



Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

shRNA: The Basics

Deborah Smith, Ph.D.

February 29, 2012

RNAi User Group Meeting

shRNA: The Basics

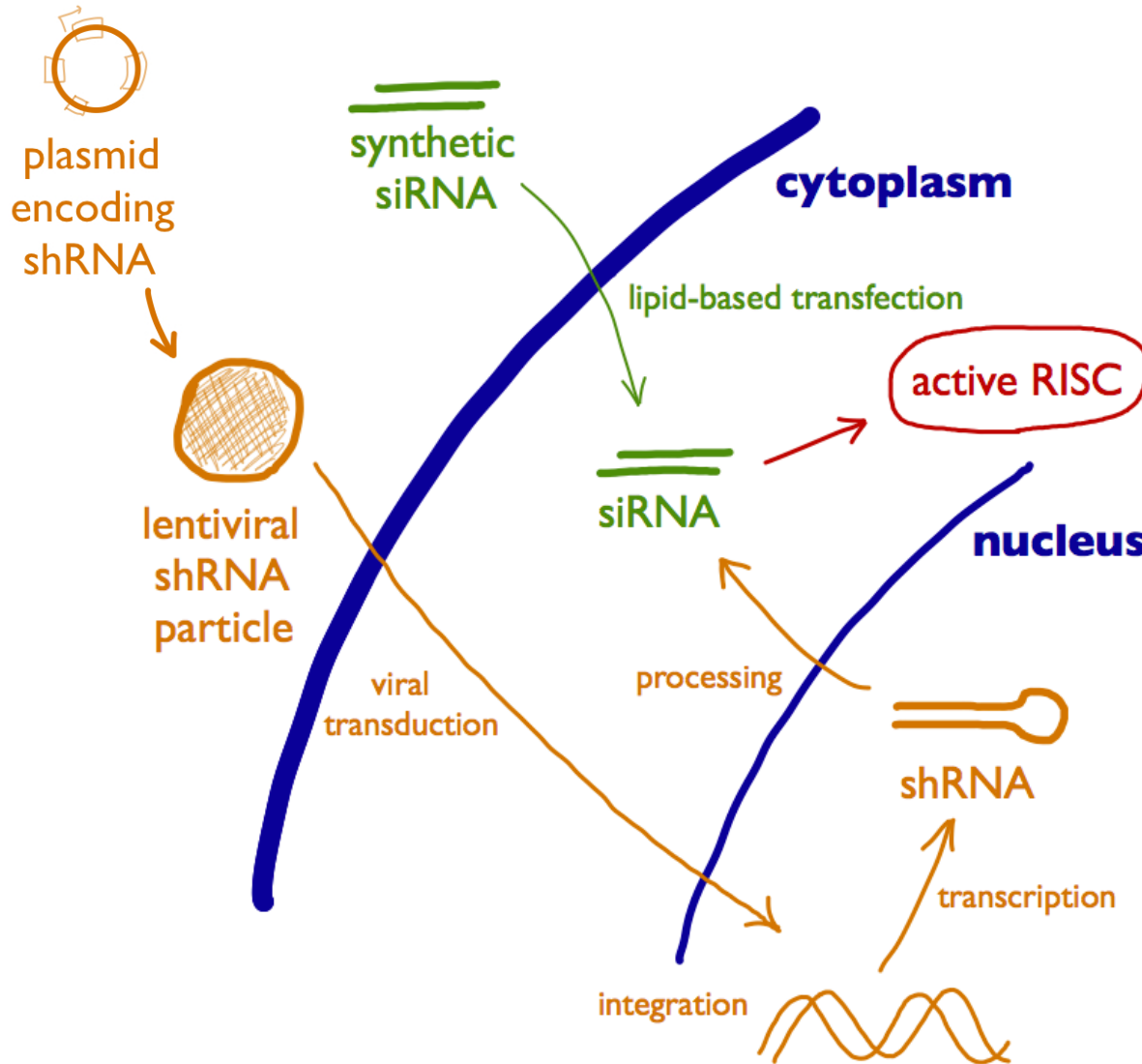
- **What is shRNA and how does it work?**
- **Off-target effects**
- **Practical aspects of choosing shRNA clones**

Goals:

Find shRNAs that effectively silence the target

Control for off-target effects

shRNA can silence gene expression

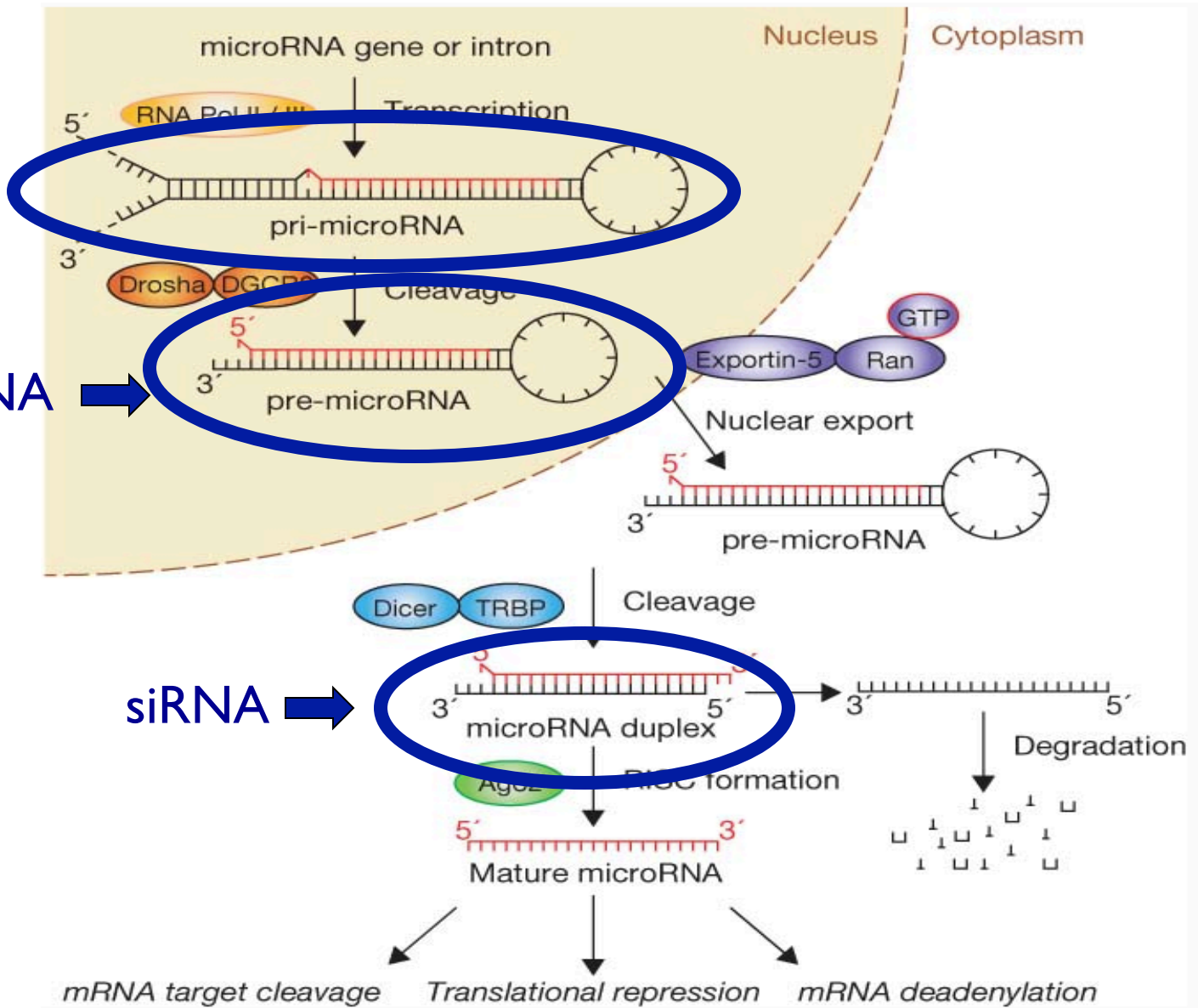


This pathway of RNAi exists primarily to process endogenous microRNAs

What is microRNA?

- **> 1000 miRNAs in Human Genome**
- **Highly conserved**
- **Each miRNA regulates 10's to 100's of genes**
- **Functions to fine-tune levels of protein expression**

shRNA-mir



shRNA

siRNA

mRNA target cleavage Translational repression mRNA deadenylation

microRNA seed region

- ```

1. UUACACUCUCUUUUAAUCCAACUCAGGGA
 | | | | | | | | | | | | | | | |
 AGUGUGA ----- ACUCCAGAGUCCCU

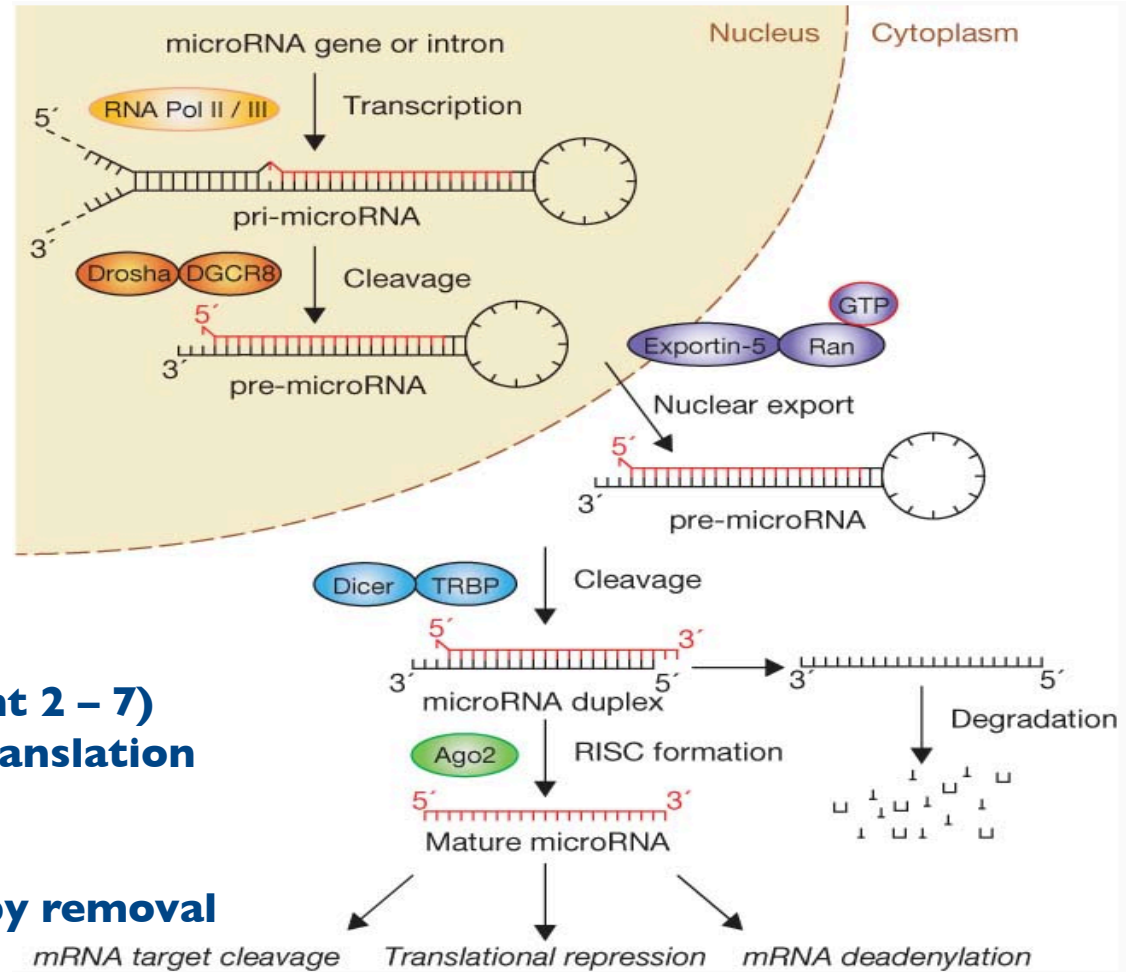
```
- ```

2. UCAUUGAACU - CAGGAAUUUCUUCUACCUCAGGGA
   | | | | | | | | | | | | | | | |
   AGUG --- UGAACUCC ----- AGAGUCCCU
            
```
- ```

3. ACUCACA --- ACCAACUCAGGGA
 | | | | | | | | | | | | | |
 AGUGUGAACUCCAGAGUCCCU

