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Product Information

MISSION® shRNA Plasmid DNA Control Vectors

Catalog Numbers **SHC001, SHC002, SHC003, SHC004, SHC005, SHC007, SHC008, SHC009, SHC010, SHC011, SHC012, SHC013, SHC014, SHC015, SHC016, SHC201, SHC202, SHC203, SHC204, SHC216, SHC312, SHC314, SHC317, SHC332, SHC334, and SHC337**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

RNA interference (RNAi) is a powerful gene-specific silencing mechanism in mammalian cells. The MISSION® product line is a viral-vector-based RNAi library against annotated mouse and human genes. shRNAs that are processed into siRNAs intracellularly are delivered by amphotrophic lentivirus particles, allowing screening in a wide range of mammalian cell types. In these cells, MISSION shRNA clones permit rapid, cost-efficient loss-of-function and genetic interaction screens.

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 (HEK293T) by co-transfection with compatible packaging plasmids, found in MISSION Lentiviral Packaging Mix, Catalog Number SHP001.[1,2] 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,[3] overcoming low transfection and integration difficulties associated with these cell types.

Figure 1 depicts the base vector for all TRC1 and TRC1.5 clones (pLKO.1-puro). Figure 2 depicts the base vector for all TRC2 clones (TRC2-pLKO-puro). The TRC2 vector has a single additional element in comparison to the TRC1 vector. This element is the WPRE,[4] or the Woodchuck Hepatitis Post-Transcriptional Regulatory Element. WPRE allows for enhanced expression of transgenes delivered by lentiviral vectors.[5]

When conducting experiments using MISSION shRNA clones, proper controls are a key element of experimental design to permit accurate interpretation of knockdown results and provide assurance of the specificity of the response observed. The MISSION Control Vectors are lentiviral-based vectors that are useful as both positive and negative controls in experiments using the MISSION shRNA library. The DNA format controls may be used in direct transfection of target cells or they may also be used to create replication-incompetent viral particles.

Sigma's recommended controls for any shRNA experiment are provided in the **Control Selection Table** and are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[6] Please consult the Control Selection Table to select the controls that are most appropriate for your shRNA experiments. The **Quick Reference Guide** provides relevant insert sequence and gene target information specific to each product.

TRC1/TRC1.5 Controls

The TRC1 and TRC1.5 pLKO.1-puro Empty Vector Control Plasmid DNA (SHC001) does not contain a hairpin insert, and is a useful negative control that will not activate the RNA-induced silencing complex, or RISC.

The TRC1 and TRC1.5 pLKO.1-puro Non-Mammalian shRNA Control Plasmid DNA (SHC002), is a negative control containing a sequence that should not target any known mammalian genes, but will engage with RISC. This control may cause some knockdown of tGFP, which should be taken into consideration when working with tGFP expressing cell lines. The TRC1 and TRC1.5 pLKO.1-puro Non-Target shRNA Control Plasmid DNA (SHC016) targets no known genes from any species. These non-mammalian and non-target controls serve as useful references for interpretation of knockdown results.

The TRC1 and TRC1.5 pLKO.1-puro-CMV-TurboGFP™ Positive Control Plasmid DNA (SHC003) contains a gene encoding TurboGFP driven by the CMV (cytomegalovirus) promoter, and can be a useful positive control for measuring transfection efficiency and optimizing shRNA delivery. Alternative fluorophore choices are available in the TRC1 and TRC1.5 pLKO.1-puro vector backbone. These fluorophores are also driven by the CMV promoter, and include TagCFP™ (SHC010), TagYFP™ (SHC011), TagRFP™ (SHC012), and TagFP635™ (SHC013).

Silencing of the CMV promoter may be a problem in some cell types.[7] For these cells, the Ubiquitin C promoter (UbC) can be a viable alternative.[8] Alternative promoter choices are available in the TRC1 and TRC1.5 pLKO.1-puro vector backbone. The UbC-TurboGFP (SHC014) and UbC-TagFP635 (SHC015) controls were generated for these types of applications. Please refer to Figure 3 for corresponding excitation and emission wavelengths.

