System Biosciences

- Lentivirus technologies
- RNAi Libraries
- MicroRNA Tools
- Stem Cell Research

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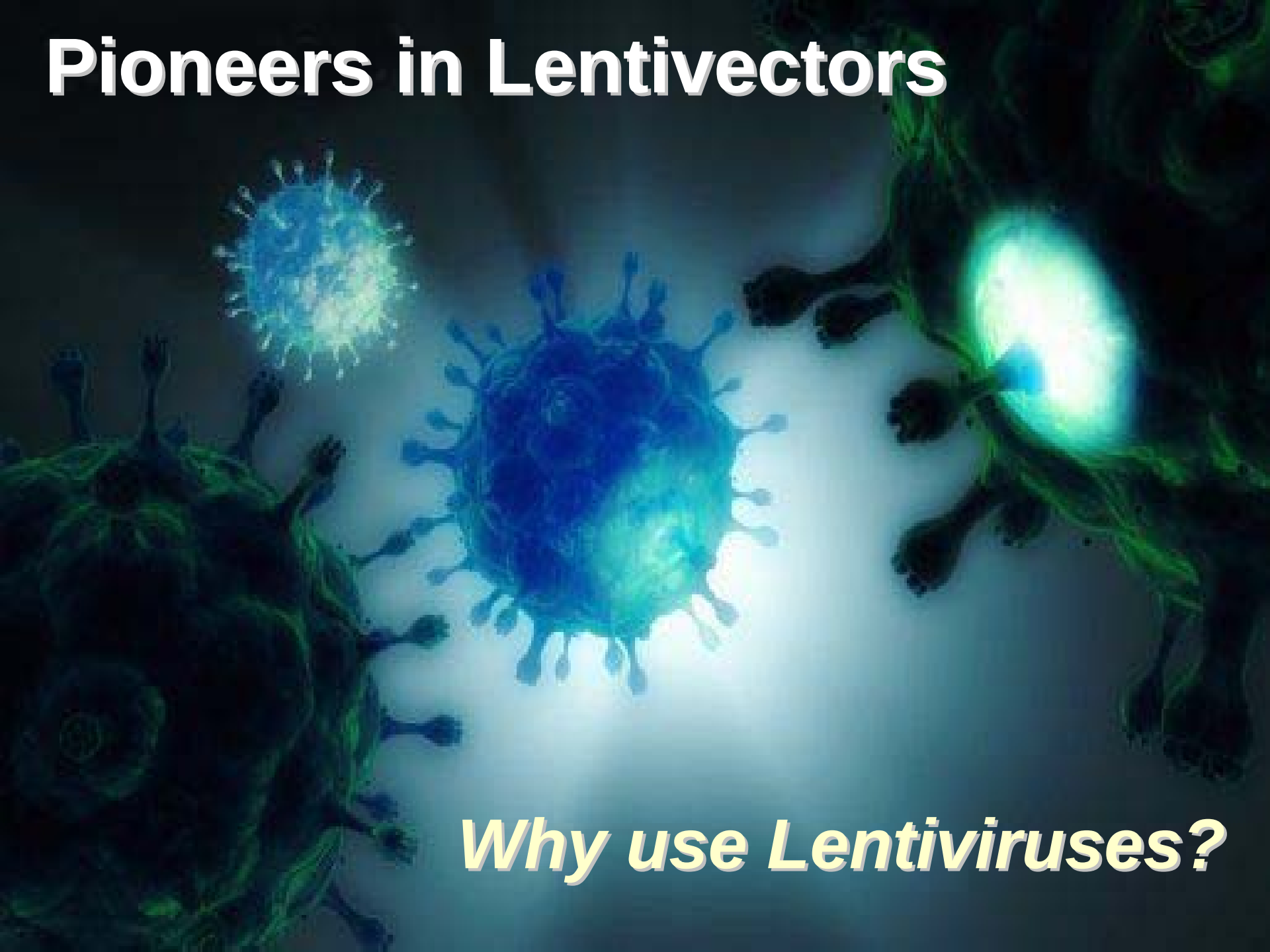
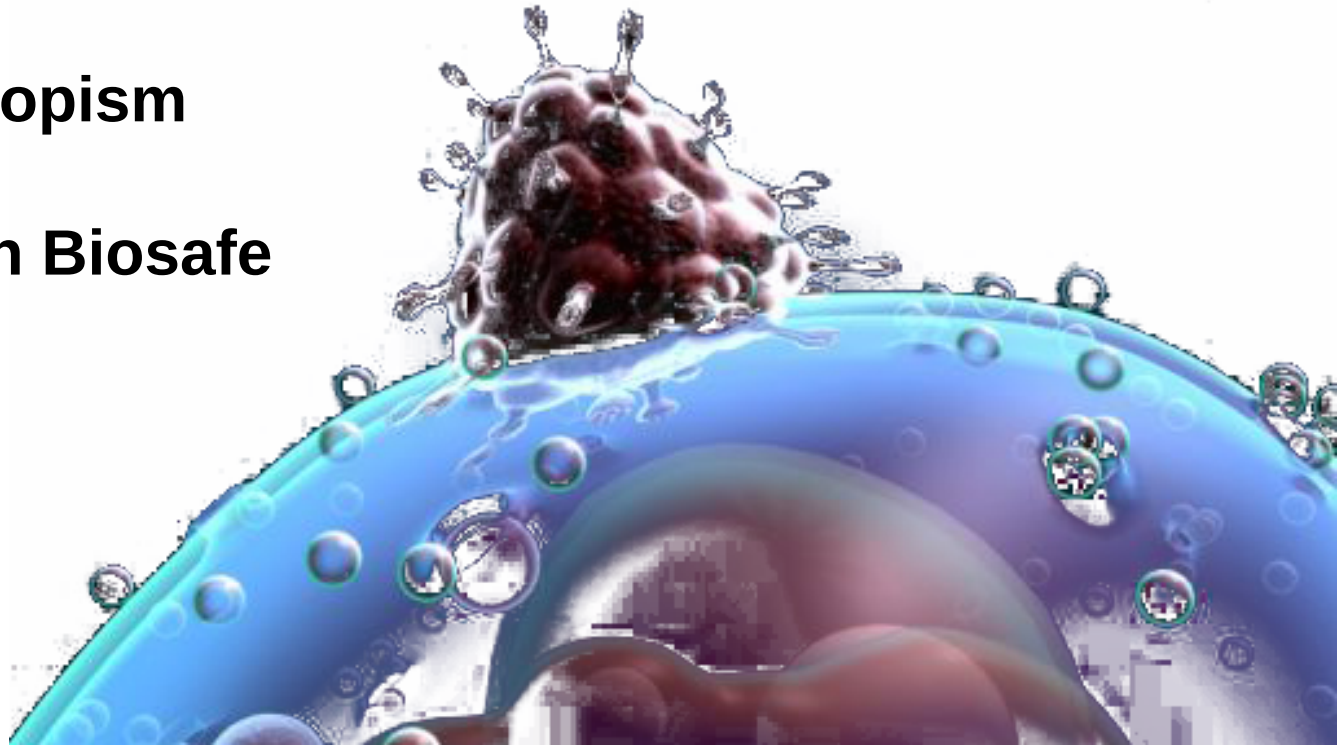
Pioneers in Lentivectors



Why use Lentiviruses?

Lentiviruses Get In

- **Dividing or Non-Dividing Cells (Retroviruses only infect dividing cells)**
- **Useful for slowly dividing Primary Cells**
- **Infect Embryonic Stem Cells and Embryoid Bodies**
- **Broad cellular tropism**
- **Third Generation Biosafe**



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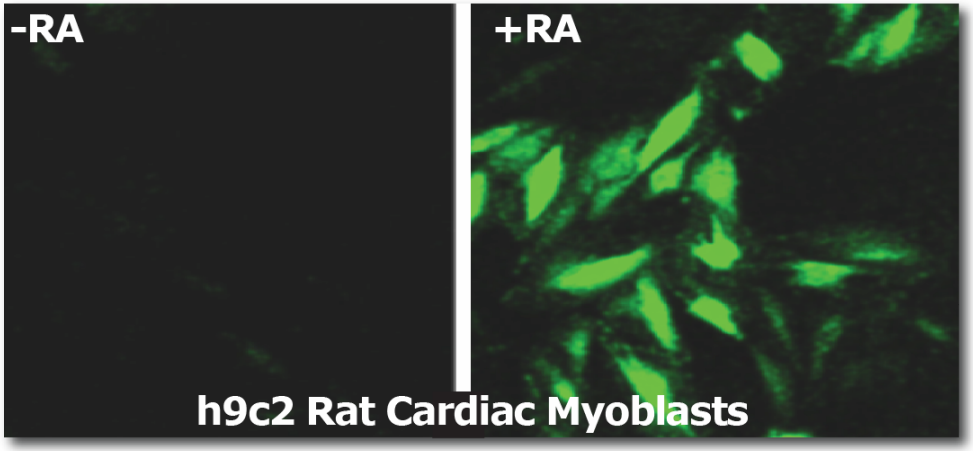
Lentiviruses Stay In

- Stable Integration of Constructs into Host Chromosome
- Good for Reporters, Knockdown & Overexpression
- Easily create Stable cell lines

Mouse Troponin Reporter
Differentiation with Retinoic Acid



Data courtesy of TJ Bartosh and R. Roque - Touro University Nevada.

