An Introduction to the shRNA Core Facility

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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

Diagram:
- plasmid encoding shRNA
- lentiviral shRNA particle
- synthetic siRNA
- lipid-based transfection
- cytoplasm
- active RISC
- nucleus
- viral transduction
- processing
- transcription
- integration
- transduction
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
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
PuroR       Puromycin resistance for selection in mammalian cells


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

PuroR   Puromycin resistance for selection in mammalian cells

LentiORF library

- 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\(\text{\textsuperscript{R}}\): Blasticidin resistance for selection in mammalian cells

Clones do not contain the naturally-occurring stop codon, to facilitate cloning of fusion tags
shRNA Facility services

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

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

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

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**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**

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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 shRNA (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)

Robotics system with biosafety enclosure
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