

Ethyl methanesulfonate (E.M.S.) mutagenesis
U. Heberlein, 1988

WARNING: EMS is a powerful mutagen and a suspected carcinogen. [Also known as methane sulfonic acid ethyl ester, Sigma #M-0880.] Wear gloves and work in a fume hood. Use disposable plastic & glassware. Inactivate EMS solutions before disposal by mixing them with an equal volume of 5% sodium thiosulfate. All pipet tips and tubes contaminated with EMS should be soaked in 5% sodium thiosulfate prior to disposal.

Collect flies

1. Collect 100 males for each treatment vial you will be setting up.
2. Place each group of 100 males into an empty vial and allow to dehydrate for 2-4 hours before beginning the EMS treatment.

Prepare treatment vials (Prepare 2 vials for each set of 100 males)

Collect: Glass vials & cotton plugs
 3MM filter paper
 GF-C filter paper

1. Place a round of GF-C filter paper on the bottom of each glass vial.
2. Cut the 3MM filter paper to snugly line the inside walls of the vials, so that no flies will be able to get stuck in the nooks and crannies.
3. Plug the vials with cotton, making sure there is a tight fit.

Prepare E.M.S. solution (Mix in a fume hood)

10X EMS buffer: 100 mM Tris (pH 7.5)
 10% sucrose
 Autoclave and store at room temperature.

4. Dilute EMS buffer to 1X. [3.5 ml 10X buffer in 35 ml of ddH₂O.]
5. Double glove each hand.
6. Add 0.111 ml of EMS (a dense, oily liquid) to the 1X buffer to attain 30 mM EMS solution. [EMS: mw = 124.2 g/ mole. ρ = 1.17 g/ml]
7. Vortex vigorously.

E.M.S. treatment (In a fume hood, on an absorbent pad)

8. Pour EMS solution into the 1st vial.
9. Transfer the same liquid into the next vial and continue until half of your prepared vials are treated with EMS. Drain all the solution out of each vial as much as possible.
10. Dry the top of each vial off with a Kimwipe and dispose in EMS waste. Do not allow the cotton plug to get wet, as this will prevent ventilation during the treatment.
11. Inactivate the remaining EMS solution by adding an equal volume of 5% sodium thiosulfate. Dispose of the top layer of gloves into the EMS waste.

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12. Transfer dehydrated flies into the treatment vials in groups of 100 males.
13. Leave flies overnight (no more than 16 hours) in the hood. (Leave a sign on the hood indicating that *toxic* EMS is in use.)
14. Transfer flies to the 2nd set of untreated vials. Allow the flies to clean themselves for 30 minutes.
15. Transfer the flies to regular fly food vials containing food and yeast paste. Let them recover and expel EMS from their digestive tract for a couple of hours.
16. Properly dispose of all EMS dry waste.
17. Mate the treated males to virgin females, and incubate at 25° C.
18. Transfer the crosses to new bottles (or vials) each day for 4 days. Dispose of the adults on the 5th day.