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Supplemental Information

RNase H1-Dependent Antisense Oligonucleotides Are Robustly Active in Directing RNA Cleavage in Both the Cytoplasm and the Nucleus

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RNase H1-dependent antisense oligonucleotides are robustly active in directing RNA cleavage in both the cytoplasm and the nucleus

Supplementary Materials

RNase H1-dependent 5-10-5 gapmer PS/MOE ASOs

These ASOs are linked with phosphorothioate backbones, with 10 deoxynucleotides in the center and 5 nucleotides at both ends modified with 2'-O-Methoxyethyl (MOE). The 2'-MOE modified nucleotides are underlined. The numbers of the ASOs are Ionis ASO ID numbers.

Human Drosha	25688	<u>GCACTTCCTTTCCTCCATCT</u>
Human Drosha	25689	<u>TCGATACGGACAGAGCTTGG</u>
Human Drosha	25690	<u>ATCCCTTTCTTCCGCATGTG</u>
Human NCL1	110074	<u>GTCATCGTCATCCTCATCAT</u>
Human Ago2	136761	<u>AGCCGGGTGTGGTGCCTCTT</u>
Human Ago2	136762	<u>AAGAGCCGGGTGTGGTGCCT</u>
Human Ago2	136765	<u>GTCGTGCCTGCTGGAATGTT</u>
Human Ago2	136785	<u>GCTGGCCATCTGTTGGTCTG</u>
Mouse SRB1	205382	<u>CACCTCTGCCACGTACAGTG</u>
Human SOD1	333632	<u>TTTCTTCATTTCCACCTTTG</u>
Human MALAT1	395254	<u>GGCATATGCAGATAAATGTTC</u>
Mouse MALAT1	399479	<u>CGGTGCAAGGCTTAGGAATT</u>
U16 snoRNA	462026	<u>CAGCAGGCAACTGTCGCTGA</u>
Human SOD1	480774	<u>AAGTGAAAAGATACATGACT</u>
Human U4 snRNA	479333	<u>GGTATTGGGAAAAGTTTTC</u>
Control ASO	129700	<u>TAGTGCGGACCTACCCACGA</u>

Human U23 ASOs:

483771	<u>ACAGAAAGGAGCTCAATGAG</u>
483772	<u>GATAGACAGAAAGGAGCTCA</u>
483773	<u>CCACTGATAGACAGAAAGGA</u>
483774	<u>AACTGCCACTGATAGACAGA</u>
483775	<u>CCATAAACTGCCACTGATAG</u>
483776	<u>CGAATCCATAAACTGCCACT</u>
483777	<u>TCGTGCGAATCCATAAACTG</u>
483778	<u>TCTTCTCGTGCGAATCCATA</u>
483779	<u>TCTTCTTCTCGTGCGAAT</u>
483780	<u>AATTCTCTTCTTCTCGTG</u>
483781	<u>CTGTGAATTCTCTTCTTCT</u>
483782	<u>TAGTTCTGTGAATTCTTCT</u>
483783	<u>AATGCTAGTTCTGTGAATTC</u>
483784	<u>AAAATAATGCTAGTTCTGTG</u>
483785	<u>AAGGTAAAATAATGCTAGTT</u>
483786	<u>GACAGAAGGTAAAATAATGC</u>
483787	<u>GTAAAGACAGAAGGTAAAAT</u>

483788 CCTCTGTAAAGACAGAAGGT
483789 ATATACCTCTGTAAAGACAG
483790 GCTAAATATACCTCTGTAAA
483791 ATACAGCTAAATATACCTCT
483792 TCACAATACAGCTAAATATA
483793 ATGTCTCACAAATACAGCTAA

Oligonucleotide probes used for northern hybridization:

XL011, 5'-TTGCTCAGTAAGAATTTTCG-3', antisense, to U16 snoRNAs
XL016, 5'-ACCACTCAGACCGCGTTCTCTCC-3', antisense, to U3 snoRNA
XL019, 5'-ATTGCCAGTGCCGACTATAT-3', antisense, to U4 snRNA
XL021, 5'-TGGAACGCTTCACGAATTTGCG-3', antisense, to U6 snRNA
XL057, 5'-CTCAGCCTCCCGAGTAGCTG-3', antisense, to wild type 7SL RNA
XL099, 5'-GAATGTCTCACAAATACAGCTAAAT-3', antisense, to U23 snoRNA
XL104, 5'-GACGCAAATTACGACATCAT-3', antisense, to U16 snoRNA
XL272, 5'-CAGCAGGCAACTGTCGCTGA-3', antisense, to both U16 and 7SLm RNAs
XL297, 5'-CTACAGCCCAGCGCAACTCAGC-3', antisense, 3' probe for 7SLm RNA
XL299, 5'-GTCGCTGACCTGGACTCAA-3', antisense, 5' probe for 7SLm RNA

siRNAs:

