RNA Interference with Guaranteed Knockdown!

shRNA, siRNA & microRNA

shRNA & siRNA mediated Knockdown

microRNA Detection with SYBR-Green qPCR Experiments

microRNA Expression

microRNA Target Validation with 3'-UTR Clones

ORIGENE

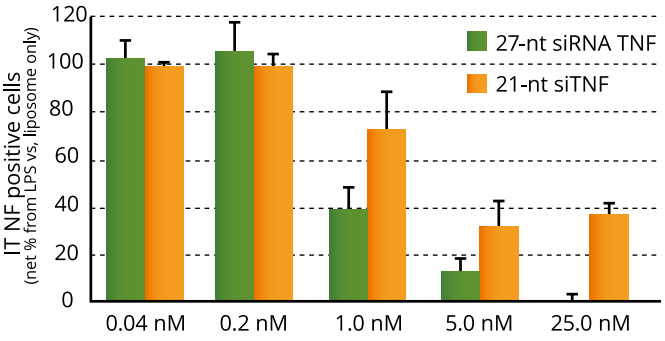
www.origene.com

Dicer-Substrate Technology for shRNA & siRNA

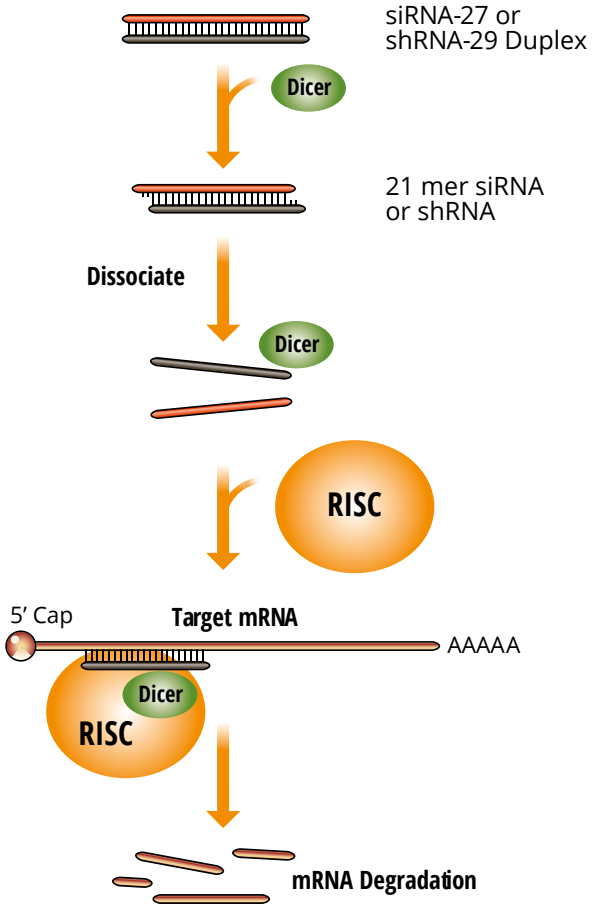
27mer-29mer delivering higher knockdown than traditional 21mer

By its optimal length, HuSH-29 shRNA & Trilencer-27 siRNA have the advantages of improved efficacy and minimal interferon response. The length and design of OriGene's RNAi substrates are important improvements over the use of traditional 21mer designs. Longer shRNA & siRNA constructs appear to enter the RNAi pathway more efficiently and result in much higher potency and specificity than shorter RNAi forms. However, in most mammalian cells, long double-stranded RNA provokes an interferon response as part of an antiviral defense. To overcome this obstacle, OriGene designs shRNAs & siRNA of less than 30 base pairs in length, which evade the mammalian immune system while still initiating strong and specific gene silencing.

A comparative study of different siRNA designs was conducted in a Nature Publication (Reference 1). According to the publication, "short RNAs that are long enough to serve as Dicer substrates (D-siRNA) can often evoke **more potent RNA interference** than the corresponding 21-nt siRNAs; this is probably a consequence of the physical handoff of the Dicer-produced siRNAs to the RNA-induced silencing complex."



