RNAi在研究實務上的整合應用技術

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20100803

Overview of presentation

- Basic Biology of RNA interference
- Application of siRNA for gene function ?
- How to study miRNA ?
- How to deliver siRNA and miRNA?
- New prospects on RNAi research

RNAi: A Historical Perspective

Plants: co-suppression

1990

Napoli et al. 1990. Plant Cell 2:279

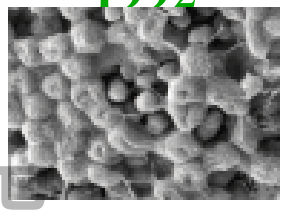
Mammals: RNAi

2001

Elbashir et al. (2001) Nature 411:494

Fungi: quelling

1992



Romano (1992) Mol Micro 6:3343

Flies: RNAi

1998

Kennerdell et al. 1998. Cell 95:1017
Hammond et al. 2000. Nature 404:293
Zamore et al. 2000. Cell 101:25

Worms: RNAi

1998

Fire et al. (1998) Nature 391:806

Discovery of microRNA

1993

2000

2001

2002

2003

2004

2005

2006

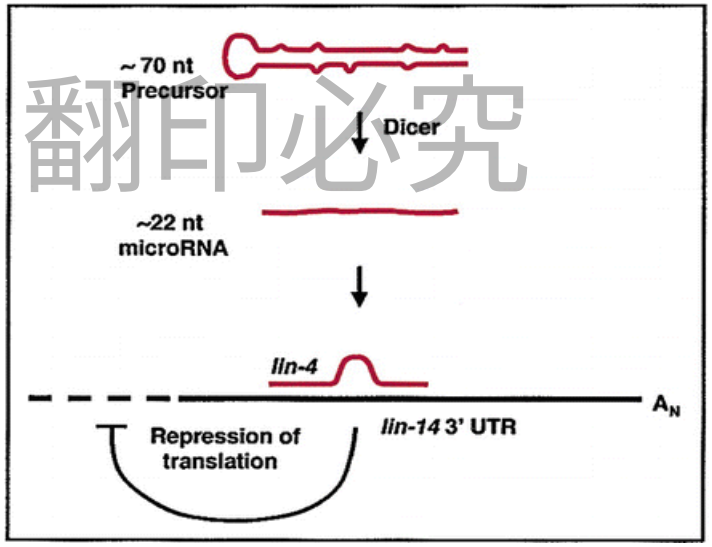
2007

Cell, Vol. 75, 843–854, December 3, 1993, Copyright ©1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

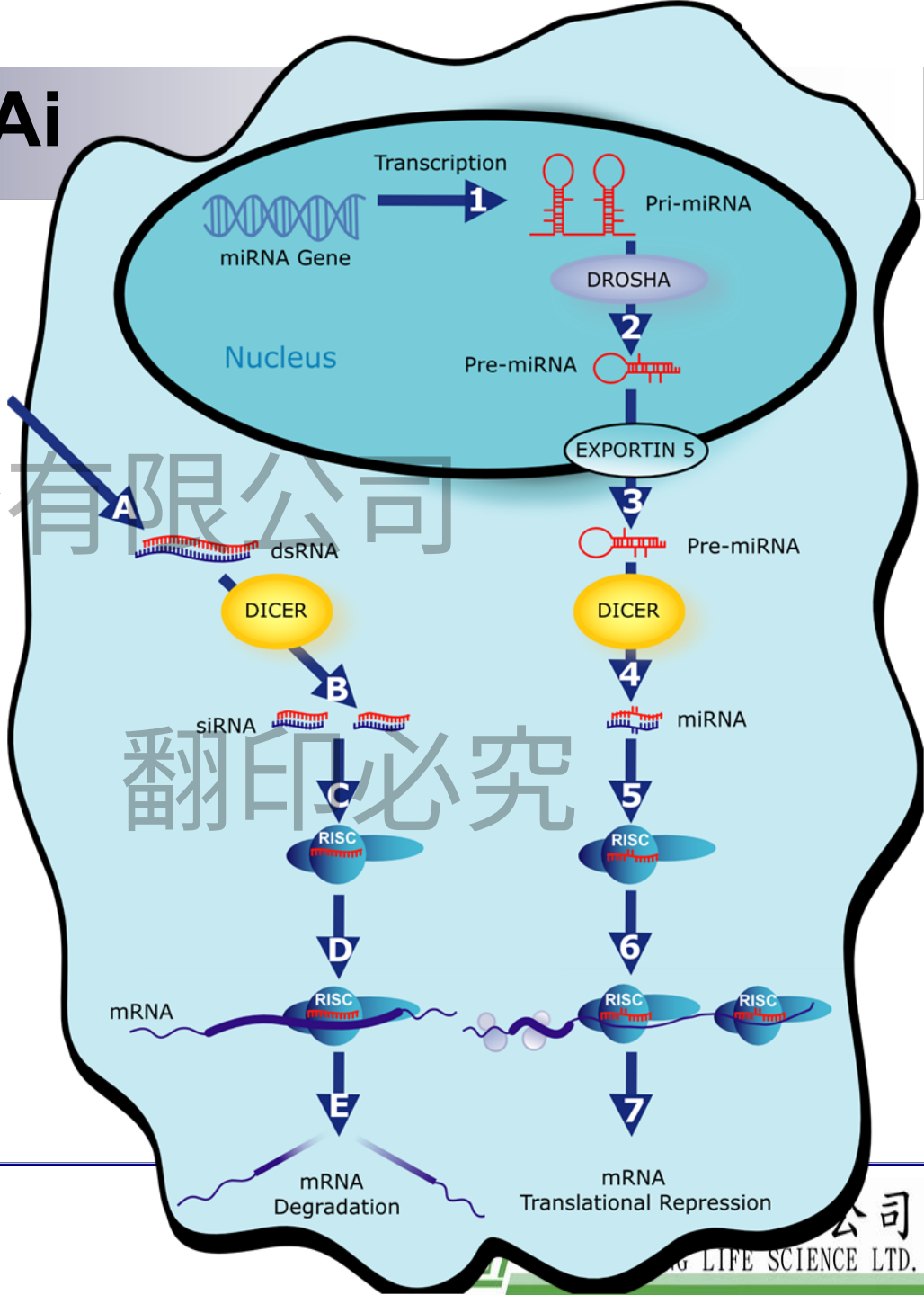
Cell, Vol. 75, 855–862, December 3, 1993, Copyright ©1993 by Cell Press

Posttranscriptional Regulation of the Heterochronic Gene *lin-14* by *lin-4* Mediates Temporal Pattern Formation in C. elegans



Mechanism of RNAi

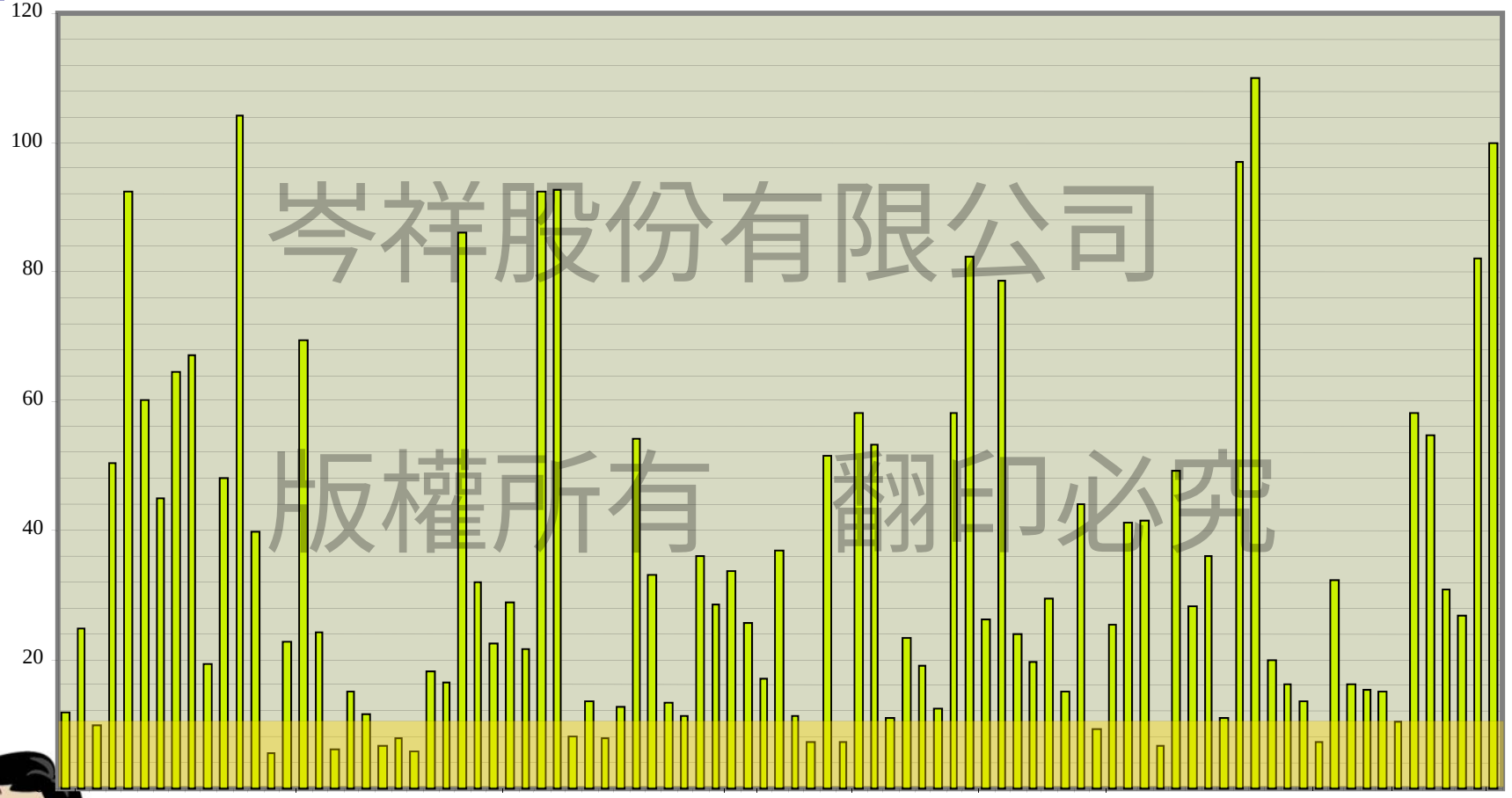
1. miRNA gene is transcribed into pri-miRNA.
2. Pri-miRNA is processed into hairpin pre-miRNA.
3. Pre-miRNAs are transported to cytoplasm.
4. Pre-miRNAs are processed into short, mature miRNA duplexes.
5. Mature miRNAs complex with RISC-like structure.
6. miRNA/RISC complex binds to target mRNA.
7. mRNA is translationally repressed or cleaved.



siRNA -- A tool to elucidate gene function

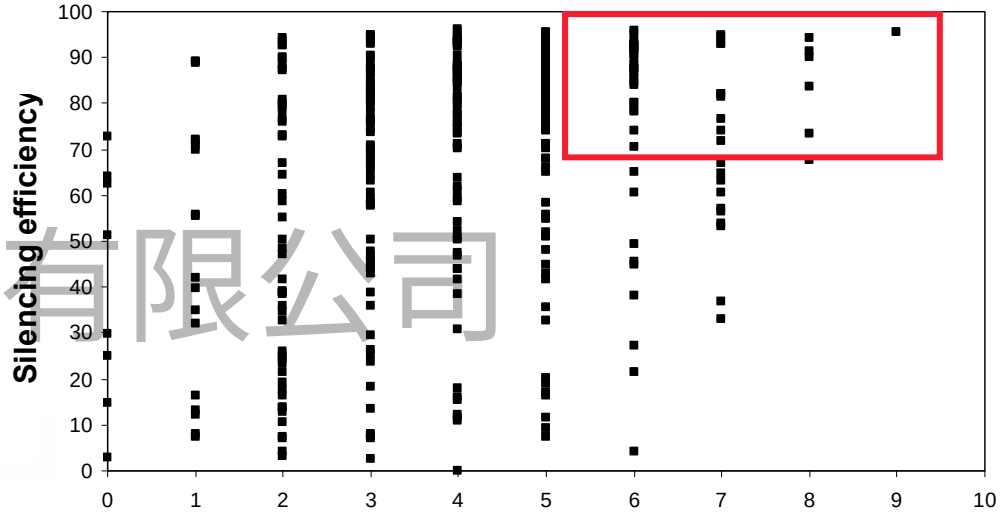
Activity of Randomly Selected siRNAs

only ~20% of randomly selected siRNAs knock-down target with efficiency 90% and higher

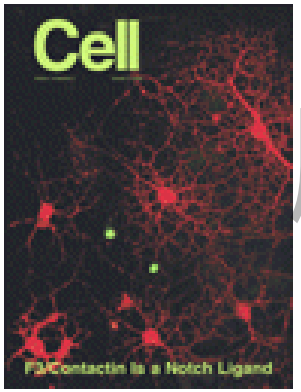


siRNA Properties Correlate with Functionality

- *Stability at terminus of each strand*
- *Overall G/C content of siRNA molecule*
- *Base preferences at specific positions*



8 parameter algorithm score



Cell (2003) 115: 209-216

Functional siRNAs and miRNAs Exhibit Strand Bias

Anastasia Khvorova,[1,2] Angela Reynolds,[1,2] and Sumedha D. Jayasena[1,*]

Nature Biotechnology (2004) 22:326-330

Rational siRNA design for RNA interference

Angela Reynolds, Devin Leake, Queta Boese, Stephen Scaringe, William S Marshall & Anastasia Khvorova

