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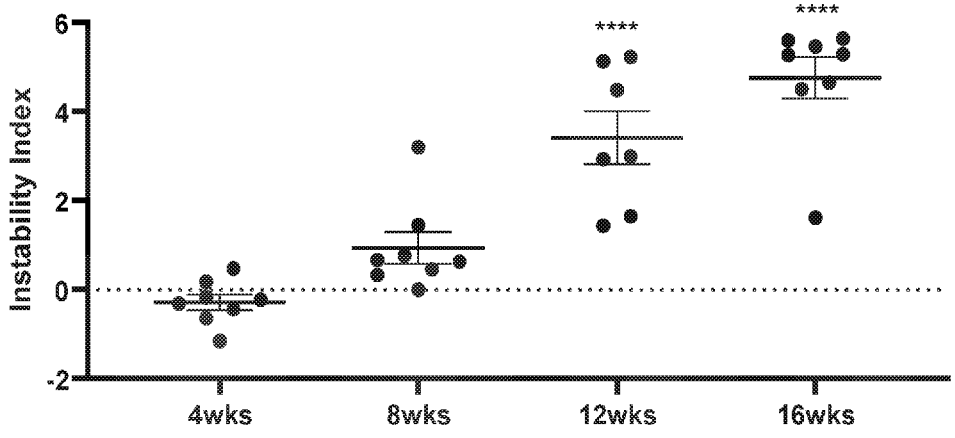


FIG. 1

(57) Abstract: The present disclosure features useful compositions and methods to treat trinucleotide repeat expansion disorders, e.g., in a subject in need thereof. In some aspects, the compositions and methods described herein are useful in the treatment of disorders associated with MSH3 activity.

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METHODS FOR THE TREATMENT OF TRINUCLEOTIDE REPEAT EXPANSION DISORDERS ASSOCIATED WITH MSH3 ACTIVITY

Incorporation by Reference of Sequence Listing

The contents of the text file named "4398.008PC03_SL_ST25.txt," which was created on November 25, 2019 and is 545,271 bytes in size, is hereby incorporated by reference in its entirety.

Background

Trinucleotide repeat expansion disorders are genetic disorders caused by trinucleotide repeat expansions. Trinucleotide repeat expansions are a type of genetic mutation where nucleotide repeats in certain genes or introns exceed the normal, stable threshold for that gene. The trinucleotide repeats can result in defective or toxic gene products, impair RNA transcription, and/or cause toxic effects by forming toxic mRNA transcripts.

Trinucleotide repeat expansion disorders are generally categorized by the type of repeat expansion. For example, Type 1 disorders such as Huntington's disease are caused by CAG repeats which result in a series of glutamine residues known as a polyglutamine tract, Type 2 disorders are caused by heterogeneous expansions that are generally small in magnitude, and Type 3 disorders such as fragile X syndrome are characterized by large repeat expansions that are generally located outside of the protein coding region of the genes. Trinucleotide repeat expansion disorders are characterized by a wide variety of symptoms such as progressive degeneration of nerve cells that is common in the Type 1 disorders.

Subjects with a trinucleotide repeat expansion disorder or those who are considered at risk for developing a trinucleotide repeat expansion disorder have a constitutive nucleotide expansion in a gene associated with disease (i.e., the trinucleotide repeat expansion is present in the gene during embryogenesis). Constitutive trinucleotide repeat expansions can undergo expansion after embryogenesis (i.e., somatic trinucleotide repeat expansion). Both constitutive trinucleotide repeat expansion and somatic trinucleotide repeat expansion can be associated with presence of disease, age at onset of disease, and/or rate of progression of disease.

Summary of the Disclosure

The present disclosure features useful compositions and methods to treat trinucleotide repeat expansion disorders, e.g., in a subject in need thereof. In some aspects, the compositions and methods described herein are useful in the treatment of disorders associated with MSH3 activity.

Oligonucleotides

Some aspects of this disclosure are directed to a single-stranded oligonucleotide of 10-30 linked nucleosides in length, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene. In some aspects, the disclosure is directed to a single-stranded oligonucleotide of 10-30 linked nucleosides in length, wherein the oligonucleotide comprises: (a) a DNA core sequence comprising linked deoxyribonucleosides; (b) a 5' flanking sequence comprising linked nucleosides; and (c) a 3' flanking

sequence comprising linked nucleosides; wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

In some aspects, the disclosure is directed to a single-stranded oligonucleotide of 10-30 linked nucleosides in length for inhibiting expression of a human MSH3 gene in a cell, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene. In some aspects, the disclosure is directed to a single-stranded oligonucleotide of 10-30 linked nucleosides in length for inhibiting expression of a human MSH3 gene in a cell, wherein the oligonucleotide comprises: (a) a DNA core comprising linked deoxyribonucleosides; (b) a 5' flanking sequence comprising linked nucleosides; and (c) a 3' flanking sequence comprising linked nucleosides; wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

In some aspects, the region of at least 10 nucleobases has at least 90% complementary to an MSH3 gene. In some aspects, the region of at least 10 nucleobases has at least 95% complementary to an MSH3 gene.

In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 355-385, 398-496, 559-589, 676-724, 762-810, 876-903, 912-974, 984-1047, 1054-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1768-1866, 2029-2063, 2087-2199, 2262-2293, 2304-2330, 2371-2410, 2432-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3073, 31323245, 3266-3306, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4074-4101, or 4281-4319 of the MSH3 gene. In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 359-385, 398-496, 559-589, 676-724, 762-810, 876-974, 984-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2262-2293, 2304-2329, 2371-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3072, 3132-3245, 3266-3303, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4076-4101, or 4281-4319 of the MSH3 gene. In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at is one or more of positions 155-196, 359-385, 413-462, 559-589, 676-724, 762-810, 876-974, 984-1096, 1114-1179, 1200-1227, 1294-1337, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2265-2293, 2378-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2712, 2727-2753, 2767-2919, 2934-3000, 3046-3071, 3144-3183, 3220-3245, 3397-3484, 3534-3575, 3591-3616, 3901-3931, or 4281-4306 of the MSH3 gene. In some aspects the region of at least 10

nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 435-462, 559-584, 763-808, 876-902, 931-958, 1001-1083, 1114-1179, 1294-1337, 1544-1578, 1835-1863, 2031-2056, 2144-2169, 2543-2577, 2590-2615, 2621-2647, 2685-2711, 2769-2795, or 2816-2868 of the MSH3 gene. In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 876-902, 930-958, 1056-1081, 1114-1139, 1154-1179, 1310-1337, 1546-1571, 1836-1862, 2141-2199, 2267-2292, 2540-2580, 2620-2647, 2686-2711, 2769-2868, 2939-2976, 3144-3169, or 3399-3424 of the MSH3 gene. In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 984-1021, 1467-1493, 1722-1747, 1767-1802, 1833-1861, 2385-2410, 2554-2581, 2816-2845, 2861-2920, or 3151-3183 of the MSH3 gene.

In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 6-2545. In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463. In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462 -2463. In some aspects, the

oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460. In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633. In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068. In some aspects, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

In some aspects, the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 6-2545. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661,

699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462-2463. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068. In some aspects, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456,1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

In some aspects, the oligonucleotide exhibits at least 50% mRNA inhibition at 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 60% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 70% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 85% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 50% mRNA inhibition at 2 nM when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least

60% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 70% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In some aspects, the oligonucleotide exhibits at least 85% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

The cell assay can comprise transfecting a mammalian cell, such as HEK293, NIH3T3, or HeLa, with oligonucleotides using Lipofectamine 2000 (Invitrogen) and measuring mRNA levels compared to a mammalian cell transfected with a mock oligonucleotide.

In some aspects, the oligonucleotide comprises at least one alternative internucleoside linkage. In some aspects, the at least one alternative internucleoside linkage is a phosphorothioate internucleoside linkage. In some aspects, the at least one alternative internucleoside linkage is a 2'-alkoxy internucleoside linkage. In some aspects, the at least one alternative internucleoside linkage is an alkyl phosphate internucleoside linkage.

In some aspects, the oligonucleotide comprises at least one alternative nucleobase. In some aspects, the alternative nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

In some aspects, the oligonucleotide comprises at least one alternative sugar moiety. In some aspects, the alternative sugar moiety is 2'-OMe or a bicyclic nucleic acid.

In some aspects, the oligonucleotide further comprises a ligand conjugated to the 5' end or the 3' end of the oligonucleotide through a monovalent or branched bivalent or trivalent linker.

In some aspects, the oligonucleotide comprises a region complementary to at least 17 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide comprises a region complementary to at least 19 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide comprises a region complementary to 19 to 23 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide comprises a region complementary to 19 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide comprises a region complementary to 20 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide is from about 15 to 25 nucleosides in length. In some aspects, the oligonucleotide is 20 nucleosides in length.

Pharmaceutical Compositions and Methods of Treatment Using the Same

In some aspects, the application is directed to a pharmaceutical composition comprising one or more of the oligonucleotides described herein and a pharmaceutically acceptable carrier or excipient.

In some aspects, the application is directed to a composition comprising one or more of the oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

In some aspects, the application is directed to a method of inhibiting transcription of MSH3 in a cell, the method comprising contacting the cell with one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex

nanoparticle, a lipoplex nanoparticle, or a liposome; for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibiting expression of the MSH3 gene in the cell.

In some aspects, the application is directed to a method of treating, preventing, or delaying the progression a trinucleotide repeat expansion disorder in a subject in need thereof, the method comprising contacting the cell with one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome; for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibiting expression of the MSH3 gene in the cell.

In some aspects, the application is directed to a method of reducing the level and/or activity of MSH3 in a cell of a subject identified as having a trinucleotide repeat expansion disorder, the method comprising contacting the cell with one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome, for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibiting expression of the MSH3 gene in the cell.

In some aspects, the application is directed to a method for inhibiting expression of an MSH3 gene in a cell comprising contacting the cell with one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome; for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibiting expression of the MSH3 gene in the cell, and maintaining the cell for a time sufficient to obtain degradation of a mRNA transcript of an MSH3 gene, thereby inhibiting expression of the MSH3 gene in the cell.

In some aspects, the application is directed to a method of decreasing trinucleotide repeat expansion in a cell, the method comprising contacting the cell with one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome; for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibiting expression of the MSH3 gene in the cell.

In some aspects, the cell is in a subject. In some aspects, the subject is a human. In some aspects, the cell is a cell of the central nervous system or a muscle cell.

In some aspects, the subject is identified as having a trinucleotide repeat expansion disorder. In some aspects, the trinucleotide repeat expansion disorder is a polyglutamine disease. In some aspects, the polyglutamine disease is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, and

Huntington's disease-like 2. In some aspects, the trinucleotide repeat expansion disorder is Huntington's disease.

In some aspects, the trinucleotide repeat expansion disorder is a non-polyglutamine disease. In some aspects, the non-polyglutamine disease is selected from the group consisting of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy. In some aspects, the trinucleotide repeat expansion disorder is Friedreich's ataxia. In some aspects, the trinucleotide repeat expansion disorder is myotonic dystrophy type 1.

In some aspects, the application is directed one or more of the oligonucleotides described herein, a pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome, for use in the prevention or treatment of a trinucleotide repeat expansion disorder. In some aspects, the one or more of the oligonucleotides described herein, the pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome is administered intrathecally.

In some aspects, the one or more of the oligonucleotides described herein, the pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome is administered intraventricularly.

In some aspects, the one or more of the oligonucleotides described herein, the pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome is administered intramuscularly.

In some aspects, the application is directed to a method of treating, preventing, or delaying progression a disorder in a subject in need thereof wherein the subject is suffering from trinucleotide repeat expansion disorder, comprising administering to said subject one or more of the oligonucleotides described herein, the pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome. In some aspects, the method of treating, preventing, or delaying progression of a disorder in a subject further comprises administering an additional therapeutic agent. In some aspects, the additional therapeutic agent is another oligonucleotide that hybridizes to an mRNA encoding the Huntingtin gene.

In some aspects, the method of treating, preventing, or delaying progression of a disorder in a subject progression delays progression of the trinucleotide repeat expansion disorder by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with a predicted progression.

In some aspects, the application is directed to one or more of the oligonucleotides described herein, the pharmaceutical composition of one or more of the oligonucleotides described herein, or the composition of one or more oligonucleotides described herein and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome for use in preventing or delaying progression of a trinucleotide repeat expansion disorder in a subject

Definitions

For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular aspects, and are not intended to limit the claimed technology, because the scope of the technology is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

In this application, unless otherwise clear from context, (i) the term “a” can be understood to mean “at least one”; (ii) the term “or” can be understood to mean “and/or”; and (iii) the terms “including” and “comprising” can be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps.

As used herein, the terms “about” and “approximately” refer to a value that is within 10% above or below the value being described. For example, the term “about 5 nM” indicates a range of from 4.5 to 5.5 nM.

The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21-nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range. “At least” is also not limited to integers (e.g., “at least 5% includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures.

As used herein, “no more than” or “less than” is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, an oligonucleotide with “no more than 3 mismatches to a target sequence” has 3, 2, 1, or 0 mismatches to a target sequence. When “no more than” is present before a series of numbers or a range, it is understood that “no more than” can modify each of the numbers in the series or range.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) can be by any appropriate route, such as one described herein.

As used herein, a “combination therapy” or “administered in combination” means that two (or more) different agents or treatments are administered to a subject as part of a defined treatment regimen for a particular disease or condition. The treatment regimen defines the doses and periodicity of administration of each agent such that the effects of the separate agents on the subject overlap. In some aspects, the delivery of the two or more agents is simultaneous or concurrent and the agents can be co-formulated. In some aspects, the two or more agents are not co-formulated and are administered in a sequential manner as part of a prescribed regimen. In some aspects, administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (e.g., synergistic). Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, one therapeutic agent of the combination can be administered by intravenous injection while an additional therapeutic agent of the combination can be administered orally.

As used herein, the term “MSH3” refers to MutS Homolog 3, a DNA mismatch repair protein, having an amino acid sequence from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native MSH3 that maintain at least one in vivo or in vitro activity of a native MSH3. The term encompasses full-length unprocessed precursor forms of MSH3 as well as mature forms resulting from post-translational cleavage of the signal peptide. MSH3 is encoded by the MSH3 gene. The nucleic acid sequence of an exemplary Homo sapiens (human) MSH3 gene is set forth in NCBI Reference NM_002439.4 or in SEQ ID NO: 1. The term “MSH3” also refers to natural variants of the wild-type MSH3 protein, such as proteins having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) to the amino acid sequence of wild-type human MSH3, which is set forth in NCBI Reference No. NP_002430.3 or in SEQ ID NO: 2. The nucleic acid sequence of an exemplary Mus musculus (mouse) MSH3 gene is set forth in NCBI Reference No. NM_010829.2 or in SEQ ID NO: 3. The nucleic acid sequence of an exemplary Rattus norvegicus (rat) MSH3 gene is set forth in NCBI Reference No. NM_001191957.1 or in SEQ ID NO: 4. The nucleic acid sequence of an exemplary Macaca fascicularis (cyno) MSH3 gene is set forth in NCBI Reference No. XM_005557283.2 or in SEQ ID NO: 5.

The term "MSH3" as used herein also refers to a particular polypeptide expressed in a cell by naturally occurring DNA sequence variations of the MSH3 gene, such as a single nucleotide polymorphism in the MSH3 gene. Numerous SNPs within the MSH3 gene have been identified and can be found at, for example, NCBI dbSNP (see, e.g., www.ncbi.nlm.nih.gov/snp). Non-limiting examples of SNPs within the MSH3 gene can be found at, NCBI dbSNP Accession Nos.: rs1650697, rs70991108, rs10168, rs26279 , rs26282, rs26779, rs26784, rs32989, rs33003, rs33008, rs33013,

rs40139, rs181747, rs184967, rs245346, rs245397, rs249633, rs380691, rs408626, rs442767, rs836802, rs836808, rs863221, rs1105525, rs1428030, rs1478834, rs1650694, rs1650737, rs1677626, rs1677658, rs1805355, rs2897298, rs3045983, rs3797897, rs4703819, rs6151627, rs6151640, rs6151662, rs6151670, rs6151735, rs6151838, rs7709909, rs7712332, rs10079641, rs12513549, and rs12522132.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an MSH3 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one aspect, the target portion of the sequence will be at least long enough to serve as a substrate for oligonucleotide-directed (e.g., antisense oligonucleotide (ASO)-directed) cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a MSH3 gene. The target sequence can be, for example, from about 9-36 nucleotides in length, e.g., about 15-30 nucleotides in length. For example, the target sequence can be 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated.

“G,” “C,” “A,” “T,” and “U” each generally stand for a naturally-occurring nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "nucleotide" can refer to an alternative nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of oligonucleotides by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured herein.

The terms “nucleobase” and “base” include the purine (e.g. adenine and guanine) and pyrimidine (e.g. uracil, thymine, and cytosine) moiety present in nucleosides and nucleotides which form hydrogen bonds in nucleic acid hybridization. The term nucleobase also encompasses alternative nucleobases which can differ from naturally-occurring nucleobases, but are functional during nucleic acid hybridization. In this context “nucleobase” refers to both naturally occurring nucleobases such as adenine, guanine, cytosine, thymidine, uracil, xanthine, and hypoxanthine, as well as alternative nucleobases. Such variants are for example described in Hirao et al (2012)

Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1.

The term "nucleoside" refers to a monomeric unit of an oligonucleotide or a polynucleotide having a nucleobase and a sugar moiety. A nucleoside can include those that are naturally-occurring as well as alternative nucleosides, such as those described herein. The nucleobase of a nucleoside can be a naturally-occurring nucleobase or an alternative nucleobase. Similarly, the sugar moiety of a nucleoside can be a naturally-occurring sugar or an alternative sugar.

The term "alternative nucleoside" refers to a nucleoside having an alternative sugar or an alternative nucleobase, such as those described herein.

In some aspects the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as an "alternative nucleobase" selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uridine, 5-bromouridine 5-thiazolo-uridine, 2-thio-uridine, pseudouridine, 1-methylpseudouridine, 5-methoxyuridine, 2′-thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine, and 2-chloro-6-aminopurine.

The nucleobase moieties can be indicated by the letter code for each corresponding nucleobase, e.g. A, T, G, C, or U, wherein each letter can include alternative nucleobases of equivalent function. In some aspects, e.g., for gapmers, 5-methyl cytosine LNA nucleosides can be used.

A "sugar" or "sugar moiety," includes naturally occurring sugars having a furanose ring. A sugar also includes an "alternative sugar," defined as a structure that is capable of replacing the furanose ring of a nucleoside. In some aspects, alternative sugars are non-furanose (or 4′-substituted furanose) rings or ring systems or open systems. Such structures include simple changes relative to the natural furanose ring, such as a six-membered ring, or can be more complicated as is the case with the non-ring system used in peptide nucleic acid. Alternative sugars can include sugar surrogates wherein the furanose ring has been replaced with another ring system such as, for example, a morpholino or hexitol ring system. Sugar moieties useful in the preparation of oligonucleotides having motifs include, without limitation, β-D-ribose, β-D-2′-deoxyribose, substituted sugars (such as 2′, 5′ and bis substituted sugars), 4′-S-sugars (such as 4′-S-ribose, 4′-S-2′-deoxyribose and 4′-S-2′-substituted ribose), bicyclic alternative sugars (such as the 2′-O—CH_2-4′ or 2′-O—$(CH_2)_2$-4′ bridged ribose derived bicyclic sugars) and sugar surrogates (such as when the ribose ring has been replaced with a morpholino or a hexitol ring system). The type of heterocyclic base and internucleoside linkage used at each position is variable and is not a factor in determining the motif. In most nucleosides having an alternative sugar moiety, the heterocyclic nucleobase is generally maintained to permit hybridization.

A "nucleotide," as used herein, refers to a monomeric unit of an oligonucleotide or polynucleotide that comprises a nucleoside and an internucleosidic linkage. The internucleosidic linkage can include a phosphate linkage. Similarly, "linked nucleosides" can be linked by phosphate linkages. Many "alternative internucleosidic linkages" are known in the art, including, but not limited to, phosphate, phosphorothioate, and boronophosphate linkages. Alternative nucleosides include

bicyclic nucleosides (BNAs) (e.g., locked nucleosides (LNAs) and constrained ethyl (cEt) nucleosides), peptide nucleosides (PNAs), phosphotriesters, phosphorothionates, phosphoramidates, and other variants of the phosphate backbone of native nucleoside, including those described herein.

An “alternative nucleotide,” as used herein, refers to a nucleotide having an alternative nucleoside or an alternative sugar, and an internucleoside linkage, which can include alternative nucleoside linkages.

The terms “oligonucleotide” and “polynucleotide,” as used herein, are defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides can be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide can be man-made. For example, the oligonucleotide can be chemically synthesized, and be purified or isolated. Oligonucleotide is also intended to include (i) compounds that have one or more furanose moieties that are replaced by furanose derivatives or by any structure, cyclic or acyclic, that can be used as a point of covalent attachment for the base moiety, (ii) compounds that have one or more phosphodiester linkages that are either modified, as in the case of phosphoramidate or phosphorothioate linkages, or completely replaced by a suitable linking moiety as in the case of formacetal or riboacetal linkages, and/or (iii) compounds that have one or more linked furanose-phosphodiester linkage moieties replaced by any structure, cyclic or acyclic, that can be used as a point of covalent attachment for the base moiety. The oligonucleotide can comprise one or more alternative nucleosides or nucleotides (e.g., including those described herein). It is also understood that oligonucleotide includes compositions lacking a sugar moiety or nucleobase but are still capable of forming a pairing with or hybridizing to a target sequence.

“Oligonucleotide” refers to a short polynucleotide (e.g., of 100 or fewer linked nucleosides).

"Chimeric" oligonucleotides or "chimeras," as used herein, are oligonucleotides which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide or nucleoside in the case of an oligonucleotide. Chimeric oligonucleotides also include “gapmers.”

The oligonucleotide can be of any length that permits specific degradation of a desired target RNA through an RNase H-mediated pathway, and can range from about 10-30 nucleosides in length, e.g., about 15-30 nucleosides in length or about 18-20 nucleosides in length, for example, about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleosides in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleosides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated.

As used herein, the term "oligonucleotide comprising a nucleobase sequence" refers to an oligonucleotide comprising a chain of nucleotides or nucleosides that is described by the sequence referred to using the standard nucleotide nomenclature.

The term “contiguous nucleobase region” refers to the region of the oligonucleotide which is complementary to the target nucleic acid. The term can be used interchangeably herein with the term “contiguous nucleotide sequence” or “contiguous nucleobase sequence.” In some aspects all the nucleotides of the oligonucleotide are present in the contiguous nucleotide or nucleoside region. In some aspects the oligonucleotide comprises the contiguous nucleotide region and can comprise further nucleotide(s) or nucleoside(s), for example a nucleotide linker region which can be used to attach a functional group to the contiguous nucleotide sequence. The nucleotide linker region can be complementary to the target nucleic acid. In some aspects the internucleoside linkages present between the nucleotides of the contiguous nucleotide region are all phosphorothioate internucleoside linkages. In some aspects, the contiguous nucleotide region comprises one or more sugar-modified nucleosides.

The term “gapmer,” as used herein, refers to an oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap or DNA core) which is flanked 5′ and 3′ by regions which comprise one or more affinity enhancing alternative nucleosides (wings or flanking sequence). Various gapmer designs are described herein. Headmers and tailmers are oligonucleotides capable of recruiting RNase H where one of the flanks is missing, i.e. only one of the ends of the oligonucleotide comprises affinity enhancing alternative nucleosides. For headmers the 3′ flanking sequence is missing (i.e. the 5′ flanking sequence comprises affinity enhancing alternative nucleosides) and for tailmers the 5′ flanking sequence is missing (i.e. the 3′ flanking sequence comprises affinity enhancing alternative nucleosides). A “mixed flanking sequence gapmer” refers to a gapmer wherein the flanking sequences comprise at least one alternative nucleoside, such as at least one DNA nucleoside or at least one 2′ substituted alternative nucleoside, such as, for example, 2′-O-alkyl-RNA, 2′-O-methyl-RNA, 2′-alkoxy-RNA, 2′-O-methoxyethyl-RNA (MOE), 2′-amino-DNA, 2′-Fluoro-RNA, 2′-F-ANA nucleoside(s), or bicyclic nucleosides (e.g., locked nucleosides or constrained ethyl (cEt) nucleosides). In some aspects the mixed flanking sequence gapmer has one flanking sequence which comprises alternative nucleosides (e.g. 5′ or 3′) and the other flanking sequence (3′ or 5′ respectfully) comprises 2′ substituted alternative nucleoside(s).

A "linker" or "linking group" is a connection between two atoms that links one chemical group or segment of interest to another chemical group or segment of interest via one or more covalent bonds. Conjugate moieties can be attached to the oligonucleotide directly or through a linking moiety (e.g. linker or tether). Linkers serve to covalently connect a third region, e.g. a conjugate moiety to an oligonucleotide (e.g. the termini of region A or C). In some aspects the conjugate or oligonucleotide conjugate can, comprise a linker region which is positioned between the oligonucleotide and the conjugate moiety. In some aspects, the linker between the conjugate and oligonucleotide is biocleavable. Phosphodiester containing biocleavable linkers are described in more detail in WO 2014/076195 (herein incorporated by reference).

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide or nucleoside sequence in relation to a second nucleotide or nucleoside sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide or nucleoside sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 °C, or 70 °C, for 12-16 hours followed by washing (see, e.g., "Molecular Cloning: A Laboratory Manual, Sambrook, et al. (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can be used. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides or nucleosides.

“Complementary” sequences, as used herein, can include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and alternative nucleotides or nucleosides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing. Complementary sequences between an oligonucleotide and a target sequence as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide or nucleoside sequence to an oligonucleotide or polynucleotide comprising a second nucleotide or nucleoside sequence over the entire length of one or both nucleotide or nucleoside sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3 or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, e.g., inhibition of gene expression via an RNase H-mediated pathway. “Substantially complementary” can refer to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (e.g., an mRNA encoding MSH3). For example, a polynucleotide is complementary to at least a part of a MSH3 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding MSH3.

As used herein, the term "region of complementarity" refers to the region on the oligonucleotide that is substantially complementary to all or a portion of a gene, primary transcript, a sequence (e.g., a target sequence, e.g., an MSH3 nucleotide sequence), or processed mRNA, so as to interfere with expression of the endogenous gene (e.g., MSH3). Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, e.g., within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the oligonucleotide.

As used herein, an “agent that reduces the level and/or activity of MSH3” refers to any polynucleotide agent (e.g., an oligonucleotide, e.g., an ASO) that reduces the level of or inhibits

expression of MSH3 in a cell or subject. The phrase "inhibiting expression of MSH3," as used herein, includes inhibition of expression of any MSH3 gene (such as, e.g., a mouse MSH3 gene, a rat MSH3 gene, a monkey MSH3 gene, or a human MSH3 gene) as well as variants or mutants of a MSH3 gene that encode a MSH3 protein. Thus, the MSH3 gene can be a wild-type MSH3 gene, a mutant MSH3 gene, or a transgenic MSH3 gene in the context of a genetically manipulated cell, group of cells, or organism.

By “reducing the activity of MSH3,” is meant decreasing the level of an activity related to MSH3 (e.g., by reducing the amount of trinucleotide repeats in a gene associated with a trinucleotide repeat expansion disorder that is related to MSH3 activity). The activity level of MSH3 can be measured using any method known in the art (e.g., by directly sequencing a gene associated with a trinucleotide repeat expansion disorder to measure the levels of trinucleotide repeats).

By “reducing the level of MSH3,” is meant decreasing the level of MSH3 in a cell or subject, e.g., by administering an oligonucleotide to the cell or subject. The level of MSH3 can be measured using any method known in the art (e.g., by measuring the levels of MSH3 mRNA or levels of MSH3 protein in a cell or a subject).

By “modulating the activity of a MutSβ heterodimer comprising MSH3,” is meant altering the level of an activity related to a MutSβ heterodimer, or a related downstream effect. The activity level of a MutSβ heterodimer can be measured using any method known in the art.

As used herein, the term “inhibitor” refers to any agent which reduces the level and/or activity of a protein (e.g., MSH3). Non-limiting examples of inhibitors include polynucleotides (e.g., oligonucleotide, e.g., ASOs). The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing," and other similar terms, and includes any level of inhibition.

The phrase "contacting a cell with an oligonucleotide," such as an oligonucleotide, as used herein, includes contacting a cell by any possible means. Contacting a cell with an oligonucleotide includes contacting a cell in vitro with the oligonucleotide or contacting a cell in vivo with the oligonucleotide. The contacting can be done directly or indirectly. Thus, for example, the oligonucleotide can be put into physical contact with the cell by the individual performing the method, or alternatively, the oligonucleotide agent can be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell in vitro can be done, for example, by incubating the cell with the oligonucleotide. Contacting a cell in vivo can be done, for example, by injecting the oligonucleotide into or near the tissue where the cell is located, or by injecting the oligonucleotide agent into another area, e.g., the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the oligonucleotide can contain and/or be coupled to a ligand, e.g., GalNAc3, that directs the oligonucleotide to a site of interest, e.g., the liver. Combinations of in vitro and in vivo methods of contacting are also possible. For example, a cell can be contacted in vitro with an oligonucleotide and subsequently transplanted into a subject.

In one aspect, contacting a cell with an oligonucleotide includes "introducing" or "delivering the oligonucleotide into the cell" by facilitating or effecting uptake or absorption into the cell.

Absorption or uptake of an ASO can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing an oligonucleotide into a cell can be in vitro and/or in vivo. For example, for in vivo introduction, oligonucleotides can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

As used herein, "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, e.g., an oligonucleotide. LNP refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). LNPs are described in, for example, U.S. Pat. Nos. 6,858,225; 6,815,432; 8,158,601; and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, e.g., one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the oligonucleotide composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the oligonucleotide composition, although in some examples, it can. Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids.

"Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

The term “antisense,” as used herein, refers to a nucleic acid comprising an oligonucleotide or polynucleotide that is sufficiently complementary to all or a portion of a gene, primary transcript, or processed mRNA, so as to interfere with expression of the endogenous gene (e.g., MSH3). “Complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides can hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other.

As used herein, the terms “effective amount,” “therapeutically effective amount,” and “a “sufficient amount” of an agent that reduces the level and/or activity of MSH3 (e.g., in a cell or a subject) described herein refer to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an “effective amount” or synonym thereto depends on the context in which it is being applied. For example, in the

context of treating a trinucleotide repeat expansion disorder, it is an amount of the agent that reduces the level and/or activity of MSH3 sufficient to achieve a treatment response as compared to the response obtained without administration of the agent that reduces the level and/or activity of MSH3. The amount of a given agent that reduces the level and/or activity of MSH3 described herein that will correspond to such an amount will vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, and/or weight) or host being treated, and the like, but can nevertheless be routinely determined by one of skill in the art. Also, as used herein, a "therapeutically effective amount" of an agent that reduces the level and/or activity of MSH3 of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a control. As defined herein, a therapeutically effective amount of an agent that reduces the level and/or activity of MSH3 of the present disclosure can be readily determined by one of ordinary skill by routine methods known in the art. Dosage regimen can be adjusted to provide the optimum therapeutic response.

"Prophylactically effective amount," as used herein, is intended to include the amount of an oligonucleotide that, when administered to a subject having or predisposed to have a trinucleotide repeat expansion disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" can vary depending on the oligonucleotide, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated. A prophylactically effective amount can refer to, for example, an amount of the agent that reduces the level and/or activity of MSH3 (e.g., in a cell or a subject) described herein or can refer to a quantity sufficient to, when administered to the subject, including a human, delay the onset of one or more of the trinucleotide repeat disorders described herein by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with the predicted onset.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount (either administered in a single or in multiple doses) of an oligonucleotide that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. Oligonucleotides employed in the methods herein can be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

As used herein, the term "region of complementarity" refers to the region on the oligonucleotide that is substantially complementary to all or a portion of a gene, primary transcript, a sequence (e.g., a target sequence, e.g., an MSH3 nucleotide sequence), or processed mRNA, so as to interfere with expression of the endogenous gene (e.g., MSH3). Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, e.g., within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the oligonucleotide.

An “amount effective to reduce trinucleotide repeat expansion” of a particular gene refers to an amount of the agent that reduces the level and/or activity of MSH3 (e.g., in a cell or a subject) described herein, orto a quantity sufficient to, when administered to the subject, including a human, to reduce the trinucleotide repeat expansion of a particular gene (e.g., a gene associated with a trinucleotide repeat expansion disorder described herein).

As used herein, the term “a subject identified as having a trinucleotide repeat expansion disorder” refers to a subject identified as having a molecular or pathological state, disease or condition of or associated with a trinucleotide repeat expansion disorder, such as the identification of a trinucleotide repeat expansion disorder or symptoms thereof, or to identification of a subject having or suspected of having a trinucleotide repeat expansion disorder who can benefit from a particular treatment regimen.

As used herein, “trinucleotide repeat expansion disorder” refers to a class of genetic diseases or disorders characterized by excessive trinucleotide repeats (e.g., trinucleotide repeats such as CAG) in a gene or intron in the subject which exceed the normal, stable threshold, for the gene or intron. Nucleotide repeats are common in the human genome and are not normally associated with disease. In some cases, however, the number of repeats expands beyond a stable threshold and can lead to disease, with the severity of symptoms generally correlated with the number of repeats. Trinucleotide repeat expansion disorders include “polyglutamine” and “non-polyglutamine” disorders.

By “determining the level of a protein” is meant the detection of a protein, or an mRNA encoding the protein, by methods known in the art either directly or indirectly. “Directly determining” means performing a process (e.g., performing an assay or test on a sample or “analyzing a sample” as that term is defined herein) to obtain the physical entity or value. “Indirectly determining” refers to receiving the physical entity or value from another party or source (e.g., a third-party laboratory that directly acquired the physical entity or value). Methods to measure protein level generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, liquid chromatography (LC)-mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry, as well as assays based on a property of a protein including, but not limited to, enzymatic activity or interaction with other protein partners. Methods to measure mRNA levels are known in the art.

“Percent (%) sequence identity” with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps (DNA core sequences), if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate

parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values can be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

By "level" is meant a level or activity of a protein, or mRNA encoding the protein (e.g., MSH3), optionally as compared to a reference. The reference can be any useful reference, as defined herein. By a "decreased level" or an "increased level" of a protein is meant a decrease or increase in protein level, as compared to a reference (e.g., a decrease or an increase by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, or more; a decrease or an increase of more than about 10%, about 15%, about 20%, about 50%, about 75%, about 100%, or about 200%, as compared to a reference; a decrease or an increase by less than about 0.01-fold, about 0.02-fold, about 0.1-fold, about 0.3-fold, about 0.5-fold, about 0.8-fold, or less; or an increase by more than about 1.2-fold, about 1.4-fold, about 1.5-fold, about 1.8-fold, about 2.0-fold, about 3.0-fold, about 3.5-fold, about 4.5-fold, about 5.0-fold, about 10-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 100-fold, about 1000-fold, or more). A level of a protein can be expressed in mass/vol (e.g., g/dL, mg/mL, µg/mL, or ng/mL) or percentage relative to total protein or mRNA in a sample.

The term "pharmaceutical composition," as used herein, represents a composition containing a compound described herein formulated with a pharmaceutically acceptable excipient, and can be manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, caplet, gelcap, or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); for intrathecal injection; for intracerebroventricular injections; for intraparenchymal injection; or in any other pharmaceutically acceptable formulation.

A "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a

patient. Excipients can include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

As used herein, the term “pharmaceutically acceptable salt” means any pharmaceutically acceptable salt of the compound of any of the compounds described herein. For example, pharmaceutically acceptable salts of any of the compounds described herein include those that are within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., J. Pharmaceutical Sciences 66:1-19, 1977 and in Pharmaceutical Salts: Properties, Selection, and Use, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting a free base group with a suitable organic acid.

The compounds described herein can have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts can be acid addition salts involving inorganic or organic acids or the salts can, in the case of acidic forms of the compounds described herein, be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases and methods for preparation of the appropriate salts are well-known in the art. Salts can be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and

magnesium, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, and ethylamine.

By a “reference” is meant any useful reference used to compare protein or mRNA levels or activity. The reference can be any sample, standard, standard curve, or level that is used for comparison purposes. The reference can be a normal reference sample or a reference standard or level. A “reference sample” can be, for example, a control, e.g., a predetermined negative control value such as a “normal control” or a prior sample taken from the same subject; a sample from a normal healthy subject, such as a normal cell or normal tissue; a sample (e.g., a cell or tissue) from a subject not having a disease; a sample from a subject that is diagnosed with a disease, but not yet treated with a compound described herein; a sample from a subject that has been treated by a compound described herein; or a sample of a purified protein (e.g., any described herein) at a known normal concentration. By “reference standard or level” is meant a value or number derived from a reference sample. A “normal control value” is a pre-determined value indicative of non-disease state, e.g., a value expected in a healthy control subject. Typically, a normal control value is expressed as a range (“between X and Y”), a high threshold (“no higher than X”), or a low threshold (“no lower than X”). A subject having a measured value within the normal control value for a particular biomarker is typically referred to as “within normal limits” for that biomarker. A normal reference standard or level can be a value or number derived from a normal subject not having a disease or disorder (e.g., a trinucleotide repeat expansion disorder); a subject that has been treated with a compound described herein. In some aspects, the reference sample, standard, or level is matched to the sample subject sample by at least one of the following criteria: age, weight, sex, disease stage, and overall health. A standard curve of levels of a purified protein, e.g., any described herein, within the normal reference range can be used as a reference.

As used herein, the term “subject” refers to any organism to which a composition can be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans). A subject can seek or be in need of treatment, require treatment, be receiving treatment, be receiving treatment in the future, or be a human or animal who is under care by a trained professional for a particular disease or condition.

As used herein, the terms "treat," "treated," and "treating" mean both therapeutic treatment and prophylactic or preventative measures wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (i.e., not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder, or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive

levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

As used herein, the terms “variant” and “derivative” are used interchangeably and refer to naturally-occurring, synthetic, and semi-synthetic analogues of a compound, peptide, protein, or other substance described herein. A variant or derivative of a compound, peptide, protein, or other substance described herein can retain or improve upon the biological activity of the original material.

The details of one or more aspects are set forth in the description below. Other features, objects, and advantages will be apparent from the description and from the claims.

Brief Description of Drawings

FIG. 1 is a distribution plot showing the somatic expansion of a human HTT transgene in the striatum as measured by the instability index in R6/2 mice at 4, 8, 12, and 16 weeks of age (4 male and 4 female per age group). The bars are mean values and error bars indicate standard deviation.

FIG. 2 is a distribution plot showing the somatic expansion of a human HTT transgene in the cerebellum as measured by the instability index in R6/2 mice at 4, 8, 12, and 16 weeks of age (4 male and 4 female per age group).

Detailed Description

The present inventors have found that inhibition or depletion of MSH3 level and/or activity in a cell is effective in the treatment of a trinucleotide repeat expansion disorder. Accordingly, useful compositions and methods to treat trinucleotide repeat expansion disorders, e.g., in a subject in need thereof are provided herein.

I. Trinucleotide Repeat Expansion Disorders

Trinucleotide repeat expansion disorders are a family of genetic disorders characterized by the pathogenic expansion of a repeat region within a genomic region. In such disorders, the number of repeats exceeds that of a gene’s normal, stable threshold, expanding into a diseased range.

Trinucleotide repeat expansion disorders generally can be categorized as “polyglutamine” or “non-polyglutamine.” Polyglutamine disorders, including Huntington's disease (HD) and several spinocerebellar ataxias, are caused by a CAG (glutamine) repeats in the protein-coding regions of specific genes. Non-polyglutamine disorders are more heterogeneous and can be caused by CAG trinucleotide repeat expansions in non-coding regions, as in Myotonic dystrophy, or by the expansion of trinucleotide repeats other than CAG that can be in coding or non-coding regions such as the CGG repeat expansion responsible for Fragile X Syndrome.

Trinucleotide repeat expansion disorders are dynamic in the sense that the number of repeats can vary from generation-to-generation, or even from cell-to-cell in the same individual. Repeat expansion is believed to be caused by polymerase "slipping" during DNA replication. Tandem repeats in the DNA sequence can "loop out" while maintaining complementary base pairing between the

parent strand and daughter strands. If the loop structure is formed from the daughter strand, the number of repeats will increase.

Conversely, if the loop structure is formed from the parent strand, the number of repeats will decrease. It appears that expansion is more common than reduction. In general, the length of repeat expansion is negatively correlated with prognosis; longer repeats are correlated with an earlier age of onset and worsened disease severity. Thus, trinucleotide repeat expansion disorders are subject to "anticipation," meaning the severity of symptoms and/or age of onset worsen through successive generations of affected families due to the expansion of these repeats from one generation to the next.

Trinucleotide repeat expansion disorders are well known in the art. Exemplary trinucleotide repeat expansion disorders and the trinucleotide repeats of the genes commonly associated with them are included in Table 1.

Table 1: Exemplary Trinucleotide Repeat Expansion Disorders

Disease	Gene	Nucleotide Repeat
ARX-nonsyndromic X-linked mental retardation (XLMR)	ARX	GCG
Baratela-Scott Syndrome	XYLT1	GGC
Blepharophimosis / Ptosis / Epicanthus inversus syndrome type II	FOXL2	GCG
Cleidocranial dysplasia (CCD)	RUNX2	GCG
Congenital central hypoventilation	PHOX-2B	GCG
Congenital central hypoventilation syndrome (CCHS)	PHOX2B	GCG
Creutzfeldt-Jakob disease	PRNP	
Dentatorubral-pallidoluysian atrophy (DRPLA) / Haw River syndrome	ATN1	CAG
Early infantile epileptic encephalopathy (Ohtahara syndrome)	ARX	GCG
FRA2A syndrome	AFF3	CGC
FRA7A syndrome	ZNF713	CGG
Fragile X mental retardation (FRAX-E)	AFF2 / FMR2	GCC
Fragile X Syndrome (FXS)	FMR1	CGG
Fragile X-associated Primary Ovarian Insufficiency (FXPOI)	FMR1	CGG
Fragile X-associated Tremor Ataxia Syndrome (FXTAS)	FMR1	CGG
Friedreich ataxia (FRDA)	FXN	GAA
Fuchs' Corneal Endothelial Dystrophy (FECD)	TCF4	CTG
Hand-foot genital syndrome (HFGS)	HOXA13	GCG
Holoprosencephaly disorder (HPE)	ZIC2	GCG
Huntington disease-like 2 (HDL2)	JPH3	CTG
Huntington's Disease (HD)	HTT	CAG

Infantile spasm syndrome / West syndrome (ISS)	ARX	GCG
Jacobsen syndrome		
KCNN3-associated (e.g., schizophrenia)	KCNN3	CAG
Multiple Skeletal dysplasias	COMP	GAC
Myotonic Dystrophy type 1 (DM1)	DMPK	CTG
Myotonic Dystrophy type 2 (DM2)	CNBP	CCTG
NCOA3-associated (e.g., increased risk of prostate cancer)	NCOA3	CAG
Neuronal intranuclear inclusion disease (NIID)	NOTCH2NLC	GGC
Oculopharyngeal Muscular Dystrophy (OPMD)	PABPN1	GCG
Spastic ataxia - Charlevoix-Saguenay		
Spinal Muscular Bulbar Atrophy (SMBA)	AR	CAG
Spinocerebellar ataxia type 1 (SCA1)	ATXN1	CAG
Spinocerebellar ataxia type 10 (SCA10)	ATXN10	ATTCT
Spinocerebellar ataxia type 12 (SCA12)	PPP2R2B	CAG
Spinocerebellar ataxia type 17 (SCA17)	TBP / ATXN17	CAG
Spinocerebellar ataxia type 2 (SCA2)	ATXN2	CAG
Spinocerebellar ataxia type 3 (SCA3) / Machado-Joseph Disease	ATXN3	CAG
Spinocerebellar ataxia type 45 (SCA45)	FAT2	CAG
Spinocerebellar ataxia type 6 (SCA6)	CACNA1A	CAG
Spinocerebellar ataxia type 7 (SCA7)	ATXN7	CAG
Spinocerebellar ataxia type 8 (SCA8)	ATXN8	CTG
Syndromic neurodevelopmental disorder with cerebellar, ocular, craniofacial, and genital features (COFG syndrome)	MAB21L1	CAG
Synpolydactyly (SPD I)	HOXD13	GCG
Synpolydactyly (SPD II)	HOXD12	GCG

The proteins associated with trinucleotide repeat expansion disorders are typically selected based on an experimental association of the protein associated with a trinucleotide repeat expansion disorder to a trinucleotide repeat expansion disorder. For example, the production rate or circulating concentration of a protein associated with a trinucleotide repeat expansion disorder can be elevated or depressed in a population having a trinucleotide repeat expansion disorder relative to a population lacking the trinucleotide repeat expansion disorder. Differences in protein levels can be assessed using proteomic techniques including but not limited to Western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA), and mass spectrometry. Alternatively, the proteins associated with trinucleotide repeat expansion disorders can be identified by obtaining gene expression profiles of the genes encoding the proteins using genomic techniques including, but not

limited to, DNA microarray analysis, serial analysis of gene expression (SAGE), and quantitative real-time polymerase chain reaction (qPCR).

II. Evidence for the Involvement of Mismatch Repair Pathway in Trinucleotide Repeat Expansion

There is growing evidence that DNA repair pathways, particularly mismatch repair (MMR), are involved in the expansion of trinucleotide repeats. A recent genome-wide association (GWA) analysis led to the identification of loci harboring genetic variations that alter the age at neurological onset of Huntington's disease (HD) (GEM-HD Consortium, Cell. 2015 Jul 30;162(3):516-26). The study identified MLH1, the human homolog of the *E. coli* DNA mismatch repair gene *mutL*. A subsequent GWA study in polyglutamine disease patients found significant association of age at onset when grouping all polyglutamine diseases (HD and SCAs) with DNA repair genes as a group, as well as significant associations for specific SNPs in *FAN1* and *PMS2* with the diseases (Bettencourt et al., (2016) *Ann. Neurol.,* 79: 983-990). These results were consistent with those from an earlier study comparing differences in repeat expansion in two different mouse models of Huntington's Disease, which identified Mlh1 and Mlh3 as novel critical modifiers of CAG instability (Pinto et al., (2013) Mismatch Repair Genes *Mlh1* and *Mlh3* Modify CAG Instability in Huntington's Disease Mice: Genome-Wide and Candidate Approaches. PLoS Genet 9(10): e1003930). Another member of the mismatch repair pathway, 8-oxo-guanine glycosylase (OGG1) has also been implicated in expansion, as somatic expansion was found to be reduced in transgenic mice lacking *OGG1* (Kovtun I. V. et al. (2007) Nature 447, 447–452). However, another study found that human subjects containing a Ser326Cys polymorphism in *hOGG1*, which results in reduced OGG1 activity, results in increased mutant huntingtin (Coppede et al., (2009) *Toxicol*., 278: 199-203). Likewise, complete inactivation of *Fan1*, another component of the DNA repair pathway, in a mouse HD model produces somatic CAG expansions (Long et al. (2018) *J. Hum Genet.,* 103: 1-9). MSH3, another component of the mismatch repair pathway, has been reported to be linked to somatic expansion: polymorphisms in *Msh3* was associated with somatic instability of the expanded CTG trinucleotide repeat in myotonic dystrophy type 1 (DM1) patients (Morales et al., (2016) *DNA Repair* 40: 57-66). Furthermore, natural polymorphisms in *Msh3* and *Mlh1* have been revealed as mediators of mouse strain specific differences in CTG•CAG repeat instability (Pinto et al. (2013) *ibid;* Tome et al., (2013) *PLoS Genet.* 9 e1003280). Further evidence of *Msh2* and *Msh3*'s involvement in expansion repeats was reported in a study in which short hairpin RNA (shRNA) knockdown of either MSH2 or MSH3 slowed, and ectopic expression of either MSH2 or MSH3 induced GAA trinucleotide repeat expansion of the Friedreich Ataxia (FRDA) gene in fibroblasts derived from FRDA patients (Halabi et al., (2012) *J. Biol. Chem. 287,* 29958–29967). In spite of some inconsistent results provided above, there is strong evidence that the MMR pathway plays some role in the expansion of trinucleotide repeats in various disorders. Moreover, they are the first to recognize that the inhibition of the MMR pathway provides for the treatment or prevention of these repeat expansion disorders; however, no therapy is currently available or in development which modulates MMR for purposes of treating or preventing these repeat expansion disorders.

III. Oligonucleotide Agents

Agents described herein that reduce the level and/or activity of MSH3 in a cell can be, for example, a polynucleotide, e.g., an oligonucleotide. These agents reduce the level of an activity related to MSH3, or a related downstream effect, or reduce the level of MSH3 in a cell or subject.

In some aspects, the agent that reduces the level and/or activity of MSH3 is a polynucleotide. In some aspects, the polynucleotide is a single-stranded oligonucleotide, e.g., that acts by way of an RNase H-mediated pathway. Oligonucleotides include DNA and DNA/RNA chimeric molecules, typically about 10 to 30 nucleotides in length, which recognize polynucleotide target sequences or sequence portions through hydrogen bonding interactions with the nucleotide bases of the target sequence (e.g., MSH3). An oligonucleotide molecule can decrease the expression level (e.g., protein level or mRNA level) of MSH3. For example, an oligonucleotide includes oligonucleotides that targets full-length MSH3. In some aspects, the oligonucleotide molecule recruits an RNase H enzyme, leading to target mRNA degradation.

In some aspects, the oligonucleotide decreases the level and/or activity of a positive regulator of function. In other aspects, the oligonucleotide increases the level and/or activity of an inhibitor of a positive regulator of function. In some aspects, the oligonucleotide increases the level and/or activity of a negative regulator of function.

In some aspects, the oligonucleotide decreases the level and/or activity or function of MSH3. In some aspects, the oligonucleotide inhibits expression of MSH3. In other aspects, the oligonucleotide increases degradation of MSH3 and/or decreases the stability (i.e., half-life) of MSH3. The oligonucleotide can be chemically synthesized.

The oligonucleotide includes an oligonucleotide having a region of complementarity (e.g., a contiguous nucleobase region) which is complementary to at least a part of an mRNA formed in the expression of a MSH3 gene. The region of complementarity can be about 30 nucleotides or less in length (e.g., about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the MSH3 gene, the oligonucleotide can inhibit the expression of the MSH3 gene (e.g., a human, a primate, a non-primate, or a bird MSH3 gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

Similarly, the region of complementarity to the target sequence can be between 10 and 30 linked nucleosides in length, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 or between 10-29, 10-28, 10-27, 10-26, 10-25, 10-24, 10-23, 10-22, 10-21, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 linked nucleosides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated.

An oligonucleotide can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

The oligonucleotide compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide comprising unnatural or alternative nucleotides can be easily prepared. Single-stranded oligonucleotides can be prepared using solution-phase or solid-phase organic synthesis or both.

In one aspect, an oligonucleotide includes a region of at least 10 contiguous nucleobases having at least 80% (e.g., at least 85%, at least 90%, at least 95%, or at least 99%) complementary to at least 10 contiguous nucleotides of a MSH3 gene. In some aspects, the oligonucleotide comprises a sequence complementary to at least 17 contiguous nucleotides, 19-23 contiguous nucleotides, 19 contiguous nucleotides, or 20 contiguous nucleotides of a MSH3 gene. The oligonucleotide sequence can be selected from the group of sequences provided in any one of SEQ ID NOs: 6-2545.

In one aspect, the sequence is substantially complementary to a sequence of an mRNA generated in the expression of a MSH3 gene. In some aspects, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 355-385, 398-496, 559-589, 676-724, 762-810, 876-903, 912-974, 984-1047, 1054-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1768-1866, 2029-2063, 2087-2199, 2262-2293, 2304-2330, 2371-2410, 2432-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3073, 3132-3245, 3266-3306, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4074-4101, and 4281-4319 of the MSH3 gene. In one aspect, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 359-385, 398-496, 559-589, 676-724, 762-810, 876-974, 984-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2262-2293, 2304-2329, 2371-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3072, 3132-3245, 3266-3303, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4076-4101, and 4281-4319 of the MSH3 gene. In one aspect, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-196, 359-385, 413-462, 559-589, 676-724, 762-810, 876-974, 984-1096, 1114-1179, 1200-1227, 1294-1337, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2265-2293, 2378-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2712, 2727-2753, 2767-2919, 2934-3000, 3046-3071, 3144-3183, 3220-3245, 3397-3484, 3534-3575, 3591-3616, 3901-3931, and 4281-4306 of the MSH3 gene. In one aspect, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 435-462, 559-584, 763-808, 876-902, 931-958, 1001-1083, 1114-1179, 1294-1337, 1544-1578, 1835-1863, 2031-2056, 2144-2169, 2543-2577, 2590-2615, 2621-2647, 2685-2711, 2769-2795, and 2816-2868 of the MSH3 gene. In one aspect, the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 876-902,

930-958, 1056-1081, 1114-1139, 1154-1179, 1310-1337, 1546-1571, 1836-1862, 2141-2199, 2267-2292, 2540-2580, 2620-2647, 2686-2711, 2769-2868, 2939-2976, 3144-3169, and 3399-3424 of the MSH3 gene. In one aspect the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 984-1021, 1467-1493, 1722-1747, 1767-1802, 1833-1861, 2385-2410, 2554-2581, 2816-2845, 2861-2920, and 3151-3183 of the MSH3 gene.

In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 6-2545. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, and 2463. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, and 2462-and 2463. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252,

1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, and 2460. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, and 1631-1633. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, and 2068. In one aspect, the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461,1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, and 1862-1869

In some aspects, the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 6-2545. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, and 2463. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745,

1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, and 2462-and 2463. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, and 2460. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, and 1631-1633. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, and 2068. In one aspect, the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, and 1862-1869.

In one aspect, the oligonucleotide exhibits at least 50% mRNA inhibition at 20 nM when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 60% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 70% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 85% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 50% mRNA inhibition at 2 nM when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 60% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 70% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell. In one aspect, the oligonucleotide exhibits at least 85% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

The cell assay can comprise transfecting mammalian cells, such as HEK293, NIH3T3, or HeLa cells, with the desired a concentration of oligonucleotide (e.g., 2 nM or 20 nM) using Lipofectamine 2000 (Invitrogen) and comparing MSH3 mRNA levels of transfected cells to MSH3 levels of control cells. Control cells can be transfected with oligonucleotides not specific to MSH3 or mock transfected. mRNA levels can be determined using RT-qPCR and MSH3 mRNA levels can be normalized to GAPDH mRNA levels. The percent inhibition can be calculated as the percent of MSH3 mRNA concentration relative to the MSH3 concentration of the control cells.

In some aspects the oligonucleotide, or contiguous nucleotide region thereof, has a gapmer design or structure also referred herein merely as "gapmer." In a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5′-flanking sequence (also known as a 5'-wing), a DNA core sequence (also known as a gap) and a 3′-flanking sequence (also known as a 3'-wing), in '5->3' orientation. In this design, the 5' and 3' flanking sequences comprise at least one alternative nucleoside which is adjacent to a DNA core sequence, and can, in some aspects, comprise a contiguous stretch of 2-7 alternative nucleosides, or a contiguous stretch of alternative and DNA nucleosides (mixed flanking sequences comprising both alternative and DNA nucleosides).

