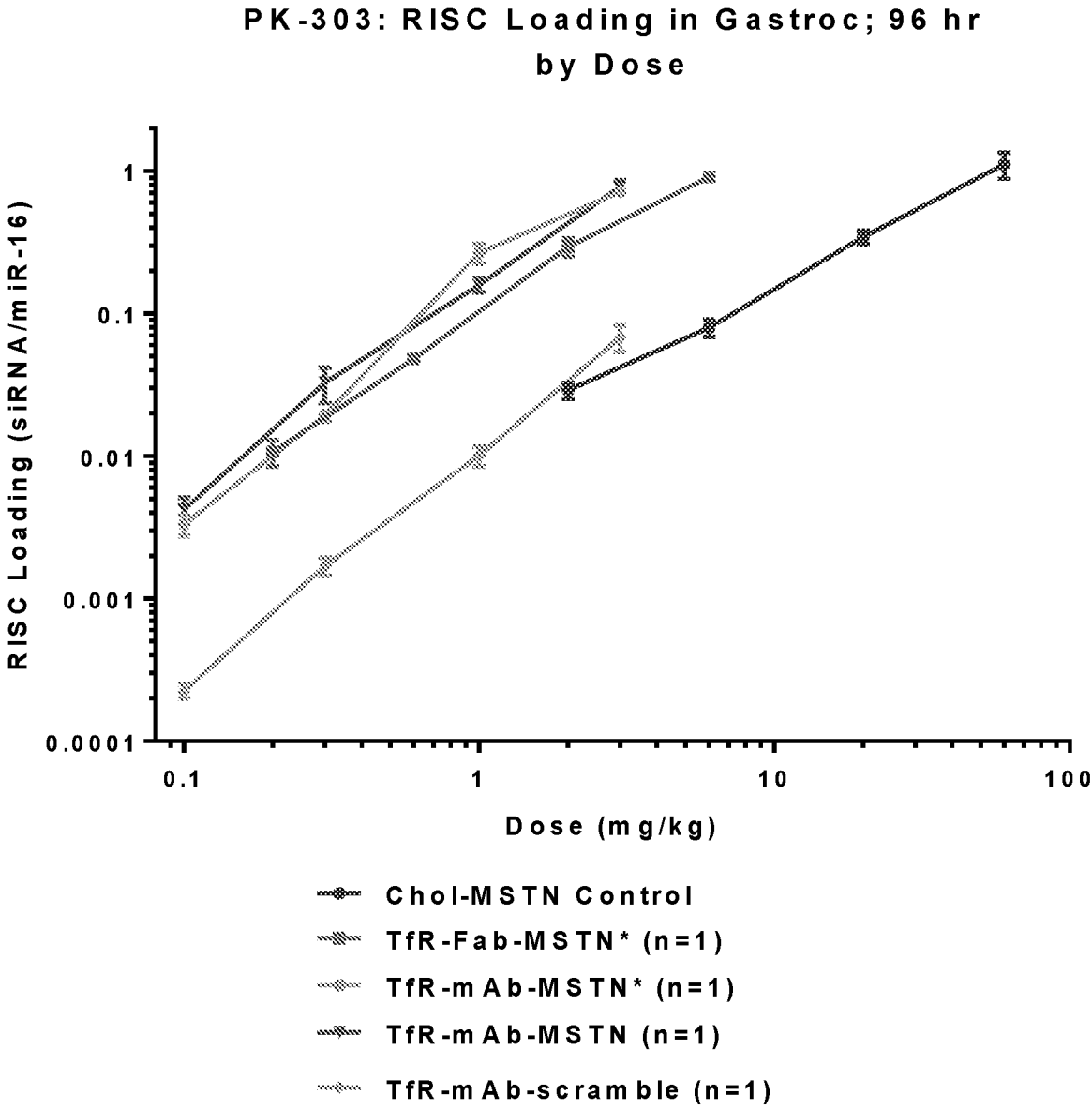


FIG. 13A

Group	Test Article	N	siRNA Dose (mg/kg)	Survival Bleed (day)	Terminal Bleed (day)	Harvest Time (day)
1	Chol-MSTN	5	50	7	14	14
2	Chol-MSTN	5	50	21	28	28
3	Chol-MSTN	5	50	35	42	42
4	Chol-MSTN	5	50	49	56	56
5	TfR-mAb-MSTN (n=1)	5	3	7	14	14
6	TfR-mAb-MSTN (n=1)	5	3	21	28	28
7	TfR-mAb-MSTN (n=1)	5	3	35	42	42
8	TfR-mAb-MSTN (n=1)	5	3	49	56	56
9	TfR-mAb-MSTN (n=2)	5	3	7	14	14
10	TfR-mAb-MSTN (n=2)	5	3	21	28	28
11	TfR-mAb-MSTN (n=2)	5	3	35	42	42
12	TfR-mAb-MSTN (n=2)	5	3	49	56	56
13	TfR-mAb-scramble (n=1)	5	3	7	14	14
14	TfR-mAb-scramble (n=1)	5	3	21	28	28
15	TfR-mAb-scramble (n=1)	5	3	35	42	42
16	TfR-mAb-scramble (n=1)	5	3	49	56	56
17	PBS Control	5	-	7	14	14
18	PBS Control	5	-	21	28	28
19	PBS Control	5	-	35	42	42
20	PBS Control	5	-	49	56	56

FIG. 13B

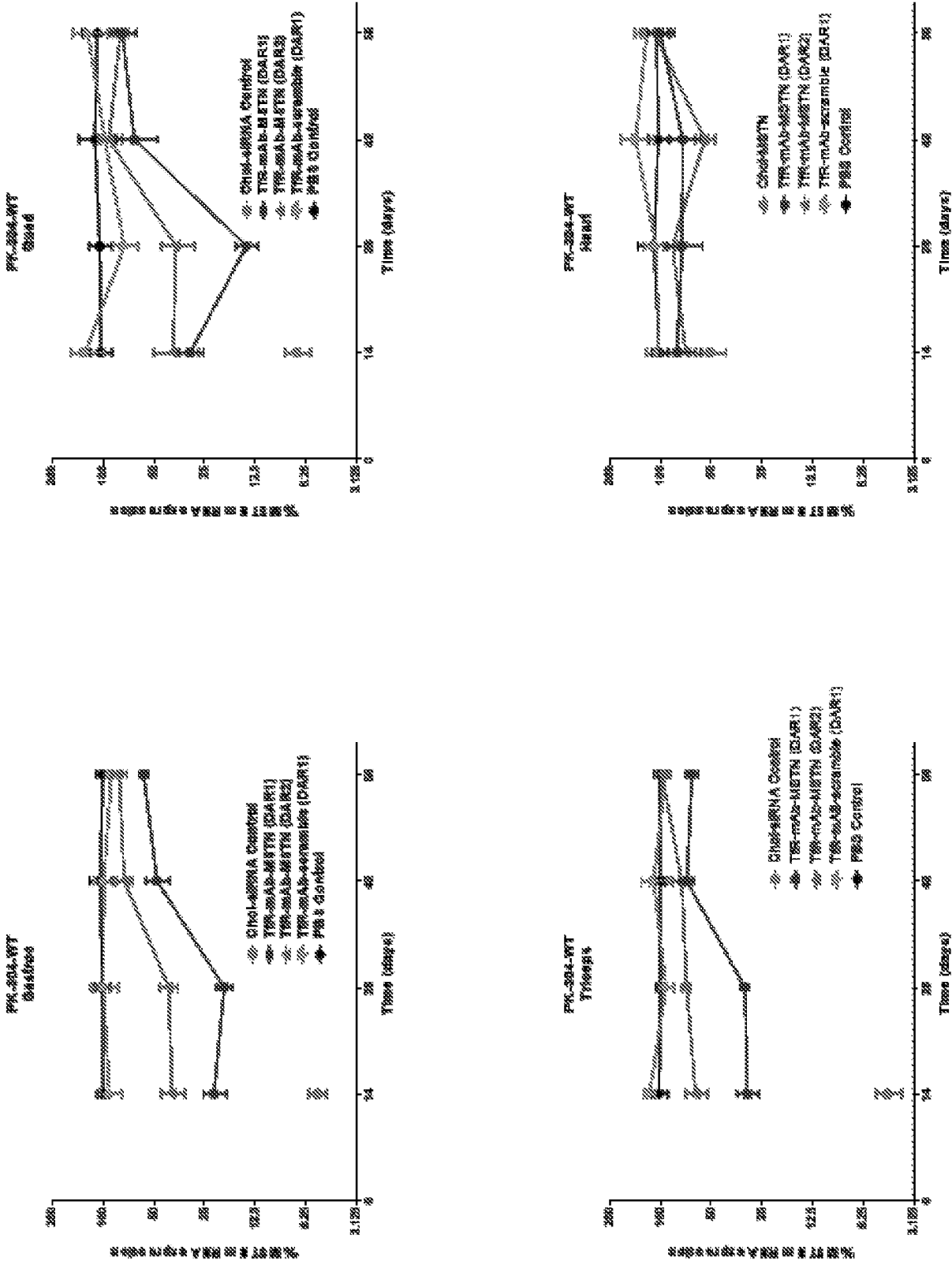


FIG. 13C

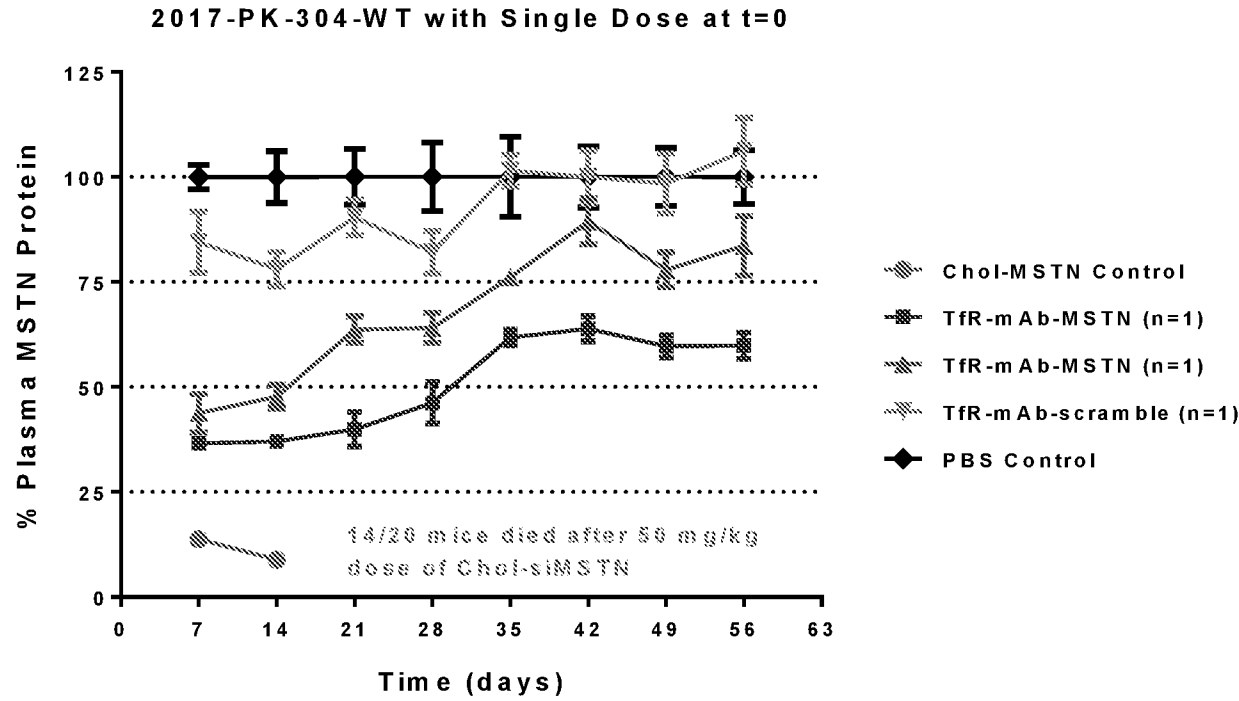


FIG. 13D

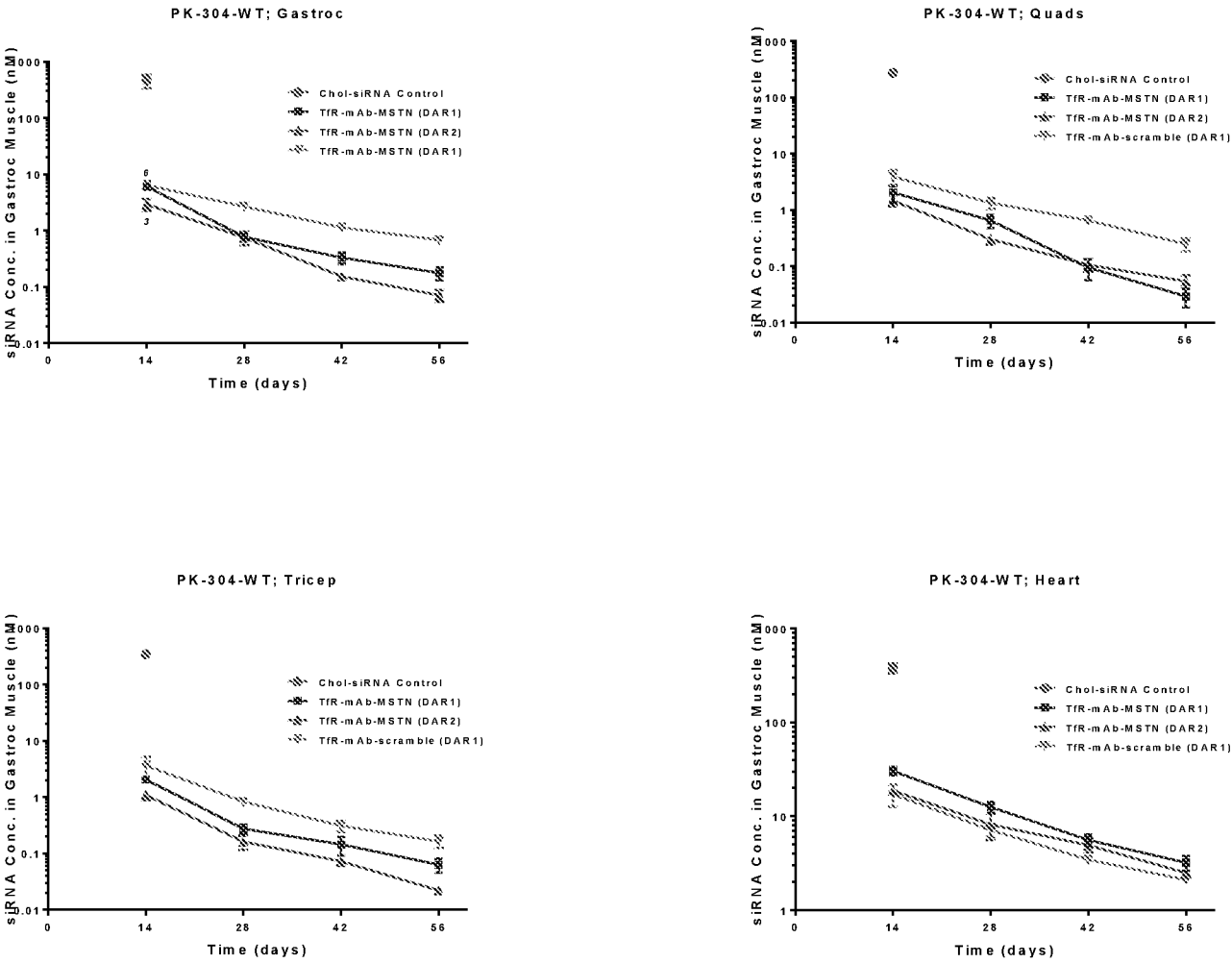


FIG. 13E

PK-304-WT; Gastroc
Risc Loading vs KD

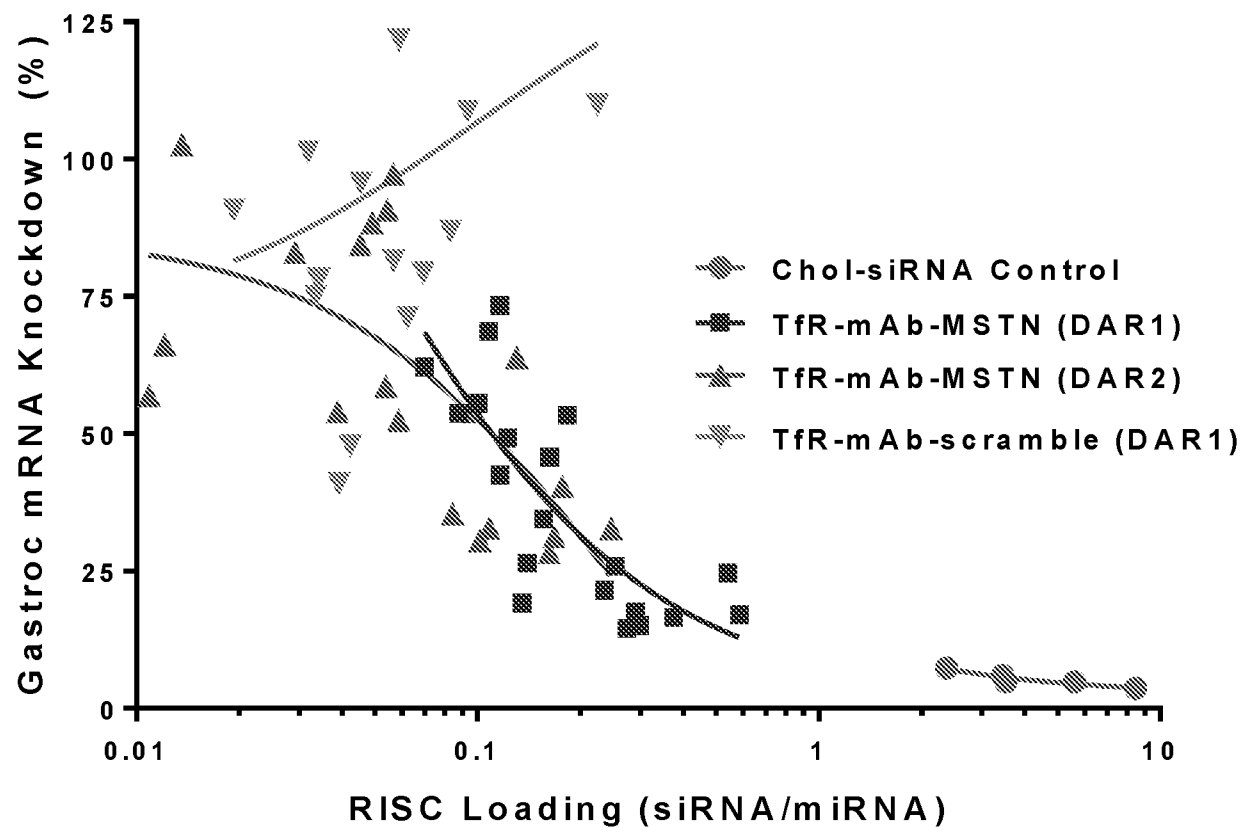


FIG. 13F

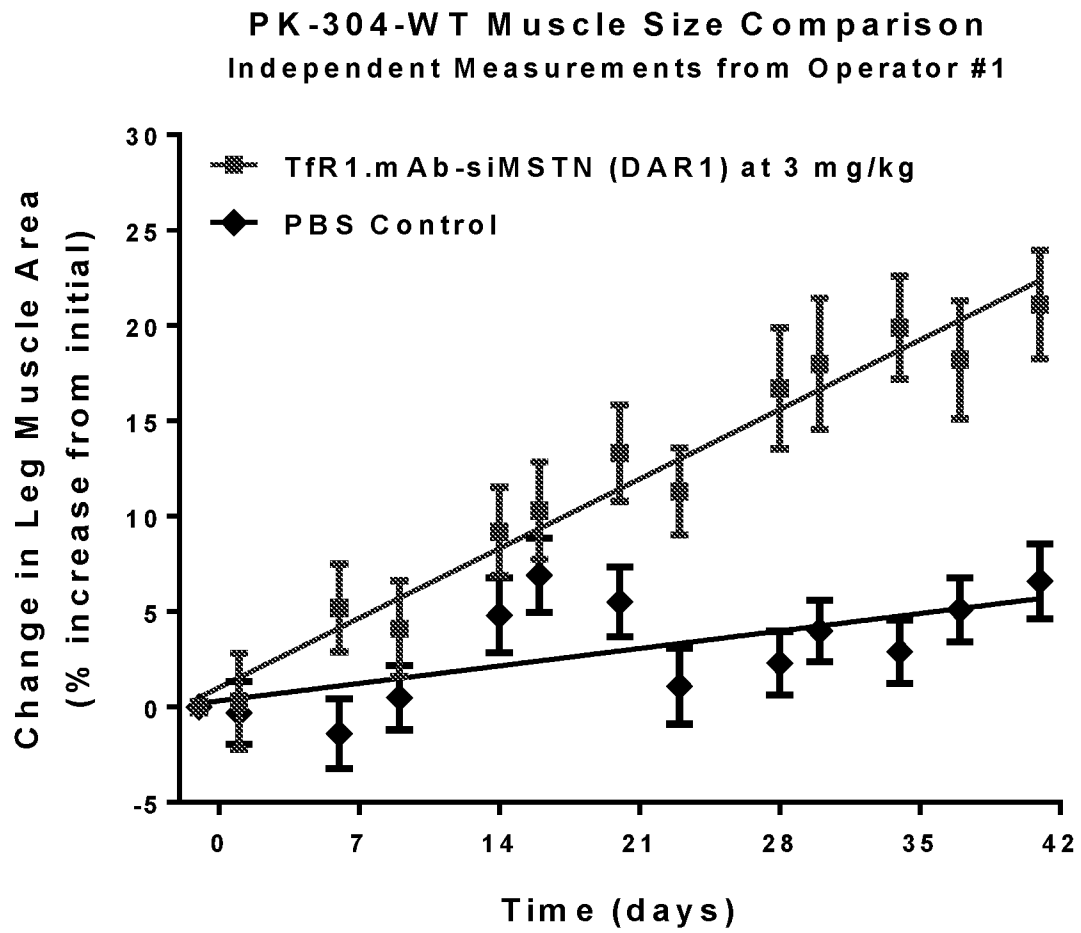


FIG. 13G

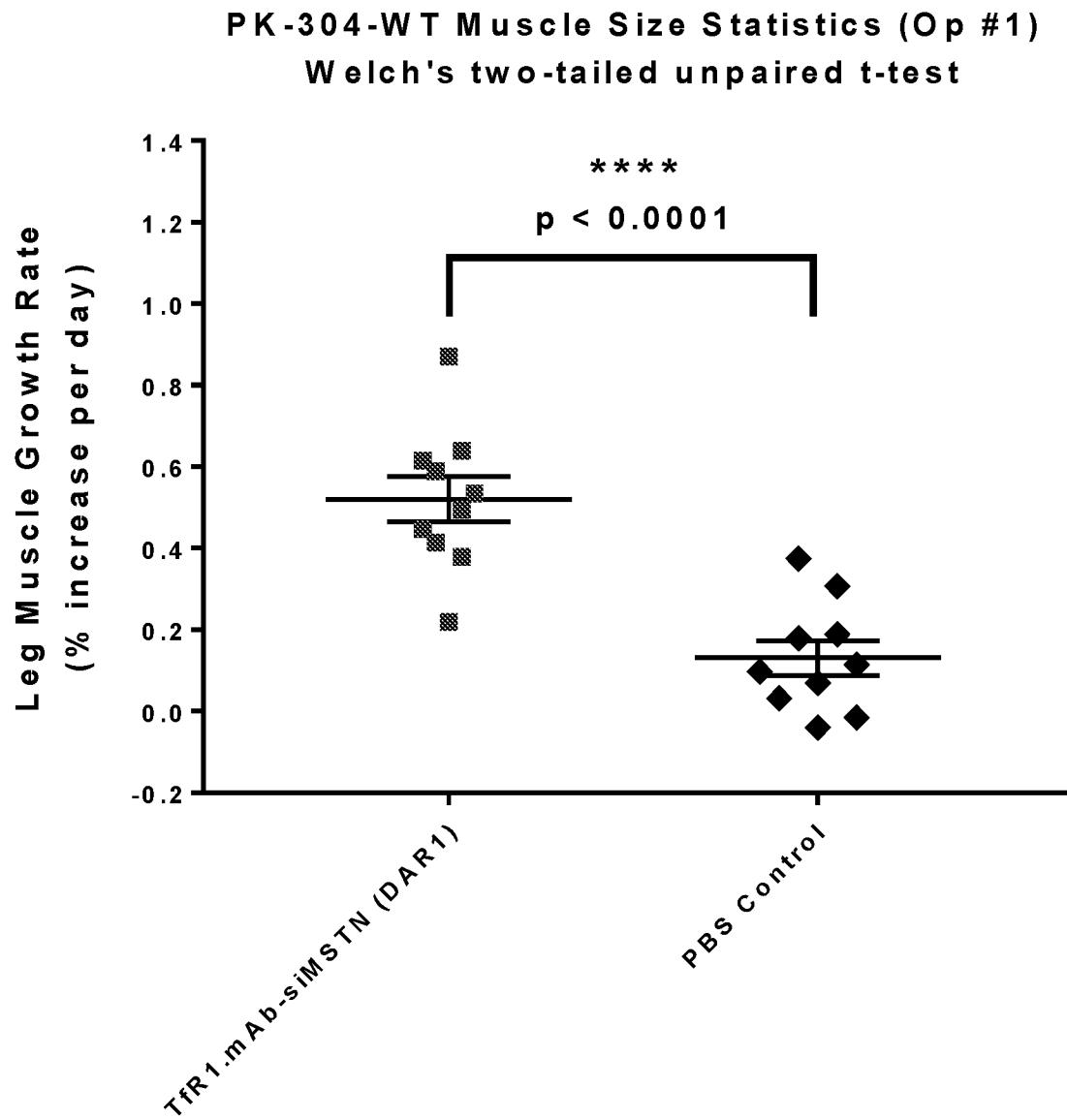


FIG. 14A

Group	Test Article	N	each siRNA Dose (mg/kg)	siRNA Mixture (mg/kg)	Harvest Time (h)	Final siRNA dose (mg/kg)
1	TfR-mAb-HPRT(n=1)	4	3		96	3
2	TfR-mAb-HPRT(n=1)	4	1		96	1
3	TfR-mAb-HPRT(n=1)	4	0.3		96	0.3
4	TfR-mAb-HPRT(n=1)	4	0.1		96	0.1
5	TfR-mAb-SSB (n=1)	4	3		96	3
6	TfR-mAb-SSB (n=1)	4	1		96	1
7	m TfR-mAb-SSB (n=1)	4	0.3		96	0.3
8	TfR-mAb-SSB (n=1)	4	0.1		96	0.1
9	TfR-mAb-HPRT (n=1) + TfR-mAb-SSB (n=1)	4	3		96	6
10	TfR-mAb-HPRT (n=1) + TfR-mAb-SSB (n=1)	4	1		96	2
11	TfR-mAb-HPRT (n=1) + TfR-mAb-SSB (n=1)	4	0.3		96	0.6
12	TfR-mAb-HPRT (n=1) + TfR-mAb-SSB (n=1)	4	0.1		96	0.2
13	TfR-mAb- HPRT/SSB(1:1) (n=2)	4		3	96	6
14	TfR-mAb- HPRT/SSB(1:1) (n=2)	4		1	96	2
15	TfR-mAb- HPRT/SSB(1:1) (n=2)	4		0.3	96	0.6
16	TfR-mAb- HPRT/SSB(1:1) (n=2)	4		0.1	96	0.2
	PBS Control	5	-		96	