The shRNA vectors designed against commonly used reporter genes: TurboGFP (SHC004), eGFP (SHC005), and Luciferase (SHC007), are useful as positive controls for knockdown, and can be particularly applicable when working with stably expressing reporter cell lines. Because these vectors do not target any known human or mouse genes, they can also be used as non-targeting controls in many shRNA experiments.

β_2-microglobulin is a MHC Class I molecule present on most cell types.[9] It is commonly used as an endogenous control due to this universal expression. The MISSION pLKO.1-puro B2M shRNA Control Plasmid DNA (SHC008) specifically targets the human β_2-microglobulin gene and reduces expression by ~80% in A549 cells via quantitative RT-PCR analysis.

Rho GDP dissociation inhibitor (GDI) alpha (ARHGDIA) is an ubiquitously expressed protein that acts on Rho GTPases, including RhoA, Rac1, and Cdc42, by keeping these proteins in an inactive state.[10,11] Complete understanding of ARHGDIA's roles is still being elucidated but it is believed to be involved in various signal transduction pathways and cellular cytoskeletal functions. The MISSION pLKO.1-puro ARHGDIA shRNA Control Plasmid DNA (SHC009) specifically targets the human ARHGDIA gene and reduces expression by 90% or more in A549 cells, as verified by both quantitative RT-PCR and Western blot analysis using Anti-Rho-GDI, Catalog Number R3025.

The selected clones for both human positive controls were identified from the existing and available target sets for these genes because they have provided consistent knockdown, which can be useful in experimental optimization.

TRC2 Controls

The MISSION TRC2 pLKO.5-puro Empty Vector Control Plasmid DNA (SHC201) does not contain a hairpin insert and is a useful negative control that will not activate the RNA-induced silencing complex or RISC.

The TRC2 pLKO.5-puro Non-Mammalian shRNA Control Plasmid DNA (SHC202) is a negative control containing a sequence that should not target any known human or mouse gene, but will engage with RISC. This non-targeting control serves as a useful reference for interpretation of knockdown results.

The TRC2 pLKO.5-puro-CMV-TurboGFP Positive Control Plasmid DNA (SHC203) contains a gene encoding TurboGFP driven by the CMV promoter and can be a useful positive control for measuring transfection efficiency and optimizing shRNA delivery.

Also available is the vector containing shRNA to TurboGFP (SHC204). This control is useful as a positive control for knockdown and can be particularly applicable when working with stably expressing reporter cell lines. Because this vector does not target any known human or mouse genes, it can also be used as a non-targeting control in many shRNA experiments.

Inducible Controls
Sigma offers IPTG-inducible shRNA vectors. The pLKO vector has been redesigned to contain a LacI (repressor) and a modified human U6 shRNA promoter with LacO (operator) sequences. In the absence of IPTG (isopropyl-β-D-thio-galactoside), an analogue of lactose, LacI binds to LacO preventing expression of the shRNA. When IPTG is present, the allosteric LacI repressor changes conformation, releasing itself from lacO modified human U6 promoter, and subsequently allows expression of the shRNA.

We are proud to offer two different IPTG inducible vectors for your research. The preferred inducible vector, pLKO_IPTG_3xLacO, contains three lac operon sequences (two in the U6 promoter and one 3′ of the promoter) affording both tight regulation and great gene silencing. Whereas, the pLKO_IPTG_1xLacO vector contains a single lac operon sequence in the U6 promoter, which allows for an advantage to shRNA expression, but looser control of the promoter when not induced.

The 1X and 3X LacO Inducible Non-Target shRNA Plasmid DNA controls (SHC312 and SHC332) are negative controls containing a sequence that should not target any genes in any known species, but will engage with RISC.