```

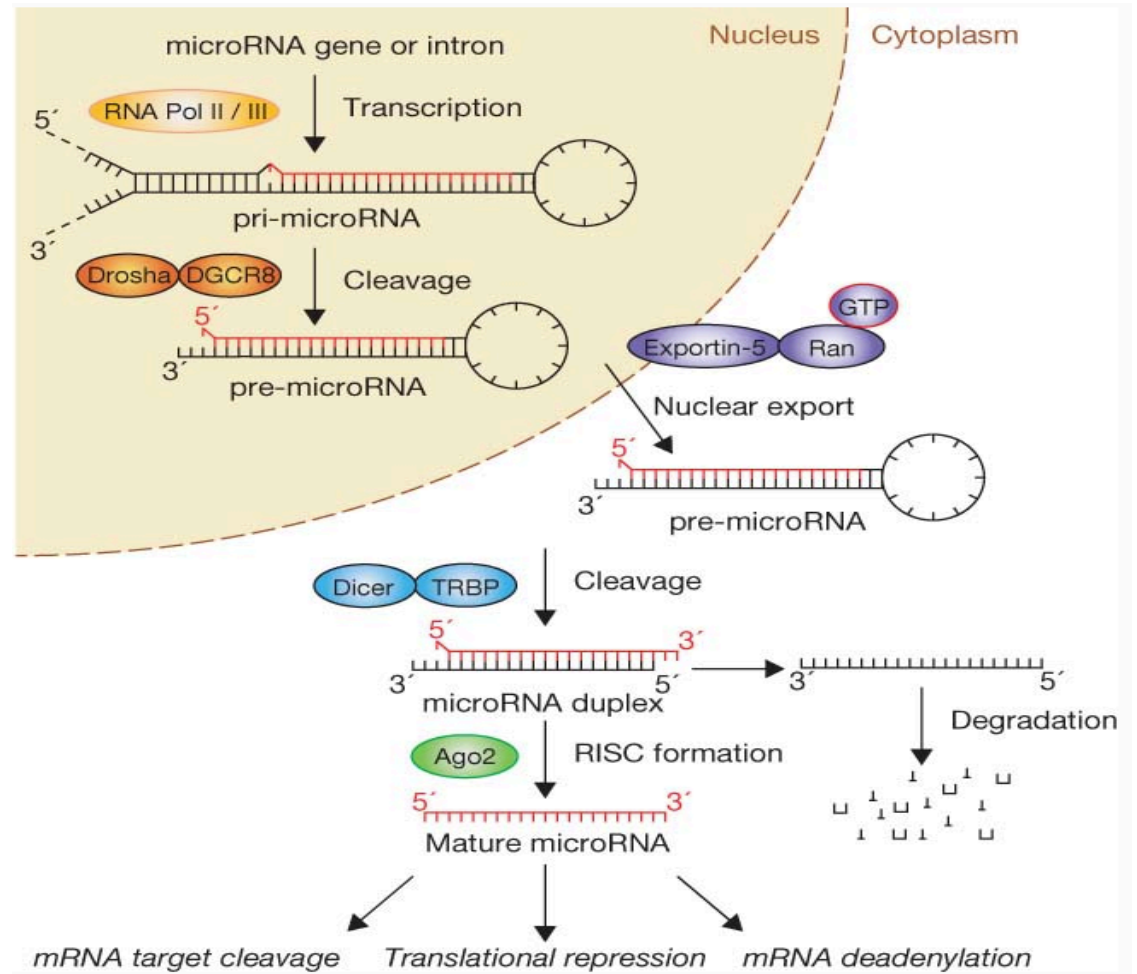
- **Pairing of seed region (nt 2 – 7) with 3'UTR results in translation repression**
- **mRNA destabilization by removal of polyA tail**
- **Full-length complementarity with mRNA leads to mRNA target cleavage**



Winter, et al. *Nature Cell Biology* 11, 228-234 (2009)

## Off-Target Effects:

The majority of sequence-specific Off-Target effects arise through SEED region of shRNA causing translational repression



## Off-Target Effects are common

| Reagent pair | Seed region | Reagent<br>(nM) | Gene signature size<br><i>P</i> < 0.01 |                   |
|--------------|-------------|-----------------|----------------------------------------|-------------------|
|              |             |                 | Up                                     | Down              |
| CDKN1A-558   | TTCTCCAA    | shRNA           | 73                                     | 17                |
| CDKN1A-558   | TTCTCCAA    | 0.5             | 29                                     | 137               |
| CDKN1A-558   | TTCTCCAA    | 1               | 65                                     | 324               |
| CDKN1A-558   | TTCTCCAA    | 2.5             | 247                                    | 468               |
| E2F1-503     | TCACTGAA    | shRNA           | 153                                    | 62                |
| E2F1-503     | TCACTGAA    | 0.5             | 266                                    | 1004 <sup>a</sup> |
| E2F1-503     | TCACTGAA    | 1               | 68                                     | 143               |
| E2F1-503     | TCACTGAA    | 2.5             | 94                                     | 273               |
| EZH2-1636    | CAAGGAAA    | shRNA           | 112                                    | 121               |
| EZH2-1636    | CAAGGAAA    | 0.5             | 157                                    | 50                |
| EZH2-1636    | CAAGGAAA    | 1               | 58                                     | 241               |
| EZH2-1636    | CAAGGAAA    | 2.5             | 324                                    | 281               |
| EZH2-2292    | ATAGGTAT    | shRNA           | 75                                     | 50                |
| EZH2-2292    | ATAGGTAT    | 0.5             | 36                                     | 30                |
| EZH2-2292    | ATAGGTAT    | 1               | NA                                     | NA                |
| EZH2-2292    | ATAGGTAT    | 2.5             | 138                                    | 258               |
| FDXR-1260    | ACAACCAT    | shRNA           | 238                                    | 170               |
| FDXR-1260    | ACAACCAT    | 0.5             | 230                                    | 254               |
| FDXR-1260    | ACAACCAT    | 1               | 136                                    | 327               |
| FDXR-1260    | ACAACCAT    | 2.5             | 226                                    | 514               |

Klinghoffer, et al. "Reduced seed region-based off-target activity with lentivirus-mediated RNAi (2010) RNA 16:879-884.



