

MGC premier human ORFeome v8.1 Library

TOH3506

Overview

MGC *premier* human ORFeome version 8.1 (hORFeome v8.1) is the newest version of the human ORFeome developed by the Center for Cancer Systems Biology (CCSB). This collection is derived from the Mammalian Gene Collection (MGC) and contains over 12,071 clonally-derived ORFs that represent 11,016 human genes. The ORF clones in this library contain the coding sequences located between the initiation and termination codons, excluding the 5' and 3' mRNA untranslated regions. Each ORF clone in the collection has been verified by next generation sequencing and is provided in a Gateway adapted entry vector for fast and convenient transfer to any compatible expression vector. The termination codon has been removed to allow for the utilization of the Gateway system to add a 3' tag to their ORF of interest if desired.

Vector information

The ORF's are cloned into the pDONR223 vector which has Spectinomycin resistance.

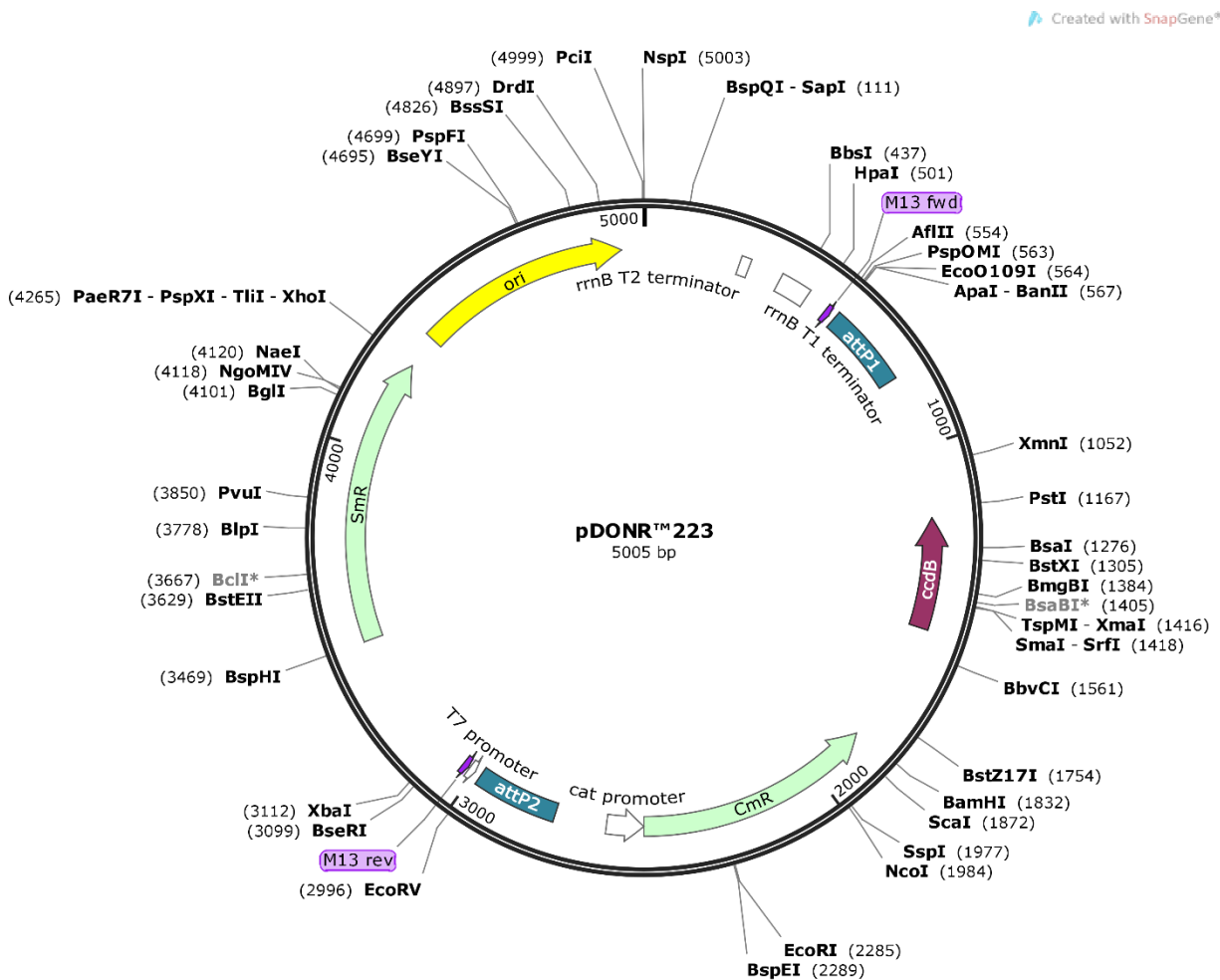


Figure 1 – pDONR223 vector map

Deliverable

The ORFeome 8.1 Library is delivered as 96-well glycerol stock plates (with lids) – 132 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 Human ORFeome Lab:

<http://horfdb.dfci.harvard.edu>

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
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 °C 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 the spectinomycin (50 µg/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 50 µg/ml spectinomycin. 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.