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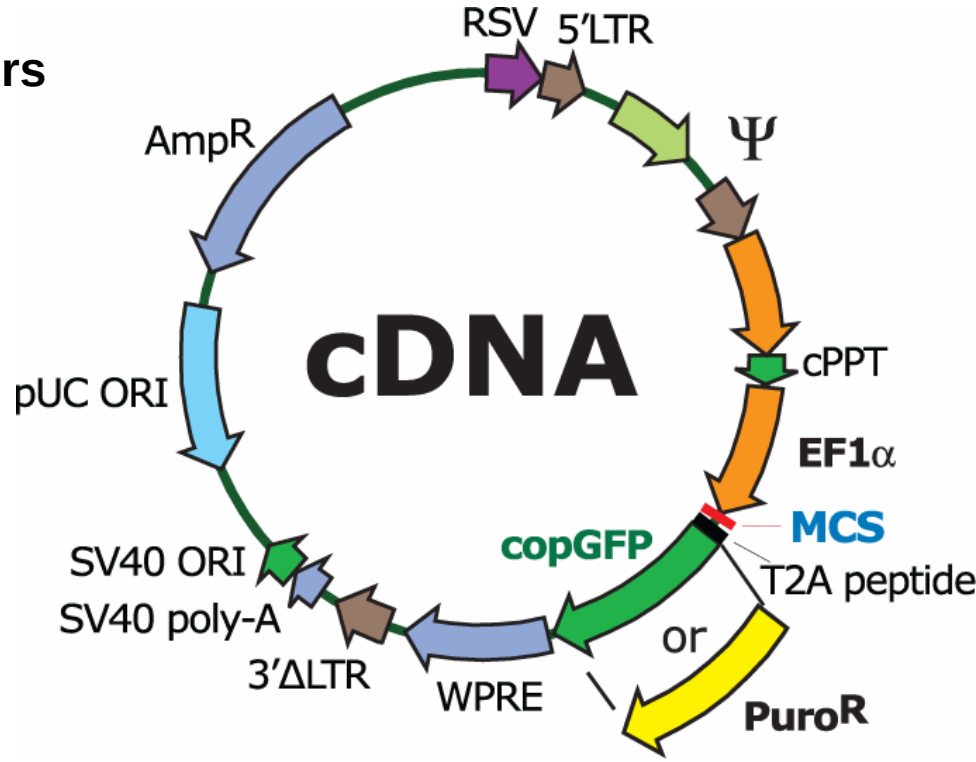
Pioneers in Lentivectors

Stably express cDNAs

Strong and ubiquitous expression of the gene of interest

- **Single or double expression cassette with choice of reporter gene**
- **Target gene expressed from CMV, EF1, or MSCV promoter**
- **Choose from FIV- or HIV-based vectors**



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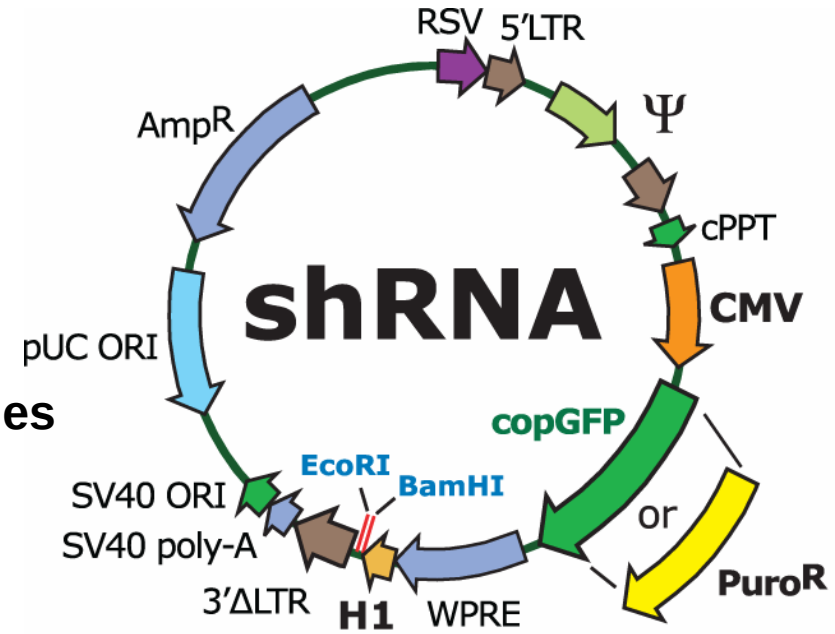
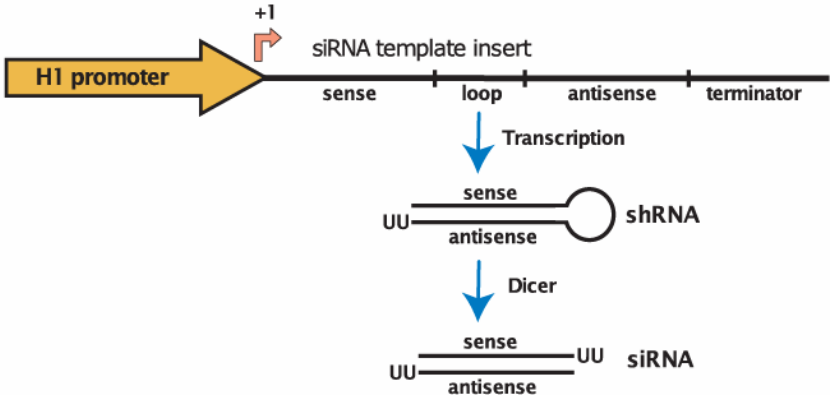
Pioneers in Lentivectors

Stably express shRNAs

Permanent, heritable gene knockdown

- **Efficient delivery and permanent Knock down**
- **Analyze the specific effects of Target genes**
- **Single or Double promoter formats**





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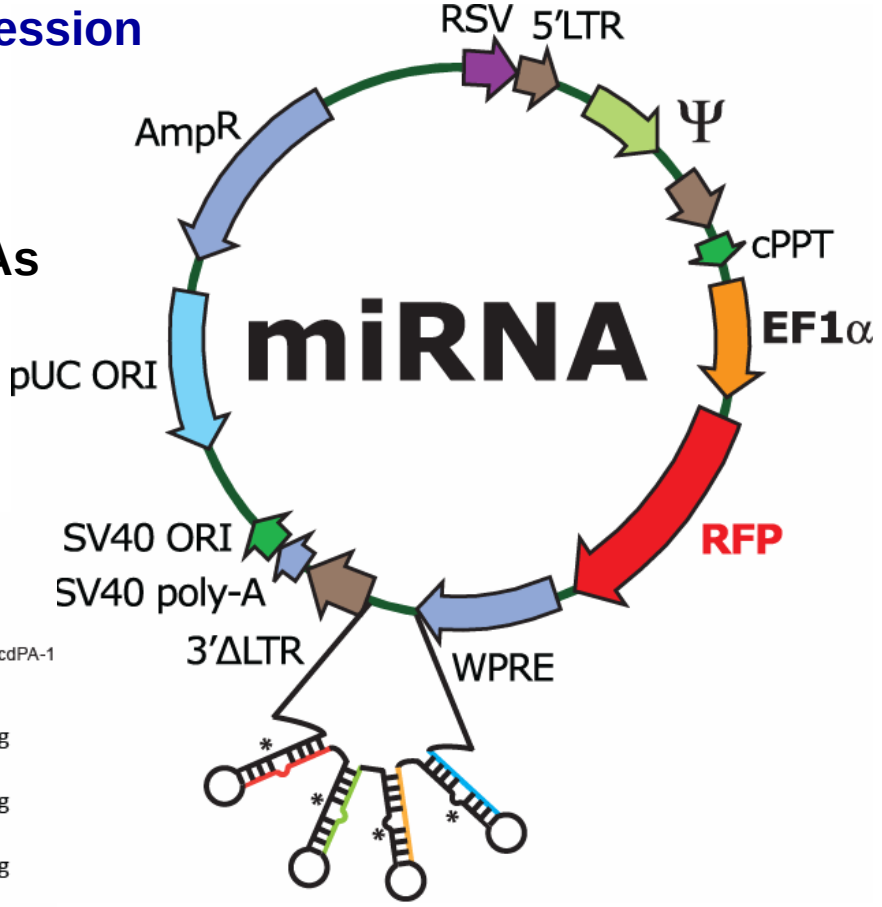
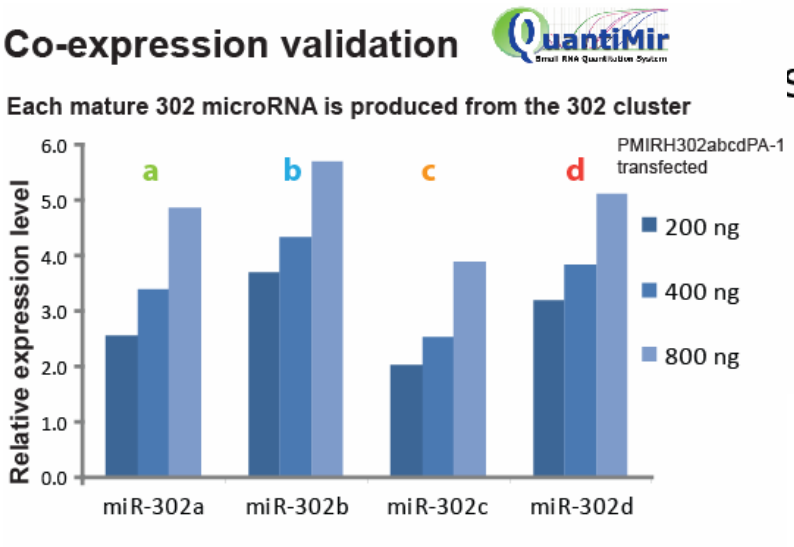
Pioneers in Lentivectors

