

Technology Review: Tools for microRNA Research

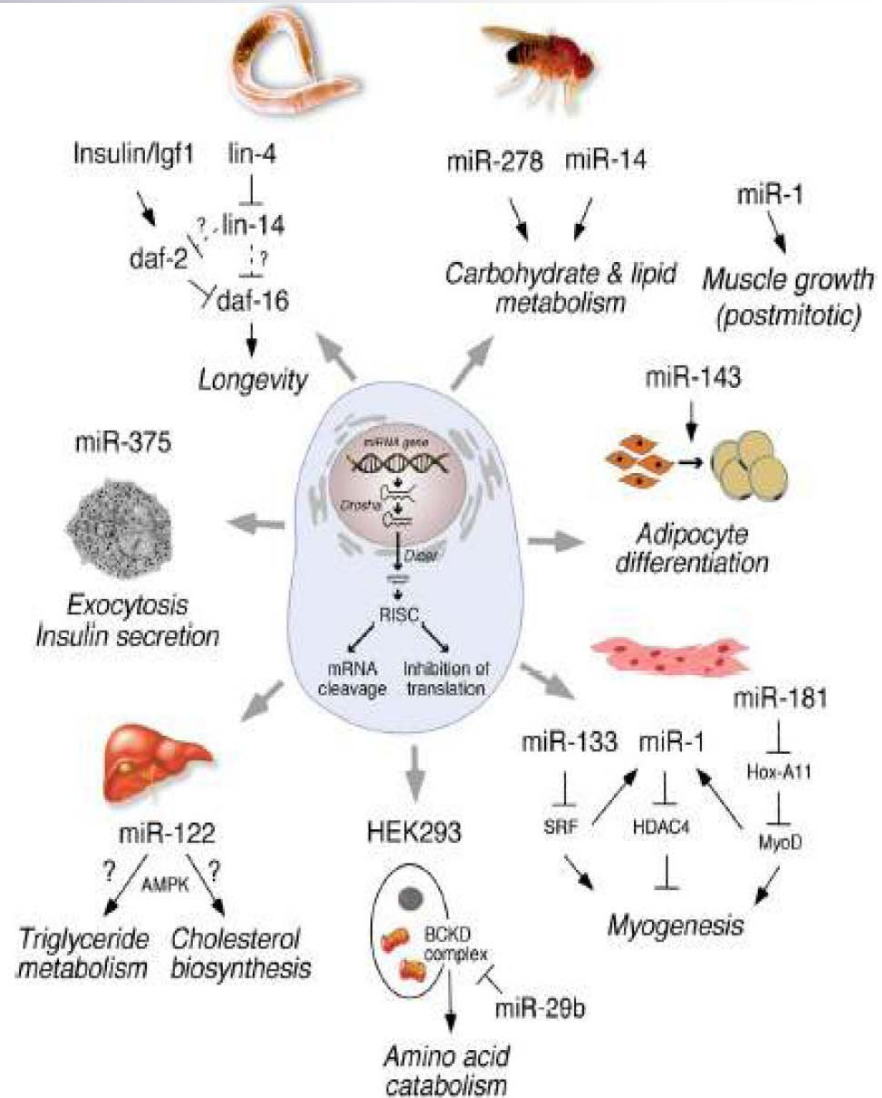
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Technical Specialist



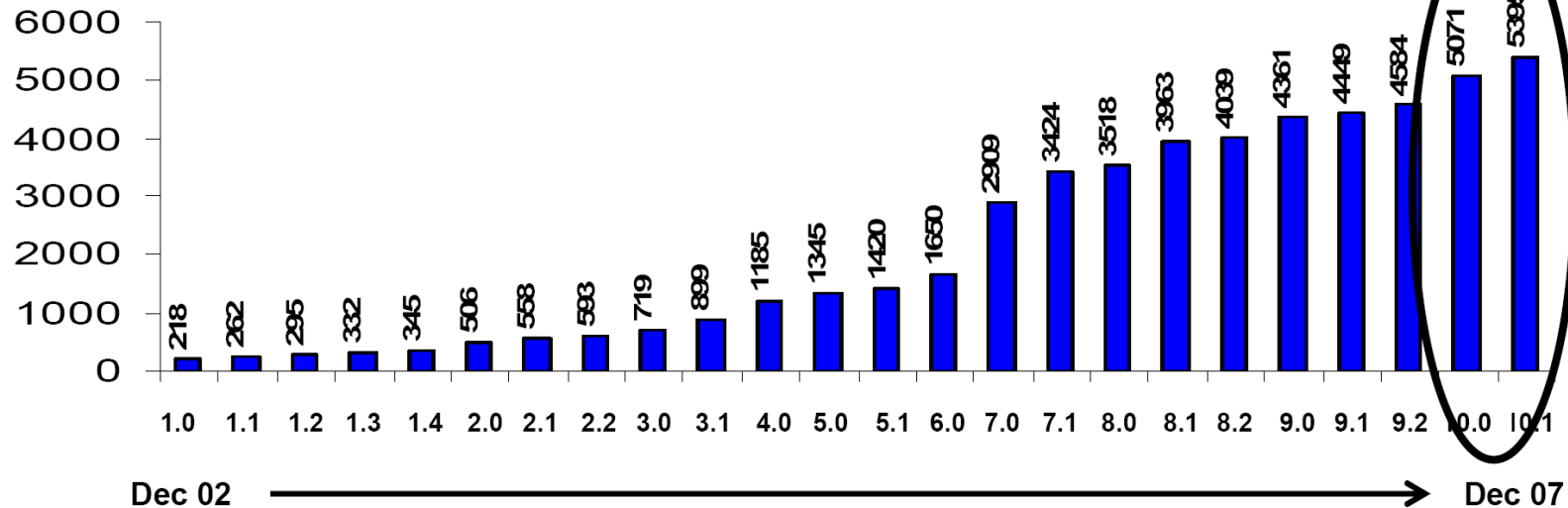
microRNA Biology

- microRNAs genes represent 1-2% of genome
(533 microRNAs identified in humans)
- Approximately 2/3 of human genome is regulated by microRNAs
- Each microRNA regulates ~200 target genes and multiple microRNAs can regulate same gene
- Patterns of microRNA expression define tissue and cell type
- microRNAs are responsible for stem cell differentiation
- microRNAs are critical in normal development and etiology of disease



