"How-to" Make a Special Delivery Morpholino Oligo

Special Delivery Morpholinos are Morpholino oligos pre-paired with partially complementary DNA. This MO-DNA heteroduplex has a net negative charge, unlike the non-charged backbone of a single-stranded Morpholino. These oligos were first described by Morcos[1] to be used in conjunction with the EPEI delivery reagent to achieve effective cytosolic delivery of Morpholinos to cells in culture. In addition, Special Delivery Morpholinos have been electroporated in chick[2] and used with standard nucleic acid transfection reagents in *Xenopus*[3].

This protocol is being made available to researchers since Gene Tools' has supplanted Special Delivery Morpholinos with Endo-Porter, a peptide based delivery reagent. Any Morpholino can be made into a Special Delivery oligo using these steps. For more information about Endo-Porter and a comparison between Special Delivery Morpholinos and Endo-Porter please see http://www.gene-tools.com/node/24. Please contact Gene Tools Customer Support (custsuppport@gene-tools.com) for any questions or clarifications.

<u>The quantities listed below are to make a standard 300 nanomole MO into 300 nanomoles of Special Delivery Morpholino.</u> If you have a different amount of MO, please adjust the quantities appropriately.

1. Obtain 250 nanomoles of a 26-mer standard-grade and desalted DNA oligomer that is partially complementary to your Morpholino.

Note: Gene Tools has found that excess salt will interfere with MO delivery. If you suspect excess salt in your DNA oligomer, please run your oligomer through an additional desalting step with a Q-Sepharose column.

The DNA oligomer has two distinct domains: 1) 10 bases are all A's to facilitate strong interaction with EPEI. 2) The second domain is complementary to 16 bases of the 3' end of the Morpholino oligo and facilitates strong interaction of the DNA with the Morpholino. See the example below.

MO Sequence
5'-CCTCTTACCTCAGTTACAATTTATA-3'
3'-AGTCAATGTTAAATATAAAAAAAAAA-5'
DNA Sequence

2. Quantitate your DNA in water via UV spectrometry at 260 nanometers.

3. Quantitate your MO in 0.1 N HCl via UV spectrometry at 265 nanometers (see http://www.gene-tools.com/files/determining_concentration.pdf for protocol).

4. Add the DNA to the MO so that the molar ratio of MO:DNA is 1.4 to 1.0. Stated another way, for every 100 nanomoles of MO add 71.4 nanomoles of DNA. The excess MO serves to saturate the DNA oligomers and allows for final delivery concentration to be determined as a function of DNA concentration.

For a typical 300 nanomoles of Morpholino oligo, add 214.3 nanomoles of DNA and add water to bring the final volume to 600 microliters. This will give you a stock solution with a concentration of 500 micromolar MO to be used with the EPEI Special Delivery protocol or 357 micromolar DNA to be used with standard DNA delivery methods (Lipofectamine, etc.).

5. Swirl well to mix.

6. Follow the EPEI Special Delivery protocol available http://www.gene-tools.com/files/special_delivery.pdf or an alternative protocol if you are not using EPEI as the delivery reagent. EPEI is available from Gene Tools.

Special Delivery Morpholinos should be stored at –20° or –80° C.

References:
1) Morcos PA. Achieving efficient delivery of morpholino oligos in cultured cells. Genesis. 2001 Jul;30(3):94-102.

2) Kos R, Tucker RP, Hall R, Duong TD, Erickson CA. Methods for introducing morpholinos into the chicken embryo. Dev Dyn. 2003 Mar;226(3):470-7.

3) Ohnuma S, Mann F, Boy S, Perron M, Harris WA. Lipofection strategy for the study of Xenopus retinal development. Methods. 2002 Dec;28(4):411-9.