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Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

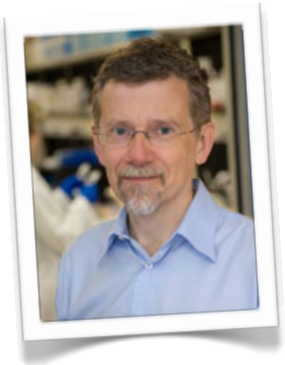
An Introduction to the shRNA Core Facility

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.

SCALE single gene → whole genome

APPLICATIONS

- specific biological question
- assay development
- target validation

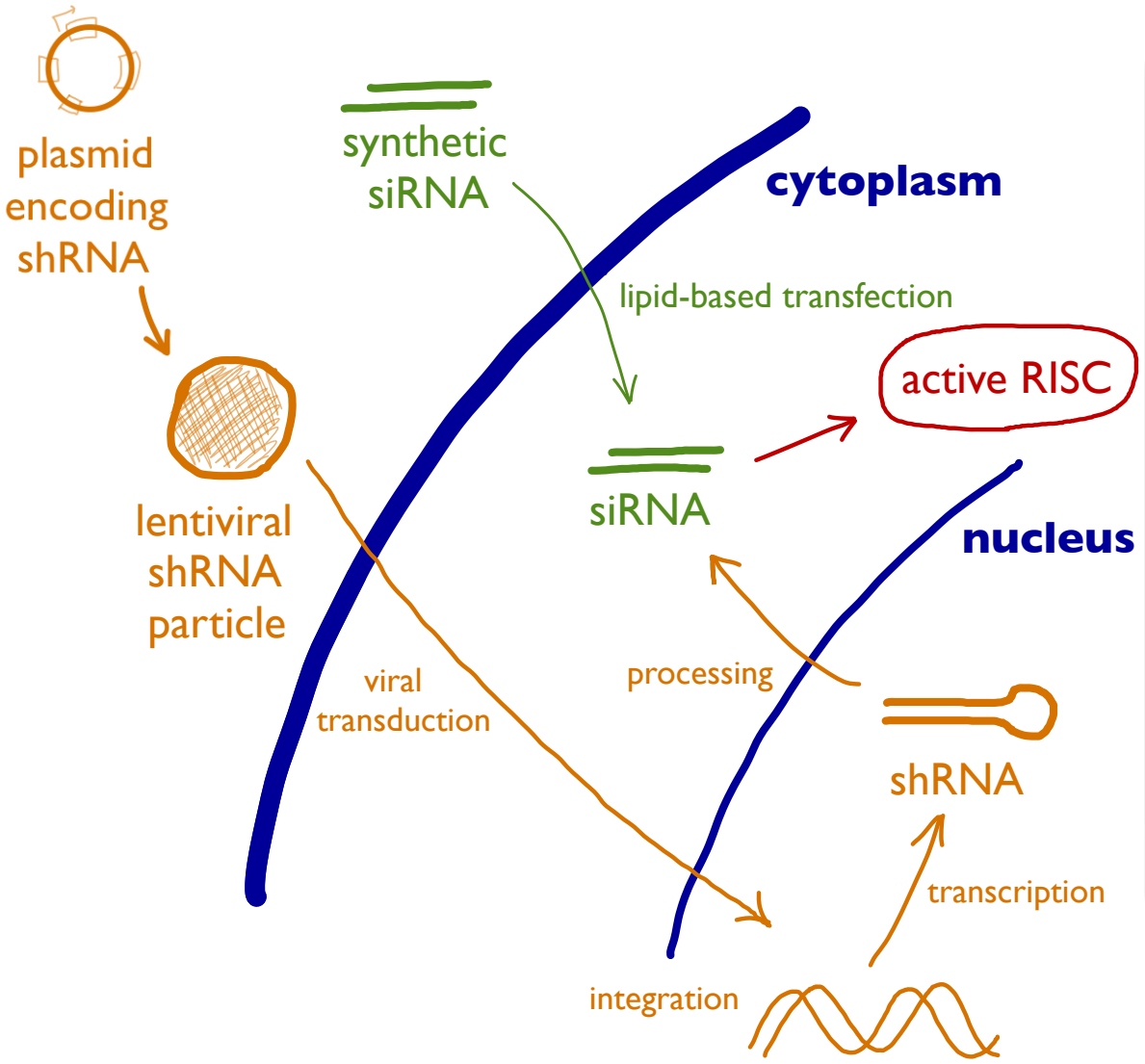
- pathway biology
- gene families
- gene prioritization

