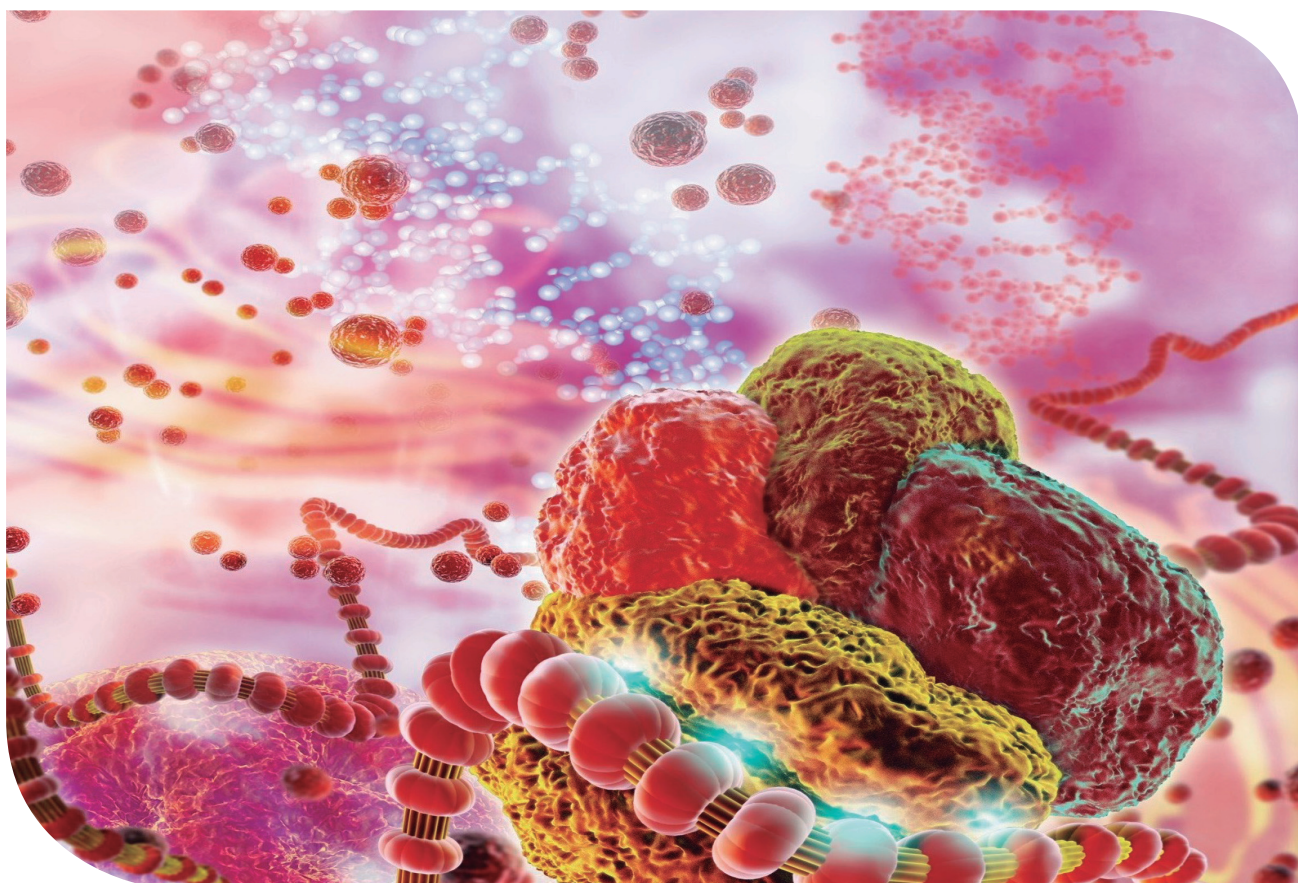


MicroRNA Solutions



Over-Expression

miExpress™ miRNA Precursors

Inhibition

miArrest™ miRNA Inhibitors

3' UTR Target Validation

miTarget™ miRNA 3' UTR Targets

Quantitation

All-in-One™ miRNA qRT-PCR Primers

All-in-One™ miRNA qRT-PCR Kits

Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level. They regulate gene expression by binding to the 3' untranslated regions (3' UTRs) of targeted mRNAs specifically, which results in either translation suppression or mRNA cleavage and degradation. Usually 21-23 nucleotides in length, microRNAs are important modulators in cellular pathways and are highly conserved in eukaryotic organisms. Irregularities in miRNA-regulated gene expression have been found to be associated with cancers, cardiovascular disorders and a variety of other diseases.

