

Product Information

MISSION® shRNA Human Gene Family Set Transduction Particles

Catalog Numbers: **SH0131, SH0231, SH0431, SH0531, SH0731, SH0831, SH1031, SH1131, SH1331, SH1831, SH1931, SH2131, SH2231, SH2331, SH2431, SH2531, SH2631, SH2731, SH2831, SH2931, and SH3031**

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. We have collected a list of reviews that highlight the importance of each gene family set.

The MISSION shRNA Transduction Particles Set allows for high throughput loss-of-function and genetic interaction screens. The set consists of VSV-G pseudotyped lentiviral particles. Each MISSION shRNA clone is constructed within the lentivirus plasmid vector pLKO.1-Puro.¹ Each gene target set consists of 3 or more constructs that have been designed against each target gene using a proprietary algorithm. Therefore, a range of knockdown 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 series of gene knockdown efficiencies. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

The lentiviral transduction particles are produced from a library of sequence-verified shRNA lentiviral plasmid vectors. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids.^{5,6}

In addition, the lentiviral transduction particles are pseudotyped with an envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G), allowing transduction of a wide variety of mammalian cells.⁷ 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 **Cell Type Table** for those cell types that have been successfully infected by pLKO.1-puro based shRNA constructs.

The lentiviral transduction particles are titered via a p24 antigen ELISA assay, and pg/ml of p24 are then converted to transducing units per ml using a conversion factor. The conversion can be viewed at: www.tronolab.com.

Components/Reagents

Each individual construct is provided as 4 × 50 µL of frozen stock containing 10⁶ lentiviral transducing particles per ml in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal bovine serum (FBS) and penicillin-streptomycin. 10% of the clones in a set are titered via an ELISA p24 assay for quality control. Fully titered sets are available on a custom basis; contact RNAi@sial.com for more information.

Lentivirus sets are packaged into a 96-well plate and labeled with 2-D barcodes for simple plate identification. A CD contains detailed clone position, 2-D barcode reference, RefSeq, locus link, gene description, gene symbol, clone ID, and hairpin sequence.

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).

Materials Suggested but Not Provided

- Hexadimethrine bromide, Catalog Number [H9268](#)
- Puromycin Ready Made Solution (10 mg/ml in H₂O), Catalog Number [P9620](#)
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- 96-well cell culture treated plates
- Mammalian cells to be transduced
- Primers, probes, and PCR mix for qRT-PCR
- Cell-based, enzymatic, or array based assay for phenotypic assay

Precautions and Disclaimer

These products are 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.

Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.⁹ Follow all published RGL-2 guidelines for handling and waste decontamination. Also, use extra caution when using lentiviral transduction particles that express shRNA targeting genes involved in cell cycle control.

Storage/Stability

All components are guaranteed to be stable for at least six months after receipt when stored at -70°C . Avoid repeated freeze/thaw cycles, as this will severely reduce transduction efficiency.

Preparation Instructions

1. Prepare mammalian cell cultures so that they are growing exponentially and are no more than 70–80% confluent before transduction.
2. Prepare a stock solution of hexadimethrine bromide (the chemical equivalent of Polybrene) at 2 mg/mL in water.

Procedure

The following protocol has been developed for high-content screening in 96-well plates with stable selection through puromycin.

Day 1.

- a. Add 1.6×10^4 cells in fresh medium to the number of wells needed for each construct in a 96-well plate. Duplicate or triplicate wells for each lentiviral construct and control should be used.
- b. Incubate 18–20 hours at 37°C in a humidified incubator in an atmosphere of 5–7% CO₂.

Note: The growth rates of cells vary greatly. Adjust the number of cells plated to accommodate a confluency of 70% upon transduction. Also account for the length of time the cells will be growing before downstream analysis when determining the plating density.

Day 2.

- a. Remove medium from wells. To each well add 110 μL medium and hexadimethrine bromide to a final concentration of 8 $\mu\text{g}/\text{mL}$. Gently swirl the plate to mix.

Note: Hexadimethrine bromide enhances transduction of most cell types. Some cells, like primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide to these types of cells. If working with a cell type for the first time, a hexadimethrine control only well should be used to determine cell sensitivity.