- novel biology
- novel drug target identification
- hypothesis generation

FORMAT

individual reagents | arrayed screens | pooled screens

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

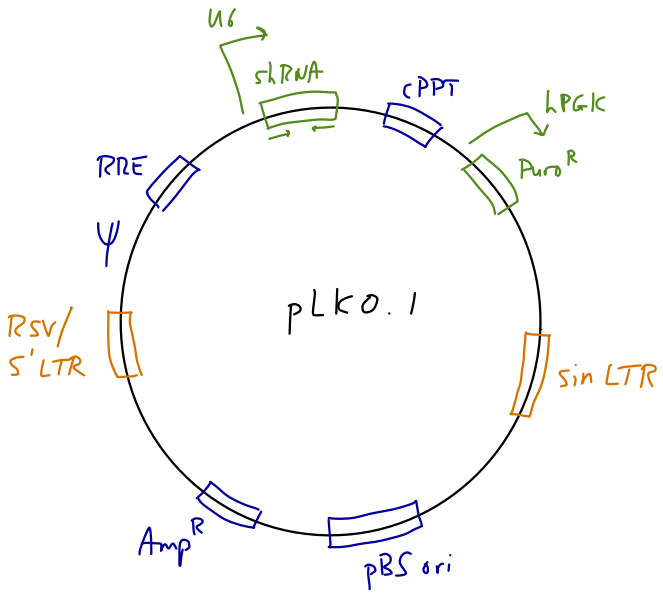
Arrayed libraries

- 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

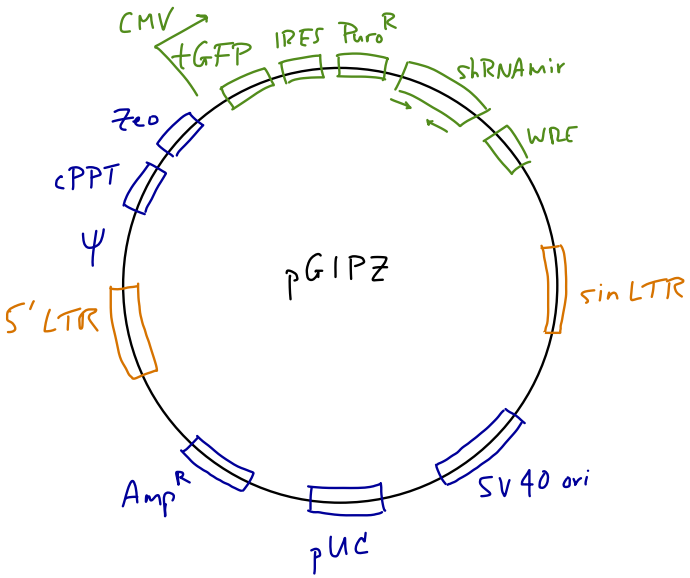
Developed by The RNAi Consortium based at the Broad Institute

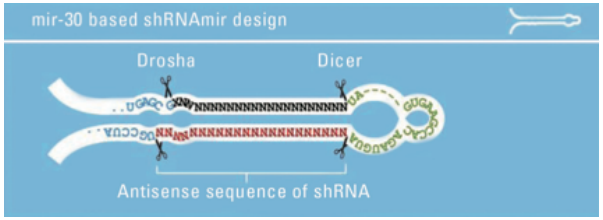
shRNA	shRNA hairpin
U6	RNA Pol III promoter
Puro^R	Puromycin resistance for selection in mammalian cells