Comparison of gene knockdown using dicer-substrate siRNA and 21 mer siRNA



Key publications on dicer-substrate technology

1. Rational design and in vitro and in vivo delivery of Dicer substrate siRNA, Nature Protocols 1, 508 - 517 (2006)
2. Principles of Dicer Substrate (D-siRNA) Design and Function, Methods in Molecular Biology, 442: 3-10

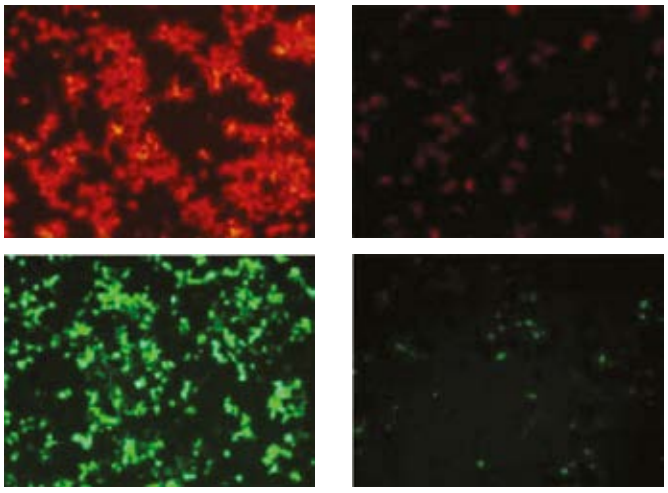
Read more about our siRNA here:
www.origene.com/sirna

Comprehensive coverage of human, mouse & rat genes

Features & Benefits

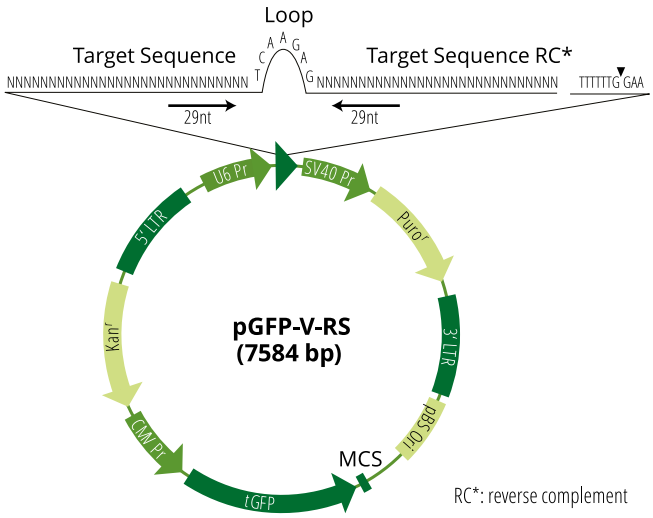
- **29mer shRNA**: higher potency & minimal interferon response
- **Lentiviral particles available:** transduce almost all cells
- **Four retroviral vectors**: including GFP & RFP for transfection monitoring and multiple selection markers to carry out double knockdown experiments
- **Transient/stable transfection** or **retroviral infection**
- **shRNA kit**: 4 gene-specific constructs + scrambled control

Double Knockdown Experiment

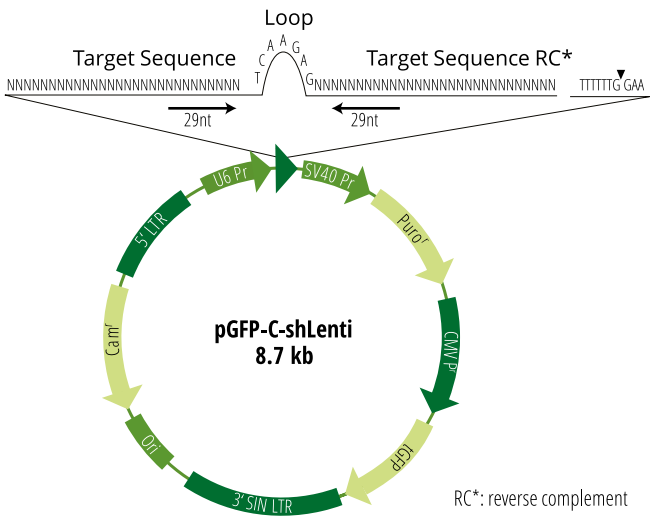


Scrambled shRNA (GFP Vector)
+
Scrambled shRNA (RFP Vector)

shRNA against RFP (GFP Vector)
+
shRNA against GFP (RFP Vector)



