

RNA Interference with Guaranteed Knockdown!

microRNA, shRNA, & siRNA



microRNA detection with SYBR-Green qPCR experiments

microRNA expression

microRNA target validation with 3'-UTR clones

shRNA & siRNA mediated knockdown

microRNA Expression Plasmids

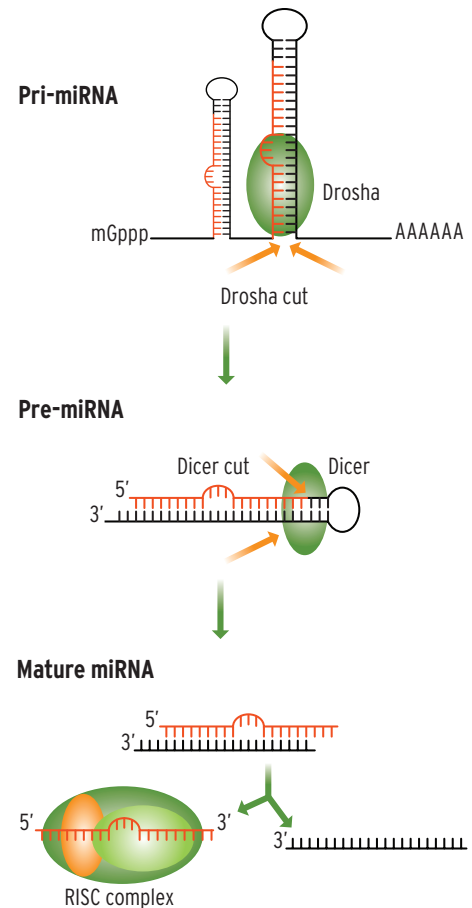
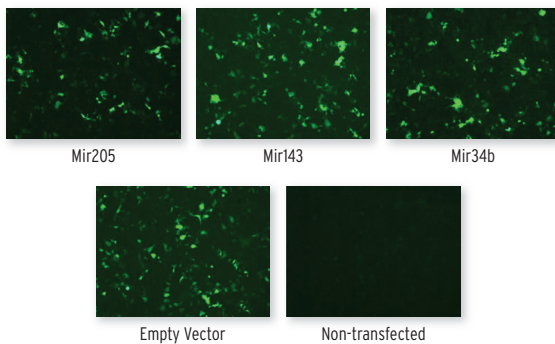
Comprehensive coverage for human and mouse

OriGene provides clones for over-expression of microRNA of your choice. OriGene's microRNA precursor contains pre-miRNA (60-70nt) with 250-300 nts up- and down-stream of flanking sequence. It was 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 – **652 human and 486 mouse**
- Sequence confirmation of the precursor microRNA
- GFP for transfection monitoring
- Neomycin selection for stable cell establishment

GFP transfection of microRNA expression plasmids in HEK293 cells



MICRORNA EXPRESSION PLASMIDS

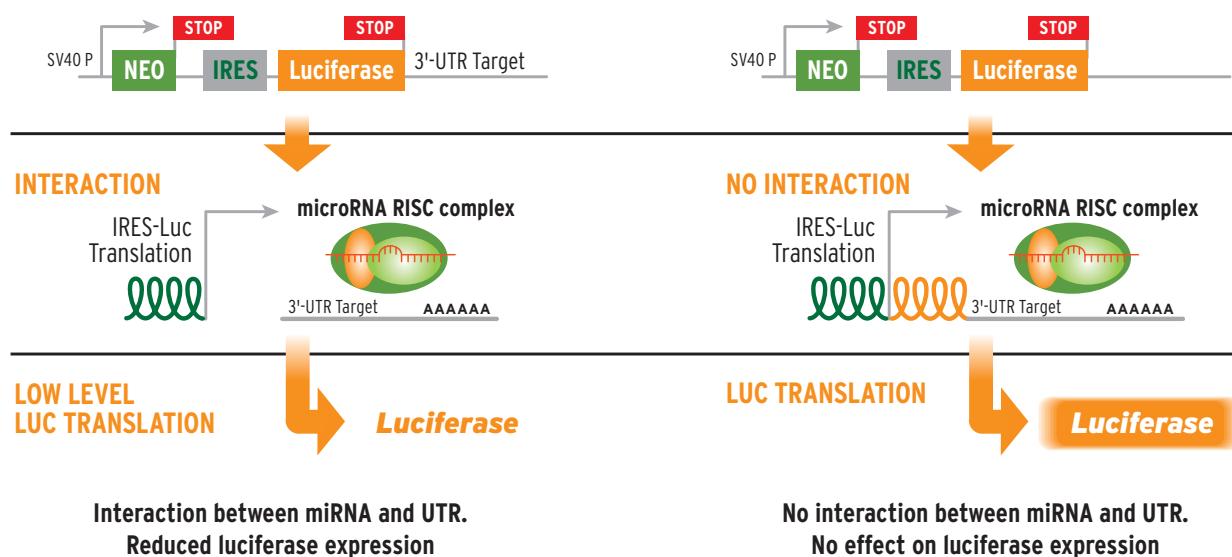
Sold individually as 10ug transfection-ready DNA or can be purchased as following sets

