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STEP BY STEP PROTOCOL FOR FEEDING RNAi

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Day 1.

Streak out culture from desired library well on LB/Amp plate (40 µg amp/ml) (optional).

Day 2.

1. Culture bacteria containing each RNAi clone in 1 ml LB medium containing 40 µg/ml ampicillin overnight.

2. Chunk a starved nematode plate and allow starved nematodes to recover on fresh *E. coli* OP50-seeded NGM plates for two days (until Day 4 of experiment).

Day 3.

In the 24 well format, 20 µl of each culture was spotted in a single well of a 24-well plate containing NGM agar, 6 mM IPTG and 25 µg/ml carbenicillin. In the 6 cm format, spread out 150 µl of culture. I usually make duplicate plates, so I have a plate ready to score a second generation.

Day 4.

Release eggs from gravid hermaphrodites on the plates chunked on Day 2 using alkaline hypochlorite solution. Following washes in M9 buffer, transfer eggs to plates seeded with *E. coli* HT115(DE3) bacteria expressing double-stranded RNA (dsRNA) (Seeded Day 3) and incubated at 23˚C. (For detailed protocol, see Hope (1999) C. elegans, A Practical Approach. Oxford Univ. Press)

Day 5.

Take the day off.

Day 6.

1. Analyze animals with light microscopy.

Primary screen:

Score the animals for clear patches using a dissecting microscopy. This is approximately 48h after hatching; animals should be young adults.

Secondary screen:

Mount the animals on agar pads using 0.08M sodium azide as an anesthetic. View DTC migration using 40X Nomarski microscopy.

2. Transfer 5 young adults to new plates seeded with RNAi bacteria.

Day 7.

Take the day off.

Day 8.

Analyze young adult progeny animals as described for Day 6. If the worms aren't old enough (likely), analyze them on **Day 9.**