The length of the 5'- flanking sequence region can be at least two nucleosides in length (e.g., at least at least 2, at least 3, at least 4, at least 5, or more nucleosides in length). The length of the 3'-flanking sequence region can be at least two nucleosides in length (e.g., at least 2, at least 3, at least at least 4, at least 5, or more nucleosides in length). The 5' and 3' flanking sequences can be symmetrical or asymmetrical with respect to the number of nucleosides they comprise. In some aspects, the DNA core sequence comprises about 10 nucleosides flanked by a 5' and a 3' flanking sequence each comprising about 5 nucleosides, also referred to as a 5-10-5 gapmer.

Consequently, the nucleosides of the 5′ flanking sequence and the 3′ flanking sequence which are adjacent to the DNA core sequence are alternative nucleosides, such as 2′ alternative nucleosides. The DNA core sequence comprises a contiguous stretch of nucleotides which are capable of recruiting RNase H, when the oligonucleotide is in duplex with the MSH3 target nucleic acid. In some aspects, the DNA core sequence comprises a contiguous stretch of 5-16 DNA nucleosides. In other aspects, the DNA core sequence comprises a region of at least 10 contiguous nucleobases having at least 80% (e.g., at least 85%, at least 90%, at least 95%, or at least 99%) complementarity to an MSH3 gene. In some aspects, the gapmer comprises a region complementary to at least 17 contiguous nucleotides, 19-23 contiguous nucleotides, or 19 contiguous nucleotides of a MSH3 gene. The gapmer is complementary to the MSH3 target nucleic acid, and can therefore be the contiguous nucleoside region of the oligonucleotide.

The 5' and 3' flanking sequences, flanking the 5′ and 3′ ends of the DNA core sequence, can comprise one or more affinity enhancing alternative nucleosides. In some aspects, the 5' and/or 3′ flanking sequence comprises at least one 2′-O-methoxyethyl (MOE) nucleoside. In some aspects, the 5' and/or 3' flanking sequences, contain at least two MOE nucleosides. In some aspects, the 5′ flanking sequence comprises at least one MOE nucleoside. In some aspects both the 5′ and 3′ flanking sequence comprise a MOE nucleoside. In some aspects, all the nucleosides in the flanking sequences are MOE nucleosides. In other aspects, the flanking sequence can comprise both MOE

nucleosides and other nucleosides (mixed flanking sequence), such as DNA nucleosides and/or non-MOE alternative nucleosides, such as bicyclic nucleosides (BNAs) (e.g., LNA nucleosides or cET nucleosides), or other 2' substituted nucleosides. In this case the DNA core sequence is defined as a contiguous sequence of at least 5 RNase H recruiting nucleosides (such as 5-16 DNA nucleosides) flanked at the 5′ and 3′ end by an affinity enhancing alternative nucleoside, such as an MOE nucleoside.

In other aspects, the 5' and/ or 3′ flanking sequence comprises at least one BNA (e.g., at least one LNA nucleoside or cET nucleoside). In some aspects, 5' and/ or 3′ flanking sequence comprises at least 2 bicyclic nucleosides. In some aspects, the 5′ flanking sequence comprises at least one BNA. In some aspects both the 5′ and 3′ flanking sequence comprise a BNA. In some aspects, all the nucleosides in the flanking sequences are BNAs. In other aspects, the flanking sequence can comprise both BNAs and other nucleosides (mixed flanking sequences), such as DNA nucleosides and/or non-BNA alternative nucleosides, such as 2′ substituted nucleosides. In this case the DNA core sequence is defined as a contiguous sequence of at least five RNase H recruiting nucleosides (such as 5-16 DNA nucleosides) flanked at the 5′ and 3′ end by an affinity enhancing alternative nucleoside, such as a BNA, such as an LNA, such as beta-D-oxy-LNA.

The 5′ flank attached to the 5' end of the DNA core sequence comprises, contains, or consists of at least one alternative sugar moiety (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative sugar moieties). In some aspects, the flanking sequence comprises or consists of from 1 to 7 alternative nucleobases, such as from 2 to 6 alternative nucleobases, such as from 2 to 5 alternative nucleobases, such as from 2 to 4 alternative nucleobases, such as from 1 to 3 alternative nucleobases, such as one, two, three or four alternative nucleobases. In some aspects, the flanking sequence comprises or consists of at least one alternative internucleoside linkage (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

The 3′ flank attached to the 3' end of the DNA core sequence comprises, contains, or consists of at least one alternative sugar moiety (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative sugar moieties). In some aspects, the flanking sequence comprises or consists of from 1 to 7 alternative nucleobases, such as from 2 to 6 alternative nucleobases, such as from 2 to 5 alternative nucleobases, such as from 2 to 4 alternative nucleobases, such as from 1 to 3 alternative nucleobases, such as one, two, three, or four alternative nucleobases. In some aspects, the flanking sequence comprises or consists of at least one alternative internucleoside linkage (e.g., at least three, at least four, at least five, at least six, at least seven, or more alternative internucleoside linkages).

In an aspect, one or more or all of the alternative sugar moieties in the flanking sequence are 2' alternative sugar moieties.

In a further aspect, one or more of the 2′ alternative sugar moieties in the wing regions are selected from 2′-O-alkyl-sugar moieties, 2′-O-methyl-sugar moieties, 2′-amino-sugar moieties, 2′-fluoro-sugar moieties, 2′-alkoxy-sugar moieties, MOE sugar moieties, LNA sugar moieties, arabino nucleic acid (ANA) sugar moieties, and 2′-fluoro-ANA sugar moieties.

In one aspect, all the alternative nucleosides in the flanking sequences are bicyclic nucleosides. In a further aspect, the bicyclic nucleosides in the flanking sequences are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET, and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof.

In some aspects, the one or more alternative internucleoside linkages in the flanking sequences are phosphorothioate internucleoside linkages. In some aspects, the phosphorothioate linkages are stereochemically pure phosphorothioate linkages. In some aspects, the phosphorothioate linkages are Sp phosphorothioate linkages. In other aspects, the phosphorothioate linkages are Rp phosphorothioate linkages. In some aspects, the alternative internucleoside linkages are 2'-alkoxy internucleoside linkages. In other aspects, the alternative internucleoside linkages are alkyl phosphate internucleoside linkages.

The DNA core sequence can comprise, contain, or consist of at least 5-16 consecutive DNA nucleosides capable of recruiting RNase H. In some aspects, all of the nucleosides of the DNA core sequence are DNA units. In further aspects, the DNA core region can consist of a mixture of DNA and other nucleosides capable of mediating RNase H cleavage. In some aspects, at least 50% of the nucleosides of the DNA core sequence are DNA, such as at least 60%, at least 70% or at least 80%, or at least 90% DNA. In some aspects, all of the nucelosides of the DNA core sequence are RNA units.

The oligonucleotide comprises a contiguous region which is complementary to the target nucleic acid. In some aspects, the oligonucleotide can further comprise additional linked nucleosides positioned 5′ and/or 3′ to either the 5' and 3' flanking sequences. These additional linked nucleosides can be attached to the 5′ end of the 5' flanking sequence or the 3′ end of the 3' flanking sequence, respectively. The additional nucleosides can, in some aspects, form part of the contiguous sequence which is complementary to the target nucleic acid, or in other aspects, can be non-complementary to the target nucleic acid.

The inclusion of the additional nucleosides at either, or both of the 5' and 3' flanking sequences can independently comprise one, two, three, four, or five additional nucleotides, which can be complementary or non-complementary to the target nucleic acid. In this respect the oligonucleotide, can in some aspects comprise a contiguous sequence capable of modulating the target which is flanked at the 5′ and/or 3′ end by additional nucleotides. Such additional nucleosides can serve as a nuclease susceptible biocleavable linker, and can therefore be used to attach a functional group such as a conjugate moiety to the oligonucleotide. In some aspects, the additional 5′ and/or 3′ end nucleosides are linked with phosphodiester linkages, and can be DNA or RNA. In another aspect, the additional 5′ and/or 3′ end nucleosides are alternative nucleosides which can for example be included to enhance nuclease stability or for ease of synthesis.

In other aspects, the oligonucleotides utilize "altimer" design and comprise alternating 2'-fluoro-ANA and DNA regions that are alternated every three nucleosides. Altimer oligonucleotides are discussed in more detail in Min, et al., Bioorganic & Medicinal Chemistry Letters, 2002, 12(18): 2651-2654 and Kalota, et al., Nuc. Acid Res. 2006, 34(2): 451-61 (herein incorporated by reference).

In other aspects, the oligonucleotides utilize “hemimer” design and comprise a single 2’-modified flanking sequence adjacent to (on either side of the 5’ or the 3’ side of) a DNA core sequence. Hemimer oligonucleotides are discussed in more detail in Geary et al., 2001, J. Pharm. Exp. Therap., 296: 898-904 (herein incorporated by reference).

In some aspects, an oligonucleotide has a nucleic acid sequence with at least 50% (e.g., at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%) sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 6-2545. In some aspects, an oligonucleotide has a nucleic acid sequence with at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 6-2545.

It will be understood that, although the sequences in SEQ ID NOs: 6-2545 are described as unmodified and/or un-conjugated sequences, the nucleosides of the oligonucleotide e.g., an oligonucleotide, can comprise any one of the sequences set forth in any one of SEQ ID NOs: 6-2545 that is an alternative nucleoside and/or conjugated as described in detail below.

The skilled person is well aware that oligonucleotides having a structure of between about 18-20 base pairs can be particularly effective in inducing RNase H-mediated degradation. However, one can appreciate that shorter or longer oligonucleotides can be effective. In the aspects described above, by virtue of the nature of the oligonucleotide sequences provided herein, oligonucleotides described herein can include shorter or longer oligonucleotide sequences. It can be reasonably expected that shorter oligonucleotides minus only a few linked nucleosides on one or both ends can be similarly effective as compared to the oligonucleotides described above. Hence, oligonucleotides having a sequence of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous linked nucleosides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a MSH3 gene by not more than about 5, 10, 15, 20, 25, or 30% inhibition from an oligonucleotide comprising the full sequence, are contemplated to be within the scope.

The oligonucleotides described herein can function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides are capable of recruiting a nuclease, such as an endonuclease like endoribonuclease (RNase) (e.g., RNase H). Examples of oligonucleotide designs which operate via nuclease mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 DNA nucleosides and are flanked on one side or both sides by affinity enhancing alternative nucleosides, for example gapmers, headmers, and tailmers.

The RNase H activity of an oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. WO01/23613 provides in vitro methods for determining RNase H activity, which can be used to determine the ability to recruit RNase H. Typically an oligonucleotide is deemed capable of recruiting RNase H if it, when provided with a complementary target nucleic acid sequence, has an initial rate, as measured in pmol/l/min, of at least 5%, such as at least 10% or more than 20% of the of the initial rate determined when using an oligonucleotide having the same base sequence as the modified oligonucleotide being tested, but containing only DNA monomers, with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91-95 of WO01/23613 (hereby incorporated by reference).

Furthermore, the oligonucleotides described herein identify a site(s) in a MSH3 transcript that is susceptible to RNase H-mediated cleavage. As used herein, an oligonucleotide is said to target within a particular site of an RNA transcript if the oligonucleotide promotes cleavage of the transcript anywhere within that particular site. Such an oligonucleotide will generally include at least about 5-10 contiguous linked nucleosides from one of the sequences provided herein coupled to additional linked nucleoside sequences taken from the region contiguous to the selected sequence in a MSH3 gene.

Inhibitory oligonucleotides can be designed by methods well known in the art. While a target sequence is generally about 10-30 linked nucleosides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA.

Oligonucleotides with homology sufficient to provide sequence specificity required to uniquely degrade any RNA can be designed using programs known in the art

Systematic testing of several designed species for optimization of the inhibitory oligonucleotide sequence can be undertaken in accordance with the teachings provided herein. Considerations when designing interfering oligonucleotides include, but are not limited to, biophysical, thermodynamic, and structural considerations, base preferences at specific positions, and homology. The making and use of inhibitory therapeutic agents based on non-coding oligonucleotides are also known in the art.

Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, e.g., *in silico*) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an oligonucleotide agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified herein represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified herein, further optimization could be achieved by systematically either adding or removing linked nucleosides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of oligonucleotides based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition.

Further still, such optimized sequences can be adjusted by, e.g., the introduction of alternative nucleosides, alternative sugar moieties, and/or alternative internucleosidic linkages as described herein or as known in the art, including alternative nucleosides, alternative sugar moieties, and/or alternative internucleosidic linkages as known in the art and/or discussed herein to further optimize the molecule (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor. An oligonucleotide agent as described herein can contain one or more mismatches to the target sequence. In one aspect, an oligonucleotide as described herein contains no more than 3 mismatches. If the oligonucleotide contains mismatches to a target sequence, in some aspects, the area of mismatch is not located in the center of the region of complementarity. If the oligonucleotide contains mismatches to the target sequence, in some aspects, the mismatch should be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 30-linked nucleoside oligonucleotide agent, the contiguous nucleobase region which is complementary to a region of a MSH3 gene, generally does not contain any mismatch within the central 5-10 linked nucleosides. The methods described herein or methods known in the art can be used to determine whether an oligonucleotide containing a mismatch to a target sequence is effective in inhibiting the expression of a MSH3 gene. Consideration of the efficacy of oligonucleotides with mismatches in inhibiting expression of a MSH3 gene is important, especially if the particular region of complementarity in a MSH3 gene is known to have polymorphic sequence variation within the population.

Construction of vectors for expression of polynucleotides can be accomplished using conventional techniques which do not require detailed explanation to one of ordinary skill in the art. For generation of efficient expression vectors, it is necessary to have regulatory sequences that control the expression of the polynucleotide. These regulatory sequences include promoter and enhancer sequences and are influenced by specific cellular factors that interact with these sequences, and are well known in the art.

A. Alternative Oligonucleosides

In one aspect, one or more of the linked nucleosides or internucleosidic linkages of the oligonucleotide, is naturally occurring, and does not comprise, e.g., chemical modifications and/or conjugations known in the art and described herein. In another aspect, one or more of the linked nucleosides or internucleosidic linkages of an oligonucleotide, is chemically modified to enhance stability or other beneficial characteristics. Without being bound by theory, it is believed that certain modifications can increase nuclease resistance and/or serum stability, or decrease immunogenicity. For example, oligonucleotides can contain nucleotides found to occur naturally in DNA or RNA (e.g., adenine, thymidine, guanosine, cytidine, uridine, or inosine) or can contain alternative nucleosides or internucleosidic linkages which have one or more chemical modifications to one or more components of the nucleotide (e.g., the nucleobase, sugar, or phospho-linker moiety). Oligonucleotides can be linked to one another through naturally occurring phosphodiester bonds, or can contain alternative linkages (e.g., covalently linked through phosphorothioate (e.g., Sp phosphorothioate or Rp

phosphorothioate), 3'-methylenephosphonate, 5'-methylenephosphonate, 3'-phosphoamidate, 2'-5' phosphodiester, guanidinium, S-methylthiourea, 2'-alkoxy, alkyl phosphate, or peptide bonds).

In some aspects, substantially all of the nucleosides or internucleosidic linkages of an oligonucleotide are alternative nucleosides. In other aspects, all of the nucleosides or internucleosidic linkages of an oligonucleotide are alternative nucleosides. Oligonucleotides in which "substantially all of the nucleosides are alternative nucleosides" are largely but not wholly modified and can include not more than five, four, three, two, or one naturally-occurring nucleosides. In still other aspects, oligonucleotides can include not more than five, four, three, two, or one alternative nucleosides.

The nucleic acids can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S. L. et al. (Edrs.), John Wiley & Sons, Inc., New York, N.Y., USA, which is hereby incorporated herein by reference. Alternative nucleotides and nucleosides include those with modifications including, for example, end modifications, e.g., 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (e.g., at the 2'-position or 4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. The nucleobase can be an isonucleoside in which the nucleobase is moved from the C1 position of the sugar moiety to a different position (e.g. C2, C3, C4, or C5). Specific examples of oligonucleotide compounds useful in the aspects described herein include, but are not limited to alternative nucleosides containing modified backbones or no natural internucleoside linkages. Nucleotides and nucleosides having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, alternative RNAs that do not have a phosphorus atom in their internucleoside backbone can be considered to be oligonucleosides. In some aspects, an oligonucleotide will have a phosphorus atom in its internucleoside backbone.

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

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 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; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265;

6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Alternative internucleoside linkages that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH_2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 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; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

In other aspects, suitable oligonucleotides include those in which both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, a mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar of a nucleoside is replaced with an amide containing backbone, in particular 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 U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the oligonucleotides are described in, for example, in Nielsen et al., Science, 1991, 254, 1497-1500.

Some aspects include oligonucleotides with phosphorothioate backbones and oligonucleotides with heteroatom backbones, and in particular $-CH_2-NH-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$ and $-N(CH_3)-CH_2-CH_2-$[wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some aspects, the oligonucleotides featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506. In other aspects, the oligonucleotides described herein include phosphorodiamidate morpholino oligomers (PMO), in which the deoxyribose moiety is replaced by a morpholine ring, and the charged phosphodiester inter-subunit linkage is replaced by an uncharged phophorodiamidate linkage, as described in Summerton, et al., Antisense Nucleic Acid Drug Dev. 1997, 7:63-70.

Alternative nucleosides and nucleotides can contain one or more substituted sugar moieties. The oligonucleotides, e.g., oligonucleotides, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nOCH_3$, $-O(CH_2)_n-NH_2$, $-O(CH_2)_nCH_3$, $-O(CH_2)_n-ONH_2$, and $-O(CH_2)_n-ON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. In other aspects, oligonucleotides include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. In some aspects, the modification includes a 2'-methoxyethoxy (2'-O-$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chin. Acta, 1995, 78:486-504) i.e., an alkoxy-alkoxy group. MOE nucleosides confer several beneficial properties to oligonucleotides including, but not limited to, increased nuclease resistance, improved pharmacokinetics properties, reduced non-specific protein binding, reduced toxicity, reduced immunostimulatory properties, and enhanced target affinity as compared to unmodified oligonucleotides.

Another exemplary alternative contains 2'-dimethylaminooxyethoxy, i.e., a $-O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-$(CH_2)_2$-O-$(CH_2)_2$-$N(CH_3)_2$. Further exemplary alternatives include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other alternatives include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can be made at other positions on the nucleosides and nucleotides of an oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides can have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An oligonucleotide can include nucleobase (often referred to in the art simply as "base") alternatives (e.g., modifications or substitutions). Unmodified or natural nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Alternative nucleobases include other synthetic and natural nucleobases such as 5-

methylcytidine, 5-hydroxymethylcytidine, 5-formylcytidine, 5-carboxycytidine, pyrrolocytidine, dideoxycytidine, uridine, 5-methoxyuridine, 5-hydroxydeoxyuridine, dihydrouridine, 4-thiourdine, pseudouridine, 1-methyl-pseudouridine, deoxyuridine, 5-hydroxybutynl-2'-deoxyuridine, xanthine, hypoxanthine, 7-deaza-xanthine, thienoguanine, 8-aza-7-deazaguanosine, 7-methylguanosine, 7-deazaguanosine, 6-aminomethyl-7-deazaguanosine, 8-aminoguanine, 2,2,7-trimethylguanosine, 8-methyladenine, 8-azidoadenine, 7-methyladenine, 7-deazaadenine, 3-deazaadenine, 2,6-diaminopurine, 2-aminopurine, 7-deaza-8-aza-adenine, 8-amino-adenine, thymine, dideoxythymine, 5-nitroindole, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouridine, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uridine and cytidine, 6-azo uridine, cytidine and thymine, 4-thiouridine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uridines and cytidines, 8-azaguanine and 8-azaadenine, and 3-deazaguanine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., (1991) Angewandte Chemie, International Edition, 30:613, and those disclosed by Sanghvi, Y S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotide. These include 5-substituted pyrimidines, 6-azapyrimidines, and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil, and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted alternative nucleobases as well as other alternative nucleobases include, but are not limited to, the above noted U.S. Pat. Nos. 3,687,808, 4,845,205; 5,130,30; 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; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

In other aspects, the sugar moiety in the nucleotide can be a ribose molecule, optionally having a 2'-O-methyl, 2'-O-MOE, 2'-F, 2'-amino, 2'-O-propyl, 2'-aminopropyl, or 2'-OH modification.

An oligonucleotide can include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In some aspects, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some aspects, an oligonucleotide can include one or more locked

nucleosides. A locked nucleoside is a nucleoside having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, a locked nucleoside is a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH_2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleosides to oligonucleotides has been shown to increase oligonucleotide stability in serum, and to reduce off-target effects (Grunweller, A. et al., (2003) Nucleic Acids Research 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In some aspects, the polynucleotide agents include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-(CH_2)$_2$-O-2' (ENA); 4'-CH(CH_3)-O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH_2OCH_3)-O-2' (and analogs thereof; see, e.g., U.S. Pat. No. 7,399,845); 4'-C(CH_3)(CH_3)-O-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,283); 4'-CH_2-N(OCH_3)-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,425); 4'-CH_2-O-N(CH_3)$_2$-2' (see, e.g., U.S. Patent Publication No. 2004/0171570); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see, e.g., U.S. Pat. No. 7,427,672); 4'-CH_2-C(H)(CH_3)-2' (see, e.g., Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134); and 4'-CH_2-C(=CH_2)-2' (and analogs thereof; see, e.g., U.S. Pat. No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and US Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

An oligonucleotide can be modified to include one or more constrained ethyl nucleosides. As used herein, a "constrained ethyl nucleoside" or "cEt" is a locked nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH_3)-O-2' bridge. In one aspect, a constrained ethyl nucleoside is in the S conformation referred to herein as "S-cEt."

An oligonucleotide can include one or more "conformationally restricted nucleosides" ("CRN"). CRN are nucleoside analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and --C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some aspects, an oligonucleotide comprises one or more monomers that are UNA (unlocked nucleoside) nucleosides. UNA is unlocked acyclic nucleoside, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (i.e. the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see Nuc. Acids Symp. Series, 52, 133-134 (2008) and Fluiter et al., Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Pat. No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

The ribose molecule can be modified with a cyclopropane ring to produce a tricyclodeoxynucleic acid (tricyclo DNA). The ribose moiety can be substituted for another sugar such as 1,5,-anhydrohexitol, threose to produce a threose nucleoside (TNA), or arabinose to produce an arabino nucleoside. The ribose molecule can be replaced with non-sugars such as cyclohexene to produce cyclohexene nucleoside or glycol to produce glycol nucleosides.

Potentially stabilizing modifications to the ends of nucleoside molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other alternatives chemistries of an oligonucleotide include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic of an oligonucleotide. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

Exemplary oligonucleotides comprise nucleosides with alternative sugar moieties and can comprise DNA or RNA nucleosides. In some aspects, the oligonucleotide comprises nucleosides comprising alternative sugar moieties and DNA nucleosides. Incorporation of alternative nucleosides into the oligonucleotide can enhance the affinity of the oligonucleotide for the target nucleic acid. In that case, the alternative nucleosides can be referred to as affinity enhancing alternative nucleotides.

In some aspects, the oligonucleotide comprises at least 1 alternative nucleoside, such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 alternative nucleosides. In other aspects, the oligonucleotides comprise from 1 to 10 alternative nucleosides, such as from 2 to 9 alternative nucleosides, such as from 3 to 8 alternative nucleosides, such as from 4 to 7 alternative nucleosides, such as 6 or 7 alternative nucleosides. In an aspect, the oligonucleotide can comprise alternatives, which are independently selected from these three types of alternatives (alternative sugar moiety, alternative nucleobase, and alternative internucleoside linkage), or a combination thereof. In one aspect, the oligonucleotide comprises one or more nucleosides comprising alternative sugar moieties, e.g., 2′ sugar alternative nucleosides. In some aspect, the oligonucleotide comprises

the one or more 2′ sugar alternative nucleoside independently selected from the group consisting of 2′-O-alkyl-RNA, 2′-O-methyl-RNA, 2′-alkoxy-RNA, 2′-O-methoxyethyl-RNA, 2′-amino-DNA, 2′-fluoro-DNA, arabino nucleic acid (ANA), 2′-fluoro-ANA, and BNA (e.g., LNA) nucleosides. In some aspects, the one or more alternative nucleoside is a BNA.

In some aspects, at least 1 of the alternative nucleosides is a BNA (e.g., an LNA), such as at least 2, such as at least 3, at least 4, at least 5, at least 6, at least 7, or at least 8 of the alternative nucleosides are BNAs. In a still further aspect, all the alternative nucleosides are BNAs.

In a further aspect the oligonucleotide comprises at least one alternative internucleoside linkage. In some aspects, the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boronophosphate internucleoside linkages. In some aspects, all the internucleotide linkages in the contiguous sequence of the oligonucleotide are phosphorothioate linkages. In some aspects, the phosphorothioate linkages are stereochemically pure phosphorothioate linkages. In some aspects, the phosphorothioate linkages are Sp phosphorothioate linkages. In other aspects, the phosphorothioate linkages are Rp phosphorothioate linkages.

In some aspects, the oligonucleotide comprises at least one alternative nucleoside which is a 2′-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 2′-MOE-RNA nucleoside units. In some aspects, the 2'-MOE-RNA nucleoside units are connected by phosphorothioate linkages. In some aspects, at least one of said alternative nucleoside is 2′-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 2′-fluoro-DNA nucleoside units. In some aspects, the oligonucleotide comprises at least one BNA unit and at least one 2′ substituted modified nucleoside. In some aspects, the oligonucleotide comprises both 2′ sugar modified nucleosides and DNA units. In some aspects, the oligonucleotide or contiguous nucleotide region thereof is a gapmer oligonucleotide.

B. Oligonucleotides Conjugated to Ligands

Oligonucleotides can be chemically linked to one or more ligands, moieties, or conjugates that enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., (1989) Proc. Natl. Acid. Sci. USA, 86: 6553-6556), cholic acid (Manoharan et al., (1994) Biorg. Med. Chem. Let., 4:1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., (1992) Ann. N.Y. Acad. Sci., 660:306-309; Manoharan et al., (1993) Biorg. Med. Chem. Let., 3:2765-2770), a thiocholesterol (Oberhauser et al., (1992) Nucl. Acids Res., 20:533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., (1991) EMBO J, 10:1111-1118; Kabanov et al., (1990) FEBS Lett., 259:327-330; Svinarchuk et al., (1993) Biochimie, 75:49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan et al., (1995) Tetrahedron Lett., 36:3651-3654; Shea et al., (1990) Nucl. Acids Res., 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., (1995) Nucleosides & Nucleotides, 14:969-973), or adamantane acetic acid (Manoharan et al., (1995) Tetrahedron Lett., 36:3651-3654), a palmityl moiety (Mishra et al., (1995) Biochim. Biophys. Acta, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., (1996) J. Pharmacol. Exp. Ther., 277:923-937).

In one aspect, a ligand alters the distribution, targeting, or lifetime of an oligonucleotide agent into which it is incorporated. In some aspects, a ligand provides an enhanced affinity for a selected target, e.g., molecule, cell or cell type, compartment, e.g., a cellular or organ compartment, tissue, organ, or region of the body, as, e.g., compared to a species absent such a ligand.

Ligands can include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that bind to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

Other examples of ligands include dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralen, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g. EDTA), lipophilic molecules, e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g. biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can include hormones and hormone receptors. They can include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent

galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose.

The ligand can be a substance, e.g., a drug, which can increase the uptake of the oligonucleotide agent into the cell, for example, by disrupting the cell's cytoskeleton, e.g., by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some aspects, a ligand attached to an oligonucleotide as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins etc. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin etc. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, e.g., oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable as ligands (e.g. as PK modulating ligands). In addition, aptamers that bind serum components (e.g. serum proteins) are also suitable for use as PK modulating ligands in the aspects described herein.

Ligand-conjugated oligonucleotides can be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide can be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates can be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art can additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

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

When using conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some aspects, the oligonucleotides or linked nucleosides are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard

phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

i. Lipid Conjugates

In one aspect, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule can bind a serum protein, e.g., human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, e.g., a non-kidney target tissue of the body. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) be used to adjust binding to a serum protein, e.g., HSA.

In another aspect, the ligand is a moiety, e.g., a vitamin, which is taken up by a target cell, e.g., a proliferating cell. Exemplary vitamins include vitamin A, E, and K.

ii. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, such as a helical cell-permeation agent. In one aspect, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. In one aspect, the helical agent is an alpha-helical agent, which can havea lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to oligonucleotide agents can affect pharmacokinetic distribution of the oligonucleotide, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (e.g., consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP. An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP containing a hydrophobic MTS can be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam et al., Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to an oligonucleotide agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties

can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods can be linear or cyclic, and can be modified, e.g., glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics can include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Some conjugates of this ligand target PECAM-1 or VEGF.

A cell permeation peptide is capable of permeating a cell, e.g., a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (e.g., LL-37 or Ceropin P1), a disulfide bond-containing peptide (e.g., α-defensin, β-defensin, or bactenecin), or a peptide containing only one or two dominating amino acids (e.g., PR-39 or indolicidin). A cell permeation peptide can include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., Nucl. Acids Res. 31:2717-2724, 2003).

iii. Carbohydrate Conjugates

In some aspects of the compositions and methods described herein, an oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated oligonucleotides are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (e.g., C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (e.g., C5, C6, C7, or C8).

In one aspect, a carbohydrate conjugate for use in the compositions and methods described herein is a monosaccharide.

In some aspects, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

Additional carbohydrate conjugates (and linkers) suitable for use include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

iv. Linkers

In some aspects, the conjugate or ligand described herein can be attached to an oligonucleotide with various linkers that can be cleavable or non-cleavable.

Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR^8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, $N(R^8)$, C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R^8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one aspect, the linker is between about 1-24, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, 8-16 or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 21, 22, 23, or 24 atoms.

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

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

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic

pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between at least two conditions, where at least one condition is selected to be indicative of cleavage in a target cell and another condition is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In some aspects, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

a. Redox Cleavable Linking Groups

In one aspect, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (--S--S--). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular oligonucleotide moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can be evaluated under conditions which are selected to mimic blood or serum conditions. In one aspect, candidate compounds are cleaved by at most about 10% in the blood. In other aspects, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

b. Phosphate-Based Cleavable Linking Groups

In another aspect, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(OR^k)-O-, -O-P(S)(OR^k)-O-, -O-P(S)(SR^k)-O-, -S-P(O)(OR^k)-O-, -O-P(O)(OR^k)-S-, -S-P(O)(OR^k)-S-, -O-P(S)(OR^k)-S-, -S-P(S)(OR^k)-O-, -O-P(O)(R^k)-O-, -O-P(S)(R^k)-O-, -S-P(O)(R^k)-O-, -S-P(S)(R^k)-O-, -S-P(O)(R^k)-S-, -O-P(S)(R^k)-S-. These candidates can be evaluated using methods analogous to those described above.

c. Acid Cleavable Linking Groups

In another aspect, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In some aspects, acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula –C=NN--, C(O)O, or --OC(O). In one aspect, the carbon is attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

d. Ester-Based Linking Groups

In another aspect, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula --C(O)O--, or --OC(O)--. These candidates can be evaluated using methods analogous to those described above.

e. Peptide-Based Cleaving Groups

In yet another aspect, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene, or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula

-NHCHR^{A}C(O)NHCHR^{B}C(O)--, where R^A and R^B are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

In one aspect, an oligonucleotide is conjugated to a carbohydrate through a linker. Linkers include bivalent and trivalent branched linker groups. Linkers for oligonucleotide carbohydrate conjugates include, but are not limited to, those described in formulas 24-35 of PCT Publication No. WO 2018/195165.

Representative U.S. patents that teach the preparation of oligonucleotide conjugates include, but are not limited to, 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; 5,591,584; 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; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. Oligonucleotide compounds that are chimeric compounds are also contemplated. Chimeric oligonucleotides typically contain at least one region wherein the RNA is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide can serve as a substrate for enzymes capable of cleaving RNA:DNA. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxy oligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the nucleotides of an oligonucleotide can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution, or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. et al., Biochem. Biophys. Res. Comm, 2007, 365(1):54-61; Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al.,

FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such oligonucleotide conjugates have been listed above. Typical conjugation protocols involve the synthesis of an oligonucleotide bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide, in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate.

IV. Pharmaceutical Uses

The oligonucleotide compositions described herein are useful in the methods described herein, and, while not bound by theory, are believed to exert their desirable effects through their ability to modulate the level, status, and/or activity of a MutSβ heterodimer comprising MSH3, e.g., by inhibiting the activity or level of the MSH3 protein in a cell in a mammal.

An aspect relates to methods of treating disorders related to DNA mismatch repair such as trinucleotide repeat expansion disorders in a subject in need thereof. Another aspect includes reducing the level of MSH3 in a cell of a subject identified as having a trinucleotide repeat expansion disorder. Still another aspect includes a method of inhibiting expression of MSH3 in a cell in a subject. Further aspects include methods of decreasing trinucleotide repeat expansion in a cell. The methods include contacting a cell with an oligonucleotide, in an amount effective to inhibit expression of MSH3 in the cell, thereby inhibiting expression of MSH3 in the cell.

Based on the above methods, an oligonucleotide, or a composition comprising such an oligonucleotide, for use in therapy, or for use as a medicament, or for use in treating disorders related to DNA mismatch repair such as repeat expansion disorders in a subject in need thereof, or for use in reducing the level of MSH3 in a cell of a subject identified as having a trinucleotide repeat expansion disorder, or for use in inhibiting expression of MSH3 in a cell in a subject, or for use in decreasing trinucleotide repeat expansion in a cell is contemplated. The uses include the contacting of a cell with the oligonucleotide, in an amount effective to inhibit expression of MSH3 in the cell, thereby inhibiting expression of MSH3 in the cell. Aspects described below in relation to the methods described herein are also applicable to these further aspects.

Contacting of a cell with an oligonucleotide can be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the oligonucleotide includes contacting a cell or group of cells within a subject, e.g., a human subject, with the oligonucleotide. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell can be direct or indirect, as discussed above. Furthermore, contacting a cell can be accomplished via a targeting ligand, including any ligand described herein or

known in the art. In some aspects, the targeting ligand is a carbohydrate moiety, e.g., a $GalNAc_3$ ligand, or any other ligand that directs the oligonucleotide to a site of interest. Cells can include those of the central nervous system, or muscle cells.

Inhibiting expression of a MSH3 gene includes any level of inhibition of a MSH3 gene, e.g., at least partial suppression of the expression of a MSH3 gene, such as an inhibition by at least about 20%. In some aspects, inhibition is by at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

The expression of a MSH3 gene can be assessed based on the level of any variable associated with MSH3 gene expression, e.g., MSH3 mRNA level or MSH3 protein level.

Inhibition can be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level can be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control).

In some aspects, surrogate markers can be used to detect inhibition of MSH3. For example, effective treatment of a trinucleotide repeat expansion disorder, as demonstrated by acceptable diagnostic and monitoring criteria with an agent to reduce MSH3 expression can be understood to demonstrate a clinically relevant reduction in MSH3.

In some aspects of the methods, expression of a MSH3 gene is inhibited by at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some aspects, the methods include a clinically relevant inhibition of expression of MSH3, e.g., as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of MSH3.

Inhibition of the expression of a MSH3 gene can be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells can be present, for example, in a sample derived from a subject) in which a MSH3 gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an oligonucleotide, or by administering an oligonucleotide to a subject in which the cells are or were present) such that the expression of a MSH3 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an oligonucleotide or not treated with an oligonucleotide targeted to the gene of interest). The degree of inhibition can be expressed in terms of:

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

In other aspects, inhibition of the expression of a MSH3 gene can be assessed in terms of a reduction of a parameter that is functionally linked to MSH3 gene expression, e.g., MSH3 protein

expression or MSH3 signaling pathways. MSH3 gene silencing can be determined in any cell expressing MSH3, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a MSH3 protein can be manifested by a reduction in the level of the MSH3 protein that is expressed by a cell or group of cells (e.g., the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells can similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that can be used to assess the inhibition of the expression of a MSH3 gene includes a cell or group of cells that has not yet been contacted with an oligonucleotide. For example, the control cell or group of cells can be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an oligonucleotide.

The level of MSH3 mRNA that is expressed by a cell or group of cells can be determined using any method known in the art for assessing mRNA expression. In one aspect, the level of expression of MSH3 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the MSH3 gene. RNA can be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNEASY™ RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, in situ hybridization, and microarray analysis. Circulating MSH3 mRNA can be detected using methods the described in PCT Publication WO2012/177906, the entire contents of which are hereby incorporated herein by reference. In some aspects, the level of expression of MSH3 is determined using a nucleic acid probe. The term "probe," as used herein, refers to any molecule that is capable of selectively binding to a specific MSH3 sequence, e.g. to an mRNA or polypeptide. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes can be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses, and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to MSH3 mRNA. In one aspect, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative aspect, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an AFFYMETRIX gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of MSH3 mRNA.

An alternative method for determining the level of expression of MSH3 in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (the experimental aspect set forth in Mullis, 1987,

U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189-193), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In some aspects, the level of expression of MSH3 is determined by quantitative fluorogenic RT-PCR (i.e., the TAQMAN™ System) or the DUAL-GLO® Luciferase assay.

The expression levels of MSH3 mRNA can be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722; 5,874,219; 5,744,305; 5,677,195; and 5,445,934, which are incorporated herein by reference. The determination of MSH3 expression level can comprise using nucleic acid probes in solution.

In some aspects, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can be used for the detection of MSH3 nucleic acids.

The level of MSH3 protein expression can be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can be used for the detection of proteins indicative of the presence or replication of MSH3 proteins.

In some aspects of the methods described herein, the oligonucleotide is administered to a subject such that the oligonucleotide is delivered to a specific site within the subject. The inhibition of expression of MSH3 can be assessed using measurements of the level or change in the level of MSH3 mRNA or MSH3 protein in a sample derived from a specific site within the subject. In some aspects, the methods include a clinically relevant inhibition of expression of MSH3, e.g., as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of MSH3.

In other aspects, the oligonucleotide is administered in an amount and for a time effective to result in one of (or more, e.g., two or more, three or more, four or more of): (a) decrease the number of repeats, (b) decrease the level of polyglutamine, (c) decreased cell death (e.g., CNS cell death and/or muscle cell death), (d) delayed onset of the disorder, (e) increased survival of subject, and (f) increased progression free survival of a subject.

Treating trinucleotide repeat expansion disorders can result in an increase in average survival time of an individual or a population of subjects treated with an oligonucleotide described herein in comparison to a population of untreated subjects. For example, the survival time of an individual or average survival time of a population is increased by more than 30 days (more than 60 days, 90 days, or 120 days). An increase in survival time of an individual or in average survival time of a population can be measured by any reproducible means. An increase in survival time of an individual can be measured, for example, by calculating for an individual the length of survival time following the initiation of treatment with the compound described herein. An increase in average survival time of a population can be measured, for example, by calculating for the average length of survival time following initiation of treatment with the compound described herein. An increase in survival time of an individual can be measured, for example, by calculating for an individual length of survival time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein. An increase in average survival time of a population can be measured, for example, by calculating for a population the average length of survival time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein.

Treating trinucleotide repeat expansion disorders can result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. For example, the mortality rate is decreased by more than 2% (e.g., more than 5%, 10%, or 25%). A decrease in the mortality rate of a population of treated subjects can be measured by any reproducible means, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with a compound or pharmaceutically acceptable salt of a compound described herein. A decrease in the mortality rate of a population can be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with a compound or pharmaceutically acceptable salt of a compound described herein.

A. Delivery of anti-MSH3 Agents

The delivery of an oligonucleotide to a cell e.g., a cell within a subject, such as a human subject e.g., a subject in need thereof, such as a subject having a trinucleotide repeat expansion disorder can be achieved in a number of different ways. For example, delivery can be performed by contacting a cell with an oligonucleotide either *in vitro* or *in vivo*. *In vivo* delivery can be performed directly by administering a composition comprising an oligonucleotide to a subject. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an oligonucleotide (see e.g., Akhtar S. and Julian R L., (1992) Trends Cell. Biol. 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). *For in vivo* delivery, factors to consider in order to deliver an oligonucleotide molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an oligonucleotide can be minimized by local administration, for example, by direct injection or implantation into a tissue or

topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the oligonucleotide to be administered.

For administering an oligonucleotide systemically for the treatment of a disease, the oligonucleotide can include alternative nucleobases, alternative sugar moieties, and/or alternative internucleoside linkages, or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the oligonucleotide by endo- and exo-nucleases *in vivo*. Modification of the oligonucleotide or the pharmaceutical carrier can permit targeting of the oligonucleotide composition to the target tissue and avoid undesirable off-target effects. Oligonucleotide molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. In an alternative aspect, the oligonucleotide can be delivered using drug delivery systems such as a nanoparticle, a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an oligonucleotide molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an oligonucleotide by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an oligonucleotide, or induced to form a vesicle or micelle that encases an oligonucleotide. The formation of vesicles or micelles further prevents degradation of the oligonucleotide when administered systemically. In general, any methods of delivery of nucleic acids known in the art may be adaptable to the delivery of the oligonucleotides described herein. Methods for making and administering cationic oligonucleotide complexes are well within the abilities of one skilled in the art (see e.g., Sorensen, D R., et al. (2003) J. Mol. Biol 327:761-766; Verma, U N. et al., (2003) Clin. Cancer Res. 9:1291-1300; Arnold, A S et al., (2007) J. Hypertens. 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of oligonucleotides include DOTAP (Sorensen, D R., et al (2003), supra; Verma, U N. et al., (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, T S. et al., (2006) Nature 441:111-114), cardiolipin (Chien, P Y. et al., (2005) Cancer Gene Ther. 12:321-328; Pal, A. et al., (2005) Int J. Oncol. 26:1087-1091), polyethyleneimine (Bonnet M E. et al., (2008) Pharm. Res. Aug 16 Epub ahead of print; Aigner, A. (2006) J. Biomed. Biotechnol. 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) Mol. Pharm. 3:472-487), and polyamidoamines (Tomalia, D A. et al., (2007) Biochem. Soc. Trans. 35:61-67; Yoo, H. et al., (1999) Pharm. Res. 16:1799-1804). In some aspects, an oligonucleotide forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of oligonucleotides and cyclodextrins can be found in U.S. Pat. No. 7,427,605, which is herein incorporated by reference in its entirety. In some aspects the oligonucleotides described herein are delivered by polyplex or lipoplex nanoparticles. Methods for administration and pharmaceutical compositions of oligonucleotides and polyplex nanoparticles and lipoplex nanoparticles can be found in U.S. Patent Application Nos. 2017/0121454; 2016/0369269; 2016/0279256; 2016/0251478; 2016/0230189; 2015/0335764;

2015/0307554; 2015/0174549; 2014/0342003; 2014/0135376; and 2013/0317086, which are herein incorporated by reference in their entirety.

i. Membranous Molecular Assembly Delivery Methods

The oligonucleotides can be delivered using a variety of membranous molecular assembly delivery methods including polymeric, biodegradable microparticle, or microcapsule delivery devices known in the art. For example, a colloidal dispersion system can be used for targeted delivery of an oligonucleotide agent described herein. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. Liposomes are artificial membrane vesicles that are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the oligonucleotide are delivered into the cell where the oligonucleotide can specifically bind to a target RNA and can mediate RNase H-mediated gene silencing. In some cases, the liposomes are also specifically targeted, e.g., to direct the oligonucleotide to particular cell types. The composition of the liposome is usually a combination of phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids can be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

A liposome containing an oligonucleotide can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and can be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The oligonucleotide preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the oligonucleotide and condense around the oligonucleotide to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield a liposomal preparation of oligonucleotide.

If necessary, a carrier compound that assists in condensation can be added during the condensation reaction, e.g., by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (e.g., spermine or spermidine). The pH can be adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as a structural component of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can include one or more aspects of exemplary methods described in Feigner, P. L. et al., (1987) Proc. Natl. Acad. Sci. USA 8:7413-7417; U.S. Pat. No. 4,897,355; U.S. Pat. No.

5,171,678; Bangham et al., (1965) M. Mol. Biol. 23:238; Olson et al., (1979) Biochim. Biophys. Acta 557:9; Szoka et al., (1978) Proc. Natl. Acad. Sci. 75: 4194; Mayhew et al., (1984) Biochim. Biophys. Acta 775:169; Kim et al., (1983) Biochim. Biophys. Acta 728:339; and Fukunaga et al., (1984) Endocrinol. 115:757. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer et al., (1986) Biochim. Biophys. Acta 858:161. Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew et al., (1984) Biochim. Biophys. Acta 775:169). These methods are readily adapted to packaging oligonucleotide preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al. (1987) Biochem. Biophys. Res. Commun., 147:980-985).

Liposomes, which are pH-sensitive or negatively charged, entrap nucleic acids rather than complex with them. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al. (1992) Journal of Controlled Release, 19:269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Feigner, (1994) J. Biol. Chem. 269:2550; Nabel, (1993) Proc. Natl. Acad. Sci. 90:11307; Nabel, (1992) Human Gene Ther. 3:649; Gershon, (1993) Biochem. 32:7143; and Strauss, (1992) EMBO J. 11:417.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising NOVASOME™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and NOVASOME™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu et al., (1994) S.T.P.Pharma. Sci., 4(6):466).

Liposomes can be sterically stabilized liposomes, comprising one or more specialized lipids that result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., (1987) FEBS Letters, 223:42; Wu et al., (1993) Cancer Research, 53:3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., (1987), 507:64) reported the ability of monosialoganglioside G^{M1}, galactocerebroside sulfate, and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., (1988), 85:6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al).

In one aspect, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver oligonucleotides to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated oligonucleotides in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of oligonucleotide (see, e.g., Feigner, P. L. et al., (1987) Proc. Natl. Acad. Sci. USA 8:7413-7417, and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. LIPOFECTIN™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive.

Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Ind.) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (TRANSFECTAM™, Promega, Madison, Wis.) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., (1991) Biochim. Biophys. Res. Commun. 179:280). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., (1991) Biochim. Biophys. Acta 1065:8). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, Calif.) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Md.). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer oligonucleotide into the skin. In some implementations, liposomes are used for delivering oligonucleotide to epidermal cells and also to enhance the penetration of oligonucleotide into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner et al., (1992) Journal of Drug Targeting, vol. 2,405-410 and du Plessis et al., (1992) Antiviral Research, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) Biotechniques 6:682-690; Itani, T. et al., (1987) Gene 56:267-276; Nicolau, C. et al. (1987) Meth. Enzymol. 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) Meth. Enzymol. 101:512-527; Wang, C. Y. and Huang, L., (1987) Proc. Natl. Acad. Sci. USA 84:7851-7855).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising NOVASOME I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and NOVASOME II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with oligonucleotides are useful for treating a dermatological disorder.

The targeting of liposomes is also possible based on, for example, organ-specificity, cell-specificity, and organelle-specificity and is known in the art. In the case of a liposomal targeted

delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. Additional methods are known in the art and are described, for example in U.S. Patent Application Publication No. 20060058255, the linking groups of which are herein incorporated by reference.

Liposomes that include oligonucleotides can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include oligonucleotides can be delivered, for example, subcutaneously by infection to deliver oligonucleotides to keratinocytes in the skin. To cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transfersomes can be self-optimizing (adaptive to the shape of pores, e.g., in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Other suitable formulations are described in U.S. provisional application Ser. No. 61/018,616, filed Jan. 2, 2008; 61/018,611, filed Jan. 2, 2008; 61/039,748, filed Mar. 26, 2008; 61/047,087, filed Apr. 22, 2008 and 61/051,528, filed May 8, 2008. PCT application No. PCT/US2007/080331, filed Oct. 3, 2007 also describes suitable. Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general, their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated

alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines, and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The oligonucleotides for use in the methods can be provided as micellar formulations. Micelles are a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

ii. Lipid Nanoparticle-Based Delivery Methods

Oligonucleotides can be fully encapsulated in a lipid formulation, e.g., a lipid nanoparticle (LNP), or other nucleic acid-lipid particle. LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Pat. Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one aspect, the lipid to drug ratio (mass/mass ratio) (e.g., lipid to oligonucleotide ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated.

Non-limiting examples of cationic lipids include N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N--(l-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N--(l-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-

Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyetetrahydro--3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yeethylazanediyedidodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise, for example, from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be, for example, from about 5 mol % to about 90 mol %, about 10 mol %, or about 60 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), or a PEG-distearyloxypropyl (C_{18}). The conjugated lipid that prevents aggregation of particles can be, for example, from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some aspects, the nucleic acid-lipid particle further includes cholesterol at, e.g., about 10 mol % to about 60 mol % or about 50 mol % of the total lipid present in the particle.

B. Combination Therapies

An oligonucleotide can be used alone or in combination with at least one additional therapeutic agent, e.g., other agents that treat trinucleotide repeat expansion disorders or symptoms associated therewith, or in combination with other types of therapies to treat trinucleotide repeat expansion disorders. In combination treatments, the dosages of one or more of the therapeutic compounds can be reduced from standard dosages when administered alone. For example, doses can be determined empirically from drug combinations and permutations or can be deduced by

isobolographic analysis (e.g., Black et al., Neurology 65:S3-S6 (2005)). In this case, dosages of the compounds when combined should provide a therapeutic effect.

In some aspects, the oligonucleotide agents described herein can be used in combination with at least one additional therapeutic agent to treat a trinucleotide repeat expansion disorder associated with gene having a trinucleotide repeat (e.g., any of the trinucleotide repeat expansion disorders and associated genes having a nucleotide repeat listed in Table 1). In some aspects, at least one of the additional therapeutic agents can be an oligonucleotide (e.g., an ASO) that hybridizes with the mRNA of gene associated with a trinucleotide repeat expansion disorder (e.g., any of the genes listed in Table 1). In some aspects, the trinucleotide repeat expansion disorder is Huntington's disease (HD). In some aspects, the gene associated with a trinucleotide repeat expansion disorder is Huntingtin (HTT). Several allelic variants of the Huntingtin gene have been implicated in the etiology of Huntington's disease. In some cases, these variants are identified on the basis of having unique HD-associated single nucleotide polymorphisms (SNPs). In some aspects, the oligonucleotide hybridizes to an mRNA of the Huntingtin gene containing any of the HD-associated SNPs known in the art (e.g., any of the HD-associated SNPs described in Skotte et al., PLoS One 2014, 9(9): e107434, Carroll et al., Mol. Ther. 2011, 19(12): 2178-85, Warby et al., Am. J. Hum. Gen. 2009, 84(3): 351-66 (herein incorporated by reference)). In some aspects, the oligonucleotide that is an additional therapeutic agent hybridizes to an mRNA of the Huntingtin gene lacking any of the HD-associated SNPs. In some of the aspects, the oligonucleotide that is an additional therapeutic agent hybridizes to an mRNA of the Huntingtin gene having any of the SNPs selected from the group of rs362307 and rs365331. In some aspects, the oligonucleotide that is an additional therapeutic agent can be a modified oligonucleotide (e.g., an oligonucleotide including any of the modifications described herein). In some aspects, the modified oligonucleotides that is an additional therapeutic agent comprise one or more phosphorothioate internucleoside linkages. In some aspects, the modified oligonucleotide comprises one or more 2'-MOE moieties. In some aspects, the oligonucleotide that is an additional therapeutic agent that hybridizes to the mRNA of the Huntingtin gene has a sequence selected from the SEQ ID NOs. 6-285 of US Patent No. 9,006,198; SEQ ID NOs. 6-8 of US Patent Application Publication No. 2017/0044539; SEQ ID NOs. 1-1565 of US Patent Application Publication 2018/0216108; and SEQ ID NOs. 1-2432 of PCT Publication WO 2017/192679, the sequences of which are hereby incorporated by reference.

In some aspects, at least one of the additional therapeutic agents is a chemotherapeutic agent (e.g., a cytotoxic agent or other chemical compound useful in the treatment of a trinucleotide repeat expansion disorder).

In some aspects, at least one of the additional therapeutic agents can be a therapeutic agent which is a non-drug treatment. For example, at least one of the additional therapeutic agents is physical therapy.

In any of the combination aspects described herein, the two or more therapeutic agents are administered simultaneously or sequentially, in either order. For example, a first therapeutic agent can be administered immediately, up to 1 hour, up to 2 hours, up to 3 hours, up to 4 hours, up to 5 hours, up to 6 hours, up to 7 hours, up to, 8 hours, up to 9 hours, up to 10 hours, up to 11 hours, up to

12 hours, up to 13 hours, 14 hours, up to hours 16, up to 17 hours, up 18 hours, up to 19 hours up to 20 hours, up to 21 hours, up to 22 hours, up to 23 hours up to 24 hours or up to 1-7, 1-14, 1-21 or 1-30 days before or after one or more of the additional therapeutic agents.

V. Pharmaceutical Compositions

The oligonucleotides described herein are formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration *in vivo*.

The compounds described herein can be used in the form of the free base, in the form of salts, solvates, and as prodrugs. All forms are within the methods described herein. In accordance with the methods described herein, the described oligonucleotides or salts, solvates, or prodrugs thereof can be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compounds described herein can be administered, for example, by oral, parenteral, intrathecal, intracerebroventricular, intraparenchymal, buccal, sublingual, nasal, rectal, patch, pump, or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, intracerebroventricular, intraparenchymal, rectal, and topical modes of administration. Parenteral administration can be by continuous infusion over a selected period of time.

A compound described herein can be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it can be enclosed in hard or soft shell gelatin capsules, or it can be compressed into tablets, or it can be incorporated directly with the food of the diet. For oral therapeutic administration, a compound described herein can be incorporated with an excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, and wafers. A compound described herein can be administered parenterally. Solutions of a compound described herein can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can be prepared in glycerol, liquid polyethylene glycols, DMSO, and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2012, 22nd ed.) and in The United States Pharmacopeia: The National Formulary (USP 41 NF 36), published in 2018. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that can be easily administered via syringe. Compositions for nasal administration can conveniently be formulated as aerosols, drops, gels, and powders. Aerosol formulations typically include a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container can be a unitary dispensing device, such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form includes an aerosol dispenser,

it will contain a propellant, which can be a compressed gas, such as compressed air or an organic propellant, such as fluorochlorohydrocarbon. The aerosol dosage forms can take the form of a pump-atomizer. Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, where the active ingredient is formulated with a carrier, such as sugar, acacia, tragacanth, gelatin, and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base, such as cocoa butter

The compounds described herein can be administered to an animal, e.g., a human, alone or in combination with pharmaceutically acceptable carriers, as noted herein, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

VI. Dosages

The dosage of the compositions (e.g., a composition including an oligonucleotide) described herein, can vary depending on many factors, such as the pharmacodynamic properties of the compound; the mode of administration; the age, health, and weight of the recipient; the nature and extent of the symptoms; the frequency of the treatment, and the type of concurrent treatment, if any; and the clearance rate of the compound in the animal to be treated. The compositions described herein can be administered initially in a suitable dosage that can be adjusted as required, depending on the clinical response. In some aspects, the dosage of a composition (e.g., a composition including an oligonucleotide) is a prophylactically or a therapeutically effective amount.

VII. Kits

Kits including (a) a pharmaceutical composition including an oligonucleotide agent that reduces the level and/or activity of MSH3 in a cell or subject described herein, and (b) a package insert with instructions to perform any of the methods described herein are contemplated. In some aspects, the kit includes (a) a pharmaceutical composition including an oligonucleotide agent that reduces the level and/or activity of MSH3 in a cell or subject described herein, (b) an additional therapeutic agent, and (c) a package insert with instructions to perform any of the methods described herein.