FIG. 14B

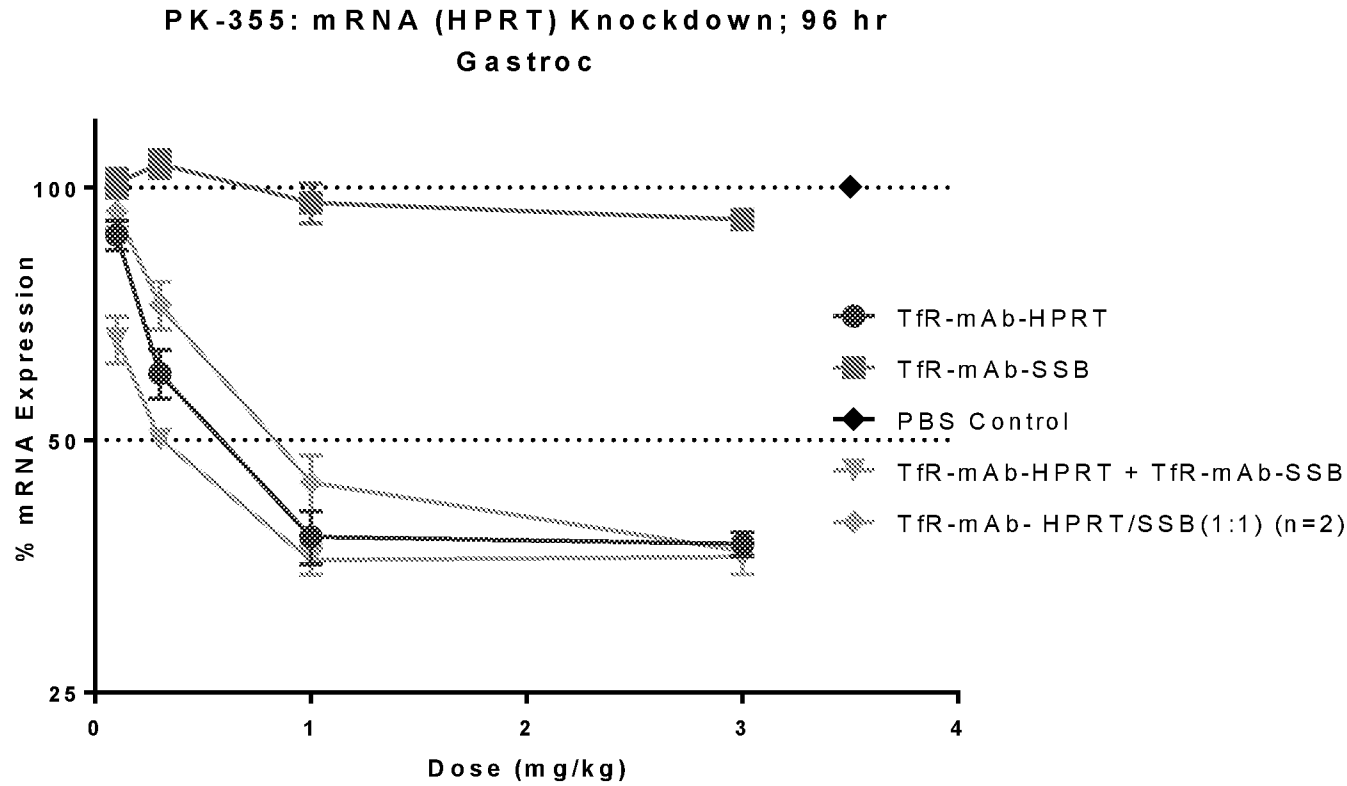


FIG. 14C

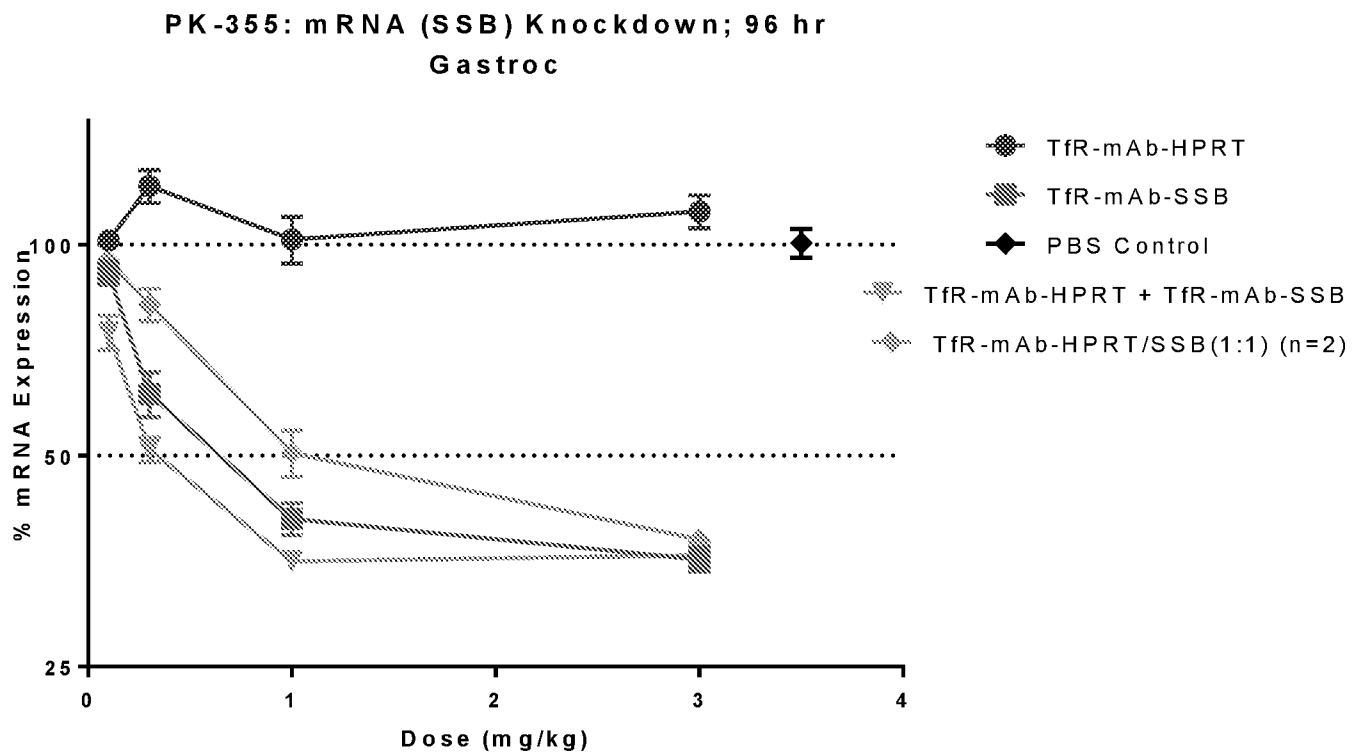


FIG. 14D

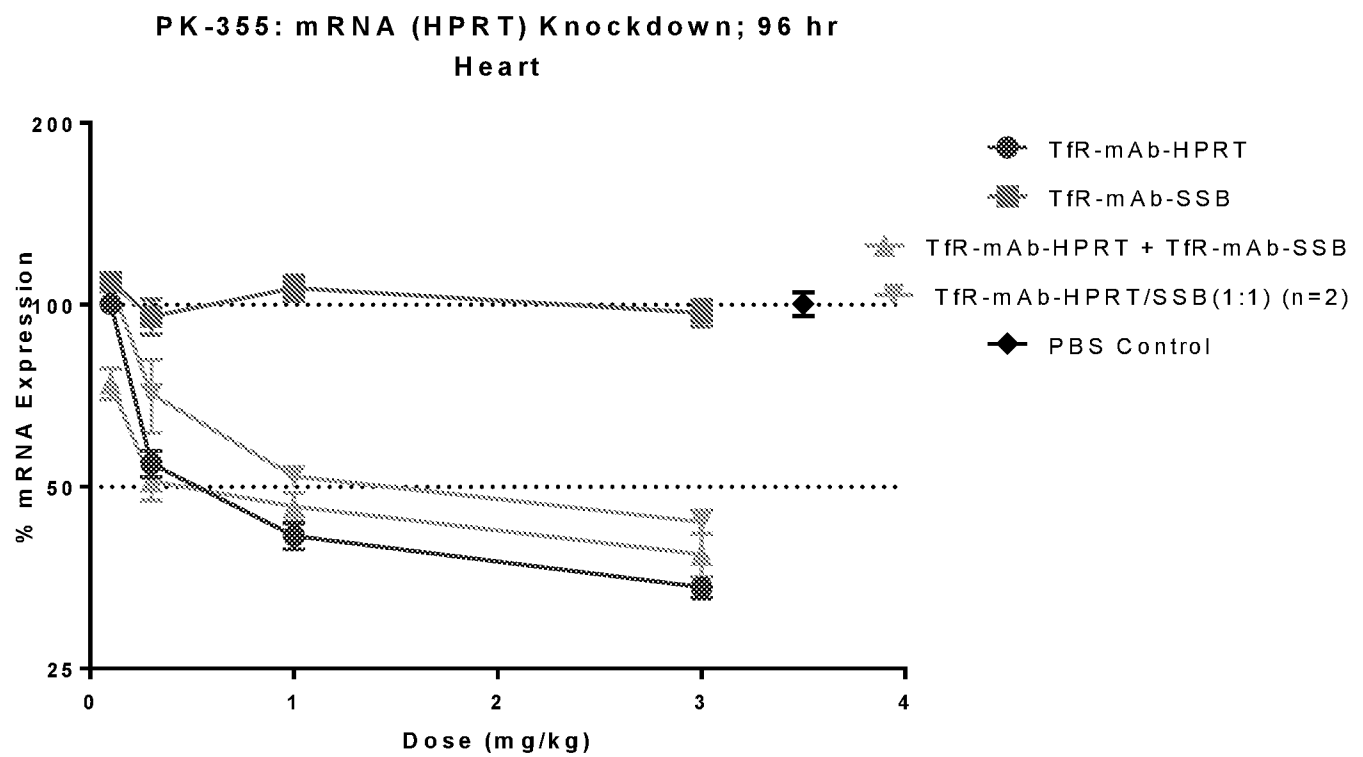


FIG. 14E

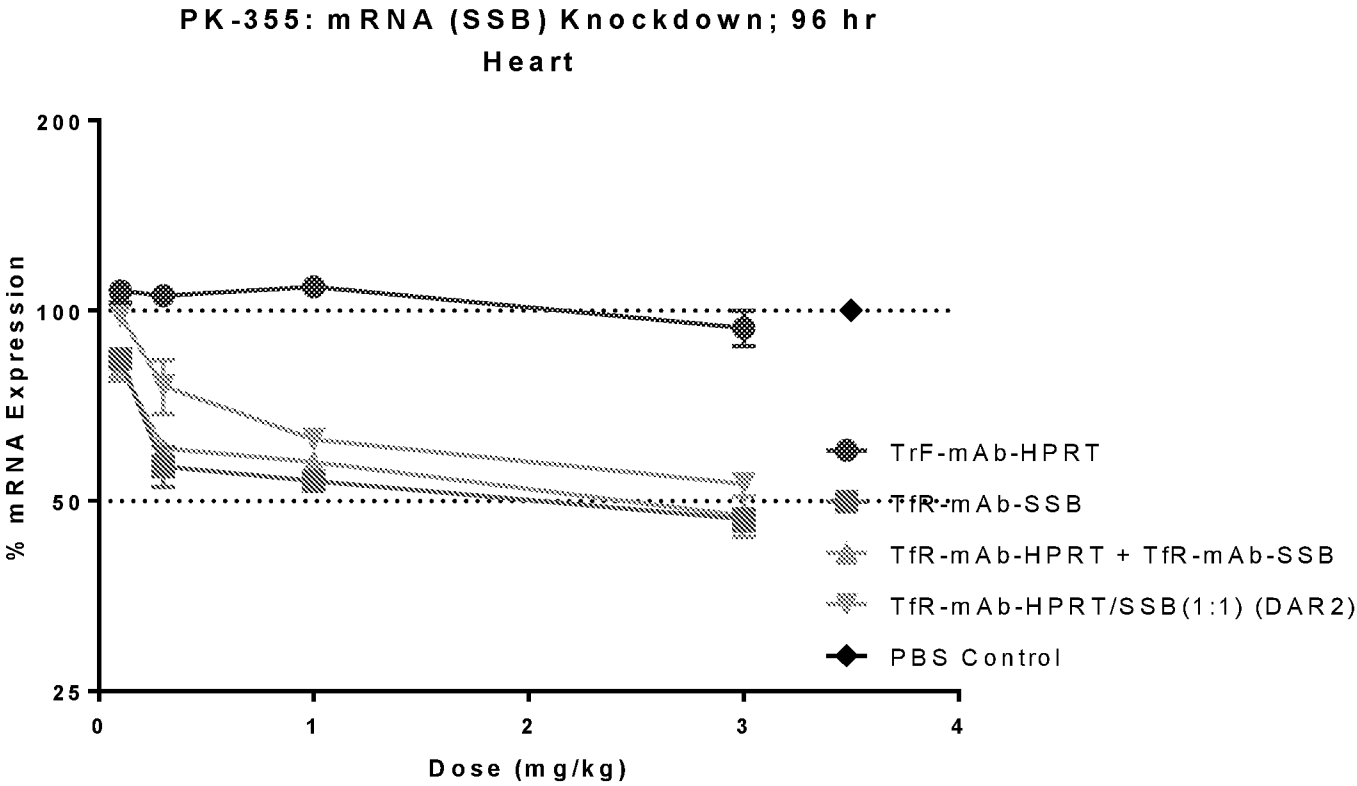


FIG. 14F

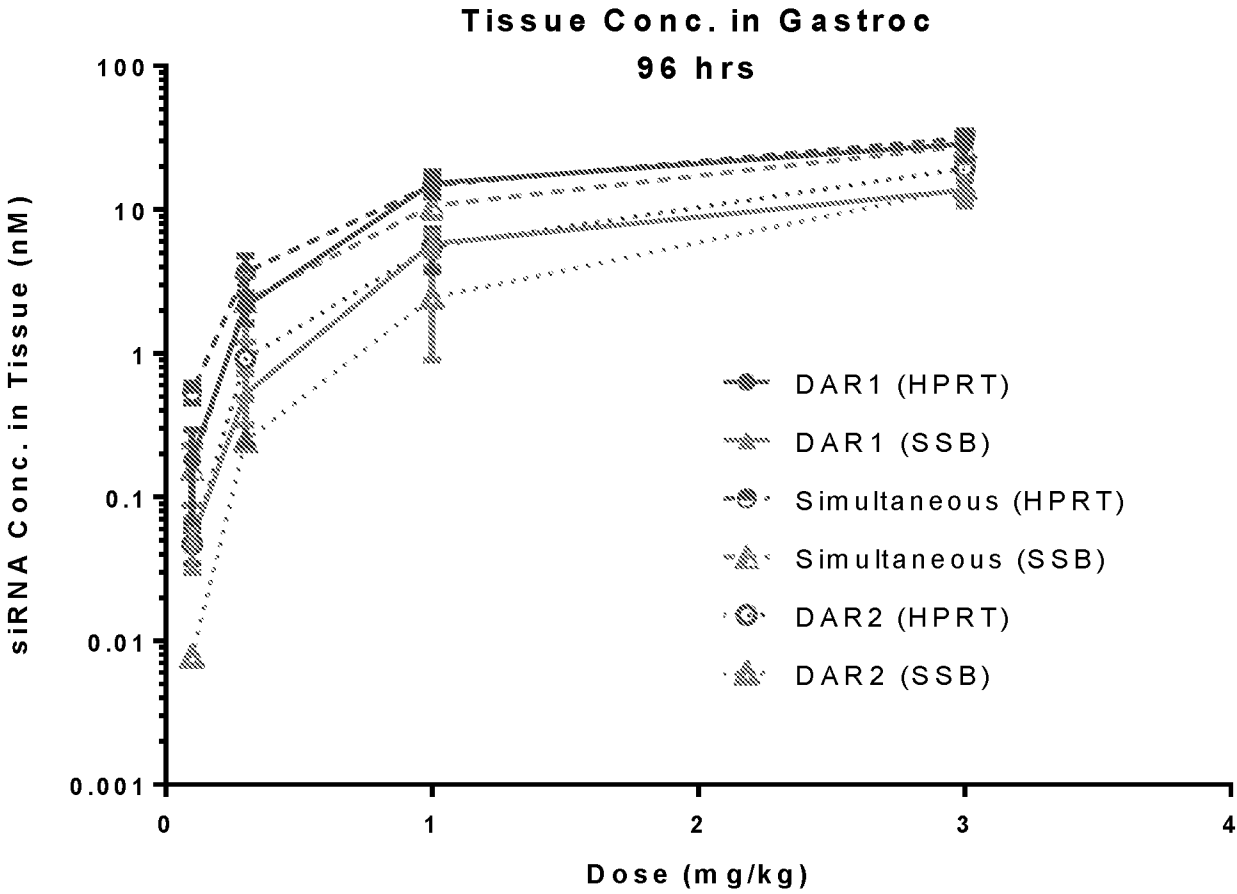


FIG. 15A

Group	Test Article	N	siRNA Dose (mg/kg)	Harvest Time (h)
1	TfR-mAb-mAtrogin#1179(n=1)	4	3	96
2	TfR-mAb-mAtrogin#1179(n=1)	4	1	96
3	TfR-mAb-mAtrogin#1179(n=1)	4	0.3	96
4	TfR-mAb-mAtrogin#1179(n=1)	4	0.1	96
5	TfR-mAb-mAtrogin#1504 (n=1)	4	3	96
6	TfR-mAb-mAtrogin#1504 (n=1)	4	1	96
7	TfR-mAb-mAtrogin#1504 (n=1)	4	0.3	96
8	TfR-mAb-mAtrogin#1504 (n=1)	4	0.1	96
9	TfR-mAb-m/hAtrogin#631 (n=1)	4	3	96
10	TfR-mAb-m/hAtrogin#631 (n=1)	4	1	96
11	TfR-mAb-m/hAtrogin#631 (n=1)	4	0.3	96
12	TfR-mAb-m/hAtrogin#631 (n=1)	4	0.1	96
13	TfR-mAb-m/hAtrogin#586 (n=1)	4	3	96
14	TfR-mAb-m/hAtrogin#586 (n=1)	4	1	96
15	TfR-mAb-m/hAtrogin#586 (n=1)	4	0.3	96
16	TfR-mAb-m/hAtrogin#586 (n=1)	4	0.1	96
17	PBS Control	5	-	96

FIG. 15B

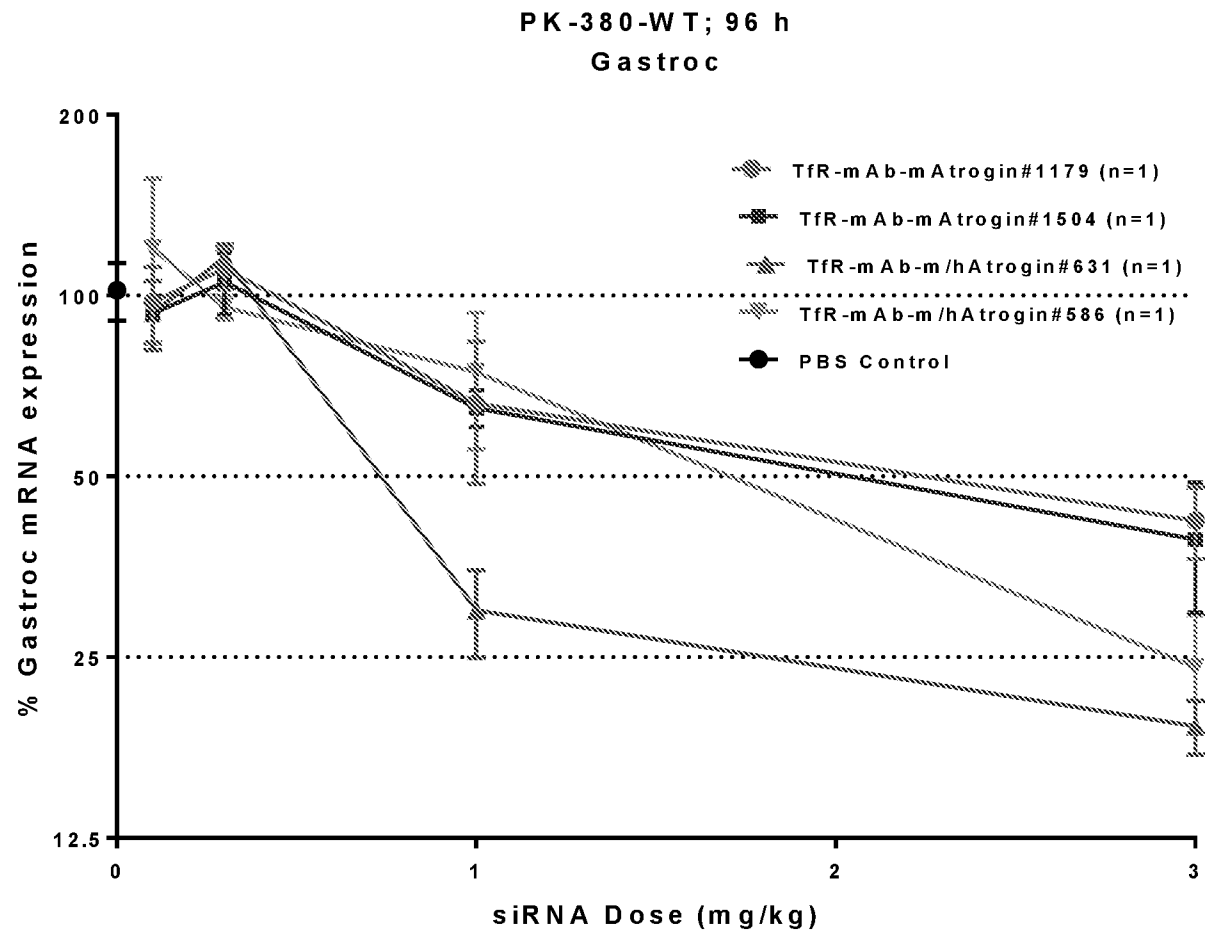


FIG. 15C

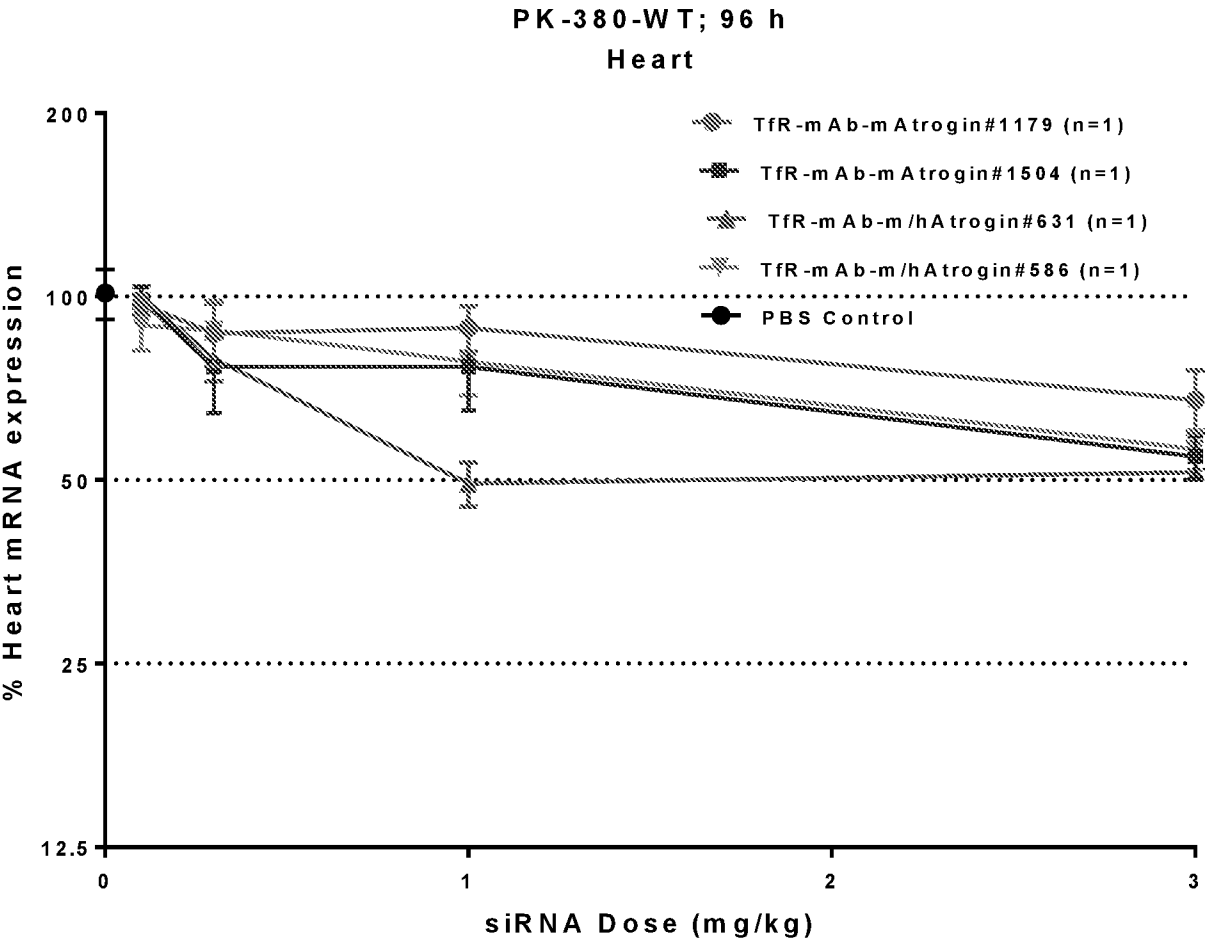


FIG. 16A

Group	Test Article	N	siRNA Dose (mg/kg)	Harvest Time (h)
1	TfR-mAb-MuRF1#651(n=1)	4	3	96
2	TfR-mAb-MuRF1#651(n=1)	4	1	96
3	TfR-mAb-MuRF1#651(n=1)	4	0.3	96
4	TfR-mAb-MuRF1#651(n=1)	4	0.1	96
5	TfR-mAb-MuRF1#1387 (n=1)	4	3	96
6	TfR-mAb-MuRF1#1387 (n=1)	4	1	96
7	TfR-mAb-MuRF1#1387 (n=1)	4	0.3	96
8	TfR-mAb-MuRF1#1387 (n=1)	4	0.1	96
9	TfR-mAb-MuRF1#1454 (n=1)	4	3	96
10	TfR-mAb-MuRF1#1454 (n=1)	4	1	96
11	TfR-mAb-MuRF1#1454 (n=1)	4	0.3	96
12	TfR-mAb-MuRF1#1454 (n=1)	4	0.1	96
13	TfR-mAb-MuRF1#1660 (n=1)	4	3	96
14	TfR-mAb-MuRF1#1660 (n=1)	4	1	96
15	TfR-mAb-MuRF1#1660 (n=1)	4	0.3	96
16	TfR-mAb-MuRF1#1660 (n=1)	4	0.1	96
17	PBS Control	5	-	96

