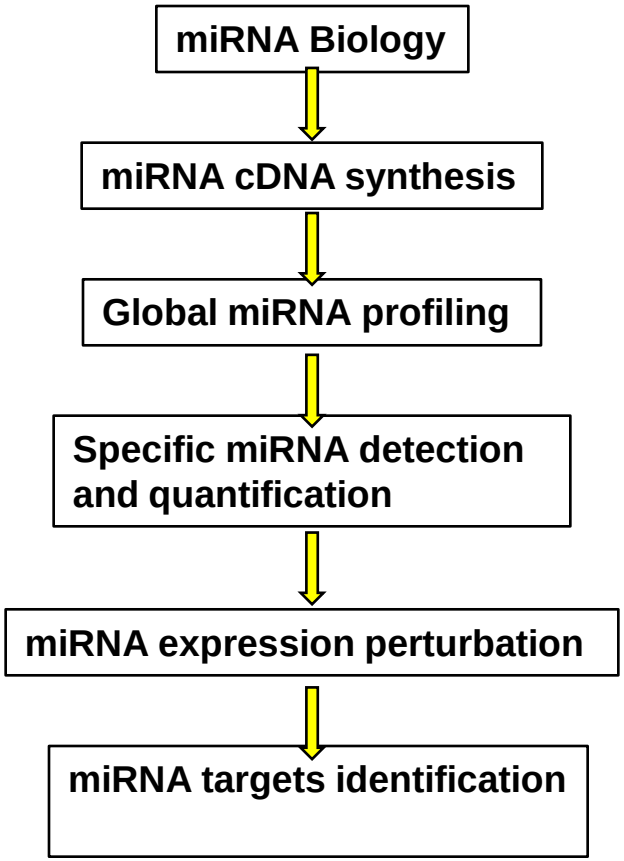
OriGene Technologies, Inc.

MicroRNA analysis: Detection, Perturbation, and Target Validation

-Optimal strategies to a successful miRNA research project

YOUR GENE COMPANY

Optimal strategies to a successful miRNA research project



Searching miRNAs involving in your research

Studying specific miRNAs

Bridging the gap between miRNA and biology by identifying protein target of miRNA

Optimal strategies to a successful miRNA research project

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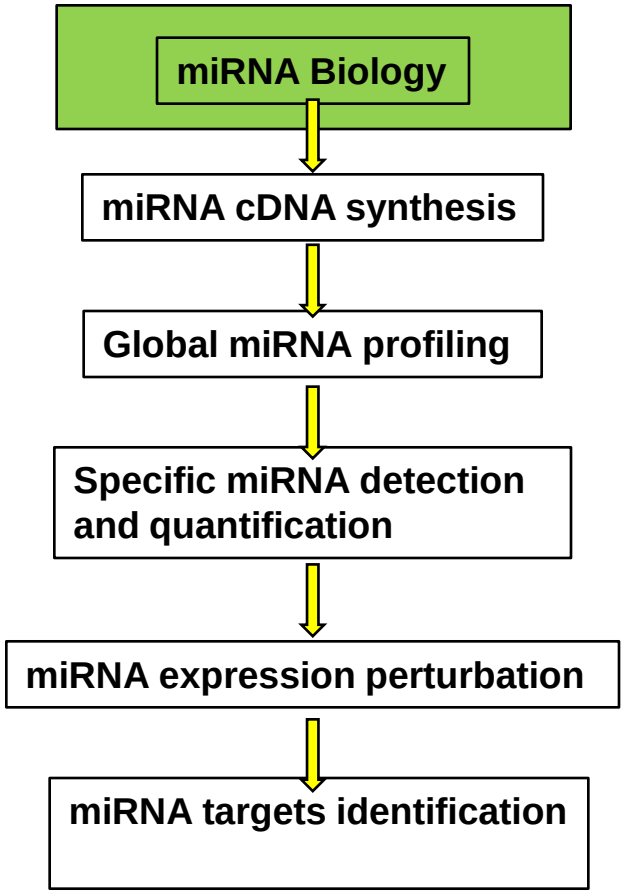

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MicroRNAs are naturally occurring non-coding RNAs

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-Mature microRNA is 21-23nt (miRNA)

First discovered in 1993 by Victor Ambros at Harvard (*lin-4*)

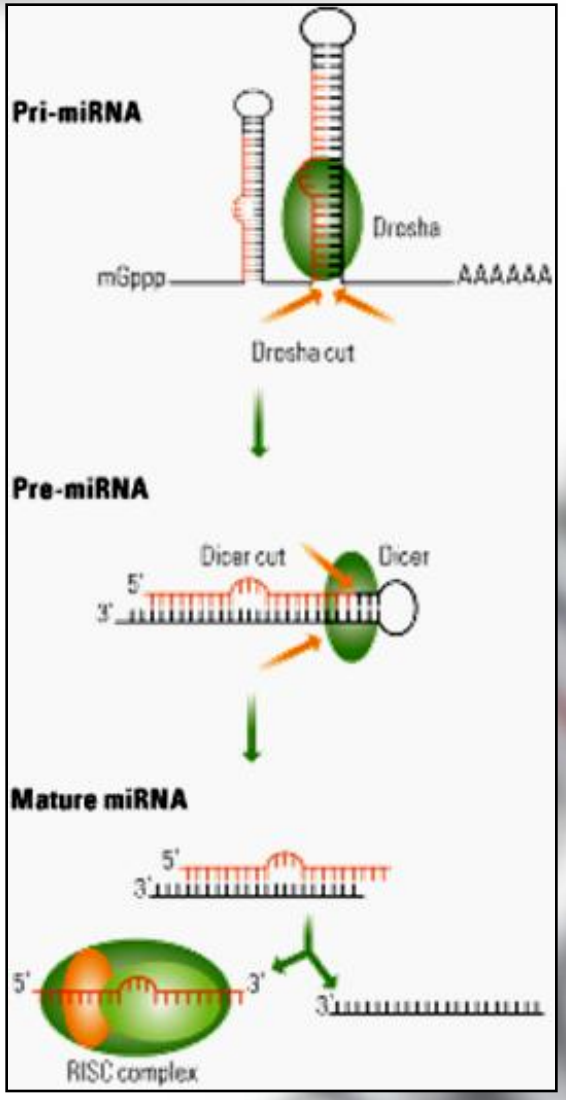
-MiRNA are processed in multiple steps:

DNA→ Pri-miRNA → Pre-miRNA→ Mature miRNA

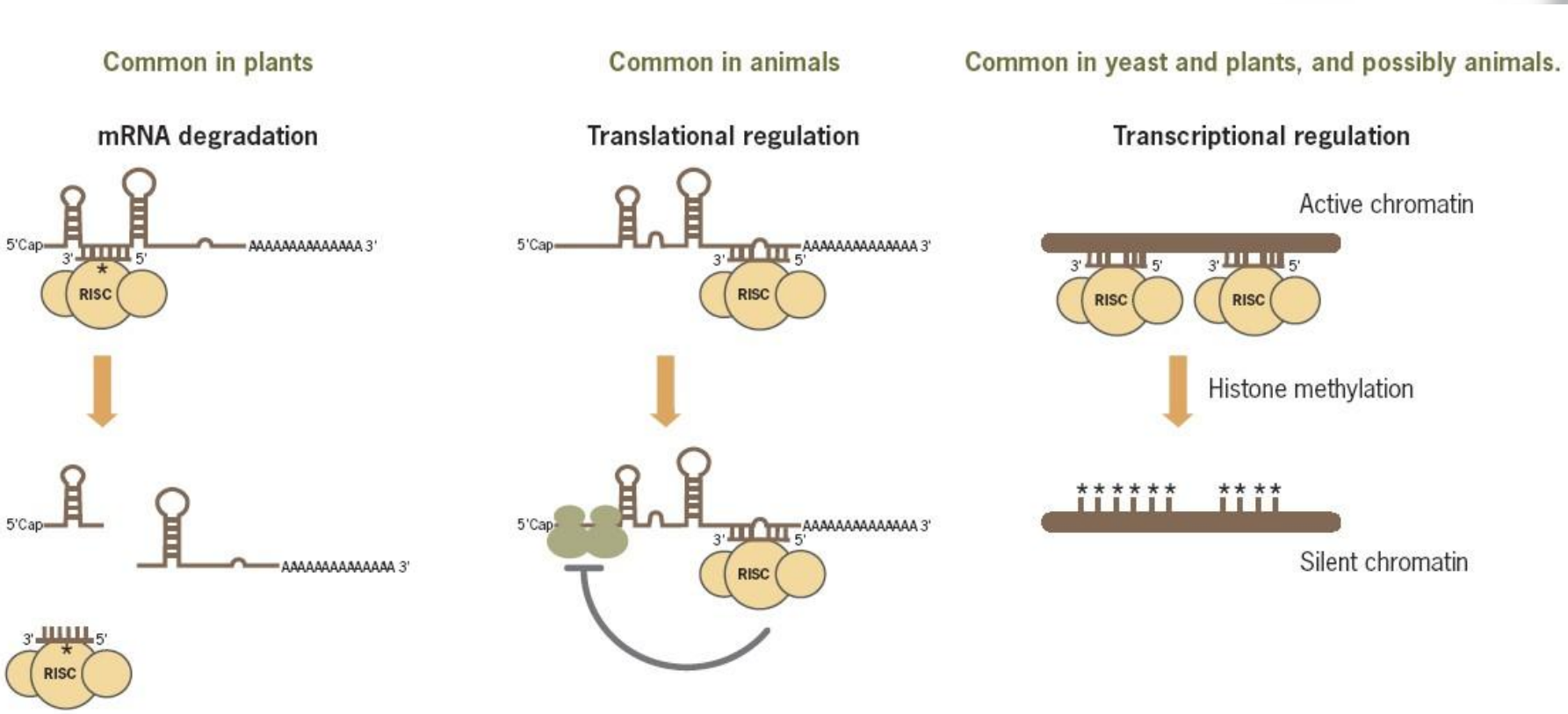
Pri-miRNA : Capped & polyadenylated full length Precursors(mRNA), transcribed by RNA polymerase II

Pre-miRNA: ~70nt hairpin precursor product of Drosha

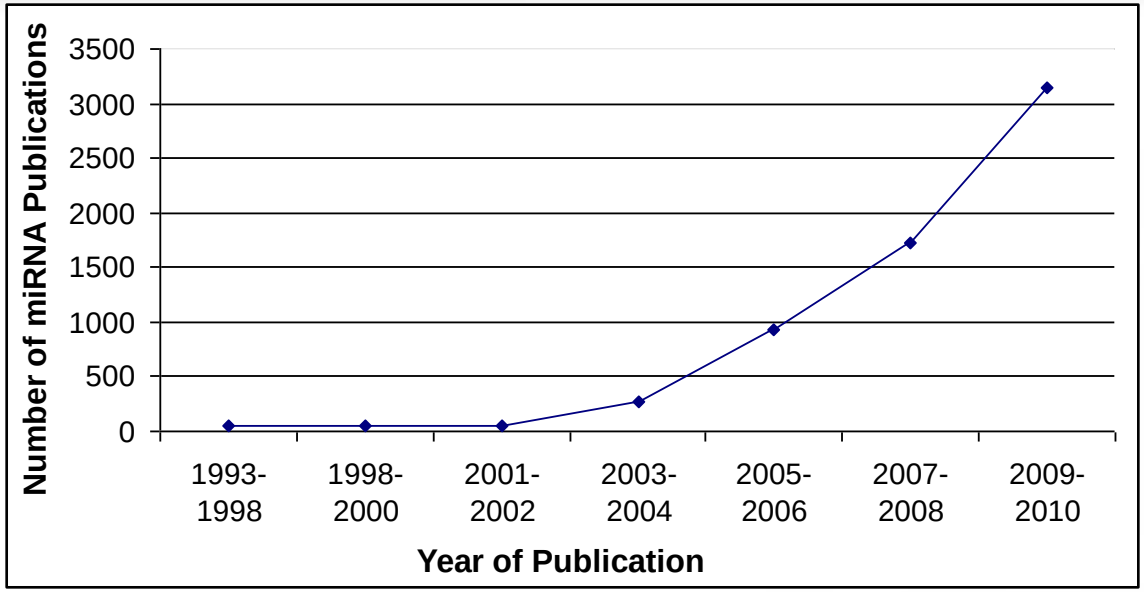
Mature miRNA: Functional RISC complex, product of Dicer



miRNAs regeulate of gene expression at translational level



miRNAs regulates many biological processes



First miRNA, *lin-14* in C. elegans, was reported by Vector Ambros in 1993. Since then miRNA has been reported:

1. Targeting most development related genes
2. stem cells maintenance and differentiation.
3. Cancer as oncomiR.
4. Heart disease.
5. Neuronal development and synapse formation.
6. Viral infections process.

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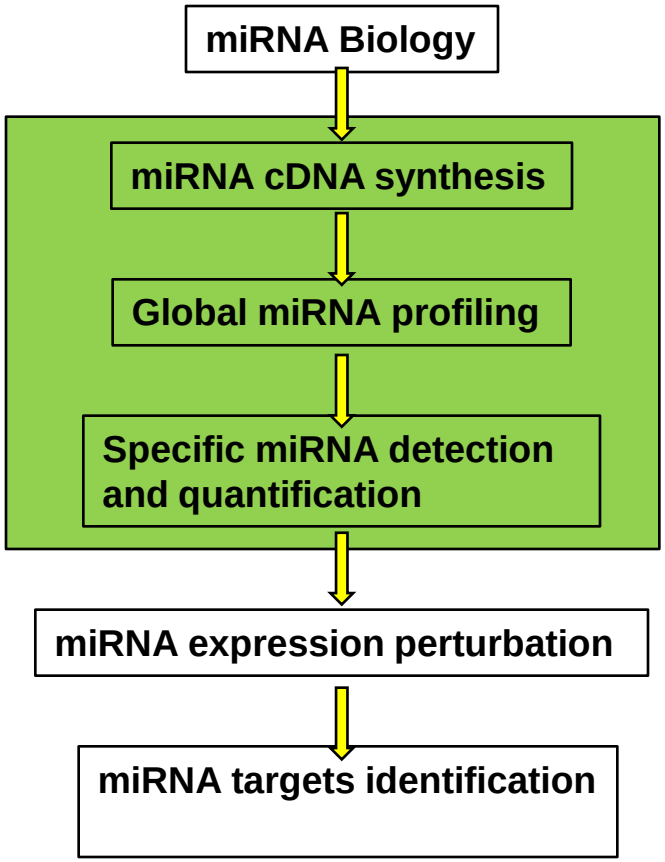

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Comparison of methods used in global miRNA profiling