Rapid increases in discovery of microRNAs

miRBase Sanger Institute, Cambridge, UK



Currently- v. 10.1

Human = 541
Rat = 287
Mouse = 443

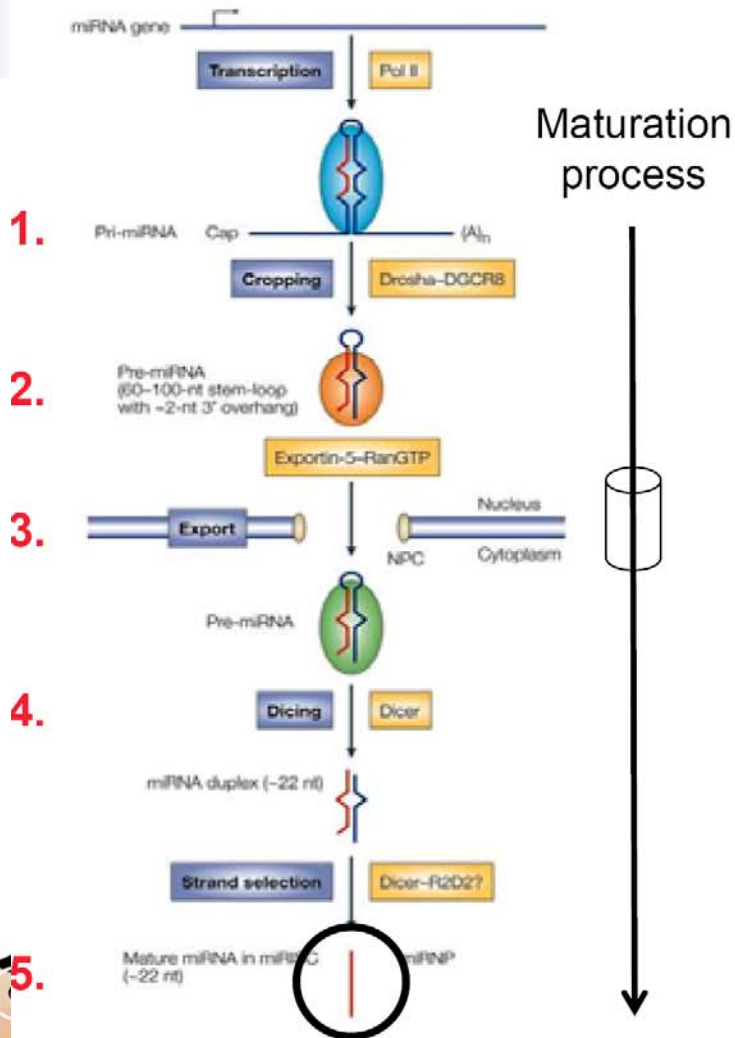
66 different organisms

- Metazoa (35)
- Mycetozoa (1)
- Protista (1)
- Viridiplantae (17)
- Virus (12)

**Most extensive update
in microRNA sequences**



MicroRNA biogenesis & maturation



1. microRNA gene → primary transcript
2. primary transcript → “cropped” to stem-loop microRNA precursor
3. microRNA precursor cytoplasm
4. microRNA precursor → “diced” into mature microRNA duplex
5. mature microRNA duplex → loaded into RISC-like complex
6. gene expression down-regulated

Kim, 2005, Nature Reviews, MCB, vol. 6: p. 376.



How to detect miRNA?

- **Unknown miRNA:**

- isolate total small RNA → cloning or computative analysis

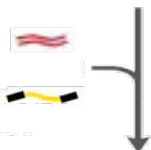
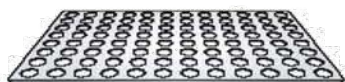
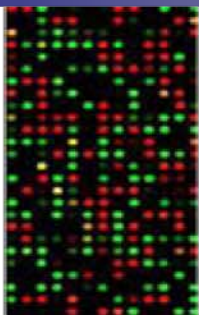
RNA → PCR → TA cloning → sequencing → Northern blotting → prediction of stem-loop sequence

