Morpholino tips, Chien lab

This protocol is a major revision of Tips For Using Morpholinos.

Original version: Chi-Bin Chien, 10/12/10

Morpholino stocks

1. The best solvent for morpholino stocks is water. DEPC can ruin morpholinos, so use DEPC-free water.

2. While GeneTools recommends 1mM stock solutions (approximately 8 ng/nl), this can be too low, especially if the morpholino requires a high dose, or is being mixed together with other morpholinos. 2 or 3 mM will give more "headroom" to make working stocks as desired.

3. It is good practice to OD your morpholino stocks. The concentration does not always correspond to the amount given by GeneTools, for unclear reasons (perhaps because of imperfect solubility). This will also let you reconstitute the stock if it accidentally dries out.

4. Do not keep morpholinos on ice on the bench top, as they may precipitate. Room temperature handling is best.

5. It is important to store stocks either frozen or in airtight containers to prevent evaporation. Standard microfuge tubes allow too much evaporation over long periods; seal them with parafilm.

6. Many labs aliquot morpholino stocks and keep them frozen. Avoid freeze-thaw cycles because they may trigger precipitation, especially for GC-rich morpholinos. After thawing stored morpholinos, heat them to 65ºC for 10 minutes to ensure that they are completely dissolved

Injecting morpholinos

7. Before injection, dilute stocks to a working concentration in injection buffer. Many labs use Danieau's, although this is an extracellular solution. A common, simpler alternative is 1% phenol red, which has the added advantage of letting you track which embryos have been injected.

8. The working concentration depends on the morpholino dose needed. When trying a morpholino for the first time, a good guess would be 2 ng /nl; you can then inject 1, 2, or 3 puffs (calibrated to 1 nl each using an eyepiece micrometer) to get doses of 2, 4, and 6 ng to assess phenotypes and toxicity.

9. Remember that the pressure injectors used by everyone in the field are difficult to calibrate for drop size. Thus, a "1 nl" drop does not necessarily correspond to "1 nl" injected in a paper, or even "1 nl" injected by the same person on a different day, or even by the same person with the same needle after it has partially clogged.

10. Nevertheless, you should calibrate as best you can. Use an eyepiece micrometer (eyepiece with a reticule in it), measure the diameter of a bolus injected into oil or into the yolk of an embryo, and calculate the volume according to 4/3 * pi * r^3. You must calibrate with the same pipette and pressure settings that you will use to inject embryos.

11. We place embryos in E2 embryo medium in 1% agarose dishes with long grooves, made by a lucite mold, e.g. the TU-1 from Adaptive Science Tools. The injection needle is held either by a micromanipulator, or freehand, and embryos are injected through their chorions.

12. When injecting morpholinos, aim either for the cytoplasm or the high yolk, just below the cytoplasm. You may need to roll the embryo in its chorion to get a good angle. Cytoplasmic streaming will then carry the MOs up into the cell. We prefer to stick with 1-4 cell embryos; after this time it is hard to be sure that the MO has gone into all the cells.

Double-injecting

13. If you are doing an experiment where you inject two components, e.g. an RNA rescue experiment where you compare MO to MO + RNA, the cleanest method is to inject embryos twice. The alternative is to have one pipette with MO only, and another pipette with MO + RNA; however, given the difficulties in calibrating injected volumes, it is impossible to be sure that the MO dose is the same for both conditions.

14. Inject all of the embryos with solution 1 (e.g. MO), no phenol red. Be sure to immediately remove uninjected or damaged embryos, so that all remaining embryos are known to be injected. Next, inject every other embryo with solution 2 (e.g. RNA), with phenol red. Separate red and non-red embryos to be the double-injected and single-injected groups, respectively.

References

Gene Tools' essential information page.

Review on using morpholinos: JS Eisen and JC Smith (2008), "Controlling morpholino experiments: don't stop making antisense". Development, 135:1735-43. (free download from Development website).