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	DNA microarray	qPCR
Throughput	whole genome on one slide	96 or 384 on one plate
Sensitivity	low	Can detect down to 2 fold changes
Sample preparation	labeling RNA	Converting RNA to cDNA
Assay time	Overnight hybridization	2hrs
Cost	expensive	inexpensive
Easy to perform	difficult	easy
equipment	Core facility	Most labs

qPCR method can provide

- Full genome-wide coverage
- High sensitivity
- Easy assay in most labs

Hairpin primer vs. Poly A tailing in miRNA cDNA synthesis

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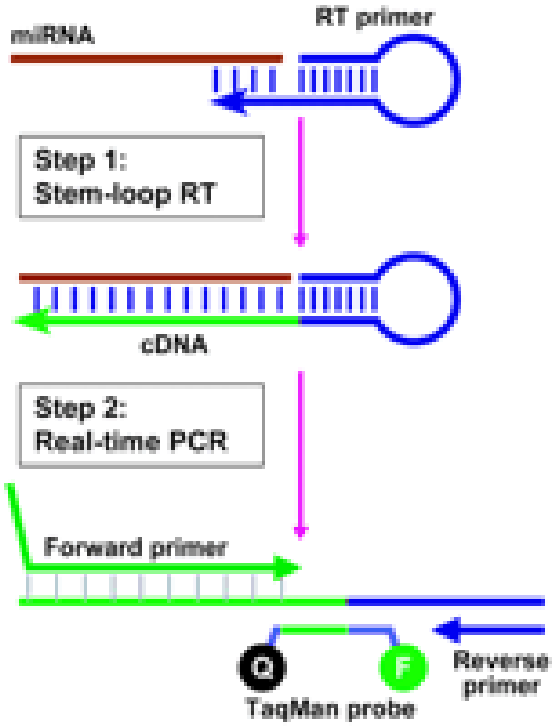
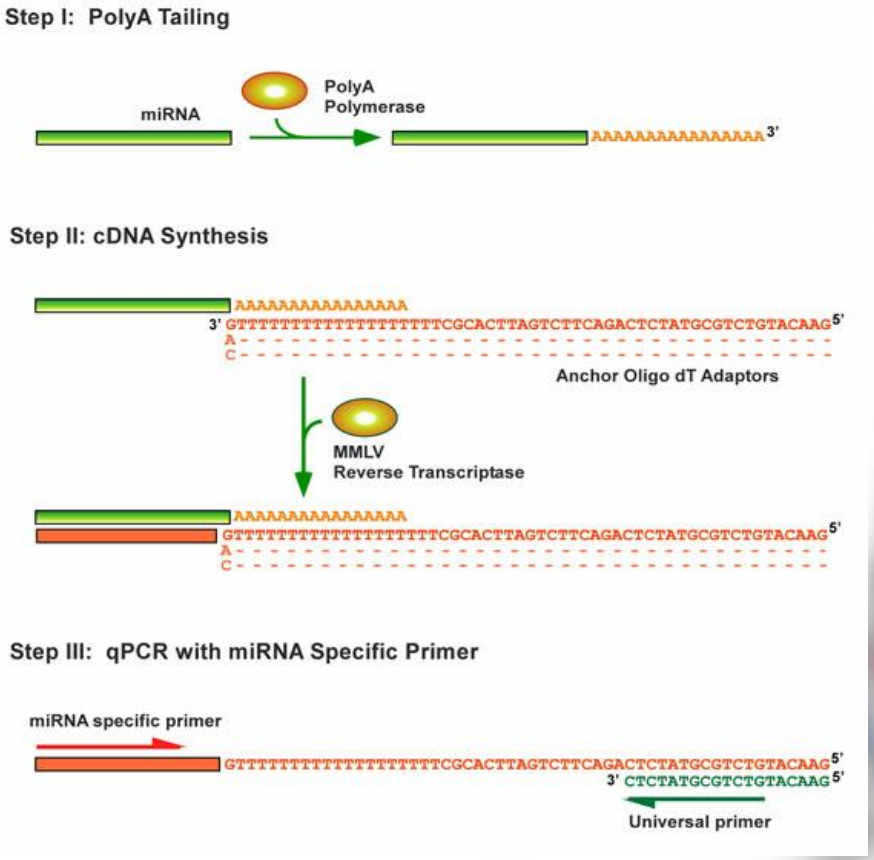
Hairpin primer



Poly A tailing



Hairpin primer vs. Poly A tailing in miRNA cDNA synthesis

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	Hairpin priming	Poly A tailing
Sensitivity for individual miRNA	Higher	Lower
Throughput	Low	High
Representation	Only detect a subset due to 3' imprecision	Fully coverage
Sample preparation includes both mRNA and miRNA	NO	Yes

qSTAR qPCR microRNA detection System from OriGene.

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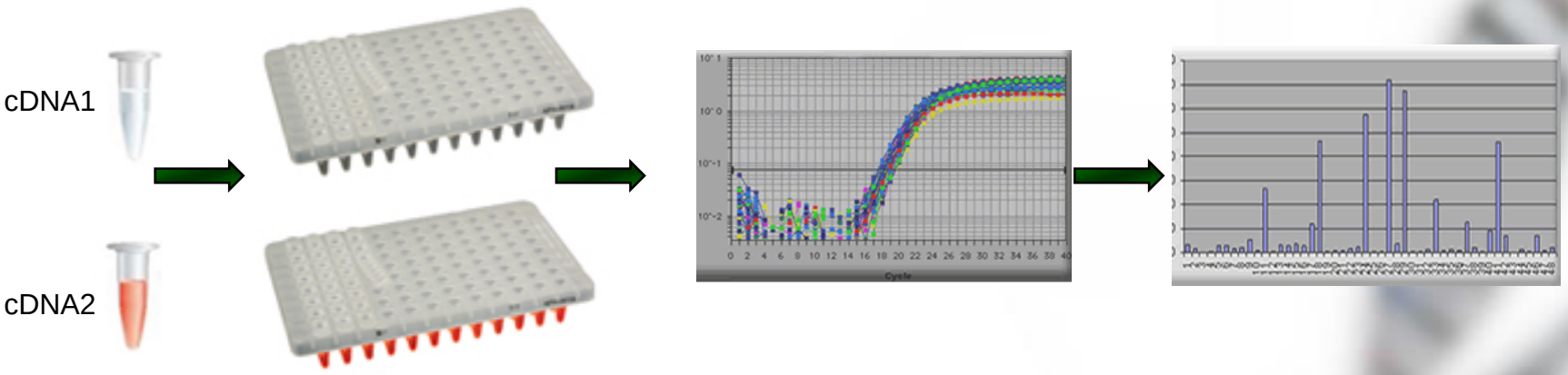
Products offered in qSTAR microRNA Detection System

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**



cDNA Synthesis

Mix cDNA with miRNA Primer Panels SYBR Green Master Mix

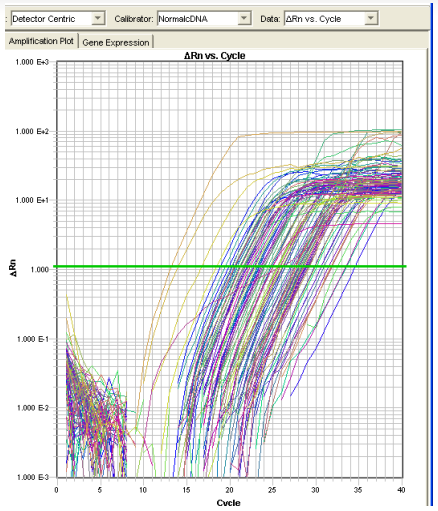
qPCR results

Data analysis

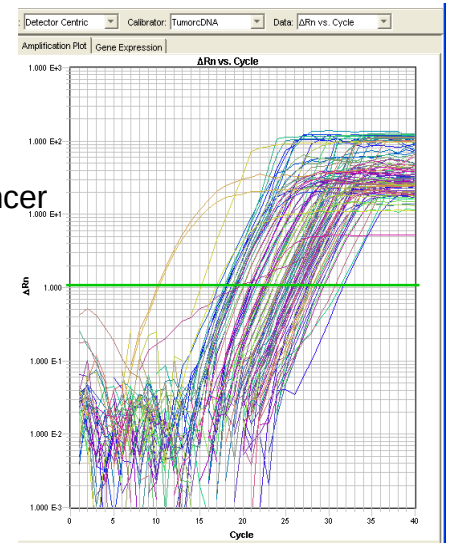
*Components 2,3 & 4 are unique and should only be used alongside OriGene's qPCR miRNA detection system

