RNA Interference (RNAi) by Bacterial Feeding

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[Abstract] There are 3 ways to perform RNAi in worms: microinjection, soaking and feeding. In the feeding protocol, RNAi is induced by cultivating worms on bacteria expressing gene-specific dsRNA. dsRNA is expressed in *E. coli* and ingested by worms. This protocol describes the feeding protocol to induce RNAi.

Materials and Reagents

1. *C. elegans* RNAi clone/libraries (Open Biosystems or Source BioScience LifeSciences)
2. Ampicillin (IBI Scientific)
3. Tetracycline
4. IPTG (Gold Biotechnology)
5. LB agar medium: Agar (BD Biosciences), tryptone (BD Biosciences), yeast extract (BD Biosciences), NaCl (Research Organics)
6. RNAi plate (NGM/IPTG/Ampicillin) (see Recipes)

Equipment

1. Aluminum foil
2. Petri dish (35 x 10 mm)

Procedure

A. Streak dsRNA-expressing *E coli* onto LB agar plate containing ampicillin (50 µg ml^{-1}) and tetracycline selection (12.5 µg ml^{-1}) and incubate at 37 °C overnight.
   Note: There are two RNAi feeding libraries. One was constructed by the Vidal and Heuvel lab and can be ordered from Open Biosystems; the other was constructed by Julie Ahringer's lab and is available at Source BioScience LifeSciences.
B. Inoculate bacteria in 3 ml LB liquid medium containing ampicillin (100 µg ml^{-1}) only and incubate at 37 °C overnight.
C. Spin down all 3 ml culture and pour off supernatant to 150 µl (concentrate culture by 20x). Resuspend pellet.

D. Transfer 50 µl of cell resuspend to center of RNAi plate (NGM/IPTG/Ampicillin). Let dry (wrapped in aluminum foil) and induce overnight at room temperature (RNAi-seeded plates can be stored at RT for 2-3 days before use).

E. Place 10-15 egg-laying worms on each plate. Incubate 2 - 6 h at 20 or 25 °C. Suck off parents and incubate at desired temperature until desired stage for further experiments.
Note: Instead of egg lay, synchronize worms by bleach and transfer starved L1 larva to RNAi plates.

Recipes

1. RNAi plate (NGM/IPTG/Ampicillin)
   Use the same recipe for making NGM agar medium but instead of adding streptomycin, add IPTG to final a concentration of 1 mM and ampicillin with a concentration of 100 µg ml^{-1}. Typically pour onto small petri dish (35 x 10 mm).

References

1. Timmons, L. and Fire, A. (1998). Specific interference by ingested dsRNA. *Nature* 395(6705): 854.
2. Rual, J. F., Ceron, J., Koreth, J., Hao, T., Nicot, A. S., Hirozane-Kishikawa, T., Vandenhaute, J., Orkin, S. H., Hill, D. E., van den Heuvel, S. and Vidal, M. (2004). Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. *Genome Res* 14(10B): 2162-2168.
3. Kamath, R. S. and Ahringer, J. (2003). *Genome-wide RNAi screening in Caenorhabditis elegans.* *Methods* 30(4): 313-321.

