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Product Information

MISSION® shRNA Mouse Gene Family Sets, Bacterial Glycerol Stocks

Catalog Numbers **SM0111, SM0411, and SM2011**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

Small interfering RNAs (siRNAs) generated from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) for extended periods of time in mammalian cells. The MISSION® product line is a viral-vector-based RNAi library against annotated mouse and human genes. MISSION shRNAs are expressed intracellularly after transduction with amphotropic lentivirus particles, allowing screening in a wide range of mammalian cell lines. In these cell lines, MISSION shRNA clones permit rapid, cost efficient loss-of-function and genetic interaction screens. These murine gene family sets were designed specifically for the most common mammalian model organism, the mouse. A collection of reviews that highlight the importance of each gene family set is included.

The MISSION shRNA Gene Family Sets allow for high throughput loss-of-function and genetic interaction screens. The glycerol stock format consists of bacterial glycerol stocks harboring sequence-verified shRNA lentiviral plasmid vectors. Each MISSION shRNA clone is constructed within the lentivirus plasmid vector pLKO.1-puro.[1] The pLKO.1-puro vector contains the ampicillin and puromycin antibiotic resistance genes for selection of inserts in bacterial or mammalian cells, respectively. The sets consist of sequence-verified shRNA lentiviral plasmid DNA. For each gene target, there are 3 or more constructs that have been designed against each target gene using a proprietary algorithm. Therefore, a range of gene silencing efficiencies, with at least one construct from each gene set being >70%, can be expected when using these clones. This allows one to examine the effect of loss of gene function over a large range of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

Bacterial cultures may be amplified from the glycerol stocks for use in purification of the shRNA plasmid DNA. Subsequently, target cell lines may be transfected with the purified plasmid for transient or stable gene silencing (puromycin selection). In addition, self-inactivating replication incompetent viral particles can be produced in packaging cells (HEK 293T) by co-transfection with compatible packaging plasmids.[2,3] Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based particles permit efficient infection and integration of the specific shRNA construct into differentiated and non-dividing cells, such as neurons and dendritic cells,[4] overcoming low transfection and integration difficulties when using these cell lines.

Please see the **Mouse Cell Type Table** for those cell types that have been successfully infected by pLKO.1-puro based shRNA constructs.

Each MISSION shRNA clone is constructed within the lentiviral plasmid vector pLKO.1-puro[4] followed by transformation into *Escherichia coli*. The pLKO.1-puro vector contains bacterial (ampicillin) and mammalian (puromycin) antibiotic resistance genes for selection of inserts in either bacterial or mammalian cell lines.

Components

The individual clones are provided as a 50 µL bacterial glycerol stock containing Terrific Broth (TB), carbenicillin (100 µg/mL), and 15% glycerol. The sets are provided in 96-well barcoded plates, along with a CD containing gene description, symbol, RefSeq, locus link, clone ID, hairpin sequence, and plate map position for each clone. The number of plates will vary between gene families; however, a target set will not be broken up between plates.

The hairpin sequence and other unique clone information may be obtained by searching the MISSION search database at www.sigma.com/yfg using:
RefSeq accession numbers, e.g., NM_027088,
unique clone identification numbers,
e.g., NM_027088.1-989s1c1, or
TRC numbers, e.g., TRCN0000030720.

Genotype of host *E. coli* strain
F^- Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17 (r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *ton*A

Catalog Number	Gene Family Set	Gene Count *	Clone Count *	Avarage Number Clones/Gene *
SM0111	Kinases	618	3916	6.3
SM0411	Phosphatases	290	1734	6.0
SM2011	Tyrosine Kinases	133	850	6.4

*The MISSION production and bio-informatics team constantly reviews and confirms the clones available for a gene family set. These numbers are very close to the actual number that will be shipped, but each researcher will receive a final plate map indicating the location and exact TRCN clone numbers.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Stable for at least six months after receipt when stored at –70 °C. Avoid repeated freeze/thaw cycles, which will severely reduce culture viability.

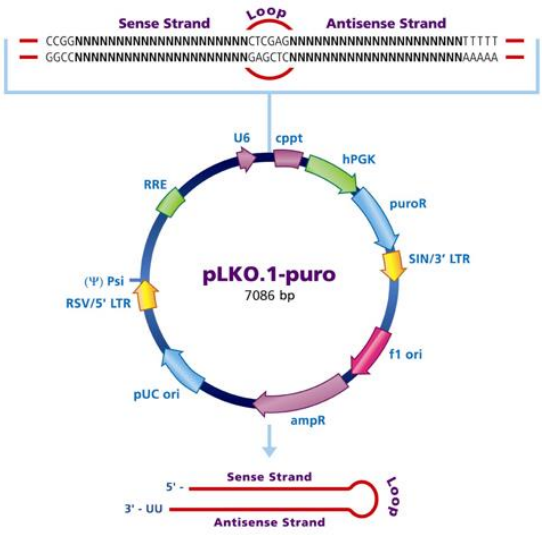
Procedure for Culturing Clonal Cell Lines
1. Remove ice splinters (50–100 µL) from the frozen bacterial glycerol stock using a sterile loop and place them into a sterile culture tube containing 0.5 mL of LB without antibiotics.
2. Incubate the culture at 37 °C with shaking for 15–30 minutes.
3. Using a sterile loop, streak 25–50 µL of the incubated culture onto freshly prepared plates containing LB agar and carbenicillin (100 µg/mL, Catalog Number C2113). Carbenicillin, an ampicillin analog, is recommended over ampicillin due to its increased stability in cultures.
4. Incubate plates in a humidified atmosphere for 15–20 hours at 37 °C.
5. Isolate a single colony from the plate and use as a source inoculum for downstream applications (e.g., plasmid DNA preparation).