## Causes of Off-Target Effects:

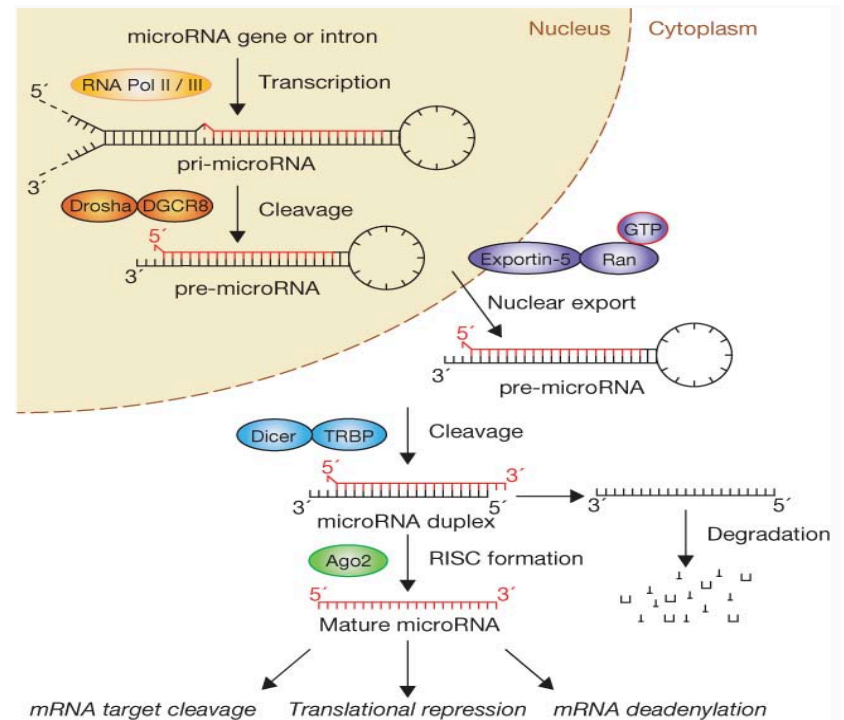
The majority of sequence-specific Off-Target effects arise through SEED region recognition of mRNA

Very high levels of shRNA can saturate the components that process miRNAs. -Often toxic

High levels of shRNA displace endogenous miRNAs from RISC

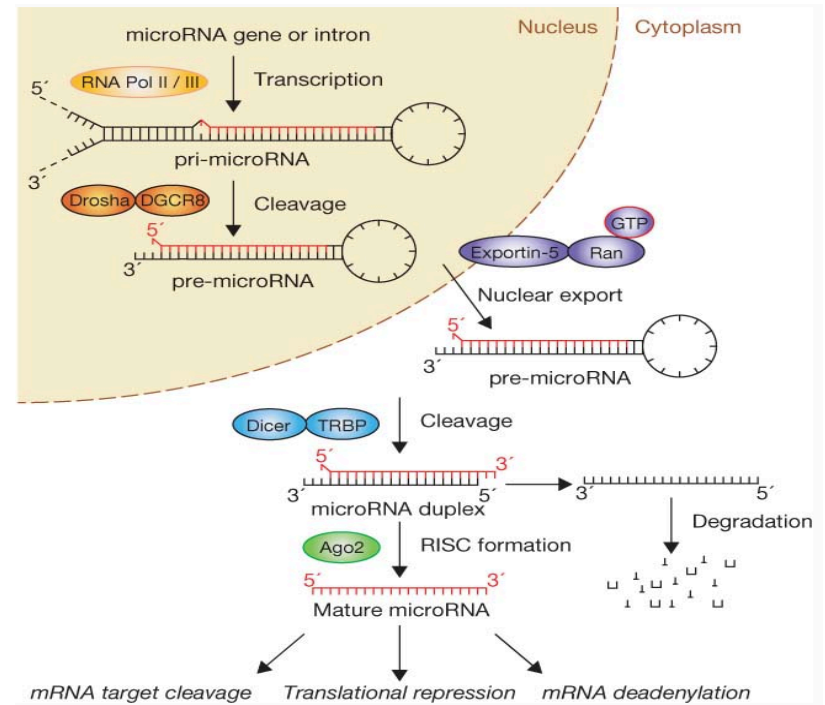
RISC loads the wrong strand as the guide strand

Induction of innate immune reaction



## Control for Off-Target Effects:

- **Demonstrate your phenotype with at least 2 independent shRNAs**
- **Rescue phenotype with a cDNA clone, if feasible**
- **Confirm result without using RNAi (gene targeting or small molecule inhibitors)**
- **Confirm knockdown and phenotype in additional cell types**



# 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

The screenshot shows the website for the shRNA Core Facility at the Albert Einstein College of Medicine. The header includes the Einstein logo and the text "Albert Einstein College of Medicine OF YESHIVA UNIVERSITY". Navigation links for "About Einstein", "Departments & Centers", "Clinical Partners", "Admissions", "Research", and "Library" are visible. A left sidebar lists "Shared Facilities" with options: home, libraries, services, ordering, instrumentation, resources, rnaI user group, faq, staff, and contact. The main content area is titled "shRNA Core Facility" and "SHRNA". It features a welcome message and a detailed description of the facility's services, including RNAi reagents, shRNA-based screening, and RNA expertise. A photograph of a green fluorescent cell culture is shown on the right. The footer contains the slogan "Science at the heart of medicine" and various links and contact information.

**EINSTEIN** Albert Einstein College of Medicine  
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Clinical Partners | Library

Shared Facilities | **shRNA Core Facility**

home | **SHRNA**  
libraries | **Welcome to the Albert Einstein College of Medicine shRNA Core Facility**  
services | **The Albert Einstein College of Medicine shRNA Core Facility is a state-of-the-art RNAi core facility dedicated to providing researchers access to reagents and infrastructure that enable loss-of-function studies at scales ranging from individual gene to genome scale. We provide RNAi reagents, shRNA-based screening services, and RNA expertise to the Einstein research community. In addition, we offer some services to members of the New York Structural Biology Center as well. The Facility houses four genome-wide [shRNA libraries](#), from which we provide individual shRNA clones, shRNA sets for arrayed screens, and shRNA pools for selection-based screening. The Facility is [located](#) in the Price Center on the Einstein campus in the Bronx, and is affiliated with the [Albert Einstein Cancer Center](#).**  
ordering |   
instrumentation  
resources  
rnaI user group  
faq  
staff  
contact

Science at the heart of medicine

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Jack and Pearl Resnick Campus | 1300 Morris Park Avenue Bronx, NY 10461

Which gene(s) are of interest to you?

