

C. elegans lab

Day 1 T/W

1. Practice picking worms
2. Crosses - Place TWO L4 dpy-17,unc-32 hermaphrodites onto male plates. The male plates will come prepared with 5 L4 WT males/plate. Set this cross up TWICE.
3. RNAi - Place TWO L4 WT hermaphrodites onto four different RNAi plates: dpy-5, unc-22, pop-1, vector. Label these as "RNAi plates #1".

Tape up your plates and label them with your names, your section, and the date before placing them in the box.

Day 2 W/Th

1. RNAi - transfer both adults onto a second RNAi plate ~ 24-30hrs later. Make sure to transfer to the correct plate (i.e. transfer the dpy-5 RNAi plate adults to a new dpy-5 plate, etc.). Label these as "RNAi plates #2".

Note of caution: Sometimes the worms will escape or burrow down into the plates. You will notice this if you can only see one or no worms on your #1 plates when you go to transfer them. In this case, take note of which #1 plates you were only able to transfer one or neither worm, because they can resurface later on and this can throw off your counting when you score for phenotypes or lethality in a couple of days.

Tape up your plates and label them with your names, your section, and the date before placing them in the box.

Day 3 Th/Fri

1. RNAi - Remove both adults from the second plates (you just flame your pick with them on it)!
2. RNAi - Count the unhatched embryos on the pop-1 and vector #1 RNAi plates.

Embryos are slightly oval in shape, clear, and tend to look a little "puffed up". Do not count oocytes, which are unfertilized eggs that look circular, dark, and "flat." You shouldn't see a lot of oocytes, if any at all. **Count ONLY the pop-1 and vector plates!!!** If the RNAi worked, you should see lots of dead embryos on the pop-1 plates and none on the vector plates.

Make sure to do this step today. Any hatched worms can grow up and start laying eggs of their own which will prevent you from getting an accurate embryonic lethality percentage. If you forgot to do this count, then just move on.

Tape up your plates and label them with your names, your section, and the date before placing them in the box.

Day 4 Fri/Sat!!

1. RNAi - Score for RNAi penetrance on all #1 RNAi plates: dpy-5, unc-22, pop-1 and vector.

The vector plates, as well as the pop-1 plates, should have only WT worms. Count the adults on these plates. Screen plates to see if any "spontaneous" mutants arise. Count the number of short and fat worms on your dpy-5 plates as well as the total number of worms (dpy + WT). Count the number of twitchy and relatively immobile worms on your unc-22 plates as well as the total number of worms (unc + WT).

Calculate penetrance for RNAi #1 plates (penetrance is reflected as a percentage):

pop-1 penetrance (embryonic lethality) = # of unhatched embryos / (# of unhatched embryos + all adults) * 100

dpy-5 penetrance = # of dpy worms / total # of worms * 100

unc-22 penetrance = # of unc worms / total # of worms * 100

Was there any embryonic lethality on the vector plates? Were there any non-WT adults on the vector plates?

Make sure to do this step today. Worms grow fast on RNAi plates, so the plate might be starved (overcrowded) if you wait too long. You can't accurately determine phenotypes if the worms are starved and sick-looking.

You won't need to return to these plates if you're confident that you've completed the phenotype scoring, so you can throw your #1 RNAi plates away!

2. RNAi - Count the unhatched embryos on the pop-1 and vector #2 RNAi plates.

Make sure to do this step today. Look at Day 3 for more detailed instructions.

Tape up your plates and label them with your names, your section, and the date before placing them in the box.

Day 5 Sat/Sun

1. RNAi - Score for RNAi penetrance on all #2 RNAi plates: dpy-5, unc-22, pop-1, and vector.

Make sure to do this step today. Look at Day 4 for more detailed instructions.

Take notice of the differences in penetrance between the first and second RNAi plates. Is there an increase or decrease on the 2nd plates?

You won't need to return to these plates if you're confident that you've completed the phenotype scoring, so you can throw your #1 RNAi plates away!

2. Crosses - Place ONE WT L4 hermaphrodite onto a fresh plate. This is called "singling". Do this for 4 plates.

Only single WT-looking worms, since these are the only ones that are going to be "cross-progeny"—offspring from the mating of the hermaphrodites and males. "Self-progeny"—offspring from the hermaphrodite alone, will look like the original dpy-17, unc-32 hermaphrodite.

Tape up your plates and label them with your names, your section, and the date before placing them in the box.

Day 6-7

Nothing!

Day 8 T/W

1. Crosses - Look at your F1 plates. Count the different types of F2 progeny coming out of each plate: # of WT, # of dpy-17, unc-32, # of dpy-17 only (these will be rare), # of unc-32 only (these will be rare).

Look at the crosses handout for the expected F2 outcomes. Do your observed numbers reflect the expected outcomes? (i.e. ~2/3 should be WT and ~1/3 dpy-17, unc-32 with the occasional single mutant)

2. Crosses - Place ONE WT L4 hermaphrodite onto a fresh plate. Do this for 4 plates.
3. Crosses - Place ONE dpy-17, unc-32 L4 hermaphrodite onto a fresh plate. Do this for 4 plates.
4. Crosses - If you find any recombinants (dpy-17 only or unc-32 only worms) pick those as well. Make sure to single them!

Day 9-11

Nothing!

Day 12 Sat/Sun

1. Crosses - Look at your F2 plates. Count/examine the different types of F3 progeny coming out of each plate.

Look at the crosses handout for guideline questions.