Examples

Example 1. Design and Selection of Antisense Oligonucleotides

Identification and selection of target transcripts: Target transcript selection and off-target scoring (below) utilized NCBI RefSeq sequences, downloaded from NCBI 21 Nov. 2018. Experimentally validated “NM” transcript models were used except for cynomolgus monkey, which only has “XM” predicted models for the large majority of genes. The longest human, mouse, rat, and cynomolgus monkey MSH3 transcript that contained all mapped internal exons was selected (SEQ IDs 1, 3, 4, and 5 for human, mouse, rat, and cynomolgus monkey, respectively, SEQ ID NO:2 is the protein sequence).

Selection of 20mer oligonucleotide sequences: All antisense 20mer sub-sequences per transcript were generated. Candidate antisense oligonucleotides (“ASOs”) were selected that met the

following thermodynamic and physical characteristics determined by the inventors: predicted melting temperature of ASO:target duplex ("T_m") of 30-65 °C, predicted melting temperature of hairpins ("$T_{hairpin}$") < 35 °C, predicted melting temperature of homopolymer formation ("T_{homo}") < 25 °C, GC content of 20-60%, no G homopolymers 4 or longer, and no A, T, or C homopolymers of 6 or longer. These selected or "preferred" oligonucleotides were further evaluated for specificity (off-target scoring, below).

Off-target scoring: The specificity of the preferred ASOs was evaluated via alignment to all unspliced RefSeq transcripts ("NM" models for human, mouse, and rat; "NM" and "XM" models for cynomolgus monkey), using the FASTA algorithm with an E value cutoff of 1000. The number of mismatches between each ASO and each transcript (per species) was tallied. An "off-target score" for each ASO in each species was calculated as the lowest number of mismatches to any transcript other than those encoded by the MSH3 gene.

Selection of ASOs for screening: A set of 480 preferred ASOs was selected for screening according to both specificity and ASO:mRNA (target) hybridization energy maximization information as follows. All candidate ASOs were evaluated for delta G of hybridization with the predicted target mRNA secondary structure ($\Delta G^{overall}$) according to Xu and Mathews (*Methods Mol Biol*. 1490:15-34 (2016)). Next, two subsets of ASOs were chosen: First, 69 ASOs that matched human, cyno, and mouse target transcripts, had off-target scores of at least 1 in three species, and negative $\Delta G^{overall}$; second, 411 ASOs that matched human and cyno target transcripts, had off-target scores of at least 2 in both species, and $\Delta G^{overall}$ less than -9.5 degrees Celsius.

The sequences, positions in human transcript, conservation in other species and species-specific off-target scores of each ASO are given in Table 2. Wherever indicated as "NC", the ASO does not match the MSH3 gene in that species, and therefore off-target scores were not generated.

ASOs were synthesized as 5-10-5 "flanking sequence--DNA core sequence-flanking sequence" antisense oligonucleotides, with ribonucleotides at positions 1-5 and 16-20 and deoxyribonucleotides at positions 6-15, and with the following generic structure:

5'-Nm$\underline{s}$Nm$\underline{s}$Nm$\underline{s}$Nm$\underline{s}$Nm$\underline{s}$ N$\underline{s}$N$\underline{s}$N$\underline{s}$N$\underline{s}$N$\underline{s}$ N$\underline{s}$N$\underline{s}$N$\underline{s}$N$\underline{s}$N$\underline{s}$ Nm$\underline{s}$Nm$\underline{s}$Nm$\underline{s}$Nm$\underline{s}$Nm-3'

wherein:

- Nm: 2'-MOE residues (including 5methyl-2'-MOE-C and 5methyl-2'-MOE-U)
- N: DNA/RNA residues
- $\underline{s}$: phosphorothioate (the backbone is fully phosphorothioate-modified)
- All "C" within the DNA core (positions 6-15) are 5'-Methyl-2'-MOE-dC
- All "T" in positions 1-5 or 16-20 are 5'-methyl-2'-MOE-U.

For primary screens at 2 nM and 20 nM, desalted oligonucleotides were used. For detailed characterization of a subset of oligonucleotides, oligonucleotides were further purified by HPLC.

Table 2: Exemplary Oligonucleotides

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
6	67	AGACATGGCAGGGCAAGGAT	2	2	NC	NC
7	134	TCAAAACCGCTTGCCTCGCA	3	NC	NC	NC
8	146	GGAAGAATCGGCTCAAAACC	2	NC	NC	NC
9	147	TGGAAGAATCGGCTCAAAAC	3	2	NC	NC
10	148	CTGGAAGAATCGGCTCAAAA	3	3	NC	NC
11	149	ACTGGAAGAATCGGCTCAAA	2	2	NC	NC
12	150	GACTGGAAGAATCGGCTCAA	2	2	NC	NC
13	151	AGACTGGAAGAATCGGCTCA	2	2	NC	NC
14	152	TAGACTGGAAGAATCGGCTC	2	2	NC	NC
15	153	GTAGACTGGAAGAATCGGCT	2	2	NC	NC
16	154	CGTAGACTGGAAGAATCGGC	3	2	NC	NC
17	155	CCGTAGACTGGAAGAATCGG	3	3	NC	NC
18	156	CCCGTAGACTGGAAGAATCG	2	3	NC	NC
19	157	TCCCGTAGACTGGAAGAATC	3	3	NC	NC
20	158	TTCCCGTAGACTGGAAGAAT	2	2	NC	NC
21	162	AGGCTTCCCGTAGACTGGAA	2	2	NC	NC
22	166	TTTCAGGCTTCCCGTAGACT	3	3	NC	NC
23	167	ATTTCAGGCTTCCCGTAGAC	2	2	NC	NC
24	168	GATTTCAGGCTTCCCGTAGA	2	2	NC	NC
25	169	GGATTTCAGGCTTCCCGTAG	3	3	NC	NC
26	170	TGGATTTCAGGCTTCCCGTA	2	2	NC	NC
27	171	GTGGATTTCAGGCTTCCCGT	2	3	NC	NC
28	173	AGGTGGATTTCAGGCTTCCC	2	3	NC	NC
29	174	GAGGTGGATTTCAGGCTTCC	2	2	NC	NC
30	175	GGAGGTGGATTTCAGGCTTC	1	2	NC	NC
31	176	AGGAGGTGGATTTCAGGCTT	2	2	NC	NC
32	177	GAGGAGGTGGATTTCAGGCT	2	2	NC	NC
33	179	AGGAGGAGGTGGATTTCAGG	2	NC	NC	NC
34	180	GAGGAGGAGGTGGATTTCAG	1	NC	NC	NC
35	181	GGAGGAGGAGGTGGATTTCA	1	NC	NC	NC
36	182	TGGAGGAGGAGGTGGATTTC	2	NC	NC	NC
37	183	GTGGAGGAGGAGGTGGATTT	1	NC	NC	NC
38	184	TGTGGAGGAGGAGGTGGATT	2	NC	NC	NC
39	312	TCAATTTCTGTAGCTATGTG	2	NC	NC	NC
40	313	GTCAATTTCTGTAGCTATGT	1	NC	NC	NC
41	314	TGTCAATTTCTGTAGCTATG	1	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
42	315	CTGTCAATTTCTGTAGCTAT	2	NC	NC	NC
43	316	TCTGTCAATTTCTGTAGCTA	2	NC	NC	NC
44	317	TTCTGTCAATTTCTGTAGCT	1	NC	NC	NC
45	318	CTTCTGTCAATTTCTGTAGC	1	NC	NC	NC
46	319	TCTTCTGTCAATTTCTGTAG	1	NC	NC	NC
47	320	TTCTTCTGTCAATTTCTGTA	1	NC	NC	NC
48	321	TTTCTTCTGTCAATTTCTGT	2	NC	NC	NC
49	322	CTTTCTTCTGTCAATTTCTG	1	NC	NC	NC
50	323	TCTTTCTTCTGTCAATTTCT	1	NC	NC	NC
51	324	TTCTTTCTTCTGTCAATTTC	1	NC	NC	NC
52	325	CTTCTTTCTTCTGTCAATTT	2	NC	NC	NC
53	326	TCTTCTTTCTTCTGTCAATT	1	NC	NC	NC
54	327	CTCTTCTTTCTTCTGTCAAT	1	NC	NC	NC
55	328	TCTCTTCTTTCTTCTGTCAA	1	NC	NC	NC
56	329	GTCTCTTCTTTCTTCTGTCA	1	NC	NC	NC
57	330	GGTCTCTTCTTTCTTCTGTC	1	NC	NC	NC
58	331	TGGTCTCTTCTTTCTTCTGT	2	NC	NC	NC
59	332	ATGGTCTCTTCTTTCTTCTG	2	NC	NC	NC
60	333	AATGGTCTCTTCTTTCTTCT	2	NC	NC	NC
61	334	CAATGGTCTCTTCTTTCTTC	2	NC	NC	NC
62	335	CCAATGGTCTCTTCTTTCTT	2	NC	NC	NC
63	336	TCCAATGGTCTCTTCTTTCT	1	NC	NC	NC
64	337	TTCCAATGGTCTCTTCTTTC	1	NC	NC	NC
65	338	TTTCCAATGGTCTCTTCTTT	1	NC	NC	NC
66	339	TTTTCCAATGGTCTCTTCTT	1	1	NC	NC
67	340	ATTTTCCAATGGTCTCTTCT	1	0	NC	NC
68	341	CATTTTCCAATGGTCTCTTC	1	1	NC	NC
69	350	CAGGCCCATCATTTTCCAAT	2	1	NC	NC
70	351	ACAGGCCCATCATTTTCCAA	2	1	NC	NC
71	352	AACAGGCCCATCATTTTCCA	2	1	NC	NC
72	353	TAACAGGCCCATCATTTTCC	2	1	NC	NC
73	354	TTAACAGGCCCATCATTTTC	2	2	NC	NC
74	355	TTTAACAGGCCCATCATTTT	2	2	NC	NC
75	356	TTTTAACAGGCCCATCATTT	1	2	NC	NC
76	357	TTTTTAACAGGCCCATCATT	2	2	NC	NC
77	358	CTTTTTAACAGGCCCATCAT	2	2	NC	NC
78	359	TCTTTTTAACAGGCCCATCA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
79	360	TTCTTTTTAACAGGCCCATC	2	2	NC	NC
80	361	TTTCTTTTTAACAGGCCCAT	2	1	NC	NC
81	362	CTTTCTTTTTAACAGGCCCA	2	2	NC	NC
82	363	ACTTTCTTTTTAACAGGCCC	2	2	NC	NC
83	364	TACTTTCTTTTTAACAGGCC	1	1	NC	NC
84	365	TTACTTTCTTTTTAACAGGC	1	1	NC	NC
85	366	TTTACTTTCTTTTTAACAGG	1	2	NC	NC
86	367	CTTTACTTTCTTTTTAACAG	2	1	NC	NC
87	373	GACTTTCTTTACTTTCTTTT	1	NC	NC	NC
88	374	GGACTTTCTTTACTTTCTTT	1	NC	NC	NC
89	375	TGGACTTTCTTTACTTTCTT	1	NC	NC	NC
90	376	TTGGACTTTCTTTACTTTCT	1	NC	NC	NC
91	377	GTTGGACTTTCTTTACTTTC	2	NC	NC	NC
92	378	TGTTGGACTTTCTTTACTTT	1	NC	NC	NC
93	379	TTGTTGGACTTTCTTTACTT	1	NC	NC	NC
94	380	TTTGTTGGACTTTCTTTACT	1	NC	NC	NC
95	381	TTTTGTTGGACTTTCTTTAC	1	NC	NC	NC
96	382	CTTTTGTTGGACTTTCTTTA	1	NC	NC	NC
97	383	CCTTTTGTTGGACTTTCTTT	2	NC	NC	NC
98	384	TCCTTTTGTTGGACTTTCTT	2	NC	NC	NC
99	385	TTCCTTTTGTTGGACTTTCT	2	NC	NC	NC
100	386	CTTCCTTTTGTTGGACTTTC	2	NC	NC	NC
101	387	CCTTCCTTTTGTTGGACTTT	2	NC	NC	NC
102	388	TCCTTCCTTTTGTTGGACTT	2	NC	NC	NC
103	389	CTCCTTCCTTTTGTTGGACT	2	NC	NC	NC
104	390	CCTCCTTCCTTTTGTTGGAC	2	NC	NC	NC
105	391	TCCTCCTTCCTTTTGTTGGA	2	NC	NC	NC
106	392	TTCCTCCTTCCTTTTGTTGG	1	2	NC	NC
107	393	CTTCCTCCTTCCTTTTGTTG	1	1	NC	NC
108	394	ACTTCCTCCTTCCTTTTGTT	1	1	NC	NC
109	395	CACTTCCTCCTTCCTTTTGT	1	1	NC	NC
110	396	TCACTTCCTCCTTCCTTTTG	2	1	NC	NC
111	397	ATCACTTCCTCCTTCCTTTT	1	1	NC	NC
112	398	GATCACTTCCTCCTTCCTTT	1	2	NC	NC
113	399	AGATCACTTCCTCCTTCCTT	1	2	NC	NC
114	400	CAGATCACTTCCTCCTTCCT	2	2	NC	NC
115	401	CCAGATCACTTCCTCCTTCC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
116	402	CCCAGATCACTTCCTCCTTC	2	1	NC	NC
117	403	TCCCAGATCACTTCCTCCTT	2	2	NC	NC
118	404	TTCCCAGATCACTTCCTCCT	2	2	NC	NC
119	405	ATTCCCAGATCACTTCCTCC	1	2	NC	NC
120	406	CATTCCCAGATCACTTCCTC	2	2	NC	NC
121	407	ACATTCCCAGATCACTTCCT	1	1	NC	NC
122	408	GACATTCCCAGATCACTTCC	2	1	NC	NC
123	409	AGACATTCCCAGATCACTTC	2	2	NC	NC
124	410	CAGACATTCCCAGATCACTT	2	2	NC	NC
125	411	CCAGACATTCCCAGATCACT	2	2	NC	NC
126	412	GCCAGACATTCCCAGATCAC	2	3	NC	NC
127	413	TGCCAGACATTCCCAGATCA	2	2	NC	NC
128	414	TTGCCAGACATTCCCAGATC	2	2	NC	NC
129	415	GTTGCCAGACATTCCCAGAT	2	2	NC	NC
130	416	AGTTGCCAGACATTCCCAGA	2	2	NC	NC
131	417	GAGTTGCCAGACATTCCCAG	2	2	NC	NC
132	418	AGAGTTGCCAGACATTCCCA	2	2	NC	NC
133	419	CAGAGTTGCCAGACATTCCC	2	2	NC	NC
134	420	TCAGAGTTGCCAGACATTCC	2	2	NC	NC
135	421	CTCAGAGTTGCCAGACATTC	2	1	NC	NC
136	422	GCTCAGAGTTGCCAGACATT	1	1	NC	NC
137	430	TTTCTTTGGCTCAGAGTTGC	1	1	NC	NC
138	431	ATTTCTTTGGCTCAGAGTTG	1	1	NC	NC
139	432	CATTTCTTTGGCTCAGAGTT	1	1	NC	NC
140	433	ACATTTCTTTGGCTCAGAGT	1	2	NC	NC
141	434	GACATTTCTTTGGCTCAGAG	1	2	NC	NC
142	435	AGACATTTCTTTGGCTCAGA	1	2	NC	NC
143	436	CAGACATTTCTTTGGCTCAG	2	2	NC	NC
144	437	TCAGACATTTCTTTGGCTCA	2	2	NC	NC
145	438	CTCAGACATTTCTTTGGCTC	2	2	NC	NC
146	439	CCTCAGACATTTCTTTGGCT	2	1	NC	NC
147	440	TCCTCAGACATTTCTTTGGC	2	2	NC	NC
148	454	TGAAACATTCCTGGTCCTCA	2	NC	NC	NC
149	455	TTGAAACATTCCTGGTCCTC	1	NC	NC	NC
150	456	TTTGAAACATTCCTGGTCCT	2	NC	NC	NC
151	457	CTTTGAAACATTCCTGGTCC	2	NC	NC	NC
152	458	ACTTTGAAACATTCCTGGTC	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
153	459	GACTTTGAAACATTCCTGGT	2	NC	NC	NC
154	460	AGACTTTGAAACATTCCTGG	1	NC	NC	NC
155	461	GAGACTTTGAAACATTCCTG	2	NC	NC	NC
156	462	AGAGACTTTGAAACATTCCT	2	NC	NC	NC
157	463	CAGAGACTTTGAAACATTCC	2	NC	NC	NC
158	464	CCAGAGACTTTGAAACATTC	2	NC	NC	NC
159	465	TCCAGAGACTTTGAAACATT	2	NC	NC	NC
160	466	TTCCAGAGACTTTGAAACAT	2	NC	NC	NC
161	467	TTTCCAGAGACTTTGAAACA	2	NC	NC	NC
162	468	TTTTCCAGAGACTTTGAAAC	1	NC	NC	NC
163	469	TTTTTCCAGAGACTTTGAAA	2	NC	NC	NC
164	470	ATTTTTCCAGAGACTTTGAA	2	NC	NC	NC
165	471	AATTTTTCCAGAGACTTTGA	2	NC	NC	NC
166	472	CAATTTTTCCAGAGACTTTG	2	NC	NC	NC
167	473	TCAATTTTTCCAGAGACTTT	2	2	NC	NC
168	474	TTCAATTTTTCCAGAGACTT	2	2	NC	NC
169	475	TTTCAATTTTTCCAGAGACT	1	2	NC	NC
170	476	CTTTCAATTTTTCCAGAGAC	1	2	NC	NC
171	477	TCTTTCAATTTTTCCAGAGA	1	2	NC	NC
172	478	TTCTTTCAATTTTTCCAGAG	1	2	NC	NC
173	479	ATTCTTTCAATTTTTCCAGA	2	2	NC	NC
174	480	AATTCTTTCAATTTTTCCAG	1	1	NC	NC
175	481	GAATTCTTTCAATTTTTCCA	1	1	NC	NC
176	482	AGAATTCTTTCAATTTTTCC	1	1	NC	NC
177	483	CAGAATTCTTTCAATTTTTC	1	0	NC	NC
178	484	GCAGAATTCTTTCAATTTTT	2	0	NC	NC
179	485	AGCAGAATTCTTTCAATTTT	2	1	NC	NC
180	486	CAGCAGAATTCTTTCAATTT	2	1	NC	NC
181	487	GCAGCAGAATTCTTTCAATT	2	NC	NC	NC
182	488	CGCAGCAGAATTCTTTCAAT	2	NC	NC	NC
183	489	TCGCAGCAGAATTCTTTCAA	2	NC	NC	NC
184	490	ATCGCAGCAGAATTCTTTCA	2	NC	NC	NC
185	491	AATCGCAGCAGAATTCTTTC	2	NC	NC	NC
186	492	GAATCGCAGCAGAATTCTTT	2	NC	NC	NC
187	493	AGAATCGCAGCAGAATTCTT	2	NC	NC	NC
188	494	CAGAATCGCAGCAGAATTCT	2	NC	NC	NC
189	495	GCAGAATCGCAGCAGAATTC	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
190	496	GGCAGAATCGCAGCAGAATT	2	NC	NC	NC
191	497	GGGCAGAATCGCAGCAGAAT	3	NC	NC	NC
192	498	AGGGCAGAATCGCAGCAGAA	2	NC	NC	NC
193	499	AAGGGCAGAATCGCAGCAGA	2	NC	NC	NC
194	504	TGAGGAAGGGCAGAATCGCA	2	NC	NC	NC
195	505	TTGAGGAAGGGCAGAATCGC	3	NC	NC	NC
196	506	TTTGAGGAAGGGCAGAATCG	2	NC	NC	NC
197	507	CTTTGAGGAAGGGCAGAATC	1	2	NC	NC
198	521	CTGTCTGGACTCTACTTTGA	1	2	NC	NC
199	522	TCTGTCTGGACTCTACTTTG	1	2	NC	NC
200	523	TTCTGTCTGGACTCTACTTT	2	2	NC	NC
201	524	ATTCTGTCTGGACTCTACTT	2	2	NC	NC
202	525	GATTCTGTCTGGACTCTACT	1	2	NC	NC
203	526	AGATTCTGTCTGGACTCTAC	2	2	NC	NC
204	527	GAGATTCTGTCTGGACTCTA	2	2	NC	NC
205	528	AGAGATTCTGTCTGGACTCT	2	2	NC	NC
206	529	CAGAGATTCTGTCTGGACTC	2	2	NC	NC
207	548	GAACTGCAAATCTCTCCTGC	1	2	NC	NC
208	549	AGAACTGCAAATCTCTCCTG	1	2	NC	NC
209	550	CAGAACTGCAAATCTCTCCT	2	2	NC	NC
210	562	AGTACATTTTGGCAGAACTG	2	2	NC	NC
211	563	CAGTACATTTTGGCAGAACT	1	2	NC	NC
212	564	TCAGTACATTTTGGCAGAAC	2	2	NC	NC
213	565	ATCAGTACATTTTGGCAGAA	2	2	NC	NC
214	566	AATCAGTACATTTTGGCAGA	2	2	NC	NC
215	567	AAATCAGTACATTTTGGCAG	2	2	NC	NC
216	568	AAAATCAGTACATTTTGGCA	2	1	NC	NC
217	572	CATCAAAATCAGTACATTTT	1	2	NC	NC
218	573	TCATCAAAATCAGTACATTT	1	1	NC	NC
219	574	ATCATCAAAATCAGTACATT	1	2	NC	NC
220	575	TATCATCAAAATCAGTACAT	1	1	NC	NC
221	576	ATATCATCAAAATCAGTACA	2	2	NC	NC
222	577	GATATCATCAAAATCAGTAC	2	2	NC	NC
223	578	TGATATCATCAAAATCAGTA	2	2	NC	NC
224	588	TGTAGAAGACTGATATCATC	1	NC	NC	NC
225	589	GTGTAGAAGACTGATATCAT	2	NC	NC	NC
226	590	CGTGTAGAAGACTGATATCA	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
227	591	GCGTGTAGAAGACTGATATC	3	NC	NC	NC
228	592	TGCGTGTAGAAGACTGATAT	2	NC	NC	NC
229	593	TTGCGTGTAGAAGACTGATA	3	NC	NC	NC
230	594	TTTGCGTGTAGAAGACTGAT	3	NC	NC	NC
231	595	CTTTGCGTGTAGAAGACTGA	2	NC	NC	NC
232	596	TCTTTGCGTGTAGAAGACTG	3	NC	NC	NC
233	597	TTCTTTGCGTGTAGAAGACT	2	NC	NC	NC
234	598	ATTCTTTGCGTGTAGAAGAC	3	NC	NC	NC
235	599	CATTCTTTGCGTGTAGAAGA	2	NC	NC	NC
236	614	CTTCAGAAGAAACTGCATTC	2	2	NC	NC
237	615	TCTTCAGAAGAAACTGCATT	1	2	NC	NC
238	625	ACGTTTCGAATCTTCAGAAG	2	NC	NC	NC
239	626	GACGTTTCGAATCTTCAGAA	3	NC	NC	NC
240	627	TGACGTTTCGAATCTTCAGA	3	NC	NC	NC
241	628	TTGACGTTTCGAATCTTCAG	3	NC	NC	NC
242	629	TTTGACGTTTCGAATCTTCA	3	NC	NC	NC
243	630	ATTTGACGTTTCGAATCTTC	3	NC	NC	NC
244	631	AATTTGACGTTTCGAATCTT	2	NC	NC	NC
245	632	TAATTTGACGTTTCGAATCT	2	NC	NC	NC
246	633	TTAATTTGACGTTTCGAATC	3	NC	NC	NC
247	634	ATTAATTTGACGTTTCGAAT	2	NC	NC	NC
248	635	GATTAATTTGACGTTTCGAA	2	NC	NC	NC
249	636	TGATTAATTTGACGTTTCGA	2	NC	NC	NC
250	637	TTGATTAATTTGACGTTTCG	2	NC	NC	NC
251	638	TTTGATTAATTTGACGTTTC	2	NC	NC	NC
252	640	CTTTTGATTAATTTGACGTT	3	NC	NC	NC
253	641	CCTTTTGATTAATTTGACGT	2	NC	NC	NC
254	642	TCCTTTTGATTAATTTGACG	2	NC	NC	NC
255	643	GTCCTTTTGATTAATTTGAC	1	NC	NC	NC
256	644	TGTCCTTTTGATTAATTTGA	2	NC	NC	NC
257	645	GTGTCCTTTTGATTAATTTG	2	NC	NC	NC
258	646	TGTGTCCTTTTGATTAATTT	2	NC	NC	NC
259	647	TTGTGTCCTTTTGATTAATT	2	NC	NC	NC
260	648	GTTGTGTCCTTTTGATTAAT	2	NC	NC	NC
261	649	TGTTGTGTCCTTTTGATTAA	2	NC	NC	NC
262	650	GTGTTGTGTCCTTTTGATTA	2	NC	NC	NC
263	651	AGTGTTGTGTCCTTTTGATT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
264	652	AAGTGTTGTGTCCTTTTGAT	2	NC	NC	NC
265	653	AAAGTGTTGTGTCCTTTTGA	1	NC	NC	NC
266	654	AAAAGTGTTGTGTCCTTTTG	1	NC	NC	NC
267	656	CAAAAAGTGTTGTGTCCTTT	1	NC	NC	NC
268	657	TCAAAAAGTGTTGTGTCCTT	1	NC	NC	NC
269	658	ATCAAAAAGTGTTGTGTCCT	1	NC	NC	NC
270	659	GATCAAAAAGTGTTGTGTCC	2	NC	NC	NC
271	660	AGATCAAAAAGTGTTGTGTC	2	NC	NC	NC
272	661	GAGATCAAAAAGTGTTGTGT	2	NC	NC	NC
273	662	TGAGATCAAAAAGTGTTGTG	2	NC	NC	NC
274	663	CTGAGATCAAAAAGTGTTGT	2	NC	NC	NC
275	664	ACTGAGATCAAAAAGTGTTG	2	NC	NC	NC
276	665	GACTGAGATCAAAAAGTGTT	2	NC	NC	NC
277	666	TGACTGAGATCAAAAAGTGT	2	NC	NC	NC
278	667	CTGACTGAGATCAAAAAGTG	2	NC	NC	NC
279	668	ACTGACTGAGATCAAAAAGT	1	NC	NC	NC
280	669	AACTGACTGAGATCAAAAAG	2	NC	NC	NC
281	670	AAACTGACTGAGATCAAAAA	2	NC	NC	NC
282	671	CAAACTGACTGAGATCAAAA	2	NC	NC	NC
283	672	CCAAACTGACTGAGATCAAA	2	NC	NC	NC
284	673	TCCAAACTGACTGAGATCAA	2	NC	NC	NC
285	674	ATCCAAACTGACTGAGATCA	2	NC	NC	NC
286	675	GATCCAAACTGACTGAGATC	1	NC	NC	NC
287	676	TGATCCAAACTGACTGAGAT	2	NC	NC	NC
288	677	ATGATCCAAACTGACTGAGA	1	NC	NC	NC
289	678	GATGATCCAAACTGACTGAG	2	NC	NC	NC
290	679	TGATGATCCAAACTGACTGA	2	2	NC	NC
291	680	TTGATGATCCAAACTGACTG	2	2	NC	NC
292	681	TTTGATGATCCAAACTGACT	2	2	NC	NC
293	682	ATTTGATGATCCAAACTGAC	2	2	NC	NC
294	683	TATTTGATGATCCAAACTGA	1	1	NC	NC
295	684	GTATTTGATGATCCAAACTG	2	2	NC	NC
296	685	TGTATTTGATGATCCAAACT	2	2	NC	NC
297	686	TTGTATTTGATGATCCAAAC	2	1	NC	NC
298	688	ACTTGTATTTGATGATCCAA	2	1	NC	NC
299	689	GACTTGTATTTGATGATCCA	2	2	NC	NC
300	690	TGACTTGTATTTGATGATCC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
301	691	ATGACTTGTATTTGATGATC	2	2	NC	NC
302	692	CATGACTTGTATTTGATGAT	2	2	NC	NC
303	693	TCATGACTTGTATTTGATGA	2	2	NC	NC
304	694	TTCATGACTTGTATTTGATG	2	2	NC	NC
305	695	TTTCATGACTTGTATTTGAT	2	2	NC	NC
306	696	TTTTCATGACTTGTATTTGA	1	2	NC	NC
307	697	ATTTTCATGACTTGTATTTG	0	1	NC	NC
308	701	GTAAATTTTCATGACTTGTA	1	2	NC	NC
309	702	TGTAAATTTTCATGACTTGT	2	2	NC	NC
310	703	CTGTAAATTTTCATGACTTG	1	2	NC	NC
311	704	TCTGTAAATTTTCATGACTT	1	2	NC	NC
312	705	TTCTGTAAATTTTCATGACT	1	2	NC	NC
313	706	TTTCTGTAAATTTTCATGAC	1	2	NC	NC
314	708	GTTTTCTGTAAATTTTCATG	1	1	NC	NC
315	721	TGATTTGGAAGCAGTTTTCT	1	NC	NC	NC
316	730	TTTGTTAGCTGATTTGGAAG	2	NC	NC	NC
317	731	GTTTGTTAGCTGATTTGGAA	2	NC	NC	NC
318	732	CGTTTGTTAGCTGATTTGGA	2	NC	NC	NC
319	733	CCGTTTGTTAGCTGATTTGG	2	NC	NC	NC
320	734	ACCGTTTGTTAGCTGATTTG	2	NC	NC	NC
321	735	GACCGTTTGTTAGCTGATTT	2	NC	NC	NC
322	736	GGACCGTTTGTTAGCTGATT	2	NC	NC	NC
323	737	TGGACCGTTTGTTAGCTGAT	2	NC	NC	NC
324	738	TTGGACCGTTTGTTAGCTGA	2	NC	NC	NC
325	739	TTTGGACCGTTTGTTAGCTG	3	NC	NC	NC
326	740	TTTTGGACCGTTTGTTAGCT	3	NC	NC	NC
327	741	CTTTTGGACCGTTTGTTAGC	3	NC	NC	NC
328	742	GCTTTTGGACCGTTTGTTAG	3	NC	NC	NC
329	743	TGCTTTTGGACCGTTTGTTA	2	NC	NC	NC
330	744	ATGCTTTTGGACCGTTTGTT	2	NC	NC	NC
331	745	GATGCTTTTGGACCGTTTGT	2	NC	NC	NC
332	746	AGATGCTTTTGGACCGTTTG	2	NC	NC	NC
333	747	TAGATGCTTTTGGACCGTTT	3	NC	NC	NC
334	748	ATAGATGCTTTTGGACCGTT	3	NC	NC	NC
335	749	TATAGATGCTTTTGGACCGT	3	NC	NC	NC
336	750	GTATAGATGCTTTTGGACCG	2	NC	NC	NC
337	751	CGTATAGATGCTTTTGGACC	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
338	752	GCGTATAGATGCTTTTGGAC	3	NC	NC	NC
339	753	GGCGTATAGATGCTTTTGGA	3	NC	NC	NC
340	754	CGGCGTATAGATGCTTTTGG	3	NC	NC	NC
341	755	GCGGCGTATAGATGCTTTTG	3	NC	NC	NC
342	756	AGCGGCGTATAGATGCTTTT	3	NC	NC	NC
343	757	TAGCGGCGTATAGATGCTTT	3	NC	NC	NC
344	758	CTAGCGGCGTATAGATGCTT	3	NC	NC	NC
345	759	TCTAGCGGCGTATAGATGCT	4	NC	NC	NC
346	760	TTCTAGCGGCGTATAGATGC	3	NC	NC	NC
347	761	ATTCTAGCGGCGTATAGATG	3	NC	NC	NC
348	762	AATTCTAGCGGCGTATAGAT	3	NC	NC	NC
349	763	TAATTCTAGCGGCGTATAGA	3	NC	NC	NC
350	764	GTAATTCTAGCGGCGTATAG	3	NC	NC	NC
351	765	TGTAATTCTAGCGGCGTATA	2	3	NC	NC
352	766	TTGTAATTCTAGCGGCGTAT	3	3	NC	NC
353	767	ATTGTAATTCTAGCGGCGTA	3	3	NC	NC
354	768	TATTGTAATTCTAGCGGCGT	3	3	NC	NC
355	769	GTATTGTAATTCTAGCGGCG	3	3	NC	NC
356	770	TGTATTGTAATTCTAGCGGC	3	3	NC	NC
357	771	ATGTATTGTAATTCTAGCGG	3	3	NC	NC
358	772	TATGTATTGTAATTCTAGCG	2	2	NC	NC
359	773	CTATGTATTGTAATTCTAGC	2	2	NC	NC
360	774	TCTATGTATTGTAATTCTAG	1	2	NC	NC
361	781	CTTCATTTCTATGTATTGTA	2	2	NC	NC
362	782	GCTTCATTTCTATGTATTGT	2	2	NC	NC
363	783	TGCTTCATTTCTATGTATTG	2	1	NC	NC
364	784	CTGCTTCATTTCTATGTATT	1	1	NC	NC
365	785	GCTGCTTCATTTCTATGTAT	2	2	NC	NC
366	786	TGCTGCTTCATTTCTATGTA	2	2	NC	NC
367	787	CTGCTGCTTCATTTCTATGT	1	1	NC	NC
368	788	GCTGCTGCTTCATTTCTATG	2	2	NC	NC
369	810	ACACACAAAACTGCATCTTT	1	2	NC	NC
370	811	CACACACAAAACTGCATCTT	1	1	NC	NC
371	812	CCACACACAAAACTGCATCT	2	1	NC	NC
372	813	TCCACACACAAAACTGCATC	2	2	NC	NC
373	814	TTCCACACACAAAACTGCAT	2	2	NC	NC
374	815	ATTCCACACACAAAACTGCA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
375	816	CATTCCACACACAAAACTGC	1	2	NC	NC
376	817	ACATTCCACACACAAAACTG	1	1	NC	NC
377	818	CACATTCCACACACAAAACT	1	1	NC	NC
378	819	CCACATTCCACACACAAAAC	2	1	NC	NC
379	820	TCCACATTCCACACACAAAA	1	0	NC	NC
380	821	ATCCACATTCCACACACAAA	1	1	NC	NC
381	822	TATCCACATTCCACACACAA	2	1	NC	NC
382	823	ATATCCACATTCCACACACA	2	2	NC	NC
383	824	TATATCCACATTCCACACAC	2	1	NC	NC
384	825	TTATATCCACATTCCACACA	1	1	NC	NC
385	826	CTTATATCCACATTCCACAC	2	2	NC	NC
386	827	ACTTATATCCACATTCCACA	1	2	NC	NC
387	828	TACTTATATCCACATTCCAC	1	2	NC	NC
388	829	ATACTTATATCCACATTCCA	1	1	NC	NC
389	830	TATACTTATATCCACATTCC	1	2	NC	NC
390	831	CTATACTTATATCCACATTC	2	2	NC	NC
391	832	TCTATACTTATATCCACATT	1	1	NC	NC
392	833	ATCTATACTTATATCCACAT	2	2	NC	NC
393	834	AATCTATACTTATATCCACA	2	2	NC	NC
394	835	GAATCTATACTTATATCCAC	2	2	NC	NC
395	836	AGAATCTATACTTATATCCA	2	2	NC	NC
396	837	AAGAATCTATACTTATATCC	1	1	NC	NC
397	840	CCAAAGAATCTATACTTATA	2	2	NC	NC
398	841	CCCAAAGAATCTATACTTAT	2	2	NC	NC
399	842	CCCCAAAGAATCTATACTTA	1	2	NC	NC
400	843	TCCCCAAAGAATCTATACTT	1	2	NC	NC
401	844	TTCCCCAAAGAATCTATACT	1	2	NC	NC
402	845	CTTCCCCAAAGAATCTATAC	2	2	NC	NC
403	854	TCTCTGCATCTTCCCCAAAG	1	1	NC	NC
404	855	ATCTCTGCATCTTCCCCAAA	1	1	NC	NC
405	856	AATCTCTGCATCTTCCCCAA	1	1	NC	NC
406	857	CAATCTCTGCATCTTCCCCA	2	2	NC	NC
407	879	TAAATATTGAGCTCTCGGGC	2	2	NC	NC
408	880	ATAAATATTGAGCTCTCGGG	2	2	NC	NC
409	881	AATAAATATTGAGCTCTCGG	2	2	NC	NC
410	893	GATCTAAATGGCAATAAATA	2	2	NC	NC
411	894	TGATCTAAATGGCAATAAAT	1	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
412	895	GTGATCTAAATGGCAATAAA	2	2	NC	NC
413	896	TGTGATCTAAATGGCAATAA	2	2	NC	NC
414	897	TTGTGATCTAAATGGCAATA	2	2	NC	NC
415	898	GTTGTGATCTAAATGGCAAT	2	2	NC	NC
416	899	AGTTGTGATCTAAATGGCAA	2	2	NC	NC
417	900	AAGTTGTGATCTAAATGGCA	2	2	NC	NC
418	901	AAAGTTGTGATCTAAATGGC	2	2	NC	NC
419	902	TAAAGTTGTGATCTAAATGG	2	2	NC	NC
420	903	ATAAAGTTGTGATCTAAATG	1	1	NC	NC
421	904	CATAAAGTTGTGATCTAAAT	2	2	NC	NC
422	905	TCATAAAGTTGTGATCTAAA	2	2	NC	NC
423	906	GTCATAAAGTTGTGATCTAA	2	2	NC	NC
424	907	TGTCATAAAGTTGTGATCTA	2	2	NC	NC
425	908	CTGTCATAAAGTTGTGATCT	1	1	NC	NC
426	909	GCTGTCATAAAGTTGTGATC	2	2	NC	NC
427	910	TGCTGTCATAAAGTTGTGAT	2	2	NC	NC
428	911	TTGCTGTCATAAAGTTGTGA	2	2	NC	NC
429	912	CTTGCTGTCATAAAGTTGTG	2	2	NC	NC
430	913	ACTTGCTGTCATAAAGTTGT	2	1	NC	NC
431	914	TACTTGCTGTCATAAAGTTG	2	2	NC	NC
432	915	ATACTTGCTGTCATAAAGTT	2	2	NC	NC
433	917	GTATACTTGCTGTCATAAAG	1	3	NC	NC
434	918	GGTATACTTGCTGTCATAAA	2	2	NC	NC
435	919	AGGTATACTTGCTGTCATAA	2	2	NC	NC
436	920	TAGGTATACTTGCTGTCATA	2	2	NC	NC
437	921	GTAGGTATACTTGCTGTCAT	2	2	NC	NC
438	922	AGTAGGTATACTTGCTGTCA	2	2	NC	NC
439	931	CAGTCTGTGAGTAGGTATAC	3	2	NC	NC
440	932	ACAGTCTGTGAGTAGGTATA	2	2	NC	NC
441	933	AACAGTCTGTGAGTAGGTAT	2	3	NC	NC
442	934	AAACAGTCTGTGAGTAGGTA	2	2	NC	NC
443	935	CAAACAGTCTGTGAGTAGGT	1	2	NC	NC
444	936	ACAAACAGTCTGTGAGTAGG	2	2	NC	NC
445	937	AACAAACAGTCTGTGAGTAG	1	2	NC	NC
446	938	GAACAAACAGTCTGTGAGTA	2	2	NC	NC
447	939	TGAACAAACAGTCTGTGAGT	2	2	NC	NC
448	940	ATGAACAAACAGTCTGTGAG	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
449	941	CATGAACAAACAGTCTGTGA	2	2	NC	NC
450	942	ACATGAACAAACAGTCTGTG	2	2	NC	NC
451	943	TACATGAACAAACAGTCTGT	2	2	NC	NC
452	944	GTACATGAACAAACAGTCTG	2	2	NC	NC
453	945	CGTACATGAACAAACAGTCT	3	3	NC	NC
454	946	GCGTACATGAACAAACAGTC	2	3	NC	NC
455	947	GGCGTACATGAACAAACAGT	3	3	NC	NC
456	948	CGGCGTACATGAACAAACAG	2	3	NC	NC
457	949	GCGGCGTACATGAACAAACA	3	3	NC	NC
458	950	GGCGGCGTACATGAACAAAC	3	3	NC	NC
459	951	AGGCGGCGTACATGAACAAA	3	3	NC	NC
460	952	CAGGCGGCGTACATGAACAA	3	3	NC	NC
461	969	TTATATCCTTTTGCCACCAG	2	2	NC	NC
462	970	CTTATATCCTTTTGCCACCA	2	2	NC	NC
463	971	CCTTATATCCTTTTGCCACC	2	2	NC	NC
464	972	ACCTTATATCCTTTTGCCAC	2	3	NC	NC
465	973	CACCTTATATCCTTTTGCCA	2	2	NC	NC
466	974	CCACCTTATATCCTTTTGCC	2	2	NC	NC
467	975	CCCACCTTATATCCTTTTGC	2	2	NC	NC
468	976	TCCCACCTTATATCCTTTTG	2	2	NC	NC
469	977	CTCCCACCTTATATCCTTTT	1	2	NC	NC
470	978	ACTCCCACCTTATATCCTTT	2	2	NC	NC
471	979	AACTCCCACCTTATATCCTT	2	2	NC	NC
472	980	CAACTCCCACCTTATATCCT	2	2	NC	NC
473	981	ACAACTCCCACCTTATATCC	2	2	NC	NC
474	982	CACAACTCCCACCTTATATC	3	2	NC	NC
475	983	TCACAACTCCCACCTTATAT	2	2	NC	NC
476	984	TTCACAACTCCCACCTTATA	2	2	NC	NC
477	985	CTTCACAACTCCCACCTTAT	2	2	NC	NC
478	986	GCTTCACAACTCCCACCTTA	2	2	NC	NC
479	987	TGCTTCACAACTCCCACCTT	2	2	2	2
480	988	TTGCTTCACAACTCCCACCT	2	2	2	2
481	989	TTTGCTTCACAACTCCCACC	2	2	2	2
482	990	GTTTGCTTCACAACTCCCAC	2	2	2	2
483	991	AGTTTGCTTCACAACTCCCA	2	2	2	2
484	992	CAGTTTGCTTCACAACTCCC	2	3	1	2
485	993	TCAGTTTGCTTCACAACTCC	2	2	1	2