FIG. 16B

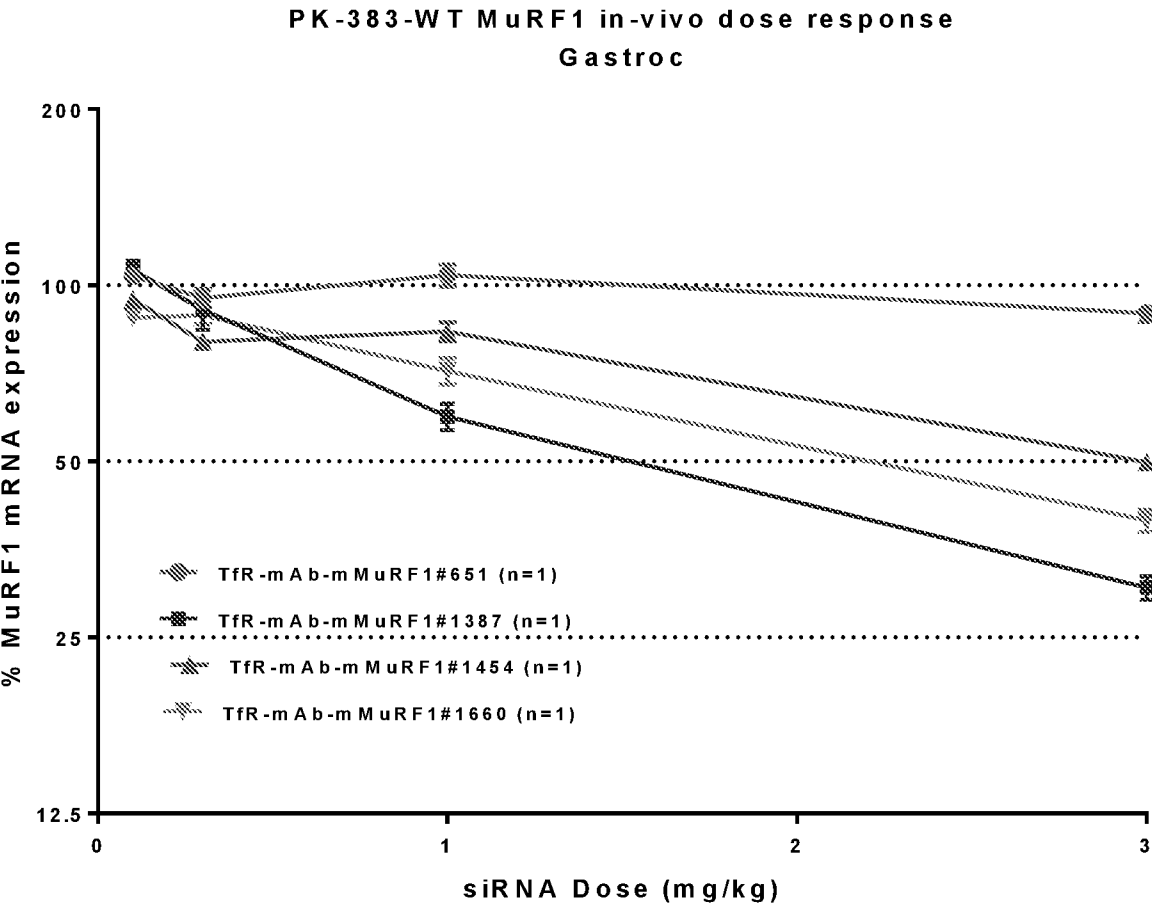


FIG. 16C

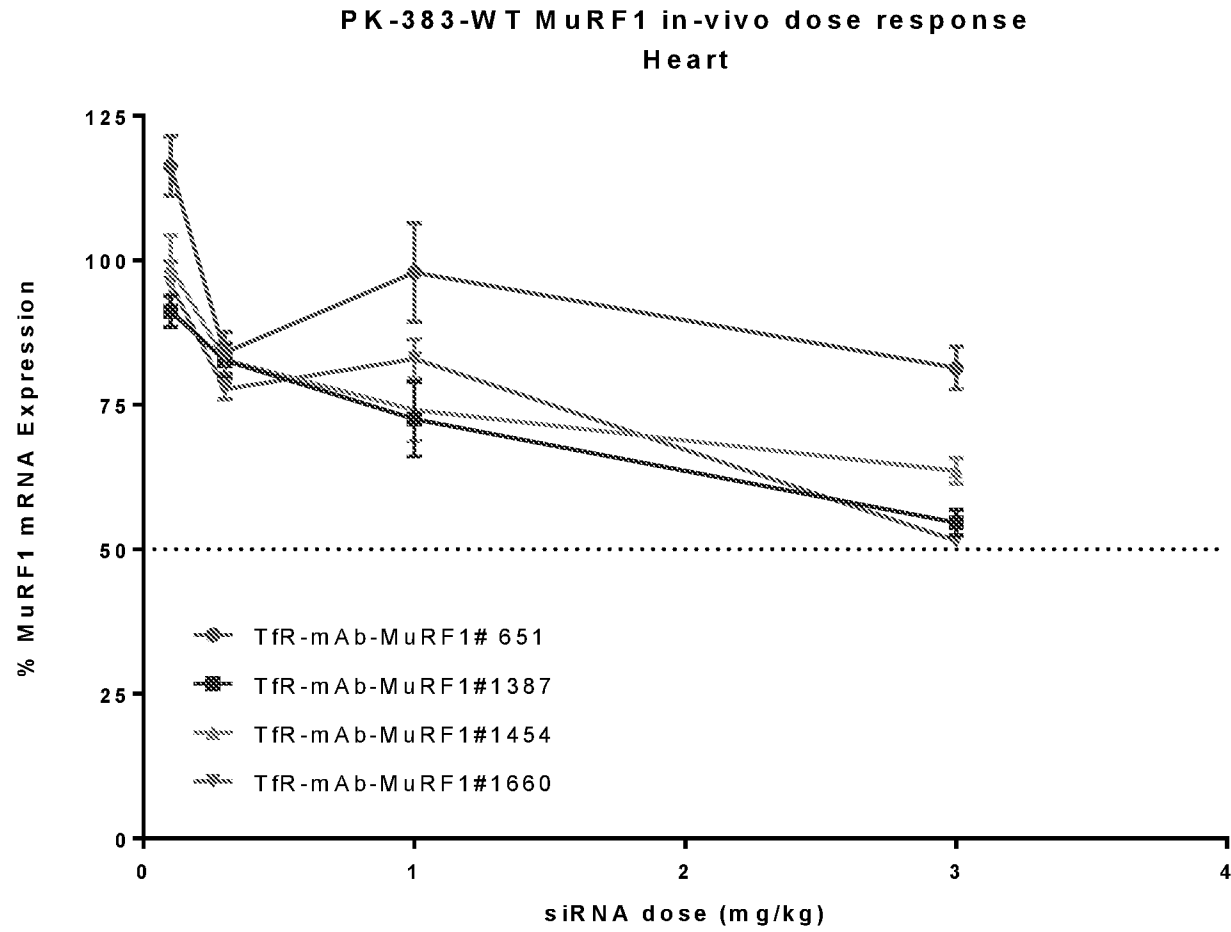


FIG. 17

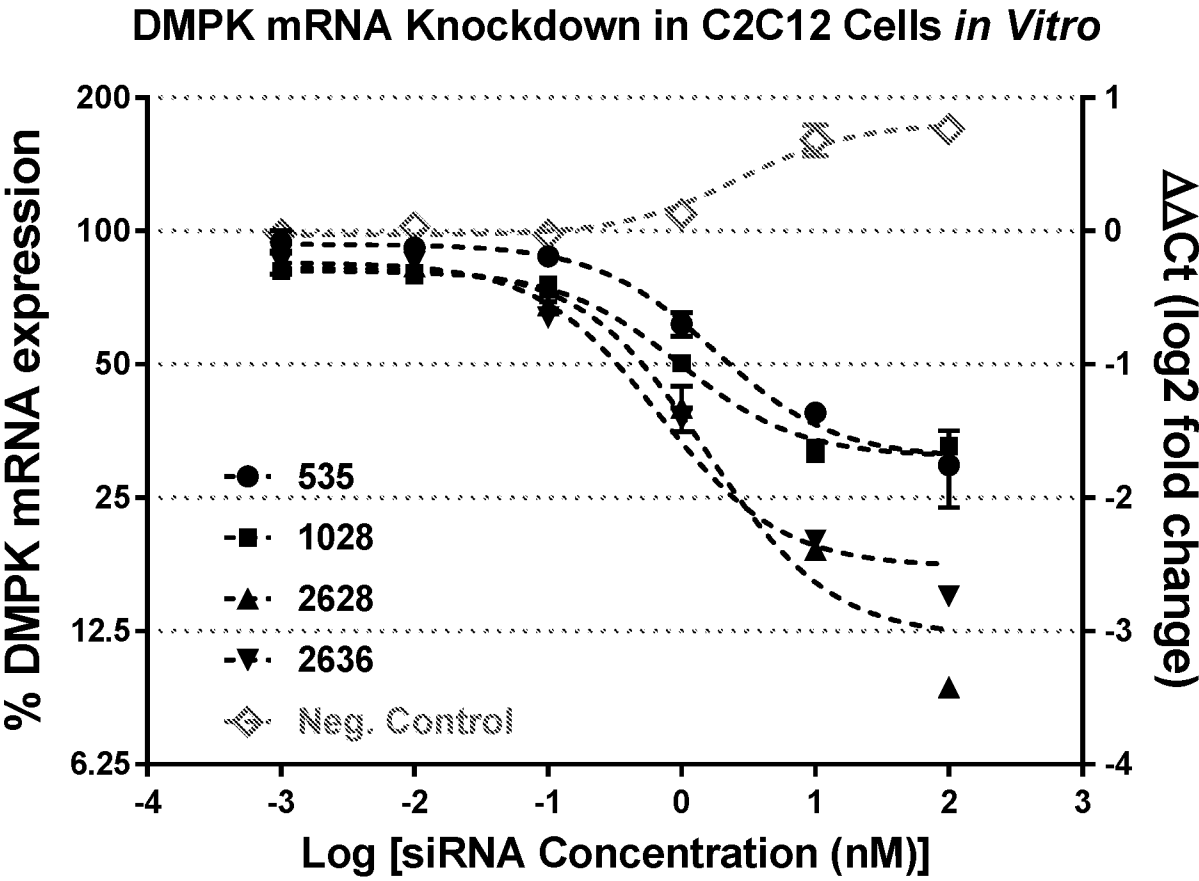


Fig. 18A

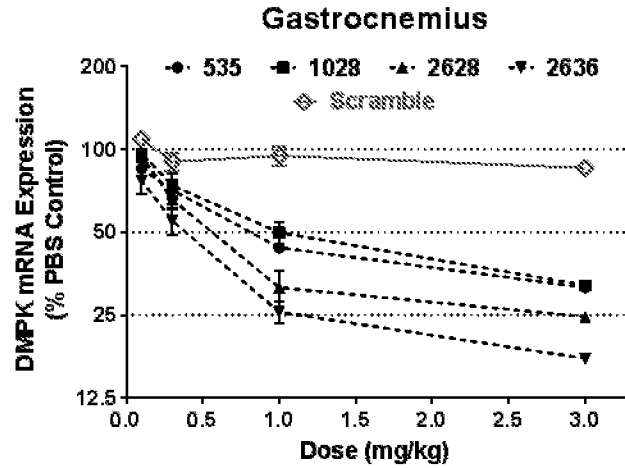


Fig. 18B

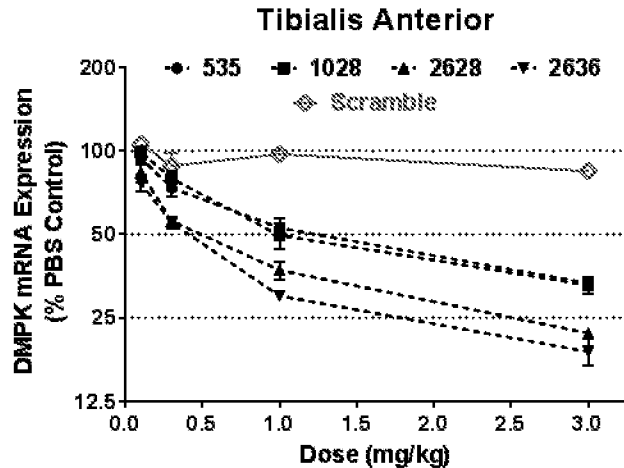


Fig. 18C

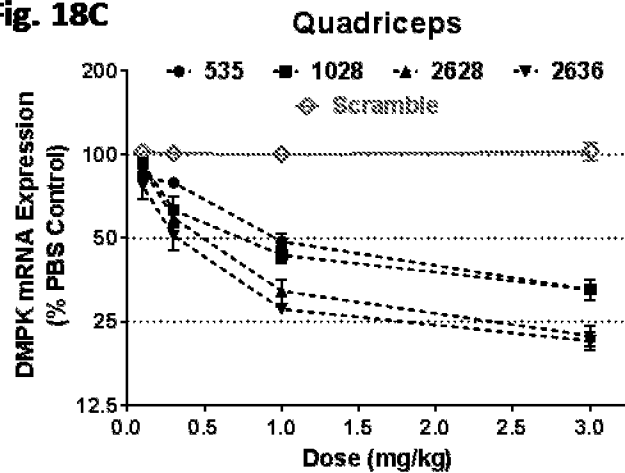


Fig. 18D

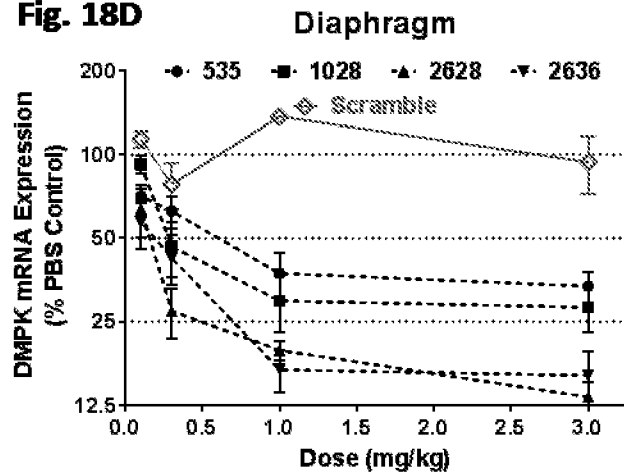


Fig. 18E

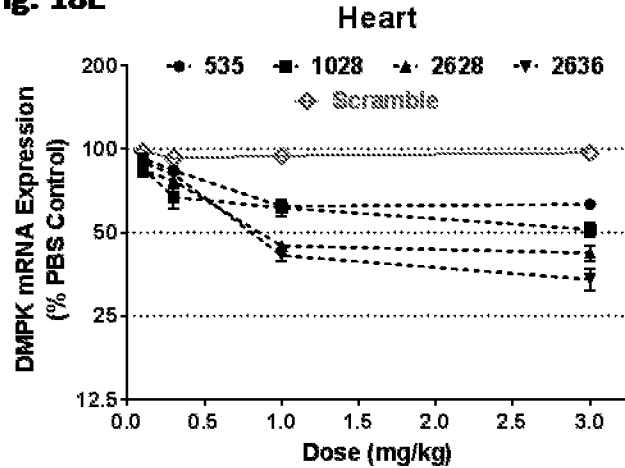


Fig. 18F

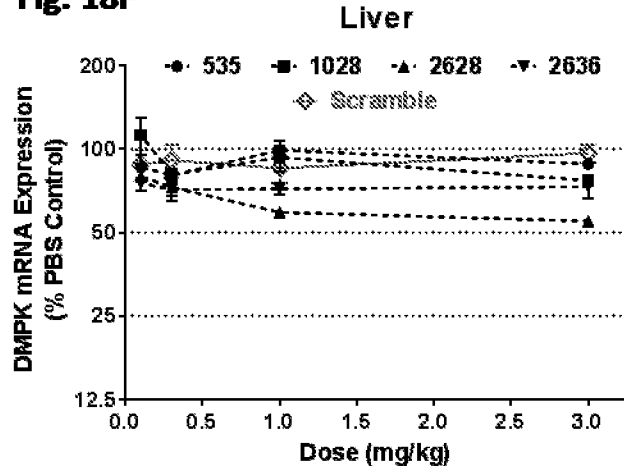


FIG. 19A

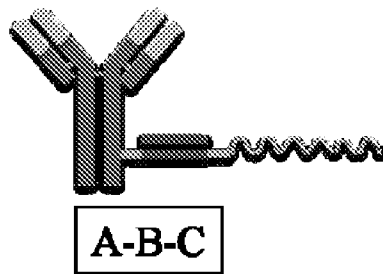


FIG. 19B

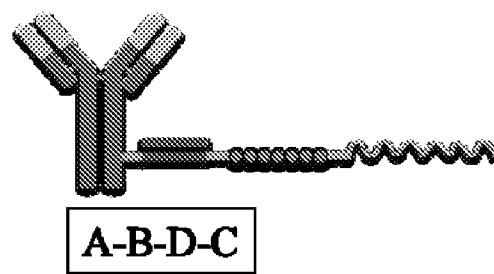


FIG. 19C

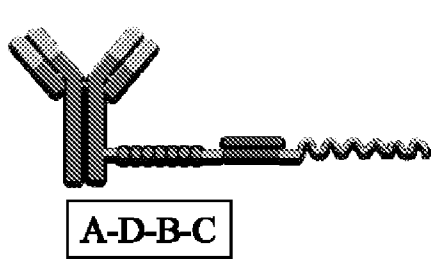


FIG. 19D

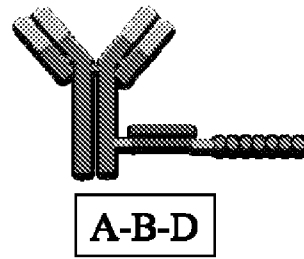


FIG. 19E

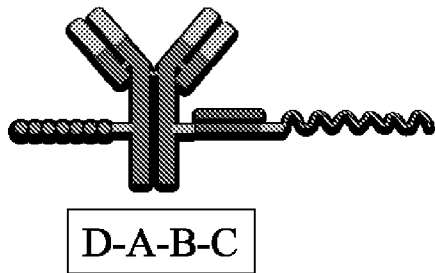


FIG. 19F

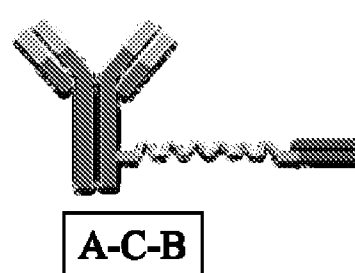


FIG. 19G

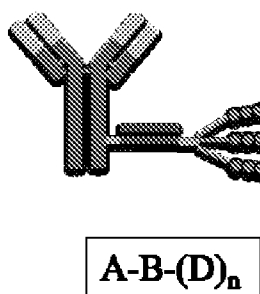


FIG. 19H

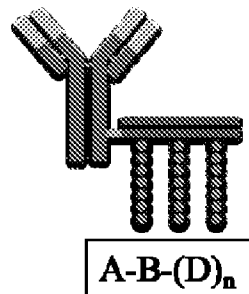


FIG. 19I

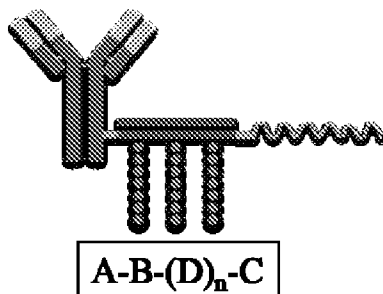


FIG. 19J

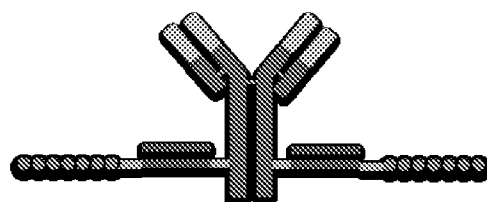


FIG. 19K

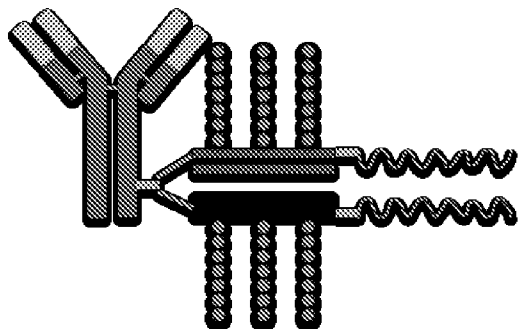


FIG. 19L

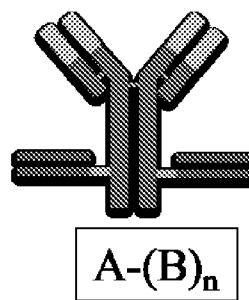


FIG. 19M

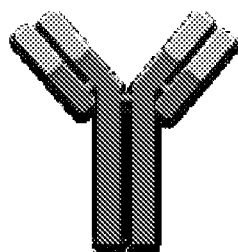


FIG. 20A

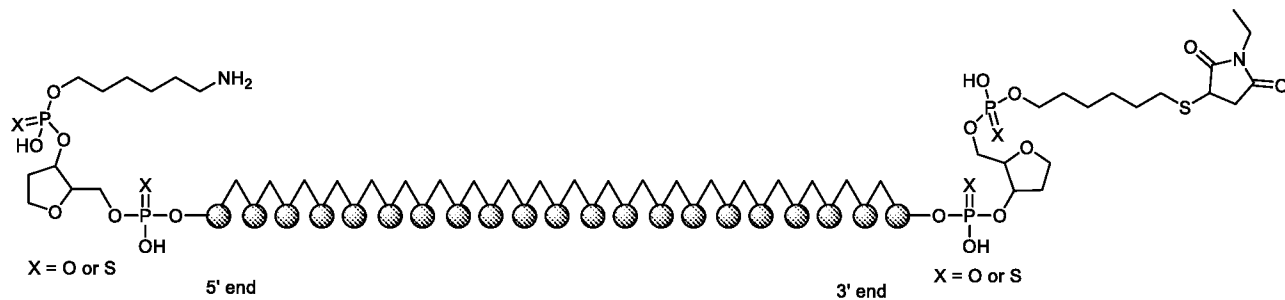


FIG. 20B

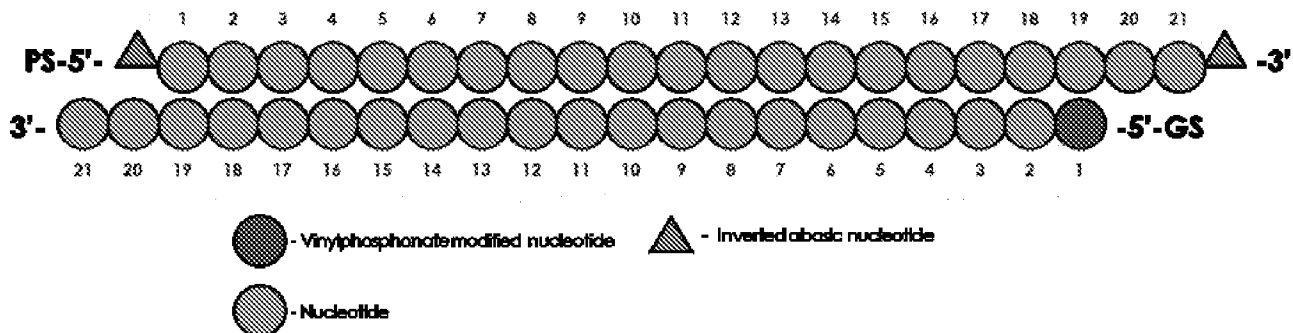


FIG. 23

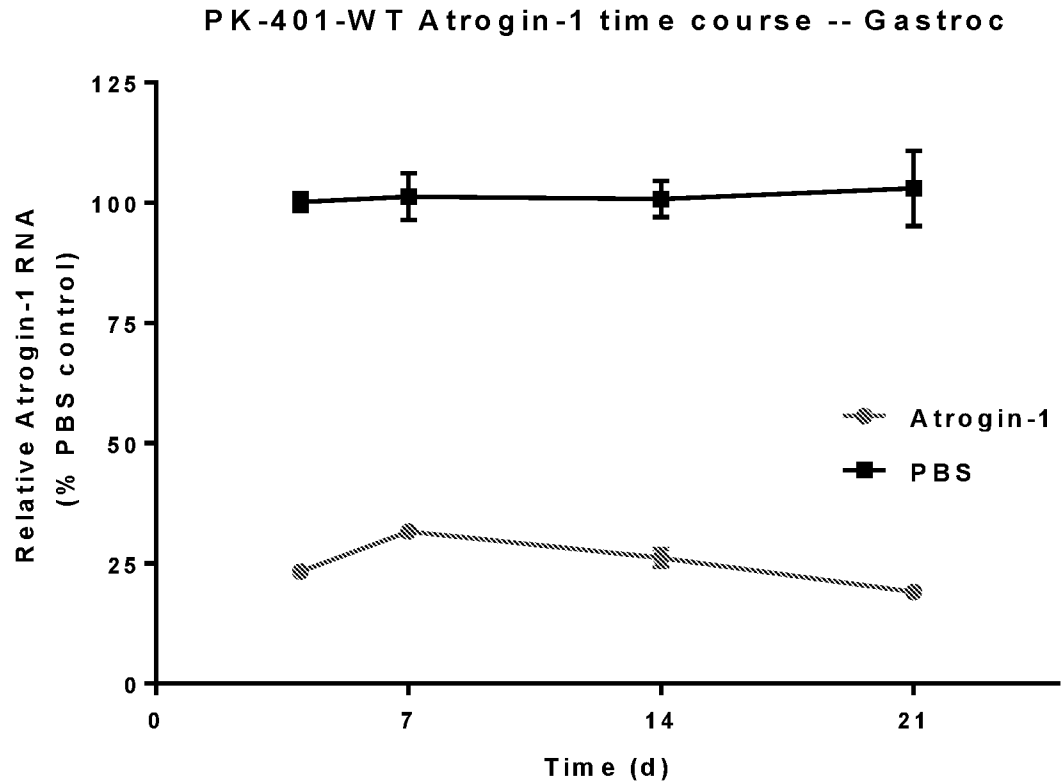


FIG. 24

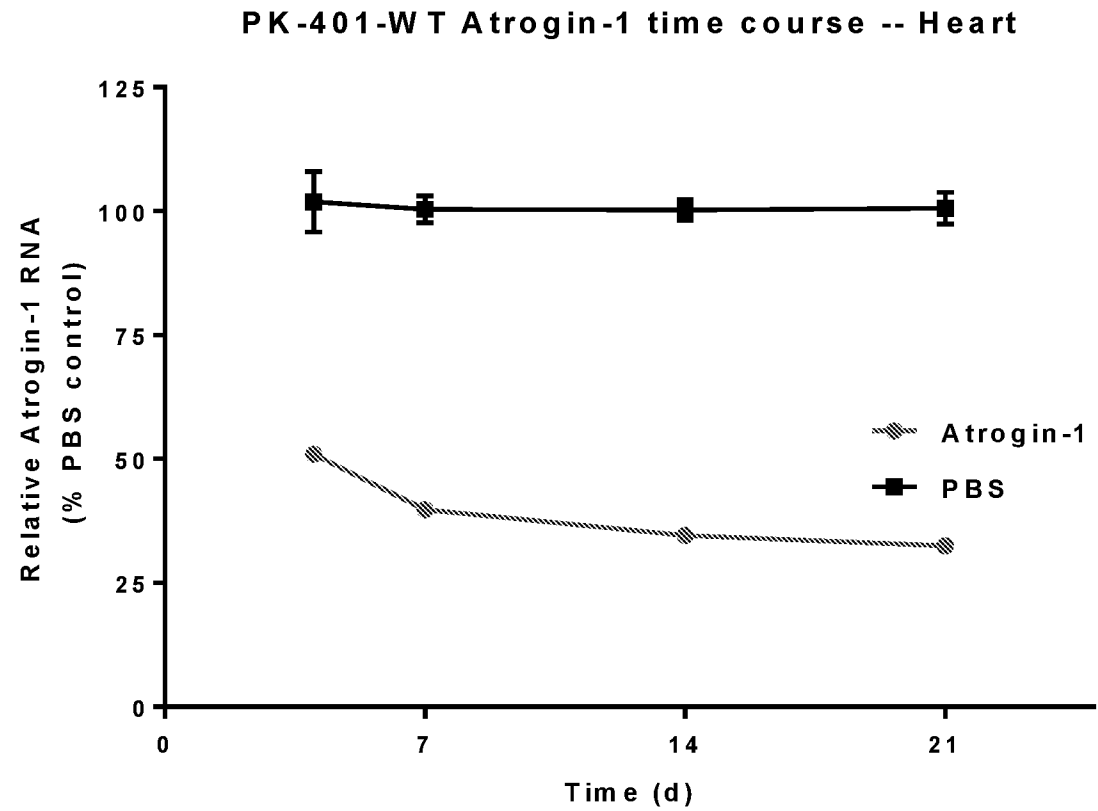


FIG. 25

Group	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	Harvest Time (h)
1	mTfR1-mAb-MuRF1(R2089) (DAR1)	4	3	5.0	96
2	mTfR1-mAb-MuRF1(R2089) (DAR1)	4	1	5.0	96
3	mTfR1-mAb-MuRF1(R2265) (DAR1)	4	3	5.0	96
4	mTfR1-mAb-MuRF1(R2265) (DAR1)	4	1	5.0	96
5	mTfR1-mAb-MuRF1(R2265) (DAR1)	4	0.3	5.0	96
6	mTfR1-mAb-MuRF1(R2265) (DAR1)	4	0.1	5.0	96
7	mTfR1-mAb-MuRF1(R2266) (DAR1)	4	3	5.0	96
8	mTfR1-mAb-MuRF1(R2266) (DAR1)	4	1	5.0	96
9	mTfR1-mAb-MuRF1(R2266) (DAR1)	4	0.3	5.0	96
10	mTfR1-mAb-MuRF1(R2266) (DAR1)	4	0.1	5.0	96
11	mTfR1-mAb-MuRF1(R2267) (DAR1)	4	3	5.0	96
12	mTfR1-mAb-MuRF1(R2267) (DAR1)	4	1	5.0	96
13	mTfR1-mAb-MuRF1(R2267) (DAR1)	4	0.3	5.0	96
14	mTfR1-mAb-MuRF1(R2267) (DAR1)	4	0.1	5.0	96
15	mTfR1-mAb-MuRF1(R2268) (DAR1)	4	3	5.0	96
16	mTfR1-mAb-MuRF1(R2268) (DAR1)	4	1	5.0	96
27	mTfR1-mAb-MuRF1(R2268) (DAR1)	4	0.3	5.0	96
18	mTfR1-mAb-MuRF1(R2268) (DAR1)	4	0.1	5.0	96
19	mTfR1-mAb-MuRF1(R2269) (DAR1)	4	3	5.0	96
20	mTfR1-mAb-MuRF1(R2269) (DAR1)	4	1	5.0	96
21	mTfR1-mAb-MuRF1(R2269) (DAR1)	4	0.3	5.0	96
22	mTfR1-mAb-MuRF1(R2269) (DAR1)	4	0.1	5.0	96
23	PBS Control	5	-	5.0	96

