TECHNICAL MANUAL

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C. elegans ORF Clone Collection

Cat. #OCE1181, OCE1182

Introduction

Dr. Marc Vidal's laboratory at the Dana-Farber Cancer Institute has generated the *C. elegans* Open Reading Frame (ceORF) clones representing over 10,000 worm genes. The *C. elegans* ORF Collection is the leading resource for functionally characterizing the worm proteome through applications such as protein-protein interactions, RNAi and protein expression.

The *C. elegans* ORFs were generated by amplification from full length cDNA libraries and cloning into the Gateway™ recombinational cloning system (pDONR201). *C. elegans* ORF clones can be sub-cloned into any expression vector, using traditional restriction enzyme and ligase cloning methods. Alternatively, the recombinational cloning system allows the target gene to be rapidly transferred in-frame into a variety of expression vectors. These Release 1.1 clones represent a pool of cloned products, thus multiple splice variants are preserved in each pool. The *C. elegans* ORF clones in Release 1.1 have been end sequence tagged (OST) to confirm the identity of the gene and the presence of at least one splicing event. Subsequent releases will be occurring as further refinements of the *C. elegans* ORF clones occur. This on-going process includes in-depth characterization by interaction mapping, RNAi screening and full insert sequencing. The *C. elegans* ORF collection, containing 10,566 clones, is also available as a complete set.

Shipping and storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. They may stored indefinitely at –80 °C. Plates are shipped on dry ice and should be stored at –80 °C.

To allow any CO_2 that may have dissolved into the media from the dry ice in shipping to dissipate, please store plates at –80 °C for at least 48 hours before thawing.

Product description

Clones are provided as bacterial cultures of *E. coli* in LB broth with an inert growth indicator + 8% glycerol + kanamycin at a concentration of 100 µg/mL.

Glycerol stock replication

Culture clones in LB broth with 8% glycerol and kanamycin at a concentration of 100 µg/mL in a 37 °C incubator for 24 hours.

Replication of individual clones

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. Grow the pure culture in LB broth + the appropriate antibiotic. Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at –80 °C.

Replication of 96-well plates

Prepare target plates

1. Dispense ~160 µL of sterile LB media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

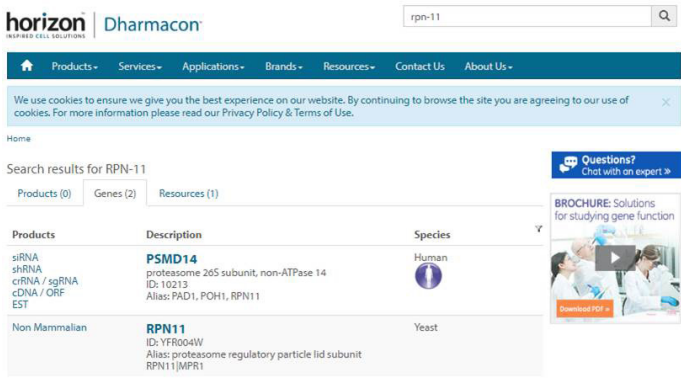
Prepare Source Plates

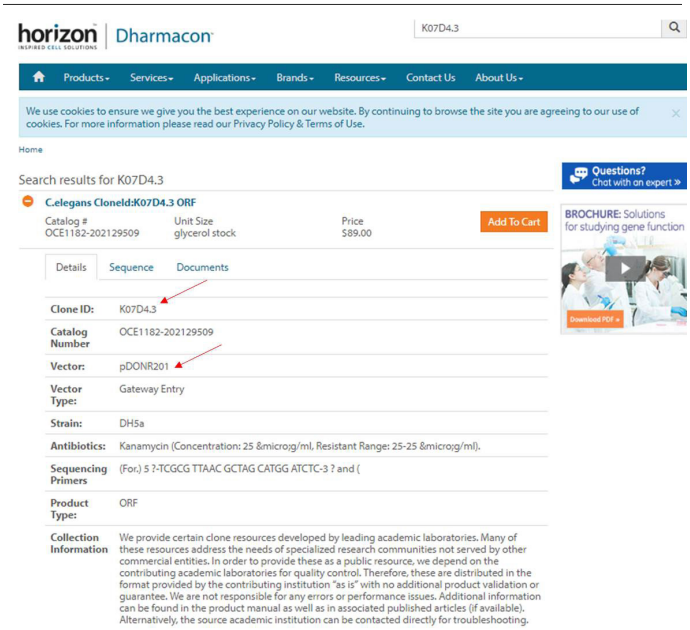
1. Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
2. Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a Kimwipe dampened with alcohol.

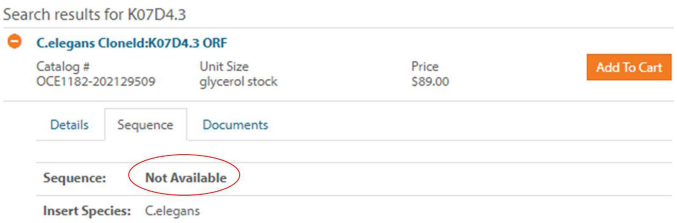
Replicate

1. Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
2. Gently remove the replicator from the source plate and gently place the replicator into the target plate. Gently move the replicator back and forth in the target plate to transfer cells.
3. Discard the replicator.
4. Place the lids back on the source plates and target plates.
5. Seal the source plates, being mindful to avoid cross contamination.
6. Repeat this process until all plates have been replicated.
7. Return the source plates to the –80 ºC freezer.
8. Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 12–24 hours.

Obtaining clone information







References

1. 1. Reboul J, Vaglio P, Rual J, Lamesch P, Martinez M, Armstrong C, Li S, Jacotot L, Bertin N, Janky R, Moore T, Hudson J, Hartley J, Brasch M, Vandenhaute J, Boulton S, Endress G, Jenna S, Chevet E, Papasotiropoulos V, Tolias P, Ptacek J, Snyder M, Huang R, Chance M, Lee H, Doucette-Stamm L, Hill D, Vidal M. 2003. *C. elegans* ORFeome version 1.1 experimental verification of the genome annotation and resource for proteome-scale protein expression. *Nat. Gen.* **34:** 35-41.
2. Reboul J, Vaglio P, Tzellas N, Thierry-Mieg N, Moore T, Jackson C, Shin-i T, Kohara Y, Thierry-Mieg D, Thierry-Mieg J, Lee H, Hitti J, Doucette-Stamm L, Hartley JL, Temple GF, Brasch MA, Vandenhaute J, Lamesch PE, Hill DE, Vidal M. 2001. Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in *C. elegans*. *Nat. Gen.* **27**:332-6

If you have any questions, contact

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