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
486	994	TTCAGTTTGCTTCACAACTC	1	1	1	2
487	995	TTTCAGTTTGCTTCACAACT	2	2	2	2
488	996	GTTTCAGTTTGCTTCACAAC	2	2	2	2
489	997	AGTTTCAGTTTGCTTCACAA	2	2	1	2
490	998	CAGTTTCAGTTTGCTTCACA	2	2	1	1
491	999	GCAGTTTCAGTTTGCTTCAC	2	2	1	2
492	1004	ATGCTGCAGTTTCAGTTTGC	2	2	NC	NC
493	1005	AATGCTGCAGTTTCAGTTTG	2	2	NC	NC
494	1006	TAATGCTGCAGTTTCAGTTT	1	2	NC	NC
495	1007	TTAATGCTGCAGTTTCAGTT	1	1	NC	NC
496	1008	TTTAATGCTGCAGTTTCAGT	1	2	NC	NC
497	1010	CCTTTAATGCTGCAGTTTCA	2	2	NC	NC
498	1011	GCCTTTAATGCTGCAGTTTC	3	2	NC	NC
499	1012	GGCCTTTAATGCTGCAGTTT	3	2	NC	NC
500	1013	TGGCCTTTAATGCTGCAGTT	2	2	NC	NC
501	1014	ATGGCCTTTAATGCTGCAGT	2	2	NC	NC
502	1015	AATGGCCTTTAATGCTGCAG	2	2	NC	NC
503	1016	CAATGGCCTTTAATGCTGCA	2	2	NC	NC
504	1017	CCAATGGCCTTTAATGCTGC	2	3	NC	NC
505	1018	TCCAATGGCCTTTAATGCTG	2	2	NC	NC
506	1019	CTCCAATGGCCTTTAATGCT	3	2	NC	NC
507	1020	TCTCCAATGGCCTTTAATGC	2	2	NC	NC
508	1021	GTCTCCAATGGCCTTTAATG	2	3	NC	NC
509	1022	TGTCTCCAATGGCCTTTAAT	2	2	NC	NC
510	1023	TTGTCTCCAATGGCCTTTAA	2	2	NC	NC
511	1024	GTTGTCTCCAATGGCCTTTA	2	2	NC	NC
512	1025	TGTTGTCTCCAATGGCCTTT	2	2	NC	NC
513	1026	CTGTTGTCTCCAATGGCCTT	2	2	NC	NC
514	1027	TCTGTTGTCTCCAATGGCCT	2	2	NC	NC
515	1028	TTCTGTTGTCTCCAATGGCC	1	2	NC	NC
516	1029	CTTCTGTTGTCTCCAATGGC	1	2	NC	NC
517	1030	ACTTCTGTTGTCTCCAATGG	1	2	NC	NC
518	1031	AACTTCTGTTGTCTCCAATG	1	2	NC	NC
519	1032	GAACTTCTGTTGTCTCCAAT	2	2	NC	NC
520	1033	TGAACTTCTGTTGTCTCCAA	2	2	NC	NC
521	1034	GTGAACTTCTGTTGTCTCCA	2	2	NC	NC
522	1035	AGTGAACTTCTGTTGTCTCC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
523	1036	GAGTGAACTTCTGTTGTCTC	3	2	NC	NC
524	1037	AGAGTGAACTTCTGTTGTCT	2	1	NC	NC
525	1038	AAGAGTGAACTTCTGTTGTC	2	2	NC	NC
526	1039	AAAGAGTGAACTTCTGTTGT	2	1	NC	NC
527	1040	AAAAGAGTGAACTTCTGTTG	2	2	NC	NC
528	1042	GGAAAAGAGTGAACTTCTGT	2	2	NC	NC
529	1043	GGGAAAAGAGTGAACTTCTG	2	2	NC	NC
530	1044	CGGGAAAAGAGTGAACTTCT	2	2	NC	NC
531	1045	CCGGGAAAAGAGTGAACTTC	2	2	NC	NC
532	1046	TCCGGGAAAAGAGTGAACTT	2	2	NC	NC
533	1047	TTCCGGGAAAAGAGTGAACT	2	2	NC	NC
534	1048	TTTCCGGGAAAAGAGTGAAC	2	3	NC	NC
535	1049	ATTTCCGGGAAAAGAGTGAA	2	3	NC	NC
536	1050	AATTTCCGGGAAAAGAGTGA	2	3	NC	NC
537	1051	CAATTTCCGGGAAAAGAGTG	2	3	NC	NC
538	1052	TCAATTTCCGGGAAAAGAGT	2	2	NC	NC
539	1053	GTCAATTTCCGGGAAAAGAG	2	2	NC	NC
540	1054	AGTCAATTTCCGGGAAAAGA	2	2	NC	NC
541	1055	CAGTCAATTTCCGGGAAAAG	2	2	NC	NC
542	1056	GCAGTCAATTTCCGGGAAAA	1	2	NC	NC
543	1057	GGCAGTCAATTTCCGGGAAA	2	3	NC	NC
544	1058	GGGCAGTCAATTTCCGGGAA	2	3	NC	NC
545	1059	AGGGCAGTCAATTTCCGGGA	2	2	NC	NC
546	1060	AAGGGCAGTCAATTTCCGGG	2	2	NC	NC
547	1061	AAAGGGCAGTCAATTTCCGG	2	2	NC	NC
548	1062	TAAAGGGCAGTCAATTTCCG	2	3	NC	NC
549	1063	ATAAAGGGCAGTCAATTTCC	2	2	NC	NC
550	1064	TATAAAGGGCAGTCAATTTC	2	2	NC	NC
551	1065	GTATAAAGGGCAGTCAATTT	2	2	NC	NC
552	1066	TGTATAAAGGGCAGTCAATT	2	2	NC	NC
553	1067	TTGTATAAAGGGCAGTCAAT	2	2	NC	NC
554	1068	TTTGTATAAAGGGCAGTCAA	2	2	NC	NC
555	1069	TTTTGTATAAAGGGCAGTCA	2	2	NC	NC
556	1070	ATTTTGTATAAAGGGCAGTC	2	2	NC	NC
557	1071	GATTTTGTATAAAGGGCAGT	2	2	NC	NC
558	1072	AGATTTTGTATAAAGGGCAG	2	2	NC	NC
559	1073	TAGATTTTGTATAAAGGGCA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
560	1074	GTAGATTTTGTATAAAGGGC	2	2	NC	NC
561	1075	TGTAGATTTTGTATAAAGGG	2	2	NC	NC
562	1076	GTGTAGATTTTGTATAAAGG	2	2	NC	NC
563	1077	AGTGTAGATTTTGTATAAAG	2	2	NC	NC
564	1082	CAATAAGTGTAGATTTTGTA	1	NC	NC	NC
565	1083	CCAATAAGTGTAGATTTTGT	2	NC	NC	NC
566	1084	TCCAATAAGTGTAGATTTTG	2	NC	NC	NC
567	1085	CTCCAATAAGTGTAGATTTT	1	NC	NC	NC
568	1086	TCTCCAATAAGTGTAGATTT	1	NC	NC	NC
569	1087	TTCTCCAATAAGTGTAGATT	2	NC	NC	NC
570	1088	CTTCTCCAATAAGTGTAGAT	1	NC	NC	NC
571	1089	TCTTCTCCAATAAGTGTAGA	1	NC	NC	NC
572	1090	ATCTTCTCCAATAAGTGTAG	3	NC	NC	NC
573	1091	CATCTTCTCCAATAAGTGTA	2	NC	NC	NC
574	1092	ACATCTTCTCCAATAAGTGT	2	NC	NC	NC
575	1093	CACATCTTCTCCAATAAGTG	2	NC	NC	NC
576	1094	TCACATCTTCTCCAATAAGT	1	NC	NC	NC
577	1095	TTCACATCTTCTCCAATAAG	2	NC	NC	NC
578	1096	ATTCACATCTTCTCCAATAA	2	NC	NC	NC
579	1097	GATTCACATCTTCTCCAATA	1	NC	NC	NC
580	1098	GGATTCACATCTTCTCCAAT	2	1	NC	NC
581	1099	GGGATTCACATCTTCTCCAA	2	1	NC	NC
582	1117	ATCATCCAGCTTGATTAGGG	3	3	NC	NC
583	1118	CATCATCCAGCTTGATTAGG	2	2	NC	NC
584	1119	GCATCATCCAGCTTGATTAG	2	3	NC	NC
585	1120	AGCATCATCCAGCTTGATTA	2	2	NC	NC
586	1127	CATTTACAGCATCATCCAGC	2	2	NC	NC
587	1128	ACATTTACAGCATCATCCAG	1	2	NC	NC
588	1129	AACATTTACAGCATCATCCA	2	2	NC	NC
589	1130	CAACATTTACAGCATCATCC	2	2	NC	NC
590	1131	TCAACATTTACAGCATCATC	2	2	NC	NC
591	1132	ATCAACATTTACAGCATCAT	2	2	NC	NC
592	1133	CATCAACATTTACAGCATCA	2	1	NC	NC
593	1134	TCATCAACATTTACAGCATC	1	1	NC	NC
594	1135	CTCATCAACATTTACAGCAT	1	1	NC	NC
595	1136	TCTCATCAACATTTACAGCA	1	2	NC	NC
596	1137	ATCTCATCAACATTTACAGC	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
597	1138	TATCTCATCAACATTTACAG	1	1	NC	NC
598	1139	TTATCTCATCAACATTTACA	2	2	NC	NC
599	1140	ATTATCTCATCAACATTTAC	1	2	NC	NC
600	1141	CATTATCTCATCAACATTTA	2	1	NC	NC
601	1142	TCATTATCTCATCAACATTT	1	1	NC	NC
602	1143	GTCATTATCTCATCAACATT	1	2	NC	NC
603	1144	AGTCATTATCTCATCAACAT	2	2	NC	NC
604	1145	CAGTCATTATCTCATCAACA	2	2	NC	NC
605	1146	TCAGTCATTATCTCATCAAC	1	2	NC	NC
606	1147	ATCAGTCATTATCTCATCAA	1	2	NC	NC
607	1148	TATCAGTCATTATCTCATCA	1	2	NC	NC
608	1149	GTATCAGTCATTATCTCATC	1	2	NC	NC
609	1150	AGTATCAGTCATTATCTCAT	1	2	NC	NC
610	1151	AAGTATCAGTCATTATCTCA	1	2	NC	NC
611	1152	GAAGTATCAGTCATTATCTC	2	2	NC	NC
612	1153	AGAAGTATCAGTCATTATCT	1	2	NC	NC
613	1154	TAGAAGTATCAGTCATTATC	2	2	NC	NC
614	1155	GTAGAAGTATCAGTCATTAT	2	2	NC	NC
615	1156	GGTAGAAGTATCAGTCATTA	2	3	NC	NC
616	1157	TGGTAGAAGTATCAGTCATT	2	2	NC	NC
617	1158	CTGGTAGAAGTATCAGTCAT	1	1	NC	NC
618	1159	GCTGGTAGAAGTATCAGTCA	1	1	NC	NC
619	1160	AGCTGGTAGAAGTATCAGTC	2	2	NC	NC
620	1161	TAGCTGGTAGAAGTATCAGT	2	2	NC	NC
621	1163	GATAGCTGGTAGAAGTATCA	2	2	NC	NC
622	1164	AGATAGCTGGTAGAAGTATC	2	2	NC	NC
623	1165	AAGATAGCTGGTAGAAGTAT	2	2	NC	NC
624	1166	GAAGATAGCTGGTAGAAGTA	2	2	NC	NC
625	1167	AGAAGATAGCTGGTAGAAGT	2	2	NC	NC
626	1168	CAGAAGATAGCTGGTAGAAG	2	2	NC	NC
627	1169	ACAGAAGATAGCTGGTAGAA	2	2	NC	NC
628	1170	CACAGAAGATAGCTGGTAGA	2	2	NC	NC
629	1171	GCACAGAAGATAGCTGGTAG	2	3	NC	NC
630	1172	TGCACAGAAGATAGCTGGTA	2	3	NC	NC
631	1173	ATGCACAGAAGATAGCTGGT	2	3	NC	NC
632	1174	GATGCACAGAAGATAGCTGG	2	2	NC	NC
633	1176	GAGATGCACAGAAGATAGCT	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
634	1177	AGAGATGCACAGAAGATAGC	2	2	NC	NC
635	1178	CAGAGATGCACAGAAGATAG	2	1	NC	NC
636	1179	TCAGAGATGCACAGAAGATA	2	2	NC	NC
637	1180	TTCAGAGATGCACAGAAGAT	2	2	NC	NC
638	1181	TTTCAGAGATGCACAGAAGA	1	1	NC	NC
639	1182	TTTTCAGAGATGCACAGAAG	2	1	NC	NC
640	1183	ATTTTCAGAGATGCACAGAA	1	1	NC	NC
641	1184	TATTTTCAGAGATGCACAGA	1	1	NC	NC
642	1185	TTATTTTCAGAGATGCACAG	1	1	NC	NC
643	1186	CTTATTTTCAGAGATGCACA	2	2	NC	NC
644	1187	CCTTATTTTCAGAGATGCAC	2	2	NC	NC
645	1188	TCCTTATTTTCAGAGATGCA	2	1	NC	NC
646	1189	TTCCTTATTTTCAGAGATGC	1	1	NC	NC
647	1190	TTTCCTTATTTTCAGAGATG	1	1	NC	NC
648	1191	TTTTCCTTATTTTCAGAGAT	1	1	NC	NC
649	1192	ATTTTCCTTATTTTCAGAGA	1	2	NC	NC
650	1193	CATTTTCCTTATTTTCAGAG	1	1	NC	NC
651	1194	ACATTTTCCTTATTTTCAGA	1	2	NC	NC
652	1195	AACATTTTCCTTATTTTCAG	1	2	NC	NC
653	1197	CTAACATTTTCCTTATTTTC	1	1	NC	NC
654	1198	CCTAACATTTTCCTTATTTT	1	1	NC	NC
655	1199	CCCTAACATTTTCCTTATTT	1	1	NC	NC
656	1200	TCCCTAACATTTTCCTTATT	1	2	NC	NC
657	1201	GTCCCTAACATTTTCCTTAT	2	1	NC	NC
658	1202	TGTCCCTAACATTTTCCTTA	2	1	NC	NC
659	1203	TTGTCCCTAACATTTTCCTT	2	2	NC	NC
660	1204	TTTGTCCCTAACATTTTCCT	1	2	NC	NC
661	1205	TTTTGTCCCTAACATTTTCC	2	2	NC	NC
662	1206	TTTTTGTCCCTAACATTTTC	1	1	NC	NC
663	1224	ATAAAAATGTTGCCCTTTTT	1	NC	NC	NC
664	1225	AATAAAAATGTTGCCCTTTT	2	NC	NC	NC
665	1226	CAATAAAAATGTTGCCCTTT	2	NC	NC	NC
666	1227	CCAATAAAAATGTTGCCCTT	2	NC	NC	NC
667	1228	GCCAATAAAAATGTTGCCCT	2	NC	NC	NC
668	1229	TGCCAATAAAAATGTTGCCC	1	NC	NC	NC
669	1230	ATGCCAATAAAAATGTTGCC	2	NC	NC	NC
670	1231	AATGCCAATAAAAATGTTGC	1	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
671	1232	CAATGCCAATAAAAATGTTG	1	NC	NC	NC
672	1233	ACAATGCCAATAAAAATGTT	2	NC	NC	NC
673	1234	CACAATGCCAATAAAAATGT	1	NC	NC	NC
674	1235	CCACAATGCCAATAAAAATG	2	NC	NC	NC
675	1236	CCCACAATGCCAATAAAAAT	2	NC	NC	NC
676	1237	TCCCACAATGCCAATAAAAA	2	1	NC	NC
677	1238	CTCCCACAATGCCAATAAAA	2	2	NC	NC
678	1239	ACTCCCACAATGCCAATAAA	2	1	NC	NC
679	1240	CACTCCCACAATGCCAATAA	2	1	NC	NC
680	1241	GCACTCCCACAATGCCAATA	2	2	NC	NC
681	1269	TCAAACACAACCTCGCCTGT	2	3	NC	NC
682	1270	ATCAAACACAACCTCGCCTG	2	2	NC	NC
683	1271	TATCAAACACAACCTCGCCT	2	2	NC	NC
684	1272	CTATCAAACACAACCTCGCC	2	2	NC	NC
685	1273	ACTATCAAACACAACCTCGC	3	3	NC	NC
686	1274	AACTATCAAACACAACCTCG	2	2	NC	NC
687	1275	AAACTATCAAACACAACCTC	1	1	NC	NC
688	1276	GAAACTATCAAACACAACCT	2	2	NC	NC
689	1277	GGAAACTATCAAACACAACC	2	2	NC	NC
690	1278	TGGAAACTATCAAACACAAC	2	2	NC	NC
691	1279	CTGGAAACTATCAAACACAA	2	2	NC	NC
692	1280	CCTGGAAACTATCAAACACA	2	1	NC	NC
693	1281	TCCTGGAAACTATCAAACAC	1	1	NC	NC
694	1282	GTCCTGGAAACTATCAAACA	1	1	NC	NC
695	1283	AGTCCTGGAAACTATCAAAC	2	2	NC	NC
696	1284	GAGTCCTGGAAACTATCAAA	1	1	NC	NC
697	1285	AGAGTCCTGGAAACTATCAA	2	2	NC	NC
698	1286	CAGAGTCCTGGAAACTATCA	2	2	NC	NC
699	1297	TGAACGAGAAGCAGAGTCCT	2	2	NC	NC
700	1298	CTGAACGAGAAGCAGAGTCC	2	2	NC	NC
701	1299	TCTGAACGAGAAGCAGAGTC	2	2	NC	NC
702	1310	GGGTTTCTAGCTCTGAACGA	3	3	NC	NC
703	1311	CGGGTTTCTAGCTCTGAACG	3	3	NC	NC
704	1312	CCGGGTTTCTAGCTCTGAAC	3	2	NC	NC
705	1313	TCCGGGTTTCTAGCTCTGAA	2	2	NC	NC
706	1314	ATCCGGGTTTCTAGCTCTGA	2	2	NC	NC
707	1315	CATCCGGGTTTCTAGCTCTG	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
708	1316	ACATCCGGGTTTCTAGCTCT	3	2	NC	NC
709	1317	GACATCCGGGTTTCTAGCTC	2	3	NC	NC
710	1318	TGACATCCGGGTTTCTAGCT	2	2	NC	NC
711	1319	TTGACATCCGGGTTTCTAGC	3	3	NC	NC
712	1320	CTTGACATCCGGGTTTCTAG	2	NC	NC	NC
713	1321	GCTTGACATCCGGGTTTCTA	3	NC	NC	NC
714	1322	GGCTTGACATCCGGGTTTCT	3	NC	NC	NC
715	1323	AGGCTTGACATCCGGGTTTC	2	NC	NC	NC
716	1324	CAGGCTTGACATCCGGGTTT	2	NC	NC	NC
717	1371	TCTGTTTGCTCGGACAAGGC	2	2	NC	NC
718	1372	CTCTGTTTGCTCGGACAAGG	2	2	NC	NC
719	1373	CCTCTGTTTGCTCGGACAAG	2	NC	NC	NC
720	1374	GCCTCTGTTTGCTCGGACAA	2	NC	NC	NC
721	1383	TGGATGAGCGCCTCTGTTTG	2	NC	NC	NC
722	1384	GTGGATGAGCGCCTCTGTTT	3	NC	NC	NC
723	1385	TGTGGATGAGCGCCTCTGTT	2	NC	NC	NC
724	1395	GATGTGGCTCTGTGGATGAG	2	2	NC	NC
725	1396	AGATGTGGCTCTGTGGATGA	2	2	NC	NC
726	1397	CAGATGTGGCTCTGTGGATG	2	2	NC	NC
727	1410	TCCTGCACACTAACAGATGT	2	2	NC	NC
728	1411	ATCCTGCACACTAACAGATG	2	2	NC	NC
729	1412	CATCCTGCACACTAACAGAT	2	2	NC	NC
730	1413	TCATCCTGCACACTAACAGA	2	2	NC	NC
731	1414	GTCATCCTGCACACTAACAG	2	2	NC	NC
732	1415	TGTCATCCTGCACACTAACA	2	2	NC	NC
733	1416	CTGTCATCCTGCACACTAAC	2	2	NC	NC
734	1417	TCTGTCATCCTGCACACTAA	2	2	NC	NC
735	1418	TTCTGTCATCCTGCACACTA	2	2	NC	NC
736	1419	ATTCTGTCATCCTGCACACT	2	2	NC	NC
737	1420	AATTCTGTCATCCTGCACAC	2	2	NC	NC
738	1421	GAATTCTGTCATCCTGCACA	2	2	NC	NC
739	1422	CGAATTCTGTCATCCTGCAC	2	2	NC	NC
740	1423	TCGAATTCTGTCATCCTGCA	2	2	NC	NC
741	1424	CTCGAATTCTGTCATCCTGC	2	2	NC	NC
742	1425	ACTCGAATTCTGTCATCCTG	2	2	NC	NC
743	1426	GACTCGAATTCTGTCATCCT	2	NC	NC	NC
744	1427	CGACTCGAATTCTGTCATCC	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
745	1428	TCGACTCGAATTCTGTCATC	3	NC	NC	NC
746	1439	TATCCATCCTTTCGACTCGA	3	NC	NC	NC
747	1440	TTATCCATCCTTTCGACTCG	3	NC	NC	NC
748	1441	GTTATCCATCCTTTCGACTC	2	NC	NC	NC
749	1442	TGTTATCCATCCTTTCGACT	2	NC	NC	NC
750	1443	ATGTTATCCATCCTTTCGAC	3	NC	NC	NC
751	1444	AATGTTATCCATCCTTTCGA	2	NC	NC	NC
752	1445	AAATGTTATCCATCCTTTCG	2	NC	NC	NC
753	1446	TAAATGTTATCCATCCTTTC	1	1	NC	NC
754	1447	ATAAATGTTATCCATCCTTT	1	2	NC	NC
755	1448	AATAAATGTTATCCATCCTT	1	2	NC	NC
756	1449	AAATAAATGTTATCCATCCT	2	1	NC	NC
757	1450	AAAATAAATGTTATCCATCC	1	2	NC	NC
758	1451	CAAAATAAATGTTATCCATC	1	2	NC	NC
759	1459	GCTGTATTCAAAATAAATGT	1	1	NC	NC
760	1460	GGCTGTATTCAAAATAAATG	2	1	NC	NC
761	1461	TGGCTGTATTCAAAATAAAT	2	2	NC	NC
762	1462	ATGGCTGTATTCAAAATAAA	2	2	NC	NC
763	1463	CATGGCTGTATTCAAAATAA	2	1	NC	NC
764	1464	GCATGGCTGTATTCAAAATA	1	1	NC	NC
765	1465	AGCATGGCTGTATTCAAAAT	2	2	NC	NC
766	1466	AAGCATGGCTGTATTCAAAA	1	1	NC	NC
767	1467	AAAGCATGGCTGTATTCAAA	1	2	2	2
768	1468	GAAAGCATGGCTGTATTCAA	2	2	2	2
769	1469	GGAAAGCATGGCTGTATTCA	2	2	2	2
770	1470	TGGAAAGCATGGCTGTATTC	2	2	2	2
771	1471	CTGGAAAGCATGGCTGTATT	1	2	2	2
772	1472	CCTGGAAAGCATGGCTGTAT	2	2	NC	NC
773	1473	GCCTGGAAAGCATGGCTGTA	2	2	NC	NC
774	1482	TCTGTAACTGCCTGGAAAGC	2	2	NC	NC
775	1483	CTCTGTAACTGCCTGGAAAG	2	2	NC	NC
776	1484	ACTCTGTAACTGCCTGGAAA	2	2	NC	NC
777	1485	AACTCTGTAACTGCCTGGAA	1	1	NC	NC
778	1486	AAACTCTGTAACTGCCTGGA	2	1	NC	NC
779	1487	AAAACTCTGTAACTGCCTGG	2	2	NC	NC
780	1488	TAAAACTCTGTAACTGCCTG	2	2	NC	NC
781	1489	ATAAAACTCTGTAACTGCCT	1	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
782	1490	CATAAAACTCTGTAACTGCC	1	2	NC	NC
783	1491	GCATAAAACTCTGTAACTGC	1	2	NC	NC
784	1492	TGCATAAAACTCTGTAACTG	1	1	NC	NC
785	1493	TTGCATAAAACTCTGTAACT	0	1	NC	NC
786	1494	TTTGCATAAAACTCTGTAAC	1	1	NC	NC
787	1495	TTTTGCATAAAACTCTGTAA	2	1	NC	NC
788	1496	CTTTTGCATAAAACTCTGTA	2	2	NC	NC
789	1497	TCTTTTGCATAAAACTCTGT	2	2	NC	NC
790	1498	ATCTTTTGCATAAAACTCTG	2	NC	NC	NC
791	1499	TATCTTTTGCATAAAACTCT	2	NC	NC	NC
792	1500	GTATCTTTTGCATAAAACTC	2	NC	NC	NC
793	1501	TGTATCTTTTGCATAAAACT	1	NC	NC	NC
794	1502	CTGTATCTTTTGCATAAAAC	2	NC	NC	NC
795	1503	ACTGTATCTTTTGCATAAAA	2	NC	NC	NC
796	1504	AACTGTATCTTTTGCATAAA	2	NC	NC	NC
797	1505	CAACTGTATCTTTTGCATAA	2	NC	NC	NC
798	1506	TCAACTGTATCTTTTGCATA	2	NC	NC	NC
799	1507	GTCAACTGTATCTTTTGCAT	2	NC	NC	NC
800	1508	TGTCAACTGTATCTTTTGCA	2	NC	NC	NC
801	1509	ATGTCAACTGTATCTTTTGC	2	NC	NC	NC
802	1510	GATGTCAACTGTATCTTTTG	2	NC	NC	NC
803	1511	TGATGTCAACTGTATCTTTT	1	NC	NC	NC
804	1512	TTGATGTCAACTGTATCTTT	2	NC	NC	NC
805	1513	TTTGATGTCAACTGTATCTT	2	NC	NC	NC
806	1514	CTTTGATGTCAACTGTATCT	2	NC	NC	NC
807	1515	CCTTTGATGTCAACTGTATC	2	NC	NC	NC
808	1516	ACCTTTGATGTCAACTGTAT	2	NC	NC	NC
809	1517	AACCTTTGATGTCAACTGTA	2	NC	NC	NC
810	1518	GAACCTTTGATGTCAACTGT	1	2	NC	NC
811	1519	AGAACCTTTGATGTCAACTG	1	2	NC	NC
812	1520	GAGAACCTTTGATGTCAACT	2	2	NC	NC
813	1521	TGAGAACCTTTGATGTCAAC	2	3	NC	NC
814	1522	TTGAGAACCTTTGATGTCAA	2	2	NC	NC
815	1523	TTTGAGAACCTTTGATGTCA	2	2	NC	NC
816	1524	ATTTGAGAACCTTTGATGTC	2	2	NC	NC
817	1525	AATTTGAGAACCTTTGATGT	2	2	NC	NC
818	1526	TAATTTGAGAACCTTTGATG	1	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
819	1527	ATAATTTGAGAACCTTTGAT	1	2	NC	NC
820	1528	AATAATTTGAGAACCTTTGA	2	1	NC	NC
821	1529	AAATAATTTGAGAACCTTTG	1	1	NC	NC
822	1530	GAAATAATTTGAGAACCTTT	1	2	NC	NC
823	1531	AGAAATAATTTGAGAACCTT	2	2	NC	NC
824	1532	CAGAAATAATTTGAGAACCT	2	2	NC	NC
825	1533	CCAGAAATAATTTGAGAACC	2	2	NC	NC
826	1534	GCCAGAAATAATTTGAGAAC	1	2	NC	NC
827	1535	TGCCAGAAATAATTTGAGAA	1	2	NC	NC
828	1536	ATGCCAGAAATAATTTGAGA	2	2	NC	NC
829	1537	AATGCCAGAAATAATTTGAG	2	2	NC	NC
830	1538	CAATGCCAGAAATAATTTGA	2	2	NC	NC
831	1539	ACAATGCCAGAAATAATTTG	2	2	NC	NC
832	1540	AACAATGCCAGAAATAATTT	2	1	NC	NC
833	1541	TAACAATGCCAGAAATAATT	2	1	NC	NC
834	1542	TTAACAATGCCAGAAATAAT	1	0	NC	NC
835	1543	GTTAACAATGCCAGAAATAA	1	1	NC	NC
836	1544	AGTTAACAATGCCAGAAATA	1	1	NC	NC
837	1545	AAGTTAACAATGCCAGAAAT	2	2	NC	NC
838	1546	TAAGTTAACAATGCCAGAAA	2	2	NC	NC
839	1547	CTAAGTTAACAATGCCAGAA	2	2	NC	NC
840	1548	TCTAAGTTAACAATGCCAGA	2	2	NC	NC
841	1549	CTCTAAGTTAACAATGCCAG	2	2	NC	NC
842	1550	TCTCTAAGTTAACAATGCCA	2	2	NC	NC
843	1551	TTCTCTAAGTTAACAATGCC	1	1	NC	NC
844	1552	CTTCTCTAAGTTAACAATGC	2	1	NC	NC
845	1553	GCTTCTCTAAGTTAACAATG	2	2	NC	NC
846	1554	GGCTTCTCTAAGTTAACAAT	2	2	NC	NC
847	1555	AGGCTTCTCTAAGTTAACAA	2	2	NC	NC
848	1556	CAGGCTTCTCTAAGTTAACA	2	2	NC	NC
849	1557	ACAGGCTTCTCTAAGTTAAC	2	2	NC	NC
850	1558	CACAGGCTTCTCTAAGTTAA	2	2	NC	NC
851	1559	TCACAGGCTTCTCTAAGTTA	2	2	NC	NC
852	1560	ATCACAGGCTTCTCTAAGTT	2	2	NC	NC
853	1561	AATCACAGGCTTCTCTAAGT	2	2	NC	NC
854	1562	AAATCACAGGCTTCTCTAAG	2	2	NC	NC
855	1563	CAAATCACAGGCTTCTCTAA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
856	1564	GCAAATCACAGGCTTCTCTA	2	2	NC	NC
857	1565	AGCAAATCACAGGCTTCTCT	1	1	NC	NC
858	1566	GAGCAAATCACAGGCTTCTC	1	1	NC	NC
859	1567	AGAGCAAATCACAGGCTTCT	2	1	NC	NC
860	1568	AAGAGCAAATCACAGGCTTC	2	2	NC	NC
861	1569	AAAGAGCAAATCACAGGCTT	2	2	NC	NC
862	1571	CCAAAGAGCAAATCACAGGC	1	2	NC	NC
863	1572	GCCAAAGAGCAAATCACAGG	2	1	NC	NC
864	1573	AGCCAAAGAGCAAATCACAG	1	1	NC	NC
865	1574	CAGCCAAAGAGCAAATCACA	1	1	NC	NC
866	1575	GCAGCCAAAGAGCAAATCAC	2	1	NC	NC
867	1576	GGCAGCCAAAGAGCAAATCA	2	1	NC	NC
868	1577	TGGCAGCCAAAGAGCAAATC	2	2	NC	NC
869	1578	ATGGCAGCCAAAGAGCAAAT	1	2	NC	NC
870	1579	GATGGCAGCCAAAGAGCAAA	1	1	NC	NC
871	1580	TGATGGCAGCCAAAGAGCAA	1	1	NC	NC
872	1581	ATGATGGCAGCCAAAGAGCA	2	2	NC	NC
873	1582	TATGATGGCAGCCAAAGAGC	2	2	NC	NC
874	1583	TTATGATGGCAGCCAAAGAG	1	2	NC	NC
875	1584	TTTATGATGGCAGCCAAAGA	1	1	NC	NC
876	1585	TTTTATGATGGCAGCCAAAG	1	1	NC	NC
877	1586	ATTTTATGATGGCAGCCAAA	2	1	NC	NC
878	1587	TATTTTATGATGGCAGCCAA	1	1	NC	NC
879	1589	GGTATTTTATGATGGCAGCC	1	1	NC	NC
880	1590	AGGTATTTTATGATGGCAGC	2	1	NC	NC
881	1591	GAGGTATTTTATGATGGCAG	2	1	NC	NC
882	1592	TGAGGTATTTTATGATGGCA	1	1	NC	NC
883	1593	TTGAGGTATTTTATGATGGC	2	2	NC	NC
884	1594	TTTGAGGTATTTTATGATGG	2	2	NC	NC
885	1595	CTTTGAGGTATTTTATGATG	2	2	NC	NC
886	1596	TCTTTGAGGTATTTTATGAT	1	1	NC	NC
887	1597	TTCTTTGAGGTATTTTATGA	1	1	NC	NC
888	1598	ATTCTTTGAGGTATTTTATG	1	1	NC	NC
889	1600	GAATTCTTTGAGGTATTTTA	2	2	NC	NC
890	1601	TGAATTCTTTGAGGTATTTT	1	1	NC	NC
891	1602	TTGAATTCTTTGAGGTATTT	2	1	NC	NC
892	1603	GTTGAATTCTTTGAGGTATT	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
893	1604	AGTTGAATTCTTTGAGGTAT	2	2	NC	NC
894	1605	AAGTTGAATTCTTTGAGGTA	2	2	NC	NC
895	1606	CAAGTTGAATTCTTTGAGGT	2	2	NC	NC
896	1607	CCAAGTTGAATTCTTTGAGG	2	2	NC	NC
897	1608	TCCAAGTTGAATTCTTTGAG	2	2	NC	NC
898	1609	TTCCAAGTTGAATTCTTTGA	1	2	NC	NC
899	1610	TTTCCAAGTTGAATTCTTTG	2	1	NC	NC
900	1611	TTTTCCAAGTTGAATTCTTT	2	2	NC	NC
901	1612	CTTTTCCAAGTTGAATTCTT	2	NC	NC	NC
902	1613	TCTTTTCCAAGTTGAATTCT	1	NC	NC	NC
903	1614	ATCTTTTCCAAGTTGAATTC	2	NC	NC	NC
904	1615	CATCTTTTCCAAGTTGAATT	2	NC	NC	NC
905	1616	GCATCTTTTCCAAGTTGAAT	2	NC	NC	NC
906	1617	AGCATCTTTTCCAAGTTGAA	2	NC	NC	NC
907	1618	GAGCATCTTTTCCAAGTTGA	2	NC	NC	NC
908	1631	TCTCAGGTTTGGAGAGCATC	3	NC	NC	NC
909	1637	TAAAATTCTCAGGTTTGGAG	1	2	NC	NC
910	1638	TTAAAATTCTCAGGTTTGGA	2	2	NC	NC
911	1639	TTTAAAATTCTCAGGTTTGG	2	1	NC	NC
912	1640	GTTTAAAATTCTCAGGTTTG	1	1	NC	NC
913	1641	TGTTTAAAATTCTCAGGTTT	2	1	NC	NC
914	1642	CTGTTTAAAATTCTCAGGTT	2	2	NC	NC
915	1643	GCTGTTTAAAATTCTCAGGT	2	2	NC	NC
916	1644	AGCTGTTTAAAATTCTCAGG	2	1	NC	NC
917	1645	TAGCTGTTTAAAATTCTCAG	1	2	NC	NC
918	1646	ATAGCTGTTTAAAATTCTCA	2	1	NC	NC
919	1647	GATAGCTGTTTAAAATTCTC	2	2	NC	NC
920	1648	TGATAGCTGTTTAAAATTCT	1	1	NC	NC
921	1649	TTGATAGCTGTTTAAAATTC	2	1	NC	NC
922	1650	CTTGATAGCTGTTTAAAATT	2	1	NC	NC
923	1651	ACTTGATAGCTGTTTAAAAT	1	2	NC	NC
924	1652	TACTTGATAGCTGTTTAAAA	1	2	NC	NC
925	1653	TTACTTGATAGCTGTTTAAA	2	2	NC	NC
926	1654	TTTACTTGATAGCTGTTTAA	2	2	NC	NC
927	1655	TTTTACTTGATAGCTGTTTA	1	1	NC	NC
928	1656	ATTTTACTTGATAGCTGTTT	2	2	NC	NC
929	1657	CATTTTACTTGATAGCTGTT	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
930	1658	CCATTTTACTTGATAGCTGT	2	2	NC	NC
931	1659	TCCATTTTACTTGATAGCTG	2	2	NC	NC
932	1660	TTCCATTTTACTTGATAGCT	2	2	NC	NC
933	1661	ATTCCATTTTACTTGATAGC	2	2	NC	NC
934	1662	AATTCCATTTTACTTGATAG	2	1	NC	NC
935	1666	CATAAATTCCATTTTACTTG	2	1	NC	NC
936	1668	GTCATAAATTCCATTTTACT	2	2	NC	NC
937	1669	TGTCATAAATTCCATTTTAC	1	1	NC	NC
938	1676	CATTAATTGTCATAAATTCC	2	1	NC	NC
939	1677	CCATTAATTGTCATAAATTC	2	1	NC	NC
940	1680	GTTCCATTAATTGTCATAAA	2	2	NC	NC
941	1681	TGTTCCATTAATTGTCATAA	2	2	NC	NC
942	1682	TTGTTCCATTAATTGTCATA	1	1	NC	NC
943	1683	GTTGTTCCATTAATTGTCAT	1	1	NC	NC
944	1684	TGTTGTTCCATTAATTGTCA	1	1	NC	NC
945	1685	ATGTTGTTCCATTAATTGTC	2	2	NC	NC
946	1686	AATGTTGTTCCATTAATTGT	1	1	NC	NC
947	1687	TAATGTTGTTCCATTAATTG	2	1	NC	NC
948	1691	TCCTTAATGTTGTTCCATTA	2	2	NC	NC
949	1693	ATTCCTTAATGTTGTTCCAT	2	2	NC	NC
950	1694	GATTCCTTAATGTTGTTCCA	2	2	NC	NC
951	1695	AGATTCCTTAATGTTGTTCC	2	2	NC	NC
952	1696	CAGATTCCTTAATGTTGTTC	2	2	NC	NC
953	1697	CCAGATTCCTTAATGTTGTT	1	2	NC	NC
954	1698	TCCAGATTCCTTAATGTTGT	1	2	NC	NC
955	1699	TTCCAGATTCCTTAATGTTG	2	2	NC	NC
956	1700	TTTCCAGATTCCTTAATGTT	2	1	NC	NC
957	1701	ATTTCCAGATTCCTTAATGT	2	1	NC	NC
958	1702	GATTTCCAGATTCCTTAATG	2	2	NC	NC
959	1703	GGATTTCCAGATTCCTTAAT	2	2	NC	NC
960	1704	AGGATTTCCAGATTCCTTAA	2	2	NC	NC
961	1705	TAGGATTTCCAGATTCCTTA	2	2	NC	NC
962	1706	GTAGGATTTCCAGATTCCTT	1	2	NC	NC
963	1715	TCTGATTCTGTAGGATTTCC	1	2	NC	NC
964	1716	GTCTGATTCTGTAGGATTTC	2	2	NC	NC
965	1717	AGTCTGATTCTGTAGGATTT	2	2	NC	NC
966	1718	CAGTCTGATTCTGTAGGATT	2	3	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
967	1719	TCAGTCTGATTCTGTAGGAT	2	2	NC	NC
968	1720	ATCAGTCTGATTCTGTAGGA	2	3	NC	NC
969	1721	TATCAGTCTGATTCTGTAGG	3	3	NC	NC
970	1722	ATATCAGTCTGATTCTGTAG	2	2	NC	NC
971	1723	CATATCAGTCTGATTCTGTA	2	2	NC	NC
972	1724	TCATATCAGTCTGATTCTGT	2	2	NC	NC
973	1725	TTCATATCAGTCTGATTCTG	1	2	2	2
974	1726	TTTCATATCAGTCTGATTCT	2	2	NC	NC
975	1727	TTTTCATATCAGTCTGATTC	2	2	NC	NC
976	1728	GTTTTCATATCAGTCTGATT	2	2	NC	NC
977	1730	TGGTTTTCATATCAGTCTGA	2	2	NC	NC
978	1731	TTGGTTTTCATATCAGTCTG	2	1	NC	NC
979	1732	TTTGGTTTTCATATCAGTCT	1	2	NC	NC
980	1733	CTTTGGTTTTCATATCAGTC	1	2	NC	NC
981	1734	CCTTTGGTTTTCATATCAGT	2	2	NC	NC
982	1735	TCCTTTGGTTTTCATATCAG	2	2	NC	NC
983	1736	TTCCTTTGGTTTTCATATCA	2	2	NC	NC
984	1737	CTTCCTTTGGTTTTCATATC	2	2	NC	NC
985	1738	ACTTCCTTTGGTTTTCATAT	1	1	NC	NC
986	1739	AACTTCCTTTGGTTTTCATA	2	2	NC	NC
987	1740	AAACTTCCTTTGGTTTTCAT	2	2	NC	NC
988	1741	CAAACTTCCTTTGGTTTTCA	2	2	NC	NC
989	1749	ACCCACAGCAAACTTCCTTT	2	2	NC	NC
990	1750	AACCCACAGCAAACTTCCTT	2	2	NC	NC
991	1751	AAACCCACAGCAAACTTCCT	1	2	NC	NC
992	1752	AAAACCCACAGCAAACTTCC	2	2	NC	NC
993	1753	TAAAACCCACAGCAAACTTC	2	2	NC	NC
994	1754	CTAAAACCCACAGCAAACTT	2	2	NC	NC
995	1755	TCTAAAACCCACAGCAAACT	2	1	NC	NC
996	1756	GTCTAAAACCCACAGCAAAC	2	2	NC	NC
997	1769	AAGTTTTAGTGTGGTCTAAA	2	2	2	NC
998	1770	GAAGTTTTAGTGTGGTCTAA	2	2	2	NC
999	1771	TGAAGTTTTAGTGTGGTCTA	2	2	2	NC
1000	1772	ATGAAGTTTTAGTGTGGTCT	2	2	2	NC
1001	1773	AATGAAGTTTTAGTGTGGTC	2	2	2	NC
1002	1774	AAATGAAGTTTTAGTGTGGT	2	2	2	NC
1003	1775	CAAATGAAGTTTTAGTGTGG	2	1	2	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1004	1776	CCAAATGAAGTTTTAGTGTG	2	2	2	NC
1005	1777	CCCAAATGAAGTTTTAGTGT	2	2	2	NC
1006	1778	TCCCAAATGAAGTTTTAGTG	2	2	2	NC
1007	1779	CTCCCAAATGAAGTTTTAGT	2	2	1	NC
1008	1780	TCTCCCAAATGAAGTTTTAG	2	2	2	NC
1009	1781	GTCTCCCAAATGAAGTTTTA	1	1	NC	NC
1010	1782	CGTCTCCCAAATGAAGTTTT	2	2	NC	NC
1011	1783	CCGTCTCCCAAATGAAGTTT	2	2	NC	NC
1012	1784	TCCGTCTCCCAAATGAAGTT	2	2	NC	NC
1013	1785	TTCCGTCTCCCAAATGAAGT	2	2	NC	NC
1014	1786	CTTCCGTCTCCCAAATGAAG	2	2	NC	NC
1015	1787	ACTTCCGTCTCCCAAATGAA	2	2	NC	NC
1016	1788	AACTTCCGTCTCCCAAATGA	2	2	NC	NC
1017	1789	TAACTTCCGTCTCCCAAATG	2	2	NC	NC
1018	1790	TTAACTTCCGTCTCCCAAAT	2	1	NC	NC
1019	1791	TTTAACTTCCGTCTCCCAAA	3	2	NC	NC
1020	1792	CTTTAACTTCCGTCTCCCAA	2	1	NC	NC
1021	1793	TCTTTAACTTCCGTCTCCCA	3	2	NC	NC
1022	1794	TTCTTTAACTTCCGTCTCCC	2	2	NC	NC
1023	1795	CTTCTTTAACTTCCGTCTCC	1	1	NC	NC
1024	1796	ACTTCTTTAACTTCCGTCTC	1	1	NC	NC
1025	1797	CACTTCTTTAACTTCCGTCT	2	2	NC	NC
1026	1798	CCACTTCTTTAACTTCCGTC	2	2	NC	NC
1027	1799	CCCACTTCTTTAACTTCCGT	2	2	NC	NC
1028	1800	ACCCACTTCTTTAACTTCCG	3	2	NC	NC
1029	1801	CACCCACTTCTTTAACTTCC	2	2	NC	NC
1030	1802	TCACCCACTTCTTTAACTTC	2	2	NC	NC
1031	1803	GTCACCCACTTCTTTAACTT	2	2	NC	NC
1032	1804	GGTCACCCACTTCTTTAACT	2	2	NC	NC
1033	1818	TTAAGGAGTGGCTGGGTCAC	2	1	NC	NC
1034	1819	TTTAAGGAGTGGCTGGGTCA	2	2	NC	NC
1035	1820	ATTTAAGGAGTGGCTGGGTC	2	2	NC	NC
1036	1821	AATTTAAGGAGTGGCTGGGT	2	2	NC	NC
1037	1822	TAATTTAAGGAGTGGCTGGG	2	1	NC	NC
1038	1823	TTAATTTAAGGAGTGGCTGG	2	2	NC	NC
1039	1824	CTTAATTTAAGGAGTGGCTG	1	2	NC	NC
1040	1836	GCATTTATTTCCCTTAATTT	2	2	2	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1041	1837	GGCATTTATTTCCCTTAATT	2	2	1	NC
1042	1838	GGGCATTTATTTCCCTTAAT	2	1	1	NC
1043	1839	CGGGCATTTATTTCCCTTAA	2	2	2	NC
1044	1840	CCGGGCATTTATTTCCCTTA	2	2	NC	NC
1045	1841	GCCGGGCATTTATTTCCCTT	2	2	NC	NC
1046	1842	AGCCGGGCATTTATTTCCCT	2	1	NC	NC
1047	1844	CAAGCCGGGCATTTATTTCC	2	2	NC	NC
1048	1845	TCAAGCCGGGCATTTATTTC	2	3	NC	NC
1049	1846	ATCAAGCCGGGCATTTATTT	2	3	NC	NC
1050	1847	CATCAAGCCGGGCATTTATT	3	3	NC	NC
1051	1848	GCATCAAGCCGGGCATTTAT	3	3	NC	NC
1052	1860	ACTTCCGATACAGCATCAAG	2	NC	NC	NC
1053	1861	AACTTCCGATACAGCATCAA	2	NC	NC	NC
1054	1862	GAACTTCCGATACAGCATCA	2	NC	NC	NC
1055	1863	AGAACTTCCGATACAGCATC	2	NC	NC	NC
1056	1864	GAGAACTTCCGATACAGCAT	3	NC	NC	NC
1057	1865	GGAGAACTTCCGATACAGCA	3	NC	NC	NC
1058	1875	GATTCTGAATGGAGAACTTC	1	2	NC	NC
1059	1876	AGATTCTGAATGGAGAACTT	1	2	NC	NC
1060	1877	TAGATTCTGAATGGAGAACT	2	2	NC	NC
1061	1878	CTAGATTCTGAATGGAGAAC	2	2	NC	NC
1062	1879	ACTAGATTCTGAATGGAGAA	2	2	NC	NC
1063	1880	CACTAGATTCTGAATGGAGA	2	3	NC	NC
1064	1881	ACACTAGATTCTGAATGGAG	2	2	NC	NC
1065	1882	CACACTAGATTCTGAATGGA	2	2	NC	NC
1066	1883	ACACACTAGATTCTGAATGG	2	2	NC	NC
1067	1884	AACACACTAGATTCTGAATG	2	2	NC	NC
1068	1885	AAACACACTAGATTCTGAAT	2	2	NC	NC
1069	1886	CAAACACACTAGATTCTGAA	1	1	NC	NC
1070	1887	CCAAACACACTAGATTCTGA	1	1	NC	NC
1071	1888	ACCAAACACACTAGATTCTG	2	2	NC	NC
1072	1889	GACCAAACACACTAGATTCT	2	2	NC	NC
1073	1890	TGACCAAACACACTAGATTC	2	2	NC	NC
1074	1891	CTGACCAAACACACTAGATT	2	2	NC	NC
1075	1892	TCTGACCAAACACACTAGAT	2	2	NC	NC
1076	1893	ATCTGACCAAACACACTAGA	2	1	NC	NC
1077	1894	TATCTGACCAAACACACTAG	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1078	1895	CTATCTGACCAAACACACTA	2	2	NC	NC
1079	1896	TCTATCTGACCAAACACACT	2	2	NC	NC
1080	1897	TTCTATCTGACCAAACACAC	2	2	NC	NC
1081	1898	TTTCTATCTGACCAAACACA	2	2	NC	NC
1082	1899	TTTTCTATCTGACCAAACAC	2	2	NC	NC
1083	1900	ATTTTCTATCTGACCAAACA	1	1	NC	NC
1084	1901	GATTTTCTATCTGACCAAAC	2	2	NC	NC
1085	1902	TGATTTTCTATCTGACCAAA	1	1	NC	NC
1086	1903	ATGATTTTCTATCTGACCAA	1	2	NC	NC
1087	1904	GATGATTTTCTATCTGACCA	1	2	NC	NC
1088	1905	AGATGATTTTCTATCTGACC	2	2	NC	NC
1089	1906	TAGATGATTTTCTATCTGAC	2	2	NC	NC
1090	1907	GTAGATGATTTTCTATCTGA	2	2	NC	NC
1091	1908	CGTAGATGATTTTCTATCTG	2	2	NC	NC
1092	1909	ACGTAGATGATTTTCTATCT	2	2	NC	NC
1093	1910	TACGTAGATGATTTTCTATC	2	2	NC	NC
1094	1911	TTACGTAGATGATTTTCTAT	2	2	NC	NC
1095	1912	TTTACGTAGATGATTTTCTA	2	2	NC	NC
1096	1913	ATTTACGTAGATGATTTTCT	2	2	NC	NC
1097	1914	AATTTACGTAGATGATTTTC	2	2	NC	NC
1098	1915	CAATTTACGTAGATGATTTT	2	2	NC	NC
1099	1916	GCAATTTACGTAGATGATTT	2	3	NC	NC
1100	1917	GGCAATTTACGTAGATGATT	2	3	NC	NC
1101	1918	GGGCAATTTACGTAGATGAT	3	NC	NC	NC
1102	1919	CGGGCAATTTACGTAGATGA	3	NC	NC	NC
1103	1920	TCGGGCAATTTACGTAGATG	2	NC	NC	NC
1104	1921	GTCGGGCAATTTACGTAGAT	2	NC	NC	NC
1105	1922	TGTCGGGCAATTTACGTAGA	2	NC	NC	NC
1106	1923	ATGTCGGGCAATTTACGTAG	2	NC	NC	NC
1107	1924	TATGTCGGGCAATTTACGTA	2	NC	NC	NC
1108	1925	CTATGTCGGGCAATTTACGT	2	NC	NC	NC
1109	1926	TCTATGTCGGGCAATTTACG	2	NC	NC	NC
1110	1927	CTCTATGTCGGGCAATTTAC	2	NC	NC	NC
1111	1928	TCTCTATGTCGGGCAATTTA	3	NC	NC	NC
1112	1929	CTCTCTATGTCGGGCAATTT	3	NC	NC	NC
1113	1930	CCTCTCTATGTCGGGCAATT	2	NC	NC	NC
1114	1931	CCCTCTCTATGTCGGGCAAT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1115	1947	TAAATGCTACAGAGTCCCCT	1	NC	NC	NC
1116	1948	ATAAATGCTACAGAGTCCCC	2	NC	NC	NC
1117	1949	GATAAATGCTACAGAGTCCC	2	NC	NC	NC
1118	1950	TGATAAATGCTACAGAGTCC	2	2	NC	NC
1119	1951	GTGATAAATGCTACAGAGTC	2	2	NC	NC
1120	1952	TGTGATAAATGCTACAGAGT	2	2	NC	NC
1121	1953	TTGTGATAAATGCTACAGAG	1	1	NC	NC
1122	1954	TTTGTGATAAATGCTACAGA	1	1	NC	NC
1123	1955	TTTTGTGATAAATGCTACAG	2	1	NC	NC
1124	1956	TTTTTGTGATAAATGCTACA	2	1	NC	NC
1125	1972	CTCTTGGGTAGAACATTTTT	1	2	NC	NC
1126	1973	ACTCTTGGGTAGAACATTTT	2	2	NC	NC
1127	1974	AACTCTTGGGTAGAACATTT	2	2	NC	NC
1128	1975	GAACTCTTGGGTAGAACATT	2	3	NC	NC
1129	1976	AGAACTCTTGGGTAGAACAT	2	3	NC	NC
1130	1977	AAGAACTCTTGGGTAGAACA	2	2	NC	NC
1131	1978	GAAGAACTCTTGGGTAGAAC	2	2	NC	NC
1132	1979	AGAAGAACTCTTGGGTAGAA	2	2	NC	NC
1133	1988	TGACAATCAAGAAGAACTCT	2	1	NC	NC
1134	1989	TTGACAATCAAGAAGAACTC	2	2	NC	NC
1135	1990	TTTGACAATCAAGAAGAACT	2	2	NC	NC
1136	1991	TTTTGACAATCAAGAAGAAC	2	2	NC	NC
1137	1992	GTTTTGACAATCAAGAAGAA	2	2	NC	NC
1138	1993	AGTTTTGACAATCAAGAAGA	2	NC	NC	NC
1139	1994	AAGTTTTGACAATCAAGAAG	2	NC	NC	NC
1140	1995	AAAGTTTTGACAATCAAGAA	2	NC	NC	NC
1141	1996	TAAAGTTTTGACAATCAAGA	2	NC	NC	NC
1142	1997	ATAAAGTTTTGACAATCAAG	2	NC	NC	NC
1143	2000	GATATAAAGTTTTGACAATC	1	NC	NC	NC
1144	2002	GTGATATAAAGTTTTGACAA	2	NC	NC	NC
1145	2003	GGTGATATAAAGTTTTGACA	2	NC	NC	NC
1146	2004	AGGTGATATAAAGTTTTGAC	2	NC	NC	NC
1147	2005	TAGGTGATATAAAGTTTTGA	1	NC	NC	NC
1148	2006	TTAGGTGATATAAAGTTTTG	1	NC	NC	NC
1149	2008	CTTTAGGTGATATAAAGTTT	2	NC	NC	NC
1150	2012	CTGACTTTAGGTGATATAAA	2	NC	NC	NC
1151	2013	TCTGACTTTAGGTGATATAA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1152	2014	TTCTGACTTTAGGTGATATA	2	1	NC	NC
1153	2015	ATTCTGACTTTAGGTGATAT	1	1	NC	NC
1154	2016	AATTCTGACTTTAGGTGATA	1	1	NC	NC
1155	2017	AAATTCTGACTTTAGGTGAT	1	1	NC	NC
1156	2018	GAAATTCTGACTTTAGGTGA	1	1	NC	NC
1157	2019	TGAAATTCTGACTTTAGGTG	1	1	NC	NC
1158	2020	TTGAAATTCTGACTTTAGGT	1	1	NC	NC
1159	2021	CTTGAAATTCTGACTTTAGG	1	1	NC	NC
1160	2022	GCTTGAAATTCTGACTTTAG	2	1	NC	NC
1161	2023	TGCTTGAAATTCTGACTTTA	1	1	NC	NC
1162	2024	TTGCTTGAAATTCTGACTTT	1	2	NC	NC
1163	2025	ATTGCTTGAAATTCTGACTT	1	1	NC	NC
1164	2026	TATTGCTTGAAATTCTGACT	2	2	NC	NC
1165	2027	TTATTGCTTGAAATTCTGAC	2	2	NC	NC
1166	2028	ATTATTGCTTGAAATTCTGA	2	1	NC	NC
1167	2029	TATTATTGCTTGAAATTCTG	1	2	NC	NC
1168	2030	GTATTATTGCTTGAAATTCT	1	1	NC	NC
1169	2031	GGTATTATTGCTTGAAATTC	1	2	NC	NC
1170	2032	AGGTATTATTGCTTGAAATT	2	2	NC	NC
1171	2033	CAGGTATTATTGCTTGAAAT	1	1	NC	NC
1172	2034	GCAGGTATTATTGCTTGAAA	2	2	NC	NC
1173	2041	ATTAACAGCAGGTATTATTG	2	2	NC	NC
1174	2042	AATTAACAGCAGGTATTATT	1	2	NC	NC
1175	2043	GAATTAACAGCAGGTATTAT	2	2	NC	NC
1176	2044	GGAATTAACAGCAGGTATTA	2	2	NC	NC
1177	2045	GGGAATTAACAGCAGGTATT	2	2	NC	NC
1178	2046	TGGGAATTAACAGCAGGTAT	1	2	NC	NC
1179	2047	GTGGGAATTAACAGCAGGTA	2	2	NC	NC
1180	2048	TGTGGGAATTAACAGCAGGT	2	NC	NC	NC
1181	2049	ATGTGGGAATTAACAGCAGG	2	NC	NC	NC
1182	2050	AATGTGGGAATTAACAGCAG	2	NC	NC	NC
1183	2051	GAATGTGGGAATTAACAGCA	2	NC	NC	NC
1184	2052	TGAATGTGGGAATTAACAGC	2	NC	NC	NC
1185	2053	CTGAATGTGGGAATTAACAG	2	NC	NC	NC
1186	2054	ACTGAATGTGGGAATTAACA	2	NC	NC	NC
1187	2055	GACTGAATGTGGGAATTAAC	3	NC	NC	NC
1188	2056	TGACTGAATGTGGGAATTAA	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1189	2057	CTGACTGAATGTGGGAATTA	2	NC	NC	NC
1190	2058	TCTGACTGAATGTGGGAATT	1	NC	NC	NC
1191	2059	GTCTGACTGAATGTGGGAAT	2	NC	NC	NC
1192	2060	AGTCTGACTGAATGTGGGAA	2	NC	NC	NC
1193	2061	AAGTCTGACTGAATGTGGGA	2	NC	NC	NC
1194	2062	CAAGTCTGACTGAATGTGGG	2	NC	NC	NC
1195	2063	GCAAGTCTGACTGAATGTGG	2	NC	NC	NC
1196	2064	AGCAAGTCTGACTGAATGTG	2	NC	NC	NC
1197	2065	GAGCAAGTCTGACTGAATGT	2	NC	NC	NC
1198	2066	GGAGCAAGTCTGACTGAATG	2	NC	NC	NC
1199	2067	CGGAGCAAGTCTGACTGAAT	2	NC	NC	NC
1200	2068	CCGGAGCAAGTCTGACTGAA	2	NC	NC	NC
1201	2069	TCCGGAGCAAGTCTGACTGA	2	NC	NC	NC
1202	2081	CTAAAATAACGGTCCGGAGC	3	NC	NC	NC
1203	2082	TCTAAAATAACGGTCCGGAG	3	NC	NC	NC
1204	2083	TTCTAAAATAACGGTCCGGA	3	NC	NC	NC
1205	2084	TTTCTAAAATAACGGTCCGG	2	NC	NC	NC
1206	2085	ATTTCTAAAATAACGGTCCG	2	NC	NC	NC
1207	2086	AATTTCTAAAATAACGGTCC	2	NC	NC	NC
1208	2087	GAATTTCTAAAATAACGGTC	2	NC	NC	NC
1209	2088	GGAATTTCTAAAATAACGGT	2	2	NC	NC
1210	2089	AGGAATTTCTAAAATAACGG	2	2	NC	NC
1211	2090	CAGGAATTTCTAAAATAACG	2	2	NC	NC
1212	2091	TCAGGAATTTCTAAAATAAC	1	2	NC	NC
1213	2093	GTTCAGGAATTTCTAAAATA	2	1	NC	NC
1214	2094	AGTTCAGGAATTTCTAAAAT	2	2	NC	NC
1215	2095	GAGTTCAGGAATTTCTAAAA	1	2	NC	NC
1216	2096	GGAGTTCAGGAATTTCTAAA	2	2	NC	NC
1217	2097	AGGAGTTCAGGAATTTCTAA	1	2	NC	NC
1218	2098	GAGGAGTTCAGGAATTTCTA	1	2	NC	NC
1219	2099	TGAGGAGTTCAGGAATTTCT	2	1	NC	NC
1220	2108	CCACTGGACTGAGGAGTTCA	2	2	NC	NC
1221	2109	TCCACTGGACTGAGGAGTTC	2	2	NC	NC
1222	2113	ATGCTCCACTGGACTGAGGA	2	2	NC	NC
1223	2114	AATGCTCCACTGGACTGAGG	3	2	NC	NC
1224	2115	TAATGCTCCACTGGACTGAG	2	2	NC	NC
1225	2116	GTAATGCTCCACTGGACTGA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1226	2117	AGTAATGCTCCACTGGACTG	2	2	NC	NC
1227	2118	AAGTAATGCTCCACTGGACT	2	2	NC	NC
1228	2119	TAAGTAATGCTCCACTGGAC	1	2	NC	NC
1229	2120	TTAAGTAATGCTCCACTGGA	2	2	NC	NC
1230	2121	TTTAAGTAATGCTCCACTGG	2	2	NC	NC
1231	2122	CTTTAAGTAATGCTCCACTG	2	2	NC	NC
1232	2123	TCTTTAAGTAATGCTCCACT	2	2	NC	NC
1233	2124	ATCTTTAAGTAATGCTCCAC	2	2	NC	NC
1234	2125	TATCTTTAAGTAATGCTCCA	1	2	NC	NC
1235	2126	GTATCTTTAAGTAATGCTCC	2	2	NC	NC
1236	2127	AGTATCTTTAAGTAATGCTC	2	2	NC	NC
1237	2128	GAGTATCTTTAAGTAATGCT	2	2	NC	NC
1238	2129	TGAGTATCTTTAAGTAATGC	2	2	NC	NC
1239	2130	TTGAGTATCTTTAAGTAATG	2	2	NC	NC
1240	2132	CATTGAGTATCTTTAAGTAA	2	2	NC	NC
1241	2133	TCATTGAGTATCTTTAAGTA	2	2	NC	NC
1242	2134	TTCATTGAGTATCTTTAAGT	2	2	NC	NC
1243	2135	GTTCATTGAGTATCTTTAAG	1	1	NC	NC
1244	2136	TGTTCATTGAGTATCTTTAA	2	2	NC	NC
1245	2137	TTGTTCATTGAGTATCTTTA	2	2	NC	NC
1246	2138	CTTGTTCATTGAGTATCTTT	2	2	NC	NC
1247	2139	GCTTGTTCATTGAGTATCTT	2	2	NC	NC
1248	2140	AGCTTGTTCATTGAGTATCT	2	2	NC	NC
1249	2141	CAGCTTGTTCATTGAGTATC	2	2	NC	NC
1250	2142	GCAGCTTGTTCATTGAGTAT	1	2	NC	NC
1251	2143	GGCAGCTTGTTCATTGAGTA	2	2	NC	NC
1252	2144	TGGCAGCTTGTTCATTGAGT	2	3	NC	NC
1253	2145	TTGGCAGCTTGTTCATTGAG	1	2	NC	NC
1254	2146	TTTGGCAGCTTGTTCATTGA	2	2	NC	NC
1255	2147	CTTTGGCAGCTTGTTCATTG	2	2	NC	NC
1256	2148	ACTTTGGCAGCTTGTTCATT	2	2	NC	NC
1257	2149	AACTTTGGCAGCTTGTTCAT	2	2	NC	NC
1258	2150	CAACTTTGGCAGCTTGTTCA	2	2	NC	NC
1259	2162	CAGTTTTATCCCCAACTTTG	2	2	NC	NC
1260	2163	TCAGTTTTATCCCCAACTTT	2	1	NC	NC
1261	2164	TTCAGTTTTATCCCCAACTT	2	1	NC	NC
1262	2165	ATTCAGTTTTATCCCCAACT	1	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1263	2166	AATTCAGTTTTATCCCCAAC	2	1	NC	NC
1264	2167	TAATTCAGTTTTATCCCCAA	2	1	NC	NC
1265	2168	ATAATTCAGTTTTATCCCCA	1	1	NC	NC
1266	2169	AATAATTCAGTTTTATCCCC	1	1	NC	NC
1267	2170	AAATAATTCAGTTTTATCCC	1	1	NC	NC
1268	2177	GGTCTTTAAATAATTCAGTT	2	2	NC	NC
1269	2178	AGGTCTTTAAATAATTCAGT	2	1	NC	NC
1270	2179	AAGGTCTTTAAATAATTCAG	2	1	NC	NC
1271	2181	GAAAGGTCTTTAAATAATTC	1	1	NC	NC
1272	2183	CAGAAAGGTCTTTAAATAAT	1	2	NC	NC
1273	2184	TCAGAAAGGTCTTTAAATAA	1	2	NC	NC
1274	2185	GTCAGAAAGGTCTTTAAATA	2	2	NC	NC
1275	2186	AGTCAGAAAGGTCTTTAAAT	2	1	NC	NC
1276	2187	AAGTCAGAAAGGTCTTTAAA	2	2	NC	NC
1277	2188	GAAGTCAGAAAGGTCTTTAA	1	2	NC	NC
1278	2189	GGAAGTCAGAAAGGTCTTTA	1	2	NC	NC
1279	2190	GGGAAGTCAGAAAGGTCTTT	2	1	NC	NC
1280	2191	AGGGAAGTCAGAAAGGTCTT	2	1	NC	NC
1281	2192	AAGGGAAGTCAGAAAGGTCT	2	2	NC	NC
1282	2193	AAAGGGAAGTCAGAAAGGTC	2	2	NC	NC
1283	2194	TAAAGGGAAGTCAGAAAGGT	2	2	NC	NC
1284	2195	TTAAAGGGAAGTCAGAAAGG	1	1	NC	NC
1285	2196	ATTAAAGGGAAGTCAGAAAG	1	1	NC	NC
1286	2197	TATTAAAGGGAAGTCAGAAA	1	2	NC	NC
1287	2198	TTATTAAAGGGAAGTCAGAA	1	2	NC	NC
1288	2199	TTTATTAAAGGGAAGTCAGA	1	1	2	1
1289	2200	TTTTATTAAAGGGAAGTCAG	1	2	2	2
1290	2201	TTTTTATTAAAGGGAAGTCA	2	2	1	2
1291	2217	ATTTCATCCTTCCTCTTTTT	1	1	NC	NC
1292	2218	AATTTCATCCTTCCTCTTTT	1	1	NC	NC
1293	2219	GAATTTCATCCTTCCTCTTT	2	2	NC	NC
1294	2220	TGAATTTCATCCTTCCTCTT	2	1	NC	NC
1295	2221	TTGAATTTCATCCTTCCTCT	1	1	NC	NC
1296	2222	CTTGAATTTCATCCTTCCTC	2	1	NC	NC
1297	2223	CCTTGAATTTCATCCTTCCT	2	2	NC	NC
1298	2224	ACCTTGAATTTCATCCTTCC	2	2	NC	NC
1299	2225	CACCTTGAATTTCATCCTTC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1300	2226	ACACCTTGAATTTCATCCTT	1	1	NC	NC
1301	2227	AACACCTTGAATTTCATCCT	2	2	NC	NC
1302	2228	TAACACCTTGAATTTCATCC	2	2	NC	NC
1303	2229	ATAACACCTTGAATTTCATC	2	NC	NC	NC
1304	2230	AATAACACCTTGAATTTCAT	2	NC	NC	NC
1305	2231	CAATAACACCTTGAATTTCA	2	NC	NC	NC
1306	2232	TCAATAACACCTTGAATTTC	2	NC	NC	NC
1307	2233	GTCAATAACACCTTGAATTT	2	NC	NC	NC
1308	2234	CGTCAATAACACCTTGAATT	2	NC	NC	NC
1309	2235	TCGTCAATAACACCTTGAAT	2	NC	NC	NC
1310	2236	CTCGTCAATAACACCTTGAA	2	NC	NC	NC
1311	2237	TCTCGTCAATAACACCTTGA	2	NC	NC	NC
1312	2238	ATCTCGTCAATAACACCTTG	2	NC	NC	NC
1313	2239	GATCTCGTCAATAACACCTT	2	NC	NC	NC
1314	2240	GGATCTCGTCAATAACACCT	2	NC	NC	NC
1315	2241	CGGATCTCGTCAATAACACC	3	NC	NC	NC
1316	2265	TTTCGTATTTCTTGCAAATG	2	2	NC	NC
1317	2266	TTTTCGTATTTCTTGCAAAT	1	1	NC	NC
1318	2267	TTTTTCGTATTTCTTGCAAA	2	2	NC	NC
1319	2268	ATTTTTCGTATTTCTTGCAA	2	2	NC	NC
1320	2269	TATTTTTCGTATTTCTTGCA	2	2	NC	NC
1321	2270	GTATTTTTCGTATTTCTTGC	2	2	NC	NC
1322	2271	AGTATTTTTCGTATTTCTTG	2	2	NC	NC
1323	2292	TATTGTGCAGAAGGATTTTT	2	2	NC	NC
1324	2293	ATATTGTGCAGAAGGATTTT	2	2	NC	NC
1325	2294	CATATTGTGCAGAAGGATTT	2	2	NC	NC
1326	2295	ACATATTGTGCAGAAGGATT	2	2	NC	NC
1327	2306	CTGATACTGTCACATATTGT	2	2	NC	NC
1328	2307	CCTGATACTGTCACATATTG	2	2	NC	NC
1329	2308	TCCTGATACTGTCACATATT	2	2	NC	NC
1330	2309	GTCCTGATACTGTCACATAT	2	2	NC	NC
1331	2310	TGTCCTGATACTGTCACATA	2	2	NC	NC
1332	2311	CTGTCCTGATACTGTCACAT	1	2	NC	NC
1333	2312	CCTGTCCTGATACTGTCACA	1	2	NC	NC
1334	2313	TCCTGTCCTGATACTGTCAC	2	1	NC	NC
1335	2314	CTCCTGTCCTGATACTGTCA	2	2	NC	NC
1336	2315	ACTCCTGTCCTGATACTGTC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1337	2316	AACTCCTGTCCTGATACTGT	2	2	NC	NC
1338	2317	AAACTCCTGTCCTGATACTG	2	2	NC	NC
1339	2318	TAAACTCCTGTCCTGATACT	1	2	NC	NC
1340	2319	ATAAACTCCTGTCCTGATAC	2	2	NC	NC
1341	2320	CATAAACTCCTGTCCTGATA	2	3	NC	NC
1342	2321	TCATAAACTCCTGTCCTGAT	2	2	NC	NC
1343	2322	ATCATAAACTCCTGTCCTGA	1	1	NC	NC
1344	2323	TATCATAAACTCCTGTCCTG	1	NC	NC	NC
1345	2324	CTATCATAAACTCCTGTCCT	2	NC	NC	NC
1346	2325	TCTATCATAAACTCCTGTCC	1	NC	NC	NC
1347	2326	TTCTATCATAAACTCCTGTC	1	NC	NC	NC
1348	2327	TTTCTATCATAAACTCCTGT	2	NC	NC	NC
1349	2328	ATTTCTATCATAAACTCCTG	2	NC	NC	NC
1350	2329	TATTTCTATCATAAACTCCT	1	NC	NC	NC
1351	2330	TTATTTCTATCATAAACTCC	1	NC	NC	NC
1352	2337	GAGTTCTTTATTTCTATCAT	1	NC	NC	NC
1353	2338	AGAGTTCTTTATTTCTATCA	2	NC	NC	NC
1354	2339	CAGAGTTCTTTATTTCTATC	2	NC	NC	NC
1355	2340	GCAGAGTTCTTTATTTCTAT	2	NC	NC	NC
1356	2341	AGCAGAGTTCTTTATTTCTA	2	NC	NC	NC
1357	2342	CAGCAGAGTTCTTTATTTCT	1	NC	NC	NC
1358	2343	ACAGCAGAGTTCTTTATTTC	2	2	NC	NC
1359	2344	TACAGCAGAGTTCTTTATTT	2	2	NC	NC
1360	2345	ATACAGCAGAGTTCTTTATT	2	2	NC	NC
1361	2346	GATACAGCAGAGTTCTTTAT	2	2	NC	NC
1362	2347	AGATACAGCAGAGTTCTTTA	2	2	NC	NC
1363	2348	AAGATACAGCAGAGTTCTTT	2	2	NC	NC
1364	2349	CAAGATACAGCAGAGTTCTT	2	2	NC	NC
1365	2350	ACAAGATACAGCAGAGTTCT	2	2	NC	NC
1366	2351	TACAAGATACAGCAGAGTTC	2	2	NC	NC
1367	2352	ATACAAGATACAGCAGAGTT	1	1	NC	NC
1368	2353	TATACAAGATACAGCAGAGT	2	2	NC	NC
1369	2354	GTATACAAGATACAGCAGAG	2	2	NC	NC
1370	2355	GGTATACAAGATACAGCAGA	2	2	NC	NC
1371	2356	TGGTATACAAGATACAGCAG	2	2	NC	NC
1372	2357	TTGGTATACAAGATACAGCA	2	2	NC	NC
1373	2374	AACCTTTACCCAATCAGTTG	3	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1374	2375	CAACCTTTACCCAATCAGTT	2	2	NC	NC
1375	2376	CCAACCTTTACCCAATCAGT	2	2	NC	NC
1376	2377	TCCAACCTTTACCCAATCAG	2	2	NC	NC
1377	2378	TTCCAACCTTTACCCAATCA	2	2	NC	NC
1378	2379	CTTCCAACCTTTACCCAATC	2	2	NC	NC
1379	2380	GCTTCCAACCTTTACCCAAT	2	2	NC	NC
1380	2381	TGCTTCCAACCTTTACCCAA	2	2	NC	NC
1381	2382	GTGCTTCCAACCTTTACCCA	2	2	NC	NC
1382	2383	TGTGCTTCCAACCTTTACCC	2	2	NC	NC
1383	2384	TTGTGCTTCCAACCTTTACC	2	2	NC	NC
1384	2385	TTTGTGCTTCCAACCTTTAC	1	1	NC	NC
1385	2386	TTTTGTGCTTCCAACCTTTA	1	2	NC	NC
1386	2387	CTTTTGTGCTTCCAACCTTT	2	2	NC	NC
1387	2388	GCTTTTGTGCTTCCAACCTT	1	2	2	2
1388	2410	AGGAGAGTGAAAGCGGCTCA	2	NC	NC	NC
1389	2411	AAGGAGAGTGAAAGCGGCTC	2	NC	NC	NC
1390	2412	AAAGGAGAGTGAAAGCGGCT	2	NC	NC	NC
1391	2413	AAAAGGAGAGTGAAAGCGGC	2	NC	NC	NC
1392	2414	TAAAAGGAGAGTGAAAGCGG	2	NC	NC	NC
1393	2415	ATAAAAGGAGAGTGAAAGCG	2	NC	NC	NC
1394	2416	AATAAAAGGAGAGTGAAAGC	2	NC	NC	NC
1395	2417	CAATAAAAGGAGAGTGAAAG	1	NC	NC	NC
1396	2418	ACAATAAAAGGAGAGTGAAA	1	NC	NC	NC
1397	2419	TACAATAAAAGGAGAGTGAA	1	NC	NC	NC
1398	2420	CTACAATAAAAGGAGAGTGA	1	NC	NC	NC
1399	2421	TCTACAATAAAAGGAGAGTG	1	NC	NC	NC
1400	2422	TTCTACAATAAAAGGAGAGT	1	NC	NC	NC
1401	2423	TTTCTACAATAAAAGGAGAG	1	NC	NC	NC
1402	2424	TTTTCTACAATAAAAGGAGA	1	NC	NC	NC
1403	2425	ATTTTCTACAATAAAAGGAG	2	NC	NC	NC
1404	2432	GTCTGTAATTTTCTACAATA	1	NC	NC	NC
1405	2433	TGTCTGTAATTTTCTACAAT	1	NC	NC	NC
1406	2434	ATGTCTGTAATTTTCTACAA	1	1	NC	NC
1407	2435	GATGTCTGTAATTTTCTACA	2	2	NC	NC
1408	2436	AGATGTCTGTAATTTTCTAC	2	2	NC	NC
1409	2448	CGGAGCTGATTCAGATGTCT	2	NC	NC	NC
1410	2449	CCGGAGCTGATTCAGATGTC	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1411	2450	CCCGGAGCTGATTCAGATGT	2	NC	NC	NC
1412	2451	TCCCGGAGCTGATTCAGATG	3	NC	NC	NC
1413	2452	CTCCCGGAGCTGATTCAGAT	2	NC	NC	NC
1414	2478	TCAGCACTGCAGTCAAGGAC	2	2	NC	NC
1415	2479	TTCAGCACTGCAGTCAAGGA	2	2	NC	NC
1416	2480	ATTCAGCACTGCAGTCAAGG	2	2	NC	NC
1417	2481	CATTCAGCACTGCAGTCAAG	2	2	NC	NC
1418	2482	CCATTCAGCACTGCAGTCAA	1	1	NC	NC
1419	2483	GCCATTCAGCACTGCAGTCA	2	2	NC	NC
1420	2484	AGCCATTCAGCACTGCAGTC	2	2	NC	NC
1421	2485	AAGCCATTCAGCACTGCAGT	1	1	NC	NC
1422	2486	CAAGCCATTCAGCACTGCAG	2	2	NC	NC
1423	2487	TCAAGCCATTCAGCACTGCA	1	2	NC	NC
1424	2488	ATCAAGCCATTCAGCACTGC	1	2	NC	NC
1425	2489	AATCAAGCCATTCAGCACTG	2	2	NC	NC
1426	2490	AAATCAAGCCATTCAGCACT	2	2	NC	NC
1427	2491	AAAATCAAGCCATTCAGCAC	2	2	NC	NC
1428	2492	GAAAATCAAGCCATTCAGCA	2	1	NC	NC
1429	2493	AGAAAATCAAGCCATTCAGC	2	2	NC	NC
1430	2494	TAGAAAATCAAGCCATTCAG	2	2	NC	NC
1431	2495	CTAGAAAATCAAGCCATTCA	2	2	NC	NC
1432	2496	TCTAGAAAATCAAGCCATTC	2	2	NC	NC
1433	2497	CTCTAGAAAATCAAGCCATT	2	2	NC	NC
1434	2498	TCTCTAGAAAATCAAGCCAT	2	2	NC	NC
1435	2499	TTCTCTAGAAAATCAAGCCA	2	2	NC	NC
1436	2509	TTCACTGAATTTCTCTAGAA	2	1	NC	NC
1437	2510	GTTCACTGAATTTCTCTAGA	2	2	NC	NC
1438	2511	TGTTCACTGAATTTCTCTAG	2	2	NC	NC
1439	2512	ATGTTCACTGAATTTCTCTA	2	2	NC	NC
1440	2513	AATGTTCACTGAATTTCTCT	1	1	NC	NC
1441	2514	TAATGTTCACTGAATTTCTC	2	2	NC	NC
1442	2515	ATAATGTTCACTGAATTTCT	2	2	NC	NC
1443	2516	GATAATGTTCACTGAATTTC	2	2	NC	NC
1444	2517	TGATAATGTTCACTGAATTT	2	2	NC	NC
1445	2525	ACAAGGAGTGATAATGTTCA	2	NC	NC	NC
1446	2538	TGCACTGCTTTACACAAGGA	2	NC	NC	NC
1447	2539	ATGCACTGCTTTACACAAGG	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1448	2540	GATGCACTGCTTTACACAAG	2	2	NC	NC
1449	2541	TGATGCACTGCTTTACACAA	2	2	NC	NC
1450	2542	GTGATGCACTGCTTTACACA	2	2	NC	NC
1451	2543	GGTGATGCACTGCTTTACAC	2	2	NC	NC
1452	2544	AGGTGATGCACTGCTTTACA	2	2	NC	NC
1453	2545	TAGGTGATGCACTGCTTTAC	2	2	NC	NC
1454	2546	CTAGGTGATGCACTGCTTTA	2	2	NC	NC
1455	2547	GCTAGGTGATGCACTGCTTT	2	2	NC	NC
1456	2548	TGCTAGGTGATGCACTGCTT	2	2	NC	NC
1457	2555	CAACAGTTGCTAGGTGATGC	2	2	NC	NC
1458	2556	TCAACAGTTGCTAGGTGATG	2	2	NC	NC
1459	2557	GTCAACAGTTGCTAGGTGAT	2	2	1	NC
1460	2558	AGTCAACAGTTGCTAGGTGA	2	2	1	NC
1461	2559	CAGTCAACAGTTGCTAGGTG	2	2	1	NC
1462	2560	GCAGTCAACAGTTGCTAGGT	2	2	NC	NC
1463	2566	GAAAATGCAGTCAACAGTTG	1	2	NC	NC
1464	2567	AGAAAATGCAGTCAACAGTT	2	1	NC	NC
1465	2568	GAGAAAATGCAGTCAACAGT	2	1	NC	NC
1466	2569	GGAGAAAATGCAGTCAACAG	2	1	NC	NC
1467	2570	GGGAGAAAATGCAGTCAACA	1	1	NC	NC
1468	2571	AGGGAGAAAATGCAGTCAAC	2	2	NC	NC
1469	2572	CAGGGAGAAAATGCAGTCAA	1	1	NC	NC
1470	2573	CCAGGGAGAAAATGCAGTCA	2	1	NC	NC
1471	2574	GCCAGGGAGAAAATGCAGTC	2	2	NC	NC
1472	2575	GGCCAGGGAGAAAATGCAGT	1	2	NC	NC
1473	2576	TGGCCAGGGAGAAAATGCAG	2	1	NC	NC
1474	2577	TTGGCCAGGGAGAAAATGCA	2	2	NC	NC
1475	2578	CTTGGCCAGGGAGAAAATGC	2	1	NC	NC
1476	2590	TTGCTTAGCGACCTTGGCCA	2	2	NC	NC
1477	2593	TCCTTGCTTAGCGACCTTGG	2	2	NC	NC
1478	2594	CTCCTTGCTTAGCGACCTTG	2	NC	NC	NC
1479	2595	TCTCCTTGCTTAGCGACCTT	2	NC	NC	NC
1480	2596	ATCTCCTTGCTTAGCGACCT	2	NC	NC	NC
1481	2597	AATCTCCTTGCTTAGCGACC	2	NC	NC	NC
1482	2598	TAATCTCCTTGCTTAGCGAC	3	NC	NC	NC
1483	2599	GTAATCTCCTTGCTTAGCGA	3	NC	NC	NC
1484	2600	AGTAATCTCCTTGCTTAGCG	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1485	2601	CAGTAATCTCCTTGCTTAGC	2	NC	NC	NC
1486	2602	GCAGTAATCTCCTTGCTTAG	1	NC	NC	NC
1487	2603	TGCAGTAATCTCCTTGCTTA	1	NC	NC	NC
1488	2604	CTGCAGTAATCTCCTTGCTT	1	NC	NC	NC
1489	2605	TCTGCAGTAATCTCCTTGCT	1	NC	NC	NC
1490	2607	GGTCTGCAGTAATCTCCTTG	2	NC	NC	NC
1491	2608	TGGTCTGCAGTAATCTCCTT	2	NC	NC	NC
1492	2609	TTGGTCTGCAGTAATCTCCT	2	NC	NC	NC
1493	2610	GTTGGTCTGCAGTAATCTCC	2	NC	NC	NC
1494	2611	AGTTGGTCTGCAGTAATCTC	2	NC	NC	NC
1495	2612	CAGTTGGTCTGCAGTAATCT	2	NC	NC	NC
1496	2622	TCTTCTTGTACAGTTGGTCT	2	2	NC	NC
1497	2623	TTCTTCTTGTACAGTTGGTC	2	2	NC	NC
1498	2624	TTTCTTCTTGTACAGTTGGT	2	2	NC	NC
1499	2625	CTTTCTTCTTGTACAGTTGG	2	2	NC	NC
1500	2626	TCTTTCTTCTTGTACAGTTG	2	2	NC	NC
1501	2627	TTCTTTCTTCTTGTACAGTT	2	1	NC	NC
1502	2628	TTTCTTTCTTCTTGTACAGT	1	1	NC	NC
1503	2629	TTTTCTTTCTTCTTGTACAG	1	1	NC	NC
1504	2630	TTTTTCTTTCTTCTTGTACA	1	1	NC	NC
1505	2631	ATTTTTCTTTCTTCTTGTAC	1	1	NC	NC
1506	2633	CAATTTTTCTTTCTTCTTGT	1	NC	NC	NC
1507	2634	ACAATTTTTCTTTCTTCTTG	1	NC	NC	NC
1508	2658	ACAGGGTGCCTTCCATTTTT	2	NC	NC	NC
1509	2659	CACAGGGTGCCTTCCATTTT	2	NC	NC	NC
1510	2660	TCACAGGGTGCCTTCCATTT	2	NC	NC	NC
1511	2661	ATCACAGGGTGCCTTCCATT	2	NC	NC	NC
1512	2662	AATCACAGGGTGCCTTCCAT	2	NC	NC	NC
1513	2663	CAATCACAGGGTGCCTTCCA	2	NC	NC	NC
1514	2664	TCAATCACAGGGTGCCTTCC	2	NC	NC	NC
1515	2665	ATCAATCACAGGGTGCCTTC	2	NC	NC	NC
1516	2666	CATCAATCACAGGGTGCCTT	2	NC	NC	NC
1517	2667	ACATCAATCACAGGGTGCCT	2	NC	NC	NC
1518	2668	CACATCAATCACAGGGTGCC	2	NC	NC	NC
1519	2669	ACACATCAATCACAGGGTGC	2	NC	NC	NC
1520	2670	AACACATCAATCACAGGGTG	2	NC	NC	NC
1521	2671	CAACACATCAATCACAGGGT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1522	2672	GCAACACATCAATCACAGGG	2	NC	NC	NC
1523	2673	AGCAACACATCAATCACAGG	2	2	NC	NC
1524	2674	CAGCAACACATCAATCACAG	1	1	NC	NC
1525	2675	CCAGCAACACATCAATCACA	2	2	NC	NC
1526	2676	CCCAGCAACACATCAATCAC	2	2	NC	NC
1527	2677	TCCCAGCAACACATCAATCA	2	2	NC	NC
1528	2678	CTCCCAGCAACACATCAATC	1	1	NC	NC
1529	2679	TCTCCCAGCAACACATCAAT	2	2	NC	NC
1530	2680	TTCTCCCAGCAACACATCAA	2	2	NC	NC
1531	2681	GTTCTCCCAGCAACACATCA	2	1	NC	NC
1532	2682	TGTTCTCCCAGCAACACATC	2	2	NC	NC
1533	2683	CTGTTCTCCCAGCAACACAT	2	1	NC	NC
1534	2684	CCTGTTCTCCCAGCAACACA	2	0	NC	NC
1535	2685	TCCTGTTCTCCCAGCAACAC	2	1	NC	NC
1536	2686	ATCCTGTTCTCCCAGCAACA	2	1	NC	NC
1537	2687	GATCCTGTTCTCCCAGCAAC	2	1	NC	NC
1538	2688	TGATCCTGTTCTCCCAGCAA	2	2	NC	NC
1539	2689	TTGATCCTGTTCTCCCAGCA	2	2	NC	NC
1540	2690	ATTGATCCTGTTCTCCCAGC	2	2	NC	NC
1541	2691	TATTGATCCTGTTCTCCCAG	2	2	NC	NC
1542	2692	ATATTGATCCTGTTCTCCCA	2	2	NC	NC
1543	2693	CATATTGATCCTGTTCTCCC	2	2	NC	NC
1544	2694	ACATATTGATCCTGTTCTCC	2	2	NC	NC
1545	2695	GACATATTGATCCTGTTCTC	2	NC	NC	NC
1546	2696	GGACATATTGATCCTGTTCT	2	NC	NC	NC
1547	2697	GGGACATATTGATCCTGTTC	2	NC	NC	NC
1548	2698	TGGGACATATTGATCCTGTT	2	NC	NC	NC
1549	2711	AATCTGTATTATTTGGGACA	1	NC	NC	NC
1550	2712	AAATCTGTATTATTTGGGAC	2	NC	NC	NC
1551	2713	TAAATCTGTATTATTTGGGA	2	NC	NC	NC
1552	2714	ATAAATCTGTATTATTTGGG	1	NC	NC	NC
1553	2715	GATAAATCTGTATTATTTGG	2	NC	NC	NC
1554	2719	CTCTGATAAATCTGTATTAT	2	NC	NC	NC
1555	2720	CCTCTGATAAATCTGTATTA	2	NC	NC	NC
1556	2721	TCCTCTGATAAATCTGTATT	1	NC	NC	NC
1557	2722	GTCCTCTGATAAATCTGTAT	2	NC	NC	NC
1558	2723	AGTCCTCTGATAAATCTGTA	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1559	2724	GAGTCCTCTGATAAATCTGT	2	2	NC	NC
1560	2725	TGAGTCCTCTGATAAATCTG	1	1	NC	NC
1561	2726	CTGAGTCCTCTGATAAATCT	1	1	NC	NC
1562	2727	TCTGAGTCCTCTGATAAATC	2	2	NC	NC
1563	2728	CTCTGAGTCCTCTGATAAAT	2	2	NC	NC
1564	2729	TCTCTGAGTCCTCTGATAAA	1	2	NC	NC
1565	2730	CTCTCTGAGTCCTCTGATAA	2	2	NC	NC
1566	2731	TCTCTCTGAGTCCTCTGATA	2	2	NC	NC
1567	2732	CTCTCTCTGAGTCCTCTGAT	2	1	NC	NC
1568	2742	ATTATCATTACTCTCTCTGA	2	2	NC	NC
1569	2743	AATTATCATTACTCTCTCTG	2	2	NC	NC
1570	2744	TAATTATCATTACTCTCTCT	2	1	NC	NC
1571	2752	TGGTCCGGTAATTATCATTA	3	NC	NC	NC
1572	2753	TTGGTCCGGTAATTATCATT	3	NC	NC	NC
1573	2754	TTTGGTCCGGTAATTATCAT	2	NC	NC	NC
1574	2755	GTTTGGTCCGGTAATTATCA	3	NC	NC	NC
1575	2756	TGTTTGGTCCGGTAATTATC	3	NC	NC	NC
1576	2757	ATGTTTGGTCCGGTAATTAT	3	NC	NC	NC
1577	2758	CATGTTTGGTCCGGTAATTA	2	NC	NC	NC
1578	2759	CCATGTTTGGTCCGGTAATT	3	NC	NC	NC
1579	2770	GCTCTTTCCACCCATGTTTG	2	2	NC	NC
1580	2771	AGCTCTTTCCACCCATGTTT	1	2	NC	NC
1581	2772	GAGCTCTTTCCACCCATGTT	2	2	NC	NC
1582	2773	GGAGCTCTTTCCACCCATGT	2	2	NC	NC
1583	2782	TTTTATGTAGGAGCTCTTTC	2	2	NC	NC
1584	2783	GTTTTATGTAGGAGCTCTTT	2	2	NC	NC
1585	2784	TGTTTTATGTAGGAGCTCTT	2	2	NC	NC
1586	2785	TTGTTTTATGTAGGAGCTCT	2	2	NC	NC
1587	2786	CTTGTTTTATGTAGGAGCTC	3	3	NC	NC
1588	2787	ACTTGTTTTATGTAGGAGCT	2	2	NC	NC
1589	2788	AACTTGTTTTATGTAGGAGC	2	2	NC	NC
1590	2789	CAACTTGTTTTATGTAGGAG	2	2	NC	NC
1591	2790	GCAACTTGTTTTATGTAGGA	2	2	NC	NC
1592	2791	TGCAACTTGTTTTATGTAGG	2	3	NC	NC
1593	2792	ATGCAACTTGTTTTATGTAG	2	2	NC	NC
1594	2793	AATGCAACTTGTTTTATGTA	2	2	NC	NC
1595	2794	CAATGCAACTTGTTTTATGT	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1596	2795	TCAATGCAACTTGTTTTATG	2	2	NC	NC
1597	2796	ATCAATGCAACTTGTTTTAT	2	2	NC	NC
1598	2797	AATCAATGCAACTTGTTTTA	1	2	NC	NC
1599	2798	TAATCAATGCAACTTGTTTT	1	1	NC	NC
1600	2799	GTAATCAATGCAACTTGTTT	2	2	NC	NC
1601	2800	GGTAATCAATGCAACTTGTT	2	2	NC	NC
1602	2801	TGGTAATCAATGCAACTTGT	2	2	NC	NC
1603	2802	ATGGTAATCAATGCAACTTG	2	2	NC	NC
1604	2803	GATGGTAATCAATGCAACTT	2	2	NC	NC
1605	2804	TGATGGTAATCAATGCAACT	2	2	NC	NC
1606	2819	AGCCAATCTGAGCCATGATG	2	2	2	NC
1607	2820	GAGCCAATCTGAGCCATGAT	2	2	2	NC
1608	2821	GGAGCCAATCTGAGCCATGA	2	2	2	NC
1609	2822	AGGAGCCAATCTGAGCCATG	2	2	2	NC
1610	2823	TAGGAGCCAATCTGAGCCAT	2	2	2	NC
1611	2824	ATAGGAGCCAATCTGAGCCA	2	2	NC	NC
1612	2825	CATAGGAGCCAATCTGAGCC	3	2	NC	NC
1613	2826	ACATAGGAGCCAATCTGAGC	2	2	NC	NC
1614	2827	AACATAGGAGCCAATCTGAG	2	2	NC	NC
1615	2828	GAACATAGGAGCCAATCTGA	2	2	NC	NC
1616	2829	GGAACATAGGAGCCAATCTG	1	2	NC	NC
1617	2830	AGGAACATAGGAGCCAATCT	1	2	NC	NC
1618	2831	CAGGAACATAGGAGCCAATC	2	2	NC	NC
1619	2832	GCAGGAACATAGGAGCCAAT	2	2	NC	NC
1620	2833	TGCAGGAACATAGGAGCCAA	2	2	NC	NC
1621	2834	CTGCAGGAACATAGGAGCCA	2	2	NC	NC
1622	2835	TCTGCAGGAACATAGGAGCC	2	2	NC	NC
1623	2836	TTCTGCAGGAACATAGGAGC	2	2	NC	NC
1624	2837	CTTCTGCAGGAACATAGGAG	2	2	NC	NC
1625	2838	TCTTCTGCAGGAACATAGGA	2	2	NC	NC
1626	2839	TTCTTCTGCAGGAACATAGG	2	2	NC	NC
1627	2840	CTTCTTCTGCAGGAACATAG	2	2	NC	NC
1628	2841	GCTTCTTCTGCAGGAACATA	2	2	NC	NC
1629	2842	CGCTTCTTCTGCAGGAACAT	2	2	NC	NC
1630	2843	TCGCTTCTTCTGCAGGAACA	1	2	NC	NC
1631	2844	GTCGCTTCTTCTGCAGGAAC	2	2	NC	NC
1632	2845	TGTCGCTTCTTCTGCAGGAA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1633	2846	TTGTCGCTTCTTCTGCAGGA	2	2	NC	NC
1634	2847	ATTGTCGCTTCTTCTGCAGG	2	2	NC	NC
1635	2848	AATTGTCGCTTCTTCTGCAG	2	2	NC	NC
1636	2849	CAATTGTCGCTTCTTCTGCA	2	2	NC	NC
1637	2850	CCAATTGTCGCTTCTTCTGC	2	3	NC	NC
1638	2851	CCCAATTGTCGCTTCTTCTG	2	2	NC	NC
1639	2852	TCCCAATTGTCGCTTCTTCT	2	2	NC	NC
1640	2853	ATCCCAATTGTCGCTTCTTC	2	3	NC	NC
1641	2854	AATCCCAATTGTCGCTTCTT	1	2	NC	NC
1642	2855	CAATCCCAATTGTCGCTTCT	2	3	NC	NC
1643	2864	TGCCATCCACAATCCCAATT	2	2	2	NC
1644	2865	ATGCCATCCACAATCCCAAT	2	2	2	NC
1645	2866	AATGCCATCCACAATCCCAA	1	1	2	NC
1646	2867	AAATGCCATCCACAATCCCA	1	1	2	NC
1647	2868	AAAATGCCATCCACAATCCC	2	2	2	NC
1648	2869	GAAAATGCCATCCACAATCC	2	2	1	NC
1649	2870	TGAAAATGCCATCCACAATC	2	2	1	NC
1650	2871	GTGAAAATGCCATCCACAAT	2	2	2	NC
1651	2872	TGTGAAAATGCCATCCACAA	1	1	2	NC
1652	2873	TTGTGAAAATGCCATCCACA	1	1	2	NC
1653	2874	CTTGTGAAAATGCCATCCAC	1	1	2	NC
1654	2875	CCTTGTGAAAATGCCATCCA	1	1	2	NC
1655	2876	TCCTTGTGAAAATGCCATCC	2	2	2	NC
1656	2877	ATCCTTGTGAAAATGCCATC	2	2	1	NC
1657	2878	CATCCTTGTGAAAATGCCAT	2	2	2	NC
1658	2879	CCATCCTTGTGAAAATGCCA	2	2	2	NC
1659	2880	CCCATCCTTGTGAAAATGCC	2	2	2	NC
1660	2881	ACCCATCCTTGTGAAAATGC	2	1	2	NC
1661	2882	CACCCATCCTTGTGAAAATG	2	2	2	NC
1662	2883	GCACCCATCCTTGTGAAAAT	1	2	2	NC
1663	2884	AGCACCCATCCTTGTGAAAA	2	2	2	NC
1664	2885	CAGCACCCATCCTTGTGAAA	1	2	2	NC
1665	2886	GCAGCACCCATCCTTGTGAA	1	2	1	NC
1666	2887	TGCAGCACCCATCCTTGTGA	1	2	1	NC
1667	2889	TCTGCAGCACCCATCCTTGT	1	2	2	NC
1668	2891	TGTCTGCAGCACCCATCCTT	2	2	2	NC
1669	2892	TTGTCTGCAGCACCCATCCT	2	2	2	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1670	2893	ATTGTCTGCAGCACCCATCC	2	1	1	NC
1671	2894	TATTGTCTGCAGCACCCATC	2	2	2	NC
1672	2895	ATATTGTCTGCAGCACCCAT	2	2	2	NC
1673	2896	TATATTGTCTGCAGCACCCA	2	3	2	NC
1674	2897	ATATATTGTCTGCAGCACCC	2	2	2	2
1675	2898	TATATATTGTCTGCAGCACC	2	3	2	2
1676	2899	ATATATATTGTCTGCAGCAC	2	2	NC	NC
1677	2900	TATATATATTGTCTGCAGCA	2	2	NC	NC
1678	2901	TTATATATATTGTCTGCAGC	2	2	NC	NC
1679	2902	TTTATATATATTGTCTGCAG	2	2	NC	NC
1680	2903	CTTTATATATATTGTCTGCA	2	1	NC	NC
1681	2904	CCTTTATATATATTGTCTGC	2	2	NC	NC
1682	2905	TCCTTTATATATATTGTCTG	1	1	NC	NC
1683	2906	GTCCTTTATATATATTGTCT	2	1	NC	NC
1684	2907	TGTCCTTTATATATATTGTC	2	NC	NC	NC
1685	2908	CTGTCCTTTATATATATTGT	2	NC	NC	NC
1686	2909	TCTGTCCTTTATATATATTG	2	NC	NC	NC
1687	2910	CTCTGTCCTTTATATATATT	1	NC	NC	NC
1688	2911	ACTCTGTCCTTTATATATAT	2	NC	NC	NC
1689	2912	TACTCTGTCCTTTATATATA	2	NC	NC	NC
1690	2913	GTACTCTGTCCTTTATATAT	2	NC	NC	NC
1691	2914	TGTACTCTGTCCTTTATATA	2	NC	NC	NC
1692	2915	ATGTACTCTGTCCTTTATAT	2	NC	NC	NC
1693	2916	AATGTACTCTGTCCTTTATA	2	NC	NC	NC
1694	2917	AAATGTACTCTGTCCTTTAT	2	NC	NC	NC
1695	2918	TAAATGTACTCTGTCCTTTA	1	NC	NC	NC
1696	2919	ATAAATGTACTCTGTCCTTT	1	NC	NC	NC
1697	2920	CATAAATGTACTCTGTCCTT	2	NC	NC	NC
1698	2921	CCATAAATGTACTCTGTCCT	2	NC	NC	NC
1699	2922	TCCATAAATGTACTCTGTCC	2	NC	NC	NC
1700	2923	TTCCATAAATGTACTCTGTC	2	NC	NC	NC
1701	2924	CTTCCATAAATGTACTCTGT	2	NC	NC	NC
1702	2925	TCTTCCATAAATGTACTCTG	1	NC	NC	NC
1703	2926	TTCTTCCATAAATGTACTCT	0	NC	NC	NC
1704	2927	GTTCTTCCATAAATGTACTC	1	2	NC	NC
1705	2928	AGTTCTTCCATAAATGTACT	1	2	NC	NC
1706	2929	CAGTTCTTCCATAAATGTAC	1	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1707	2930	TCAGTTCTTCCATAAATGTA	1	2	NC	NC
1708	2931	GTCAGTTCTTCCATAAATGT	1	1	NC	NC
1709	2932	AGTCAGTTCTTCCATAAATG	2	1	NC	NC
1710	2933	CAGTCAGTTCTTCCATAAAT	1	2	NC	NC
1711	2934	TCAGTCAGTTCTTCCATAAA	2	1	NC	NC
1712	2935	GTCAGTCAGTTCTTCCATAA	2	1	NC	NC
1713	2936	TGTCAGTCAGTTCTTCCATA	2	2	NC	NC
1714	2937	GTGTCAGTCAGTTCTTCCAT	2	2	NC	NC
1715	2938	TGTGTCAGTCAGTTCTTCCA	2	1	NC	NC
1716	2939	CTGTGTCAGTCAGTTCTTCC	2	2	NC	NC
1717	2940	GCTGTGTCAGTCAGTTCTTC	2	2	NC	NC
1718	2941	TGCTGTGTCAGTCAGTTCTT	2	2	NC	NC
1719	2942	CTGCTGTGTCAGTCAGTTCT	2	2	NC	NC
1720	2943	TCTGCTGTGTCAGTCAGTTC	2	2	NC	NC
1721	2944	TTCTGCTGTGTCAGTCAGTT	2	2	NC	NC
1722	2945	TTTCTGCTGTGTCAGTCAGT	2	2	NC	NC
1723	2946	ATTTCTGCTGTGTCAGTCAG	2	1	NC	NC
1724	2947	TATTTCTGCTGTGTCAGTCA	2	2	NC	NC
1725	2948	TTATTTCTGCTGTGTCAGTC	2	1	NC	NC
1726	2949	ATTATTTCTGCTGTGTCAGT	1	1	NC	NC
1727	2950	GATTATTTCTGCTGTGTCAG	2	2	NC	NC
1728	2951	TGATTATTTCTGCTGTGTCA	2	2	NC	NC
1729	2952	CTGATTATTTCTGCTGTGTC	2	2	NC	NC
1730	2953	TCTGATTATTTCTGCTGTGT	2	2	NC	NC
1731	2954	TTCTGATTATTTCTGCTGTG	2	2	NC	NC
1732	2955	TTTCTGATTATTTCTGCTGT	1	2	NC	NC
1733	2956	TTTTCTGATTATTTCTGCTG	1	2	NC	NC
1734	2957	CTTTTCTGATTATTTCTGCT	1	1	NC	NC
1735	2958	GCTTTTCTGATTATTTCTGC	1	1	NC	NC
1736	2959	TGCTTTTCTGATTATTTCTG	1	1	NC	NC
1737	2960	TTGCTTTTCTGATTATTTCT	1	1	NC	NC
1738	2961	GTTGCTTTTCTGATTATTTC	2	1	NC	NC
1739	2962	TGTTGCTTTTCTGATTATTT	1	1	NC	NC
1740	2963	ATGTTGCTTTTCTGATTATT	2	2	NC	NC
1741	2964	GATGTTGCTTTTCTGATTAT	2	2	NC	NC
1742	2965	TGATGTTGCTTTTCTGATTA	1	1	NC	NC
1743	2966	GTGATGTTGCTTTTCTGATT	1	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1744	2967	TGTGATGTTGCTTTTCTGAT	1	1	NC	NC
1745	2968	CTGTGATGTTGCTTTTCTGA	2	2	NC	NC
1746	2969	ACTGTGATGTTGCTTTTCTG	2	2	NC	NC
1747	2970	GACTGTGATGTTGCTTTTCT	2	2	NC	NC
1748	2971	GGACTGTGATGTTGCTTTTC	1	2	NC	NC
1749	2972	AGGACTGTGATGTTGCTTTT	1	2	NC	NC
1750	2973	AAGGACTGTGATGTTGCTTT	1	2	NC	NC
1751	2974	CAAGGACTGTGATGTTGCTT	2	2	NC	NC
1752	2975	CCAAGGACTGTGATGTTGCT	2	2	NC	NC
1753	2976	ACCAAGGACTGTGATGTTGC	2	2	NC	NC
1754	2977	AACCAAGGACTGTGATGTTG	2	2	NC	NC
1755	2978	TAACCAAGGACTGTGATGTT	2	2	NC	NC
1756	2979	ATAACCAAGGACTGTGATGT	2	2	NC	NC
1757	2980	GATAACCAAGGACTGTGATG	3	3	NC	NC
1758	2981	AGATAACCAAGGACTGTGAT	2	2	NC	NC
1759	2982	AAGATAACCAAGGACTGTGA	1	1	NC	NC
1760	2983	CAAGATAACCAAGGACTGTG	1	1	NC	NC
1761	2984	CCAAGATAACCAAGGACTGT	1	2	NC	NC
1762	2985	TCCAAGATAACCAAGGACTG	2	2	NC	NC
1763	2986	ATCCAAGATAACCAAGGACT	2	2	NC	NC
1764	2987	CATCCAAGATAACCAAGGAC	2	2	NC	NC
1765	2988	TCATCCAAGATAACCAAGGA	2	2	NC	NC
1766	2989	TTCATCCAAGATAACCAAGG	2	2	NC	NC
1767	2990	GTTCATCCAAGATAACCAAG	2	NC	NC	NC
1768	2991	AGTTCATCCAAGATAACCAA	2	NC	NC	NC
1769	2992	TAGTTCATCCAAGATAACCA	2	NC	NC	NC
1770	2993	CTAGTTCATCCAAGATAACC	2	NC	NC	NC
1771	2994	CCTAGTTCATCCAAGATAAC	2	NC	NC	NC
1772	2995	TCCTAGTTCATCCAAGATAA	2	NC	NC	NC
1773	2996	TTCCTAGTTCATCCAAGATA	2	NC	NC	NC
1774	2997	CTTCCTAGTTCATCCAAGAT	2	NC	NC	NC
1775	2998	TCTTCCTAGTTCATCCAAGA	2	NC	NC	NC
1776	2999	CTCTTCCTAGTTCATCCAAG	2	NC	NC	NC
1777	3000	CCTCTTCCTAGTTCATCCAA	2	NC	NC	NC
1778	3001	CCCTCTTCCTAGTTCATCCA	2	NC	NC	NC
1779	3002	TCCCTCTTCCTAGTTCATCC	2	NC	NC	NC
1780	3003	GTCCCTCTTCCTAGTTCATC	3	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1781	3004	CGTCCCTCTTCCTAGTTCAT	2	NC	NC	NC
1782	3005	TCGTCCCTCTTCCTAGTTCA	2	NC	NC	NC
1783	3006	CTCGTCCCTCTTCCTAGTTC	2	NC	NC	NC
1784	3007	GCTCGTCCCTCTTCCTAGTT	2	NC	NC	NC
1785	3008	TGCTCGTCCCTCTTCCTAGT	2	NC	NC	NC
1786	3010	AGTGCTCGTCCCTCTTCCTA	2	NC	NC	NC
1787	3013	ATGAGTGCTCGTCCCTCTTC	2	NC	NC	NC
1788	3014	CATGAGTGCTCGTCCCTCTT	2	NC	NC	NC
1789	3015	TCATGAGTGCTCGTCCCTCT	2	NC	NC	NC
1790	3016	ATCATGAGTGCTCGTCCCTC	2	NC	NC	NC
1791	3017	CATCATGAGTGCTCGTCCCT	2	NC	NC	NC
1792	3019	TCCATCATGAGTGCTCGTCC	2	NC	NC	NC
1793	3020	TTCCATCATGAGTGCTCGTC	3	NC	NC	NC
1794	3021	ATTCCATCATGAGTGCTCGT	2	NC	NC	NC
1795	3022	AATTCCATCATGAGTGCTCG	2	NC	NC	NC
1796	3023	CAATTCCATCATGAGTGCTC	2	NC	NC	NC
1797	3024	GCAATTCCATCATGAGTGCT	2	NC	NC	NC
1798	3025	GGCAATTCCATCATGAGTGC	2	NC	NC	NC
1799	3049	ATACTCAAGTGTAGCATAGG	3	3	NC	NC
1800	3050	AATACTCAAGTGTAGCATAG	2	2	NC	NC
1801	3051	AAATACTCAAGTGTAGCATA	2	2	NC	NC
1802	3052	GAAATACTCAAGTGTAGCAT	1	1	NC	NC
1803	3053	TGAAATACTCAAGTGTAGCA	1	1	NC	NC
1804	3054	ATGAAATACTCAAGTGTAGC	1	1	NC	NC
1805	3055	GATGAAATACTCAAGTGTAG	1	2	NC	NC
1806	3056	TGATGAAATACTCAAGTGTA	2	2	NC	NC
1807	3057	CTGATGAAATACTCAAGTGT	1	2	NC	NC
1808	3058	TCTGATGAAATACTCAAGTG	1	2	NC	NC
1809	3059	CTCTGATGAAATACTCAAGT	1	2	NC	NC
1810	3060	TCTCTGATGAAATACTCAAG	1	2	NC	NC
1811	3061	ATCTCTGATGAAATACTCAA	2	2	NC	NC
1812	3063	ACATCTCTGATGAAATACTC	2	2	NC	NC
1813	3064	CACATCTCTGATGAAATACT	2	2	NC	NC
1814	3065	TCACATCTCTGATGAAATAC	2	1	NC	NC
1815	3066	TTCACATCTCTGATGAAATA	1	1	NC	NC
1816	3067	TTTCACATCTCTGATGAAAT	1	1	NC	NC
1817	3069	GATTTCACATCTCTGATGAA	1	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1818	3070	GGATTTCACATCTCTGATGA	2	2	NC	NC
1819	3071	AGGATTTCACATCTCTGATG	2	2	NC	NC
1820	3072	AAGGATTTCACATCTCTGAT	2	2	NC	NC
1821	3073	TAAGGATTTCACATCTCTGA	2	2	NC	NC
1822	3074	TTAAGGATTTCACATCTCTG	2	2	NC	NC
1823	3075	GTTAAGGATTTCACATCTCT	2	2	NC	NC
1824	3076	GGTTAAGGATTTCACATCTC	2	2	NC	NC
1825	3077	GGGTTAAGGATTTCACATCT	2	2	NC	NC
1826	3078	AGGGTTAAGGATTTCACATC	2	2	NC	NC
1827	3079	CAGGGTTAAGGATTTCACAT	2	2	NC	NC
1828	3080	ACAGGGTTAAGGATTTCACA	2	2	NC	NC
1829	3081	AACAGGGTTAAGGATTTCAC	2	2	NC	NC
1830	3082	AAACAGGGTTAAGGATTTCA	2	2	NC	NC
1831	3083	CAAACAGGGTTAAGGATTTC	2	2	NC	NC
1832	3084	ACAAACAGGGTTAAGGATTT	1	2	NC	NC
1833	3085	GACAAACAGGGTTAAGGATT	2	2	NC	NC
1834	3086	TGACAAACAGGGTTAAGGAT	1	1	NC	NC
1835	3087	GTGACAAACAGGGTTAAGGA	1	2	NC	NC
1836	3088	GGTGACAAACAGGGTTAAGG	1	2	NC	NC
1837	3089	GGGTGACAAACAGGGTTAAG	1	2	NC	NC
1838	3090	TGGGTGACAAACAGGGTTAA	1	2	NC	NC
1839	3091	ATGGGTGACAAACAGGGTTA	2	2	NC	NC
1840	3092	AATGGGTGACAAACAGGGTT	2	2	NC	NC
1841	3093	TAATGGGTGACAAACAGGGT	2	2	NC	NC
1842	3094	ATAATGGGTGACAAACAGGG	1	2	NC	NC
1843	3095	GATAATGGGTGACAAACAGG	2	2	NC	NC
1844	3096	GGATAATGGGTGACAAACAG	2	2	NC	NC
1845	3097	CGGATAATGGGTGACAAACA	2	2	NC	NC
1846	3098	GCGGATAATGGGTGACAAAC	2	3	NC	NC
1847	3099	GGCGGATAATGGGTGACAAA	2	2	NC	NC
1848	3100	TGGCGGATAATGGGTGACAA	2	3	NC	NC
1849	3101	CTGGCGGATAATGGGTGACA	3	2	NC	NC
1850	3102	ACTGGCGGATAATGGGTGAC	3	2	NC	NC
1851	3103	AACTGGCGGATAATGGGTGA	2	1	NC	NC
1852	3104	AAACTGGCGGATAATGGGTG	3	2	NC	NC
1853	3105	CAAACTGGCGGATAATGGGT	3	3	NC	NC
1854	3106	ACAAACTGGCGGATAATGGG	3	3	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1855	3107	CACAAACTGGCGGATAATGG	3	3	NC	NC
1856	3108	TCACAAACTGGCGGATAATG	3	3	NC	NC
1857	3109	TTCACAAACTGGCGGATAAT	2	NC	NC	NC
1858	3110	GTTCACAAACTGGCGGATAA	3	NC	NC	NC
1859	3135	ACCTGGTGTGAGTAATTTTT	2	2	NC	NC
1860	3146	GGTAATTCCCCACCTGGTGT	2	2	NC	NC
1861	3147	TGGTAATTCCCCACCTGGTG	2	3	NC	NC
1862	3154	TCCCATGTGGTAATTCCCCA	3	2	2	NC
1863	3155	ATCCCATGTGGTAATTCCCC	2	2	2	NC
1864	3156	AATCCCATGTGGTAATTCCC	2	3	2	NC
1865	3157	GAATCCCATGTGGTAATTCC	2	2	2	NC
1866	3158	AGAATCCCATGTGGTAATTC	2	2	1	NC
1867	3159	AAGAATCCCATGTGGTAATT	2	2	1	NC
1868	3160	CAAGAATCCCATGTGGTAAT	2	2	2	NC
1869	3161	CCAAGAATCCCATGTGGTAA	1	1	2	NC
1870	3164	TGACCAAGAATCCCATGTGG	2	2	2	NC
1871	3165	CTGACCAAGAATCCCATGTG	2	2	NC	NC
1872	3166	ACTGACCAAGAATCCCATGT	2	NC	NC	NC
1873	3167	CACTGACCAAGAATCCCATG	2	NC	NC	NC
1874	3168	TCACTGACCAAGAATCCCAT	2	NC	NC	NC
1875	3169	CTCACTGACCAAGAATCCCA	2	NC	NC	NC
1876	3170	CCTCACTGACCAAGAATCCC	2	NC	NC	NC
1877	3171	TCCTCACTGACCAAGAATCC	2	NC	NC	NC
1878	3172	ATCCTCACTGACCAAGAATC	2	NC	NC	NC
1879	3173	CATCCTCACTGACCAAGAAT	2	NC	NC	NC
1880	3174	TCATCCTCACTGACCAAGAA	2	NC	NC	NC
1881	3175	TTCATCCTCACTGACCAAGA	2	NC	NC	NC
1882	3176	TTTCATCCTCACTGACCAAG	2	NC	NC	NC
1883	3177	CTTTCATCCTCACTGACCAA	2	NC	NC	NC
1884	3178	GCTTTCATCCTCACTGACCA	2	NC	NC	NC
1885	3179	TGCTTTCATCCTCACTGACC	2	NC	NC	NC
1886	3180	TTGCTTTCATCCTCACTGAC	2	NC	NC	NC
1887	3181	TTTGCTTTCATCCTCACTGA	2	NC	NC	NC
1888	3182	GTTTGCTTTCATCCTCACTG	1	NC	NC	NC
1889	3183	AGTTTGCTTTCATCCTCACT	2	NC	NC	NC
1890	3184	CAGTTTGCTTTCATCCTCAC	2	NC	NC	NC
1891	3185	CCAGTTTGCTTTCATCCTCA	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1892	3186	TCCAGTTTGCTTTCATCCTC	2	2	NC	NC
1893	3187	ATCCAGTTTGCTTTCATCCT	2	2	NC	NC
1894	3188	GATCCAGTTTGCTTTCATCC	2	2	NC	NC
1895	3189	GGATCCAGTTTGCTTTCATC	2	2	NC	NC
1896	3190	TGGATCCAGTTTGCTTTCAT	2	2	NC	NC
1897	3205	TTGTTCTGCTGCGCCTGGAT	2	NC	NC	NC
1898	3213	TCAGGGACTTGTTCTGCTGC	2	NC	NC	NC
1899	3214	ATCAGGGACTTGTTCTGCTG	2	NC	NC	NC
1900	3215	AATCAGGGACTTGTTCTGCT	2	NC	NC	NC
1901	3216	AAATCAGGGACTTGTTCTGC	2	NC	NC	NC
1902	3217	AAAATCAGGGACTTGTTCTG	2	NC	NC	NC
1903	3218	CAAAATCAGGGACTTGTTCT	2	2	NC	NC
1904	3219	ACAAAATCAGGGACTTGTTC	2	2	NC	NC
1905	3220	GACAAAATCAGGGACTTGTT	2	2	NC	NC
1906	3221	TGACAAAATCAGGGACTTGT	2	2	NC	NC
1907	3222	GTGACAAAATCAGGGACTTG	2	2	NC	NC
1908	3223	GGTGACAAAATCAGGGACTT	2	2	NC	NC
1909	3224	AGGTGACAAAATCAGGGACT	1	1	NC	NC
1910	3225	AAGGTGACAAAATCAGGGAC	1	2	NC	NC
1911	3226	GAAGGTGACAAAATCAGGGA	1	2	NC	NC
1912	3227	GGAAGGTGACAAAATCAGGG	1	2	NC	NC
1913	3228	AGGAAGGTGACAAAATCAGG	1	2	NC	NC
1914	3229	AAGGAAGGTGACAAAATCAG	1	1	NC	NC
1915	3230	AAAGGAAGGTGACAAAATCA	1	1	NC	NC
1916	3231	TAAAGGAAGGTGACAAAATC	2	2	NC	NC
1917	3232	GTAAAGGAAGGTGACAAAAT	1	2	NC	NC
1918	3233	GGTAAAGGAAGGTGACAAAA	2	1	NC	NC
1919	3234	TGGTAAAGGAAGGTGACAAA	1	2	NC	NC
1920	3235	TTGGTAAAGGAAGGTGACAA	1	2	NC	NC
1921	3236	TTTGGTAAAGGAAGGTGACA	1	2	NC	NC
1922	3237	ATTTGGTAAAGGAAGGTGAC	2	1	NC	NC
1923	3238	TATTTGGTAAAGGAAGGTGA	2	2	NC	NC
1924	3239	TTATTTGGTAAAGGAAGGTG	1	1	NC	NC
1925	3240	GTTATTTGGTAAAGGAAGGT	2	2	NC	NC
1926	3241	AGTTATTTGGTAAAGGAAGG	2	2	NC	NC
1927	3242	TAGTTATTTGGTAAAGGAAG	2	2	NC	NC
1928	3243	CTAGTTATTTGGTAAAGGAA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1929	3244	TCTAGTTATTTGGTAAAGGA	2	2	NC	NC
1930	3245	CTCTAGTTATTTGGTAAAGG	1	1	NC	NC
1931	3246	CCTCTAGTTATTTGGTAAAG	2	2	NC	NC
1932	3247	TCCTCTAGTTATTTGGTAAA	1	2	NC	NC
1933	3248	TTCCTCTAGTTATTTGGTAA	1	1	NC	NC
1934	3249	ATTCCTCTAGTTATTTGGTA	1	1	NC	NC
1935	3250	AATTCCTCTAGTTATTTGGT	2	2	NC	NC
1936	3251	CAATTCCTCTAGTTATTTGG	2	1	NC	NC
1937	3252	GCAATTCCTCTAGTTATTTG	2	2	NC	NC
1938	3253	TGCAATTCCTCTAGTTATTT	1	2	NC	NC
1939	3254	CTGCAATTCCTCTAGTTATT	1	1	NC	NC
1940	3255	GCTGCAATTCCTCTAGTTAT	2	1	NC	NC
1941	3256	TGCTGCAATTCCTCTAGTTA	3	2	NC	NC
1942	3257	TTGCTGCAATTCCTCTAGTT	2	1	NC	NC
1943	3258	CTTGCTGCAATTCCTCTAGT	2	1	NC	NC
1944	3259	CCTTGCTGCAATTCCTCTAG	2	1	NC	NC
1945	3260	TCCTTGCTGCAATTCCTCTA	2	2	NC	NC
1946	3261	CTCCTTGCTGCAATTCCTCT	1	1	NC	NC
1947	3262	ACTCCTTGCTGCAATTCCTC	2	2	NC	NC
1948	3263	AACTCCTTGCTGCAATTCCT	2	2	NC	NC
1949	3264	TAACTCCTTGCTGCAATTCC	1	1	NC	NC
1950	3265	ATAACTCCTTGCTGCAATTC	1	1	NC	NC
1951	3266	CATAACTCCTTGCTGCAATT	1	1	NC	NC
1952	3267	CCATAACTCCTTGCTGCAAT	2	2	NC	NC
1953	3268	TCCATAACTCCTTGCTGCAA	2	2	NC	NC
1954	3269	ATCCATAACTCCTTGCTGCA	2	2	NC	NC
1955	3270	AATCCATAACTCCTTGCTGC	2	2	NC	NC
1956	3271	TAATCCATAACTCCTTGCTG	2	2	NC	NC
1957	3272	TTAATCCATAACTCCTTGCT	2	1	NC	NC
1958	3273	TTTAATCCATAACTCCTTGC	2	1	NC	NC
1959	3274	ATTTAATCCATAACTCCTTG	2	1	NC	NC
1960	3275	CATTTAATCCATAACTCCTT	2	1	NC	NC
1961	3276	ACATTTAATCCATAACTCCT	1	2	NC	NC
1962	3277	CACATTTAATCCATAACTCC	2	2	NC	NC
1963	3278	CCACATTTAATCCATAACTC	2	2	NC	NC
1964	3279	GCCACATTTAATCCATAACT	2	2	NC	NC
1965	3280	AGCCACATTTAATCCATAAC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
1966	3281	TAGCCACATTTAATCCATAA	2	2	NC	NC
1967	3282	TTAGCCACATTTAATCCATA	2	2	NC	NC
1968	3283	TTTAGCCACATTTAATCCAT	2	1	NC	NC
1969	3284	GTTTAGCCACATTTAATCCA	2	2	NC	NC
1970	3285	AGTTTAGCCACATTTAATCC	2	2	NC	NC
1971	3286	TAGTTTAGCCACATTTAATC	2	2	NC	NC
1972	3287	CTAGTTTAGCCACATTTAAT	2	3	NC	NC
1973	3298	AGGAACATCTGCTAGTTTAG	2	NC	NC	NC
1974	3299	CAGGAACATCTGCTAGTTTA	2	NC	NC	NC
1975	3300	CCAGGAACATCTGCTAGTTT	2	NC	NC	NC
1976	3301	TCCAGGAACATCTGCTAGTT	2	NC	NC	NC
1977	3302	CTCCAGGAACATCTGCTAGT	2	NC	NC	NC
1978	3303	TCTCCAGGAACATCTGCTAG	2	NC	NC	NC
1979	3304	TTCTCCAGGAACATCTGCTA	2	NC	NC	NC
1980	3305	TTTCTCCAGGAACATCTGCT	1	NC	NC	NC
1981	3306	ATTTCTCCAGGAACATCTGC	2	NC	NC	NC
1982	3307	AATTTCTCCAGGAACATCTG	1	NC	NC	NC
1983	3308	AAATTTCTCCAGGAACATCT	2	NC	NC	NC
1984	3309	AAAATTTCTCCAGGAACATC	2	NC	NC	NC
1985	3310	CAAAATTTCTCCAGGAACAT	1	NC	NC	NC
1986	3311	TCAAAATTTCTCCAGGAACA	1	NC	NC	NC
1987	3312	TTCAAAATTTCTCCAGGAAC	2	NC	NC	NC
1988	3313	CTTCAAAATTTCTCCAGGAA	2	1	NC	NC
1989	3314	TCTTCAAAATTTCTCCAGGA	2	1	NC	NC
1990	3315	TTCTTCAAAATTTCTCCAGG	1	2	NC	NC
1991	3316	TTTCTTCAAAATTTCTCCAG	1	1	NC	NC
1992	3317	CTTTCTTCAAAATTTCTCCA	1	2	NC	NC
1993	3318	GCTTTCTTCAAAATTTCTCC	2	2	NC	NC
1994	3319	TGCTTTCTTCAAAATTTCTC	2	2	NC	NC
1995	3320	CTGCTTTCTTCAAAATTTCT	2	2	NC	NC
1996	3321	GCTGCTTTCTTCAAAATTTC	2	2	NC	NC
1997	3322	AGCTGCTTTCTTCAAAATTT	1	1	NC	NC
1998	3323	GAGCTGCTTTCTTCAAAATT	2	1	NC	NC
1999	3324	TGAGCTGCTTTCTTCAAAAT	2	1	NC	NC
2000	3325	GTGAGCTGCTTTCTTCAAAA	2	2	NC	NC
2001	3326	TGTGAGCTGCTTTCTTCAAA	2	2	NC	NC
2002	3327	TTGTGAGCTGCTTTCTTCAA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2003	3328	CTTGTGAGCTGCTTTCTTCA	1	1	NC	NC
2004	3329	ACTTGTGAGCTGCTTTCTTC	2	2	NC	NC
2005	3330	GACTTGTGAGCTGCTTTCTT	2	2	NC	NC
2006	3331	TGACTTGTGAGCTGCTTTCT	2	2	NC	NC
2007	3332	TTGACTTGTGAGCTGCTTTC	2	2	NC	NC
2008	3333	TTTGACTTGTGAGCTGCTTT	1	2	NC	NC
2009	3334	TTTTGACTTGTGAGCTGCTT	2	2	NC	NC
2010	3335	CTTTTGACTTGTGAGCTGCT	2	2	NC	NC
2011	3336	TCTTTTGACTTGTGAGCTGC	2	2	NC	NC
2012	3337	CTCTTTTGACTTGTGAGCTG	2	2	NC	NC
2013	3340	CAGCTCTTTTGACTTGTGAG	2	3	NC	NC
2014	3341	CCAGCTCTTTTGACTTGTGA	2	2	NC	NC
2015	3342	TCCAGCTCTTTTGACTTGTG	2	2	NC	NC
2016	3343	TTCCAGCTCTTTTGACTTGT	2	2	NC	NC
2017	3344	CTTCCAGCTCTTTTGACTTG	1	2	NC	NC
2018	3345	CCTTCCAGCTCTTTTGACTT	1	1	NC	NC
2019	3346	TCCTTCCAGCTCTTTTGACT	2	2	NC	NC
2020	3347	ATCCTTCCAGCTCTTTTGAC	2	2	NC	NC
2021	3348	AATCCTTCCAGCTCTTTTGA	1	1	NC	NC
2022	3349	TAATCCTTCCAGCTCTTTTG	1	2	NC	NC
2023	3350	TTAATCCTTCCAGCTCTTTT	1	2	NC	NC
2024	3351	ATTAATCCTTCCAGCTCTTT	1	1	NC	NC
2025	3352	TATTAATCCTTCCAGCTCTT	2	2	NC	NC
2026	3353	TTATTAATCCTTCCAGCTCT	2	2	NC	NC
2027	3354	TTTATTAATCCTTCCAGCTC	2	2	NC	NC
2028	3355	ATTTATTAATCCTTCCAGCT	1	2	NC	NC
2029	3356	TATTTATTAATCCTTCCAGC	2	2	NC	NC
2030	3357	GTATTTATTAATCCTTCCAG	1	1	NC	NC
2031	3358	CGTATTTATTAATCCTTCCA	1	2	NC	NC
2032	3359	TCGTATTTATTAATCCTTCC	2	2	NC	NC
2033	3360	TTCGTATTTATTAATCCTTC	1	2	NC	NC
2034	3363	CTTTTCGTATTTATTAATCC	2	2	NC	NC
2035	3369	CTCTTTCTTTTCGTATTTAT	1	NC	NC	NC
2036	3370	TCTCTTTCTTTTCGTATTTA	1	NC	NC	NC
2037	3371	GTCTCTTTCTTTTCGTATTT	2	NC	NC	NC
2038	3372	AGTCTCTTTCTTTTCGTATT	2	NC	NC	NC
2039	3373	GAGTCTCTTTCTTTTCGTAT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2040	3374	TGAGTCTCTTTCTTTTCGTA	2	NC	NC	NC
2041	3375	TTGAGTCTCTTTCTTTTCGT	2	NC	NC	NC
2042	3376	CTTGAGTCTCTTTCTTTTCG	1	NC	NC	NC
2043	3377	ACTTGAGTCTCTTTCTTTTC	1	NC	NC	NC
2044	3378	TACTTGAGTCTCTTTCTTTT	2	NC	NC	NC
2045	3379	ATACTTGAGTCTCTTTCTTT	2	NC	NC	NC
2046	3380	AATACTTGAGTCTCTTTCTT	2	NC	NC	NC
2047	3381	AAATACTTGAGTCTCTTTCT	2	NC	NC	NC
2048	3382	AAAATACTTGAGTCTCTTTC	2	NC	NC	NC
2049	3383	CAAAATACTTGAGTCTCTTT	2	NC	NC	NC
2050	3384	GCAAAATACTTGAGTCTCTT	1	2	NC	NC
2051	3385	TGCAAAATACTTGAGTCTCT	1	2	NC	NC
2052	3386	TTGCAAAATACTTGAGTCTC	2	2	NC	NC
2053	3387	TTTGCAAAATACTTGAGTCT	1	1	NC	NC
2054	3388	CTTTGCAAAATACTTGAGTC	1	1	NC	NC
2055	3389	ACTTTGCAAAATACTTGAGT	2	2	NC	NC
2056	3390	AACTTTGCAAAATACTTGAG	1	2	NC	NC
2057	3391	TAACTTTGCAAAATACTTGA	2	1	NC	NC
2058	3392	ATAACTTTGCAAAATACTTG	2	2	NC	NC
2059	3393	CATAACTTTGCAAAATACTT	2	2	NC	NC
2060	3394	CCATAACTTTGCAAAATACT	1	1	NC	NC
2061	3395	TCCATAACTTTGCAAAATAC	2	2	NC	NC
2062	3396	GTCCATAACTTTGCAAAATA	2	2	NC	NC
2063	3397	CGTCCATAACTTTGCAAAAT	2	2	NC	NC
2064	3398	TCGTCCATAACTTTGCAAAA	2	2	NC	NC
2065	3399	ATCGTCCATAACTTTGCAAA	2	3	NC	NC
2066	3400	CATCGTCCATAACTTTGCAA	3	2	NC	NC
2067	3401	GCATCGTCCATAACTTTGCA	2	2	NC	NC
2068	3402	TGCATCGTCCATAACTTTGC	2	2	NC	NC
2069	3403	ATGCATCGTCCATAACTTTG	2	2	NC	NC
2070	3404	TATGCATCGTCCATAACTTT	3	2	NC	NC
2071	3405	TTATGCATCGTCCATAACTT	3	3	NC	NC
2072	3406	ATTATGCATCGTCCATAACT	2	2	NC	NC
2073	3407	CATTATGCATCGTCCATAAC	2	3	NC	NC
2074	3427	CCACTTCTGCAGGTCTTGTG	1	2	NC	NC
2075	3428	TCCACTTCTGCAGGTCTTGT	2	2	NC	NC
2076	3429	GTCCACTTCTGCAGGTCTTG	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2077	3430	TGTCCACTTCTGCAGGTCTT	2	2	NC	NC
2078	3431	CTGTCCACTTCTGCAGGTCT	2	2	NC	NC
2079	3432	TCTGTCCACTTCTGCAGGTC	2	2	NC	NC
2080	3433	CTCTGTCCACTTCTGCAGGT	1	1	NC	NC
2081	3435	TCCTCTGTCCACTTCTGCAG	2	1	NC	NC
2082	3436	CTCCTCTGTCCACTTCTGCA	1	2	NC	NC
2083	3437	ACTCCTCTGTCCACTTCTGC	2	2	NC	NC
2084	3438	AACTCCTCTGTCCACTTCTG	1	1	NC	NC
2085	3439	GAACTCCTCTGTCCACTTCT	2	2	NC	NC
2086	3440	TGAACTCCTCTGTCCACTTC	1	NC	NC	NC
2087	3441	TTGAACTCCTCTGTCCACTT	1	NC	NC	NC
2088	3442	GTTGAACTCCTCTGTCCACT	2	NC	NC	NC
2089	3443	TGTTGAACTCCTCTGTCCAC	2	NC	NC	NC
2090	3444	ATGTTGAACTCCTCTGTCCA	2	NC	NC	NC
2091	3445	CATGTTGAACTCCTCTGTCC	2	NC	NC	NC
2092	3446	CCATGTTGAACTCCTCTGTC	2	NC	NC	NC
2093	3447	TCCATGTTGAACTCCTCTGT	2	NC	NC	NC
2094	3448	TTCCATGTTGAACTCCTCTG	2	NC	NC	NC
2095	3449	CTTCCATGTTGAACTCCTCT	2	NC	NC	NC
2096	3450	TCTTCCATGTTGAACTCCTC	2	NC	NC	NC
2097	3451	TTCTTCCATGTTGAACTCCT	2	NC	NC	NC
2098	3452	TTTCTTCCATGTTGAACTCC	2	NC	NC	NC
2099	3453	GTTTCTTCCATGTTGAACTC	2	NC	NC	NC
2100	3454	TGTTTCTTCCATGTTGAACT	2	NC	NC	NC
2101	3455	GTGTTTCTTCCATGTTGAAC	2	NC	NC	NC
2102	3456	TGTGTTTCTTCCATGTTGAA	1	NC	NC	NC
2103	3457	CTGTGTTTCTTCCATGTTGA	1	NC	NC	NC
2104	3458	TCTGTGTTTCTTCCATGTTG	2	NC	NC	NC
2105	3459	GTCTGTGTTTCTTCCATGTT	2	NC	NC	NC
2106	3460	AGTCTGTGTTTCTTCCATGT	2	NC	NC	NC
2107	3461	AAGTCTGTGTTTCTTCCATG	2	NC	NC	NC
2108	3462	GAAGTCTGTGTTTCTTCCAT	2	2	NC	NC
2109	3463	AGAAGTCTGTGTTTCTTCCA	1	1	NC	NC
2110	3464	GAGAAGTCTGTGTTTCTTCC	2	2	NC	NC
2111	3466	AAGAGAAGTCTGTGTTTCTT	2	1	NC	NC
2112	3467	GAAGAGAAGTCTGTGTTTCT	1	1	NC	NC
2113	3468	AGAAGAGAAGTCTGTGTTTC	1	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2114	3469	AAGAAGAGAAGTCTGTGTTT	2	NC	NC	NC
2115	3470	GAAGAAGAGAAGTCTGTGTT	1	NC	NC	NC
2116	3471	TGAAGAAGAGAAGTCTGTGT	1	NC	NC	NC
2117	3472	ATGAAGAAGAGAAGTCTGTG	1	NC	NC	NC
2118	3473	AATGAAGAAGAGAAGTCTGT	2	NC	NC	NC
2119	3474	TAATGAAGAAGAGAAGTCTG	2	NC	NC	NC
2120	3475	TTAATGAAGAAGAGAAGTCT	2	NC	NC	NC
2121	3476	TTTAATGAAGAAGAGAAGTC	2	NC	NC	NC
2122	3477	TTTTAATGAAGAAGAGAAGT	1	NC	NC	NC
2123	3478	ATTTTAATGAAGAAGAGAAG	1	NC	NC	NC
2124	3495	TCACAAATGTAGTCTTCATT	2	NC	NC	NC
2125	3496	TTCACAAATGTAGTCTTCAT	2	NC	NC	NC
2126	3497	GTTCACAAATGTAGTCTTCA	2	NC	NC	NC
2127	3498	TGTTCACAAATGTAGTCTTC	1	NC	NC	NC
2128	3499	TTGTTCACAAATGTAGTCTT	2	NC	NC	NC
2129	3521	GGTATTTTTAATTCTCCATT	1	1	NC	NC
2130	3522	TGGTATTTTTAATTCTCCAT	1	1	NC	NC
2131	3523	TTGGTATTTTTAATTCTCCA	1	1	NC	NC
2132	3524	GTTGGTATTTTTAATTCTCC	2	2	NC	NC
2133	3525	AGTTGGTATTTTTAATTCTC	1	1	NC	NC
2134	3526	CAGTTGGTATTTTTAATTCT	1	1	NC	NC
2135	3527	ACAGTTGGTATTTTTAATTC	2	1	NC	NC
2136	3529	GTACAGTTGGTATTTTTAAT	1	1	NC	NC
2137	3530	TGTACAGTTGGTATTTTTAA	1	1	NC	NC
2138	3531	TTGTACAGTTGGTATTTTTA	2	2	NC	NC
2139	3532	TTTGTACAGTTGGTATTTTT	1	1	NC	NC
2140	3533	TTTTGTACAGTTGGTATTTT	2	1	NC	NC
2141	3534	ATTTTGTACAGTTGGTATTT	2	1	NC	NC
2142	3535	TATTTTGTACAGTTGGTATT	1	1	NC	NC
2143	3536	TTATTTTGTACAGTTGGTAT	2	2	NC	NC
2144	3537	GTTATTTTGTACAGTTGGTA	3	2	NC	NC
2145	3538	AGTTATTTTGTACAGTTGGT	3	2	NC	NC
2146	3539	GAGTTATTTTGTACAGTTGG	2	2	NC	NC
2147	3540	AGAGTTATTTTGTACAGTTG	2	2	NC	NC
2148	3541	GAGAGTTATTTTGTACAGTT	2	1	NC	NC
2149	3542	GGAGAGTTATTTTGTACAGT	2	2	NC	NC
2150	3543	TGGAGAGTTATTTTGTACAG	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2151	3544	CTGGAGAGTTATTTTGTACA	1	2	NC	NC
2152	3545	ACTGGAGAGTTATTTTGTAC	1	2	NC	NC
2153	3546	TACTGGAGAGTTATTTTGTA	1	1	NC	NC
2154	3547	TTACTGGAGAGTTATTTTGT	1	1	NC	NC
2155	3548	GTTACTGGAGAGTTATTTTG	1	2	NC	NC
2156	3549	TGTTACTGGAGAGTTATTTT	2	2	NC	NC
2157	3550	CTGTTACTGGAGAGTTATTT	2	2	NC	NC
2158	3551	GCTGTTACTGGAGAGTTATT	2	2	NC	NC
2159	3552	GGCTGTTACTGGAGAGTTAT	2	2	NC	NC
2160	3553	AGGCTGTTACTGGAGAGTTA	2	2	NC	NC
2161	3554	TAGGCTGTTACTGGAGAGTT	1	2	NC	NC
2162	3555	ATAGGCTGTTACTGGAGAGT	1	2	NC	NC
2163	3556	GATAGGCTGTTACTGGAGAG	1	NC	NC	NC
2164	3557	AGATAGGCTGTTACTGGAGA	2	NC	NC	NC
2165	3558	AAGATAGGCTGTTACTGGAG	2	NC	NC	NC
2166	3559	AAAGATAGGCTGTTACTGGA	2	NC	NC	NC
2167	3560	CAAAGATAGGCTGTTACTGG	2	NC	NC	NC
2168	3561	ACAAAGATAGGCTGTTACTG	1	NC	NC	NC
2169	3562	CACAAAGATAGGCTGTTACT	2	NC	NC	NC
2170	3563	ACACAAAGATAGGCTGTTAC	2	NC	NC	NC
2171	3564	CACACAAAGATAGGCTGTTA	2	NC	NC	NC
2172	3565	TCACACAAAGATAGGCTGTT	2	NC	NC	NC
2173	3566	GTCACACAAAGATAGGCTGT	2	NC	NC	NC
2174	3567	TGTCACACAAAGATAGGCTG	2	NC	NC	NC
2175	3568	ATGTCACACAAAGATAGGCT	2	NC	NC	NC
2176	3569	CATGTCACACAAAGATAGGC	1	NC	NC	NC
2177	3570	ACATGTCACACAAAGATAGG	2	NC	NC	NC
2178	3571	CACATGTCACACAAAGATAG	2	NC	NC	NC
2179	3572	TCACATGTCACACAAAGATA	2	NC	NC	NC
2180	3573	CTCACATGTCACACAAAGAT	2	NC	NC	NC
2181	3574	GCTCACATGTCACACAAAGA	1	NC	NC	NC
2182	3575	TGCTCACATGTCACACAAAG	1	NC	NC	NC
2183	3576	ATGCTCACATGTCACACAAA	1	1	NC	NC
2184	3577	TATGCTCACATGTCACACAA	1	1	NC	NC
2185	3578	TTATGCTCACATGTCACACA	1	1	NC	NC
2186	3579	TTTATGCTCACATGTCACAC	1	1	NC	NC
2187	3580	TTTTATGCTCACATGTCACA	1	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2188	3581	ATTTTATGCTCACATGTCAC	2	2	NC	NC
2189	3582	AATTTTATGCTCACATGTCA	1	2	NC	NC
2190	3583	TAATTTTATGCTCACATGTC	2	1	NC	NC
2191	3584	ATAATTTTATGCTCACATGT	2	1	NC	NC
2192	3585	CATAATTTTATGCTCACATG	1	1	NC	NC
2193	3594	TACCATGGTCATAATTTTAT	2	2	NC	NC
2194	3595	ATACCATGGTCATAATTTTA	2	2	NC	NC
2195	3596	TATACCATGGTCATAATTTT	1	2	NC	NC
2196	3597	ATATACCATGGTCATAATTT	1	2	NC	NC
2197	3598	AATATACCATGGTCATAATT	1	2	NC	NC
2198	3599	GAATATACCATGGTCATAAT	2	2	NC	NC
2199	3600	GGAATATACCATGGTCATAA	1	2	NC	NC
2200	3601	AGGAATATACCATGGTCATA	0	1	NC	NC
2201	3602	TAGGAATATACCATGGTCAT	1	2	NC	NC
2202	3603	ATAGGAATATACCATGGTCA	2	NC	NC	NC
2203	3604	AATAGGAATATACCATGGTC	2	NC	NC	NC
2204	3605	CAATAGGAATATACCATGGT	2	NC	NC	NC
2205	3606	CCAATAGGAATATACCATGG	2	NC	NC	NC
2206	3618	ACCTCTCTGTTTCCAATAGG	2	NC	NC	NC
2207	3619	AACCTCTCTGTTTCCAATAG	2	NC	NC	NC
2208	3620	AAACCTCTCTGTTTCCAATA	1	NC	NC	NC
2209	3621	AAAACCTCTCTGTTTCCAAT	2	NC	NC	NC
2210	3622	AAAAACCTCTCTGTTTCCAA	1	NC	NC	NC
2211	3623	GAAAAACCTCTCTGTTTCCA	1	2	NC	NC
2212	3624	AGAAAAACCTCTCTGTTTCC	1	2	NC	NC
2213	3625	CAGAAAAACCTCTCTGTTTC	1	1	NC	NC
2214	3630	GTCTTCAGAAAAACCTCTCT	2	1	NC	NC
2215	3631	TGTCTTCAGAAAAACCTCTC	2	2	NC	NC
2216	3643	CTTGAAAAAGACTGTCTTCA	2	NC	NC	NC
2217	3644	ACTTGAAAAAGACTGTCTTC	2	NC	NC	NC
2218	3645	AACTTGAAAAAGACTGTCTT	1	NC	NC	NC
2219	3646	AAACTTGAAAAAGACTGTCT	2	NC	NC	NC
2220	3647	GAAACTTGAAAAAGACTGTC	2	NC	NC	NC
2221	3648	AGAAACTTGAAAAAGACTGT	2	NC	NC	NC
2222	3649	CAGAAACTTGAAAAAGACTG	2	NC	NC	NC
2223	3650	ACAGAAACTTGAAAAAGACT	2	NC	NC	NC
2224	3651	GACAGAAACTTGAAAAAGAC	1	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2225	3652	AGACAGAAACTTGAAAAAGA	1	NC	NC	NC
2226	3653	AAGACAGAAACTTGAAAAAG	1	NC	NC	NC
2227	3654	GAAGACAGAAACTTGAAAAA	1	NC	NC	NC
2228	3655	GGAAGACAGAAACTTGAAAA	1	NC	NC	NC
2229	3656	AGGAAGACAGAAACTTGAAA	1	NC	NC	NC
2230	3657	TAGGAAGACAGAAACTTGAA	1	NC	NC	NC
2231	3658	TTAGGAAGACAGAAACTTGA	1	NC	NC	NC
2232	3659	GTTAGGAAGACAGAAACTTG	1	NC	NC	NC
2233	3660	AGTTAGGAAGACAGAAACTT	2	NC	NC	NC
2234	3661	AAGTTAGGAAGACAGAAACT	2	NC	NC	NC
2235	3662	AAAGTTAGGAAGACAGAAAC	2	NC	NC	NC
2236	3663	AAAAGTTAGGAAGACAGAAA	2	NC	NC	NC
2237	3664	GAAAAGTTAGGAAGACAGAA	2	NC	NC	NC
2238	3665	AGAAAAGTTAGGAAGACAGA	1	NC	NC	NC
2239	3666	TAGAAAAGTTAGGAAGACAG	2	2	NC	NC
2240	3667	GTAGAAAAGTTAGGAAGACA	2	NC	NC	NC
2241	3668	CGTAGAAAAGTTAGGAAGAC	2	NC	NC	NC
2242	3669	ACGTAGAAAAGTTAGGAAGA	2	NC	NC	NC
2243	3670	TACGTAGAAAAGTTAGGAAG	2	NC	NC	NC
2244	3671	ATACGTAGAAAAGTTAGGAA	2	NC	NC	NC
2245	3672	TATACGTAGAAAAGTTAGGA	2	NC	NC	NC
2246	3673	TTATACGTAGAAAAGTTAGG	2	NC	NC	NC
2247	3674	TTTATACGTAGAAAAGTTAG	2	NC	NC	NC
2248	3675	GTTTATACGTAGAAAAGTTA	2	NC	NC	NC
2249	3676	TGTTTATACGTAGAAAAGTT	2	NC	NC	NC
2250	3677	GTGTTTATACGTAGAAAAGT	2	NC	NC	NC
2251	3678	AGTGTTTATACGTAGAAAAG	2	NC	NC	NC
2252	3679	GAGTGTTTATACGTAGAAAA	2	NC	NC	NC
2253	3680	AGAGTGTTTATACGTAGAAA	2	NC	NC	NC
2254	3681	AAGAGTGTTTATACGTAGAA	2	NC	NC	NC
2255	3682	CAAGAGTGTTTATACGTAGA	2	NC	NC	NC
2256	3683	TCAAGAGTGTTTATACGTAG	2	NC	NC	NC
2257	3684	TTCAAGAGTGTTTATACGTA	2	NC	NC	NC
2258	3685	ATTCAAGAGTGTTTATACGT	2	NC	NC	NC
2259	3686	TATTCAAGAGTGTTTATACG	2	NC	NC	NC
2260	3687	CTATTCAAGAGTGTTTATAC	2	1	NC	NC
2261	3688	TCTATTCAAGAGTGTTTATA	2	1	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2262	3689	GTCTATTCAAGAGTGTTTAT	2	2	NC	NC
2263	3690	AGTCTATTCAAGAGTGTTTA	2	2	NC	NC
2264	3691	AAGTCTATTCAAGAGTGTTT	2	2	NC	NC
2265	3692	GAAGTCTATTCAAGAGTGTT	2	2	NC	NC
2266	3693	GGAAGTCTATTCAAGAGTGT	2	2	NC	NC
2267	3694	TGGAAGTCTATTCAAGAGTG	2	2	NC	NC
2268	3696	AGTGGAAGTCTATTCAAGAG	2	1	NC	NC
2269	3697	AAGTGGAAGTCTATTCAAGA	2	2	NC	NC
2270	3698	AAAGTGGAAGTCTATTCAAG	2	2	NC	NC
2271	3699	CAAAGTGGAAGTCTATTCAA	2	2	NC	NC
2272	3700	ACAAAGTGGAAGTCTATTCA	2	2	NC	NC
2273	3701	TACAAAGTGGAAGTCTATTC	1	2	NC	NC
2274	3702	TTACAAAGTGGAAGTCTATT	2	2	NC	NC
2275	3703	ATTACAAAGTGGAAGTCTAT	2	2	NC	NC
2276	3704	AATTACAAAGTGGAAGTCTA	2	2	NC	NC
2277	3705	TAATTACAAAGTGGAAGTCT	2	1	NC	NC
2278	3706	CTAATTACAAAGTGGAAGTC	2	2	NC	NC
2279	3707	TCTAATTACAAAGTGGAAGT	2	2	NC	NC
2280	3708	TTCTAATTACAAAGTGGAAG	2	2	NC	NC
2281	3709	TTTCTAATTACAAAGTGGAA	2	1	NC	NC
2282	3710	TTTTCTAATTACAAAGTGGA	1	1	NC	NC
2283	3711	ATTTTCTAATTACAAAGTGG	1	1	NC	NC
2284	3722	CTGTCCATAAAATTTTCTAA	2	NC	NC	NC
2285	3723	ACTGTCCATAAAATTTTCTA	2	NC	NC	NC
2286	3724	TACTGTCCATAAAATTTTCT	2	NC	NC	NC
2287	3725	TTACTGTCCATAAAATTTTC	2	NC	NC	NC
2288	3726	CTTACTGTCCATAAAATTTT	2	NC	NC	NC
2289	3727	ACTTACTGTCCATAAAATTT	1	NC	NC	NC
2290	3738	GGCTTTACTGGACTTACTGT	2	1	NC	NC
2291	3739	AGGCTTTACTGGACTTACTG	2	1	NC	NC
2292	3740	AAGGCTTTACTGGACTTACT	2	1	NC	NC
2293	3741	TAAGGCTTTACTGGACTTAC	2	2	NC	NC
2294	3742	TTAAGGCTTTACTGGACTTA	2	2	NC	NC
2295	3743	CTTAAGGCTTTACTGGACTT	2	2	NC	NC
2296	3744	ACTTAAGGCTTTACTGGACT	3	2	NC	NC
2297	3745	CACTTAAGGCTTTACTGGAC	2	3	NC	NC
2298	3746	CCACTTAAGGCTTTACTGGA	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2299	3756	TTATATTCTGCCACTTAAGG	2	2	NC	NC
2300	3757	ATTATATTCTGCCACTTAAG	2	2	NC	NC
2301	3758	AATTATATTCTGCCACTTAA	2	2	NC	NC
2302	3759	GAATTATATTCTGCCACTTA	2	2	NC	NC
2303	3760	GGAATTATATTCTGCCACTT	2	2	NC	NC
2304	3761	GGGAATTATATTCTGCCACT	3	3	NC	NC
2305	3762	TGGGAATTATATTCTGCCAC	2	2	NC	NC
2306	3763	TTGGGAATTATATTCTGCCA	2	2	NC	NC
2307	3764	CTTGGGAATTATATTCTGCC	3	2	NC	NC
2308	3765	GCTTGGGAATTATATTCTGC	2	3	NC	NC
2309	3766	AGCTTGGGAATTATATTCTG	2	2	NC	NC
2310	3767	AAGCTTGGGAATTATATTCT	1	2	NC	NC
2311	3768	AAAGCTTGGGAATTATATTC	1	2	NC	NC
2312	3769	AAAAGCTTGGGAATTATATT	2	2	NC	NC
2313	3770	CAAAAGCTTGGGAATTATAT	2	2	NC	NC
2314	3780	TATCACCCTCCAAAAGCTTG	2	2	NC	NC
2315	3781	ATATCACCCTCCAAAAGCTT	1	2	NC	NC
2316	3782	TATATCACCCTCCAAAAGCT	2	2	NC	NC
2317	3783	TTATATCACCCTCCAAAAGC	2	2	NC	NC
2318	3784	TTTATATCACCCTCCAAAAG	2	1	NC	NC
2319	3785	TTTTATATCACCCTCCAAAA	1	1	NC	NC
2320	3786	TTTTTATATCACCCTCCAAA	2	1	NC	NC
2321	3787	ATTTTTATATCACCCTCCAA	2	2	NC	NC
2322	3788	AATTTTTATATCACCCTCCA	2	2	NC	NC
2323	3789	AAATTTTTATATCACCCTCC	2	2	NC	NC
2324	3790	TAAATTTTTATATCACCCTC	2	2	NC	NC
2325	3791	GTAAATTTTTATATCACCCT	3	2	NC	NC
2326	3792	AGTAAATTTTTATATCACCC	2	2	NC	NC
2327	3818	CTGAACTGAAACAAATAAAA	1	1	NC	NC
2328	3819	TCTGAACTGAAACAAATAAA	1	1	NC	NC
2329	3820	ATCTGAACTGAAACAAATAA	1	1	NC	NC
2330	3821	TATCTGAACTGAAACAAATA	2	1	NC	NC
2331	3822	TTATCTGAACTGAAACAAAT	1	1	NC	NC
2332	3823	ATTATCTGAACTGAAACAAA	1	1	NC	NC
2333	3824	AATTATCTGAACTGAAACAA	1	1	NC	NC
2334	3825	CAATTATCTGAACTGAAACA	2	2	NC	NC
2335	3826	CCAATTATCTGAACTGAAAC	2	2	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2336	3827	GCCAATTATCTGAACTGAAA	2	2	NC	NC
2337	3828	TGCCAATTATCTGAACTGAA	2	2	NC	NC
2338	3829	TTGCCAATTATCTGAACTGA	2	NC	NC	NC
2339	3830	GTTGCCAATTATCTGAACTG	2	NC	NC	NC
2340	3831	AGTTGCCAATTATCTGAACT	2	NC	NC	NC
2341	3832	CAGTTGCCAATTATCTGAAC	2	NC	NC	NC
2342	3833	CCAGTTGCCAATTATCTGAA	2	NC	NC	NC
2343	3834	CCCAGTTGCCAATTATCTGA	2	NC	NC	NC
2344	3835	ACCCAGTTGCCAATTATCTG	2	NC	NC	NC
2345	3836	CACCCAGTTGCCAATTATCT	2	NC	NC	NC
2346	3837	TCACCCAGTTGCCAATTATC	3	NC	NC	NC
2347	3838	TTCACCCAGTTGCCAATTAT	2	NC	NC	NC
2348	3839	ATTCACCCAGTTGCCAATTA	2	NC	NC	NC
2349	3840	GATTCACCCAGTTGCCAATT	3	NC	NC	NC
2350	3841	AGATTCACCCAGTTGCCAAT	2	NC	NC	NC
2351	3842	CAGATTCACCCAGTTGCCAA	2	NC	NC	NC
2352	3843	CCAGATTCACCCAGTTGCCA	2	NC	NC	NC
2353	3845	TGCCAGATTCACCCAGTTGC	2	NC	NC	NC
2354	3846	CTGCCAGATTCACCCAGTTG	1	NC	NC	NC
2355	3847	CCTGCCAGATTCACCCAGTT	1	NC	NC	NC
2356	3848	TCCTGCCAGATTCACCCAGT	2	NC	NC	NC
2357	3849	TTCCTGCCAGATTCACCCAG	2	1	NC	NC
2358	3850	ATTCCTGCCAGATTCACCCA	2	2	NC	NC
2359	3851	GATTCCTGCCAGATTCACCC	2	2	NC	NC
2360	3852	AGATTCCTGCCAGATTCACC	2	2	NC	NC
2361	3853	TAGATTCCTGCCAGATTCAC	2	NC	NC	NC
2362	3854	ATAGATTCCTGCCAGATTCA	2	NC	NC	NC
2363	3855	GATAGATTCCTGCCAGATTC	2	NC	NC	NC
2364	3866	TTAGTTCAATGGATAGATTC	2	NC	NC	NC
2365	3867	TTTAGTTCAATGGATAGATT	2	NC	NC	NC
2366	3868	TTTTAGTTCAATGGATAGAT	1	NC	NC	NC
2367	3869	ATTTTAGTTCAATGGATAGA	1	NC	NC	NC
2368	3870	TATTTTAGTTCAATGGATAG	2	NC	NC	NC
2369	3888	CTGGTTGCATAATAAAATTA	2	NC	NC	NC
2370	3889	ACTGGTTGCATAATAAAATT	2	NC	NC	NC
2371	3890	AACTGGTTGCATAATAAAAT	1	NC	NC	NC
2372	3891	AAACTGGTTGCATAATAAAA	1	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2373	3892	TAAACTGGTTGCATAATAAA	2	NC	NC	NC
2374	3893	ATAAACTGGTTGCATAATAA	2	NC	NC	NC
2375	3894	GATAAACTGGTTGCATAATA	2	NC	NC	NC
2376	3895	GGATAAACTGGTTGCATAAT	2	NC	NC	NC
2377	3896	TGGATAAACTGGTTGCATAA	2	NC	NC	NC
2378	3897	GTGGATAAACTGGTTGCATA	2	NC	NC	NC
2379	3898	GGTGGATAAACTGGTTGCAT	2	NC	NC	NC
2380	3899	TGGTGGATAAACTGGTTGCA	2	NC	NC	NC
2381	3900	TTGGTGGATAAACTGGTTGC	3	NC	NC	NC
2382	3901	CTTGGTGGATAAACTGGTTG	2	NC	NC	NC
2383	3902	TCTTGGTGGATAAACTGGTT	2	NC	NC	NC
2384	3903	TTCTTGGTGGATAAACTGGT	2	NC	NC	NC
2385	3904	GTTCTTGGTGGATAAACTGG	2	2	NC	NC
2386	3905	TGTTCTTGGTGGATAAACTG	1	1	NC	NC
2387	3906	ATGTTCTTGGTGGATAAACT	1	1	NC	NC
2388	3907	TATGTTCTTGGTGGATAAAC	2	2	NC	NC
2389	3908	TTATGTTCTTGGTGGATAAA	2	1	NC	NC
2390	3909	CTTATGTTCTTGGTGGATAA	2	2	NC	NC
2391	3910	TCTTATGTTCTTGGTGGATA	2	2	NC	NC
2392	3911	TTCTTATGTTCTTGGTGGAT	2	2	NC	NC
2393	3912	ATTCTTATGTTCTTGGTGGA	2	2	NC	NC
2394	3913	AATTCTTATGTTCTTGGTGG	2	2	NC	NC
2395	3914	AAATTCTTATGTTCTTGGTG	2	2	NC	NC
2396	3915	AAAATTCTTATGTTCTTGGT	2	1	NC	NC
2397	3916	AAAAATTCTTATGTTCTTGG	2	1	NC	NC
2398	3935	CCAATTCTTTCTACTTATAA	1	NC	NC	NC
2399	3936	GCCAATTCTTTCTACTTATA	2	NC	NC	NC
2400	3937	GGCCAATTCTTTCTACTTAT	2	NC	NC	NC
2401	3938	TGGCCAATTCTTTCTACTTA	2	NC	NC	NC
2402	3939	CTGGCCAATTCTTTCTACTT	2	NC	NC	NC
2403	3940	CCTGGCCAATTCTTTCTACT	2	NC	NC	NC
2404	3941	GCCTGGCCAATTCTTTCTAC	2	NC	NC	NC
2405	3942	TGCCTGGCCAATTCTTTCTA	1	1	NC	NC
2406	3943	ATGCCTGGCCAATTCTTTCT	1	0	NC	NC
2407	3944	CATGCCTGGCCAATTCTTTC	1	0	NC	NC
2408	3945	CCATGCCTGGCCAATTCTTT	1	0	NC	NC
2409	4018	GTCTTGAACTCCTGACCTCA	NA	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2410	4022	GCTGGTCTTGAACTCCTGAC	NA	NC	NC	NC
2411	4023	GGCTGGTCTTGAACTCCTGA	NA	NC	NC	NC
2412	4024	AGGCTGGTCTTGAACTCCTG	NA	NC	NC	NC
2413	4025	CAGGCTGGTCTTGAACTCCT	NA	NC	NC	NC
2414	4061	ATATTTTTAGTAAAGATGGG	0	NC	NC	NC
2415	4076	GTAGAGATGTACTTTATATT	2	2	NC	NC
2416	4077	AGTAGAGATGTACTTTATAT	2	2	NC	NC
2417	4078	TAGTAGAGATGTACTTTATA	2	2	NC	NC
2418	4079	TTAGTAGAGATGTACTTTAT	2	2	NC	NC
2419	4080	TTTAGTAGAGATGTACTTTA	2	1	NC	NC
2420	4081	TTTTAGTAGAGATGTACTTT	1	0	NC	NC
2421	4082	TTTTTAGTAGAGATGTACTT	1	0	NC	NC
2422	4083	ATTTTTAGTAGAGATGTACT	1	0	NC	NC
2423	4084	TATTTTTAGTAGAGATGTAC	1	0	NC	NC
2424	4085	GTATTTTTAGTAGAGATGTA	0	0	NC	NC
2425	4086	CGTATTTTTAGTAGAGATGT	NA	NC	NC	NC
2426	4087	TCGTATTTTTAGTAGAGATG	NA	NC	NC	NC
2427	4088	TTCGTATTTTTAGTAGAGAT	NA	NC	NC	NC
2428	4089	TTTCGTATTTTTAGTAGAGA	NA	NC	NC	NC
2429	4090	TTTTCGTATTTTTAGTAGAG	NA	NC	NC	NC
2430	4107	ACCATGCCCAGCTAATTTTT	NA	NC	NC	NC
2431	4108	CACCATGCCCAGCTAATTTT	NA	NC	NC	NC
2432	4109	CCACCATGCCCAGCTAATTT	NA	NC	NC	NC
2433	4110	GCCACCATGCCCAGCTAATT	NA	NC	NC	NC
2434	4160	AAGAGATTCTCCTGCCTCAG	NA	0	NC	NC
2435	4161	CAAGAGATTCTCCTGCCTCA	NA	0	NC	NC
2436	4162	TCAAGAGATTCTCCTGCCTC	NA	0	NC	NC
2437	4163	TTCAAGAGATTCTCCTGCCT	NA	0	NC	NC
2438	4164	GTTCAAGAGATTCTCCTGCC	NA	0	NC	NC
2439	4165	GGTTCAAGAGATTCTCCTGC	NA	0	NC	NC
2440	4166	AGGTTCAAGAGATTCTCCTG	NA	0	NC	NC
2441	4167	CAGGTTCAAGAGATTCTCCT	NA	0	NC	NC
2442	4168	CCAGGTTCAAGAGATTCTCC	NA	0	NC	NC
2443	4169	CCCAGGTTCAAGAGATTCTC	NA	0	NC	NC
2444	4170	TCCCAGGTTCAAGAGATTCT	NA	0	NC	NC
2445	4171	CTCCCAGGTTCAAGAGATTC	NA	0	NC	NC
2446	4172	CCTCCCAGGTTCAAGAGATT	NA	0	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2447	4173	GCCTCCCAGGTTCAAGAGAT	NA	NC	NC	NC
2448	4201	GACGTGATCTCGGCTCATTG	0	NC	NC	NC
2449	4209	AGTGCAGTGACGTGATCTCG	0	NC	NC	NC
2450	4210	GAGTGCAGTGACGTGATCTC	0	NC	NC	NC
2451	4211	GGAGTGCAGTGACGTGATCT	NA	NC	NC	NC
2452	4212	TGGAGTGCAGTGACGTGATC	NA	NC	NC	NC
2453	4213	CTGGAGTGCAGTGACGTGAT	NA	NC	NC	NC
2454	4216	AAGCTGGAGTGCAGTGACGT	0	NC	NC	NC
2455	4232	TCTTGCTCTGTTGCCCAAGC	0	NC	NC	NC
2456	4233	GTCTTGCTCTGTTGCCCAAG	0	NC	NC	NC
2457	4234	AGTCTTGCTCTGTTGCCCAA	NA	NC	NC	NC
2458	4245	TTTTGAGATGGAGTCTTGCT	NA	NC	NC	NC
2459	4246	TTTTTGAGATGGAGTCTTGC	NA	NC	NC	NC
2460	4284	GCTTGATAATTCTATTTCTT	2	2	NC	NC
2461	4285	AGCTTGATAATTCTATTTCT	1	2	NC	NC
2462	4286	AAGCTTGATAATTCTATTTC	2	2	NC	NC
2463	4297	CTAGTTTTTAAAAGCTTGAT	2	2	NC	NC
2464	4298	TCTAGTTTTTAAAAGCTTGA	2	NC	NC	NC
2465	4299	CTCTAGTTTTTAAAAGCTTG	2	NC	NC	NC
2466	4300	GCTCTAGTTTTTAAAAGCTT	2	NC	NC	NC
2467	4301	TGCTCTAGTTTTTAAAAGCT	1	NC	NC	NC
2468	4302	GTGCTCTAGTTTTTAAAAGC	1	NC	NC	NC
2469	4303	TGTGCTCTAGTTTTTAAAAG	1	NC	NC	NC
2470	4304	CTGTGCTCTAGTTTTTAAAA	1	NC	NC	NC
2471	4305	TCTGTGCTCTAGTTTTTAAA	1	NC	NC	NC
2472	4306	TTCTGTGCTCTAGTTTTTAA	1	NC	NC	NC
2473	4307	CTTCTGTGCTCTAGTTTTTA	1	NC	NC	NC
2474	4308	CCTTCTGTGCTCTAGTTTTT	1	NC	NC	NC
2475	4309	TCCTTCTGTGCTCTAGTTTT	1	NC	NC	NC
2476	4310	TTCCTTCTGTGCTCTAGTTT	1	NC	NC	NC
2477	4311	ATTCCTTCTGTGCTCTAGTT	1	NC	NC	NC
2478	4312	TATTCCTTCTGTGCTCTAGT	1	NC	NC	NC
2479	4313	TTATTCCTTCTGTGCTCTAG	2	NC	NC	NC
2480	4314	CTTATTCCTTCTGTGCTCTA	2	NC	NC	NC
2481	4315	CCTTATTCCTTCTGTGCTCT	1	NC	NC	NC
2482	4316	ACCTTATTCCTTCTGTGCTC	2	NC	NC	NC
2483	4317	GACCTTATTCCTTCTGTGCT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2484	4318	TGACCTTATTCCTTCTGTGC	2	NC	NC	NC
2485	4319	ATGACCTTATTCCTTCTGTG	2	NC	NC	NC
2486	4320	CATGACCTTATTCCTTCTGT	2	NC	NC	NC
2487	4321	TCATGACCTTATTCCTTCTG	2	NC	NC	NC
2488	4322	TTCATGACCTTATTCCTTCT	1	NC	NC	NC
2489	4323	TTTCATGACCTTATTCCTTC	2	NC	NC	NC
2490	4324	ATTTCATGACCTTATTCCTT	2	NC	NC	NC
2491	4325	AATTTCATGACCTTATTCCT	2	NC	NC	NC
2492	4326	AAATTTCATGACCTTATTCC	1	NC	NC	NC
2493	4327	TAAATTTCATGACCTTATTC	1	2	NC	NC
2494	4331	CTTTTAAATTTCATGACCTT	1	NC	NC	NC
2495	4332	CCTTTTAAATTTCATGACCT	2	NC	NC	NC
2496	4333	ACCTTTTAAATTTCATGACC	2	NC	NC	NC
2497	4334	AACCTTTTAAATTTCATGAC	2	NC	NC	NC
2498	4348	CTATGACAATATTTAACCTT	2	NC	NC	NC
2499	4349	CCTATGACAATATTTAACCT	2	NC	NC	NC
2500	4350	TCCTATGACAATATTTAACC	2	NC	NC	NC
2501	4351	ATCCTATGACAATATTTAAC	2	NC	NC	NC
2502	4355	CTTAATCCTATGACAATATT	2	NC	NC	NC
2503	4356	GCTTAATCCTATGACAATAT	2	NC	NC	NC
2504	4357	TGCTTAATCCTATGACAATA	2	NC	NC	NC
2505	4358	CTGCTTAATCCTATGACAAT	2	NC	NC	NC
2506	4359	ACTGCTTAATCCTATGACAA	2	NC	NC	NC
2507	4360	AACTGCTTAATCCTATGACA	2	NC	NC	NC
2508	4361	AAACTGCTTAATCCTATGAC	2	NC	NC	NC
2509	4362	TAAACTGCTTAATCCTATGA	2	NC	NC	NC
2510	4363	TTAAACTGCTTAATCCTATG	2	NC	NC	NC
2511	4364	TTTAAACTGCTTAATCCTAT	2	NC	NC	NC
2512	4365	CTTTAAACTGCTTAATCCTA	2	NC	NC	NC
2513	4366	TCTTTAAACTGCTTAATCCT	2	2	NC	NC
2514	4367	ATCTTTAAACTGCTTAATCC	2	NC	NC	NC
2515	4368	AATCTTTAAACTGCTTAATC	2	NC	NC	NC
2516	4369	CAATCTTTAAACTGCTTAAT	2	NC	NC	NC
2517	4370	ACAATCTTTAAACTGCTTAA	2	NC	NC	NC
2518	4371	AACAATCTTTAAACTGCTTA	2	NC	NC	NC
2519	4372	CAACAATCTTTAAACTGCTT	2	NC	NC	NC
2520	4373	CCAACAATCTTTAAACTGCT	2	NC	NC	NC