- b. Add 2–15 μL of lentiviral particles to appropriate wells. Gently swirl the plate to mix. Incubate 18–20 hours at 37°C in a humidified incubator in an atmosphere of 5–7% CO₂. Cells may be incubated for as little as 4 hours before changing the medium containing lentiviral particles. Overnight incubation may be avoided when toxicity of the lentiviral particles are a concern.

Note: When transducing a lentiviral construct into a cell line for the first time, a range of volume, or Multiplicity of Infection (MOI), should be tested. 2, 5, 10, and 15 μL of lentiviral particles per 1.6×10^4 cells or MOIs of 0.5, 1, 2, and 5 should be used to determine the optimal transduction efficiency and knockdown for each cell line. Transduction efficiency can be optimized using the TurboGFP™ Control Transduction Particles (SHC003V).

Multiplicity of Infection (MOI):

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.

To calculate:

$$\text{(Total number of cells per well)} \times \text{(Desired MOI)} = \text{Total transducing units needed (TU)}$$

$$\text{(Total TU needed)} / \text{(TU/ml reported on C of A)} = \text{Total mL of lentiviral particles to add to each well}$$
Day 3.

Remove the medium containing lentiviral particles from wells. Add fresh medium to a volume of 120 μL to each well.

Note: For cell types that do not strongly adhere to the plate, 100 μL of medium may be removed and replaced with 100 μL fresh medium.

Day 4.

Remove medium from wells. Add fresh medium containing puromycin.

Note: The appropriate concentration of puromycin for each cell type will vary. If the appropriate concentration for the desired cell type is unknown, a titration experiment, or kill curve, must be performed. Typically, 2–10 $\mu\text{g/ml}$ are sufficient to kill most untransduced mammalian cell types.

Puromycin Titration:

Puromycin titration (kill curve) should be performed when working with a new cell type.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 μL fresh medium.
2. The next day add 500–10,000 ng/mL of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3–14 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the medium containing puromycin every 3 days. The minimum concentration of puromycin that causes complete cell death after the desired time should be used for that cell type and experiment.

Note: Excess puromycin can cause many undesired phenotypic responses in most cell types.

Day 5 and on

- a. Replace medium with fresh puromycin containing medium every 3–4 days until resistant colonies can be identified.
- b. Pick a minimum of 5 puromycin-resistant colonies and expand each clone to assay for knockdown of the target gene.
- c. A variety of phenotypic, enzymatic, or gene expression assays may be performed. Each assay should be optimized prior to the high-content screen with both negative and positive controls.

Note: Due to the random integration of the lentivirus into the host genome, varying levels of target gene knockdown may be seen with different puromycin resistant colonies. Testing a number of colonies will allow the optimal degree of knockdown to be determined.

References

1. Demedts, I.K. *et al.*, Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respiratory Research*, **7**, 53 (2006).
2. Fink, S.K. *et al.*, Apoptosis, Pyroptosis, and Necrosis: Mechanistic Description of Dead and Dying Eukaryotic Cells. *Infection and Immunity*, **73**(4), 1907-1916 (2005).
3. Miller, M.A. *et al.*, Caspase 8L, a novel inhibitory isoform of caspase 8, is associated with undifferentiated neuroblastoma. *Apoptosis*, **11**, 15-24 (2006).
4. Stewart, S.A. *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
5. Zufferey, R. *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* **15**, 871-85 (1997).
6. Zufferey, R. *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J Virol.*, **72**, 9873-80 (1998).
7. Burns, J.C. *et al.*, Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors: Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA*, **90**, 8033-8037 (1993).
8. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).
9. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (<http://www4.od.nih.gov/oba>)

