TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

# CCSB-Broad Lentiviral Expression Library

Cat. #OHS6085, OHS6269, OHS6270, OHS6271

## Product description

The Center for Cancer Systems Biology (CCSB)-Broad Lentiviral Expression Library is a genome-scale expression collection developed by researchers at Dana-Farber Cancer Institute and The Broad Institute to provide a sequence confirmed collection of human open reading frames (ORFs) in an expression-ready lentiviral system. This library was created from the hORFeome v8.1 to enable targeted experiments and large-scale screening in diverse cell types.

More information for the creation of this library can be found in the following publication: A public genome-scale lentiviral expression library of human ORFs. *Nat Methods*. 2011 June 26; **8**:659-61

CCSB-Broad Lentiviral Expression clones are shipped as bacterial cultures of DH5α in LB broth with 8% glycerol and ampicillin (50 µg/mL).

## Background

The Human ORFeome v8.1 Library was created by first taking a collection of Mammalian Genome Collection (MGC) cDNAs and using directed PCR to create Gateway™-entry clones while removing the stop codons from the coding sequences. These pools of PCR products were cloned into recombination entry vectors, clonal isolates were collected, and clones were sent for next generation sequencing. Clone sequences were analyzed, and clones that passed the QC criteria were arrayed, creating the Human ORFeome v8.1 library (hORFeome v8.1). Lastly, this hORFeome v8.1 was transferred to the pLX304 vector, thereby creating the CCSB-Broad Lentiviral Expression Library.

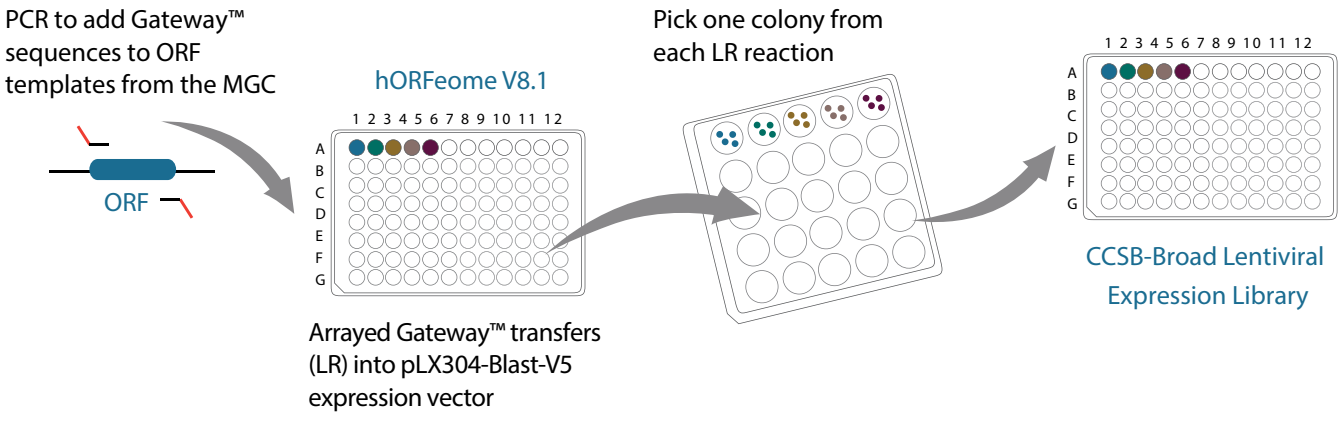


**Figure 1. Schematic of the creation of the CCSB-Broad Lentiviral Expression Library.**

- The available collection of MGC cDNAs were transferred to Gateway™ ORF templates in polyclonal by PCR.
- Clonal plasmid isolates were derived from single bacterial colonies, sequenced, and filtered to determine clones to be included in hORFeome v8.1 collection.
- Using Gateway™ recombination, the entry clone collection was transferred into the PLX304-Blast-v5 destination vector.
- A single colony was isolated from each recombination reaction, resulting in the CCSB-Broad Lentiviral Expression Library.