Stably express microRNAs

Permanent, heritable microRNA overexpression

- **Efficient delivery and robust expression**
- **Analyze the specific effects of MicroRNAs**
- **Express single microRNA precursors or clusters**





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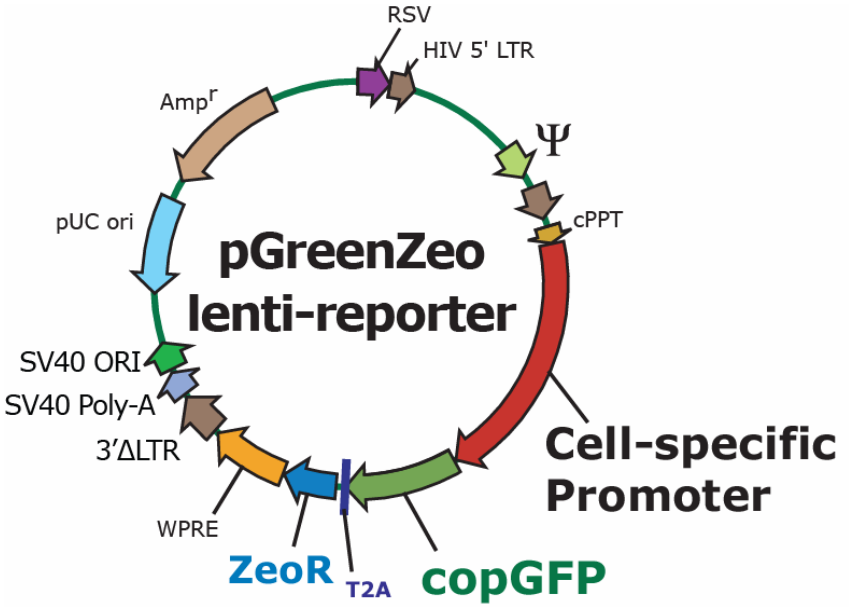
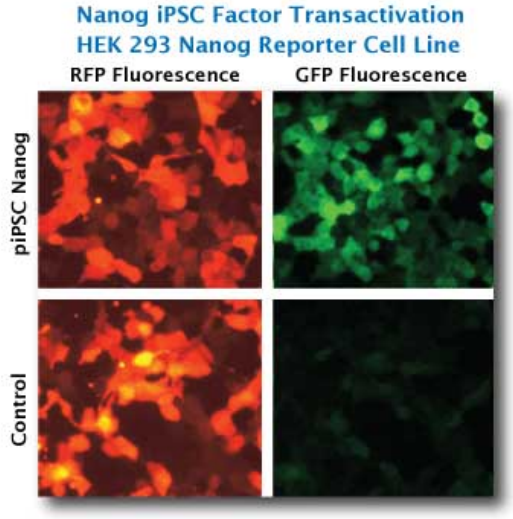
Pioneers in Lentivectors

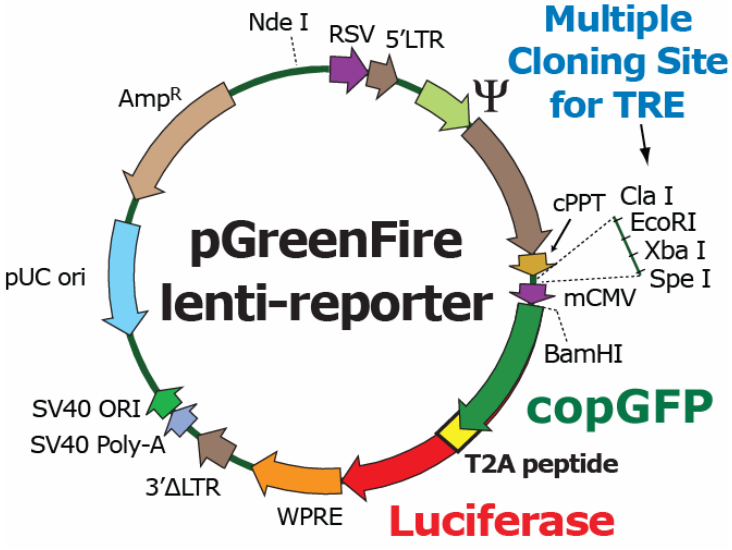
Efficiently create reporter cell lines

Sort for GFP/RFP or Zeo/Puro Selection

- **Report transcription network activity**
- **Track cell differentiation**
- **Quantify transcription response**







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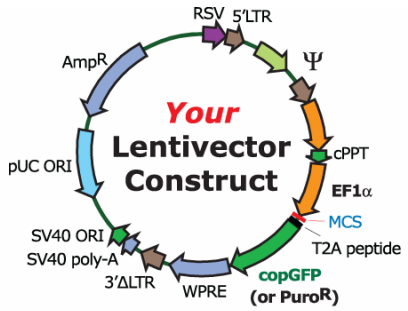
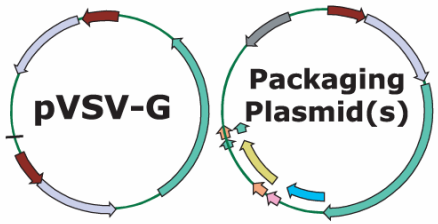
High-titer Virus Production

