Biol-311L Cell Biology Laboratory

Bacterial-mediated Feeding RNAi Protocol

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Day –n

Initiate a culture of *C. elegans* that will yield gravid (with embryos) adults on **Day 0**

Day –1

Obtain a plate of bacterial colonies.
Pick a colony and *grow* overnight in **2ml** Luria Broth with:
200ug/ml ampicillin (1ul per ml), 0.2% glucose (dextrose, 10ul per ml). **Mix well!**

Day 0

Plate 100ul of overnight bacterial culture on:
Bacterial Feeding RNAi plate (M9 lactose plate), dry thoroughly.
Centrifuge, *decant* supernatant and *freeze* pellet from overnight culture.
Wash off gravid adults and *bleach* to recover embryos.
Plate embryos on RNAi plates.

Day 2 or 3

Observe RNAi worms versus controls and *document* novel phenotypes.

Hints, Tips and Tricks

Initiating a culture of *C. elegans* with various numbers of worms of various ages will give you the necessary worms on Day O. Some protocols: The time between L1-adult is about 2 days, so lots of L1 (or L2s) washed off of a starving plate and re-plated will yield gravid adults in two days. Single adults will lay lots of eggs over the course of a day as well, so ~15 laying adults will yield lots of gravid adults in three or four days.

Cool your bacterial inoculating loop before touching a colony by waiting a few seconds and then sticking the agar someplace besides the colony first.

Freeze the remaining overnight culture by spinning in the clinical at the highest setting for five minutes. Pour the used media down a sink and put the tube in the freezer.

After plating the RNAi bacteria, ***dry*** down the lawn in the hood if necessary.

Plate embryos on a spot on the RNAi plate ***away*** from the dried lawn. The hatchlings will find the bacteria without a problem.

When bleaching, lots of adults yield lots of embryos.