Gene Symbol: **SOX11**

gene ID: **6664**



species: **human**

| Human shRNA clones |         |   |                   |                |                 |                       |                               |           |             |        |                             |           |                                         |           |         |
|--------------------|---------|---|-------------------|----------------|-----------------|-----------------------|-------------------------------|-----------|-------------|--------|-----------------------------|-----------|-----------------------------------------|-----------|---------|
| Gene Symbol        | Gene ID | # | Catalog Number    | Oligo ID       | Production Note | Sense Sequence        | Top BLAST hit to desired mRNA | Alignment | Target site | Region | Top BLAST hit to other mRNA | Alignment | Top BLAST hit to other RNA (e.g. ncRNA) | Alignment | Library |
| SOX11              | 6664    | 1 | RHS4430-101065732 | V3LHS_386176   |                 | AGCGAGAAGATCCCGTTCA   | NM_003108.3                   | 19/19     | 332 - 350   | CDS    | NM_001040272.5              | 15/19 S   | None in results                         | #N/A      | Hs_GIPZ |
| SOX11              | 6664    | 2 | RHS4430-98842890  | V2LHS_153351   |                 | GTTATACTCCATTCTCATT   | NM_003108.3                   | 19/19     | 7907 - 7925 | 3'UTR  | NM_001135111.1              | 15/19     | NR_024072.2                             | 15/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 3 | RHS4430-98896113  | V2LHS_153346   |                 | GCCGCAAGTGTGGACCTCT   | NM_003108.3                   | 19/19     | 3569 - 3587 | 3'UTR  | NM_001372.3                 | 15/19 S   | NR_038932.1                             | 13/19     | Hs_GIPZ |
| SOX11              | 6664    | 4 | RHS4430-98913178  | V2LHS_153348   |                 | CCATTGGTGGTACATTAT    | NM_003108.3                   | 19/19     | 5072 - 5090 | 3'UTR  | NM_025142.1                 | 15/19     | XR_109277.1                             | 13/19     | Hs_GIPZ |
| SOX11              | 6664    | 5 | RHS4430-101064588 | V3LHS_386174   |                 | TCCGACCTGGTGTTCACAT   | NM_003108.3                   | 19/19     | 1358 - 1376 | CDS    | NM_002836.3                 | 14/19     | NR_038925.1                             | 13/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 1 | RHS3979-9586582   | TRCN0000019174 |                 | GCTCATAATGTTCCATGTATA | NM_003108.3                   | 21/21     | 7305 - 7325 | 3'UTR  | NM_001077619.1              | 15/21 S   | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 2 | RHS3979-9586583   | TRCN0000019175 |                 | CCGCCTACTACAGTCCAA    | NM_003108.3                   | 21/21     | 982 - 1002  | CDS    | NM_178160.2                 | 16/21     | NR_040448.1                             | 14/21     | Hs_TRC  |
| SOX11              | 6664    | 3 | RHS3979-9586584   | TRCN0000019176 |                 | CTGGTGGATAAGGATTTGGAT | NM_003108.3                   | 21/21     | 1235 - 1255 | CDS    | NM_031244.3                 | 14/21     | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 4 | RHS3979-9586585   | TRCN0000019177 |                 | GTTCATGGTATGGCCAAGAT  | NM_003108.3                   | 21/21     | 220 - 240   | CDS    | NM_004189.3                 | 15/21     | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 5 | RHS3979-9586586   | TRCN0000019178 |                 | CGCCAGCCAGAGCCAGAGAA  | NM_003108.3                   | 21/21     | 454 - 474   | CDS    | NM_173542.3                 | 17/21 S   | XM_001717531.3                          | 15/21     | Hs_TRC  |
| SOX11              | 6664    | 6 | RMM3981-9579791   | TRCN0000012102 |                 | GTTCGACCTGAGCTGAATTT  | NM_003108.3                   | 21/21     | 1141 - 1161 | CDS    | NM_004263.3                 | 15/21     | NR_036614.1                             | 13/21     | Mm_TRC  |

## Examine the sequence present in the shRNA.

| Human shRNA clones |         |   |                   |                |                 |                        |                               |             |             |       |                             |         |                                         |         |         |
|--------------------|---------|---|-------------------|----------------|-----------------|------------------------|-------------------------------|-------------|-------------|-------|-----------------------------|---------|-----------------------------------------|---------|---------|
| Gene Symbol        | Gene ID | # | Catalog Number    | Oligo ID       | Production Note | Sense Sequence         | Top BLAST hit to desired mRNA |             |             |       | Top BLAST hit to other mRNA |         | Top BLAST hit to other RNA (e.g. ncRNA) |         | Library |
|                    |         |   |                   |                |                 |                        | Alignment                     | Target site | Region      |       | Alignment                   |         | Alignment                               |         |         |
| SOX11              | 6664    | 1 | RHS4430-101065732 | V3LHS_386176   |                 | AGCGAGAAGATCCCGTTCA    | NM_003108.3                   | 19/19       | 332 - 350   | CDS   | NM_001040272.5              | 15/19 S | None in results                         | #N/A    | Hs_GIPZ |
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| SOX11              | 6664    | 3 | RHS4430-98896113  | V2LHS_153346   |                 | GCCGCAGTGTGGACCTCT     | NM_003108.3                   | 19/19       | 3569 - 3587 | 3'UTR | NM_001372.3                 | 15/19 S | NR_038932.1                             | 13/19   | Hs_GIPZ |
| SOX11              | 6664    | 4 | RHS4430-98913178  | V2LHS_153348   |                 | CCATTGGGTACATTAT       | NM_003108.3                   | 19/19       | 5072 - 5090 | 3'UTR | NM_025142.1                 | 15/19   | XR_109277.1                             | 13/19   | Hs_GIPZ |
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| SOX11              | 6664    | 1 | RHS3979-9586582   | TRCN0000019174 |                 | GTCATAATGTTCCATGTATA   | NM_003108.3                   | 21/21       | 7305 - 7325 | 3'UTR | NM_001077619.1              | 15/21 S | None in results                         | #N/A    | Hs_TRC  |
| SOX11              | 6664    | 2 | RHS3979-9586583   | TRCN0000019175 |                 | CCGCCTCTACTACAGTTCFAA  | NM_003108.3                   | 21/21       | 982 - 1002  | CDS   | NM_178160.2                 | 16/21   | NR_040448.1                             | 14/21   | Hs_TRC  |
| SOX11              | 6664    | 3 | RHS3979-9586584   | TRCN0000019176 |                 | CTGGTGGATAAGGATTTGGAT  | NM_003108.3                   | 21/21       | 1235 - 1255 | CDS   | NM_031244.3                 | 14/21   | None in results                         | #N/A    | Hs_TRC  |
| SOX11              | 6664    | 4 | RHS3979-9586585   | TRCN0000019177 |                 | GTTTCATGGTATGGTCCAAGAT | NM_003108.3                   | 21/21       | 220 - 240   | CDS   | NM_004189.3                 | 15/21   | None in results                         | #N/A    | Hs_TRC  |
| SOX11              | 6664    | 5 | RHS3979-9586586   | TRCN0000019178 |                 | CGCCAGCCAGAGCCCAAGAA   | NM_003108.3                   | 21/21       | 454 - 474   | CDS   | NM_173542.3                 | 17/21 S | XM_001717531.3                          | 15/21   | Hs_TRC  |
| SOX11              | 6664    | 6 | RMM3981-9579791   | TRCN0000012102 |                 | GTTTCGACCTGAGCTGAATTT  | NM_003108.3                   | 21/21       | 1141 - 1161 | CDS   | NM_004263.3                 | 15/21   | NR_036614.1                             | 13/21   | Mm_TRC  |

