

# Chemical Mutagenesis

(Source: S. Johnson, D. Grunwald, W. Driever, and M. Mullins)

Because chemical mutagens may be potent carcinogens, these agents that are used to induce mutations are potentially dangerous to the researcher using them as well as to the surrounding environment. Consequently, precautions must be taken to limit this risk. We use several strategies to ensure safe use of chemical mutagens: (1) containment of the mutagen in a fume hood during the experiment, (2) inactivation of the mutagen after it is used and (3) isolation of treated fish after mutagenesis to limit the number of workers potentially exposed to the mutagen.

## Mutagens

Currently, two chemical mutagens are used in zebrafish laboratories: EMS and ENU. EMS is available as an oily liquid that can be pipetted directly from the bottle. Wrap the bottle with Parafilm and store at 4°C or -20°C between experiments. EMS is readily soluble in water, but may require vigorous shaking to get it into solution. By contrast, ENU is a powder. To avoid the hazards of weighing powdered mutagens, we use sealed 1 g "isopacs" (Sigma # N 3385). Solvent is injected into the isopac with a syringe (100 ml H<sub>2</sub>O or 10 ml EtOH). After the powder has dissolved, the required amount is drawn off in the syringe and the remainder disposed of as outlined below.

Both EMS and ENU are alkylating agents, and can be inactivated similarly by treatment with high pH and sodium thiosulfate (10% sodium thiosulfate, 1% sodium hydroxide). The half-life of EMS in 10% sodium thiosulfate is 17 minutes at 37°C and 1.4 hours at 20°C, while the half-life of ENU in 10% sodium thiosulfate in basic solution is about 10 minutes at 37°C. Other means of inactivation may be required for other chemical mutagens.

## Rules for containment and inactivation of mutagens

1. Handle mutagen only in the fume hood.
2. Keep a hazardous waste bag in the fume hood. Dispose of most objects (gloves, towels, pipettes, etc.) in this bag (which might have come in contact with the mutagen). Arrange with Environmental Health and Safety for disposal of this bag following the experiment.
3. Dedicate nondisposable equipment to mutagenesis. Keep these in the fume hood. These include several mouse cages, beakers, a 500 l pipetter and a small dip net. Although these items are decontaminated (see below) after use, they are still left in the hood.
4. Cover the working surfaces of the fume hood and the floor in front of the fume hood with absorbent paper. Dispose of these papers in the hazardous waste bag after the experiment.
5. Wear gloves at all times and change them frequently. Specifically, change your gloves whenever you suspect contact with the mutagen or whenever you leave the fume hood. Wash your hands (and face) periodically during the experiment.
6. Inactivate the mutagen following the experiment by pouring the mutagen and rinses into an equal volume of 20% sodium thiosulfate, 1% NaOH. Make a separate inactivation bath to soak items (beakers, mouse cages, pipette tips, tubes, etc.) that came into direct contact with the mutagen. Allow these baths to sit for 24 hours, then pour the liquid down the cup sink at the back of the hood.

Treatment of animals during and after mutagenesis

## Embryos

1. Mutagenize embryos either in beakers or Petri dishes.
2. Following mutagenesis, wash embryos three times, then transfer them into fresh beakers that have not been exposed to mutagen.
3. Transfer the embryos into the nursery for normal rearing.

## Adults

1. Mutagenize in beakers or mouse cages.
2. Dispose of animals that die during the first 24 hpf by placing them into the sodium thiosulfate inactivation bath (see rule 6, above).
3. Following mutagenesis, wash adults by catching them in a small dip net and passing them briefly through two changes of clean water.
4. Transfer mutagenized animals into fish tanks. Label all tanks used with a prominent notice, e.g. "MUTAGEN-TREATED FISH, PLEASE DO NOT SCRUB, SIPHON, OR REMOVE DEAD FISH. PLEASE FEED NORMALLY". Also, write your name and the date of treatment on the notice. After the first 24 hpf, dead fish can be disposed of in a normal manner. Keep a dedicated siphon and dip net in the hood for this purpose. After one month remove the notice and return the fish to normal care.
5. Personally inform the workers who maintain the tanks of these changes.