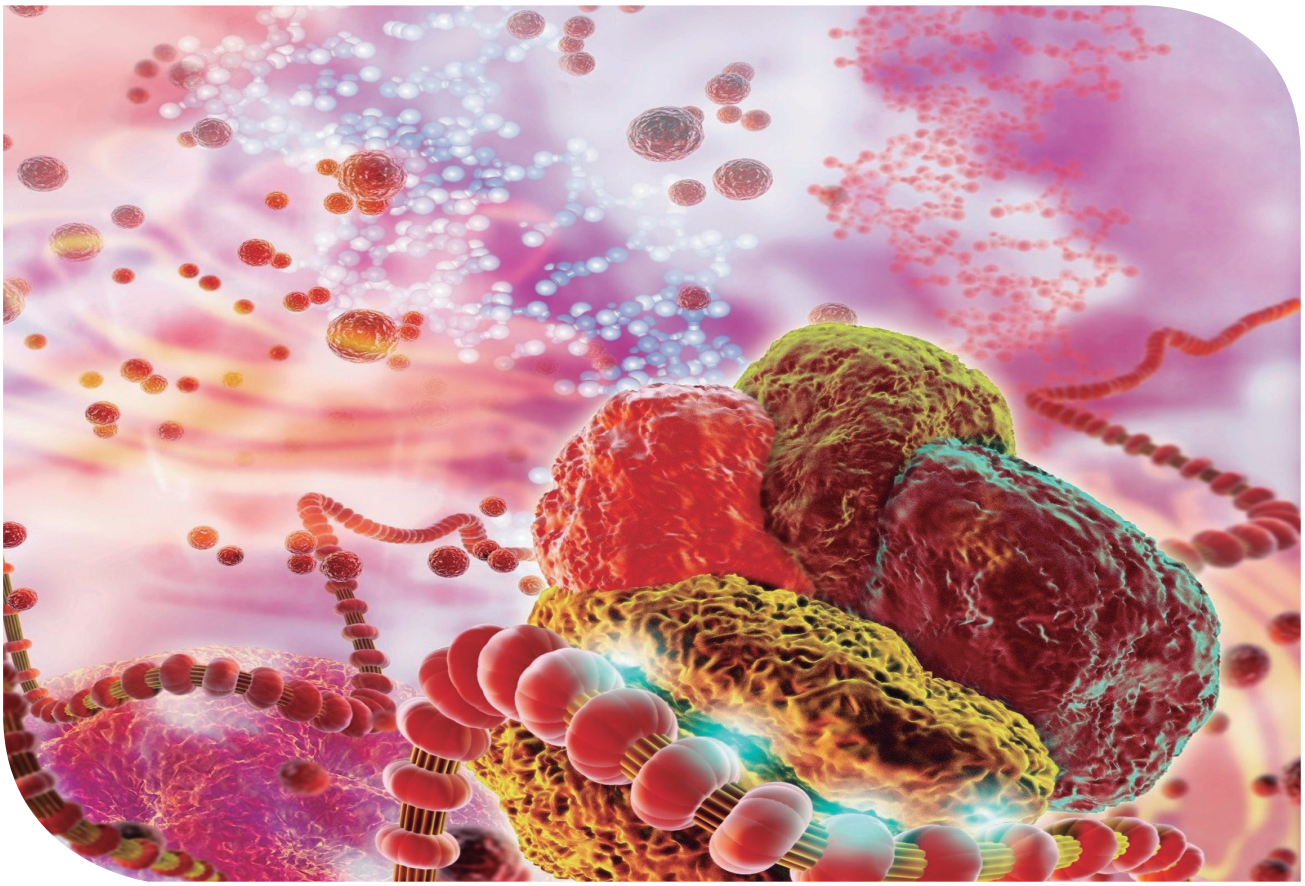
MicroRNA Solutions



OverExpression
miExpress™ miRNA Precursor Clones

Inhibition
miArrest™ miRNA Inhibitor Clones/Oligos

Target Validation
miTarget™ miRNA 3' UTR Target Clones

Quantitation and Profiling
miProfile™ miRNA qPCR Arrays
All-in-One™ miRNA qPCR Primers
All-in-One™ miRNA qRT-PCR Kits