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

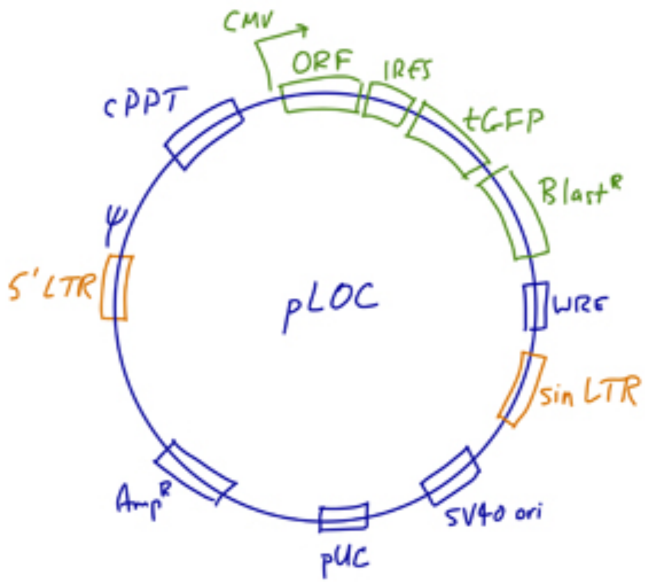
Rat (from human and mouse libraries)
- 10,000 genes
- ~2 shRNA clones per gene

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 cells

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

Source (ORF) Clone Library Information			ORF and ORF vector sequence transferred with ORF during Gateway cloning into LentiORF vector (Variable by source clone)			LentiORF Vector Sequence		
Clone Source	**Donor vector**	**Entry vector**	**ORF-**	**Vector sequence (from ORF clone)**	**att site**	**Vector**	**Fusion tag cloning**	**Synthetic STOP**
DFCI-CCSB	pDONR223	pENTR223 SpnR	ORF-	TGC C	CCA ACT TTC TTG TAC P T F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DFCI-CCSB	pDONR223	pENTR223 SpnR	ORF-	TAC Y	CCA ACT TTC TTG TAC P T F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DFCI-CCSB (ORFeome 5)	pDONR223	pENTR223 SpnR	ORF-	TTG L	CCA ACT TTC TTG TAC P T F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DFCI-CCSB (ORFeome 1&3)	pDONR223	pENTR223 SpnR	ORF-		CCA ACT TTC TTG TAC P T F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
WTSI	pDONR223	pENTR223 SpnR	ORF-		CCA ACT TTC TTG TAC P T F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
HIP	pDONR221§	pENTR221 KnR	ORF-	TTG GAC L D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
Kazusa	pDONR201§	pENTR201 KnR	ORF-	TAC GTA GAC Y V D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	pDONR221§	pENTR221 (KnR)	ORF-	TGG ATC CAC W I H	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	pDONR221§	pENTR221 (KnR)	ORF-	AAG CTT GAC K L D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	pDONR221§	pENTR221 (KnR)	ORF-	TGT ATT CAC C I H	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	pDONR221§	pENTR221 (KnR)	ORF-	GGC GAC G D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	pDONR201§	pENTR201 (KnR)	ORF-	GGC GAC G D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
MGC Synthetic	pDONR223.1	pENTR223.1 SpnR	ORF-	TCA GGC CTC ATG GGC S G L M G	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *
DKFZ	None	pENTR/D- (KnR)	ORF-	AGG GGT GGG CGC GCC GAC K G G R A D	CCA GCT TTC TTG TAC P A F L Y	AAA GTG GTT K V V	GCT AGC A S	TAA TGA * *