Avoid picking duplicate or near-duplicate sequence targets.

| Sense Sequence I                                                                  |                                           |
|-----------------------------------------------------------------------------------|-------------------------------------------|
|                                                                                   | AGCGAGAAGATCCCGTCA                        |
|  | T A T A C T C C A T T C T C A T T T       |
|                                                                                   | G C C G C A G T G T T T G A C C T C T     |
|  | A T A C T C C A T T C T C A T T T A T     |
|                                                                                   | T C C G A C C T G G T G T T C A C A T     |
|                                                                                   | G C T C A T A A T G T T C C A T G T A T A |
|                                                                                   | C C G C C T C T A C T A C A T T C A C A A |
|                                                                                   | C T G G T G G A T A A G G A T T T G G A T |
|                                                                                   | G T T C A T G G T A T G G T C C A A G A T |
|                                                                                   | C G C G A G A C A G A G C C C A G A G A A |
|                                                                                   | G T T C G A C C T G A G C T T G A A T T T |

## Where in the mRNA are the target sequences located?

| Human shRNA clones |         |   |                   |                |                 |                        |                               |           |             |        |                             |           |                                         |           |         |
|--------------------|---------|---|-------------------|----------------|-----------------|------------------------|-------------------------------|-----------|-------------|--------|-----------------------------|-----------|-----------------------------------------|-----------|---------|
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| SOX11              | 6664    | 3 | RHS4430-98896113  | V2LHS_153346   |                 | GCCGCAGTGTTTGACCTCT    | NM_003108.3                   | 19/19     | 3569 - 3587 | 3'UTR  | NM_001372.3                 | 15/19 S   | NR_038932.1                             | 13/19     | Hs_GIPZ |
| SOX11              | 6664    | 4 | RHS4430-98913178  | V2LHS_153348   |                 | CCATTGGTGATACATTAT     | NM_003108.3                   | 19/19     | 5072 - 5090 | 3'UTR  | NM_025142.1                 | 15/19     | XR_109277.1                             | 13/19     | Hs_GIPZ |
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What's better: shRNA targeting coding sequence (CDS) or 3'UTR?

Gene Symbol: **SOX11** gene ID: **6664**

| Alignment | Target site1 | Region1 |
|-----------|--------------|---------|
| 19/19     | 332 - 350    | CDS     |
| 19/19     | 7907 - 7925  | 3'UTR   |
| 19/19     | 3569 - 3587  | 3'UTR   |
| 19/19     | 5072 - 5090  | 3'UTR   |
| 19/19     | 1358 - 1376  | CDS     |
|           |              |         |
| 21/21     | 7305 - 7325  | 3'UTR   |
| 21/21     | 982 - 1002   | CDS     |
| 21/21     | 1235 - 1255  | CDS     |
| 21/21     | 220 - 240    | CDS     |
| 21/21     | 454 - 474    | CDS     |
| 21/21     | 1141 - 1161  | CDS     |



## Are other genes targeted by this sequence?

| Human shRNA clones |         |   |                   |                |                 |                        |                               |           |             |        |                             |           |                                         |           |         |
|--------------------|---------|---|-------------------|----------------|-----------------|------------------------|-------------------------------|-----------|-------------|--------|-----------------------------|-----------|-----------------------------------------|-----------|---------|
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| SOX11              | 6664    | 2 | RHS3979-9586583   | TRCN0000019175 |                 | CGGCCTCTACTACAGCTTCAA  | NM_003108.3                   | 21/21     | 982 - 1002  | CDS    | NM_178160.2                 | 16/21     | NR_040448.1                             | 14/21     | Hs_TRC  |
| SOX11              | 6664    | 3 | RHS3979-9586584   | TRCN0000019176 |                 | CTGGTGGATAAGGATTTGGAT  | NM_003108.3                   | 21/21     | 1235 - 1255 | CDS    | NM_031244.3                 | 14/21     | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 4 | RHS3979-9586585   | TRCN0000019177 |                 | GTTTCATGGTATGGTCCAAGAT | NM_003108.3                   | 21/21     | 220 - 240   | CDS    | NM_004189.3                 | 15/21     | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 5 | RHS3979-9586586   | TRCN0000019178 |                 | CGCCAGCCAGAGCCAGAGAA   | NM_003108.3                   | 21/21     | 454 - 474   | CDS    | NM_173542.3                 | 17/21 S   | XM_001717531.3                          | 15/21     | Hs_TRC  |
| SOX11              | 6664    | 6 | RMM3981-9579791   | TRCN0000012102 |                 | GTTCGACCTGAGCTTGAATTT  | NM_003108.3                   | 21/21     | 1141 - 1161 | CDS    | NM_001362.2                 | 15/21     | NR_036614.1                             | 13/21     | Mm_TRC  |

Confirm that shRNA does not have perfect complementarity to any other gene product

| Top BLAST hit to other mRNA1 | Alignment2 |
|------------------------------|------------|
| NM_001040272.5               | 15/19 S    |
| NM_001135111.1               | 15/19      |
| NM_001372.3                  | 15/19 S    |
| NM_025142.1                  | 15/19      |
| NM_002836.3                  | 14/19      |
| NM_001077619.1               | 15/21 S    |
| NM_178160.2                  | 16/21      |
| NM_031244.3                  | 14/21      |
| NM_004189.3                  | 15/21      |
| NM_173542.3                  | 17/21 S    |
| NM_004263.3                  | 15/21      |

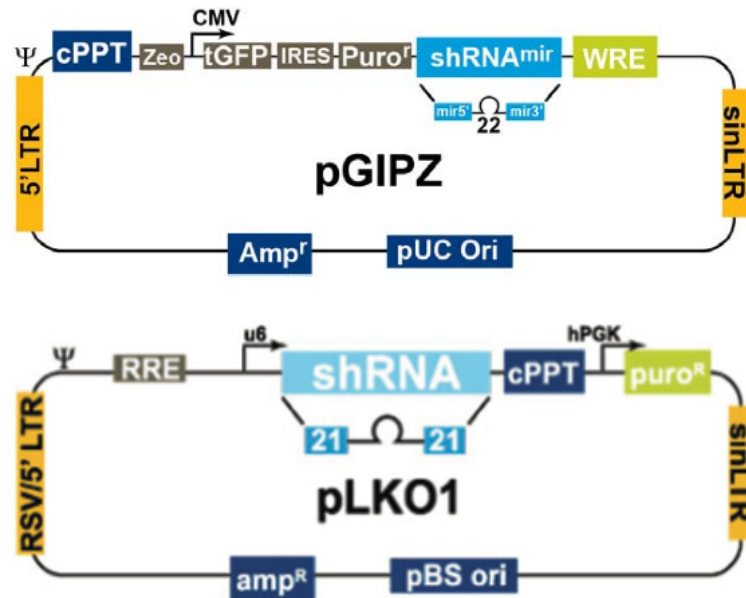
## Which Library is the shRNA from?