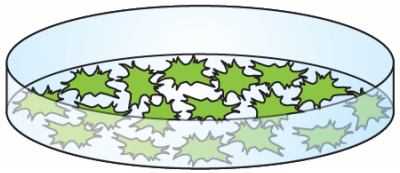


+

pPACK™ Packaging Mix

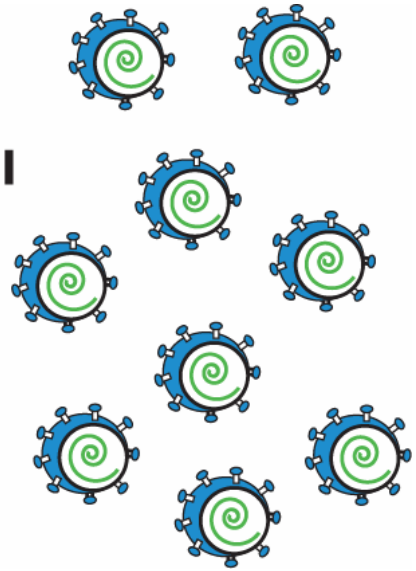


293TN Producer Cells

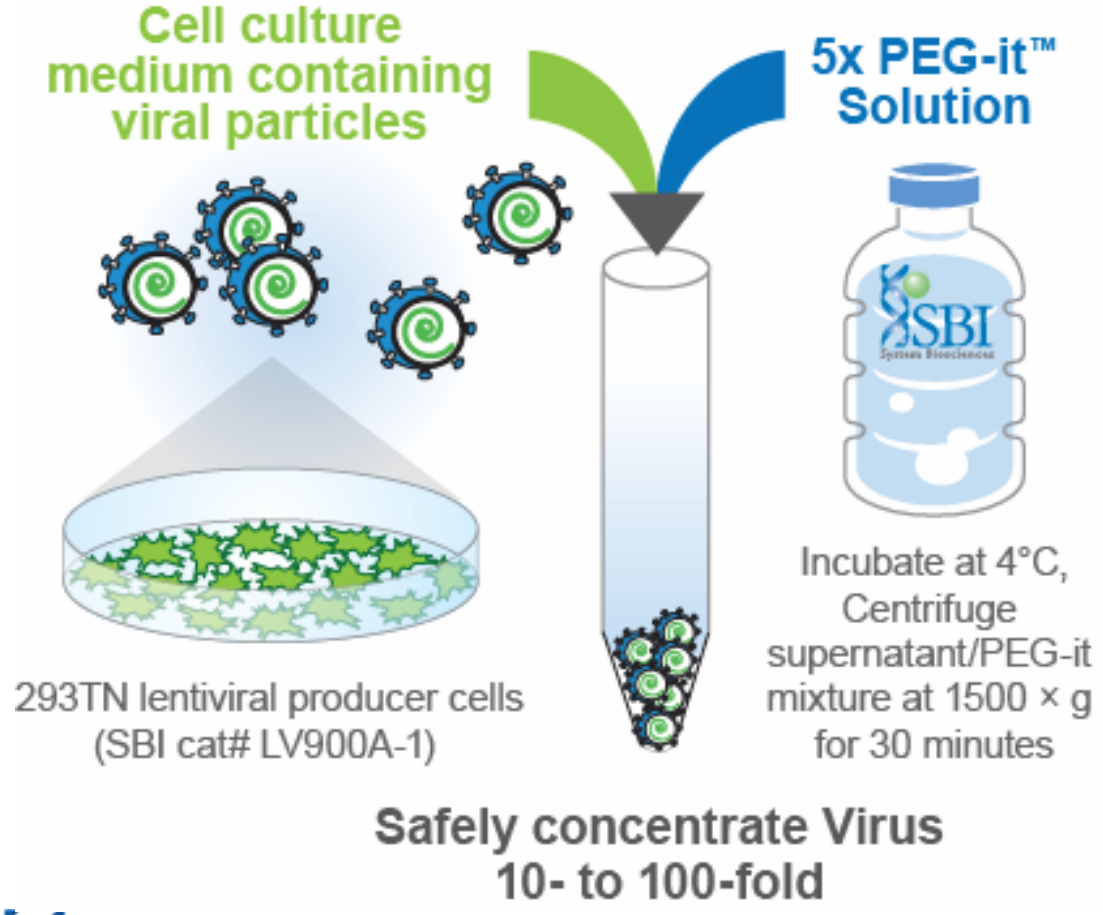


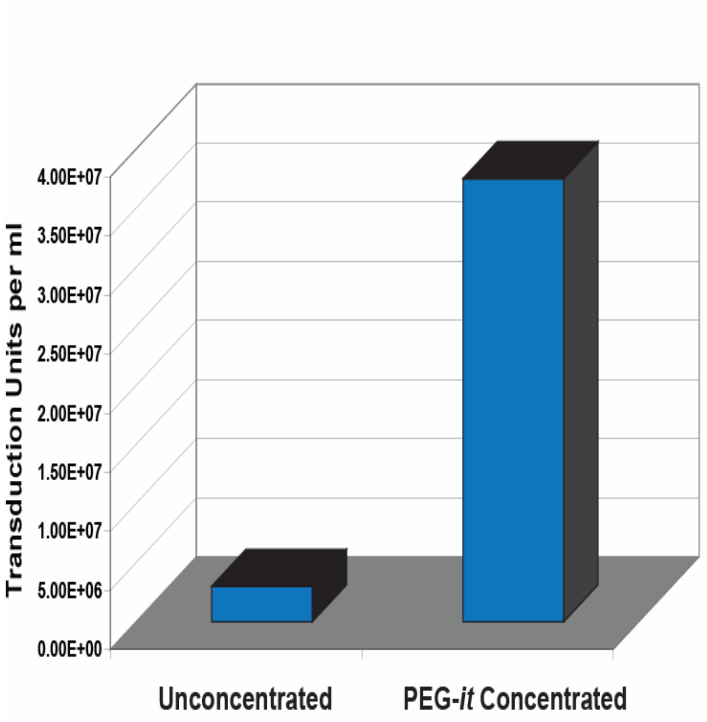
- **Produce highly efficient, transduction-ready, and replication-incompetent FIV or HIV-based pseudoviral particles containing lentiviral constructs**
- **Introduce and stably express lentiviral constructs in virtually any mammalian cell, including hard-to-transfect primary cells, neuronal cells and stem cells**
- **SBI's 293TN producer cell line is optimized for high pseudoviral titers**

Pseudoviral Particles



Virus Concentration – *PEG-it*





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Virus Titering – *UltraRapid Titer*

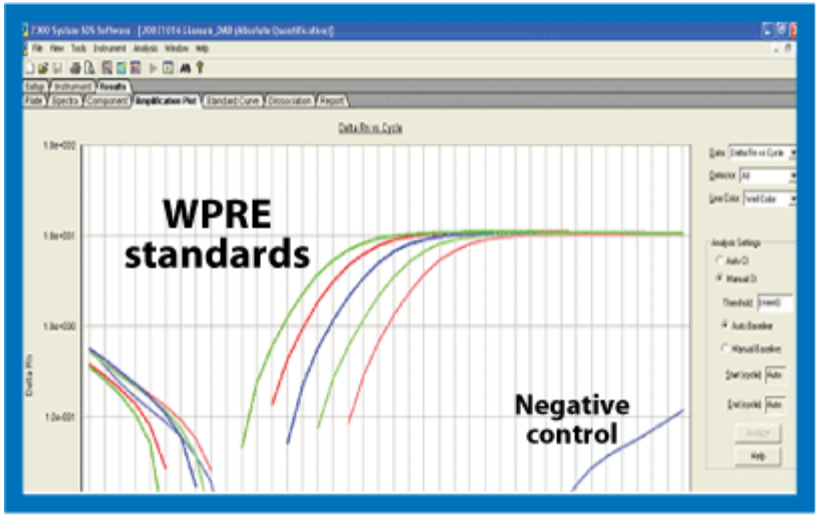
Lyse infected cells
(2 minutes, 95°C)

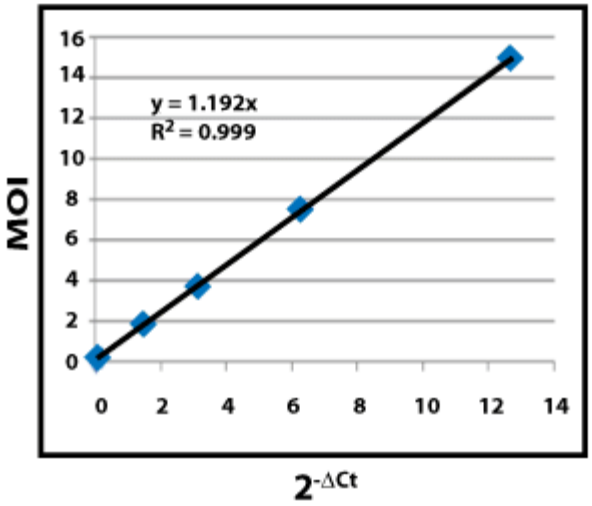
↓

Directly add cleared lysate sample to qPCR Mastermix

↓

Real-time PCR run
(2 hours)





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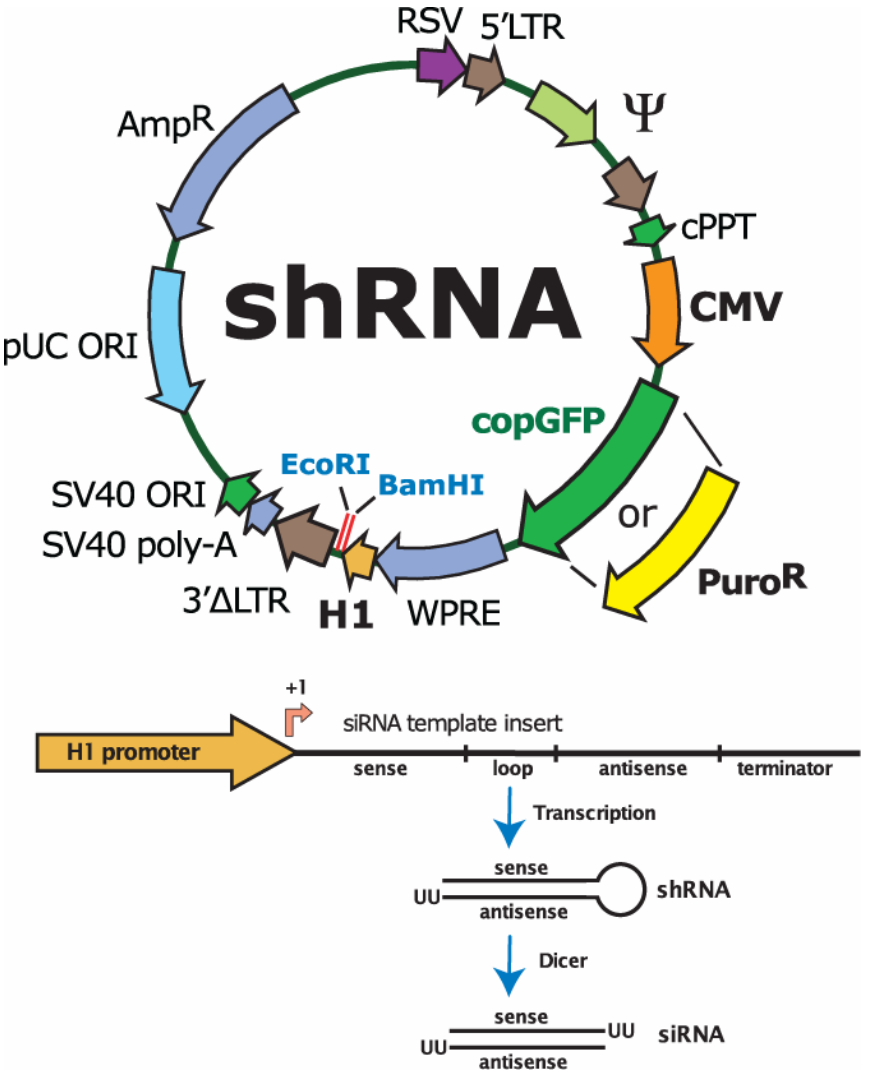
Lentivector-based RNAi

GeneNet™ shRNA Libraries

- **High-throughput gene knockdown studies on a genome-wide or pathway-focused basis.**
- **Simultaneously identify multiple genes that alter a specific cellular phenotype—in a single experiment.**



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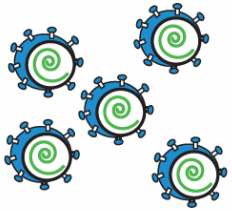
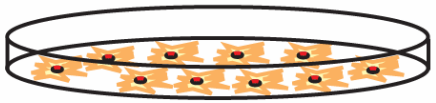
How to use Lentivirus Libraries