- **Known miRNA:**

- miRNA microarray



Multi-pronged approach to identify the role of microRNAs



microRNA expression profiling



Phenotypic screen with microRNA mimics and/or inhibitors

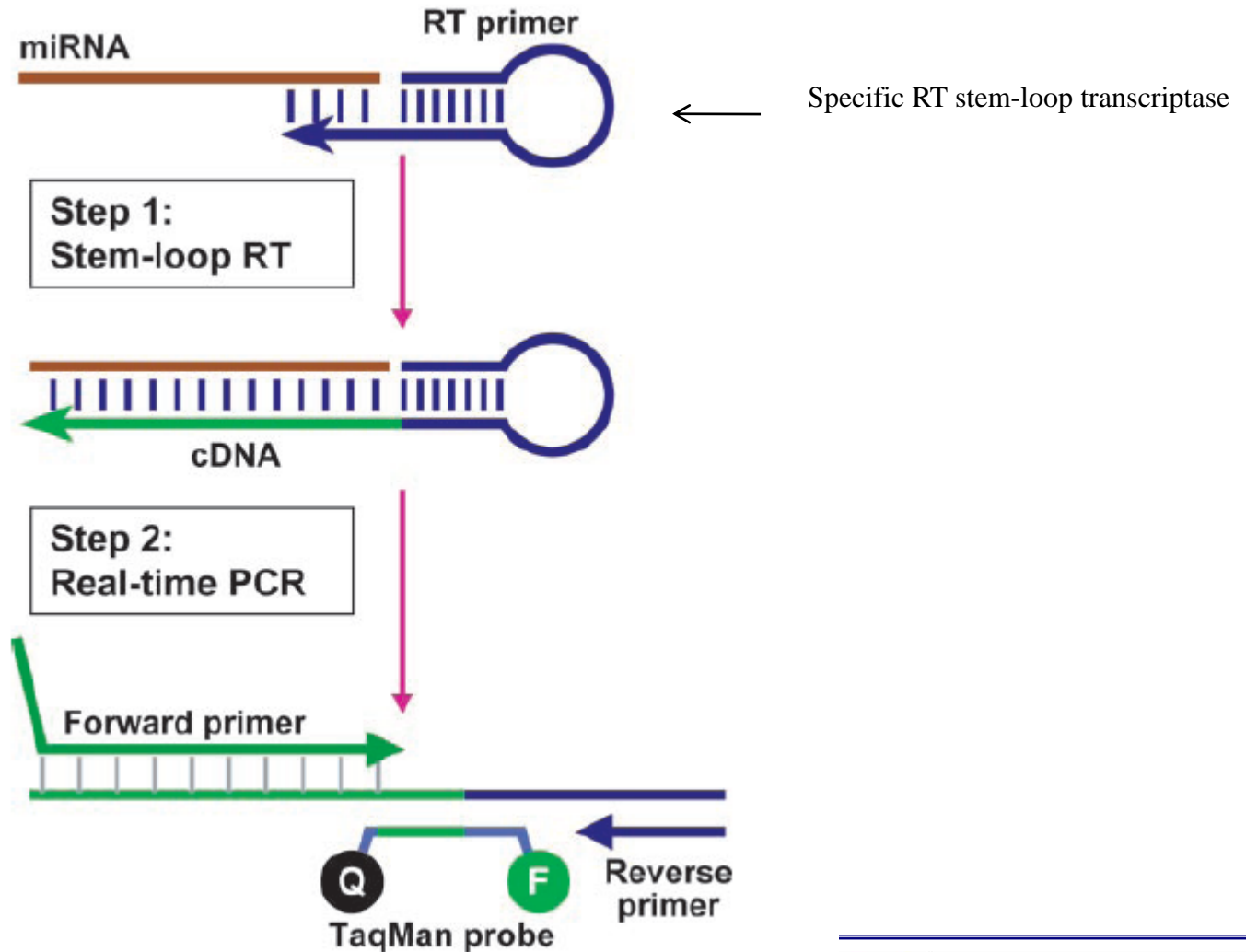


siRNA-induced knockdown

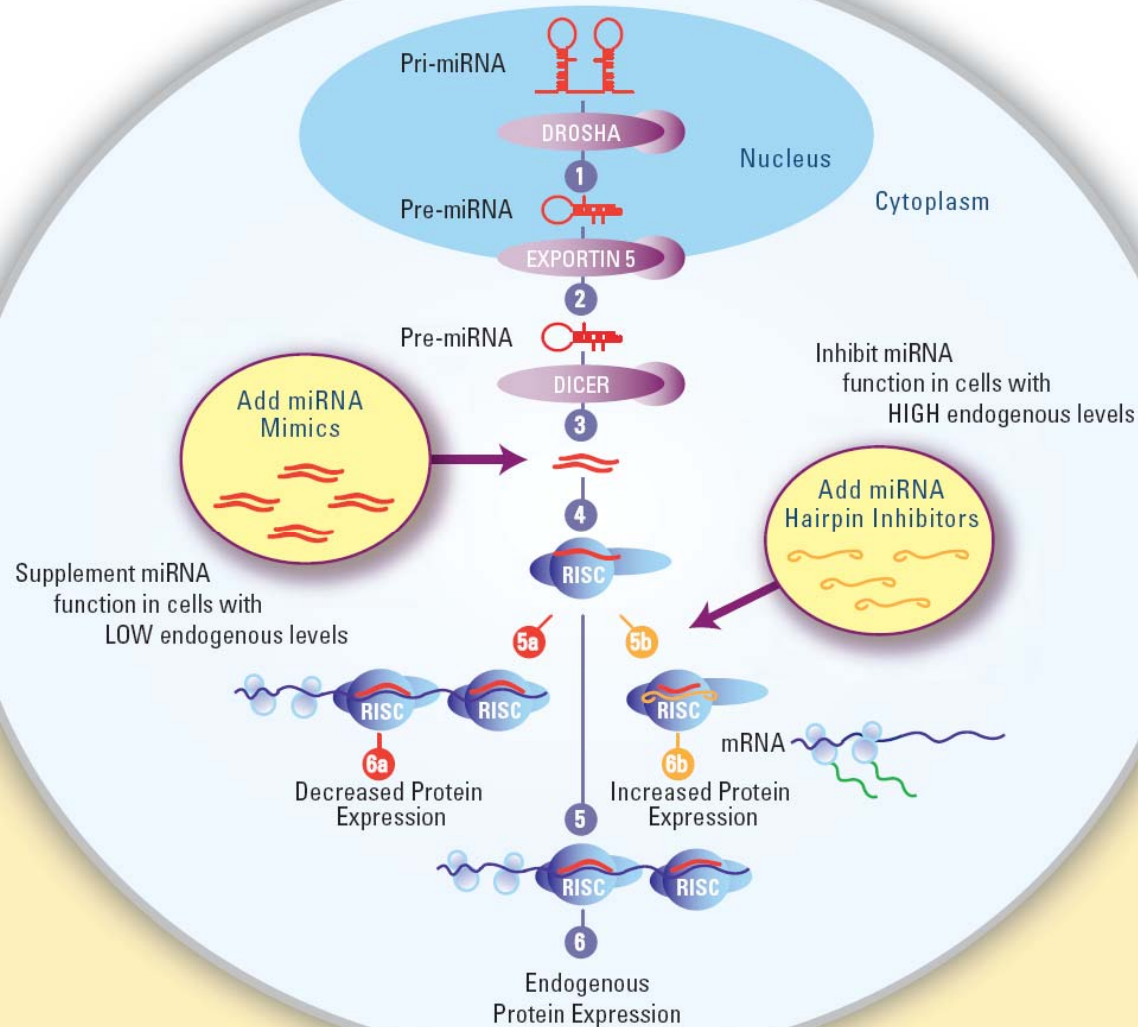
validation of microRNAs in ...



Quantification of miRNA (Q-PCR)



Get into microRNA functional assay : miRIDIAN miRNA Mimics and Inhibitors



Product Details: miRIDIAN microRNA Mimic

- Double-stranded synthetic RNA oligonucleotide
 - Intended to mimic function of endogenous miRNA , chemically modified with ON-TARGET®
- Chemically modified to:
 - Enter miRNA pathway with active strand
 - Exclude passenger strand from loading
 - Minimize interferon response
 - Improve target binding specificity & efficiency
- New! miRIDIAN Mimic designs now updated to Sanger miRBase 10.1 (released Nov 2007) Still be the maximum product line



Applications of miRIDIAN microRNA Mimics

- Supplement miRNA activity to study gain-of-function effects
- Screen for miRNAs that regulate gene expression and affect cellular pathways
- Elucidate miRNA involvement in normal biological and disease pathways
- Identify and validate miRNA targets



miRIDIAN microRNA Mimics: Optimal Design

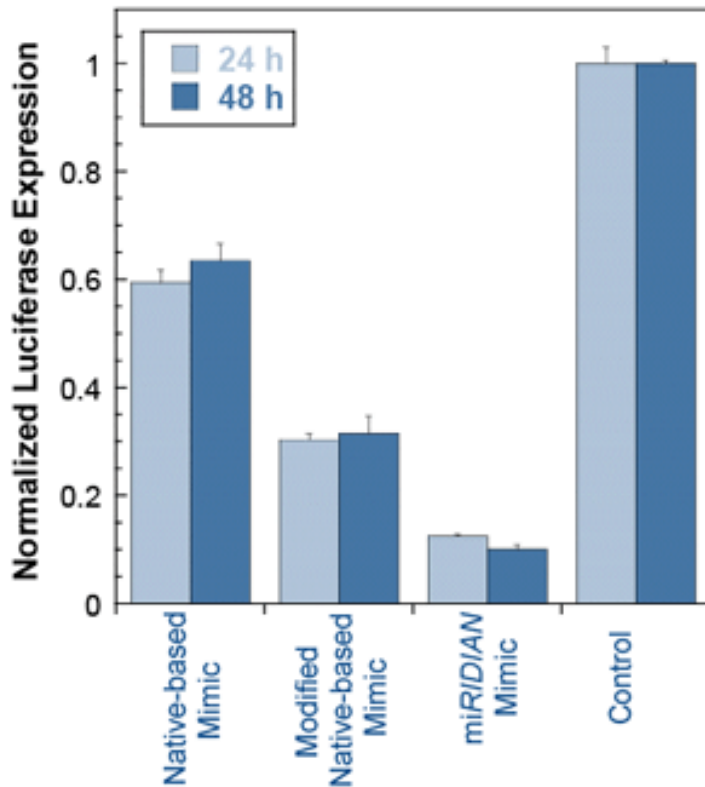


Figure . miRNA mimic function was assayed in HeLa cells 24 and 48 h after transfection of 10 nM indicated miRNA mimics.

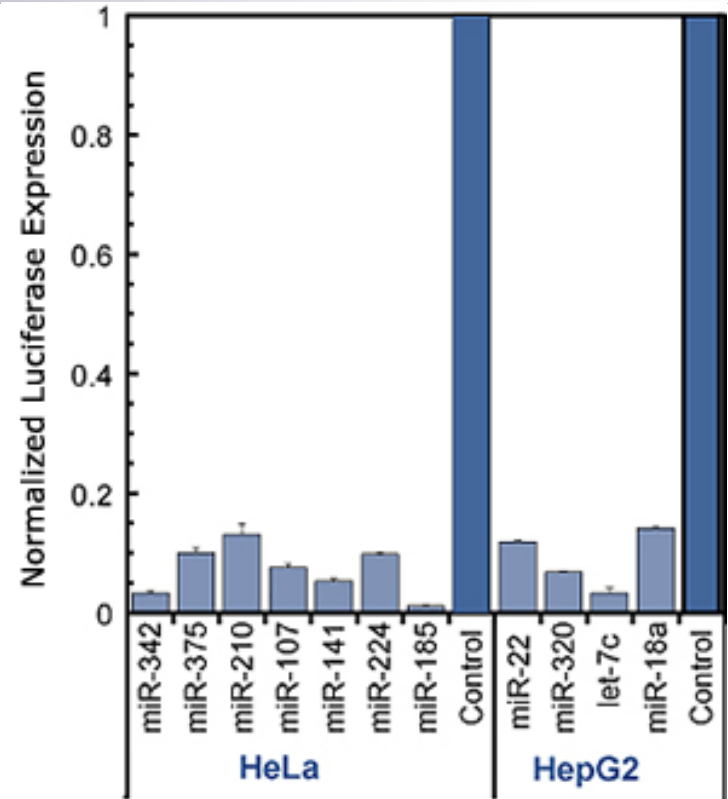


Figure . miRIDIAN microRNA Mimic function for 11 human miRNAs was assayed in HeLa and HepG2 cells 48 h after transfection of 10 nM indicated mimic