Mechanism

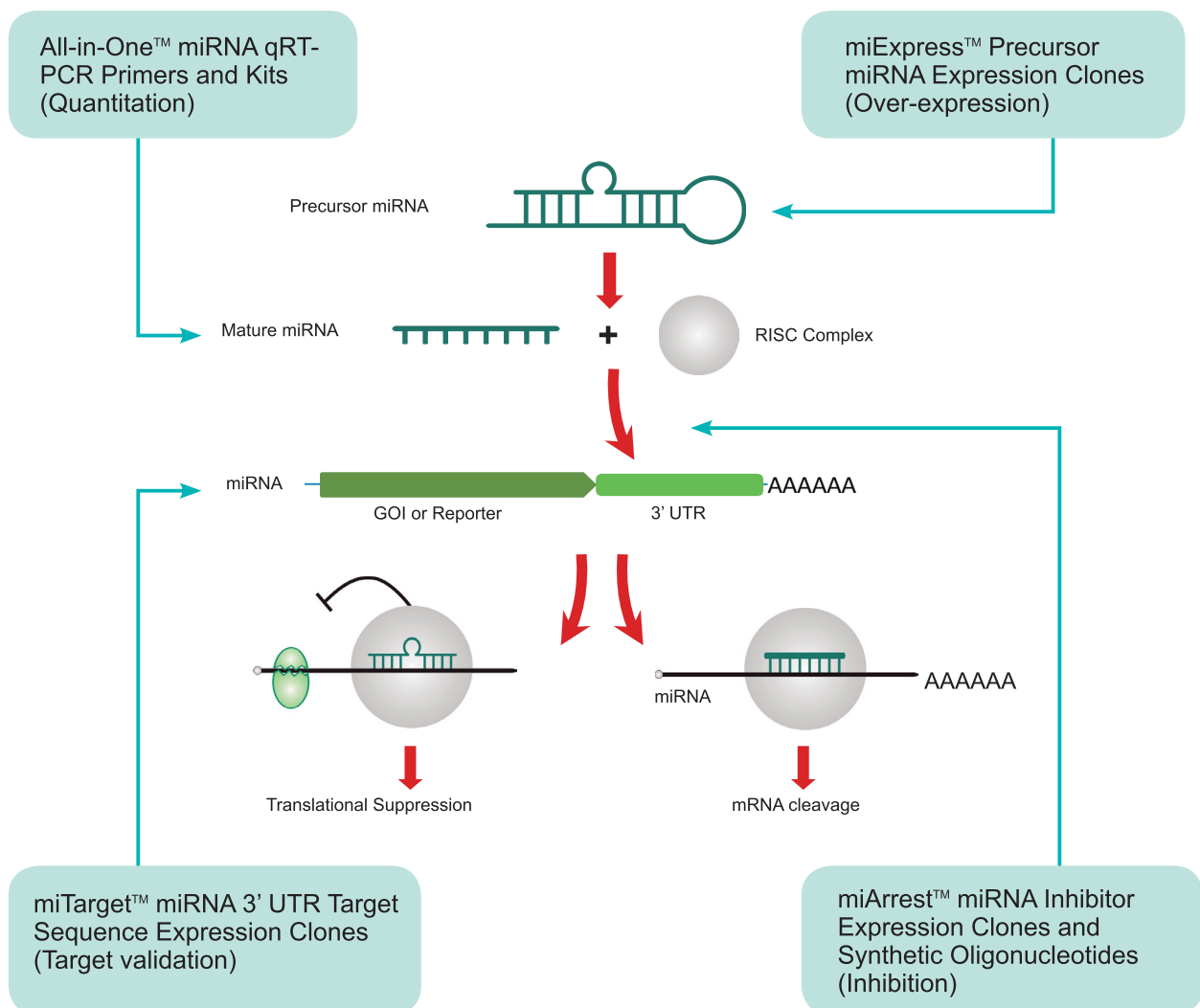


Figure 1. miRNA mechanism and GeneCopoeia comprehensive miRNA solutions. GOI: gene of interest.