SEQ ID NO	Position	Sequence	Off-target Score			
			Human	Cyno	Mouse	Rat
2521	4374	TCCAACAATCTTTAAACTGC	1	NC	NC	NC
2522	4375	ATCCAACAATCTTTAAACTG	2	NC	NC	NC
2523	4376	CATCCAACAATCTTTAAACT	2	NC	NC	NC
2524	4377	TCATCCAACAATCTTTAAAC	2	NC	NC	NC
2525	4378	TTCATCCAACAATCTTTAAA	2	NC	NC	NC
2526	4379	TTTCATCCAACAATCTTTAA	2	NC	NC	NC
2527	4380	ATTTCATCCAACAATCTTTA	2	NC	NC	NC
2528	4381	AATTTCATCCAACAATCTTT	2	NC	NC	NC
2529	4382	TAATTTCATCCAACAATCTT	1	NC	NC	NC
2530	4383	ATAATTTCATCCAACAATCT	2	NC	NC	NC
2531	4384	AATAATTTCATCCAACAATC	1	NC	NC	NC
2532	4386	CAAATAATTTCATCCAACAA	1	NC	NC	NC
2533	4387	ACAAATAATTTCATCCAACA	1	NC	NC	NC
2534	4388	GACAAATAATTTCATCCAAC	2	NC	NC	NC
2535	4389	TGACAAATAATTTCATCCAA	2	1	NC	NC
2536	4390	ATGACAAATAATTTCATCCA	2	1	NC	NC
2537	4391	AATGACAAATAATTTCATCC	2	2	NC	NC
2538	4392	GAATGACAAATAATTTCATC	2	NC	NC	NC
2539	4399	CTTGAATGAATGACAAATAA	1	NC	NC	NC
2540	4400	ACTTGAATGAATGACAAATA	2	NC	NC	NC
2541	4401	TACTTGAATGAATGACAAAT	2	NC	NC	NC
2542	4402	TTACTTGAATGAATGACAAA	1	NC	NC	NC
2543	4403	ATTACTTGAATGAATGACAA	2	NC	NC	NC
2544	4404	TATTACTTGAATGAATGACA	1	NC	NC	NC
2545	4405	TTATTACTTGAATGAATGAC	1	NC	NC	NC