shRNA Facility services

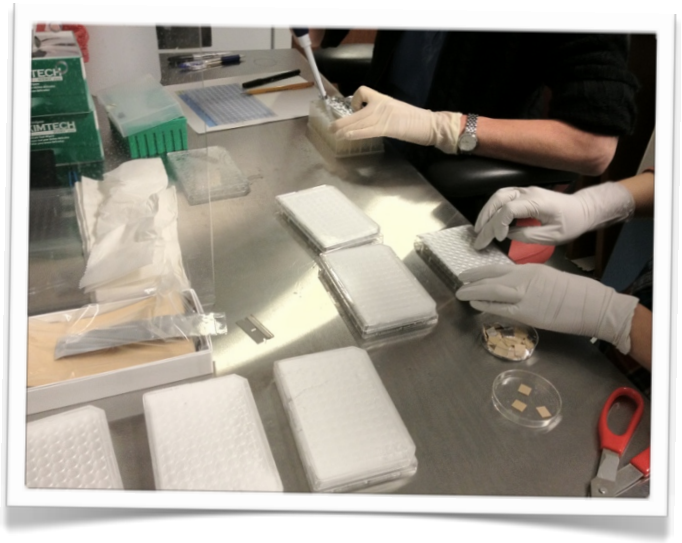
CURRENT

Reagent distribution from our shRNA and LentiORF libraries

- Glycerol stocks
- Plasmid DNA
- Lentivirus
  - Viral supernatant (>10^6 TU/ml)
  - High-titer viral stock (>10^8 TU/ml)
- Assessment of knockdown or overexpression by branched DNA assay

RNAi expertise

- Experimental design and data analysis



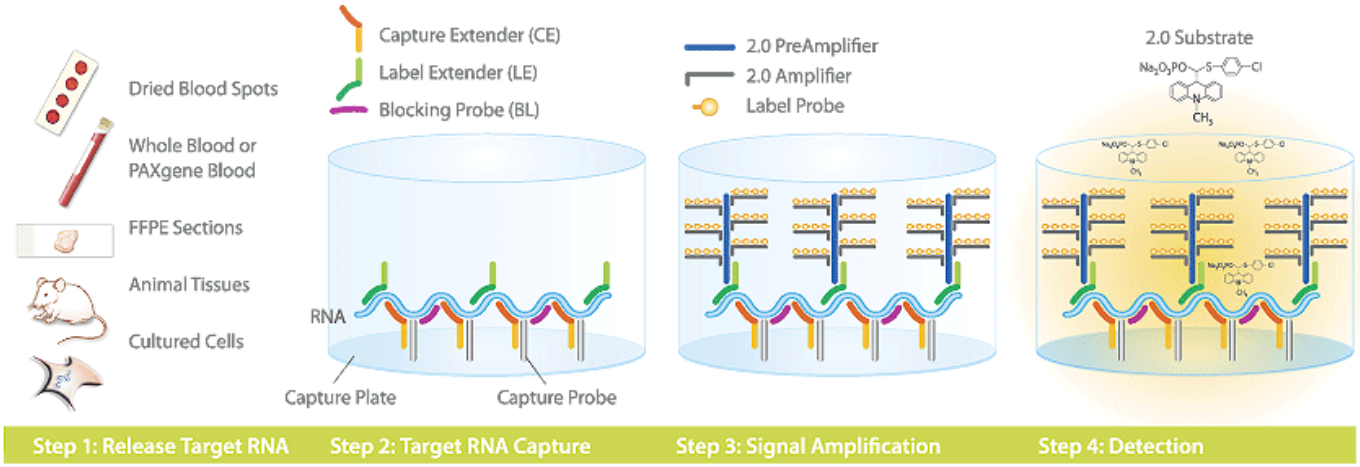
FUTURE

Screening services

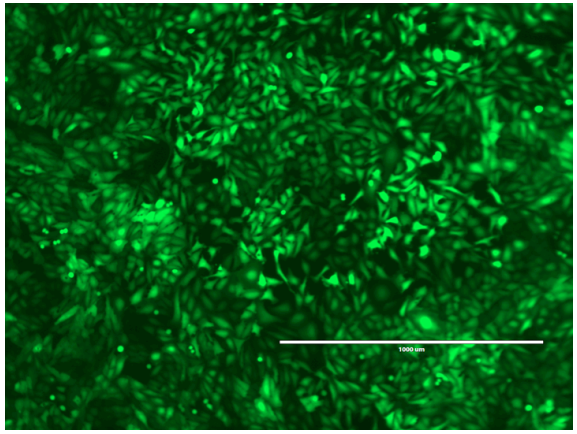
- Arrayed shRNA sets, provided as lentiviral preps
  - Pre-defined shRNA sets (e.g. to common pathways)
  - Custom sets
- Pooled screens
  - Genome-wide or large subset screens using pooled libraries
  - Custom pooled screening sets
- Assay support for screens
  - Plate reader and high content assays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

