Oligonucleotides with LNA and targeting of biologically important RNAs

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Reference: Guterstam P, Lindgren M, Johansson H, Tedebark U (GE Healthcare), Wengel J (South Danish University), EL Andaloussi S, Langel Ü
Splice switching efficiency and specificity for oligonucleotides with locked nucleic acid monomers
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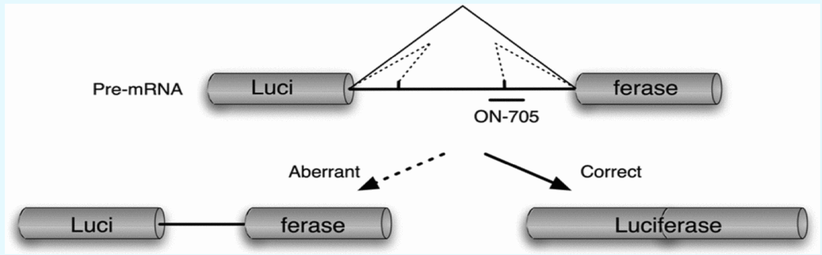
Cells expressing luciferase pre-mRNA interrupted by an aberrantly spliced β-globin intron, HeLa pLuc705, were used to monitor the splice-switching activity of modified oligonucleotides (ONs) by detection of the expression of functional luciferase. It was observed that phosphorothioate 2'-O-Methyl RNA (2OMe) 18mer ONs containing locked nucleic acid (LNA) monomers provide outstanding splice-switching activity. However, similar ONs with several mismatches do not impede splice-switching activity. The activity is abolished when mismatches are introduced at several positions with LNA monomers suggesting that LNA monomers in such long mixmers have to be positioned with care in order to achieve desired mismatch discrimination for target pre-mRNA. By shortening the sequence length specificity for LNA containing splice-switching oligonucleotides (SSOs) is restored without severely compromising with activity. Hence, activity of RNA-targeting antisense oligonucleotides increases when introducing LNA monomers, implying that an 18 nucleotides long LNA / 2OMe mixmer SSO can be shortened to 12mer and have similar activity and specificity as a 18mer 2OMe SSO. Positioning of LNA monomers has to be carefully considered when utilizing the potent LNA monomers in RNA-targeting antisense oligonucleotides.



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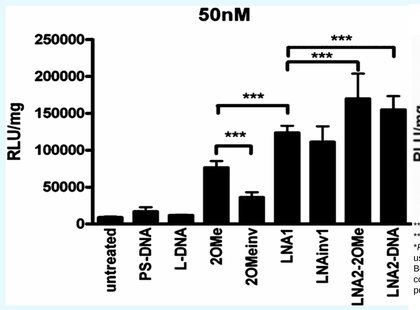
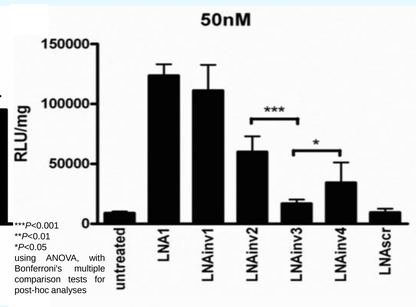
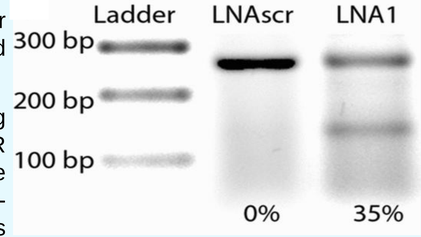
Above: Reporter system for splice-switching based on a plasmid carrying a luciferase-coding sequence with insertion of intron 2 from β-globin pre-mRNA containing an aberrant splice-site that activates a cryptic splice-site







Above: Luciferase activity after treatment with 50 nM SSO and Lipofectamine 2000 for 16h.

Right: RT–PCR analysis after treating cells with 100 nM SSO. PCR fragments derived from luciferase mRNA with and without splice-switching are 142 and 268 bps respectively. An estimation of the proportion of correctly spliced mRNA is shown below each lane.

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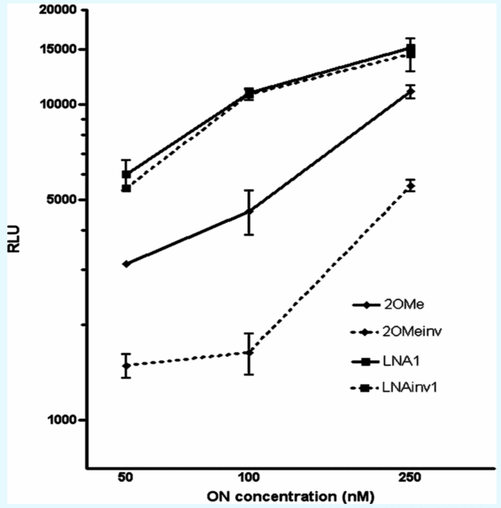
Conclusions

- Increased splice-switching activity when using LNA / 2OMe mixmers compared to 2OMe ONs
- Splice-switching activity is further increased with increased proportion of LNA.
- 12mer LNA / 2OMe mixmers induce similar activity and specificity as 18mer 2OMe ONs.

