Einstein shRNA Core Facility
REAGENTS AND INFORMATION

The following table summarizes shRNA and ORF libraries and some of their features (note that TRIPZ clones are not available from the shRNA Core Facility but can be ordered from Open Biosystems):

Library	Library format	Vector backbone	Bacterial selection	Plasmid copy no.	Packaging mix	Fluorescent marker	Mammalian selection	Mammalian promotor(s)
GIPZ shRNA	arrayed	pGIPZ	amp	high	2nd Gen	GFP	puro	CMV
TRIPZ shRNA	arrayed	pTRIPZ	amp	high	2nd Gen	RFP	puro	CMV
TRC shRNA	arrayed	pLKO.1	amp	low	3rd Gen	none	puro	U6, hPGK
Precision LentiORF	arrayed	pLOC	amp	high	2nd Gen	tGFP	blast	CMV
hORFeome V8.1	arrayed	pLX304	amp	low	3rd Gen	none	blast	CMV
Decipher shRNA	pooled	pRSI9	amp	high	3rd Gen	RFP	puro	UbiC
Decipher shRNA	pooled	pRSI12	amp	high	3rd Gen	RFP	puro	UbiC
Decode shRNA	pooled	pGIPZ	amp	high	2nd Gen	GFP	puro	CMV

REAGENT SOURCES

For general information on packaging systems, see http://www.addgene.org/lentiviral/packaging/. Listed here are some places to get 293T cells and packaging reagents for making shRNA or ORF lentivirus:

HEK293T cells

From ATTC:	CRL-11268	HEK 293T/17 cell line	$279
From Open Biosystems (this is what we use):	HCL4517	HEK293T cell line	$272

You can also get 293T cells from Open Biosystems as part of a packaging kit (see below).

Packaging plasmids

GIPZ, TRIPZ, and Precision LentiORF clones can use 2nd-generation packaging plasmids. They DO NOT work with 3rd-generation plasmids. They also work with Open Biosystems reagents (see below).

Two 2nd-generation packaging options from Addgene (each plasmid costs $60):
From the Trono lab: psPAX2 and pMD2.g
From the Weinberg lab: pCMV-dR8.2 and pCMV-VSVG

NOTE: With this system, recombination to produce an infectious HIV particle is possible so you must maintain BSL2+ standards and check your viral preps for virus that can replicate on its own.

TRC and hORFeome V8.1 clones can use either 2nd- or 3rd-generation packaging systems. Since the 3rd-generation system is much safer, this is preferable.

3rd-generation plasmids from Addgene (each plasmid costs $60):
pMDLg/pRRE , pRSV-Rev and pMD2.G

Both types of clones can also be grown with the packaging kit from Open Biosystems.

Trans-Lentiviral Packaging Kit from Open Biosystems:

There are several versions available online:
https://www.openbiosystems.com/Viral%20Packaging/TransLentiviral%20Packaging%20Syst/shRNA/

The most cost-effective for a few small preps are these:

TLP5912	Translentiviral Packaging Kit with Ca Phos — 10 rxn	$750
TLP5917	Translentiviral Packaging Kit with Ca Phos and HEK cells — 10 rxn	$850

PROTOCOLS AND CONTROLS

Here is some information about control and empty vectors for the different libraries we offer:

GIPZ

For the pGIPZ libraries, there are two controls:

- pGIPZ-GAPDH, a positive control, which expresses an shRNA that targets both human, mouse, and rat GAPDH, with demonstrated effectiveness; and
- pGIPZ-NonSilencing, a negative control, which expresses an shRNA designed to not target any mammalian gene. However, every shRNA, including this one, has off-target effects. If the off-target effects of the NonSilencing shRNA affect your assay system, we can advise you on finding a better negative control.

Here are two web sites with some information about pGIPZ:
https://www.openbiosystems.com/rnai/shrnamirlibraries/gipzlentiviralshrnamir/
http://addgene.org/vector-database/2918/

The annotated vector sequence can be found here:
http://www.bioxys.com/i_openbio/pdf/rnai/pGIPZ%20lentiviral%20non-silencing%20shRNAmir.pdf
Note: The hairpin sequence in the above file is *not* the sequence of the non-silencing control. The GIPZ non-silencing shRNAmir control hairpin sequence is as follows: TGCTGTTGACAGTGAGCGATCTCGCTTGGGCGAG AGTAAGTAGTGAAGCCACAGATGTACTTACTCTCGCCCAAGCGAGAGTGCCTACTGCCTCGGA
22mer sense: ATCTCGCTTGGGCGAGAGTAAG 22mer antisense: CTTACTCTCGCCCAAGCGAGAG

Some protocols from Open Biosystems can be found here:
https://www.openbiosystems.com/Viral%20Packaging/TransLentiviral%20Packaging%20Syst/shRNA/

Some additional useful information is available on the Dharmacon website (Open Biosystems and Dharamacon are both part of Thermo Scientific): http://dharmacon.com/empty.aspx?id=3812&imageid=1965

TRC

The TRC library has an empty vector control, i.e. the vector pLKO.1 without any shRNA insert. The second control that is available has an shRNA against GFP. For most cell lines, this can work as a negative control, with the usual caveat that there is always the possibility of off-target effects. For cell lines that have been engineered to express GFP, this would be a positive control.

Info on pLKO.1 is here:
http://addgene.org/8453/

Other info on the TRC library, including protocols, is here (TRC stands for The RNAi Consortium):
http://www.broadinstitute.org/rnai/trc/lib

Precision LentiORF

There is one control available for the Precision LentiORF library, a vector encoding RFP. This vector expresses both GFP (as the normal fluorescent marker) and RFP (as a control ORF). These are cloned into the vecotr pLOC. A map of this vector is here:

https://www.openbiosystems.com/collateral/genomics/pi/Precision%20LentiORFs/Precision_LentiORF_Technical_Manual.pdf

hORFeome V8.1

The hORFeome V8.1 library has a negative control, the pLX304 vector. A useful website for plasmid map and sequence information is here:

http://www.addgene.org/browse/sequence/12128/

The pLX304 vector is part of Invitrogen's Gateway Cloning System. To make a new clone, you must first insert your ORF into an Entry vector. From there, it can be transferred to pLX304 using lamba recombination sites. All necessary reagents can be obtained from Invitrogen. More information about the Gateway system can be found here:

http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cloning/Gateway-Cloning.html