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(71) Demandeurs/Applicants:

RANA THERAPEUTICS INC., US;  
THE GENERAL HOSPITAL CORPORATION D/B/A  
MASSACHUSETTS GENERAL HOSPITAL, US

(72) Inventeurs/Inventors:

KRIEG, ARTHUR M., US;  
SUBRAMANIAN, ROMESH, US;  
MCSWIGGEN, JAMES, US;  
LEE, JEANNIE T., US

(74) Agent: RIDOUT & MAYBEE LLP

(54) Titre : COMPOSITIONS ET METHODES POUR MODULER L'EXPRESSION DE MECP2

(54) Title: COMPOSITIONS AND METHODS FOR MODULATING MECP2 EXPRESSION

(57) **Abrégé/Abstract:**

Aspects of the invention provide single stranded oligonucleotides for activating or enhancing expression of MECP2. Further aspects provide compositions and kits comprising single stranded oligonucleotides for activating or enhancing expression of MECP2. Methods for modulating expression of MECP2 using the single stranded oligonucleotides are also provided. Further aspects of the invention provide methods for selecting a candidate oligonucleotide for activating or enhancing expression of MECP2.

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(71) Applicants: RANA THERAPEUTICS, INC. [US/US]; 790 Memorial Drive, Suite 203, Cambridge, MA 02139 (US). THE GENERAL HOSPITAL CORPORATION D/B/A MASSACHUSETTS GENERAL HOSPITAL [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: KRIEG, Arthur, M.; 173 Winding River Road, Wellesley, MA 02482 (US). SUBRAMANIAN, Romesh; 9 Alan Street, Framingham, MA 01701 (US). MCSWIGGEN, James; 15 Beacon Street, Unit A, Arlington, MA 02474 (US). LEE, Jeannie, T.; 185 Cambridge Street, Boston, MA 02114 (US).

(74) Agent: YOUNG, Daniel, W.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).
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(54) Title: COMPOSITIONS AND METHODS FOR MODULATING MECP2 EXPRESSION

(57) Abstract: Aspects of the invention provide single stranded oligonucleotides for activating or enhancing expression of MECP2. Further aspects provide compositions and kits comprising single stranded oligonucleotides for activating or enhancing expression of MECP2. Methods for modulating expression of MECP2 using the single stranded oligonucleotides are also provided. Further aspects of the invention provide methods for selecting a candidate oligonucleotide for activating or enhancing expression of MECP2.

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**COMPOSITIONS AND METHODS FOR MODULATING MECP2 EXPRESSION****CROSS-REFERENCE TO RELATED APPLICATIONS**

5           This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/648,051, entitled “COMPOSITIONS AND METHODS FOR MODULATING MECP2 EXPRESSION”, filed May 16, 2012, which is incorporated herein by reference in its entirety.

**FIELD OF THE INVENTION**

10           The invention relates to oligonucleotide based compositions, as well as methods of using oligonucleotide based compositions for treating disease.

**BACKGROUND OF THE INVENTION**

15           Rett syndrome is a developmental disorder of the brain occurring mostly in females characterized by normal early development, followed by a slowing of development resulting in loss of control of the hands, loss of speech, breathing problems, slowed brain and head growth, ambulatory problems, seizures, and mental retardation. Rett syndrome affects approximately 1 in 10,000 live female births. Most cases of Rett syndrome are caused by a  
20           mutation in the methyl CpG binding protein 2, or MECP2 gene, on the X chromosome that causes reduced activity or inactivation of MECP2.

          MECP2 is a transcriptional repressor that binds to methylated DNA and is present in large quantities in mature nerve cells. MECP2 represses transcription from methylated gene promoters through interaction with histone deacetylase and the corepressor SIN3A. Many of  
25           the genes that are known to be regulated by the MECP2 protein play a role in normal brain function, particularly the maintenance of synapses. Mouse studies have demonstrated MECP2 mutations cause defects in synaptic function, especially in synaptic plasticity.

**SUMMARY OF THE INVENTION**

30           Aspects of the invention disclosed herein provide methods and compositions that are useful for upregulating MECP2 in cells. In some embodiments, single stranded oligonucleotides are provided that target a PRC2-associated region of a MECP2 gene (e.g., human MECP2) and thereby cause upregulation of the gene. In some embodiments, single

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stranded oligonucleotides are provided that target a PRC2-associated region of the gene encoding MECP2. In some embodiments, these single stranded oligonucleotides activate or enhance expression of MECP2 by relieving or preventing PRC2 mediated repression of MECP2.

5 Upregulation of MECP2 offers a treatment for diseases associated with decreased MECP2 activity. Aspects of the invention disclosed herein provide methods and compositions that are useful for upregulating MECP2 for the treatment and/or prevention of Rett Syndrome. MECP2 is also associated with MECP2-related severe neonatal encephalopathy, Angelman syndrome, and PPM-X syndrome. Accordingly, certain aspects  
10 of the invention disclosed herein provide methods and compositions that are useful for upregulating MECP2 for the treatment and/or prevention of MECP2-related severe neonatal encephalopathy, Angelman syndrome, and PPM-X syndrome.

Further aspects of the invention provide methods for selecting oligonucleotides for activating or enhancing expression of MECP2. In some embodiments, methods are provided  
15 for selecting a set of oligonucleotides that is enriched in candidates (*e.g.*, compared with a random selection of oligonucleotides) for activating or enhancing expression of MECP2. Accordingly, the methods may be used to establish sets of clinical candidates that are enriched in oligonucleotides that activate or enhance expression of MECP2. Such libraries may be utilized, for example, to identify lead oligonucleotides for developing therapeutics to  
20 treat MECP2. Furthermore, in some embodiments, oligonucleotide chemistries are provided that are useful for controlling the pharmacokinetics, biodistribution, bioavailability and/or efficacy of the single stranded oligonucleotides for activating expression of MECP2.

According to some aspects of the invention single stranded oligonucleotides are provided that have a region of complementarity that is complementary with (*e.g.*, at least 8  
25 consecutive nucleotides of ) a PRC2-associated region of a MECP2 gene, *e.g.*, a PRC2-associated region of the nucleotide sequence set forth as SEQ ID NO: 1 or 2. In some embodiments, the oligonucleotide has at least one of the following features: a) a sequence that is 5'X-Y-Z, in which X is any nucleotide and in which X is at the 5' end of the oligonucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a human  
30 seed sequence of a microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length; b) a sequence that does not comprise three or more consecutive guanosine

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nucleotides; c) a sequence that has less than a threshold level of sequence identity with every sequence of nucleotides, of equivalent length to the second nucleotide sequence, that are between 50 kilobases upstream of a 5'-end of an off-target gene and 50 kilobases downstream of a 3'-end of the off-target gene; d) a sequence that is complementary to a PRC2-associated region that encodes an RNA that forms a secondary structure comprising at least two single stranded loops; and e) a sequence that has greater than 60% G-C content. In some embodiments, the single stranded oligonucleotide has at least two of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has at least three of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has at least four of features a), b), c), d), and e), each independently selected. In some embodiments, the single stranded oligonucleotide has each of features a), b), c), d), and e). In certain embodiments, the oligonucleotide has the sequence 5'-X-Y-Z, in which the oligonucleotide is 8-50 nucleotides in length.

According to some aspects of the invention, single stranded oligonucleotides are provided that have a sequence X-Y-Z, in which X is any nucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a seed sequence of a human microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length, in which the single stranded oligonucleotide is complementary with a PRC2-associated region of a MECP2 gene, e.g., a PRC2-associated region of the nucleotide sequence set forth as SEQ ID NO: 1 or 2. In some aspects of the invention, single stranded oligonucleotides are provided that have a sequence 5'-X-Y-Z, in which X is any nucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a seed sequence of a human microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length, in which the single stranded oligonucleotide is complementary with at least 8 consecutive nucleotides of a PRC2-associated region of a MECP2 gene, e.g., a PRC2-associated region of the nucleotide sequence set forth as SEQ ID NO: 1 or 2. In some embodiments, Y is a sequence selected from Table 1. In some embodiments, the PRC2-associated region is a sequence listed in any one of SEQ ID NOS: 5 to 118.

In some embodiments, the single stranded oligonucleotide comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 83660, or a fragment thereof that is at least 8 nucleotides. In some embodiments, the single stranded oligonucleotide comprises a

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nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 83660, in which the 5' end of the nucleotide sequence provided is the 5' end of the oligonucleotide. In some embodiments, the region of complementarity (*e.g.*, the at least 8 consecutive nucleotides) is also present within the nucleotide sequence set forth as SEQ ID NO: 3 or 4.

5           In some embodiments, the single stranded oligonucleotide comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 83660. In some embodiments, the single stranded oligonucleotide comprises a fragment of at least 8 nucleotides of a nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 83660.

10           In some embodiments, the PRC2-associated region is a sequence listed in any one of SEQ ID NOS: 5 to 84. In some embodiments, the single stranded oligonucleotide comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 47653 or a fragment thereof that is at least 8 nucleotides. In some embodiments, the single stranded oligonucleotide comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 119 to 47653, wherein the 5' end of the nucleotide sequence provided in any one of SEQ ID  
15           NOS: 119 to 47653 is the 5' end of the oligonucleotide. In some embodiments, the at least 8 consecutive nucleotides are also present within the nucleotide sequence set forth as SEQ ID NO: 3.

          In some embodiments, the PRC2-associated region is a sequence listed in any one of SEQ ID NOS: 85 to 118. In some embodiments, the single stranded oligonucleotide  
20           comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 47462 to 83660 or a fragment thereof that is at least 8 nucleotides. In some embodiments, the single stranded oligonucleotide comprises a nucleotide sequence as set forth in any one of SEQ ID NOS: 47462 to 83660, wherein the 5' end of the nucleotide sequence provided in any one of SEQ ID NOS: 47462 to 83660 is the 5' end of the oligonucleotide. In some embodiments, the at  
25           least 8 consecutive nucleotides are present within the nucleotide sequence set forth as SEQ ID NO: 4.

          In some embodiments, a single stranded oligonucleotide comprises a nucleotide sequence as set forth in Table 3. In some embodiments, the single stranded oligonucleotide comprises a fragment of at least 8 nucleotides of a nucleotide sequence as set forth in Table 3.

In some embodiments, a single stranded oligonucleotide consists of a nucleotide sequence as set forth in Table 3.

In some embodiments, the single stranded oligonucleotide does not comprise three or more consecutive guanosine nucleotides. In some embodiments, the single stranded

oligonucleotide does not comprise four or more consecutive guanosine nucleotides.

In some embodiments, the single stranded oligonucleotide is 8 to 30 nucleotides in length. In some embodiments, the single stranded oligonucleotide is up to 50 nucleotides in length. In some embodiments, the single stranded oligonucleotide is 8 to 10 nucleotides in length and all but 1, 2, or 3 of the nucleotides of the complementary sequence of the PRC2-associated region are cytosine or guanosine nucleotides.

In some embodiments, the single stranded oligonucleotide is complementary with at least 8 consecutive nucleotides of a PRC2-associated region of a MECP2 gene, e.g., a PRC2-associated region of a nucleotide sequence set forth as SEQ ID NO: 1 or 2, in which the nucleotide sequence of the single stranded oligonucleotide comprises one or more of a nucleotide sequence selected from the group consisting of

(a) (X)XXXXXX, (X)xXXXXX, (X)xxXXXX, (X)xxxXXX, (X)xxxxXX and (X)xxxxX,

(b) (X)XXXXXX, (X)XxXXXX, (X)XxxXXX, (X)XxxxXX, (X)XxxxxX, (X)xXXXXX, (X)xXxXXX, (X)xXxxXX, (X)xXxxxX, (X)xxXXXX, (X)xxXxXX, (X)xxXXxX, (X)xxxXXX, (X)xxxXxX and (X)xxxxXX,

(c) (X)XXXxxx, (X)xXXXxx, (X)xxXXXx, (X)xxxXXX, (X)XXxXxx, (X)XXxxXx, (X)XXxxxX, (X)xXXxXx, (X)xXXxxX, (X)xxXXxX, (X)XxXXxx, (X)XxxXXx, (X)XxxxXX, (X)xXxXXx, (X)xXxxXX, (X)xxXxXX, (X)xXxXxX and (X)XxXxXx,

(d) (X)xxXXX, (X)xXxXXX, (X)xXXxXX, (X)xXXXxX, (X)xXXXXX, (X)XxxXXXX, (X)XxXxXX, (X)XxXXxX, (X)XxXXXx, (X)XXxxXX, (X)XXxXxX, (X)XXxXXx, (X)XXXxxX, (X)XXXxXx, and (X)XXXXxx,

(e) (X)xXXXXX, (X)XxXXXX, (X)XXxXXX, (X)XXXxXX, (X)XXXXxX and (X)XXXXXx, and

(f) XXXXXX, XxXXXXX, XXxXXXX, XXXxXXX, XXXXxXX, XXXXXxX and XXXXXXx, wherein "X" denotes a nucleotide analogue, (X) denotes an optional nucleotide analogue, and "x" denotes a DNA or RNA nucleotide unit.

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In some embodiments, at least one nucleotide of the oligonucleotide is a nucleotide analogue. In some embodiments, the at least one nucleotide analogue results in an increase in  $T_m$  of the oligonucleotide in a range of 1 to 5 °C compared with an oligonucleotide that does not have the at least one nucleotide analogue.

5 In some embodiments, at least one nucleotide of the oligonucleotide comprises a 2' O-methyl. In some embodiments, each nucleotide of the oligonucleotide comprises a 2' O-methyl. In some embodiments, the oligonucleotide comprises at least one ribonucleotide, at least one deoxyribonucleotide, or at least one bridged nucleotide. In some embodiments, the bridged nucleotide is a LNA nucleotide, a cEt nucleotide or a ENA modified nucleotide. In  
10 some embodiments, each nucleotide of the oligonucleotide is a LNA nucleotide.

In some embodiments, the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and 2'-fluoro-deoxyribonucleotides. In some embodiments, the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and 2'-O-methyl nucleotides. In some embodiments, the nucleotides of the oligonucleotide comprise  
15 alternating deoxyribonucleotides and ENA nucleotide analogues. In some embodiments, the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and LNA nucleotides. In some embodiments, the 5' nucleotide of the oligonucleotide is a deoxyribonucleotide. In some embodiments, the nucleotides of the oligonucleotide comprise alternating LNA nucleotides and 2'-O-methyl nucleotides. In some embodiments, the 5'  
20 nucleotide of the oligonucleotide is a LNA nucleotide. In some embodiments, the nucleotides of the oligonucleotide comprise deoxyribonucleotides flanked by at least one LNA nucleotide on each of the 5' and 3' ends of the deoxyribonucleotides.

In some embodiments, the single stranded oligonucleotide comprises modified internucleotide linkages (*e.g.*, phosphorothioate internucleotide linkages or other linkages)  
25 between at least two, at least three, at least four, at least five or more nucleotides. In some embodiments, the single stranded oligonucleotide comprises modified internucleotide linkages (*e.g.*, phosphorothioate internucleotide linkages or other linkages) between between all nucleotides.

In some embodiments, the nucleotide at the 3' position of the oligonucleotide has a 3'  
30 hydroxyl group. In some embodiments, the nucleotide at the 3' position of the oligonucleotide has a 3' thiophosphate. In some embodiments, the single stranded

oligonucleotide has a biotin moiety or other moiety conjugated to its 5' or 3' nucleotide. In some embodiments, the single stranded oligonucleotide has cholesterol, Vitamin A, folate, sigma receptor ligands, aptamers, peptides, such as CPP, hydrophobic molecules, such as lipids, ASGPR or dynamic polyconjugates and variants thereof at its 5' or 3' end.

5           According to some aspects of the invention compositions are provided that comprise any of the oligonucleotides disclosed herein, and a carrier. In some embodiments, compositions are provided that comprise any of the oligonucleotides in a buffered solution. In some embodiments, the oligonucleotide is conjugated to the carrier. In some embodiments, the carrier is a peptide. In some embodiments, the carrier is a steroid. According to some  
10           aspects of the invention pharmaceutical compositions are provided that comprise any of the oligonucleotides disclosed herein, and a pharmaceutically acceptable carrier.

          According to other aspects of the invention, kits are provided that comprise a container housing any of the compositions disclosed herein.

          According to some aspects of the invention, methods of increasing expression of  
15           MECP2 in a cell are provided. In some embodiments, the methods involve delivering any one or more of the single stranded oligonucleotides disclosed herein into the cell. In some embodiments, delivery of the single stranded oligonucleotide into the cell results in a level of expression of MECP2 that is greater (*e.g.*, at least 50% greater) than a level of expression of MECP2 in a control cell that does not comprise the single stranded oligonucleotide.

20           According to some aspects of the invention, methods of increasing levels of MECP2 in a subject are provided. According to some aspects of the invention, methods of treating a condition (*e.g.*, Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, PPM-X syndrome) associated with decreased levels of MECP2 in a subject are provided. In some embodiments, the methods involve administering any one or more of the  
25           single stranded oligonucleotides disclosed herein to the subject.

### BRIEF DESCRIPTION OF TABLES

**Table 1:** Hexamers that are not seed sequences of human miRNAs

**Table 2:** A listing of oligonucleotide modifications

30           **Table 3:** Formatted oligonucleotide sequences made for testing showing nucleotide modifications. The table shows the sequence of the modified nucleotides, where InaX

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represents an LNA nucleotide with 3' phosphorothioate linkage, omeX is a 2'-O-methyl nucleotide, dX is a deoxy nucleotide. An s at the end of a nucleotide code indicates that the nucleotide had a 3' phosphorothioate linkage. The "-Sup" at the end of the sequence marks the fact that the 3' end lacks either a phosphate or thiophosphate on the 3' linkage. The

5 Formatted Sequence column shows the sequence of the oligonucleotide, including modified nucleotides.

### DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

Aspects of the invention provided herein relate to the discovery of polycomb

10 repressive complex 2 (PRC2)-interacting RNAs. Polycomb repressive complex 2 (PRC2) is a histone methyltransferase and a known epigenetic regulator involved in silencing of genomic regions through methylation of histone H3. Among other functions, PRC2 interacts with long noncoding RNAs (lncRNAs), such as RepA, Xist, and Tsix, to catalyze trimethylation of histone H3-lysine27. PRC2 contains four subunits, Eed, Suz12, RbAp48,

15 and Ezh2. Aspects of the invention relate to the recognition that single stranded oligonucleotides that bind to PRC2-associated regions of RNAs (*e.g.*, lncRNAs) that are expressed from within a genomic region that encompasses or that is in functional proximity to the MECP2 gene can induce or enhance expression of MECP2. In some embodiments, this upregulation is believed to result from inhibition of PRC2 mediated repression of

20 MECP2.

As used herein, the term "PRC2-associated region" refers to a region of a nucleic acid that comprises or encodes a sequence of nucleotides that interact directly or indirectly with a component of PRC2. A PRC2-associated region may be present in a RNA (*e.g.*, a long non-coding RNA (lncRNA)) that that interacts with a PRC2. A PRC2-associated region may be

25 present in a DNA that encodes an RNA that interacts with PRC2. In some cases, the PRC2-associated region is equivalently referred to as a PRC2-interacting region.

In some embodiments, a PRC2-associated region is a region of an RNA that crosslinks to a component of PRC2 in response to *in situ* ultraviolet irradiation of a cell that expresses the RNA, or a region of genomic DNA that encodes that RNA region. In some

30 embodiments, a PRC2-associated region is a region of an RNA that immunoprecipitates with an antibody that targets a component of PRC2, or a region of genomic DNA that encodes that

RNA region. In some embodiments, a PRC2-associated region is a region of an RNA that immunoprecipitates with an antibody that binds specifically to SUZ12, EED, EZH2 or RBBP4 (which as noted above are components of PRC2), or a region of genomic DNA that encodes that RNA region.

5 In some embodiments, a PRC2-associated region is a region of an RNA that is protected from nucleases (*e.g.*, RNases) in an RNA-immunoprecipitation assay that employs an antibody that targets a component of PRC2, or a region of genomic DNA that encodes that protected RNA region. In some embodiments, a PRC2-associated region is a region of an RNA that is protected from nucleases (*e.g.*, RNases) in an RNA-immunoprecipitation assay  
10 that employs an antibody that targets SUZ12, EED, EZH2 or RBBP4, or a region of genomic DNA that encodes that protected RNA region.

