

Thermo Scientific Open Biosystems Human ORFeome Clones and Collection

Catalog #: OHS1770, OHS1771

Product Description

The Human ORFeome Library from Open Biosystems contains cloned open reading frames (ORFs) derived from fully sequenced Mammalian Gene Collection (MGC) full-length cDNA clones inserted into recombinational entry vectors. The ORF clones contain the coding sequences located exactly between the initiation and termination codons, excluding the 5' and 3' mRNA untranslated regions (UTRs). 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. In the human ORFeome v1.1, each clone represents a mini-pool of PCR amplified inserts cloned into the pDONR223 vector, not a single unique insert.

Each pool is expected to contain the source ORF, but it is formally possible that various by-products might have contaminated the pool during the various cloning steps. For example, although PCR conditions were optimized (high proof reading DNA polymerase and limited number of cycles) mutations will still occur at low frequency during the PCR amplification. Out of ~70,000 insert nucleotides sequenced, the misincorporation rate was 1 mutation every 35,000 nucleotides.

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

Background

Version 1.1 of the human ORF collection utilizes the Mammalian Gene Collection (http://www.openbiosystems.com/full_length_human_cdnas.php) as a starting point. Future additions will be added as the MGC collection is expanded. Bioinformatic analysis revealed that the MGC collection corresponds to over 10,000 distinct full-length ORFs. Eliminated were i) clones for which the corresponding ORFs were reported as "partial coding sequence" at NCBI (no 5' ATG detected); ii) multiple copies of identical cDNA clones; and iii) clones that only differ in their 5' or 3' UTRs or both but not in the coding sequence. Also discarded were clones for which the ORF length is smaller than 100 nucleotides, following a convention similar to that initiated during the annotation of the yeast genome. The remaining MGC clones were arrayed by increasing length and this resource constituted the starting point for cloning. ORF cloning was counted as successful if the ORF Sequence Tag (OST) obtained matched the sequence of the expected ORF insert. The ORFs that were successfully cloned and sequenced were then arrayed by increasing length, thereby generating version 1.1 of the human ORFeome (hORFeome v1.1). In total, human ORFeome v1.1 constitutes a resource large enough to constitute a platform for reverse proteomics (protein expression, localization, interactions).

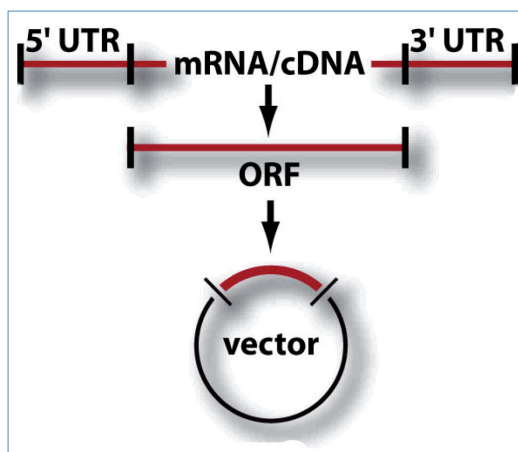


Figure 1. Cloning Schematic

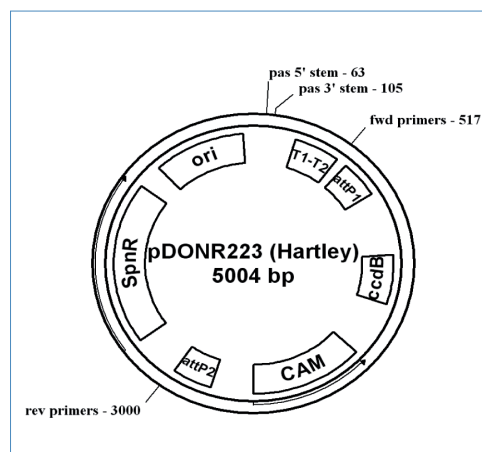


Figure 2. pDONR223 Vector Map

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.

Plates are shipped on dry ice and should be stored at -80°C.

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

Glycerol Stock Replication

Culture clones in LB broth with 8% glycerol and spectinomycin (50 µg/ml) at 37° 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. 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 media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates

- Remove the foil seals 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 Kimwipe dampened with alcohol.

Replicate

- Gently place a disposable replicator 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 plate of the well.
- Gently 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

What is the sequencing primer for the pDONR223 vector?

5' - TTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGT - 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 (TAATACGACTCACTATAGGG) matches perfectly.

What strain of *E.coli* are the Human ORF clones in (Vidal collection)?

DH5alpha.

How can I find the sequence of a Human ORFeome clone from the Vidal collection?

In order to find the sequence of a Human ORFeome clone, search our gene query using the clone ID or catalog number of your clone. The accession associated with the clone represents the 5' end sequence performed by the Vidal lab to confirm that the ORF from the MGC clone template was present. If you click on this accession, the resulting description will read: "Full Length cDNA from the Mammalian Gene Collection Homo sapiens cDNA 5- similar to _____" and will then list a BC accession. If you take this accession, enter it into NCBI and search for the corresponding nucleotide result, you will find the coding sequence information for the MGC clone from which the Human ORFeome clone was created. The Human ORFeome clone contains the coding sequence from the MGC clone, with the stop codon removed. To view this coding sequence for the MGC clone, click on the "CDS" link in blue on the left side of the BC accession result. This is the CDS or ORF of the MGC clone, and the

Human ORFeome clone contains this sequence, minus the stop codon.

Do the Human ORFeome clone have a stop codon?

No.

For answers to questions that are not addressed here, please email technical support at openbiosystems@thermofisher.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.

Alternatively, you can search our online FAQ database: <https://www.openbiosystems.com/Help/FAQ/>

References

Jean-Francois Rual, Tomoko Hirozane-Kishikawa, Tong Hao, Nicolas Bertin, Siming Li, Amélie Dricot, Ning Li, Jennifer Rosenberg, Philippe Lamesch, Pierre-Olivier Vidalain, Tracey R. Clingingsmith, James L. Hartley, Dominic Esposito, David Cheo, Troy Moore, Blake Simmons, Reynaldo Sequerra, Stephanie Bosak, Lynn Doucette-Stamm, Christian Le Peuch, Jean Vandenhaute, Michael E. Cusick, Joanna S. Albalá, David E. Hill, and Marc Vidal. Human ORFeome Version 1.1: A Platform for Reverse Proteomics. *Genome Research* 2004 14:2128–2135

Walhout, A.J., G.F. Temple, M.A. Brasch, J.L. Hartley, M.A. Lorson, S. van den Heuvel, and M. Vidal. 2000b. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol* 328: 575-592

Goffeau *et al.* *Science*. 1996 Oct 25;274(5287):546, 563-7.

Hartley, J.L., G.F. Temple, and M.A. Brasch. 2000. DNA cloning using in vitro sitespecific recombination. *Genome Res* 10: 1788-1795.

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/>

Contact Information

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