shRNA: The Basics

Deborah Smith, Ph.D.

February 29, 2012

RNAi User Group Meeting

shRNA: The Basics

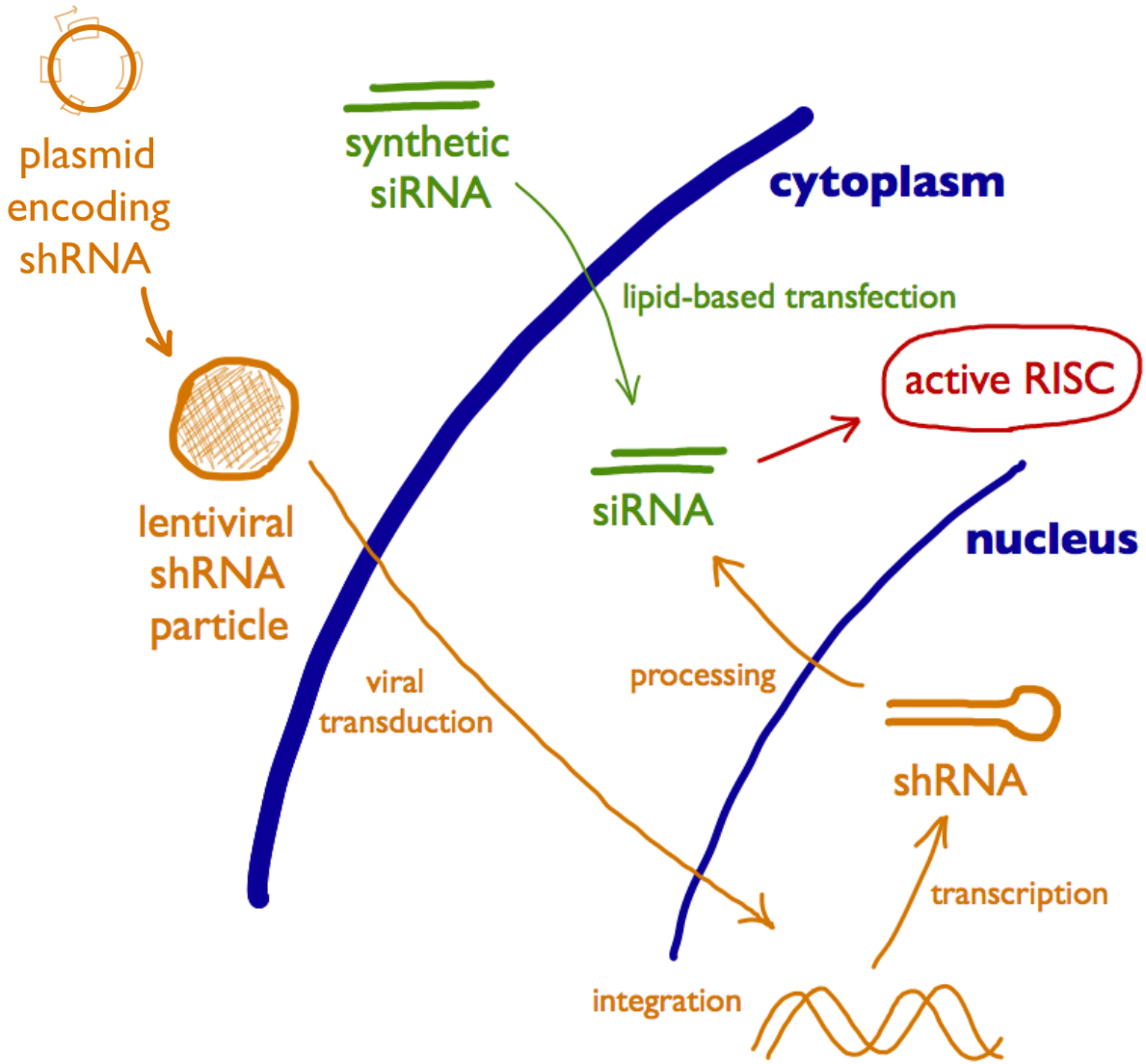
- **What is shRNA and how does it work?**
- **Off-target effects**
- **Practical aspects of choosing shRNA clones**