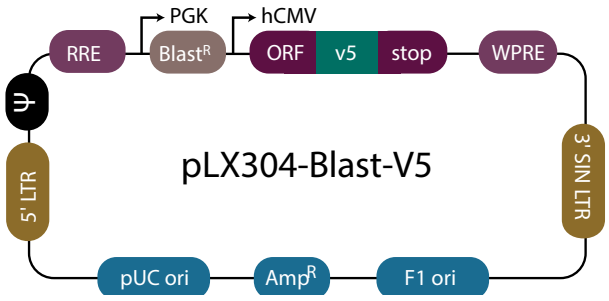


**Figure 2. pLX304 Vector Schematic.**

**Table 1.**

| ORF cassette elements | Function |
|---|---|
| PGK Promoter | Human phosphoglycerate kinase (PGK) promoter |
| Bsd$^R$ | Blasticidin resistance gene |
| hCMV Promoter | RNA Polymerase II |
| ORF | Open Reading Frame Insert |
| V5 | V5 Epitope Tag |
| **Lentiviral elements** | **Function** |
| 3' SIN-LTR | Increases safety - 3' Self inactivating long terminal repeat |
| 5' LTR | 5' long terminal |
| WPRE | Enhances the stability and translation of transcripts |
| cPPT (Central Polypurine Tract) | Increases translocation into the nucleus of non-dividing cells |
| RRE (Rev Response Element) | Increases efficient packaging full-length viral genomes |
| hCMV Promoter | RNA Polymerase II |
| **Plasmid backbone elements** | **Function** |
| pUC ori | High copy replication and maintenance in *E. coli* |
| AMPr$^R$ | Ampicillin bacterial selectable marker |

**Note: In order to provide the maximum gene-coverage for this collection, we have chosen to make the following types of clones available at a discount:**

- CCSB-Broad Lentiviral Expression Mutant Clones (OHS6269) are fully sequenced and have been found to contain more than 1 mutation within the open reading frames
- CCSB-Broad Lentiviral Expression Unsequenced Clones (OHS6270) have not been fully sequenced
- CCSB-Broad Lentiviral Expression Partially Sequenced Clones (OHS6271) have been partially sequenced, and the total number of mutations is unknown. For all CCSB-Broad clones, the empirical sequencing results for individual clones can be viewed at the Broad Institute's Public TRC Portal: broadinstitute.org/rnai/public/clone/search

The CCSB-Broad Lentiviral Expression Library contains ~15,000 arrayed clones, representing at least 11,000 unique gene IDs. These ORFs, cloned into the pLOX304-Blast-v5 vector, can be used to create lentiviral particles for delivery of ORF content, allowing over-expression screening in virtually any cell type.

## 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 (Fisher Scientific Cat #BP2291) and ampicillin (100 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 + ampicillin (100 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.

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## Replication of 96-well plates

**Prepare target plates**

1. Dispense ~160 µL of sterile LB medium into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and ampicillin (100 µg/mL).

**Prepare source plates**

1. 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 70% ethanol.

**Replicate**

1. 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 well.
2. 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.
3. Discard the replicator.
4. Place the lids back on the source plate and target plates.
5. Seal the source plates, being mindful to avoid cross contamination.
6. Repeat this process until all plate have been replicated.
7. Return the source plates to the -80 °C freezer.
8. Place the inoculated target plates in a 30 °C incubator. Incubate the plates for 24 hours.

## FAQS/troubleshooting

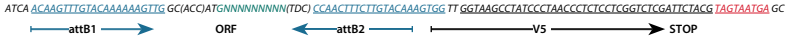
**What are the sequencing primers for the pLX304 Vector?**
5'-end forward: CGCAAATGGGCGGTAGGCGTG
3'-end reverse: TACGGGAAGCAATAGCATGA
To ensure verifiable experimental results, it is recommended that you verify clone identity by sequencing prior to starting your experiment.

**What strain of *E. coli* are the CCSB-Broad Lentiviral Expression clones?**
DH5α.

**What type of recombination sites are present in the CCSB-Broad Lentiviral Expression clones?**
Gateway™ attB1 and attB2 sites flank the ORF insert (see figure below).


**What is the sequence flanking the ORF inserts present in the CCSB-Broad Lentiviral Expression clones?**
The sequence is as follows, the location of the ORF insert is represented by "NNNNNN"
5'-TCAACAAGTTTGTACAAAAAAGTTGGCNNNNNNNNNNNN(TDC)
CCAACTTTCTTGTA-3'
()=codon can be variable

**Do the CCSB-Broad Lentiviral Expression clones have a native stop codon?**
No, all of the clones in the CCSB-Broad Lentiviral Expression Library contain a C-terminal V5 epitope tag fused to the ORF of interest. There is a stop codon located at the 3' end of the V5 epitope tag (see figure below).




**How do I package the CCSB-Broad Lentiviral Expression clones?**
The Dharmacon™ Trans-Lentiviral™ ORF Packaging Kit (Cat #TLP5916, TLP5918) is an effective method for packaging these clones, more information can be found at dharmacon.horizondiscovery.com.

**How can I transfect the CCSB-Broad Lentiviral Expression clones?**
DharmaFECT kb (Catalog #T-2006) can be used to transfect these plasmids into mammalian cells, more information can be found at dharmacon.horizondiscovery.com.

**How can I find the sequence of the ORF insert for a CCSB-Broad Lentiviral Expression clone?**
For each CCSB-Broad Lentiviral Expression 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 CCSB-Broad Lentiviral Expression clone, you can search the Public TRC Portal using the ccsbBroad clone ID (portals.broadinstitute.org/gpp/public/clone/search). Click on the Clone ID link for ORF sequence details.