The 1X and 3X LacO Inducible shRNA vectors, designed against commonly used reporter genes: TurboGFP (SHC314 and SHC334) and Luciferase (SHC317 and SHC337), are useful as positive controls for knockdown and can be particularly applicable when working with stably expressing reporter cell lines. Because these vectors do not target any known human or mouse genes, they can also be used as non-targeting controls in many shRNA experiments.

Components/Reagents
Each MISSION Control Vector is provided as 10 μg of purified plasmid DNA at a concentration of ~500 ng/μL in 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA.

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.

Storage/Stability
These products are guaranteed to be stable for at least one year after receipt when stored at –20 °C.

Materials suggested but not provided
- Mammalian cells to be transfected or transduced
- ESCORT™ II Transfection Reagent, Catalog Number L6037
- Minimum Essential Medium containing 10% fetal calf serum or growth medium optimized for the specific cell line
- Puromycin dihydrochloride, cell culture tested, Catalog Number P8833
- MISSION Lentiviral Packaging Mix, Catalog Number SHP001
- Anti-Rho-GDI, Catalog Number R3025

Procedures

Transfection

Transfection reagents that exhibit high performance in delivery of plasmid DNA are recommended. Sigma offers ESCORT II Transfection Reagent, Catalog Number L6037.

Seed cells and transfect according to the transfection reagent manufacturer's instructions. Cells should be healthy, free of contamination, proliferating well, and plated at an appropriate density.

Incubation Time Post-Transfection

Incubation time depends on the cell line and the protein being expressed, as well as the vector construct. Untransfected control cells under puromycin selection can be used to determine the post-transfection incubation time required to eliminate non-resistant cells for complete selection. Optimal puromycin concentration for selection should be determined by performing a titration, or Puromycin Kill Curve, in your cell line.

Lentiviral Production

Controls may be co-transfected in packaging cells (HEK293T) with MISSION Lentiviral Packaging Mix (Catalog Number SHP001) to produce self-inactivating replication incompetent viral particles. Seed cells and co-transfect according to the MISSION Lentiviral Packaging Mix Technical Bulletin.

Puromycin Kill Curve

Prior to beginning experiments, determine the concentration of puromycin for target cells by performing a Puromycin Kill Curve.

1. Plate 1.6×10^4 cells into wells of a 96-well plate with 120 µL of fresh medium.
2. The next day replace the medium in the wells with medium containing varying concentrations of puromycin (0, 2, 4, 6, 8, 10 µg/mL).
3. Examine viability of cells 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.

Images

Cells that express fluorescent proteins should be imaged in a darkroom with a microscope capable of detecting fluorescence. Best images are acquired when corresponding channels are used with the microscope.

References

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9. Schardijn, Gus H.C., and L.W. Statius Van Eps, β_2-microglobulin: Its significance in the evaluation of renal function. Kidney International, Vol. 32, pp. 635-641 (1987).
10. Couchman, J.R. et al., RhoGDI: multiple functions in the regulation of Rho family GTPase activities. Biochem J., **390**, 1-9 (2005).
11. Meyer, A-K. et al., Defects in cytokinesis, actin reorganization and the contractile vacuole in cells deficient in RhoGDI. EMBO, **21**(**17)**, 4539-4549 (2002).