Example 2. Antisense Inhibition of MSH3

Inhibition or knockdown of MSH3 can be demonstrated using a cell-based assay. For example, HEK293, NIH3T3, or Hela or another available mammalian cell line with oligonucleotides targeting MSH3 identified above in Example 1 using at least five different dose levels, using transfection reagents such as lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Cells are harvested at multiple time points up to 7 days post transfection for either mRNA or protein analyses. Knockdown of mRNA and protein are determined by RT-qPCR or western blot analyses respectively, using standard molecular biology techniques as previously described (see, for example, as described in Drouet et al., 2014, PLOS One 9(6): e99341). The relative levels of the MSH3 mRNA and protein at the different oligonucleotide levels are compared with a mock oligonucleotide control. The most potent oligonucleotides (for example, those which are

capable of at least 90% at least 95%, at least 97%, at least 98%, at or at least 99% or more, reduction in protein levels when compared with controls) are selected for subsequent studies, for example, as described in the examples below.

Human cell lines

HeLa cells were obtained from ATCC (ATCC in partnership with LGC Standards, Wesel, Germany, cat.# ATCC-CRM-CCL-2) and cultured in HAM's F12 (#FG0815, Biochrom, Berlin, Germany), supplemented to contain 10% fetal calf serum (1248D, Biochrom GmbH, Berlin, Germany), and 100U/ml Penicillin/100µg/ml Streptomycin (A2213, Biochrom GmbH, Berlin, Germany) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator. For transfection of HeLa cells with ASOs, cells were seeded at a density of 15,000 cells / well into 96-well tissue culture plates (#655180, GBO, Germany).

Transfections

In HeLa cells, transfection of ASOs was carried out with Lipofectamine 2000 (Invitrogen/Life Technologies, Karlsruhe, Germany) according to manufacturer's instructions for reverse transfection with 0.25 µL Lipofectamine 2000 per well.

The dual dose screen was performed with ASOs in quadruplicates at 20 nM and 2 nM, respectively, with two ASOs targeting AHSA1 (one MOE-ASO and one 2'oMe-ASO) as unspecific controls and a mock transfection. Dose-response experiments were done with ASOs in 5 concentrations transfected in quadruplicates, starting at 20 nM in 5-6-fold dilutions steps down to ~15-32 pM. Mock transfected cells served as control in dose-response curve (DRC) experiments.

Analysis and Quantitation

After 24h of incubation with ASOs, medium was removed and cells were lysed in 150µl Medium-Lysis Mixture (1 volume lysis mixture, 2 volumes cell culture medium) and then incubated at 53°C for 30 minutes. bDNA assay was performed according to manufacturer's instructions. Luminescence was read using 1420 Luminescence Counter (WALLAC VICTOR Light, Perkin Elmer, Rodgau-Jügesheim, Germany) following 30 minutes incubation at RT in the dark.

The two Ahsa1-ASOs (one 2'-OMe and one MOE-modified) served at the same time as unspecific controls for respective target mRNA expression and as a positive control to analyze transfection efficiency with regards to Ahsa1 mRNA level. By hybridization with an Ahsa1 probeset, the mock transfected wells served as controls for Ahsa1 mRNA level. Transfection efficiency for each 96-well plate and both doses in the dual dose screen were calculated by relating Ahsa1-level with Ahsa1-ASO (normalized to GapDH) to Ahsa1-level obtained with mock controls.

For each well, the target mRNA level was normalized to the respective GAPDH mRNA level. The activity of a given ASO was expressed as percent mRNA concentration of the respective target (normalized to GAPDH mRNA) in treated cells, relative to the target mRNA concentration (normalized to GAPDH mRNA) averaged across control wells.

The results of the dual-dose screen of ~480 ASOs targeting MSH3, as well as IC_{20}, IC_{50} and IC_{80} values of approximately 42 positive ASOs from the dual dose screen, are shown in Table 3 below.