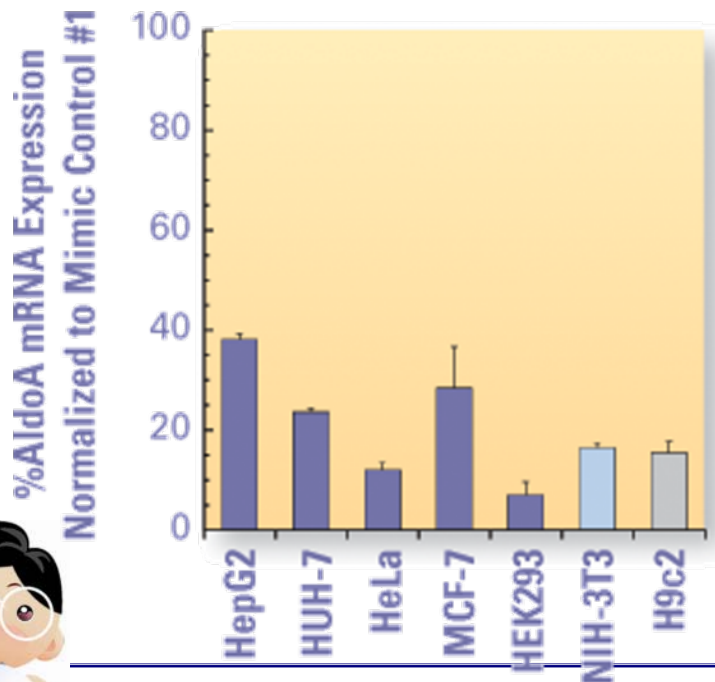
miRIDIAN microRNA Mimic Positive Controls (I)

Endogenous miRNA positive control

(幾乎多數細胞都有表現的內源性 miRNA)

miR-122 ↔ Aldolase A

- Validated miRIDIAN microRNA Mimic that targets Aldolase A in human, mouse, and rat
- Provides the ability to optimize assay conditions by monitoring mimic function on an endogenous gene target (Aldolase A) with a conserved miR-122 binding site



Many cells lines express low to moderate levels of miR-122. Aldolase A is a predicted target of miR-122 and the 3' UTR is conserved in human, mouse and rat at the 8-mer miR-122 predicted seed site. miRIDIAN microRNA Mimics designed to modulate endogenous miR-122 was transfected at 50 nM (Huh-7 at 40 nM) using DharmaFECT 1 into the indicated cell lines and assessed for their ability to decrease AldoA mRNA levels. AldoA downregulation was determined using the Quantigene branched DNA assay(Panomics) at 3 days (HepG2 at 5 days) post-transfection.

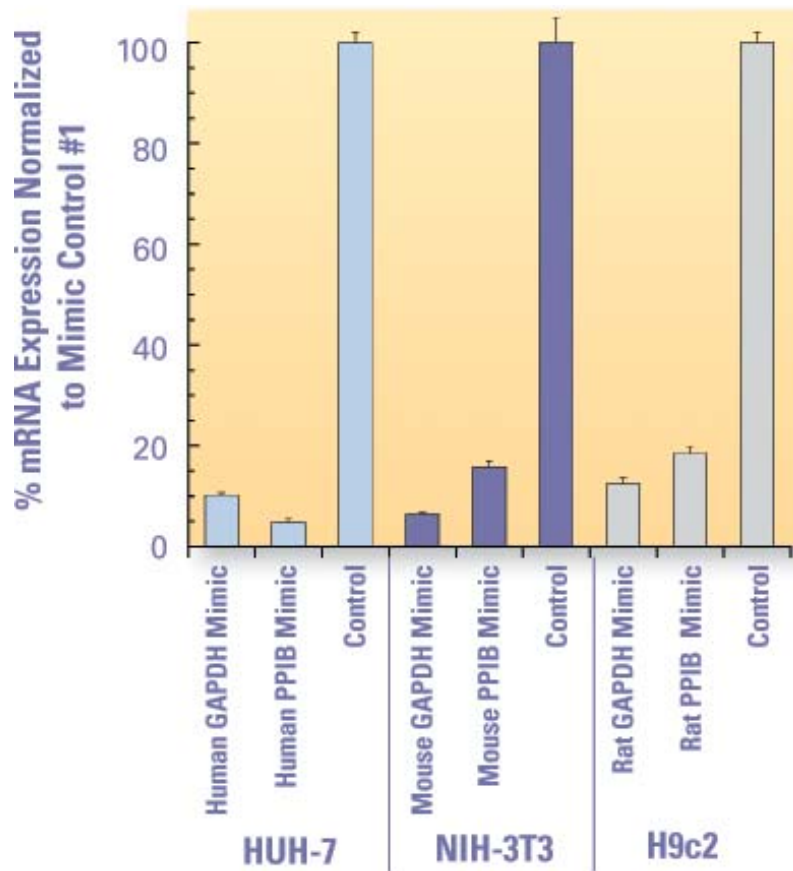


miRIDIAN microRNA Mimic Positive Controls (II)

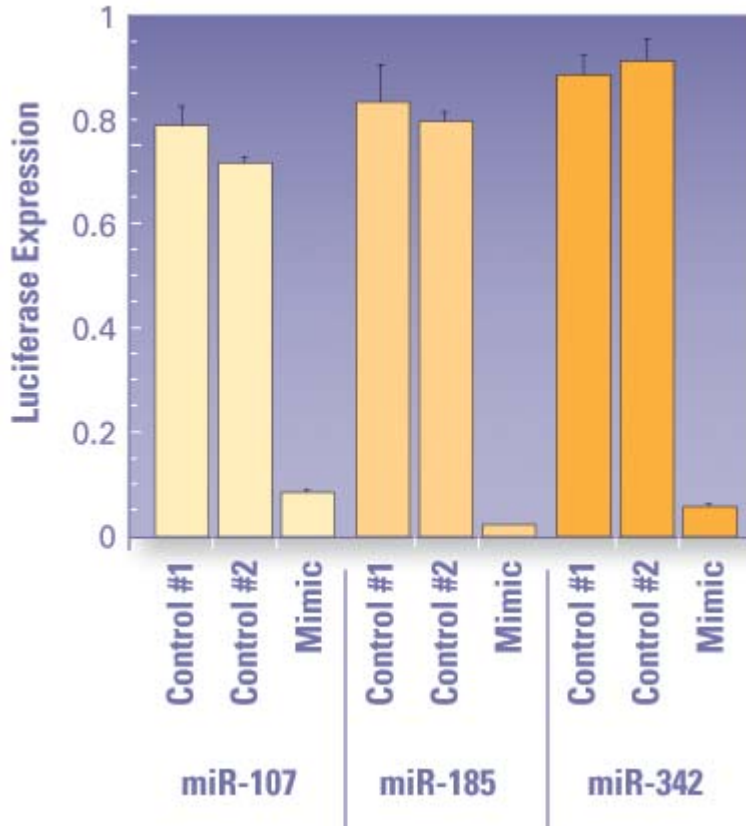
(人工設計出來的miRNA 序列，可以target到GAPDH和PPIB的3'UTR)

Housekeeping miRNA positive control

- Target the 3' UTR (untranslated region) of the standard housekeeping genes, PPIB or GAPDH
- Employ the same structure and design as experimental miRIDIAN microRNA Mimics



miRIDIAN microRNA Negative Control



- Negative control sequence based on cel-miR-67 (#1) and cel-miR-239b
- Identical design and modifications as miRIDIAN microRNA Mimics and Hairpin Inhibitors
- No identifiable effects on tested miRNA function in human, mouse and rat cell lines.

Effects of miRIDIAN microRNA Mimic Negative Controls on the function of three human miRNAs were assayed at 24 hours after transfection of 10 nM mimic or negative control in HeLa cells using a dual luciferase reporter system.



