

RNA Interference (RNAi)

Background

Gene phenotypes can be assessed in *C. elegans* via RNA interference (RNAi; Fire et al. *Nature*. 1998; Figure 1).

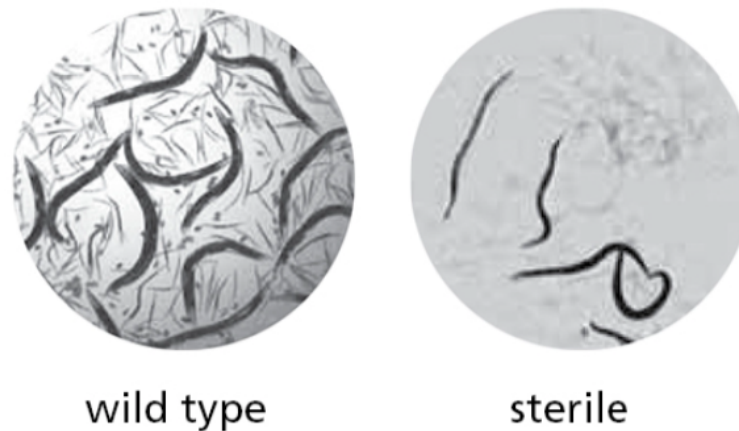


Figure 1. RNAi Phenotype - Worms on the right were fed bacteria that express RNA interference molecules against a gene required for embryonic development. The worms on the left were fed non-RNAi control bacteria. Note the lack of eggs and small worms in the RNAi-treated large worms. Image from Alberts et al., *Molecular Biology of the Cell*, 7th edition.

This conserved cellular process involves the targeting of specific mRNAs by a protein complex that incorporates a complementary small interfering RNA (siRNA). siRNA's are derived from double-stranded RNA (dsRNA), and are used by Argonaute and other proteins to silence gene expression via 3 distinct mechanisms: 1) cleavage of target mRNA, 2) translational inhibition of target mRNA, or 3) heterochromatin silencing of the target mRNA's gene sequence (Figure 2).

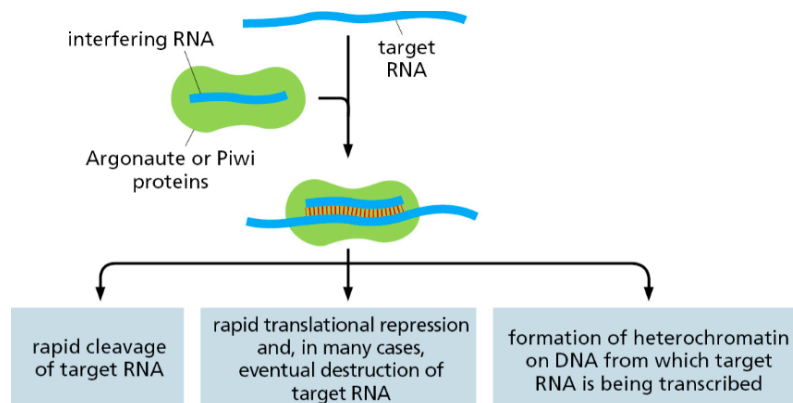


Figure 2. Mechanisms of RNA Interference - Argonaute proteins and the siRNA combine to target the cellular mRNA via one of three distinct mechanisms. Image from Alberts et al., *Molecular Biology of the Cell*, 7th edition.

RNAi can be performed simply in worms by feeding them bacteria that express a double-stranded RNA corresponding to the gene of interest. The small interfering RNA segment is engineered into a special plasmid (L4440) that has an origin of replication, antibiotic resistance gene, and a multicloning site flanked by two T7 bacteriophage promoters (Figure 3).

