TECHNICAL MANUAL

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Dharmacon™ CCSB Human ORFeome clones and collection

Cat #OHS1770, OHS4103, OHS4108, OHS4112, OHS4297, OHS4838, OHS4910, OHS4913, OHS4922, OHS4925, OHS4929, OHS4939, OHS4941, OHS5016, OHS5029, OHS5030,OHS5031, OHS5177

Product description

The Center for Cancer Systems Biology of the Dana-Farber Institute has created a collection of human open reading frames (ORFs) cloned into a Gateway™-entry vector, known as the CCSB Human ORFeome Collection. The Gateway-adapted ORFs are ideal for easily moving gene content into compatible destination vectors for various proteomics studies. The native stop codons have been removed from the ORFs for maximum flexibility.

Human ORF clones are shipped as a bacterial cultures of DH5α in LB broth with 8% glycerol and spectinomycin (50 µg/mL).

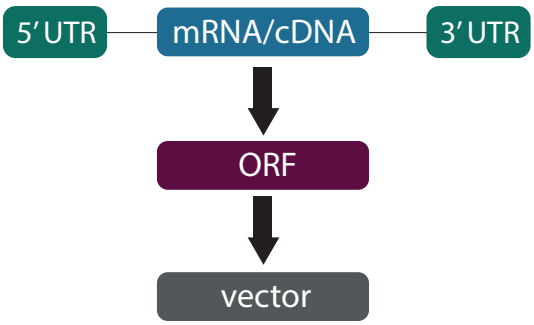


Figure 1. Cloning Schematic.

Background

This collection, also known as hORFeome v5.1, contains over 15,000 individual clones representing more than 12,000 unique genes. These clones are derived from fully sequenced Mammalian Gene Collection (MGC) full-length cDNAs and have been subsequently cloned into a Gateway recombinational entry vector. Each coding sequence (CDS) was amplified for only 25 cycles with gene-specific primers and high-fidelity polymerase, minimizing the risk of PCR-induced mutations. The human ORFeome version 5.1 (hORFeome v5.1) includes previous human ORFeome releases (v1.1 and 3.1), as well as approximately 3,000 additional constructs.

Mammalian Gene Collection

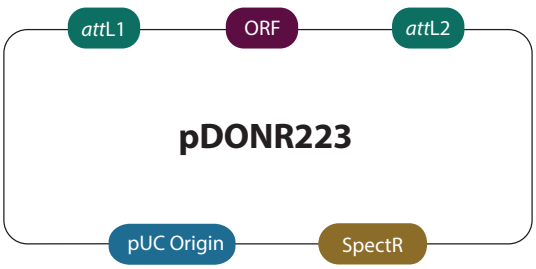
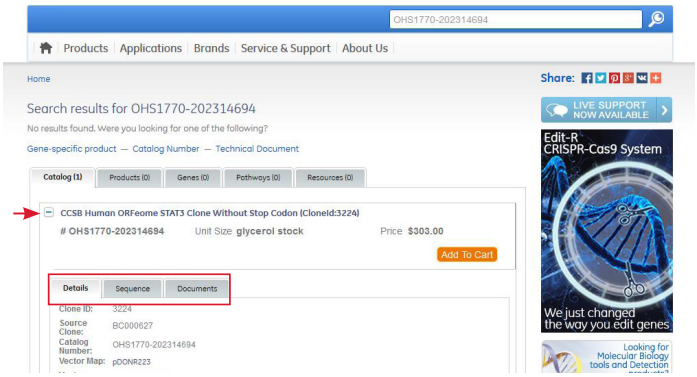


Figure 2. pDONR223 Vector Schematic.



Obtaining clone information

The Dharmacon Search provides a rapid means of obtaining clone information. Enter the Clone ID or catalog number of your clone in the query box. Expand the clone information by clicking on the "+" symbol on the left of the clone description. The vector information is in the "Details" tab and the sequence accession number is proviced in the "Sequence" tab. The accession link will take you to NCBI for the sequence of the MGC clone from which your Human ORFeome clone was created. The Human ORFeome clone contains only the coding sequence (CDS) of the MGC clone without the stop codon.

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 CO_2 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 °C for 18–24 hours.

*Glycerol can be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at –80 °C, 8% glycerol is required.

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 + 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.

**Testing of 3-5 colonies is recommended.

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.

*Glycerol can be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at -80 °C, 8% glycerol is required.

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 paper wipe 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 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 12–24 hours.

FAQS/troubleshooting

What is the sequencing primer for the pDONR223 vector?
M13R. Also, there is a 20 bp match between the T7 promoter and the "reverse primers" region of pDONR223. The so-called "T7 short" primer sequence (TAATACGACTCACTATAGGG) matches perfectly.

What strain of *E.coli* are the CCSB Human ORFeome Collection clones in?
DH5alpha.

How can I find the sequence of a CCSB Human ORFeome Collection clone?
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 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 CCSB Human ORFeome clones have a stop codon?
No.

For answers to questions that are not addressed here, please email technical support at ts.dharmacon@horizondiscovery.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. Rual *et al.* Human ORFeome Version 1.1: A platform for reverse proteomics. *Genome Research* **2004 14**:2128–2135
2. Walhout *et al.* GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol 2000* **328**: 575-592
3. Goffeau *et al.* Life with 6000 genes. Science 1996 274(5287): 546, 563-537.
4. Hartley, J.L., G.F. Temple, and M.A. Brasch.DNA cloning using *in vitro* sitespecific recombination. *Genome Res 2000* **10**: 1788-1795.

Useful websites

CCSB Human ORFeome 5.1

The Mammalian Gene Collection

Restriction Mapper

NCBI

Disclaimer

Horizon Discovery (formerly Dharmacon) is a distributor of multiple gene expression clone collections (cDNAs and ORFs). These clone collections were generated by groups outside of Dharmacon and thus the quality of the collections is largely dependent upon what was received from these groups. Specific clone information and plate coordinates were provided by the suppliers of these clone collections. We have not sequence verified each individual clone from these collections. These collections and individual clones are distributed "as is" with no additional product validation or guarantees. Our site has established quality procedures to ensure that individual clones are picked from the identified well in a plate, grown on the correct antibiotic, and are free of phage contamination. Due to the quality of the information provided by the originator of the collection(s), the clone you receive might not match the expected clone. If this occurs, please contact Technical Support (ts.dharmacon@horizondiscovery.com)

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t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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