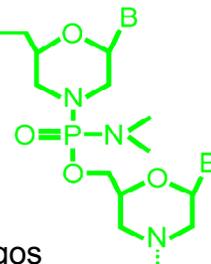


Morpholino oligos can block translation or nuclear processing of mRNA

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Antisense that Works



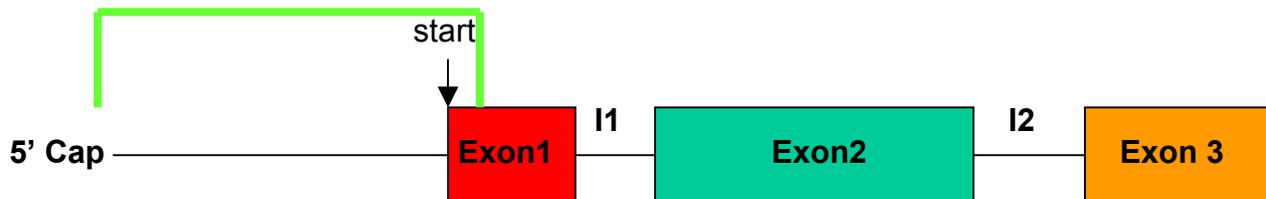
Designing Morpholino oligos is easier than designing DNA, S-DNA or siRNA oligos because Morpholinos have higher RNA binding affinities and a simple steric-blocking mechanism of action. High RNA affinity allows Morpholinos to invade RNA secondary structure and vastly increases the probability an oligo will work. Morpholinos act by steric-blocking instead of by RNase H-mediated or RISC-mediated mRNA degradation; by binding to RNA, Morpholinos block access of the ribosomal initiation complex or of splicing proteins, with results more predictable than the complex enzyme-mediated pathways. High affinity and simplicity of mechanism mean designing one oligo usually suffices to knock down a target protein. Gene Tools will design your oligo for no cost.

Blocking Translation

In order to sterically block the translation initiation complex, one must **select an oligo that targets sequence in the post-spliced mRNA in the region from the 5' cap to about 25 bases 3' to the AUG translational start site**. Morpholinos targeted more than about 30 bases 3' to the AUG translational start site do not appreciably block translation. A typical mRNA (shown below) has enough 5' target sequence to design two or more highly effective Morpholinos to block translation. Unlike DNA, S-DNA or siRNA oligos, which may require many tries to have success, in most cases one Morpholino oligo annealing in this target region will yield the desired antisense results.