Product Portfolios

Committed to serve researchers in the functional genomics and proteomics area, GeneCopoeia provides comprehensive tools and services for miRNA research.

Product	Expressway to Discovery
miExpress™ Precursor miRNA Expression Clones	1048 human, 672 mouse, 408 rat Over-express miRNA for gain of function studies
miArrest™ miRNA Inhibitor Expression Clones and Synthetic Oligonucleotides	1048 human, 672 mouse, 408 rat Inhibit miRNA for loss of function studies
miTarget™ miRNA 3' UTR Target Sequence Expression Clones	40,000 human, mouse, rat and custom 3'UTR clones Validate miRNAs and their gene targets (3'UTR)
All-in-One™ miRNA qRT-PCR Primers	Validated human, mouse and rat primers Amplify mature miRNAs to study their expression profiles
All-in-One™ miRNA qRT-PCR Detection Kits	SYBR® Green-based qRT-PCR kits Detect mature miRNAs and study their expression profiles
Luc-Pair™ miR Luciferase Assay Kits	Sequential analysis of dual luciferase reporters, optimized for use with miTarget™ miRNA 3' UTR target expression clones Detect changes in luciferase expression for miRNA target validation

Benefits

Complete solutions

for human, mouse and rat miRNA functional analysis and quantitation

Flexible delivery options

for choices of long-term or short-term miRNA study in virtually all cell types

Reliable design and validated tools

for effective modulation and accurate quantitation of miRNA function

■ ■ ■ Precursors

Over-expression of miRNA in addition to endogenous miRNA enhances miRNA regulation and suppresses target protein translation.

miExpress™ Precursor miRNA Expression Clones

Available in both viral and non-viral vectors, miExpress™ precursor miRNA expression clones allow stable transduction or transient transfection of miRNA into virtually all cell types, including difficult-to-transfect and non-dividing cells.

Vector	Promoter	Selection marker	Reporter gene	Viral type
pEZX-MR01	H1	Neomycin	eGFP	Lenti
pEZX-MR03	CMV	Puromycin	eGFP	Lenti
pEZX-MR04	CMV	Puromycin	eGFP	N/A

Full coverage

- Cover all known human, mouse and rat miRNAs in the miRBase database.

Flexible delivery systems

- Lentiviral vectors allow efficient transduction and stable integration into the host genome of non-dividing and difficult-to-transfect target cells.
- Non-viral vectors are available for transient transfection studies.

Optimized expression cassettes

- Expression cassettes have been fully sequenced and optimized for high expression of precursor miRNA and maturation of miRNA inside cells.

Selection markers and reporters

- An eGFP reporter is designed to monitor transduction/transfection efficiencies.
- A neomycin or puromycin marker enables stable cell line selection.

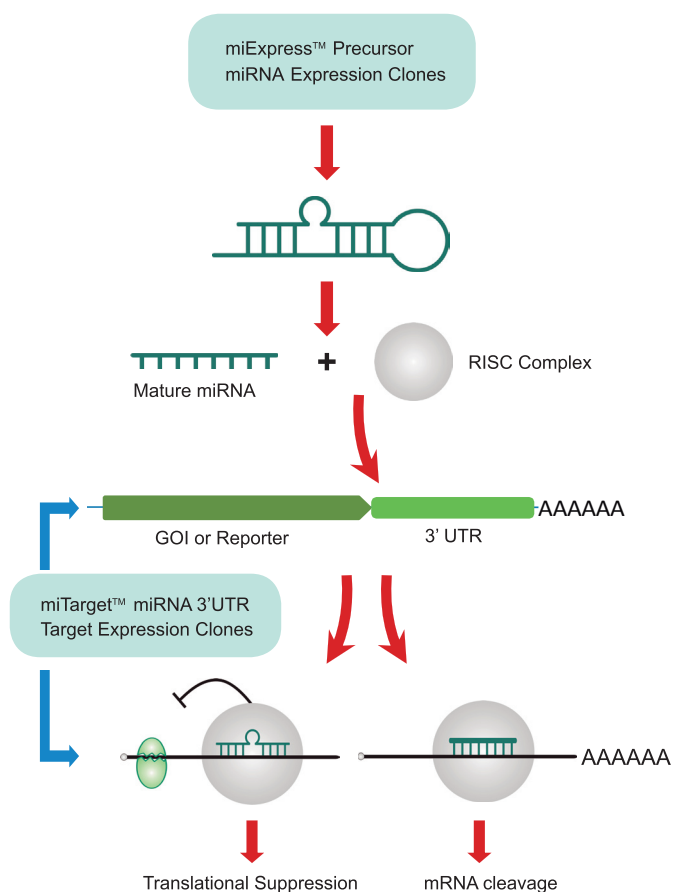


Figure 2. Roles of miExpress™ precursor miRNA and miTarget™ 3' UTR target clones in miRNA regulation studies.

