TECHNICAL MANUAL

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# *C. elegans* RNAi feeding clones & v1.1 feeding library

Cat. #RCE1181, RCE1182

## Product description

*C. elegans* ORFeome library v1.1, the *C. elegans* RNAi collection provides comprehensive coverage for screening with over 11,000 RNAi clones. These constructs are cloned into the pL4440-dest-RNAi Destination vector and are archived as glycerol stocks of the *E. coli* feeding strain HT115(DE3).

The parental *C. elegans* ORFeome Library was originally derived from *C. elegans* cDNA clones that were PCR-amplified to remove the 3' untranslated regions (UTRs) and add Gateway™ recombination sites (Figure 1).[1] These ORF clones were then transferred using high-throughput recombination cloning into the PL4440-Dest-RNAi feeding vector. Each clone of the resulting *C. elegans* RNAi collection targets a single gene, and with introns removed, these clones provide a larger amount of template for in vivo siRNA production than previously described genomic DNA fragments.

The C. elegans ORF-RNAi Feeding clones are provided as stock cultures of *E. coli* in LB broth with an inert growth indicator, 8% glycerol, ampicillin at a concentration of 100 µg/mL, and tetracycline at a concentration of 12.5 µg/mL.

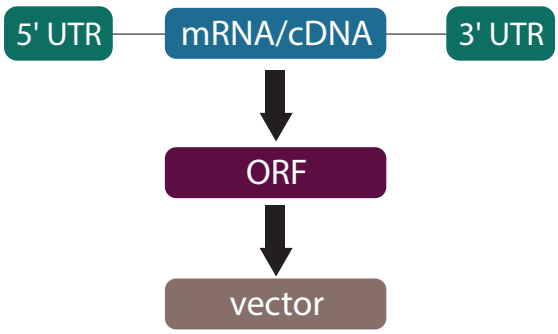


**Figure 1. *C. elegans* open reading frames were PCR amplified from mRNA/cDNA, during which the 5'- and 3'- UTRs were replaced with Gateway recombinational sites.**

## Selectable marker

The HT115 (DE3) host carries a tetracycline marker conferred by a transposon. The clones can be grown in ampicillin only since the tetracycline marker is very stable.

## Clone storage

Individual *C. elegans* ORF-RNAi feeding clones are shipped at ambient temperature. The clones may be stored at 4 °C for up to one week. Long-term storage should be at –80 °C.

## Primers

The following pairs of universal primers can be used for PCR amplification and sequencing: pL4440-dest-RNAi-FOR (5' GTTTTCCCAGTCACGACGTT 3'); pL4440-dest-RNAi-REV (5' TGGATAACCGTATTACCGCC 3')

## Verification

The *C. elegans* ORFeome clones that have been transferred into the RNAi feeding vector have been end-sequence verified. A sampling of 100 RNAi clones has been randomly picked and sequenced. Ninety-six percent of the RNAi clones contained the expected sequence. It is strongly suggested that you sequence your clones prior to an experiment if you use less than 100 RNAi clones. If you perform a genome scale analysis, it is suggested you sequence verify your "hits" following the RNAi screening.

## Making a stock culture

Once the clone has been streak isolated and the identity of the strain has been confirmed, we recommend making a stock of the pure culture.

1. Grow the pure culture in LB broth plus ampicillin (100 µg/mL) and tetracycline (12.5 µg/mL). Addition of tetracycline is optional.
2. Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution.
3. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at –80 °C.

## Replication of plates

1. Prepare target plates by dispensing ~ 160 µL of LB medium, 8% glycerol, ampicillin at a concentration of 100 µg/mL, and tetracycline at a concentration of 12.5 µg/mL into each well. Addition of tetracycline is optional.
2. Remove the lids of the first source plate and target plate, allowing the source plate to thaw before you begin replication.
3. Gently place the disposable 96-pin replicator (Fisher Scientific Cat #NC9584102) into the source plate and lightly move the replicator around inside the well to stir the culture. Make sure to scrape the bottom of the well.
4. Pull the replicator out of the source plate and gently place into target plate and mix gently in the same manner.
5. Dispose of replicator into a biohazard container.
6. Replace the lids of the source and target plates.
7. Repeat steps 1–6 until all plates have been replicated.
8. Return the source plates to the freezer.
9. Place the inoculated target plates inside a 10" × 12" zip-lock freezer bag (maximum of 10 plates per bag). Place the bagged plates in a 37 °C incubator for 24 hours.
10. Check the target plates for growth on the following day.
11. After plates are frozen, seal all of the source and target plates by placing an aluminum plate seal over the frozen plate and securing the seal with a rolling device.

If you do not have a disposable 96-pin replicator, you can use a multichannel pipettor to transfer 10 µL of culture from each well of the source plate to the target plate.

## Background

The *C. elegans* ORFeome v1.1 library contains 11,942 ORF clones, comprising 10,623 ORFs cloned "in frame" plus 1,319 ORFs cloned out of frame. ORFs were cloned out of frame because of mis-predictions of their Start or Stop codons. Only the in-frame ORFs can be used for protein expression, but both sets of clones can be used for RNAi. In all, over 11,800 RNAi clones were generated. These clones were archived as glycerol stocks of transformed *E. coli* strain HT115(DE3) for RNAi feeding protocols and as templates for in vitro dsRNA synthesis for soaking or injection protocols.

In the *C. elegans* ORFeome v1.1, each clone represents a mini-pool of PCR amplified inserts cloned into the pDONR223 vector, not a single unique insert. Each pool is expected to contain the source ORF, but it is formally possible that various by-products might have contaminated the pool during the various cloning steps. For example, although PCR conditions were optimized (high proof reading DNA polymerase and limited number of cycles) mutations will still occur at low frequency during the PCR amplification. Out of 70,000 insert nucleotides sequenced, the mis-incorporation rate was 1 mutation every 35,000 nucleotides. Following transfer to pL4440, a sampling of 100 RNAi clones have been randomly picked and sequenced resulting in 96% of the RNAi clones revealing the expected sequence.

## Reference


1. A.J. Walhout, G.F. Temple, GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol.* **328**, 575-592 (2000b).





**If you have any questions contact**

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