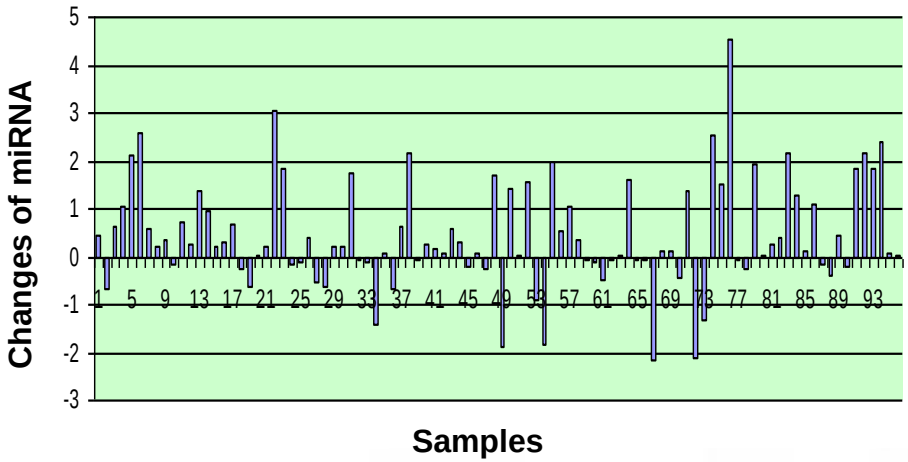
miRNA profiling in Ovarian Cancer
with OriGene's miRNA primer panel

Normal



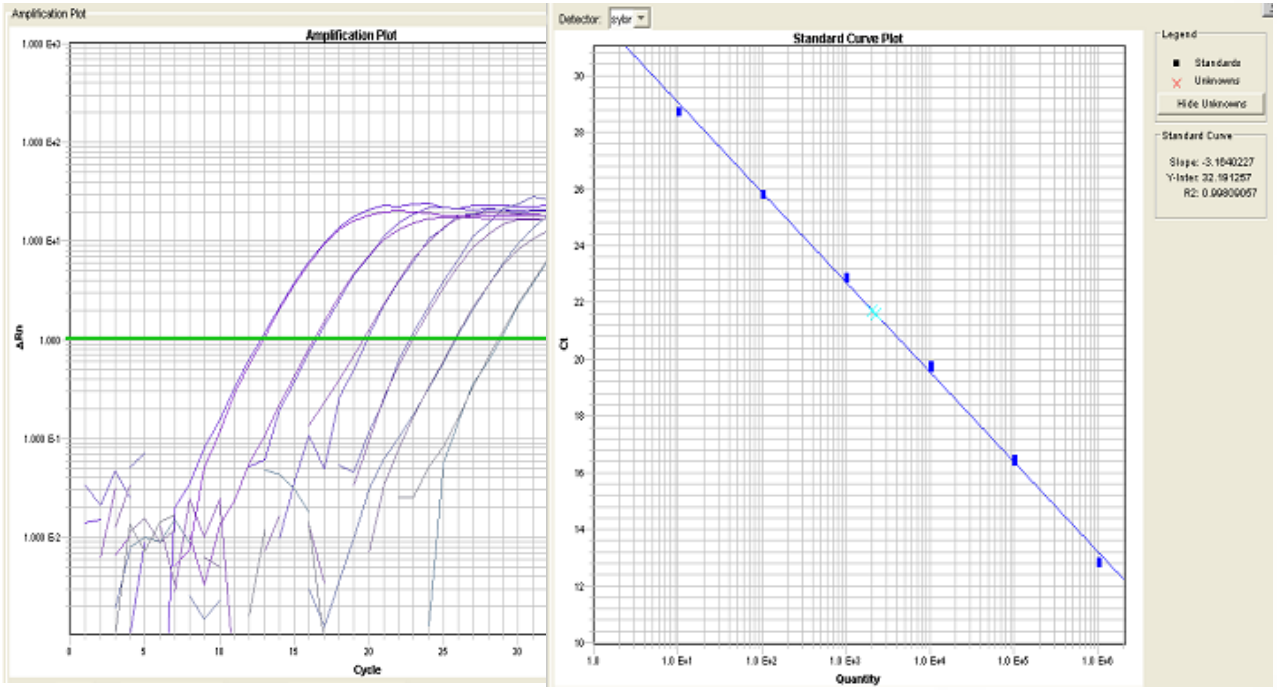
Ovarian Cancer





1. **Over 90% of the miRNAs were successfully identified in normal sample**
2. **Ovarian cancer cDNA showed differential miRNA profiling compare to normal cDNA**

miRNA template standards



Copy number standards were used to determine the absolute transcript copy number of an experiment sample using the standard curve method

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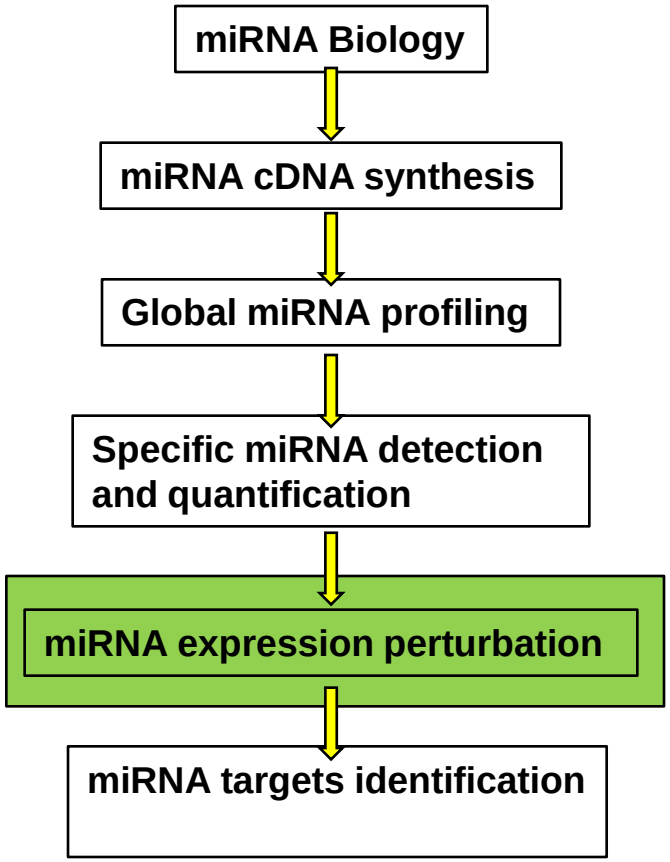

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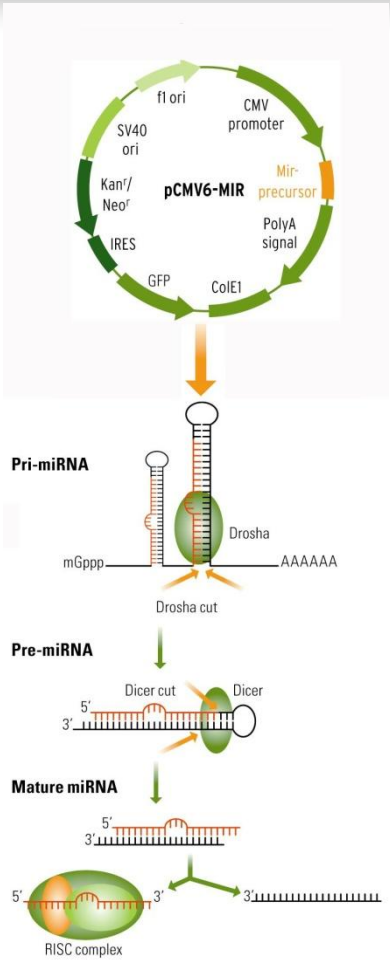
miRNA expression perturbation