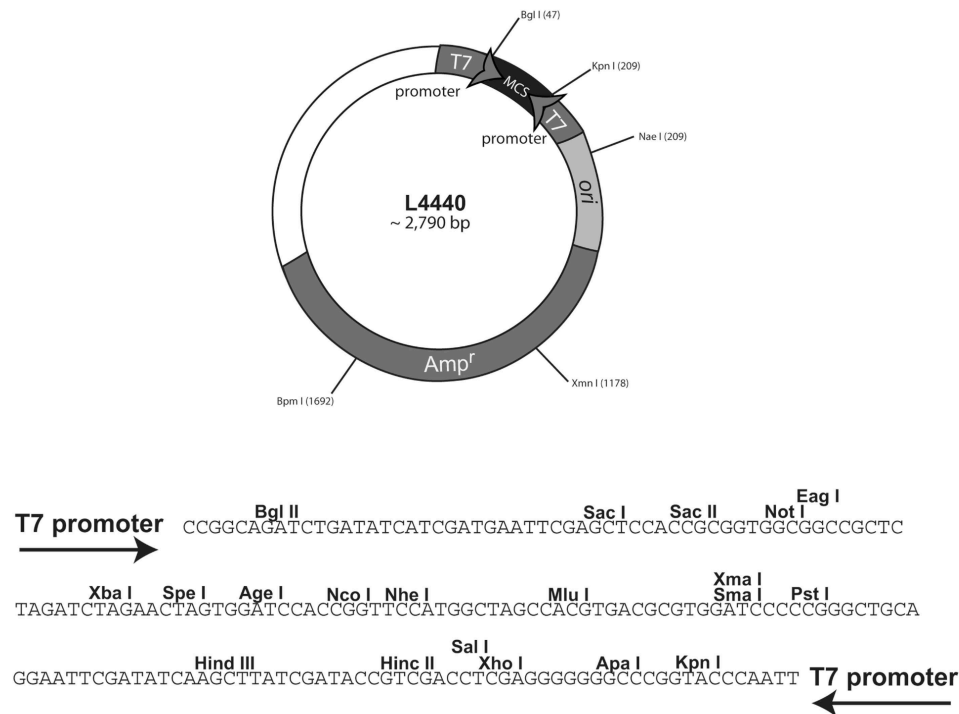


Figure 3. RNAi Feeding Plasmid - The plasmid contains an origin of replication, antibiotic (ampicillin) resistance gene, and a cloning site flanked by inducible T7 promoters. Plasmid map from Andrew Fire Lab.

After inserting the gene of interest into the MCS, the plasmid is transformed into a bacterial strain (HT115(DE3)) which contains an IPTG-inducible T7 bacteriophage polymerase. By growing the bacteria on plates with ampicillin selection and IPTG induction, the bacteria will produce double-stranded RNA from the T7 promoters. The worms then eat the bacteria, allowing the double-stranded RNA to enter the cells of the worm and induce the RNAi response (Fig 4).

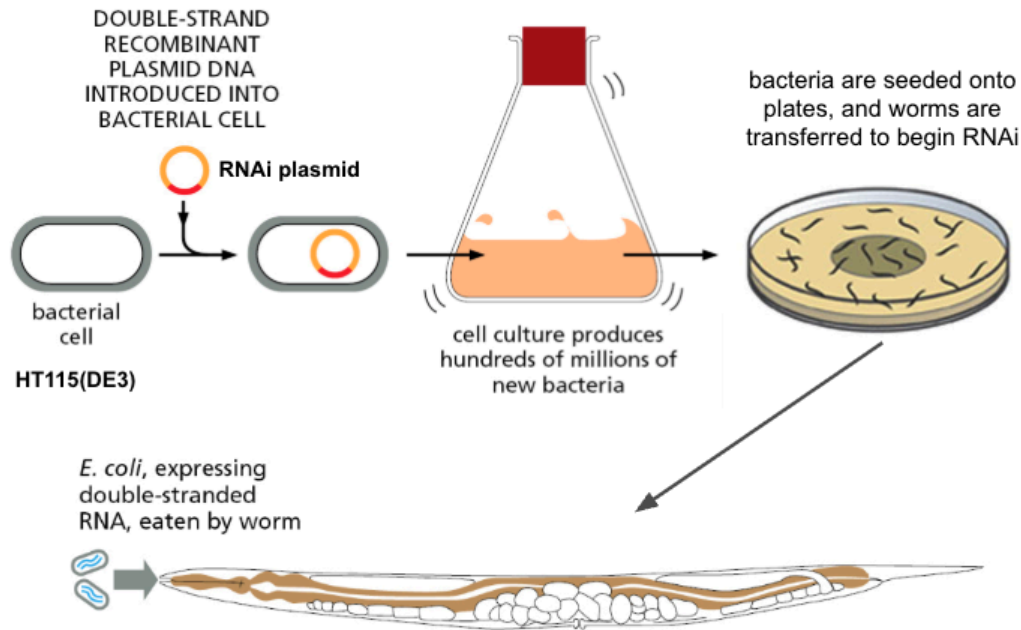


Figure 4. RNAi Procedure - The RNAi feeding plasmid is engineered with a gene of interest, cloned into the HT115(DE3) bacterial strain, and grown to high-density in a liquid culture. The bacteria are then plated on NGM with ampicillin and IPTG to induce double-stranded RNA expression. When worms eat the bacteria, the dsRNA will enter the cells and initiate the RNAi response. Adapted from Alberts et al. *Molecular Biology of the Cell*, 7th ed.

Various phenotypes can be induced by RNAi, including developmental (Fig 1) and behavioral effects. Some tissues are resistant to RNAi, but can be sensitized with various mutations (*eri-1*, *lin-15b*, *sid-1*, etc.). Additionally, genetic engineering approaches have made it possible to sensitize only a specific cell- or tissue-type to RNAi, allowing for an investigation of cellular requirements of gene function (Fig 5).