Catalog Number	Gene Family Set	Gene Count*	Clone Count*	Average Number Clones/Gene*
SH1931	Apoptosis Pathway	443	3512	7.9
SH2931	B-Cell Activation	99	661	6.7
SH2231	Cell Adhesion Genes	368	2396	6.5
SH0831	Cytokine and Chemokine	106	538	5.1
SH1331	Cytokine and Chemokine Receptors	93	584	6.3
SH2331	Cytoskeleton Genes	275	1991	7.2
SH3031	Epigenetic Regulators	10	59	5.9
SH1831	DNA Repair Pathway	117	837	7.2
SH0731	Ubiquitin Hydrolases (DUBS)	127	830	6.5
SH2531	Extracellular Matrix Genes	331	1968	5.9
SH0231	G-Protein Coupled Receptors (GPCRs)	541	2864	5.3
SH2631	Helicase	136	909	6.7
SH1031	Ion Channel	277	1479	5.3
SH2731	JAK-STAT Pathway	190	1358	7.1
SH0131	Kinases, complete	678	7607	11.2
SH1131	Nuclear Hormone Receptors	218	1448	6.6
SH2431	p53 Pathway	242	1865	7.7
SH0431	Phosphatases	320	2099	6.6
SH2831	T-Cell Activation	242	1469	6.1
SH0531	Tumor Suppressors	73	575	7.9
SH2131	Ubiquitin Ligases (E1, E2, E3)	349	2151	6.2

*The MISSION Production and Bio-informatics Team constantly reviews and confirms the clones available for each 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 exact TRCN clone numbers and their plate location.

Troubleshooting Guide

Problem	Cause	Solution
Low levels of target gene knockdown due to low transduction efficiency.	Hexadimethrine bromide not included during transduction.	Transduce in the presence of hexadimethrine bromide.
	Non-dividing cell type used.	Transduce at a higher MOI.
	MOI is too low.	Transduce at a higher MOI.
	Cells were harvested and assayed too soon after transduction.	The shRNA must be permitted to accumulate in cells. Harvest 48–72 hours after transduction. Alternatively, knockdown results may be improved by placing cells under puromycin selection because untransduced cells will be killed.
No gene knockdown is observed.	Viral stock stored incorrectly.	Store stocks at –70 °C. Do not freeze/thaw more than 3 times.
	MOI is too low.	Transduce at a higher MOI.
Cytotoxic effects observed after transduction.	Target gene is essential for cell viability.	Be sure that target gene is not essential for cell growth or viability.
	Hexadimethrine bromide was used during transduction.	Be sure that cells are not sensitive to hexadimethrine bromide. Omit the hexadimethrine bromide during the transduction.
	Too much puromycin was used for selection.	Determine the puromycin sensitivity of the cells by performing a kill curve and use the minimum concentration required to kill the untransduced cells.

Control Selection Table

The recommended controls for any shRNA experiment are described in the **Control Selection Table** and are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.⁸

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will provide a reference point for comparing all other samples.
Negative Control: Transduction with empty viral particles, containing no shRNA insert	MISSION pLKO.1-puro Control Transduction Particles, Catalog No. SHC001V The empty viral particles, produced from pLKO.1-puro, are a useful negative control that will not activate the RNAi pathway because they do not contain an shRNA insert. It will allow for observation of cellular effects of the transduction process. Cells transduced with the empty viral particles provide a useful reference point for comparing specific knockdown.
Negative Control: Transduction with non-targeting shRNA	MISSION Non-Target shRNA Control Transduction Particles, Catalog No. SHC002V This non-targeting shRNA is a useful negative control that will activate RISC and the RNAi pathway, but does not target any human or mouse genes. The short-hairpin sequence contains 5 base pair mismatches to any known human or mouse gene. This allows for examination of the effects of shRNA transduction on gene expression. Cells infected with the non-target shRNA will also provide a useful reference for interpretation of knockdown.
Positive Control: Transduction with positive reporter viral particles	MISSION TurboGFP Control Transduction Particles, Catalog No. SHC003V This is a useful positive control for measuring transduction efficiency and optimizing shRNA delivery. The TurboGFP Control transduction particles are produced from the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Transfection of this control provides fast visual confirmation of successful transduction.
Positive Control: Transduction with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Transduction Particles, Catalog Number SHC004V The TurboGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1-Puro vector containing shRNA that targets TurboGFP (Catalog # SHC004). These particles can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this shRNA targets TurboGFP, and it does not target any human or mouse genes, it can also be used as a negative non-target control in shRNA experiments