Product Quick Reference Guide

Catalog Number Description	**Vector Backbone**	**Insert**	**Insert Sequence / Vector Description**
SHC001 MISSION pLKO.1-puro Empty Vector Control	TRC1/1.5	No hairpin	No shRNA Insert
SHC201 MISSION TRC2 pLKO.5-puro Empty Vector Control	TRC2	No hairpin	No shRNA Insert
SHC002 MISSION pLKO.1-puro Non-Mammalian shRNA Control	TRC1/1.5	Non human or mouse shRNA	CCGG**CAACAAGATGAAGAGCACCAA**CTC-GAG**TTGGTGCTCTTCATCTTGTTG**TTTTT
SHC202 MISSION TRC2 pLKO.5-puro Non-Mammalian shRNA Control	TRC2	Non human or mouse shRNA	CCGG**CAACAAGATGAAGAGCACCAA**CTC-GAG**TTGGTGCTCTTCATCTTGTTG**TTTTT
SHC003 MISSION pLKO.1-puro-CMV-TurboGFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TurboGFP gene, under the control of the CMV promoter. TurboGFP is an improved variant of the green fluorescent protein copGFP cloned from the copepoda *Pontellina plumata*.
SHC203 MISSION TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control	TRC2	No hairpin	No shRNA insert. Contains TurboGFP gene, under the control of the CMV promoter. TurboGFP is an improved variant of the green fluorescent protein copGFP cloned from the copepoda *Pontellina plumata*.
SHC004 MISSION pLKO.1-puro TurboGFP™ shRNA Control	TRC1/1.5	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC204 MISSION TRC2 pLKO.5-puro TurboGFP™ shRNA Control	TRC2	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATC**TTTTT
SHC005 MISSION pLKO.1-puro eGFP shRNA Control	TRC1/1.5	shRNA targeting eGFP	CCGG**TACAACAGCCACAACGTCTAT**CTC-GAG**ATAGACGTTGTGGCTGTTGTA**TTTTT
SHC007 MISSION pLKO.1-puro Luciferase shRNA Control	TRC1/1.5	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT
SHC008 MISSION pLKO.1-puro B2M shRNA Control	TRC1/1.5	shRNA targeting human β_2-micro-globulin	CCGG**CAGCAGAGAATGGAAAGTCAA**CTC-GAG**TTGACTTTCCATTCTCTGCTG**TTTTT
SHC009 MISSION pLKO.1-puro ARHGDIA shRNA Control Plasmid	TRC1/1.5	shRNA targeting human ARHGDIA	CCGG**CAAGATTGACAAGACTGACTA**CTC-GAG**TAGTCAGTCTTGTCAATCTTG**TTTTT
SHC010 MISSION pLKO.1-puro-CMV-TagCFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagCFP gene under the control of the CMV promoter.
SHC011 MISSION pLKO.1-puro-CMV-TagYFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagYFP gene under the control of the CMV promoter.
SHC012 MISSION pLKO.1-puro-CMV-TagRFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagRFP gene under the control of the CMV promoter.

Product Quick Reference Guide (continued)

Catalog Number Description	**Vector Backbone**	**Insert**	**Insert Sequence / Vector Description**
SHC013 MISSION pLKO.1-puro-CMV-TagFP635™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagFP635 gene under the control of the CMV promoter.
SHC014 MISSION pLKO.1-puro-UbC-TurboGFP™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TurboGFP gene under the control of the UbC promoter.
SHC015 MISSION pLKO.1-puro-UbC-TagFP635™ Positive Control	TRC1/1.5	No hairpin	No shRNA insert. Contains TagFP635 gene under the control of the UbC promoter.
SHC016 MISSION pLKO.1-puro Non-Target shRNA Control	TRC1/1.5	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC216 MISSION TRC2 pLKO.5-puro Non-Target shRNA Control	TRC2	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC312 MISSION 1X LacO Inducible Non-Target shRNA Control	IPTG Inducible	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC332 MISSION 3X LacO Inducible Non-Target shRNA Control	IPTG Inducible	Non-target shRNA	CCGG**GCGCGATAGCGCTAATAATTT**CTC-GAG**AAATTATTAGCGCTATCGCGC**TTTTT
SHC314 MISSION 1X LacO Inducible TurboGFP™ shRNA Control	IPTG Inducible	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC334 MISSION 3X LacO Inducible TurboGFP™ shRNA Control	IPTG Inducible	shRNA targeting TurboGFP	CCGG**CGTGATCTTCACCGACAAGAT**CTC-GAG**ATCTTGTCGGTGAAGATCACG**TTTTT
SHC317 MISSION 1X LacO Inducible Luciferase shRNA Control	IPTG Inducible	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT
SHC337 MISSION 3X LacO Inducible Luciferase shRNA Control	IPTG Inducible	shRNA targeting Luciferase	CCGG**CGCTGAGTACTTCGAAATGTC**CTC-GAG**GACATTTCGAAGTACTCAGCG**TTTTT