QuantiGene branched DNA assay from Panomics

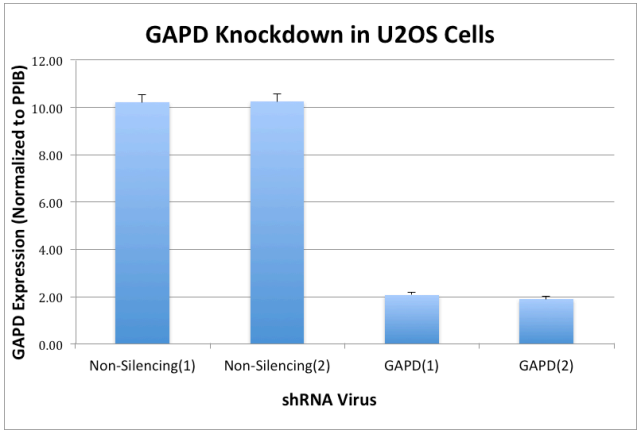
For quantitation of mRNA following shRNA-mediated knockdown



panomics.com



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

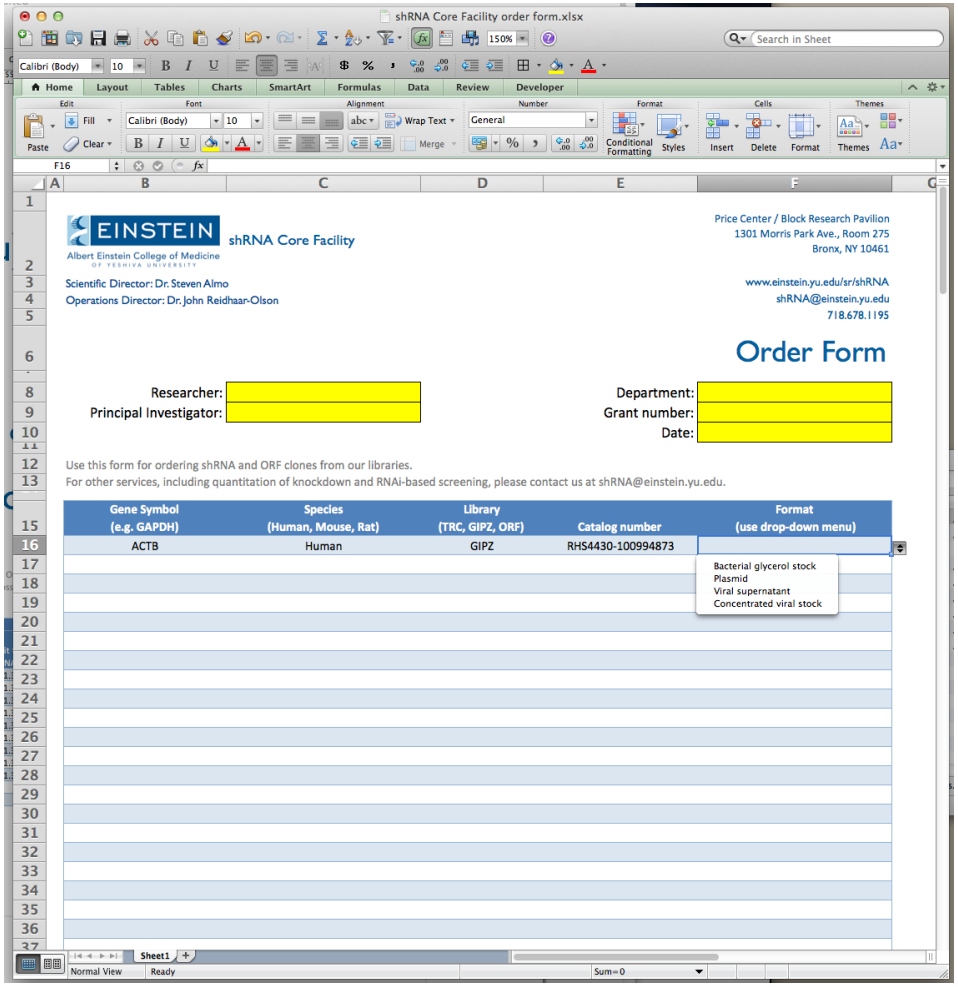
Einstein shRNA Core Facility

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

Gene Symbol	Gene ID	Catalog Number	Oligo ID	Production Note	Sense Sequence	Top BLAST hit to desired mRNA	Alignment	Target site	Top BLAST hit to other mRNA	Alignment	Top BLAST hit to other 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 - 1596	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 - 1066	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 - 1483	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