Naturally Generated siRNA Pools in Model Organisms

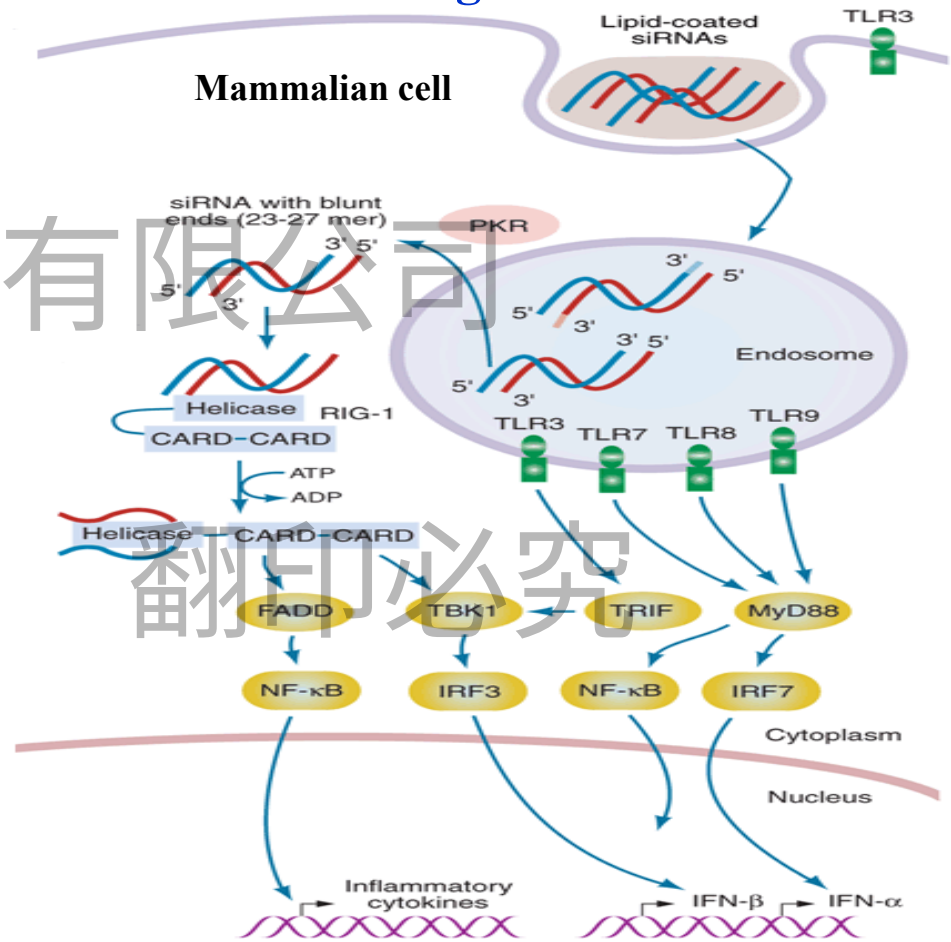
Long dsRNA

Dicer

siRNA Pool

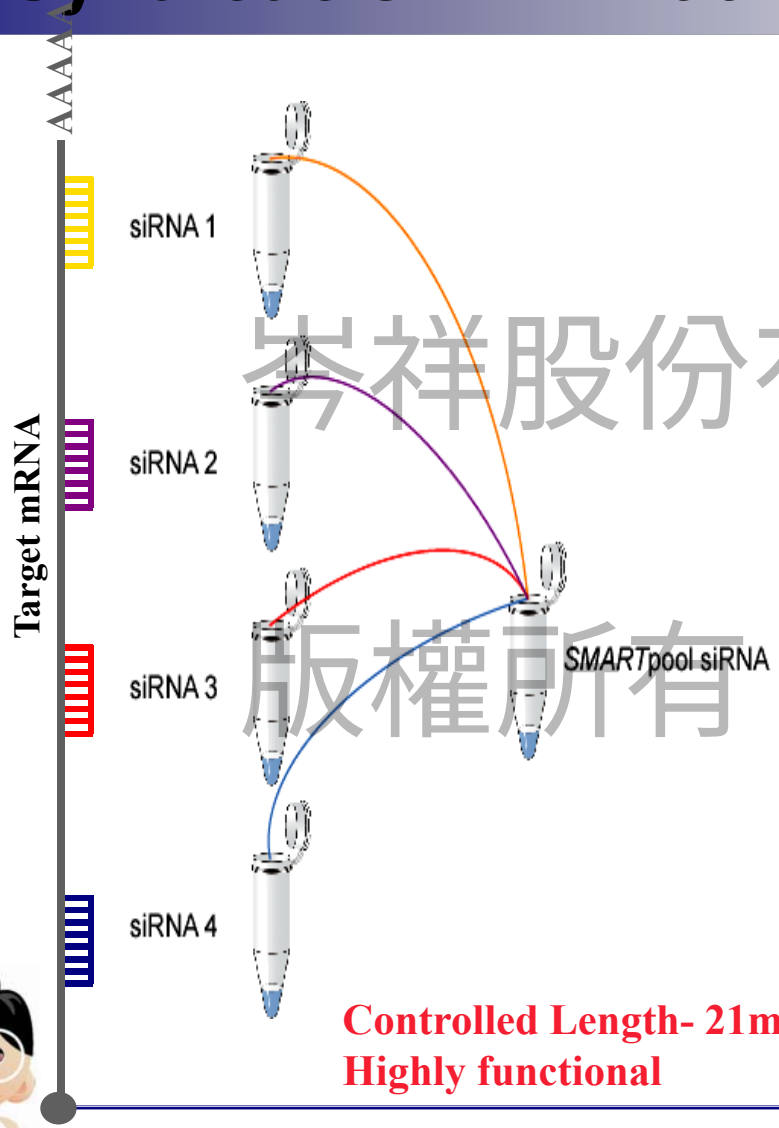
Different activities
Different specificities
Different length

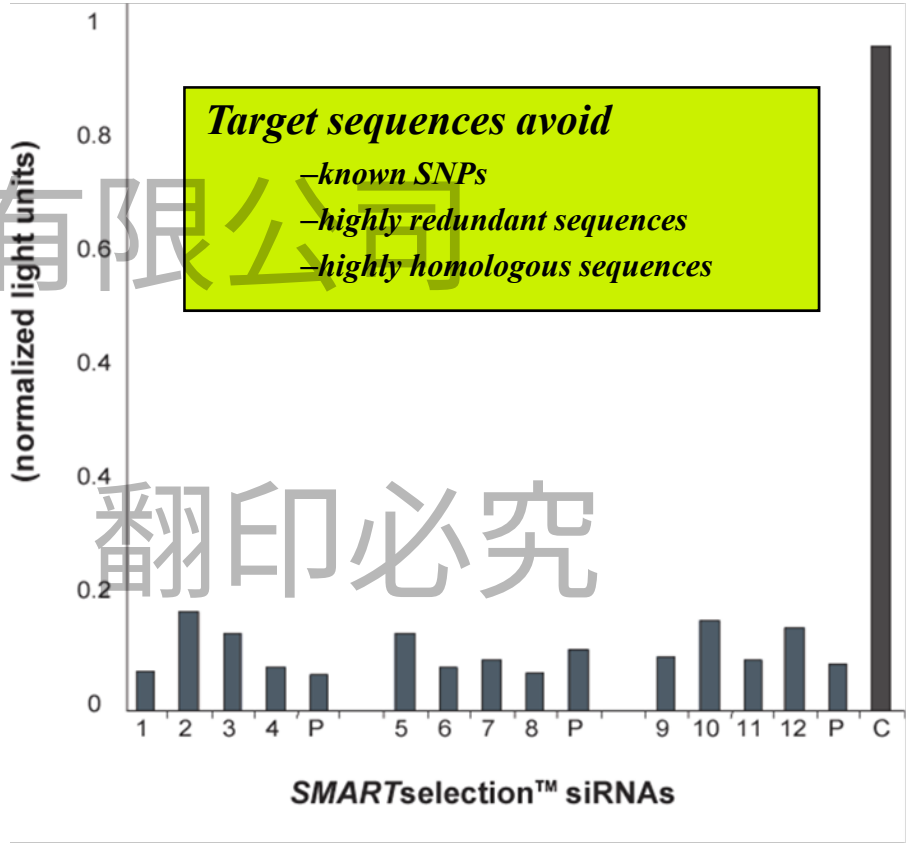
Long dsRNA



Adapted from Sioud (2006) *Nature Biotechnology*, 24: 521-522.

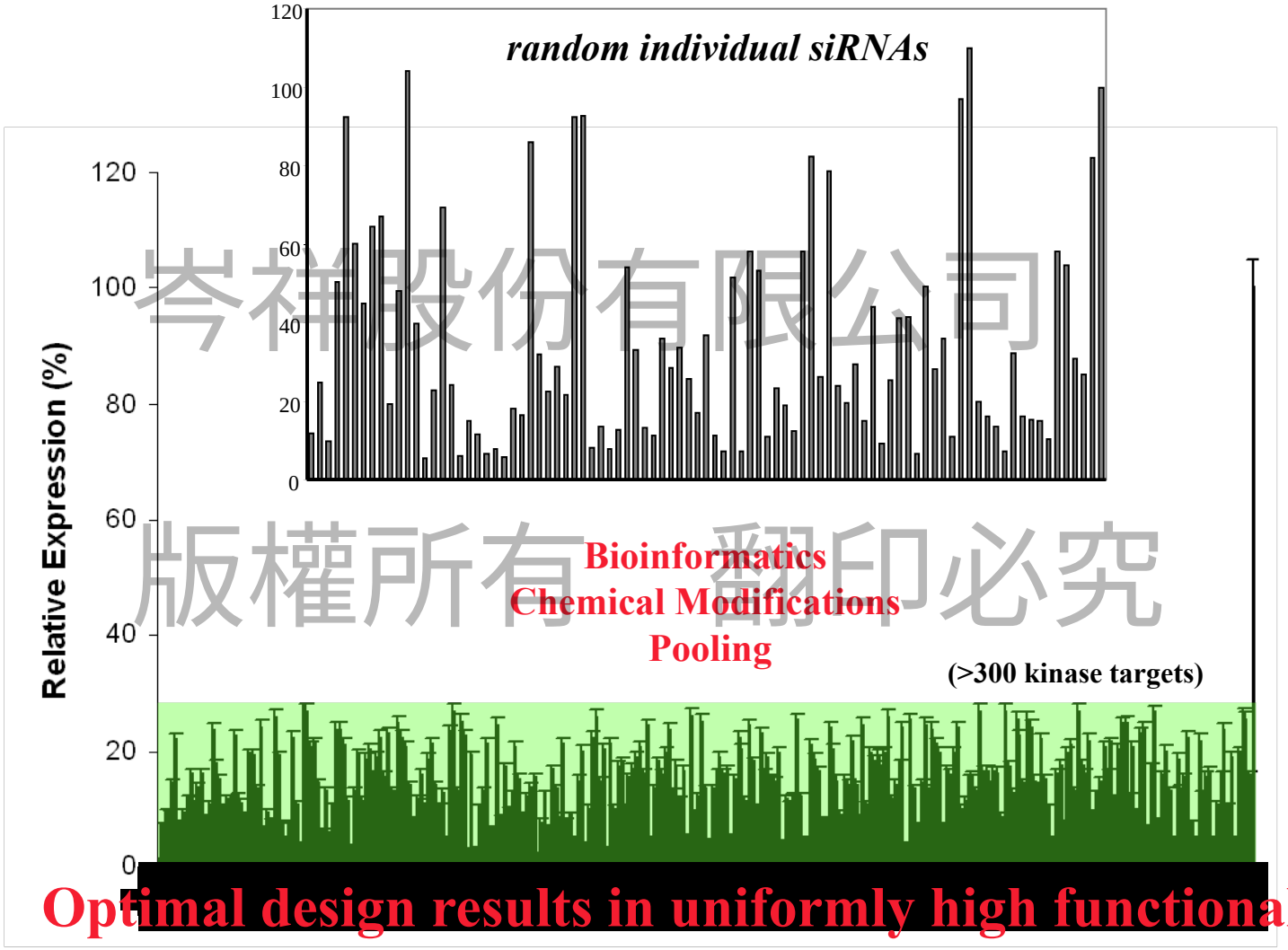
Synthetic siRNA Pools Ensure Robust Silencing





Controlled Length- 21mers
Highly functional

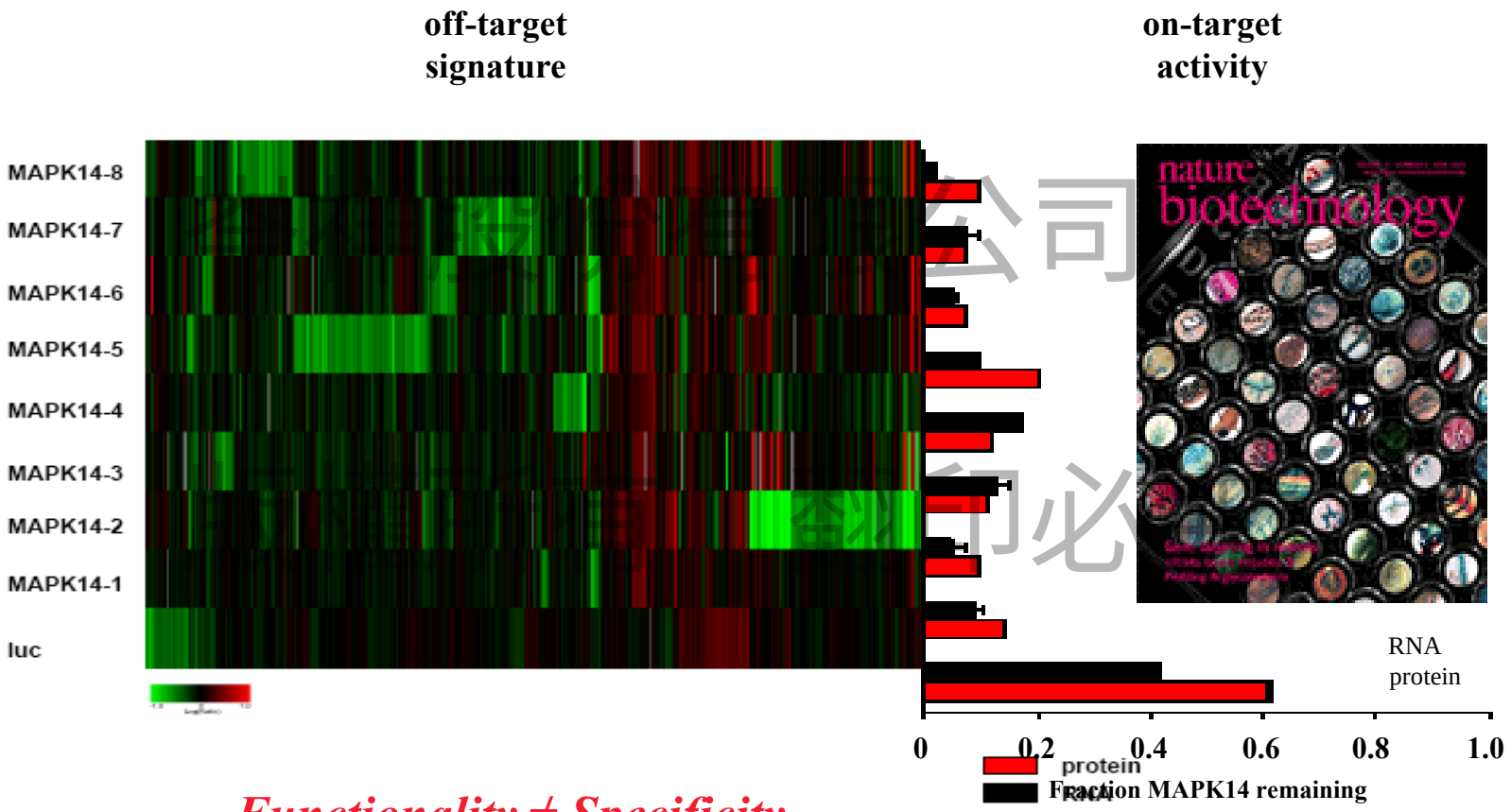
Validation of SMART*pool*™ Technology



Optimal design results in uniformly high functionality

Collaboration with Joan Brugge's laboratory, Harvard Medical School

Functional siRNAs Induce Off-target Effects



Functionality ≠ Specificity

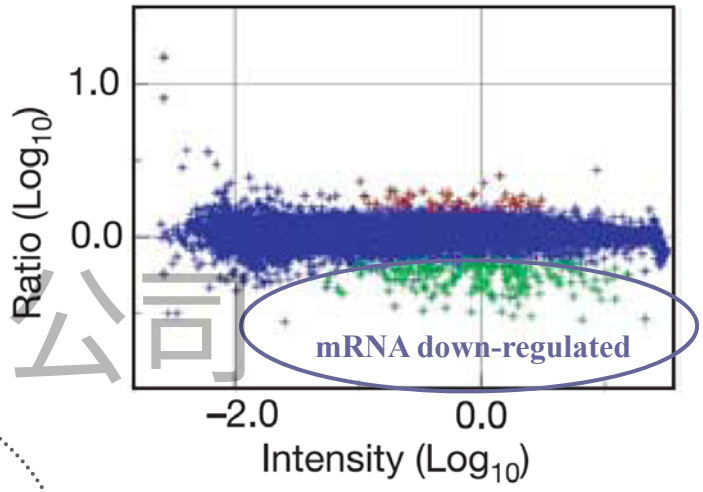
Adapted from Jackson et al., (2003) *Nature Biotechnology*, 21: 635-638.