GeneCopoeia™
Expressway to Discovery

Mechanism

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

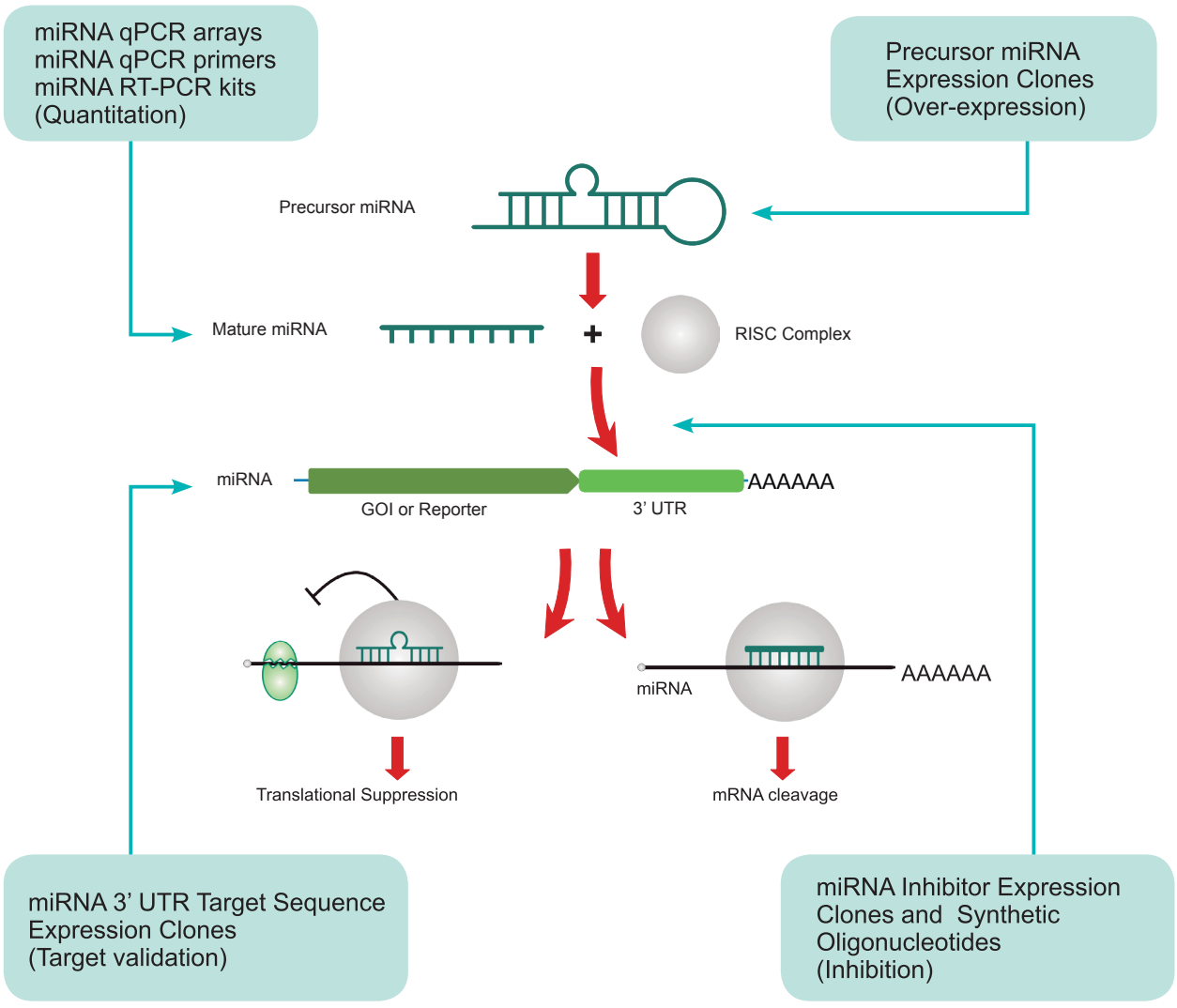


Figure 1. miRNA mechanism and GeneCopoeia comprehensive miRNA solutions. GOI: gene of interest.

