**RNA interference in C. elegans by *E. coli* feeding**

**PROTOCOL:** This protocol allows the user to knock down the expression of a given gene target in *C. elegans*. *E. coli* containing a specific expression vector can be induced to transcribe a genetic fragment from both the 5’ and 3’ ends. When the resulting double-stranded RNA molecule is ingested by *C. elegans*, an RNA interference reaction targets endogenous transcripts of the same sequence for destruction. In this protocol, the method of testing embryonic lethality (% hatching) is described but observations can also be made in the parent as well.

**STEPS:**

1. Obtain *E. coli* strain.
2. Day 1 & 2: Grow, induce, and seed *E. coli*
3. Day 3: Move worms onto *E. coli* seeded plates
4. Day 4: Capture laid eggs
5. Day 5: Measure embryonic viability

**KEY CONTROLS:**

* Positive control: *pop-1* RNAi is a standard positive control that should produce 100% embryonic lethality.
* Negative control: Empty vector (L4440) is a common negative control that should result in near 0 % embryonic lethality.
* Question specific controls: Other controls can be selected for the exact question of interest. For example, the researcher is interested in embryonic lethality due to intestine-specific failure, *elt-2* RNAi (intestine regulatory gene) is a typical control.

**PROTOCOL:**

1. Obtain *E. coli* strains

* Streak out strains from the *E. coli* feeding collection onto LB Carb (100 mg/ L) and/or Tet (12.5 mg/ L) plates. Grow at 37 C over night – 2 nights until colonies emerge.
* Store resulting single colonies in a personal -80C stock for future reference.
* Sequence the resulting vectors to ensure the appropriate transcript will be targeted.
* Stock plates of *E. coli* can be stored at 4 C for 4 – 6 weeks after which time, a fresh batch from the -80 C is recommended for best dsRNA activity.

1. Grow and Induce *E. coli*

DAY 1

* Inoculate 5 ml LB (100 mg/L Carb) liquid cultures with each *E. coli* strain including controls.
* Grow overnight at 37 C, shaking.
* If 6 cm NGM + Carb plates have not been prepared in advance, pour those plates now. Keep track of how many plates were poured with every 500 ml of culture to calculate roughly the volume of culture per plate.

DAY2

* No more than 18 hours after initial *E. coli* inoculation dilute overnight cultures. Add 40 ul of each overnight culture into 6 ml fresh LB (100 mg / L Carb).
* Grow shaking at 37 C for 3 hours.
* Induce dsRNA transcription by adding IPTG to 1 mM final concentration to each culture.
* Organiz e 6 cm RNAi plates by labeling them.
* Add IPTG to 1 mM final concentration per plate (use calculation above).
* 3 hours after *E. coli* induction, plate 500 ul of each culture on a 6 cm RNAi plate.
* Let plates dry overnight at room temp.

DAY3

\*Store plates up to 2 weeks at 4 C or use immediately in step 3.

1. Move worms onto *E. coli* seeded plates

DAY3 or LATER

* Move 1 – 2 worms onto an *E. coli* seeded plate. L4 worms can be used or very young adult worms.
* This step can be optimized in a variety of ways. N2 worms or RNAi sensitive worms can be used. The age of the worm can be optimized for each transcript. Just try to stay consistent with the age of each worm added within a given experiment.
* Allow worms to grow on plates for 24 hours at 20 C.

1. Capture laid eggs

DAY4

* 24 hours after first *E. coli* exposure, obtain freshly laid eggs by moving worms to a new *E. coli*-seeded plate (of the same dsRNA expression). This is called the “24 hr. Capture Plate”.
* Let the worms lay on the capture plate for 2 hrs – 24 hours depending on their egg-laying rate. Try to capture at least 50 eggs.
* Move worms to a new plate (of the same dsRNA type) and return to 20 C. Count the eggs on the capture plate.

1. Measure embryonic viability.

DAY5

* 24 hours after the adult worms were moved off of the capture plate, count the # embryos and # hatched worms.
* Compare the total numbers with the number embryos counted the day before.
* If there are discrepancies, look for dead worms on the lip of the plate and try to count those.
* If counting is challenging, count each plate twice.
* Tabulate numbers both 1) by hand in the lab notebook and 2) in excel spreadsheet form.
* Calculate the % viability and % lethality for each strain.