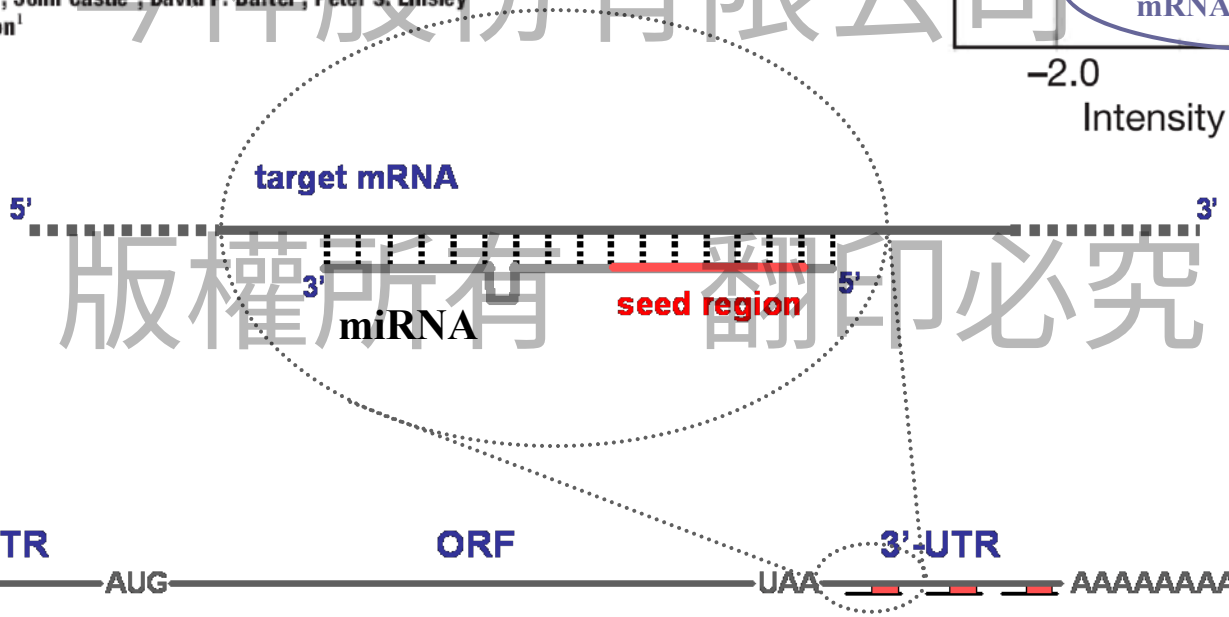
miRNA Gene Targeting

Nature (2005) 433:769

Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs

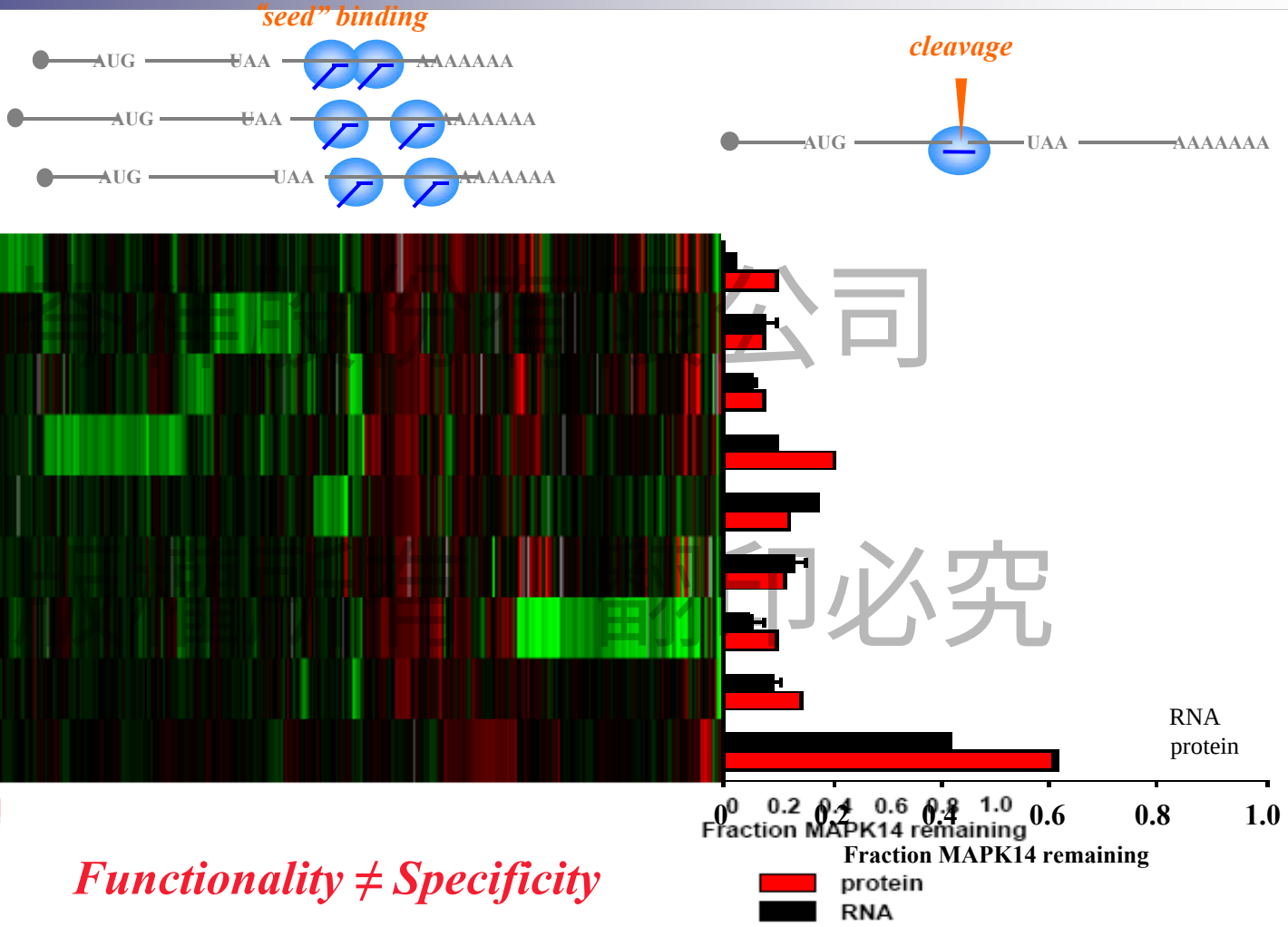
Lee P. Lim[1], Nelson C. Lau[2], Philip Garrett-Engele[1], Andrew Grimson[2], Janell M. Schelter[1], John Castle[1], David P. Bartel[2], Peter S. Linsley[1] & Jason M. Johnson[1]





miRNA can down-regulate multiple gene targets

Functional siRNAs Induce Off-target Effects



Functionality ≠ Specificity

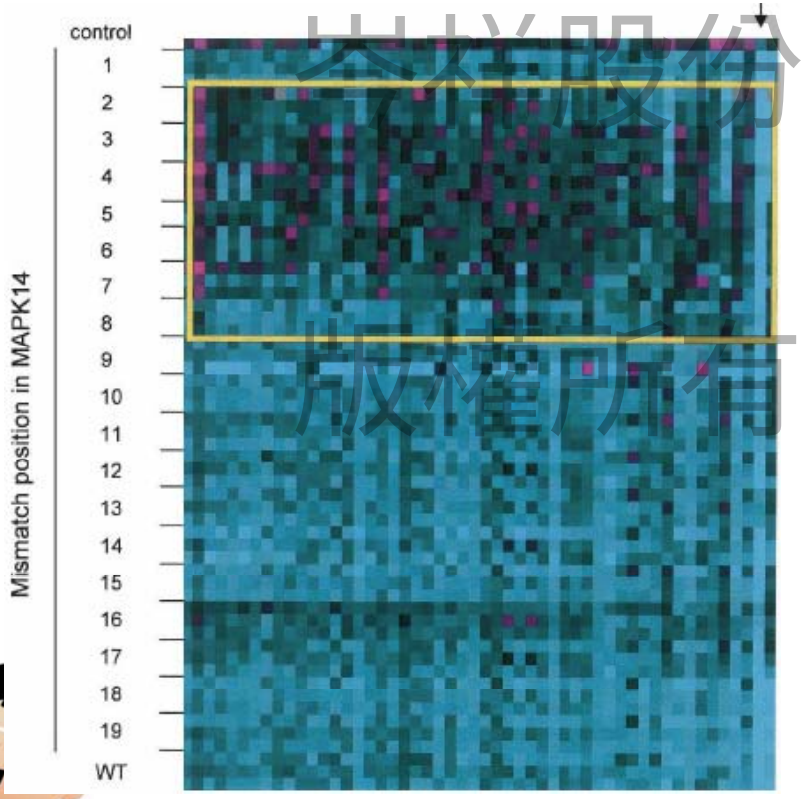
Adapted from Jackson et al., (2003) *Nature Biotechnology*, 21: 635-638.

Seed Region is Responsible for Off-target Effects

Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity

RNA (2006) 21:635

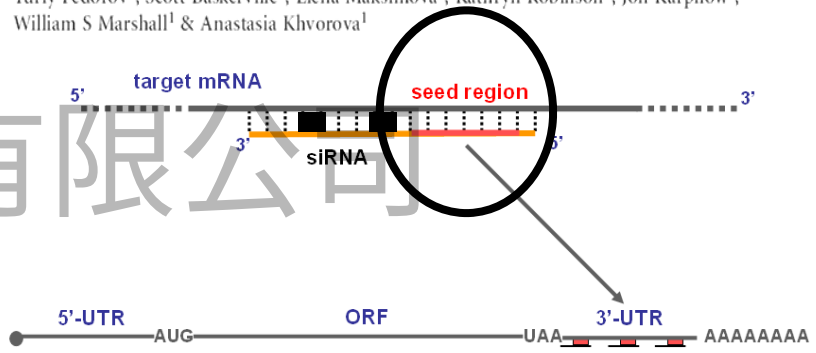
AIMEE L. JACKSON, JULJA BURCHARD, JANELL SCHELTER, B. NELSON CHAU, MICHELE CLEARY, LEE LIM, and PETER S. LINSLEY
Rosetta Inpharmatics, LLC, a wholly owned subsidiary of Merck & Co., Inc., Seattle, Washington 98109, USA

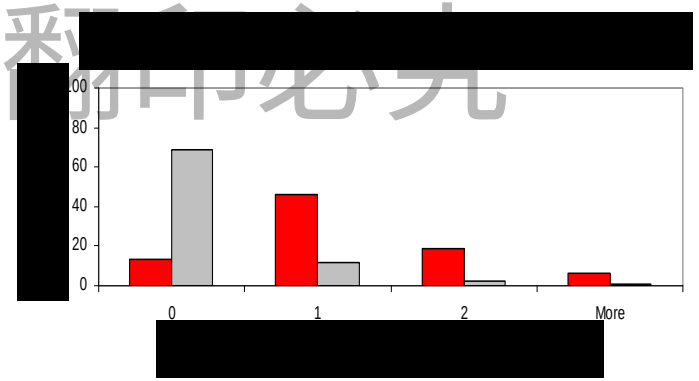


3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets

NATURE METHODS | VOL.3 NO.3 | MARCH 2006 | 199

Amanda Birmingham[1], Emily M Anderson[1], Angela Reynolds[1], Diane Ilsley-Tyree[2], Devin Leake[1], Yuriy Fedorov[1], Scott Baskerville[1], Elena Maksimova[1], Kathryn Robinson[1], Jon Karpilow[1], William S Marshall[1] & Anastasia Khvorova[1]