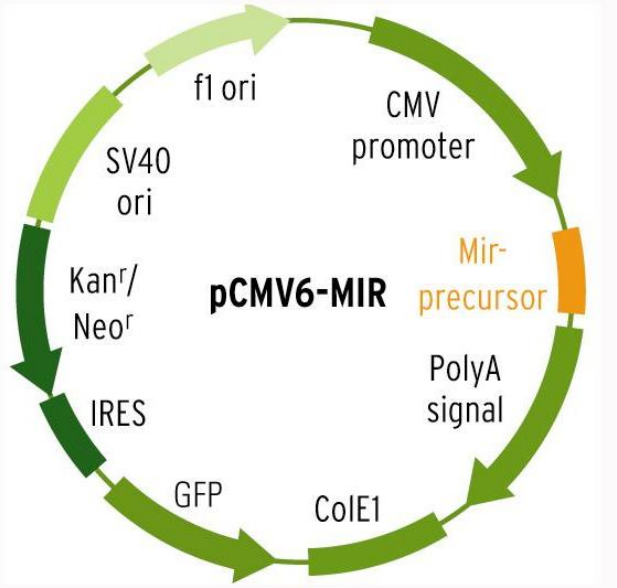
	Pre-miRNA precursor	miRNA mimics	miRNA inhibitors
perturbation	gain-of-function	gain-of-function	loss function
product	plasmid DNA	synthetic small RNA	
stability	very stable, good for studying long term function	relative instable, only good for days	
mimic native condition	Native substrate for Drosha and Dicer.	artificial, doesn't reflect the 3' end imprecision	

MicroRNA Expression Plasmids



Features of OriGene's pCMV6-MIR.

1. miRNAs and 300 bps of flanking genomic sequences are expressed by strong CMV promoter.
2. PolyA signal is added to facilitate pri-miRNA processing by Drosha.
3. SV40 drive IRES-GFP expression serves great transfection marker.
4. Neomycin selection enables to select stable transfections.



Validation of pCMV6-MIR system

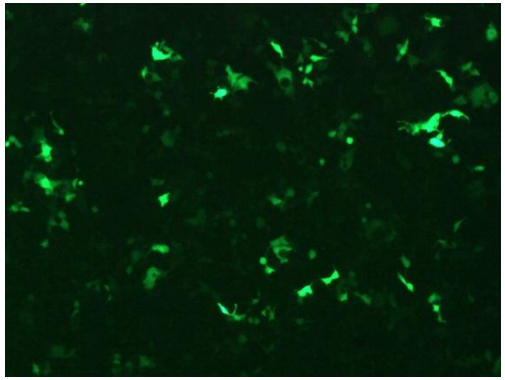
1. Sequence confirmation of the precursor sequence
   - All sequences are displayed on the website

2. Verify the GFP expression in the transfected cells

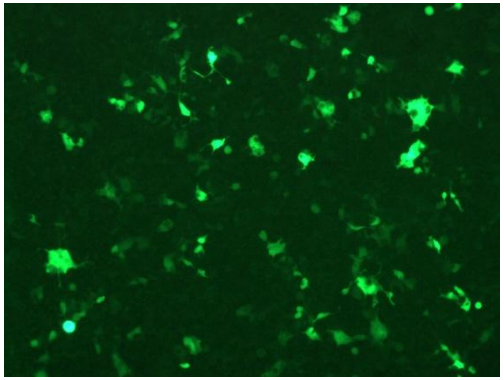
3. Demonstrate the over-expression of the intended miRNA

4. Demonstrate the gain-of-function of the intended miRNA

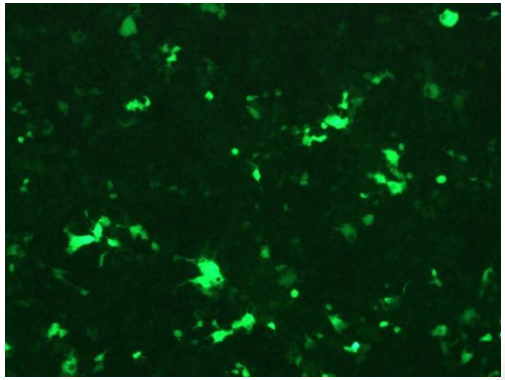
Verify the GFP expression



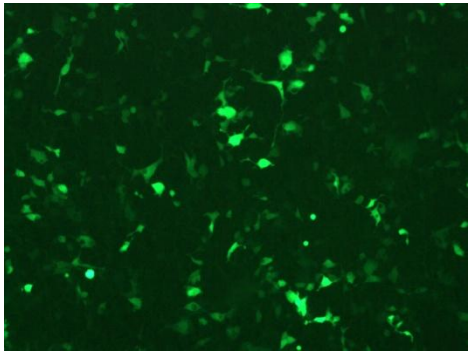
Mir205



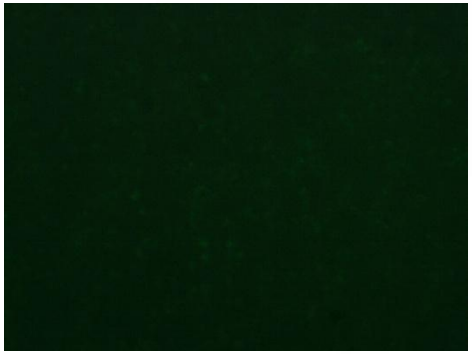
Mir143



Mir34b



Empty Vector



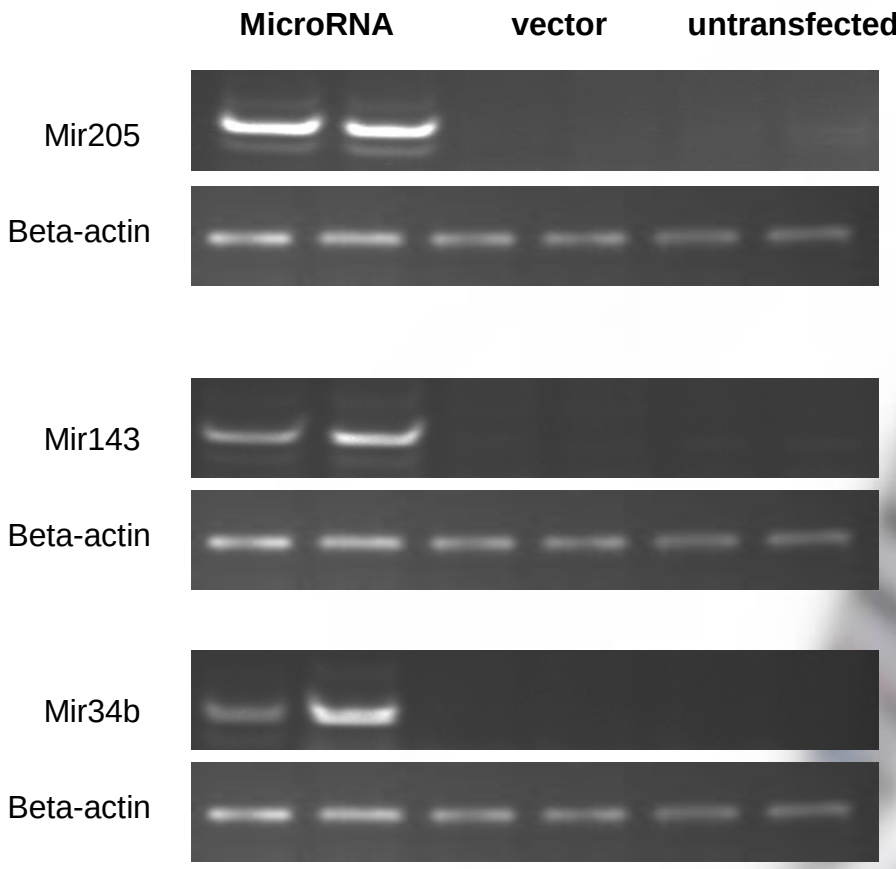
Non-transfected

24hr after transfection

Demonstrate the over-expression

Method: HEK293 cells were transfected with miRNA expression plasmids and the total RNA were isolated 48 hrs later. miRNA were detected using Poly A tailing RT_PCR.