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

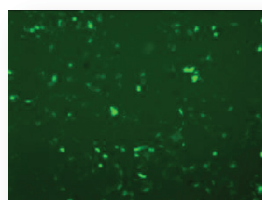
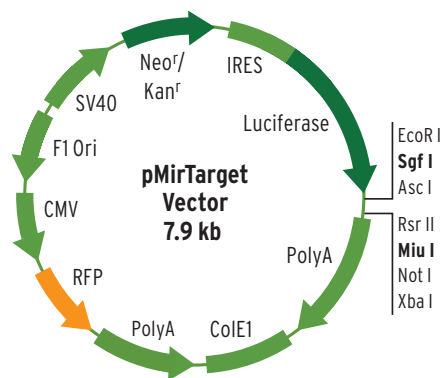
Luciferase reporter assays for human genome

The 3'-UTR plasmids provide quantitative assessment of the inhibitory effect between miRNAs and their potential target genes. The 3'-UTR sequence of a gene was cloned downstream of the firefly luciferase gene. The chimeric transcript level is regulated by its interaction with miRNA(s), which results in varied luciferase activity quantifiable by a colorimetric assay. MicroRNAs and their 3'-UTR targets can be assayed for endogenous microRNA activity or for overexpressed microRNA in a co-transfection experiment.

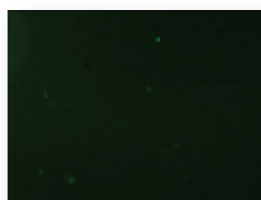


FEATURES & BENEFITS

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



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.

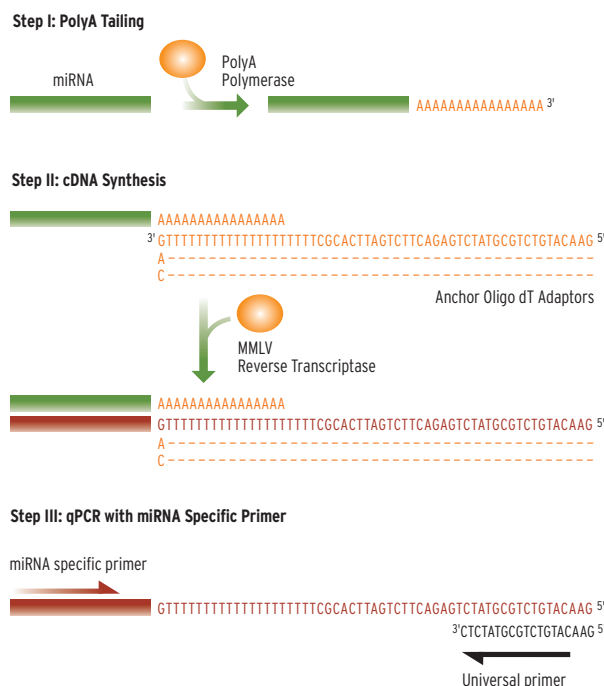
qSTAR microRNA qPCR Detection Assays

Quantify your results down to the absolute copy number!