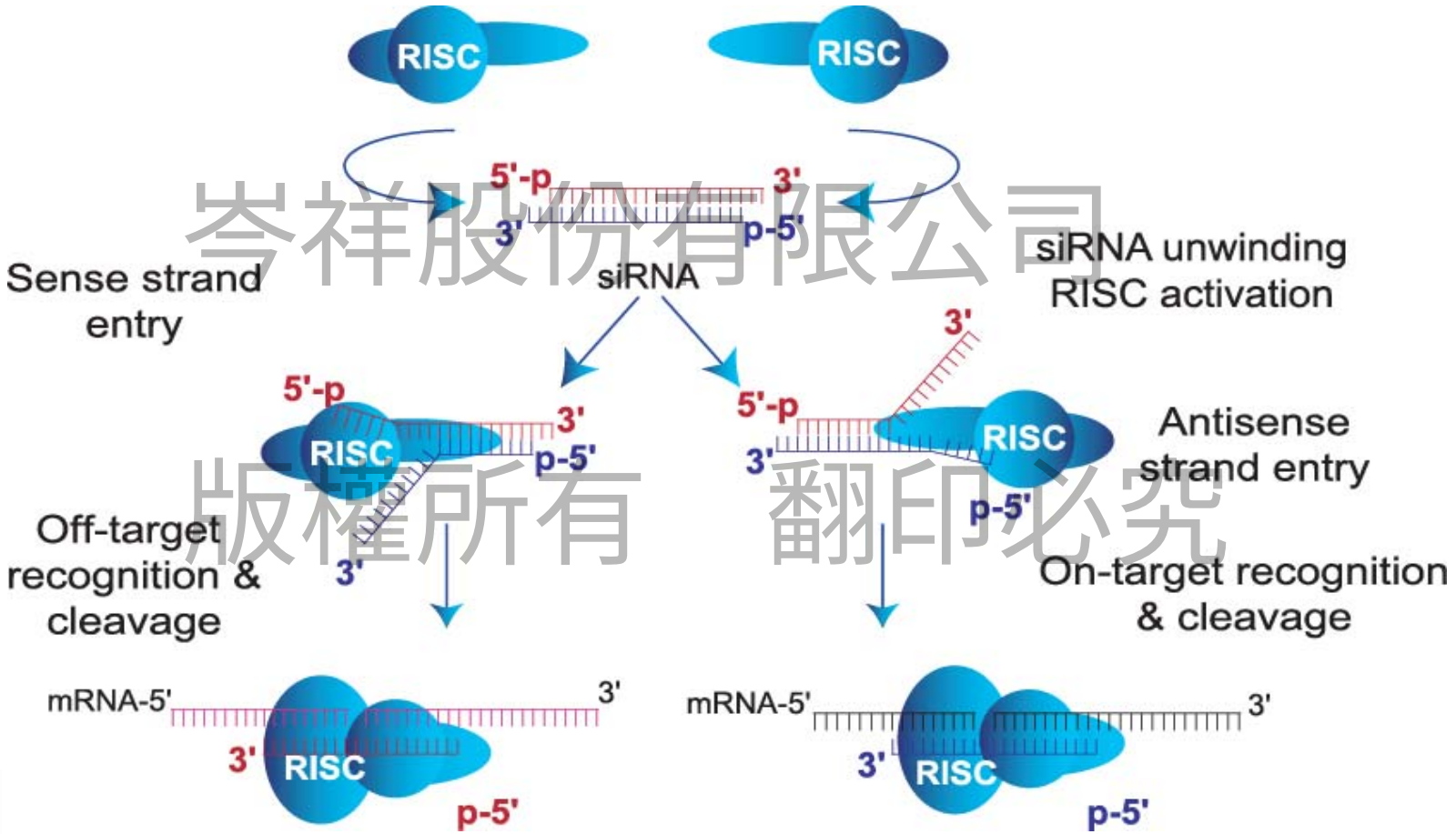




P-value = 2.82×10^{-16} <<<0.05
(using χ^2 test of independence)

RISC Associated Helicase Activity

Conventional siRNA RISC Process



Modification Applied to ON-TARGET*plus*™ siRNA Reagents

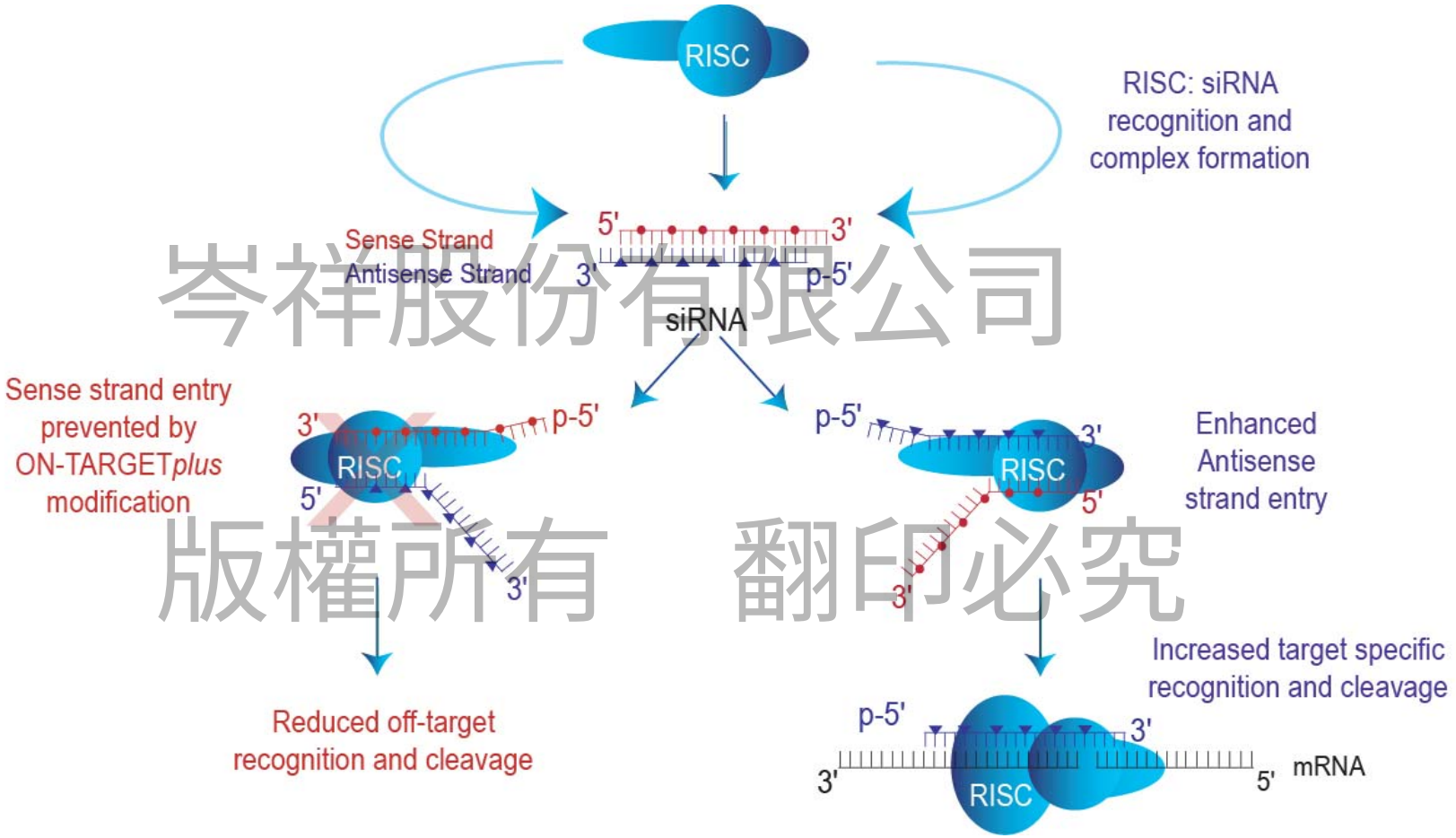
Chemical modifications to ***both*** the sense and antisense strands of the siRNA duplex:

- Sense strand is deterred from entering RISC
- Antisense strand requires more rigorous complementarity with target mRNA

patent pending

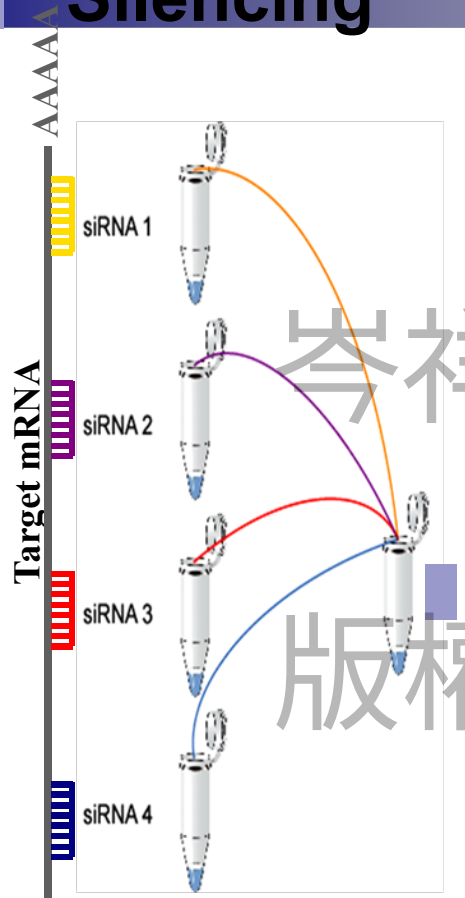
Jackson AL *et al.* (2006) RNA 12: 1197-1205.

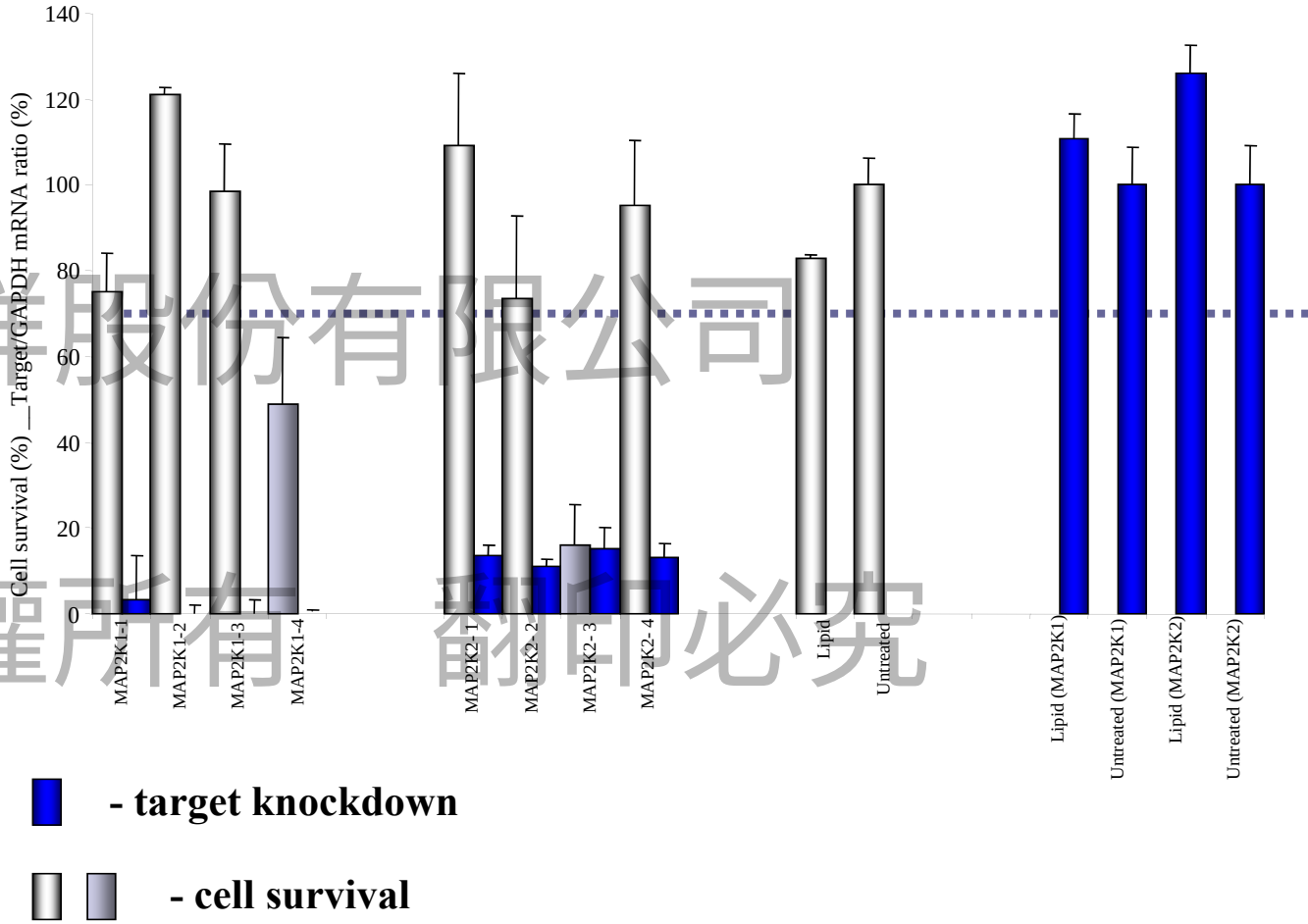
Chemical Modifications Eliminate Sense Strand-Mediated RNAi and Enhance Antisense Strand Specificity



patent pending

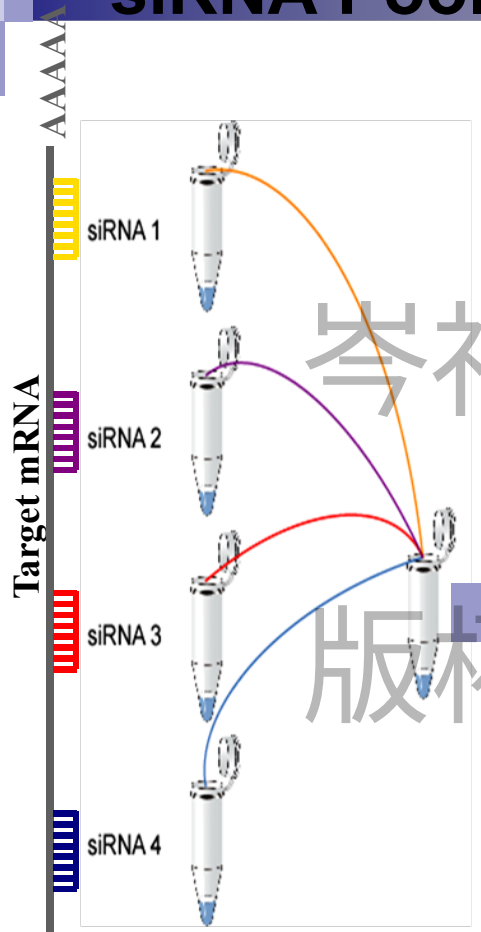
Off-target Phenotype is Independent of Target Silencing