Product Details: miRIDIAN microRNA Hairpin Inhibitors

- Most effective inhibition of endogenous mature microRNA function by means of proprietary, innovative design
- Patent-pending molecule combines chemical modifications and completely novel secondary structure motif
- Superior potency and longevity in comparison to any other synthetic product offered commercially
- Enhanced potency and longevity allows for multiplexed microRNA inhibition at very low nanomolar concentrations with minimal toxicity



Applications of miRIDIAN microRNA HP Inhibitors

- Suppress miRNA activity to study loss-of-function effects
- Screen for miRNAs that regulate gene expression and affect cellular processes
- Elucidate miRNA involvement in normal biological and disease pathways
- Identify and validate miRNA targets



miRIDIAN microRNA HP Inhibitor : Potent design

From Vermeulen et al., RNA (2007) *Double-stranded regions are essential design components of potent inhibitors of RISC function*

A

Flanking nucleotides

0 (RC)

2

4

6

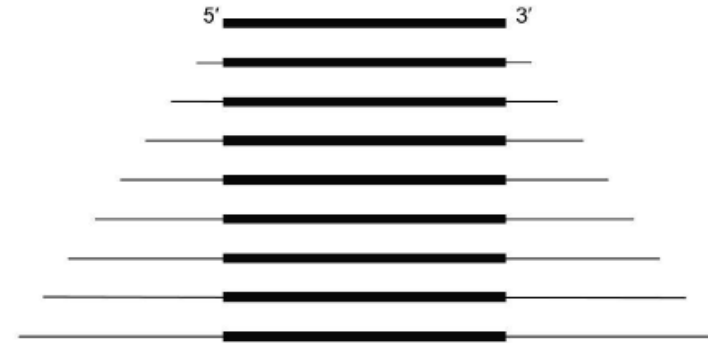
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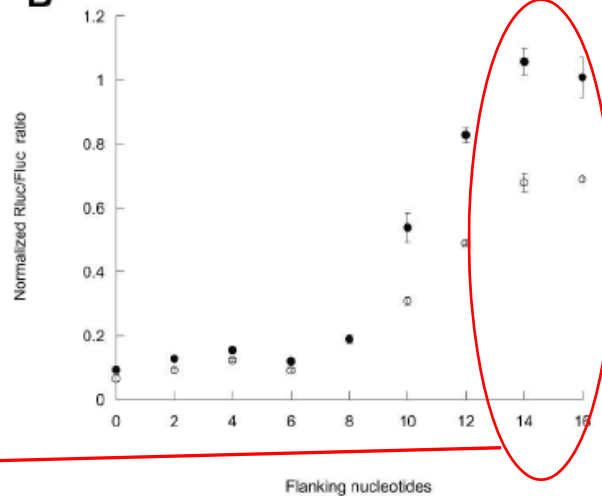
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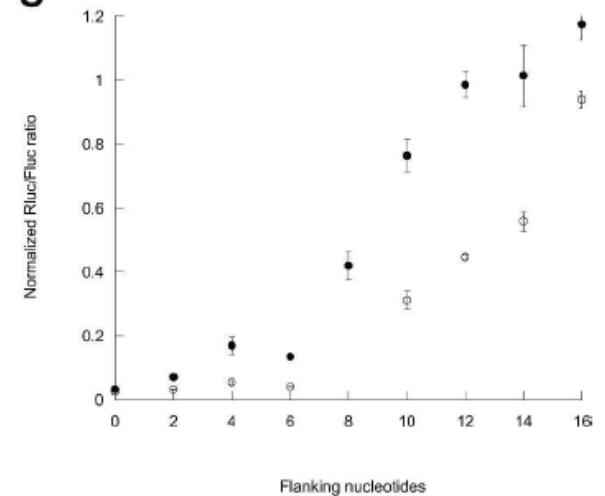
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B



C



More Flanking nucleotides improves inhibitor function



NEW! The miRIDIAN Hairpin Inhibitor

- Next generation, patent-pending microRNA inhibitor combines
 - Chemical modifications
 - Secondary structure motif
 - Flanking region is the same for all inhibitors and is designed to fold back as hairpins on each end
 - Demonstrated to greatly enhance functionality
- Based on work published in Vermeulen et al. (2007) RNA 13:723-730
- Competes with (and in some cases outperforms) LNA inhibitors in potency and longevity in benchmarking