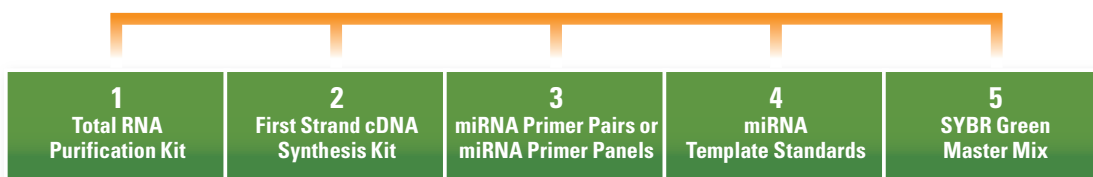
OriGene's unique primer-based, SYBR Green qPCR microRNA detection system not only offers researchers a fast and simple method for profiling miRNA expression levels, but also provides means to quantify the results down to the absolute copy number of miRNA.

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 microRNA DETECTION SYSTEM



*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 kit

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

microRNA Primers & Panels

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

microRNA Copy Number Standards

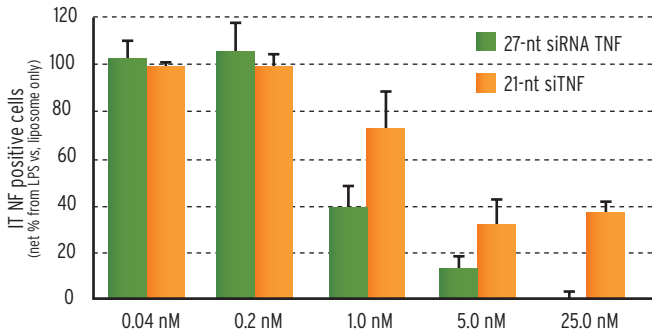
- Unique offering only from OriGene
- Determine the absolute transcript copy number of an experiment sample using the standard curve method

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."