Control Selection Table

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 Empty Vector Control, Catalog No. SHC001. MISSION TRC2 pLKO.5-puro Empty Vector Control, Catalog No. SHC201. MISSION pLKO.1-puro-CMV-TurboGFP™ Positive Control, Catalog No. SHC003. MISSION TRC2 pLKO.5-puro-CMV-TurboGFP™ Positive Control, Catalog No. SHC203. MISSION pLKO.1-puro-CMV-TagCFP™ Positive Control, Catalog No. SHC010. MISSION pLKO.1-puro-CMV-TagYFP™ Positive Control, Catalog No. SHC011. MISSION pLKO.1-puro-CMV-TagRFP™ Positive Control, Catalog No. SHC012. MISSION pLKO.1-puro-CMV-TagFP635™ Positive Control, Catalog No. SHC013. MISSION pLKO.1-puro-UbC-TurboGFP™ Positive Control, Catalog No. SHC014. MISSION pLKO.1-puro-UbC-TagFP635™ Positive Control, Catalog No. SHC015.
	These vectors can serve as useful negative controls that will not activate the RNAi pathway because they do not contain an shRNA insert. They will allow for observation of cellular effects of the transfection process. Cells transfected with these vectors provide a useful reference point for comparing specific knockdown.
Negative Control: Transfection with non-targeting shRNA	MISSION pLKO.1-puro Non-Mammalian shRNA Control, Catalog No. SHC002. MISSION TRC2 pLKO.5-puro Non-Mammalian shRNA Control, Catalog No. SHC202. MISSION pLKO.1-puro Non-Target shRNA Control, Catalog No. SHC016. MISSION TRC2 pLKO.5-puro Non-Target shRNA Control, Catalog No. SHC216. MISSION 1X LacO Inducible Non-Target shRNA Control, Catalog No. SHC312. MISSION 3X LacO Inducible Non-Target shRNA Control, Catalog No. SHC332.
	The Non-Target shRNA vectors are produced from the sequence-verified lentiviral plasmid vectors containing non-targeting shRNAs. These non-targeting shRNAs are useful negative controls that should activate RISC and the RNAi pathway, but should not target any known human or mouse genes. This allows for examination of the effects of transfection on gene expression. Cells infected with the non-target shRNA will also provide a useful reference for interpretation of knockdown.
Positive Control for transfection: Transfection with positive reporter viral particles	MISSION Control Vectors, Catalog Nos. SHC003, SHC010, SHC011, SHC012, SHC013, SHC014, and SHC015. MISSION TRC2-pLKO-puro CMV-TurboGFP, Catalog No. SHC203.
	These are useful positive controls for measuring transfection efficiency and optimizing shRNA delivery.
Positive Controls for knockdown: Transfection with shRNA targeting reporter gene	MISSION TurboGFP shRNA Control Vector, Catalog No. SHC004. MISSION TRC2-pLKO-puro TurboGFP shRNA Control Vector, Catalog No. SHC204. MISSION 1X LacO Inducible TurboGFP™ shRNA Control, Catalog No. SHC314. MISSION 3X LacO Inducible TurboGFP™ shRNA Control, Catalog No. SHC334.
	The TurboGFP shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets TurboGFP (this TurboGFP shRNA has been experimentally shown to reduce GFP expression by 99.6% in HEK293T cells after 24 hours. Because this shRNA targets TurboGFP, and it does not target any known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.
	MISSION eGFP shRNA Control Vector, Catalog No. SHC005.
	The eGFP shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets eGFP. Because this shRNA targets eGFP (GenBank Accession No. pEGFP U55761), and it does not target any known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.