double-stranded
region

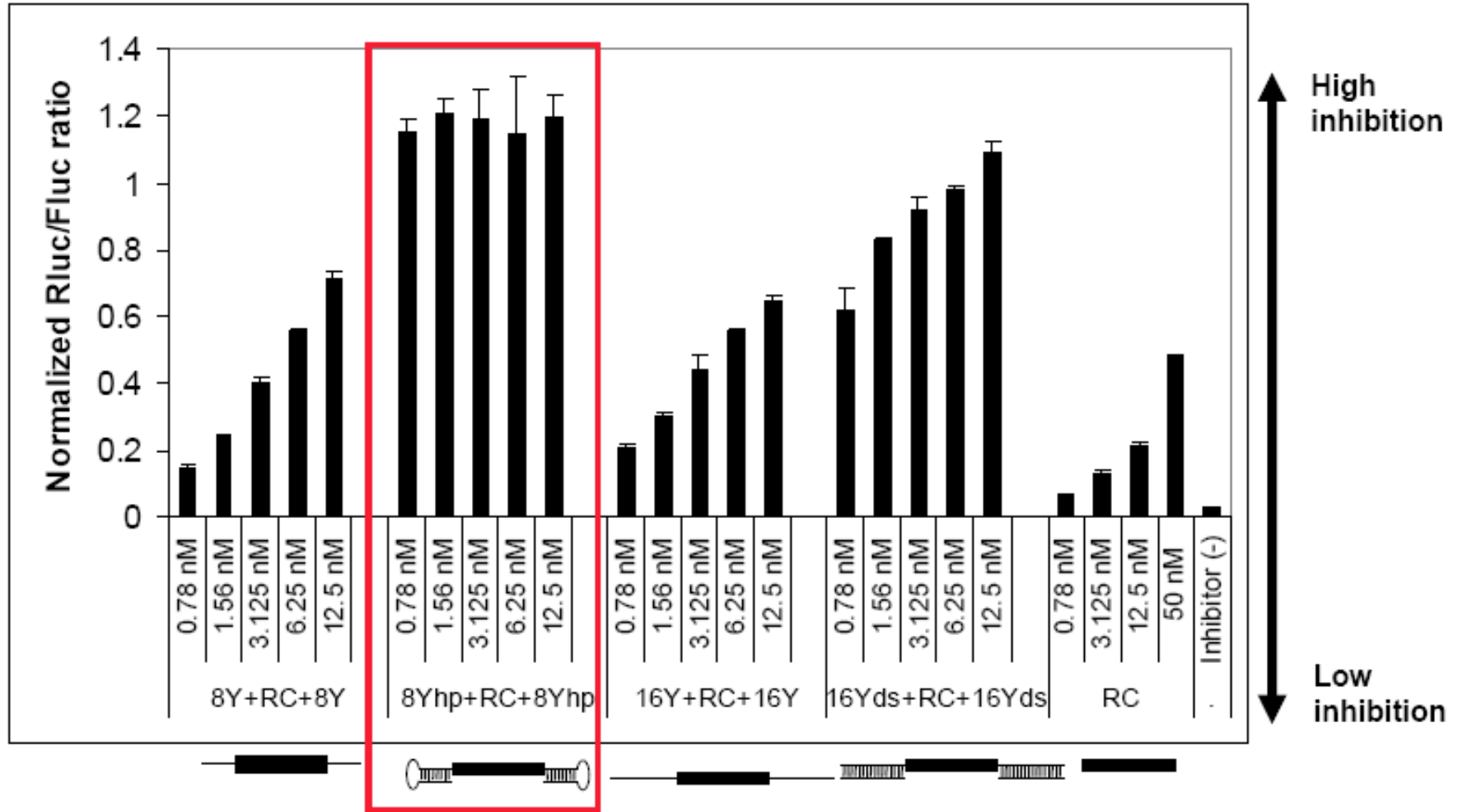
double-stranded
region



RC



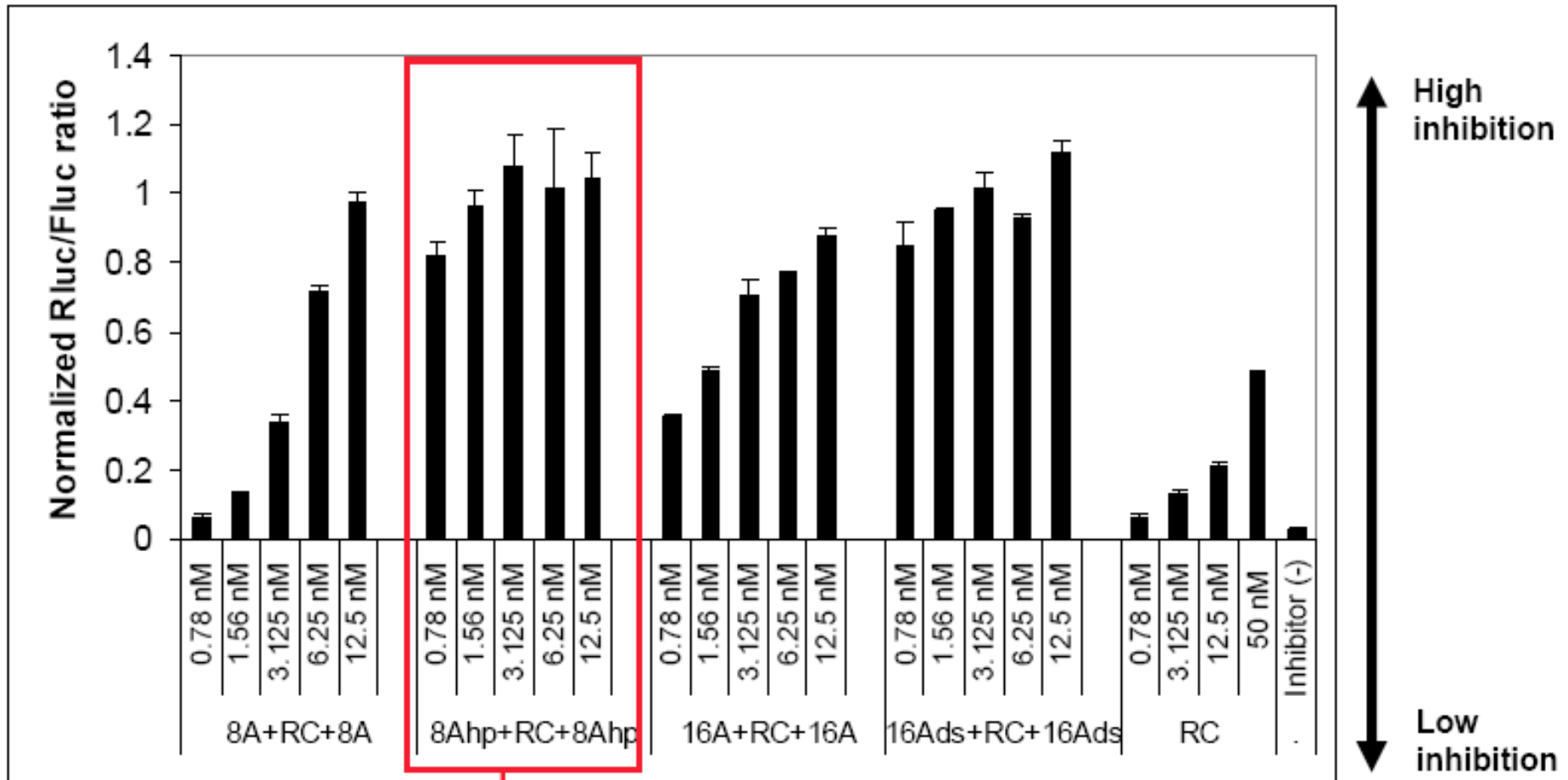
Importance of secondary structure , polypyrimidine (C's and U's) for the flanking sequences



Pyrimidine flanking sequences, miR-21, HeLa dual-luc reporter

We tried several different structures, and the hairpin gave the best inhibition

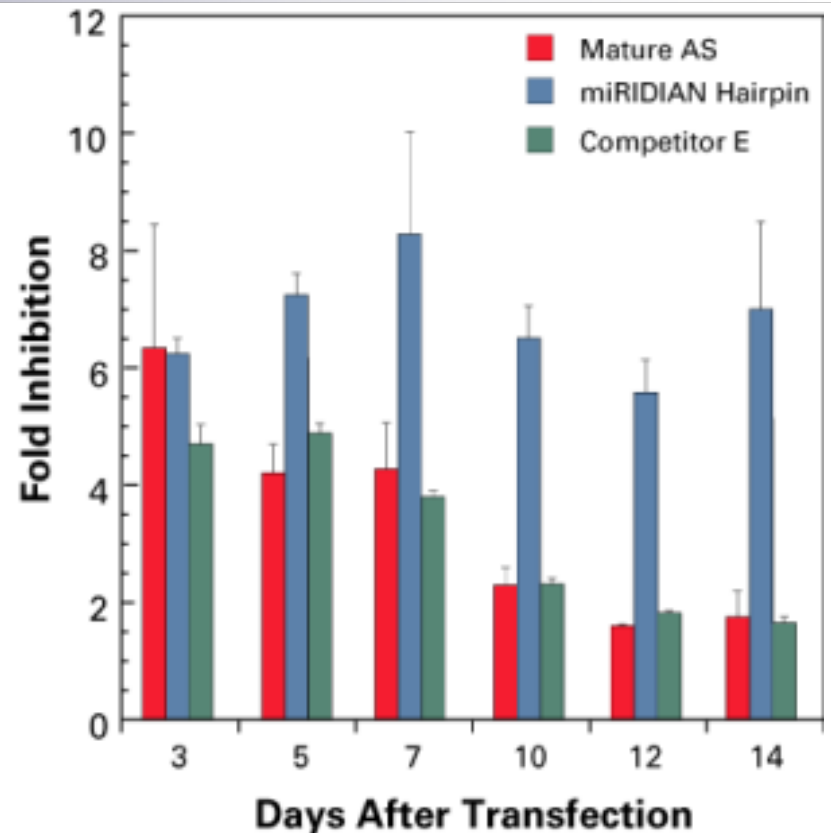
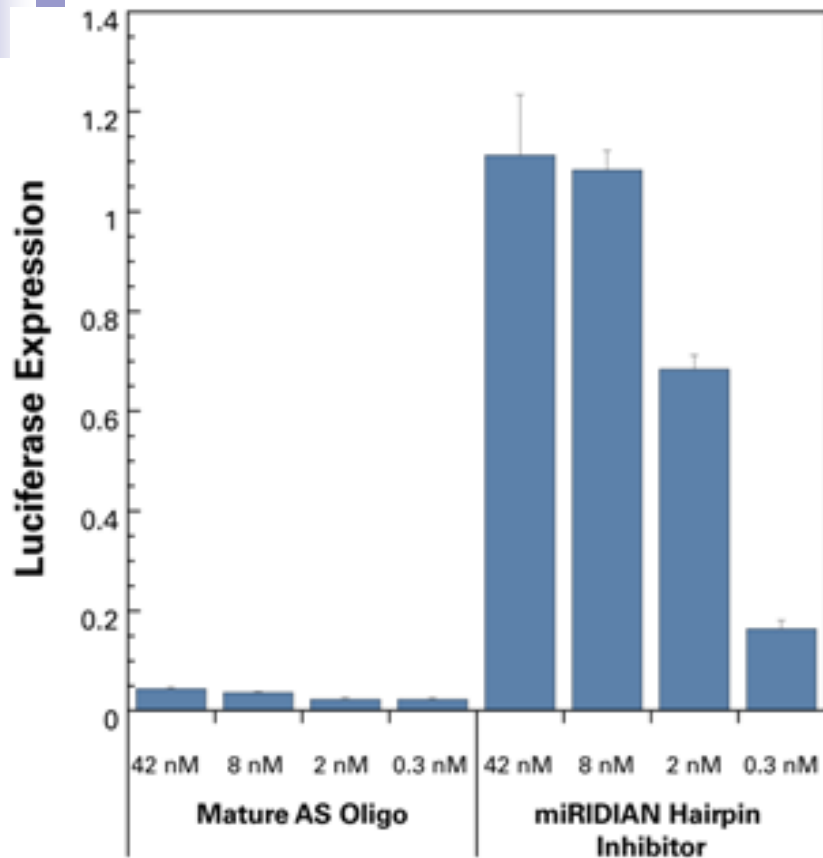
Importance of secondary structure, arbitrary bases as the flanking sequences



