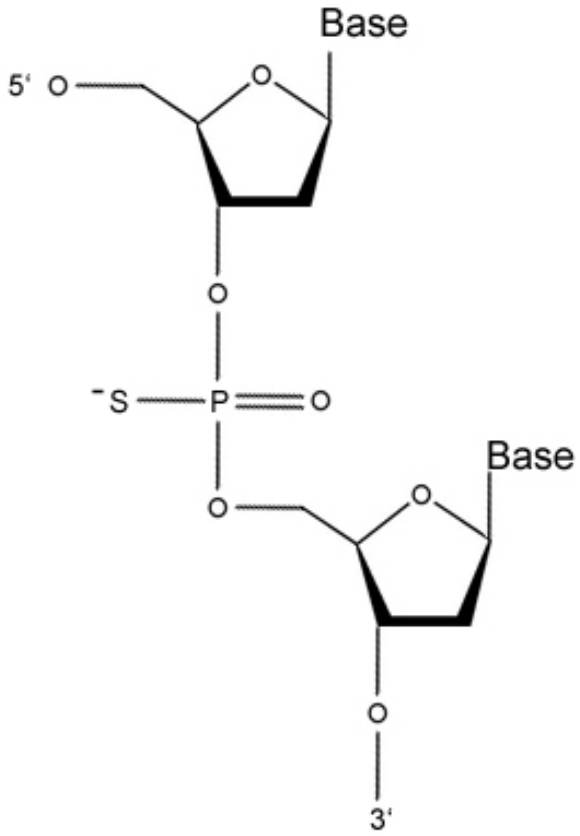
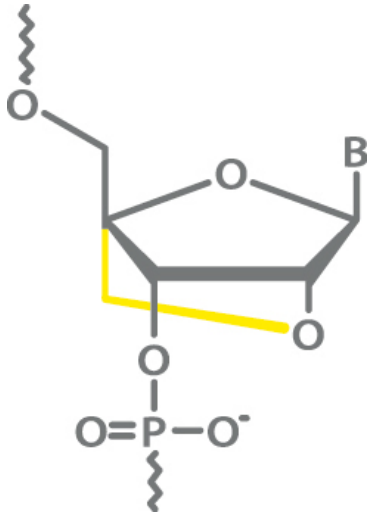
Supplementary Materials

Barrey et al. 2020

Figure S1: Oligonuclotide modifications used in ASO (A) and in antisense LNA GapmeR synthesis (B) to increase their stability, resistance to nucleases and specificity.



A: Phosphorothioate (PTO)