All experiments were conducted in duplicates. Beta-actin were used as normalization control.



24hr after transfection

Gain-of-function of miR205

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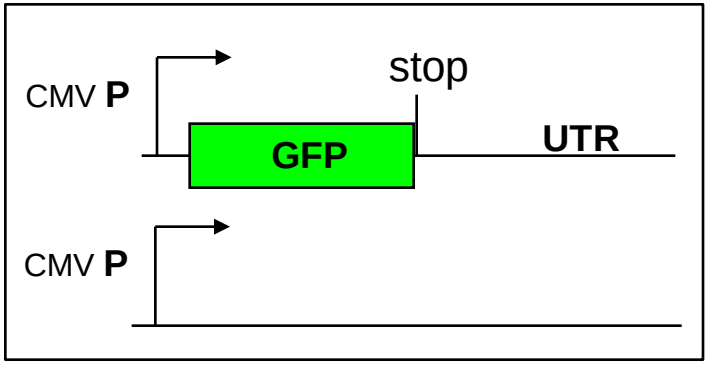
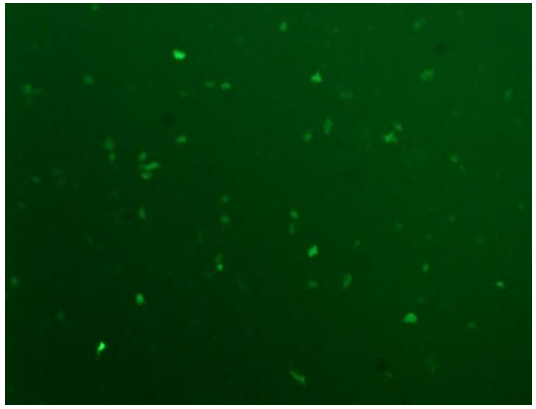
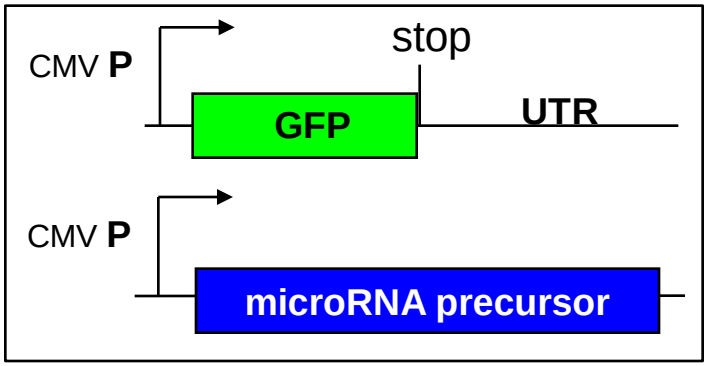
NM_032440

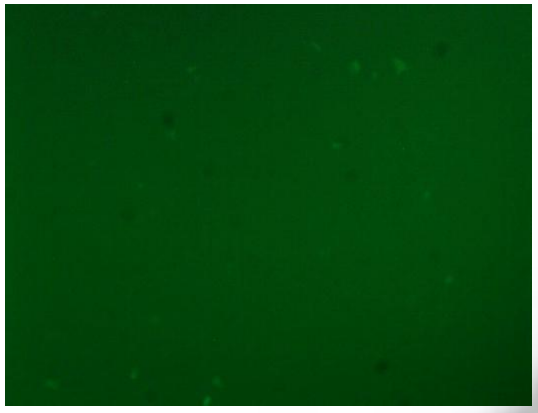
Control





Mir205





24hr after transfection

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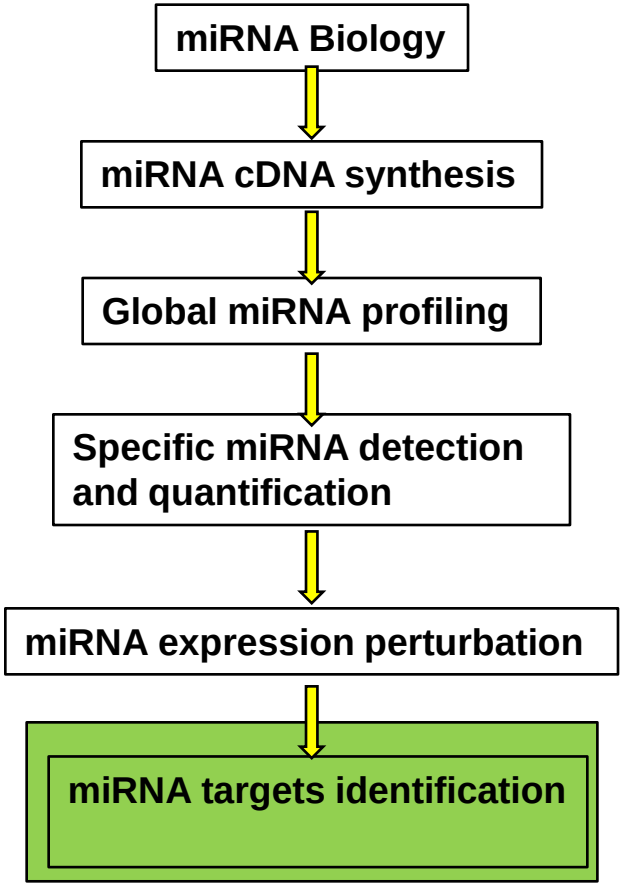

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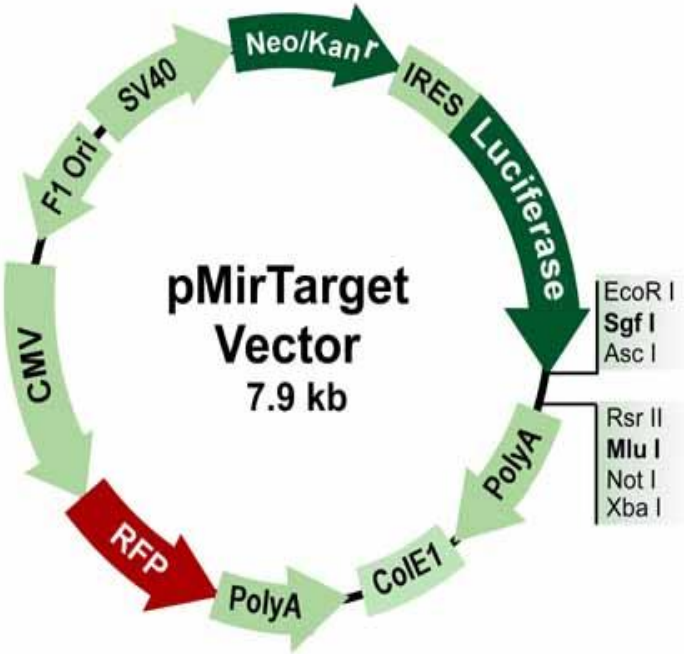
miRNA targets analysis

Methods	pros	cons
Luciferase reporter	Easy, Sensitive and reliable	Require premade reporter construct
Western blot	Less sensitive, very definitive	Most antibodies are not available
DNA microarray	Whole genome coverage, high throughput	Changes in mRNA level have poor co-relation to miRNA function
In silica predication	Easy	Error-prone

Luciferase reporter assays are the most widely used methods in miRNA target identification