Arbitrary flanking sequences, miR-21, HeLa, dual-luc reporter

We tried several different structures, and the hairpin gave the best inhibition 中祥股份有限公司
 CHUNG HSIANG LIFE SCIENCE LTD.





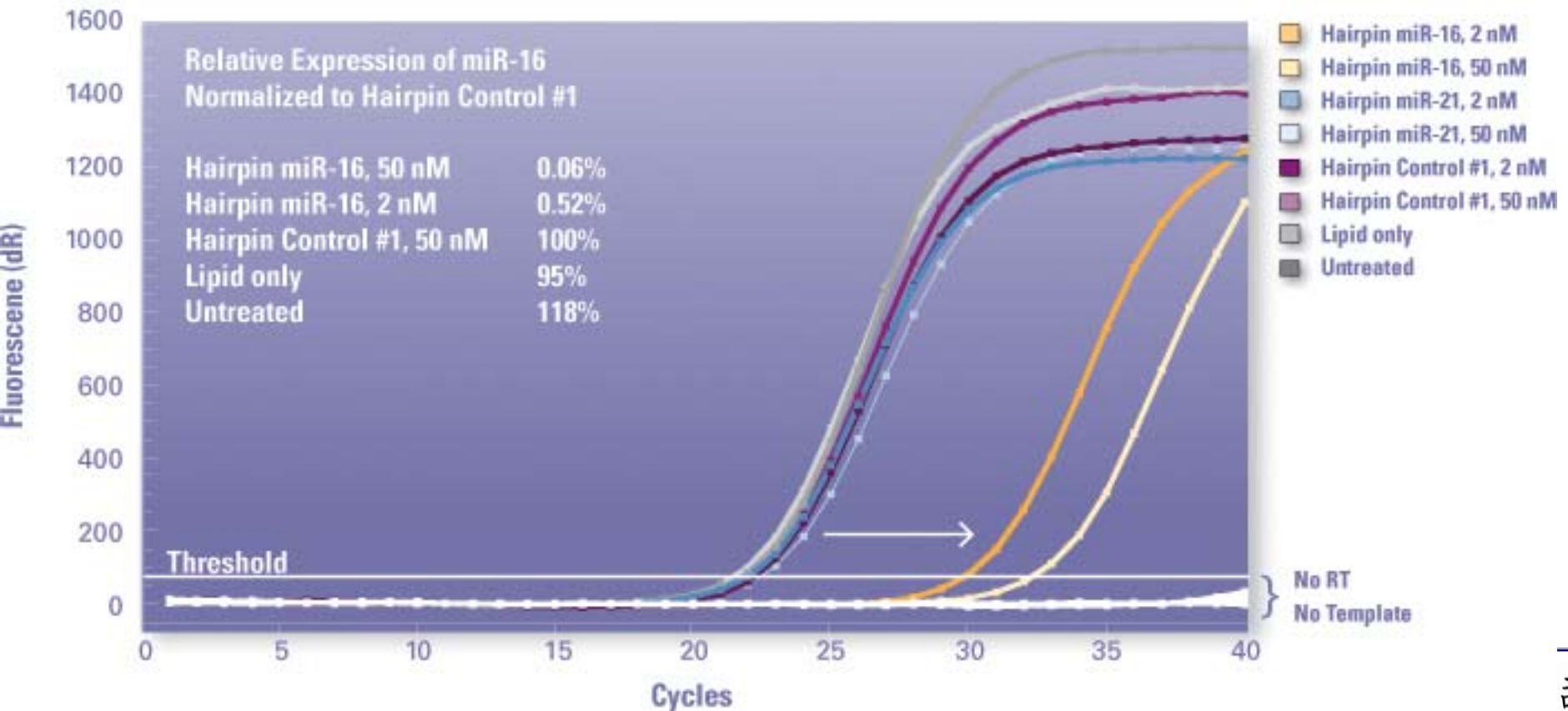
microRNA Inhibitor function for miR-21 miRNA was assayed in HeLa cells, 48 h after transfection using a dual-luciferase reporter system. Each sample was normalized to matching treatment in empty dual-luciferase reporter.

Time course of inhibition by three different designs targeting endogenous miR-23 in a MCF-7 reporter cell line from a stably-integrated luciferase construct. Inhibitors were transfected using Dharmafect-1 at 200 nM for Mature AS and 40 nM for miRIDIAN Hairpin and Competitor E designs. Data was normalized to cells with no inhibitor, so no inhibition is equal to 1.



miRIDIAN microRNA HP Inhibitor Positive Controls

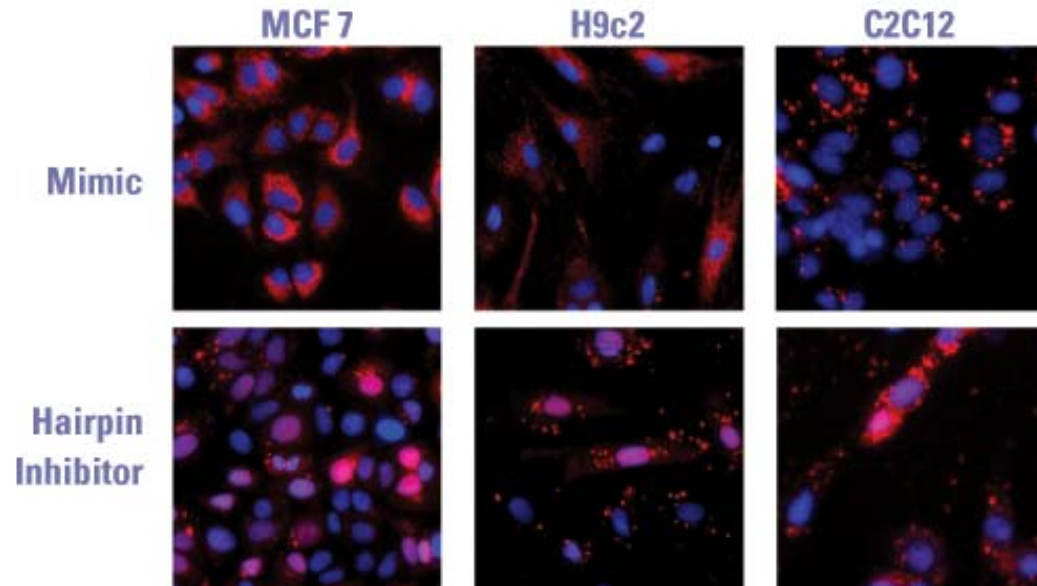
Targets miR-16 in human, mouse, and rat cells resulting in reduced miR-16 activity