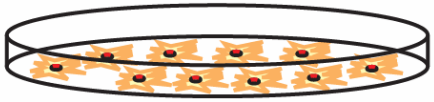
1 Transduction
with shRNA Library





Infected Target Cells

2 Treatment
to induce phenotype



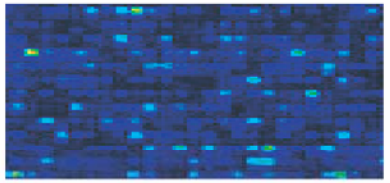
Treated Cells

3 Selection
for phenotype

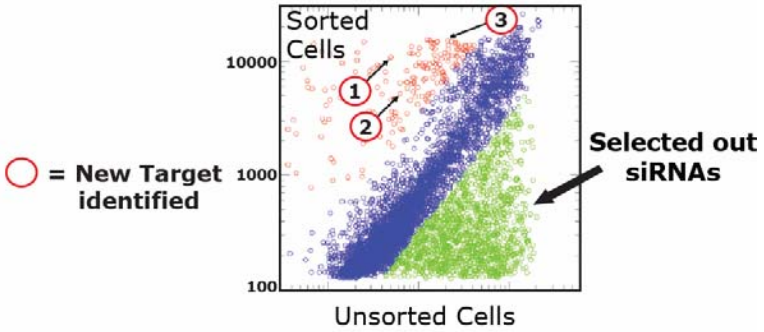
Cells with Specific Phenotype

4 Amplification
of shRNA effectors from selected cells

5 Hybridization
to Affymetrix GeneChip®
or sequence inserts



6 Analyze
with statistical software



SBI's RNAi Libraries

Genome-wide

GeneNet™ shRNA Libraries

HIV-based Libraries	Transcripts Targeted	# shRNAs
Human 50K	47,400	200,000
Mouse 40K	39,000	150,000
FIV-based Libraries		
Human 50K	47,400	200,000
Human 50K	47,400	200,000
Mouse 40K	39,000	150,000

Pathway-focused

GeneNet™ focused shRNA Libraries

HIV-based Libraries	Targeted Genes	# shRNAs
Human Apoptosis	579	6,876
Human Kinase	897	10,453
Human Phophatase	244	2,719

How it works

Knockdown
Each mRNA targeted by 4-5 separate shRNAs

Screen
All genes simultaneously

Dissect
Signaling pathways and cellular responses

Discover
New drug targets and diagnostic markers

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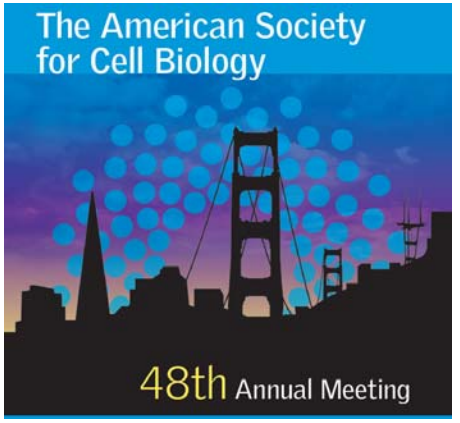
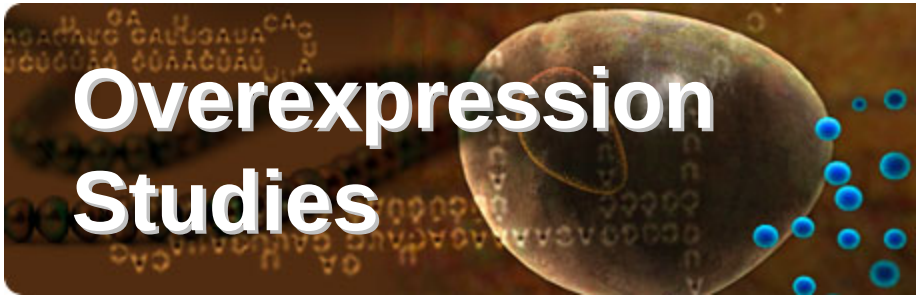
Study and Analysis of MicroRNAs

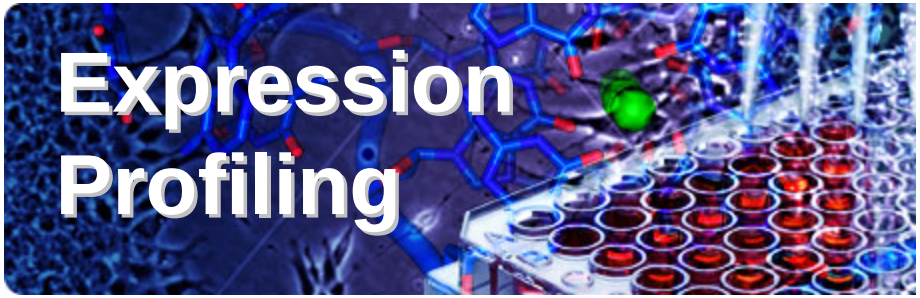
Travis J. Antes, Ph.D.
System Biosciences (SBI)

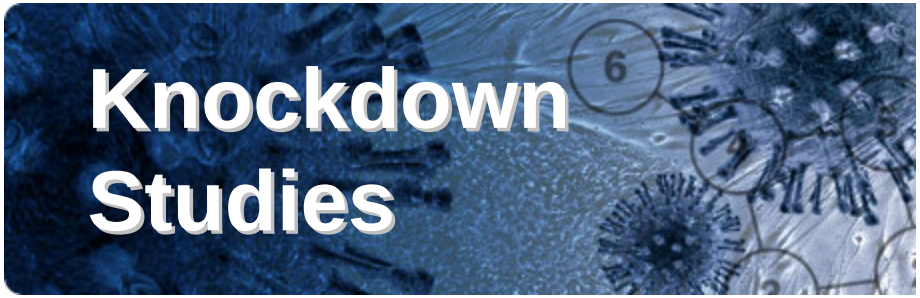
ASCB 2008

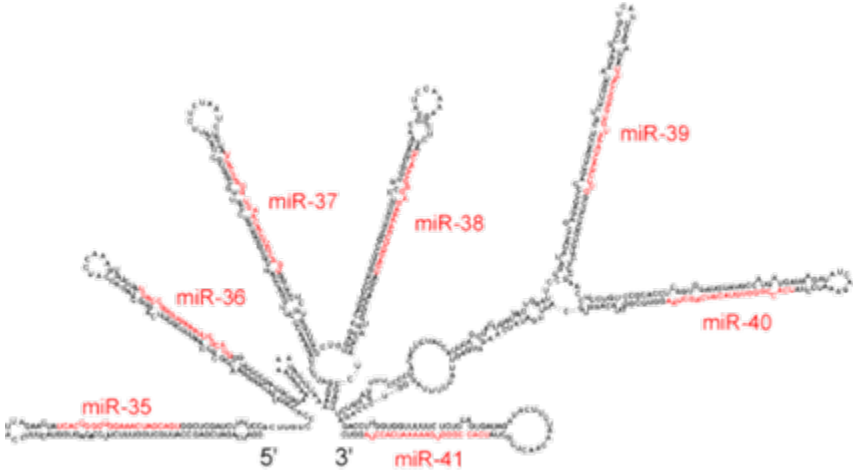


Areas of Investigation









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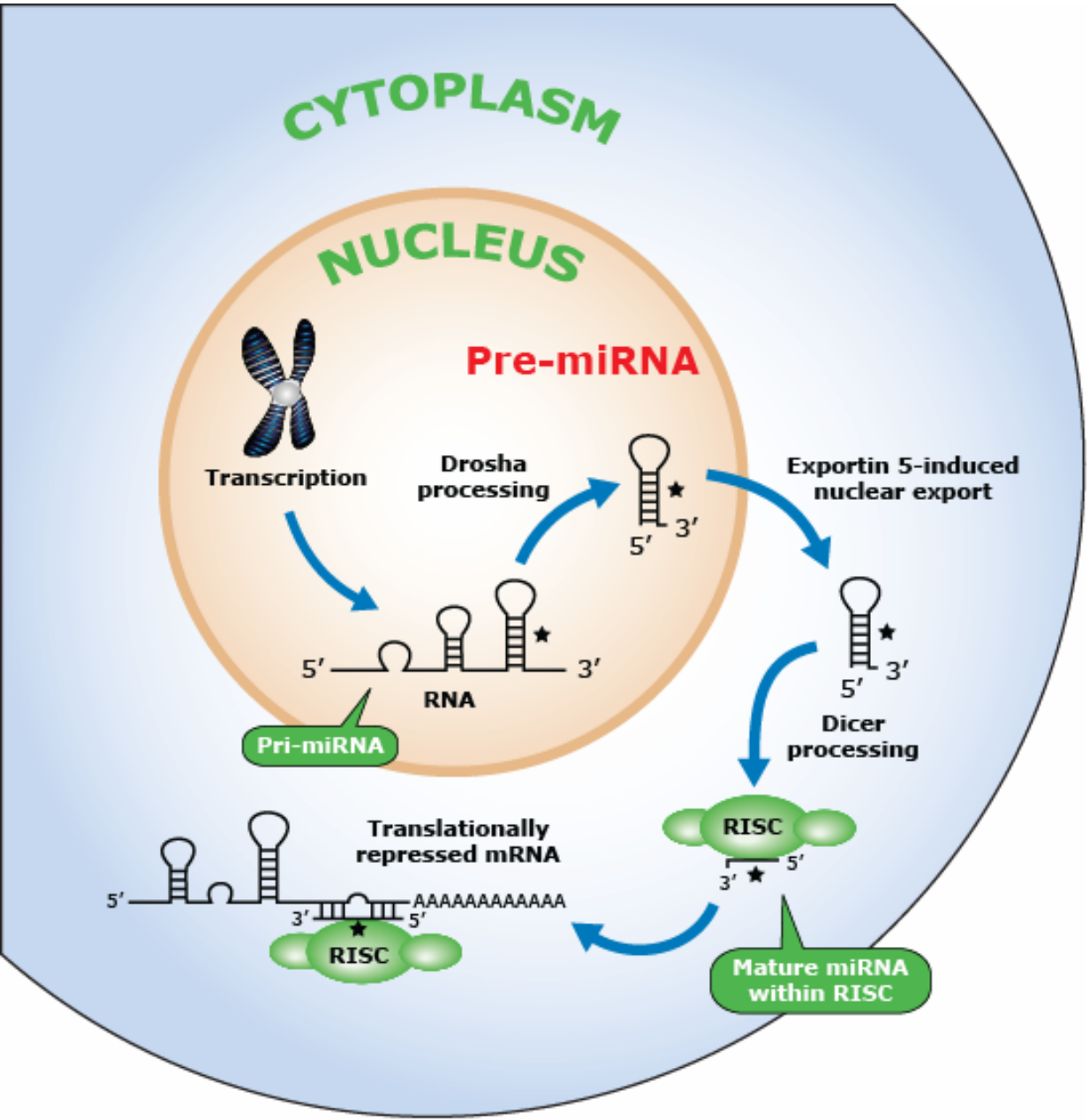
MicroRNA Biogenesis