3' UTR Targets

miRNAs regulate target gene expressions by binding to the specific sequences in the 3' UTR regions of target mRNAs. When the 3' UTR target sequence is fused downstream to a luciferase reporter and expressed in vitro, the luciferase expression is regulated by the miRNA that binds to the downstream 3' UTR specially. Therefore luciferase activity can be analyzed to study miRNA-target regulation and specificity.

miTarget™ miRNA 3' UTR Target Sequence Expression Clones

Constructed in a single vector system with dual reporters, miTarget™ 3' UTR target expression clones enable convenient and accurate study using one reporter for regulatory detection and the other one for internal control and signal normalization.

Vector	Reporter gene	Tracking gene	Advantage
pEZX-MT01	Firefly luciferase	<i>Renilla</i> luciferase	Perform assays on cell lysates
pEZX-MT02	<i>Gaussia</i> luciferase	Alkaline phosphatase	Perform enzymatic assays without destroying target cells

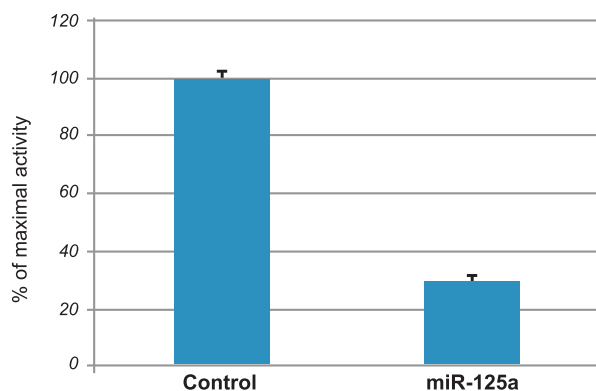


Figure 3. The inhibitory regulation of miRNA on a 3' UTR target -fLuc expression clone. HEK 293 cells were transfected with either miRNA control clone or miR-125a precursor miRNA clone, together with Lin28 3'UTR miRNA target clone. Both firefly luciferase and Renilla luciferase activities expressed from Lin28 3' UTR miRNA target clone were measured using GeneCopoeia's Luc-Pair™ miR Luciferase Assay Kit. Renilla luciferase activity was used to normalize firefly luciferase signal in the same well. In the presence of miR-125a miRNA, the firefly luciferase activity was significantly down-regulated.

Simple systematic design

- The 3' UTR target sequence is fused downstream to either a firefly or *Gaussia* luciferase. Expression of the luciferase is suppressed when a miRNA complex binds specifically to the 3' UTR target.

Easy quantification

- Luciferase activities can be easily quantified using either cell lysate (firefly luciferase, non-secreted) or supernatant without destroying target cells (*Gaussia* luciferase, secreted).

Convenient dual-reporter vector

- Single vector with dual reporters - one for regulatory detection and one for internal control and signal normalization.
- Eliminate the need for transfecting two separate reporter plasmids.

Full coverage

- Genome-wide coverage of human, mouse and rat.

Inhibitors

miRNA inhibitors block miRNA regulation of target gene expression. They are designed and optimized for miRNA loss of function study.

miArrest™ miRNA Inhibitor Expression Clones and Synthetic Oligonucleotides

Available as lentiviral and non-viral vector-based expression clones or synthetic oligonucleotides, miArrest™ miRNA inhibitors bind specifically to their target miRNA, allowing transient or stable blockage of the miRNA regulation.

Vector	Promoter	Selection marker	Reporter gene	Viral type
pEZX-AM03 or AM04	H1 or U6	Hygromycin	mCherry	Lenti
pEZX-AM01 or AM02	H1 or U6	Puromycin	mCherry	N/A

Full coverage

- Cover all known human, mouse and rat miRNAs available in the miRBase database.