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	Over-express miRNAs for gain of function studies
miArrest™ miRNA Inhibitor Expression Clones and Synthetic Oligonucleotides	Inhibit miRNAs for loss of function studies
miTarget™ miRNA 3' UTR Target Sequence Expression Clones	human, mouse, rat and custom 3'UTR clones Validate miRNAs and their gene targets (3'UTR)
miProfile™ miRNA qPCR Arrays	Whole-genome or focused group profiling of miRNA expression using validated primers and robust RT-PCR conditions
All-in-One™ miRNA qPCR primers	Validated human, mouse and rat primers Amplify mature miRNAs to quantitate and study their expression
All-in-One™ miRNA qRT-PCR Detection Kits	SYBR® Green-based qRT-PCR kits Detect mature miRNAs and study their expression profiles
Secrete-Pair™ Dual Luminescence Assay Kits	Analyze the activities of Gaussia Luciferase and Secreted Alkaline Phosphatase of a dual-reporter system side-by-side using the same sample from the cell culture medium. Optimized for use with miTarget 3' UTR target clones
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

miExpress™ Precursor miRNA Expression Clones

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

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

miRNA clones can be purchased as either individual clones or clone sets.

Full coverage
- Human, mouse and rat

Flexible delivery
- Lentiviral or non-viral vectors

Optimized expression
- Fully sequence-verified
- Optimized for high expression of precursor miRNAs and mature miRNAs inside cells

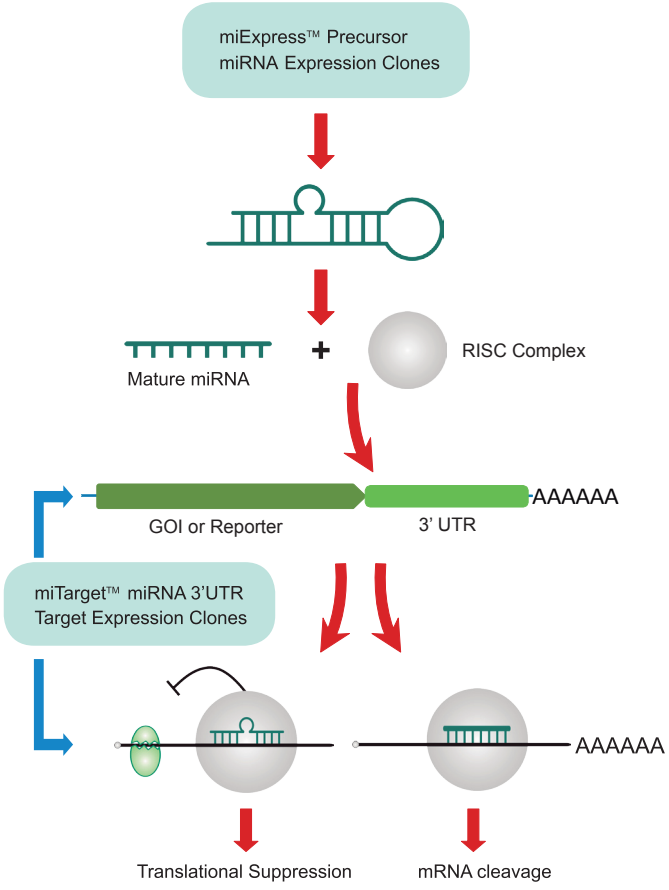


Figure 2. Roles of miExpress™ precursor miRNA and miTarget™ 3' UTR target clones in miRNA regulation studies.

Inhibitors

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. They are designed and optimized for miRNA loss of function study.

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

miRNA inhibitor clones can be purchased as either individual clones or clone sets.

