

Dharmacon™ Human ORFeome v8.1 Clones and Collection

Cat. #OHS6086, OHS6084, OHS6090

The Human ORFeome v8.1 represents a sequence-verified version of the Human ORFeome from the Center for Cancer Systems Biology at Dana-Farber Cancer Institute (CCSB-DFCI). Originally derived from Mammalian Gene Collection (MGC) cDNA clones, these fully sequenced open reading frames (ORFs) have been Gateway adapted to enable efficient transfer of the ORF construct into compatible expression vectors.

Product Description:

The Human ORFeome v8.1 Library from Dharmacon contains cloned ORFs derived from fully sequenced MGC, full-length cDNA clones and inserted into recombinational entry vectors (Figure 1). The ORF clones contain the coding sequences located between the initiation and termination codons, excluding the 5' and 3' mRNA untranslated regions (UTRs; 1). The termination codon has been engineered out of these clones to allow the researcher to utilize the Gateway system to add a 3' tag to their ORF of interest if desired (Figure 2; 2). In the human ORFeome v8.1, each clone represents a single unique insert, verified by next generation sequencing to match the peptide-sequence at 100% consensus.

Human ORF clones are shipped as bacterial cultures of DH5 α in LB broth with 8% glycerol and spectinomycin (50 μ g/mL).

Background:

Previous versions of the Human ORFeome have been assembled by first taking a collection of MGC cDNAs and using directed PCR to create Gateway-entry clones while removing the stop codons from the coding sequences (2, 3). These earlier versions of the Human ORFeome were derived from cloned pools of PCR products, and are neither clonal nor sequence verified. In order to improve upon the quality of the previous Human ORFeome releases, clonal isolates were collected from the historical Human ORFeome, and subjected to next generation sequencing. Sequences were analyzed and clones that passed the QC criteria were arrayed, creating the Human ORFeome v8.1 library (hORFeome v8.1).

The sequence-verified hORFeome v8.1 collection contains ~11,300 arrayed clones, representing at least 11,000 unique gene IDs. These ORFs, cloned into the pDONR223 vector, are a high-quality source for ORF content, and can be efficiently transferred by recombination into compatible expression vectors (4).

Shipping and Storage:

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. They may be stored indefinitely at -80 °C.

96-well plates are shipped on dry ice and should be stored at -80 °C.

To allow any CO₂ to dissipate that may have dissolved into the medium from the dry ice in shipping, please store plates at -80 °C for at least 48 hours before thawing.

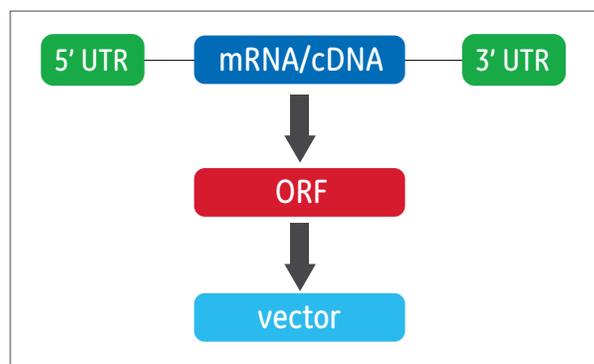


Figure 1. Cloning Schematic.

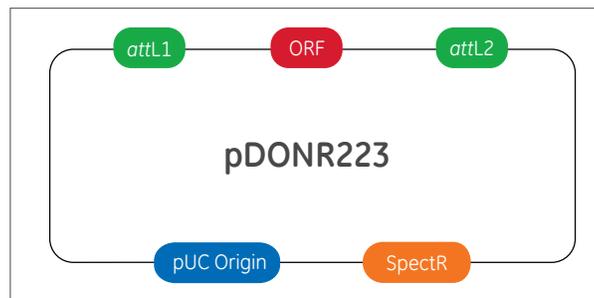


Figure 2. pDONR223 Vector Schematic.