FIG. 26

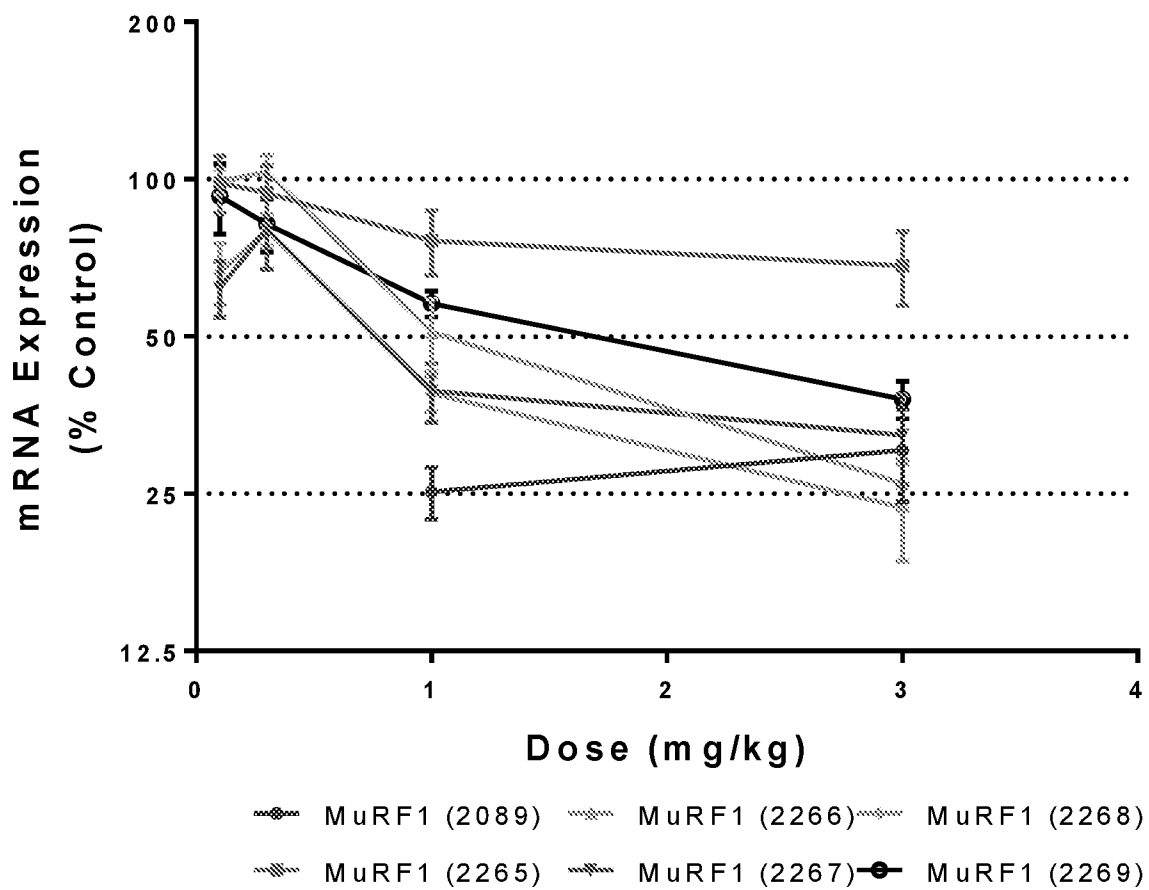
Gastroc

FIG. 27

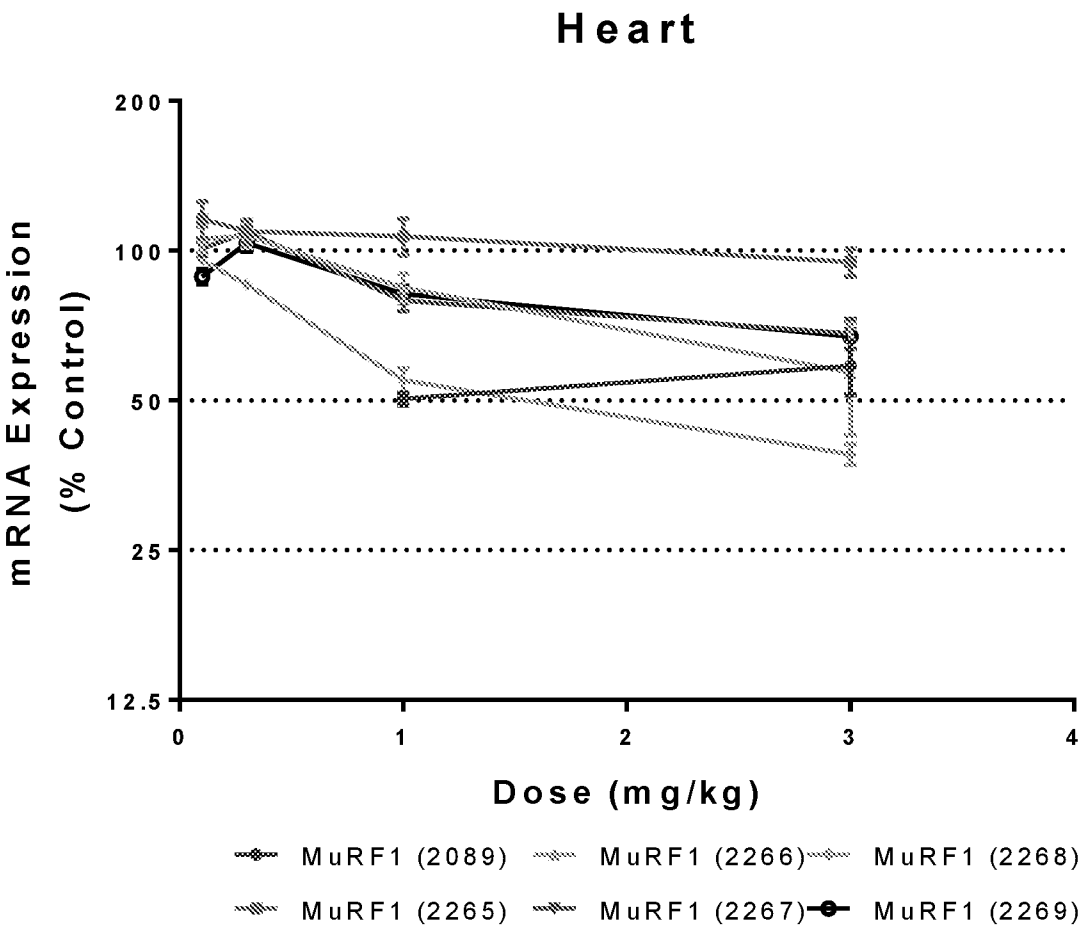


FIG. 28

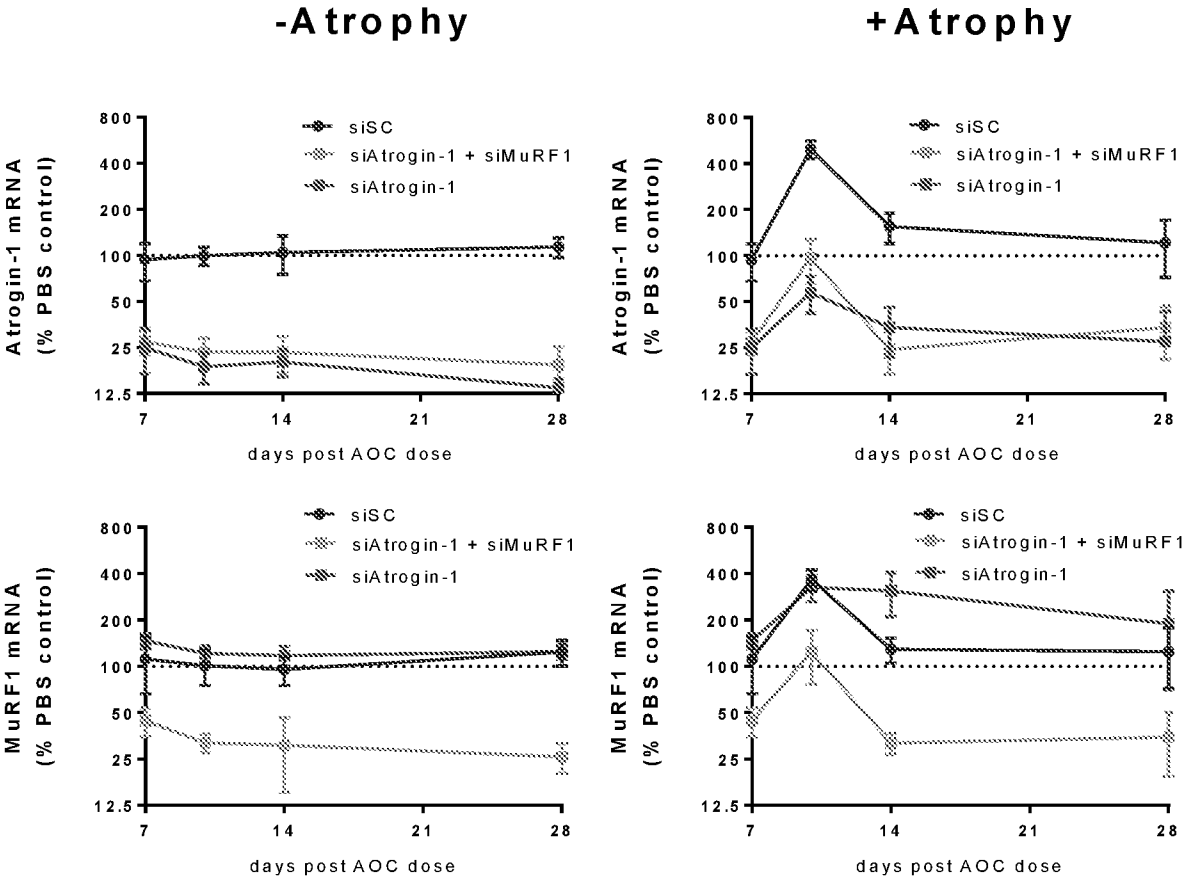


FIG. 29

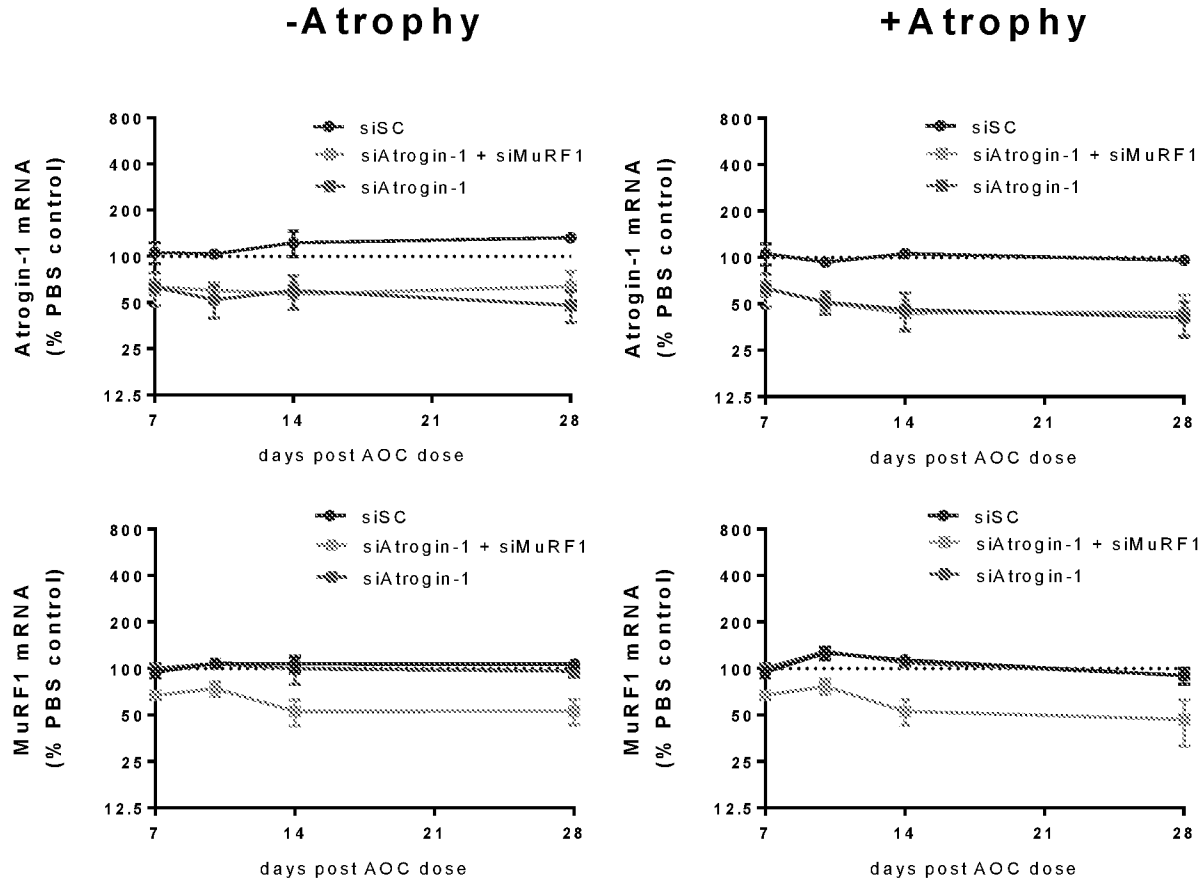


FIG. 30

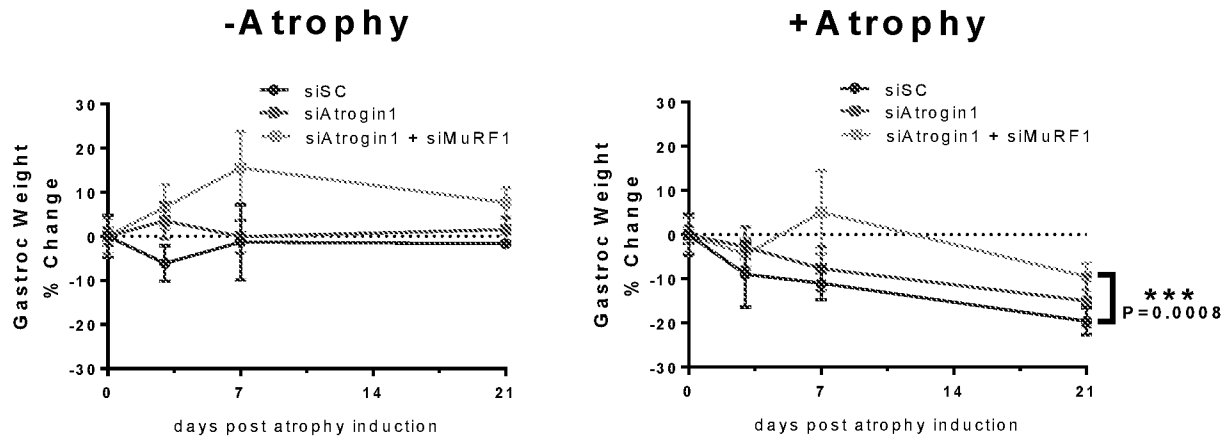


FIG. 31

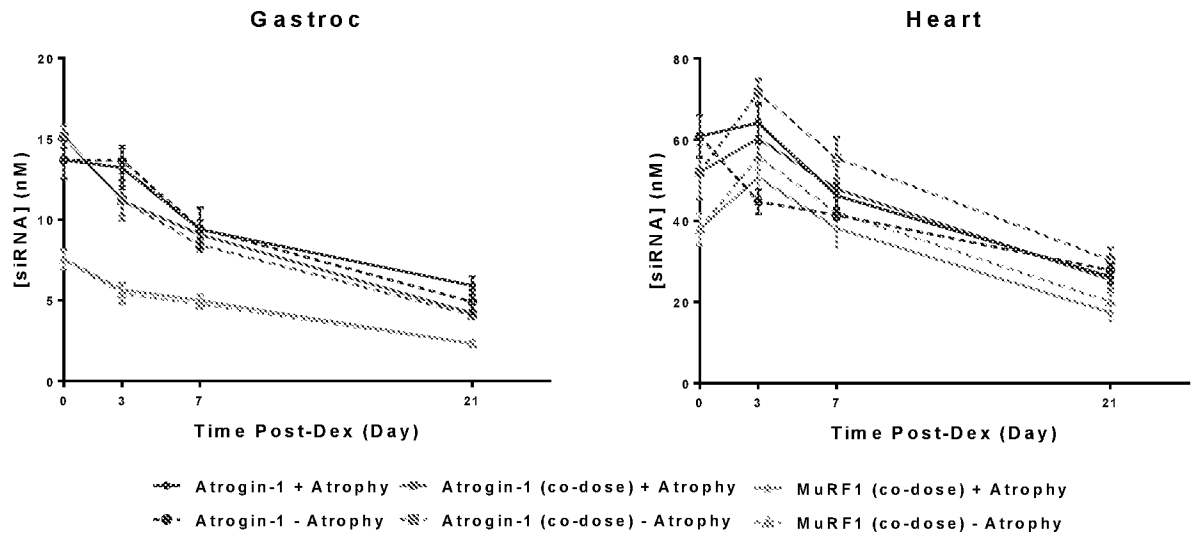


FIG. 32

Group	ASC	Atrophy	N	siRNA Dose (mg/kg)	siRNA: mAb Ratio (mol/mol)	Dose Volume (mL/kg)	# of Doses	Harvest Time (day post Dex)
1	TfR1-Atrogin-1 DAR1		4	9	1.0	5.0	1	0
2	TfR1-Atrogin-1 DAR1	+ PBS	4	9	1.0	5.0	1	3
3	TfR1-Atrogin-1 DAR1	+ Dex	4	9	1.0	5.0	1	3
4	TfR1-Atrogin-1 DAR1		4	3	1.0	5.0	1	0
5	TfR1-Atrogin-1 DAR1	+ PBS	4	3	1.0	5.0	1	3
6	TfR1-Atrogin-1 DAR1	+ Dex	4	3	1.0	5.0	1	3
7	TfR1-Atrogin-1 DAR1		4	1	1.0	5.0	1	0
8	TfR1-Atrogin-1 DAR1	+ PBS	4	1	1.0	5.0	1	3
9	TfR1-Atrogin-1 DAR1	+ Dex	4	1	1.0	5.0	1	3
10	TfR1-Scramble DAR1		4	9	1.0	5.0	1	0
11	TfR1-Scramble DAR1	+ PBS	4	9	1.0	5.0	1	3
12	TfR1-Scramble DAR1	+ Dex	4	9	1.0	5.0	1	3
13	TfR1-Scramble DAR1		4	3	1.0	5.0	1	0
14	TfR1-Scramble DAR1	+ PBS	4	3	1.0	5.0	1	3
15	TfR1-Scramble DAR1	+ Dex	4	3	1.0	5.0	1	3
16	PBS Control		5	-	-	5.0	1	0

FIG. 33

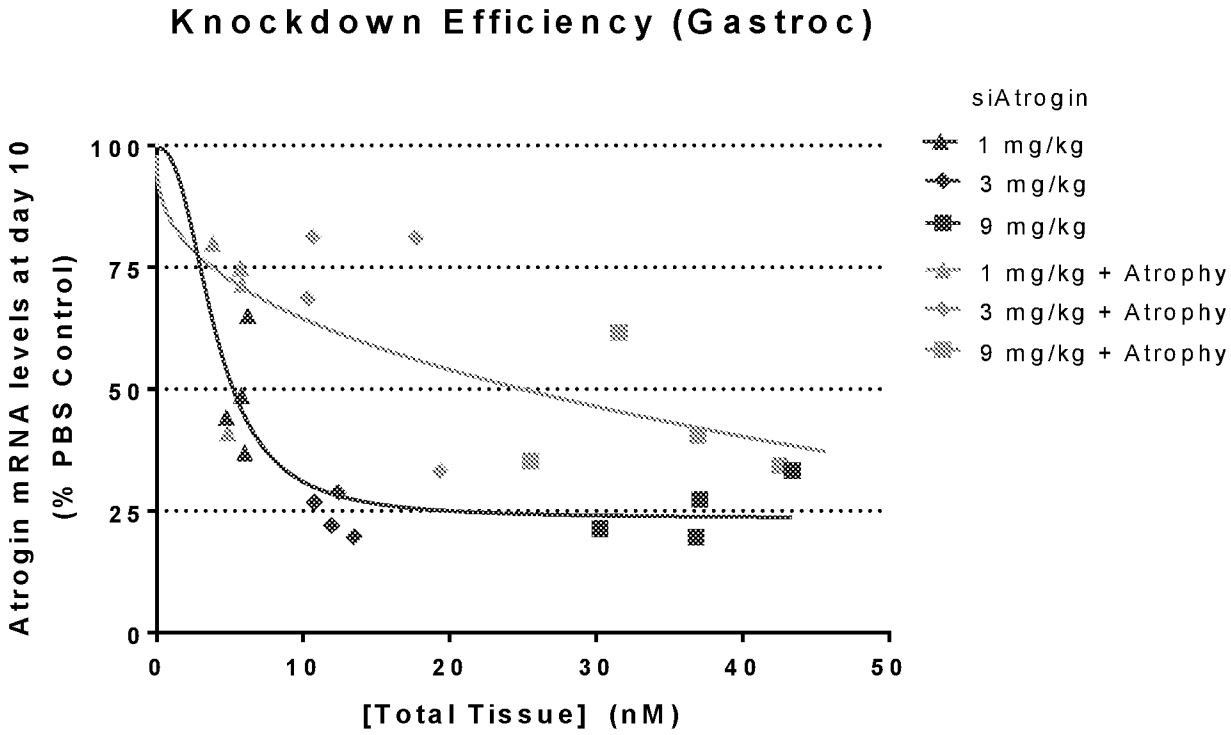


FIG. 34

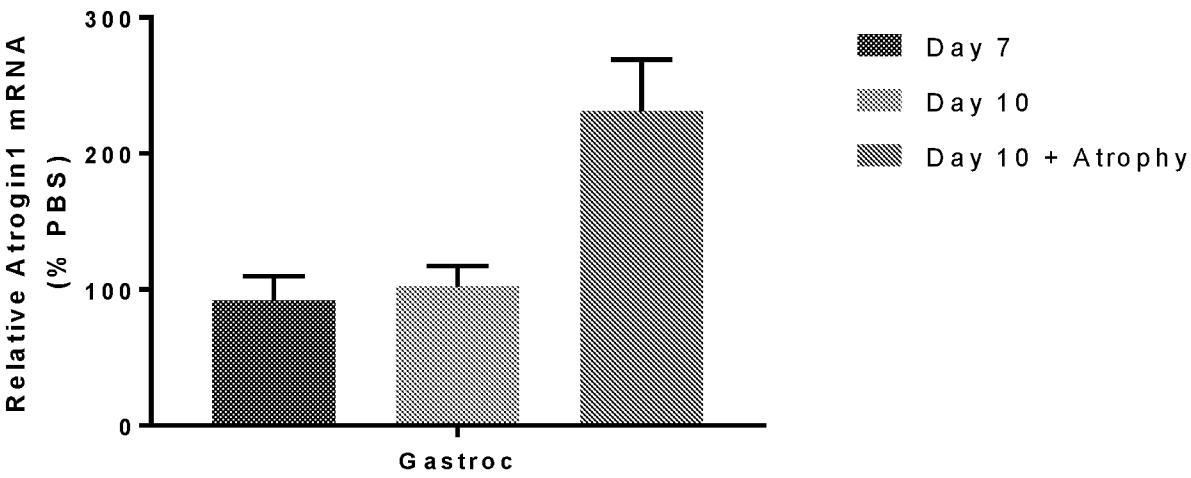


FIG. 35

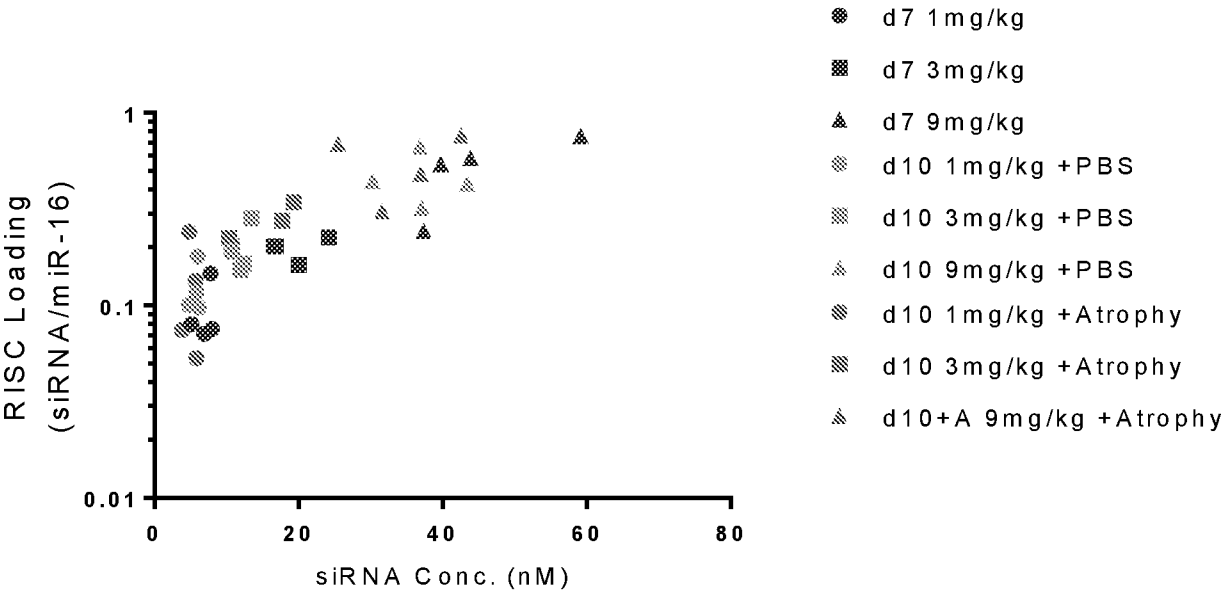


FIG. 36

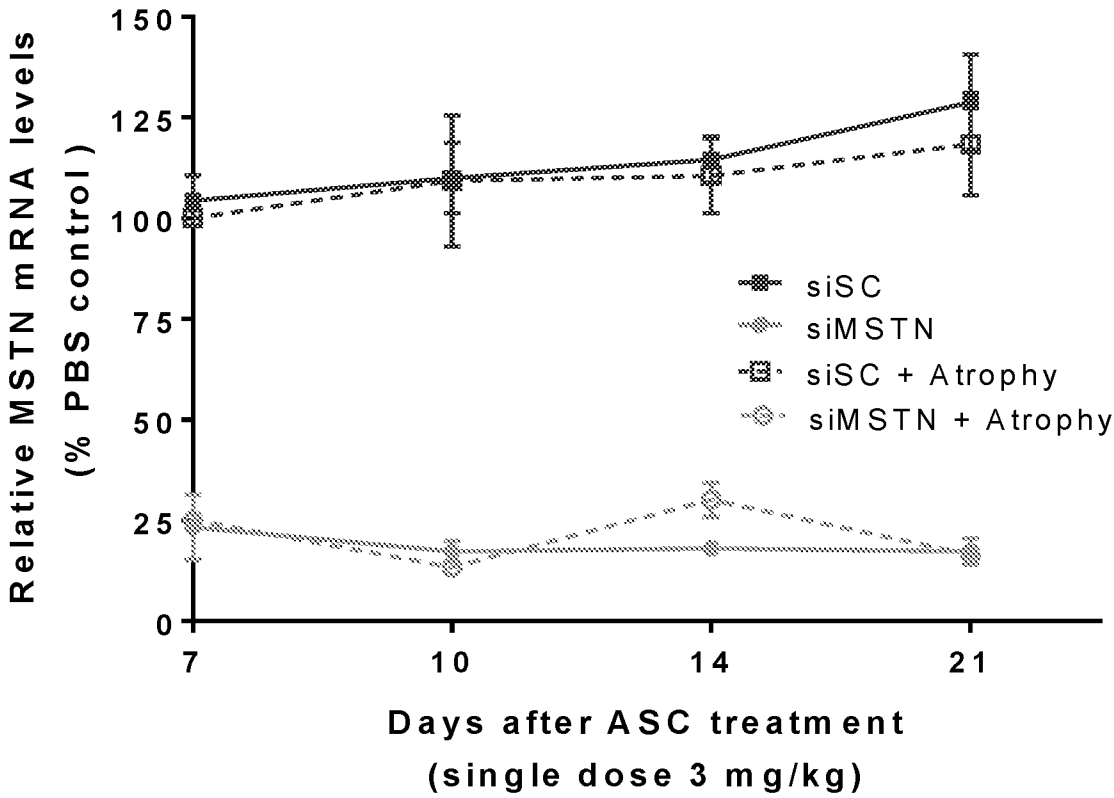


FIG. 37

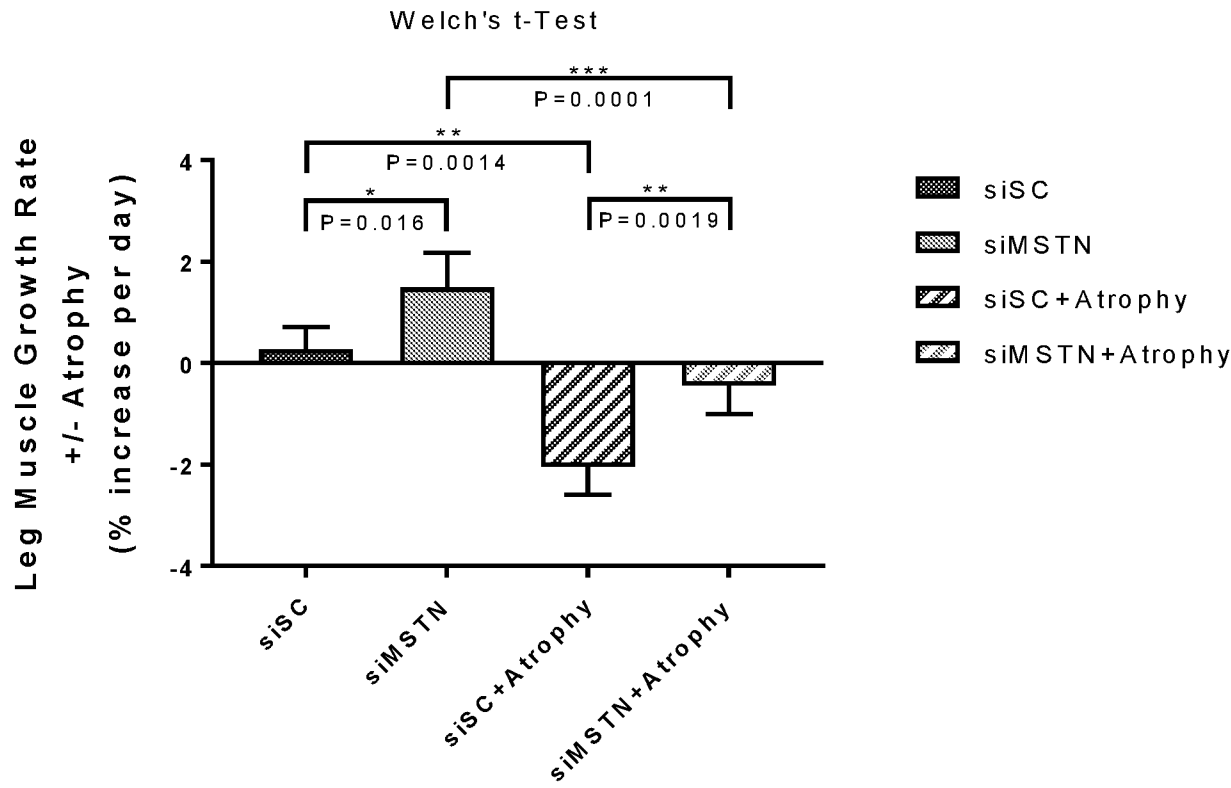


FIG. 38

Group	ASC	N	Total siRNA Dose (mg/kg)	Dose Volume (mL/kg)	Harvest Time (day post ASC)	Harvest Time (day post DEN)
1	TfR1-Atrogin-1 DAR1	4	4.5	5.0	7	0
2	TfR1-Atrogin-1 DAR1	4	4.5	5.0	10	3
3	TfR1-Atrogin-1 DAR1	4	4.5	5.0	17	10
4	TfR1-Atrogin-1 DAR1	8	4.5	5.0	28	21
5	TfR1-MuRF1 DAR1	4	4.5	5.0	7	0
6	TfR1-MuRF1 DAR1	4	4.5	5.0	10	3
7	TfR1-MuRF1 DAR1	4	4.5	5.0	17	10
8	TfR1-MuRF1 DAR1	8	4.5	5.0	28	21
9	TfR1-Atrogin-1 + TfR1-MuRF1 DAR1	4	9	5.0	7	0
10	TfR1-Atrogin-1 + TfR1-MuRF1 DAR1	4	9	5.0	10	3
11	TfR1-Atrogin-1 + TfR1-MuRF1 DAR1	4	9	5.0	17	10
12	TfR1-Atrogin-1 + TfR1-MuRF1 DAR1	8	9	5.0	28	21
13	TfR1-SC DAR1	4	4.5	5.0	7	0
14	TfR1-SC DAR1	4	4.5	5.0	10	3
15	TfR1-SC DAR1	4	4.5	5.0	17	10
16	TfR1-SC DAR1	8	4.5	5.0	28	21
17	PBS Control	5	-	5.0	7	0