Cell Type Table

The cell types listed below have been successfully infected by pLKO.1-puro based shRNA constructs

Cell lines, human	Cell Type	Cell lines, human	Cell Type	Primary cells human	Cell Type
HEK293	embryonic kidney cells	A431	epidermal carcinoma	dendritic	immature dendritic
HeLa	cervical adenocarcinoma	THP1	monocytic	T-cells	lymphocytes
A549	lung adenocarcinoma	RAW264.7	macrophage	epithelial	prostate
H1299	lung carcinoma	SH-SY5Y	brain neuroblastoma	fibroblasts	primary mammary
HT29-D4	colon carcinoma	HCN-1A	brain cortical neuron	Primary cells, other species	Cell Type
HepG2	hepatocellular carcinoma	SupT1	T-cells	ECS	mouse embryonic stem cells
HCT116	colon carcinoma	BJ-TERT	diploid fibroblasts	fibroblasts	mouse embryonic fibroblasts
MCF7	breast carcinoma	Cell lines, mouse	Cell Type	MC3T3-E1	mouse bone marrow derived
MCF10A	breast carcinoma	NIH3T3	fibroblast	molar mesenchymal	mouse embryonic mesenchymal
Panc-1	pancreatic epithelioid carcinoma	Primary cells, human	Cell Type	cardiomyocytes	rat neonatal cardiomyocytes
PC3	prostate carcinoma	astrocytes	normal		
DU145	prostate carcinoma	C3H10T1/2	mesenchymal		

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

Apoptosis Pathway

1. Krysko, D.V. *et al.*, Apoptosis and necrosis: detection, discrimination and phagocytosis. *Methods*, **44**, 205-21 (2008).
2. Howley, B., and Fearnhead, H.O., Caspases as therapeutic targets. *J. Cell Mol. Med.*, Feb 24 [Epub ahead of print] (2008)
3. Logue, S.E., and Martin, S.J., Caspase activation cascades in apoptosis. *Biochem. Soc. Trans.*, **36 (Pt 1)**, 1-9 (2008).

B Cell Activation

1. Tolar, P. *et al.*, Viewing the antigen-induced initiation of B-cell activation in living cells. *Immunol Rev.*, **221**, 64-76 (2008).
2. Youinou, P., B cell conducts the lymphocyte orchestra. *J. Autoimmun.*, **28**, 143-51. (2007).

Cell Adhesion

1. Ebnet, K., Organization of multiprotein complexes at cell-cell junctions. *Histochem. Cell Biol.*, Mar 26 [Epub ahead of print] (2008).
2. Basson, M.D., An intracellular signal pathway that regulates cancer cell adhesion in response to extracellular forces. *Cancer Res.*, **68**, 2-4 (2008).
3. Mousa, S.A., Cell adhesion molecules: potential therapeutic & diagnostic implications. *Mol. Biotechnol.*, **38**, 33-40. (2008).

Cytokine and Chemokine Receptors

1. Callewaere, C. *et al.*, Chemokines and chemokine receptors in the brain: implication in neuroendocrine regulation. *J. Mol. Endocrinol.*, **38**, 355-63 (2007)
2. Allen, S.J. *et al.*, Chemokine: receptor structure, interactions, and antagonism. *Annu. Rev. Immunol.*, **25**, 787-820 (2007).
3. Zlotnik, A. *et al.*, The chemokine and chemokine receptor superfamilies and their molecular evolution. *Genome Biol.*, **7**, 243 (2006).
4. Mantovani, A. *et al.*, Regulatory pathways in inflammation. *Autoimmun. Rev.*, **7**, 8-11 (2007).

Cytokines and Chemokines

1. Anderson, P. Post-transcriptional control of cytokine production. *Nat. Immunol.*, **9**, 353-9 (2008).
2. Tayal, V., and Kalra, B.S., Cytokines and anti-cytokines as therapeutics--an update. *Eur. J. Pharmacol.*, **579**,1-12 (2008).

Cytoskeleton

1. Dalby, M.J., and Yarwood, S.J., Analysis of focal adhesions and cytoskeleton by custom microarray. *Methods Mol. Biol.*, **370**, 121-34 (2007).
2. Dustin, M.L., Cell adhesion molecules and actin cytoskeleton at immune synapses and kinapses. *Curr. Opin. Cell Biol.*, **19**, 529-33 (2007).