Potential translational blocking targets in a typical pre-mRNA

Target region for translation blocking

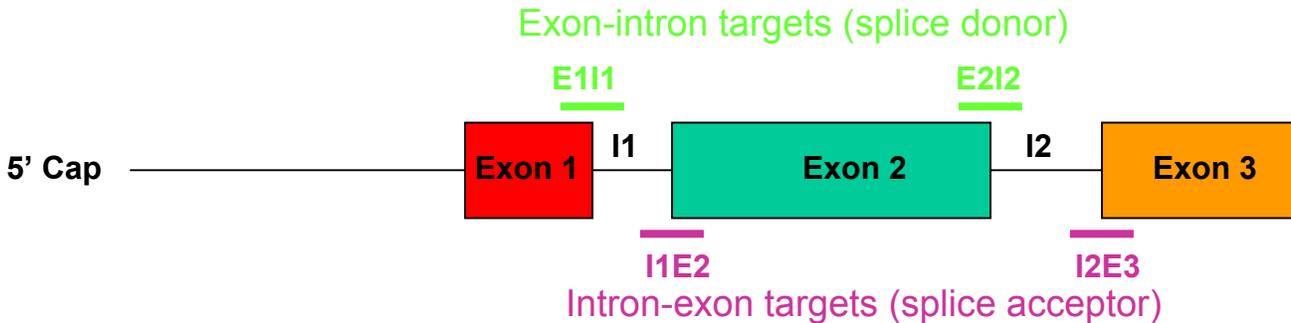


Blocking Nuclear Processing

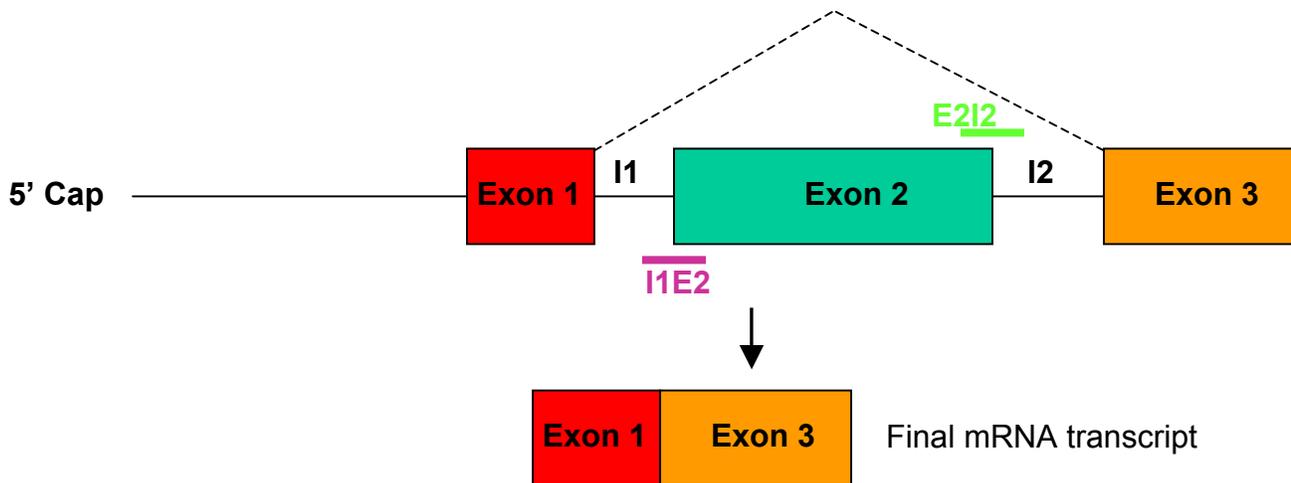
Morpholino oligos can block nuclear processing events, pre-mRNA processing in particular. The power of high specificity and steric blocking allows one to specifically and reproducibly delete exons of choice by blocking access of the splicing machinery to the pre-mRNA. **This technology, not possible with RNase-dependent or RISC-dependent oligos (phosphorothioates, RNAi and others)**, not only allows characterizing specific exon function and creating loss-of-function deletions or insertions but it also allows researchers to eliminate a specific splice variant while leaving another splice variant of the same gene intact. Many papers have now been published using Morpholinos to block splicing by targeting exon-intron or intron-exon boundaries.

Targeting an exon-intron boundary (splice donor) or intron-exon boundary (splice acceptor) usually results in deletion of the included exon. Occasionally blocking an existing site activates a cryptic splice site, giving unpredictable results. Fortunately, the products of Morpholino-targeted mRNA processing events can be **easily characterized using RT-PCR**; therefore splice-blocking is the best method for using Morpholinos if the researcher has no antibodies or other means of characterizing antisense effects.

Potential splice targets in a typical multi-exon pre-mRNA



The most predictive targets are I1E2 or E2I2, both of which alone should result in a deletion of Exon 2 as shown below.



What constitutes an optimal Morpholino oligo for translation or splice blocking?

Once you've found a region you would like to target, the optimal Morpholino oligo will be a 25-base oligo with a relatively high GC content (i.e. 40-60%) without stretches of 4 or more contiguous G, which can cause solubility problems in all antisense types. The oligo should also not be significantly complementary to itself. If working with organisms or cells at temperatures below 37 °C, reducing the length of the antisense oligos may be desirable to maintain the exquisite specificity possible with Morpholino oligos.