Estimated number of MicroRNAs in Humans = ~ 3000

Number of MicroRNA entries in miRBase
Human = 868
Mouse = 627

miRBase

http://microrna.sanger.ac.uk/

Functional differences between miRNA and siRNA

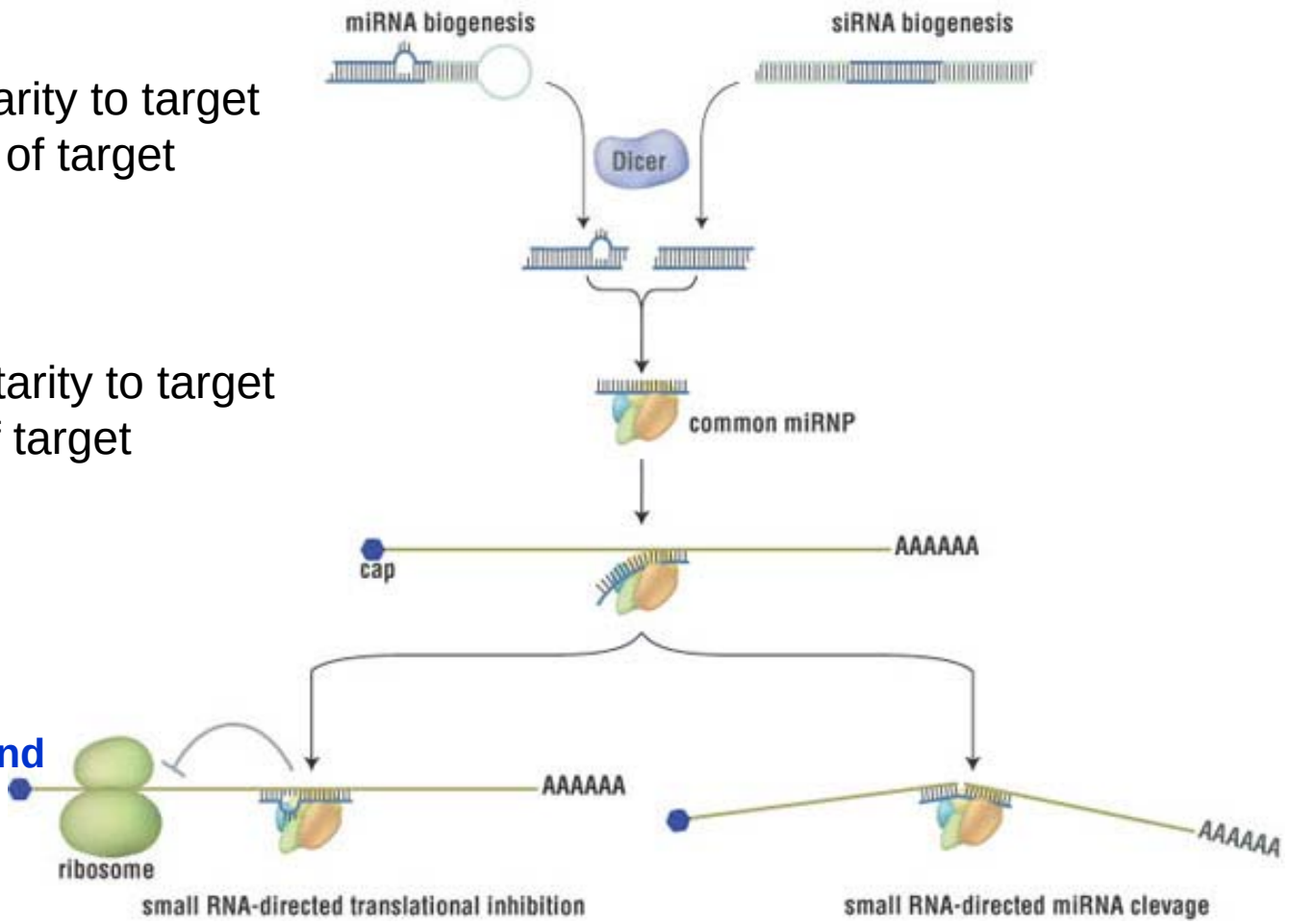
miRNA

- Partial complementarity to target
- Repress translation of target

siRNA

- Perfect complementarity to target
- Elicit degradation of target

- **Both are involved in: Apoptosis, Differentiation & Cancer**
- **Demonstrate tissue and temporal-specific expression**



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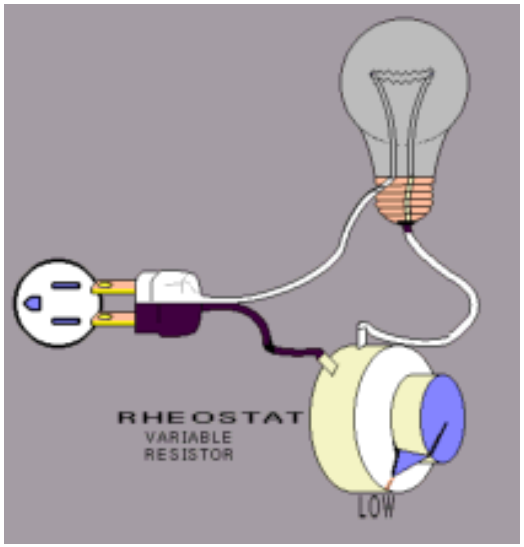
New Paradigm

Translation

mRNA → **Protein**



Negative regulation

- **miRNA - inhibit translation**
- **siRNA – mRNA degradation**

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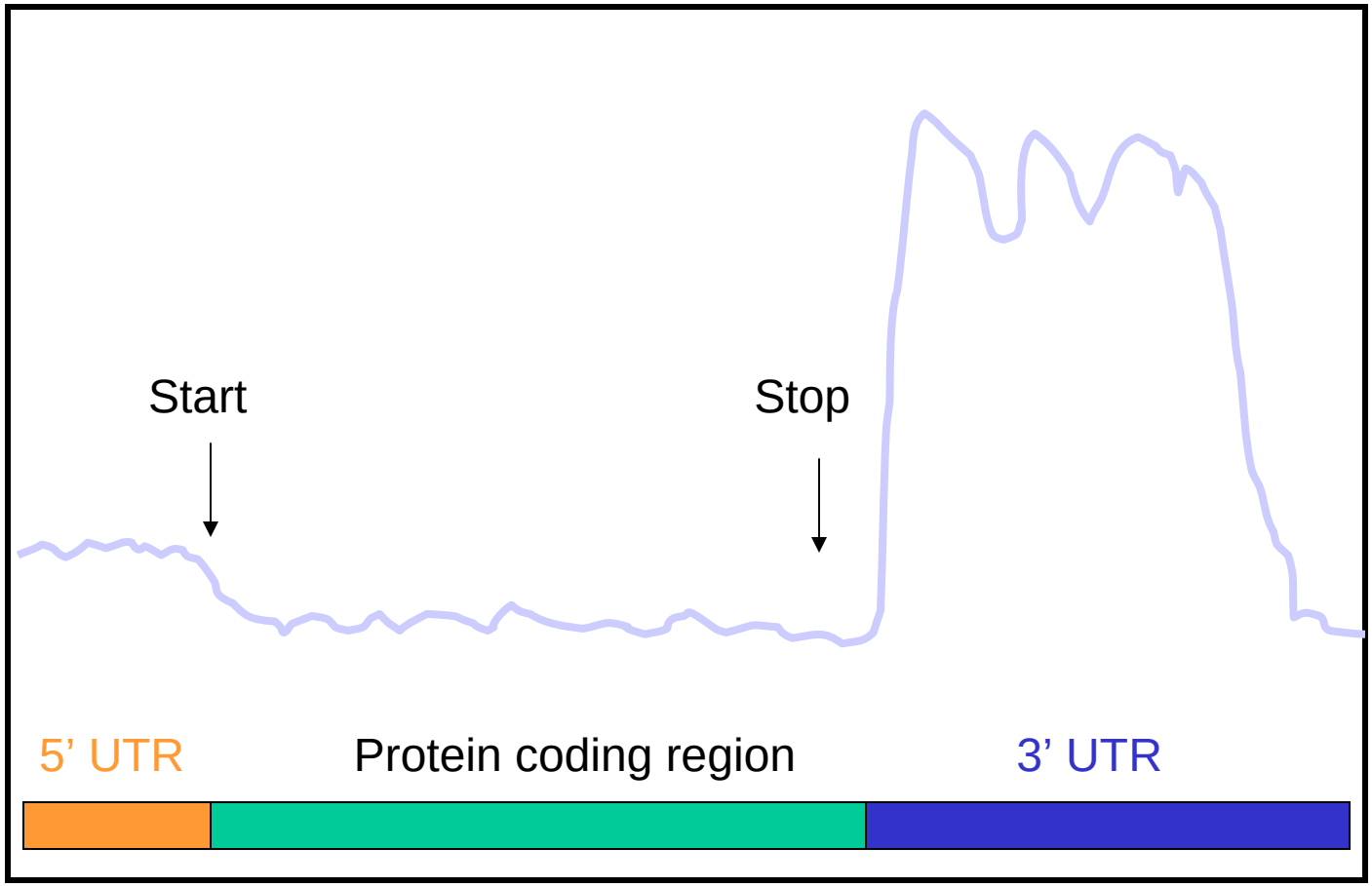
MicroRNAs bind to 3’ UTRs of messenger RNAs