Flexible delivery systems

- Lentiviral vectors allow efficient transduction and stable integration into the host genome of non-dividing and difficult-to-transfect target cells.
- Non-viral vectors and chemically synthesized oligonucleotides are available for transient transfection.

Advanced and reliable design

- Expression cassettes have been designed using a proprietary algorithm and tested rigorously by carefully designed experiments.

Superior performance

- Superior potency, long-lasting inhibition and extremely low cell toxicity compared to other chemically synthesized miRNA inhibitors.
- Choices of H1 or U6 promoter allow constitutive expression of inhibitors in virtually all mammalian cell types.

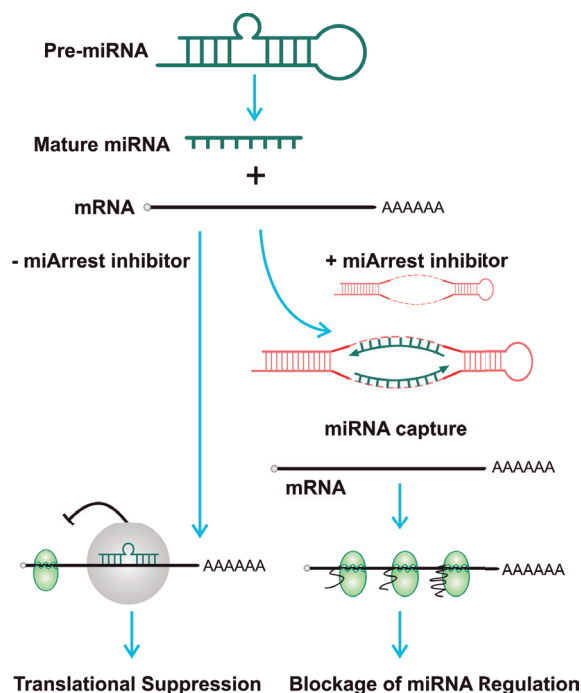
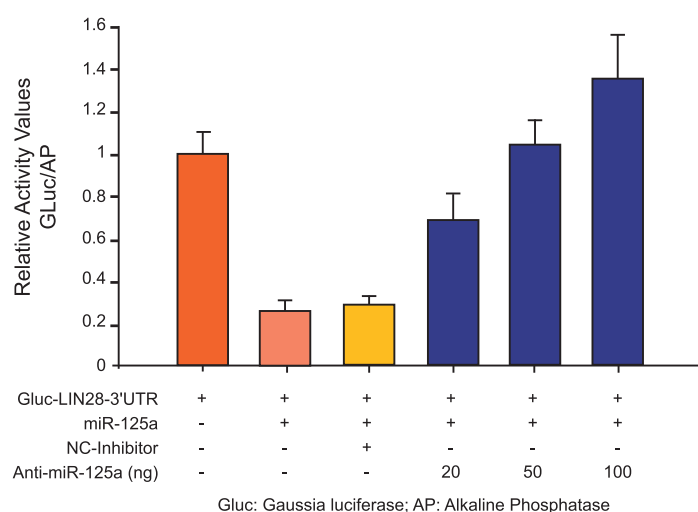


Figure 4. Mechanism of miArrest™ miRNA inhibitor expression clones.

Vector-Based vs. Synthetic

Feature	Vector-based inhibitor	Synthetic 2'-OMe inhibitor
Inhibition	+++++	++
Specificity	+++++	+++
Stability	+++++	+
Durability	Long term	Transient
Cell toxicity	-	-
Delivery to resting and hard-to-transfect cells	+++++	-

Example



Gluc-LIN28-3'UTR:

3'UTR sequence of LIN28 in *Gaussia* Luciferase-Alkaline Phosphatase dual reporter expression vector. LIN28 is a known target gene for miR-125a .

