

BIO 251

Lab Prep Week 11

Inhibition of *unc-22* function using double stranded RNA mediated interference (dsRNAi) by feeding

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Day -2:

Streak out cells for L4440 and pLT76 (L4440/*unc-22*) to LB- Amp/Tet plates (100ug/ml Ampicillin, 12.5 ug/ml tetracycline). Grow 37°C overnight.

Pour NGM agar plates with 100 ug/ml carbenicillin and 1mM IPTG. Allow plates to dry in hood.

Make sure sufficient N2 plates (2 per bench) will be ready by lab day (try to time them so plates have many L4's).

Day -1:

Inoculate single colonies to 2 mls LB + Amp (100 ug/ml) to make enough culture for all plates needed (eg. 4.8 mls needed, use 3 x 2mls for each cell line). Grow 8 hrs at 37°C.

Seed plates with 100 ul of culture (prepare 2 plates of each culture per group). Room temperature O/N.

Lab day:

At each bench:

- 2 L4440 seeded plates
- 2 L4440/*unc-22* (pLT76) seeded plates
- 2 worm pics
- 2 N2 plates
- 1 alcohol burner
- 1 book matches
- 2 dissecting microscopes
- 1 fine point Sharpie pen

Instructor bench:

- Instructor demo microscope
- Monitor and microscope camera system
- Plate with N2 worms to demo L4's

GENERAL COMMENTS/SUGGESTIONS:

- Consider using coded labeling so students don't know which plates are controls or experimentals until after results scored.

- Have students come back to score plates outside of class (or during a lecture period later in week) so that plates don't sit for a week at 16°C. Results better when plates kept at 20°C.
- A more 'inquiry driven' version of this lab for an upper level class could start with a list of possible genes (for which you have RNAi constructs available). The students would then have to decide what gene they wanted to work on, research it, come up with hypotheses and predictions and then run the experiment. If they meet more often or are used to coming in on their own time, they could also be required to set up their own culture plates and maintain their own N2 stocks.