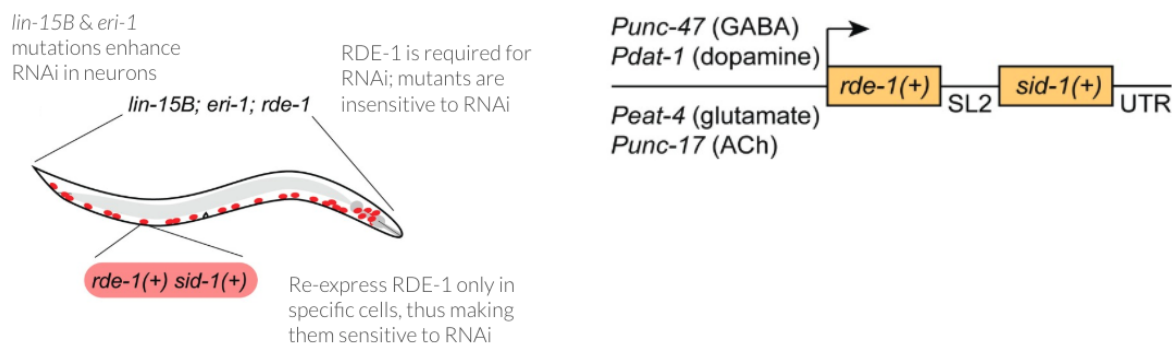


Figure 5. Tissue-Specific RNAi - Worms engineered to sensitize only specific tissues; the *rde-1* gene is required for RNAi. Introducing a wild type copy of *rde-1* expressed under a tissue-specific promoter in an *rde-1* mutant background will allow for RNAi in only the tissue of interest. Other mutations enhance RNAi. From Firnhaber and Hammarlund (2013).

Procedure (Adapted from Conte Jr. et al. *Curr Protoc Mol Biol*. 2017)

The RNAi procedure requires several distinct steps: 1) preparing the plates, 2) seeding the plates, and 3) plating the worms. The general steps for each are detailed below, but the details may need to be modified for different experiments.

I. Plate Preparation

- A. Prepare 500 mL of standard NGM media as described (here).
- B. After cooling the liquid NGM to 65° C, add 500 uL of 5 mg/mL **cholesterol** (in ethanol) for a final concentration of 5 ug/mL.
- C. Add 500 uL of **ampicillin** stock (100 mg/mL) for a final concentration of 100 ug/mL.
- D. Add 1 mL of **IPTG** stock (500 mM) for a final concentration of 1 mM.
- E. Swirl the flask while maintaining sterile conditions.
- F. Pour 8-12 mL of liquid media into each 6 cm plate, producing approximately 45-50 RNAi plates.
- G. Allow the plates to dry for 1-2 days depending on humidity levels.
- H. Store the plates at 4° C for up to 1 month.

II. Bacterial Culture

- A. Start an overnight culture of the specific bacteria containing the RNAi clone of interest.
 1. The culture should come from a verified single colony - either from the -80° C frozen stock, or a recent overnight culture.
 2. At least two control RNAi clones are generally prepared in parallel to your gene of interest: L4440 empty vector; pos-1, which is an indicator of embryonic phenotypes, or unc-22, which is an indicator of adult-specific phenotypes.
- B. Add 5 mL of LB+ampicillin to a round-bottom flask for each clone that you will culture.
- C. Add a single bacterial colony or 5 uL of liquid culture into the flask. Repeat for each additional RNAi clone.
- D. Grow the cultures overnight at 37° C.
- E. Ensure that the cultures appear cloudy, indicating bacterial growth.

- F. Seed 5 - 25 RNAi plates with each of the specific RNAi clones. Apply only a single drop of food and swirl the plate to allow the bacteria to dry quickly (within an hour).
- G. Let the plates sit at room temperature overnight.
- H. Use the plates immediately or store at 4° C for up to 3 weeks.

III. Worm Plating

- A. Worms can be plated on the RNA plates by either picking or washing.
 - a. Worms can be synchronized by using starved L1's (washing), or by picking L4's.
 - b. If picking, serial transfers using the RNAi bacteria can minimize OP50 contamination.
 - c. If washing from unstarved plates, make sure to do several rounds of M9 followed by centrifugation to minimize OP50 contamination.
- B. Some phenotypes can be observed in the first generation of worms put on the plates, especially if those worms were plated at the L1 stage and adult phenotypes are examined. However, embryonic phenotypes will be observed in the second generation of worms.
- C. Generally, phenotypes will be stronger the longer that worms are maintained on the RNAi plates. Progeny from the first or second generation can be picked to new RNAi plates to maintain worms that are constantly exposed to the RNAi.