miR-125a:

miR-125a precursor expression plasmid

NC-Inhibitor:

negative control, no inhibitor

Anti-miR-125a :

miR-125a inhibitor expression plasmid

Figure 5. Effect of vector-based inhibitor against miR-125a. A miR-125a inhibitor expression plasmid was transfected into HEK 293 cells with 1) a miR-125a precursor expression plasmid and 2) a 3'UTR sequence of LIN28 in *Gaussia* Luciferase-Alkaline Phosphatase dual reporter expression vector. Both the GLuc activity and an internal control AP activity were determined 24 hours post-transfection. For normalization purpose, the activity ratio of GLuc to AP was set to 1 for LIN28 3'-UTR target clone only transfection (first bar from the left). The result shows that mir-125a suppressed the luciferase activity from the Gluc-LIN28-3'-UTR clone by more than 70% (second bar from the left). This suppression effect was blocked by the introduction of varying amount of miArrest™ inhibitor clone against miR-125a in a dose-dependent manner. At the highest dose, the reporter GLuc activity is higher than the control (first bar from the right). This could be attributed to the fact that this vector-based inhibitor may have blocked the regulatory effect of endogenous miR-125a, which would result in increased translational activity of GLuc-LIN28-3'-UTR transcript.

Profiling Kits

All-in-One™ miRNA qRT-PCR Detection Kit

The All-in-One™ miRNA qRT-PCR detection kit combines PCR technology and SYBR® Green to provide fast and accurate quantitation of mature miRNAs from total RNA samples as small as 100 pg.

The All-in-One™ uses a two-step method and makes detection and quantification of multiple miRNAs from one cDNA synthesis easy and efficient.

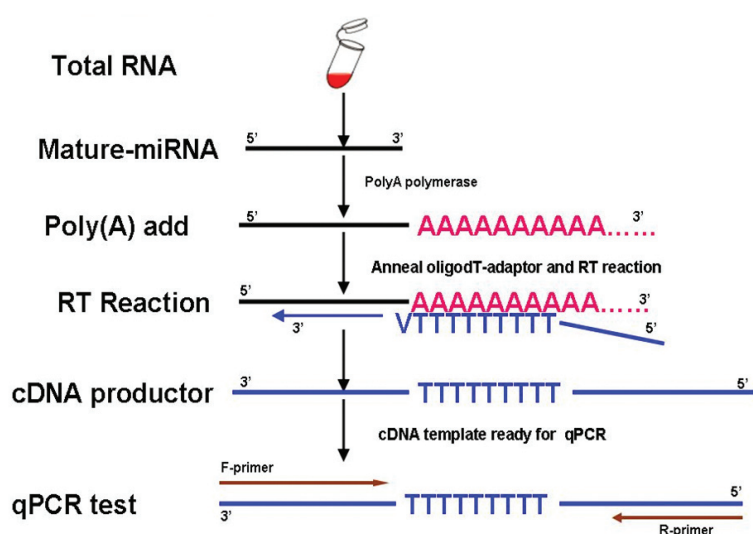


Figure 6. Overview of steps involved in the All-in-One™ miRNA qRT-PCR detection kit. During 3' polyadenylation, M-MLV RTase and a unique oligo dT adaptor primer reverse transcribe the poly A miRNAs. The qRT-PCR mix in the kit, containing SYBR® Green specifically, detects the reverse transcribed miRNAs.

The All-in-One™ reverse transcriptase mix contains a novel optimized blend of poly A polymerase and reverse transcriptase in a RT buffer that was specifically developed for the maximum activity of both enzymes, which gives robust and reproducible performance.

Catalog#	Product	Description
AOMD-Q020	All-in-One™ miRNA qRT-PCR Detection Kit (20 RT and 200 qPCR reactions)	Poly A polymerase, RTase mix, qPCR mix, ROX reference dye, universal adaptor PCR primer and other buffers
AOMD-Q050	All-in-One™ miRNA qRT-PCR Detection Kit (50 RT and 500 qPCR reactions)	Poly A polymerase, RTase mix, qPCR mix, ROX reference dye, universal adaptor PCR primer and other buffers
Variable	All-in-One miRNA qRT-PCR Primer (20 µl x 500 reactions)	Validated human, mouse and rat mature miRNA primers

Validated Primers

All-in-One™ qRT-PCR miRNA Primers

The All-in-One™ qRT-PCR mature miRNA primers are designed using a proprietary algorithm and experimentally validated. When used in combination with the All-in-One™ SYBR® Green miRNA qRT-PCR kit, the All-in-One™ miRNA primers deliver reliable and reproducible high performance.

