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Product Information

MISSION® TRC3 Human Whole Genome Lentiviral ORF Pool

Catalog Number **ORFPOOLWG**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description
An open reading frame (ORF) is a continuous segment of DNA beginning with an initiation codon, methionine ATG, and ending with one of the three termination codons, TAA, TAG, or TGA, that is coded into a polypeptide chain or a protein. An ORF contains the coding sequence of a gene (CDS) and lacks both the 5′ and 3′ UTRs. These ORFs are inserted into expression vectors (containing an artificial stop codon) and their expression may be modulated (overexpressed) to understand the corresponding gene/protein function. ORFs also play an important role in RNAi and CRISPR rescue experiments, where gene expression is restored (gain-of-function) for gene validation.

Sigma-Aldrich offers the largest collection of fully sequenced and pre-cloned human open reading frames (ORFs) to-date. Our ready-to-use MISSION® TRC3 Human LentiORFs allow for stable integration, enrichment of cells, and long-term gene expression in difficult-to-transfect cell lines utilizing our best-in-class lentiviral manufacturing. This collection provides researchers with the unique tools to gain insights into gene function through modulating gene and protein expression. The Complete Mission TRC3 Human LentiORF collection consists of over 50,000 pre-cloned ORFs, spanning over 14,000 human genes.

Whether looking to perform gene overexpression studies or validate gene knockdown/knockout results from RNAi/CRISPR experiments, LentiORFs are the ideal shortcuts to protein expression and tools for gene analysis. Researchers need to validate “hits” from a screen to eliminate false positives. This means confirming whether the observed phenotype is due the target gene of interest. Re-expressing the gene of interest through ORFs is the best and most accepted way to validate a gene target.

These libraries were introduced in the 2011 *Nature Methods* paper: "A public genome-scale lentiviral expression library of human ORFs." (DOI: 10.1038/nmeth.1638). Supplementary Table 4, containing a summarized view of the contents of the library, is available in two formats (pdf, xls). For protocols detailing the use of this library, see the Protocols section found below.

The MISSION TRC3 Human Lentiviral ORF Pool consists of plasmid-based, puromycin resistant ORF constructs expressing over 14,000 human genes. On average, there are 1.2 ORFs for each gene in the ORFeome pool. The ORF plasmids are further processed into lentiviral particles to facilitate stable gene expression in both dividing and quiescent cells.

Gene Overexpression Studies
While RNA interference technology allows scientists to “turn off” or silence certain genes, ORFs can be used to “turn on” or “overexpress” particular genes. Flipping the switches on genes one at a time can help reveal the functions of individual genes, such as those that play a role in cancer. The lentiviral expression system has been used in several studies where gene screening, expression analysis, and genetic rescue are important. Overexpression of genes and proteins is widely used in functional genomics, proteomics, and system biology studies.

Some of the studies include:
1. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation

2. KRAS and YAP1 converge to regulate EMT and tumor survival

The MISSION TRC3 Human Whole Genome Lentiviral ORF Pool is a lentiviral pool produced using a proprietary process. Assay for representation of each individual ORF ensures robust library coverage. The pool is provided in ready-to-use lentiviral format at titers of at least 5×10^8 TU/ml via p24 assay. See Table 1 for approximate number of ORF constructs.

Table 1.
Clone and Gene Coverage by Human Whole Genome Pooled ORF Library

Product	Approximate Total Clone Coverage	Approximate Number of Genes
ORFPOOLWG	17,000+	14,000+

Quality Control
Each MISSION TRC3 Human Whole Genome Lentiviral ORF Pool has been tested for representation of each individual ORF through an internal QC process. The pool has also been tested via p24 assay and has demonstrated a titer of at least 5×10^8 TU/ml.

Components
The MISSION Human Whole Genome Lentiviral ORF Pool includes eight aliquots - enough virus to perform multiple screens. Each aliquot is provided at 5×10^8 TU/ml or higher in a volume of 25 µl. Please refer to the Certificate of Analysis for the exact titer.

Additionally, each ORF Pool kit will include a USB Flash Drive containing the MISSION Human ORF pool platemap file and the complete list of ORF clone information.

Note: Primers for amplification and sequencing of MISSION ORF are not included, but sequence information is provided. See Table 2 for details.

Included Materials
Positive Target Overexpression Controls and Transduction Efficiency Optimization

- ORF Lenti Human Puro BFP Control Virus High Titer, Catalog Number ORFBFPH

  This positive control can be used for measuring transduction efficiency and optimizing ORF delivery in cell lines that do not survive any puromycin exposure. The BFP Control contains a gene encoding BFP driven by the EF1a promoter. This control provides fast visual confirmation of successful transduction.

- ORF Lenti Human Puro TurboGFP Control High Titer, Catalog Number ORFGFPH

  This positive control can be used for measuring transduction efficiency and optimizing ORF delivery in cell lines that do not survive any puromycin exposure. The TurboGFP Control contains a gene encoding GFP driven by the EF1a promoter. This control provides fast visual confirmation of successful transduction.