Control Selection Table (continued)

Recommended Control	Objective
Positive Controls for knockdown: Transfection with shRNA targeting reporter gene (Continued)	MISSION Luciferase shRNA Control Vector, Catalog No. SHC007. MISSION 1X LacO Inducible Luciferase shRNA Control, Catalog No. SHC317. MISSION 3X LacO Inducible Luciferase shRNA Control, Catalog No. SHC337.
	The MISSION Luciferase shRNA vector consists of the pLKO.1-puro vector, containing an shRNA that targets the luciferase from North American Firefly, *Photinus pyralis* (GenBank Accession No. M15077). Because the shRNA targets firefly luciferase, and it does not target any known human or mouse genes, it can also be used as a negative non-targeting control in shRNA experiments.
Positive Controls for knockdown: Transfection with shRNA targeting gene	MISSION shRNA Human Positive Control Vector #1, Catalog No. SHC008.
	The β_2-microglobulin shRNA control consists of the sequence-verified lentiviral plasmid pLKO.1–puro vector containing shRNA that targets human β_2-microglobulin (Catalog No. SHC008). This control will provide clear and measurable knockdown of the human target, typically 80–90% in A549 cells, a human epithelial lung carcinoma cell line.
	MISSION shRNA Human Positive Control Vector #2, Catalog No. SHC009.
	The ARHGDIA shRNA control consists of the sequence-verified lentiviral plasmid pLKO.1–puro vector containing shRNA that targets human Rho GDP Dissociation Inhibitor alpha (Catalog No. SHC009). This control will provide clear and measurable knockdown of the human target, typically 80–90% in A549 cells, a human epithelial lung carcinoma cell line.

Figure 1. TRC1 and TRC1.5 Lentiviral Plasmid Vector pLKO.1-puro Features

Name	Description
U6	U6 Promoter
cppt	Central polypurine tract
hPGK	Human 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

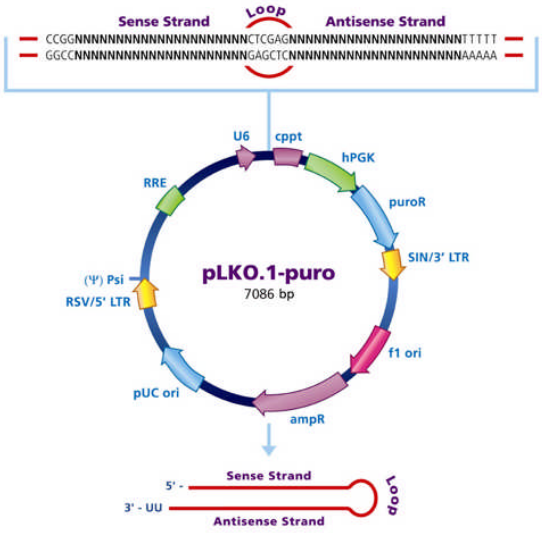


Figure 2. TRC2 Lentiviral Plasmid Vector TRC2-pLKO-puro Features

Name	Description
U6	U6 Promoter
cppt	Central polypurine tract
hPGK	Human phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
WPRE	Woodchuck Hepatitis Post-Transcriptional Regulatory Element
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

