

Albert Einstein College of Medicine OF YESHIVA UNIVERSITY

An Introduction to the shRNA Core Facility John Reidhaar-Olson, Ph.D.

January 25, 2012 RNAi User Group Meeting

shRNA Core Facility

Price 268 • www.einstein.yu.edu/sr/shRNA • shRNA@einstein.yu.edu



John Reidhaar-Olson, Ph.D.

- Ph.D. in Biochemistry from MIT
- Post-doc at UCSF
- >15 years in biotech and pharma, most recently at Roche
- Expertise in RNAi-based screening, RNA therapeutics, genomics





Deborah Smith, Ph.D.

- Ph.D. in Molecular Biology from NYU
- Post-doc at Sloan-Kettering
- Worked at high-throughput screening centers at Columbia and Yale
- Expertise in assay development, screening, and RNAi

Deyan Tong, M.S.

- Master's degree from University of Wisconsin-Madison
- Experience with lentivirus cloning, preparation and transduction

Applications

The shRNA Core Facility is dedicated to providing researchers with access to reagents, expertise, and infrastructure to enable RNAi-based loss-of-function studies at scales ranging from individual genes to genome scale.



siRNA and shRNA as means to silence gene expression



siRNA

- straightforward process
- highly effective knockdown
- effect is transient
- some cell types cannot be transfected
- limited to arrayed screens

shRNA

- more involved process
- broadly applicable to most cell types
- stable knockdown
- less off-target silencing
- allows for marker to follow transduction
- compatible with both arrayed screens and pooled selections

shRNA libraries at Einstein

Arrayed libraries

- Human
 - The RNAi Consortium (TRC) genome-wide shRNA collection
 - GIPZ lentiviral shRNAmir library (release 6.1–6.28)
 - Human Precision LentiORF Library
- Mouse
 - TRC genome-wide shRNA collection
 - GIPZ lentiviral shRNAmir library (release 7.1–7.17)

Pooled libraries

- Human
 - Decode RNAi-GIPZ whole genome pooled screening library
 - Decipher Human shRNA Libraries, modules 1, 2, and 3



TRC libraries



Human

- 18,000 genes
- ~4 shRNA clones per gene

Mouse

- 15,000 genes
- ~4–5 shRNA clones per gene

Rat (from human and mouse libraries)

- 10,000 genes
- ~2 shRNA clones per gene

Developed by The RNAi Consortium based at the Broad Institute

shRNA	shRNA hairpin
U6	RNA Pol III promoter
Puro ^R	Puromycin resistance for
	selection in mammalian cells

Root, D. E., Hacohen, N., Hahn, W. C., Lander, E. S. and Sabatini, D. M. (2006) Genome-scale loss-of-function screening with a lentiviral library. *Nature Methods* 3:715–719.

Moffat J, Grueneberg DA, Yang X, Kim SY, Kloepfer AM, Hinkle G, Piqani B, Eisenhaure TM, Luo B, Grenier JK, Carpenter AE, Foo SY, Stewart SA, Stockwell BR, Hacohen N, Hahn WC, Lander ES, Sabatini DM, Root DE. (2006) A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 124:1283.

GIPZ library





Human

- 18,000 genes
- ~5 shRNA clones per gene

Mouse

- 15,800 genes
- ~3 shRNA clones per gene

Rat (from human and mouse libraries)

- 10,000 genes
- ~2 shRNA clones per gene

Developed by Greg Hannon and Steven Elledge

- shRNAmir shRNA hairpin with mir-30 loop and context sequences to improve Drosha processing
- tGFP turbo-GFP marker to track expression in transduced cells
- CMV RNA Pol II promoter
- Puro^R Puromycin resistance for selection in mammalian cells

Silva JM, Li MZ, Chang K, Ge W, Golding MC, Rickles RJ, Siolas D, Hu G, Paddison PJ, Schlabach MR, Sheth N, Bradshaw J, Burchard J, Kulkarni A, Cavet G, Sachidanandam R, McCombie WR, Cleary MA, Elledge SJ, Hannon GJ. (2005) Second-generation shRNA libraries covering the mouse and human genomes. Nature Genetics 11:1281.

LentiORF library



Human

- Currently 6, 192 genes
- One ORF clones per gene

Based on content from the sequence-validated **ORFeome Collaboration collection**

Gateway vector format

ORF	Open reading frame
CMV	RNA Pol II promoter
tGFP	turboGFP marker
Blast ^R	Blasticidin resistance for selection in
	mammalian celis

Clones do not contain the naturally-occuring stop codon, to facilitate cloning of fusion tags

Sc	ource (ORF) Clone L	ibrary Information				cle	oning Variat	into Le ble by	entiORF v source clo	vector one)							Lenti	ORF V	ector Se	equence	
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DFCI-CCSB	pDONR223	pENTR223	ORF-						TAC	CCA	ACT			TAC	AAA			GCT	AGC		
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		(KnR)						G						Y	К			А	s		
MGC	pDONR223.1	pENTR223.1	ORF-	1	TCA	GGC	СТС	ATG	GGC	CCA	GCT			TAC	AAA			GCT	AGC		
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shRNA Facility services

Reagent distribution from our shRNA and LentiORF libraries

- Glycerol stocks
- Plasmid DNA
- Lentivirus
 - Viral supernatant (>10⁶ TU/ml)
 - High-titer viral stock (>10⁸ TU/ml)
- Assessment of knockdown or overexpression by branched DNA assay

RNAi expertise

• Experimental design and data analysis

Screening services

- Arrayed shRNA sets, provided as lentiviral preps
 - Pre-defined shRNA sets (e.g. to common pathways)
 - Custom sets
- Pooled screens
 - Genome-wide or large subset screens using pooled libraries
 - Custom pooled screening sets
- Assay support for screens
 - Plate reader and high content assays
- Hit identification assistance for pooled screens



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QuantiGene branched DNA assay from Panomics For quantitation of mRNA following shRNA-mediated knockdown





U2OS cells transduced with lentivirus containing the pGIPZ vector encoding GFP and shRNA targeting GAPDH



shRNA-mediated GAPDH knockdown in U2OS cells. Error bars represent standard deviations of technical triplicates.

