MGC premier human ORFeome Collaboration Library

TOH3507

Overview

The MGC premier human ORFeome Collaboration Library currently represents 9,804 genes with 16,581 clones. The majority of the ORFeome collaboration targets were generated by the Dana Farber Cancer Institute-Center for Cancer Systems Biology (DFCI-CCSB). These full-length, annotated and sequence verified ORFs originate from the existing collection of MGC premier full-length human cDNA clones and have been transferred into Gateway Entry vectors as a ready to use resource for recombinant protein expression. For additional convenience and versatility, the ORF clones are available in two formats, with and without stop codons. The ORF clones without stop codons facilitate the synthesis of either C- or N- terminal fusion proteins and clones with stop codons enable the synthesis of native proteins in addition to N-terminal fusion proteins.

Vector information

The ORF's are cloned into a few different Gateway Entry vectors and the maps can be found on our website:

http://www.transomic.com/Products/Gene-Expression/MGC-premier-ORF-Clones/Documents.aspx

Deliverable

The ORFeome Collaboration Library is delivered as 96-well glycerol stock plates (with lids) – 198 plates total. Plates are sealed with aluminum sealing tape and are shipped on dry ice for next day delivery. The plates should be stored at -80C upon arrival.

QC of library

When making replica copies of the ORF library plates, Transomic ensures successful growth of each clone by comparing the growth results of each replica plate copy to the same master stock plate. In order to pass QC, each replica plate should have the same growth pattern as the master stock plate.

Useful websites:

The ORFeome Collaboration:

http://www.orfeomecollaboration.org/

The Mammalian Gene Collection:

http://mgc.nci.nih.gov

SnapGene:

http://www.snapgene.com/

NCBI:

http://www.ncbi.nlm.nih.gov

Replication protocols

Materials for individual and plate replication

LB-Lennox Broth (low salt)	VWR EM1.00547.0500
Glycerol	VWR EM-4760
Spectinomycin	VWR 101454-194
Kanamycin	VWR 80502-840
96-well plates	VWR 62407-174
Aluminum seals	VWR 29445-082
Disposable replicators	Genetix X5054

Grow all clones at 37°C for 18 hours or until even growth is observed in all wells.
Autoclave broth to sterilize and allow cooling to ~60℃ before adding the antibiotic.

Individual ORF clones

E. coli carrying ORF plasmids are best propagated in LB broth or LB broth 8% glycerol for freezing.

1. Grow the ORF clone in LB broth with appropriate antibiotic:
    a. spectinomycin (50 µg/ml)
    b. kanamycin (25ug/ml)
2. 2-10 ml starter cultures for plasmid purification can be inoculated using 2 to 10 µl.

Alternatively

1. Pick a single starter colony from a freshly streaked LB agar plate containing the antibiotic and inoculate into the desired volume of LB broth for plasmid purification.
2. Grow for 18 hours at 37°C with vigorous shaking (~300 rpm).

Replication of plates

Prepare target plates by dispensing ~200 µl of LB-Lennox media supplemented with 8% glycerol and appropriate antibiotic. If a lower-volume 96-well plate is substituted, then fill each well ~50% with media. Glycerol can be omitted from the media if you are culturing for a plasmid DNA extraction.

Prepare source plates

1. Remove foil seals while the source plates are still frozen. This minimizes cross-contamination.
2. Wipe any condensation underneath the lid with a paper wipe dampened with ethanol.
3. Thaw the source plates with the lid on.

Replicate

1. Gently place a disposable replicator in 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 in the target plate and mix in the same manner to transfer cells.

3. Dispose of the replicator.
4. Place the lids back on the source plates and target plates.
5. Repeat steps 1-4 until all plates have been replicated.
6. Return the source plates to the -80°C freezer.
7. Place the inoculated target plates in a 37°C incubator for 18 hours or until even growth is observed in all wells.

Minimize thawed condition of plates where possible.
Always store plates at -80°C. It is recommended that an archival copy is made as soon as possible. Glycerol stocks kept at -80°C are stable indefinitely as long as freeze/thaw cycles are kept to a minimum.