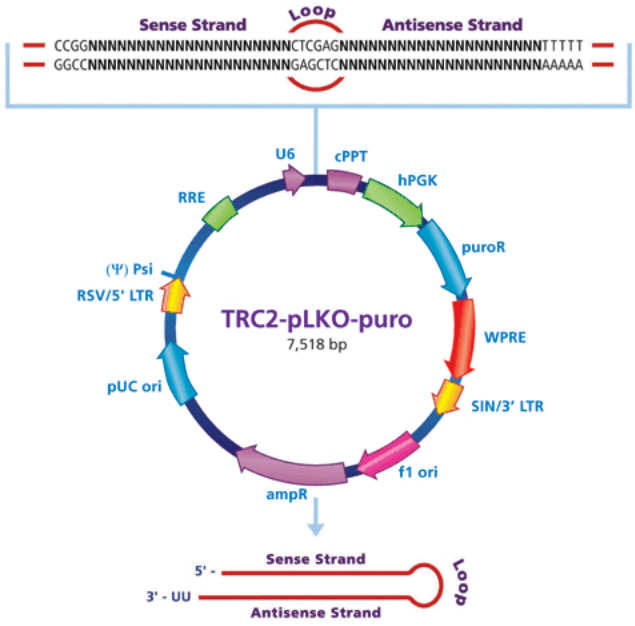
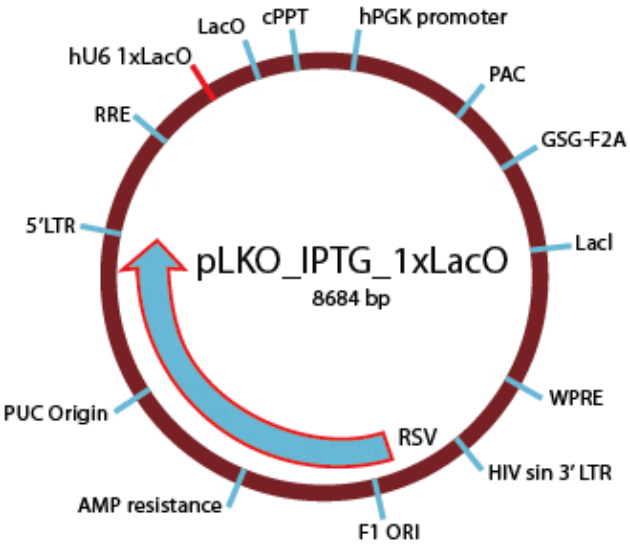


Figure 3. Inducible shRNA Vectors



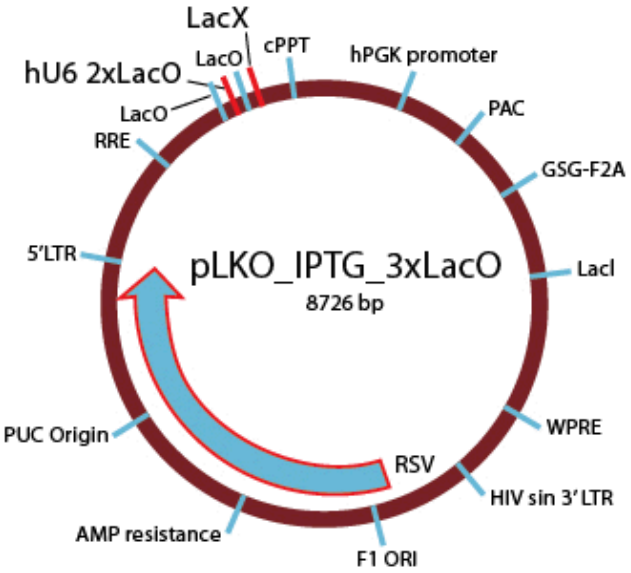
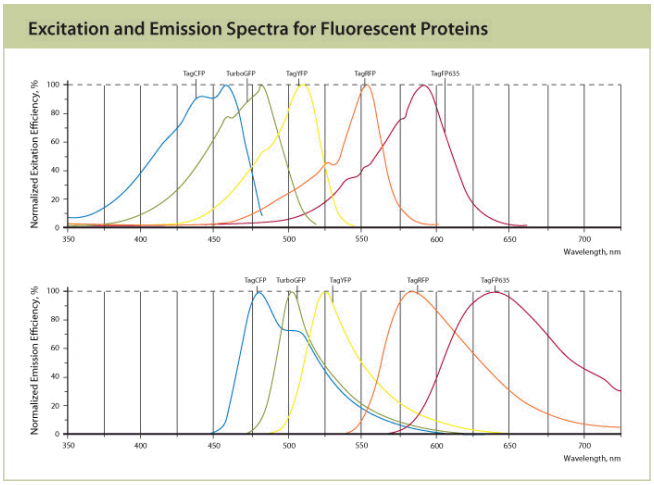


