FUNCTIONAL GENOMICS FACILITY
UNIVERSITY OF COLORADO CANCER CENTER SHARED RESOURCE

Frequently Asked Questions (CCSB LentiORF)

General Information about CCSB-Broad Lentiviral Expression Library

Disclaimer

The Functional Genomics Facility is a distributor of LentiORF (clone ID. eg. ccsb304_xxxx) collection for gene expression clone collection only. This clone collection was purchased from GE Healthcare Dharmacon (Thermo at the time of purchase) and was generated by Dana Farber and The Broad Institute. Thus, the quality of the collections is largely dependent upon what the Facility received from these groups. Specific clone information and plate coordinates were provided to the Functional Genomics Facility by the suppliers of these clone collections. Neither the Facility nor Dharmacon has sequence verified each individual clone from these collections. These collections and individual clones are distributed "as is" with no additional product validation or guarantees.

The FAQ section is partially borrowed from GE Healthcare Dharmacon.

The 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 ORFs in an expression-ready lentiviral system. This library was derived from the hORFeome v8.1 and created to enable targeted experiments and large-scale screening in diverse cell types.

More information on the creation of this library can be found in the following publication: X. Yang, et al., A public genome-scale lentiviral expression library of human ORFs. Nat Methods 8(8), 659-661 (2011).

Highlights

• CCSB-Broad Lentiviral Expression Clones (OHS6085) were fully sequenced, and match their accessions with no more than a single mutation within the open reading frame
• Lentiviral expression vector – allows creation of lentiviral particles for transduction of almost any mammalian cell type
• CMV promoter drives strong expression of the ORF construct
• Blasticidin resistance allows for multiplex experiments in cells containing Puromycin or Neomycin resistance
• V5 tag facilitates pull-downs and co-immunoprecipitation experiments in order to determine protein-protein interactions, also allows for labeling of expressed ORFs in cells via immunofluorescence

Note

The following types of clones* available:
• CCSB-Broad Lentiviral Expression Mutant Clones (OHS6269) were 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: http://www.broadinstitute.org/rnai/public/clone/search

How was the CCSB-Broad Lenti Expression Library developed?
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).
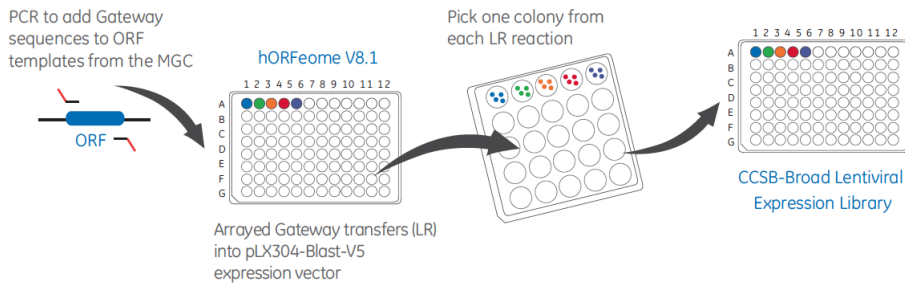
Figure 1: Schematic diagram of CCSB-Broad LentiORF Expression library development

The CCSB-Broad Lentiviral Expression Library contains ~15,000 arrayed clones, representing at least 11,000 unique gene IDs. These ORFs, cloned into the pLX304_Blast_V5 vector, can be used to create lentiviral particles for delivery of ORF content, allowing over-expression screening in virtually any cell type.

What elements are present in the pLX304 vector?

Figure 2 shows a schematic of the pLX304 vector, and Table 1 gives a comprehensive description of the various elements in the pLX304 vector.
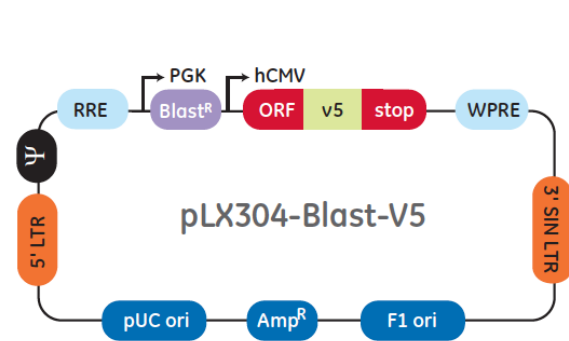
Table 1:

Figure 2: Schematic of PLX304

ORF Cassette Elements	Function
PGK	Promoter Human phosphoglycerate kinase (PGK) promoter
BsdR	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

In what format was the CCSB-Broad Lenti Expression Library provided to the Facility (96-well plate catalog number, seal, etc.)?

Library collections were shipped as bacterial cultures of E. coli DH5a 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 (OHS6087) 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.

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 1 clones?

DH5α.

What types of recombination sites are present in the CCSB-Broad Lentiviral Expression clones?

Gateway™ attB1 and attB2 sites flank the ORF insert (Figure 3).

Figure 3: Schematic of recombination sites present in the CCSB-Broad Lentiviral Expression clones

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 (Figure 3).

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. The Functional Genomics Facility-optimized protocol using the pD8.9 and pVSVG also works quite well. A detailed protocol to package CCSB-Broad Lenti Expression ORF can be found at Transfection and Transduction of CCSB-Broad Lenti ORF

How can I transfect the CCSB-Broad Lentiviral Expression clones?
Thermo Scientific™ TurboFect™ Transfection Reagent (Catalog #R0532) can be used to transfect these plasmids into mammalian cells.

How can I find the sequence of the ORF insert for a CCSB-Broad Lentiviral Expression clone?
For each CCSB-Broad Lentiviral Expression clone, Dharmacon™ 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 (broadinstitute.org/rnai/public/clone/search). Click on the Clone ID link for ORF sequence details.

Where can I find the map of pLX304?
The pLX304 vector sequence data can found on Addgene (http://www.addgene.org/25890/). More information about the pLX304 vector can also be found at pLX304_sequence.

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.

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.

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?
The empty pLX304 vector can purchased from Addgene (http://www.addgene.org/25890/).

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.

Useful Websites:

CCSB-Broad Lentiviral Expression Collection @ Dharmacon
http://dharmacon.gelifesciences.com/mammalian-orf/ccsb-broad-lentiviral-orfs/

Public TRC Portal, Clone Search
broadinstitute.org/rnai/public/clone/search/

The Broad Institute
broadinstitute.org/rnai/orfs

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/

References:
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).