Ordering shRNA reagents

Contact us (shRNA@einstein.yu.edu) with a list of genes you are interested in targeting

- Species (human, mouse, or rat)
- Library (GIPZ or TRC)
- Gene provide gene symbol (e.g. GAPDH or ACTB) and gene ID (e.g. 2597 or 60)

We will send you a list of available clones and an order form

Einstein shRNA Core Facility

Click Oligo ID for more information from TRC or Open Biosystems websites. Click accession numbers to open RefSeq records. An "S" in the "Alignment" column indicates a possible off-target effect arising from the **sense** strand. **Do not delete hidden columns**

Human clones													
						Top BLAST hit to			Fop BLAST hit to other		Top BLAST hit to other		
Gene Symbol	Gene ID	Catalog Number	Oligo ID	Production Note	Sense Sequence	desired mRNA	Alignment	Target site	mRNA	Alignment	RNA (e.g. ncRNA)	Alignment	Library
ACTB	60	RHS4430-100994873	V3LHS_358210		AGCACAATGAAGATCAAGA	NM_001101.3	19/19	1051 - 1069	NM_001199954.1	18/19	NR_037688.1	18/19	Hs_GIPZ
ACTB	60	RHS4430-98854611	V2LHS_233110		CATTGCTTTCGTGTAAATT	NM_001101.3	19/19	1578 - 1595	NM_005708.3	16/19	XR_108735.2	19/19 S	Hs_GIPZ
ACTB	60	RHS4430-99139928	V2LHS_94242		GAAATCGTGCGTGACATTA	NM_001101.3	19/19	703 - 721	NM_001099771.2	17/19	XM_929706.4	17/19	Hs_GIPZ
ACTB	60	RHS4430-100991313	V3LHS_358211		TGGGACGACATGGAGAAAA	NM_001101.3	19/19	319 - 337	NM_001199954.1	17/19	NR_037688.1	17/19	Hs_GIPZ
ACTB	60	RHS4430-100995794	V3LHS_358208		CCCAGCACAATGAAGATCA	NM_001101.3	19/19	1048 - 106 <mark>6</mark>	NM_024068.3	16/19 S	NR_037688.1	18/19	Hs_GIPZ
ACTB	60	RHS4430-98521746	V2LHS_262423		CAAATATGAGATGCATTGT	NM_001101.3	18/19	1465 - 148 <mark>3</mark>	NM_006197.3	15/19	XR_108735.2	18/19 S	Hs_GIPZ
ACTB	60	RHS3979-9596821	TRCN0000029413		CGAAACTACCTTCAACTCCAT	NM_001101.3	21/21	909 - 929	NM_001099771.2	20/21	NR_004845.1	21/21	Hs_TRC
ACTB	60	RHS3979-9596818	TRCN0000029410		CGTGCGTGACATTAAGGAGAA	NM_001101.3	21/21	708 - 728	NM_017450.2	17/21	NR_045673.1	15/21	Hs_TRC
ACTB	60	RHS3979-9596820	TRCN0000029412		GTTGCTATCCAGGCTGTGCTA	NM_001101.3	21/21	484 - 504	NM_003511.2	15/21	NR_002929.2	14/21	Hs_TRC
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Ordering shRNA reagents

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Bacterial glycerol stock

- Individual clones: \$20 each
- Multi-clone discount: \$10 per clone

Plasmid DNA (>1 µg)

- Individual clones: \$80 each
- Multi-clone discount: \$40 per clone

Viral supernatant (200 μ l at >106 TU/ml; titer will be determined for each clone)

- Individual clones: \$120 each
- Multi-clone discount: \$60 per clone

Concentrated viral stock (50 μl at >108 TU/ml;

titer will be determined for each clone)

- Individual clone: \$300 each
- Multi-clone discount: \$150 per clone

Assessment of knockdown

- Per-gene probe set charge: \$120
- Per-plate branched DNA assay charge: \$700

A **multi-clone discount** is offered when four or more clones are ordered in a given format for the same gene target from the same library.

Controls are available at the multi-clone discount price, regardless of the number of other clones ordered. Prices subject to change. Check website for current pricing.

Available controls

TRC libraries

- Empty pLKO.I vector (no shRNA insert)
- pLKO.1 clone targeting eGFP (can function as positive control or as non-targeting control)

GIPZ libraries

- Non-silencing control
- GAPDH shRNAmir (positive control)

LentiORF library

• pLOC clone encoding RFP (in addition to the GFP reporter)

Suggested practices

Test several shRNAs for each gene of interest

- Aim for at least two giving the phenotype of interest
- Make sure the functional shRNAs are independent (i.e. non-sequence-overlapping)

Include appropriate negative controls

- Non-targeting shRNA
- Non-transduced cells

Confirm knockdown at the protein or mRNA level

• Western blot, qRT-PCR, branched DNA

Confirm knockdown and phenotype in additional cell types, when possible

Consider rescue experiments when feasible

• Use an shRNA-resistant lentiORF to test for reversal of phenotype

shRNA Core Facility equipment





EnVision plate reader



High-content imaging system (later in 2012)





Contact

john.olson@einstein.yu.edu or shRNA@einstein.yu.edu 718-678-1195 • Price 275 (office), 268 (lab) www.einstein.yu.edu/sr/shRNA