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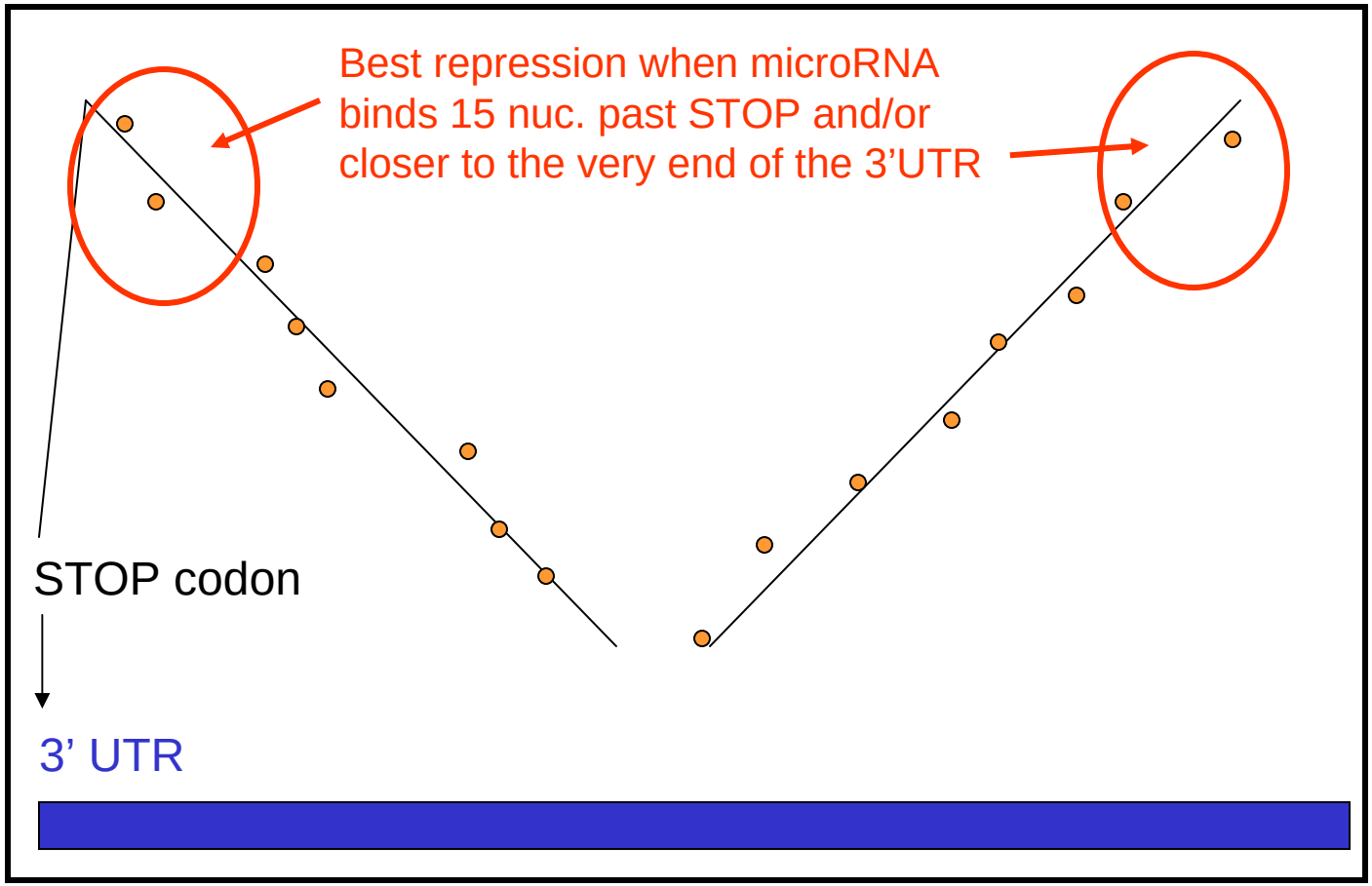
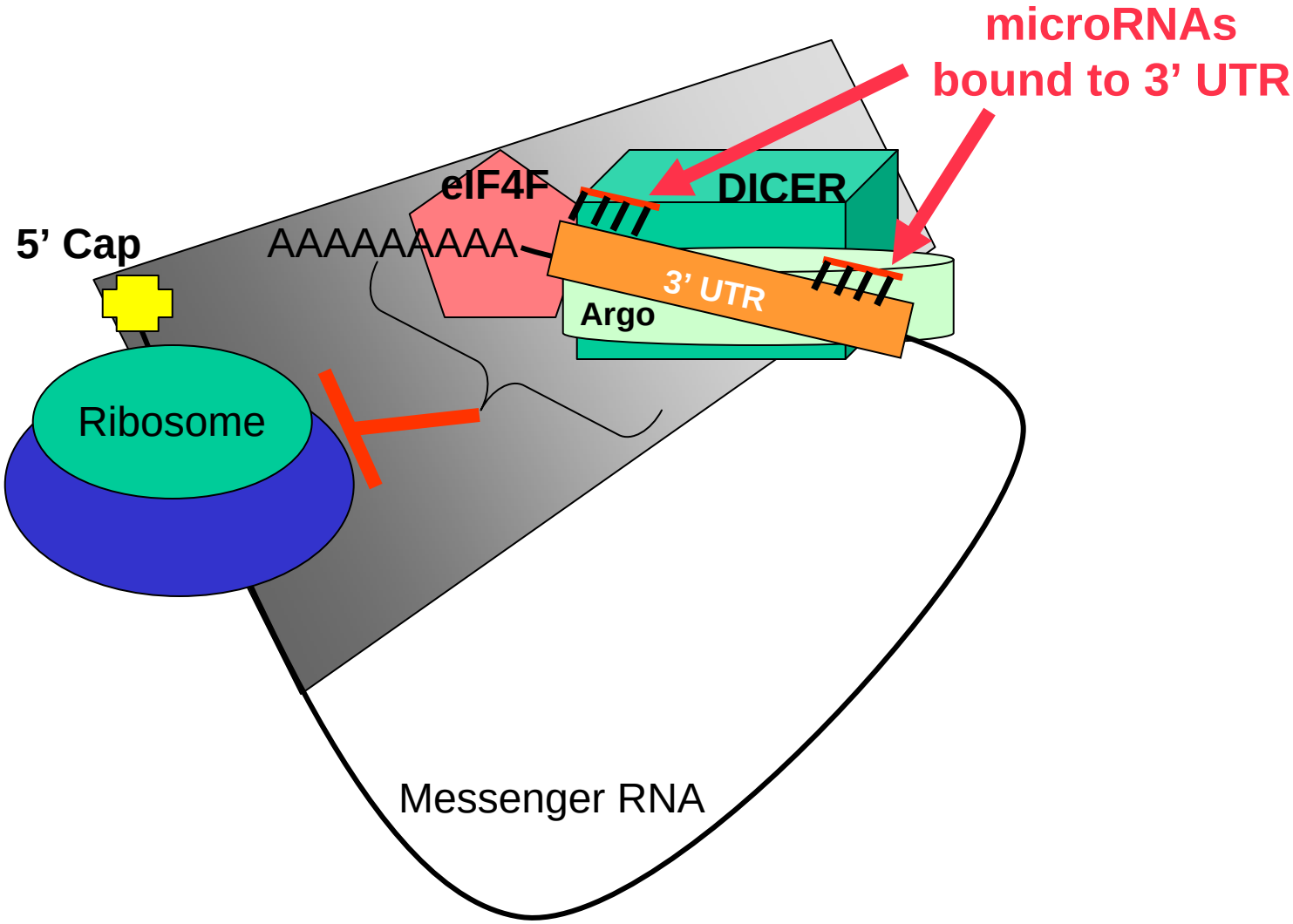
MicroRNA Targeting Specificity in Mammals: Determinants Beyond Seed Pairing. Grimson, A. & Bartel, D. Molecular Cell 27, 91–105, July 6, 2007.

Preferred binding positions within 3’ UTRs



Spatial preferences of microRNA targets in 3' untranslated regions. Majoros WH, Ohler U. BMC Genomics. 2007 Jun 7;8:152.