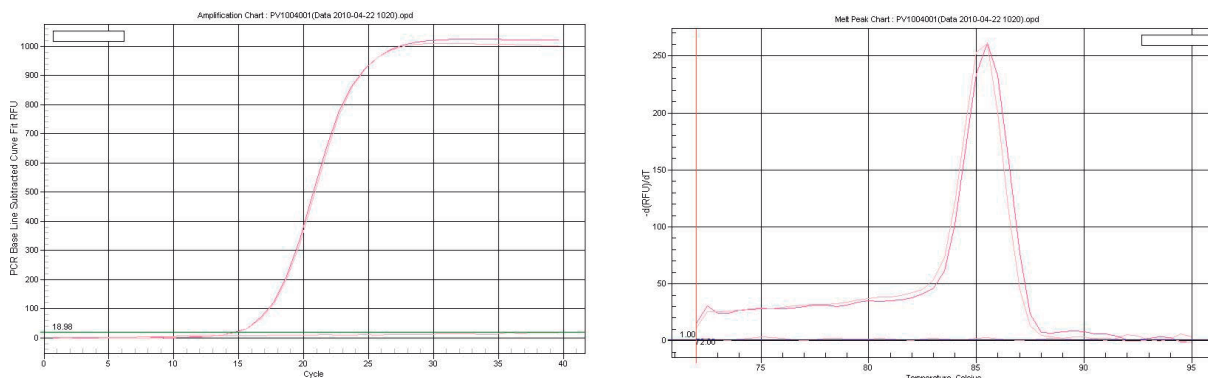


Figure 7. The All-in-One™ miRNA qPCR primers are validated to generate a single amplification of the correct size for the targeted miRNA and to yield a single dissociation curve peak. A cDNA pool, containing reverse transcribed products from 10 human tissue total RNA samples was used as the validation template. qPCR was performed using 0.2 μ M primer with 2X All-in-One™ qPCR Mix. (Left) Validated result for amplification curve. (Right) Validated result for melting curve.

Validated primer design

- Mature miRNA-specific primers are designed using a proprietary algorithm and experimentally validated.

Full coverage

- Primers for all known human, mouse and rat miRNAs are available.

Robust and sensitive

- A robust formulation provides more universal reaction condition.
- Detect single-base differences.
- Detect mature miRNA in 100 pg of total RNA.

Convenient and reproducible

- A two-step method makes detection and quantification of multiple miRNAs from one cDNA synthesis easy and efficient.
- Deliver reliable and reproducible results.

Assays

Luc-Pair™ miR Luciferase Assay Kit

The Luc-Pair™ miR luciferase assay kit is optimized for use with miTarget™ miRNA 3' UTR target expression clones for easy, cost-effective and high-throughput validation of miRNA-target interactions. It provides an efficient system by measuring firefly and *Renilla* luciferases sequentially in a convenient 96-well plate format.

Catalog #	Product	Description
LPFR-M010	Luc-Pair miR Luciferase Assay Kit (1)	Substrates and buffers for 100 reactions
LPFR-M030	Luc-Pair miR Luciferase Assay Kit (3)	Substrates and buffers for 300 reactions
LPFR-M100	Luc-Pair miR Luciferase Assay Kit (10)	Substrates and buffers for 1000 reactions