Luciferase 3' UTR reporter system from OriGene



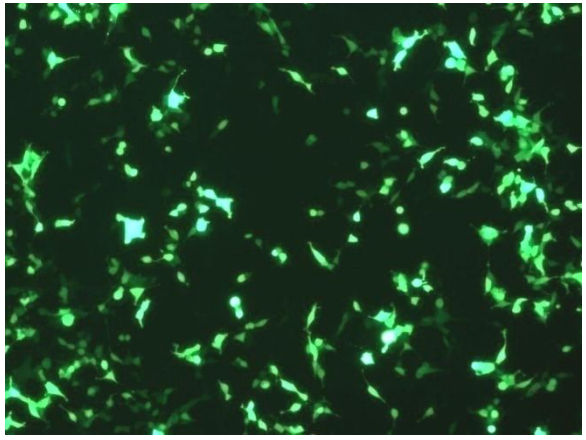
- High sensitivity of Luciferase assay due to IRES-driven luciferase cassette
- RFP as a reporter for transfection monitoring and normalization
- Neomycin selection marker for stable cell establishment
- Can serve as a control for target validation experiments.
- 3-UTR Clones for MicroRNA target validation
- OriGene provides human genome wide 3' UTR clones

Comparison of different luciferase reporter assays

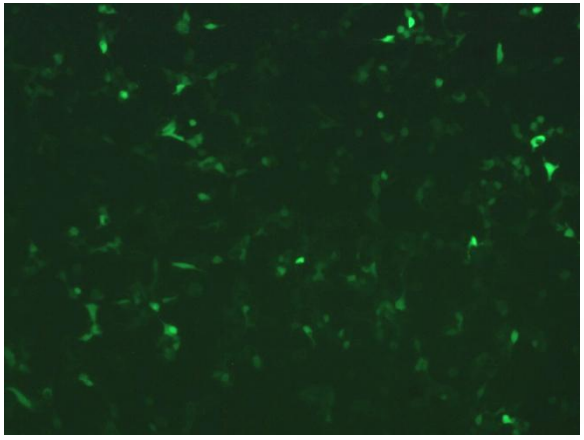
	pMIR-Report (Ambion)	Dual-Luciferase reporter (Promega)	pMir_Target (OriGene)
Sensitivity	low	low	high
Reporter	firefly luciferase	firefly luciferase	firefly luciferase
Promoter	CMV (strong)	PGK (weaker)	SV40 (strong)
Transfection marker and internal control	non	SV40 drives second luciferase	CMV drives RFP
Methods to weaken reporter expression	non	Weaker promoter	IRES weakening the translation
Compatibility with OriGene's pMir miRNA system	yes	yes	yes
Stable transfection	no	no	yes

Why introduce IRES?

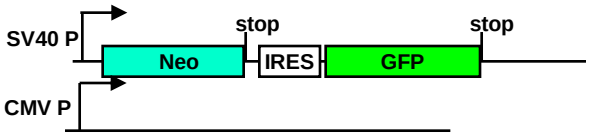
pSV40-GFP



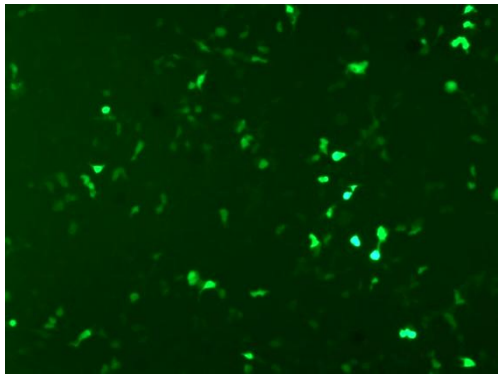
pSV40-IRES-GFP

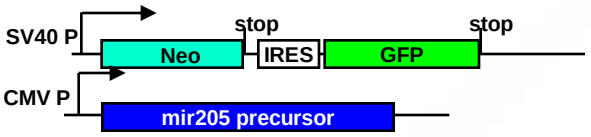


OriGene's design works

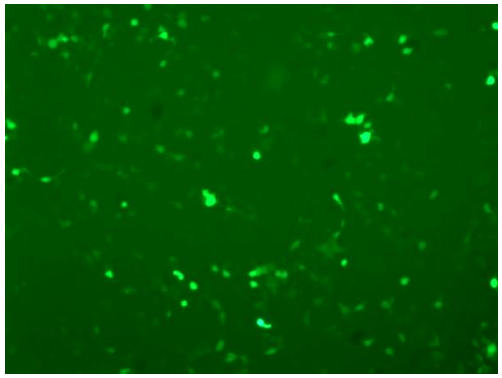


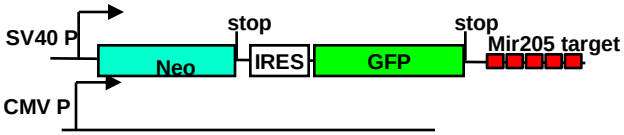
Empty reporter pCMV-Mir



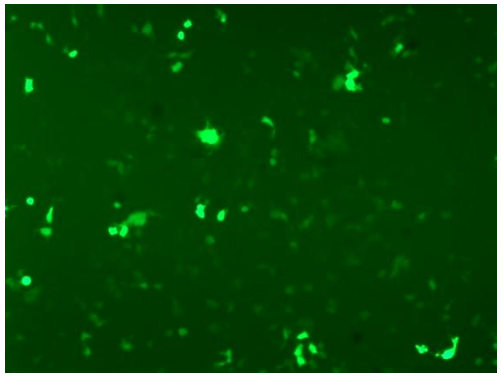


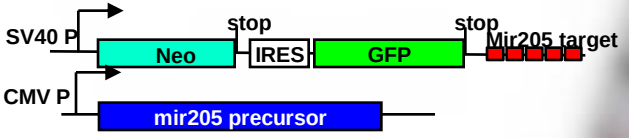
Empty reporter Mir205



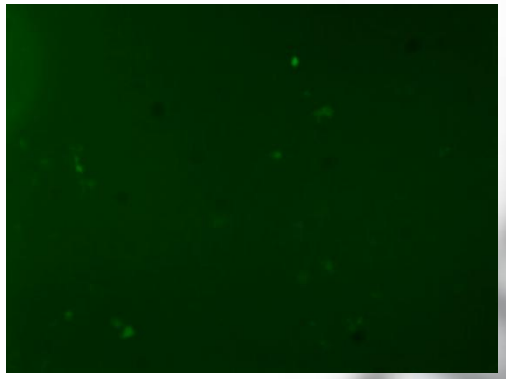


Mir205 target pCMV-Mir





Mir205 target Mir205



pMir-Target system validation

-Interaction of mir205 with its target genes

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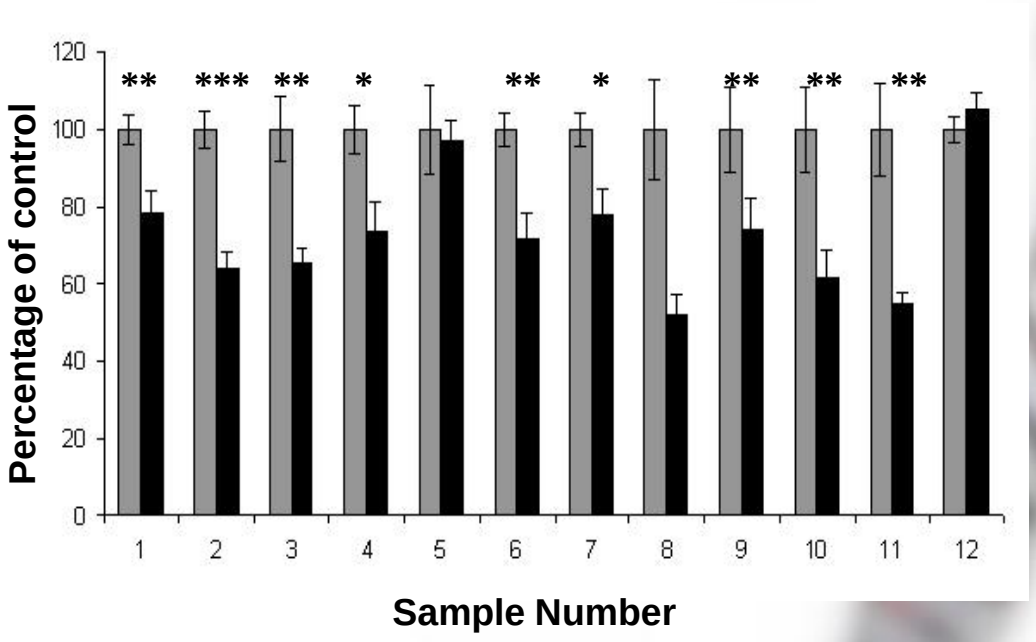
Table 1. 3'UTR-luciferase reporter clones in mir205 targets study