RC*: reverse complement



RC*: reverse complement

Schematic diagram of 2 available GFP vectors
Both vectors have been validated through publications.
Cat# TR30007 (left) and Cat# TR30023 (right)

Exact-shRNA: Custom shRNA Design Service

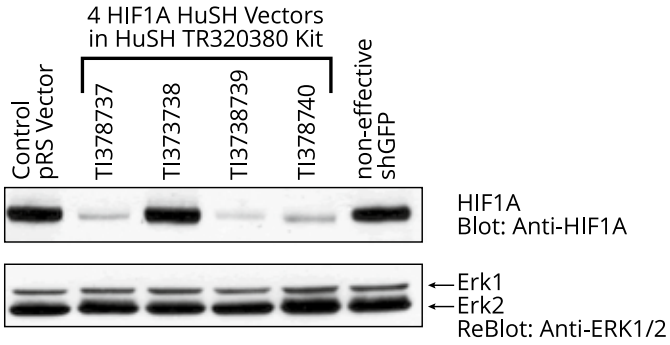
Design any shRNA or miRNA with our synthesis service

The same superior design that is available for our pre-designed HuSH-29 sets is also offered through our **Exact-shRNA** service.

- Self-design or let OriGene design it for you
- Target species other than human, mouse or rat
- Integrate an effective siRNA sequence into an shRNA vector
- Reproduce the result of a published shRNA sequence

HuSH-29 shRNA & Trilener-27 siRNA

In vitro assessment of shRNA targeting HIF1A



Downregulation of HIF1A Expression by HuSH Constructs

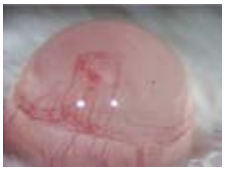
Key publications

1. Suppression of Sproutys Has a Therapeutic Effect for a Mouse Model of Ischemia by Enhancing Angiogenesis, PLoS ONE. 2009; 4(5): e5467 [In vivo application]
2. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements, Nature Genetics (4 July 2010) doi:10.1038/ng.613 Article [shRNA targeting TOP2B in GFP vector]
3. EMX2 is epigenetically silenced and suppresses growth in human lung cancer, Oncogene (9 August 2010) doi:10.1038/onc.2010.330 Short Communication [shRNA targeting EMX2 in RFP Vector]

Learn more about our shRNA at
www.origene.com/shrna

In vivo effects of shRNA targeting Sprouty4

(Cat# TR509780) PLoS ONE. 2009; 4(5): e5467



Control shRNA

Spry2/Spry4 shRNA

WT

Spry 4 KO

Trilencer-27 siRNA
Guaranteed gene silencing for human genes

Similar to HuSH-29 shRNA, OriGene's Trilencer-27 siRNA utilizes a 27mer Dicer-Substrate design that as the advantages over traditional 21mer of improved efficacy and minimal interferon response.

Features & Benefits

- **Genomewide coverage** against human, mouse and rat
- **Higher potency & minimal interferon response**
- **siRNA kit**: 3 gene-specific siRNAs + 1 negative control

siTRAN 1.0 siRNA transfection reagent

- Dual purpose reagent—transfect both siRNA duplex and corresponding cDNA clone
- High transfection efficiency and low cytotoxicity
- Cat # TT300001, TT300002 & TT300003