Negative Target Overexpression Controls

- ORF Lenti Human Puro TurboGFP Control High Titer, Catalog Number ORFGFPH

- ORF Lenti Human Puro BFP Control High Titer, Catalog Number ORFBFPH

  Either the BFP or GFP control can also be used as a negative control in cell lines that don't already express either fluorophore. The control virus serves as a negative control of lentivirus treatment for the same lentiviral backbone. It allows for validation of the specificity of target expression in target cells, and observation of potential cellular effects due to the transduction process.

Materials And Reagents Required But Not Provided
For Transduction of MISSION TRC3 Human Whole Genome Lentiviral ORF Pool into Target Cells

- Hexadimethrine bromide (chemical equivalent of Polybrene), Catalog Number H9268
- Tissue Culture Plates and Related Tissue Culture Supplies
- Puromycin dihydrochloride, Catalog Number P9620

For Purification of Genomic DNA

- GenElute™ Mammalian Genomic DNA Miniprep Kit 70 preps, Catalog Number G1N70

For PCR Amplification of ORF Inserts

- JumpStart™ *Taq* ReadyMix™, Catalog Number P2893
- Magnesium chloride solution, Catalog Number M8787
- 1% 1× TAE Agarose gel
- DirectLoad™ PCR 100 bp Low Ladder, Catalog Number D3687
- Thermal Cycler

For Purification of Amplified ORF Inserts

- GenElute PCR Clean-Up Kit, Catalog Number NA1020

Precautions And Disclaimers
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Though the lentiviral transduction particles produced are replication incompetent, it is recommended they be treated as Risk Group Level 2 (RGL-2) organisms for laboratory handling. Follow all published RGL-2 guidelines for laboratory handling and waste decontamination.

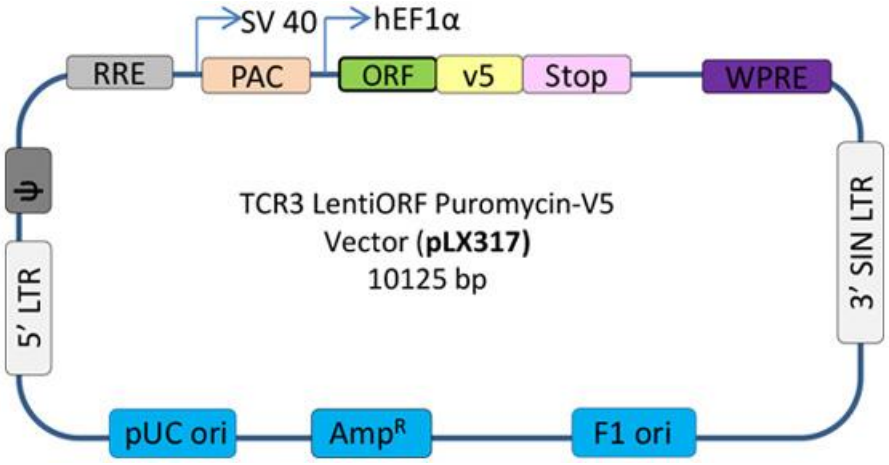
Storage and Stability
All components are stable for at least 6 months after receipt when stored at –70 °C. Avoid repeated freeze/thaw cycles, which will severely reduce transduction efficiency.

Procedures
Please refer to the Pooled shRNA Technical Bulletin for more information:
http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/shph01bul.pdf

Table 2.
Suggested Materials (**Not Included**) – Primers and Positive Control

Description	Sequence	Minimum Volume	Approximate Concentration
Amplification Primer 1 (Forward)	cttgtacaaagtggttgatatc	750 µl	20 µM
Amplification Primer 2 (Reverse)	caactcctcataaagagacag	750 µl	20 µM
Sequencing Primer	ttgtacaaagtggttgatatc	750 µl	5 µM

Figure 1.
Mission TRC3 LentiORF Expression Vector Map



The puromycin based LentiORF expression vector systems allow for additional peptide sequences or tags to modify properties of the native protein. Affinity tags can be used to purify the protein further for further structural and characterization studies. Epitope tags (such as V5 found in the blasticidin and puromycin vectors) allow for simple detection or purification via antibodies. The V5 epitope tag is also useful for Western blotting, immunofluoresence, and immunoprecipation experiments. The addition of reporters and fluorescent tags also allows for gene expression monitoring and cellular localization.

Vector Label	Function
attR1	Gateway recombination site 1
ORF	Open Reading Frame Insert
attR2	Gateway recombination site 2
pUC ori	High copy replication and maintenance in *E. coli*
Spect	Spectinomycin resistance gene
RRE (Rev Response Element)	Increased full-length viral packaging
SV40 Promoter	Simian virus 40 (SV40) promoter
PAC	Puromycin resistance gene
PGK Promoter	Human phosphoglycerate kinase (PGK) promoter
Bsd	Blasticidin resistance gene
hEF1α Promoter	RNA Polymerase II
hCMV Promoter	RNA Polymerase II
V5	V5 Epitope Tag
WPRE (Woodchuck Posttranscriptional Regulatory Element)	Enhance transcription of transgenes
5' LTR	5' long terminal repeat
3' SIN LTR	*Safety* 3' Self inactivating long terminal repeat
F1 ori	Phage-derived ORI that allows for the replication and packaging of ssDNA into phage particles
AmpR	Ampicillin bacterial selection marker
Ψ	Psi viral genome packaging sequence
*Barcode (not shown)	Pooled Screening application barcode-Puromycin only

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