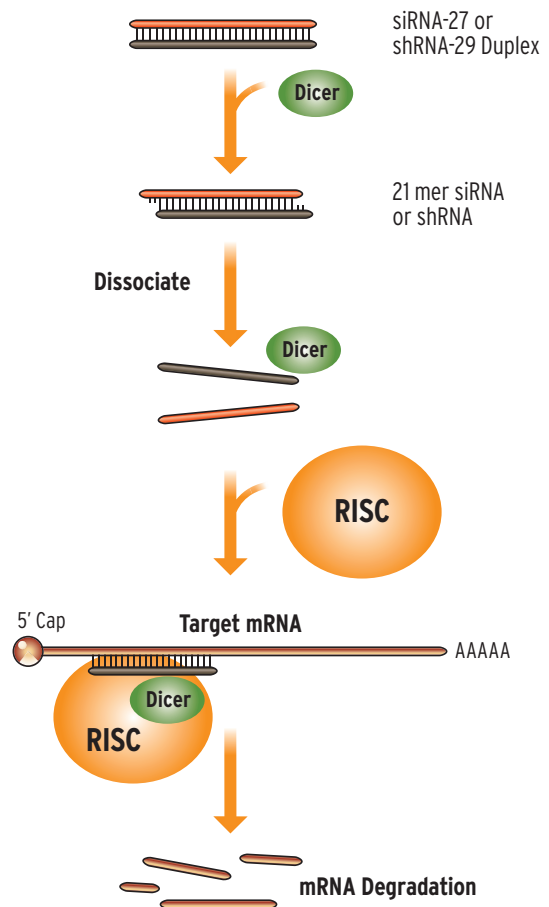


Comparison of gene knockdown using dicer-substrate siRNA and 21 mer siRNA

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

HOW DICER-SUBSTRATE RNAi WORKS



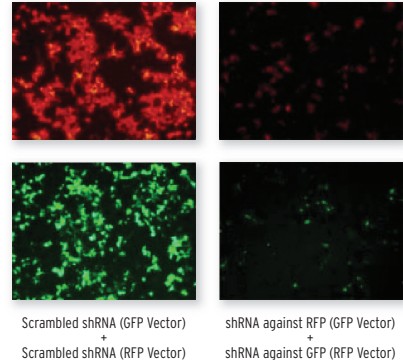
HuSH-29 shRNA–Guaranteed Gene Knockdown

Comprehensive coverage of human, mouse & rat genes

FEATURES & BENEFITS

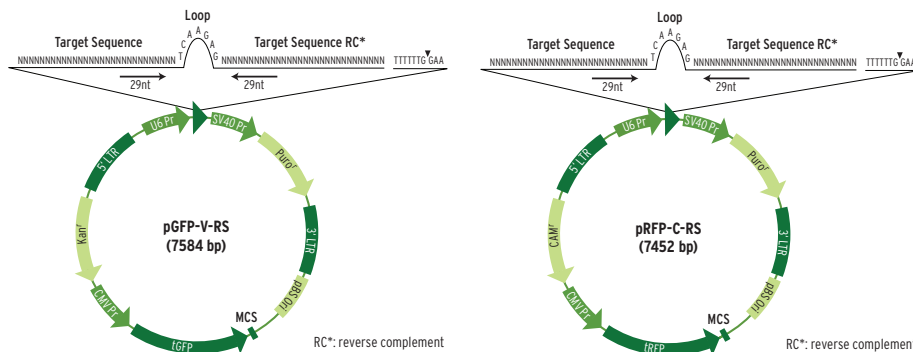
- **29mer shRNA:** higher potency, minimal interferon response
- **Four retroviral vectors**, including GFP & RFP for transfection monitoring and multiple selection markers to carry out double knockdown experiments
- **Transient or stable transfection** or **retroviral infection**
- **shRNA kit:** 4 gene-specific constructs + 2 negative controls

DOUBLE KNOCKDOWN EXPERIMENT



SCHEMATIC DIAGRAM OF GFP & RFP VECTORS

Both vectors have been validated through publications.



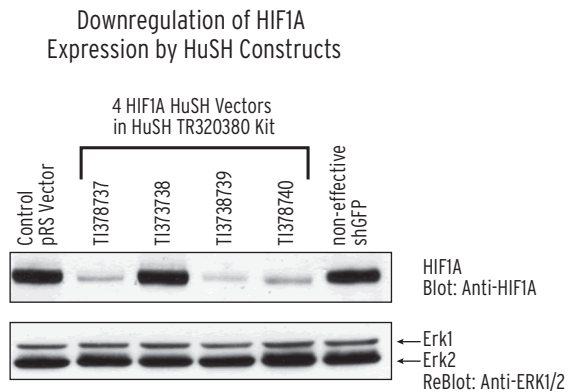
Exact-shRNA Service

Design any shRNA or miRNA with custom synthesis service

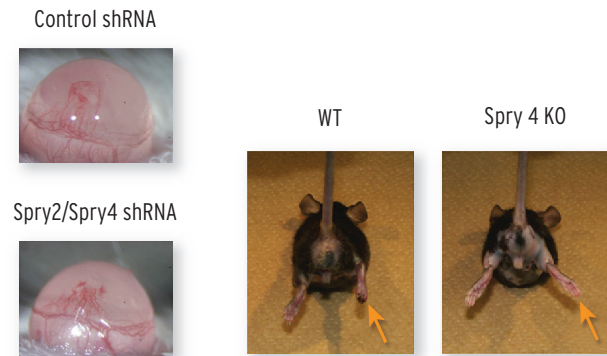
The same superior design that is available for our pre-designed HuSH-29 sets is also offered through our **Exact-shRNA** service.

- Self-design or let OriGene design 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

In vitro assessment of shRNA targeting HIF1A



In vivo effects of shRNA targeting Sprouty4 (Cat# TR509780) PLoS ONE. 2009; 4(5): e5467



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]

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 has the advantages over traditional 21mer of improved efficacy and minimal interferon response.

FEATURES & BENEFITS

- **Genomewide coverage** against human
- **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

OriGene, Your Partner in Gene Research and Beyond

KEY TECHNOLOGIES AND PRODUCTS

- Full-length cDNA clones, ORF clones in expression-ready vectors
- Gene synthesis: any gene, any variant, any vector
- RNAi research reagents: shRNA, siRNA, and miRNA function and detection
- SYBR Green qPCR assays for mRNA and miRNA detection, primer panels
- Recombinant human proteins and over-expression lysates
- TrueMAB™ monoclonal antibodies
- Luminex multiplex immunoassays
- Cancer tissue biorepository, TMA, RPPA, and TissueScan qPCR arrays
- UltraMAB™ validated IHC antibodies



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