DNA Repair Pathway

1. Hinkal, G., and Donehower, L.A., How does suppression of IGF-1 signaling by DNA damage affect aging and longevity? *Mech. Ageing Dev.*, **129**, 243-53 (2008).
2. Hakem, R., DNA-damage repair; the good, the bad, and the ugly. *EMBO J.*, **27**, 589-605 (2008).
3. Harper, J.W., and Elledge, S.J., The DNA damage response: ten years after. *Mol. Cell.*, **28**, 739-45 (2007).

DUBS - Ubiquitin Hydrolyases

1. Nicholson, B. *et al.*, Deubiquitinating enzymes as novel anticancer targets. *Future Oncol.*, **3**, 191-9 (2007).
2. Millard, S.M., and Wood, S.A., Riding the DUBway: regulation of protein trafficking by deubiquitylating enzymes. *J. Cell Biol.*, **173**, 463-8 (2006).
3. Amerik, A.Y., and Hochstrasser, M., Mechanism and function of deubiquitinating enzymes. *Biochim. Biophys. Acta*, **1695**, 189-207 (2004).

Epigenetic Regulators

1. Esteller, M., Epigenetics in cancer. *N. Engl. J. Med.*, **358**, 1148-59. Review (2008).
2. Grønbaek, K. *et al.*, Epigenetic changes in cancer. *APMIS*, **115**, 1039-59 (2007).

Extracellular Matrix

1. Rees, M.D. *et al.*, Oxidative damage to extracellular matrix and its role in human pathologies. *Free Radic. Biol. Med.*, Apr 8 (2008). [Epub ahead of print]
2. Adair-Kirk, T.L., and Senior, R.M., Fragments of extracellular matrix as mediators of inflammation. *Int. J. Biochem. Cell Biol.*, **40**, 1101-10 (2008).
3. Daley, W.P. *et al.*, Extracellular matrix dynamics in development and regenerative medicine. *J. Cell Sci.*, **121(Pt 3)**, 255-64 (2008).

G-Protein-Coupled Receptors:

1. Thompson, M.D. *et al.*, G protein-coupled receptors disrupted in human genetic disease. *Methods Mol. Biol.*; **448**, 109-37 (2008).
2. Milligan, G., New aspects of G-protein-coupled receptor signalling and regulation. *Trends Endocrinol. Metab.*, **9**, 13-9 (1998).

Helicases

1. Ha, T., Need for speed: mechanical regulation of a replicative helicase. *Cell*, **129**, 1249-50 (2007).
2. Singleton, M.R. *et al.*, Structure and mechanism of helicases and nucleic acid translocases. *Annu. Rev. Biochem.*, **76**, 23-50 (2007).
3. Xi, X.G., Helicases as antiviral and anticancer drug targets. *Curr. Med. Chem.*, **14**, 883-915 (2007).

Ion Channels

1. Cannon, S.C., Physiologic principles underlying ion channelopathies. *Neurotherapeutics*, **4**, 174-83 (2007).

JAK-STAT Pathway

1. Murray, P.J., The JAK-STAT signaling pathway: input and output integration. *J. Immunol.*, **178**, 2623-9 (2007).
2. O'Sullivan, L.A. *et al.*, Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol. Immunol.*, **44**, 2497-506 (2007).

Kinases

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

Nuclear Hormone Receptors

1. Kininis, M., and Kraus, W.L., A global view of transcriptional regulation by nuclear receptors: gene expression, factor localization, and DNA sequence analysis. *Nucl. Recept. Signal*, **6**, e005 (2008).

p53 Pathway

1. Bose, I., and Ghosh, B., The p53-MDM2 network: from oscillations to apoptosis. *J. Biosci.*, **32**, 991-7 (2007).
2. Efeyan, A., and Serrano, M., p53: guardian of the genome and policeman of the oncogenes. *Cell Cycle*, **6**, 1006-10 (2007).
3. Kastan, M.B., Wild-type p53: tumors can't stand it. *Cell*, **128**, 837-40 (2007).