**How do I create a map of the pLX304 vector with my ORF of interest?**
To generate an approximate map of the pLX304 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. For a more specific look at the junctions, we recommend sequencing the clone from both ends of the insert to confirm the sequence. The sequencing primers given on our website are FWD: 5' - CGCAAATGGGCGGTAGGCGTG - 3'
REV: 5'- TACGGGAAGCAATAGCATGA - 3'

**What is the purpose of the V5 epitope tag? How do I detect V5 epitope tag?**
The V5 tag is a short peptide based on a viral protein. Antibodies specific to the V5 epitope can easily be produced and some are commercially available. The V5 tag facilitates pull-downs and co-immunoprecipitation experiments in order to determine protein-protein interactions. It also allows for labeling of expressed ORFs in cells via immunofluorescence.

For In-Cell Western (ICW) assay of V5 tagged ORF clones, please see the protocol on the Broad Institute website. (broadinstitute.org/rnai/public/static/protocols/TRC%20V5%20Staining%20protocol%20201210.pdf).

**How many amino acids were added to the parental ORFs (including the attB2) in the CCSB-Broad Lentiviral Collection?**
In general, there are 9 amino acids between the last ORF codon and the first V5 codon as a result of the attB2 site. The V5 epitope tag is 14 amino acids long, so there are a total of 23 amino acids added to the 3' end of each ORF before the stop codon.

**Where do you purchase *Blasticidin S*?**
*Blasticidin S* is available from Fisher Scientific (Cat #BP2647-25).

**How do I know what concentration of *Blasticidin S* to use for mammalian selection?**
The pLX304 vector confers resistance to *Blasticidin S* in transduced or transfected cells. In order to generate stable cell lines, it is important to determine the minimum amount of *Blasticidin S* required to kill non-transfected or non-transduced cells by generating a *Blasticidin S* kill curve.

**Where do I purchase the pLX304 empty vector?**
We do not have the empty pLX304 vector available for sale.

**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.

**What are mutant, unsequenced and partially sequenced clones?**
CCSB-Broad Lentiviral Expression Mutant Clones (OHS6269) are fully sequenced and have been found to contain more than 1 mutation within the open reading frames. CCSB-Broad Lentiviral Expression Unsequenced Clones (OHS6270) have not been fully sequenced. CCSB-Broad Lentiviral Expression Partially Sequenced Clones (OHS6271) have been partially sequenced, and the total number of mutations is unknown.

## For customers who purchase library collections

**How are libraries provided (96-well plate catalog number, seal, etc.)?**
Library collections are shipped as bacterial cultures of *E. coli* DH5α glycerol stocks arrayed into 96-well plates (96-well microplates Fisher Scientific #12-565-363) sealed with aluminum seals (Fisher Scientific # 07-200-684).

**Does the CCSB-Broad Lentiviral Expression Library contain the unsequenced (OHS6270), partially sequenced (OHS6271), and mutant clones (OHS6269) as well as the ones that had verified sequence matches (OHS6085)?**
Yes, the CCSB-Broad Lentiviral Expression Library contains clones with known point mutations, unsequenced clones, and partially sequenced clones since these clones are also of value to researchers. The majority of the clones in the collection are categorized as fully sequenced clones (OHS6085) and contain no known mutations.

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.

## Useful websites

**Public TRC Portal, Clone Search**
portals.broadinstitute.org/gpp/public/clone/search

**The Broad Institute**
broadinstitute.org

**The RNAi Consortium (TRC) Broad Institute Protocols**
broadinstitute.org/rnai/public/resources/protocols

**Dana-Farber Cancer Institute CCSB hORFeome Database**
horfdb.dfci.harvard.edu/index.php?page=toolsdata

**Restriction Mapper**
restrictionmapper.org/

**NCBI**
ncbi.nlm.nih.gov/

## References


1. Yang X, Boehm JS, Yang X, Salehi-Ashtiani K, Hao T, Shen Y, Lubonja R, Thomas SR, Alkan O, Bhimdi T, Green TM, Johannessen CM, Silver SJ, Nguyen C, Murray RR, Hieronymus H, Balcha D, Fan C, Lin C, Ghamsari L, Vidal M, Hahn WC, Hill DE, Root DE. A public genome-scale lentiviral expression library of human ORFs. *Nature Methods*. **8**, 659-61 (2011).


Horizon Discovery Group is a distributor of multiple gene expression clone collections (cDNAs and ORFs). These clone collections were generated by outside groups 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. We have 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 outside group, the clone you receive might not match the expected clone. If this occurs, please contact Technical Support (ts.dharmacon@horizondiscovery.com)


**If you have any questions, contact**

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