In some embodiments, a PRC2-associated region is a region of an RNA within which occur a relatively high frequency of sequence reads in a sequencing reaction of products of an RNA-immunoprecipitation assay that employs an antibody that targets a component of PRC2,  
15 or a region of genomic DNA that encodes that RNA region. In some embodiments, a PRC2-associated region is a region of an RNA within which occur a relatively high frequency of sequence reads in a sequencing reaction of products of an RNA-immunoprecipitation assay that employs an antibody that binds specifically to SUZ12, EED, EZH2 or RBBP4, or a region of genomic DNA that encodes that protected RNA region. In such embodiments, the  
20 PRC2-associated region may be referred to as a “peak.”

In some embodiments, a PRC2-associated region comprises a sequence of 40 to 60 nucleotides that interact with PRC2 complex. In some embodiments, a PRC2-associated region comprises a sequence of 40 to 60 nucleotides that encode an RNA that interacts with PRC2. In some embodiments, a PRC2-associated region comprises a sequence of up to 5kb  
25 in length that comprises a sequence (*e.g.*, of 40 to 60 nucleotides) that interacts with PRC2. In some embodiments, a PRC2-associated region comprises a sequence of up to 5kb in length within which an RNA is encoded that has a sequence (*e.g.*, of 40 to 60 nucleotides) that is known to interact with PRC2. In some embodiments, a PRC2-associated region comprises a sequence of about 4kb in length that comprise a sequence (*e.g.*, of 40 to 60  
30 nucleotides) that interacts with PRC2. In some embodiments, a PRC2-associated region comprises a sequence of about 4kb in length within which an RNA is encoded that includes a

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sequence (*e.g.*, of 40 to 60 nucleotides) that is known to interact with PRC2. In some embodiments, a PRC2-associated region has a sequence as set forth in any one of SEQ ID NOS: 5 to 118.

In some embodiments, single stranded oligonucleotides are provided that specifically  
5 bind to, or are complementary to, a PRC2-associated region in a genomic region that encompasses or that is in proximity to the MECP2 gene. In some embodiments, single stranded oligonucleotides are provided that specifically bind to, or are complementary to, a PRC2-associated region that has a sequence as set forth in any one of SEQ ID NOS: 5 to 118. In some embodiments, single stranded oligonucleotides are provided that specifically bind to,  
10 or are complementary to, a PRC2-associated region that has a sequence as set forth in any one of SEQ ID NOS: 5 to 118 combined with up to 2kb, up to 5kb, or up to 10kb of flanking sequences from a corresponding genomic region to which these SEQ IDs map (*e.g.*, in a human genome). In some embodiments, single stranded oligonucleotides have a sequence as set forth in any one of SEQ ID NOS: 119 to 83660. In some embodiments, single stranded  
15 oligonucleotides have a sequence as set forth in Table 3.

Without being bound by a theory of invention, these oligonucleotides are able to interfere with the binding of and function of PRC2, by preventing recruitment of PRC2 to a specific chromosomal locus. For example, a single administration of single stranded oligonucleotides designed to specifically bind a PRC2-associated region lncRNA can stably  
20 displace not only the lncRNA, but also the PRC2 that binds to the lncRNA, from binding chromatin. After displacement, the full complement of PRC2 is not recovered for up to 24 hours. Further, lncRNA can recruit PRC2 in a *cis* fashion, repressing gene expression at or near the specific chromosomal locus from which the lncRNA was transcribed.

Methods of modulating gene expression are provided, in some embodiments, that may  
25 be carried out *in vitro*, *ex vivo*, or *in vivo*. It is understood that any reference to uses of compounds throughout the description contemplates use of the compound in preparation of a pharmaceutical composition or medicament for use in the treatment of condition (*e.g.*, Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, PPM-X syndrome) associated with decreased levels or activity of MECP2. Thus, as one nonlimiting  
30 example, this aspect of the invention includes use of such single stranded oligonucleotides in

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the preparation of a medicament for use in the treatment of disease, wherein the treatment involves upregulating expression of MECP2.

In further aspects of the invention, methods are provided for selecting a candidate oligonucleotide for activating expression of MECP2. The methods generally involve  
5 selecting as a candidate oligonucleotide, a single stranded oligonucleotide comprising a nucleotide sequence that is complementary to a PRC2-associated region (*e.g.*, a nucleotide sequence as set forth in any one of SEQ ID NOS: 5 to 118). In some embodiments, sets of oligonucleotides may be selected that are enriched (*e.g.*, compared with a random selection of oligonucleotides) in oligonucleotides that activate expression of MECP2.

#### *Single Stranded Oligonucleotides for Modulating Expression of MECP2*

In one aspect of the invention, single stranded oligonucleotides complementary to the PRC2-associated regions are provided for modulating expression of MECP2 in a cell. In some embodiments, expression of MECP2 is upregulated or increased. In some  
15 embodiments, single stranded oligonucleotides complementary to these PRC2-associated regions inhibit the interaction of PRC2 with long RNA transcripts such that gene expression is upregulated or increased. In some embodiments, single stranded oligonucleotides complementary to these PRC2-associated regions inhibit the interaction of PRC2 with long RNA transcripts, resulting in reduced methylation of histone H3 and reduced gene  
20 inactivation, such that gene expression is upregulated or increased. In some embodiments, this interaction may be disrupted or inhibited due to a change in the structure of the long RNA that prevents or reduces binding to PRC2. The oligonucleotide may be selected using any of the methods disclosed herein for selecting a candidate oligonucleotide for activating expression of MECP2.

25 The single stranded oligonucleotide may comprise a region of complementarity that is complementary with a PRC2-associated region of a nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4. The region of complementarity of the single stranded oligonucleotide may be complementary with at least 6, *e.g.*, at least 7, at least 8, at least 9, at least 10, at least 15 or more consecutive nucleotides of the PRC2-associated region.

30 The PRC2-associated region may map to a position in a chromosome between 50 kilobases upstream of a 5'-end of the MECP2 gene and 50 kilobases downstream of a 3'-end

of the MECP2 gene. The PRC2-associated region may map to a position in a chromosome between 25 kilobases upstream of a 5'-end of the MECP2 gene and 25 kilobases downstream of a 3'-end of the MECP2 gene. The PRC2-associated region may map to a position in a chromosome between 12 kilobases upstream of a 5'-end of the MECP2 gene and 12 kilobases downstream of a 3'-end of the MECP2 gene. The PRC2-associated region may map to a position in a chromosome between 5 kilobases upstream of a 5'-end of the MECP2 gene and 5 kilobases downstream of a 3'-end of the MECP2 gene.

The genomic position of the selected PRC2-associated region relative to the MECP2 gene may vary. For example, the PRC2-associated region may be upstream of the 5' end of the MECP2 gene. The PRC2-associated region may be downstream of the 3' end of the MECP2 gene. The PRC2-associated region may be within an intron of the MECP2 gene. The PRC2-associated region may be within an exon of the MECP2 gene. The PRC2-associated region may traverse an intron-exon junction, a 5'-UTR-exon junction or a 3'-UTR-exon junction of the MECP2 gene.

The single stranded oligonucleotide may comprise a sequence having the formula X-Y-Z, in which X is any nucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a human seed sequence of a microRNA, and Z is a nucleotide sequence of varying length. In some embodiments X is the 5' nucleotide of the oligonucleotide. In some embodiments, when X is anchored at the 5' end of the oligonucleotide, the oligonucleotide does not have any nucleotides or nucleotide analogs linked 5' to X. In some embodiments, other compounds such as peptides or sterols may be linked at the 5' end in this embodiment as long as they are not nucleotides or nucleotide analogs. In some embodiments, the single stranded oligonucleotide has a sequence 5'-X-Y-Z and is 8-50 nucleotides in length. Oligonucleotides that have these sequence characteristics are predicted to avoid the miRNA pathway. Therefore, in some embodiments, oligonucleotides having these sequence characteristics are unlikely to have an unintended consequence of functioning in a cell as a miRNA molecule. The Y sequence may be a nucleotide sequence of 6 nucleotides in length set forth in Table 1.

The single stranded oligonucleotide may have a sequence that does not contain guanosine nucleotide stretches (*e.g.*, 3 or more, 4 or more, 5 or more, 6 or more consecutive guanosine nucleotides). In some embodiments, oligonucleotides having guanosine nucleotide

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stretches have increased non-specific binding and/or off-target effects, compared with oligonucleotides that do not have guanosine nucleotide stretches.

The single stranded oligonucleotide may have a sequence that has less than a threshold level of sequence identity with every sequence of nucleotides, of equivalent length, that map to a genomic position encompassing or in proximity to an off-target gene. For example, an oligonucleotide may be designed to ensure that it does not have a sequence that maps to genomic positions encompassing or in proximity with all known genes (*e.g.*, all known protein coding genes) other than MECP2. In a similar embodiment, an oligonucleotide may be designed to ensure that it does not have a sequence that maps to any other known PRC2-associated region, particularly PRC2-associated regions that are functionally related to any other known gene (*e.g.*, any other known protein coding gene). In either case, the oligonucleotide is expected to have a reduced likelihood of having off-target effects. The threshold level of sequence identity may be 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% sequence identity.

The single stranded oligonucleotide may have a sequence that is complementary to a PRC2-associated region that encodes an RNA that forms a secondary structure comprising at least two single stranded loops. It has been discovered that, in some embodiments, oligonucleotides that are complementary to a PRC2-associated region that encodes an RNA that forms a secondary structure comprising one or more single stranded loops (*e.g.*, at least two single stranded loops) have a greater likelihood of being active (*e.g.*, of being capable of activating or enhancing expression of a target gene) than a randomly selected oligonucleotide. In some cases, the secondary structure may comprise a double stranded stem between the at least two single stranded loops. Accordingly, the region of complementarity between the oligonucleotide and the PRC2-associated region may be at a location of the PRC2 associated region that encodes at least a portion of at least one of the loops. In some cases, the region of complementarity between the oligonucleotide and the PRC2-associated region may be at a location of the PRC2-associated region that encodes at least a portion of at least two of the loops. In some cases, the region of complementarity between the oligonucleotide and the PRC2-associated region may be at a location of the PRC2 associated region that encodes at least a portion of the double stranded stem. In some embodiments, a PRC2-associated region (*e.g.*, of an lncRNA) is identified (*e.g.*, using RIP-

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Seq methodology or information derived therefrom). In some embodiments, the predicted secondary structure RNA (*e.g.*, lncRNA) containing the PRC2-associated region is determined using RNA secondary structure prediction algorithms, *e.g.*, RNAfold, mfold. In some embodiments, oligonucleotides are designed to target a region of the RNA that forms a secondary structure comprising one or more single stranded loop (*e.g.*, at least two single stranded loops) structures which may comprise a double stranded stem between the at least two single stranded loops.

The single stranded oligonucleotide may have a sequence that is has greater than 30% G-C content, greater than 40% G-C content, greater than 50% G-C content, greater than 60% G-C content, greater than 70% G-C content, or greater than 80% G-C content. The single stranded oligonucleotide may have a sequence that has up to 100% G-C content, up to 95% G-C content, up to 90% G-C content, or up to 80% G-C content. In some embodiments in which the oligonucleotide is 8 to 10 nucleotides in length, all but 1, 2, 3, 4, or 5 of the nucleotides of the complementary sequence of the PRC2-associated region are cytosine or guanosine nucleotides. In some embodiments, the sequence of the PRC2-associated region to which the single stranded oligonucleotide is complementary comprises no more than 3 nucleotides selected from adenine and uracil.

The single stranded oligonucleotide may be complementary to a chromosome of a different species (*e.g.*, a mouse, rat, rabbit, goat, monkey, *etc.*) at a position that encompasses or that is in proximity to that species' homolog of MECP2. The single stranded oligonucleotide may be complementary to a human genomic region encompassing or in proximity to the MECP2 gene and also be complementary to a mouse genomic region encompassing or in proximity to the mouse homolog of MECP2. For example, the single stranded oligonucleotide may be complementary to a sequence as set forth in SEQ ID NO: 1 or 2, which is a human genomic region encompassing or in proximity to the MECP2 gene, and also be complementary to a sequence as set forth in SEQ ID NO: 3 or 4, which is a mouse genomic region encompassing or in proximity to the mouse homolog of the MECP2 gene. Oligonucleotides having these characteristics may be tested *in vivo* or *in vitro* for efficacy in multiple species (*e.g.*, human and mouse). This approach also facilitates development of clinical candidates for treating human disease by selecting a species in which an appropriate animal exists for the disease.

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In some embodiments, the region of complementarity of the single stranded oligonucleotide is complementary with at least 8 to 15, 8 to 30, 8 to 40, or 10 to 50, or 5 to 50, or 5 to 40 bases, *e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive nucleotides of a PRC2-associated region. In some embodiments, the region of complementarity is complementary with at least 8 consecutive nucleotides of a PRC2-associated region. In some embodiments the sequence of the single stranded oligonucleotide is based on an RNA sequence that binds to PRC2, or a portion thereof, said portion having a length of from 5 to 40 contiguous base pairs, or about 8 to 40 bases, or about 5 to 15, or about 5 to 30, or about 5 to 40 bases, or about 5 to 50 bases.

Complementary, as the term is used in the art, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of PRC2-associated region, then the single stranded nucleotide and PRC2-associated region are considered to be complementary to each other at that position. The single stranded nucleotide and PRC2-associated region are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides that can hydrogen bond with each other through their bases. Thus, “complementary” is a term which is used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the single stranded nucleotide and PRC2-associated region. For example, if a base at one position of a single stranded nucleotide is capable of hydrogen bonding with a base at the corresponding position of a PRC2-associated region, then the bases are considered to be complementary to each other at that position. 100% complementarity is not required.

The single stranded oligonucleotide may be at least 80% complementary to (optionally one of at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% complementary to) the consecutive nucleotides of a PRC2-associated region. In some embodiments the single stranded oligonucleotide may contain 1, 2 or 3 base mismatches compared to the portion of the consecutive nucleotides of a PRC2-associated region. In some embodiments the single stranded oligonucleotide may have up to 3 mismatches over 15 bases, or up to 2 mismatches over 10 bases.

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It is understood in the art that a complementary nucleotide sequence need not be 100% complementary to that of its target to be specifically hybridizable. In some embodiments, a complementary nucleic acid sequence for purposes of the present disclosure is specifically hybridizable when binding of the sequence to the target molecule (*e.g.*,  
5 lncRNA) interferes with the normal function of the target (*e.g.*, lncRNA) to cause a loss of activity (*e.g.*, inhibiting PRC2-associated repression with consequent up-regulation of gene expression) and there is a sufficient degree of complementarity to avoid non-specific binding of the sequence to non-target sequences under conditions in which avoidance of non-specific binding is desired, *e.g.*, under physiological conditions in the case of *in vivo* assays or  
10 therapeutic treatment, and in the case of *in vitro* assays, under conditions in which the assays are performed under suitable conditions of stringency.

In some embodiments, the single stranded oligonucleotide is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 or more nucleotides in length. In a preferred embodiment, the oligonucleotide is 8 to 30 nucleotides  
15 in length.

In some embodiments, the PRC2-associated region occurs on the same DNA strand as a gene sequence (sense). In some embodiments, the PRC2-associated region occurs on the opposite DNA strand as a gene sequence (anti-sense). Oligonucleotides complementary to a PRC2-associated region can bind either sense or anti-sense sequences. Base pairings may  
20 include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (*e.g.*, Wobble base pairing and Hoogsteen base pairing). It is understood that for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as 3-nitropyrrole or 5-nitroindole can hybridize to  
25 and are considered complementary to any A, C, U, or T. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U or T.

In some embodiments, any one or more thymidine (T) nucleotides (or modified nucleotide thereof) or uridine (U) nucleotides (or a modified nucleotide thereof) in a sequence provided herein, including a sequence provided in the sequence listing, may be  
30 replaced with any other nucleotide suitable for base pairing (*e.g.*, via a Watson-Crick base pair) with an adenosine nucleotide. In some embodiments, any one or more thymidine (T)

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nucleotides (or modified nucleotide thereof) or uridine (U) nucleotides (or a modified nucleotide thereof) in a sequence provided herein, including a sequence provided in the sequence listing, may be suitably replaced with a different pyrimidine nucleotide or vice versa. In some embodiments, any one or more thymidine (T) nucleotides (or modified nucleotide thereof) in a sequence provided herein, including a sequence provided in the sequence listing, may be suitably replaced with a uridine (U) nucleotide (or a modified nucleotide thereof) or vice versa. In some embodiments, GC content of the single stranded oligonucleotide is preferably between about 30-60 %. Contiguous runs of three or more Gs or Cs may not be preferable in some embodiments. Accordingly, in some embodiments, the oligonucleotide does not comprise a stretch of three or more guanosine nucleotides.

In some embodiments, the single stranded oligonucleotide specifically binds to, or is complementary to an RNA that is encoded in a genome (e.g., a human genome) as a single contiguous transcript (e.g., a non-spliced RNA). In some embodiments, the single stranded oligonucleotide specifically binds to, or is complementary to an RNA that is encoded in a genome (e.g., a human genome), in which the distance in the genome between the 5' end of the coding region of the RNA and the 3' end of the coding region of the RNA is less than 1 kb, less than 2 kb, less than 3 kb, less than 4 kb, less than 5 kb, less than 7 kb, less than 8 kb, less than 9 kb, less than 10 kb, or less than 20 kb.

It is to be understood that any oligonucleotide provided herein can be excluded. In some embodiments, it has been found that single stranded oligonucleotides disclosed herein may increase expression of mRNA corresponding to the gene by at least about 50% (i.e. 150% of normal or 1.5 fold), or by about 2 fold to about 5 fold. In some embodiments, expression may be increased by at least about 15 fold, 20 fold, 30 fold, 40 fold, 50 fold or 100 fold, or any range between any of the foregoing numbers. It has also been found that increased mRNA expression has been shown to correlate to increased protein expression.

In some or any of the embodiments of the oligonucleotides described herein, or processes for designing or synthesizing them, the oligonucleotides will upregulate gene expression and may specifically bind or specifically hybridize or be complementary to the PRC2 binding RNA that is transcribed from the same strand as a protein coding reference gene. The oligonucleotide may bind to a region of the PRC2 binding RNA that originates within or overlaps an intron, exon, intron exon junction, 5' UTR, 3' UTR, a translation

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initiation region, or a translation termination region of a protein coding sense strand of a reference gene (refGene).

In some or any of the embodiments of oligonucleotides described herein, or processes for designing or synthesizing them, the oligonucleotides will upregulate gene expression and may specifically bind or specifically hybridize or be complementary to a PRC2 binding RNA that transcribed from the opposite strand (the antisense strand) of a protein coding reference gene. The oligonucleotide may bind to a region of the PRC2 binding RNA that originates within or overlaps an intron, exon, intron exon junction, 5' UTR, 3' UTR, a translation initiation region, or a translation termination region of a protein coding antisense strand of a reference gene

The oligonucleotides described herein may be modified, *e.g.*, comprise a modified sugar moiety, a modified internucleoside linkage, a modified nucleotide and/or combinations thereof. In addition, the oligonucleotides can exhibit one or more of the following properties: do not induce substantial cleavage or degradation of the target RNA; do not cause substantially complete cleavage or degradation of the target RNA; do not activate the RNase H pathway; do not activate RISC; do not recruit any Argonaute family protein; are not cleaved by Dicer; do not mediate alternative splicing; are not immune stimulatory; are nuclease resistant; have improved cell uptake compared to unmodified oligonucleotides; are not toxic to cells or mammals; may have improved endosomal exit; do interfere with interaction of lncRNA with PRC2, preferably the Ezh2 subunit but optionally the Suz12, Eed, RbAp46/48 subunits or accessory factors such as Jarid2; do decrease histone H3 lysine27 methylation and/or do upregulate gene expression.

Oligonucleotides that are designed to interact with RNA to modulate gene expression are a distinct subset of base sequences from those that are designed to bind a DNA target (*e.g.*, are complementary to the underlying genomic DNA sequence from which the RNA is transcribed).

