

Reverse Genetics and RNAi

RNAi is a natural cellular process in many eukaryotes which scientists have taken advantage of in the lab as a valuable reverse genetics mechanism for regulating gene expression. RNAi involves double-stranded RNA (dsRNA) interfering with the expression of genes with sequences complementary to the dsRNA. By administering a specific dsRNA to a cell culture or multicellular organism, RNAi can be induced to selectively reduce expression of that specific gene in the cells or organism.

RNAi has been particularly well studied in *C. elegans*, in which the RNAi gene silencing phenotype is heritable (passed from parent to progeny in subsequent generations) and the dsRNA is easily administered. *E. coli* bacteria carrying RNAi plasmids that produce the desired dsRNA can be fed to worms, and the dsRNA is transferred to the worm via the intestinal tract. RNAi plasmids typically consist of DNA coding sequence from the intended target gene cloned between two T7/*lac* promoters. The plasmid also has a selectable marker that confers resistance to an antibiotic, in this case ampicillin. In 7.15, you will use the *E. coli* strain HT115 carrying various L4440 plasmids, each containing a different cloned gene sequence. HT115 is an RNase III-deficient *E. coli* strain with IPTG-inducible T7 polymerase activity. To induce dsRNA production from these plasmids, the HT115 bacteria is grown on special RNAi NGM feeding plates that contain IPTG and the ampicillin analog carbenicillin. Carbenicillin is preferred over ampicillin because it tends to be more stable.

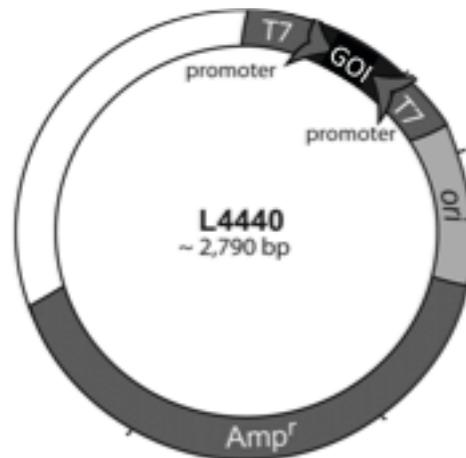


Figure 1. Map of L4440 vector commonly used for RNAi experiments in *C. elegans*. A fragment from the gene of interest (GOI) is amplified by PCR and cloned into the L4440 double-T7 vector, which has two T7 promoters in inverted orientation flanking the multiple cloning site. Cloned plasmids are transformed into HT115 (DE3), an RNase III-deficient *E. coli* strain with IPTG-inducible expression of T7 polymerase. Adapted from <https://www.addgene.org/1654/>.

Protocol

A. Preparing feeding plates

- 1) Inoculate 3mL of LB containing 50 µg/mL ampicillin with individual desired bacterial strain. Grow overnight at 37°C.
- 2) Seed NGM agar feeding plates (containing 25 µg/mL carbenicillin and 1mM IPTG) by pipetting 150 µL of LB bacterial culture into the center of the plate. You should have three RNAi feeding plates:
 - a. One plate will be seeded with HT115 bacteria carrying the “empty” L4440 vector (no *C. elegans* gene is cloned in the vector).
 - b. Another plate will be seeded with HT115 bacteria carrying a portion of the *unc-22* gene cloned into the L4440 vector. The phenotype resulting from RNAi of *unc-22* is well-established and is reliably reproducible under the experimental conditions you are using today.
 - c. A third plate will be seeded with HT115 bacteria carrying a portion of your gene of interest cloned into the L4440 vector. We will use *dpy10* as our gene of interest.
- 3) Let the plates dry overnight at room temperature.

B. Transfer N2 L4 worms

- 1) Transfer two L4 hermaphrodites from the N2 stock plate to each of the RNAi feeding plates, minimizing the amount of OP50 bacteria transferred. Try to avoid bringing any younger contaminating worms along with the L4 worms you are transferring.
- 2) Incubate the plates until the progeny reach the desired age (Note: it takes ~4 days for a *C. elegans* egg to grow into a gravid adult when grown at 16°C).

C. Scoring RNAi phenotypes

- 1) Observe RNAi controls. Start by looking at the L4440 RNAi plates - what phenotype(s) would you expect to see on these plates? Next, look at the *unc-22* RNAi plates – what phenotype(s) would you expect to see on these plates? Record all your observations in your notebook. If you do not see the expected phenotypes on your control plates, this may indicate your RNAi experiment was set-up incorrectly.
- 2) Observe the RNAi phenotypes for the experimental RNAi construct and record all your observations in your notebook. Examine how the observed phenotypes compare with the corresponding null mutant phenotypes in the same gene (the WormBase database has information on previously characterized RNAi and null mutant phenotypes).

Note: All the progeny laid by fed mothers are scored, including progeny laid early and late. This may result in a range of RNAi knockdowns, from weak to strong.

References

Ahringer, J., ed. Reverse genetics (April 6, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.47.1, <http://www.wormbook.org>.

Timmons, L., Fire, A.(1998). Specific interference by ingested dsRNA. *Nature* 395, 854.

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7.15 Experimental Molecular Genetics
Spring 2015

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