Phosphatases

1. Hendriks, W.J. *et al.*, Protein tyrosine phosphatases: functional inferences from mouse models and human 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).

T Cell Activation

1. Won, J., and Lee, G.H., T-cell-targeted signaling inhibitors. *Int. Rev. Immunol.*, **27**, 19-41 (2008).
2. Brenner, D. *et al.*, Concepts of activated T cell death. *Crit. Rev. Oncol. Hematol.*, **66**, 52-64 (2008).
3. Seminario, M.C., and Bunnell, S.C., Signal initiation in T-cell receptor microclusters. *Immunol. Rev.*, **221**, 90-106 (2008).
4. Lämmermann, T., and Sixt, M., The microanatomy of T-cell responses. *Immunol. Rev.*, **221**, 26-43 (2008).

Tumor Suppressors

1. Vatteemi, E., and Claudio, P.P., Tumor suppressor genes as cancer therapeutics. *Drug News Perspect*, **20**, 511-20 (2007).
2. Berger, J.C. *et al.*, Metastasis suppressor genes: from gene identification to protein function and regulation. *Cancer Biol. Ther.*, **4**, 805-12 (2005).

Ubiquitin Ligases (E1, E2, E3)

1. Cardozo, T., and Pagano, M., Wrenches in the works: drug discovery targeting the SCF ubiquitin ligase and APC/C complexes. *BMC Biochem.*, **8 Suppl 1**, S9 (2007).
2. Newton, K., and Vucic, D., Ubiquitin ligases in cancer: ushers for degradation. *Cancer Invest.*, **25**, 502-13 (2007).
3. Sun, Y., Overview of approaches for screening for ubiquitin ligase inhibitors. *Methods Enzymol.*, **399**, 654-63 (2005).
4. Hershko, A., The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. *Cell Death Differ.*, **12**, 1191-7 (2005).

Limited Use Licenses:

Use of this product for Commercial Purposes requires a license from Sigma-Aldrich Corporation. The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration, but excludes not-for-profit core facilities providing services within their own research institutions at cost. Core facilities are invited to join Sigma-Aldrich's RNAi Partnership Program. Details of Sigma-Aldrich's RNAi Partnership Program can be found at www.sigma.com/rpp.

The MISSION shRNA Library of The RNAi Consortium is produced and distributed under license from the Massachusetts Institute of Technology.

Licensed under Carnegie Institution US Patent 6,506,559 and Massachusetts Institute of Technology and for laboratory and commercial research use only.

This product is licensed under U.S. Pat. Nos. 5,817,491; 5,591,624; 5,716,832; 6,312,682; 6,669,936; 6,235,522; 6,924,123 and foreign equivalents from Oxford BioMedica (UK) Ltd., Oxford, UK, and is provided for use in academic and commercial *in vitro* and *in vivo* research for elucidating gene function, and for validating potential gene products and pathways for drug discovery and development, but excludes any use of LentiVector® technology for: creating transgenic birds for the purpose of producing useful or valuable proteins in the eggs of such transgenic birds, the delivery of gene therapies, and for commercial production of therapeutic, diagnostic or other commercial products not intended for research use where such products do not consist of or incorporate a lentiviral vector.

Information about licenses for commercial uses excluded under this license is available from Oxford BioMedica (UK), Ltd., Medawar Center, Oxford Science Park, Oxford OX4 4GA UK enquiries@oxfordbiomedica.co.uk or BioMedica Inc., 11622 El Camino Real #100, San Diego CA 92130-2049 USA. LentiVector is a registered US and European Community trademark of Oxford BioMedica plc.

This product is licensed under agreement with Benitec Australia Ltd. and CSIRO as co-owners of U.S. Pat. No. 6,573,099 and foreign counterparts, for use in research to understand, diagnose, monitor, treat and prevent human diseases and disorders, including the use of animals for such research use, except that use of ddRNAi as a therapeutic agent or as a method of disease treatment, prevention, diagnosis or for disease monitoring is excluded. Information regarding licenses to these patents for use of ddRNAi as a therapeutic agent or for other uses excluded under this license is available from Benitec at licensing@benitec.com. Information about licenses for the use of ddRNAi in other fields, is available from CSIRO at pi.csiro.au/RNAi.