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

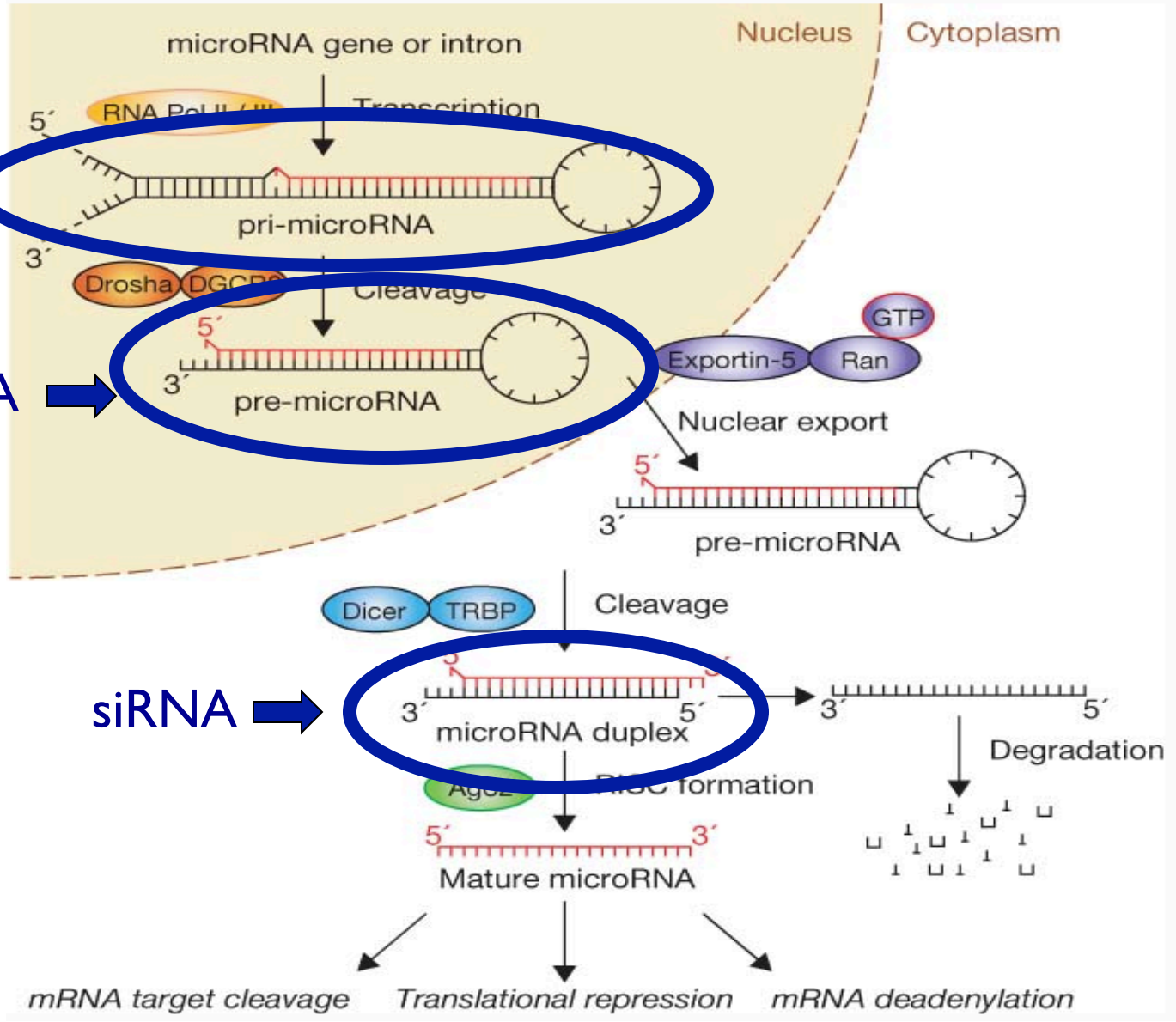
What is microRNA?