miRIDIAN microRNA Mimic and Inhibitor Transfection Controls

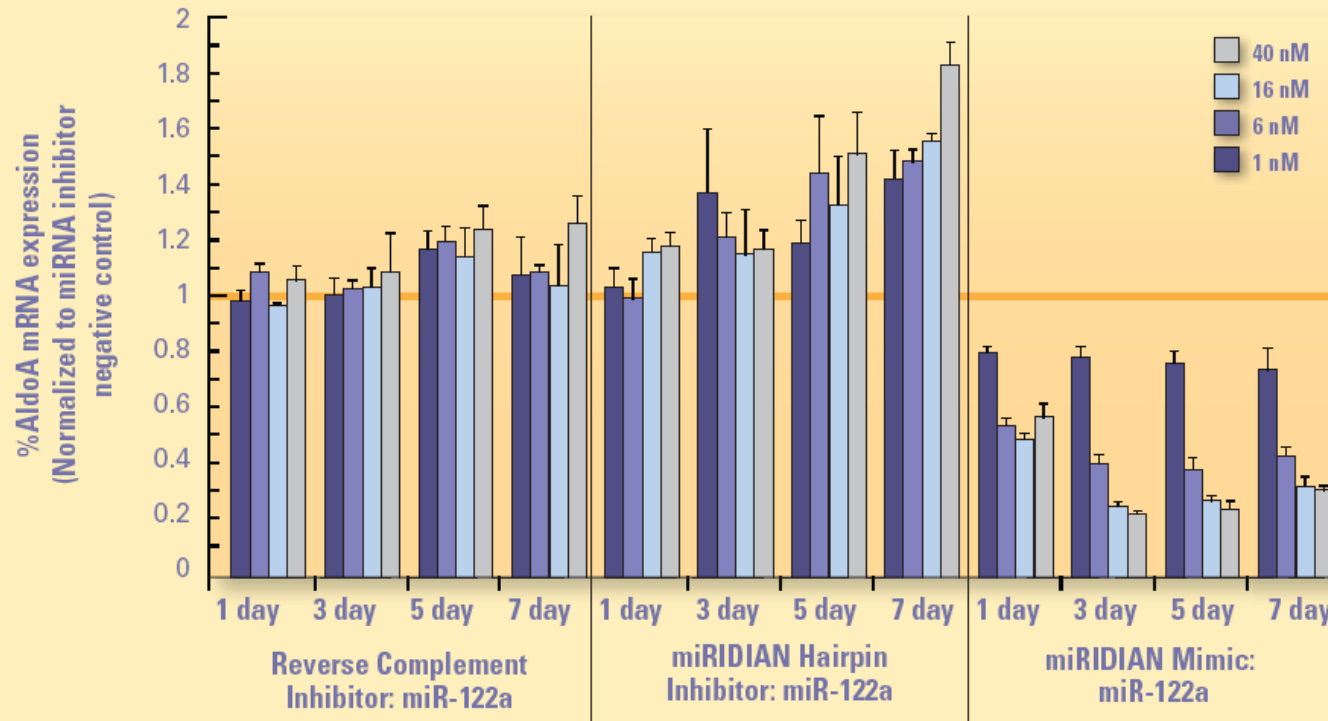
The miRIDIAN microRNA Hairpin Inhibitor Transfection Control is a Dy547-labeled Hairpin Inhibitor based on *C. elegans* miRNA (cel-miR-67) for monitoring delivery into human, mouse and rat cells.

- Non-targeting miRIDIAN Hairpin Inhibitors labeled with Thermo Scientific Pierce Dy547 (absorbance/emission max: 557/570 nm).
- Control designs and modifications are identical to experimental miRIDIAN microRNA Hairpin Inhibitors.



Potent and long-lived tools to validate microRNA targets

Extended duration of effect enables microRNA target analysis



Modulation of AldoA mRNA levels in Hih-7 cell line using miRNA miRIDIAN Mimics & Inhibitors. Orange line indicates AldoA expression level as a result of endogenous miR-122 regulation. AldoA mRNA expression values >1 indicates inhibitor of miR-122, while values <1 indicates additional down regulation of AldoA by miRNA. The miRIDIAN miRNA Inhibitor resulted in an increase in Aldo A mRNA levels even at 7 days, whereas a standard 2'-oMe ENA oligo complementary to mature miR-122 showed a small effect. The miRIDIAN miRNA Mimic immediately reduced Aldo A mRNA levels and sustained suppression throughout the experiment.



miRIDIAN microRNA Summary

Mimics

- Gain-of-function experiments
- Supplement existing endogenous levels of miRNA
- Simulate miRNA levels where there are none

- Protein expression: ↓

inhibitors

- Loss-of-function experiments
- Bind endogenous miRNAs and prevent them from hybridizing to target mRNA

- Protein expression: ↑



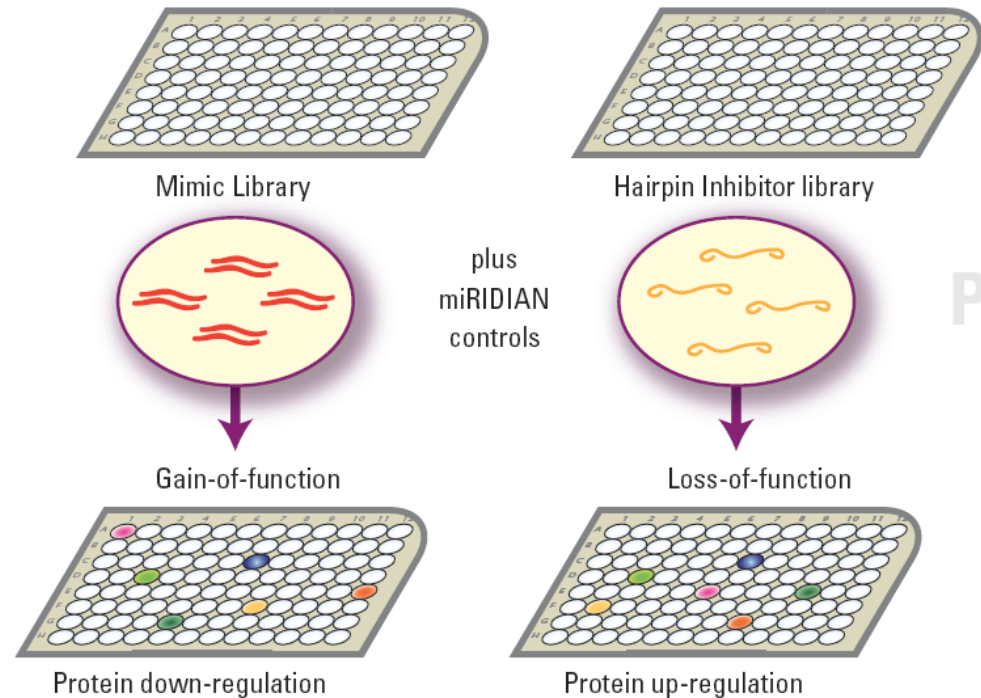
miRIDIAN microRNA Mimic and Hairpin Inhibitor Libraries

- Complete libraries of miRIDIAN microRNA Mimics and Hairpin Inhibitors to promote research on the roles of miRNAs in biological pathways such as:
 - – Metabolic Pathways
 - – Developmental Pathways
 - – Cell Growth
 - – Tissue Development
 - – Cancer Cell Differentiation and Development
 - – Stem Cell Differentiation
- Libraries contain microRNA Mimics and Hairpin Inhibitors for every Human, Mouse, and Rat miRNA in the [miRBase Sequence Database](#) (version 10.1)
- Packaged in 96-well plates for convenient high-throughput screening



High-throughput Phenotypic Screening: miRIDIAN microRNA Mimic and Inhibitor Libraries

- Perform phenotypic high-throughput screening
- Find potential biomarkers of normal or diseased cellular processes
- Couple microRNA screening with high-content analysis for multi-parametric data
- Identify microRNAs that synergize with drugs of interest for increased therapeutic benefit



miRNA Library

- Regular products
- Custom library



Thank You for Your Attention!

