

MISSION™ shRNA Libraries

Join The RNAi Consortium (TRC) and Sigma-Aldrich on a Path to Discovery

The MISSION shRNA Libraries, developed by The RNAi Consortium (TRC) at the Broad Institute of MIT and Harvard, are a comprehensive collection of 150,000 pre-cloned shRNA constructs targeting 15,000 annotated human genes and 15,000 annotated mouse genes for RNA interference-mediated transcriptional silencing. At this time, approximately 35,000 clones are currently available targeting 5,300 human and 2,200 mouse genes with additional releases to be made on a quarterly basis. The mission of Sigma-Aldrich and TRC is to make tools for functional genomics research broadly available to scientists worldwide.

Features

- **Comprehensive** — Average of 3–5 shRNA constructs per target gene
- **Economical** — Vector-based system provides a renewable resource compared to siRNA
- **Flexible** — Transient or stable silencing (puromycin selection) for long term expression or phenotypic studies
- **Solutions** — Lentiviral system for transduction of difficult cell lines (non-dividing cells and primary cells)
- **Quality** — All clones are sequence verified and handled by state-of-the-art robotics

The shRNA clones are designed to include a hairpin comprised primarily of 21 base sense/anti-sense regions and a 6 base loop. The hairpin sequences are each cloned into the pLKO.1 vector and sequence verified. Typically, 3–5 shRNA constructs are created for each target gene to provide

varying levels of knockdown (see Figures 1 and 2) and to provide adequate coverage of the target gene.

Features of the pLKO.1-Puro vector allow for transient or stable transfection of the shRNA as well as production of lentiviral particles. Stable gene silencing is selected using the puromycin selectable marker, while viral particles may be produced via co-transfection with packaging plasmids into appropriate cell lines. Compared to siRNA and other vector-based systems, pLKO.1 provides solutions for long-term knockdown and phenotypic observation, transfection of difficult or sensitive cell lines (non-dividing cells or primary cells), and is an economical renewable resource.

Sigma-Aldrich is committed to providing unmatched infrastructure and service for RNAi. With the addition of state-of-the-art robotics, liquid handling, LIMS (Laboratory Inventory Management System), and dedicated laboratories in our new Life Science & High Technology Center, our goal is to provide superior quality and convenient service for your RNAi research needs.

Silencing of MAPK1 Using MISSION shRNA Constructs

Quantitative RT-PCR Results Normalized to GAPDH

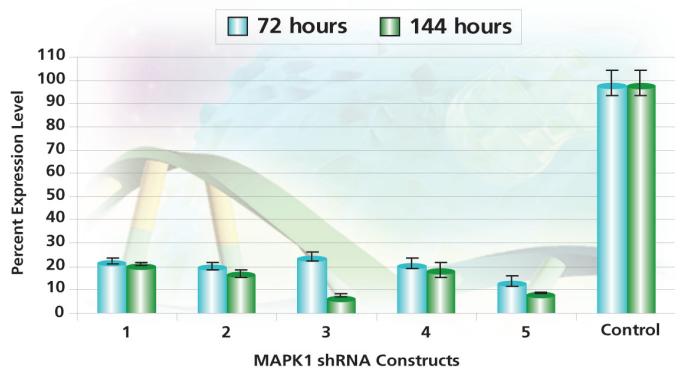


Figure 1. The Human Mitogen-Activated Protein Kinase 1 (MAPK 1, NM_138957) MISSION shRNA set (5 individual hairpins) was used to generate lentiviral particles in packaging cells. HEK 293T cells were infected with the lentiviral particles containing the shRNA sequences. 72 and 144 hours post-infection, total RNA was purified and analyzed with the appropriate TaqMan™ Gene Expression Assay. Results were normalized for input RNA using GAPDH quantitative RT-PCR results. The difference in Ct between infected samples and uninfected control cells along with the efficiency of PCR were used to generate percentage expression levels. Percentage is expressed as a level of the control (100%).

Assays were performed using Sigma's Quantitative RT-PCR ReadyMix™ (QR 0200) supplemented with additional MgCl₂ (M 8787) along with other Sigma reagents (MMLV-RT, M 1427; water, W 1754).



Silencing of JAK 1 Using MISSION shRNA Constructs

Quantitative RT-PCR Results Normalized to GAPDH

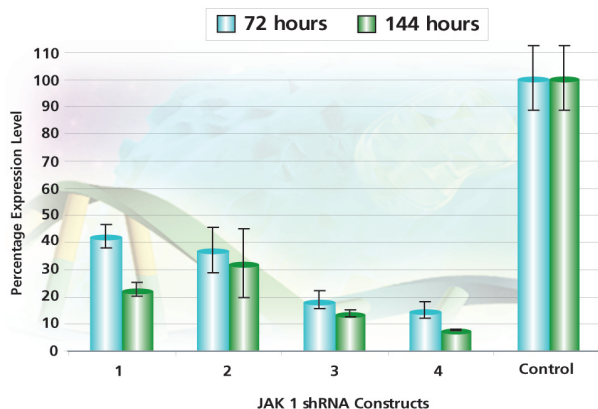


Figure 2. The Human Janus Kinase 1 (JAK 1, NM_002227) MISSION shRNA set (4 individual hairpins) was used to generate lentiviral particles and infect HEK 293T cells as in Figure 1. Total RNA from 72 and 144 hours post-infection was also analyzed using the same procedures as in Figure 1. The results shown indicate the shRNA constructs produce JAK 1 gene silencing compared to control levels.

Glycerol Format Ordering Information:

The libraries are currently offered in a standard frozen bacterial glycerol stock format and are available for ordering online. To search the database, please visit us online at sigma-aldrich.com/missionsearch and enter RefSeq, TRC number, gene symbol, or gene description information for your targets of interest. Information located on these pages will direct you through the simple order process and provide contact information should you need assistance regarding the product or the database.

For immediate questions or concerns, please e-mail us at: RNAi@sial.com.

Check back periodically for additional formats, gene family sets, and updates to the Web search capabilities.

Current Libraries by Gene Family or Functional Class

Gene Family	Human (~5,300 Genes)	Mouse (~2,200 Genes)
Acetylase/deacetylase	33	40
Aminase	21	5
Androgen Receptors	42	-
Carboxylase	4	2
Cyclase	14	9
Cyclin	32	14
Dehydrogenase	212	1
DUB	48	46
GPCR	445	289
G-protein	64	47
Hox	137	18
Ion Channels	30	6
Kinase	508	814
Lipase	13	7
Methylase/demethylase	40	39
NHR	46	48
Oxidase	5	1
Oxygenase	1	1
PH Control	187	-
Phosphatase	198	144
Protein Degradation	392	563
Reductase	33	2
Spliceosome	42	-
Synthase	36	14
Transcription Factors	1,698	77
Transferase	406	-
Transporters	297	3
Tumor Suppressors	86	38
UB(E1/E2/E3)	252	69



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