- > 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

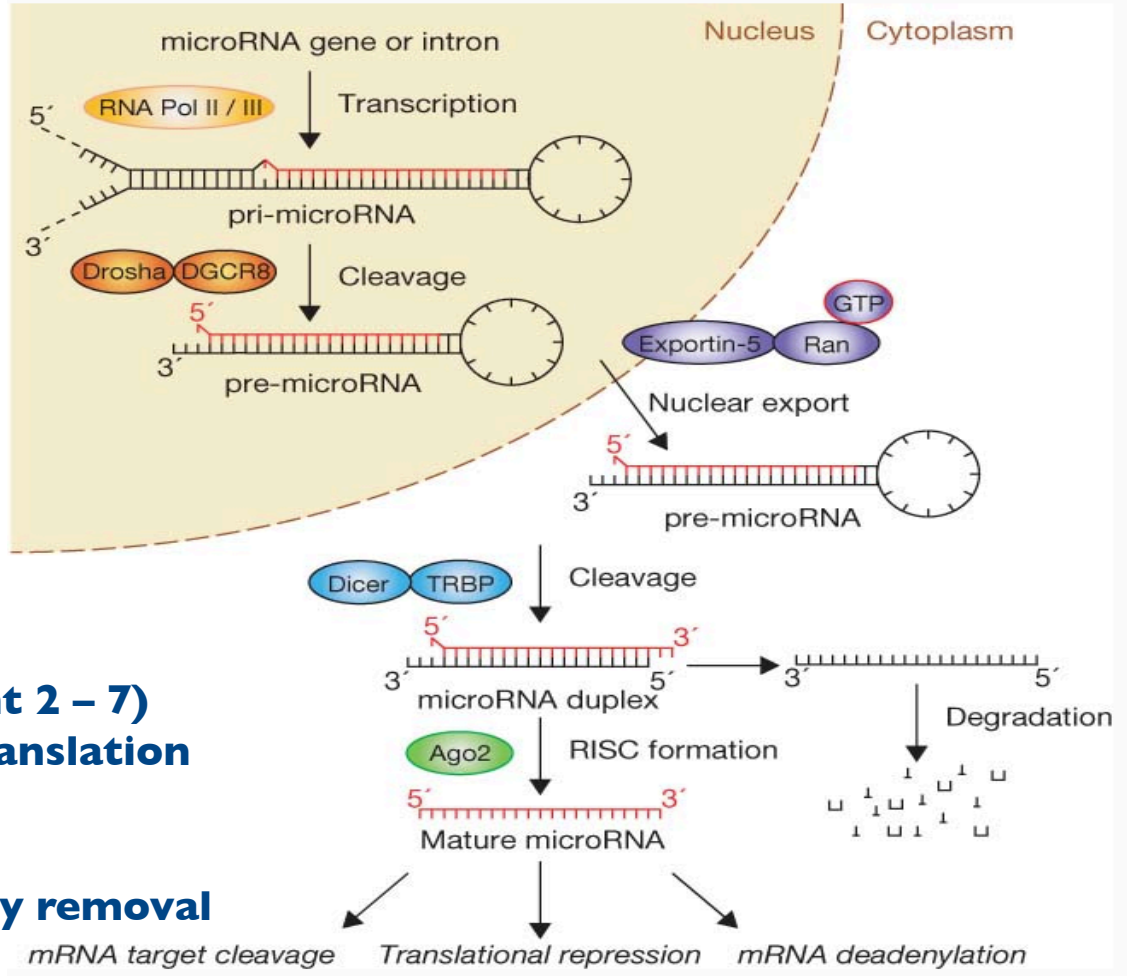


microRNA seed region

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

2. UCAUUGAACU - CAGGAAUUUCUUCUAC CUCAGGGA
   AGUG - - - UGAACUCC - - - - - - - - - - - - AGAGUCCCU

3. ACUCACA - - - ACCAACUCAGGGA
   AGUGUGAACUCCAGAGUCCCU

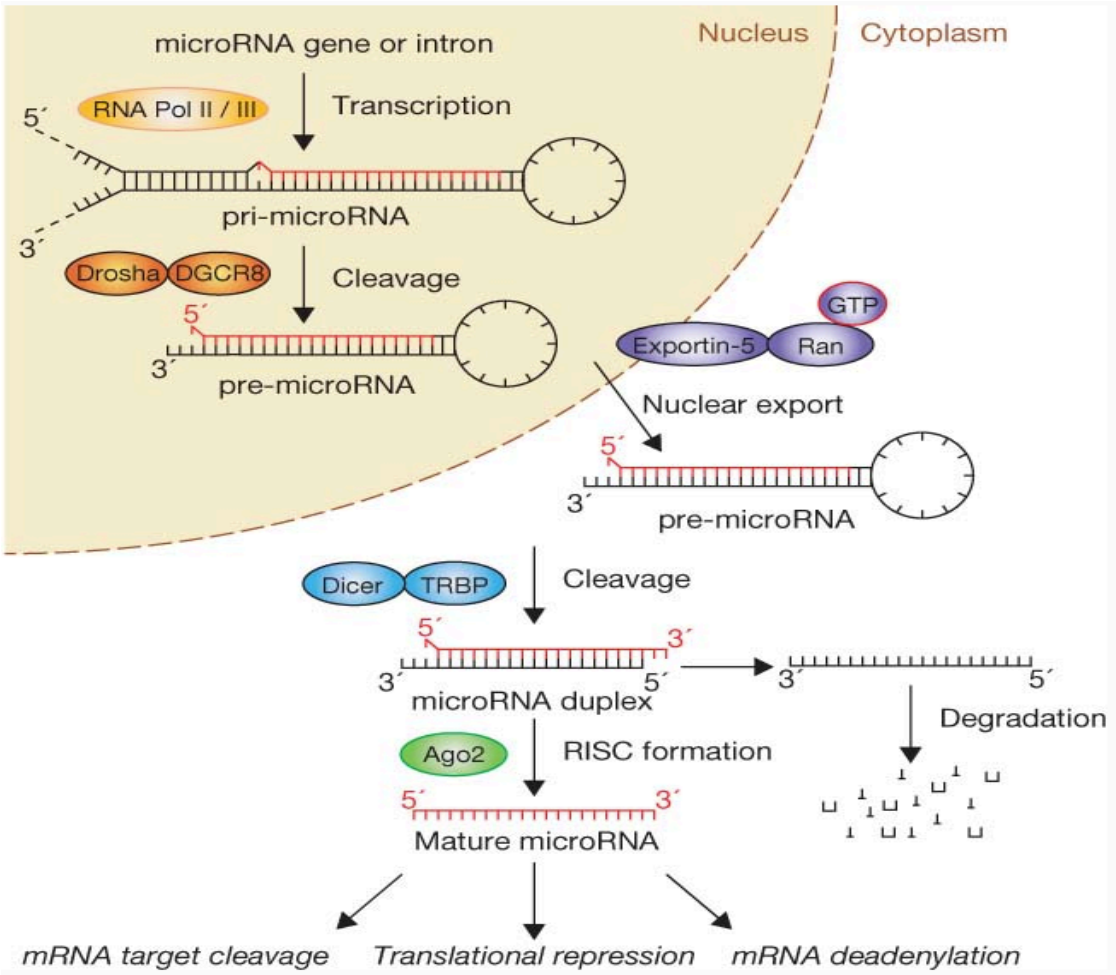


- **Pairing of seed region (nt 2 – 7) with 3'UTR results in translation repression**
- **mRNA destabilization by removal of polyA tail**
- **Full-length complementarity with mRNA leads to mRNA target cleavage**

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 (nM)	Gene signature size $P < 0.01$	
			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[a]
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:

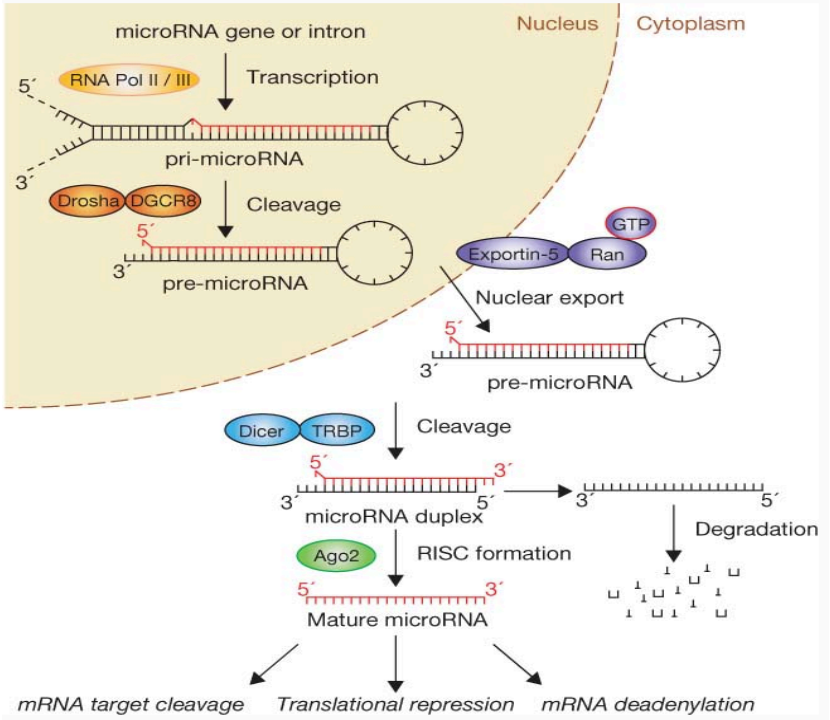
The majority of sequence-specific Off-Target effects arise through SEED region recognition of mRNA

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

High levels of shRNA displace endogenous miRNAs from RISC

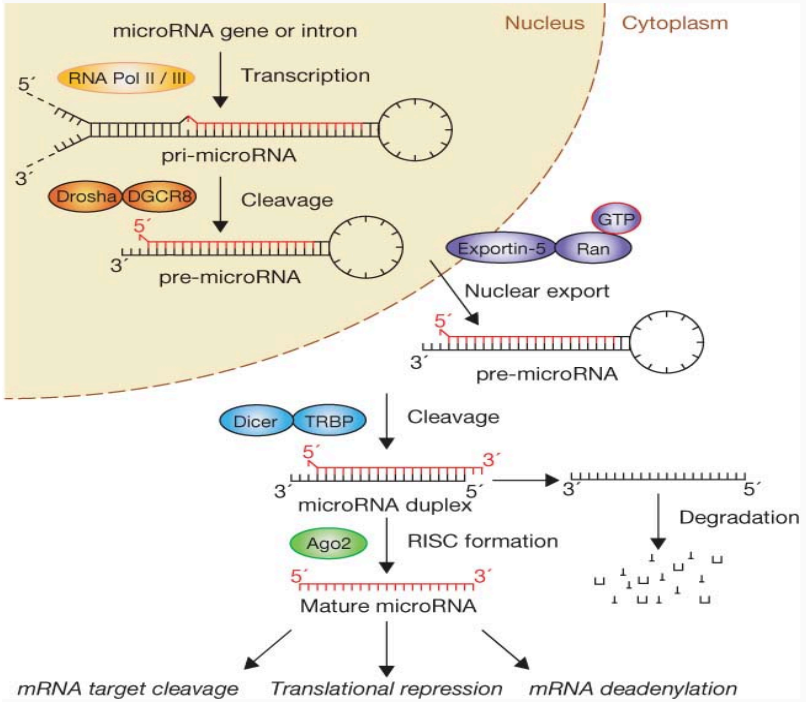
RISC loads the wrong strand as the guide strand

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

Which gene(s) are of interest to you?