Any of the oligonucleotides disclosed herein may be linked to one or more other oligonucleotides disclosed herein by a linker, *e.g.*, a cleavable linker.

*Method for Selecting Candidate Oligonucleotides for Activating Expression of MECP2*

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Methods are provided herein for selecting a candidate oligonucleotide for activating or enhancing expression of MECP2. The target selection methods may generally involve steps for selecting single stranded oligonucleotides having any of the structural and functional characteristics disclosed herein. Typically, the methods involve one or more steps aimed at  
5 identifying oligonucleotides that target a PRC2-associated region that is functionally related to MECP2, for example a PRC2-associated region of a lncRNA that regulates expression of MECP2 by facilitating (*e.g.*, in a *cis*-regulatory manner) the recruitment of PRC2 to the MECP2 gene. Such oligonucleotides are expected to be candidates for activating expression of MECP2 because of their ability to hybridize with the PRC2-associated region of a nucleic  
10 acid (*e.g.*, a lncRNA). In some embodiments, this hybridization event is understood to disrupt interaction of PRC2 with the nucleic acid (*e.g.*, a lncRNA) and as a result disrupt recruitment of PRC2 and its associated co-repressors (*e.g.*, chromatin remodeling factors) to the MECP2 gene locus.

Methods of selecting a candidate oligonucleotide may involve selecting a PRC2-  
15 associated region (*e.g.*, a nucleotide sequence as set forth in any one of SEQ ID NOS: 5 to 118) that maps to a chromosomal position encompassing or in proximity to the MECP2 gene (*e.g.*, a chromosomal position having a sequence as set forth in any one of SEQ ID NOS: 1 to 4). The PRC2-associated region may map to the strand of the chromosome comprising the sense strand of the MECP2 gene, in which case the candidate oligonucleotide is  
20 complementary to the sense strand of the MECP2 gene (*i.e.*, is antisense to the MECP2 gene). Alternatively, the PRC2-associated region may map to the strand of the first chromosome comprising the antisense strand of the MECP2 gene, in which case the oligonucleotide is complementary to the antisense strand (the template strand) of the MECP2 gene (*i.e.*, is sense to the MECP2 gene).

25 Methods for selecting a set of candidate oligonucleotides that is enriched in oligonucleotides that activate expression of MECP2 may involve selecting one or more PRC2-associated regions that map to a chromosomal position that encompasses or that is in proximity to the MECP2 gene and selecting a set of oligonucleotides, in which each oligonucleotide in the set comprises a nucleotide sequence that is complementary with the  
30 one or more PRC2-associated regions. As used herein, the phrase, “a set of oligonucleotides that is enriched in oligonucleotides that activate expression of” refers to a set of

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oligonucleotides that has a greater number of oligonucleotides that activate expression of a target gene (*e.g.*, MECP2) compared with a random selection of oligonucleotides of the same physicochemical properties (*e.g.*, the same GC content,  $T_m$ , length *etc.*) as the enriched set.

Where the design and/or synthesis of a single stranded oligonucleotide involves  
5 design and/or synthesis of a sequence that is complementary to a nucleic acid or PRC2-associated region described by such sequence information, the skilled person is readily able to determine the complementary sequence, *e.g.*, through understanding of Watson Crick base pairing rules which form part of the common general knowledge in the field.

In some embodiments design and/or synthesis of a single stranded oligonucleotide  
10 involves manufacture of an oligonucleotide from starting materials by techniques known to those of skill in the art, where the synthesis may be based on a sequence of a PRC2-associated region, or portion thereof.

Methods of design and/or synthesis of a single stranded oligonucleotide may involve one or more of the steps of:

15 Identifying and/or selecting PRC2-associated region;

Designing a nucleic acid sequence having a desired degree of sequence identity or complementarity to a PRC2-associated region or a portion thereof;

Synthesizing a single stranded oligonucleotide to the designed sequence;

Purifying the synthesized single stranded oligonucleotide; and

20 Optionally mixing the synthesized single stranded oligonucleotide with at least one pharmaceutically acceptable diluent, carrier or excipient to form a pharmaceutical composition or medicament.

Single stranded oligonucleotides so designed and/or synthesized may be useful in method of modulating gene expression as described herein.

25 Preferably, single stranded oligonucleotides of the invention are synthesized chemically. Oligonucleotides used to practice this invention can be synthesized *in vitro* by well-known chemical synthesis techniques.

Oligonucleotides of the invention can be stabilized against nucleolytic degradation such as by the incorporation of a modification, *e.g.*, a nucleotide modification. For example,  
30 nucleic acid sequences of the invention include a phosphorothioate at least the first, second, or third internucleotide linkage at the 5' or 3' end of the nucleotide sequence. As another

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example, the nucleic acid sequence can include a 2'-modified nucleotide, *e.g.*, a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O--N-methylacetamido (2'-O--NMA).

5 As another example, the nucleic acid sequence can include at least one 2'-O-methyl-modified nucleotide, and in some embodiments, all of the nucleotides include a 2'-O-methyl modification. In some embodiments, the nucleic acids are "locked," *i.e.*, comprise nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom and the 4'-C atom.

10 It is understood that any of the modified chemistries or formats of single stranded oligonucleotides described herein can be combined with each other, and that one, two, three, four, five, or more different types of modifications can be included within the same molecule.

In some embodiments, the method may further comprise the steps of amplifying the synthesized single stranded oligonucleotide, and/or purifying the single stranded  
15 oligonucleotide (or amplified single stranded oligonucleotide), and/or sequencing the single stranded oligonucleotide so obtained.

As such, the process of preparing a single stranded oligonucleotide may be a process that is for use in the manufacture of a pharmaceutical composition or medicament for use in the treatment of disease, optionally wherein the treatment involves modulating expression of  
20 a gene associated with a PRC2-associated region.

In the methods described above a PRC2-associated region may be, or have been, identified, or obtained, by a method that involves identifying RNA that binds to PRC2.

Such methods may involve the following steps: providing a sample containing nuclear ribonucleic acids, contacting the sample with an agent that binds specifically to PRC2 or a  
25 subunit thereof, allowing complexes to form between the agent and protein in the sample, partitioning the complexes, synthesizing nucleic acid that is complementary to nucleic acid present in the complexes.

Where the single stranded oligonucleotide is based on a PRC2-associated region, or a portion of such a sequence, it may be based on information about that sequence, *e.g.*,  
30 sequence information available in written or electronic form, which may include sequence information contained in publicly available scientific publications or sequence databases.

*Nucleotide Analogues*

In some embodiments, the oligonucleotide may comprise at least one ribonucleotide, at least one deoxyribonucleotide, and/or at least one bridged nucleotide. In some  
5 embodiments, the oligonucleotide may comprise a bridged nucleotide, such as a locked nucleic acid (LNA) nucleotide, a constrained ethyl (cEt) nucleotide, or an ethylene bridged nucleic acid (ENA) nucleotide. Examples of such nucleotides are disclosed herein and known in the art. In some embodiments, the oligonucleotide comprises a nucleotide analog disclosed in one of the following United States Patent or Patent Application Publications: US  
10 7,399,845, US 7,741,457, US 8,022,193, US 7,569,686, US 7,335,765, US 7,314,923, US 7,335,765, and US 7,816,333, US 20110009471, the entire contents of each of which are incorporated herein by reference for all purposes. The oligonucleotide may have one or more 2' O-methyl nucleotides. The oligonucleotide may consist entirely of 2' O-methyl nucleotides.

15 Often the single stranded oligonucleotide has one or more nucleotide analogues. For example, the single stranded oligonucleotide may have at least one nucleotide analogue that results in an increase in  $T_m$  of the oligonucleotide in a range of 1°C, 2 °C, 3°C, 4 °C, or 5°C compared with an oligonucleotide that does not have the at least one nucleotide analogue. The single stranded oligonucleotide may have a plurality of nucleotide analogues that results  
20 in a total increase in  $T_m$  of the oligonucleotide in a range of 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C or more compared with an oligonucleotide that does not have the nucleotide analogue.

The oligonucleotide may be of up to 50 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30, 2 to 40, 2 to 45, or more  
25 nucleotides of the oligonucleotide are nucleotide analogues. The oligonucleotide may be of 8 to 30 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30 nucleotides of the oligonucleotide are nucleotide analogues. The oligonucleotide may be of 8 to 15 nucleotides in length in which 2 to 4, 2 to 5, 2 to 6, 2 to 7, 2 to 8, 2 to 9, 2 to 10, 2 to 11, 2 to 12, 2 to 13, 2 to 14 nucleotides of the oligonucleotide  
30 are nucleotide analogues. Optionally, the oligonucleotides may have every nucleotide except 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides modified.

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The oligonucleotide may consist entirely of bridged nucleotides (*e.g.*, LNA nucleotides, cEt nucleotides, ENA nucleotides). The oligonucleotide may comprise alternating deoxyribonucleotides and 2'-fluoro-deoxyribonucleotides. The oligonucleotide may comprise alternating deoxyribonucleotides and 2'-O-methyl nucleotides. The oligonucleotide may comprise alternating deoxyribonucleotides and ENA nucleotide analogues. The oligonucleotide may comprise alternating deoxyribonucleotides and LNA nucleotides. The oligonucleotide may comprise alternating LNA nucleotides and 2'-O-methyl nucleotides. The oligonucleotide may have a 5' nucleotide that is a bridged nucleotide (*e.g.*, a LNA nucleotide, cEt nucleotide, ENA nucleotide). The oligonucleotide may have a 5' nucleotide that is a deoxyribonucleotide.

The oligonucleotide may comprise deoxyribonucleotides flanked by at least one bridged nucleotide (*e.g.*, a LNA nucleotide, cEt nucleotide, ENA nucleotide) on each of the 5' and 3' ends of the deoxyribonucleotides. The oligonucleotide may comprise deoxyribonucleotides flanked by 1, 2, 3, 4, 5, 6, 7, 8 or more bridged nucleotides (*e.g.*, LNA nucleotides, cEt nucleotides, ENA nucleotides) on each of the 5' and 3' ends of the deoxyribonucleotides. The 3' position of the oligonucleotide may have a 3' hydroxyl group. The 3' position of the oligonucleotide may have a 3' thiophosphate.

The oligonucleotide may be conjugated with a label. For example, the oligonucleotide may be conjugated with a biotin moiety, cholesterol, Vitamin A, folate, sigma receptor ligands, aptamers, peptides, such as CPP, hydrophobic molecules, such as lipids, ASGPR or dynamic polyconjugates and variants thereof at its 5' or 3' end.

Preferably the single stranded oligonucleotide comprises one or more modifications comprising: a modified sugar moiety, and/or a modified internucleoside linkage, and/or a modified nucleotide and/or combinations thereof. It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the modifications described herein may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide.

In some embodiments, the single stranded oligonucleotides are chimeric oligonucleotides that contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region of modified nucleotides that confers one or more beneficial properties (such as, for example, increased

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nuclease resistance, increased uptake into cells, increased binding affinity for the target) and a region that is a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. Chimeric single stranded oligonucleotides of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures comprise, but are not limited to, US patent nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

In some embodiments, the single stranded oligonucleotide comprises at least one nucleotide modified at the 2' position of the sugar, most preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA. Such modifications are routinely incorporated into oligonucleotides and these oligonucleotides have been shown to have a higher  $T_m$  (i.e., higher target binding affinity) than 2'-deoxyoligonucleotides against a given target.

A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide; these modified oligos survive intact for a longer time than unmodified oligonucleotides. Specific examples of modified oligonucleotides include those comprising modified backbones, for example, phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are oligonucleotides with phosphorothioate backbones and those with heteroatom backbones, particularly  $\text{CH}_2\text{-NH-O-CH}_2$ ,  $\text{CH}_2\text{-N(CH}_3\text{)-O-CH}_2$  (known as a methylene(methylimino) or MMI backbone,  $\text{CH}_2\text{-O-N(CH}_3\text{)-CH}_2$ ,  $\text{CH}_2\text{-N(CH}_3\text{)-N(CH}_3\text{)-CH}_2$  and  $\text{O-N(CH}_3\text{)-CH}_2\text{-CH}_2$  backbones, wherein the native phosphodiester backbone is represented as  $\text{O-P-O-CH}_2$ ); amide backbones (see De Mesmaeker et al. *Ace. Chem. Res.* 1995, 28:366-374); morpholino backbone structures (see Summerton and Weller, U.S. Pat. No. 5,034,506); peptide nucleic

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acid (PNA) backbone (wherein the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen et al., Science 1991, 254, 1497).

Phosphorus-containing linkages include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

Morpholino-based oligomeric compounds are described in Dwaine A. Braasch and David R. Corey, Biochemistry, 2002, 41(14), 4503-4510; Genesis, volume 30, issue 3, 2001; Heasman, J., Dev. Biol., 2002, 243, 209-214; Nasevicius et al., Nat. Genet., 2000, 26, 216-220; Lacerra et al., Proc. Natl. Acad. Sci., 2000, 97, 9591-9596; and U.S. Pat. No. 5,034,506, issued Jul. 23, 1991. In some embodiments, the morpholino-based oligomeric compound is a phosphorodiamidate morpholino oligomer (PMO) (*e.g.*, as described in Iverson, Curr. Opin. Mol. Ther., 3:235-238, 2001; and Wang et al., J. Gene Med., 12:354-364, 2010; the disclosures of which are incorporated herein by reference in their entireties).

Cyclohexenyl nucleic acid oligonucleotide mimetics are described in Wang et al., J. Am. Chem. Soc., 2000, 122, 8595-8602.

Modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These comprise those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing

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backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts; see US patent nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264, 562; 5, 264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596, 086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623, 070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

Modified oligonucleotides are also known that include oligonucleotides that are based on or constructed from arabinonucleotide or modified arabinonucleotide residues.

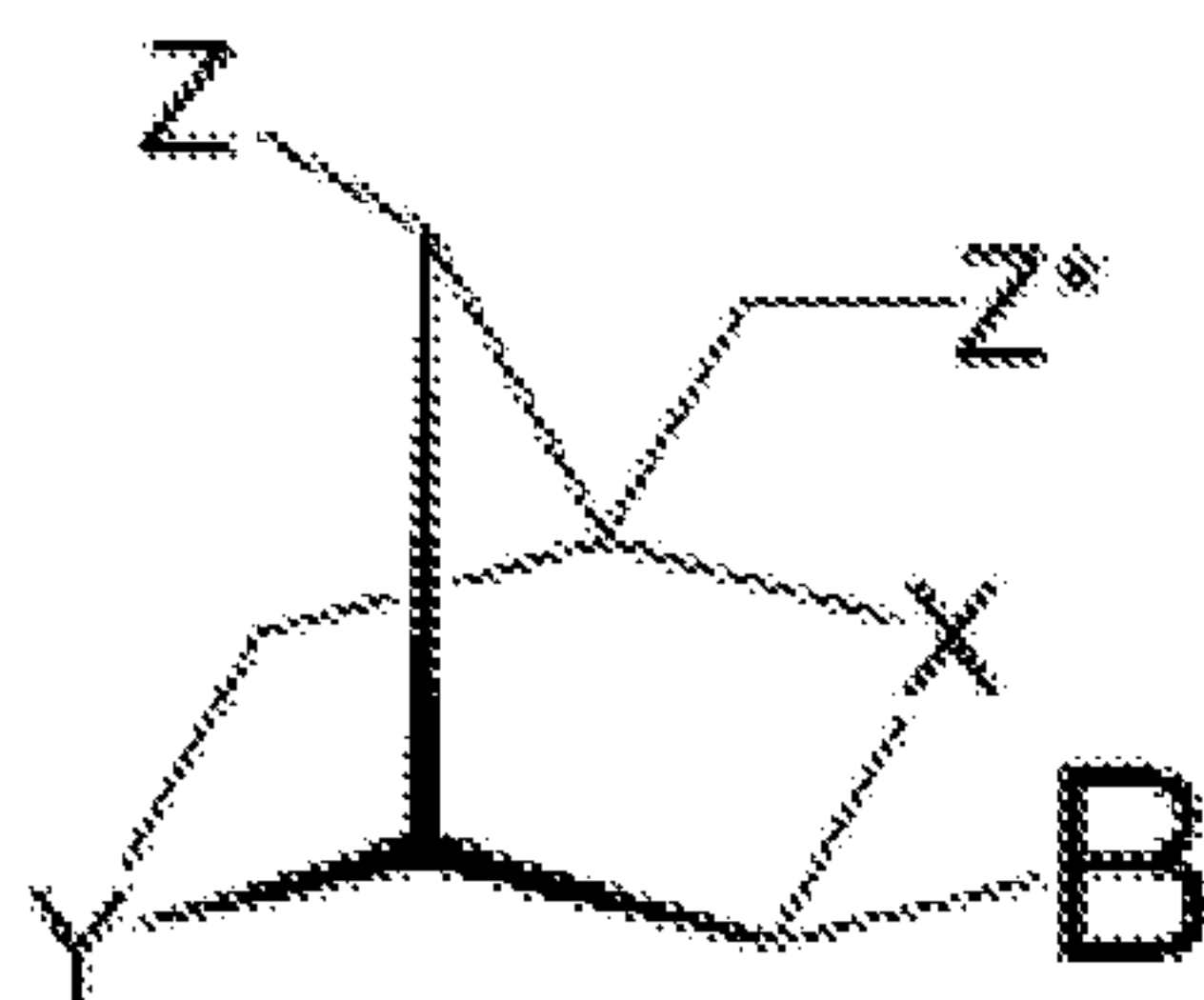
Arabinonucleosides are stereoisomers of ribonucleosides, differing only in the configuration at the 2'-position of the sugar ring. In some embodiments, a 2'-arabino modification is 2'-F arabino. In some embodiments, the modified oligonucleotide is 2'-fluoro-D-arabinonucleic acid (FANA) (as described in, for example, Lon et al., *Biochem.*, 41:3457-3467, 2002 and Min et al., *Bioorg. Med. Chem. Lett.*, 12:2651-2654, 2002; the disclosures of which are incorporated herein by reference in their entireties). Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on a 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide.

PCT Publication No. WO 99/67378 discloses arabinonucleic acids (ANA) oligomers and their analogues for improved sequence specific inhibition of gene expression via association to complementary messenger RNA.

Other preferred modifications include ethylene-bridged nucleic acids (ENAs) (*e.g.*, International Patent Publication No. WO 2005/042777, Morita et al., *Nucleic Acid Res.*, Suppl 1:241-242, 2001; Surono et al., *Hum. Gene Ther.*, 15:749-757, 2004; Koizumi, *Curr. Opin. Mol. Ther.*, 8:144-149, 2006 and Horie et al., *Nucleic Acids Symp. Ser (Oxf)*, 49:171-172, 2005; the disclosures of which are incorporated herein by reference in their entireties). Preferred ENAs include, but are not limited to, 2'-O,4'-C-ethylene-bridged nucleic acids.

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Examples of LNAs are described in WO/2008/043753 and include compounds of the following general formula.



5

where X and Y are independently selected among the groups -O-,

-S-, -N(H)-, N(R)-, -CH<sub>2</sub>- or -CH- (if part of a double bond),

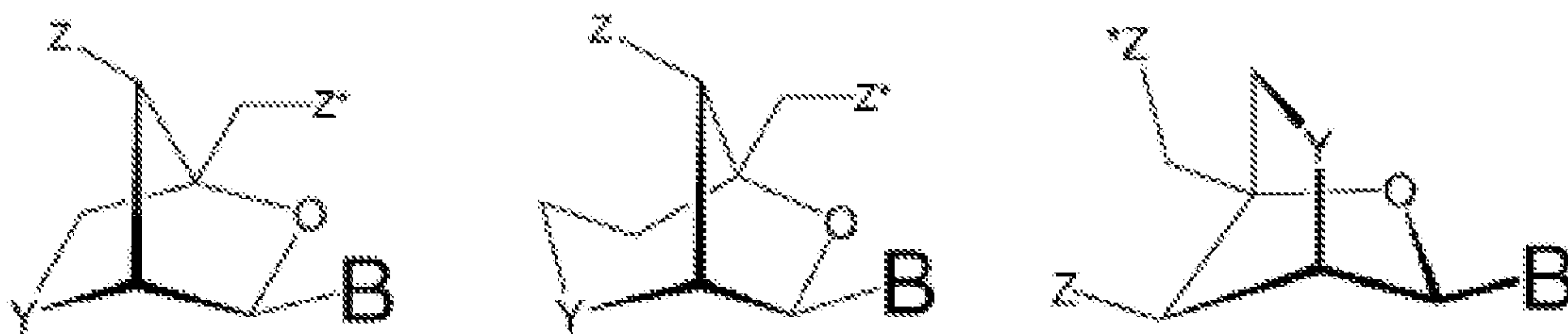
-CH<sub>2</sub>-O-, -CH<sub>2</sub>-S-, -CH<sub>2</sub>-N(H)-, -CH<sub>2</sub>-N(R)-, -CH<sub>2</sub>-CH<sub>2</sub>- or -CH<sub>2</sub>-CH- (if part of a double bond),

10

-CH=CH-, where R is selected from hydrogen and C<sub>1-4</sub>-alkyl; Z and Z\* are independently selected among an internucleoside linkage, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety; and the asymmetric groups may be found in either orientation.

