

Biology 423L

**Oct.6/7 and Oct. 13/14 and
other days in those two weeks.**

***C. elegans* genetics and RNAi**

Report due: Oct. 27/28.

Reading: *C. elegans*: Hartwell Chapter 8 pp. 255-256.
Epigenetics: Hartwell Chapter 18 pp. 665-668, 726-728.
Verdel et al 2009 Int. J. Dev. Biol. 53:245-257.
Eymery et al., 2009 Int. J. Dev. Biol. 53: 259-268.
(the papers above are available on course web site)

Objectives:

Become familiar with *C. elegans* development
Discriminate between wild-type and mutant worms
Learn how to interpret mutant analysis
Perform a genetic cross with *C. elegans* and identify dominant vs. recessive mutations
Use RNAi to phenocopy genetic mutations in *C. elegans*

Materials:

T.A. Prepare in advance:

4 tubes containing 2ml of LB+ 50µg/ml ampicillin+ 12µg/ml tetracycline to grow cultures of RNAi bacteria (strains described below). Liquid cultures of OP50 bacteria (used for regular worm culturing) are kept at 4°C – check with Ahmed lab to get bacterial strains and to get *C. elegans* strains. They also have a plate pourer machine to help with making NGM and RNAi plates.

Recipe for 1 litre of LB:

10 g Tryptone
5 g Yeast extract
5g NaCl
Add water to 1 liter and autoclave.

NGM plates: Need 1 glue plate per group. For mating: 2 plates for parental cross, 4 for F1s, 8 for F2s (all these will be seeded with OP50 bacteria).

RNAi plates: 1 seeded with each type of bacteria for RNAi including a plate of vector only control.

Recipe for NGM plates: 1 liter should make 200 35mm plates

- 17 g agar
 - 2.5 g bacto-peptone
 - 3g NaCl
 - 1 ml Cholesterol (5mg/ml in EtOH)
 - Water to 1 L
- Autoclave, cool down and add (sterile)
- CaCl₂ to 1mM

- MgSO₄ to 1mM
 - Potassium phosphate buffer pH 6.0 to 25mM
- For RNAi plates also add:
- ampicillin to 50µg/ml
 - tetracycline to 12µg/ml
 - IPTG to 1mM (make IPTG fresh from powder stored at -20°C)

Picks for everyone made from short Pasteur pipettes and platinum wire

TA: At least 1 week before first lab day:

Pour NGM and RNAi plates and let them solidify at room temp overnight. You can use the Ahmed lab plate pourer machine. Seed glue plates with OP50 bacteria the next day.

TA: Two days before the lab day:

Streak RNAi bacteria (as described below) from frozen stocks on LB+amp+tet plates and grow overnight at 37°C.

Be sure *C. elegans* strains will be ready

TA: Day before first lab:

Set up liquid cultures containing LB+amp+tet (as described above) from single colonies of RNAi bacterial strains and grow for 6-8 hours shaking at 37°C.

Spread the appropriate bacteria on RNAi plates and OP50 on NGM plates. Use 50 – 100µl per plate. The plates then need to dry for one day.

We will have a strain with the RNAi vector without an insert as a negative control and, three strains to block gene expression

1. *Dpy*
2. *Prz*
3. Embryonic lethal (*pop1*)
4. Empty vector

Each group needs 1 plate with each type of bacteria.

Each group needs at least 1 extra plate with no bacteria.

Each group needs a glue plate. This is a NGM agar plate seeded with OP50 bacteria one week before.

For crosses: Day 1 Need 2 plates per group seeded with OP50 bacteria.

Day 3 Need 4 plates per group seeded with OP50 bacteria.

Day 8 Need 8 plates per group seeded with OP50 bacteria

C. elegans strains:

Need wild type hermaphrodites staged to L4 for day 1. Get help from Ahmed lab.

Need double mutant *dpy17;unc32* hermaphrodite and wild type males (all staged at L4) for crosses on day 1. Note for 07: we can use very strong alleles of two unlinked mutations. Ask Shawn Ahmed for help with the choice.

Need mutants of *dpy*, *prz*, and wild type for day 8 to compare phenotypes.

For RNAi experiments, use a mutant *C. elegans* in which the DNA dependent DNA polymerase is deficient. This makes worms more sensitive to RNAi processing.

Materials for class:

Wild-type *C. elegans* hermaphrodites, *dpy17;unc32* mutant hermaphrodites and wild type males staged to L4. If possible, separate wild type males out for the students (10 per plate and one plate per group). Be sure the correct genotype of worms is used for RNAi experiments.

Mutant strains to demonstrate phenotypes expected for loss of function of RNAi target genes.

RNAi plates with bacterial strains expressing dsRNAs for Dpy, Prz, and Embryonic lethal phenotypes (4 plates each per group) and vector (4 plates per group)

Worm picks (platinum wire and Pasteur pipets)

Bunsen burner needed to make picks

Dissecting microscopes with base illumination.

