

# EMS mutagenesis

by Michael Koelle (4/6/94), edited by Erik Andersen (3/9/2010)

1. Wash worms off plates with M9, using sterile glass pipettes. Want to use plates with a lot of early L4 larvae.
2. Transfer to a 15 ml sterile plastic centrifuge tube. Spin down (1000 rpm in a clinical centrifuge for 30 sec) and remove supernatant.
3. Add M9 to 3 ml of worm suspension in the 15 ml sterile plastic centrifuge tube.
4. Place 1 ml M9 in a separate 15 ml tube.
5. Wearing gloves, in a hood, using a special pipette used only for EMS, add 20  $\mu$ l EMS (methanesulfonic acid, ethyl ester, Sigma #M-0880). EMS is stored at room temperature in the hood. Keep a special jar for sharps (pasteur pipettes and pipettman tips) and another one for gloves that have been exposed to EMS. Shake the EMS suspension until it is no longer cloudy.
6. Transfer the 3 ml of worms into the tube of EMS. Parafilm the top. Place the tube on a spinning wheel at 20°C for four hours. The final concentration of EMS is 47 mM.
7. Spin the worms down and remove the supernatant to a waste 15 ml plastic centrifuge tube.
8. Wash the worms twice with 3 ml M9, transferring the supernatants to the waste 15 ml tube.
9. Transfer the worms, in a few drops of M9, using a sterile glass pipette, to the edge of the bacterial lawn on plate. Do not ever transfer worms with a plastic yellow tip—they stick to the inside.
10. Kill the EMS in the waste liquid from the washes by adding a few pellets of KOH. Mix a little, label the tube, and leave in the hood for a day. Then pour down the sink, and discard the tube in the trash.
11. Let the worms sit for 15-20 minutes (some wait 2 hours). Pick off the healthy looking late L4 animals (P<sub>0</sub>'s). Mutagenizing too early (before much germline proliferation) will theoretically give fewer independently mutagenized genomes and give jackpots from those mutations that do occur. Mutagenizing too late will be ineffective. Some people pick late L4's to mutagenize, so that the animals are very young adults at the end of the EMS treatment. It is somewhat difficult to recognize L4's after the EMS treatment; because the animals are starved, the white crescent normally visible in the vulval region of well fed L4's is not very apparent. The "dot" that appears within the crescent at the very end of L4 is still visible in starved animals.