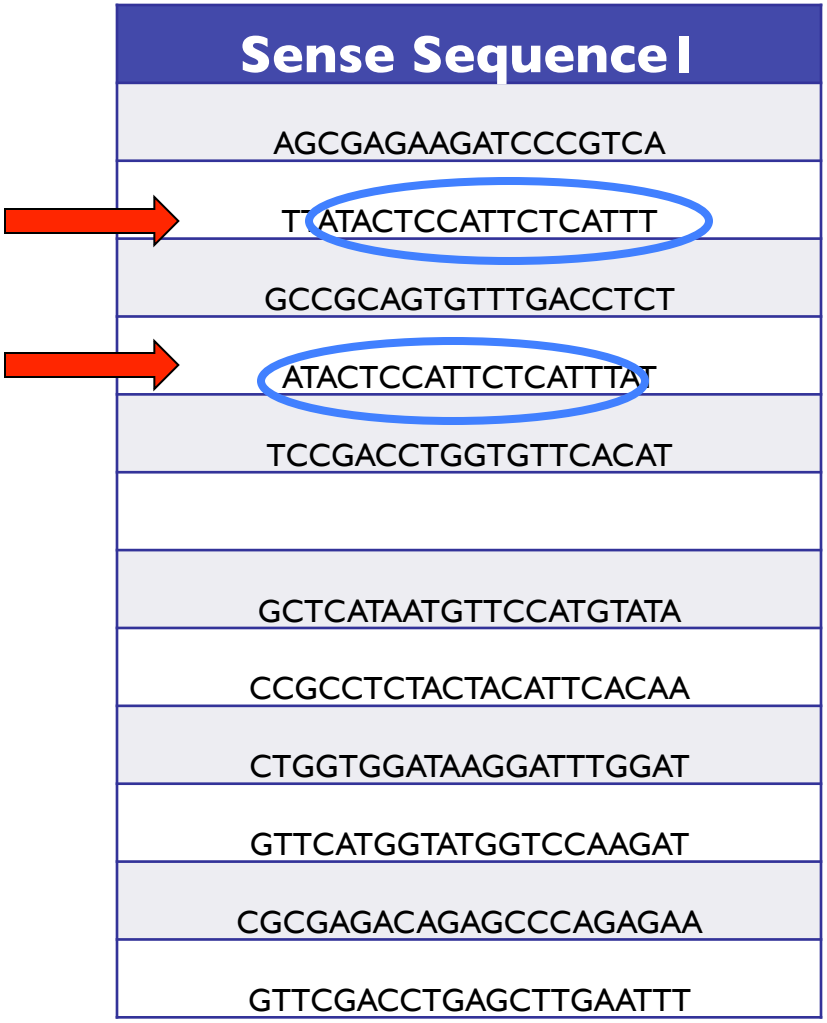
Gene Symbol: SOX11 gene ID: 6664 species: human

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		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		CCATTGGTGGTACATTTAT	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		CCGCCTCTACTACAGCTTCAA	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		GTTCATGGTATGGTCCAAGAT	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		CGCCAGCCAGAGCCCAGAGAA	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_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 Not	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		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		CCATTGGTGGTACATTTAT	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		CCGCCTCTACTACAGCTTCAA	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		GTTCATGGTATGGTCCAAGAT	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		CGCCAGCCAGAGCCCAGAGAA	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_004263.3	15/21	NR_036614.1	13/21	Mm_TRC

Avoid picking duplicate or near-duplicate sequence targets.



Where in the mRNA are the target sequences located?