Table 3

SEQ ID NO	Position	Sequence	Off-target Score				mean % mRNA remaining		SD % mRNA remaining		IC20 (nM)	IC50 (nM)	IC80 (nM)
			Human	Cyno	Mouse	Rat	2 nM	20 nM	2 nM	20 nM			
20	158	TTCCCGTAGACTGGAAGAAT	2	2	NC	NC	30.06	24.97	3.97	1.36	NA	NA	NA
22	166	TTTCAGGCTTCCCGTAGACT	3	3	NC	NC	32.44	39.78	3.32	7.92	NA	NA	NA
23	167	ATTTCAGGCTTCCCGTAGAC	2	2	NC	NC	47.70	52.74	3.41	3.24	NA	NA	NA
24	168	GATTTCAGGCTTCCCGTAGA	2	2	NC	NC	43.01	51.58	2.43	2.46	NA	NA	NA
25	169	GGATTTCAGGCTTCCCGTAG	3	3	NC	NC	25.33	54.39	0.63	5.43	NA	NA	NA
26	170	TGGATTTCAGGCTTCCCGTA	2	2	NC	NC	25.24	35.58	4.01	3.13	NA	NA	NA
27	171	GTGGATTTCAGGCTTCCCGT	2	3	NC	NC	21.74	44.38	3.11	5.66	NA	NA	NA
28	173	AGGTGGATTTCAGGCTTCCC	2	3	NC	NC	22.86	29.47	3.63	1.81	NA	NA	NA
29	174	GAGGTGGATTTCAGGCTTCC	2	2	NC	NC	24.05	28.78	2.55	3.32	NA	NA	NA
31	176	AGGAGGTGGATTTCAGGCTT	2	2	NC	NC	31.37	42.25	7.19	6.92	NA	NA	NA
32	177	GAGGAGGTGGATTTCAGGCT	2	2	NC	NC	26.64	31.62	0.60	1.35	NA	NA	NA
77	358	CTTTTTAACAGGCCCATCAT	2	2	NC	NC	59.62	48.15	3.76	5.94	NA	NA	NA
78	359	TCTTTTTAACAGGCCCATCA	2	2	NC	NC	45.61	49.28	2.20	12.27	NA	NA	NA
81	362	CTTTCTTTTTAACAGGCCCA	2	2	NC	NC	22.04	18.27	2.73	4.55	NA	NA	NA
82	363	ACTTTCTTTTTAACAGGCCC	2	2	NC	NC	16.43	24.75	2.06	1.78	NA	NA	NA
114	400	CAGATCACTTCCTCCTTCCT	2	2	NC	NC	52.23	70.33	3.26	5.36	NA	NA	NA
115	401	CCAGATCACTTCCTCCTTCC	2	2	NC	NC	36.69	70.06	2.89	7.89	NA	NA	NA
117	403	TCCCAGATCACTTCCTCCTT	2	2	NC	NC	40.90	72.21	4.42	5.91	NA	NA	NA
118	404	TTCCCAGATCACTTCCTCCT	2	2	NC	NC	58.27	82.25	12.58	6.99	NA	NA	NA
120	406	CATTCCCAGATCACTTCCTC	2	2	NC	NC	82.15	101.53	8.33	18.80	NA	NA	NA
130	416	AGTTGCCAGACATTCCCAGA	2	2	NC	NC	28.37	36.19	3.13	2.95	NA	NA	NA
132	418	AGAGTTGCCAGACATTCCCA	2	2	NC	NC	34.75	60.80	4.42	3.63	NA	NA	NA

133	419	CAGAGTTGCCAGACATTCCC	2	2	NC	NC	21.81	40.77	0.55	4.31	NA	NA	NA
134	420	TCAGAGTTGCCAGACATTCC	2	2	NC	NC	25.37	37.98	2.10	7.04	NA	NA	NA
144	437	TCAGACATTTCTTTGGCTCA	2	2	NC	NC	25.10	34.55	3.26	5.27	NA	NA	NA
145	438	CTCAGACATTTCTTTGGCTC	2	2	NC	NC	11.87	27.07	0.98	18.87	NA	NA	NA
147	440	TCCTCAGACATTTCTTTGGC	2	2	NC	NC	12.32	19.35	0.96	1.47	NA	NA	NA
167	473	TCAATTTTTCCAGAGACTTT	2	2	NC	NC	47.74	79.79	9.49	3.67	NA	NA	NA
168	474	TTCAATTTTTCCAGAGACTT	2	2	NC	NC	35.67	65.30	7.37	7.81	NA	NA	NA
173	479	ATTCTTTCAATTTTTCCAGA	2	2	NC	NC	58.81	67.93	12.18	7.76	NA	NA	NA
210	562	AGTACATTTTGGCAGAACTG	2	2	NC	NC	14.38	34.64	1.54	3.66	NA	NA	NA
212	564	TCAGTACATTTTGGCAGAAC	2	2	NC	NC	24.12	21.13	3.41	1.47	NA	NA	NA
213	565	ATCAGTACATTTTGGCAGAA	2	2	NC	NC	23.76	33.72	7.51	2.12	NA	NA	NA
214	566	AATCAGTACATTTTGGCAGA	2	2	NC	NC	39.25	37.45	5.65	3.07	NA	NA	NA
215	567	AAATCAGTACATTTTGGCAG	2	2	NC	NC	20.31	20.26	2.66	1.32	NA	NA	NA
290	679	TGATGATCCAAACTGACTGA	2	2	NC	NC	26.26	21.63	2.50	0.77	NA	NA	NA
291	680	TTGATGATCCAAACTGACTG	2	2	NC	NC	27.04	24.63	0.80	2.66	NA	NA	NA
292	681	TTTGATGATCCAAACTGACT	2	2	NC	NC	32.31	27.81	2.68	5.91	NA	NA	NA
293	682	ATTTGATGATCCAAACTGAC	2	2	NC	NC	30.17	28.45	2.10	1.04	NA	NA	NA
295	684	GTATTTGATGATCCAAACTG	2	2	NC	NC	28.21	33.08	1.83	14.09	NA	NA	NA
296	685	TGTATTTGATGATCCAAACT	2	2	NC	NC	34.76	29.03	5.19	7.05	NA	NA	NA
299	689	GACTTGTATTTGATGATCCA	2	2	NC	NC	19.56	31.89	9.42	2.64	NA	NA	NA
300	690	TGACTTGTATTTGATGATCC	2	2	NC	NC	18.41	19.69	3.19	1.64	NA	NA	NA
301	691	ATGACTTGTATTTGATGATC	2	2	NC	NC	27.62	29.49	5.93	2.77	NA	NA	NA
302	692	CATGACTTGTATTTGATGAT	2	2	NC	NC	31.13	26.78	5.49	2.36	NA	NA	NA
303	693	TCATGACTTGTATTTGATGA	2	2	NC	NC	23.35	27.19	3.48	2.78	NA	NA	NA
304	694	TTCATGACTTGTATTTGATG	2	2	NC	NC	22.42	18.03	4.97	2.27	NA	NA	NA

305	695	TTTCATGACTTGTATTTGAT	2	2	NC	NC	36.78	45.87	12.49	10.13	NA	NA
309	702	TGTAAATTTTCATGACTTGT	2	2	NC	NC	22.97	19.81	13.48	2.58	NA	NA
351	765	TGTAATTCTAGCGGCGTATA	2	3	NC	NC	24.71	25.94	5.57	7.09	NA	NA
352	766	TTGTAATTCTAGCGGCGTAT	3	3	NC	NC	19.28	12.68	1.64	1.02	NA	NA
353	767	ATTGTAATTCTAGCGGCGTA	3	3	NC	NC	16.00	34.34	2.96	1.85	NA	NA
354	768	TATTGTAATTCTAGCGGCGT	3	3	NC	NC	17.26	24.00	2.53	9.90	NA	NA
355	769	GTATTGTAATTCTAGCGGCG	3	3	NC	NC	18.21	38.10	1.32	5.85	NA	NA
356	770	TGTATTGTAATTCTAGCGGC	3	3	NC	NC	27.37	36.47	21.05	5.97	NA	NA
357	771	ATGTATTGTAATTCTAGCGG	3	3	NC	NC	23.27	35.90	8.67	5.68	NA	NA
358	772	TATGTATTGTAATTCTAGCG	2	2	NC	NC	20.35	29.01	5.26	4.90	NA	NA
359	773	CTATGTATTGTAATTCTAGC	2	2	NC	NC	20.97	17.94	2.16	2.89	NA	NA
361	781	CTTCATTTCTATGTATTGTA	2	2	NC	NC	27.17	48.73	3.43	8.15	NA	NA
362	782	GCTTCATTTCTATGTATTGT	2	2	NC	NC	25.22	35.27	5.40	1.77	NA	NA
365	785	GCTGCTTCATTTCTATGTAT	2	2	NC	NC	13.38	44.30	0.75	5.44	NA	NA
366	786	TGCTGCTTCATTTCTATGTA	2	2	NC	NC	13.29	26.28	1.52	3.60	NA	NA
368	788	GCTGCTGCTTCATTTCTATG	2	2	NC	NC	19.60	38.50	16.21	3.51	NA	NA
407	879	TAAATATTGAGCTCTCGGGC	2	2	NC	NC	8.50	13.95	1.86	1.49	0.16	0.40	1.67
408	880	ATAAATATTGAGCTCTCGGG	2	2	NC	NC	12.35	14.43	2.49	0.95	0.07	0.35	2.29
409	881	AATAAATATTGAGCTCTCGG	2	2	NC	NC	17.73	21.81	3.28	2.89	NA	NA	NA
432	915	ATACTTGCTGTCATAAAGTT	2	2	NC	NC	23.32	27.35	3.55	2.60	NA	NA	NA
437	921	GTAGGTATACTTGCTGTCAT	2	2	NC	NC	19.95	28.11	4.07	7.40	NA	NA	NA
438	922	AGTAGGTATACTTGCTGTCA	2	2	NC	NC	19.44	47.18	6.70	1.53	NA	NA	NA
439	931	CAGTCTGTGAGTAGGTATAC	3	2	NC	NC	12.94	18.17	5.71	0.91	NA	NA	NA
440	932	ACAGTCTGTGAGTAGGTATA	2	2	NC	NC	11.65	26.21	0.91	0.40	NA	NA	NA
441	933	AACAGTCTGTGAGTAGGTAT	2	3	NC	NC	10.55	16.21	2.20	2.38	0.28	0.57	1.97

442	934	AAACAGTCTGTGAGTAGGTA	2	2	NC	NC	10.61	12.58	2.44	1.80	0.27	2.12	11.55
444	936	ACAAACAGTCTGTGAGTAGG	2	2	NC	NC	12.83	14.75	2.71	1.94	0.18	0.45	2.14
459	951	AGGCGGCGTACATGAACAAA	3	3	NC	NC	46.19	30.59	8.20	5.86	NA	NA	NA
460	952	CAGGCGGCGTACATGAACAA	3	3	NC	NC	43.41	21.98	7.88	2.01	NA	NA	NA
479	987	TGCTTCACAACTCCCACCTT	2	2	2	2	19.06	24.79	4.38	4.94	NA	NA	NA
482	990	GTTTGCTTCACAACTCCCAC	2	2	2	2	17.17	21.52	3.38	2.03	NA	NA	NA
483	991	AGTTTGCTTCACAACTCCCA	2	2	2	2	19.13	24.84	3.46	5.32	NA	NA	NA
484	992	CAGTTTGCTTCACAACTCCC	2	3	1	2	15.94	21.52	1.66	1.91	NA	NA	NA
485	993	TCAGTTTGCTTCACAACTCC	2	2	1	2	21.17	27.81	1.95	5.73	NA	NA	NA
486	994	TTCAGTTTGCTTCACAACTC	1	1	1	2	22.96	42.91	6.39	7.91	NA	NA	NA
487	995	TTTCAGTTTGCTTCACAACT	2	2	2	2	39.21	76.31	2.23	18.80	NA	NA	NA
488	996	GTTTCAGTTTGCTTCACAAC	2	2	2	2	21.50	31.61	2.03	4.52	NA	NA	NA
489	997	AGTTTCAGTTTGCTTCACAA	2	2	1	2	23.61	39.27	11.21	4.30	NA	NA	NA
490	998	CAGTTTCAGTTTGCTTCACA	2	2	1	1	29.78	50.09	3.60	13.00	NA	NA	NA
491	999	GCAGTTTCAGTTTGCTTCAC	2	2	1	2	19.62	34.41	1.57	4.47	NA	NA	NA
492	1004	ATGCTGCAGTTTCAGTTTGC	2	2	NC	NC	14.69	27.18	5.48	1.65	NA	NA	NA
493	1005	AATGCTGCAGTTTCAGTTTG	2	2	NC	NC	35.38	50.31	2.27	12.47	NA	NA	NA
497	1010	CCTTTAATGCTGCAGTTTCA	2	2	NC	NC	24.32	41.47	1.78	5.60	NA	NA	NA
498	1011	GCCTTTAATGCTGCAGTTTC	3	2	NC	NC	18.64	28.39	0.91	4.18	NA	NA	NA
500	1013	TGGCCTTTAATGCTGCAGTT	2	2	NC	NC	13.95	17.48	7.01	2.47	NA	NA	NA
501	1014	ATGGCCTTTAATGCTGCAGT	2	2	NC	NC	22.76	41.41	3.70	11.74	NA	NA	NA
503	1016	CAATGGCCTTTAATGCTGCA	2	2	NC	NC	20.09	22.88	1.78	6.09	NA	NA	NA
504	1017	CCAATGGCCTTTAATGCTGC	2	3	NC	NC	14.55	24.20	6.47	9.83	NA	NA	NA
505	1018	TCCAATGGCCTTTAATGCTG	2	2	NC	NC	19.49	18.66	4.15	5.64	NA	NA	NA
506	1019	CTCCAATGGCCTTTAATGCT	3	2	NC	NC	25.15	34.45	4.26	4.79	NA	NA	NA

507	1020	TCTCCAATGGCCTTTAATGC	2	2	NC	NC	43.44	66.75	13.44	15.71	NA	NA
508	1021	GTCTCCAATGGCCTTTAATG	2	3	NC	NC	23.31	30.90	3.66	9.70	NA	NA
509	1022	TGTCTCCAATGGCCTTTAAT	2	2	NC	NC	29.99	38.55	2.92	13.42	NA	NA
510	1023	TTGTCTCCAATGGCCTTTAA	2	2	NC	NC	25.71	18.28	12.62	2.93	NA	NA
511	1024	GTTGTCTCCAATGGCCTTTA	2	2	NC	NC	11.93	24.30	4.26	2.85	NA	NA
512	1025	TGTTGTCTCCAATGGCCTTT	2	2	NC	NC	25.31	10.97	31.38	0.78	NA	NA
543	1057	GGCAGTCAATTTCCGGGAAA	2	3	NC	NC	64.26	33.26	99.75	2.75	NA	NA
544	1058	GGGCAGTCAATTTCCGGGAA	2	3	NC	NC	12.70	24.66	8.93	1.23	NA	NA
545	1059	AGGGCAGTCAATTTCCGGGA	2	2	NC	NC	8.42	17.29	1.38	1.89	0.03	0.09	0.44
546	1060	AAGGGCAGTCAATTTCCGGG	2	2	NC	NC	9.58	23.44	0.73	6.88	NA	NA	NA
547	1061	AAAGGGCAGTCAATTTCCGG	2	2	NC	NC	12.65	27.02	0.95	2.17	NA	NA	NA
548	1062	TAAAGGGCAGTCAATTTCCG	2	3	NC	NC	83.92	15.94	133.95	2.40	NA	NA	NA
549	1063	ATAAAGGGCAGTCAATTTCC	2	2	NC	NC	26.77	37.76	5.76	10.21	NA	NA	NA
550	1064	TATAAAGGGCAGTCAATTTC	2	2	NC	NC	45.72	27.89	1.78	3.80	NA	NA	NA
551	1065	GTATAAAGGGCAGTCAATTT	2	2	NC	NC	94.84	121.02	10.78	9.88	NA	NA	NA
552	1066	TGTATAAAGGGCAGTCAATT	2	2	NC	NC	79.73	33.75	38.34	2.32	NA	NA	NA
553	1067	TTGTATAAAGGGCAGTCAAT	2	2	NC	NC	42.40	20.47	4.44	1.09	NA	NA	NA
554	1068	TTTGTATAAAGGGCAGTCAA	2	2	NC	NC	35.80	18.68	2.66	3.12	NA	NA	NA
555	1069	TTTTGTATAAAGGGCAGTCA	2	2	NC	NC	33.34	17.73	3.60	3.66	NA	NA	NA
556	1070	ATTTTGTATAAAGGGCAGTC	2	2	NC	NC	20.01	20.95	1.74	2.52	NA	NA	NA
557	1071	GATTTTGTATAAAGGGCAGT	2	2	NC	NC	26.22	38.90	1.73	3.61	NA	NA	NA
558	1072	AGATTTTGTATAAAGGGCAG	2	2	NC	NC	24.63	26.08	2.03	1.17	NA	NA	NA
559	1073	TAGATTTTGTATAAAGGGCA	2	2	NC	NC	34.94	32.54	2.53	3.19	NA	NA	NA
560	1074	GTAGATTTTGTATAAAGGGC	2	2	NC	NC	22.45	29.79	2.51	2.88	NA	NA	NA
561	1075	TGTAGATTTTGTATAAAGGG	2	2	NC	NC	60.77	51.45	6.24	4.07	NA	NA	NA

562	1076	GTGTAGATTTTGTATAAAGG	2	2	NC	NC	35.71	33.49	3.56	5.62	NA	NA
563	1077	AGTGTAGATTTTGTATAAAG	2	2	NC	NC	68.25	60.14	4.25	5.39	NA	NA
582	1117	ATCATCCAGCTTGATTAGGG	3	3	NC	NC	13.93	13.80	1.84	1.30	0.23	0.59	NA	2.29
583	1118	CATCATCCAGCTTGATTAGG	2	2	NC	NC	19.44	15.57	1.05	2.57	NA	NA	NA	
584	1119	GCATCATCCAGCTTGATTAG	2	3	NC	NC	16.80	35.91	2.64	3.38	NA	NA	NA	
585	1120	AGCATCATCCAGCTTGATTA	2	2	NC	NC	20.33	30.80	8.01	2.56	NA	NA	NA	
588	1129	AACATTTACAGCATCATCCA	2	2	NC	NC	40.28	69.60	3.82	13.68	NA	NA	NA	
589	1130	CAACATTTACAGCATCATCC	2	2	NC	NC	25.71	65.14	2.21	17.75	NA	NA	NA	
590	1131	TCAACATTTACAGCATCATC	2	2	NC	NC	38.63	79.78	2.77	3.39	NA	NA	NA	
591	1132	ATCAACATTTACAGCATCAT	2	2	NC	NC	39.61	72.61	2.46	7.61	NA	NA	NA	
598	1139	TTATCTCATCAACATTTACA	2	2	NC	NC	61.27	86.61	5.47	9.19	NA	NA	NA	
603	1144	AGTCATTATCTCATCAACAT	2	2	NC	NC	20.98	40.74	5.43	9.81	NA	NA	NA	
604	1145	CAGTCATTATCTCATCAACA	2	2	NC	NC	14.28	31.74	1.61	10.40	NA	NA	NA	
611	1152	GAAGTATCAGTCATTATCTC	2	2	NC	NC	28.18	33.13	10.48	14.23	NA	NA	NA	
613	1154	TAGAAGTATCAGTCATTATC	2	2	NC	NC	25.57	42.56	3.05	10.29	NA	NA	NA	
614	1155	GTAGAAGTATCAGTCATTAT	2	2	NC	NC	29.00	33.44	5.21	9.86	NA	NA	NA	
615	1156	GGTAGAAGTATCAGTCATTA	2	3	NC	NC	15.98	39.39	1.60	2.23	NA	NA	NA	
616	1157	TGGTAGAAGTATCAGTCATT	2	2	NC	NC	13.05	15.05	1.39	3.61	0.12	0.51		2.41
659	1203	TTGTCCCTAACATTTTCCTT	2	2	NC	NC	19.61	43.69	3.69	10.29	NA	NA	NA	
661	1205	TTTTGTCCCTAACATTTTCC	2	2	NC	NC	24.98	42.77	2.16	8.71	NA	NA	NA	
699	1297	TGAACGAGAAGCAGAGTCCT	2	2	NC	NC	20.10	13.93	7.03	2.75	NA	NA	NA	
700	1298	CTGAACGAGAAGCAGAGTCC	2	2	NC	NC	21.05	14.88	1.55	1.89	NA	NA	NA	
702	1310	GGGTTTCTAGCTCTGAACGA	3	3	NC	NC	14.81	38.11	1.59	5.25	NA	NA	NA	
705	1313	TCCGGGTTTCTAGCTCTGAA	2	2	NC	NC	8.72	12.58	1.33	1.55	0.13	0.38		8.72
706	1314	ATCCGGGTTTCTAGCTCTGA	2	2	NC	NC	8.41	13.64	1.27	1.53	0.04	0.21		1.43

707	1315	CATCCGGGTTTCTAGCTCTG	2	2	NC	NC	8.17	13.49	1.36	1.85	0.09	0.31	1.71
724	1395	GATGTGGCTCTGTGGATGAG	2	2	NC	NC	39.91	43.65	5.72	3.75	NA	NA	NA
725	1396	AGATGTGGCTCTGTGGATGA	2	2	NC	NC	41.36	53.60	7.68	10.44	NA	NA	NA
770	1470	TGGAAAGCATGGCTGTATTC	2	2	2	2	15.79	20.14	7.80	2.10	NA	NA	NA
771	1471	CTGGAAAGCATGGCTGTATT	1	2	2	2	15.31	15.99	2.42	1.07	NA	NA	NA
812	1520	GAGAACCTTTGATGTCAACT	2	2	NC	NC	16.84	34.79	2.00	3.80	NA	NA	NA
813	1521	TGAGAACCTTTGATGTCAAC	2	3	NC	NC	16.82	26.10	2.07	2.78	NA	NA	NA
814	1522	TTGAGAACCTTTGATGTCAA	2	2	NC	NC	15.39	19.95	0.70	6.17	NA	NA	NA
815	1523	TTTGAGAACCTTTGATGTCA	2	2	NC	NC	27.25	37.99	8.32	5.27	NA	NA	NA
816	1524	ATTTGAGAACCTTTGATGTC	2	2	NC	NC	23.08	35.38	4.24	4.05	NA	NA	NA
838	1546	TAAGTTAACAATGCCAGAAA	2	2	NC	NC	29.18	45.78	3.00	9.41	NA	NA	NA
839	1547	CTAAGTTAACAATGCCAGAA	2	2	NC	NC	14.39	19.14	1.15	1.44	NA	NA	NA
840	1548	TCTAAGTTAACAATGCCAGA	2	2	NC	NC	13.64	26.77	2.16	5.05	NA	NA	NA
841	1549	CTCTAAGTTAACAATGCCAG	2	2	NC	NC	9.38	17.85	1.13	4.30	0.04	0.23	1.88
842	1550	TCTCTAAGTTAACAATGCCA	2	2	NC	NC	13.09	33.96	1.05	8.54	NA	NA	NA
845	1553	GCTTCTCTAAGTTAACAATG	2	2	NC	NC	22.42	40.81	1.00	1.79	NA	NA	NA
846	1554	GGCTTCTCTAAGTTAACAAT	2	2	NC	NC	23.90	37.58	1.55	1.52	NA	NA	NA
847	1555	AGGCTTCTCTAAGTTAACAA	2	2	NC	NC	25.15	30.95	2.56	3.60	NA	NA	NA
848	1556	CAGGCTTCTCTAAGTTAACA	2	2	NC	NC	21.08	13.75	2.12	3.59	NA	NA	NA
849	1557	ACAGGCTTCTCTAAGTTAAC	2	2	NC	NC	22.83	31.48	2.74	1.92	NA	NA	NA
850	1558	CACAGGCTTCTCTAAGTTAA	2	2	NC	NC	23.12	34.35	4.05	4.67	NA	NA	NA
851	1559	TCACAGGCTTCTCTAAGTTA	2	2	NC	NC	29.38	46.70	2.38	5.97	NA	NA	NA
852	1560	ATCACAGGCTTCTCTAAGTT	2	2	NC	NC	37.59	47.94	2.35	7.39	NA	NA	NA
855	1563	CAAATCACAGGCTTCTCTAA	2	2	NC	NC	68.40	93.31	13.78	9.55	NA	NA	NA
856	1564	GCAAATCACAGGCTTCTCTA	2	2	NC	NC	24.37	31.77	5.94	4.88	NA	NA	NA

883	1593	TTGAGGTATTTTATGATGGC	2	2	NC	NC	19.16	31.84	2.64	4.39	NA	NA
884	1594	TTTGAGGTATTTTATGATGG	2	2	NC	NC	30.45	48.27	4.12	5.65	NA	NA
885	1595	CTTTGAGGTATTTTATGATG	2	2	NC	NC	26.53	23.68	3.37	5.69	NA	NA
889	1600	GAATTCTTTGAGGTATTTTA	2	2	NC	NC	24.17	28.63	0.35	3.07	NA	NA
893	1604	AGTTGAATTCTTTGAGGTAT	2	2	NC	NC	22.44	34.76	3.90	8.71	NA	NA
894	1605	AAGTTGAATTCTTTGAGGTA	2	2	NC	NC	34.01	46.42	4.13	4.49	NA	NA
895	1606	CAAGTTGAATTCTTTGAGGT	2	2	NC	NC	31.48	22.43	23.75	3.28	NA	NA
896	1607	CCAAGTTGAATTCTTTGAGG	2	2	NC	NC	25.90	19.17	3.83	3.77	NA	NA
897	1608	TCCAAGTTGAATTCTTTGAG	2	2	NC	NC	26.01	17.09	4.08	1.24	NA	NA
900	1611	TTTTCCAAGTTGAATTCTTT	2	2	NC	NC	72.34	99.69	9.24	4.86	NA	NA
936	1668	GTCATAAATTCCATTTTACT	2	2	NC	NC	19.32	40.31	3.36	5.48	NA	NA
940	1680	GTTCCATTAATTGTCATAAA	2	2	NC	NC	16.14	33.06	0.56	1.18	NA	NA
941	1681	TGTTCCATTAATTGTCATAA	2	2	NC	NC	32.89	51.92	2.28	5.56	NA	NA
945	1685	ATGTTGTTCCATTAATTGTC	2	2	NC	NC	26.77	49.20	3.35	9.23	NA	NA
948	1691	TCCTTAATGTTGTTCCATTA	2	2	NC	NC	37.79	79.84	5.05	9.09	NA	NA
949	1693	ATTCCTTAATGTTGTTCCAT	2	2	NC	NC	53.10	103.25	6.35	20.66	NA	NA
950	1694	GATTCCTTAATGTTGTTCCA	2	2	NC	NC	31.53	46.45	3.95	9.99	NA	NA
955	1699	TTCCAGATTCCTTAATGTTG	2	2	NC	NC	37.91	75.62	3.39	18.66	NA	NA
959	1703	GGATTTCCAGATTCCTTAAT	2	2	NC	NC	36.98	65.94	2.46	2.92	NA	NA
960	1704	AGGATTTCCAGATTCCTTAA	2	2	NC	NC	35.62	62.15	3.70	2.11	NA	NA
961	1705	TAGGATTTCCAGATTCCTTA	2	2	NC	NC	38.52	22.41	1.94	3.91	NA	NA
965	1717	AGTCTGATTCTGTAGGATTT	2	2	NC	NC	19.85	35.08	2.05	1.84	NA	NA
966	1718	CAGTCTGATTCTGTAGGATT	2	3	NC	NC	19.92	26.87	1.89	3.94	NA	NA
967	1719	TCAGTCTGATTCTGTAGGAT	2	2	NC	NC	20.97	29.52	1.85	5.49	NA	NA
968	1720	ATCAGTCTGATTCTGTAGGA	2	3	NC	NC	21.33	33.93	0.53	3.72	NA	NA

972	1724	TCATATCAGTCTGATTCTGT	2	2	NC	NC	26.53	46.80	0.78	4.01	NA	NA
973	1725	TTCATATCAGTCTGATTCTG	1	2	2	2	24.44	48.84	1.86	3.13	NA	NA
998	1770	GAAGTTTTAGTGTGGTCTAA	2	2	2	NC	61.82	71.92	2.67	11.43	NA	NA
999	1771	TGAAGTTTTAGTGTGGTCTA	2	2	2	NC	51.74	45.10	9.13	11.06	NA	NA
1000	1772	ATGAAGTTTTAGTGTGGTCT	2	2	2	NC	53.70	53.39	8.43	12.09	NA	NA
1007	1779	CTCCCAAATGAAGTTTTAGT	2	2	1	NC	46.87	75.13	6.37	23.97	NA	NA
1008	1780	TCTCCCAAATGAAGTTTTAG	2	2	2	NC	54.23	78.26	6.39	22.53	NA	NA
1016	1788	AACTTCCGTCTCCCAAATGA	2	2	NC	NC	48.29	64.35	3.86	20.38	NA	NA
1017	1789	TAACTTCCGTCTCCCAAATG	2	2	NC	NC	45.46	70.65	5.54	18.54	NA	NA
1019	1791	TTTAACTTCCGTCTCCCAAA	3	2	NC	NC	41.03	68.88	3.15	13.89	NA	NA
1021	1793	TCTTTAACTTCCGTCTCCCA	3	2	NC	NC	45.70	77.67	3.35	12.15	NA	NA
1022	1794	TTCTTTAACTTCCGTCTCCC	2	2	NC	NC	43.00	86.71	3.06	15.68	NA	NA
1034	1819	TTTAAGGAGTGGCTGGGTCA	2	2	NC	NC	58.34	62.03	12.32	7.53	NA	NA
1035	1820	ATTTAAGGAGTGGCTGGGTC	2	2	NC	NC	53.90	51.73	2.92	3.24	NA	NA
1036	1821	AATTTAAGGAGTGGCTGGGT	2	2	NC	NC	68.88	48.51	4.89	5.09	NA	NA
1040	1836	GCATTTATTTCCCTTAATTT	2	2	2	NC	47.93	46.43	9.49	5.50	NA	NA
1041	1837	GGCATTTATTTCCCTTAATT	2	2	1	NC	20.55	32.67	2.58	2.25	NA	NA
1042	1838	GGGCATTTATTTCCCTTAAT	2	1	1	NC	12.99	29.64	2.63	2.26	NA	NA
1043	1839	CGGGCATTTATTTCCCTTAA	2	2	2	NC	11.77	17.60	2.40	5.52	0.06	0.22	ND
1044	1840	CCGGGCATTTATTTCCCTTA	2	2	NC	NC	12.67	13.81	1.92	1.24	0.02	0.12	1.19
1045	1841	GCCGGGCATTTATTTCCCTT	2	2	NC	NC	13.68	37.50	0.67	5.43	NA	NA	NA
1047	1844	CAAGCCGGGCATTTATTTCC	2	2	NC	NC	28.96	18.47	5.83	2.58	NA	NA	NA
1096	1913	ATTTACGTAGATGATTTTCT	2	2	NC	NC	53.08	52.94	5.84	3.65	NA	NA	NA
1170	2032	AGGTATTATTGCTTGAAATT	2	2	NC	NC	28.28	48.33	1.61	9.36	NA	NA	NA
1172	2034	GCAGGTATTATTGCTTGAAA	2	2	NC	NC	12.55	27.77	1.79	0.97	NA	NA	NA

1173	2041	ATTAACAGCAGGTATTATTG	2	2	NC	NC	48.21	62.50	5.76	11.58	NA	NA	NA
1211	2090	CAGGAATTTCTAAAATAACG	2	2	NC	NC	49.13	57.36	4.32	11.92	NA	NA	NA
1214	2094	AGTTCAGGAATTTCTAAAAT	2	2	NC	NC	79.03	91.21	6.69	8.43	NA	NA	NA
1216	2096	GGAGTTCAGGAATTTCTAAA	2	2	NC	NC	18.29	36.80	2.38	1.83	NA	NA	NA
1222	2113	ATGCTCCACTGGACTGAGGA	2	2	NC	NC	16.69	18.24	1.23	5.14	NA	NA	NA
1232	2123	TCTTTAAGTAATGCTCCACT	2	2	NC	NC	61.50	77.12	3.42	8.51	NA	NA	NA
1233	2124	ATCTTTAAGTAATGCTCCAC	2	2	NC	NC	51.63	71.96	2.70	2.75	NA	NA	NA
1235	2126	GTATCTTTAAGTAATGCTCC	2	2	NC	NC	31.68	25.10	1.93	8.80	NA	NA	NA
1239	2130	TTGAGTATCTTTAAGTAATG	2	2	NC	NC	51.48	85.95	11.45	18.61	NA	NA	NA
1240	2132	CATTGAGTATCTTTAAGTAA	2	2	NC	NC	48.01	46.09	5.81	4.38	NA	NA	NA
1241	2133	TCATTGAGTATCTTTAAGTA	2	2	NC	NC	42.00	38.76	5.01	5.01	NA	NA	NA
1242	2134	TTCATTGAGTATCTTTAAGT	2	2	NC	NC	38.54	40.10	1.83	5.16	NA	NA	NA
1244	2136	TGTTCATTGAGTATCTTTAA	2	2	NC	NC	36.89	28.49	4.46	6.15	NA	NA	NA
1245	2137	TTGTTCATTGAGTATCTTTA	2	2	NC	NC	32.83	36.71	3.31	7.84	NA	NA	NA
1246	2138	CTTGTTCATTGAGTATCTTT	2	2	NC	NC	23.96	19.15	2.75	4.55	NA	NA	NA
1247	2139	GCTTGTTCATTGAGTATCTT	2	2	NC	NC	19.12	40.62	1.95	6.61	NA	NA	NA
1248	2140	AGCTTGTTCATTGAGTATCT	2	2	NC	NC	16.10	24.45	0.87	4.66	NA	NA	NA
1249	2141	CAGCTTGTTCATTGAGTATC	2	2	NC	NC	15.53	18.08	2.34	2.37	NA	NA	NA
1251	2143	GGCAGCTTGTTCATTGAGTA	2	2	NC	NC	21.08	38.35	4.02	3.26	NA	NA	NA
1252	2144	TGGCAGCTTGTTCATTGAGT	2	3	NC	NC	22.01	19.89	3.22	2.62	0.07	0.24	ND
1254	2146	TTTGGCAGCTTGTTCATTGA	2	2	NC	NC	35.31	24.10	12.62	3.80	NA	NA	NA
1255	2147	CTTTGGCAGCTTGTTCATTG	2	2	NC	NC	26.81	10.55	6.01	1.36	0.07	0.26	1.63
1256	2148	ACTTTGGCAGCTTGTTCATT	2	2	NC	NC	44.60	38.11	6.07	6.73	NA	NA	NA
1257	2149	AACTTTGGCAGCTTGTTCAT	2	2	NC	NC	46.26	27.82	10.50	3.03	NA	NA	NA
1258	2150	CAACTTTGGCAGCTTGTTCA	2	2	NC	NC	36.21	15.35	5.88	2.61	NA	NA	NA

1259	2162	CAGTTTTATCCCCAACTTTG	2	2	NC	NC	27.53	23.74	5.12	3.44	NA	NA
1268	2177	GGTCTTTAAATAATTCAGTT	2	2	NC	NC	20.61	27.40	3.96	3.35	0.13	0.41	ND
1316	2265	TTTCGTATTTCTTGCAAATG	2	2	NC	NC	37.10	41.93	5.75	10.06	NA	NA	NA
1318	2267	TTTTTCGTATTTCTTGCAAA	2	2	NC	NC	52.26	31.85	10.93	1.81	NA	NA	NA
1319	2268	ATTTTTCGTATTTCTTGCAA	2	2	NC	NC	39.03	20.85	10.26	2.62	NA	NA	NA
1320	2269	TATTTTTCGTATTTCTTGCA	2	2	NC	NC	47.22	45.11	9.89	3.25	NA	NA	NA
1321	2270	GTATTTTTCGTATTTCTTGC	2	2	NC	NC	20.41	20.13	2.93	3.87	0.05	0.23	1.25
1322	2271	AGTATTTTTCGTATTTCTTG	2	2	NC	NC	39.26	27.58	5.11	4.16	NA	NA	NA
1328	2307	CCTGATACTGTCACATATTG	2	2	NC	NC	55.01	32.97	9.75	5.81	NA	NA	NA
1329	2308	TCCTGATACTGTCACATATT	2	2	NC	NC	52.74	42.90	5.89	4.78	NA	NA	NA
1373	2374	AACCTTTACCCAATCAGTTG	3	2	NC	NC	47.07	34.37	6.35	5.00	NA	NA	NA
1374	2375	CAACCTTTACCCAATCAGTT	2	2	NC	NC	44.40	67.92	15.96	9.89	NA	NA	NA
1375	2376	CCAACCTTTACCCAATCAGT	2	2	NC	NC	45.07	67.35	4.71	13.26	NA	NA	NA
1376	2377	TCCAACCTTTACCCAATCAG	2	2	NC	NC	64.01	68.26	9.83	13.95	NA	NA	NA
1377	2378	TTCCAACCTTTACCCAATCA	2	2	NC	NC	71.45	74.59	18.83	13.07	NA	NA	NA
1378	2379	CTTCCAACCTTTACCCAATC	2	2	NC	NC	59.21	56.53	14.56	15.52	NA	NA	NA
1379	2380	GCTTCCAACCTTTACCCAAT	2	2	NC	NC	45.57	31.39	21.69	8.62	NA	NA	NA
1380	2381	TGCTTCCAACCTTTACCCAA	2	2	NC	NC	55.11	23.26	12.44	8.05	NA	NA	NA
1381	2382	GTGCTTCCAACCTTTACCCA	2	2	NC	NC	45.92	28.32	13.54	4.29	NA	NA	NA
1382	2383	TGTGCTTCCAACCTTTACCC	2	2	NC	NC	35.78	30.38	8.52	3.88	NA	NA	NA
1383	2384	TTGTGCTTCCAACCTTTACC	2	2	NC	NC	61.02	38.15	15.49	8.42	NA	NA	NA
1386	2387	CTTTTGTGCTTCCAACCTTT	2	2	NC	NC	51.63	28.91	14.78	5.58	NA	NA	NA
1387	2388	GCTTTTGTGCTTCCAACCTT	1	2	2	2	42.27	23.42	20.49	3.09	NA	NA	NA
1407	2435	GATGTCTGTAATTTTCTACA	2	2	NC	NC	42.27	43.98	3.38	9.09	NA	NA	NA
1408	2436	AGATGTCTGTAATTTTCTAC	2	2	NC	NC	42.86	29.19	6.25	3.78	NA	NA	NA

1427	2491	AAAATCAAGCCATTCAGCAC	2	2	NC	NC	101.49	73.07	11.62	7.85	NA	NA	NA
1433	2497	CTCTAGAAAATCAAGCCATT	2	2	NC	NC	37.31	22.56	1.98	3.80	NA	NA	NA
1434	2498	TCTCTAGAAAATCAAGCCAT	2	2	NC	NC	31.11	26.43	10.17	5.34	NA	NA	NA
1435	2499	TTCTCTAGAAAATCAAGCCA	2	2	NC	NC	41.92	29.52	5.61	5.70	NA	NA	NA
1450	2542	GTGATGCACTGCTTTACACA	2	2	NC	NC	27.37	23.56	2.70	2.65	NA	NA	NA
1451	2543	GGTGATGCACTGCTTTACAC	2	2	NC	NC	18.24	27.08	1.20	3.69	0.15	0.45	1.49
1454	2546	CTAGGTGATGCACTGCTTTA	2	2	NC	NC	19.72	4.95	2.00	0.28	0.08	0.27	1.10
1455	2547	GCTAGGTGATGCACTGCTTT	2	2	NC	NC	15.25	13.19	12.00	1.18	0.06	0.18	0.88
1456	2548	TGCTAGGTGATGCACTGCTT	2	2	NC	NC	8.73	11.44	0.93	3.02	0.03	0.25	2.06
1457	2555	CAACAGTTGCTAGGTGATGC	2	2	NC	NC	23.09	14.17	3.60	2.89	0.37	0.73	2.61
1458	2556	TCAACAGTTGCTAGGTGATG	2	2	NC	NC	25.90	17.87	2.81	1.52	0.04	0.30	3.21
1459	2557	GTCAACAGTTGCTAGGTGAT	2	2	1	NC	21.74	17.34	3.10	2.04	0.19	0.67	ND
1460	2558	AGTCAACAGTTGCTAGGTGA	2	2	1	NC	27.41	21.03	7.21	1.14	0.13	0.61	15.80
1461	2559	CAGTCAACAGTTGCTAGGTG	2	2	1	NC	26.55	25.31	7.23	4.23	NA	NA	NA
1476	2590	TTGCTTAGCGACCTTGGCCA	2	2	NC	NC	55.38	15.90	32.59	2.50	NA	NA	NA
1477	2593	TCCTTGCTTAGCGACCTTGG	2	2	NC	NC	41.61	12.54	9.35	4.32	NA	NA	NA
1496	2622	TCTTCTTGTACAGTTGGTCT	2	2	NC	NC	32.56	29.70	10.48	7.68	NA	NA	NA
1497	2623	TTCTTCTTGTACAGTTGGTC	2	2	NC	NC	20.80	17.03	5.21	3.06	0.27	0.63	2.04
1498	2624	TTTCTTCTTGTACAGTTGGT	2	2	NC	NC	29.53	13.66	9.50	2.56	0.03	0.25	2.13
1499	2625	CTTTCTTCTTGTACAGTTGG	2	2	NC	NC	22.83	9.13	4.31	0.69	0.18	0.52	ND
1532	2682	TGTTCTCCCAGCAACACATC	2	2	NC	NC	47.46	27.14	15.61	6.25	NA	NA	NA
1538	2688	TGATCCTGTTCTCCCAGCAA	2	2	NC	NC	24.66	7.17	4.41	1.88	0.08	0.36	1.68
1539	2689	TTGATCCTGTTCTCCCAGCA	2	2	NC	NC	19.33	10.62	2.91	4.26	0.05	0.25	1.49
1540	2690	ATTGATCCTGTTCTCCCAGC	2	2	NC	NC	31.91	22.82	16.29	6.38	NA	NA	NA
1541	2691	TATTGATCCTGTTCTCCCAG	2	2	NC	NC	42.07	33.37	6.78	5.96	NA	NA	NA

1542	2692	ATATTGATCCTGTTCTCCCA	2	2	NC	NC	53.44	53.86	6.38	3.85	NA	NA	NA
1543	2693	CATATTGATCCTGTTCTCCC	2	2	NC	NC	64.40	68.94	7.59	8.23	NA	NA	NA
1544	2694	ACATATTGATCCTGTTCTCC	2	2	NC	NC	82.01	70.33	16.88	6.35	NA	NA	NA
1565	2730	CTCTCTGAGTCCTCTGATAA	2	2	NC	NC	42.55	23.58	4.51	3.32	NA	NA	NA
1566	2731	TCTCTCTGAGTCCTCTGATA	2	2	NC	NC	36.03	29.18	5.36	7.30	NA	NA	NA
1568	2742	ATTATCATTACTCTCTCTGA	2	2	NC	NC	61.55	59.56	12.39	11.34	NA	NA	NA
1569	2743	AATTATCATTACTCTCTCTG	2	2	NC	NC	50.91	71.82	8.91	31.14	NA	NA	NA
1579	2770	GCTCTTTCCACCCATGTTTG	2	2	NC	NC	27.13	22.73	3.88	8.89	NA	NA	NA
1581	2772	GAGCTCTTTCCACCCATGTT	2	2	NC	NC	21.28	13.11	3.94	0.74	0.07	0.15	1.15
1582	2773	GGAGCTCTTTCCACCCATGT	2	2	NC	NC	13.33	17.05	1.66	2.62	0.05	0.10	ND
1583	2782	TTTTATGTAGGAGCTCTTTC	2	2	NC	NC	61.29	41.99	13.48	12.94	NA	NA	NA
1584	2783	GTTTTATGTAGGAGCTCTTT	2	2	NC	NC	44.03	19.45	7.88	2.33	NA	NA	NA
1585	2784	TGTTTTATGTAGGAGCTCTT	2	2	NC	NC	47.18	21.48	3.54	3.10	NA	NA	NA
1586	2785	TTGTTTTATGTAGGAGCTCT	2	2	NC	NC	42.62	16.00	3.79	1.17	NA	NA	NA
1587	2786	CTTGTTTTATGTAGGAGCTC	3	3	NC	NC	26.47	17.43	2.23	3.41	0.05	0.25	1.91
1588	2787	ACTTGTTTTATGTAGGAGCT	2	2	NC	NC	29.82	20.90	2.38	3.99	NA	NA	NA
1589	2788	AACTTGTTTTATGTAGGAGC	2	2	NC	NC	40.86	21.42	8.58	3.85	NA	NA	NA
1590	2789	CAACTTGTTTTATGTAGGAG	2	2	NC	NC	58.69	57.40	6.17	5.46	NA	NA	NA
1591	2790	GCAACTTGTTTTATGTAGGA	2	2	NC	NC	25.04	23.92	3.31	8.78	NA	NA	NA
1594	2793	AATGCAACTTGTTTTATGTA	2	2	NC	NC	72.20	69.11	9.11	6.88	NA	NA	NA
1597	2796	ATCAATGCAACTTGTTTTAT	2	2	NC	NC	85.81	95.46	9.79	10.59	NA	NA	NA
1600	2799	GTAATCAATGCAACTTGTTT	2	2	NC	NC	51.51	41.06	21.54	5.60	NA	NA	NA
1601	2800	GGTAATCAATGCAACTTGTT	2	2	NC	NC	24.57	22.71	2.39	1.25	0.13	0.37	ND
1602	2801	TGGTAATCAATGCAACTTGT	2	2	NC	NC	21.39	20.55	2.20	5.18	0.05	0.22	1.41
1603	2802	ATGGTAATCAATGCAACTTG	2	2	NC	NC	28.82	22.62	2.65	6.53	NA	NA	NA

1604	2803	GATGGTAATCAATGCAACTT	2	2	NC	NC	34.01	28.75	4.52	2.73	NA	NA	NA
1605	2804	TGATGGTAATCAATGCAACT	2	2	NC	NC	43.81	33.91	4.91	5.35	NA	NA	NA
1606	2819	AGCCAATCTGAGCCATGATG	2	2		2	NC	19.96	12.26	3.24	1.31	0.07	0.27	3.67
1607	2820	GAGCCAATCTGAGCCATGAT	2	2		2	NC	23.76	14.02	9.32	0.30	0.05	0.31	3.71
1610	2823	TAGGAGCCAATCTGAGCCAT	2	2		2	NC	20.83	11.89	4.80	1.26	0.10	0.35	1.90
1625	2838	TCTTCTGCAGGAACATAGGA	2	2	NC		NC	50.65	19.34	7.07	2.32	NA	NA	NA
1627	2840	CTTCTTCTGCAGGAACATAG	2	2	NC		NC	51.09	20.35	5.80	2.45	NA	NA	NA
1628	2841	GCTTCTTCTGCAGGAACATA	2	2	NC		NC	35.57	19.49	3.46	1.81	NA	NA	NA
1629	2842	CGCTTCTTCTGCAGGAACAT	2	2	NC		NC	39.30	20.59	7.46	2.38	NA	NA	NA
1631	2844	GTCGCTTCTTCTGCAGGAAC	2	2	NC		NC	19.48	14.60	1.86	1.86	0.03	0.16	1.58
1632	2845	TGTCGCTTCTTCTGCAGGAA	2	2	NC		NC	21.76	13.58	1.40	1.13	0.06	0.28	7.37
1633	2846	TTGTCGCTTCTTCTGCAGGA	2	2	NC		NC	26.21	13.75	8.35	2.95	0.08	0.28	1.61
1634	2847	ATTGTCGCTTCTTCTGCAGG	2	2	NC		NC	40.04	17.98	2.96	5.57	NA	NA	NA
1635	2848	AATTGTCGCTTCTTCTGCAG	2	2	NC		NC	46.92	20.59	3.14	1.18	NA	NA	NA
1636	2849	CAATTGTCGCTTCTTCTGCA	2	2	NC		NC	44.66	17.42	2.95	3.88	NA	NA	NA
1637	2850	CCAATTGTCGCTTCTTCTGC	2	3	NC		NC	41.59	19.68	3.88	3.23	NA	NA	NA
1638	2851	CCCAATTGTCGCTTCTTCTG	2	2	NC		NC	32.04	26.69	11.53	5.23	NA	NA	NA
1639	2852	TCCCAATTGTCGCTTCTTCT	2	2	NC		NC	41.35	44.29	1.88	9.55	NA	NA	NA
1640	2853	ATCCCAATTGTCGCTTCTTC	2	3	NC		NC	65.20	70.02	13.92	11.22	NA	NA	NA
1643	2864	TGCCATCCACACAATCCCAATT	2	2		2	NC	41.83	48.79	13.98	7.95	NA	NA	NA
1644	2865	ATGCCATCCACACAATCCCAAT	2	2		2	NC	63.97	52.82	8.09	14.01	NA	NA	NA
1645	2866	AATGCCATCCACACAATCCCAA	1	1		2	NC	59.66	67.08	13.70	12.42	NA	NA	NA
1646	2867	AAATGCCATCCACACAATCCCA	1	1		2	NC	78.71	76.59	22.83	16.29	NA	NA	NA
1647	2868	AAAATGCCATCCACACAATCCC	2	2		2	NC	93.25	110.00	18.07	33.29	NA	NA	NA
1648	2869	GAAAATGCCATCCACACAATCC	2	2		1	NC	90.33	100.23	17.01	18.40	NA	NA	NA

1649	2870	TGAAAATGCCATCCACAATC	2	2	1	NC	113.95	103.92	65.68	21.09	NA	NA
1650	2871	GTGAAAATGCCATCCACAAT	2	2	2	NC	45.14	32.65	7.68	5.98	NA	NA
1651	2872	TGTGAAAATGCCATCCACAA	1	1	2	NC	40.78	19.87	9.71	2.27	NA	NA
1652	2873	TTGTGAAAATGCCATCCACA	1	1	2	NC	44.41	19.62	5.40	3.76	NA	NA
1653	2874	CTTGTGAAAATGCCATCCAC	1	1	2	NC	48.65	24.91	1.73	8.71	NA	NA
1654	2875	CCTTGTGAAAATGCCATCCA	1	1	2	NC	40.26	26.83	3.89	7.25	NA	NA
1655	2876	TCCTTGTGAAAATGCCATCC	2	2	2	NC	32.86	39.36	2.21	11.81	NA	NA
1656	2877	ATCCTTGTGAAAATGCCATC	2	2	1	NC	46.43	75.07	7.64	13.35	NA	NA
1657	2878	CATCCTTGTGAAAATGCCAT	2	2	2	NC	41.96	71.11	6.97	16.53	NA	NA
1658	2879	CCATCCTTGTGAAAATGCCA	2	2	2	NC	40.33	60.89	9.79	11.35	NA	NA
1659	2880	CCCATCCTTGTGAAAATGCC	2	2	2	NC	39.42	66.58	4.92	12.41	NA	NA
1660	2881	ACCCATCCTTGTGAAAATGC	2	1	2	NC	48.54	80.91	7.11	17.46	NA	NA
1661	2882	CACCCATCCTTGTGAAAATG	2	2	2	NC	53.01	81.62	5.57	14.54	NA	NA
1662	2883	GCACCCATCCTTGTGAAAAT	1	2	2	NC	63.00	50.52	17.43	4.00	NA	NA
1663	2884	AGCACCCATCCTTGTGAAAA	2	2	2	NC	83.48	40.78	10.03	6.56	NA	NA
1664	2885	CAGCACCCATCCTTGTGAAA	1	2	2	NC	67.26	46.89	7.73	5.09	NA	NA
1665	2886	GCAGCACCCATCCTTGTGAA	1	2	1	NC	49.70	35.28	3.93	3.66	NA	NA
1668	2891	TGTCTGCAGCACCCATCCTT	2	2	2	NC	49.54	16.39	3.49	2.77	NA	NA
1669	2892	TTGTCTGCAGCACCCATCCT	2	2	2	NC	55.67	31.03	3.82	8.49	NA	NA
1670	2893	ATTGTCTGCAGCACCCATCC	2	1	1	NC	45.50	26.60	2.04	4.51	NA	NA
1671	2894	TATTGTCTGCAGCACCCATC	2	2	2	NC	48.24	22.99	5.75	5.61	NA	NA
1672	2895	ATATTGTCTGCAGCACCCAT	2	2	2	NC	55.40	26.44	6.04	5.69	NA	NA
1673	2896	TATATTGTCTGCAGCACCCA	2	3	2	NC	63.58	22.39	6.37	2.86	NA	NA
1674	2897	ATATATTGTCTGCAGCACCC	2	2	2	2	46.55	29.58	8.36	2.45	NA	NA
1675	2898	TATATATTGTCTGCAGCACC	2	3	2	2	56.94	32.12	3.10	4.06	NA	NA

1713	2936	TGTCAGTCAGTTCTTCCATA	2	2	NC	NC	38.51	31.85	2.04	4.00	NA	NA
1714	2937	GTGTCAGTCAGTTCTTCCAT	2	2	NC	NC	29.33	22.59	2.08	1.96	NA	NA
1716	2939	CTGTGTCAGTCAGTTCTTCC	2	2	NC	NC	37.78	53.84	5.88	49.02	NA	NA
1717	2940	GCTGTGTCAGTCAGTTCTTC	2	2	NC	NC	25.54	25.11	4.03	8.61	NA	NA
1718	2941	TGCTGTGTCAGTCAGTTCTT	2	2	NC	NC	29.89	21.07	1.44	4.46	NA	NA
1719	2942	CTGCTGTGTCAGTCAGTTCT	2	2	NC	NC	25.76	18.43	2.31	1.49	0.14	0.46	ND
1720	2943	TCTGCTGTGTCAGTCAGTTC	2	2	NC	NC	28.57	21.19	2.04	2.12	NA	NA	NA
1721	2944	TTCTGCTGTGTCAGTCAGTT	2	2	NC	NC	29.39	18.60	1.64	2.41	0.07	0.31	6.67
1722	2945	TTTCTGCTGTGTCAGTCAGT	2	2	NC	NC	39.28	22.93	5.12	4.54	NA	NA	NA
1724	2947	TATTTCTGCTGTGTCAGTCA	2	2	NC	NC	51.45	41.86	5.98	7.72	NA	NA	NA
1727	2950	GATTATTTCTGCTGTGTCAG	2	2	NC	NC	37.06	25.29	5.86	4.85	NA	NA	NA
1728	2951	TGATTATTTCTGCTGTGTCA	2	2	NC	NC	29.42	20.64	4.83	3.41	NA	NA	NA
1729	2952	CTGATTATTTCTGCTGTGTC	2	2	NC	NC	28.67	21.32	4.45	1.95	NA	NA	NA
1730	2953	TCTGATTATTTCTGCTGTGT	2	2	NC	NC	21.19	16.41	2.77	1.76	0.09	0.33	ND
1731	2954	TTCTGATTATTTCTGCTGTG	2	2	NC	NC	29.96	18.56	12.13	3.24	0.06	0.46	11.81
1740	2963	ATGTTGCTTTTCTGATTATT	2	2	NC	NC	57.50	67.22	8.03	30.02	NA	NA	NA
1741	2964	GATGTTGCTTTTCTGATTAT	2	2	NC	NC	60.65	42.71	7.69	8.70	NA	NA	NA
1745	2968	CTGTGATGTTGCTTTTCTGA	2	2	NC	NC	29.03	19.64	2.51	2.17	NA	NA	NA
1746	2969	ACTGTGATGTTGCTTTTCTG	2	2	NC	NC	71.34	49.89	9.61	3.05	NA	NA	NA
1747	2970	GACTGTGATGTTGCTTTTCT	2	2	NC	NC	41.02	35.87	5.17	9.28	NA	NA	NA
1751	2974	CAAGGACTGTGATGTTGCTT	2	2	NC	NC	30.19	24.05	1.81	2.64	NA	NA	NA
1752	2975	CCAAGGACTGTGATGTTGCT	2	2	NC	NC	26.91	22.69	1.86	3.46	NA	NA	NA
1753	2976	ACCAAGGACTGTGATGTTGC	2	2	NC	NC	31.72	26.81	5.62	8.26	NA	NA	NA
1754	2977	AACCAAGGACTGTGATGTTG	2	2	NC	NC	29.04	25.79	4.23	4.06	NA	NA	NA
1755	2978	TAACCAAGGACTGTGATGTT	2	2	NC	NC	54.12	26.12	5.33	4.43	NA	NA	NA

1799	3049	ATACTCAAGTGTAGCATAGG	3	3	NC	NC	61.97	29.64	9.76	5.83	NA	NA
1800	3050	AATACTCAAGTGTAGCATAG	2	2	NC	NC	66.45	32.84	6.70	4.05	NA	NA
1801	3051	AAATACTCAAGTGTAGCATA	2	2	NC	NC	83.11	40.50	10.34	9.22	NA	NA
1859	3135	ACCTGGTGTGAGTAATTTTT	2	2	NC	NC	39.74	33.52	4.06	3.70	NA	NA
1860	3146	GGTAATTCCCCACCTGGTGT	2	2	NC	NC	30.16	46.33	3.99	22.16	NA	NA
1861	3147	TGGTAATTCCCCACCTGGTG	2	3	NC	NC	29.88	18.10	12.44	4.67	0.04	0.17	ND
1862	3154	TCCCATGTGGTAATTCCCCA	3	2	2	NC	38.85	33.98	4.14	3.58	NA	NA	NA
1863	3155	ATCCCATGTGGTAATTCCCC	2	2	2	NC	45.13	42.96	16.88	5.88	NA	NA	NA
1864	3156	AATCCCATGTGGTAATTCCC	2	3	2	NC	46.56	56.15	1.87	7.55	NA	NA	NA
1865	3157	GAATCCCATGTGGTAATTCC	2	2	2	NC	43.98	36.52	4.54	3.54	NA	NA	NA
1866	3158	AGAATCCCATGTGGTAATTC	2	2	1	NC	50.81	39.97	4.17	3.24	NA	NA	NA
1867	3159	AAGAATCCCATGTGGTAATT	2	2	1	NC	70.78	68.91	8.85	8.07	NA	NA	NA
1868	3160	CAAGAATCCCATGTGGTAAT	2	2	2	NC	57.30	34.39	6.47	1.13	NA	NA	NA
1869	3161	CCAAGAATCCCATGTGGTAA	1	1	2	NC	38.91	22.81	4.39	1.10	NA	NA	NA
1892	3186	TCCAGTTTGCTTTCATCCTC	2	2	NC	NC	76.01	88.11	9.13	11.04	NA	NA	NA
1893	3187	ATCCAGTTTGCTTTCATCCT	2	2	NC	NC	81.36	92.92	11.09	5.38	NA	NA	NA
1894	3188	GATCCAGTTTGCTTTCATCC	2	2	NC	NC	58.44	41.92	12.70	2.97	NA	NA	NA
1895	3189	GGATCCAGTTTGCTTTCATC	2	2	NC	NC	55.68	30.10	6.00	2.80	NA	NA	NA
1896	3190	TGGATCCAGTTTGCTTTCAT	2	2	NC	NC	53.87	33.06	4.88	7.44	NA	NA	NA
1903	3218	CAAAATCAGGGACTTGTTCT	2	2	NC	NC	62.87	90.64	5.52	61.69	NA	NA	NA
1904	3219	ACAAAATCAGGGACTTGTTC	2	2	NC	NC	54.02	54.01	5.45	0.88	NA	NA	NA
1905	3220	GACAAAATCAGGGACTTGTT	2	2	NC	NC	34.95	38.02	3.58	2.61	NA	NA	NA
1906	3221	TGACAAAATCAGGGACTTGT	2	2	NC	NC	50.22	36.75	10.72	4.79	NA	NA	NA
1907	3222	GTGACAAAATCAGGGACTTG	2	2	NC	NC	56.19	39.89	13.13	0.56	NA	NA	NA
1908	3223	GGTGACAAAATCAGGGACTT	2	2	NC	NC	45.29	29.23	3.97	0.69	NA	NA	NA