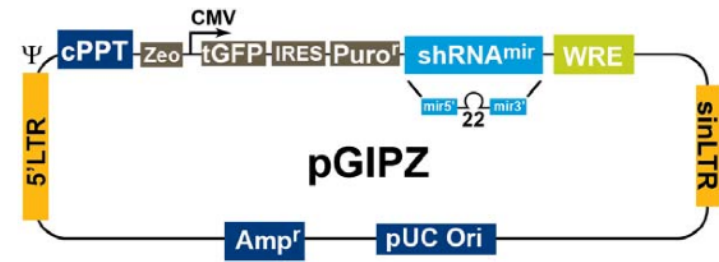
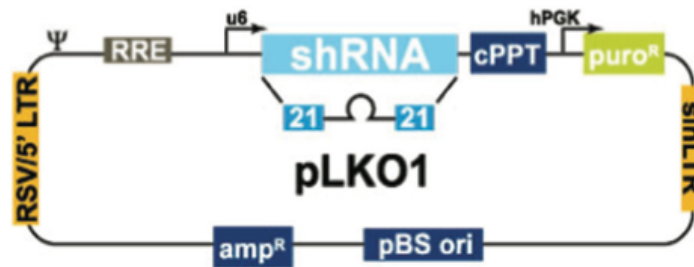
Gene Symbol: **SOX11** gene ID: **6664**

| Human shRNA clones |         |   |                   |                |                 |                        |                               |           |             |        |                             |           |                                         |           |         |
|--------------------|---------|---|-------------------|----------------|-----------------|------------------------|-------------------------------|-----------|-------------|--------|-----------------------------|-----------|-----------------------------------------|-----------|---------|
| Gene Symbol        | Gene ID | # | Catalog Number    | Oligo ID       | Production Note | Sense Sequence         | Top BLAST hit to desired mRNA | Alignment | Target site | Region | Top BLAST hit to other mRNA | Alignment | Top BLAST hit to other RNA (e.g. ncRNA) | Alignment | Library |
| SOX11              | 6664    | 1 | RHS4430-101065732 | V3LHS_386176   |                 | AGCGAGAAGATCCCGTTCA    | NM_003108.3                   | 19/19     | 332 - 350   | CDS    | NM_001040272.5              | 15/19 S   | None in results                         | #N/A      | Hs_GIPZ |
| SOX11              | 6664    | 2 | RHS4430-98842890  | V2LHS_153351   |                 | GTTATACTCCATTCTCATT    | NM_003108.3                   | 19/19     | 7907 - 7925 | 3'UTR  | NM_001135111.1              | 15/19 S   | NR_024072.2                             | 15/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 3 | RHS4430-98896113  | V2LHS_153346   |                 | GCCGCAGTGTGGTACCTCT    | NM_003108.3                   | 19/19     | 3569 - 3587 | 3'UTR  | NM_0011372.3                | 15/19 S   | NR_038932.1                             | 13/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 4 | RHS4430-98913178  | V2LHS_153348   |                 | CCATTGGTGGTACATTAT     | NM_003108.3                   | 19/19     | 5072 - 5090 | 3'UTR  | NM_025142.1                 | 15/19 S   | XR_109277.1                             | 13/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 5 | RHS4430-101064588 | V3LHS_386174   |                 | TCCGACCTGGTGTCCACAT    | NM_003108.3                   | 19/19     | 1358 - 1376 | CDS    | NM_002836.3                 | 14/19 S   | NR_038925.1                             | 13/19 S   | Hs_GIPZ |
| SOX11              | 6664    | 1 | RHS3979-9586582   | TRCN0000019174 |                 | GCTCATAATGTTCCATGTATA  | NM_003108.3                   | 21/21     | 7305 - 7325 | 3'UTR  | NM_001077619.1              | 15/21 S   | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 2 | RHS3979-9586583   | TRCN0000019175 |                 | CGGCCTCTACTACAGCTTCAA  | NM_003108.3                   | 21/21     | 982 - 1002  | CDS    | NM_178160.2                 | 16/21 S   | NR_040448.1                             | 14/21 S   | Hs_TRC  |
| SOX11              | 6664    | 3 | RHS3979-9586584   | TRCN0000019176 |                 | CTGGTGATAAGGATTTGGAT   | NM_003108.3                   | 21/21     | 1235 - 1255 | CDS    | NM_031244.3                 | 14/21 S   | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 4 | RHS3979-9586585   | TRCN0000019177 |                 | GTTTCATGGTATGGTCCAAGAT | NM_003108.3                   | 21/21     | 220 - 240   | CDS    | NM_004189.3                 | 15/21 S   | None in results                         | #N/A      | Hs_TRC  |
| SOX11              | 6664    | 5 | RHS3979-9586586   | TRCN0000019178 |                 | CGCCAGCCAGAGCCAGAGAA   | NM_003108.3                   | 21/21     | 454 - 474   | CDS    | NM_173542.3                 | 17/21 S   | XM_001717531.3                          | 15/21 S   | Hs_TRC  |
| SOX11              | 6664    | 6 | RMM3981-9579791   | TRCN0000012102 |                 | GTTCGACCTGAGCTTGAATT   | NM_003108.3                   | 21/21     | 1141 - 1161 | CDS    | NM_004263.3                 | 15/21 S   | NR_036614.1                             | 13/21 S   | Mm_TRC  |