15

Preferably, the LNA used in the oligonucleotides described herein comprises at least one LNA unit according any of the formulas



20

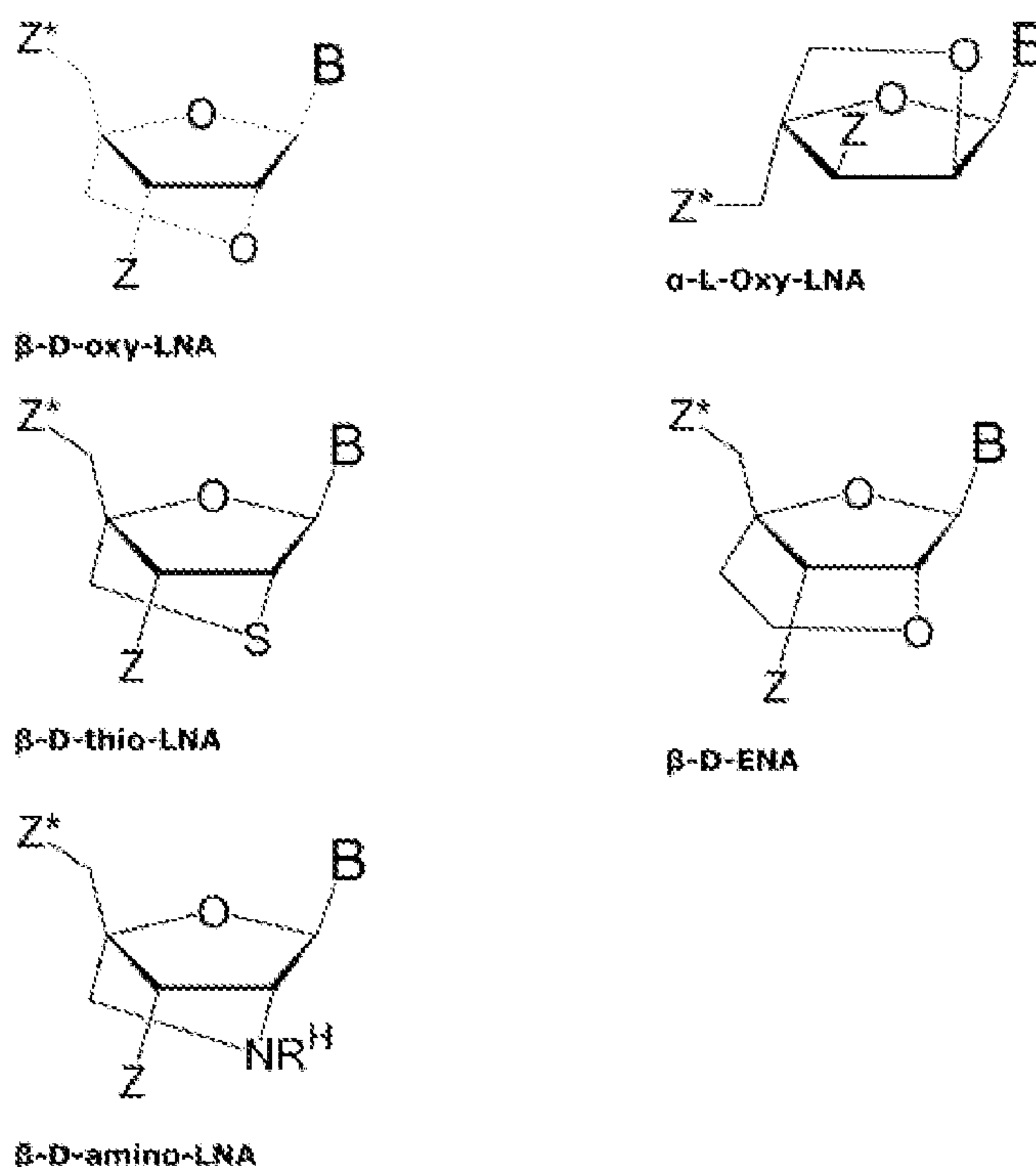
wherein Y is -O-, -S-, -NH-, or N(R<sup>H</sup>); Z and Z\* are independently selected among an internucleoside linkage, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base moiety, and RH is selected from hydrogen and C<sub>1-4</sub>-alkyl.

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In some embodiments, the Locked Nucleic Acid (LNA) used in the oligonucleotides described herein comprises at least one Locked Nucleic Acid (LNA) unit according any of the formulas shown in Scheme 2 of PCT/DK2006/000512.

In some embodiments, the LNA used in the oligomer of the invention comprises internucleoside linkages selected from -O-P(O)<sub>2</sub>-O-, -O-P(O,S)-O-, -O-P(S)<sub>2</sub>-O-, -S-P(O)<sub>2</sub>-O-, -S-P(O,S)-O-, -S-P(S)<sub>2</sub>-O-, -O-P(O)<sub>2</sub>-S-, -O-P(O,S)-S-, -S-P(O)<sub>2</sub>-S-, -O-PO(R<sup>H</sup>)-O-, O-PO(OCH<sub>3</sub>)-O-, -O-PO(NR<sup>H</sup>)-O-, -O-PO(OCH<sub>2</sub>CH<sub>2</sub>S-R)-O-, -O-PO(BH<sub>3</sub>)-O-, -O-PO(NHR<sup>H</sup>)-O-, -O-P(O)<sub>2</sub>-NR<sup>H</sup>-, -NR<sup>H</sup>-P(O)<sub>2</sub>-O-, -NR<sup>H</sup>-CO-O-, where R<sup>H</sup> is selected from hydrogen and C<sub>1-4</sub>-alkyl.

Specifically preferred LNA units are shown in scheme 2:



**Scheme 2**

The term "thio-LNA" comprises a locked nucleotide in which at least one of X or Y in the general formula above is selected from S or -CH<sub>2</sub>-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.

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The term "amino-LNA" comprises a locked nucleotide in which at least one of X or Y in the general formula above is selected from -N(H)-, N(R)-, CH<sub>2</sub>-N(H)-, and -CH<sub>2</sub>-N(R)- where R is selected from hydrogen and C<sub>1-4</sub>-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

5 The term "oxy-LNA" comprises a locked nucleotide in which at least one of X or Y in the general formula above represents -O- or -CH<sub>2</sub>-O-. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ena-LNA" comprises a locked nucleotide in which Y in the general formula above is -CH<sub>2</sub>-O- (where the oxygen atom of -CH<sub>2</sub>-O- is attached to the 2'-position relative to  
10 the base B).

LNAs are described in additional detail herein.

One or more substituted sugar moieties can also be included, *e.g.*, one of the following at the 2' position: OH, SH, SCH<sub>3</sub>, F, OCN, OCH<sub>3</sub>, OCH<sub>3</sub>, OCH<sub>3</sub> O(CH<sub>2</sub>)<sub>n</sub> CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub> NH<sub>2</sub> or O(CH<sub>2</sub>)<sub>n</sub> CH<sub>3</sub> where n is from 1 to about 10; C<sub>1</sub> to C<sub>10</sub> lower alkyl,  
15 alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF<sub>3</sub>; OCF<sub>3</sub>; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH<sub>3</sub>; SO<sub>2</sub> CH<sub>3</sub>; ONO<sub>2</sub>; NO<sub>2</sub>; N<sub>3</sub>; NH<sub>2</sub>; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an  
20 oligonucleotide and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy [2'-O-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, also known as 2'-O-(2-methoxyethyl)] (Martin et al, *Helv. Chim. Acta*, 1995, 78, 486). Other preferred modifications include 2'-methoxy (2'-O-CH<sub>3</sub>), 2'-propoxy (2'-OCH<sub>2</sub> CH<sub>2</sub>CH<sub>3</sub>) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3'  
25 position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

Single stranded oligonucleotides can also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As  
30 used herein, "unmodified" or "natural" nucleobases include adenine (A), guanine (G),

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thymine (T), cytosine (C) and uracil (U). Modified nucleobases include nucleobases found only infrequently or transiently in natural nucleic acids, *e.g.*, hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, isocytosine, pseudoisocytosine, as well as synthetic nucleobases, *e.g.*, 2-aminoadenine, 2- (methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalkylamino)adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 5-propynyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl)adenine, 6-aminopurine, 2-aminopurine, 2-chloro-6-aminopurine and 2,6-diaminopurine or other diaminopurines. See, *e.g.*, Kornberg, "DNA Replication," W. H. Freeman & Co., San Francisco, 1980, pp75-77; and Gebeyehu, G., et al. Nucl. Acids Res., 15:4513 (1987)). A "universal" base known in the art, *e.g.*, inosine, can also be included. 5-Me-C substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, in Crooke, and Lebleu, eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and may be used as base substitutions.

It is not necessary for all positions in a given oligonucleotide to be uniformly modified, and in fact more than one of the modifications described herein may be incorporated in a single oligonucleotide or even at within a single nucleoside within an oligonucleotide.

In some embodiments, both a sugar and an internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, for example, an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by

reference. Further teaching of PNA compounds can be found in Nielsen et al, Science, 1991, 254, 1497-1500.

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

Further, nucleobases comprise those disclosed in United States Patent No. 3,687,808, those disclosed in "The Concise Encyclopedia of Polymer Science And Engineering", pages 858-859, Kroschwitz, ed. John Wiley & Sons, 1990; those disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, page 613, and those disclosed by Sanghvi, Chapter 15, Antisense Research and Applications," pages 289- 302, Crooke, and Lebleu, eds., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, comprising 2-aminopropyladenine, 5-propynyluracil and 5- propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, et al., eds, "Antisense Research and Applications," CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications. Modified nucleobases are described in US patent nos. 3,687,808, as well as 4,845,205; 5,130,302; 5,134,066; 5,175, 273; 5, 367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177;

5,525,711; 5,552,540; 5,587,469; 5,596,091; 5,614,617; 5,750,692, and 5,681,941, each of which is herein incorporated by reference.

In some embodiments, the single stranded oligonucleotides are chemically linked to one or more moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. For example, one or more single stranded oligonucleotides, of the same or different types, can be conjugated to each other; or single stranded oligonucleotides can be conjugated to targeting moieties with enhanced specificity for a cell type or tissue type. Such moieties include, but are not limited to, lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060), a thioether, *e.g.*, hexyl-S- tritylthiol (Manoharan et al, Ann. N. Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49- 54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl- rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Mancharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-t oxysterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937). See also US patent nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552, 538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082, 830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5, 245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391, 723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5, 565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599, 928 and 5,688,941, each of which is herein incorporated by reference.

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These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application No. PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, which are incorporated herein by reference. Conjugate moieties include, but are not limited to, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, *e.g.*, hexyl-5-tritylthiol, a thiocholesterol, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a phospholipid, *e.g.*, di-hexadecyl-rac- glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxy cholesterol moiety. See, *e.g.*, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

In some embodiments, single stranded oligonucleotide modification include modification of the 5' or 3' end of the oligonucleotide. In some embodiments, the 3' end of the oligonucleotide comprises a hydroxyl group or a thiophosphate. It should be appreciated that additional molecules (*e.g.* a biotin moiety or a fluorophor) can be conjugated to the 5' or

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3' end of the single stranded oligonucleotide. In some embodiments, the single stranded oligonucleotide comprises a biotin moiety conjugated to the 5' nucleotide.

In some embodiments, the single stranded oligonucleotide comprises locked nucleic acids (LNA), ENA modified nucleotides, 2'-O-methyl nucleotides, or 2'-fluoro-deoxyribonucleotides. In some embodiments, the single stranded oligonucleotide comprises alternating deoxyribonucleotides and 2'-fluoro-deoxyribonucleotides. In some embodiments, the single stranded oligonucleotide comprises alternating deoxyribonucleotides and 2'-O-methyl nucleotides. In some embodiments, the single stranded oligonucleotide comprises alternating deoxyribonucleotides and ENA modified nucleotides. In some embodiments, the single stranded oligonucleotide comprises alternating deoxyribonucleotides and locked nucleic acid nucleotides. In some embodiments, the single stranded oligonucleotide comprises alternating locked nucleic acid nucleotides and 2'-O-methyl nucleotides.

In some embodiments, the 5' nucleotide of the oligonucleotide is a deoxyribonucleotide. In some embodiments, the 5' nucleotide of the oligonucleotide is a locked nucleic acid nucleotide. In some embodiments, the nucleotides of the oligonucleotide comprise deoxyribonucleotides flanked by at least one locked nucleic acid nucleotide on each of the 5' and 3' ends of the deoxyribonucleotides. In some embodiments, the nucleotide at the 3' position of the oligonucleotide has a 3' hydroxyl group or a 3' thiophosphate.

In some embodiments, the single stranded oligonucleotide comprises phosphorothioate internucleotide linkages. In some embodiments, the single stranded oligonucleotide comprises phosphorothioate internucleotide linkages between at least two nucleotides. In some embodiments, the single stranded oligonucleotide comprises phosphorothioate internucleotide linkages between all nucleotides.

It should be appreciated that the single stranded oligonucleotide can have any combination of modifications as described herein.

The oligonucleotide may comprise a nucleotide sequence having one or more of the following modification patterns.

- (a) (X)XXXXXX, (X)xXXXXX, (X)xxXXXX, (X)xxxXXX, (X)xxxxXx and (X)xxxxxX,
- (b) (X)XXXXXX, (X)XxXXXX, (X)XxxXXX, (X)XxxxXx, (X)XxxxxX, (X)xXXxxx,
- (X)xXxXxx, (X)xXxxXx, (X)xXxxxX, (X)xxXXxx, (X)xxXxXx, (X)xxXxxX, (X)xxxXXx,
- (X)xxxXxX and (X)xxxxXX,

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(c) (X)XXXXxx, (X)xXXXXxx, (X)xxXXXXx, (X)xxxXXX, (X)XXxXxx, (X)XXxxXx,  
 (X)XXxxxX, (X)xXXxXx, (X)xXXxxX, (X)xxXXxX, (X)XxXXxx, (X)XxxXXx  
 (X)XxxxXX, (X)xXxXXx, (X)xXxxXX, (X)xxXxXX, (X)xXxXxX and (X)XxXxXx,

(d) (X)xxXXX, (X)xXxXXX, (X)xXXxXX, (X)xXXXxX, (X)xXXXXx,  
 5 (X)XxxXXXX, (X)XxXxXX, (X)XxXXxX, (X)XxXXx, (X)XXxxXX, (X)XXxXxX,  
 (X)XXxXXx, (X)XXXxxX, (X)XXXxXx, and (X)XXXXxx,

(e) (X)xXXXXX, (X)XxXXXX, (X)XXxXXX, (X)XXXxXX, (X)XXXXxX and  
 (X)XXXXXx, and

(f) XXXXXX, XxXXXXX, XXxXXXX, XXXxXXX, XXXXxXX, XXXXXxX and  
 10 XXXXXXx, in which “X” denotes a nucleotide analogue, (X) denotes an optional nucleotide  
 analogue, and “x” denotes a DNA or RNA nucleotide unit. Each of the above listed patterns  
 may appear one or more times within an oligonucleotide, alone or in combination with any of  
 the other disclosed modification patterns.

### 15 *Methods for Modulating Gene Expression*

In one aspect, the invention relates to methods for modulating gene expression in a  
 cell (*e.g.*, a cell for which MECP2 levels are reduced) for research purposes (*e.g.*, to study the  
 function of the gene in the cell). In another aspect, the invention relates to methods for  
 modulating gene expression in a cell (*e.g.*, a cell for which MECP2 levels are reduced) for  
 20 gene or epigenetic therapy. The cells can be *in vitro*, *ex vivo*, or *in vivo* (*e.g.*, in a subject who  
 has a disease resulting from reduced expression or activity of MECP2. In some  
 embodiments, methods for modulating gene expression in a cell comprise delivering a single  
 stranded oligonucleotide as described herein. In some embodiments, delivery of the single  
 stranded oligonucleotide to the cell results in a level of expression of gene that is at least 5%,  
 25 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200% or more greater than a level  
 of expression of gene in a control cell to which the single stranded oligonucleotide has not  
 been delivered. In certain embodiments, delivery of the single stranded oligonucleotide to  
 the cell results in a level of expression of gene that is at least 50% greater than a level of  
 expression of gene in a control cell to which the single stranded oligonucleotide has not been  
 30 delivered.

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In another aspect of the invention, methods comprise administering to a subject (*e.g.* a human) a composition comprising a single stranded oligonucleotide as described herein to increase protein levels in the subject. In some embodiments, the increase in protein levels is at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or more, higher than the amount of a protein in the subject before administering.

As another example, to increase expression of MECP2 in a cell, the methods include introducing into the cell a single stranded oligonucleotide that is sufficiently complementary to a PRC2-associated region (*e.g.*, of a long non-coding RNA) that maps to a genomic position encompassing or in proximity to the MECP2 gene.

In another aspect of the invention provides methods of treating a condition (*e.g.*, Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, PPM-X syndrome) associated with decreased levels of expression of MECP2 in a subject, the method comprising administering a single stranded oligonucleotide as described herein.

A subject can include a non-human mammal, *e.g.* mouse, rat, guinea pig, rabbit, cat, dog, goat, cow, or horse. In preferred embodiments, a subject is a human. Single stranded oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Single stranded oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

For therapeutics, an animal, preferably a human, suspected of having Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, or PPM-X syndrome is treated by administering single stranded oligonucleotide in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of a single stranded oligonucleotide as described herein.

#### *Formulation, Delivery, And Dosing*

The oligonucleotides described herein can be formulated for administration to a subject for treating a condition (*e.g.*, Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, PPM-X syndrome) associated with decreased levels of

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MECP2. It should be understood that the formulations, compositions and methods can be practiced with any of the oligonucleotides disclosed herein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active  
5 ingredient (*e.g.*, an oligonucleotide or compound of the invention) which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration, *e.g.*, intradermal or inhalation. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a  
10 therapeutic effect, *e.g.* tumor regression.

Pharmaceutical formulations of this invention can be prepared according to any method known to the art for the manufacture of pharmaceuticals. Such formulations can contain sweetening agents, flavoring agents, coloring agents and preserving agents. A formulation can be admixed with nontoxic pharmaceutically acceptable excipients which  
15 are suitable for manufacture. Formulations may comprise one or more diluents, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, lyophilized powders, sprays, creams, lotions, controlled release formulations, tablets, pills, gels, on patches, in implants, etc.

A formulated single stranded oligonucleotide composition can assume a variety of  
20 states. In some examples, the composition is at least partially crystalline, uniformly crystalline, and/or anhydrous (*e.g.*, less than 80, 50, 30, 20, or 10% water). In another example, the single stranded oligonucleotide is in an aqueous phase, *e.g.*, in a solution that includes water. The aqueous phase or the crystalline compositions can, *e.g.*, be incorporated into a delivery vehicle, *e.g.*, a liposome (particularly for the aqueous phase) or a particle (*e.g.*,  
25 a microparticle as can be appropriate for a crystalline composition). Generally, the single stranded oligonucleotide composition is formulated in a manner that is compatible with the intended method of administration.

In some embodiments, the composition is prepared by at least one of the following methods: spray drying, lyophilization, vacuum drying, evaporation, fluid bed drying, or a  
30 combination of these techniques; or sonication with a lipid, freeze-drying, condensation and other self-assembly.