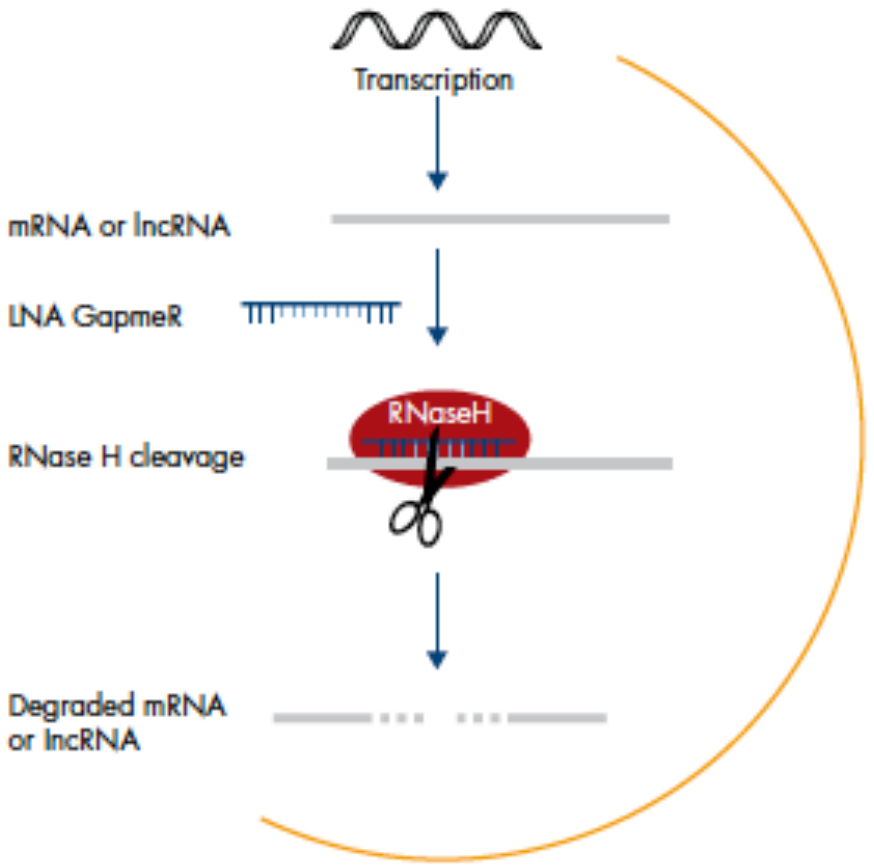


B: Locked Nucleic Acid (LNA) the sugar ring locked in the 3'-end conformation

K. Bondensgaard *et al.*, *Chem. Eur. J.* **2000**, *6*, 2687
M. Petersen *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 5974

Figure S2: Principle of antisense LNA GapmeRs for silencing the viral RNA targets. The conventional ASO (PTO modified) have the same activity. (Figure adapted from Exiqon - Qiagen document).

- 14-16 nt LNA™/DNA – strand specific for hybridizing the viral RNA target.
- DNA/RNA heteroduplex recruits RNase H1 in the nucleus and cytosol.
- RNAse H1 digests the RNA in the middle of the target sequence.
- The cleaved RNA will be digest by exonucleases.
- LNA Gapmer is released and can go on and catalyze degradation of another RNA target.



Wheeler TM; Nature. 2012 Aug 2;488(7409):111-115

Figure S3: Comparison of relative positions of the antisense LNA GapmeRs obtained on two different SARS-Cov-2 sequences NC_045512.2 (MN908947) and MN988668. The candidates are scored from 1 (best) to 10 on each fragmement of each sequence.