FIG. 39A

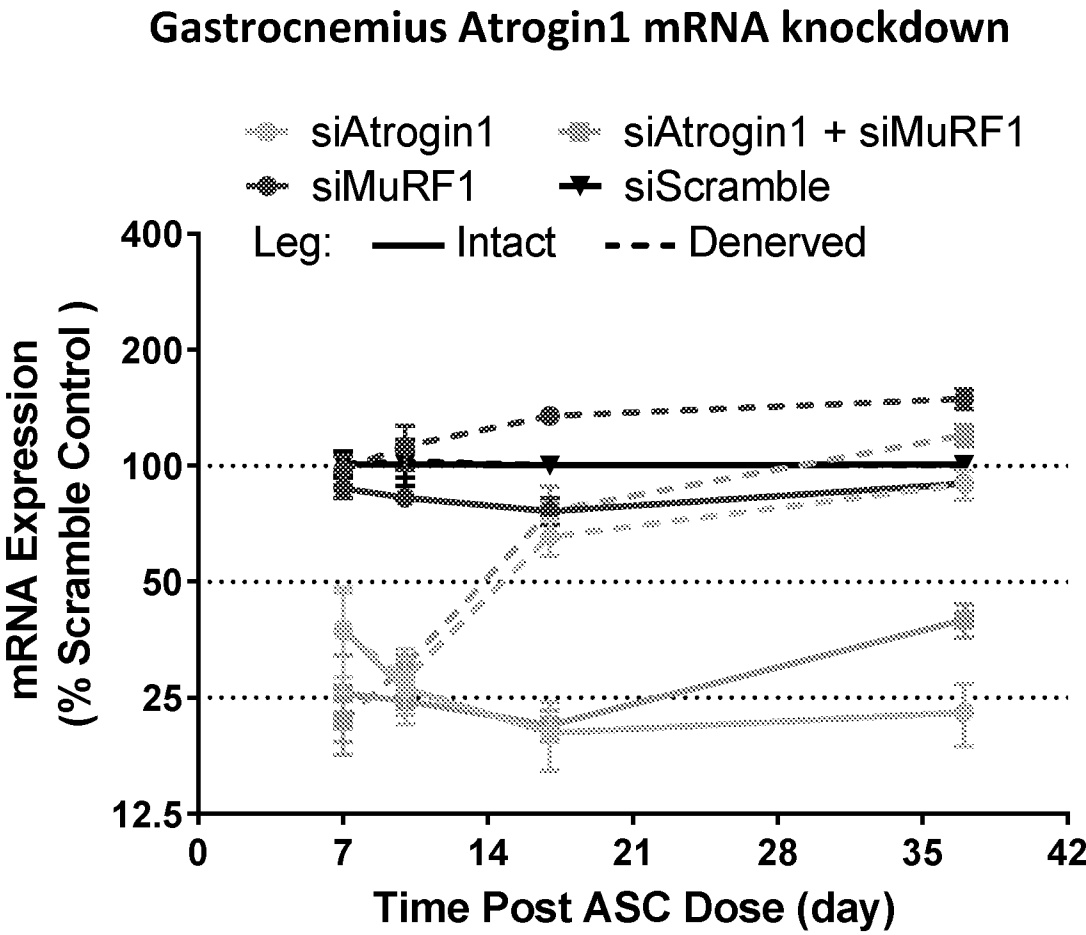


FIG. 39B

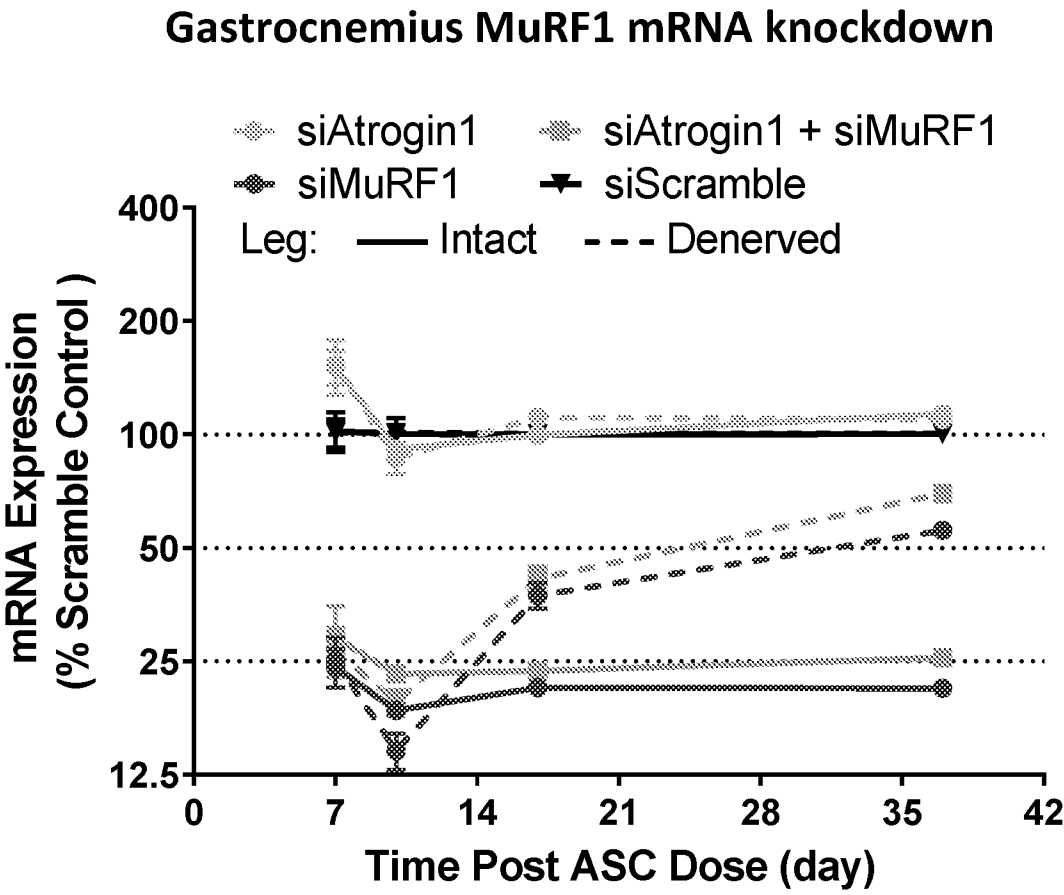


FIG. 39C

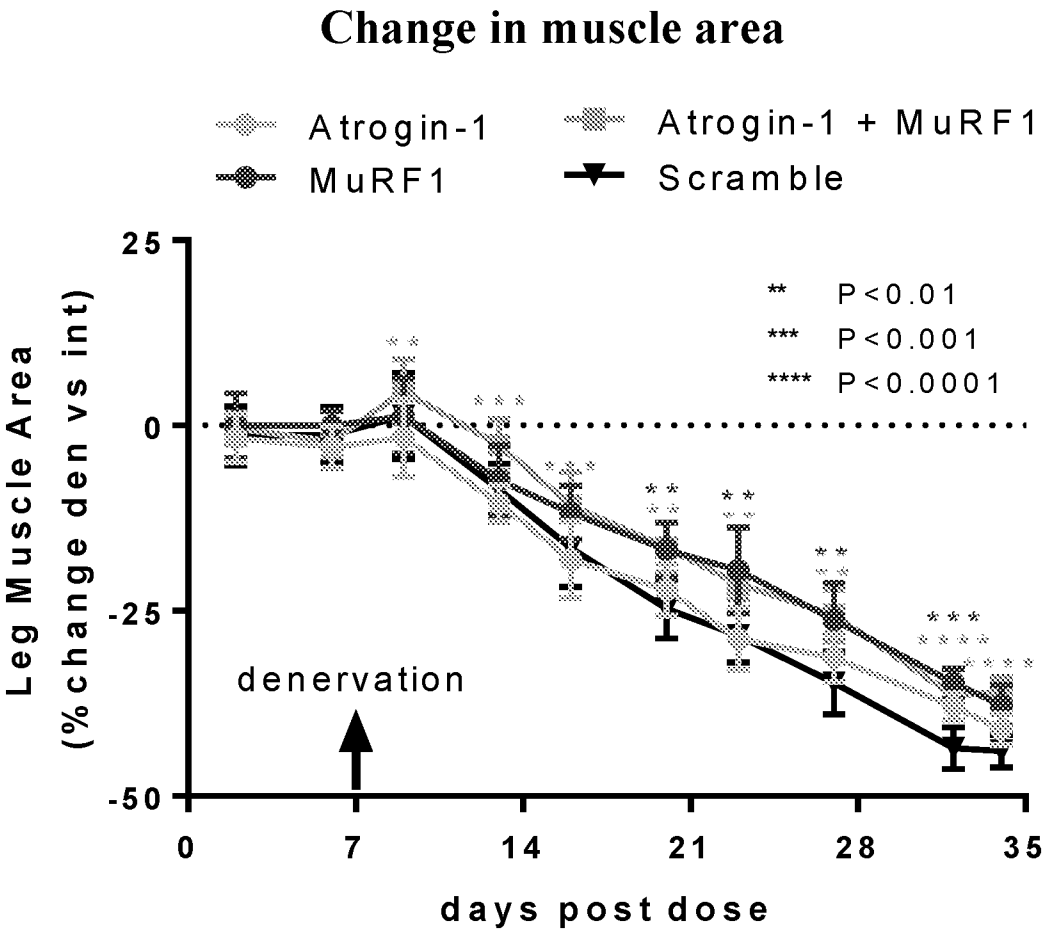


FIG. 39D

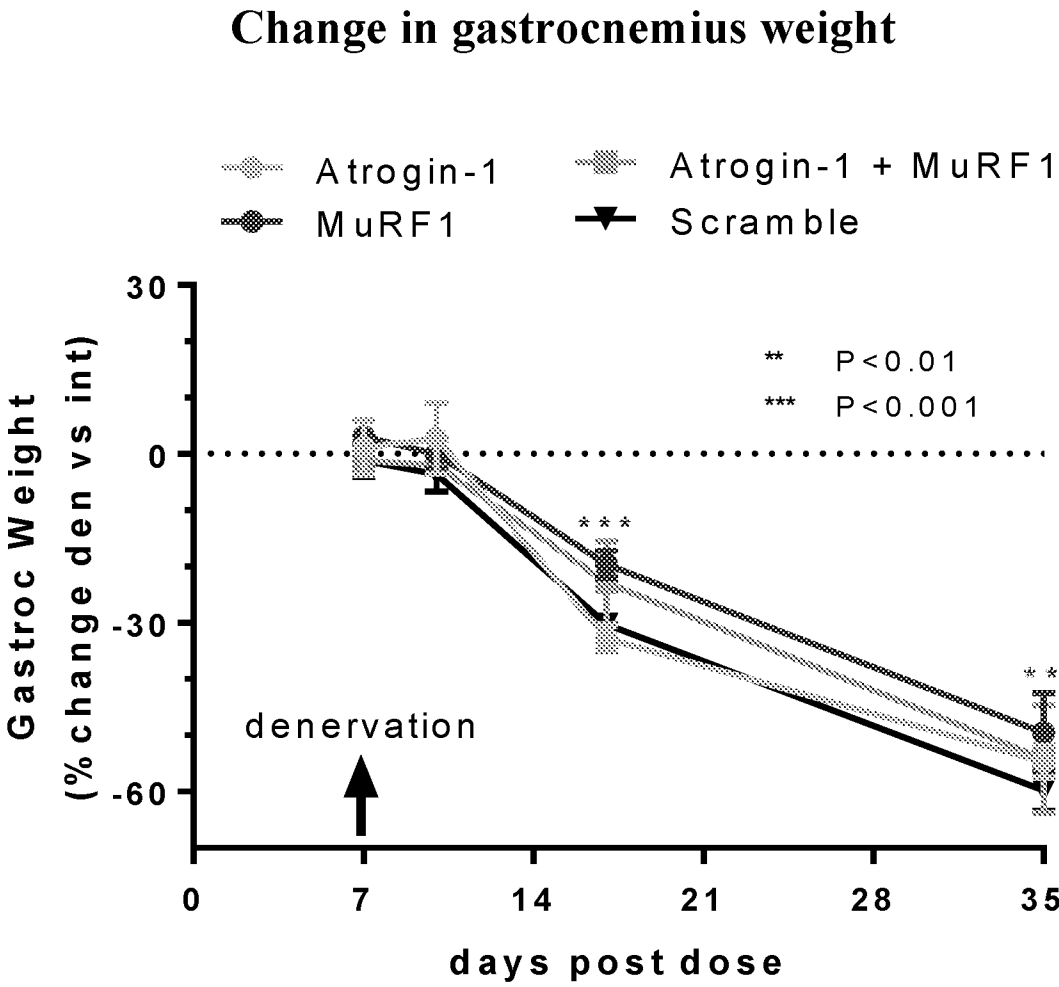


FIG. 39E

**Treatment-induced % Sparing of Muscle Wasting
(Leg muscle area)**

days post dose	days post denervation	MuRF1	Atrogin-1 + MuRF1
16	9	29.0	34.7
20	13	32.0	33.4
23	16	31.0	24.5
27	20	24.5	26.3
32	25	20.2	16.2
34	27	14.2	18.2

FIG. 39F

**Treatment-induced % Sparing of Muscle Wasting
(Gastrocnemius weight)**

days post dose	days post denervation	MuRF1
17	10	35.2
35	28	17.1

45532-722_601_SL.txt
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<120> COMPOSITIONS AND METHODS OF TREATING MUSCLE ATROPHY

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<151> 2018-08-31

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<151> 2017-12-06

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1				5					10					15	

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							20

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<211> 24

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Met Ile Asp Gly Trp Tyr Gly Cys
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<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
peptide

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1 5 10 15

Met Ile Trp Asp Tyr Gly Ser Gly Ser Cys Gly
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Gly Leu Phe Glu Ala Ile Glu Gly Phe Ile Glu Asn Gly Trp Glu Gly
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Gly	Leu	Phe	Glu	Ala	Ile	Glu	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
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Met	Ile	Trp	Asp	Tyr	Gly	Ser	Gly	Ser	Cys	Lys
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Cys	Leu	Ile	Gly	Ala	Ile	Leu	Lys	Val	Leu	Ala	Thr	Gly	Leu	Pro	Thr
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Leu	Ile	Ser	Trp	Ile	Lys	Asn	Lys	Arg	Lys	Gln
			20					25		

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Gly	Ile	Gly	Ala	Val	Leu	Lys	Val	Leu	Thr	Thr	Gly	Leu	Pro	Ala	Leu
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Ile	Ser	Trp	Ile	Lys	Arg	Lys	Arg	Gln	Gln

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Phe Phe Gly His Leu Phe Lys Leu Ala Thr Lys Ile Ile Pro Ser Leu
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Phe Gln

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Lys Glu Thr Trp Trp Glu Thr Trp Trp Thr Glu Trp Ser Gln Pro Lys
1 5 10 15

Lys Lys Arg Lys Val
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Val Thr Gly Lys Gly Asp Pro Lys Pro Asp
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Lys	Ala	Leu	Ala	Ala	Leu	Ala	Lys	Lys	Ile	Leu
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Ala	Gly	Tyr	Leu	Leu	Gly	Lys	Ile	Asn	Leu	Lys	Ala	Leu	Ala	Ala	Leu
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Ala	Lys	Lys	Ile	Leu
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Gly	Ala	Leu	Phe	Leu	Gly	Phe	Leu	Gly	Ala	Ala	Gly	Ser	Thr	Met	Gly
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His	Gly	Leu	Ala	Ser	Thr	Leu	Thr	Arg	Trp	Ala	His	Tyr	Asn	Ala	Leu
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Gly	Leu	Trp	Arg	Ala	Leu	Trp	Arg	Leu	Leu	Arg	Ser	Leu	Trp	Arg	Leu
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1 5 10 15

Met Ile Asp Gly Trp Tyr Gly Cys
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Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
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Met	Ile	Asp	Gly	Arg	Gln	Ile	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Met
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Lys	Trp	Lys	Lys
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Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
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Met	Ile	Asp	Gly	Ser	Ser	Lys	Lys	Lys	Lys

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1 5 10 15

Met Ile Asp Gly Gly Gly Tyr Cys
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<210> 3449

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<210> 3482

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19

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<210> 3497

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<210> 3498

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<210> 3501
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<210> 3504

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<210> 3507

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<210> 3508

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<210> 3509

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<210> 3511
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<210> 3512
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<210> 3513
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<210> 3518

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<210> 3519

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<210> 3520

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<210> 3521

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<210> 3522

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<210> 3523

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<210> 3524

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<210> 3528
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<210> 3529

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<210> 3530

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<210> 3531

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<210> 3532

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 <210> 3533
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 <210> 3534
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 <210> 3535
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<210> 3536

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<210> 3537

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<210> 3538

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<210> 3539

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<210> 3540

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<210> 3541

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<210> 3542

<211> 19

<212> RNA

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19

<210> 3543

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19

<210> 3544

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19

<210> 3545

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<400> 3545

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19

<210> 3546

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19

<210> 3547
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<210> 3548
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<210> 3549
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<210> 3550
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<210> 3551
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<210> 3552
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<210> 3553
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<210> 3554
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<210> 3555

<211> 19

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<210> 3556

<211> 19

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19

<210> 3557

<211> 19

<212> RNA

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19

<210> 3558
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 <210> 3559
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 <210> 3560
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 <210> 3561
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<210> 3562
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<210> 3563
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19

<210> 3566

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<210> 3567

<211> 19

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19

<210> 3568

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19

<210> 3569

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<210> 3570

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19

<210> 3571

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<210> 3572

<211> 19

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<210> 3573
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ccccggccccg gggaggggc 19

<210> 3574
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oligonucleotide

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cccggccccg ggaggggcc 19

<210> 3575
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<210> 3576
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oligonucleotide

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19

<210> 3577

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 3578

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 3579

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 3579

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19

<210> 3580

<211> 19
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<400> 3580
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19

<210> 3581
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<400> 3581
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19

<210> 3582
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<400> 3582
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19

<210> 3583
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<400> 3583

gggagggggcc auggugcug

19

<210> 3584

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 3584

ggagggggcca uggugcugc

19

<210> 3585

<211> 19

<212> RNA

<213> Artificial Sequence

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<400> 3585

gagggggccau ggugcugcc

19

<210> 3586

<211> 19

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oligonucleotide

<400> 3586

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19

<210> 3587

<211> 19

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oligonucleotide

<400> 3587

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19

<210> 3588

<211> 19

<212> RNA

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<400> 3588

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19

<210> 3589

<211> 19

<212> RNA

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<400> 3589

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19

<210> 3590

<211> 19

<212> RNA

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oligonucleotide

<400> 3590

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19

<210> 3591

<211> 19

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oligonucleotide

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ccauggugcu gccugucca 19

<210> 3592
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oligonucleotide

<400> 3592
cauggugcug ccuguccaa 19

<210> 3593
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oligonucleotide

<400> 3593
auggugcugc cuguccaac 19

<210> 3594
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oligonucleotide

<400> 3594
uggugcugcc uguccaaca 19

<210> 3595
 <211> 19
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<220>
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 oligonucleotide

<400> 3595
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<210> 3596
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<220>
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 oligonucleotide

<400> 3596
 gugcugccug uccaacau 19

<210> 3597
 <211> 19
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<220>
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 oligonucleotide

<400> 3597
 ugcugccugu ccaacau 19

<210> 3598
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oligonucleotide

<400> 3598
gcugccuguc caacauguc 19

<210> 3599
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oligonucleotide

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cugccugucc aacauguca 19

<210> 3600
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oligonucleotide

<400> 3600
ugccugucca acaugucag 19

<210> 3601
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oligonucleotide

<400> 3601
gccuguccaa caugucagc 19

<210> 3602
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oligonucleotide

<400> 3602

ccuguccaac augucagcc

19

<210> 3603

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<400> 3603

cuguccaaca ugucagccg

19

<210> 3604

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oligonucleotide

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uguccaacaug gucagccga

19

<210> 3605

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oligonucleotide

<400> 3605

guccaacaug ucagccgag

19

<210> 3606
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<400> 3606
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<210> 3607
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<210> 3608
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<400> 3608
 caacauguca gccgaggug 19

<210> 3609
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<400> 3609
aacaugucag ccgaggugc 19

<210> 3610
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oligonucleotide

<400> 3610
acaugucagc cgaggugcg 19

<210> 3611
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oligonucleotide

<400> 3611
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<210> 3625

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<210> 3629

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<210> 3630

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<210> 3632

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<210> 3635

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<210> 3636

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<210> 3637

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<210> 3638

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<210> 3639

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<210> 3642
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<210> 3643
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<210> 3644
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<210> 3646
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<210> 3647

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<210> 3648

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19

<210> 3649

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19

<210> 3650

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<210> 3651

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<210> 3652

<211> 19

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19

<210> 3653

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19

<210> 3654
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<210> 3656
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<210> 3661
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19

<210> 3662

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19

<210> 3663

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19

<210> 3664

<211> 19

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<210> 3665

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<210> 3666

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<210> 3667

<211> 19

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<210> 3668

<211> 19

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<210> 3671
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19

<210> 3673

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19

<210> 3674

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<212> RNA

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<210> 3675

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19

<210> 3676

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<210> 3677

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oligonucleotide

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19

<210> 3678

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<210> 3679

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<210> 3680

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<210> 3681

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<210> 3682

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<210> 3683

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<210> 3684

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<210> 3685

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<210> 3686

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<210> 3687

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<210> 3688

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<210> 3689

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<210> 3690

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<210> 3691
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<210> 3693
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<210> 3694
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oligonucleotide

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<210> 3695
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<210> 3696
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<210> 3697
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<210> 3698
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19

<210> 3699

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19

<210> 3700

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19

<210> 3701

<211> 19

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19

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<210> 3703
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<400> 3703
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<210> 3704
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<210> 3705
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<210> 3706
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oligonucleotide

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<210> 3707
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<210> 3708
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oligonucleotide

<400> 3708
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<210> 3709
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<400> 3709

accaggagcu gggcgccuc

19

<210> 3710

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19

<210> 3711

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<400> 3711

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19

<210> 3712

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<212> RNA

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19

<210> 3713

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19

<210> 3714

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oligonucleotide

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gagcugggcg ccuccgaac

19

<210> 3715

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<400> 3715

agcugggcgc cuccgaacu

19

<210> 3716

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oligonucleotide

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<210> 3717
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<400> 3717
cugggcgccu ccgaacugg 19

<210> 3718
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oligonucleotide

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<210> 3719
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<210> 3720
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ggcgccuccg aacuggccc

19

<210> 3721

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gcgccuccga acuggccca

19

<210> 3722

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19

<210> 3723

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gccuccgaac uggcccagg

19

<210> 3724

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19

<210> 3725
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19

<210> 3726
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<210> 3758

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<210> 3759

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<210> 3762

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<210> 3763

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<210> 3769

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<210> 3771

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<210> 3777

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19

<210> 3782

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19

<210> 3806

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oligonucleotide

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19

<210> 3807

<211> 19

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19

<210> 3808

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19

<210> 3809

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<210> 3810

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<210> 3811

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<210> 3812

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<400> 3813
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<210> 3814
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<210> 3815
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<210> 3816
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<210> 3817

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<210> 3818

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19

<210> 3819

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<212> RNA

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<210> 3820

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<210> 3823
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19

<210> 3824

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oligonucleotide

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19

<210> 3825

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19

<210> 3826

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19

<210> 3827

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oligonucleotide

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<210> 3828

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19

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<210> 3830

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oligonucleotide

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19

<210> 3831

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19

<210> 3832

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19

<210> 3833

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19

<210> 3834

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oligonucleotide

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19

<210> 3835
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<210> 3836
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<210> 3837
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<210> 3838
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oligonucleotide

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<210> 3840
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<210> 3872

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<210> 3920

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<210> 3923

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<210> 3925

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<210> 3926

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<210> 3927

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<210> 3928

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<210> 3930

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<210> 3936
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19

<210> 3939

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19

<210> 3940

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19

<210> 3941

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19

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<210> 3947
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<210> 3948
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<210> 3949
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oligonucleotide

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19

<210> 3950

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gucgugcuuc cgugaggag

19

<210> 3951

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19

<210> 3952

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cgugcuuccg ugaggagag

19

<210> 3953

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19

<210> 3954

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19

<210> 3955

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<210> 3985
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<210> 3986
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<210> 3987

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<213> Artificial Sequence

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<210> 3988

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19

<210> 3989

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<212> RNA

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<210> 3990
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<210> 3992
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<210> 3993
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<210> 3994
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<210> 3995
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<210> 3996
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<210> 3997
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<210> 3998

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<210> 3999

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<210> 4000

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<210> 4001

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<210> 4002

<211> 19

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<210> 4003

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<210> 4004

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<210> 4009

<211> 19

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<210> 4010

<211> 19

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<210> 4011

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<210> 4012

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<210> 4014
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<210> 4015
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<210> 4016

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19

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<210> 4022

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<210> 4023

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<210> 4028
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<210> 4030
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19

<210> 4031

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19

<210> 4032

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gagaacuacc uguaccugg

19

<210> 4033

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<210> 4034

<211> 19

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19

<210> 4035

<211> 19

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oligonucleotide

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19

<210> 4036

<211> 19

<212> RNA

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19

<210> 4037

<211> 19

<212> RNA

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19

<210> 4038
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<210> 4039
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<210> 4040
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<210> 4041
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<210> 4042
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oligonucleotide

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<210> 4043
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oligonucleotide

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<210> 4044
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<400> 4044
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<210> 4045
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oligonucleotide

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19

<210> 4046

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19

<210> 4047

<211> 19

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19

<210> 4048

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4049

<211> 19

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oligonucleotide

<400> 4049

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19

<210> 4050

<211> 19

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oligonucleotide

<400> 4050

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19

<210> 4051

<211> 19

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oligonucleotide

<400> 4051

ucauggagua uuacguggg

19

<210> 4052

<211> 19

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oligonucleotide

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<210> 4053
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oligonucleotide

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<210> 4054
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<210> 4055
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<210> 4056
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oligonucleotide

<400> 4056

gaguauuacg ugggcgggg

19

<210> 4057

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oligonucleotide

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aguauuacgu gggcgggga

19

<210> 4058

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oligonucleotide

<400> 4058

guauuacgug ggcggggac

19

<210> 4059

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oligonucleotide

<400> 4059

uauuacgugg gcggggacc

19

<210> 4060

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<400> 4060

auuacguggg cggggaccu

19

<210> 4061

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<213> Artificial Sequence

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<400> 4061

uuacgugggc ggggaccug

19

<210> 4062

<211> 19

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<400> 4062

uacgugggcg gggaccugc

19

<210> 4063

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19

<210> 4064

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4064

cgugggcggg gaccugcug

19

<210> 4065

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oligonucleotide

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gugggcgggg accugcuga

19

<210> 4066

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

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ugggcgggga ccugcugac

19

<210> 4067

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<213> Artificial Sequence

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gggcggggac cugcugaca

19

<210> 4068

<211> 19

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oligonucleotide

<400> 4068

ggcggggacc ugcugacac

19

<210> 4069

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gcgggggaccu gcugacacu

19

<210> 4070

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<210> 4084

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<210> 4085

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<210> 4086
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<210> 4094

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<210> 4095

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<210> 4096

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<210> 4097

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<210> 4098

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<210> 4099

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<210> 4100

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<210> 4103
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<210> 4105

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<210> 4106

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19

<210> 4107

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<210> 4108

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<210> 4111
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19

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<210> 4114

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<210> 4117

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<210> 4118

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<210> 4119

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<210> 4120

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<210> 4121

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19

<210> 4122

<211> 19

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<210> 4132

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<210> 4142

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<210> 4148

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<210> 4151
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<210> 4153

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<210> 4155

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oligonucleotide

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<210> 4156

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<210> 4160

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<210> 4161

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<210> 4162

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19

<210> 4163

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19

<210> 4164

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19

<210> 4165

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19

<210> 4166

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19

<210> 4167

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oligonucleotide

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<210> 4168

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19

<210> 4169

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19

<210> 4170

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oligonucleotide

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<210> 4171
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<400> 4171
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<210> 4172
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<400> 4172
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<210> 4173
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 oligonucleotide

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<210> 4174
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oligonucleotide

<400> 4174

ggcuuggcua cgugcacag

19

<210> 4175

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oligonucleotide

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gcuuggcuac gugcacagg

19

<210> 4176

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19

<210> 4177

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oligonucleotide

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19

<210> 4178

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19

<210> 4179

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19

<210> 4180

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oligonucleotide

<400> 4180

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19

<210> 4181

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19

<210> 4182
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<400> 4182
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<210> 4183
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<400> 4183
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<210> 4184
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<210> 4240

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<210> 4250

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<210> 4251

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<210> 4258

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<210> 4263

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<210> 4266

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<210> 4277

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19

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<210> 4286

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<210> 4287

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<210> 4288

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<210> 4296
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<210> 4297

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19

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<210> 4489

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<210> 4491

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<210> 4501

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<210> 4502

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<210> 4503

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<212> RNA

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<210> 4504

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<210> 4505

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<210> 4506

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<210> 4509
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<210> 4510
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ugguggacga agggguccc 19

<210> 4514
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19

<210> 4515

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<210> 4516

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<210> 4517

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19

<210> 4526

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ggucccugag gaggcucga

19

<210> 4527

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19

<210> 4528

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ucccugagga ggcucgaga

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<210> 4530

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<210> 4532

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<210> 4537

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<210> 4538

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<210> 4539

<211> 19

<212> RNA

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<210> 4540

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<210> 4542
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<210> 4543
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<210> 4544

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<210> 4545

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<210> 4546

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<210> 4547

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<210> 4548

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19

<210> 4549

<211> 19

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<210> 4550

<211> 19

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<213> Artificial Sequence

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<210> 4551

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<213> Artificial Sequence

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<210> 4552

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 4553

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4554

<211> 19

<212> RNA

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<210> 4555
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<210> 4556
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<210> 4557
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<210> 4558
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19

<210> 4559

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4560

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19

<210> 4561

<211> 19

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19

<210> 4562

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4563

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<213> Artificial Sequence

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19

<210> 4564

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4565

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4565

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19

<210> 4566
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<210> 4567
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<210> 4568
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<210> 4569
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<210> 4570
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<210> 4571
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<210> 4572
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<210> 4573
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19

<210> 4574

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4575

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4576

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4577

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<212> RNA

<213> Artificial Sequence

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19

<210> 4578

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4578

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19

<210> 4579

<211> 19

<212> RNA

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<220>

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oligonucleotide

<400> 4579

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<210> 4580

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 4581
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<210> 4582
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<210> 4583
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oligonucleotide

<400> 4583
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<210> 4584
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<400> 4584

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19

<210> 4585

<211> 19

<212> RNA

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19

<210> 4586

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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<400> 4586

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19

<210> 4587

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4588

<211> 19

<212> RNA

<213> Artificial Sequence

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gccggggugg agcaggcga

19

<210> 4589

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<212> RNA

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oligonucleotide

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ccggggugga gcaggcgac

19

<210> 4590

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4591

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<212> RNA

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19

<210> 4592

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4593

<211> 19

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<213> Artificial Sequence

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19

<210> 4594

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oligonucleotide

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19

<210> 4595

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19

<210> 4596

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19

<210> 4597

<211> 19

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oligonucleotide

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19

<210> 4598

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4599

<211> 19

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oligonucleotide

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19

<210> 4600

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4601

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4602

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4603
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<210> 4604
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<210> 4605
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<210> 4606
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oligonucleotide

<400> 4606

acuuccggac acaucccuu

19

<210> 4607

<211> 19

<212> RNA

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oligonucleotide

<400> 4607

cuuccggaca caucccuuc

19

<210> 4608

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4608

uuccggacac aucccuuc

19

<210> 4609

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4610

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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ccggacacau cccuucuuc

19

<210> 4611

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cggacacau cccuucuuc

19

<210> 4612

<211> 19

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<213> Artificial Sequence

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oligonucleotide

<400> 4612

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19

<210> 4613

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4613

gacacauccc uucuucuuu

19

<210> 4614
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 oligonucleotide

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<210> 4615
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<400> 4615
 cacaucccu cuucuuugg 19

<210> 4616
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<400> 4616
 acaucccuuc uucuuuggc 19

<210> 4617
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<400> 4617
caucccuucu ucuuuggcc 19

<210> 4618
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oligonucleotide

<400> 4618
aucccuucuu cuuuggccu 19

<210> 4619
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oligonucleotide

<400> 4619
ucccuucuc uuuggccuc 19

<210> 4620
<211> 19
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<213> Artificial Sequence

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oligonucleotide

<400> 4620
cccuucucu uuggccucg 19

<210> 4621
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oligonucleotide

<400> 4621

ccuucuucuu ugccucga

19

<210> 4622

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4622

cuucuucuuu ggccucgac

19

<210> 4623

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4623

uucuucuuug gccucgacu

19

<210> 4624

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4624

ucuucuuugg ccucgacug

19

<210> 4625

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4625

cuucuuuggc cucgacugg

19

<210> 4626

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4626

uucuuuggcc ucgacuggg

19

<210> 4627

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4627

ucuuuuggccu cgacuggga

19

<210> 4628

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4628
cuuuggccuc gacugggau 19

<210> 4629
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oligonucleotide

<400> 4629
uuuggccucg acugggau 19

<210> 4630
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oligonucleotide

<400> 4630
uuggccucga cugggaug 19

<210> 4631
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oligonucleotide

<400> 4631
uggccucgac uggauggu 19

<210> 4632
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oligonucleotide

<400> 4632

ggccucgacu gggaugguc

19

<210> 4633

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oligonucleotide

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gccucgacug ggauggucu

19

<210> 4634

<211> 19

<212> RNA

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oligonucleotide

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ccucgacugg gauggucuc

19

<210> 4635

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cucgacuggg auggucucc

19

<210> 4636

<211> 19
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 oligonucleotide

<400> 4636
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<210> 4637
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 oligonucleotide

<400> 4637
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<210> 4638
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 oligonucleotide

<400> 4638
 gacugggaug gucuccggg 19

<210> 4639
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 oligonucleotide

<400> 4639

acugggaugg ucuccggga

19

<210> 4640

<211> 19

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oligonucleotide

<400> 4640

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19

<210> 4641

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<212> RNA

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oligonucleotide

<400> 4641

ugggaugguc uccgggaca

19

<210> 4642

<211> 19

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<210> 4670

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<210> 4672

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<210> 4681

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<210> 4682

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<210> 4683

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<210> 4684

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<210> 4691

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<210> 4693

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<210> 4694

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<210> 4695

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<210> 4700
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19

<210> 4704

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<210> 4705

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<210> 4706

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19

<210> 4707

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19

<210> 4708

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<210> 4709

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<210> 4716
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19

<210> 4718

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19

<210> 4719

<211> 19

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<210> 4720

<211> 19

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<210> 4721
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<210> 4723
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<210> 4724
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<210> 4725
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<210> 4726
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<210> 4727
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<210> 4728
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19

<210> 4729

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oligonucleotide

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19

<210> 4730

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19

<210> 4731

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oligonucleotide

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19

<210> 4732

<211> 19
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19

<210> 4733
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19

<210> 4734
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19

<210> 4735
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19

<210> 4736

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19

<210> 4737

<211> 19

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19

<210> 4738

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oligonucleotide

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19

<210> 4739

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4740

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4741

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4742

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4743

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<212> RNA
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<210> 4744
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<210> 4745
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oligonucleotide

<400> 4745
gacacuguc gacauucgg 19

<210> 4746
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oligonucleotide

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<210> 4747
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<210> 4748
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<210> 4749
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 oligonucleotide

<400> 4749
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<210> 4750
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oligonucleotide

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ugucggacau ucgggaagg

19

<210> 4751

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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gucggacauu cggaaggu

19

<210> 4752

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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ucggacauuc gggaaggug

19

<210> 4753

<211> 19

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oligonucleotide

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cggacauucg ggaaggugc

19

<210> 4754

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4754

ggacauucgg gaaggugcg

19

<210> 4755

<211> 19

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<213> Artificial Sequence

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oligonucleotide

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gacauucggg aaggugcgc

19

<210> 4756

<211> 19

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oligonucleotide

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acauucggga aggugcgcc

19

<210> 4757

<211> 19

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19

<210> 4758
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<210> 4759
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<210> 4760
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<210> 4761
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<210> 4769