Vector-Based vs. Synthetic Oligonucleotides

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	+++++	-

Full coverage
- Human, mouse and rat

Flexible delivery
- Lentiviral or non-viral vectors

Superior performance
- Constitutive expression with H1 or U6 promoter
- Superior potency, long lasting inhibition
- Extremely low cell toxicity

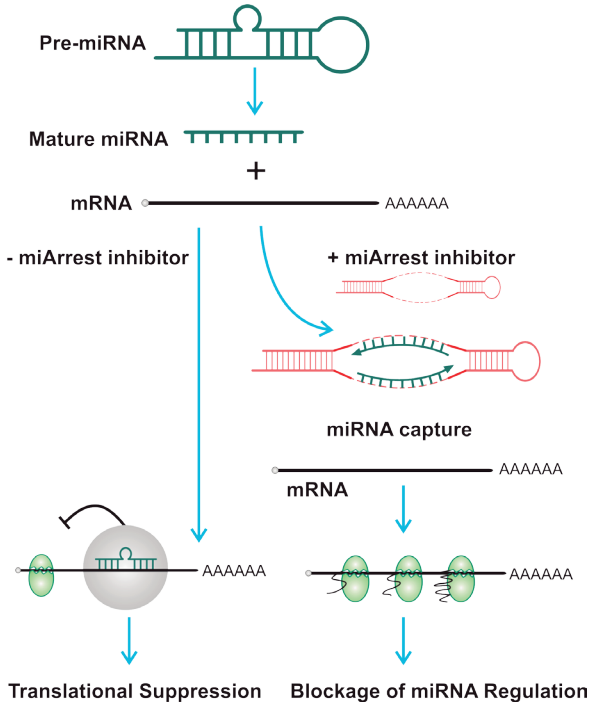


Figure 3. Mechanism of miArrest™ miRNA inhibitor expression clones.

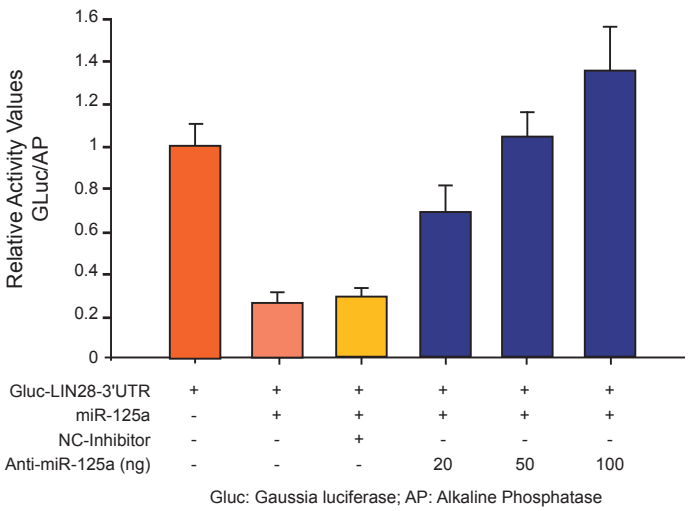
3' UTR Targets

miTarget™miRNA 3' UTR Target Sequence Expression Clones

miRNAs regulate target gene expression 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 specifically. Therefore, luciferase activity can be analyzed to study miRNA-target regulation and specificity.

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-MT05	*Gaussia* luciferase	Alkaline phosphatase	Live cell assays
pEZX-MT01	Firefly luciferase	*Renilla* luciferase	Assays on cell lysates



Gluc: Gaussia luciferase; AP: Alkaline Phosphatase

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 4. 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 purposes, 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 amounts 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 the 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.

Luciferase Assays

Luciferase activities of miTarget miRNA 3' UTR Target Clones can be analyzed using the **Secrete-Pair™ Dual Luminescence Assay Kit** or **LucPair™ miR Dual Luciferase Assay Kit**. Both kits measure dual reporter signals and allow transfection normalization.