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		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		CCATTGGTGGTACATTTAT	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		CCGCCTCTACTACAGCTTCAA	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		GTTCATGGTATGGTCCAAGAT	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		CGCCAGCCAGAGCCCAGAGAA	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_004263.3	15/21	NR_036614.1	13/21	Mm_TRC

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																
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		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		CCATTGGTGGTACATTTAT	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		CCGCCTCTACTACAGCTTCAA	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		GTTCATGGTATGGTCCAAGAT	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		CGCCAGCCAGAGCCCAGAGAA	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	[illegible]	[illegible]	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

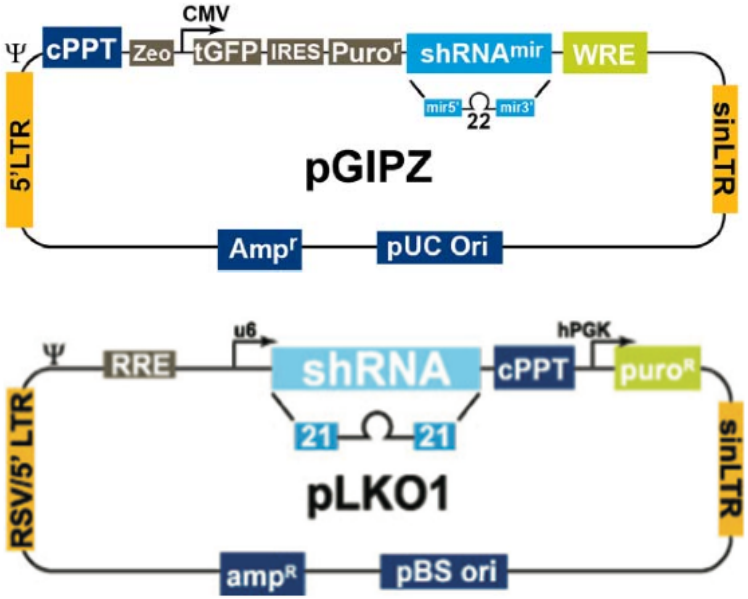
Which Library is the shRNA from?

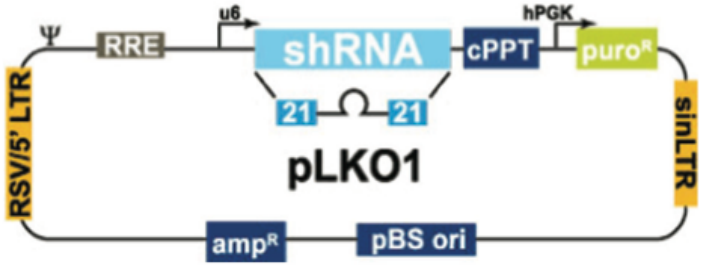
Gene Symbol: SOX11 gene ID: 6664

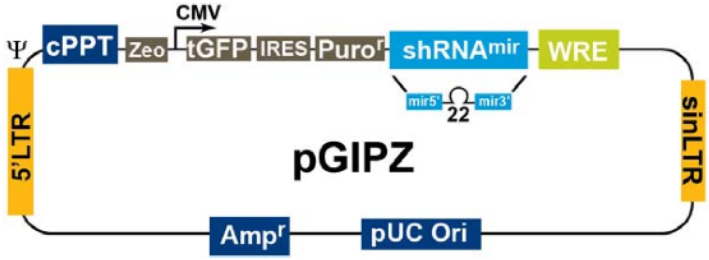
Human shRNA clones															
Gene Symbol	Gene ID	#	Catalog Number	Oligo ID	Production Note	Sense Sequence	Top BLAST hit to desired mRNA	Alignment	Target site	Region	Top BLAST hit to other mRNA	Alignment	Top BLAST hit to other RNA (e.g. ncRNA)	Alignment	Library
SOX11	6664	1	RHS4430-101065732	V3LHS_386176		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	NR_024072.2	15/19	Hs_GIPZ
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	13/1	Hs_GIPZ
SOX11	6664	4	RHS4430-98913178	V2LHS_153348		CCATTGGTGGTACATTTAT	NM_003108.3	19/19	5072 - 5090	3'UTR	NM_025142.1	15/19	XR_109277.1	13/1	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	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		CCGCCTCTACTACAGCTTCAA	NM_003108.3	21/21	982 - 1002	CDS	NM_178160.2	16/21	NR_040448.1	14/2	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		GTTCATGGTATGGTCCAAGAT	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		CGCCAGCCAGAGCCCAGAGAA	NM_003108.3	21/21	454 - 474	CDS	NM_173542.3	17/21 S	XM_001717531.3	15/2	Hs_TRC
SOX11	6664	6	RMM3981-9579791	TRCN0000012102		GTTCGACCTGAGCTTGAATTT	NM_003108.3	21/21	1141 - 1161	CDS	NM_004263.3	15/21	NR_036614.1	13/2	Mm_TRC