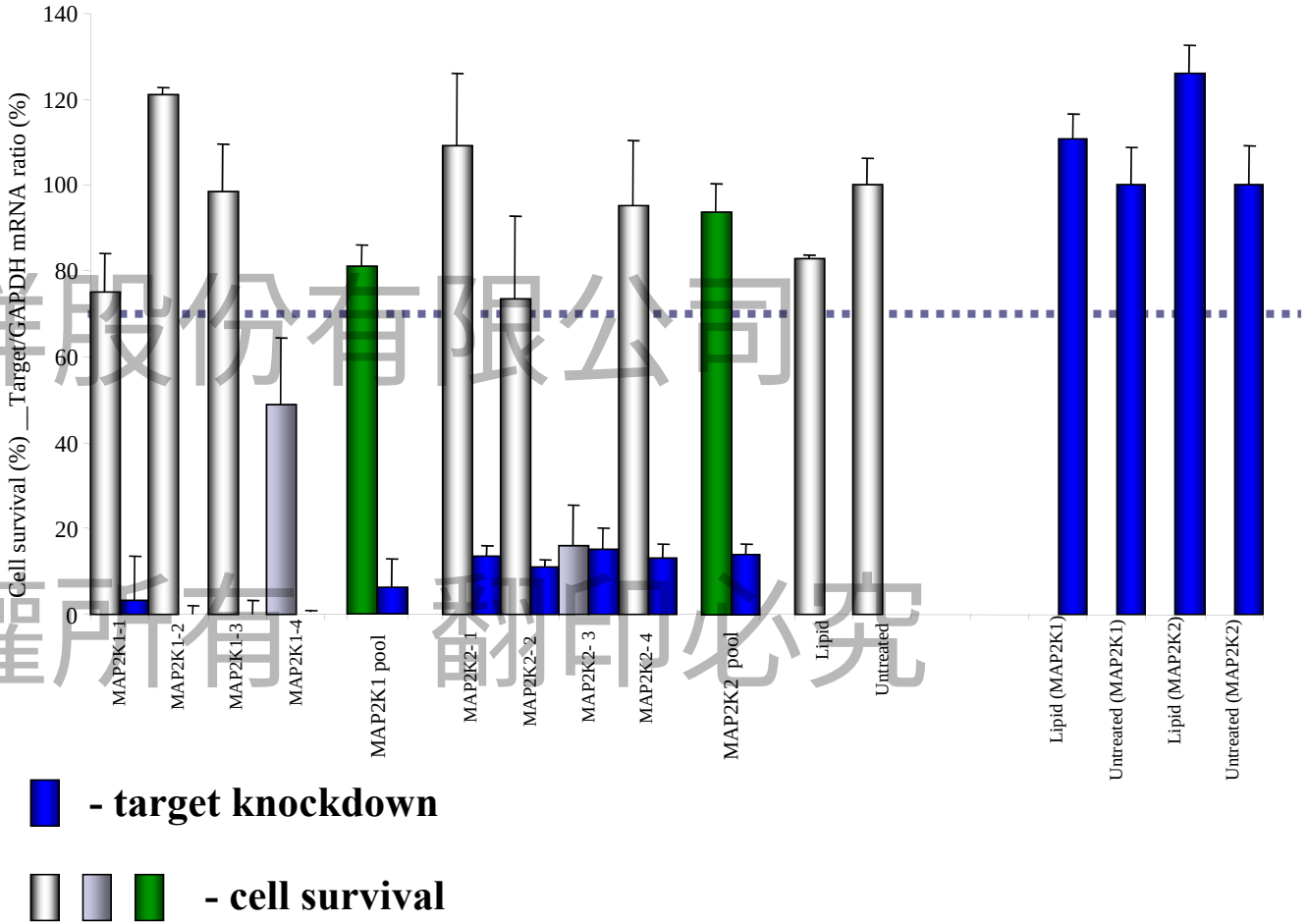




Adapted from Fedorov et al., (2006) *RNA*, 12:1188

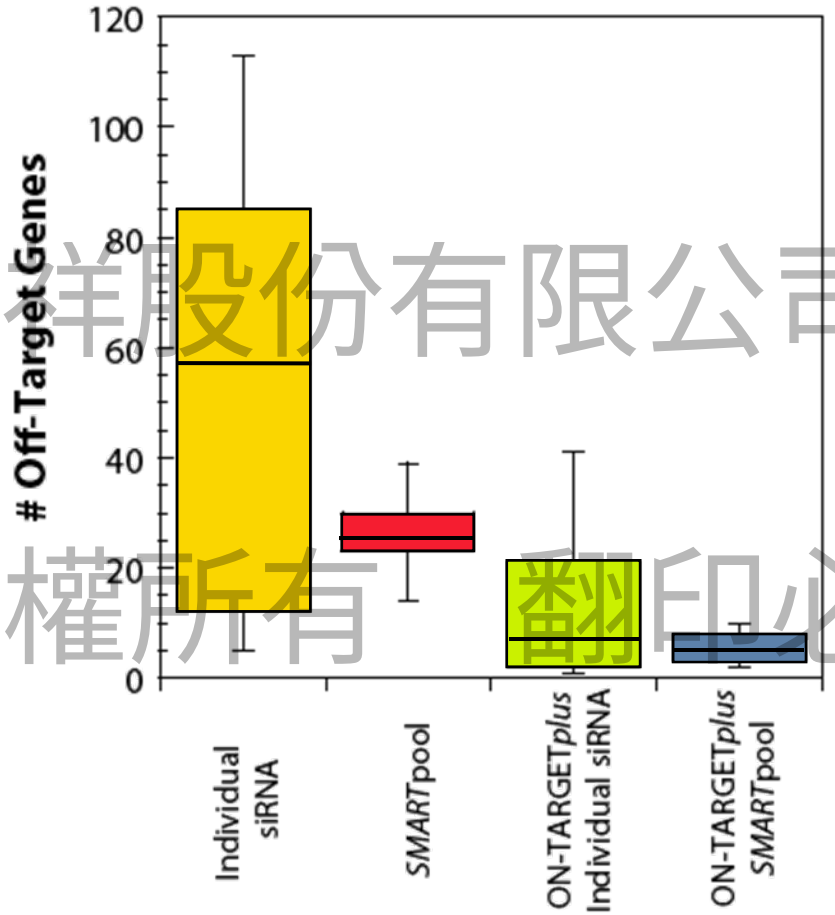
Off-target Phenotype is Eliminated in the Context of siRNA Pools





Adapted from Fedorov et al., (2006) *RNA*, 12:1188

ON-TARGET*plus*™ *SMART*pool® Reagents Provide Maximal Specificity

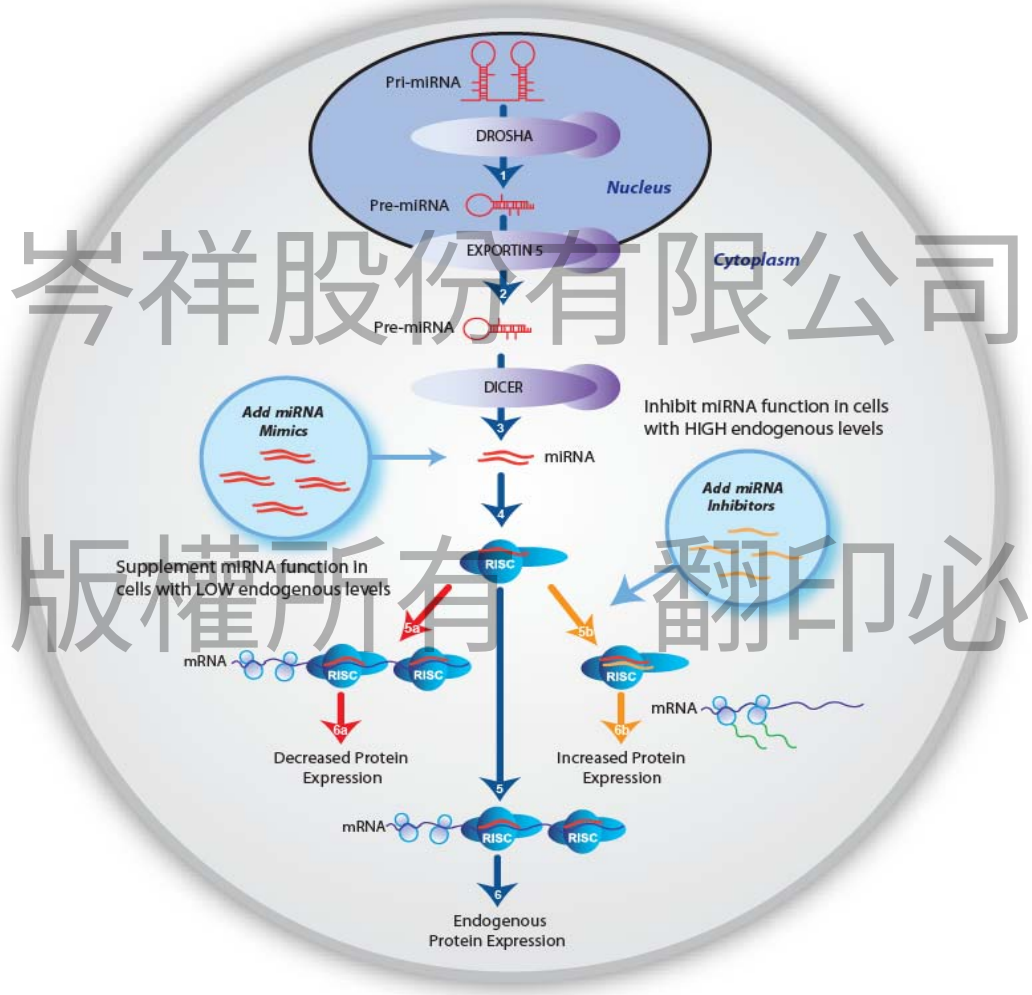


Collaboration with Agilent Technologies

How to study miRNA?

Functional Assay

miRIDIAN™ MicroRNA Mimics and Inhibitors: What Are The Biological Outcomes?



Product Details: miRIDIAN microRNA Mimic

- Double-stranded synthetic RNA oligonucleotide
  - Intended to mimic function of endogenous miRNA , chemically modified
- Chemically modified to:
  - Enter miRNA pathway with active strand
  - Exclude passenger strand from loading
  - Minimize interferon response
  - Improve target binding specificity & efficiency
- miRIDIAN Mimic designs now updated to Sanger miRBase 13.1

Applications of miRIDIAN microRNA Mimics

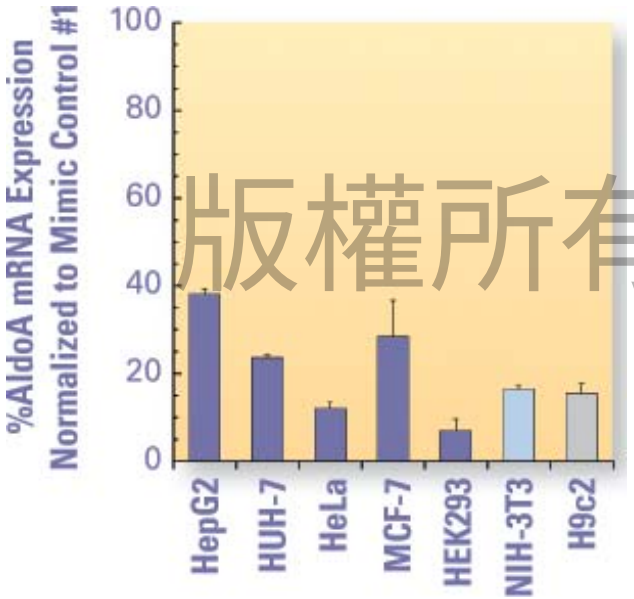
- Supplement miRNA activity to study gain-of-function effects
- Screen for miRNAs that regulate gene expression and affect cellular pathways
- Elucidate miRNA involvement in normal biological and disease pathways
- Identify and validate miRNA targets

miRIDIAN microRNA Mimic Positive Controls

Endogenous miRNA positive control (幾乎多數細胞都有表現的內源性miRNA)

miR-122 ⟷ Aldolase A

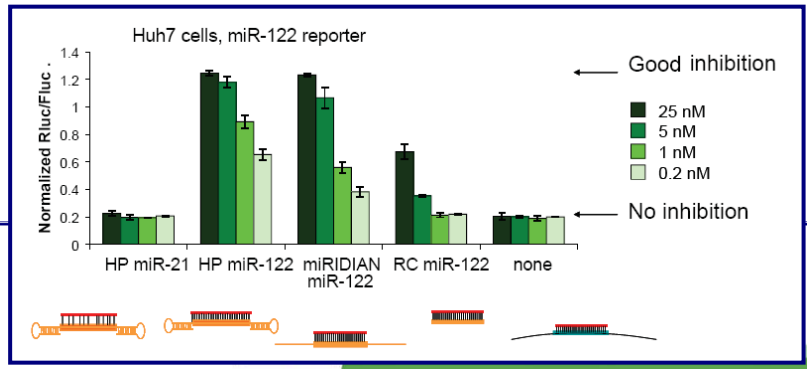
- Validated miRIDIAN microRNA Mimic that targets Aldolase A in human, mouse, and rat
- Provides the ability to optimize assay conditions by monitoring mimic function on an endogenous gene target (Aldolase A) with a conserved miR-122 binding site



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 at 3 days (HepG2 at 5 days) post-transfection.