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<210> 4770

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<210> 4771

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<210> 4772

<211> 19

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<210> 4777

<211> 19

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<210> 4778

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<210> 4779

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<210> 4780

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<210> 4781
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<210> 4782
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<210> 4783
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<210> 4784

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<210> 4785

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<210> 4786

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<210> 4787

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<210> 4788

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19

<210> 4789

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19

<210> 4790

<211> 19

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19

<210> 4791

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<210> 4793
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<210> 4794
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<210> 4795
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<210> 4796
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<210> 4797
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<400> 4797
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<210> 4798
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oligonucleotide

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19

<210> 4799

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19

<210> 4800

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19

<210> 4801

<211> 19

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19

<210> 4802

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<212> RNA

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19

<210> 4803

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4804

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 4805

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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<400> 4805

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19

<210> 4806
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<210> 4807
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<210> 4808
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<400> 4808
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<210> 4809
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<400> 4809
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<210> 4810
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<210> 4811
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<400> 4811
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<210> 4812
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<210> 4813
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<400> 4813

ccugcauggc ccucagga

19

<210> 4814

<211> 19

<212> RNA

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<400> 4814

cugcauggcc cucaggac

19

<210> 4815

<211> 19

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<400> 4815

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19

<210> 4816

<211> 19

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<400> 4816

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19

<210> 4817

<211> 19

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19

<210> 4818

<211> 19

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19

<210> 4819

<211> 19

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19

<210> 4820

<211> 19

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<210> 4821
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<210> 4822
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oligonucleotide

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<210> 4823
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<210> 4824
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19

<210> 4825

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4826

<211> 19

<212> RNA

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cagggacagu gagguccca

19

<210> 4827

<211> 19

<212> RNA

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agggacagug aggucccg

19

<210> 4828

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19

<210> 4829
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19

<210> 4830
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19

<210> 4831
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acagugaggu cccaggccc

19

<210> 4832

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19

<210> 4833

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19

<210> 4834

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19

<210> 4835

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19

<210> 4836

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19

<210> 4837

<211> 19

<212> RNA

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4837

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19

<210> 4838

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4839

<211> 19

<212> RNA

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oligonucleotide

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gucccaggcc ccacacca

19

<210> 4840

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4841

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4841

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19

<210> 4842

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4843
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<210> 4844
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 oligonucleotide

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 aggccccaca cccauggaa 19

<210> 4845
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<210> 4846
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oligonucleotide

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19

<210> 4847

<211> 19

<212> RNA

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oligonucleotide

<400> 4847

ccccacaccc auggaacug

19

<210> 4848

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cccacaccca uggaacugg

19

<210> 4849

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4849

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19

<210> 4850

<211> 19

<212> RNA

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oligonucleotide

<400> 4850

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19

<210> 4851

<211> 19

<212> RNA

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<220>

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oligonucleotide

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19

<210> 4852

<211> 19

<212> RNA

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oligonucleotide

<400> 4852

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19

<210> 4853

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oligonucleotide

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19

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<210> 4855
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 oligonucleotide

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<210> 4856
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<210> 4857
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<210> 4858
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oligonucleotide

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<210> 4859
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oligonucleotide

<400> 4859
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<210> 4860
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oligonucleotide

<400> 4860
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<210> 4861
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oligonucleotide

<400> 4861

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19

<210> 4862

<211> 19

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oligonucleotide

<400> 4862

acuggaggcc gagcagcug

19

<210> 4863

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oligonucleotide

<400> 4863

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19

<210> 4864

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oligonucleotide

<400> 4864

uggaggccga gcagcugcu

19

<210> 4865
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oligonucleotide

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<210> 4866
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oligonucleotide

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<210> 4867
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oligonucleotide

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<210> 4868
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oligonucleotide

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<210> 4869
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oligonucleotide

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<210> 4870
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oligonucleotide

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<210> 4871
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oligonucleotide

<400> 4871
cgagcagcug cuugagcca 19

<210> 4872
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19

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<211> 19

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<210> 4874

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<223> Description of Artificial Sequence: Synthetic
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<210> 4875

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<210> 4876

<211> 19
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<210> 4877
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<210> 4878
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19

<210> 4879
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<210> 4880

<211> 19

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oligonucleotide

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<210> 4881

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<210> 4882

<211> 19

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<210> 4883

<211> 19

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oligonucleotide

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<210> 4884

<211> 19

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<213> Artificial Sequence

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<210> 4885

<211> 19

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<210> 4886

<211> 19

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<213> Artificial Sequence

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oligonucleotide

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<210> 4887

<211> 19

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oligonucleotide

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<210> 4888

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
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<210> 4889

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 4890

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 4891
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<210> 4892
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<210> 4893
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<210> 4894
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<210> 4898
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<400> 4898

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<210> 4899

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<210> 4900

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oligonucleotide

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19

<210> 4901

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4902
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<220>
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<210> 4903
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<400> 4903
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<210> 4904
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<400> 4904
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<210> 4905
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<400> 4905
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<210> 4906
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<400> 4906
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<210> 4907
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<210> 4908
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<210> 4909
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oligonucleotide

<400> 4909

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19

<210> 4910

<211> 19

<212> RNA

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<210> 4911

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 4912

<211> 19

<212> RNA

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19

<210> 4913
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<400> 4913
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<210> 4914
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<210> 4915
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<400> 4916
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<210> 4917
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<210> 4918
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<400> 4918
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<210> 4919
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<210> 4920
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<400> 4920

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19

<210> 4921

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<400> 4921

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19

<210> 4922

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<400> 4922

gucccccacag gaugaaaca

19

<210> 4923

<211> 19

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<400> 4923

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19

<210> 4924

<211> 19
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<400> 4924
ccccacagga ugaaacagc 19

<210> 4925
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<400> 4925
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<210> 4926
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ccacaggaug aaacagcug 19

<210> 4927
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oligonucleotide

<400> 4927

cacaggauga aacagcuga

19

<210> 4928

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oligonucleotide

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19

<210> 4929

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4930

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4931

<211> 19

<212> RNA

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19

<210> 4932

<211> 19

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19

<210> 4933

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19

<210> 4934

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<212> RNA

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oligonucleotide

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19

<210> 4935

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oligonucleotide

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gaaacagcug aaguggcag

19

<210> 4936

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<212> RNA

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oligonucleotide

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aaacagcuga aguggcagu

19

<210> 4937

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oligonucleotide

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19

<210> 4938

<211> 19

<212> RNA

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19

<210> 4939
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<210> 4940
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<210> 4941
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<210> 4942
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oligonucleotide

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19

<210> 4943

<211> 19

<212> RNA

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oligonucleotide

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ugaaguggca guuccagcg

19

<210> 4944

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<212> RNA

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19

<210> 4945

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 4946

<211> 19

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oligonucleotide

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19

<210> 4947

<211> 19

<212> RNA

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oligonucleotide

<400> 4947

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19

<210> 4948

<211> 19

<212> RNA

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oligonucleotide

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uggcaguucc agcggcugu

19

<210> 4949

<211> 19

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oligonucleotide

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ggcaguucca gcggcuguc

19

<210> 4950
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<210> 4951
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<210> 4952
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<400> 4952
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<210> 4953
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guuccagcgg cugucccug 19

<210> 4954
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oligonucleotide

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<210> 4955
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oligonucleotide

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<210> 4956
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oligonucleotide

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<210> 4957
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oligonucleotide

<400> 4957

cagcggcugu cccugcggc

19

<210> 4958

<211> 19

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<213> Artificial Sequence

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oligonucleotide

<400> 4958

agcggcuguc ccugcggca

19

<210> 4959

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4959

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19

<210> 4960

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4960

cggcuguccc ugcggcaga

19

<210> 4961

<211> 19

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<213> Artificial Sequence

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oligonucleotide

<400> 4961

ggcugucccu gcggcagag

19

<210> 4962

<211> 19

<212> RNA

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oligonucleotide

<400> 4962

gcugucccug cggcagagg

19

<210> 4963

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4963

cugucccugc ggcagaggc

19

<210> 4964

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<212> RNA

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oligonucleotide

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<210> 4965
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oligonucleotide

<400> 4965
gucccugcgg cagaggcug 19

<210> 4966
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oligonucleotide

<400> 4966
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<210> 4967
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oligonucleotide

<400> 4967
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<210> 4968
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oligonucleotide

<400> 4968

ccugcggcag aggcugagg

19

<210> 4969

<211> 19

<212> RNA

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oligonucleotide

<400> 4969

cugcggcaga ggcugaggc

19

<210> 4970

<211> 19

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<213> Artificial Sequence

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oligonucleotide

<400> 4970

ugcggcagag gcugaggcc

19

<210> 4971

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4971

gcggcagagg cugaggccg

19

<210> 4972

<211> 19
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<400> 4972
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<210> 4973
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<400> 4973
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<210> 4974
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<400> 4974
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<210> 4975
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<220>
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<400> 4975

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19

<210> 4976

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4976

agaggcugag gccgaggug

19

<210> 4977

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4977

gaggcugagg ccgagguga

19

<210> 4978

<211> 19

<212> RNA

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oligonucleotide

<400> 4978

aggcugaggc cgaggugac

19

<210> 4979

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4979

ggcugaggcc gaggugacg

19

<210> 4980

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 4980

gcugaggccg aggugacgc

19

<210> 4981

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 4981

cugaggccga ggugacgcu

19

<210> 4982

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4982

ugaggccgag gugacgcug

19

<210> 4983

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4983

gaggccgagg ugacgcugc

19

<210> 4984

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 4984

aggccgaggu gacgcugcg

19

<210> 4985

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 4985

ggccgaggug acgcugcgg

19

<210> 4986

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 5010

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<210> 5011

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<210> 5018

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<210> 5019

<211> 19

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<210> 5024

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<210> 5025

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<210> 5026

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19

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19

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<210> 5030

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19

<210> 5031

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19

<210> 5032

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19

<210> 5033

<211> 19

<212> RNA

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19

<210> 5034

<211> 19

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19

<210> 5035
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<210> 5036
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<210> 5039

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<210> 5040

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<210> 5041

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<212> RNA

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<210> 5042

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<210> 5043

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<210> 5044

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19

<210> 5045

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<210> 5048
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<210> 5049
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19

<210> 5054

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19

<210> 5055

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19

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19

<210> 5057

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19

<210> 5058

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19

<210> 5059

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<210> 5060

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<210> 5062
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ggaggccauc cgcacggac 19

<210> 5064
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oligonucleotide

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19

<210> 5065

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5066

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5067

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5068

<211> 19
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<210> 5070
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 auccgcacgg acaaccaga 19

<210> 5071
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uccgcacgga caaccagaa

19

<210> 5072

<211> 19

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ccgcacggac aaccagaac

19

<210> 5073

<211> 19

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oligonucleotide

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cgcacggaca accagaacu

19

<210> 5074

<211> 19

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gcacggacaa ccagaacuu

19

<210> 5075

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19

<210> 5076

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19

<210> 5077

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19

<210> 5078

<211> 19

<212> RNA

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19

<210> 5079

<211> 19

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oligonucleotide

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19

<210> 5080

<211> 19

<212> RNA

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oligonucleotide

<400> 5080

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19

<210> 5081

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5081

caaccagaac uucgccagu

19

<210> 5082

<211> 19

<212> RNA

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oligonucleotide

<400> 5082

aaccagaacu ucgccaguc

19

<210> 5083
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 accagaacuu cgccaguca 19

<210> 5084
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<400> 5084
 ccagaacuuc gccagucaa 19

<210> 5085
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 oligonucleotide

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<210> 5086
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oligonucleotide

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<210> 5087
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oligonucleotide

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<210> 5088
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oligonucleotide

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<210> 5089
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oligonucleotide

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<210> 5090
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oligonucleotide

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cuucgccagu caacuacgc

19

<210> 5091

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19

<210> 5092

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19

<210> 5093

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19

<210> 5094
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<210> 5095
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<210> 5096
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<210> 5097
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<210> 5098
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<210> 5100
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<210> 5101
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oligonucleotide

<400> 5101

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<210> 5198

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19

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<210> 5247

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<210> 5248

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<210> 5249

<211> 19

<212> RNA

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<210> 5250

<211> 19

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<210> 5251

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<210> 5252

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<210> 5255
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<210> 5257

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<210> 5258

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<210> 5259

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<210> 5262
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<210> 5263
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<210> 5264

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<210> 5265

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<210> 5266

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<210> 5267

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<210> 5268

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19

<210> 5269

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19

<210> 5270

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19

<210> 5271

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<210> 5272

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<210> 5273

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<210> 5274

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<210> 5277
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<210> 5278
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19

<210> 5279

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<210> 5280

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<210> 5281

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<210> 5282

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<210> 5283

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<210> 5284

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<210> 5285

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<210> 5288
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<210> 5289
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<210> 5290
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<210> 5291
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<210> 5292
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<210> 5293
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19

<210> 5294

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acgccggcca acucaccgc

19

<210> 5295

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<212> RNA

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19

<210> 5296

<211> 19

<212> RNA

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oligonucleotide

<400> 5296

gccggccaac ucaccgcag

19

<210> 5297

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5297

ccggccaacu caccgcagu

19

<210> 5298

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5299

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5300

<211> 19

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<210> 5301
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<210> 5302
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<210> 5303
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<400> 5303
aacucaccgc agucuggcg 19

<210> 5304
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oligonucleotide

<400> 5304

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19

<210> 5305

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5306

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5306

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19

<210> 5307

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5307

caccgcaguc uggcgccgc

19

<210> 5308

<211> 19
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oligonucleotide

<400> 5308
accgcagucu ggcgccgcc 19

<210> 5309
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oligonucleotide

<400> 5309
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<210> 5310
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oligonucleotide

<400> 5310
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<210> 5311
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oligonucleotide

<400> 5311

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19

<210> 5312

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5312

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19

<210> 5313

<211> 19

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5313

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19

<210> 5314

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5314

gucuggcgcc gcccaggag

19

<210> 5315

<211> 19

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5315

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19

<210> 5316

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5316

cuggcgccgc ccaggagcc

19

<210> 5317

<211> 19

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19

<210> 5318

<211> 19

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19

<210> 5319

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5319

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19

<210> 5320

<211> 19

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oligonucleotide

<400> 5320

cgccgcccag gagccgcc

19

<210> 5321

<211> 19

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oligonucleotide

<400> 5321

gccgcccagg agccgccc

19

<210> 5322

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<400> 5322

ccgcccagga gccgcccgc

19

<210> 5323
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oligonucleotide

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<210> 5324
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oligonucleotide

<400> 5324
gcccaggagc cgcccgcgc 19

<210> 5325
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cccaggagcc gcccgcgcu 19

<210> 5326
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oligonucleotide

<400> 5326
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<210> 5327
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<400> 5327
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<210> 5328
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<400> 5328
aggagccgcc cgcgcuccc 19

<210> 5329
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<400> 5329
ggagccgccc gcgcucccu 19

<210> 5330
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<213> Artificial Sequence

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oligonucleotide

<400> 5330

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<210> 5395

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19

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19

<210> 5477

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<210> 5486

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<210> 5487

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<210> 5488

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<210> 5489

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19

<210> 5491

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<210> 5498

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<210> 5499

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<210> 5505

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<210> 5508

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<210> 5510

<211> 19

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<210> 5511

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<210> 5512

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<210> 5513

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<210> 5514

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<210> 5519

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<210> 5520

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<210> 5521

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<210> 5522

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<210> 5523

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<210> 5524

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<210> 5525

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gcggccggcg aacggggcu 19

<210> 5530
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<210> 5531
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<210> 5532
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19

<210> 5534

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<210> 5535

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19

<210> 5536

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<400> 5536

gcgaacgggg cucgaagg

19

<210> 5537

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<210> 5538

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oligonucleotide

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gaacggggcu cgaagggu

19

<210> 5539

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19

<210> 5540

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<210> 5541
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oligonucleotide

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<210> 5542
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<210> 5543
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oligonucleotide

<400> 5543
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<210> 5544
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<400> 5544

ggcucgaagg guccuugua

19

<210> 5545

<211> 19

<212> RNA

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gcucgaaggg uccuuguag

19

<210> 5546

<211> 19

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oligonucleotide

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19

<210> 5547

<211> 19

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<400> 5547

ucgaaggguc cuuguagcc

19

<210> 5548

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<210> 5549
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<400> 5549
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<210> 5550
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<400> 5550
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<210> 5551
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<400> 5551

aggguccuug uagccggga

19

<210> 5552

<211> 19

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oligonucleotide

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ggguccuugu agccgggaa

19

<210> 5553

<211> 19

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19

<210> 5554

<211> 19

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guccuuguag ccgggaug

19

<210> 5555

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19

<210> 5556

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19

<210> 5557

<211> 19

<212> RNA

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cuuguagccg ggaaugcug

19

<210> 5558

<211> 19

<212> RNA

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uuguagccgg gaaugcugc

19

<210> 5559

<211> 19

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<210> 5582

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<210> 5583

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<210> 5584

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<210> 5585

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<210> 5586

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<210> 5587

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<210> 5589
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<210> 5590
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<210> 5591
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<210> 5592
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19

<210> 5593

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19

<210> 5594

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19

<210> 5595

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<210> 5596

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<210> 5597
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<400> 5597
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<210> 5598
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<210> 5599
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<210> 5600

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<210> 5601

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<210> 5602

<211> 19

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<210> 5603

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19

<210> 5604

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19

<210> 5605

<211> 19

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oligonucleotide

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<210> 5606

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<212> RNA

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<400> 5606

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19

<210> 5607

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 <400> 5607
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<210> 5608
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 <400> 5608
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<210> 5609
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<210> 5610
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<210> 5611
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<210> 5612
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<400> 5612
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<210> 5613
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<400> 5613
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<210> 5614
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oligonucleotide

<400> 5614

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<210> 5615

<211> 19

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<210> 5616

<211> 19

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<210> 5617

<211> 19

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<400> 5617

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<210> 5618

<211> 19

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<210> 5619

<211> 19

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<210> 5620

<211> 19

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<210> 5621

<211> 19

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<210> 5622
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<210> 5625
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<210> 5628
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<210> 5629
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19

<210> 5630

<211> 19

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19

<210> 5631

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19

<210> 5632

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19

<210> 5633

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19

<210> 5634

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oligonucleotide

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19

<210> 5635

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19

<210> 5636

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oligonucleotide

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<210> 5637
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<210> 5638
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oligonucleotide

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aucacagacc auuucuuuc 19

<210> 5639
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<210> 5640
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oligonucleotide

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19

<210> 5641

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oligonucleotide

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19

<210> 5642

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<212> RNA

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oligonucleotide

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19

<210> 5643

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

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agaccuuuc uuucuuucg

19

<210> 5644

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5645

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<212> RNA

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oligonucleotide

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19

<210> 5646

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oligonucleotide

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19

<210> 5647

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oligonucleotide

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19

<210> 5648

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oligonucleotide

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19

<210> 5649

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oligonucleotide

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19

<210> 5650

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oligonucleotide

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<210> 5651

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oligonucleotide

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19

<210> 5652

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oligonucleotide

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19

<210> 5653

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oligonucleotide

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uuucuuucgg ccaggcuga

19

<210> 5654

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oligonucleotide

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19

<210> 5655

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oligonucleotide

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19

<210> 5656

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oligonucleotide

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cuuucggcca ggcugaggc

19

<210> 5657

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oligonucleotide

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uuucggccag gcugaggcc

19

<210> 5658

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oligonucleotide

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uucggccagg cugaggccc

19

<210> 5659
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 oligonucleotide

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<210> 5660
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<210> 5661
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 oligonucleotide

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<210> 5662
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oligonucleotide

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<210> 5663
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oligonucleotide

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<210> 5664
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oligonucleotide

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<210> 5665
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oligonucleotide

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<210> 5666
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oligonucleotide

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ggcugaggcc cugacgugg

19

<210> 5667

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oligonucleotide

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gcugaggccc ugacgugga

19

<210> 5668

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oligonucleotide

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cugaggcccu gacguggau

19

<210> 5669

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<212> RNA

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oligonucleotide

<400> 5669

ugaggcccug acguggaug

19

<210> 5670
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<400> 5670
 gaggccuga cguggaugg 19

<210> 5671
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 aggccugac guggauggg 19

<210> 5672
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<210> 5673
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<210> 5678

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<210> 5679

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<210> 5680

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<210> 5682

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<210> 5683

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<210> 5684

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<210> 5689

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<210> 5690

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<210> 5691

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<210> 5692

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<210> 5693
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<210> 5695
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<210> 5696

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<210> 5697

<211> 19

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<210> 5698

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oligonucleotide

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<210> 5699

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<210> 5700

<211> 19

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<210> 5701

<211> 19

<212> RNA

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<210> 5702

<211> 19

<212> RNA

<213> Artificial Sequence

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<210> 5703

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

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19

<210> 5704

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

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19

<210> 5705

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

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19

<210> 5706

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5707
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<220>
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<210> 5708
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<220>
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<400> 5708
 aggcagcaag ccgggccgu 19

<210> 5709
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<210> 5710
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oligonucleotide

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<210> 5711
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oligonucleotide

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<210> 5712
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oligonucleotide

<400> 5712
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<210> 5713
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<210> 5714
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<210> 5715

<211> 19

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oligonucleotide

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19

<210> 5716

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5717

<211> 19

<212> RNA

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oligonucleotide

<400> 5717

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19

<210> 5718
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<220>
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<210> 5719
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<220>
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<400> 5719
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<210> 5720
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 <212> RNA
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<220>
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<400> 5720
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<210> 5721
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<210> 5722
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oligonucleotide

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<210> 5723
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oligonucleotide

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<210> 5724
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oligonucleotide

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cguccguguu ccauccucc 19

<210> 5725
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oligonucleotide

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guccguguuc cauccucca

19

<210> 5726

<211> 19

<212> RNA

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oligonucleotide

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uccguguucc auccuccac

19

<210> 5727

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5728

<211> 19

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oligonucleotide

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cguguuccau ccuccacgc

19

<210> 5729

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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guguuccauc cuccacgca

19

<210> 5730

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5731

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5732

<211> 19

<212> RNA

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oligonucleotide

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oligonucleotide

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uccauccucc acgcacccc 19

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oligonucleotide

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<210> 5735
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oligonucleotide

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oligonucleotide

<400> 5736

auccuccacg cccccac

19

<210> 5737

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<212> RNA

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oligonucleotide

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uccuccacgc acccccacc

19

<210> 5738

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<212> RNA

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oligonucleotide

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19

<210> 5739

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5740

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<220>
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<210> 5741
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<210> 5742
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<210> 5743
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acgcaccccc accuauugu

19

<210> 5744

<211> 19

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oligonucleotide

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19

<210> 5745

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<212> RNA

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oligonucleotide

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19

<210> 5746

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5747

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<212> RNA

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oligonucleotide

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19

<210> 5748

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5749

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5750

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5751

<211> 19

<212> RNA
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 oligonucleotide

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<210> 5752
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 oligonucleotide

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<210> 5753
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 oligonucleotide

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<210> 5754
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 oligonucleotide

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<210> 5755
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 oligonucleotide

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<210> 5756
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 oligonucleotide

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 uaucguuggu ucgcaaagu 19

<210> 5757
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 oligonucleotide

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<210> 5758
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oligonucleotide

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<210> 5759
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oligonucleotide

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<210> 5760
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oligonucleotide

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<210> 5761
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oligonucleotide

<400> 5761
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<210> 5762
<211> 19
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oligonucleotide

<400> 5762

ugguucgcaa agugcaaag

19

<210> 5763

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5763

gguucgcaaa gugcaaagc

19

<210> 5764

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5764

guucgcaaag ugcaaagcu

19

<210> 5765

<211> 19

<212> RNA

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<220>

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oligonucleotide

<400> 5765

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19

<210> 5766
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 oligonucleotide

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<210> 5767
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<220>
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 oligonucleotide

<400> 5767
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<210> 5768
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<220>
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 oligonucleotide

<400> 5768
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<210> 5769
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 oligonucleotide

<400> 5769
caaagugcaa agcuuucuu 19

<210> 5770
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oligonucleotide

<400> 5770
aaagugcaaa gcuuucuug 19

<210> 5771
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oligonucleotide

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<210> 5772
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oligonucleotide

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<210> 5773
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<220>

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oligonucleotide

<400> 5773

gugcaaagcu uucuugugc

19

<210> 5774

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5774

ugcaaagcuu ucuugugca

19

<210> 5775

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5775

gcaaagcuuu cuugugcau

19

<210> 5776

<211> 19

<212> RNA

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oligonucleotide

<400> 5776

caaagcuuuc uugugcaug

19

<210> 5777

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<212> RNA

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oligonucleotide

<400> 5777

aaagcuuucu ugugcauga

19

<210> 5778

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oligonucleotide

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aagcuuucuu gugcaugac

19

<210> 5779

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<212> RNA

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oligonucleotide

<400> 5779

agcuuucuug ugcaugacg

19

<210> 5780

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5780
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<210> 5781
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<212> RNA
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oligonucleotide

<400> 5781
cuuucuugug caugacgcc 19

<210> 5782
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oligonucleotide

<400> 5782
uuucuugugc augacgccc 19

<210> 5783
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oligonucleotide

<400> 5783
uucuugugca ugacgccc 19

<210> 5784
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oligonucleotide

<400> 5784

ucuugugcau gacgccug

19

<210> 5785

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5785

cuugugcaug acgccugc

19

<210> 5786

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<210> 5800

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<210> 5801

<211> 19

<212> RNA

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<210> 5802

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<210> 5803
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<210> 5804
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<210> 5805
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<210> 5807

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<210> 5808

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<210> 5809

<211> 19

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<210> 5810

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<210> 5811

<211> 19

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<213> Artificial Sequence

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<210> 5812

<211> 19

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<210> 5813

<211> 19

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<210> 5816
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<210> 5817
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<210> 5818
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<210> 5819
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<400> 5819
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<210> 5820
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19

<210> 5822

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19

<210> 5823

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19

<210> 5824

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<212> RNA

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19

<210> 5825

<211> 19

<212> RNA

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19

<210> 5826

<211> 19

<212> RNA

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19

<210> 5827

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<210> 5828

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<210> 5830
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oligonucleotide

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oligonucleotide

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19

<210> 5833

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oligonucleotide

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19

<210> 5834

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oligonucleotide

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19

<210> 5835

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oligonucleotide

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19

<210> 5836

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<210> 5837
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19

<210> 5838
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19

<210> 5839
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<223> Description of Artificial Sequence: Synthetic
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19

<210> 5840

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5841

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19

<210> 5842

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<210> 5843

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<210> 5844

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19

<210> 5845

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<210> 5846

<211> 19

<212> RNA

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19

<210> 5847

<211> 19

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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auuugcuuuu gccaaaccc

19

<210> 5848

<211> 19

<212> RNA

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oligonucleotide

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uuugcuuuug ccaaaccg

19

<210> 5849

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5850

<211> 19

<212> RNA

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oligonucleotide

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19

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<210> 5853
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oligonucleotide

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19

<210> 5855

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19

<210> 5856

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19

<210> 5858

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oligonucleotide

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19

<210> 5859

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19

<210> 5860

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oligonucleotide

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aaacccgcuu uuucgggga

19

<210> 5861

<211> 19

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oligonucleotide

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19

<210> 5862
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<210> 5863
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<210> 5864
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<210> 5865
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cgcuuuuucg gggaucccg 19

<210> 5866
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oligonucleotide

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gcuuuuucgg ggaucccg 19

<210> 5867
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oligonucleotide

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<210> 5868
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oligonucleotide

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uuuuucgggg aucccgcg 19

<210> 5869
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oligonucleotide

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uuuucgggga ucccgcgcc

19

<210> 5870

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oligonucleotide

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uuucggggau cccgcgccc

19

<210> 5871

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oligonucleotide

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19

<210> 5872

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oligonucleotide

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19

<210> 5873

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oligonucleotide

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cggggauccc gcgcccccc

19

<210> 5874

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oligonucleotide

<400> 5874

ggggaucccg cgccccccu

19

<210> 5875

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oligonucleotide

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19

<210> 5876

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oligonucleotide

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<210> 5877
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oligonucleotide

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<210> 5878
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oligonucleotide

<400> 5878
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<210> 5879
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oligonucleotide

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<210> 5880
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oligonucleotide

<400> 5880

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19

<210> 5881

<211> 19

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oligonucleotide

<400> 5881

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19

<210> 5882

<211> 19

<212> RNA

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oligonucleotide

<400> 5882

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19

<210> 5883

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5883

gcgccccccu ccucacuug

19

<210> 5884

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oligonucleotide

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19

<210> 5885
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oligonucleotide

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19

<210> 5886
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oligonucleotide

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<210> 5887
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oligonucleotide

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ccccuccuc acuugcgcu

19

<210> 5888

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oligonucleotide

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ccccuccuca cuugcgcu

19

<210> 5889

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19

<210> 5893

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oligonucleotide

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19

<210> 5894

<211> 19

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 5894

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19

<210> 5895

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 5895

ucacuugcgc ugcucucgg

19

<210> 5896

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cacuugcgcu gcucucgga

19

<210> 5897

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<212> RNA

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oligonucleotide

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acuugcgcu cucucggag

19

<210> 5898

<211> 19

<212> RNA

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19

<210> 5899
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<210> 5905

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<210> 5918

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<210> 5923

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<210> 5924

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<210> 5929

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<210> 5930

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<210> 5931

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<210> 5932

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<210> 5933
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<210> 5935
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<210> 5936

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<210> 5937

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<210> 5939

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<210> 5940

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<210> 5941

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<210> 5943

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<210> 5947
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<210> 5948
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<210> 5949
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<210> 5956

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19

<210> 5957

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<210> 5959
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<210> 5961
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<210> 5962
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<210> 5963
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<210> 5964
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19

<210> 5966

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19

<210> 5967

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<212> RNA

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19

<210> 5968

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<212> RNA

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19

<210> 5969

<211> 19

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19

<210> 5970

<211> 19

<212> RNA

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19

<210> 5971

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19

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<210> 5975
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<210> 5976
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oligonucleotide

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19

<210> 5977

<211> 19

<212> RNA

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19

<210> 5978

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5979

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5980

<211> 19
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oligonucleotide

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19

<210> 5981
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oligonucleotide

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<210> 5982
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oligonucleotide

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aggcuacagg acccccaac

19

<210> 5983
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oligonucleotide

<400> 5983

ggcuacagga cccccaaca

19

<210> 5984

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oligonucleotide

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19

<210> 5985

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<212> RNA

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oligonucleotide

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19

<210> 5986

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<210> 5987

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<212> RNA

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oligonucleotide

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acaggacccc caacaacc

19

<210> 5988

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<212> RNA

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oligonucleotide

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caggaccccc aacaacccc

19

<210> 5989

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5990

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5991

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5992

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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accccccaaca accccaau

19

<210> 5993

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 5994

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 5995
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<210> 5996
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 oligonucleotide

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 ccaacaaccc caauccacg 19

<210> 5997
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 oligonucleotide

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 caacaacccc aauccacgu 19

<210> 5998
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oligonucleotide

<400> 5998
aacaacccca auccacguu 19

<210> 5999
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oligonucleotide

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acaaccccaa uccacguuu 19

<210> 6000
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oligonucleotide

<400> 6000
caaccccaau ccacguuuu 19

<210> 6001
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oligonucleotide

<400> 6001
aaccccaauc cacguuuug 19

<210> 6002
<211> 19
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oligonucleotide

