

EMS Protocol

double glove! - double glove! - double glove! - double glove! - double glove!

1. Put ~60 male flies that have been deprived of females for 2-3 days into an empty bottle. Leave for 2 hours.
Also, for each ~60 males put 50 virgin females in a separate vial.
2. After 1 1/2 hours **put on 2 sets of gloves.**
3. Mix 30 ml 1% sucrose + 79.1 μ l EMS in an Erlenmeyer flask. Mix well, so that there are no bubbles of EMS floating around.
-1% sucrose solution should be filter-sterilized
-This will make 25mM EMS from 9.42M Sigma stock in 1% sucrose
4. Wad up 2 Kimwipes and put in a glass bottle. Add 7 ml EMS/sucrose solution to the Kimwipes using a disposable pipette. Put pipette in the EMS waste.
5. Inactivate remaining solution with "ME" = mercaptoacetic acid
1.02 ml 98% Thioglycolic acid + 199 ml 1M NaOH. Pour into the liquid EMS waste container. ME should inactivate the EMS in a 1:1 molar ratio so the EMS should be way inactive after you clean up.
-1M NaOH + 0.5% Thioglycolic acid (mercaptoacetic acid)
6. Transfer flies into EMS bottles.
7. Allow flies to eat EMS for 18-24 hours.
8. Transfer flies into fresh food bottles containing yeast paste, allowing the flies to crawl up into the new bottles. Not all the flies will enter the new bottle, which is OK. Quickly recap the remaining EMS'd flies, stick the bottles in the freezer; later, thaw, inactivate the bottles with ME, and toss it all in the EMS waste.
9. Add the 50 virgins previously separated and let them party! (Please, don't watch)
10. Transfer flies to new bottles every 3 days so that there are 2 bottles of embryos per mutagenesis.