microRNA Expression Plasmids

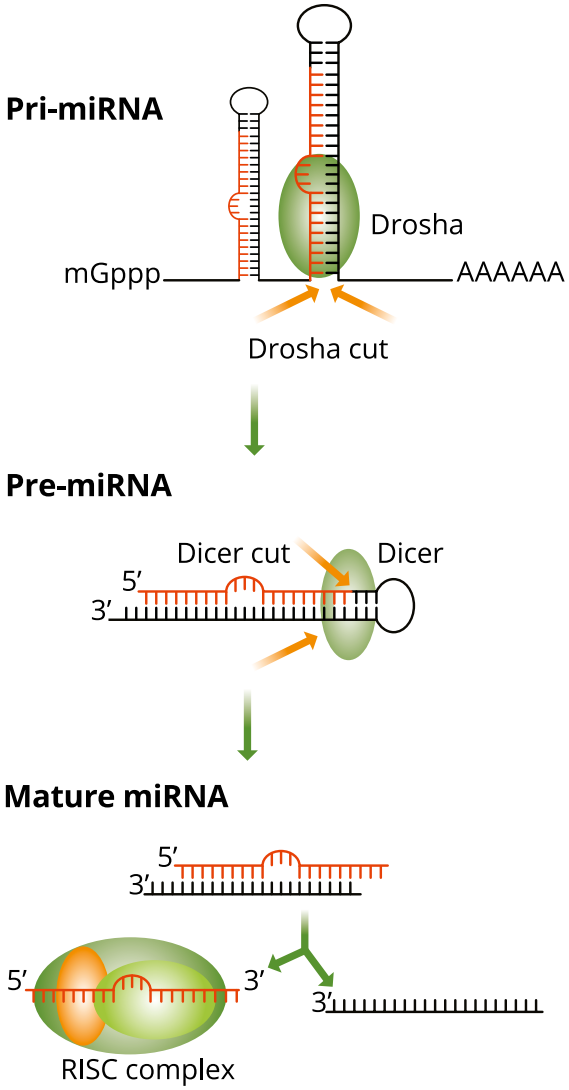
Comprehensive coverage for human, mouse, and rat genomes

OriGene provides clones for over-expression of the microRNA (miRNA) of your choice. OriGene's miRNA precursor contains pre-miRNA (60-70nt) with 250-300 nts up- and down-stream of the flanking sequence. It is amplified from human genomic DNA and cloned into OriGene's pCMV6-Mir Vector. Upon transfection, the cellular machinery will process the CMV-driven expression of miRNA precursor into mature miRNA and cellular function can be analyzed.

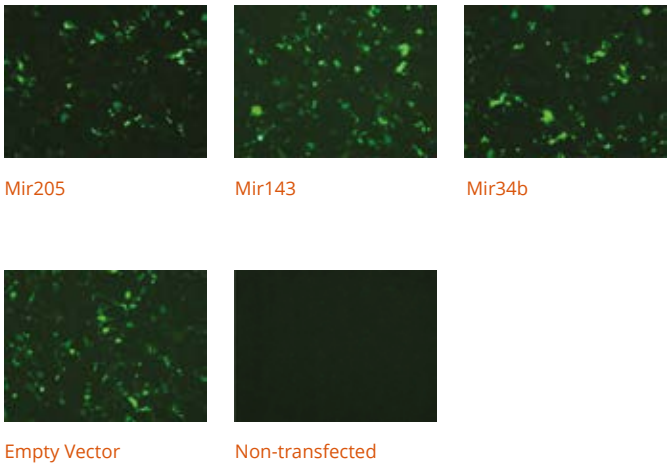
Features & Benefits

- Genome wide miRNA coverage — 1829 human, 1160 mouse, and 436 rat
- Sequence confirmation of the precursor miRNA
- GFP for transfection monitoring
- Neomycin selection for stable cell establishment GFP transfection of microRNA expression plasmids in HEK293 cells