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A single stranded oligonucleotide preparation can be formulated or administered (together or separately) in combination with another agent, *e.g.*, another therapeutic agent or an agent that stabilizes a single stranded oligonucleotide, *e.g.*, a protein that complexes with single stranded oligonucleotide. Still other agents include chelators, *e.g.*, EDTA (*e.g.*, to  
5 remove divalent cations such as  $Mg^{2+}$ ), salts, RNase inhibitors (*e.g.*, a broad specificity RNase inhibitor such as RNasin) and so forth.

In one embodiment, the single stranded oligonucleotide preparation includes another single stranded oligonucleotide, *e.g.*, a second single stranded oligonucleotide that modulates expression of a second gene or a second single stranded oligonucleotide that modulates  
10 expression of the first gene. Still other preparation can include at least 3, 5, ten, twenty, fifty, or a hundred or more different single stranded oligonucleotide species. Such single stranded oligonucleotides can mediated gene expression with respect to a similar number of different genes. In one embodiment, the single stranded oligonucleotide preparation includes at least a second therapeutic agent (*e.g.*, an agent other than an oligonucleotide).

### *Route of Delivery*

A composition that includes a single stranded oligonucleotide can be delivered to a subject by a variety of routes. Exemplary routes include: intravenous, intradermal, topical, rectal, parenteral, anal, intravaginal, intranasal, pulmonary, ocular. The term "therapeutically  
20 effective amount" is the amount of oligonucleotide present in the composition that is needed to provide the desired level of MECP2 expression in the subject to be treated to give the anticipated physiological response. The term "physiologically effective amount" is that amount delivered to a subject to give the desired palliative or curative effect. The term "pharmaceutically acceptable carrier" means that the carrier can be administered to a subject  
25 with no significant adverse toxicological effects to the subject.

The single stranded oligonucleotide molecules of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of single stranded oligonucleotide and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is  
30 intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with

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pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

5           The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or  
10   intraventricular administration.

          The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the single stranded oligonucleotide in aerosol form. The vascular endothelial cells could be targeted by coating a  
15   balloon catheter with the single stranded oligonucleotide and mechanically introducing the oligonucleotide.

          Topical administration refers to the delivery to a subject by contacting the formulation directly to a surface of the subject. The most common form of topical delivery is to the skin, but a composition disclosed herein can also be directly applied to other surfaces of the body,  
20   *e.g.*, to the eye, a mucous membrane, to surfaces of a body cavity or to an internal surface. As mentioned above, the most common topical delivery is to the skin. The term encompasses several routes of administration including, but not limited to, topical and transdermal. These modes of administration typically include penetration of the skin's permeability barrier and efficient delivery to the target tissue or stratum. Topical administration can be used as a  
25   means to penetrate the epidermis and dermis and ultimately achieve systemic delivery of the composition. Topical administration can also be used as a means to selectively deliver oligonucleotides to the epidermis or dermis of a subject, or to specific strata thereof, or to an underlying tissue.

          Formulations for topical administration may include transdermal patches, ointments,  
30   lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional

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pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Transdermal delivery is a valuable route for the administration of lipid soluble therapeutics. The dermis is more permeable than the epidermis and therefore absorption is much more rapid through abraded, burned or denuded skin. Inflammation and other physiologic conditions that increase blood flow to the skin also enhance transdermal adsorption. Absorption via this route may be enhanced by the use of an oily vehicle (inunction) or through the use of one or more penetration enhancers. Other effective ways to deliver a composition disclosed herein via the transdermal route include hydration of the skin and the use of controlled release topical patches. The transdermal route provides a potentially effective means to deliver a composition disclosed herein for systemic and/or local therapy. In addition, iontophoresis (transfer of ionic solutes through biological membranes under the influence of an electric field), phonophoresis or sonophoresis (use of ultrasound to enhance the absorption of various therapeutic agents across biological membranes, notably the skin and the cornea), and optimization of vehicle characteristics relative to dose position and retention at the site of administration may be useful methods for enhancing the transport of topically applied compositions across skin and mucosal sites.

Both the oral and nasal membranes offer advantages over other routes of administration. For example, oligonucleotides administered through these membranes may have a rapid onset of action, provide therapeutic plasma levels, avoid first pass effect of hepatic metabolism, and avoid exposure of the oligonucleotides to the hostile gastrointestinal (GI) environment. Additional advantages include easy access to the membrane sites so that the oligonucleotide can be applied, localized and removed easily.

In oral delivery, compositions can be targeted to a surface of the oral cavity, *e.g.*, to sublingual mucosa which includes the membrane of ventral surface of the tongue and the floor of the mouth or the buccal mucosa which constitutes the lining of the cheek. The sublingual mucosa is relatively permeable thus giving rapid absorption and acceptable bioavailability of many agents. Further, the sublingual mucosa is convenient, acceptable and easily accessible.

A pharmaceutical composition of single stranded oligonucleotide may also be administered to the buccal cavity of a human being by spraying into the cavity, without

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inhalation, from a metered dose spray dispenser, a mixed micellar pharmaceutical formulation as described above and a propellant. In one embodiment, the dispenser is first shaken prior to spraying the pharmaceutical formulation and propellant into the buccal cavity.

Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, slurries, emulsions, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents can be added.

Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, intrathecal or intraventricular administration. In some embodiments, parental administration involves administration directly to the site of disease (*e.g.* injection into a tumor).

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic.

Any of the single stranded oligonucleotides described herein can be administered to ocular tissue. For example, the compositions can be applied to the surface of the eye or nearby tissue, *e.g.*, the inside of the eyelid. For ocular administration, ointments or droppable liquids may be delivered by ocular delivery systems known to the art such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or poly(vinyl alcohol), preservatives such as sorbic acid, EDTA or benzylchromium chloride, and the usual quantities of diluents and/or carriers. The single stranded oligonucleotide can also be administered to the interior of the eye, and can be introduced by a needle or other delivery device which can introduce it to a selected area or structure.

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Pulmonary delivery compositions can be delivered by inhalation by the patient of a dispersion so that the composition, preferably single stranded oligonucleotides, within the dispersion can reach the lung where it can be readily absorbed through the alveolar region directly into blood circulation. Pulmonary delivery can be effective both for systemic  
5 delivery and for localized delivery to treat diseases of the lungs.

Pulmonary delivery can be achieved by different approaches, including the use of nebulized, aerosolized, micellular and dry powder-based formulations. Delivery can be achieved with liquid nebulizers, aerosol-based inhalers, and dry powder dispersion devices. Metered-dose devices are preferred. One of the benefits of using an atomizer or inhaler is  
10 that the potential for contamination is minimized because the devices are self-contained. Dry powder dispersion devices, for example, deliver agents that may be readily formulated as dry powders. A single stranded oligonucleotide composition may be stably stored as lyophilized or spray-dried powders by itself or in combination with suitable powder carriers. The delivery of a composition for inhalation can be mediated by a dosing timing element which  
15 can include a timer, a dose counter, time measuring device, or a time indicator which when incorporated into the device enables dose tracking, compliance monitoring, and/or dose triggering to a patient during administration of the aerosol medicament.

The term "powder" means a composition that consists of finely dispersed solid particles that are free flowing and capable of being readily dispersed in an inhalation device  
20 and subsequently inhaled by a subject so that the particles reach the lungs to permit penetration into the alveoli. Thus, the powder is said to be "respirable." Preferably the average particle size is less than about 10  $\mu\text{m}$  in diameter preferably with a relatively uniform spheroidal shape distribution. More preferably the diameter is less than about 7.5  $\mu\text{m}$  and most preferably less than about 5.0  $\mu\text{m}$ . Usually the particle size distribution is between  
25 about 0.1  $\mu\text{m}$  and about 5  $\mu\text{m}$  in diameter, particularly about 0.3  $\mu\text{m}$  to about 5  $\mu\text{m}$ .

The term "dry" means that the composition has a moisture content below about 10% by weight (% w) water, usually below about 5% w and preferably less it than about 3% w. A dry composition can be such that the particles are readily dispersible in an inhalation device to form an aerosol.

30 The types of pharmaceutical excipients that are useful as carrier include stabilizers such as human serum albumin (HSA), bulking agents such as carbohydrates, amino acids and

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polypeptides; pH adjusters or buffers; salts such as sodium chloride; and the like. These carriers may be in a crystalline or amorphous form or may be a mixture of the two.

Suitable pH adjusters or buffers include organic salts prepared from organic acids and bases, such as sodium citrate, sodium ascorbate, and the like; sodium citrate is preferred.

5 Pulmonary administration of a micellar single stranded oligonucleotide formulation may be achieved through metered dose spray devices with propellants such as tetrafluoroethane, heptafluoroethane, dimethylfluoropropane, tetrafluoropropane, butane, isobutane, dimethyl ether and other non-CFC and CFC propellants.

Exemplary devices include devices which are introduced into the vasculature, *e.g.*,  
10 devices inserted into the lumen of a vascular tissue, or which devices themselves form a part of the vasculature, including stents, catheters, heart valves, and other vascular devices. These devices, *e.g.*, catheters or stents, can be placed in the vasculature of the lung, heart, or leg.

Other devices include non-vascular devices, *e.g.*, devices implanted in the peritoneum, or in organ or glandular tissue, *e.g.*, artificial organs. The device can release a  
15 therapeutic substance in addition to a single stranded oligonucleotide, *e.g.*, a device can release insulin.

In one embodiment, unit doses or measured doses of a composition that includes single stranded oligonucleotide are dispensed by an implanted device. The device can include a sensor that monitors a parameter within a subject. For example, the device can  
20 include pump, *e.g.*, and, optionally, associated electronics.

Tissue, *e.g.*, cells or organs can be treated with a single stranded oligonucleotide, *ex vivo* and then administered or implanted in a subject. The tissue can be autologous, allogeneic, or xenogeneic tissue. *E.g.*, tissue can be treated to reduce graft v. host disease. In other embodiments, the tissue is allogeneic and the tissue is treated to treat a disorder  
25 characterized by unwanted gene expression in that tissue. *E.g.*, tissue, *e.g.*, hematopoietic cells, *e.g.*, bone marrow hematopoietic cells, can be treated to inhibit unwanted cell proliferation. Introduction of treated tissue, whether autologous or transplant, can be combined with other therapies. In some implementations, the single stranded oligonucleotide treated cells are insulated from other cells, *e.g.*, by a semi-permeable porous barrier that  
30 prevents the cells from leaving the implant, but enables molecules from the body to reach the

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cells and molecules produced by the cells to enter the body. In one embodiment, the porous barrier is formed from alginate.

In one embodiment, a contraceptive device is coated with or contains a single stranded oligonucleotide. Exemplary devices include condoms, diaphragms, IUD  
5 (implantable uterine devices, sponges, vaginal sheaths, and birth control devices.

### *Dosage*

In one aspect, the invention features a method of administering a single stranded oligonucleotide (e.g., as a compound or as a component of a composition) to a subject (e.g., a  
10 human subject). In one embodiment, the unit dose is between about 10 mg and 25 mg per kg of bodyweight. In one embodiment, the unit dose is between about 1 mg and 100 mg per kg of bodyweight. In one embodiment, the unit dose is between about 0.1 mg and 500 mg per kg of bodyweight. In some embodiments, the unit dose is more than 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 25, 50 or 100 mg per kg of bodyweight.

15 The defined amount can be an amount effective to treat or prevent a disease or disorder, e.g., a disease or disorder associated with the MECP2. The unit dose, for example, can be administered by injection (e.g., intravenous or intramuscular), an inhaled dose, or a topical application.

In some embodiments, the unit dose is administered daily. In some embodiments, less  
20 frequently than once a day, e.g., less than every 2, 4, 8 or 30 days. In another embodiment, the unit dose is not administered with a frequency (e.g., not a regular frequency). For example, the unit dose may be administered a single time. In some embodiments, the unit dose is administered more than once a day, e.g., once an hour, two hours, four hours, eight hours, twelve hours, etc.

25 In one embodiment, a subject is administered an initial dose and one or more maintenance doses of a single stranded oligonucleotide. The maintenance dose or doses are generally lower than the initial dose, e.g., one-half less of the initial dose. A maintenance regimen can include treating the subject with a dose or doses ranging from 0.0001 to 100 mg/kg of body weight per day, e.g., 100, 10, 1, 0.1, 0.01, 0.001, or 0.0001 mg per kg of  
30 bodyweight per day. The maintenance doses may be administered no more than once every 1, 5, 10, or 30 days. Further, the treatment regimen may last for a period of time which will

vary depending upon the nature of the particular disease, its severity and the overall condition of the patient. In some embodiments the dosage may be delivered no more than once per day, *e.g.*, no more than once per 24, 36, 48, or more hours, *e.g.*, no more than once for every 5 or 8 days. Following treatment, the patient can be monitored for changes in his condition and for alleviation of the symptoms of the disease state. The dosage of the oligonucleotide may either be increased in the event the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disease state is observed, if the disease state has been ablated, or if undesired side-effects are observed.

The effective dose can be administered in a single dose or in two or more doses, as desired or considered appropriate under the specific circumstances. If desired to facilitate repeated or frequent infusions, implantation of a delivery device, *e.g.*, a pump, semi-permanent stent (*e.g.*, intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir may be advisable.

In some embodiments, the oligonucleotide pharmaceutical composition includes a plurality of single stranded oligonucleotide species. In another embodiment, the single stranded oligonucleotide species has sequences that are non-overlapping and non-adjacent to another species with respect to a naturally occurring target sequence (*e.g.*, a PRC2-associated region). In another embodiment, the plurality of single stranded oligonucleotide species is specific for different PRC2-associated regions. In another embodiment, the single stranded oligonucleotide is allele specific. In some cases, a patient is treated with a single stranded oligonucleotide in conjunction with other therapeutic modalities.

Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the compound of the invention is administered in maintenance doses, ranging from 0.0001 mg to 100 mg per kg of body weight.

The concentration of the single stranded oligonucleotide composition is an amount sufficient to be effective in treating or preventing a disorder or to regulate a physiological condition in humans. The concentration or amount of single stranded oligonucleotide administered will depend on the parameters determined for the agent and the method of administration, *e.g.* nasal, buccal, pulmonary. For example, nasal formulations may tend to

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require much lower concentrations of some ingredients in order to avoid irritation or burning of the nasal passages. It is sometimes desirable to dilute an oral formulation up to 10-100 times in order to provide a suitable nasal formulation.

Certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a single stranded oligonucleotide can include a single treatment or, preferably, can include a series of treatments. It will also be appreciated that the effective dosage of a single stranded oligonucleotide used for treatment may increase or decrease over the course of a particular treatment. For example, the subject can be monitored after administering a single stranded oligonucleotide composition. Based on information from the monitoring, an additional amount of the single stranded oligonucleotide composition can be administered.

Dosing is dependent on severity and responsiveness of the disease condition to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of MECP2 expression levels in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compounds, and can generally be estimated based on EC50s found to be effective in in vitro and in vivo animal models. In some embodiments, the animal models include transgenic animals that express a human MECP2. In another embodiment, the composition for testing includes a single stranded oligonucleotide that is complementary, at least in an internal region, to a sequence that is conserved between MECP2 in the animal model and the MECP2 in a human.

In one embodiment, the administration of the single stranded oligonucleotide composition is parenteral, *e.g.* intravenous (*e.g.*, as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary, intranasal, urethral or ocular. Administration can be provided by the subject or by another person, *e.g.*, a health care provider. The composition can be provided in measured

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doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

### *Kits*

5           In certain aspects of the invention, kits are provided, comprising a container housing a composition comprising a single stranded oligonucleotide. In some embodiments, the composition is a pharmaceutical composition comprising a single stranded oligonucleotide and a pharmaceutically acceptable carrier. In some embodiments, the individual components of the pharmaceutical composition may be provided in one container. Alternatively, it may  
10   be desirable to provide the components of the pharmaceutical composition separately in two or more containers, *e.g.*, one container for single stranded oligonucleotides, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be  
15   combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

The present invention is further illustrated by the following Examples, which in no way should be construed as further limiting. The entire contents of all of the references  
20   (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

### EXAMPLES

25           The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

### ***MATERIALS AND METHODS:***

#### **Real Time PCR**

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RNA is harvested from the cells using Promega SV 96 Total RNA Isolation system or Trizol omitting the DNase step. RNA harvested from cells is normalized so that equal RNA is input to each reverse transcription reaction. Reverse transcriptase reactions are performed using the Superscript II kit and real time PCR performed on cDNA samples using icycler  
5 SYBR green chemistry (Biorad). A baseline level of mRNA expression for MECP2 is determined through quantitative PCR as outlined above. Baseline levels are also determined for mRNA of various housekeeping genes which are constitutively expressed. A “control” housekeeping gene with approximately the same level of baseline expression as the target gene is chosen for comparison purposes.

10

### Cell Culture

Human hepatocyte Hep3B, human hepatocyte HepG2 cells, mouse hepatoma Hepa1-6 cells, and human renal proximal tubule epithelial cells (RPTEC) are cultured using conditions known in the art (see, *e.g.* Current Protocols in Cell Biology).

15

### Oligonucleotide design

Oligonucleotides were designed within PRC2-interacting regions in order to upregulate MECP2. The sequence and structure of each oligonucleotide is shown in Table 3. The following table provides a description of the nucleotide analogs, modifications and  
20 intranucleotide linkages used for certain oligonucleotides tested and described in Table 2.

### *In vitro* transfection of cells with oligonucleotides

Oligonucleotides are designed within PRC2-interacting regions in order to upregulate MECP2. Cells are seeded into each well of 24-well plates at a density of 25,000 cells per  
25 500uL and transfections are performed with Lipofectamine and the single stranded oligonucleotides. Control wells contain Lipofectamine alone. At 48 hours post-transfection, approximately 200 uL of cell culture supernatants are stored at -80 C for ELISA. At 48 hours post-transfection, RNA is harvested from the cells and quantitative PCR is carried out as outlined above. The percent induction of target mRNA expression by each oligonucleotide is  
30 determined by normalizing mRNA levels in the presence of the oligonucleotide to the mRNA

levels in the presence of control (Lipofectamine alone). This is compared side-by-side with the increase in mRNA expression of the “control” housekeeping gene.

### **In vitro delivery of single stranded oligonucleotides**

5 Oligonucleotides are designed as candidates for upregulating MECP2 expression. The single stranded oligonucleotides are designed to be complementary to a PRC2-interacting region within a sequence as set forth in SEQ ID NO: 1 or 2. The oligonucleotides are tested in at least duplicate. Briefly, cells are transfected in vitro with each of the oligonucleotides as described above. MECP2 expression in cells following treatment is  
10 evaluated by qRT-PCR. Oligonucleotides that upregulate MECP2 expression are identified.