48 – hour time point

* In addition, a 48 hour time point is often useful. Start another capture plate for 48 hours after first *E. coli* exposure and repeat the embryonic viability calculation.

**MEDIA:**

**NGM Plates (500 ml)**

* Mix the following reagents:

**1.5 g NaCl**

**1.25 g Bacto Peptone**

**8.5 g Bacto Agar**

**Add water to 487.5 ml**

* Add a stir bar
* Autoclave media for at least 30 minutes to sterilize
* Cool media to 50 – 65 C.
* Using sterile technique, add:

**0.5 ml 5 mg/ml Cholesterol**

**0.5 ml 1 M CaCl2**

**0.5 ml 1M MgSO4**

**12.5 ml 1 M Potassium Phosphate Solution (pH 6)**

* To make NGM+CARB plates, add Carbenicillin to a final concentration of 100 mg / L:

**0.5 ml 100 mg/ml Carbenicillin in 500 ml total volume**

* To make 1 mM NGM+IPTG+CARB plates, add:

**0.5 ml 1M IPTG (alternatively, IPTG can be top-spread onto each plate prior to use)**

* Once all ingredients have been added, stir media thoroughly and then pour into 6 cm plates.
* Try to pour the same amount of media into each plate.
* At the end of pouring, count all the plates and note the average volume per plate.

**Carbenicillin Stock Solution (100 mg /ml)**

**1 g Carbenicillin**

**10 ml H2O**

* Mix thoroughly in a 15 ml conical vial.
* Filter sterile the solution through a 0.22 um filter.
* Freeze in 1 ml aliquots.
* Clearly label, date, and then store at -20C. Can be stored up to several decades.
* Use at a final concentration of 100 mg/L -- 1/1000 dil.

**Tetracycline Stock Solution (5 mg/ml)**

**50 mg Tetracycline**

**10 ml 100 % Ethanol**

* Mix thoroughly in a 15 ml conical.
* Freeze in 1 ml aliquots.
* Clearly label, date, and then store at -20C. Can be stored up to several decades.
* Use at a final concentration of 12.5 mg/L -- 1/400 dil.

**1 M IPTG**

**2.38 g Isopropyl-β-D-thiogalactopyranoside (IPTG)**

**10 ml H2O**

* Mix thoroughly in a 15 ml conical vial.
* Filter sterilize through a 0.22 um pore filter.
* Freeze 1 ml aliqiuots.
* Clearly label, date, and then store at 20 C. Can be stored up to several decades.
* Use in concentrations ranging fro 0.1 mM – 2 mM final concentrations.

UPDATES TO THE PROTOCOL:

Hi Erin,

The few changes to the original protocol were made from recommendations from the Ahringer lab’s currect protocol found here,

[http://www2.gurdon.cam.ac.uk/~ahringerlab/data/RNAi\_Feeding\_Protocol.doc](https://outlook.unc.edu/owa/redir.aspx?C=d_6cuUgWKEK-7JpNsR0fq0U_C8crptEIw3EDB-p0BlzYdRdsUZYZNf06Bg7bAsDvSZFLEEWVTyM.&URL=http%3a%2f%2fwww2.gurdon.cam.ac.uk%2f%7eahringerlab%2fdata%2fRNAi_Feeding_Protocol.doc" \t "_blank)

The main differences are some concentrations and choices of antibiotic. The major change is the top spreading of Carb/IPTG; also IPTG is not added to liquid culture.

1.       Grow E.coli in 100 mg/L Carb LB liquid overnight.

2.       Inoculate 6 mL fresh LB-Carb with 40 ul of overnight culture.

3.       Grow fresh LB Carb for 6 hours shaking at 37C

4.       Top spread NGM plates with 100 mg/L Carb and 1 M IPTG. I premix 1:1 solution of our Carb and IPTG stocks then pipette 1ul Carb-IPTG mix per 1 ml medium. Assuming 6 cm plates are 12.5 mL and 10 cm plates at 25 mL total. Spread with the L-shaped glass pipette, with ethanol/flaming in between.

5.       Briefly let the 25-50 uL of topspread Carb-IPTG mix soak in.

6.       Seed plates with 500-1000 ul 6 hour culture.

7.       Allow plates to dry and induce overnight.

8.       Store at 4 degrees.

Jay