Read more about miRNA at
www.origene.com/microRNA



GFP transfection of miRNA expression plasmids in HEK293 cells



miRNA expression plasmids

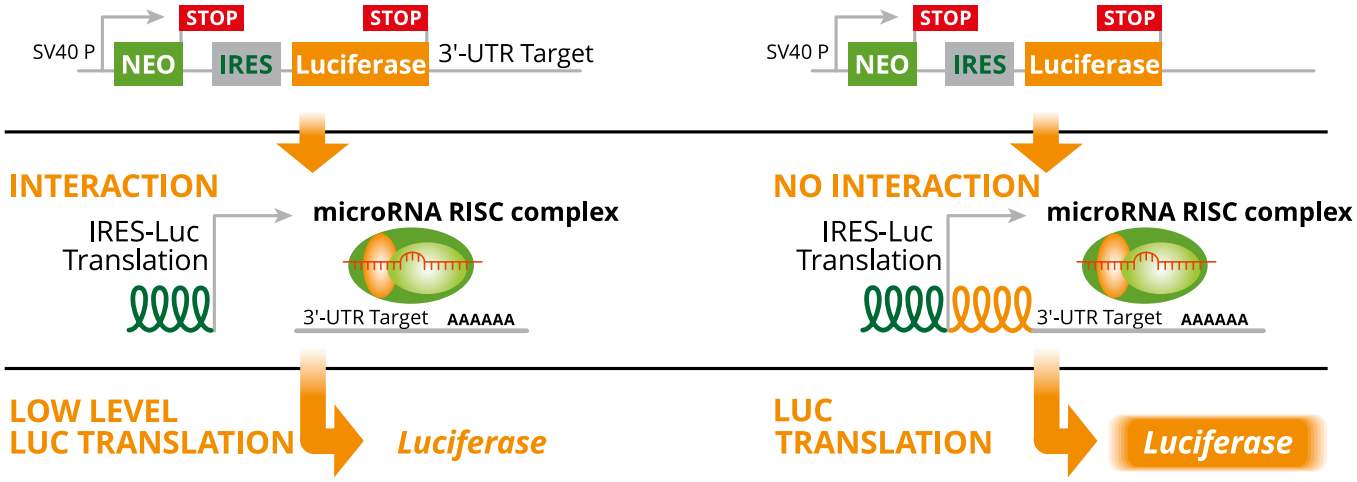
Sold individually as 10ug transfection-ready DNA or can be purchased as following sets

Catalog No.	Description
SC410001	Mouse miRNA expression plasmid set (486 vectors, 10ug each in 2-D bar coded tubes)
SC420001	Human miRNA expression plasmid set (652 vectors, 10ug each in 2-D bar coded tubes)
SC410002	Mouse miRNA expression plasmid set (486 vectors, 2ug each in 96-well plates)
SC420002	Human miRNA expression plasmid set (652 vectors, 2ug each in 96-well plates)

3'-UTR Reporter Clones for miRNA Target Validation

Luciferase reporter assays for the human genome

The 3' UTR plasmids provide a convenient solution for quantitative assessment of the inhibitory effect between miRNAs and their potential gene targets. OriGene's 3' UTR clones were designed by cloning the 3' UTR sequence of a gene of interest, downstream of the firefly luciferase gene. The chimeric transcript level is then regulated by its interaction with miRNA(s), which results in varied luciferase activity quantifiable by a colorimetric assay.



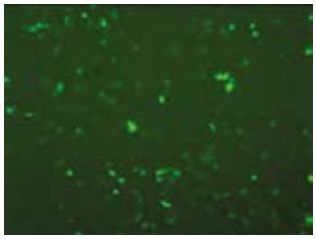
Interaction between miRNA and UTR. Reduced luciferase expression

No interaction between miRNA and UTR. No effect on luciferase expression

Features & Benefits

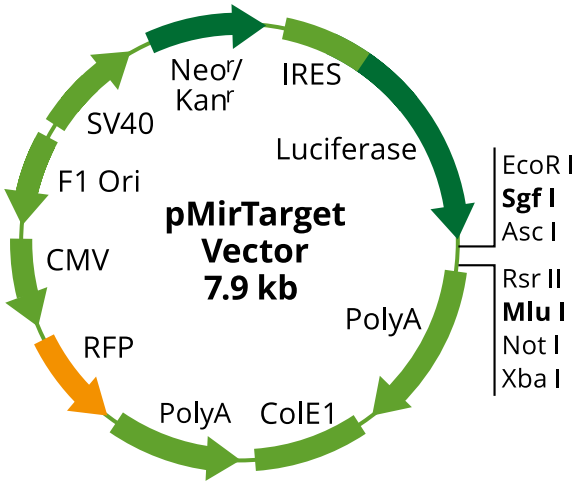
- Genome wide coverage (>20,000 human genes)
- Firefly luciferase as the easy-to-assay reporter
- RFP for transfection monitoring
- High sensitivity from IRES-driven translation of the expression cassette