### TABLE

**Table 1:** Hexamers that are not seed sequences of human miRNAs

AAAAAA, AAAAAG, AAAACA, AAAAGA, AAAAGC, AAAAGG, AAAAUA, AAACAA, AAACAC, AAACAG,  
15 AAACAU, AAACCC, AAACCU, AAACGA, AAACGC, AAACGU, AAACUA, AAACUC, AAACUU, AAAGAU,  
AAAGCC, AAAGGA, AAAGGG, AAAGUC, AAUAC, AAUAU, AAUUCG, AAUUCU, AAUUGC, AAUUGU,  
AAUUUA, AAUUUG, AACAAC, AACAAG, AACAAU, AACACA, AACACG, AACAGA, AACAGC, AACAGG,  
AACAUC, AACAUG, AACCAA, AACCAC, AACCAG, AACCAU, AACCCC, AACCCG, AACCGA, AACCGC,  
AACCGG, AACCUA, AACCUU, AACGAA, AACGAC, AACGAG, AACGAU, AACGCU, AACGGG, AACGGU,  
20 AACGUA, AACGUC, AACGUG, AACGUU, AACUAU, AACUCA, AACUCC, AACUCG, AACUGA, AACUGC,  
AACUGU, AACUUA, AACUUC, AACUUG, AACUUU, AAGAAA, AAGAAG, AAGAAU, AAGACG, AAGAGA,  
AAGAGC, AAGAGG, AAGAGU, AAGAUU, AAGCAA, AAGCAC, AAGCAG, AAGCAU, AAGCCA, AAGCCC,  
AAGCCG, AAGCCU, AAGCGA, AAGCGG, AAGCGU, AAGCUA, AAGGAA, AAGGAC, AAGGCU, AAGGGC,  
AAGGGU, AAGGUU, AAGUAA, AAGUAC, AAGUAU, AAGUCC, AAGUCG, AAGUGA, AAGUGG, AAGUUA,  
25 AAGUUU, AAUAAA, AAUAAC, AAUAAG, AAUAU, AAUACA, AAUACC, AAUACG, AAUAGA, AAUAGC,  
AAUAGG, AAUAGU, AAUAUC, AAUAUU, AAUCAA, AAUCAU, AAUCCA, AAUCCC, AAUCCG, AAUCGA,  
AAUCGC, AAUCGU, AAUCUA, AAUCUG, AAUCUU, AAUGAA, AAUGAC, AAUGAG, AAUGAU, AAUGCG,  
AAUGCU, AAUGGA, AAUGGU, AAUGUA, AAUGUC, AAUGUG, AAUUAA, AAUUAC, AAUUAG, AAUUCC,  
AAUUCG, AAUUGA, AAUUGG, AAUUGU, AAUUUC, AAUUUG, ACAAAA, ACAAAC, ACAAAG, ACAAU,  
30 ACAACC, ACAACG, ACAACU, ACAAGA, ACAAGC, ACAAGU, ACAAUC, ACA AUG, ACAAUU, ACACAG,  
ACACCA, ACACCC, ACACCG, ACACCU, ACACGA, ACACGC, ACACGU, ACACUC, ACACUG, ACACUU,  
ACAGAA, ACAGAC, ACAGCC, ACAGCG, ACAGCU, ACAGGG, ACAGUC, ACAGUG, ACAGUU, ACAUAA,  
ACAUAC, ACAUCC, ACAUCG, ACAUCU, ACAUGA, ACAUGC, ACAUGU, ACAUUG, ACAUUU, ACCAAA,  
ACCAAC, ACCAAG, ACCAAU, ACCACC, ACCACG, ACCAGA, ACCAGU, ACCAUA, ACCAUG, ACCAUU,  
35 ACCCAA, ACCCAC, ACCCCA, ACCCCG, ACCCGA, ACCCGC, ACCCUA, ACCCUC, ACCCUU, ACCGAA,

ACCGAC, ACCGAU, ACCGCA, ACCGCC, ACCGCG, ACCGCU, ACCGGA, ACCGGC, ACCGGU, ACCGUA,  
 ACCGUC, ACCGUG, ACCGUU, ACCUAA, ACCUAC, ACCUAG, ACCUAU, ACCUCA, ACCUCC, ACCUCG,  
 ACCUCU, ACCUGA, ACCUGC, ACCUGU, ACCUUA, ACCUUC, ACCUUU, ACGAAA, ACGAAC, ACGAAG,  
 ACGAAU, ACGACA, ACGACC, ACGACG, ACGACU, ACGAGA, ACGAGC, ACGAGG, ACGAGU, ACGAUA,  
 5 ACGAUC, ACGAUG, ACGAUU, ACGCAA, ACGCAG, ACGCAU, ACGCCC, ACGCCG, ACGCCU, ACGCGA,  
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GUCCAA, GUCCAC, GUCCAU, GUCCCC, GUCCCU, GUCCGA, GUCCGC, GUCCGG, GUCCGU, GUCCUA,  
GUCCUG, GUCCUU, GUCGAA, GUCGAC, GUCGAG, GUCGAU, GUCGCA, GUCGCC, GUCGCG, GUCGCU,  
GUCGGA, GUCGGC, GUCGGG, GUCGGU, GUCGUA, GUCGUC, GUCGUU, GUCUAA, GUCUAG, GUCUCA,  
20 GUCUCC, GUCUCG, GUCUGA, GUCUGG, GUCUGU, GUCUUC, GUCUUU, GUGAAA, GUGAAC, GUGAAG,  
GUGACC, GUGACG, GUGAGA, GUGAGC, GUGAGU, GUGAUC, GUGAUG, GUGAUU, GUGCAC,  
GUGCAU, GUGCCC, GUGCCG, GUGCGA, GUGCGG, GUGCGU, GUGCUA, GUGCUC, GUGCUG,  
GUGGAG, GUGGCG, GUGGCU, GUGGGU, GUGGUC, GUGGUG, GUGUAA, GUGUAG, GUGUCG,  
GUGUGA, GUGUGC, GUGUGU, GUGUUG, GUGUUU, GUUAAA, GUUAAC, GUUAAG, GUUACA,  
25 GUUACC, GUUACG, GUUACU, GUUAGA, GUUAGC, GUUAGU, GUUAUA, GUUAUC, GUUAUG,  
GUUAUU, GUUCA, GUUCAC, GUUCAG, GUUCCA, GUUCCG, GUUCGA, GUUCGC, GUUCGG, GUUCGU,  
GUUCUA, GUUCUG, GUUGAA, GUUGAC, GUUGAG, GUUGAU, GUUGCG, GUUGCU, GUUGGA,  
GUUGGC, GUUGGU, GUUGUC, GUUGUG, GUUGUU, GUUUA, GUUUAC, GUUUAG, GUUUAU,  
GUUUCA, GUUUCC, GUUUUC, GUUUGA, GUUUGC, GUUUGG, GUUUGU, GUUUUA, GUUUUC,  
30 GUUUUU, UAAAAA, UAAAAC, UAAAAG, UAAAAU, UAAACA, UAAACC, UAAACG, UAAACU, UAAAGA,  
UAAAGG, UAAAGU, UAA AUA, UAA AUC, UAA AUG, UAA AUU, UAACAA, UAACAC, UAACAG, UAACCA,  
UAACCC, UAACCG, UAACCU, UAACGA, UAACGC, UAACGG, UAACGU, UAACUA, UAACUG, UAACUU,  
UAAGAG, UAAGAU, UAAGCA, UAAGCC, UAAGCG, UAAGCU, UAAGGA, UAAGGC, UAAGGG, UAAGGU,  
UAAGUA, UAAGUC, UAAGUG, UAAGUU, UAAUAA, UAAUCA, UAAUCC, UAAUCG, UAAUCU, UAAUGA,  
35 UAAUGG, UAAUGU, UAAUUA, UAAUUC, UAAUUG, UACAAC, UACAAG, UACAAU, UACACC, UACACG,  
UACACU, UACAGA, UACAGC, UACAUA, UACAUC, UACAUU, UACCAA, UACCAC, UACCAG, UACCAU,  
UACCCC, UACCCG, UACCCU, UACCGA, UACCGC, UACCGG, UACCGU, UACCUA, UACCUG, UACGAA,  
UACGAC, UACGAG, UACGAU, UACGCA, UACGCC, UACGCG, UACGCU, UACGGC, UACGGG, UACGGU,  
UACGUA, UACGUC, UACGUG, UACGUU, UACUAA, UACUAC, UACUAG, UACUAU, UACUCA, UACUCC,  
40 UACUCG, UACUCU, UACUGA, UACUGC, UACUGG, UACUUA, UACUUG, UACUUU, UAGAAA, UAGAAG,  
UAGAAU, UAGACA, UAGACG, UAGAGA, UAGAGC, UAGAGU, UAG AUA, UAG AUC, UAG AUG, UAGCAU,

UAGCCC, UAGCCG, UAGCCU, UAGCGA, UAGCGC, UAGCGU, UAGCUA, UAGCUC, UAGCUG, UAGGAA,  
UAGGAU, UAGGCG, UAGGCU, UAGGGU, UAGGUC, UAGGUG, UAGGUU, UAGUAA, UAGUAC,  
UAGUAG, UAGUAU, UAGUCA, UAGUCG, UAGUGU, UAGUUA, UAGUUC, UAGUUG, UAGUUU,  
UAUAAC, UAUAAG, UAUACU, UAUAGA, UAUAGC, UAUAGG, UAUAGU, UAUAUA, UAUAUC, UAUAUG,  
5 UAUAUU, UAUCAA, UAUCAC, UAUCAU, UAUCCA, UAUCCC, UAUCCG, UAUCCU, UAUCGA, UAUCGC,  
UAUCGG, UAUCGU, UAUCUA, UAUCUC, UAUCUG, UAUCUU, UAUGAA, UAUGAC, UAUGAG,  
UAUGAU, UAUGCA, UAUGCG, UAUGCU, UAUGGA, UAUGGC, UAUGUC, UAUGUG, UAUGUU,  
UAUUAG, UAUUCA, UAUUCC, UAUUCG, UAUUCU, UAUUGA, UAUUGG, UAUUUA, UAUUUC,  
UAUUUG, UAUUUU, UCAAAA, UCAAAC, UCAAAG, UCAACC, UCAACU, UCAAGA, UCAAGC, UCAAUA,  
10 UCAAUC, UCAAUG, UCAAUU, UCACCC, UCACCG, UCACCU, UCACGA, UCACGC, UCACGG, UCACGU,  
UCACUA, UCACUC, UCACUU, UCAGAA, UCAGAC, UCAGAG, UCAGCG, UCAGCU, UCAGGA, UCAGGC,  
UCAGGU, UCAGUC, UCAGUU, UCAUAA, UCAUCA, UCAUCC, UCAUCG, UCAUGC, UCAUGG, UCAUGU,  
UCAUUA, UCAUUG, UCCAAA, UCCAAC, UCCAAG, UCCAAU, UCCACA, UCCACC, UCCACG, UCCAGC,  
UCCAGG, UCCAUA, UCCAUC, UCCAUU, UCCCAA, UCCCAG, UCCCAU, UCCCCC, UCCCCG, UCCCCU,  
15 UCCCGA, UCCCGC, UCCCGG, UCCCGU, UCCCUA, UCCCUC, UCCGAA, UCCGAC, UCCGAG, UCCGAU,  
UCCGCA, UCCGCC, UCCGGA, UCCGGC, UCCGGU, UCCGUA, UCCGUC, UCCGUG, UCCUAA, UCCUCA,  
UCCUCG, UCCUCU, UCCUGC, UCCUGU, UCCUUA, UCCUUC, UCCUUU, UCGAAA, UCGAAC, UCGAAG,  
UCGAAU, UCGACA, UCGACC, UCGACG, UCGACU, UCGAGA, UCGAGC, UCGAGG, UCGAUA, UCGAUC,  
UCGAUG, UCGAUU, UCGCAA, UCGCAC, UCGCAG, UCGCAU, UCGCCA, UCGCCC, UCGCCG, UCGCCU,  
20 UCGCGA, UCGCGC, UCGCGU, UCGCUA, UCGCUC, UCGGAA, UCGGAC, UCGGAG, UCGGAU, UCGGCA,  
UCGGCU, UCGGGG, UCGGGU, UCGGUC, UCGGUG, UCGGUU, UCGUAA, UCGUAC, UCGUAG,  
UCGUAU, UCGUCA, UCGUCC, UCGUCG, UCGUCU, UCGUGA, UCGUGU, UCGUUA, UCGUUC, UCGUUG,  
UCGUUU, UCUAAC, UCUAAG, UCUAAU, UCUACA, UCUACC, UCUACG, UCUACU, UCUAGC, UCUAGG,  
UCUAGU, UCUAUA, UCUAUC, UCUAUG, UCUAUU, UCUCAG, UCUCAU, UCUCCG, UCUCGC, UCUCGG,  
25 UCUCGU, UCUCUC, UCUGAA, UCUGAU, UCUGCA, UCUGCG, UCUGCU, UCUGGC, UCUGGU, UCUGUC,  
UCUGUG, UCUGUU, UCUUAA, UCUUAC, UCUUAG, UCUUAU, UCUUCA, UCUUCC, UCUUCG, UCUUCU,  
UCUUGC, UCUUGG, UCUUGU, UCUUUA, UCUUUC, UCUUUG, UCUUUU, UGAAAA, UGAAAC,  
UGAACA, UGAACC, UGAAGG, UGA AUC, UGA AUG, UGACAA, UGACAC, UGACAG, UGACCA, UGACCC,  
UGACCG, UGACGA, UGACGC, UGACGG, UGACGU, UGACUA, UGACUC, UGACUU, UGAGAG, UGAGAU,  
30 UGAGCA, UGAGCC, UGAGCU, UGAGGC, UGAGGU, UGAGUA, UGAGUU, UGAUAC, UGAUAG,  
UGAUAU, UGAUCA, UGAUCG, UGAUCU, UGAUGA, UGAUGC, UGAUGG, UGAUGU, UGAUUA,  
UGAUUC, UGAUUG, UGAUUU, UGCAAC, UGCAAG, UGCACA, UGCACG, UGCAGG, UGCAGU, UGCAUC,  
UGCCCA, UGCCCC, UGCCCG, UGCCGA, UGCCGC, UGCCGG, UGCCGU, UGCCUA, UGCCUC, UGCCUG,  
UGCCUU, UGCGAA, UGCGAC, UGCGAU, UGCGCC, UGCGCG, UGCGCU, UGCGGC, UGCGGG, UGCGGU,  
35 UGCGUA, UGCGUC, UGCGUG, UGCGUU, UGCUAC, UGCUAU, UGCUCC, UGCU CG, UGCU GC, UGCU GG,  
UGCUGU, UGCUUA, UGCUUU, UGGAAC, UGGAAG, UGGAGC, UGGAUC, UGGAUU, UGGCAA,  
UGGCAC, UGGCAG, UGGCCG, UGGCCU, UGGCGA, UGGCGC, UGGCGU, UGGCUA, UGGCUC, UGGCUU,  
UGGGAA, UGGGCA, UGGGCC, UGGGGC, UGGGUC, UGGUAA, UGGUAG, UGGUAU, UGGUCC,  
UGGUCG, UGGUCU, UGGUGA, UGGUGC, UGGUGG, UGGUGU, UGGUUA, UGGUUG, UGUAAA,  
40 UGU AAC, UGU AAG, UGU ACC, UGU ACG, UGU ACU, UGU AGA, UGU AGC, UGU AGU, UGU AUC,  
UGUAUU, UGUCAA, UGUCAC, UGUCAG, UGUCAU, UGUCCA, UGUCCC, UGUCCG, UGUCGA, UGUCGC,

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UGUCGG, UGUCGU, UGUCUA, UGUCUC, UGUGAC, UGUGAG, UGUGAU, UGUGCA, UGUGGU,  
 UGUGUA, UGUGUU, UGUUAC, UGUUAG, UGUUAU, UGUUCA, UGUUCC, UGUUCG, UGUUGG,  
 UGUUGU, UGUUUA, UGUUUC, UGUUUG, UGUUUU, UUAAAA, UUAAAC, UUAAAG, UUAAAU,  
 UUAACC, UUAACG, UUAACU, UUAAGU, UUAUAU, UUAUAC, UUAUAG, UUAUAA, UUAUCC,  
 5 UUAUCG, UUAUCU, UUAUGA, UUAUGG, UUAUGU, UUAUUA, UUAUUC, UUAUUG, UUAUUU,  
 UUCAAC, UUCAAU, UUCACA, UUCACC, UUCACG, UUCACU, UUCAGC, UUCAGG, UUCAGU, UUCAUA,  
 10 UUCAUC, UUCAUG, UUCAUU, UUCCAA, UUCCCA, UUCCCG, UUCCGA, UUCCGU, UUCCUU, UUCGAA,  
 UUCGAC, UUCGAG, UUCGAU, UUCGCA, UUCGCC, UUCGCG, UUCGCU, UUCGGA, UUCGGC, UUCGGG,  
 UUCGGU, UUCGUA, UUCGUC, UUCGUG, UUCGUU, UUCUAC, UUCUAG, UUCUCA, UUCUCG,  
 UUCUGG, UUCUUA, UUCUUU, UUGAAA, UUGAAC, UUGAAG, UUGAAU, UUGACC, UUGACG,  
 UUGACU, UUGAGA, UUGAGC, UUGAGU, UUGAUA, UUGAUC, UUGAUG, UUGAUU, UUGCAA,  
 15 UUGCAC, UUGCAG, UUGCAU, UUGCCC, UUGCCG, UUGC GA, UUGC GC, UUGC GG, UUGC GU, UUGC UA,  
 UUGC UC, UUGC UG, UUGC UU, UUGGAA, UUGGAG, UUGGCC, UUGGCG, UUGGCU, UUGGGC,  
 UUGGGU, UUGGUA, UUGGUG, UUGUAA, UUGUAC, UUGUCA, UUGUCG, UUGUCU, UUGUGC,  
 UUGUGG, UUGUUA, UUGUUG, UUGUUU, UUUAAA, UUUAAC, UUUAAAG, UUUAAU, UUUACA,  
 UUUACC, UUUACG, UUUACU, UUUAGA, UUUAGC, UUUAGG, UUUAGU, UUUUAU, UUUUAC,  
 20 UUUUAG, UUUUAU, UUUCAU, UUUCCA, UUUCCG, UUUCCU, UUU CGA, UUU CGC, UUU CGG,  
 UUU CGU, UUU CUA, UUU CUC, UUU CUG, UUU CUU, UUU GAA, UUU GAC, UUU GAG, UUU GAU,  
 UUU GCC, UUU GCU, UUU GGA, UUU GGC, UUU GGG, UUU GGU, UUU GUA, UUU GUC, UUU GUU,  
 UUU UAA, UUU UAG, UUU UAU, UUU UCC, UUU UCG, UUU UCU, UUU UGA, UUU UGC, UUU UGG,  
 UUU UGU, UUU UUA, UUU UUC, UUU UUU

25 **Table 2:** A listing of oligonucleotide modifications

| Symbol | Feature Description          |
|--------|------------------------------|
| bio    | 5' biotin                    |
| dAs    | DNA w/3' thiophosphate       |
| dCs    | DNA w/3' thiophosphate       |
| dGs    | DNA w/3' thiophosphate       |
| dTs    | DNA w/3' thiophosphate       |
| dG     | DNA                          |
| enaAs  | ENA w/3' thiophosphate       |
| enaCs  | ENA w/3' thiophosphate       |
| enaGs  | ENA w/3' thiophosphate       |
| enaTs  | ENA w/3' thiophosphate       |
| fluAs  | 2'-fluoro w/3' thiophosphate |
| fluCs  | 2'-fluoro w/3' thiophosphate |
| fluGs  | 2'-fluoro w/3' thiophosphate |

| Symbol    | Feature Description                   |
|-----------|---------------------------------------|
| fluUs     | 2'-fluoro w/3' thiophosphate          |
| lnaAs     | LNA w/3' thiophosphate                |
| lnaCs     | LNA w/3' thiophosphate                |
| lnaGs     | LNA w/3' thiophosphate                |
| lnaTs     | LNA w/3' thiophosphate                |
| omeAs     | 2'-Ome w/3' thiophosphate             |
| omeCs     | 2'-Ome w/3' thiophosphate             |
| omeGs     | 2'-Ome w/3' thiophosphate             |
| omeTs     | 2'-Ome w/3' thiophosphate             |
| lnaAs-Sup | LNA w/3' thiophosphate at 3' terminus |
| lnaCs-Sup | LNA w/3' thiophosphate at 3' terminus |
| lnaGs-Sup | LNA w/3' thiophosphate at 3' terminus |
| lnaTs-Sup | LNA w/3' thiophosphate at 3' terminus |
| lnaA-Sup  | LNA w/3' OH at 3' terminus            |
| lnaC-Sup  | LNA w/3' OH at 3' terminus            |
| lnaG-Sup  | LNA w/3' OH at 3' terminus            |
| lnaT-Sup  | LNA w/3' OH at 3' terminus            |
| omeA-Sup  | 2'-Ome w/3' OH at 3' terminus         |
| omeC-Sup  | 2'-Ome w/3' OH at 3' terminus         |
| omeG-Sup  | 2'-Ome w/3' OH at 3' terminus         |
| omeU-Sup  | 2'-Ome w/3' OH at 3' terminus         |
| dAs-Sup   | DNA w/3' thiophosphate at 3' terminus |
| dCs-Sup   | DNA w/3' thiophosphate at 3' terminus |
| dGs-Sup   | DNA w/3' thiophosphate at 3' terminus |
| dTs-Sup   | DNA w/3' thiophosphate at 3' terminus |
| dA-Sup    | DNA w/3' OH at 3' terminus            |
| dC-Sup    | DNA w/3' OH at 3' terminus            |
| dG-Sup    | DNA w/3' OH at 3' terminus            |
| dT-Sup    | DNA w/3' OH at 3' terminus            |