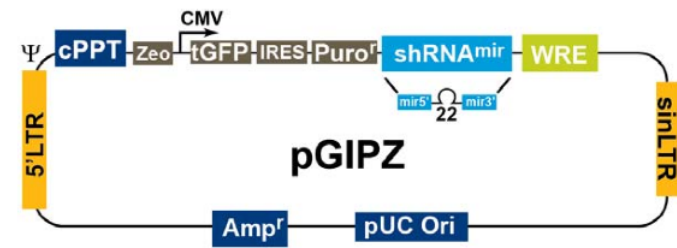
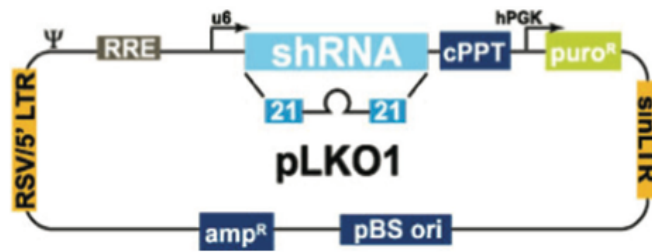
Which vector is better?

| Library1 |
|----------|
| Hs_GIPZ  |
| Hs_GIPZ  |
| Hs_GIPZ  |
| Hs_GIPZ  |
| Hs_GIPZ  |
| Hs_GIPZ  |
| Hs_TRC   |
| Hs_TRC   |
| Hs_TRC   |
| Hs_TRC   |
| Hs_TRC   |
| Hs_TRC   |
| Mm_TRC   |





|                    | <b>TRC Vector</b>                                       | <b>GIPZ Vector</b>                                                                                                                                                                                                                                                 |
|--------------------|---------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Making Plasmid     | Low Copy                                                | High Copy                                                                                                                                                                                                                                                          |
| Making Virus       | Make with 3 <sup>rd</sup> Generation Packaging Plasmids | Requires 2 <sup>nd</sup> Generation Packaging Plasmids <ul style="list-style-type: none"> <li>• Higher chance of recombination</li> <li>• BSL2+</li> </ul> Or use Open Biosystems Packaging Mix <ul style="list-style-type: none"> <li>• More Expensive</li> </ul> |
| Titering Virus     | No Marker.                                              | GFP expression allows easy visualization of transduced cells.                                                                                                                                                                                                      |
| Available Controls | Empty Vector<br>eGFP shRNA                              | Non-Silencing shRNA<br>GAPDH shRNA                                                                                                                                                                                                                                 |



|                  | <b>TRC Vector</b>                                                                                                    | <b>GIPZ Vector</b>                                             |
|------------------|----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| shRNA            | Conventional                                                                                                         | Mir-based                                                      |
| shRNA Expression | U6 Promotor (Pol III)<br>High level expression can saturate shRNA processing pathway - Increases Off-target effects. | CMV Promotor (Pol II)<br>Not expressed well in all cell types. |

# Summary

## Find shRNA clones that effectively knockdown the target

- Test multiple clones, transfecting plasmid DNA if possible
- May need to screen multiple viruses
- Confirm knockdown at protein or mRNA level

## Control for off-target effects

- Show phenotype with at least 2 independent clones
- Rescue phenotype with a cDNA, if feasible
- Confirm result without using RNAi (gene targeting or small molecule inhibitors)
- Confirm knockdown and phenotype in additional cell types



shRNA Core Facility

Mount Sinai College of Medicine

at SIKSKIP UNIVERSITY

Scientific Director: Dr. Steven Almo

Operations Director: Dr. John Reidhaar-Olson

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shRNA@einstein.yu.edu

718.678.1195

## Available Clone List and Order Form

Use this form for ordering shRNA and ORF clones, and associated controls, from our libraries.  
For other services, including quantitation of knockdown and RNAi-based screening, please contact us at shRNA@einstein.yu.edu.  
Use the drop-down menu in the "Order" column to specify the format for clones you wish to order.  
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 that the hit is to the sense strand of the shRNA.  
\*\*Do not delete hidden columns\*\*

Researcher:

Email address:

Phone number:

Principal Investigator:

Department:

Grant number:

Date:

Comments or special instructions:

Email completed form to shRNA@einstein.yu.edu

| Order (use drop-down menu) | Gene ID | Gene Symbol | Species | Library | Catalog Number    | Oligo ID       | Notes | Sense Sequence         | Top BLAST hit to desired mRNA | Alignment | Target site | Region |
|----------------------------|---------|-------------|---------|---------|-------------------|----------------|-------|------------------------|-------------------------------|-----------|-------------|--------|
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-98851790  | V2LHS_19709    |       | CATATGAATTGTACAGAAT    | NM_005030.3                   | 19/19     | 2086 - 2104 | 3'UTR  |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-99141498  | V2LHS_19711    |       | GTTCTTACTTCTGGCTAT     | NM_005030.3                   | 19/19     | 962 - 980   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-99148662  | V2LHS_241437   |       | CTCCTTAATATTTCCGCA     | NM_005030.3                   | 19/19     | 1485 - 1503 | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-99148998  | V2LHS_19708    |       | GTGTGGGACTCCTAATTAC    | NM_005030.3                   | 19/19     | 686 - 704   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-99290546  | V2LHS_262328   |       | CTGTGTGGGACTCCTAATT    | NM_005030.3                   | 19/19     | 684 - 702   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-101127346 | V3LHS_311459   |       | CCGAGGTGCTGAGCAAGAA    | NM_005030.3                   | 19/19     | 712 - 730   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-101128102 | V3LHS_311462   |       | AGCAGCTGCACAGTGCAA     | NM_005030.3                   | 19/19     | 1189 - 1207 | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_GIPZ | RHS4430-101132430 | V3LHS_311463   |       | GCGGGCAAGATTGTGCTA     | NM_005030.3                   | 19/19     | 291 - 309   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-9575123   | TRCN0000006246 |       | CCAACCATTAACGAGCTGCTT  | NM_005030.3                   | 21/21     | 933 - 953   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-9575124   | TRCN0000006247 |       | CGATACTACCTACGGCAAATT  | NM_005030.3                   | 21/21     | 513 - 533   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-9575125   | TRCN0000006248 |       | CGCCTCATCCTCTACAATGAT  | NM_005030.3                   | 21/21     | 1374 - 1394 | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-9575126   | TRCN0000006249 |       | CCGGATCAAGAAGAAATGAATA | NM_005030.3                   | 21/21     | 836 - 856   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-98488725  | TRCN0000121072 |       | CCTCCTCACTCCACCTGCAT   | NM_005030.3                   | 21/21     | 1893 - 1913 | 3'UTR  |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-98488732  | TRCN0000121073 |       | CGAGCTGCTTAATGACGAGTT  | NM_005030.3                   | 21/21     | 944 - 964   | CDS    |
|                            | 5347    | PLK1        | Human   | Hs_TRC  | RHS3979-98488652  | TRCN0000121074 |       | CAATGACTCAACACGCCAT    | NM_005030.3                   | 21/21     | 1361 - 1381 | CDS    |