Find out more at
www.origene.com/3-utr-clones



pCMV - Mir + Mir205 target

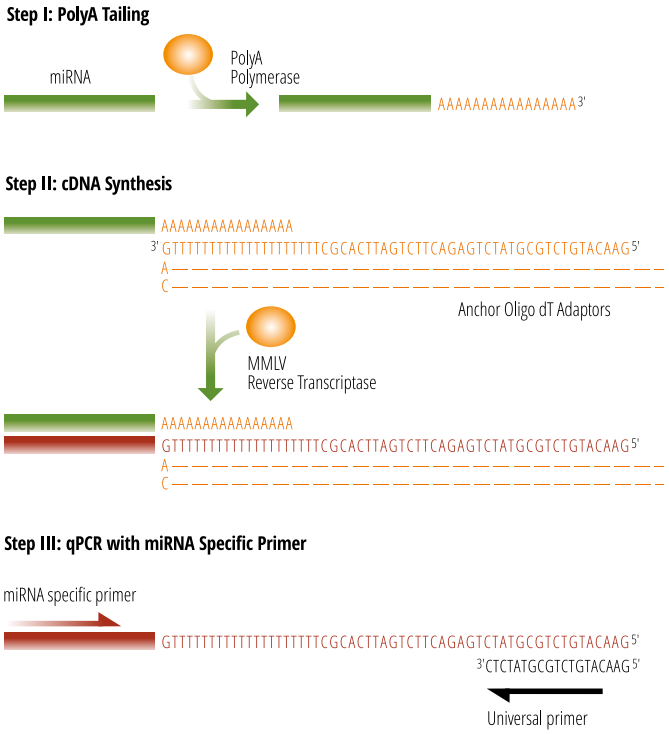
pCMV - Mir205 + Mir205 target



OriGene has used a new design adapted from C.P.Petersen et al. 2006, to dramatically increase the sensitivity of detection by decreasing the 3'UTR-luciferase reporter expression to a very low level.

Quantify your results down to the absolute copy number!

OriGene's unique primer-based, SYBR Green qPCR miRNA detection system not only offers researchers a fast and simple method for profiling miRNA expression levels, but also provides means to quantify the results down to absolute copy number of miRNA.



Features & Benefits

- Genome wide coverage of human and mouse miRNA
- Determine absolute copy number of your miRNA with template standards
- Detect miRNA directly from total RNA samples

Products offered in qPCR miRNA detection system

1	2	3	4	5
Total RNA Purification Kit	First Strand cDNA Synthesis Kit	miRNA Primer Pairs or miRNA Primer Panels	miRNA Template Standards	SYBR Green Master Mix

*Components 2,3 & 4 are unique and should only be used alongside OriGene's qPCR miRNA detection system

First-strand cDNA Synthesis Kit

Two-step protocol

- Addition of poly (A) tail to RNA sample
- Use of anchor linker oligo dT to synthesize first-strand cDNA
- Cat# NP100041 & NP100042

miRNA Primers & Panels

- Offered as individual primers, genome wide panels, & custom-mixed panels
- Pre-validated in an experiment against normal and ovarian cancer samples

miRNA Copy Number Standards

- Unique offering only from OriGene
- Determine the absolute transcript copy number of an experiment sample using the standard curve method

Find more information at
www. origene.com/qpcr-mirna

OriGene, Your Partner in Research, Diagnostics and Beyond

- cDNA Clones/Lenti & AAV Particles
- CRISPR/Cas9/sgRNA
- Expression Vectors
- Recombinant Proteins
- Antibodies
- RNAi
- Normal & Cancer Tissues

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