Figure 4.
Excitation and Emission Wavelengths for Fluorescent Proteins

Catalog Number	Fluorophore	Excitation (nm)	Emission (nm)
SHC003/ SHC014	TurboGFP	482	502
SHC010	TagCFP	458	480
SHC011	TagYFP	508	524
SHC012	TagRFP	555	584
SHC013/ SHC015	TagFP635	588	635



Troubleshooting Guide

Problem	Possible Cause	Suggested Solutions
Low transfection efficiency	Volume of transfection cocktail	For optimization, compare transfection performance when different volumes of transfection cocktail are added to the wells (e.g., 75, 100, 120, and 150 µL/well).
	Contaminated DNA	Use a high-quality plasmid preparation method yielding an $OD_{260/280}$ = 1.8–1.85.
		Use endotoxin free DNA. For endotoxin removal, use Endotoxin Removal Solution, Catalog Number E4274.
	Sub-optimal DNA/ Transfection Reagent ratio	Transfection efficiency may be increased by changing the ratio of µg DNA/µL transfection reagent.
	Vector used	In order to achieve an optimal expression rate of the transfected gene, the promoter should be compatible with the cell type.
		Low transfection efficiency results in low expression rates. On the other hand, very high exogenous protein expression levels may be cytotoxic.
		Perform a control transfection.
	Cell growth conditions	If cells have a high passage number, start a new culture from stocks of a lower passage number.
		See that cells were not dramatically stressed during plating procedure or while incubated. See that the medium and serum used are optimal for cell growth.
		Check for the presence of mycoplasma in the cells.
		Ensure that the cells are plated at the optimal density.
	Assay	Use a positive control to ensure that the assay works properly.
Signs of cell cytotoxicity	Expressed protein is toxic to the cells at the current expression level.	If the particular cell type is obligatory, try to express the gene under a different promoter.
	Volume of transfection cocktail	For optimization, compare transfection performance when different volumes of transfection cocktail are added to the wells (e.g., 75, 100, 120, and 150 µL/well). Substitute the medium containing the transfection cocktail with fresh medium 6–24 hours post transfection.
	Contaminated DNA	Use a high-quality plasmid vector.
		For endotoxin free DNA, use Endotoxin Removal Solution, Catalog No. E4274.
	Cells are stressed	Ensure that cells are not dramatically stressed during plating procedure or while incubated.
	Mycoplasma contamination	Check for the presence of mycoplasma in the cells.

Troubleshooting Guide (continued)

Problem	Possible Cause	Suggested Solutions
Transfection efficiency varies between repeats within the same experiment	Cell density and incubation conditions	The density of the cells in the different wells could vary due to clump formation or seeding cells without mixing. Avoid clump formation following trypsinization by repeatedly pipetting the cells. Verify that the plate placed in the incubator is perfectly horizontal and not adjacent to the incubator wall.
	Mycoplasma contamination	Prepare new cells.
	Cell passage number too high	Prepare new cells.
No fluorescent protein detected	Cells need more time to express the fluorescent protein	Protein expression times are cell line dependent; continue viewing fluorescence daily with media changes as needed. Approximately 6 days may be needed to view protein expression.
	Cells need to be imaged in a darkroom	Cells that express fluorescent proteins should be imaged in a darkroom with a microscope capable of detecting fluorescence. Best images are acquired when corresponding channels are used with the microscope.
	Transfection of DNA	Sigma recommends producing virus from the DNA fluorescent protein control vectors in order to view fluorescence post-transduction.

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