Luciferase siRNA: sense sequence: 5'-CGUACGCGGAAUACUUCGAtt
Drosha siRNA was purchased from Invitrogen, ID No: HSS178991
RNase H1 siRNA was purchased from Ambion (ID No: S48357, Cat. No: 4390826)
RNase H2 siRNA was purchased from Ambion (ID No: S20658, Cat. No. 4392420)
XRN2 siRNA was purchased from Invitrogen (ID No: HSS117664)

Primer probe sets used for qRT-PCR:

Human NCL1 mRNA

Forward: 5'-GCTTGGCTTCTTCTGGACTCA-3'
Reverse: 5'-TCGCGAGCTTCACCATGA-3'
Probe: 5'-CGCCACTTGTCCGCTTCACACTCC-3'

Human DROSHA mRNA:

Forward: 5'-CAAGCTCTGTCCGTATCGATCA
Reverse: 5'-TGGACGATAATCGGAAAAGTAATCA
Probe: 5'-CTGGATCGTGAACAGTTCAACCCCGAT

Human MALAT1 RNA:

Forward: 5'-GCTTGGCTTCTTCTGGACTCA -3'
Reverse: 5'-TCGCGAGCTTCACCATGA -3'
Probe: 5'-CGCCACTTGTCCGCTTCACACTCC-3'

Human U16 RNA:

Forward: 5'-CTTGCAATGATGTCGTAATTTGC-3'

Reverse: 5'-TCGTCAACCTTCTGTACCAGCTT-3'
Probe: 5'-TTACTCTGTTCTCAGCGACAGTTGCCTGC-3'

Mouse MALAT1:

Forward: 5'-TGGGTTAGAGAAGGCGTGTACTG-3'
Reverse: 5'-TCAGCGGCAACTGGGAAA-3'
Probe: 5'-CGTTGGCACGACACCTTCAGGGACT-3'

Mouse SRB1 mRNA:

Forward: 5'-TGACAACGACACCGTGTCT-3'
Reverse: 5'-ATGCGACTTGTCAGGCTGG-3'
Probe: 5'-CGTGGAGAACCGCAGCCTCCATT-3'

Human SOD1 mRNA:

Forward: 5'-CTCTCAGGAGACCATTGCATCA-3'
Reverse: 5'-TCCTGTCTTTGTACTTTCTTCATTTCC-3'
Probe: 5'-CCGCACACTGGTGGTCCATGAAAA-3'

Human SOD1, pre-mRNA (E4/I4):

Forward: 5'-TCTGTGATCTCACTCTCAGGAG-3'
Reverse: 5'-TGGATCTTTAGAAACCGCGAC-3'
Probe: 5'-CATTGCATCATTGGCCGCACACT-3'

Human SOD1, pre-mRNA (I1/E2)

Forward: 5'-TTTTCCACTCCCAAGTCTGG-3'
Reverse: 5'-AATGCTTCCCCACACCTTC-3'
Probe: 5'-CTGTGAGGGGTAAAGGTAAATCAGCTGT-3'

Human NCL1 pre-mRNA (E13):

Forward: 5'-CTCTGTCACTGGTATCTTTTCCC-3'
Reverse: 5'-CAAAACCAAACCTAGAACACCAAATG-3'
Probe: 5'-CAAGGCTACTTTCTGTGGGATGGCT-3'

Human NCL1 pre-mRNA (E3):

Forward: 5'-GGCTGGACTTACTGGTTTGG-3'
Reverse: 5'-TGCTTTCTTGGCTGGTGTG-3'
Probe: 5'-AAGAAGGTGGTCGTTTCCCCAACA-3'

Drosha pre-mRNA:

Forward: 5'-GATTATGACCGAGGGAGAACAC-3'
Reverse: 5'-ATGCCCTACTGGATCCTTTTG-3'
Probe: 5'-TAAATGGAGAATGACCGTGCCTGGG-3'

Ago2 pre-mRNA:

Forward: 5'-ACGGACAATCAGACCTCAAC-3'
Reverse: 5'-TGGATTCCACAGGGCAGC-3'
Probe: 5'-CCGGGACTGACACTCACCAATTTGCT-3'

RNase H1 mRNA:

Forward: 5'-CCTGTACTTACTGGTGTGGAAAATAGC-3'

Reverse: 5'-CCGTGTGAAAGACGCATCTG-3'

Probe: 5'-TGCAGGTAGGACCATTGCAGTGATGG-3'

RNase H2 mRNA:

Forward: 5'-CCTGTACTTACTGGTGTGGAAAATAGC-3'

Reverse: 5'-CCGTGTGAAAGACGCATCTG-3'

Probe: 5'-TGCAGGTAGGACCATTGCAGTGATGG-3'

28S rRNA:

Forward: 5'- CAGGTCTCCAAGGTGAACAG -3'

Reverse: 5'- CTTAGAGCCAATCCTTATCCCG-3'

Probe: 5'- TCCCTTACCTACATTGTTCCAACATGCC -3'

7SL RNA:

Forward: 5'- GCACTAAGTTCGGCATCAATATG -3'

Reverse: 5'- AGTGCAGTGGCTATTCACAG-3'

Probe: 5'- TCGGAAACGGAGCAGGTCAAACACT -3'