Troubleshooting Guide

Problem	Cause	Solution
No growth of bacterial culture on selection plates	Incorrect carbenicillin concentration	Re-check the carbenicillin concentration or pour fresh plates containing 100 µg/ml of carbenicillin.
	Insufficient inoculum volume from frozen culture	Remove a larger volume of culture from the frozen glycerol.
	Insufficient storage temperature of frozen culture	Storage temperature must be –70 °C or lower. Obtain new stock.
	Multiple freeze-thaw cycles	Avoid freeze thawing the culture more than 2 times.
Low plasmid yield	Difficult construct	Perform larger purifications (midi or maxi preps) on constructs that produce low yields.
	Failure to use a single colony for inoculation	Use an isolated colony for inoculation of cultures for DNA preps

Features of Lentiviral Plasmid Vector pLKO.1-puro

Name	Description
U6	U6 Promoter
cppt	Central polypurine tract
hPGK	Murine phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
SIN/3′ LTR	3′ self inactivating long terminal repeat
f1 ori	f1 origin of replication
ampR	Ampicillin resistance gene for bacterial selection
pUC ori	pUC origin of replication
5′ LTR	5′ long terminal repeat
Psi	RNA packaging signal
RRE	Rev response element



Control Selection Table
Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[5]

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will provide a reference point for comparing all other samples.
Negative Control: Transfection with empty vector, containing no shRNA insert	MISSION pLKO.1-puro Control Vector, Catalog Number SHC001 The empty vector, pLKO.1-puro, is a useful negative control that will not activate the RNAi pathway because it does not contain an shRNA insert. It will allow for observation of cellular effects of the transfection process and the delivery of the lentiviral vector. Cells transfected with the empty vector provide a useful reference point for comparing specific knockdown.
Negative Control: Transfection with non-targeting shRNA	MISSION Non-Target shRNA Control Vector, Catalog Number SHC002 This non-targeting shRNA vector is a useful negative control that will activate RISC and the RNAi pathway, but does not target any mouse genes. The short-hairpin sequence contains 5 base pair mismatches to any known mouse gene. This allows for examination of the effects of shRNA transfection on gene expression. Cells transfected with the non-target shRNA vector will also provide a useful reference for interpretation of knockdown.
Positive Control: Transfection with positive reporter vector	MISSION TurboGFP™ Control Vector, Catalog Number SHC003 This vector is a useful positive control for measuring transfection efficiency and optimizing shRNA delivery. The TurboGFP Control Vector consists of the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Transfection of this vector provides fast visual confirmation of successful transfection and delivery.
Positive Control: Transfection with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Vector, Catalog Number SHC004 The TurboGFP shRNA vector consists of the pLKO.1-puro vector, containing shRNA that targets TurboGFP, and can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA Control Vector has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this vector targets TurboGFP, and it does not target any mouse genes, it can also be used as a negative non-target control in shRNA experiments

Mouse Cell Type Table
The mouse cell types listed below have been successfully infected by pLKO.1-puro based shRNA constructs

Cell lines	**Cell Type**
NIH 3T3	fibroblast
Primary cells	**Cell Type**
astrocytes	normal
C3H10T1/2	mesenchymal
ECS	mouse embryonic stem cells
fibroblasts	mouse embryonic fibroblasts
MC3T3-E1	mouse bone marrow derived
molar mesenchymal	mouse embryonic mesenchymal

Reviews Indicating the Importance of Each of the Gene Family Sets:

Kinases
1. Gomase, V.S. *et al*., *Curr. Drug Metab*., **9**, 255-8 (2008).

Phosphatases
1. Hendriks, W.J. *et al*., Protein tyrosine phosphatases: functional inferences from mouse models and Murine diseases. *FEBS J*., **275**, 816-30 (2008).
2. Tremblay, M.L., and Giguère, V., Phosphatases at the heart of FoxO metabolic control. *Cell Metab.,* **7**, 101-3 (2008).
3. Heideker, J. *et al*., Phosphatases, DNA damage checkpoints and checkpoint deactivation. *Cell Cycle*, **6**, 3058-64 (2007).
4. Sawyer, T.K. *et al*., Protein phosphorylation and signal transduction modulation: chemistry perspectives for small-molecule drug discovery. *Med. Chem*., **1**, 293-319 (2005).

Tyrosine Kinases
1. Annerén, C., Tyrosine kinase signaling in embryonic stem cells. *Clin. Sci. (Lond)*, **115**, 43-55 (2008).
2. Comoglio, P.M. *et al.,* Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat. Rev. Drug Discov.*, **7**, 504-16 (2008).
3. Ren, H. *et al.,* Receptor tyrosine kinases as therapeutic targets in malignant glioma cells. *Rev. Recent Clin. Trials,* **2**, 87-101 (2007).

References

1. Demedts, I.K. *et al.*, Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respiratory Research*, **7**, 53 (2006).
2. Zufferey, R. *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.*, **15**, 871-85 (1997).
3. Zufferey, R. *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J. Virol*., **72**, 9873-80 (1998).
4. Stewart, S.A. *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
5. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).

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