Assay kit	Description	Target clone vector type	Reporter gene	Tracking gene
Secrete-Pair Dual Luminescence Assay Kit	Live cell assay Dual reporter assay Signal normalization	pEZX-MT05	Gaussia luciferase	Secreted alkaline phosphatase
Luc-Pair miR Dual Luciferase Assay Kit	Cell lysis required Dual reporter assay Signal normalization	pEZX-MT01	Firefly luciferase	Renilla luciferase

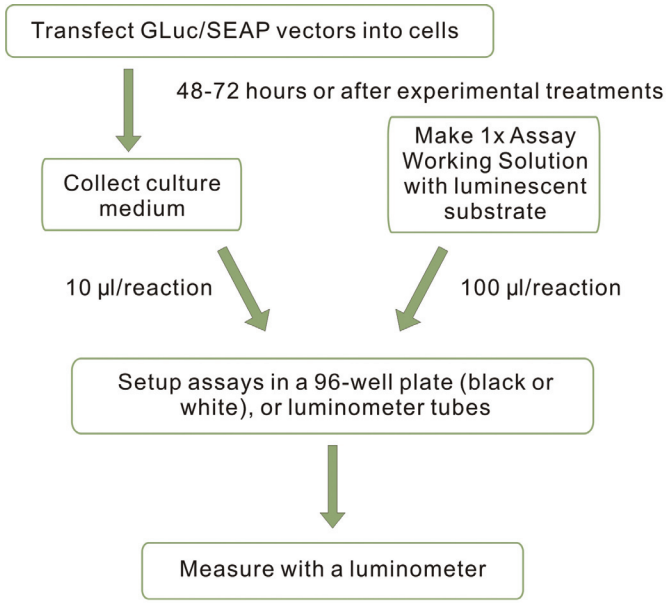


Figure 5. Simple and convenient workflow of Secrete-Pair™ Dual Luminescence Assay Kit

Dual reporter system

- Measure the activities of reporter gene and tracking gene from a single sample

Convenient live cell assay

- Live cell analysis available for pEZX-MT05 constructs with secreted reporters

Robust performance

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

qPCR Arrays

miProfile™ miRNA qPCR Arrays

The miProfile miRNA qPCR Arrays are designed for profiling miRNA expression in tissues or cells of interest to discover the miRNAs that are specifically important to your research. Each 96-well plate contains up to 84 pairs of PCR primers (forward: miRNA-specific primer; reverse: universal primer), which are pre-deposited in each well. The reverse-transcription and universal PCR assay conditions have been optimized using the All-in-One™ miRNA First-Strand cDNA Synthesis Kit and All-in-One™ qPCR Mix.

Cat. No.	Array type	Number of miRNAs	Number of plates
PAM-HG96	Human miRNome	1,565	19 x 96-well plates
PAM-HC96	Human cancer	520	5 x 96-well plates
PAM-HCN96	Human brain cancer	84	1 x 96-well plate
PAM-HCB96	Human breast cancer	168	2 x 96-well plates
PAM-HCX96	Human leukemia	168	2 x 96-well plates
PAM-HCL96	Human lung cancer	168	2 x 96-well plates
PAM-HCO96	Human ovarian cancer	168	2 x 96-well plates
PAM-HCY96	Human bladder cancer	79	1 x 96-well plate
PAM-HCC96	Human colorectal cancer	84	1 x 96-well plate
PAM-HCE96	Human endometrial cancer	84	1 x 96-well plate
PAM-HCG96	Human gastric cancer	80	1 x 96-well plate
PAM-HCH96	Human hepatocellular carcinoma	168	2 x 96-well plates
PAM-HCZ96	Human lymphoma	84	1 x 96-well plate
PAM-HCM96	Human melanoma	84	1 x 96-well plate
PAM-HCT96	Human head and neck cancer	84	1 x 96-well plate
PAM-HCP96	Human pancreatic cancer	84	1 x 96-well plate
PAM-HCQ96	Human prostate cancer	84	1 x 96-well plate
PAM-HI96	Human immunopathology	84	1 x 96-well plate
PAM-HX96	Human serum and Plasma	168	2 x 96-well plates
PAM-HF96	Human inflammatory	84	1 x 96-well plate
PAM-HH96	Human heart disease	84	1 x 96-well plate
PAM-HM96	Human muscle disease	84	1 x 96-well plate
PAM-HT96	Human toxicology related	84	1 x 96-well plate
PAM-HK96	Human IPS (stem cell)	168	2 x 96-well plates

