

### ENU mutagenesis

1 gram from Sigma (N 3385) in a rubber stopper bottle. Makes enough for 9 to 10 mutagenesis containers at 3 to 3.3 mM in 300 ml volume.

The ENU does not dissolve very easily in the 10mM acetic acid (which should not contain phosphate buffer). It will be clumpy. It's easiest to dissolve when the ENU is at a concentration of around 80 to 90mM. We purchase the ENU from sigma in 1 gram quantity. The ENU contains about 30% water and 1.7% acetic acid (it will tell you this on the label) making the total weight about 1.5 grams. We dissolve everything at once--much too dangerous to weigh out the powder. Make sure you base all your calculations on the 1 gram of ENU---and not 1.5 grams (which includes the weight of the water).

To add the 10mM acetic acid solution to the ENU, place a 10cc syringe with a pink needle (22guage) filled with the acetic acid through the rubber stopper of the ENU container. Place second 10cc syringe with pink needle, plunger removed and syringe filled with sponge or gauze 2/3 full to allow air in bottle to be displaced by liquid. Sponge serves to prevent powder from being dispelled into air (although this usually does not happen). Once the ENU powder is immersed in liquid, then carefully open the rubber stopper and add a magnetic stir bar (ENU-dedicated) to stir (usually 1 hour or so). Do all of this in the HOOD. Wear lab coat, gloves, and glasses. I usually double glove my hands. If a glove becomes contaminanted, remove immediately and place in ENU waste container. Re-double glove.

I always measure the O.D. of the ENU stock solution to determine the exact molarity (wavelength=238, E=5830 at pH=6.0). Dilute 5 microliters into 5 ml of pH6 phosphate buffer and measure OD in (ENU mutagenesis dedicated) quartz cuvette. The 3 mM amount we use for the mutagenesis is on the border of being very lethal for the fish---so small differences in the concentration can dramatically affect survival.

The solution is very stable in the 10mM acetic acid. You can check the O.D. later too to test for degradation. I have frozen and thawed it 3 years later with no problem.

We dilute the ENU into a 10mm phosphate buffer to yield a pH6.6 solution. The ENU becomes increasingly mutagenic with increasing pH, so the correct pH is crucial to mutagenizing effectively and not over-mutagenizing (which can be lethal or cause sterility). The acetic acid doesn't affect the final pH. So, if the phosphate buffer is pH6.6 after diluting it to 10mM, then the ENU will be as well. Usually we use a stock of 0.5M phosphate buffer at pH 6.0, which when diluted in water is pH 6.6 (the pH of phosphate buffers depends on its concentration). Use 6 mls, 0.5M phosphate buffer/300 ml fish water. We

typically place 5-6 fish in 300 mls of ENU solution. The ENU has a half life of 6-7 hours at this pH, so use immediately. See Current Biology article (Mullins et al., 1994) for description of containers and more info on the mutagenesis procedure.