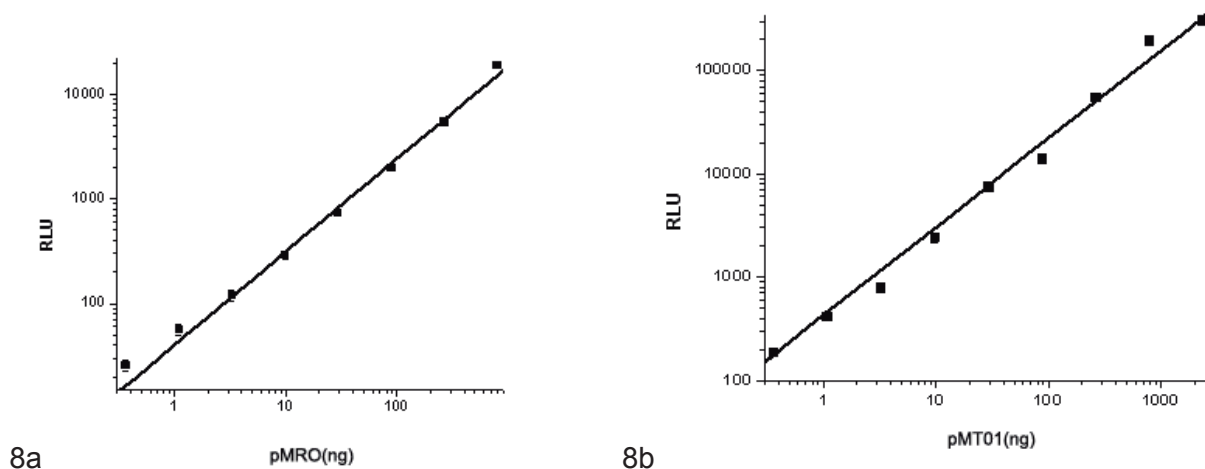


Figure 8. Linear relationship between emitted light and amount of luciferase expression vector transfected in HEK293. HEK 293 cells were plated on a 6-well plate. On the second day, the cells were transfected with different amounts of GeneCopoeia pMRO or pEZX-MT01 miRNA 3' UTR target expression clone as indicated in the figure. The cells were transferred to a 96-well plate 18 hours after transfection and cultured for another 24 hours. Firefly luciferase assay was then performed on pMRO vector (Fig. 8a) and *Renilla* luciferase assay was performed on pEZX-MT01 vector (Fig. 8b)

Dual reporter system

- Measuring the activities of firefly and *Renilla* luciferases sequentially from a single sample.

Single-tube or high-throughput format

- Suitable for both single-tube and 96-well high-throughput formats.

Robust performance

- Reliable and linear results for a large dynamic range.
- Very-limited background luminescence. No subtraction is required from readings.

Complete Solutions

GeneCopoeia offers complete solutions for microRNA research. The related products were developed to use with GeneCopoeia miRNA precursor, inhibitor and target expression clones. They have been tested and validated to provide robust and reproducible performance.

Category	Product	Description
Lentiviral System	Lentifect™ Lentivirus Production Services	High-titer crude or purified lentiviral particles produced by experts and ready-for-transduction
	Lenti-Pac™ Lentiviral Packaging Kits	<ul style="list-style-type: none"> Optimized lentiviral packaging plasmid mix eGFP control clone LentiFect™, a new transfection reagent developed to work with lentiviral-based constructs TiterBoost™, a reagent that further increases the titers by 5-10 folds
	Lenti-Pac™ 293Ta Lentiviral Packaging Cell Line	For high-titer lentiviral production using Lenti-Pac™ lentiviral packaging kits
CustomCloning	De Novo Gene Synthesis and Cloning Services	For any miRNA precursors, inhibitors, and 3'UTR target sequences that are not on our premade product list
Transfection Reagents	EndoFectin™ Lenti EndoFectin™ CHO EndoFectin™ Plus EndoFectin™ MAX	Fully optimized and validated for specific cell types
ORF cDNA Expression Clones	OmicsLink™ Expression-Ready ORF cDNA Clones	<ul style="list-style-type: none"> Genome-wide coverage of human and mouse Largest selection of vector types and fusion tags Fully sequenced and thousands are expression-tested

GeneCopoeia Headquarters, USA

9620 Medical Center Drive, Suite 101
Rockville, MD 20850, USA

Email inquiry@genecopoeia.com
Tel +1 (301) 762-0888
Toll free +1 (866) 360-9531
Fax +1 (301) 762-3888
Website www.genecopoeia.com

Fulengen Co., Ltd., P.R. China

8F, Building D
#3 Juquan Road
Guangzhou Science Park
Guangzhou, China 510663

Email sales@fulengen.com
Tel +86 (20) 3205-2376
Fax +86 (20) 3205-2877
Website www.fulengen.com

Distributors

Australia & New Zealand	United Bioresearch Products	www.unitedbioresearch.com.au
Europe	ImaGenes GmbH	www.imagenes-bio.de
	LabOmics S.A.	www.labomics.com
India	Life Technologies (India) Pvt. Ltd.	www.lifetechindia.com
Israel	ORNAT Biochemicals and Laboratory Equipment Ltd.	www.ornat.co.il
Japan	Cosmo Bio	www.cosmobio.co.jp
	Sanct Corporation	www.sanct.co.jp
Singapore	Centrogen Technologies Pte Ltd.	www.centrogen.com.sg
South Korea	Cosmo Genetech Co., Ltd.	www.cosmo4.com
Taiwan	Integrated Bio Ltd.	www.integrated-bio.com