This product (based upon the lentikat system) is sub-licensed from Invitrogen Corporation under U.S. Patent Nos. 5,686,279, 5,834,256, 5,858,740; 5,994,136; 6,013,516; 6,051,427, 6,165,782, and 6,218,187 and corresponding patents and applications in other countries for internal research purposes only. Use of this technology for gene therapy applications or bioprocessing other than for nonhuman research use requires a license from Cell Genesys, Inc. Please contact Cell Genesys, Inc. at 342 Lakeside Drive, Foster City, California 94404. Use of this technology to make or sell products or offer services for consideration in the research market requires a license from Invitrogen Corporation, 1600 Faraday Ave., Carlsbad, CA 92008.

This product is for non-clinical research use only. It is not to be used for commercial purposes. Use of this product to produce products for sale or for diagnostic, therapeutic or high throughput drug discovery purposes (the screening of more than 10,000 compounds per day) is prohibited. This product is sold under license from Invitrogen Corporation. In order to obtain a license to use this product for these commercial purposes, contact The Regents of the University of California. This product or the use of this product is covered by U.S. Patent No. 5,624,803 owned by The Regents of the University of California.

This Product is covered by US and foreign patent applications or patents and other proprietary intellectual property rights owned by CSHL ("CSHL shRNA IP Rights"), including U.S. Patent Nos. 8,153,776, 8,202,846, 8,383,599, 8,829,264, and EP1546174.

Subject to acceptance and all terms and conditions of this License, sale of the Product to Buyer by Sigma-Aldrich, Co. (acting under its license, an "Authorized Sale") conveys to Buyer only the nonexclusive, nontransferable right (with no right to sublicense) under the shRNA IP Rights to use the Product solely for Customer's internal research purposes, and only at its facility where the Product is delivered by Sigma-Aldrich, Co.

The Product is for research use only and may not be used *in vitro* or *in vivo* for any diagnostic, preventative, therapeutic or vaccine application, or used (directly or indirectly) in humans for any purpose.

Non-Profit Buyers. If Buyer is a Non-Profit Entity, then the following additional restrictions will apply:

Customer obtains no right to use, develop or otherwise exploit the product for any commercial purpose.

Commercial Buyers. If Buyer is a Commercial Entity, then the following additional restrictions will apply:

A Product sale is an Authorized Sale only if Buyer has already entered into a separate written agreement that has been executed by CSHL or Hairpin Technologies, that covers the CSHL shRNA IP Rights, and that is then currently in effect. Any delivery or transfer of Product to Customer outside of an Authorized Sale is void, conveys no implied or express right under this license and Customer will immediately return Product to Sigma-Aldrich for a refund.

"Commercial Entity" means any entity or organization other than a Non-Profit Entity.

"CSHL" means Cold Spring Harbor Laboratory.

"Hairpin Technologies" means Hairpin Technologies, Inc. located at 2200 Smithtown Avenue, Ronkonkoma, NY 11779, www.hairpintechnologies.com.

"Non-Profit Entity" means any college, university or governmental entity (including without limitation, governmental and quasi-governmental institutes and research laboratories), or any non-profit scientific, research or educational organization of the type described in section 501(c)(3) of the Internal Revenue Code or qualified under a state non-profit organization statute.

"Product" means a product (including, without limitation, expression vectors encoding a shRNA, the design, manufacture or use of which (in whole or in part) is the subject of the shRNA IP Rights, and is deemed to include all components, progeny, reproductions, modified versions and other derivatives thereof.

This license is subject to a license from CSHL or Hairpin Technologies, and CSHL and Hairpin Technologies reserves all other rights under its license. For information on licensing rights for Commercial Entities, including use of this product for purposes other than research and trial licenses, please contact Hairpin Technologies, Inc. at info@hairpintechnologies.com or call (631) 881-0844.

MISSION is a registered trademark of Sigma-Aldrich Co. LLC.

TurboGFP is a trademark of Evrogen Co.

PHC,MAM 08/17-1