Sample number	3'UTR reporter clones in pMir-Target
1	NM_001982
2	NM_030751
3	NM_001567
4	NM_014795
5	NM_001002814
6	NM_001025376
7	NM_005433
8	NM_014962
9	NM_024830
10	NM_019084
11	Mir205 Rev. comp.
12	Empty reporter



Mir205 down-regulated luciferase activity when the luciferase was fused to mir205 target sequences (grey bar, control with 3' UTR reporter clones; black bar, mir205 with 3' UTR reporter clones.)

pMir-Target system validation

-Interaction of mir205 with its target genes

Seed sequence of mir205 in the 3' UTR of ERBB3

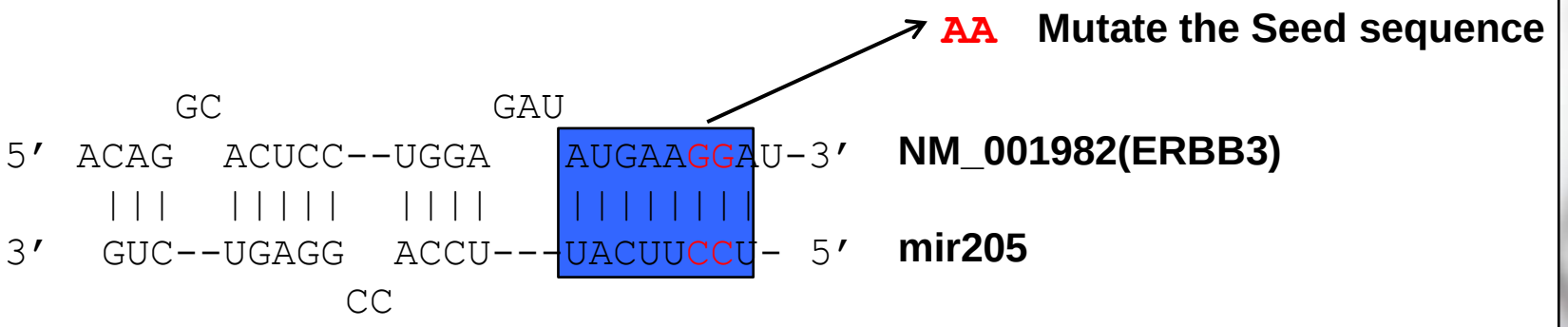


Table of experiment set-ups

Transfections	Control	Exp1	Exp2
pMir-Target-3UTR	+	+	
pMir-Target-3UTR mutant			+
MiR in pMir		+	+
PMir(Empty Vector)	+		

pMir-Target system validation

-Interaction of mir205 with its target genes

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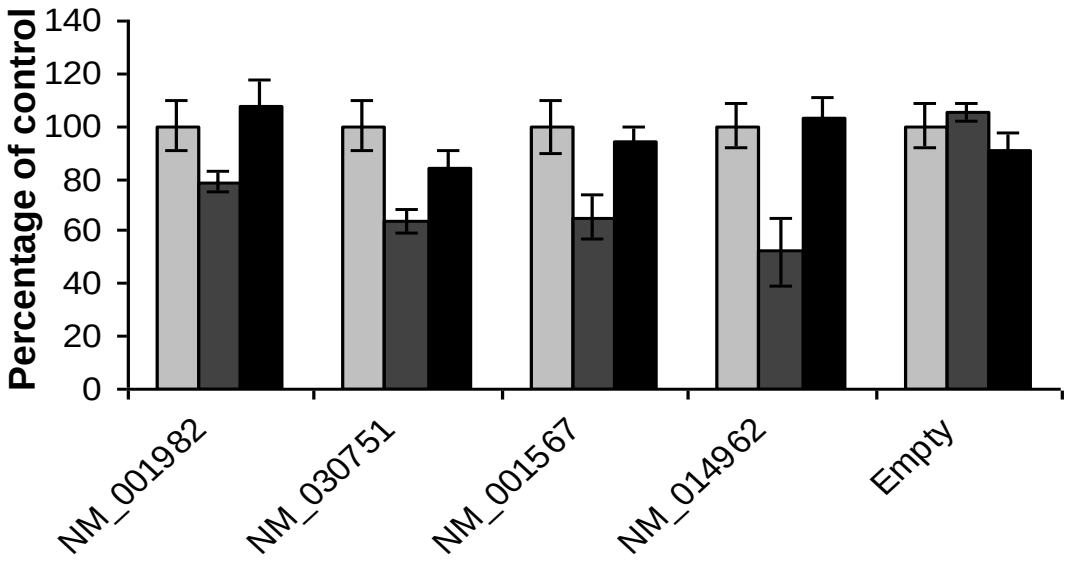
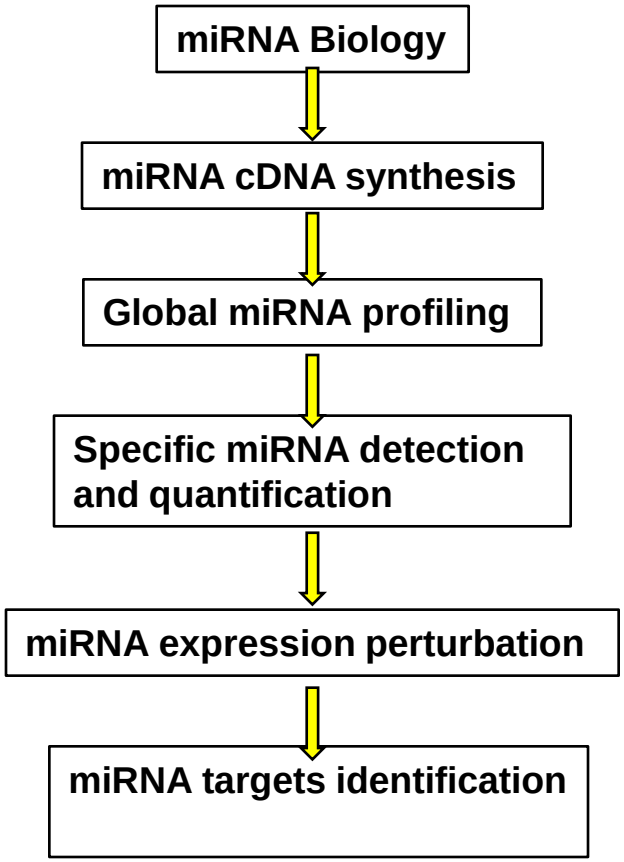
Transfections	Control	Exp1	Exp2
pMir-Target-3UTR	+	+	
pMir-Target-3UTR mutant			+
MiR in pMir		+	+
PMir(Empty Vector)	+		



Abolishment of Mir205 effects by mutated seeding sequences in the 3'UTR-luciferase reporter clones (grey bar, control with 3' UTR reporter clones; darker grey bar, mir205 with 3' UTR reporter clones; black bar, mir205 with mutated 3' UTR reporter clones.)

Optimal strategies to a successful miRNA research project



Searching miRNAs involving in your research

Studying specific miRNAs

Bridging the gap between miRNA and biology by identifying protein target of miRNA