Ordering shRNA reagents



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 µl 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.1 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

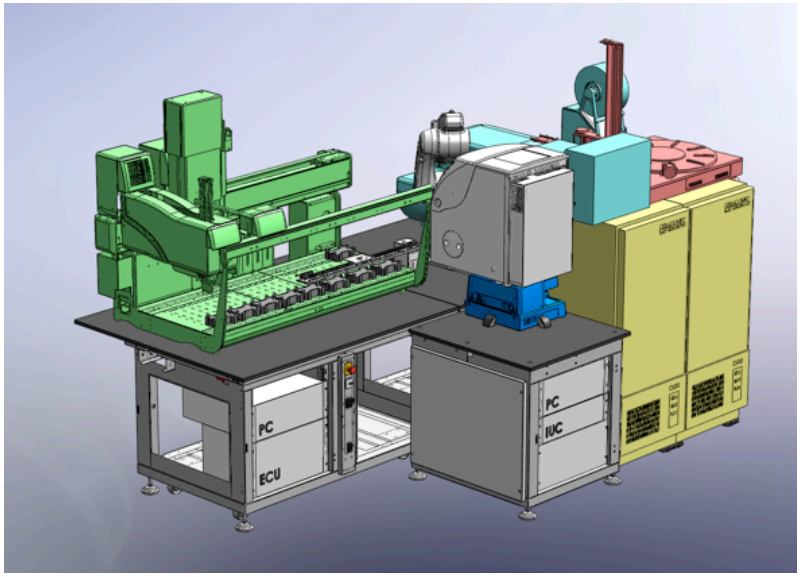
- Western blot, qRT-PCR, branched DNA

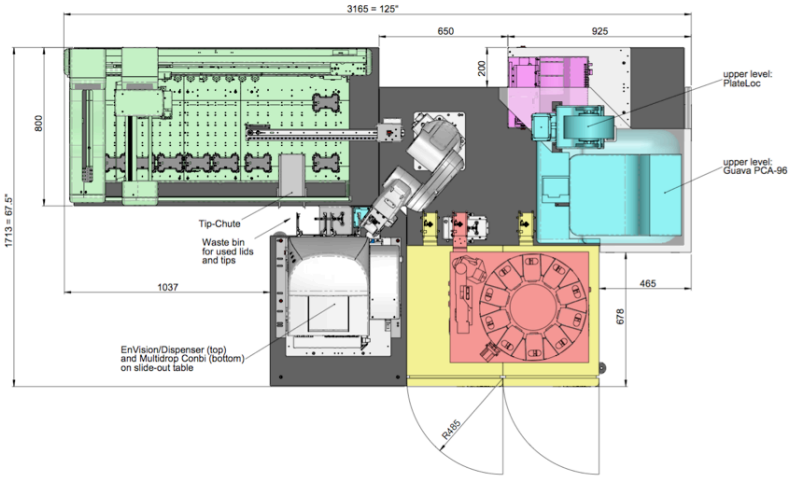
Confirm knockdown and phenotype in additional cell types, when possible

Consider rescue experiments when feasible

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

shRNA Core Facility equipment

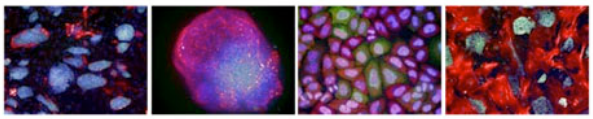




Robotics system with biosafety enclosure

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