MGC *premier* Lentiviral ORF Library

TOH7500

Overview

This human lentiviral ORF expression library was developed through the collaborative efforts of Dana-Farber Cancer Institute, The Broad Institute and the Center for Cancer Systems Biology (CCSB). The collection, which includes over 13,000 ORFs that represent approximately 11,373 genes, provides the most fully sequenced and annotated version of the human ORFeome available. For added convenience the lentiviral ORF expression vector was created to enable expression of a protein of interest with a V5 fusion tag for western blot detection, purification, co-immunoprecipitation, protein localization and FACS analysis.

Vector information

The ORF's are cloned into lentiviral vector pLX304 which has ampicillin resistance.

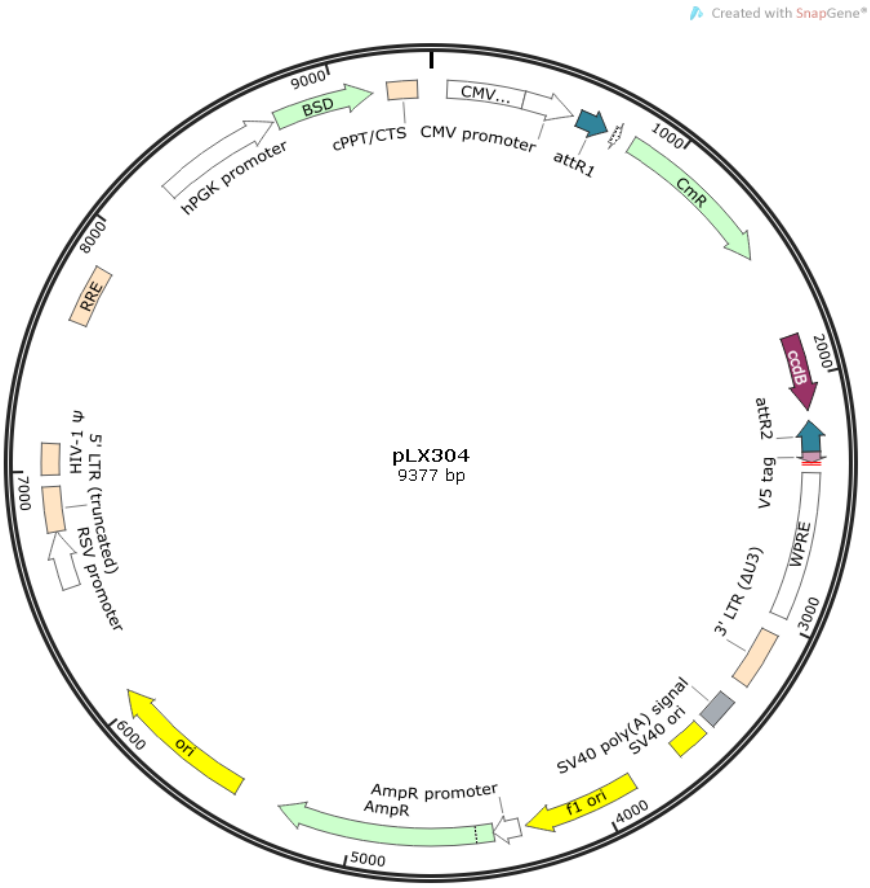

Figure 1 – pLX304 vector map

www.transomic.com 866-833-0712 support@transomic.com

Deliverable

The human lentiviral ORF expression library is delivered as 96-well glycerol stock plates (with lids) – 169 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

For information on the QC of the creation of the lentiviral ORF expression library, please visit:

https://www.broadinstitute.org/scientific-community/science/platforms/gpp/horfeome-v81-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.

Please note that the collection contains a small percentage of clones that have been found to contain a mutation within the open reading frame and contains clones that have not been fully sequenced. These clones may prove to be useful in many experiments and are clearly marked in the data file for the collection.

Useful websites:

The Broad hORFeome V8.1 Library website:
https://www.broadinstitute.org/scientific-community/science/platforms/gpp/horfeome-v81-library

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
Ampicillin	VWR 80055-786
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 ampicillin 100 ug/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 30°C incubator for 30 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.