**Table 3:** Formatted oligonucleotide sequences made for testing showing nucleotide modifications.

| Oligo Name   | Base Sequence       | RaNA Sequence                                                                                | SeqID |
|--------------|---------------------|----------------------------------------------------------------------------------------------|-------|
| MECP2-01 m01 | CGACGTCGCGC<br>CCCG | lnaCs;omeGs;lnaAs;omeCs;lnaGs;omeUs;lnaCs;omeGs;lnaCs;omeGs;lnaCs;omeCs;lnaCs;omeCs;lnaG-Sup | 1438  |
| MECP2-02 m01 | CCGACGTCGCG<br>CCCC | lnaCs;omeCs;lnaGs;omeAs;lnaCs;omeGs;lnaTs;omeCs;lnaGs;omeCs;lnaGs;omeCs;lnaCs;omeCs;lnaC-Sup | 1439  |
| MECP2-       | CCATTTTCCGG         | lnaCs;omeCs;lnaAs;omeUs;lnaTs;omeUs;lnaTs;omeC                                               | 1521  |

| Oligo Name   | Base Sequence       | RaNA Sequence                                                                                | SeqID |
|--------------|---------------------|----------------------------------------------------------------------------------------------|-------|
| 03 m01       | ACGG                | s;lnaCs;omeGs;lnaGs;omeAs;lnaCs;omeGs;lnaG-Sup                                               |       |
| MECP2-04 m01 | GCCATTTTCCG<br>GACG | lnaGs;omeCs;lnaCs;omeAs;lnaTs;omeUs;lnaTs;omeUs;lnaCs;omeCs;lnaGs;omeGs;lnaAs;omeCs;lnaG-Sup | 1522  |
| MECP2-05 m01 | GCGGCCATTTT<br>CCGG | lnaGs;omeCs;lnaGs;omeGs;lnaCs;omeCs;lnaAs;omeUs;lnaTs;omeUs;lnaTs;omeCs;lnaCs;omeGs;lnaG-Sup | 1525  |
| MECP2-06 m01 | GGCGGCCATTT<br>TCCG | lnaGs;omeGs;lnaCs;omeGs;lnaGs;omeCs;lnaCs;omeAs;lnaTs;omeUs;lnaTs;omeUs;lnaCs;omeCs;lnaG-Sup | 1526  |
| MECP2-07 m01 | GCCAGCCGTGT<br>CGTC | lnaGs;omeCs;lnaCs;omeAs;lnaGs;omeCs;lnaCs;omeGs;lnaTs;omeGs;lnaTs;omeCs;lnaGs;omeUs;lnaC-Sup | 1683  |
| MECP2-08 m01 | ATCCGCCAGCC<br>GTGT | lnaAs;omeUs;lnaCs;omeCs;lnaGs;omeCs;lnaCs;omeAs;lnaGs;omeCs;lnaCs;omeGs;lnaTs;omeGs;lnaT-Sup | 1687  |
| MECP2-09 m01 | GATCGCCGGCG<br>AAGG | lnaGs;omeAs;lnaTs;omeCs;lnaGs;omeCs;lnaCs;omeGs;lnaGs;omeCs;lnaGs;omeAs;lnaAs;omeGs;lnaG-Sup | 2463  |
| MECP2-10 m01 | GATCCCGAGCG<br>CTCC | lnaGs;omeAs;lnaTs;omeCs;lnaCs;omeCs;lnaGs;omeAs;lnaGs;omeCs;lnaGs;omeCs;lnaTs;omeCs;lnaC-Sup | 2532  |
| MECP2-11 m01 | TGTGGCGACCA<br>AGTA | lnaTs;omeGs;lnaTs;omeGs;lnaGs;omeCs;lnaGs;omeAs;lnaCs;omeCs;lnaAs;omeAs;lnaGs;omeUs;lnaA-Sup | 7070  |
| MECP2-12 m01 | AGATCACCAGT<br>TCCG | lnaAs;omeGs;lnaAs;omeUs;lnaCs;omeAs;lnaCs;omeCs;lnaAs;omeGs;lnaTs;omeUs;lnaCs;omeCs;lnaG-Sup | 7117  |
| MECP2-13 m01 | TTGTACCTATA<br>CGCA | lnaTs;omeUs;lnaGs;omeUs;lnaAs;omeCs;lnaCs;omeUs;lnaAs;omeUs;lnaAs;omeCs;lnaGs;omeCs;lnaA-Sup | 14460 |
| MECP2-14 m01 | TTTGTACCTATA<br>CGC | lnaTs;omeUs;lnaTs;omeGs;lnaTs;omeAs;lnaCs;omeCs;lnaTs;omeAs;lnaTs;omeAs;lnaCs;omeGs;lnaC-Sup | 14461 |
| MECP2-15 m01 | CCCAAACAGCG<br>GCGC | lnaCs;omeCs;lnaCs;omeAs;lnaAs;omeAs;lnaCs;omeAs;lnaGs;omeCs;lnaGs;omeGs;lnaCs;omeGs;lnaC-Sup | 65064 |
| MECP2-16 m01 | CCAAACAGCGG<br>CGCT | lnaCs;omeCs;lnaAs;omeAs;lnaAs;omeCs;lnaAs;omeGs;lnaCs;omeGs;lnaGs;omeCs;lnaGs;omeCs;lnaT-Sup | 65065 |
| MECP2-17 m01 | CAAACAGCGGC<br>GCTC | lnaCs;omeAs;lnaAs;omeAs;lnaCs;omeAs;lnaGs;omeCs;lnaGs;omeGs;lnaCs;omeGs;lnaCs;omeUs;lnaC-Sup | 65066 |
| MECP2-18 m01 | AAACAGCGGC<br>GCTCC | lnaAs;omeAs;lnaAs;omeCs;lnaAs;omeGs;lnaCs;omeGs;lnaGs;omeCs;lnaGs;omeCs;lnaTs;omeCs;lnaC-Sup | 65067 |
| MECP2-19 m01 | AACAGCGGCG<br>CTCCA | lnaAs;omeAs;lnaCs;omeAs;lnaGs;omeCs;lnaGs;omeGs;lnaCs;omeGs;lnaCs;omeUs;lnaCs;omeCs;lnaA-Sup | 65068 |
| MECP2-20 m01 | TCCATCATCCG<br>TGAC | lnaTs;omeCs;lnaCs;omeAs;lnaTs;omeCs;lnaAs;omeUs;lnaCs;omeCs;lnaGs;omeUs;lnaGs;omeAs;lnaC-Sup | 65079 |
| MECP2-21 m01 | GGACCCATGTA<br>TGAT | lnaGs;omeGs;lnaAs;omeCs;lnaCs;omeCs;lnaAs;omeUs;lnaGs;omeUs;lnaAs;omeUs;lnaGs;omeAs;lnaT-Sup | 65097 |
| MECP2-22 m01 | CTTTCGCTCTAA<br>AGT | lnaCs;omeUs;lnaTs;omeUs;lnaCs;omeGs;lnaCs;omeUs;lnaCs;omeUs;lnaAs;omeAs;lnaAs;omeGs;lnaT-Sup | 65972 |
| MECP2-23 m01 | TCGCTCTAAAG<br>TGGA | lnaTs;omeCs;lnaGs;omeCs;lnaTs;omeCs;lnaTs;omeAs;lnaAs;omeAs;lnaGs;omeUs;lnaGs;omeGs;lnaA-Sup | 65975 |
| MECP2-24 m01 | GACTTCACGGT<br>AACT | lnaGs;omeAs;lnaCs;omeUs;lnaTs;omeCs;lnaAs;omeCs;lnaGs;omeGs;lnaTs;omeAs;lnaAs;omeCs;lnaT-Sup | 66045 |

| Oligo Name   | Base Sequence       | RaNA Sequence                                                                                | SeqID |
|--------------|---------------------|----------------------------------------------------------------------------------------------|-------|
| MECP2-25 m01 | CTTCACGGTAA<br>CTGG | InaCs;omeUs;InaTs;omeCs;InaAs;omeCs;InaGs;omeGs;InaTs;omeAs;InaAs;omeCs;InaTs;omeGs;InaG-Sup | 66047 |
| MECP2-26 m01 | CAAACGCCCCG<br>GCAG | InaCs;omeAs;InaAs;omeAs;InaCs;omeGs;InaCs;omeCs;InaCs;omeCs;InaGs;omeGs;InaCs;omeAs;InaG-Sup | 66323 |
| MECP2-27 m01 | GAAGCGAAAA<br>GCTGA | InaGs;omeAs;InaAs;omeGs;InaCs;omeGs;InaAs;omeAs;InaAs;omeAs;InaGs;omeCs;InaTs;omeGs;InaA-Sup | 66338 |
| MECP2-28 m01 | AAGAAAGCCGT<br>GAAG | InaAs;omeAs;InaGs;omeAs;InaAs;omeAs;InaGs;omeCs;InaCs;omeGs;InaTs;omeGs;InaAs;omeAs;InaG-Sup | 66432 |
| MECP2-29 m01 | CATCAAGAAGC<br>GCAA | InaCs;omeAs;InaTs;omeCs;InaAs;omeAs;InaGs;omeAs;InaAs;omeGs;InaCs;omeGs;InaCs;omeAs;InaA-Sup | 66485 |
| MECP2-30 m01 | CAATGTCTTTG<br>CGCT | InaCs;omeAs;InaAs;omeUs;InaGs;omeUs;InaCs;omeUs;InaTs;omeUs;InaGs;omeCs;InaGs;omeCs;InaT-Sup | 27322 |
| MECP2-31 m01 | GGCGTCCGGCT<br>GTCC | InaGs;omeGs;InaCs;omeGs;InaTs;omeCs;InaCs;omeGs;InaGs;omeCs;InaTs;omeGs;InaTs;omeCs;InaC-Sup | 27377 |
| MECP2-32 m01 | TTTGCAATCCG<br>CTCC | InaTs;omeUs;InaTs;omeGs;InaCs;omeAs;InaAs;omeUs;InaCs;omeCs;InaGs;omeCs;InaTs;omeCs;InaC-Sup | 27422 |
| MECP2-33 m01 | GCCACGAAACT<br>CTAA | InaGs;omeCs;InaCs;omeAs;InaCs;omeGs;InaAs;omeAs;InaAs;omeCs;InaTs;omeCs;InaTs;omeAs;InaA-Sup | 27548 |
| MECP2-34 m01 | AGCGCAGGTAT<br>ATAT | InaAs;omeGs;InaCs;omeGs;InaCs;omeAs;InaGs;omeGs;InaTs;omeAs;InaTs;omeAs;InaTs;omeAs;InaT-Sup | 28519 |
| MECP2-35 m01 | TTCGGAGCTTC<br>GTGG | InaTs;omeUs;InaCs;omeGs;InaGs;omeAs;InaGs;omeCs;InaTs;omeUs;InaCs;omeGs;InaTs;omeGs;InaG-Sup | 28565 |
| MECP2-36 m01 | GACAAACACG<br>GTTTT | InaGs;omeAs;InaCs;omeAs;InaAs;omeAs;InaCs;omeAs;InaCs;omeGs;InaGs;omeUs;InaTs;omeUs;InaT-Sup | 29761 |
| MECP2-37 m01 | TTGCTACCACG<br>GCCT | InaTs;omeUs;InaGs;omeCs;InaTs;omeAs;InaCs;omeCs;InaAs;omeCs;InaGs;omeGs;InaCs;omeCs;InaT-Sup | 72740 |
| MECP2-38 m01 | TGCTACCACGG<br>CCTC | InaTs;omeGs;InaCs;omeUs;InaAs;omeCs;InaCs;omeAs;InaCs;omeGs;InaGs;omeCs;InaCs;omeUs;InaC-Sup | 72741 |
| MECP2-39 m01 | ATCAATAACAG<br>CCGC | InaAs;omeUs;InaCs;omeAs;InaAs;omeUs;InaAs;omeAs;InaCs;omeAs;InaGs;omeCs;InaCs;omeGs;InaC-Sup | 72783 |
| MECP2-40 m01 | CAATAACAGCC<br>GCTC | InaCs;omeAs;InaAs;omeUs;InaAs;omeAs;InaCs;omeAs;InaGs;omeCs;InaCs;omeGs;InaCs;omeUs;InaC-Sup | 72785 |
| MECP2-41 m01 | ACTCAATGTGT<br>GCCG | InaAs;omeCs;InaTs;omeCs;InaAs;omeAs;InaTs;omeGs;InaTs;omeGs;InaTs;omeGs;InaCs;omeCs;InaG-Sup | 72848 |
| MECP2-42 m01 | CAATGTGTGCC<br>GAGC | InaCs;omeAs;InaAs;omeUs;InaGs;omeUs;InaGs;omeUs;InaGs;omeCs;InaCs;omeGs;InaAs;omeGs;InaC-Sup | 72851 |
| MECP2-43 m01 | CAAACGCGTCA<br>CTTA | InaCs;omeAs;InaAs;omeAs;InaCs;omeGs;InaCs;omeGs;InaTs;omeCs;InaAs;omeCs;InaTs;omeUs;InaA-Sup | 36117 |
| MECP2-44 m01 | ATCGAGAATGC<br>TAAC | InaAs;omeUs;InaCs;omeGs;InaAs;omeGs;InaAs;omeAs;InaTs;omeGs;InaCs;omeUs;InaAs;omeAs;InaC-Sup | 36165 |
| MECP2-45 m01 | CGCACGAGGCC<br>GGCT | InaCs;omeGs;InaCs;omeAs;InaCs;omeGs;InaAs;omeGs;InaGs;omeCs;InaCs;omeGs;InaGs;omeCs;InaT-Sup | 76153 |
| MECP2-46 m01 | GAGTCAGTGTC<br>CGCG | InaGs;omeAs;InaGs;omeUs;InaCs;omeAs;InaGs;omeUs;InaGs;omeUs;InaCs;omeCs;InaGs;omeCs;InaG-    | 76180 |

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| Oligo Name   | Base Sequence       | RaNA Sequence                                                                                | SeqID |
|--------------|---------------------|----------------------------------------------------------------------------------------------|-------|
|              |                     | Sup                                                                                          |       |
| MECP2-47 m01 | GTGTCCGCGGA<br>CCCG | lnaGs;omeUs;lnaGs;omeUs;lnaCs;omeCs;lnaGs;omeCs;lnaGs;omeGs;lnaAs;omeCs;lnaCs;omeCs;lnaG-Sup | 76186 |
| MECP2-48 m01 | AGCACTTCTTG<br>TACG | lnaAs;omeGs;lnaCs;omeAs;lnaCs;omeUs;lnaTs;omeCs;lnaTs;omeUs;lnaGs;omeUs;lnaAs;omeCs;lnaG-Sup | 76316 |
| MECP2-49 m01 | TTCTTGTACGA<br>GGTG | lnaTs;omeUs;lnaCs;omeUs;lnaTs;omeGs;lnaTs;omeAs;lnaCs;omeGs;lnaAs;omeGs;lnaGs;omeUs;lnaG-Sup | 76321 |
| MECP2-50 m01 | TGTGCCGCTTC<br>TACA | lnaTs;omeGs;lnaTs;omeGs;lnaCs;omeCs;lnaGs;omeCs;lnaTs;omeUs;lnaCs;omeUs;lnaAs;omeCs;lnaA-Sup | 76349 |
| MECP2-51 m01 | CCGCCGACTGG<br>TGCC | lnaCs;omeCs;lnaGs;omeCs;lnaCs;omeGs;lnaAs;omeCs;lnaTs;omeGs;lnaGs;omeUs;lnaGs;omeCs;lnaC-Sup | 76385 |
| MECP2-52 m01 | TGGTGCCAGTT<br>CGGT | lnaTs;omeGs;lnaGs;omeUs;lnaGs;omeCs;lnaCs;omeAs;lnaGs;omeUs;lnaTs;omeCs;lnaGs;omeGs;lnaT-Sup | 76393 |
| MECP2-53 m01 | TGATCGTGCGC<br>GACC | lnaTs;omeGs;lnaAs;omeUs;lnaCs;omeGs;lnaTs;omeGs;lnaCs;omeGs;lnaCs;omeGs;lnaAs;omeCs;lnaC-Sup | 76490 |
| MECP2-54 m01 | GATCGTGCGCG<br>ACCA | lnaGs;omeAs;lnaTs;omeCs;lnaGs;omeUs;lnaGs;omeCs;lnaGs;omeCs;lnaGs;omeAs;lnaCs;omeCs;lnaA-Sup | 76491 |
| MECP2-55 m01 | TCGTGCGCGAC<br>CAGA | lnaTs;omeCs;lnaGs;omeUs;lnaGs;omeCs;lnaGs;omeCs;lnaGs;omeAs;lnaCs;omeCs;lnaAs;omeGs;lnaA-Sup | 76493 |
| MECP2-56 m01 | GCGGCTGTGCG<br>AGCG | lnaGs;omeCs;lnaGs;omeGs;lnaCs;omeUs;lnaGs;omeUs;lnaGs;omeCs;lnaGs;omeAs;lnaGs;omeCs;lnaG-Sup | 76515 |
| MECP2-57 m01 | GGATCAACCGC<br>AACG | lnaGs;omeGs;lnaAs;omeUs;lnaCs;omeAs;lnaAs;omeCs;lnaCs;omeGs;lnaCs;omeAs;lnaAs;omeCs;lnaG-Sup | 76565 |
| MECP2-58 m01 | GATCAACCGCA<br>ACGC | lnaGs;omeAs;lnaTs;omeCs;lnaAs;omeAs;lnaCs;omeCs;lnaGs;omeCs;lnaAs;omeAs;lnaCs;omeGs;lnaC-Sup | 76566 |
| MECP2-59 m01 | CGACTGGACAT<br>CCTT | lnaCs;omeGs;lnaAs;omeCs;lnaTs;omeGs;lnaGs;omeAs;lnaCs;omeAs;lnaTs;omeCs;lnaCs;omeUs;lnaT-Sup | 78887 |
| MECP2-60 m01 | CCCGAGGAGTA<br>CATC | lnaCs;omeCs;lnaCs;omeGs;lnaAs;omeGs;lnaGs;omeAs;lnaGs;omeUs;lnaAs;omeCs;lnaAs;omeUs;lnaC-Sup | 79422 |

### BRIEF DESCRIPTION OF THE SEQUENCE LISTING

| SEQ ID | Chrom | Gene  | Chr. Start | Chr. End  | Strand |
|--------|-------|-------|------------|-----------|--------|
| 1      | chrX  | MECP2 | 153275263  | 153375188 | -      |
| 2      | chrX  | MECP2 | 153275263  | 153375188 | +      |
| 3      | chrX  | Mecp2 | 71260160   | 71342932  | -      |
| 4      | chrX  | Mecp2 | 71260160   | 71342932  | +      |
| 5      | chrX  | MECP2 | 153278064  | 153278111 | -      |
| 6      | chrX  | MECP2 | 153278111  | 153278156 | -      |
| 7      | chrX  | MECP2 | 153278706  | 153278747 | -      |
| 8      | chrX  | MECP2 | 153279512  | 153279556 | -      |