qPCR Arrays

Advantages

Genome-wide coverage, pre-arranged groups, or customized groups

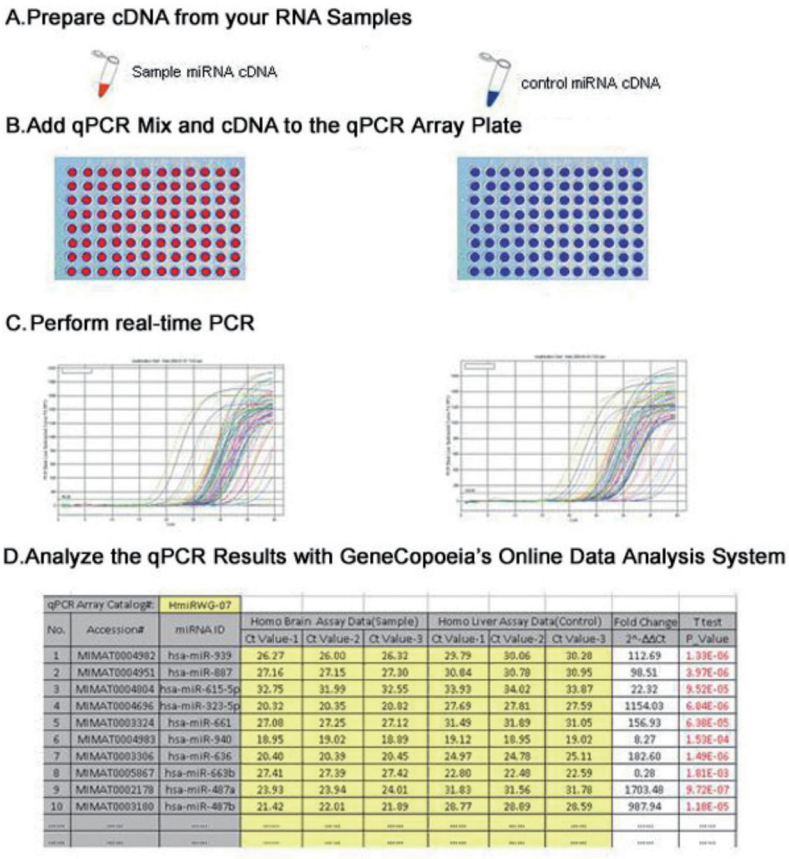
- Largest genome-wide miRNA coverage
- Cancer or other disease-related groups
- Customized miRNA arrays for focused study

Robust performance

- **Sensitive** – Detect miRNA from as little as 10 pg of small RNA or 20 pg of total RNA
- **Specific** – Be able to distinguish miRNAs with single nucleotide mismatches. Each primer set has been experimentally validated for specific amplification
- **Broad linearity**– Allow miRNAs at various expression levels to be detected simultaneously
- **Reproducible** – High reproducibility (R2>0.99) for inter-array and intra-array replicates

Validated miRNA primers

- Each miRNA primer is designed using a proprietary algorithm and experimentally validated



