**Setting up worm crosses**

**MATERIALS**

L4 stage to very young adult N2 worms

Worm strains to be crossed

Water bath

Small NGM OP50 plates

Mating plates (Want a small OP50 lawn to get the hermaphrodites and males into close proximity!):

Small NGM plates spotted with 10 – 15 uL of OP50

Actual mating plates spotted with 5 uL of OP50

**PROTOCOL**

*C. elegans* has six chromosomes: five autosomes (I – V) and an X chromosome. Hermaphrodites are diploid for all six, whereas males are diploid for the autosomes but are haploid for the sex chromosome—designated XO.

Males (XO) naturally occur at only a low frequency (~0.02%) in wild-type populations. As such, we need to make and maintain our own stocks of males.

Need to become familiar with the *C. elegans* genetics nomenclature. Diagram

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Make males

1. Turn on water bath to 33°C.
2. Pick 3 to 4 L4 stage N2 hermaphrodites (XX) onto 1 small NGM/OP50 plate.
   * Repeat for a total of ~3 plates.
3. Parafilm the plates. (Need them to be waterproof!)
4. Heat shock hermaphrodites by placing the plates, lid down, in the 33°C water bath for 3 hours.
5. Post heat shock, take the plates out of the water bath. Unwrap the parafilm and swap the plate lid for a fresh one (will have a lot of precipitation).
6. Place plates in the 20°C incubator. Check the plates over the next 2 to 3 days for males.

Diagram

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1. Pick about 4 to 5 young males (XO) onto fresh mating plates with 2 to 3 L4 stage hermaphrodites (XX) to continue propagating male progeny.
   * Male progeny will occur at a much greater frequency (~50%) now because their sperm is preferred over self-fertilization.

Diagram, schematic

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1. It is advisable to make worm stocks of males once you get a few plates going.

Setting up a cross

1. Develop the mating scheme.
   * Need to know what strains you are crossing and how you will select for the cross progeny of interest. Will you rely on a fluorescent marker or visible phenotype for selection?

Example strains to cross. We are interested in developing a heterozygous worm for *elt-2*p::GFP and *dlg-1*p::GFP.

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Example scheme:

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1. Once mating scheme is developed, take 4 to 5 young N2 males and move them onto a mating plate with 2 to 3 L4 stage hermaphrodites of the strain you would like to cross.
2. Then select the male heterozygous cross progeny of interest via a selection marker (i.e., fluorescence, visible phenotype (i.e., roller, dumpy), antibiotic resistance).
3. Now set up the second cross by plating 4 to 5 young male heterozygous cross progeny with the second strain you would like to cross.
4. Then select hermaphrodite cross progeny of interest via the selection marker. Move individual hermaphrodites onto fresh, small NGM OP50 plates (1 per plate) and let them self-fertilize.
5. Now screen the F1 progeny of selected hermaphrodites for desired phenotype. Select desired F1 worms and move them onto individual plates.
6. Continue screening subsequent progeny (i.e., F2, F3) for maintenance of desired phenotype. This may take a few generations.
7. Once the phenotype seems stable, confirm genotype and make worm stocks of the new worm strain.

**REFERENCES & RESOURCES**

<http://wormbook.org/chapters/www_introandbasics/introandbasics.html#d0e450>

<https://cgc.umn.edu/strain/search>