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| SEQ ID | Chrom | Gene  | Chr. Start | Chr. End  | Strand |
|--------|-------|-------|------------|-----------|--------|
| 9      | chrX  | MECP2 | 153279613  | 153279658 | -      |
| 10     | chrX  | MECP2 | 153281486  | 153281531 | -      |
| 11     | chrX  | MECP2 | 153283707  | 153283737 | -      |
| 12     | chrX  | MECP2 | 153284059  | 153284105 | -      |
| 13     | chrX  | MECP2 | 153287944  | 153287992 | -      |
| 14     | chrX  | MECP2 | 153288681  | 153288722 | -      |
| 15     | chrX  | MECP2 | 153290087  | 153290134 | -      |
| 16     | chrX  | MECP2 | 153290216  | 153290263 | -      |
| 17     | chrX  | MECP2 | 153290364  | 153290414 | -      |
| 18     | chrX  | MECP2 | 153291585  | 153291633 | -      |
| 19     | chrX  | MECP2 | 153292312  | 153292362 | -      |
| 20     | chrX  | MECP2 | 153292731  | 153292774 | -      |
| 21     | chrX  | MECP2 | 153293138  | 153293185 | -      |
| 22     | chrX  | MECP2 | 153293331  | 153293377 | -      |
| 23     | chrX  | MECP2 | 153293427  | 153293469 | -      |
| 24     | chrX  | MECP2 | 153293568  | 153293614 | -      |
| 25     | chrX  | MECP2 | 153293715  | 153293764 | -      |
| 26     | chrX  | MECP2 | 153293792  | 153293878 | -      |
| 27     | chrX  | MECP2 | 153293901  | 153293948 | -      |
| 28     | chrX  | MECP2 | 153294420  | 153294467 | -      |
| 29     | chrX  | MECP2 | 153297927  | 153297972 | -      |
| 30     | chrX  | MECP2 | 153315466  | 153315571 | -      |
| 31     | chrX  | MECP2 | 153343401  | 153343447 | -      |
| 32     | chrX  | MECP2 | 153344298  | 153344339 | -      |
| 33     | chrX  | MECP2 | 153348654  | 153348702 | -      |
| 34     | chrX  | MECP2 | 153348997  | 153349021 | -      |
| 35     | chrX  | MECP2 | 153349179  | 153349222 | -      |
| 36     | chrX  | MECP2 | 153349694  | 153349734 | -      |
| 37     | chrX  | MECP2 | 153350493  | 153350518 | -      |
| 38     | chrX  | MECP2 | 153356667  | 153356713 | -      |
| 39     | chrX  | MECP2 | 153356742  | 153356795 | -      |
| 40     | chrX  | MECP2 | 153357047  | 153357106 | -      |
| 41     | chrX  | MECP2 | 153357161  | 153357204 | -      |
| 42     | chrX  | MECP2 | 153361085  | 153361163 | -      |
| 43     | chrX  | MECP2 | 153361423  | 153361467 | -      |
| 44     | chrX  | MECP2 | 153362464  | 153362527 | -      |
| 45     | chrX  | MECP2 | 153276064  | 153280111 | -      |
| 46     | chrX  | MECP2 | 153276111  | 153280156 | -      |

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| SEQ ID | Chrom | Gene  | Chr. Start | Chr. End  | Strand |
|--------|-------|-------|------------|-----------|--------|
| 47     | chrX  | MECP2 | 153276706  | 153280747 | -      |
| 48     | chrX  | MECP2 | 153277512  | 153281556 | -      |
| 49     | chrX  | MECP2 | 153277613  | 153281658 | -      |
| 50     | chrX  | MECP2 | 153279486  | 153283531 | -      |
| 51     | chrX  | MECP2 | 153281707  | 153285737 | -      |
| 52     | chrX  | MECP2 | 153282059  | 153286105 | -      |
| 53     | chrX  | MECP2 | 153285944  | 153289992 | -      |
| 54     | chrX  | MECP2 | 153286681  | 153290722 | -      |
| 55     | chrX  | MECP2 | 153288087  | 153292134 | -      |
| 56     | chrX  | MECP2 | 153288216  | 153292263 | -      |
| 57     | chrX  | MECP2 | 153288364  | 153292414 | -      |
| 58     | chrX  | MECP2 | 153289585  | 153293633 | -      |
| 59     | chrX  | MECP2 | 153290312  | 153294362 | -      |
| 60     | chrX  | MECP2 | 153290731  | 153294774 | -      |
| 61     | chrX  | MECP2 | 153291138  | 153295185 | -      |
| 62     | chrX  | MECP2 | 153291331  | 153295377 | -      |
| 63     | chrX  | MECP2 | 153291427  | 153295469 | -      |
| 64     | chrX  | MECP2 | 153291568  | 153295614 | -      |
| 65     | chrX  | MECP2 | 153291715  | 153295764 | -      |
| 66     | chrX  | MECP2 | 153291792  | 153295878 | -      |
| 67     | chrX  | MECP2 | 153291901  | 153295948 | -      |
| 68     | chrX  | MECP2 | 153292420  | 153296467 | -      |
| 69     | chrX  | MECP2 | 153295927  | 153299972 | -      |
| 70     | chrX  | MECP2 | 153313466  | 153317571 | -      |
| 71     | chrX  | MECP2 | 153341401  | 153345447 | -      |
| 72     | chrX  | MECP2 | 153342298  | 153346339 | -      |
| 73     | chrX  | MECP2 | 153346654  | 153350702 | -      |
| 74     | chrX  | MECP2 | 153346997  | 153351021 | -      |
| 75     | chrX  | MECP2 | 153347179  | 153351222 | -      |
| 76     | chrX  | MECP2 | 153347694  | 153351734 | -      |
| 77     | chrX  | MECP2 | 153348493  | 153352518 | -      |
| 78     | chrX  | MECP2 | 153354667  | 153358713 | -      |
| 79     | chrX  | MECP2 | 153354742  | 153358795 | -      |
| 80     | chrX  | MECP2 | 153355047  | 153359106 | -      |
| 81     | chrX  | MECP2 | 153355161  | 153359204 | -      |
| 82     | chrX  | MECP2 | 153359085  | 153363163 | -      |
| 83     | chrX  | MECP2 | 153359423  | 153363467 | -      |
| 84     | chrX  | MECP2 | 153360464  | 153364527 | -      |

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| SEQ ID | Chrom | Gene  | Chr. Start | Chr. End  | Strand |
|--------|-------|-------|------------|-----------|--------|
| 85     | chrX  | MECP2 | 153279614  | 153279660 | +      |
| 86     | chrX  | MECP2 | 153281662  | 153281720 | +      |
| 87     | chrX  | MECP2 | 153281946  | 153281988 | +      |
| 88     | chrX  | MECP2 | 153284367  | 153284448 | +      |
| 89     | chrX  | MECP2 | 153284489  | 153284534 | +      |
| 90     | chrX  | MECP2 | 153288786  | 153288832 | +      |
| 91     | chrX  | MECP2 | 153289895  | 153289940 | +      |
| 92     | chrX  | MECP2 | 153292315  | 153292365 | +      |
| 93     | chrX  | MECP2 | 153292496  | 153292548 | +      |
| 94     | chrX  | MECP2 | 153297642  | 153297688 | +      |
| 95     | chrX  | MECP2 | 153297723  | 153297765 | +      |
| 96     | chrX  | MECP2 | 153300816  | 153300879 | +      |
| 97     | chrX  | MECP2 | 153315579  | 153315621 | +      |
| 98     | chrX  | MECP2 | 153316595  | 153316640 | +      |
| 99     | chrX  | MECP2 | 153348783  | 153348830 | +      |
| 100    | chrX  | MECP2 | 153349199  | 153349250 | +      |
| 101    | chrX  | MECP2 | 153358221  | 153358285 | +      |
| 102    | chrX  | MECP2 | 153277614  | 153281660 | +      |
| 103    | chrX  | MECP2 | 153279662  | 153283720 | +      |
| 104    | chrX  | MECP2 | 153279946  | 153283988 | +      |
| 105    | chrX  | MECP2 | 153282367  | 153286448 | +      |
| 106    | chrX  | MECP2 | 153282489  | 153286534 | +      |
| 107    | chrX  | MECP2 | 153286786  | 153290832 | +      |
| 108    | chrX  | MECP2 | 153287895  | 153291940 | +      |
| 109    | chrX  | MECP2 | 153290315  | 153294365 | +      |
| 110    | chrX  | MECP2 | 153290496  | 153294548 | +      |
| 111    | chrX  | MECP2 | 153295642  | 153299688 | +      |
| 112    | chrX  | MECP2 | 153295723  | 153299765 | +      |
| 113    | chrX  | MECP2 | 153298816  | 153302879 | +      |
| 114    | chrX  | MECP2 | 153313579  | 153317621 | +      |
| 115    | chrX  | MECP2 | 153314595  | 153318640 | +      |
| 116    | chrX  | MECP2 | 153346783  | 153350830 | +      |
| 117    | chrX  | MECP2 | 153347199  | 153351250 | +      |
| 118    | chrX  | MECP2 | 153356221  | 153360285 | +      |

**Single Strand Oligonucleotides (Antisense Strand of Target Gene)**

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SeqID range: 119-47653

SeqIDs w/o G Runs:

119-142, 157-202, 217-263, 279-293, 307-350, 364-544, 559-609, 623-677, 691-711, 726-787, 803-839, 854-915, 930-1055, 1070-1081, 1095-1103, 1118-1149, 1163, 1186-1189, 1204-1236, 1259-1301, 1343-1452, 1466-1476, 1490-1610, 1624-1658, 1672-1694, 1730-1740, 1769-1790, 1804-1814, 1843, 1881-1910, 1924-1947, 1978-1980, 2038-2052, 2066-2092, 2108-2114, 2142-2147, 2175-2192, 2207-2261, 2275-2294, 2308-2336, 2350-2379, 2394-2431, 2463-2515, 2529-2631, 2645-2771, 2786-2994, 3009-3291, 3305-3395, 3409-3463, 3477-3515, 3529-3566, 3581-3599, 3613-3686, 3701-3718, 3732-3830, 3844-3861, 3875-3970, 3984-4133, 4157-4235, 4251-4254, 4268-4474, 4488-4575, 4589-4608, 4627-4739, 4745-4756, 4780-4812, 4826-5007, 5034-5123, 5137-5239, 5253-5345, 5353-5371, 5384-5401, 5406-5477, 5503-5517, 5531-5635, 5649-5867, 5881-6171, 6185-6214, 6228-6231, 6245-6265, 6280-6406, 6420-6490, 6505-6661, 6684-6784, 6799-6964, 6979-7024, 7045-7174, 7188-7310, 7337-7388, 7402-7410, 7424-7474, 7489-7729, 7756-7797, 7811-7974, 7988-8049, 8063-8448, 8462-8671, 8686-8728, 8742-8854, 8869-8872, 8893-9071, 9076-9363, 9384-9399, 9423-9613, 9619-9706, 9715-9810, 9818-9931, 9951-9956, 9970-10101, 10116-10148, 10153-10230, 10244-10288, 10292-10379, 10386-10412, 10426-10639, 10680-10979, 11005-11060, 11072-11159, 11173-11198, 11204-11226, 11240-11566, 11580-11763, 11777-12069, 12083-12178, 12183-12197, 12213-12235, 12247-12257, 12268-12315, 12342-12351, 12373-12387, 12402-12409, 12431-12443, 12457-12466, 12485, 12510-12524, 12547, 12561-12802, 12817-12828, 12843-12854, 12868, 12882-12936, 12956-13001, 13015-13044, 13058-13087, 13106-13116, 13139-13141, 13155-13163, 13178-13612, 13634-13823, 13838-13847, 13857-13866, 13877-14524, 14538-14555, 14569-14754, 14768-14791, 14804-14806, 14820-14830, 14842-14851, 14865-14891, 14895-15008, 15022-15295, 15299-15317, 15332-15643, 15656-15658, 15670-15697, 15701-15748, 15765-15872, 15886-15941, 15964-15985, 15992-16007, 16021-16058, 16062-16084, 16098-16202, 16216-16428, 16439-16487, 16500-16758, 16772-16857, 16871-16875, 16891-16933, 16947-16990, 16999-17030, 17057-17072, 17096-17143, 17149-17150, 17165-17200, 17235-17246, 17260-17466, 17480-17545, 17566-17572, 17586-17592, 17638-17675, 17689-18203, 18217-18417, 18431-18540, 18554-18642, 18656-18669, 18683-18724, 18739-18828, 18842-19079, 19110-19222, 19236-19260, 19275-19321, 19335-19336, 19350-19415, 19430-19530, 19555-19666, 19681, 19695-19717, 19731-19841, 19856-19864, 19878-19905, 19945-20156, 20180-20184, 20218-20297, 20349-20360, 20374-20388, 20402-20409, 20423-20435, 20449-20456, 20470-20505, 20519-20524, 20564-20574, 20589-20605, 20620-20641, 20655-20673, 20687-20717, 20745-20752, 20767-20790, 20804-20808, 20832-20834, 20859-20871, 20885-20922, 20936-20972, 20995-21005, 21020-21075, 21089-21097, 21111-21120, 21134-21161, 21175-21210, 21224-21241, 21256-21263, 21277-21283, 21297-21331, 21345-21417, 21431-21526, 21540-21592, 21606-21693, 21707-21746, 21760-21783, 21808-21870, 21884-21896, 21911-21947, 21961-22177, 22191-22199, 22213, 22229-22539, 22553-22572, 22587-22603, 22617-22680, 22719-22733, 22755-22777, 22791-22804, 22818-22848, 22862-

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22877, 22891-22909, 22923-22938, 22959-22995, 23009-23017, 23034-  
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 28824-28897, 28920-28934, 28952-28960, 28974-28997, 29011-29057,  
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20 The foregoing written specification is considered to be sufficient to enable one skilled  
in the art to practice the invention. The present invention is not to be limited in scope by  
examples provided, since the examples are intended as a single illustration of one aspect of  
the invention and other functionally equivalent embodiments are within the scope of the  
invention. Various modifications of the invention in addition to those shown and described  
25 herein will become apparent to those skilled in the art from the foregoing description and fall  
within the scope of the appended claims. The advantages and objects of the invention are not  
necessarily encompassed by each embodiment of the invention.

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### CLAIMS

What is claimed is:

1. A single stranded oligonucleotide having a sequence 5'-X-Y-Z, wherein X is any nucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a seed  
5 sequence of a human microRNA, and Z is a nucleotide sequence of 1-23 nucleotides in length, wherein the single stranded oligonucleotide is complementary with at least 8 consecutive nucleotides of a PRC2-associated region of a MECP2 gene.
2. The single stranded oligonucleotide of claim 1, wherein the oligonucleotide does not comprise three or more consecutive guanosine nucleotides.
- 10 3. The single stranded oligonucleotide of claim 1 or 2, wherein the oligonucleotide does not comprise four or more consecutive guanosine nucleotides.
4. The single stranded oligonucleotide of any one of claims 1 to 3, wherein the oligonucleotide is 8 to 30 nucleotides in length.
5. The single stranded oligonucleotide of any one of claims 1 to 4, wherein the  
15 oligonucleotide is 8 to 10 nucleotides in length and all but 1, 2, or 3 of the nucleotides of the complementary sequence of the PRC2-associated region are cytosine or guanosine nucleotides.
6. The single stranded oligonucleotide of any one of claims 1 to 5, wherein at least one nucleotide of the oligonucleotide is a nucleotide analogue.
- 20 7. The single stranded oligonucleotide of claim 7, wherein the at least one nucleotide analogue results in an increase in T<sub>m</sub> of the oligonucleotide in a range of 1 to 5 °C compared with an oligonucleotide that does not have the at least one nucleotide analogue.
8. The single stranded oligonucleotide of any one of claims 1 to 7, wherein at least one nucleotide of the oligonucleotide comprises a 2' O-methyl.
- 25 9. The single stranded oligonucleotide of any one of claims 1 to 8, wherein each nucleotide of the oligonucleotide comprises a 2' O-methyl.

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10. The single stranded oligonucleotide of any one of claims 1 to 8, wherein the oligonucleotide comprises at least one ribonucleotide, at least one deoxyribonucleotide, or at least one bridged nucleotide.

11. The single strand oligonucleotide of claim 10, wherein the bridged nucleotide  
5 is a LNA nucleotide, a cEt nucleotide or a ENA modified nucleotide.

12. The single stranded oligonucleotide of any one of claims 1 to 6, wherein each nucleotide of the oligonucleotide is a LNA nucleotide.

13. The single stranded oligonucleotide of any one of claims 1 to 6, wherein the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and 2'-fluoro-  
10 deoxyribonucleotides.

14. The single stranded oligonucleotide of any one of claims 1 to 6, wherein the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and 2'-O-methyl nucleotides.

15. The single stranded oligonucleotide of any one of claims 1 to 6, wherein the  
15 nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and ENA nucleotide analogues.

16. The single stranded oligonucleotide of any one of claims 1 to 6, wherein the nucleotides of the oligonucleotide comprise alternating deoxyribonucleotides and LNA nucleotides.

17. The single stranded oligonucleotide of any one of claims 13 to 16, wherein the  
20 5' nucleotide of the oligonucleotide is a deoxyribonucleotide.

18. The single stranded oligonucleotide of any one of claims 1 to 6, wherein the nucleotides of the oligonucleotide comprise alternating LNA nucleotides and 2'-O-methyl nucleotides.

19. The single stranded oligonucleotide of claim 18, wherein the 5' nucleotide of  
25 the oligonucleotide is a LNA nucleotide.

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20. The single stranded oligonucleotide of any one of claims 1 to 8, wherein the nucleotides of the oligonucleotide comprise deoxyribonucleotides flanked by at least one LNA nucleotide on each of the 5' and 3' ends of the deoxyribonucleotides.

21. The single stranded oligonucleotide of any one of claims 1 to 20, further comprising phosphorothioate internucleotide linkages between at least two nucleotides.

22. The single stranded oligonucleotide of claim 21, further comprising phosphorothioate internucleotide linkages between all nucleotides.

23. The single stranded oligonucleotide of any one of claims 1 to 22, wherein the nucleotide at the 3' position of the oligonucleotide has a 3' hydroxyl group.

24. The single stranded oligonucleotide of any one of claims 1 to 22, wherein the nucleotide at the 3' position of the oligonucleotide has a 3' thiophosphate.

25. The single stranded oligonucleotide of any one of claims 1 to 24, further comprising a biotin moiety conjugated to the 5' nucleotide.

26. A single stranded oligonucleotide comprising a region of complementarity that is complementary with at least 8 consecutive nucleotides of a PRC2-associated region of a MECP2 gene, wherein the oligonucleotide has at least one of:

a) a sequence that is 5'X-Y-Z, wherein X is any nucleotide and wherein X is anchored at the 5' end of the oligonucleotide, Y is a nucleotide sequence of 6 nucleotides in length that is not a human seed sequence of a microRNA, and Z is a nucleotide sequence of 1 to 23 nucleotides in length;

b) a sequence that does not comprise three or more consecutive guanosine nucleotides;

c) a sequence that has less than a threshold level of sequence identity with every sequence of nucleotides, of equivalent length to the second nucleotide sequence, that are between 50 kilobases upstream of a 5'-end of an off-target gene and 50 kilobases downstream of a 3'-end of the off-target gene;

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d) a sequence that is complementary to a PRC2-associated region that encodes an RNA that forms a secondary structure comprising at least two single stranded loops; and/or

e) a sequence that has greater than 60% G-C content.

27. The single stranded oligonucleotide of claim 26, wherein the oligonucleotide  
5 has the sequence 5'X-Y-Z and wherein the oligonucleotide is 8-50 nucleotides in length.

28. A composition comprising a single stranded oligonucleotide of any one of claims 1 to 27 and a carrier.

29. A composition comprising a single stranded oligonucleotide of any one of claims 1 to 27 in a buffered solution.

10 30. A composition of claim 28, wherein the oligonucleotide is conjugated to the carrier.

31. The composition of claim 30, wherein the carrier is a peptide.

32. The composition of claim 30, wherein the carrier is a steroid.

15 33. A pharmaceutical composition comprising a composition of any one of claims 28 to 32 and a pharmaceutically acceptable carrier.

34. A kit comprising a container housing the composition of any one of claims 28 to 33.

20 35. A method of increasing expression of a MECP2 gene in a cell, the method comprising delivering the single stranded oligonucleotide of any one of claims 1 to 27 into the cell.

36. The method of claim 35, wherein delivery of the single stranded oligonucleotide into the cell results in a level of expression of the MECP2 gene that is at least 50% greater than a level of expression of the MECP2 gene in a control cell that does not comprise the single stranded oligonucleotide.

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37. A method increasing levels of a MECP2 gene in a subject, the method comprising administering the single stranded oligonucleotide of any one of claims 1 to 27 to the subject.

38. A method of treating a condition associated with decreased levels of a MECP2  
5 gene in a subject, the method comprising administering the single stranded oligonucleotide of any one of claims 1 to 27 to the subject.

39. The method of claim 38, wherein the condition is Rett Syndrome, MECP2-related severe neonatal encephalopathy, Angelman syndrome, or PPM-X syndrome.