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

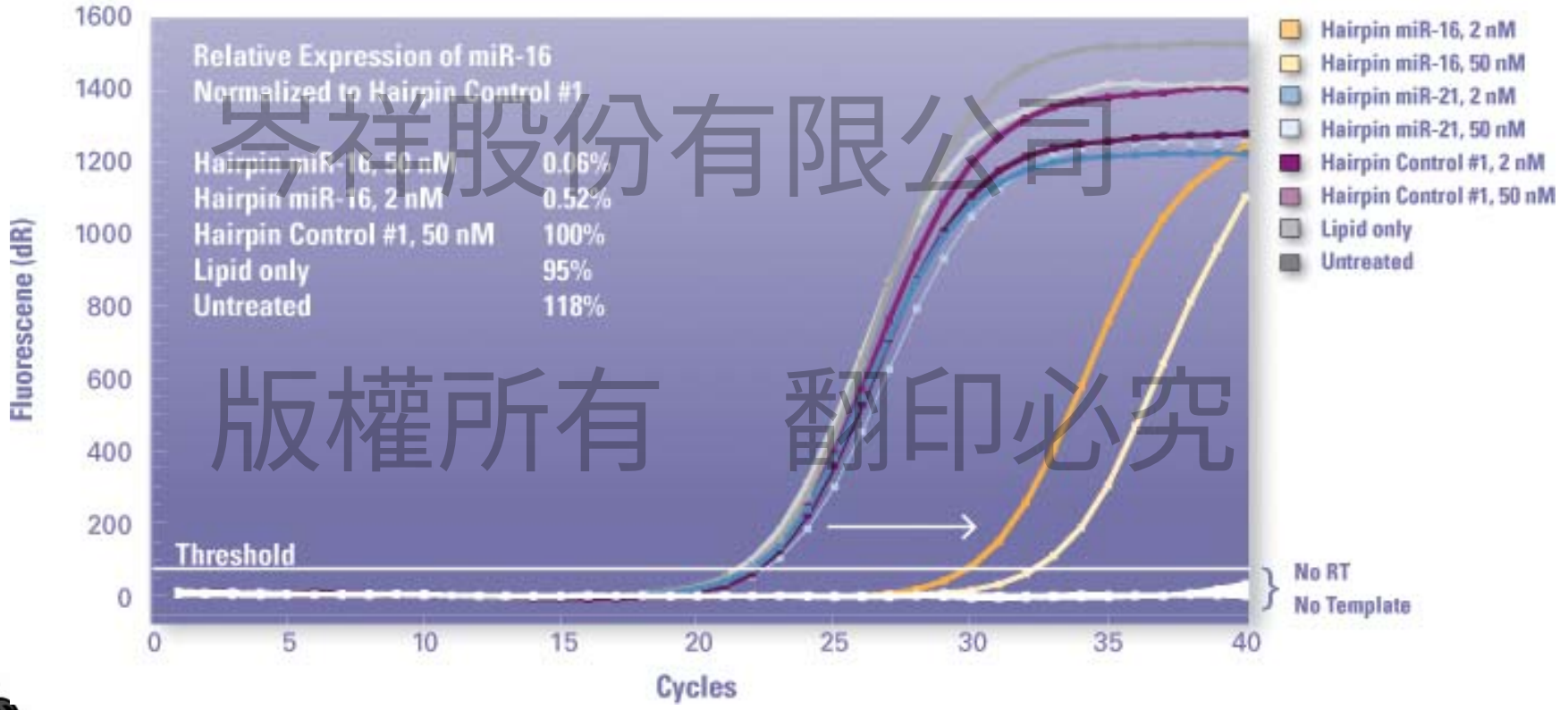


Applications of miRIDIAN microRNA HP Inhibitors

- 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 Positive Controls

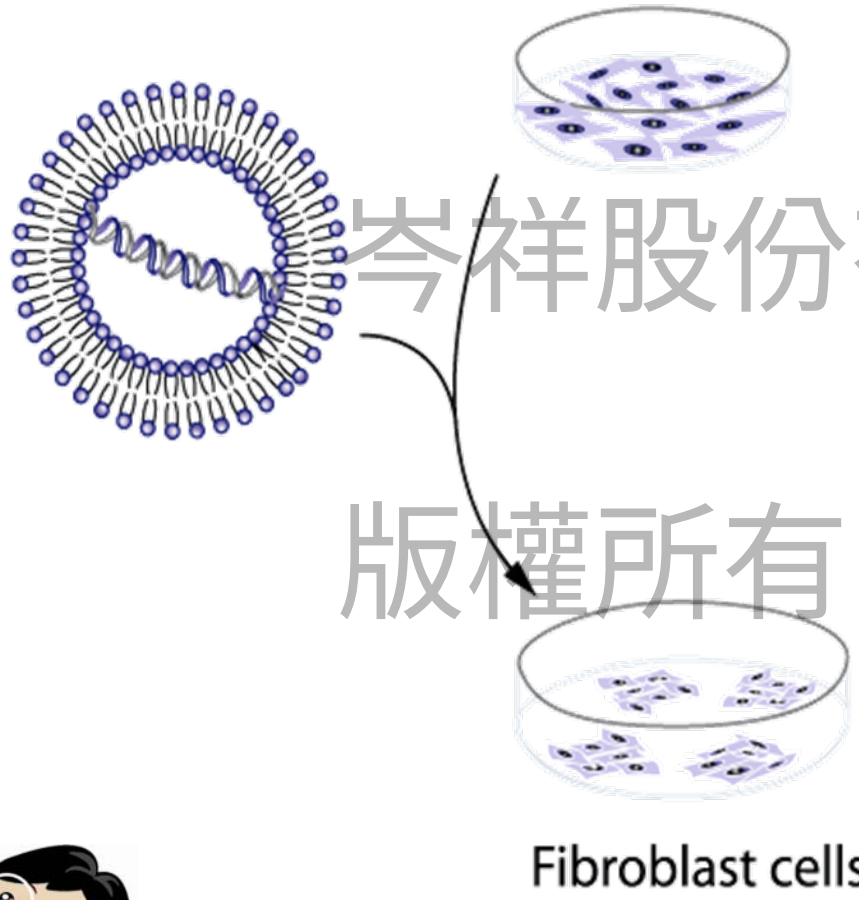
Targets miR-16 in human, mouse, and rat cells resulting in reduced miR-16 activity

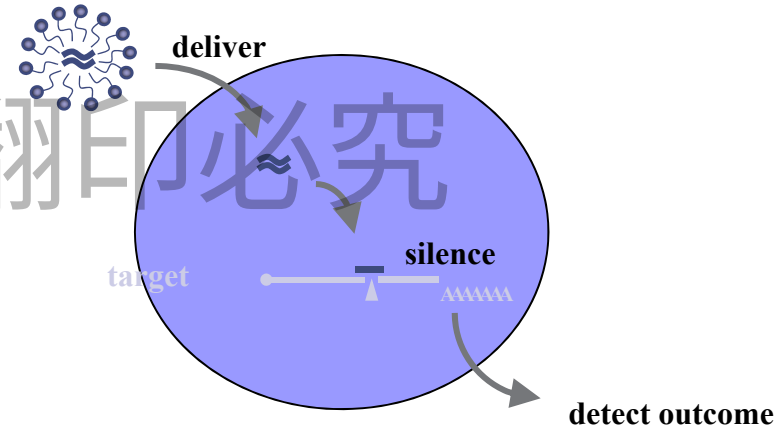


Delivery of siRNA or miRNA & experimental tips

Transfection: Definition

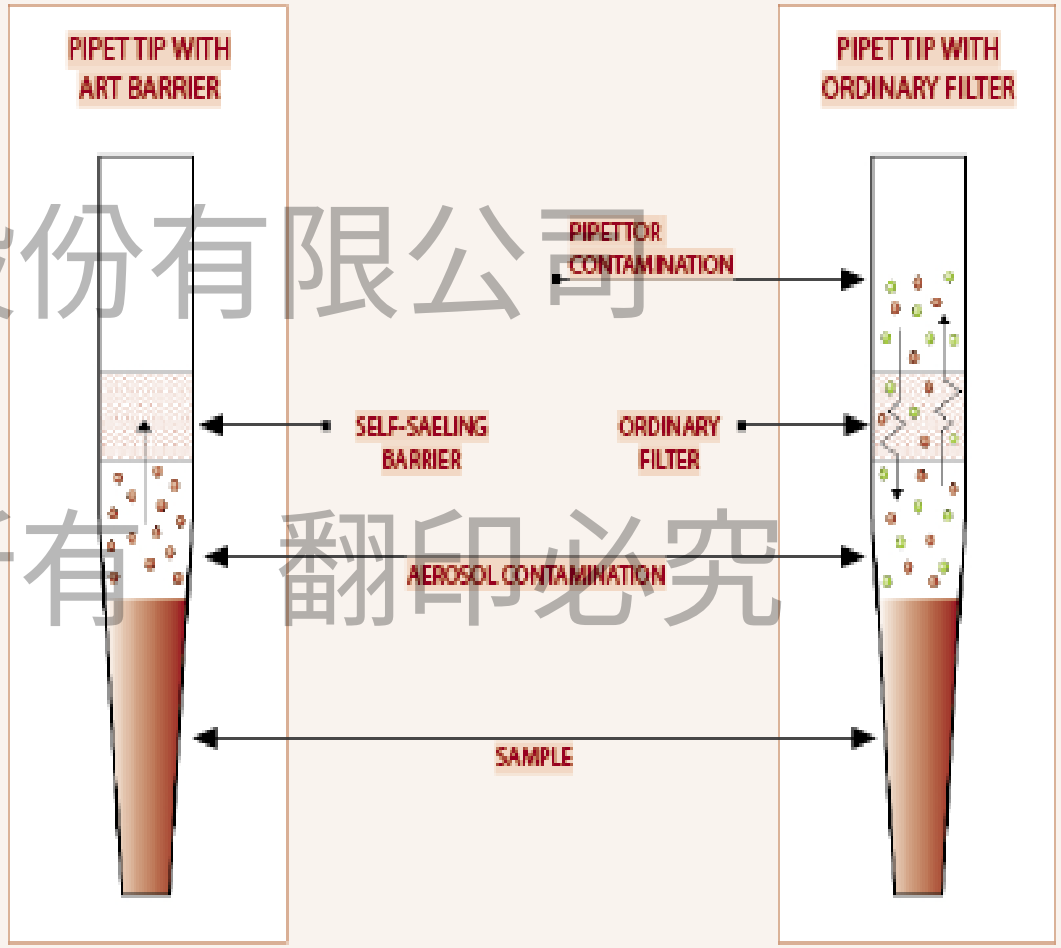
A method for delivering DNA or RNA into mammalian cells, using a complexing reagent such as cationic lipids.





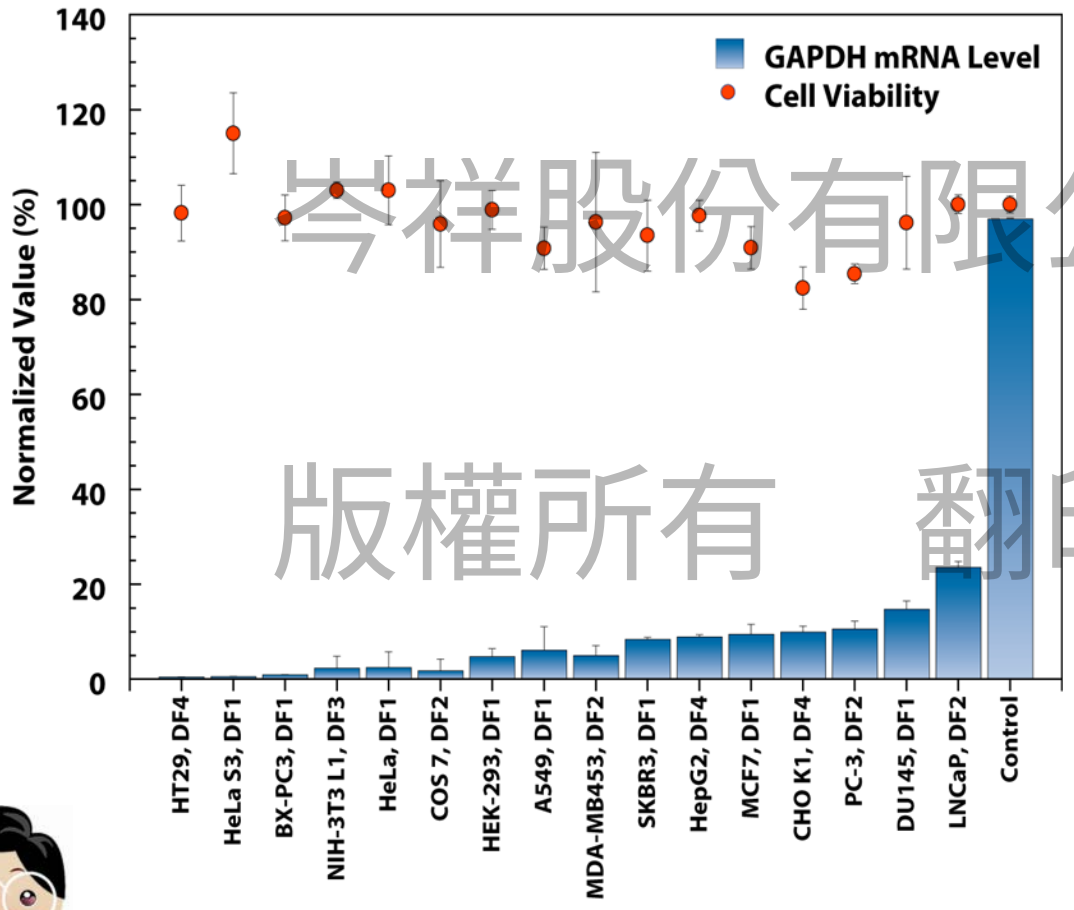
SMART tip : barrier tip ≠ filter tip

This diagram shows the difference between ART self-sealing barrier tips and ordinary filter tips. ART barriers prevent cross-contamination while contaminants pass freely through ordinary filters.

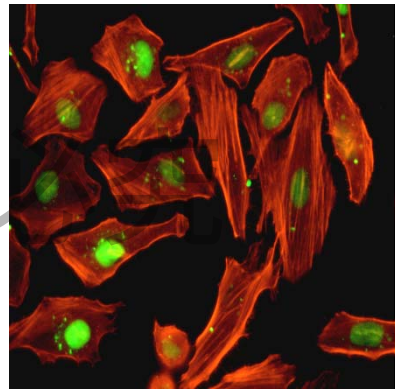


Transfection and Viability

Efficient siRNA Delivery with Minimal Effect on Cell Viability



- Broad spectrum of cell types
- Efficient delivery
- Low toxicity
- Robust silencing

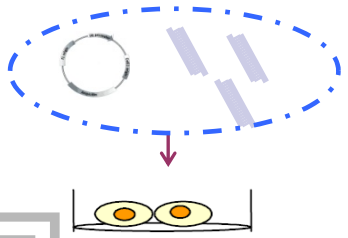


si*GLO* Green

Applications of gene profiling

RNAi in Protein screening

Experimental design of Cytokine Protein Arrays



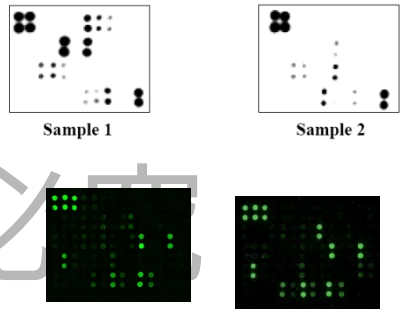
Cells treated with drugs/siRNA/miRNA of gene-of-interest or not

↓

Collect supernatant or cell lysate

↓

Profile protein expression by Protein Array



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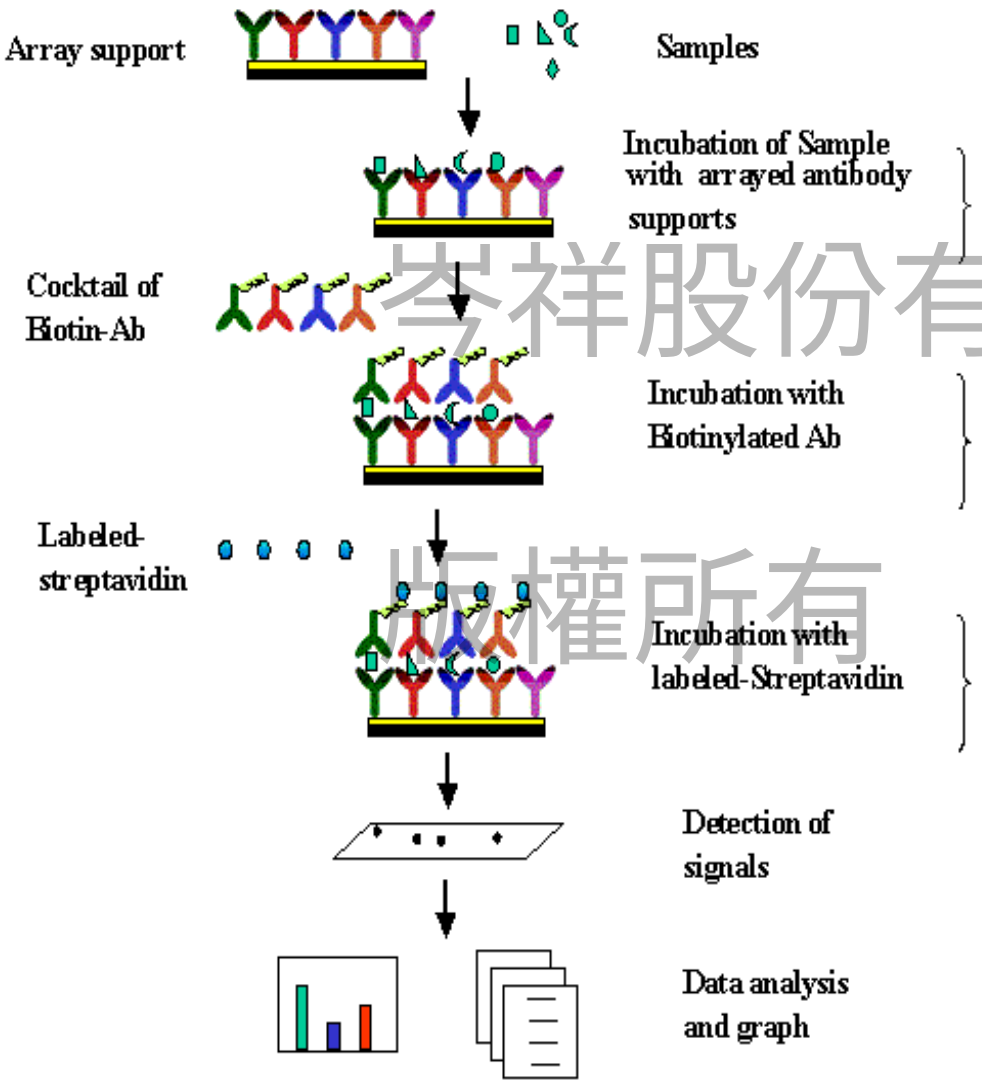
Data analysis

↓

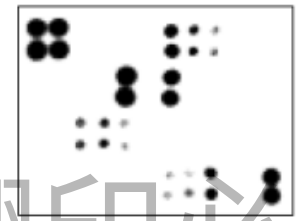
Discover pathway-of-interest or biomarker

How to profiling gene expression by RNAi?