<400> 6002

accccaaucc acguuuugg

19

<210> 6003

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6004

<211> 19

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oligonucleotide

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cccaauccac guuuuggau

19

<210> 6005

<211> 19

<212> RNA

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oligonucleotide

<400> 6005

ccaauccacg uuuuggaug

19

<210> 6006
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<210> 6007
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<220>
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 aauccacguu uuggaugca 19

<210> 6008
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<210> 6009
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<210> 6010
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oligonucleotide

<400> 6010
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<210> 6011
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oligonucleotide

<400> 6011
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<210> 6012
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<400> 6012
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<210> 6013
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cguuuuggau gcacugaga

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<210> 6014

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<210> 6020

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<210> 6025

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<210> 6026

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<210> 6027

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<210> 6028

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<210> 6029
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<210> 6030
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<210> 6031
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<210> 6032

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<210> 6033

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<210> 6035

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<210> 6036

<211> 19

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<210> 6037

<211> 19

<212> RNA

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<210> 6038

<211> 19

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<213> Artificial Sequence

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<210> 6039

<211> 19

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<210> 6040

<211> 19

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<210> 6041

<211> 19

<212> RNA

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<210> 6042

<211> 19

<212> RNA

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<400> 6042

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<210> 6043
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<210> 6044
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<400> 6044
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<210> 6045
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<210> 6046
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<210> 6047

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<212> RNA

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oligonucleotide

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<210> 6048

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<210> 6049

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<210> 6050

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<212> RNA

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19

<210> 6051

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<212> RNA

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oligonucleotide

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19

<210> 6052

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oligonucleotide

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19

<210> 6053

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6054
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<210> 6055
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<210> 6056
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<210> 6057
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<210> 6058
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oligonucleotide

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<210> 6059
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oligonucleotide

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<210> 6060
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oligonucleotide

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<210> 6061
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oligonucleotide

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19

<210> 6062

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 6062

ccccaccuag gacccccac

19

<210> 6063

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 6063

cccaccuagg acccccacc

19

<210> 6064

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6065

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6066

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

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accuaggacc cccaccccc

19

<210> 6067

<211> 19

<212> RNA

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<220>

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19

<210> 6068

<211> 19

<212> RNA

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oligonucleotide

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<210> 6069
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oligonucleotide

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<210> 6070
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oligonucleotide

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oligonucleotide

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<210> 6072
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oligonucleotide

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19

<210> 6073

<211> 19

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oligonucleotide

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19

<210> 6074

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oligonucleotide

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19

<210> 6075

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6076

<211> 19
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<220>
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<210> 6077
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 ccacccccga cccucgcga 19

<210> 6078
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<210> 6079
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<220>
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acccccgacc cucgcgaau

19

<210> 6080

<211> 19

<212> RNA

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oligonucleotide

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<210> 6081

<211> 19

<212> RNA

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oligonucleotide

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<210> 6082

<211> 19

<212> RNA

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19

<210> 6083

<211> 19

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oligonucleotide

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<210> 6084

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6085

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6086

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6087

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cccucgcgaa uaaaaggcc

19

<210> 6088

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6089

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6090

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 6090

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19

<210> 6091
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<210> 6092
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<220>
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<210> 6093
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<210> 6094
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oligonucleotide

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gaauaaaagg ccuccauc 19

<210> 6095
<211> 19
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oligonucleotide

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aauaaaaggc ccuccaucu 19

<210> 6096
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oligonucleotide

<400> 6096
auaaaaggcc cuccaucug 19

<210> 6097
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oligonucleotide

<400> 6097
uaaaaggccc uccaucugc 19

<210> 6098
<211> 19
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<213> Artificial Sequence

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oligonucleotide

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aaaaggcccu ccaucugcc

19

<210> 6099

<211> 19

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oligonucleotide

<400> 6099

aaaggcccuc caucugccc

19

<210> 6100

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 6101

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6101

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19

<210> 6102
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 ggccuccau cugccaaa 19

<210> 6103
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<400> 6103
 gccuccauc ugcccaaag 19

<210> 6104
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 oligonucleotide

<400> 6104
 ccuccaucu gcccaaagc 19

<210> 6105
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<400> 6105
ccuccaucug cccaaagcu 19

<210> 6106
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<212> RNA
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oligonucleotide

<400> 6106
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<210> 6107
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oligonucleotide

<400> 6107
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<210> 6108
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oligonucleotide

<400> 6108
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<210> 6109
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oligonucleotide

<400> 6109

caucugccca aagcucugg

19

<210> 6110

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6110

aucugcccaa agcucugga

19

<210> 6111

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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19

<210> 6112

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6112

cccuugguc cagcccccc

19

<210> 6113
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oligonucleotide

<400> 6113
accccuuggu ccagcccc 19

<210> 6114
<211> 19
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oligonucleotide

<400> 6114
caccccuugg uccagcccc 19

<210> 6115
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oligonucleotide

<400> 6115
ccaccccuug guccagccc 19

<210> 6116
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oligonucleotide

<400> 6116
cccaccccuu gguccagcc 19

<210> 6117
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oligonucleotide

<400> 6117
ccccaccccu ugguccagc 19

<210> 6118
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oligonucleotide

<400> 6118
uccccacccc ugguccag 19

<210> 6119
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oligonucleotide

<400> 6119
cuccccaccc cuuggucca 19

<210> 6120
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oligonucleotide

<400> 6120

ucucucccacc ccuuggucc

19

<210> 6121

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6121

uucucucccac cccuugguc

19

<210> 6122

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cuucucuccca ccccuuggu

19

<210> 6123

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 6123

ccuucucuccc accccuugg

19

<210> 6124

<211> 19
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<220>

<223> Description of Artificial Sequence: Synthetic
 oligonucleotide

<400> 6124
 cccuucucc caccuuug

19

<210> 6125
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 <212> RNA
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<220>

<223> Description of Artificial Sequence: Synthetic
 oligonucleotide

<400> 6125
 cccuucucc ccacccuu

19

<210> 6126
 <211> 19
 <212> RNA
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<220>

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 oligonucleotide

<400> 6126
 ucccuucuc cccacccu

19

<210> 6127
 <211> 19
 <212> RNA
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<223> Description of Artificial Sequence: Synthetic
 oligonucleotide

<400> 6127

CUCCCCUUCU CCCCACCCC

19

<210> 6128

<211> 19

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oligonucleotide

<400> 6128

CCUCCCCUUC UCCCCACCC

19

<210> 6129

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6129

UCCUCCCCUU CUCCCCACC

19

<210> 6130

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<210> 6169

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<210> 6200
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<210> 6206

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<210> 6207

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<210> 6208

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<210> 6210

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<210> 6211

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<210> 6212

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<210> 6214
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<210> 6219

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<210> 6222
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<210> 6223
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<210> 6224

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<210> 6225

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<210> 6226

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<210> 6227

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19

<210> 6228

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19

<210> 6229

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19

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oligonucleotide

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19

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19

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<210> 6241

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<210> 6242

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19

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<210> 6244

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<210> 6389

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<210> 6422

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19

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<210> 6435

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<210> 6449

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<210> 6450

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<210> 6459

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<210> 6472

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19

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<210> 6474

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<210> 6505

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<210> 6506

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<210> 6507

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<210> 6508

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<210> 6509
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<210> 6512

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<210> 6513

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<210> 6514

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<210> 6516

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<210> 6518

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oligonucleotide

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<210> 6525
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19

<210> 6531

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oligonucleotide

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19

<210> 6532

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oligonucleotide

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19

<210> 6533

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oligonucleotide

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oligonucleotide

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oligonucleotide

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19

<210> 6542

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19

<210> 6543

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19

<210> 6544

<211> 19

<212> RNA

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19

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<210> 6547
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<210> 6548
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oligonucleotide

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<210> 6549
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oligonucleotide

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cucgcugaac gccccgcgu 19

<210> 6550
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oligonucleotide

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ccucgcugaa cgccccgcg 19

<210> 6551
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oligonucleotide

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<210> 6552
<211> 19
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oligonucleotide

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19

<210> 6553

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6554

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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gcuaccucgc ugaacgccc

19

<210> 6555

<211> 19

<212> RNA

<213> Artificial Sequence

<220>

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oligonucleotide

<400> 6555

cgcuaaccucg cugaacgcc

19

<210> 6556

<211> 19
<212> RNA
<213> Artificial Sequence

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oligonucleotide

<400> 6556
ccgcuaccuc gcugaacgc

19

<210> 6557
<211> 19
<212> RNA
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oligonucleotide

<400> 6557
accgcuaccu cgcugaacg

19

<210> 6558
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<212> RNA
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oligonucleotide

<400> 6558
uaccgcuacc ucgcugaac

19

<210> 6559
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oligonucleotide

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19

<210> 6560

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oligonucleotide

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19

<210> 6561

<211> 19

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oligonucleotide

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19

<210> 6562

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19

<210> 6563

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 6564

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<212> RNA

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oligonucleotide

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19

<210> 6565

<211> 19

<212> RNA

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oligonucleotide

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ucuucacuac cgcuaaccuc

19

<210> 6566

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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aucuucacua ccgcuaccu

19

<210> 6567

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<212> RNA
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oligonucleotide

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caucuucacu accgcuacc 19

<210> 6568
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oligonucleotide

<400> 6568
ucaucuucac uaccgcuac 19

<210> 6569
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oligonucleotide

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uucaucuca cuaccgcu 19

<210> 6570
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oligonucleotide

<400> 6570
cuucaucuuc acuaccgcu 19

<210> 6571
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<210> 6572
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<210> 6573
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<210> 6574
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oligonucleotide

<400> 6574

ucugcuucau cuucacuac

19

<210> 6575

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<212> RNA

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oligonucleotide

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gucugcuuca ucuucacua

19

<210> 6576

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6576

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19

<210> 6577

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 6577

ccgucugcuu caucuucac

19

<210> 6578

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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cccgucugcu ucaucuca

19

<210> 6579

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oligonucleotide

<400> 6579

gcccgucugc uucaucuuc

19

<210> 6580

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<212> RNA

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oligonucleotide

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ggcccgucug cuucaucu

19

<210> 6581

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<212> RNA

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oligonucleotide

<400> 6581

uggcccgu cuucaucu

19

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<210> 6583
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<210> 6584
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<210> 6585
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caccuggccc gucugcuuc 19

<210> 6586
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oligonucleotide

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<210> 6587
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oligonucleotide

<400> 6587
uacaccuggc cgcugucu 19

<210> 6588
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oligonucleotide

<400> 6588
auacaccugg cccgucugc 19

<210> 6589
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oligonucleotide

<400> 6589

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19

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19

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<210> 6702
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<210> 6704

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<210> 6734

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<210> 6735

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19

<210> 6737

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19

<210> 6738

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<210> 6740

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19

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19

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<210> 6749

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19

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19

<210> 6762

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<210> 6768

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<210> 6769

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<210> 6770

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<210> 6771

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<210> 6783

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<210> 6785

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<210> 6786

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19

<210> 6787

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<210> 6788

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<210> 6793

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19

<210> 6794

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19

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<210> 6796

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19

<210> 6797

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19

<210> 6798

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19

<210> 6799

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19

<210> 6800

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<210> 6801

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<210> 6802

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<210> 6803

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<210> 6808

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<210> 6810

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19

<210> 6811
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oligonucleotide

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gcgccaucuc ggccggaau

19

<210> 6815

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<210> 6816

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gcgcgccauc ucggccgga

19

<210> 6817

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19

<210> 6818

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aagcgcgcca ucucggccg

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<210> 6830

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<210> 6842

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<210> 6843

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<210> 6844

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<210> 6846
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<210> 6848

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19

<210> 6849

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<210> 6850

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19

<210> 6851

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19

<210> 6852

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19

<210> 6853

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<210> 6854

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<210> 6855

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 <210> 6858
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<210> 6859
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<210> 6861
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<210> 6863

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<210> 6864

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<210> 6865

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<210> 6866

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<210> 6867

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19

<210> 6868

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<210> 6869

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<210> 6870
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<210> 6878

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<210> 6880

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<210> 6881

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<210> 6882

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<210> 6883

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<210> 6884

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<210> 6889

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<210> 6890

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<210> 6891

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<210> 6892

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19

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<210> 6897

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19

<210> 6898

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<210> 6899

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<210> 6900

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<210> 6901

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<210> 6902

<211> 19

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<210> 6903

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<210> 6904

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<210> 6905

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<210> 6906

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<210> 6908
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<210> 6909
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<210> 6910
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<210> 6911
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<210> 6912
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<210> 6913
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<400> 6913
agcgguccag caggauguu 19

<210> 6914
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19

<210> 6915

<211> 19

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19

<210> 6916

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19

<210> 6917

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<210> 6918
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<210> 6919
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<210> 6920
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<210> 6921
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<210> 6922
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<210> 6923
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19

<210> 6926

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19

<210> 6927

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19

<210> 6928

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19

<210> 6929

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aggcggaugu ggccacagc

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<210> 6930

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caggcggaug uggccacag

19

<210> 6931

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<212> RNA

<213> Artificial Sequence

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ccaggcggaug guggccaca

19

<210> 6932

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<212> RNA

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<210> 6933
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ggccaggcgg auguggcca 19

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<210> 6947

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<210> 6950

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<210> 6959

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<210> 6963

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<210> 6964

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<210> 6969
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<210> 6974

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<210> 6979

<211> 19

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<210> 6980

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<210> 6985

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<210> 6986

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<210> 6992

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<210> 6993

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<210> 6998

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<210> 6999

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<210> 7002

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<210> 7007

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<210> 7008

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<210> 7009

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<210> 7010

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<210> 7011

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<210> 7012

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19

<210> 7013

<211> 19

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<210> 7014
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<210> 7015
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<210> 7016
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<210> 7017
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<210> 7018
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<210> 7019
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<210> 7022

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<210> 7023

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<210> 7024

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<210> 7025

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19

<210> 7026

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<210> 7027

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<210> 7028

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<210> 7031
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<210> 7032
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19

<210> 7033

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<210> 7034

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19

<210> 7035

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<212> RNA

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<210> 7036

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gcccacagcc ugcaggauc 19

<210> 7039
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<210> 7040

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19

<210> 7041

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<210> 7042

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<210> 7043

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<210> 7044

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19

<210> 7045

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19

<210> 7046

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19

<210> 7047

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19

<210> 7048

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<210> 7377

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<210> 7378

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<210> 7379

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<210> 7380

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<210> 7381

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<210> 7382

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<210> 7383

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19

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19

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19

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19

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19

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<210> 7499
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<210> 7500
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<210> 7501
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19

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19

<210> 7503

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19

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19

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<210> 7506
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<210> 7539

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19

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<210> 7583

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<210> 7584

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<210> 7586

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<210> 7587

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<210> 7588

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<210> 7589

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<210> 7590
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19

<210> 7598

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<210> 7600

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<210> 7601

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<210> 7602

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<212> RNA

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19

<210> 7603

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19

<210> 7604

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19

<210> 7609

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19

<210> 7610

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19

<210> 7611

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<210> 7612

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19

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19

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<210> 7615
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guggggacac cgagggcuc

19

<210> 7616

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19

<210> 7617

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<210> 7618

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<210> 7619

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<210> 7621

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<210> 7806

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<210> 7809

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<210> 7811

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<210> 7812

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<210> 7813

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<210> 7814

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<210> 7815

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<210> 7823

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<210> 7824

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<210> 7825

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<210> 7826

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<210> 7827

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<212> RNA

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<210> 7828

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<210> 7829

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<210> 7833
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<210> 7834
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<210> 7835
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<210> 7836
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<210> 7837
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19

<210> 7838

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oligonucleotide

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19

<210> 7839

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19

<210> 7840

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19

<210> 7841

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19

<210> 7842

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19

<210> 7843

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19

<210> 7844

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<210> 7845
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<210> 7846
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<210> 7848
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19

<210> 7849

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19

<210> 7850

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<210> 7903

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<210> 7934

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<210> 7935

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19

<210> 7936

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<210> 7937

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<210> 7940

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<210> 7944
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<210> 7945

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<210> 7946

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<210> 7947

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<210> 7949

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<210> 7950

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<210> 7951

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<210> 7952

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<210> 7955

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<210> 7956

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<210> 7957

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19

<210> 7958

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19

<210> 7959

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<210> 7960

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<210> 7961

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19

<210> 7962

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19

<210> 7963
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<210> 7993

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<210> 7995

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<210> 7996

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<210> 7998
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<210> 7999
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<210> 8000

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<210> 8002

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<210> 8003

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<210> 8004

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<210> 8005

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<210> 8006

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<210> 8007

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<210> 8008

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<210> 8009

<211> 19

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<210> 8010

<211> 19

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<210> 8011
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<210> 8012
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<210> 8013
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<210> 8014
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<210> 8015

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<210> 8016

<211> 19

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<210> 8017

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<210> 8018

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<210> 8019

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<210> 8020

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19

<210> 8021

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<210> 8024
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<210> 8025
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<210> 8030

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<210> 8031

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<210> 8032

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<210> 8033

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<210> 8034

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<210> 8035

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<210> 8036

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19

<210> 8041

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19

<210> 8042

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<210> 8043

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<210> 8044

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<210> 8047
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19

<210> 8048

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<210> 8049

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<210> 8050

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<210> 8051

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<210> 8052

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<210> 8053

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<210> 8054

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<210> 8055

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<210> 8057

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<210> 8058

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oligonucleotide

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<210> 8061
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<210> 8062
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oligonucleotide

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ccccggaguc gaagacagu

19

<210> 8063

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19

<210> 8065

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19

<210> 8066

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ggggccccgg agucgaaga

19

<210> 8067

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<210> 8068

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<210> 8069

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19

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<210> 8072
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<210> 8073
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uuccaacggg gccccggag 19

<210> 8074
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ucuuccaacg gggccccgg 19

<210> 8076
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oligonucleotide

<400> 8076
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<210> 8077
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oligonucleotide

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agucuuccaa cggggcccc

19

<210> 8078

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<210> 8130

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<210> 8175

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<210> 8180

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<210> 8187

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19

<210> 8222

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19

<210> 8223

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<210> 8224

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19

<210> 8225

<211> 19

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<210> 8226

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<210> 8227

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<210> 8228

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<210> 8231
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<210> 8233

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19

<210> 8234

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<210> 8235

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19

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19

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19

<210> 8240

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<210> 8241

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<210> 8242

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19

<210> 8243

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19

<210> 8244

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19

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<210> 8246

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19

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19

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19

<210> 8249

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<210> 8250

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19

<210> 8251
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oligonucleotide

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cccggcuaca aggacccuu

19

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19

<210> 8256

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19

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19

<210> 8258

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19

<210> 8259

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<212> RNA

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19

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19

<210> 8261

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<210> 8262
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<210> 8263
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<210> 8268
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oligonucleotide

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<210> 8269
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oligonucleotide

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19

<210> 8270

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19

<210> 8271

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19

<210> 8272

<211> 19

<212> RNA

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19

<210> 8273

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<212> RNA

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19

<210> 8274

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<212> RNA

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oligonucleotide

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gcagcagcag cagcagcau

19

<210> 8275

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<212> RNA

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19

<210> 8276

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<210> 8277
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oligonucleotide

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<210> 8278
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oligonucleotide

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<210> 8279
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oligonucleotide

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<210> 8280
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oligonucleotide

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19

<210> 8281

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agcagcagca gcagcagca

19

<210> 8282

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19

<210> 8283

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19

<210> 8284

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19

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<210> 8287
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oligonucleotide

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agcagcagca gcagcagca

19

<210> 8288

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oligonucleotide

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19

<210> 8289

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gcagcagcag cagcagcag

19

<210> 8290

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19

<210> 8291

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19

<210> 8292

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19

<210> 8293

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oligonucleotide

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agcagcagca gcagcagca

19

<210> 8294

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oligonucleotide

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19

<210> 8295

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<210> 8296
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oligonucleotide

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<210> 8297
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oligonucleotide

<400> 8297
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<210> 8298
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<400> 8298
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<210> 8299
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<400> 8299
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<210> 8300
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<210> 8301
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<400> 8301
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<210> 8302
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oligonucleotide

<400> 8302

agcagcagca gcagcagca

19

<210> 8303

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oligonucleotide

<400> 8303

cagcagcagc agcagcagc

19

<210> 8304

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oligonucleotide

<400> 8304

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19

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<210> 8379

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<210> 8384

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<210> 8385

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<210> 8386

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<210> 8389

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<210> 8390

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<210> 8391

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<210> 8397
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<210> 8398
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oligonucleotide

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19

<210> 8399

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<210> 8400

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<210> 8401

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<210> 8402

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<210> 8403

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19

<210> 8404

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<210> 8405

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oligonucleotide

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19

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<210> 8408
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<210> 8412
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<210> 8413
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19

<210> 8414

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19

<210> 8415

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19

<210> 8416

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 <210> 8419
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<210> 8462

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<210> 8463

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<210> 8464

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<210> 8465

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19

<210> 8466

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<210> 8467

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<210> 8476

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<210> 8512

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19

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<210> 8559

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<210> 8562

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<210> 8569

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<210> 8571

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<210> 8576

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<210> 8578

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<210> 8579

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<210> 8580

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<210> 8581

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<210> 8582

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<210> 8583

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<210> 8584

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<210> 8585

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<212> RNA

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<210> 8586

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<210> 8587
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<210> 8588
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<210> 8589
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<210> 8590
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<210> 8591

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<210> 8593

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19

<210> 8594

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<210> 8595

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<210> 8596

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agagcagcgc aagugagga

19

<210> 8597

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<210> 8600
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<210> 8601
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oligonucleotide

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oligonucleotide

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19

<210> 8606

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19

<210> 8607

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19

<210> 8608

<211> 19

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19

<210> 8609

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oligonucleotide

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<210> 8610

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<212> RNA

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oligonucleotide

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<210> 8611

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<210> 8612

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<210> 8614
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oligonucleotide

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<210> 8615
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<210> 8616
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oligonucleotide

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19

<210> 8617

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19

<210> 8618

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19

<210> 8619

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<210> 8620

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<210> 8621
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<210> 8622
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19

<210> 8623
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19

<210> 8624

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19

<210> 8625

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 8626

<211> 19

<212> RNA

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oligonucleotide

<400> 8626

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19

<210> 8627

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oligonucleotide

<400> 8627

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19

<210> 8628

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oligonucleotide

<400> 8628

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19

<210> 8629

<211> 19

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<213> Artificial Sequence

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oligonucleotide

<400> 8629

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19

<210> 8630

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 8630

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19

<210> 8631

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 8631

aaccgccgaa gcgggcgga

19

<210> 8632

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 8632

aaaccgccga agcgggcgg

19

<210> 8633

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 8633

caaaccgccg aagcgggcg

19

<210> 8634

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 8635
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<210> 8636
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<400> 8636
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<210> 8637
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<400> 8637
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<210> 8638
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oligonucleotide

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<210> 8639
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oligonucleotide

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<210> 8640
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oligonucleotide

<400> 8640
aaaauuccaa accgccga 19

<210> 8641
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oligonucleotide

<400> 8641
uaaaauucca aaccgccga 19

<210> 8642
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oligonucleotide

<400> 8642

auaaaauaucc aaaccgccg

19

<210> 8643

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oligonucleotide

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aauaaaauauc caaacgcc

19

<210> 8644

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oligonucleotide

<400> 8644

cauaaaauau ccaaaccgc

19

<210> 8645

<211> 19

<212> RNA

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oligonucleotide

<400> 8645

ucauaaaaua ucaaaccg

19

<210> 8646
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 oligonucleotide

<400> 8646
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<210> 8647
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<210> 8697
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<210> 8703

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<210> 8704

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<210> 8705

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<210> 8714

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<210> 8715

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<210> 8724

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<210> 8725

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<210> 8726

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<210> 8727

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<210> 8730
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<210> 8731
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<210> 8732
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<210> 8739

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<210> 8740

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19

<210> 8741

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19

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<210> 8745
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<210> 8746
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<210> 8748
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oligonucleotide

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19

<210> 8750

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oligonucleotide

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19

<210> 8751

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oligonucleotide

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19

<210> 8752

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oligonucleotide

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19

<210> 8753

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<210> 8754

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19

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<210> 8756

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<210> 8761

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<210> 8762

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<210> 8819

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<210> 8824

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<210> 8825

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<210> 8833

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<210> 8834

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<210> 8848

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19

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<210> 8851

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<210> 8864

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19

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19

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<210> 8946

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19

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19

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<210> 8961

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<210> 8962

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<210> 8964

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19

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19

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19

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19

<210> 8976

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19

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19

<210> 8978

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<210> 8980

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19

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<210> 8991

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<210> 9200

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<210> 9202

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<210> 9203

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<210> 9205

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<210> 9206

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<210> 9207

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<210> 9208

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<210> 9209

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<210> 9218
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<210> 9219

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<210> 9220

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<210> 9674

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<210> 9701

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<210> 9710

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<210> 9711

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<210> 9712

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<210> 9713

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<210> 9714

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<210> 9715

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<210> 9716

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<210> 9724

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<210> 9734

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<210> 9747

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<210> 9748

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<210> 9749

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<210> 9752
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<210> 9757
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19

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19

<210> 9759

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19

<210> 9760

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<210> 9761

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<210> 9762

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oligonucleotide

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19

<210> 9763

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oligonucleotide

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<210> 9764

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oligonucleotide

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<210> 9766
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<210> 9767
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<210> 9768
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19

<210> 9769

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19

<210> 9770

<211> 19

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19

<210> 9771

<211> 19

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19

<210> 9772

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<210> 9773
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19

<210> 9774
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oligonucleotide

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19

<210> 9775
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oligonucleotide

<400> 9775

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19

<210> 9776

<211> 19

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oligonucleotide

<400> 9776

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19

<210> 9777

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oligonucleotide

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19

<210> 9778

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oligonucleotide

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19

<210> 9779

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oligonucleotide

<400> 9779

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19

<210> 9780

<211> 19

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oligonucleotide

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<210> 9781

<211> 19

<212> RNA

<213> Artificial Sequence

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19

<210> 9782

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 9783

<211> 19

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oligonucleotide

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19

<210> 9784

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19

<210> 9785

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oligonucleotide

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19

<210> 9786

<211> 19

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oligonucleotide

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19

<210> 9787
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<210> 9788
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<210> 9789
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<210> 9790
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oligonucleotide

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<210> 9791
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<210> 9792
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<210> 9793
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<210> 9963

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<210> 9986

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<210> 9989

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<210> 9998

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<210> 9999

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<210> 10000

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<210> 10001

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<210> 10002

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<210> 10003

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<210> 10004

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<210> 10008
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19

<210> 10009

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<210> 10010

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19

<210> 10011

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oligonucleotide

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<210> 10012

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<210> 10014
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<210> 10015
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<210> 10016

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<210> 10018

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<210> 10019

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<210> 10020

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19

<210> 10021

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<210> 10105

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<210> 10106

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<210> 10107

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<210> 10108

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<210> 10112

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<210> 10113

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<210> 10114

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<210> 10115

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<210> 10116

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<210> 10117

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<210> 10118

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<210> 10119

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<210> 10122
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<210> 10124
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<210> 10125
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<210> 10132

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<210> 10133

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<210> 10135
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<210> 10164

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<210> 10167

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<210> 10178

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<210> 10180

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19

<210> 10192

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<210> 10193

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<210> 10194

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<210> 10195

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<210> 10196

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<210> 10201

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<210> 10202

<211> 19

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19

<210> 10203

<211> 19

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oligonucleotide

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<210> 10204

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oligonucleotide

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<210> 10208

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<210> 10209

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<210> 10210

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<210> 10211

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<210> 10212

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<210> 10213

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<210> 10214

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19

<210> 10215

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oligonucleotide

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<210> 10216

<211> 19

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19

<210> 10217

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19

<210> 10218

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<210> 10219
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<210> 10220
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<210> 10221
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<210> 10222
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oligonucleotide

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19

<210> 10223

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<210> 10224

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19

<210> 10225

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19

<210> 10226

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<400> 10226

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19

<210> 10227

<211> 19

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19

<210> 10228

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oligonucleotide

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19

<210> 10229

<211> 19

<212> RNA

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oligonucleotide

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<210> 10231
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<210> 10232
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 cucaggga gugguaga 19

<210> 10233
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<210> 10234
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<210> 10235
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<210> 10236
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<210> 10237
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oligonucleotide

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19

<210> 10238

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oligonucleotide

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19

<210> 10239

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oligonucleotide

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19

<210> 10240

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19

<210> 10241

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19

<210> 10242

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oligonucleotide

<400> 10242

gugagguccc aggccccaa

19

<210> 10243

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19

<210> 10244

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oligonucleotide

<400> 10244
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<210> 10245
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oligonucleotide

<400> 10245
aggucccagg cccacaca 19

<210> 10246
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oligonucleotide

<400> 10246
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<210> 10247
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oligonucleotide

<400> 10247
gucccaggcc ccacacca 19

<210> 10248
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oligonucleotide

<400> 10248

ucccaggccc cacacccaa

19

<210> 10249

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 10249

cccaggcccc acacccaau

19

<210> 10250

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<210> 10297

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<210> 10364
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<210> 10404

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<210> 10405

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<210> 10406

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<210> 10415

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<210> 10461
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oligonucleotide

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<210> 10463

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oligonucleotide

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<210> 10464

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<210> 10466

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<210> 10467

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<210> 10511

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<210> 10512

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<210> 10515

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<210> 10516

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<210> 10517

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<210> 10528

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<210> 10531

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<210> 10538

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<210> 10550

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<210> 10559

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<210> 10560

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<210> 10561

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<210> 10563

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<210> 10570
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<210> 10575

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oligonucleotide

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<210> 10576

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oligonucleotide

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<210> 10577

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<210> 10578

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19

<210> 10579

<211> 19

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oligonucleotide

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<210> 10580

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19

<210> 10585

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<210> 10586

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19

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19

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19

<210> 10590
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19

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<210> 10603
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<210> 10613

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<210> 10624

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<210> 10625

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<210> 10626

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<210> 10627

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<210> 10632
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19

<210> 10633

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19

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<210> 10635

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19

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<210> 10648

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19

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<210> 10650

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19

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<210> 10656

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<210> 10657

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19

<210> 10659

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19

<210> 10660

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<210> 10661

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19

<210> 10670

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<210> 10671

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<210> 10672

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<210> 10673

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<210> 10674

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oligonucleotide

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19

<210> 10675

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<210> 10676

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oligonucleotide

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<210> 10678
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oligonucleotide

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<210> 10679
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oligonucleotide

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<210> 10680
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<210> 10681

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19

<210> 10682

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19

<210> 10683

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<210> 10684

<211> 19
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19

<210> 10687
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19

<210> 10688

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<210> 10689

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<210> 10690

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<210> 10691

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19

<210> 10692

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oligonucleotide

<400> 10692

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19

<210> 10693

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oligonucleotide

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19

<210> 10694

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oligonucleotide

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19

<210> 10695

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<213> Artificial Sequence

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oligonucleotide

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19

<210> 10696

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<212> RNA

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oligonucleotide

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uggcccacgc cggccaaca

19

<210> 10697

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oligonucleotide

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gcccacgcc ggccaacua

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<210> 10698

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<210> 10700
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<210> 10701
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 oligonucleotide