Name	Sequence		# Mismatches	
			Total	LNA
2OMe	5'- CCU CUU ACC UCA GUU ACA		0	0
2OMeinv	5'- CCU CUU ACA CUC GUU ACA		4	0
LNA2-2OMe	5'- CcU cUt AcC tCa GtU aCa	50% LNA	0	0
LNA2-DNA	5'- CcU cUt AcC tCa GtU aCa	50% LNA	0	0
DNA	5'- CCT CTT ACC TCA GTT ACA		0	0
L-DNA	5'- CCT CTT ACC TCA GTT ACA		0	0
LNA1	5'- cCU cUU aCC UcA GUt ACa	33% LNA	0	0
LNAinv1	5'- cCU cUU aCA CtC GUt ACa	33% LNA	4	1
LNAinv2	5'- cCU cUU aGA CtC CUt ACa	33% LNA	6	1
LNAinv3	5'- cCU aCU cCA UtC GUt ACa	33% LNA	6	3
LNAinv4	5'- cCU cUU aGA CtC CUt CAa	33% LNA	8	1
LNAtr1	5'- cCU cUU aCC UcA GUt	33% LNA	0	0
LNAtrinv1	5'- cCU cUU aCA CtC GUt	33% LNA	4	1
LNAtr2	5'- cUU aCC UcA GUt	33% LNA	0	0
LNAtrinv2	5'- cUU aCA CtC GUt	33% LNA	4	1
LNAtr3	5'- cUU aCC UcA	33% LNA	0	0
LNAtrinv3	5'- cUU aCA CtC	33% LNA	4	1
LNAscr	5'- tCA gAU tCC AtC ACc UUc	33% LNA	14	6

Sequence table: LNA ONs are LNA / 2OMe mixmers where the LNA monomers are indicated as small letters. 2'-OMe positions are capitals. For DNA, capitals indicate DNA positions, and for L-DNA capitals indicate L-DNA positions (spiegelmer). Inverted stretches are underlined. Mismatches are in red. Observe, for LNAinv3, the stretch of nine inverted monomers give rise to only six mismatches.



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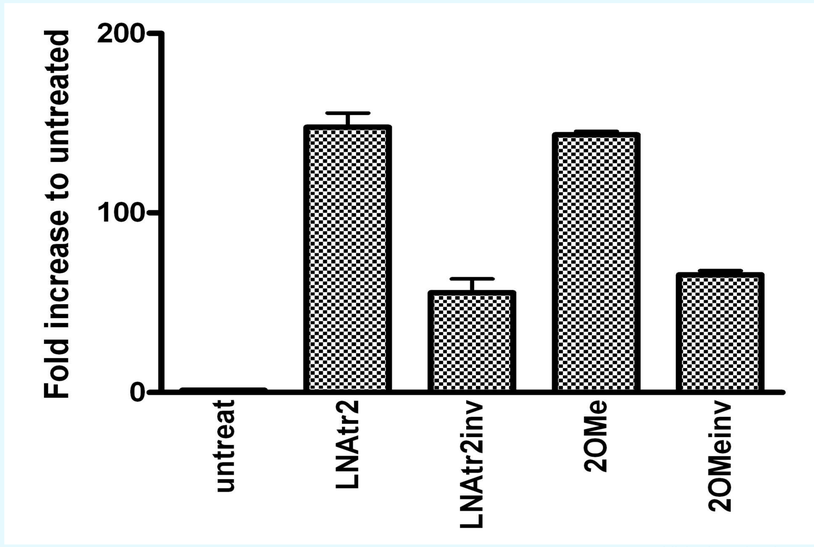
Left: The 18mer, LNAinv1, with 4 mismatches, induces splice-switching to the same extend as the correct sequence, LNA1. The LNA mixmers are more efficient than the 2OMe at low concentrations, 50 and 100 nM.

Below: Luciferase activity after SSO treatment at 250 nM with lLpofectamine 2000 for 16 h. The 12mer LNA / 2OMe mixmer, LNAtr2, display similar splice-switching activity and specificity as corresponding 18mer 2OMe SSO.



Data not published previously