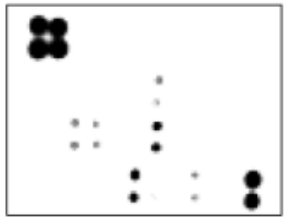


Antibody Array

- Semi-quantitative
- Quantitative



Sample 1



Sample 2

How to analyze?

Array membrane

↓

Experimental Procedure

↓

Visualization of signal

↓

Measurement of signal intensity

↓

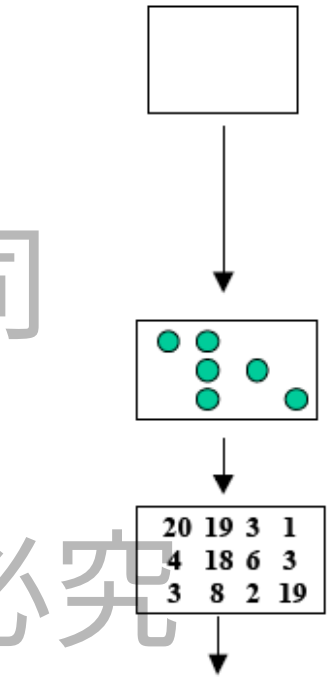
Importing data to RayBio® Antibody array Analysis tool

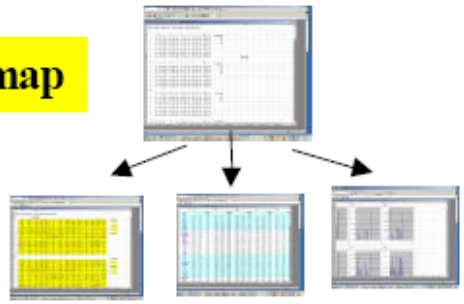
↓

Aligning data into RayBio® Antibody array map

↓

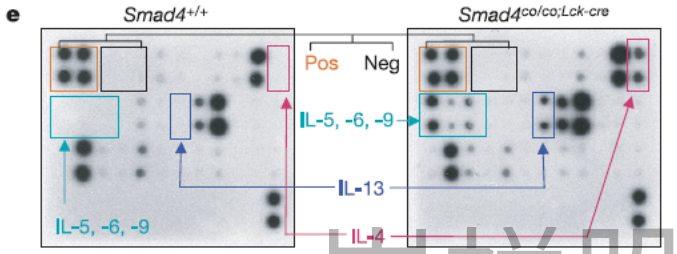
Obtain result

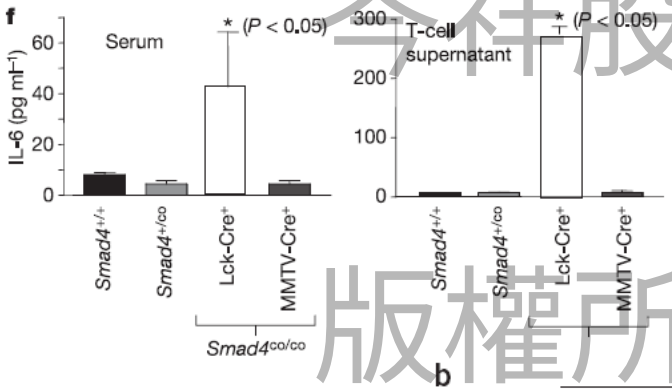




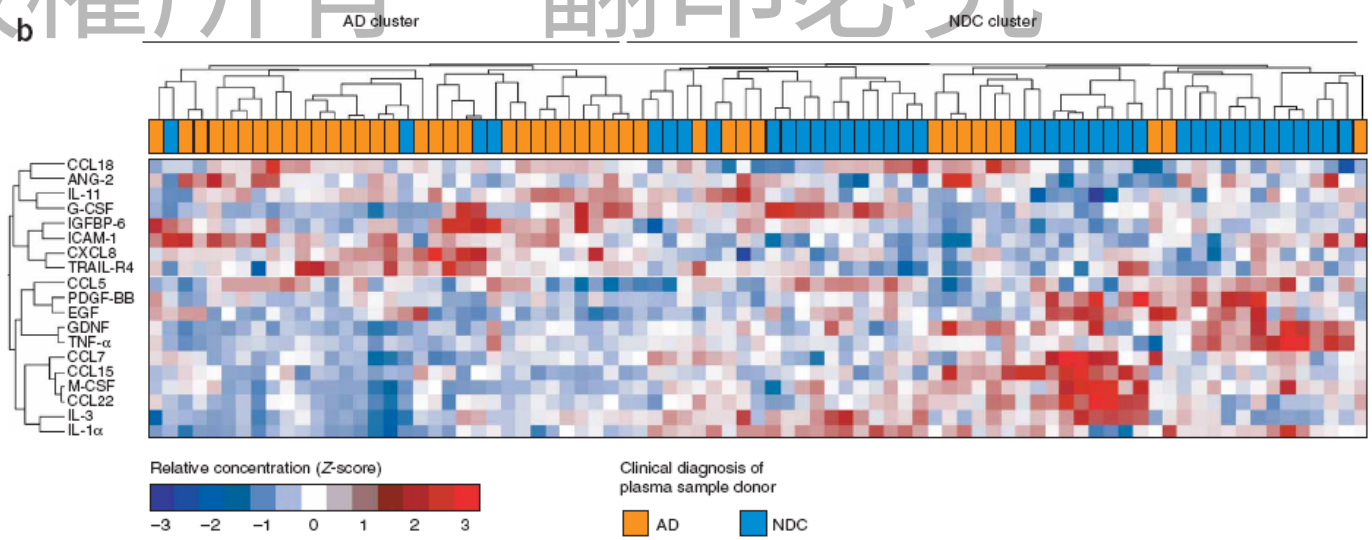
Data presentation

Nature Vol 441|22 June 2006

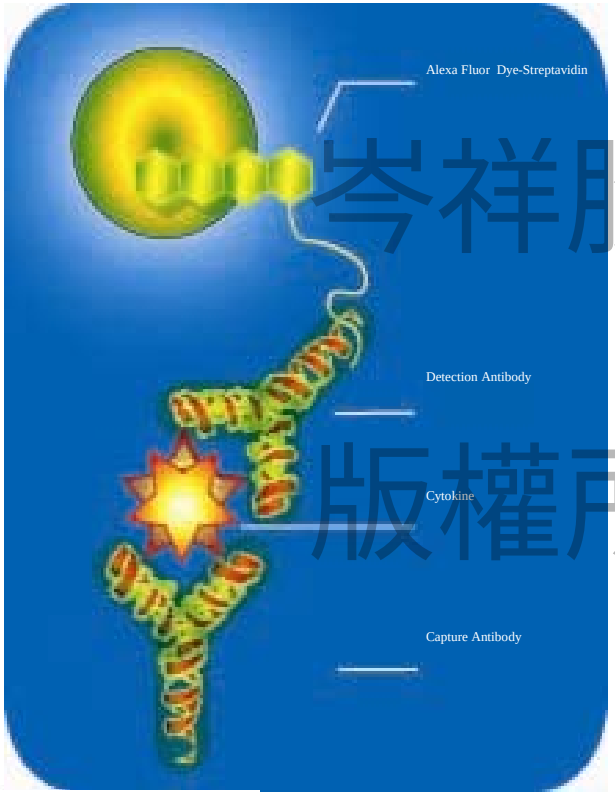




Nature Medicine,Oct 2007

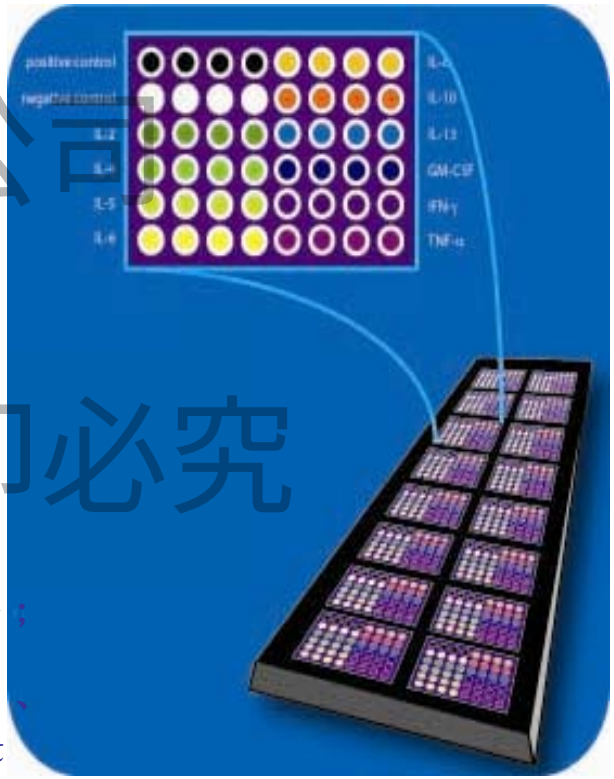


Quantibody Array – Multiple ELISA platform



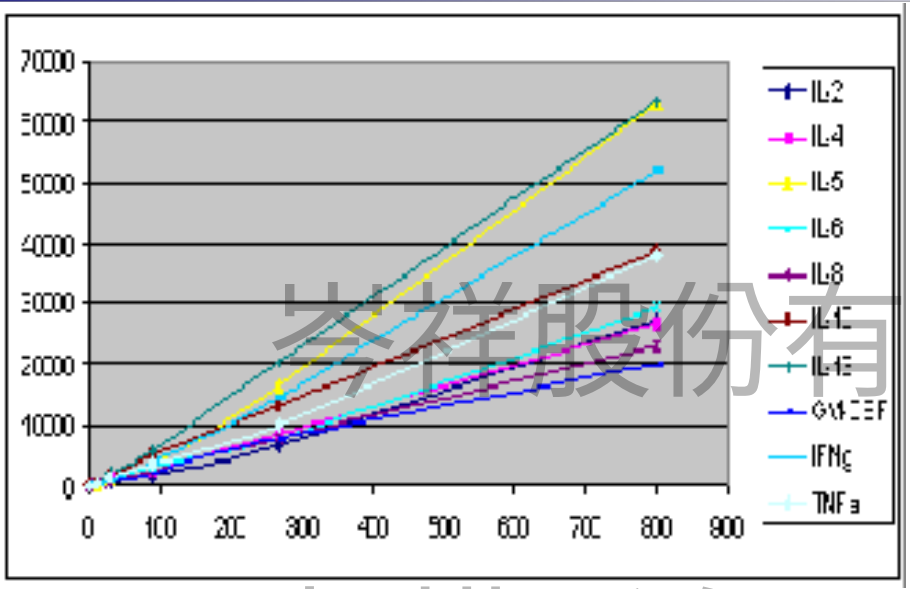
Sandwich-based ELISA

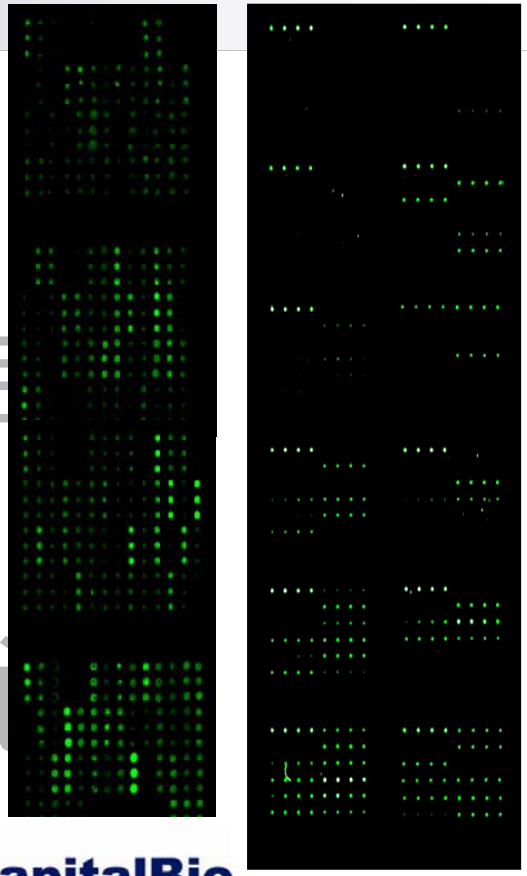
Quantibody Array使用三明治法ELISA原理：利用固定於玻片載體上的專一 capture antibodies，依序加入待測檢體、detection antibodies、和螢光標定物，最後以Laser Scanner的cy3波長判讀分析。



10~40 cytokines for one sample

一張slide上有16個sub-array；每個sub-array上有10到20個cytokine以及positive control、negative control。每個target都四重複，確保最佳實驗再現性。





Format: Standard glass slide with 16 removable wells
Sample Volume: 50-100 ul
Detection Range: 0.5-3000 pg/ml
Standard Curve Range: 5-800 pg/ml
Signal Duration: 6 hours
Reproducibility: <20% well-to-well CV
Detection Method: Fluorescence. (Cy3)