Which vector is better?

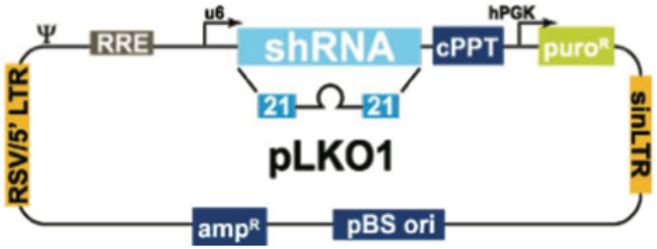
Library1
Hs_GIPZ
Hs_GIPZ
Hs_GIPZ
Hs_GIPZ
Hs_GIPZ
Hs_TRC
Hs_TRC
Hs_TRC
Hs_TRC
Hs_TRC
Mm_TRC

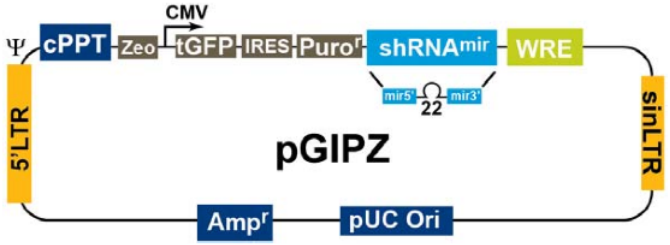






	TRC Vector		GIPZ Vector
Making Plasmid	Low Copy		High Copy
Making Virus	Make with 3rd Generation Packaging Plasmids		Requires 2nd Generation Packaging Plasmids • Higher chance of recombination • BSL2+ Or use Open Biosystems Packaging Mix • More Expensive
Titering Virus	No Marker.		GFP expression allows easy visualization of transduced cells.
Available Controls	Empty Vector eGFP shRNA		Non-Silencing shRNA GAPDH shRNA





	TRC Vector		GIPZ Vector
shRNA	Conventional		Mir-based
shRNA Expression	U6 Promotor (Pol III) High level expression can saturate shRNA processing pathway - Increases Off-target effects.		CMV Promotor (Pol II) 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

EINSTEIN
Albert Einstein College of Medicine
shRNA Core Facility

Scientific Director: Dr. Steven Almo
Operations Director: Dr. John Reidhaar-Olson

Price Center / Block Research Pavilion
1301 Morris Park Ave., Room 275
Bronx, NY 10461

www.einstein.yu.edu/sr/shRNA
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

shRNA clones

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	T
	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		GTTCTTTACTTCTGGCTAT	NM_005030.3	19/19	962 - 980	CDS	
	5347	PLK1	Human	Hs_GIPZ	RHS4430-99148662	V2LHS_241437		CTCCTTAAATATTTCCGCA	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		AGCAGCTGCACAGTGTCAA	NM_005030.3	19/19	1189 - 1207	CDS	
	5347	PLK1	Human	Hs_GIPZ	RHS4430-101132430	V3LHS_311463		GCGGGCAAGATTGTGCCTA	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		CCGGATCAAGAAGAATGAATA	NM_005030.3	21/21	836 - 856	CDS	
	5347	PLK1	Human	Hs_TRC	RHS3979-98488725	TRCN0000121072		CCTCCTCACTCCCACCTGCAT	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		CAATGACTCAACACGCCTCAT	NM_005030.3	21/21	1361 - 1381	CDS	