Sterile tips

15°C incubator

20°C incubator

alcohol lamps

matches

Method: (Students start here)

All manipulations have to be done **sterily**. Sterilize your pick by burning the wire in a flame until it glows red. Let it cool. This should only take a few seconds. All plates should be properly labeled with the worm genotype, the date and your initials.

Day 1 (2h)

RNAi:

1. Collect worms: Use the sterile metal pick to transfer worms from the plates provided to a fresh NGM plate (OP50 bacteria). Touch the worm pick to the glue plate and gently scrape it along the bacteria. This will pick up some sticky bacteria. Then touch the pick onto a worm, gently. The worm should stick to the pick. Transfer the worm to the fresh plate. Check to see if it is still moving.
2. Transfer 2 worms (P0) onto each fresh RNAi plate; Choose animals in the last larval stage (L4). L4's can be recognized by a white semi-circle near the center of the worm (lengthwise) which is the developing vulva. (The TAs will help you to recognize these worms).
3. Incubate the plates at 15°C for three days. We will need to use the incubator in Fordham Hall.

Crosses:

1. Take a plate seeded with OP50 bacteria on which the assistant has transferred 10 male wild type *C. elegans*. You may try to prepare this yourself if the assistant agrees. Transfer 2 *dpy17;unc32* hermaphrodites onto the plate. Incubate at 20°C.

Day 4 (30 min) - Friday for both groups if possible. If necessary, we will make a scheduled time on Saturday for the Wednesday lab.

RNAi: The plates will be brought to the lab room at a time agreed on by the group. Use the pick to remove the 2 adults you originally put on. Burn them in your alcohol lamp flame to dispose of them. Examine the small worms (progeny) for viability and movement. Put the plates back at 15°C.

Crosses: Pick one L4 hermaphrodite (smaller than the adults, with white patch) from the F1 progeny on the original (P0) crosses plate to a fresh NGM plate (seeded with OP50 bacteria). Prepare 3 or 4 plates with one F1 per plate. *C. elegans* hermaphrodites can fertilize themselves. Incubate at 20°C.

Day 8:

RNAi: Examine the worms for phenotypes and compare them to the genetic mutants provided from the Ahmed lab. To score the progeny: count the total number of worms displaying the appropriate phenotype and those displaying a wild-type phenotype (see Report below). We have hand counters to help you score large numbers of worms. Count up to 100 worms and express the relative proportion of phenotypes as percentages of total number of worms counted. Drawing a large cross (to section it in to fours) on the bottom of the plate may make counting easier.

For the embryonic lethal mutation: estimate the number of worms on the wild type plate by counting a small area of the plate. Compare this to the number of worms on the embryonic lethal plates.

Crosses: Examine the F1 plates. You should see the original adult (wild type phenotype) and F2 worms of both parental types. Count the relative proportions of each mutant type. Count up to 100 worms if possible. Transfer individual *dpy17;unc32* F2 types to a fresh plate and individual F2 wild types to a fresh plate. Make 4 plates of each. Do you see any recombinants, *dpy* or *unc* only? If so, transfer some of them as individuals to fresh plates.

Day 12:

Crosses: Examine the progeny of each F2 worm (the F3 generation). Are they true breeding or a mixture? Are the mutations we used dominant or recessive? Count up to 100 worms. Make a table with the proportion of mutant to wild type for each plate you have prepared.

Report: Results:

For RNAi: On each plate estimate the proportion of mutant worms to the total number of worms (mutant + wild type) by counting the worms on your plates. Make a small table to illustrate the results. This proportion is the “penetrance” of the RNAi induced mutations.

For the crosses: Make a table: record the phenotype of the F1 worms. Record the relative proportions of phenotypes in the F2 generation as percents. Record the relative proportions of F3 worms on each plate.

Discussion: Discuss the observations. For the mating, which alleles are recessive? Are the *dpy17* and *unc32* mutations linked or unlinked? Explain your answer, a Chi-square test is not necessary.

Compare the “penetrance” of each type of RNAi mutation (Penetrance is usually defined as the proportion of individuals with a given genotype that exhibit the expected trait. It is usually used to discuss genetically influenced diseases in which a person may have the alleles that usually lead to a disease but for some reason, they don't develop the symptoms of the disease).

Genome sequencing projects identify many DNA sequences that could encode a protein. In some cases sequences with homology to known genes are found. RNAi can be used in most eukaryotes and it is becoming a popular tool to determine the possible functions of genes encoded by a DNA sequence. Another tool for the same purpose is making gene “knockouts” or complete loss-of-function mutations (Hartwell Figure 20.5 p. 723). Discuss the advantages and disadvantages of using RNAi or knockouts to learn the function of a gene. Are there situations in which one method is much better than the other?