1925	3240	GTTATTTGGTAAAGGAAGGT	2	2	NC	NC	57.29	52.34	22.76	5.96	NA	NA	NA
1935	3250	AATTCCTCTAGTTATTTGGT	2	2	NC	NC	58.09	58.38	4.71	6.93	NA	NA	NA
1954	3269	ATCCATAACTCCTTGCTGCA	2	2	NC	NC	36.60	34.46	3.20	9.69	NA	NA	NA
1962	3277	CACATTTAATCCATAACTCC	2	2	NC	NC	73.57	99.41	2.49	5.56	NA	NA	NA
1963	3278	CCACATTTAATCCATAACTC	2	2	NC	NC	62.10	100.32	14.47	8.69	NA	NA	NA
1964	3279	GCCACATTTAATCCATAACT	2	2	NC	NC	27.86	49.22	4.72	6.22	NA	NA	NA
1965	3280	AGCCACATTTAATCCATAAC	2	2	NC	NC	30.97	48.86	3.37	0.84	NA	NA	NA
1966	3281	TAGCCACATTTAATCCATAA	2	2	NC	NC	25.37	38.04	5.36	8.00	NA	NA	NA
1967	3282	TTAGCCACATTTAATCCATA	2	2	NC	NC	61.79	60.00	4.25	7.29	NA	NA	NA
1969	3284	GTTTAGCCACATTTAATCCA	2	2	NC	NC	62.58	47.39	6.33	3.81	NA	NA	NA
1970	3285	AGTTTAGCCACATTTAATCC	2	2	NC	NC	81.56	74.86	4.07	1.02	NA	NA	NA
1971	3286	TAGTTTAGCCACATTTAATC	2	2	NC	NC	83.42	80.09	12.41	4.87	NA	NA	NA
2025	3352	TATTAATCCTTCCAGCTCTT	2	2	NC	NC	76.70	80.33	8.30	7.62	NA	NA	NA
2026	3353	TTATTAATCCTTCCAGCTCT	2	2	NC	NC	101.93	72.29	38.99	5.36	NA	NA	NA
2027	3354	TTTATTAATCCTTCCAGCTC	2	2	NC	NC	83.62	61.86	7.60	4.09	NA	NA	NA
2066	3400	CATCGTCCATAACTTTGCAA	3	2	NC	NC	33.22	23.89	3.20	2.08	NA	NA	NA
2067	3401	GCATCGTCCATAACTTTGCA	2	2	NC	NC	30.13	24.58	3.62	5.37	NA	NA	NA
2068	3402	TGCATCGTCCATAACTTTGC	2	2	NC	NC	26.51	15.88	3.42	2.55	0.12	0.47	ND
2069	3403	ATGCATCGTCCATAACTTTG	2	2	NC	NC	46.65	27.73	6.24	3.68	NA	NA	NA
2070	3404	TATGCATCGTCCATAACTTT	3	2	NC	NC	75.29	35.45	10.66	3.70	NA	NA	NA
2075	3428	TCCACTTCTGCAGGTCTTGT	2	2	NC	NC	35.98	21.28	6.82	4.55	NA	NA	NA
2076	3429	GTCCACTTCTGCAGGTCTTG	2	2	NC	NC	32.19	18.86	2.52	2.42	NA	NA	NA
2077	3430	TGTCCACTTCTGCAGGTCTT	2	2	NC	NC	35.15	34.84	6.67	4.98	NA	NA	NA
2078	3431	CTGTCCACTTCTGCAGGTCT	2	2	NC	NC	35.87	26.34	6.79	1.84	NA	NA	NA
2079	3432	TCTGTCCACTTCTGCAGGTC	2	2	NC	NC	37.04	24.80	3.68	1.87	NA	NA	NA

2108	3462	GAAGTCTGTGTTTCTTCCAT	2	2	NC	NC	31.09	25.62	7.10	3.54	NA	NA	NA
2138	3531	TTGTACAGTTGGTATTTTTA	2	2	NC	NC	48.13	36.80	8.86	7.67	NA	NA	NA
2143	3536	TTATTTTGTACAGTTGGTAT	2	2	NC	NC	48.18	50.26	14.21	3.39	NA	NA	NA
2144	3537	GTTATTTTGTACAGTTGGTA	3	2	NC	NC	38.42	24.67	3.62	2.01	NA	NA	NA
2145	3538	AGTTATTTTGTACAGTTGGT	3	2	NC	NC	38.93	29.43	4.93	3.92	NA	NA	NA
2146	3539	GAGTTATTTTGTACAGTTGG	2	2	NC	NC	47.46	32.24	10.39	4.53	NA	NA	NA
2147	3540	AGAGTTATTTTGTACAGTTG	2	2	NC	NC	61.10	41.21	5.97	8.65	NA	NA	NA
2156	3549	TGTTACTGGAGAGTTATTTT	2	2	NC	NC	68.06	56.63	14.65	8.01	NA	NA	NA
2157	3550	CTGTTACTGGAGAGTTATTT	2	2	NC	NC	45.90	48.42	13.51	12.26	NA	NA	NA
2158	3551	GCTGTTACTGGAGAGTTATT	2	2	NC	NC	38.58	24.20	8.97	3.62	NA	NA	NA
2159	3552	GGCTGTTACTGGAGAGTTAT	2	2	NC	NC	30.97	22.33	4.43	4.80	NA	NA	NA
2160	3553	AGGCTGTTACTGGAGAGTTA	2	2	NC	NC	28.83	22.37	10.30	4.03	NA	NA	NA
2193	3594	TACCATGGTCATAATTTTAT	2	2	NC	NC	38.71	19.75	5.86	2.34	NA	NA	NA
2194	3595	ATACCATGGTCATAATTTTA	2	2	NC	NC	40.08	30.60	4.87	3.21	NA	NA	NA
2299	3756	TTATATTCTGCCACTTAAGG	2	2	NC	NC	62.64	32.32	8.92	2.78	NA	NA	NA
2300	3757	ATTATATTCTGCCACTTAAG	2	2	NC	NC	76.61	37.79	7.95	13.87	NA	NA	NA
2302	3759	GAATTATATTCTGCCACTTA	2	2	NC	NC	76.68	65.77	15.50	3.10	NA	NA	NA
2312	3769	AAAAGCTTGGGAATTATATT	2	2	NC	NC	100.82	44.55	22.85	3.63	NA	NA	NA
2313	3770	CAAAAGCTTGGGAATTATAT	2	2	NC	NC	81.54	37.94	15.16	0.76	NA	NA	NA
2385	3904	GTTCTTGGTGGATAAACTGG	2	2	NC	NC	37.40	25.72	10.73	2.86	NA	NA	NA
2388	3907	TATGTTCTTGGTGGATAAAC	2	2	NC	NC	50.84	35.79	9.14	2.56	NA	NA	NA
2390	3909	CTTATGTTCTTGGTGGATAA	2	2	NC	NC	35.97	27.90	5.01	1.29	NA	NA	NA
2391	3910	TCTTATGTTCTTGGTGGATA	2	2	NC	NC	36.07	32.37	9.77	2.38	NA	NA	NA
2392	3911	TTCTTATGTTCTTGGTGGAT	2	2	NC	NC	34.28	32.94	5.66	0.84	NA	NA	NA
2393	3912	ATTCTTATGTTCTTGGTGGA	2	2	NC	NC	49.37	44.48	2.24	1.61	NA	NA	NA

2394	3913	AATTCTTATGTTCTTGGTGG	2	2	NC	NC	36.70	33.41	3.18	2.71	NA	NA	NA
2395	3914	AAATTCTTATGTTCTTGGTG	2	2	NC	NC	31.32	34.05	2.76	6.78	NA	NA	NA
2416	4077	AGTAGAGATGTACTTTATAT	2	2	NC	NC	48.81	43.76	7.27	5.62	NA	NA	NA
2417	4078	TAGTAGAGATGTACTTTATA	2	2	NC	NC	51.73	45.33	6.97	14.13	NA	NA	NA
2418	4079	TTAGTAGAGATGTACTTTAT	2	2	NC	NC	44.05	32.62	5.14	4.84	NA	NA	NA
2460	4284	GCTTGATAATTCTATTTCTT	2	2	NC	NC	28.78	27.45	3.64	4.24	NA	NA	NA
2462	4286	AAGCTTGATAATTCTATTTC	2	2	NC	NC	71.05	33.54	8.25	2.08	NA	NA	NA
2463	4297	CTAGTTTTTAAAAGCTTGAT	2	2	NC	NC	64.80	32.74	13.18	2.68	NA	NA	NA

Example 3. In Vitro Screen for Reduced Expansion

Expansion of DNA triplet repeats can be replicated *in vitro* using patient-derived cells lines and DNA-damaging agents. Human fibroblasts from Huntington's (GM04281, GM04687 and GM04212) or Friedreich's Ataxia patients (GM03816 and GM02153) or Myotonic Dystrophy1 (GM04602, GM03987 and GM03989) are purchased from Coriell Cell Repositories and are maintained in medium following the manufacturer's instructions (Kovtum et al., 2007 *Nature*, 447(7143): 447–452; Li et al., 2016 *Biopreservation and Biobanking* 14(4):324-29; Zhang et al., 2013 *Mol Ther* 22(2): 312-320). To induce CAG-repeat expansion *in vitro*, fibroblast cells are treated with oxidizing agents such as hydrogen peroxide (H_2O_2), potassium chromate (K_2CrO_4) or potassium bromate ($KBrO_3$) for up to 2 hrs (Kovtum et al., ibid). Cells are washed, and medium replace to allow cells to recover for 3 days. The treatment is repeated up to twice more before cells are harvested and DNA isolated. CAG repeat length is determined using methods described below.

Expansion of DNA triplet repeats can be replicated *in vitro* using patient-derived cell lines. Induced pluripotent stem cells (iPSC) derived from Human fibroblasts from Huntington's Patients (CS09iHD-109n1) are purchased from Cedars-Sinai RMI Induced Pluripotent Stem Cell Core and are maintained following the manufacturer's recommendations (https://www.cedars-sinai.org/content/dam/cedars-sinai/research/documents/biomanufacturing/recommended-guidelines-for-handling-ipscsv1.pdf). The CAG repeat from an iPSC line with 109 CAGs shows an increase in CAG repeat size over time, with an average expansion of 4 CAG repeats over 70 days in dividing iPS cells (Goold et al., 2019 Human Molecular Genetics Feb 15; 28(4): 650–661).

CS09iHD-109n1 iPSC are treated with either LNP-formulated siRNA or ASO for continuous knockdown of target mRNA and CAG repeat expansion is determined by DNA fragment analysis described below. SiRNAs or ASOs are added to cells in varying concentrations every 3 to 15 days and knockdown of mRNA is determined by RT-qPCR using standard molecular biology techniques. DNA and mRNA are isolated from cells according to standard techniques at t= 0,14 days, 28 days, 42 days, 56 days and 80 days. Lines represent linear regression best fits. The differences in expansion between treatment and control are compared according to a linear repeated-measures model, and at each time point according to Tukey's post-hoc tests.

Example 4.

Genomic DNA Extraction and Quantitation of CAG Repeat Length by Small Pool-PCR (sp-PCR) Analyses

Genomic DNA is purified using standard Proteinase K digestions and extracted using DNAzol (Invitrogen) following the manufacturer's instructions. CAG repeat length is determined by small pool-PCR analyses as previously described (Mario Gomes-Pereira and Darren Monckton, 2017, *Front Cell Neuro* 11:153). In brief, DNA is digested with HindIII, diluted to a final concentration between 1-6pg/µl and approximately 10 pg was used in subsequent PCR reactions. Primer flaking Exon 1 of the human HTT are used to amplify the CAG alleles and the PCR product is resolved by electrophoresis. Subsequently, Southern blot hybridization is performed, and the CAG alleles are observed by autoradiography OR visualized with ethidium bromide staining. CAG length can be measured directly

by sequencing on a MiSeQ or appropriate machine. The change in CAG repeat number in various treatment groups in comparison with controls is calculated using simple descriptive statistics (e.g. mean ± standard deviation).

Genomic DNA Extraction and Quantitation of CAG Repeat Length by DNA Fragment Analyses

Genomic DNA is purified using DNAeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. DNA is quantified by Qubit dsDNA assay (ThemoScientific) and CAG repeat length is determined by fragment analysis by Laragen (Culver City, CA).

Example 5. Mouse Studies

Natural history studies in HD mouse models:

The HD mouse R6/2 line is transgenic for the 5' end of the human HD gene (HTT) carrying approximately 120 CAG repeat expansions. HTT is ubiquitously expressed. Transgenic mice exhibit a progressive neurological phenotype that mimics many of the pathological features of HD, including choreiform-like movements, involuntary stereotypic movements, tremor, and epileptic seizures, as well as nonmovement disorder components, including unusual vocalization. They urinate frequently and exhibit loss of body weight and muscle bulk through the course of the disease. Neurologically these mice develop Neuronal Intranuclear Inclusions (NII) which contain both the huntingtin and ubiquitin proteins. Previously unknown, these NII have subsequently been identified in HD patients. The age of onset for development of HD symptoms in R6/2 mice has been reported to occur between 9 and 11 weeks (Mangiarini et al., 1996 Cell 87: 493-506).

Somatic expansions were reported in R6/2 mice striatum, cortex and liver. Somatic instability increased with higher constitutive length (Larson et al, Neurobiology of Disease 76 (2015) 98–111). A natural history study in R6/2 mice with 120 CAG repeats was performed. Their genotype and length of CAG expansion was determined. R6/2 mice at 4, 8, 12 and 16 weeks of age (4 male and 4 female mice per age group) were sacrificed. Striatum, cerebellum, cortex, liver, kidney, heart, spleen, lung, duodenum, colon, quadricep, CSF and plasma were collected and snap frozen in liquid nitrogen. Genomic DNA was extracted, the length of CAG repeats measured, and the instability index was calculated from striatum, cerebellum, cortex, liver and kidney according to Lee et al. BMC Systems Biology 2010, 4:29). At 12 and 16 weeks of age, the striatum showed a significant increase of somatic expansion as measured by the instability index (****$p<0.0001$, One-way ANOVA) (FIG. 1). No changes in somatic expansion were observed across all ages in the R6/2 mouse cerebellum (FIG. 2)

Mouse models recapitulating many of the features of trinucleotide repeat expansion diseases including, HD, FA and DM1, are readily available from commercial venders and academic institutions (Polyglutamine Disorders, Advances in Experimental Medicine and Biology, Vol 1049, 2018: Editors Clevio Nobrega and Lois Pereira de Almeida, Springer). All mouse experiments are conducted in accordance with local IACUC guidelines. Three examples of different diseased mouse models and how they could be used to investigate the usefulness of pharmacological intervention against MSH3 for somatic expansion are included below.

In Huntington's research, several transgenic and knock-in mouse models were generated to investigate the underlying pathological mechanisms involved in the disease. For example, the R6/2 transgenic mouse contains a transgene of ~1.9kb of human HTT containing 144 copies of the CAG repeat (Mangiarini et al., 1996 Cell 87: 493-506) while the HdhQ111 model was generated by replacing the mouse HTT exon 1 with a human exon1 containing 111 copies of the CAG repeat (Wheeler et al., 2000 Hum Mol Genet 9:503-513). Both the R6/2 and HdhQ111 models replicate many of the features of human HD including motor and behavioral dysfunctions, neuronal loss, as well as the expansion of CAG repeats in the striatum (Pouladi et al., 2013, Nature Reviews Neuroscience 14: 708-721; Mangiarini et al., 1997 Nature Genet 15: 197-200; Wheeler et al., Hum Mol Genet 8: 115-122).

R6/2 mice are genotyped using DNA derived from tail snips at weaning and the CAG repeat size is determined. Mice are randomized into groups (n=12/group) at weaning at 4 wks old and dosed with monthly (week 4 and 8) ICV injection of either PBS (control) or up to a 500 µg dose of oligos targeting MSH3. A series of oligos targeting different regions of MSH3 can be tested to identify the most efficacious oligo sequence in vivo. At 12 weeks of age, mice are euthanized, and tissues extracted for analyses. The list of tissues includes, but not restricted to, striatum, cortex, cerebellum, and liver. Genomic DNA is extracted and the length of CAG repeats measured as described below. CSF and plasma are collected for biomarker analysis. Additional pertinent mouse models of HD can be considered.

In Friedreich Ataxia, the YG8 FRDA transgenic mouse model is commonly used to understand the pathology (Al-Mahdawi et al., 2006 Genomics 88(5)580-590; Bourn et al., 2012 PLOS One 7(10); e47085). This model was generated through the insertion of a human YAC transgenic containing in the background of a null FRDA mouse. The YG8 model demonstrates somatic expansion of the GAA triplet repeat expansion in neuronal tissues with only mild motor defects. YG8 FRDA mice are genotyped using DNA derived from tail snips at weaning and the CAG repeat size is determined using methods. To determine if MSH3 plays a role in somatic expansion of the disease allele, hemizygous YG8 FRDA animals are administered ICV with oligos targeting knockdown of MSH3 identified above.

Approximately 2 months later, animals are euthanized and tissues collected for molecular analyses. Suitable tissues are heart, quadriceps, dorsal root ganglia (DRG's), cerebellum, kidney, and liver. Genomic DNA is extracted, and the length of CAG repeats measured as described above in Example 4.

In Myotonic Dystrophy, the DM300-328 transgenic mouse model is suitable for investigating the pathology behind DM1. This mouse model has a large human genomic sequence (~45 kb) containing over 300 CTG repeats and displays both the somatic expansion and degenerative muscle changes observed in human DM1 (Seznec et al., 2000; Tome et al., 2009 PLOS Genetics 5(5): e1000482; Pandey et al., 2015 J Pharmacol Exp Ther 355:329-340). DM300-328 mice are genotyped using DNA derived from tail snips at weaning and the CAG repeat size is determined. To determine if MSH3 plays a role in somatic expansion of the disease allele in myotonic dystrophy, DM300-328 transgenic animals are administered ASOs targeting knockdown of MSH3 by either

subcutaneous injections (sc), intraperitoneal (ip) or intravenous tail injections (iv). Mice are administered ASOs up to 2x/week for maximum 8 weeks of treatment. Animals are euthanized at multiple time points and tissues collected for molecular analyses. Suitable tissues are quadriceps, heart, diaphragm, cortex, cerebellum, sperm, kidney, and liver. Genomic DNA is extracted and the length of CAG repeats measured and compared with parallel controls.

The HdhQ111 mouse model for Huntington Disease is a heterozygous knock-in line, in which the majority of exon 1 and part of intron 1 on one allele of the huntingtin gene (i.e., HTT or Huntington Disease gene) are replaced with human DNA containing ~111 CAG repeats. In this example, ASOs to knock down MSH3 activity or levels is administered. After a treatment period, brain tissue from treated or untreated mice is isolated (e.g., striatum tissue) and analyzed using qRT-PCR as previously described to determine RNA levels of MSH3. Huntingtin gene repeat analysis is performed using mouse tissues (e.g., striatum tissue) after a treatment period using a human-specific PCR assay that amplifies the HTT CAG repeat from the knock-in allele but does not amplify the mouse sequence (i.e., the wild type allele). In this protocol, the forward primer is fluorescently labeled (e.g., with 6-FAM as described previously, for example Pinto RM, Dragileva E, Kirby A, et al. Mismatch repair genes MLH1 and MSH3 modify CAG instability in Huntington's disease mice: genome-wide and candidate approaches. PLoS Genet. 2013;9(10):e1003930.), and products can be resolved using an analyzer with comparison against an internal size standard to generate CAG repeat size distribution traces. Repeat size is determined from the peak with the greatest intensity from a control tissue (e.g., tail tissue in a mouse) and from an affected tissue (e.g., brain striatum tissue or brain cortex tissue). Immunohistochemistry is carried out with polyclonal anti-huntingtin antibody (e.g., EM48) on paraffin-embedded or otherwise prepared sections of brain tissue and can be quantified using a standardized staining index to capture both nuclear staining intensity and number of stained nuclei. A decrease in repeat size in affected tissue indicates that the agent that reduces the level and/or activity of MSH3 is capable of decreasing the repeat which are responsible for the toxic and/or defective gene products in Huntington's disease.

Other Aspects

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

While the invention has been described in connection with specific aspects thereof, it will be understood that invention is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and can be applied to the essential features hereinbefore set forth, and follows in the scope of the claimed.

In addition to the various aspects described herein, the present disclosure includes the following aspects numbered E1 through E90. This list of aspects is presented as an exemplary list and the application is not limited to these particular.

E1. A single-stranded oligonucleotide of 10-30 linked nucleosides in length, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene.

E2. The oligonucleotide of E1, wherein the oligonucleotide comprises: (a) a DNA core sequence comprising linked deoxyribonucleosides; (b) a 5' flanking sequence comprising linked nucleosides; and (c) a 3' flanking sequence comprising linked nucleosides; wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

E3. A single-stranded oligonucleotide of 10-30 linked nucleosides in length for inhibiting expression of a human MSH3 gene in a cell, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene.

E4. The oligonucleotide of E3, wherein the oligonucleotide comprises: (a) a DNA core comprising linked deoxyribonucleosides; (b) a 5' flanking sequence comprising linked nucleosides; and (c) a 3' flanking sequence comprising linked nucleosides; wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

E5. The oligonucleotide of any one of E1-E4, wherein the region of at least 10 nucleobases has at least 90% complementary to an MSH3 gene.

E6. The oligonucleotide of any one of E1-E5, wherein the region of at least 10 nucleobases has at least 95% complementary to an MSH3 gene.

E7. The oligonucleotide of any one of E1-E6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 355-385, 398-496, 559-589, 676-724, 762-810, 876-903, 912-974, 984-1047, 1054-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1768-1866, 2029-2063, 2087-2199, 2262-2293, 2304-2330, 2371-2410, 2432-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3073, 31323245, 3266-3306, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4074-4101, or 4281-4319 of the MSH3 gene.

E8. The oligonucleotide of any one of E1-E7, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 359-385, 398-496, 559-589, 676-724, 762-810, 876-974, 984-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1834-1866,

2029-2056, 2093-2199, 2262-2293, 2304-2329, 2371-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3072, 3132-3245, 3266-3303, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4076-4101, or 4281-4319of the MSH3 gene.

E9. The oligonucleotide of any one of E1-E6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-196, 359-385, 413-462, 559-589, 676-724, 762-810, 876-974, 984-1096, 1114-1179, 1200-1227, 1294-1337, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2265-2293, 2378-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2712, 2727-2753, 2767-2919, 2934-3000, 3046-3071, 3144-3183, 3220-3245, 3397-3484, 3534-3575, 3591-3616, 3901-3931, or 4281-4306 of the MSH3 gene.

E10. The oligonucleotide of any one of E1-E6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 435-462, 559-584, 763-808, 876-902, 931-958, 1001-1083, 1114-1179, 1294-1337, 1544-1578, 1835-1863, 2031-2056, 2144-2169, 2543-2577, 2590-2615, 2621-2647, 2685-2711, 2769-2795, or 2816-2868 of the MSH3 gene.

E11. The oligonucleotide of any one of E1-E6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 876-902, 930-958, 1056-1081, 1114-1139, 1154-1179, 1310-1337, 1546-1571, 1836-1862, 2141-2199, 2267-2292, 2540-2580, 2620-2647, 2686-2711, 2769-2868, 2939-2976, 3144-3169, or 3399-3424 of the MSH3 gene.

E12. The oligonucleotide of any one of E1-E6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 984-1021, 1467-1493, 1722-1747, 1767-1802, 1833-1861, 2385-2410, 2554-2581, 2816-2845, 2861-2920, or 3151-3183 of the MSH3 gene.

E13. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 6-2545.

E14. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-

2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463.

E15. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462 -2463.

E16. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460.

E17. The oligonucleotide of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633.

E18. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068.

E19. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

E20. The oligonucleotide of any one of E1-E6, wherein the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 6-2545.

E21. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463.

E22. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462-2463.

E23. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604,

1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460.

E24. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633.

E25. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068.

E26. The oligonucleotide of any one of E1-E6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

E27. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 50% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E28. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 60% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E29. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 70% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E30. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 85% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E31. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 50% mRNA inhibition at a 2 nM when determined using a cell assay when compared with a control cell.

E32. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 60% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E33. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 70% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E34. The oligonucleotide of any one of E1-E26, wherein the oligonucleotide exhibits at least 85% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

E35. The oligonucleotide of any one of E1-E34, wherein the oligonucleotide comprises at least one alternative internucleoside linkage.

E36. The oligonucleotide of E35, wherein the at least one alternative internucleoside linkage is a phosphorothioate internucleoside linkage.

E37. The oligonucleotide of E35, wherein the at least one alternative internucleoside linkage is a 2'-alkoxy internucleoside linkage.

E38. The oligonucleotide of E35, wherein the at least one alternative internucleoside linkage is an alkyl phosphate internucleoside linkage.

E39. The oligonucleotide of any one of E1-E38, wherein the oligonucleotide comprises at least one alternative nucleobase.

E40. The oligonucleotide of E39, wherein the alternative nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

E41. The modified oligonucleotide of any one of E1-E40, wherein the oligonucleotide comprises at least one alternative sugar moiety.

E42. The modified oligonucleotide of E41, wherein the alternative sugar moiety is 2'-OMe or a bicyclic nucleic acid.

E43. The oligonucleotide of any one of E1-E42, wherein the oligonucleotide further comprises a ligand conjugated to the 5' end or the 3' end of the oligonucleotide through a monovalent or branched bivalent or trivalent linker.

E44. The oligonucleotide of any one of E1-E43, wherein oligonucleotide comprises a region complementary to at least 17 contiguous nucleotides of a MSH3 gene.

E45. The oligonucleotide of any one of E1-E43, wherein oligonucleotide comprises a region complementary to at least 19 contiguous nucleotides of a MSH3 gene.

E46. The oligonucleotide of any one of E1-E43, wherein the oligonucleotide comprises a region complementary to 19 to 23 contiguous nucleotides of a MSH3 gene.

E47. The oligonucleotide of any one of E1-E43, wherein the oligonucleotide comprises a region complementary to 19 contiguous nucleotides of a MSH3 gene.

E48. The oligonucleotide of any one of E1-E43, wherein the oligonucleotide comprises a region complementary to 20 contiguous nucleotides of a MSH3 gene.

E49. The oligonucleotide of any one of E1-E43, wherein the oligonucleotide is from about 15 to 25 nucleosides in length.

E50. The oligonucleotide of any one of E1-E43, wherein the oligonucleotide is 20 nucleosides in length.

E51. A pharmaceutical composition comprising one or more of the oligonucleotides of any one of E1-E50 and a pharmaceutically acceptable carrier or excipient.

E52. A composition comprising one or more of the oligonucleotides of any one of E1-E50 and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

E53. A method of inhibiting transcription of MSH3 in a cell, the method comprising contacting the cell with one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical

composition of E51, or the composition of E52 for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibits expression of the MSH3 gene in the cell.

E54. A method of treating, preventing, or delaying the progression a trinucleotide repeat expansion disorder in a subject in need thereof, the method comprising administering to the subject one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52.

E55. A method of reducing the level and/or activity of MSH3 in a cell of a subject identified as having a trinucleotide repeat expansion disorder, the method comprising contacting the cell with one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52.

E56. A method for inhibiting expression of an MSH3 gene in a cell comprising contacting the cell with one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52 and maintaining the cell for a time sufficient to obtain degradation of a mRNA transcript of an MSH3 gene, thereby inhibiting expression of the MSH3 gene in the cell.

E57. A method of decreasing trinucleotide repeat expansion in a cell, the method comprising contacting the cell with one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52.

E58. The method of E56 or E57, wherein the cell is in a subject.

E59. The method of any one of E54, E55, and E58, wherein the subject is a human.

E60. The method of any one of E54-E58, wherein the cell is a cell of the central nervous system or a muscle cell.

E61. The method of any one of E54, E55, and E58-60, wherein the subject is identified as having a trinucleotide repeat expansion disorder.

E62. The method of any one of E54, E55, and E57-61, wherein the trinucleotide repeat expansion disorder is a polyglutamine disease.

E63. The method of E62, wherein the polyglutamine disease is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, and Huntington's disease-like 2.

E64. The method of any one of E54-E61, wherein the trinucleotide repeat expansion disorder is a non-polyglutamine disease.

E65. The method of E64, wherein the non-polyglutamine disease is selected from the group consisting of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

E66. One or more oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52, for use in the prevention or treatment of a trinucleotide repeat expansion disorder.

E67. The oligonucleotide, pharmaceutical composition, or composition of E65, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

E68. The oligonucleotide, pharmaceutical composition, or composition of E66 or E67, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

E69. The oligonucleotide, pharmaceutical composition, or composition for the use of E66 or E67, wherein the trinucleotide repeat expansion disorder is Friedreich's ataxia.

E70. The oligonucleotide, pharmaceutical composition, or composition for the use of E66 or E67, wherein the trinucleotide repeat expansion disorder is myotonic dystrophy type 1.

E71. The oligonucleotide, pharmaceutical composition, or composition for the use of any of E66-E70, wherein the modified oligonucleotide, pharmaceutical composition, or composition is administered intrathecally.

E72. The oligonucleotide, pharmaceutical composition, or composition of any of E66-E70, wherein the modified oligonucleotide, pharmaceutical composition, or composition is administered intraventricularly.

E73. The oligonucleotide, pharmaceutical composition, or composition of any of E66-E70, wherein the oligonucleotide, pharmaceutical composition, or composition is administered intramuscularly.

E74. A method of treating, preventing, or delaying progression a disorder in a subject in need thereof wherein the subject is suffering from trinucleotide repeat expansion disorder, comprising administering to said subject one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52.

E75. The method of E74, further comprising administering an additional therapeutic agent.

E76. The method of E75, wherein the additional therapeutic agent is another oligonucleotide that hybridizes to an mRNA encoding the Huntingtin gene.

E77. A method of preventing or delaying the progression of a trinucleotide repeat expansion disorder in a subject, the method comprising administering to the subject one or more of the oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52 in an amount effective to delay progression of a trinucleotide repeat expansion disorder of the subject.

E78. The method of E77, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type

17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

E79. The method of E77 or E78, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

E80. The method of E77 or E78, wherein the trinucleotide repeat expansion disorder is Friedrich's ataxia.

E81. The method of E77 or E78, wherein the trinucleotide repeat expansion disorder is myotonic Dystrophy type 1.

E82. The method of E77 or E78, further comprising administering an additional therapeutic agent.

E83. The method of E82, wherein the additional therapeutic agent is an oligonucleotide that hybridizes to an mRNA encoding the Huntingtin gene.

E84. The method of any of E77-E83, wherein progression of the trinucleotide repeat expansion disorder is delayed by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with a predicted progression.

E85. One or more oligonucleotides of any one of E1-E50, the pharmaceutical composition of E51, or the composition of E52, for use in preventing or delaying progression of a trinucleotide repeat expansion disorder in a subject.

E86. The oligonucleotide, pharmaceutical composition, or composition of E85, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

E87. The oligonucleotide, pharmaceutical composition, or composition of E85 or E86, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

E88. The oligonucleotide, pharmaceutical composition, or composition of E85 or E86, wherein the trinucleotide repeat expansion disorder is Friedrich's ataxia.

E89. The oligonucleotide, pharmaceutical composition, or composition of E85 or E86, wherein the trinucleotide repeat expansion disorder is myotonic Dystrophy type 1.

E90. The oligonucleotide, pharmaceutical composition, or composition of any one of E85-E89, wherein progression of the trinucleotide repeat expansion disorder is delayed by at least 120

days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with a predicted progression.

CLAIMS

1. A single-stranded oligonucleotide of 10-30 linked nucleosides in length, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene.

2. The oligonucleotide of claim 1, wherein the oligonucleotide comprises:

(a) a DNA core sequence comprising linked deoxyribonucleosides;

(b) a 5' flanking sequence comprising linked nucleosides; and

(c) a 3' flanking sequence comprising linked nucleosides;

wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

3. A single-stranded oligonucleotide of 10-30 linked nucleosides in length for inhibiting expression of a human MSH3 gene in a cell, wherein the oligonucleotide comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene.

4. The oligonucleotide of claim 3, wherein the oligonucleotide comprises:

(a) a DNA core comprising linked deoxyribonucleosides;

(b) a 5' flanking sequence comprising linked nucleosides; and

(c) a 3' flanking sequence comprising linked nucleosides;

wherein the DNA core comprises a region of at least 10 contiguous nucleobases having at least 80% complementarity to an MSH3 gene and is positioned between the 5' flanking sequence and the 3' flanking sequence; wherein the 5' flanking sequence and the 3' flanking sequence each comprises at least two linked nucleosides; and wherein at least one nucleoside of each flanking sequence comprises an alternative nucleoside.

5. The oligonucleotide of any one of claims 1-4, wherein the region of at least 10 nucleobases has at least 90% complementary to an MSH3 gene

6. The oligonucleotide of any one of claims 1-5, wherein the region of at least 10 nucleobases has at least 95% complementary to an MSH3 gene.

7. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 355-385, 398-496, 559-589, 676-724, 762-810, 876-903, 912-974, 984-1047, 1054-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493,

1517-1630, 1665-1747, 1768-1866, 2029-2063, 2087-2199, 2262-2293, 2304-2330, 2371-2410, 2432-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3073, 31323245, 3266-3306, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4074-4101, or 4281-4319 of the MSH3 gene.

8. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-199, 359-385, 398-496, 559-589, 676-724, 762-810, 876-974, 984-1098, 1114-1179, 1200-1227, 1294-1337, 1392-1417, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2262-2293, 2304-2329, 2371-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2713, 2727-2753, 2767-2920, 2933-3000, 3046-3072, 3132-3245, 3266-3303, 3397-3484, 3528-3575, 3591-3617, 3753-3792, 3901-3936, 4076-4101, or 4281-4319 of the MSH3 gene.

9. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 155-196, 359-385, 413-462, 559-589, 676-724, 762-810, 876-974, 984-1096, 1114-1179, 1200-1227, 1294-1337, 1467-1493, 1517-1630, 1665-1747, 1834-1866, 2029-2056, 2093-2199, 2265-2293, 2378-2410, 2433-2458, 2494-2521, 2539-2647, 2679-2712, 2727-2753, 2767-2919, 2934-3000, 3046-3071, 3144-3183, 3220-3245, 3397-3484, 3534-3575, 3591-3616, 3901-3931, or 4281-4306 of the MSH3 gene.

10. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 435-462, 559-584, 763-808, 876-902, 931-958, 1001-1083, 1114-1179, 1294-1337, 1544-1578, 1835-1863, 2031-2056, 2144-2169, 2543-2577, 2590-2615, 2621-2647, 2685-2711, 2769-2795, or 2816-2868 of the MSH3 gene.

11. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 876-902, 930-958, 1056-1081, 1114-1139, 1154-1179, 1310-1337, 1546-1571, 1836-1862, 2141-2199, 2267-2292, 2540-2580, 2620-2647, 2686-2711, 2769-2868, 2939-2976, 3144-3169, or 3399-3424 of the MSH3 gene

12. The oligonucleotide of any one of claims 1-6, wherein the region of at least 10 nucleobases is complementary to an MSH3 gene corresponding to a sequence of reference mRNA NM_002439.4 at one or more of positions 984-1021, 1467-1493, 1722-1747, 1767-1802, 1833-1861, 2385-2410, 2554-2581, 2816-2845, 2861-2920, or 3151-3183 of the MSH3 gene.

13. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 6-2545.

14. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463.

15. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462 -2463.

16. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-

1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460.

17. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633.

18. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068.

19. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide comprises the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

20. The oligonucleotide of any one of claims 1-6, wherein the nucleobase sequence of the oligonucleotide consists of any one of SEQ ID NOs: 6-2545.

21. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22-29, 31-32, 77-78, 81-82, 115, 117, 130, 132-134, 144-145, 147, 167-168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-512, 543-550, 552-560, 562, 582-585, 588-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724-725, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 999, 1007, 1016-1017, 1019, 1021-1022, 1036, 1040-1045, 1047, 1170, 1172-1173, 1211, 1216, 1222, 1235, 1240-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1322, 1328-1329, 1373-1375, 1379-1383, 1386-1387, 1407-1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1589, 1591, 1600-1607, 1610, 1625, 1627-1629, 1631-1639, 1643, 1650-1660, 1663-1665, 1668-1675, 1713-1714, 1716-1722, 1724, 1727-1731, 1741, 1745-1747, 1751-1755, 1799-1801, 1859-1866, 1868-1869, 1894-1896, 1905-1908, 1954, 1964-1966, 1969, 2066-2070, 2075-2079, 2108, 2138, 2143-2147, 2157-2160, 2193-2194, 2299-2300, 2312-2313, 2385, 2388, 2390-2395, 2416-2418, 2460, 2462, or 2463.

22. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 22, 25-29, 31-32, 81-82, 115, 130, 132-134, 144, 145, 147, 168, 210, 212-215, 290-293, 295-296, 299-305, 309, 351-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 459-460, 479, 482-493, 497-498, 500-501, 503-506, 508-512, 543-550, 552-560, 562, 582-585, 589-591, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 724, 770-771, 812-816, 838-842, 845-852, 856, 883-885, 889, 893-897, 936, 940-941, 945, 948, 950, 955, 959-961, 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1241-1242, 1244-1249, 1251-1252, 1254-1259, 1268, 1316, 1318-1319, 1321-1322, 1328, 1373, 1379-1383, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1541, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1607, 1610, 1625, 1627-1629, 1631-1638, 1650-1655, 1659, 1665, 1668-1675, 1713-1714, 1716-1722, 1727-1731, 1745, 1747, 1751-1755, 1799-1800, 1859, 1861-1862, 1865-1866, 1868-1869, 1895-1896, 1905-1908, 1954, 1694-1966, 2066-2070, 2075-2079, 2108, 2138, 2144-2146, 2158-2160, 2193-2194, 2299, 2300, 2313, 2385, 2388, 2390-2392, 2394-2395, 2418, 2460, or 2462-2463.

23. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 20, 25-29, 32, 81-82, 130, 133-134, 144-145, 147, 210, 212-213, 215, 290-293, 295-296, 299-304, 309, 351, 352-359, 361-362, 365-366, 368, 407-409, 432, 437-442, 444, 460, 479, 482-486, 488-492, 497-498, 500-501, 503-506, 508-512, 544-550, 553-558, 560, 582-585, 589, 603-604, 611, 613-616, 659, 661, 699-700, 702, 705-707, 770-771, 812-816, 838-842, 845-851, 856, 883, 885, 889, 893, 895-897, 936, 940, 945, 961. 965-968, 972-973, 1041-1045, 1047, 1170, 1172, 1216, 1222, 1235, 1244, 1246-1249, 1251-1252, 1254-1255, 1257-1259, 1268, 1319, 1321-1322, 1380-1381, 1386-1387, 1408, 1433-1435, 1450-1451, 1454-1461, 1476-1477, 1496-1499, 1532, 1538-1540, 1565-1566, 1579, 1581-1582, 1584-1589, 1591, 1601-1604, 1606-1607, 1610, 1625, 1627-1629, 1631-1638, 1651-1654, 1668, 1670-1674, 1714, 1717-1722, 1727-1731, 1745, 1751-1755, 1799, 1861, 1869, 1908, 1964, 1966, 2066-2069, 2075-2076, 2078-2079, 2108, 2144-2145, 2158-2160, 2193, 2385, 2390, or 2460.

24. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 145, 147, 210, 352, 365, 366, 407, 408, 439-442, 444, 492, 500, 504, 511, 512, 544-547, 582, 604, 616, 699, 700, 702, 705-707, 839-842, 848, 1042-1045, 1172, 1255, 1454-1457, 1477-1499, 1538, 1539, 1581, 1582, 1606, 1607, 1610, or 1631-1633.

25. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 407, 408, 441, 442, 444, 545, 582, 616, 705-707, 841, 1043, 1044, 1252, 1255, 1268, 1321, 1451, 1454-1460, 1497-1499, 1538, 1539, 1581, 1582, 1587, 1601, 1602, 1606, 1607, 1610, 1631-1633, 1719, 1721, 1730, 1731, 1861, or 2068.

26. The oligonucleotide of any one of claims 1-6, wherein the oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 479, 482-491, 770, 771, 973, 998-1000, 1007, 1008, 1040-1043, 1387, 1454, 1456, 1459-1461, 1538, 1539, 1606, 1607, 1610, 1643-1665, 1668-1675, or 1862-1869.

27. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 50% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

28. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 60% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

29. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 70% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

30. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 85% mRNA inhibition at a 20 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

31. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 50% mRNA inhibition at a 2 nM when determined using a cell assay when compared with a control cell.

32. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 60% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

33. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 70% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

34. The oligonucleotide of any one of claims 1-26, wherein the oligonucleotide exhibits at least 85% mRNA inhibition at a 2 nM oligonucleotide concentration when determined using a cell assay when compared with a control cell.

35. The oligonucleotide of any one of claims 1-34, wherein the oligonucleotide comprises at least one alternative internucleoside linkage.

36. The oligonucleotide of claim 35, wherein the at least one alternative internucleoside linkage is a phosphorothioate internucleoside linkage.

37. The oligonucleotide of claim 35, wherein the at least one alternative internucleoside linkage is a 2'-alkoxy internucleoside linkage.

38. The oligonucleotide of claim 35, wherein the at least one alternative internucleoside linkage is an alkyl phosphate internucleoside linkage.

39. The oligonucleotide of any one of claims 1-38, wherein the oligonucleotide comprises at least one alternative nucleobase.

40. The oligonucleotide of claim 39, wherein the alternative nucleobase is 5'-methylcytosine, pseudouridine, or 5-methoxyuridine.

41. The modified oligonucleotide of any one of claims 1-40, wherein the oligonucleotide comprises at least one alternative sugar moiety.

42. The modified oligonucleotide of claim 41, wherein the alternative sugar moiety is 2'-OMe or a bicyclic nucleic acid.

43. The oligonucleotide of any one of claims 1-42, wherein the oligonucleotide further comprises a ligand conjugated to the 5' end or the 3' end of the oligonucleotide through a monovalent or branched bivalent or trivalent linker.

44. The oligonucleotide of any one of claims 1-43, wherein oligonucleotide comprises a region complementary to at least 17 contiguous nucleotides of a MSH3 gene.

45. The oligonucleotide of any one of claims 1-43, wherein oligonucleotide comprises a region complementary to at least 19 contiguous nucleotides of a MSH3 gene.

46. The oligonucleotide of any one of claims 1-43, wherein the oligonucleotide comprises a region complementary to 19 to 23 contiguous nucleotides of a MSH3 gene.

47. The oligonucleotide of any one of claims 1-43, wherein the oligonucleotide comprises a region complementary to 19 contiguous nucleotides of a MSH3 gene.

48. The oligonucleotide of any one of claims 1-43, wherein the oligonucleotide comprises a region complementary to 20 contiguous nucleotides of a MSH3 gene.

49. The oligonucleotide of any one of claims 1-43, wherein the oligonucleotide is from about 15 to 25 nucleosides in length.

50. The oligonucleotide of any one of claims 1-43, wherein the oligonucleotide is 20 nucleosides in length.

51. A pharmaceutical composition comprising one or more of the oligonucleotides of any one of claims 1-50 and a pharmaceutically acceptable carrier or excipient.

52. A composition comprising one or more of the oligonucleotides of any one of claims 1-50 and a lipid nanoparticle, a polyplex nanoparticle, a lipoplex nanoparticle, or a liposome.

53. A method of inhibiting transcription of MSH3 in a cell, the method comprising contacting the cell with one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52 for a time sufficient to obtain degradation of an mRNA transcript of a MSH3 gene, inhibits expression of the MSH3 gene in the cell.

54. A method of treating, preventing, or delaying the progression a trinucleotide repeat expansion disorder in a subject in need thereof, the method comprising administering to the subject one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52.

55. A method of reducing the level and/or activity of MSH3 in a cell of a subject identified as having a trinucleotide repeat expansion disorder, the method comprising contacting the cell with one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52.

56. A method for inhibiting expression of an MSH3 gene in a cell comprising contacting the cell with one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52 and maintaining the cell for a time sufficient to obtain degradation of a mRNA transcript of an MSH3 gene, thereby inhibiting expression of the MSH3 gene in the cell.

57. A method of decreasing trinucleotide repeat expansion in a cell, the method comprising contacting the cell with one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52.

58. The method of claim 56 or 57, wherein the cell is in a subject.

59. The method of any one of claims 54, 55, and 58, wherein the subject is a human.

60. The method of any one of claims 54-58, wherein the cell is a cell of the central nervous system or a muscle cell.

61. The method of any one of claims 54, 55, and 58-60, wherein the subject is identified as having a trinucleotide repeat expansion disorder.

62. The method of any one of claims 54, 55, and 57-61, wherein the trinucleotide repeat expansion disorder is a polyglutamine disease.

63. The method of claim 62, wherein the polyglutamine disease is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, and Huntington's disease-like 2.

64. The method of any one of claims 54-61, wherein the trinucleotide repeat expansion disorder is a non-polyglutamine disease.

65. The method of claim 64, wherein the non-polyglutamine disease is selected from the group consisting of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

66. One or more oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52 for use in the prevention or treatment of a trinucleotide repeat expansion disorder.

67. The oligonucleotide, pharmaceutical composition, or composition for the use of claim 68, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

68. The oligonucleotide, pharmaceutical composition, or composition for the use of claim 66 or 67, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

69. The oligonucleotide, pharmaceutical composition, or composition of claim 66 or 67, wherein the trinucleotide repeat expansion disorder is Friedreich's ataxia.

70. The oligonucleotide, pharmaceutical composition, or composition for the use of claim 66 or 67, wherein the trinucleotide repeat expansion disorder is myotonic dystrophy type 1.

71. The oligonucleotide, pharmaceutical composition, or composition of any of claims 66-70, wherein the modified oligonucleotide, pharmaceutical composition, or composition is administered intrathecally.

72. The oligonucleotide, pharmaceutical composition, or composition of any of claims 66-70, wherein the modified oligonucleotide, pharmaceutical composition, or composition is administered intraventricularly.

73. The oligonucleotide, pharmaceutical composition, or composition of any of claims 66-70, wherein the oligonucleotide, pharmaceutical composition, or composition is administered intramuscularly.

74. A method of treating, preventing, or delaying the progression a disorder in a subject in need thereof wherein the subject is suffering from trinucleotide repeat expansion disorder, comprising administering to said subject one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52.

75. The method of claim 74, further comprising administering an additional therapeutic agent.

76. The method of claim 75, wherein the additional therapeutic agent is another oligonucleotide that hybridizes to an mRNA encoding the Huntingtin gene.

77. A method of preventing or delaying the progression of a trinucleotide repeat expansion disorder in a subject, the method comprising administering to the subject one or more of the oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52 in an amount effective to delay progression of a trinucleotide repeat expansion disorder of the subject.

78. The method of claim 77, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar

ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

79. The method of claim 77 or 78, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

80. The method of claim 77 or 78, wherein the trinucleotide repeat expansion disorder is Friedrich's ataxia.

81. The method of claim 77 or 78, wherein the trinucleotide repeat expansion disorder is myotonic Dystrophy type 1.

82. The method of claim 77 or 78, further comprising administering an additional therapeutic agent.

83. The method of claim 82, wherein the additional therapeutic agent is an oligonucleotide that hybridizes to an mRNA encoding the Huntingtin gene.

84. The method of any of claims 77-83, wherein progression of the trinucleotide repeat expansion disorder is delayed by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with a predicted progression.

85. One or more oligonucleotides of any one of claims 1-50, the pharmaceutical composition of claim 51, or the composition of claim 52, for use in preventing or delaying progression of a trinucleotide repeat expansion disorder in a subject.

86. The oligonucleotide, pharmaceutical composition, or composition of claim 85, wherein the trinucleotide repeat expansion disorder is selected from the group consisting of dentatorubropallidoluysian atrophy, Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 3, spinocerebellar ataxia type 6, spinocerebellar ataxia type 7, spinocerebellar ataxia type 17, Huntington's disease-like 2, fragile X syndrome, fragile X-associated tremor/ataxia syndrome, fragile XE mental retardation, Friedreich's ataxia, myotonic dystrophy type 1, spinocerebellar ataxia type 8, spinocerebellar ataxia type 12, oculopharyngeal muscular dystrophy, Fragile X-associated premature ovarian failure, FRA2A syndrome, FRA7A syndrome, and early infantile epileptic encephalopathy.

87. The oligonucleotide, pharmaceutical composition, or composition of claim 85 or 86, wherein the trinucleotide repeat expansion disorder is Huntington's disease.

88. The oligonucleotide, pharmaceutical composition, or composition of claim 85 or 86, wherein the trinucleotide repeat expansion disorder is Friedrich's ataxia.

89. The oligonucleotide, pharmaceutical composition, or composition of claim 85 or 86, wherein the trinucleotide repeat expansion disorder is myotonic Dystrophy type 1.

90. The oligonucleotide, pharmaceutical composition, or composition of any one of claims 85-89, wherein progression of the trinucleotide repeat expansion disorder is delayed by at least 120 days, for example, at least 6 months, at least 12 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 10 years or more, when compared with a predicted progression.

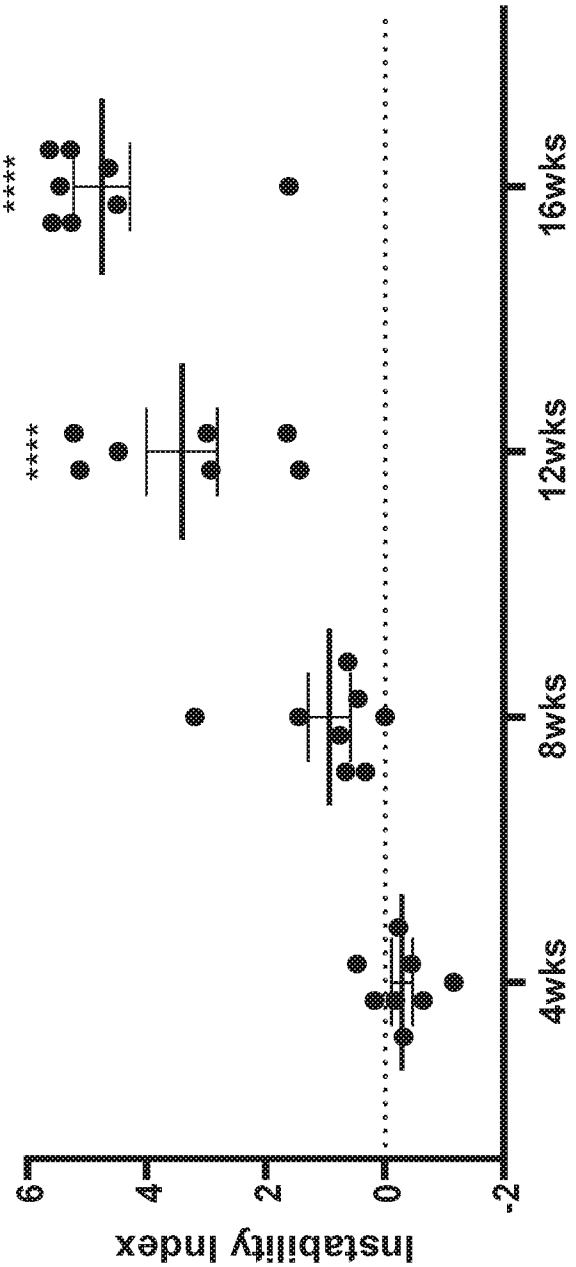


FIG. 1

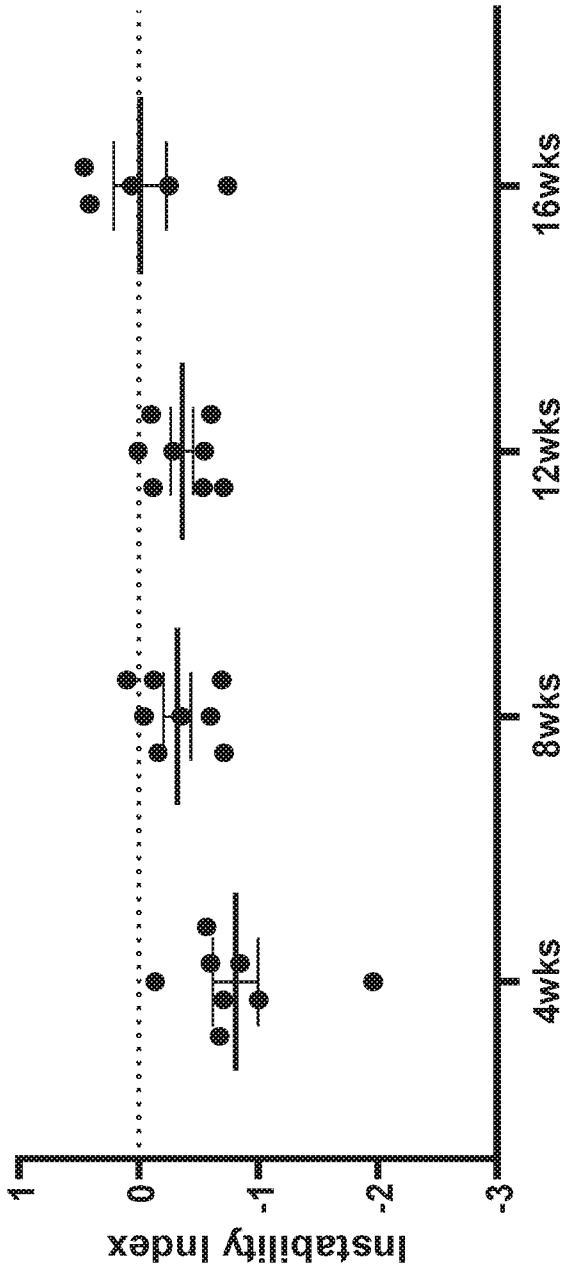


FIG. 2

SEQUENCE LISTING

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<120> METHODS FOR THE TREATMENT OF TRINUCLEOTIDE REPEAT EXPANSION DISORDERS ASSOCIATED WITH MSH3 ACTIVITY

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Pro Gly Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Pro Pro
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Pro Ala Val Asn Ser His Ile Gln Ser Asp Leu Leu Arg Thr Val Ile
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