MGC premier human ORFeome v8.1 Library

Catalog number: TOH3506
Format: Glycerol stock, 96-well plate format

This manual provides information for the storage and propagation of the **MGC premier human ORFeome v8.1 Library**. Appendix 1 contains information regarding how to locate the specific vector map for your clone and Appendix 2 contains basic safety information for handling of bacterial cultures. Review local safety guidelines for complete regulations.

Section 1: Introduction

The 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 the ORF of interest, if desired.

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.

Section 2: Vector information

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

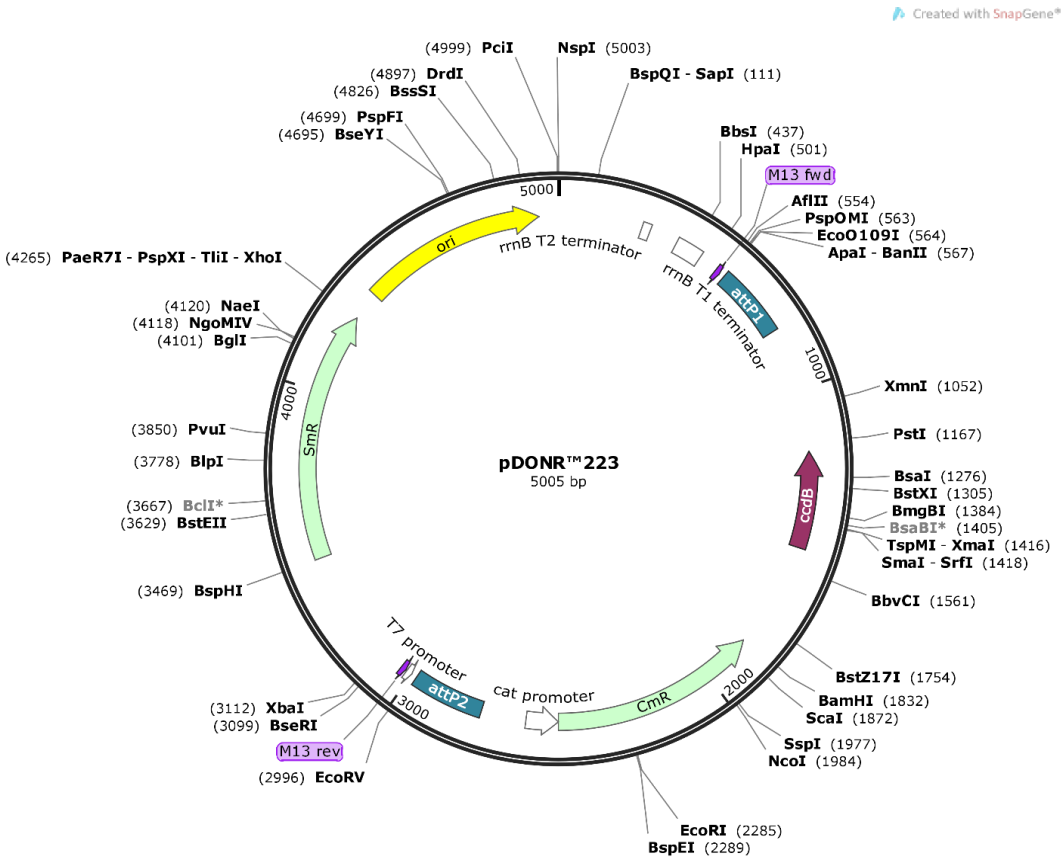

Figure 1. Schematic depicting options for the **pDONR223 vector**

Section 3: Replication protocols

Materials

LB-Lennox Broth (low salt)	VWR 90003-112
Glycerol	VWR EM-4760
Spectinomycin*	VWR 101454-194
96-well plates	VWR 62407-174
Aluminum seals	VWR 29445-082
Disposable replicators	Scinomix SCI-4010-OS

** Autoclave broth to sterilize and allow cooling to ~60°C before adding the antibiotic.*

Individual clone propagation

Cultures should be propagated in LB broth with **spectinomycin** (50 µg/ml) at 37°C for 18 hours or until the culture appears turbid. A 4 ml starter culture can be inoculated using 5 µl of the glycerol stock provided. Once turbid, place 920 µl of culture into a polypropylene tube and add 80 µl sterile glycerol (8% glycerol). Mix well and store at –80˚C. Glycerol stocks kept at -80°C are stable indefinitely if freeze/thaw cycles are minimized.
It is a good practice to streak isolate and quality control test the plasmids by restriction digest.

Replication of plates

Prepare target plates:

- Dispense ~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.

Appendices

Appendix 1 – Vector information

Full vector sequences and maps can be found at this link: https://www.transomic.com/cms/Product-Support/Vector-Maps-and-Sequences/ORF-Vector-Maps.aspx

Appendix 2 – Safety and handling

Local health and safety regulations should be determined for each institution.

For more information on Biosafety Level agents and practices, download Biosafety in Microbiological and Biomedical Laboratories (BMBL), Fifth Edition (Revised December 2009) published by the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and NIH. The publication can be found here: http://www.cdc.gov/biosafety/publications/bmbl5/.

Standard microbiological practices

- Limit access to work area
- Post biohazard warning signs
- Minimize production of aerosols
- Decontaminate potentially infectious wastes before disposal
- Use precautions with sharps (e.g., syringes, blades)
- Review biosafety manual defining any needed waste decontamination or medical surveillance policies

Safety equipment

- Biological Safety Cabinet, preferably a Class II BSC/laminar flow hood (with a HEPA microfilter) used for all manipulations of agents that cause splashes or aerosols of infectious materials; exhaust air is not recirculated
- Protective personal equipment includes: protective laboratory coats, gloves, face protection if needed

Facilities

- Autoclave available for waste decontamination
- Chemical disinfectants available for spills

Appendix 3 – References and recommended reading

- 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

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