<400> 10701
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<210> 10702
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oligonucleotide

<400> 10702

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19

<210> 10703

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19

<210> 10704

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oligonucleotide

<400> 10704

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19

<210> 10705

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oligonucleotide

<400> 10705

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19

<210> 10706

<211> 19

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oligonucleotide

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cggccaacuc accgcagua

19

<210> 10707

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oligonucleotide

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ggccaacuca ccgcagua

19

<210> 10708

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<210> 10769

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<210> 10799

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<210> 10800

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<210> 10801

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<210> 10809
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<210> 10814

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<210> 10819

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<210> 10820

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<210> 10916

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<210> 10924

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<210> 10925

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<210> 10937
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<210> 10969

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<210> 10970

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<210> 10972

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<210> 10975
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19

<210> 10976

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<210> 10977

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<210> 10979

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<210> 10980

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<210> 10982

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<210> 10983

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<210> 10984

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<210> 10985

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<210> 10986

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<210> 10987
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<210> 10992

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<210> 10993

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<210> 10994

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<210> 10996

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<210> 10997

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<210> 10998
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<210> 11006

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<210> 11007

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<210> 11008

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<210> 11011

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<210> 11012

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<210> 11014
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<210> 11015
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19

<210> 11017

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<210> 11018

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<210> 11019

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<210> 11023
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<210> 11024

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<210> 11025

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<210> 11026

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<210> 11027

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<210> 11028

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<210> 11029

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19

<210> 11030

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<210> 11031

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<210> 11032

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<210> 11033

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ugcugcuggg gggaucaaca

19

<210> 11034

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<210> 11035
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<210> 11036
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<210> 11037
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<210> 11038
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oligonucleotide

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cuggggggau cacagacca

19

<210> 11039

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uggggggau acagaccaa

19

<210> 11040

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19

<210> 11041

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19

<210> 11042

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19

<210> 11043

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19

<210> 11044

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19

<210> 11045

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oligonucleotide

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gaucacagac cauuucuua

19

<210> 11046
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<210> 11047
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<400> 11047
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<210> 11048
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<210> 11049
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<210> 11081

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<210> 11082

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<210> 11091

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<210> 11092

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<210> 11093

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<210> 11102

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<210> 11103

<211> 19

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19

<210> 11104

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<212> RNA

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19

<210> 11105

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<210> 11106

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<210> 11107

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<210> 11108

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<210> 11113

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<210> 11114

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<210> 11115

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<210> 11116

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<210> 11118
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<210> 11119
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<210> 11120

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<210> 11121

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<210> 11122

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<210> 11123

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<210> 11124

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<210> 11125

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19

<210> 11126

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<210> 11127

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19

<210> 11128

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19

<210> 11129

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<210> 11132
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<210> 11133
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<210> 11134
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uccguguucc auccuccaa

19

<210> 11135

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oligonucleotide

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19

<210> 11136

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19

<210> 11137

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19

<210> 11138

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19

<210> 11139

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19

<210> 11140

<211> 19

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19

<210> 11141

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<212> RNA

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oligonucleotide

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<210> 11143
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<210> 11144
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<210> 11145
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<210> 11146
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<210> 11147
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<210> 11148
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oligonucleotide

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<210> 11149
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oligonucleotide

<400> 11149

ccacgcacccc ccaccuaua

19

<210> 11150

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oligonucleotide

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19

<210> 11151

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oligonucleotide

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acgcaccccc accuauca

19

<210> 11152

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19

<210> 11153

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19

<210> 11154

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cacccccacc uaucguuga

19

<210> 11155

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19

<210> 11156

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<210> 11158
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cccaccuauc guugguua 19

<210> 11159
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ccaccuaucg uugguucga 19

<210> 11160
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oligonucleotide

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19

<210> 11161

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19

<210> 11162

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19

<210> 11163

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<210> 11198

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<210> 11202

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<210> 11210

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<210> 11211

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<210> 11212

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<210> 11216

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<210> 11217

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<210> 11220

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<210> 11223

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oligonucleotide

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<210> 11232

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<210> 11233

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<210> 11234

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oligonucleotide

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oligonucleotide

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19

<210> 11246

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oligonucleotide

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19

<210> 11247

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oligonucleotide

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<210> 11248

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oligonucleotide

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<210> 11249

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oligonucleotide

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<210> 11250

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<210> 11251

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oligonucleotide

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<210> 11252

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oligonucleotide

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<210> 11255
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oligonucleotide

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<210> 11256
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oligonucleotide

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19

<210> 11257

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oligonucleotide

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19

<210> 11258

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19

<210> 11259

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oligonucleotide

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<210> 11260

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<210> 11261
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oligonucleotide

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<210> 11262
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oligonucleotide

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<210> 11263
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oligonucleotide

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uugccaaacc cgcuuuuua

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<210> 11264

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oligonucleotide

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<210> 11265

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oligonucleotide

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<210> 11266

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oligonucleotide

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19

<210> 11267

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oligonucleotide

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19

<210> 11268

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19

<210> 11269

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oligonucleotide

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19

<210> 11270

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oligonucleotide

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19

<210> 11271

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oligonucleotide

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19

<210> 11272

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oligonucleotide

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19

<210> 11273

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19

<210> 11274

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oligonucleotide

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19

<210> 11275
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<210> 11276
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<210> 11277
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<210> 11309
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<210> 11310
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<210> 11312

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<210> 11314

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<210> 11316

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<210> 11317

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<210> 11318

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<210> 11324
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<210> 11325
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<210> 11328

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19

<210> 11329

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<210> 11330

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oligonucleotide

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<210> 11331

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oligonucleotide

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19

<210> 11332

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19

<210> 11333

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oligonucleotide

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<210> 11334
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<210> 11342

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<210> 11343

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<210> 11344

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<210> 11345

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<210> 11346

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<210> 11347

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19

<210> 11348

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oligonucleotide

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19

<210> 11353

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19

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19

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<210> 11356

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19

<210> 11357
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19

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19

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 oligonucleotide

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19

<210> 11360

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19

<210> 11361

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19

<210> 11362

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19

<210> 11363

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19

<210> 11364

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oligonucleotide

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19

<210> 11365

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oligonucleotide

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19

<210> 11366

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oligonucleotide

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<210> 11367

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oligonucleotide

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oligonucleotide

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<210> 11369
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oligonucleotide

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<210> 11370
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oligonucleotide

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<210> 11371
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<210> 11372
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<210> 11373
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<210> 11374
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oligonucleotide

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<210> 11375
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oligonucleotide

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<210> 11376
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oligonucleotide

<400> 11376
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<210> 11377
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oligonucleotide

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<210> 11378
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oligonucleotide

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19

<210> 11379

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19

<210> 11380

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oligonucleotide

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19

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<210> 11384
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<210> 11386
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<210> 11388
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19

<210> 11390

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aggcuacagg accccccaa

19

<210> 11391

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oligonucleotide

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<210> 11429

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19

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<210> 11525

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<210> 11534

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<210> 11538

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<210> 11539

<211> 19

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<210> 11540

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19

<210> 11545

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<210> 11546

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<210> 11547

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<210> 11548

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19

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19

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19

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<210> 11555

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<210> 11556

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<210> 11557

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<210> 11558

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<210> 11559

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<210> 11560

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<210> 11561

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19

<210> 11562

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19

<210> 11563
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<210> 11564
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oligonucleotide

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<210> 11571

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19

<210> 11572

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oligonucleotide

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19

<210> 11573

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19

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<210> 11578
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<210> 11582

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19

<210> 11583

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19

<210> 11584

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19

<210> 11585

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19

<210> 11586

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19

<210> 11587

<211> 19

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oligonucleotide

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<210> 11588

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<212> RNA

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<210> 11589
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oligonucleotide

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<210> 11590
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oligonucleotide

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<210> 11591
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oligonucleotide

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oligonucleotide

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19

<210> 11593

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oligonucleotide

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19

<210> 11594

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oligonucleotide

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19

<210> 11595

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19

<210> 11596

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oligonucleotide

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<210> 11597
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oligonucleotide

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<210> 11598
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oligonucleotide

<400> 11598
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<210> 11599
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oligonucleotide

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ugccuguccc uggcugucc

19

<210> 11600

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oligonucleotide

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19

<210> 11601

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 11602

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<212> RNA

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oligonucleotide

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19

<210> 11603

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<212> RNA

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19

<210> 11604

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oligonucleotide

<400> 11604

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19

<210> 11605

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<212> RNA

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oligonucleotide

<400> 11605

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19

<210> 11606

<211> 19

<212> RNA

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oligonucleotide

<400> 11606

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19

<210> 11607

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<212> RNA

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oligonucleotide

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19

<210> 11608

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oligonucleotide

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19

<210> 11609

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<212> RNA

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oligonucleotide

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19

<210> 11610

<211> 19

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oligonucleotide

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<210> 11611
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<210> 11613
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<210> 11614
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oligonucleotide

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19

<210> 11615

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oligonucleotide

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19

<210> 11616

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oligonucleotide

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ugcccuggcu gcaugucug

19

<210> 11617

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oligonucleotide

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19

<210> 11618

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oligonucleotide

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ugagcccugg cugcauguc

19

<210> 11619

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oligonucleotide

<400> 11619

uggagcccug gcugcaugu

19

<210> 11620

<211> 19

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<400> 11620

uuggagcccu ggcugcaug

19

<210> 11621

<211> 19

<212> RNA

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oligonucleotide

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ucuggagccc uggcugcau

19

<210> 11622
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<210> 11714

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<210> 11786

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<210> 11787

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<210> 11788

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<210> 11793

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19

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<210> 11802

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<210> 11805
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<210> 11810

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<210> 11812

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<210> 11813

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<210> 11815
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<210> 11818
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<210> 11819
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<210> 11822

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19

<210> 11823

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19

<210> 11825

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19

<210> 11826

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19

<210> 11827

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19

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<210> 11832
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19

<210> 11833

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<210> 11834

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19

<210> 11835

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19

<210> 11836

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<210> 11838
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<210> 11839
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19

<210> 11840

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19

<210> 11841

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<210> 11842

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<210> 11845

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<210> 11846

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19

<210> 11847

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<210> 11849
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<210> 11850
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<210> 11851
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<210> 11852
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<210> 11940

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<210> 11943

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<210> 11944

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<210> 11946

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<210> 11949
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<210> 11950
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<210> 11952

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<210> 11953

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<210> 11954

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19

<210> 11966

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19

<210> 11967

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19

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19

<210> 12048

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19

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<210> 12050

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19

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19

<210> 12052

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19

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19

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19

<210> 12062

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19

<210> 12063

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<210> 12064

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19

<210> 12066

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19

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<210> 12068

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<210> 12073

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19

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<210> 12080

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19

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<210> 12197

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<210> 12199
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<210> 12200
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<210> 12207

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<210> 12208

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<210> 12209

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<210> 12210

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<210> 12211

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<210> 12217

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<210> 12218

<211> 19

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<210> 12219

<211> 19

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<210> 12220

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<210> 12221
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<210> 12223
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<210> 12224

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<210> 12225

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<210> 12226

<211> 19

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<210> 12227

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oligonucleotide

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<210> 12228

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<210> 12229

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<210> 12230

<211> 19

<212> RNA

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<210> 12231

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<210> 12232

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19

<210> 12233

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oligonucleotide

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19

<210> 12234

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<210> 12235
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<210> 12236
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<210> 12238
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oligonucleotide

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<210> 12240
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<210> 12241
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<210> 12242
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19

<210> 12243

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19

<210> 12244

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19

<210> 12245

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oligonucleotide

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<210> 12246
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<210> 12247
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<210> 12248
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<210> 12249
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<210> 12250
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<210> 12251
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19

<210> 12254

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19

<210> 12255

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19

<210> 12256

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<212> RNA

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19

<210> 12257

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<212> RNA

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19

<210> 12258

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oligonucleotide

<400> 12258

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19

<210> 12259

<211> 19

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oligonucleotide

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19

<210> 12260

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oligonucleotide

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19

<210> 12265

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19

<210> 12266

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<212> RNA

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19

<210> 12267

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 12268

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oligonucleotide

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<210> 12270
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<210> 12271
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<210> 12272

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19

<210> 12273

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19

<210> 12274

<211> 19

<212> RNA

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19

<210> 12275

<211> 19

<212> RNA

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19

<210> 12276

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12277

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12278

<211> 19

<212> RNA

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oligonucleotide

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uuagccaagc cggugcacc

19

<210> 12279

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12280

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12281

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12282

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12283
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<210> 12284
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<210> 12285
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<210> 12286
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oligonucleotide

<400> 12286

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19

<210> 12287

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12287

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19

<210> 12288

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12288

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19

<210> 12289

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 12290

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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uucccugugc acguagcca

19

<210> 12291

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 12292

<211> 19

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oligonucleotide

<400> 12292

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19

<210> 12293

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oligonucleotide

<400> 12293

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19

<210> 12294
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<210> 12295
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<210> 12296
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<210> 12297
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<400> 12297
uuuugauguc ccugugcac 19

<210> 12298
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oligonucleotide

<400> 12298
uguuugaugu cccugugca 19

<210> 12299
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oligonucleotide

<400> 12299
ugguuugaug ucccugugc 19

<210> 12300
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oligonucleotide

<400> 12300
uggguuugau gucccugug 19

<210> 12301
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oligonucleotide

<400> 12301

ucggguuuga uguccug

19

<210> 12302

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oligonucleotide

<400> 12302

uucggguug auguccug

19

<210> 12303

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oligonucleotide

<400> 12303

ugucggguu gauguccu

19

<210> 12304

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oligonucleotide

<400> 12304

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19

<210> 12305

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oligonucleotide

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19

<210> 12306

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<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12306

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19

<210> 12307

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12307

uuguugucgg guuugaugu

19

<210> 12308

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12308
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<210> 12309
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<220>
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oligonucleotide

<400> 12309
ugauguuguc ggguuugau 19

<210> 12310
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oligonucleotide

<400> 12310
uggauguugu cgguuuga 19

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<210> 12514

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19

<210> 12515

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<210> 12516

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<210> 12517

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<210> 12518

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<210> 12519

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<210> 12520

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<210> 12546

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<210> 12547

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oligonucleotide

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<210> 12554

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<210> 12555

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<210> 12556

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<210> 12557
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<210> 12558
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<210> 12559
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<210> 12561

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<210> 12563

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<210> 12565

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<210> 12566

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<210> 12567

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oligonucleotide

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<210> 12568

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19

<210> 12569

<211> 19

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19

<210> 12570

<211> 19

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<210> 12571
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<210> 12574
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19

<210> 12575

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<210> 12576

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19

<210> 12577

<211> 19

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<210> 12578

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<212> RNA

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19

<210> 12579

<211> 19

<212> RNA

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19

<210> 12580

<211> 19

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19

<210> 12581

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 12582
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<210> 12583
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<210> 12584
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19

<210> 12590

<211> 19

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19

<210> 12591

<211> 19

<212> RNA

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19

<210> 12592

<211> 19

<212> RNA

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19

<210> 12593

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19

<210> 12594

<211> 19

<212> RNA

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19

<210> 12595

<211> 19

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oligonucleotide

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19

<210> 12596

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oligonucleotide

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oligonucleotide

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19

<210> 12601

<211> 19

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oligonucleotide

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<210> 12602

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 12603

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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<210> 12604

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<210> 12605
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<210> 12606
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<400> 12606
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<210> 12607
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19

<210> 12608

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oligonucleotide

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<210> 12609

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oligonucleotide

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<210> 12610

<211> 19

<212> RNA

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<210> 12611

<211> 19

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<210> 12612

<211> 19

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<210> 12613

<211> 19

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19

<210> 12614

<211> 19

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<210> 12615

<211> 19

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oligonucleotide

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19

<210> 12616

<211> 19

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oligonucleotide

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19

<210> 12617

<211> 19

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<400> 12617

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19

<210> 12618

<211> 19

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<400> 12618

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<210> 12619
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<210> 12620
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<210> 12621
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oligonucleotide

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19

<210> 12623

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oligonucleotide

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19

<210> 12624

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oligonucleotide

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19

<210> 12625

<211> 19

<212> RNA

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oligonucleotide

<400> 12625

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19

<210> 12626

<211> 19

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19

<210> 12627

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19

<210> 12628

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oligonucleotide

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19

<210> 12629

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19

<210> 12630
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<210> 12631
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 oligonucleotide

<400> 12631
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<210> 12632
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<400> 12632
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<210> 12633
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<400> 12633
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<210> 12634
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oligonucleotide

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<210> 12635
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oligonucleotide

<400> 12635
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<210> 12636
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oligonucleotide

<400> 12636
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<210> 12637
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oligonucleotide

<400> 12637

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19

<210> 12638

<211> 19

<212> RNA

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 12638

ucgagccucc ucagggacc

19

<210> 12639

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 12639

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19

<210> 12640

<211> 19

<212> RNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 12640

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19

<210> 12641

<211> 19

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oligonucleotide

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19

<210> 12642

<211> 19

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oligonucleotide

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19

<210> 12643

<211> 19

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oligonucleotide

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19

<210> 12644

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oligonucleotide

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<210> 12645
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oligonucleotide

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<210> 12647
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oligonucleotide

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<210> 12648
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oligonucleotide

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19

<210> 12649

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<212> RNA

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oligonucleotide

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19

<210> 12650

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oligonucleotide

<400> 12650

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19

<210> 12651

<211> 19

<212> RNA

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oligonucleotide

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19

<210> 12652

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<210> 12653
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<210> 12654
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<400> 12654
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<210> 12655
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<210> 12690

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19

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<210> 12719

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<210> 12720

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19

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<210> 12728
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<210> 12731
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<210> 12733
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19

<210> 12734

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19

<210> 12735

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19

<210> 12736

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19

<210> 12737

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19

<210> 12738

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19

<210> 12739

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<210> 12740

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<210> 12743
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<210> 12745

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19

<210> 12746

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<210> 12747

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19

<210> 12748

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19

<210> 12749
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19

<210> 12750
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19

<210> 12752

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oligonucleotide

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19

<210> 12753

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oligonucleotide

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19

<210> 12754

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19

<210> 12755

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19

<210> 12756

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19

<210> 12757

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19

<210> 12758

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19

<210> 12759

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19

<210> 12760

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19

<210> 12761

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oligonucleotide

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19

<210> 12762

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oligonucleotide

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19

<210> 12763
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<210> 12764
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<210> 12765
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<210> 12766
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oligonucleotide

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19

<210> 12767

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19

<210> 12768

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<210> 12769

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19

<210> 12770

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<210> 12801

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<210> 12805

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<210> 12807

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<210> 12809
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<210> 12818

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<210> 12819

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<210> 12830

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<210> 12834

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<210> 12836

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<210> 12842

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<210> 12860
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<210> 12861
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<210> 12862
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19

<210> 12863

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19

<210> 12864

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<210> 12865

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19

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<210> 12867

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<210> 12868

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<210> 12869

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19

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19

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19

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<210> 12969
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<210> 12974

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<210> 12975

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<210> 12976

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<210> 12977

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<210> 12978

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<210> 12979

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<210> 12980

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<210> 12981
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<210> 12983
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<210> 12984
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19

<210> 12985

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<210> 12986

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<210> 12987

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<210> 12988

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<210> 12990
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19

<210> 12992

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19

<210> 12993

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19

<210> 12994

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<210> 12995

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<210> 12996

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19

<210> 12997

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19

<210> 12998

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19

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<210> 13001

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<210> 13002

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<210> 13008

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<210> 13010

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<210> 13011

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<210> 13012

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<210> 13013

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<210> 13014
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<210> 13023

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<210> 13024

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<210> 13025

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<210> 13026

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<210> 13027

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<210> 13028

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<210> 13033

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19

<210> 13034

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<210> 13035

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<210> 13036

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<210> 13037
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<210> 13039
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<210> 13040

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<210> 13041

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<210> 13042

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<210> 13043

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<210> 13044

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19

<210> 13045

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<210> 13046

<211> 19

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<210> 13047

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<210> 13049
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<210> 13050
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<210> 13051
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<210> 13052
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<210> 13053
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<210> 13054
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oligonucleotide

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19

<210> 13055

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19

<210> 13056

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<210> 13057

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<210> 13058

<211> 19

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<210> 13059

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oligonucleotide

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19

<210> 13060

<211> 19

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19

<210> 13061

<211> 19

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oligonucleotide

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19

<210> 13062
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<210> 13063
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<210> 13064
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<210> 13069
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19

<210> 13070

<211> 19

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19

<210> 13071

<211> 19

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19

<210> 13072

<211> 19

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oligonucleotide

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19

<210> 13073

<211> 19

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19

<210> 13074

<211> 19

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19

<210> 13075

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19

<210> 13076

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<210> 13079
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oligonucleotide

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<210> 13080
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<400> 13080

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19

<210> 13081

<211> 19

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<213> Artificial Sequence

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oligonucleotide

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19

<210> 13082

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

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19

<210> 13083

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 13083

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19

<210> 13084

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<210> 13085
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<210> 13086
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<210> 13087
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19

<210> 13088

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19

<210> 13089

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<210> 13090

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<210> 13091

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oligonucleotide

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19

<210> 13092

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oligonucleotide

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19

<210> 13093

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oligonucleotide

<400> 13093

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19

<210> 13094

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<212> RNA

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oligonucleotide

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19

<210> 13095

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oligonucleotide

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<210> 13096
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oligonucleotide

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<210> 13097
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oligonucleotide

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<210> 13098
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oligonucleotide

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<210> 13099
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oligonucleotide

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<210> 13100
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oligonucleotide

<400> 13100
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<210> 13101
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oligonucleotide

<400> 13101
ucuccgag cgaccuc 19

<210> 13102
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oligonucleotide

<400> 13102

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19

<210> 13103

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<212> RNA

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oligonucleotide

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19

<210> 13104

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<212> RNA

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oligonucleotide

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19

<210> 13105

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oligonucleotide

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19

<210> 13106

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oligonucleotide

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19

<210> 13107

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19

<210> 13108

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19

<210> 13109

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<212> RNA

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oligonucleotide

<400> 13109

uuccuggagc ucccgcgc

19

<210> 13110
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<400> 13110
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<210> 13111
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 oligonucleotide

<400> 13111
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<210> 13113
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uggcuuccug gagcucccg 19

<210> 13114
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<210> 13253

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<210> 13254
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<210> 13257
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<210> 13262

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<210> 13263

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<212> RNA

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19

<210> 13264

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<212> RNA

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<210> 13265

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19

<210> 13266

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<210> 13267

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<210> 13268

<211> 19

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<210> 13273

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19

<210> 13274

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19

<210> 13275

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<210> 13276

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19

<210> 13277
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19

<210> 13278
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19

<210> 13279
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19

<210> 13280

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<210> 13281

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<210> 13282

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<210> 13283

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<210> 13284

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<210> 13285

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<210> 13286

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<210> 13287

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<210> 13288

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<210> 13289

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19

<210> 13290

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<210> 13291
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<210> 13292
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oligonucleotide

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<210> 13299

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19

<210> 13300

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<210> 13301

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<210> 13302
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<210> 13303
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<400> 13303
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<210> 13304
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<210> 13305
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<210> 13306
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oligonucleotide

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<400> 13307
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<210> 13308
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<210> 13309
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<400> 13309

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19

<210> 13310

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oligonucleotide

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<210> 13311

<211> 19

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oligonucleotide

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19

<210> 13312

<211> 19

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19

<210> 13313

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19

<210> 13314

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19

<210> 13315

<211> 19

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19

<210> 13316

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<400> 13317
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<210> 13318
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<400> 13318
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<210> 13319
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<400> 13319
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<210> 13320
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oligonucleotide

<400> 13320

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19

<210> 13321

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oligonucleotide

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19

<210> 13322

<211> 19

<212> RNA

<213> Artificial Sequence

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oligonucleotide

<400> 13322

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19

<210> 13323

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<212> RNA

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oligonucleotide

<400> 13323

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19

<210> 13324

<211> 19
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<400> 13324
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19

<210> 13325
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 oligonucleotide

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19

<210> 13326
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 oligonucleotide

<400> 13326
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19

<210> 13327
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<400> 13327

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19

<210> 13328

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19

<210> 13329

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<210> 13330

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<210> 13331

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19

<210> 13332

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19

<210> 13333

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<210> 13334

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19

<210> 13335

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19

<210> 13336

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19

<210> 13337

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19

<210> 13338

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oligonucleotide

<400> 13338

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19

<210> 13339
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<400> 13339
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<210> 13340
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<400> 13340
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<210> 13341
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<210> 13342
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<400> 13342

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19

<210> 13343

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ucaacggcga acaggagca

19

<210> 13344

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<210> 13383

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<210> 13388
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<210> 13391

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<210> 13392

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<210> 13393

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<210> 13394

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19

<210> 13396

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<210> 13397

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19

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<210> 13409

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<210> 13411

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<210> 13412

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<210> 13417

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<210> 13418

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<210> 13419

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<210> 13420

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<210> 13421
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<210> 13424

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<210> 13425

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19

<210> 13426

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<210> 13427

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<210> 13429

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<210> 13430

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<210> 13431

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<210> 13432

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19

<210> 13433

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19

<210> 13434

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19

<210> 13435
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<210> 13436
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<210> 13437
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<210> 13438
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oligonucleotide

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<210> 13439

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<210> 13440

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<210> 13441

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<210> 13442

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<210> 13443

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<210> 13444

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<210> 13445

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<210> 13446
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<210> 13447
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<210> 13448
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19

<210> 13454

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uguucuaggg uucagggag

19

<210> 13455

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19

<210> 13456

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oligonucleotide

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19

<210> 13457

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19

<210> 13458

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<210> 13489

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<210> 13490

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<210> 13491

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<210> 13503

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<210> 13505

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<210> 13506

<211> 19

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<210> 13507

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<210> 13508

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<210> 13515

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<210> 13521

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<210> 13526

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<210> 13527

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<210> 13530

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19

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<210> 13538

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<210> 13539

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19

<210> 13540

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<210> 13541

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<210> 13547
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<210> 13548
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<210> 13549
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<210> 13550

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19

<210> 13551

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<210> 13552

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<210> 13553

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<210> 13554

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<210> 13555

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<210> 13556

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<210> 13560
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<210> 13561

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<210> 13562

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<210> 13563

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<210> 13566
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<210> 13567
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19

<210> 13568

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<210> 13569

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19

<210> 13570

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19

<210> 13571

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<210> 13572

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<210> 13652

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<210> 13659

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19

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<210> 13664

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<210> 13666

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<210> 13667

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19

<210> 13668

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<210> 13669

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<210> 13670

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<210> 13671

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<210> 13674
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<210> 13676
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<210> 13677
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<210> 13678
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<210> 13679
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<210> 13680
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<210> 13681
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<210> 13682
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19

<210> 13683

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19

<210> 13684

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19

<210> 13685

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19

<210> 13686
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<210> 13767

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<210> 13769

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<210> 13775

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<210> 13776

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<210> 13777

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<210> 13778

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<210> 13779

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<210> 13780

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<210> 13781

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19

<210> 13794

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19

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19

<210> 13796

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<210> 13810

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<210> 13812

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<210> 13814

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<210> 13815

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<210> 13817
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<210> 13818
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<210> 13824

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<210> 13825

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<210> 13826

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<210> 13827

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<210> 13828

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<210> 13829

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<210> 13830
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<210> 13831
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<210> 13832
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<210> 13833
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<210> 13836
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<210> 13838

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<210> 13839

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<210> 13840

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<210> 13841

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<210> 13842

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<210> 13843

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<210> 13844

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<210> 13845
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<210> 13848
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<210> 13849

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<210> 13850

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<212> RNA

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19

<210> 13851

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19

<210> 13852

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<210> 13853
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<210> 13854
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19

<210> 13855
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<210> 13856

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<210> 13857

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<210> 13858

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<210> 13859

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<210> 13860

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<210> 13861

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<210> 13862

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19

<210> 13863

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<210> 13864

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<210> 13865

<211> 19

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19

<210> 13866

<211> 19

<212> RNA

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<210> 13867
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<210> 13868
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<210> 13869
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<210> 13870
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19

<210> 13871

<211> 19

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<210> 13872

<211> 19

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<210> 13873

<211> 19

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<210> 13874

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19

<210> 13875

<211> 19

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19

<210> 13876

<211> 19

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19

<210> 13877

<211> 19

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19

<210> 13878
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<210> 13879
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<210> 13880
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<210> 13881
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<210> 13882
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oligonucleotide

<400> 13882
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<210> 13883
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oligonucleotide

<400> 13883
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<210> 13884
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<400> 13884
uacaagaaag cuuugcacu 19

<210> 13885
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<400> 13885

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<210> 13886

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<210> 13887

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<212> RNA

<213> Artificial Sequence

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<400> 13887

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<210> 13888

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<210> 13889

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<400> 13889

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19

<210> 13890

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<400> 13890

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19

<210> 13891

<211> 19

<212> RNA

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<400> 13891

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19

<210> 13892

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<212> RNA

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<210> 13893
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<400> 13893
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<210> 13894
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oligonucleotide

<400> 13894
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<210> 13895
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<400> 13895
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<210> 13896
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<400> 13896

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19

<210> 13897

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<400> 13897

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19

<210> 13898

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<400> 13898

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19

<210> 13899

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<212> RNA

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oligonucleotide

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19

<210> 13900

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<210> 13901
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<400> 13901
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<210> 13902
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<400> 13902
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<210> 13903
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 oligonucleotide

<400> 13903

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<210> 13904
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<210> 13905
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<210> 13906
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<400> 13906
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<210> 13907
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<400> 13907

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<210> 13908

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19

<210> 13909

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<400> 13909

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19

<210> 13910

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oligonucleotide

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19

<210> 13911

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<400> 13911
ugacgcuccc cagagcagg 19

<210> 13912
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<210> 13913
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<210> 13949
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<210> 13951
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<210> 13952

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<210> 13959

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<210> 13961

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<210> 13962

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<210> 13963
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<210> 13970

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<210> 13972

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<210> 14005

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<210> 14006

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<210> 14007

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<210> 14008

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<210> 14009

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<210> 14010

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<210> 14011
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<210> 14013
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<210> 14014
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oligonucleotide

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<210> 14015

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<210> 14016

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<210> 14017

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<210> 14018

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<210> 14025
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<210> 14026
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<210> 14027
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<210> 14054

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19

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<210> 14058

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<210> 14063

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<210> 14064

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<210> 14065

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<210> 14066

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<210> 14068

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<210> 14069

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<210> 14070
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<210> 14078

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<210> 14079

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<210> 14080

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<210> 14081

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<210> 14082

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<210> 14083

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<210> 14104

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<210> 14112

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<210> 14113

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<210> 14114

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19

<210> 14116

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19

<210> 14117

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oligonucleotide

<400> 14125

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19

<210> 14126

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oligonucleotide

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19

<210> 14127

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<210> 14137

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19

<210> 14139

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<210> 14140

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19

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<210> 14199

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19

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<210> 14202

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19

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<210> 14208

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<210> 14210

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<210> 14211

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<210> 14222

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<210> 14224

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<210> 14227
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21

<210> 14229

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<210> 14230

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21

<210> 14231

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21

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