Replication from Individual Glycerol Stock:

Culture clones in LB broth with 8% glycerol (Fisher Scientific Cat #BP2291) and spectinomycin (50 µg/mL) at 30 °C for 18-24 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 (spectinomycin, 50 µg/mL). 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

- Dispense ~ 160 µL of sterile LB medium into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic (50 µg/mL spectinomycin).

Prepare Source Plates

- Remove the foil seals (Fisher Scientific Cat #12-565-475) from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a paper wipe dampened with ethanol.

Replicate

- Gently place a disposable replicator (Fisher Scientific Cat #NC9584102) 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 well.
- Carefully 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.
- Discard the replicator.
- Place the lids back on the source plates and target plates.
- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the -80 °C freezer.
- Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 24 hours.

FAQS/Troubleshooting:

Clone Verification

To ensure optimal experimental results, it is recommended that you verify clone identity by sequencing, prior to starting your experiment.

What is the sequencing primer for the pDONR223 vector?

5'-TTTTCCAGTCACGACGTTGTAAACGACGGCCAGT-3'

Also, there is a 20 bp match between the T7 promoter and the "reverse primers" region of pDONR223. The so-called "T7 short" primer (<http://cancer-seqbase.uchicago.edu/primers.html>) sequence (5'-TAATACGACTCACTATAGGG-3') matches perfectly.

What strain of *E. coli* are the Human ORFeome v8.1 clones?

DH5α.

What type of recombination sites are present in the Human ORFeome v8.1 clones?

Gateway attL1 and attL2 sites flank the ORF insert (see figure below).



Do the hORFeome v8.1 clones have a stop codon?

No. These clones are intended to be cloned into expression vectors, and native stop codons have been removed to allow for the inclusion of a C-terminal fusion tag.

How can I find the sequence of the ORF insert for a Human ORFeome v8.1 clone?

For each Human ORFeome v8.1 clone, our website lists the CCSB-Broad Clone ID number and the BC accession number for the MGC clone from which the CCSB-Broad Lentiviral Expression clone was created. To find the insert sequence of a Human ORFeome v8.1 clone, you can search the Public TRC Portal using the ccsbBroad clone ID (<http://www.broadinstitute.org/scientific-community/science/platforms/gpp/horfeome-v81-library>). Click on the Clone ID link for ORF sequence details.

How do I create a map of the pDONR223 vector with my ORF of interest?

To generate an approximate map of the pDONR223 vector containing your ORF of interest, replace the sequence between the two Gateway™ sites with the sequence of your ORF, starting at the start codon (ATG) of the ORF and ending with the last codon before the native stop codon.

Where can I obtain Spectinomycin?

Spectinomycin can be purchased from Calbiochem (part# 567570).

What type of quality control for growth is conducted with these clones?

All cultures are checked for growth prior to shipment using a growth indicator.

How are libraries provided (96-well plate catalog number, seal, etc.)?

Library collections are shipped as bacterial cultures of *E. coli* DH5a glycerol stocks arrayed into 96-well plates (96-well microplates Fisher Scientific Cat #12-565-363) sealed with aluminum seals (Fisher Scientific Cat # 07-200-684).

For answers to questions that are not addressed here, please email technical support at ts.dharmacon@ge.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

References:

1. A.J. Walhout, G.F. Temple, GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol.* **328**, 575-592 (2000).
2. J. Rual, T. Hirozane-Kishikawa, Human ORFeome Version 1.1: A Platform for Reverse Proteomics. *Genome Res.* **14**, 2128-2135 (2004).
3. X. Yang, J.S. Boehm, Yang X, Vidal M, Hahn WC, Hill DE, Root DE. A public genome-scale lentiviral expression library of human ORFs. *Nature Methods.* **8**, 659-61 (2011).

Useful Websites

The Human ORFeome Lab
<http://horfdb.dfci.harvard.edu>

The Mammalian Gene Collection
<http://mgc.nci.nih.gov>

Restriction Mapper
<http://www.restrictionmapper.org>

NCBI
<http://www.ncbi.nlm.nih.gov>

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1.800.235.9880; 303.604.9499 if you have any questions.