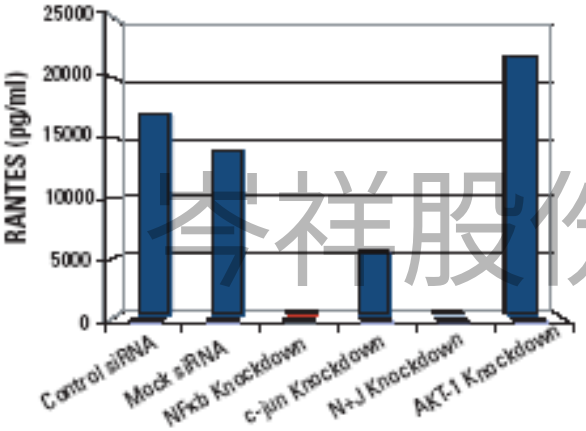
CapitalBio

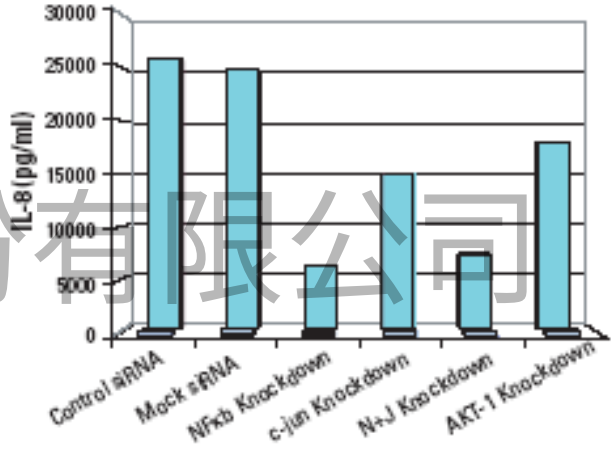
Microarray Scanner

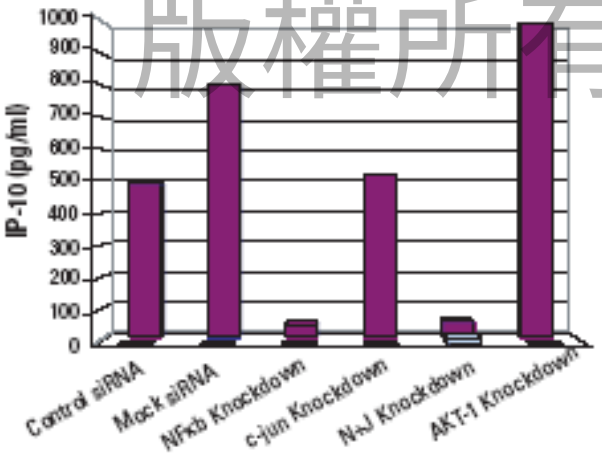
LuxScan™-10K

產品特點

Data Performance

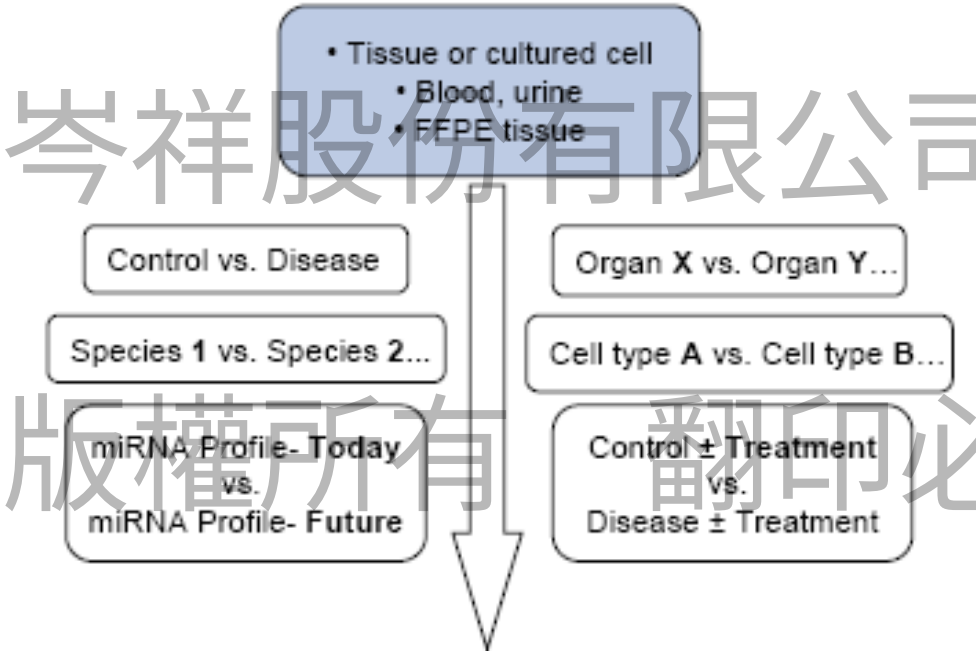




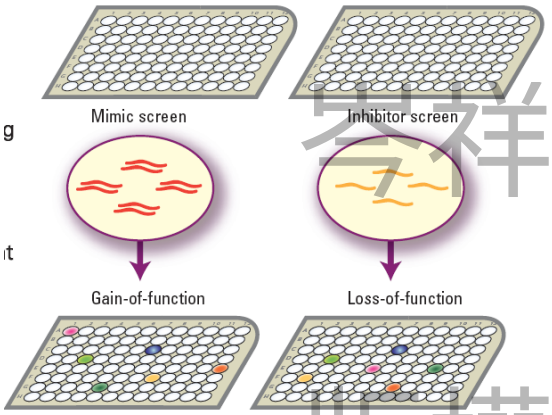


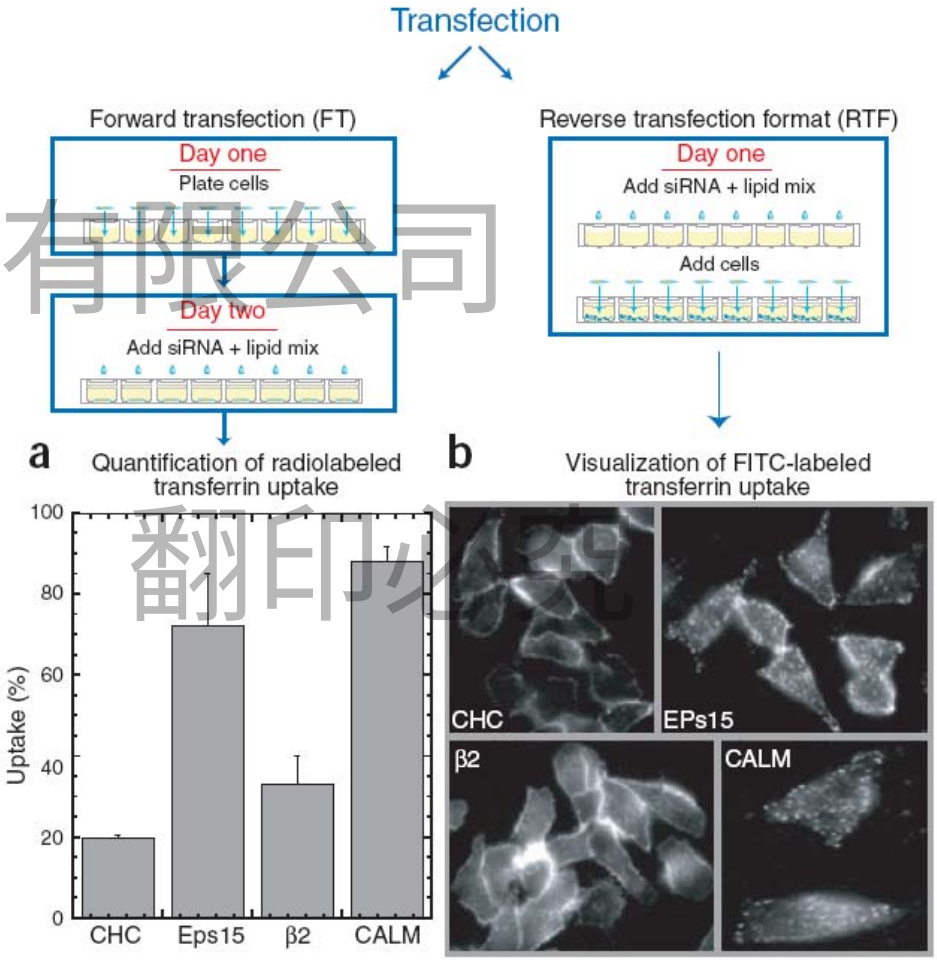
A549 cell expression levels of IL-8, IP-10 and RANTES are altered by TNFα stimulation and siRNA transfection.

Reverse phase: Profiling gene function by RNAi Library



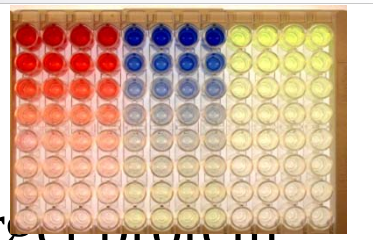
MicroRNA expression profiling to biomarker discovery



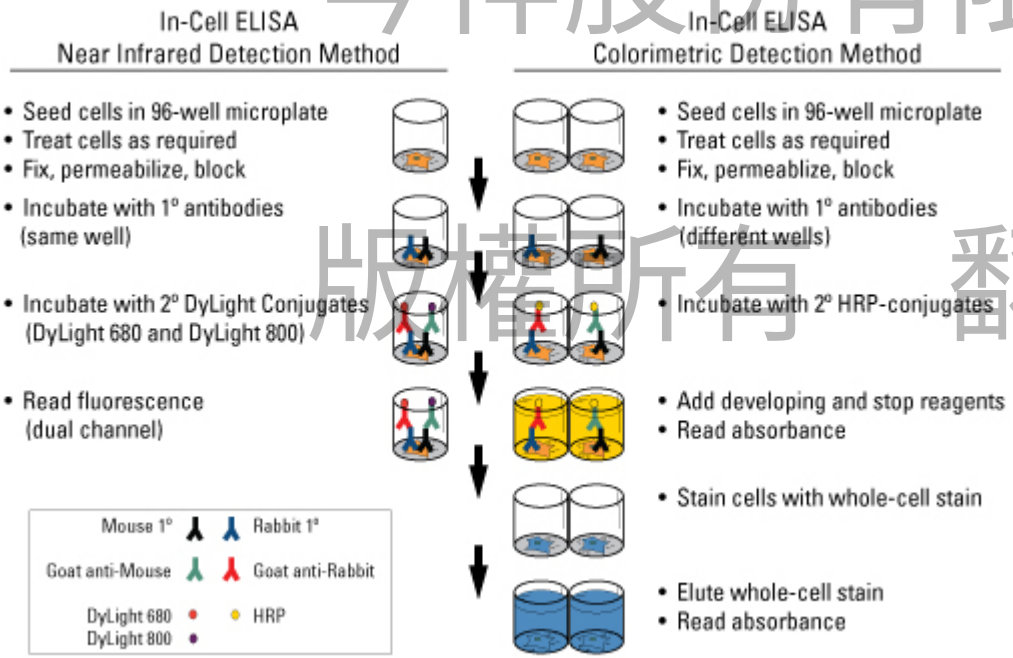


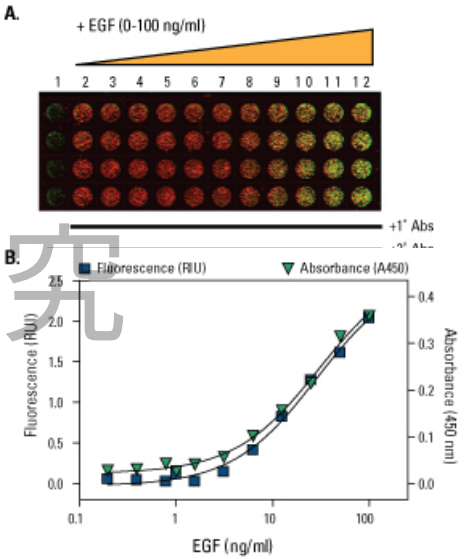
Detect phenotypes to get hints

- ELISA assay for downstream protein expression



Collect supernatant for determination of specific target protein
Fix cell lysate on 96-well plate for In-Cell-ELISA

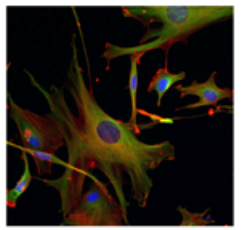




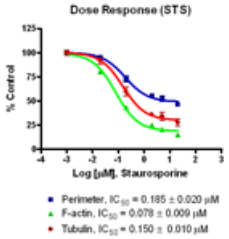
Detect phenotypes to get hints (continued)

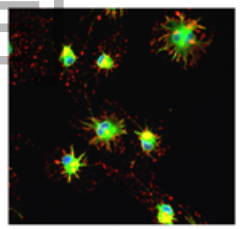
- Staining of Specific Marker as phenotype (IHC/IF)

  -- cell survival or proliferation
  -- cell morphology
  -- cytotoxicity or apoptosis
  -- cell migration
  -- cell stress or DNA damage

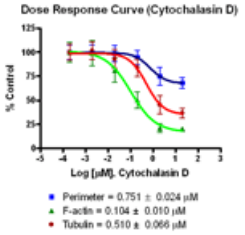


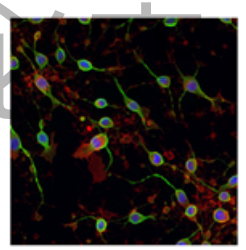
Non-treated



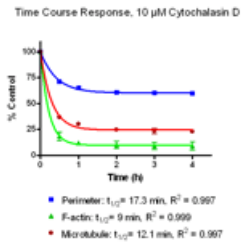


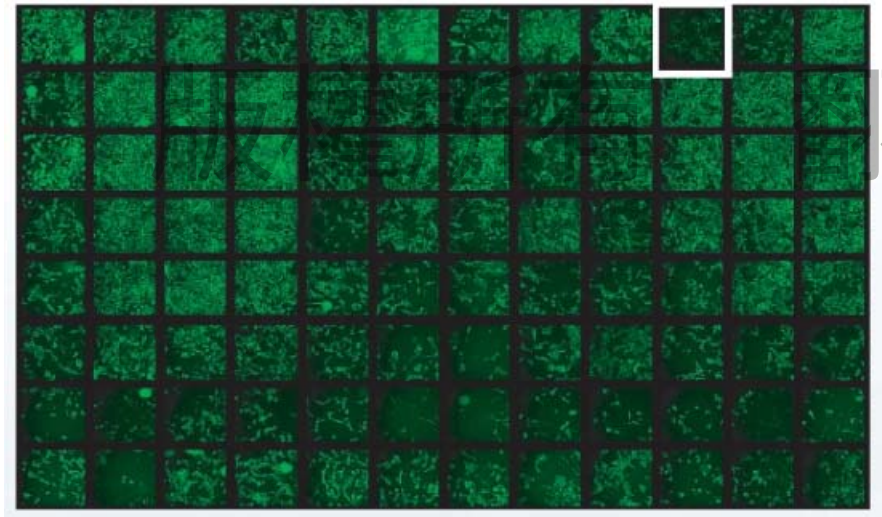
Cytochalasin D





Staurosporine





岑祥股份有限公司

Thank you for attention!