qPCR Array Catalog#:		HmiRWG-07								
No.	Accession#	miRNA ID	Homo Brain Assay Data(Sample)			Homo Liver Assay Data(Control)			Fold Change	Ttest
			Ct Value-1	Ct Value-2	Ct Value-3	Ct Value-1	Ct Value-2	Ct Value-3	2^-ΔΔCt	P_Value
1	MIMAT0004982	hsa-miR-939	26.27	26.00	26.32	29.79	30.06	30.28	112.69	1.93E-06
2	MIMAT0004951	hsa-miR-887	27.16	27.15	27.30	30.84	30.78	30.95	98.51	3.97E-06
3	MIMAT0004804	hsa-miR-615-5p	32.75	31.99	32.55	33.93	34.02	33.87	22.32	9.52E-05
4	MIMAT0004696	hsa-miR-323-5p	20.32	20.35	20.02	27.69	27.81	27.59	1154.03	6.04E-06
5	MIMAT0003324	hsa-miR-661	27.08	27.25	27.12	31.49	31.89	31.05	156.93	6.30E-05
6	MIMAT0004983	hsa-miR-940	18.95	19.02	18.89	19.12	18.95	19.02	8.27	1.53E-04
7	MIMAT0003306	hsa-miR-636	20.40	20.39	20.45	24.97	24.78	25.11	182.60	1.49E-06
8	MIMAT0005867	hsa-miR-663b	27.41	27.39	27.42	22.80	22.48	22.59	0.28	1.81E-03
9	MIMAT0002178	hsa-miR-487a	23.93	23.94	24.01	31.83	31.56	31.78	1703.48	9.72E-07
10	MIMAT0003180	hsa-miR-487b	21.42	22.01	21.89	28.77	28.69	28.59	987.94	1.18E-05

Figure 6. Simple and convenient workflow of Secrete-Pair™ Dual Luminescence Assay Kit

Profiling Kits

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

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

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.

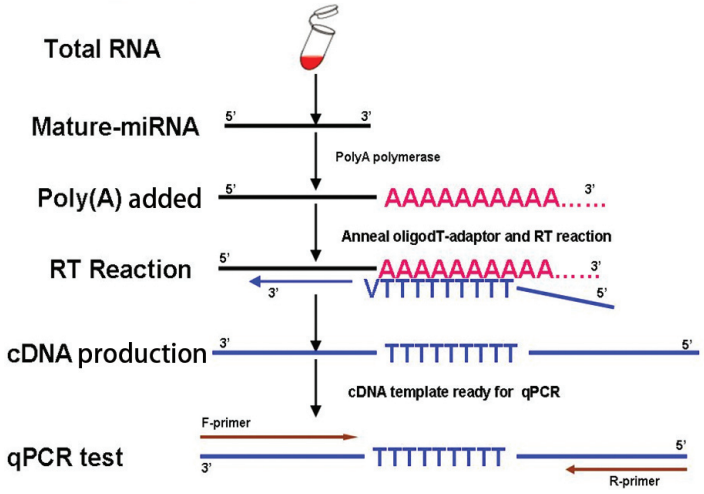


Figure 7. Overview of steps involved in the All-in-One™ miRNA qRT-PCR detection kit. During 3' polyadenylation, M-MLV RTase and a unique oligo dT adaptor primer reverse transcribe the poly(A) miRNAs. The qRT-PCR mix in the kit containing SYBR® Green specifically detects the reverse transcribed miRNAs.

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 results.

Catalog#	Product	Description
AMRT-0020	All-in-One™ miRNA First-Strand cDNA Synthesis Kit (20 RT reactions)	Poly A Polymerase, RTase Mix, PAP/ RT buffer, spike-in control (for use with miRNA qPCR arrays)
AMRT-0060	All-in-One™ miRNA First-Strand cDNA Synthesis Kit (60 RT reactions)	Poly A Polymerase, RTase Mix, PAP/ RT buffer, spike-in control (for use with miRNA qPCR arrays)
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 primers (20 µl x 500 reactions)	Validated human, mouse and rat mature miRNA primers

Complete Solutions

GeneCopoeia offers complete solutions for microRNA research. The related products were developed for 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	• 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 fold
	Lenti-Pac™ 293Ta Lentiviral Packaging Cell Line	For high-titer lentiviral production using Lenti-Pac™ lentiviral packaging kits
Custom Cloning	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	• Genome-wide coverage of human and mouse • Largest selection of vector types and fusion tags • Fully sequenced and thousands are expression-tested

GeneCopoeia™

Expressway to Discovery

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