Designing and Ordering Morpholinos & Vivo-Morpholinos 5 Apr 2016

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SELECTING MORPHOLINO ANTISENSE OLIGOS

GENE TOOLS Design Request Website

Click on the %Ordering+tab of our website and then the GENE TOOLS design request link to submit RNA sequence for our oligo design service, which we provide free-of-charge for our customers.

In order to use this design service, either:

- A. For translational blocking oligos, provide a GenBank or EMBL accession number for your sequence, OR provide the actual 5' UTR sequence through the 25th base of the coding region. Please mark the start codon with parenthesis () and provide ONLY sequence upstream of the 25th base of coding sequence. Sequence downstream of the 25th base can not be used for translational blocking.
- B. **For splice blocking oligos**, please provide 50 bases of exon-intron or intron-exon boundary sequence with exon sequence in UPPER CASE and intron sequence in lower case.
- C. For miRNA inhibiting oligos, provide the miRBase ID of the miRNA and the desired target site (for details, see http://www.genetools.com/node/31).
- D. For an oligo to block another molecule from binding to an RNA, send about 50 bases of upper-case sequence centered on the binding site. Show the binding site in lower case bases.

Customer-selected Morpholino Oligo Sequences

Customers who wish to select their own oligo sequences should refer to the targeting guidelines on our web site; click on the %Groducts and Applications+tab and the %Ghoosing the Optimal Target+link.

Note: Morpholino oligos block translation by steric blocking of the translation initiation complex, unlike RNase-H competent antisense oligos (DNA, S-DNA) or siRNA, which prevent translation by degrading the mRNA. Targeting Morpholinos is generally different from targeting with these other oligo types and the same oligo sequence may not work for all of these systems.

Suggested Morpholino Control Oligos

Control experiments involving one or more control oligos are generally performed along with experiments using the targeted custom-sequence oligo. A set of controls usually involves a negative control and a specificity control.

Negative controls: We offer two pre-made negative control sequences: the Standard Control (with or without fluorescent moieties) and the Random Control Oligo 25-N (a mixture of sequences). 100 nanomole vials of these oligos are available at reduced prices. While the standard oligo and the oligo mixture perform very well as negative controls, they are not expected to match the base composition of your experimental oligo.

<u>Specificity controls</u>: Specificity control oligos are usually either second non-overlapping targeted oligos (preferred) or are five-mispair oligos.

Experiments using second non-overlapping targeted oligo are usually one of three types.

The two non-overlapping translation-blocking oligos experiment: This experiment compares the phenotype induced by injection of two different oligos targeted to block translation of the same mRNA. If both sequences induce the same phenotype, that supports the hypothesis that the observed phenotype is due to knockdown of the targeted gene.

One splice blocker, one translation blocker: This uses a splice blocking Morpholino to produce the same phenotype as the translation blocking oligo. It is crucial to determine which exon to target in order to knock down the activity of the protein and phenocopy the translation blocker's effect.

Two splice blockers: In the two-splice-blocker experiment, the same exon is targeted in separate experiments by a splice donor blocker and a splice acceptor blocker. If both of the oligos individually cause clean excisions of the targeted exon, the phenotypes induced by the two oligos should be identical and, again, the hypothesis that the phenotype was triggered by specific excision of the exon is supported.

Antisense sequences with 5 mispairs appropriately distributed along the sequence are sometimes used to test sequence specificity. A typical mispair control is illustrated below.

Antisense: 5'-AAA CCC GGG TTT ACG AAC CGG TTT A

Mispaired control: 5'-AAA gCC cGG TTT AgG AAC CcG TaT A

When to use Vivo-Morpholinos

A Vivo-Morpholino is a Morpholino oligo with a covalently linked octaguanidinium dendrimer. The guanidiniums resemble the side chains of the arginines in an arginine-rich cell penetrating peptide. While standard Morpholinos might not enter cells effectively in animals, Vivo-Morpholinos can enter cells when administered i.v, i.p., i.c.v. or by local injection. Vivo-Morpholino are also effective in cell cultures, requiring no separate delivery system. The sequence of a Vivo-Morpholino is designed just as for a standard Morpholino, so a validated Morpholino sequence can be made as a Vivo-Morpholino.

OLIGO END MODIFICATIONS

Structures, masses and other characteristics of optional Morpholino end modifications can be viewed on our website. Click on the %Products and Applications+tab and the %Morpholino Custom Control and End Modification Options+link, then click on %End Modifications+in the table of contents. Available end modifications include fluorochromes, quenchers, affinity tags, radioisotope carriers and functional handles for coupling oligos to other moieties; most are available as 3qmodifications and a few are also available attached at the oligospend.

QUANTITY

Morpholino oligos		
Typical package size	Larger amounts available	
300 nanomoles (about 2.5 mg or 75 OD units for 25-mer)	1000 nanomoles, 6000 nanomoles, ¼ g, ½ g, 1g, etc.	
Note: The quantities above are the measured and delivered amounts of lyophilized, sterile Morpholino oligos.		

Vivo-Morpholino oligos		
Typical package size	Larger amounts available	
400 nanomoles (about 4 mg or 100 OD units for 25-mer)	2000 nanomoles, 10000 nanomoles	
Note: The quantities above are the measured and delivered amounts of lyophilized, sterile Vivo-Morpholino oligos.		

For current pricing go to the GENE TOOLS website (www.gene-tools.com), click on the %Grdering+tab and then the %Gurrent Price List+link.

ORDERING AND SHIPPING

For the fastest and most reliable service with an automatic confirmation of your order, we strongly recommend that you order online at the GENE TOOLS website. If this method is unavailable, you may also place your order by FAX or mail using the form available under the % ordering tab of our website. We accept purchase orders or Master Card, Visa or American Express credit card orders.

Please include a phone number, and your email address with your order. Be sure to include both a shipping address and a billing address.

During checkout on our online store, international customers may enter a VAT number which will be printed on the commercial invoice reviewed by customs on entry into the destination country. Some countries require a separate VAT form be included during shipment; if you are in one of these countries, please email or FAX the VAT forms once you have placed your order, indicating the order number (or, if necessary, PO number) to which we should attach the VAT form when we ship the product.

Morpholinos are typically shipped within 10 days to two weeks after ordering. If the synthesis fails quality control, this can add a week to remake the oligo. We recommend you do not prepare animals until the oligos have arrived.

Customers are responsible for payment of any duties, taxes and tariffs incurred during shipment. We recommend providing a FedEx account number with your order so that taxes can immediately be charged to your FedEx account and you will be billed by FedEx later. Otherwise, couriers may ask for payment on delivery of any taxes incurred during shipment.

DISCLAIMER

While our and othersqexperience indicate that Morpholino oligos typically outperform other antisense structural types by a wide margin, nonetheless, because of the great variability among genes and cells, GENE TOOLS makes no warranty as to the performance of either GENE TOOLS-designed or customer-selected Morpholino oligos in any given biological system.