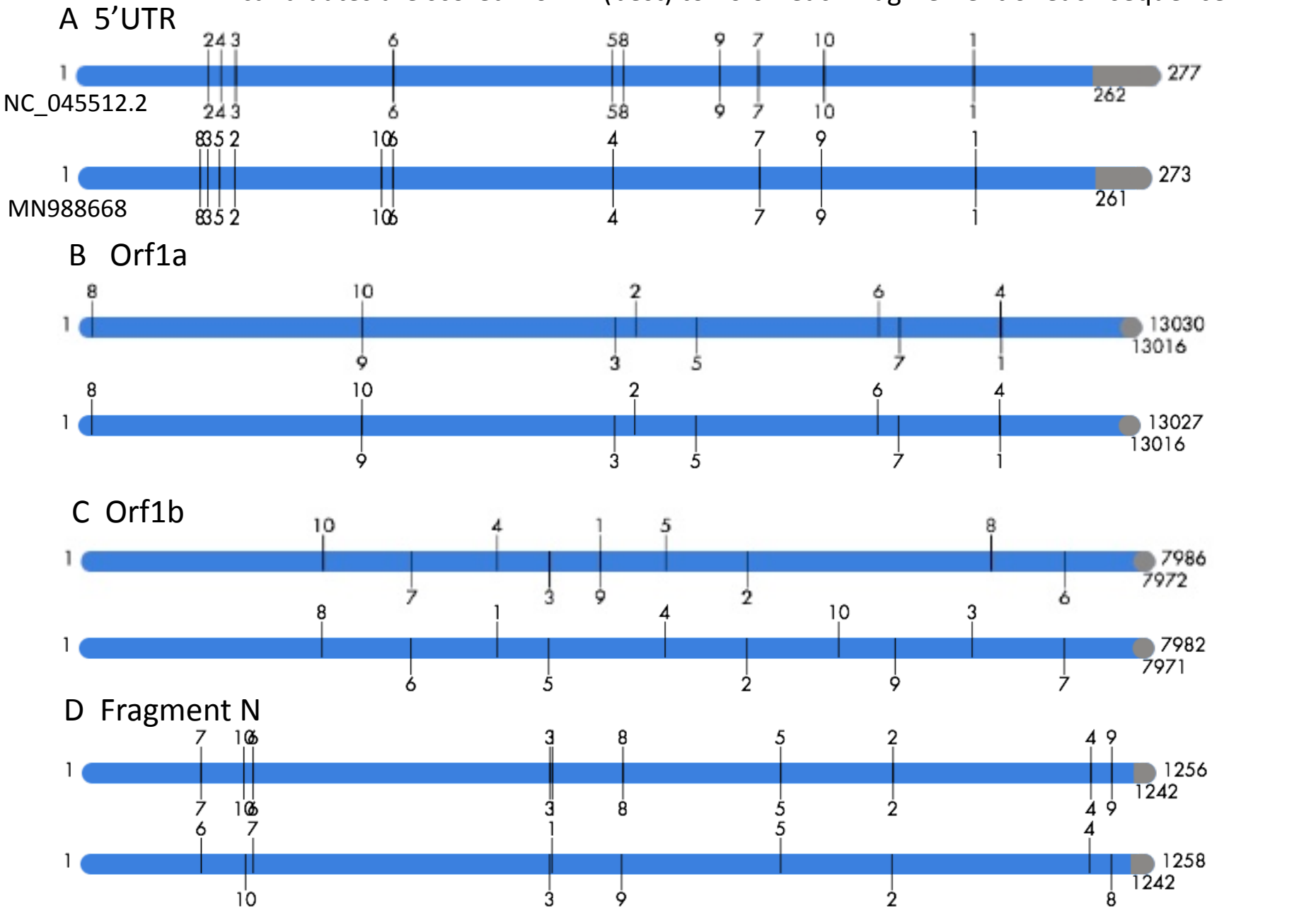


Table S1

ASO #	Fragment target	Score	Position	Sequence	Self annealing	Self annealing alignment	Self end-annealing	GC content	Melt. temp.	Secondary structure score	Secondary structure
ASO1 *	ORF1a	86.13	1936 - 1950	TTCTGTGCGTGTTTT	6	TTCTGTGCGTGTTTT | | TTTTGTGCGTGTCTT	0	0.4	48.90614	3	number of base pairs = 1, sequence = TTCTGTGCGTGTTTT, bracket notation = ::(:::)::::::: , score = 3
ASO2 *	ORF1a	85.94	5917- 5931	GGATGGTGTTGTTTG	4	GGATGGTGTTGTTTG || GTTTGTTGTGGTAGG	0	0.466667	50.35599	2	number of base pairs = 1, sequence = GGATGGTGTTGTTTG, bracket notation = ::(:::)::::::: , score = 2
ASO3 *	ORF1a	85.52	3051- 3066	AGGATGAAGAAGAAGG	4	AGGATGAAGAAGAAGG | | GGAAGAAGAAGTAGGA	0	0.4375	49.72196	2	number of base pairs = 1, sequence = AGGATGAAGAAGAAGG, bracket notation = (:::)::::::::: , score = 2
ASO4 *	ORF1b	83.31	20163- 20177	AGTTGATGGTGTTGT	8	AGTTGATGGTGTTGT | || | TGTTGTGGTAGTTGA	2	0.4	49.34404	4	number of base pairs = 2, sequence = AGTTGATGGTGTTGT, bracket notation = (::::(:::):):::: , score = 4
ASO5 *	N	78.93	28818-28832	CCTCTTCTCGTTCCT	6	CCTCTTCTCGTTCCT | | TCCTTGCTCTTCTCC	0	0.533333	50.58786	3	number of base pairs = 1, sequence = CCTCTTCTCGTTCCT, bracket notation = :::::::::(:::): , score = 3
ASO6	N	78.06	29108-29122	CCAGAACAAACCCAA	6	CCAGAACAAACCCAA | | AACCCAAACAAGACC	0	0.466667	49.83777	3	number of base pairs = 1, sequence = CCAGAACAAACCCAA, bracket notation = :::(::::::):::: , score = 3
ASO7	ORF1b	82.92	16457-16471	CACATAAACCACCCA	4	CACATAAACCACCCA | | ACCCACCAAATACAC	2	0.466667	50.68232	2	number of base pairs = 1, sequence = CACATAAACCACCCA, bracket notation = ::::(:::::):::: , score = 2
ASO8	5'UTR	94.10	26 - 40	AACAAACCAACCAAC	0	AACAAACCAACCAAC CAACCAACCAAACAA	0	0.4	48.76263	0	number of base pairs = 0, sequence = AACAAACCAACCAAC, bracket notation = :::::::::::::: , score = -1
ASO9	ORF1b	88.26	20487- 20501	GTGTGTGTGTTCTGT	6	GTGTGTGTGTTCTGT | | TGTCTTGTGTGTGTG	0	0.466667	50.50611	3	number of base pairs = 1, sequence = GTGTGTGTGTTCTGT, bracket notation = ::::::(::::):::: , score = 3