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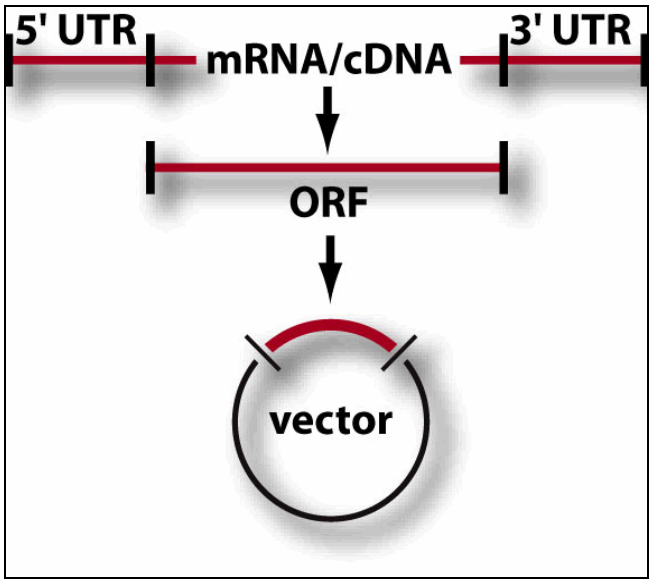
C. elegans RNAi Feeding Clones

Catalog #: RCE1182

Product description

The *C. elegans* RNAi Library includes clones derived from the *C. elegans* ORFeome Library v1.1 (http://www.openbiosystems.com/c_elegans_orf_clones_release_1_1.php). The Open Reading Frame (ORF) clones contain the coding sequences located exactly between the initiation and termination codons, excluding the 5' and 3' mRNA untranslated regions (UTRs)[1]. Each clone targets a single gene. Cloned ORFs provide an alternative to the genomic DNA fragments previously described and as ORFs are free of introns, they provide more template for *in vivo* siRNA production. High-throughput recombinational cloning protocols were then used to transfer the *C. elegans* ORFeome into the pL4440-Dest RNAi feeding vector.



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 (red cap) at a concentration of 100µg/ml, and tetracycline at a concentration of 12.5 µg/ml.

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.

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.

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 920µl of culture into a polypropylene tube and add 80µl 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^{o}C.

Background

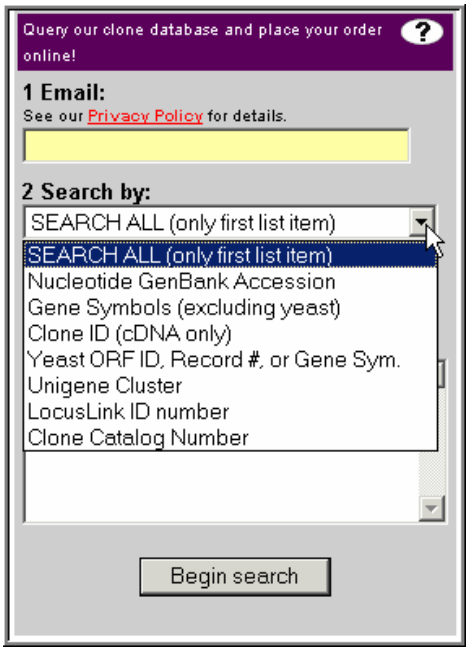
The *C. elegans* ORFeome v1.1 library contains 11,942 ORF clones, comprising 10,623 ORFs cloned "in frame" plus 1,319 ORFs cloned out of frame. ORFs were cloned out of frame because of mis-predictions of their Start or Stop codons. Only the in-frame ORFs can be used for protein expression but both sets 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.

Finding further information on clones

The Open Biosystems Clone Query provides a rapid means of locating relevant clone information. In step 2 of the query, choose the appropriate search criteria from the drop-down menu (Figure 2).



Example of search criteria:

Clone ID number: C25E10.11

Figure 2: Open Biosystems Clone Query

In the box for step 3, enter your search list. Click “Begin Search”. Example search criteria and detailed instructions are available, if necessary, by clicking the question mark icon in the

upper right corner of the query (Figure 3).

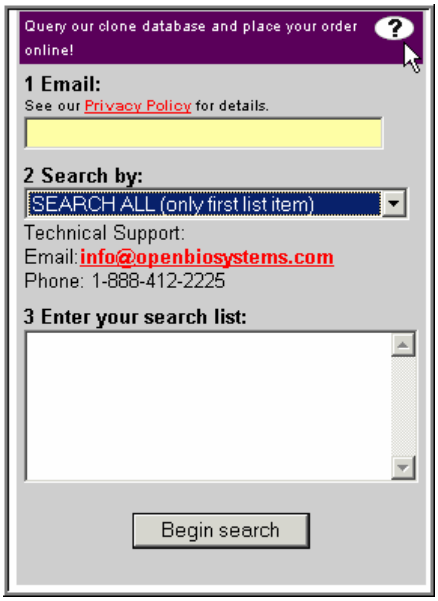


Figure 3: Query Assistance

Clicking the "View" link on the query result page will display the clone information page containing vector and host information (Figure 4).

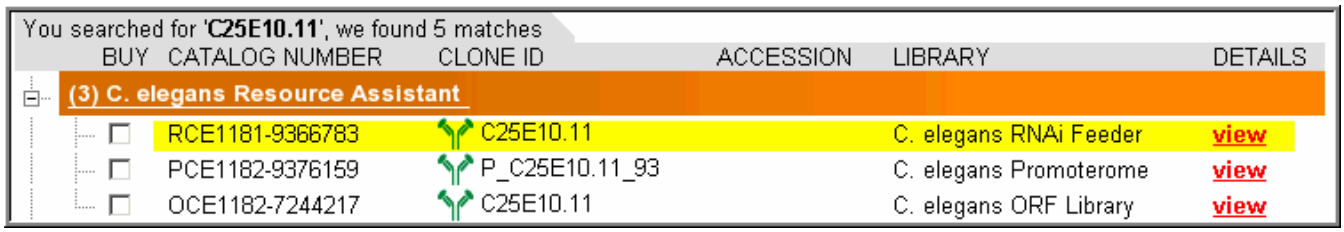


Figure 4: Open Biosystems Clone Query Results

Useful websites

- Source for *C. elegans* genome and RNAi data. http://www.wormbase.org
- Source for *C. elegans* ORFeome data. http://worfdb.dfci.harvard.edu
- Source for *C. elegans* RNAi data. http://nematoda.bio.nyu.edu

Reference

1 Walhout, A.J., G.F. Temple, M.A. Brasch, J.L. Hartley, M.A. Lorson, S. van den Heuvel, and M. Vidal. 2000b. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol* **328:** 575-592

Additional references

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. Towards Improving *Caenorhabditis elegans* Phenome Mapping With an ORFeome-Based RNAi Library. *Genome Research* 2004 Oct;14(10B): 2162-2168.

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

Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., et al. 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 421: 231-237.

Reboul, J., Vaglio, P., Rual, J.F., Lamesch, P., Martinez, M., Armstrong, C.M., Li, S., Jacotot, L., Bertin, N., Janky, R., et al. 2003. *C. elegans* ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression. *Nat. Genet.* 34: 35-41.