Theory: “Ribosomal Shadow”



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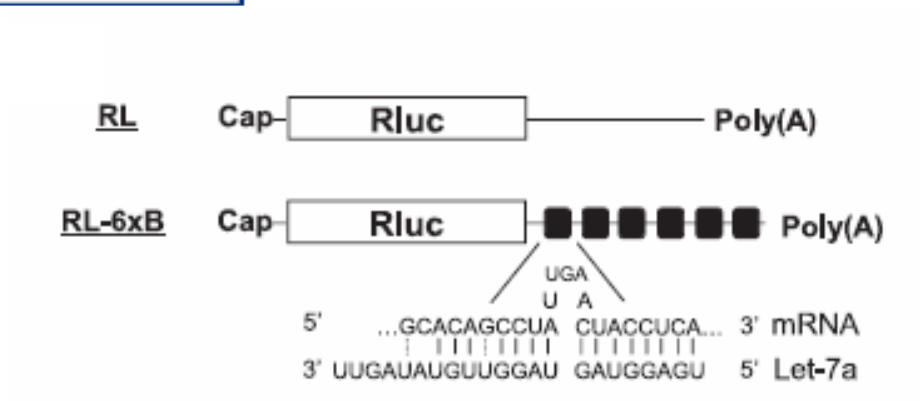
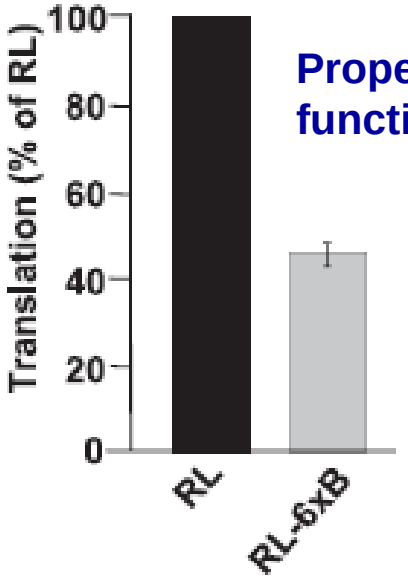
5’ Cap Requirement

Science
AAAS

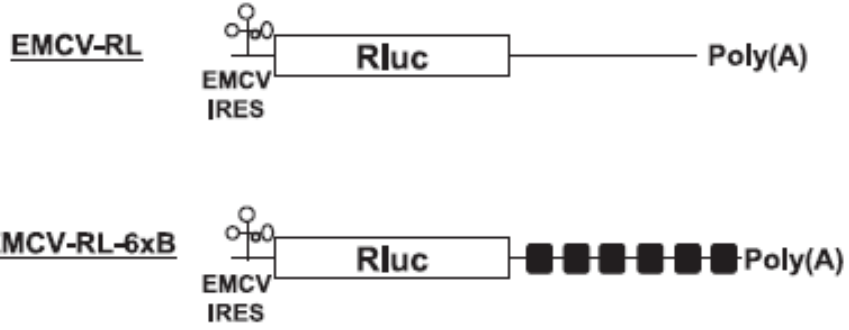
MicroRNA Inhibition of Translation Initiation in Vitro by Targeting the Cap-Binding Complex eIF4F
Géraldine Mathonnet, *et al.*
Science **317**, 1764 (2007);
DOI: 10.1126/science.1146067

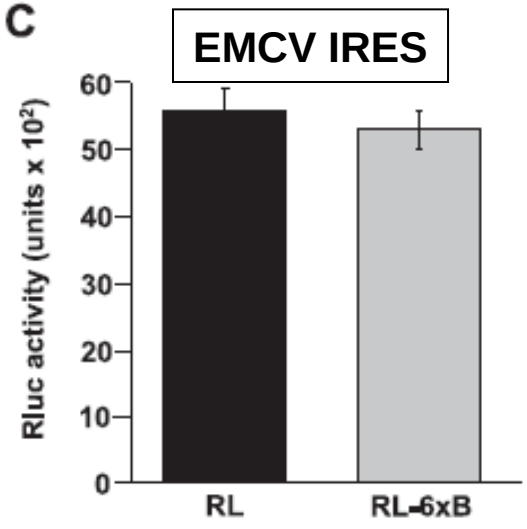




Proper function

No effect





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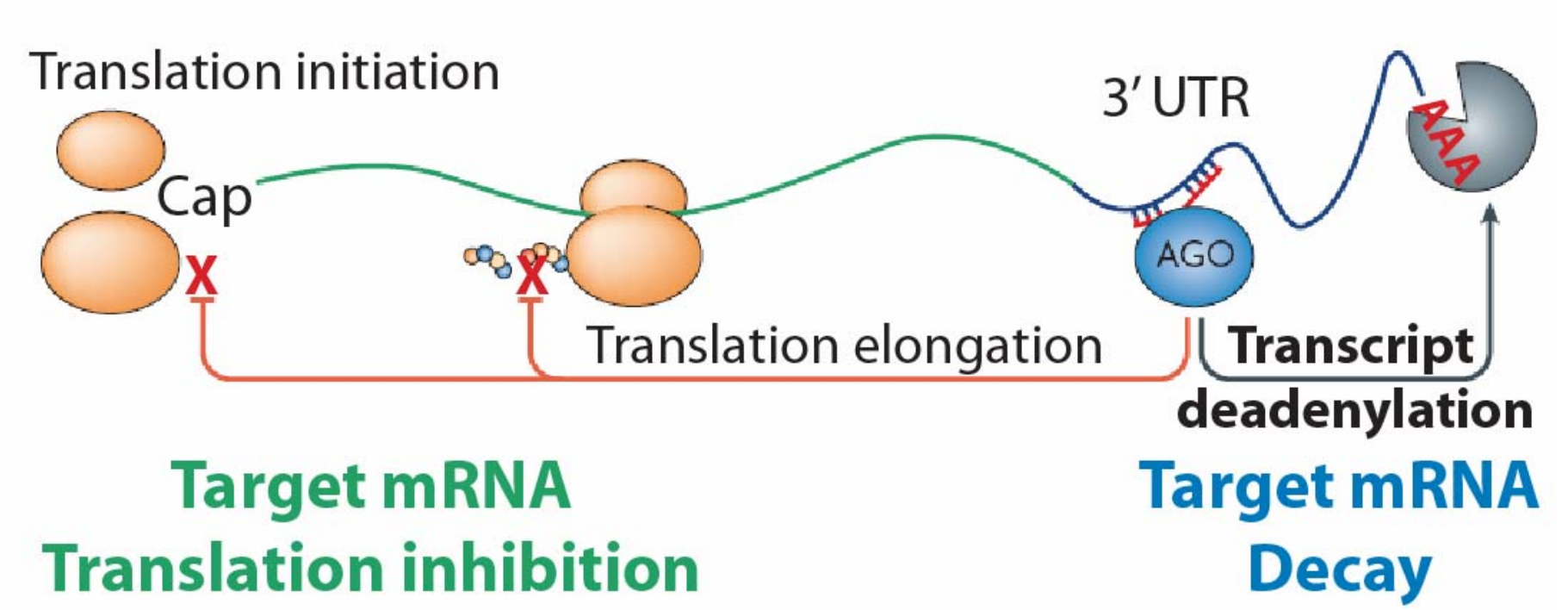
Mechanisms of MicroRNA Interference



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Expression Profiling

- Why profiling matters
- Example study of MicroRNA Dysregulation in Kidney Cancer
- Tools for expression profiling

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Why Profiling Matters

REVIEWS

Priority Repo

MicroRNA Gene Expression Deregulation in Human Breast Cancer

Marilena V. Iorio,[1] Manuela Ferracin,[2] Chang-Gong Liu,[1] Angelo Veronese,[2] Riccardo Spizzo,[2] Silvia Sabbioni,[2] Eros Magri,[2] Massimo Pedriali,[2] Muller Fabbri,[1] Manuela Campiglio,[3] Sylvie Ménard,[3] Juan P. Palazzo,[4] Anne Rosenberg,[5] Piero Musiani,[6] Stefano Volinia,[1] Italo Nenci,[2] George A. Calin,[1] Patrizia Querzoli,[2] Massimo Negrini,[2] and Carlo M. Croce[1]

[1]Comprehensive Cancer Center, Ohio State University, Columbus, Ohio; [2]Dipartimento di Medicina Sper Interdipartimentale per la Ricerca sul Cancro, Università di Ferrara, Ferrara, Italy; [3]Molecular Targeting Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy; Departments of [4]Pathology, Anatomy a and [5]Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania; and [6]Ce.S.I. Aging Research Cent

Oncomirs — microRNAs with a role in cancer

Aurora Esquela-Kerscher and Frank J. Slack

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Mounting evidence indicates microRNAs can be causative agents in Cancer = Potential Diagnostic development

A MicroRNA Signature Associated with Prognosis and Progression in Chronic Lymphocytic Leukemia

George Adrian Calin, M.D., Ph.D., Manuel
Amelia Cimmino, M.D., Ph.D., Gianpierc
Masayoshi Shimizu, B.S., Sylwia E. Wojcik, M.Sc.,
Rosa Visone, Ph.D., Nurettin Ilfer Sever, Ph.D.
Rodolfo Iuliano, Ph.D., Tiziana Palumbo, Ph.D.,
Claudia Roldo, M.D., Ramiro Garzon, M.D., Ci
Laura Rassenti, Ph.D., Hansjuerg Alder, Ph.D.,
Chang-gong Liu, Ph.D., Thomas J. Kipp
Massimo Negrini, Ph.D., and Carlo M

Cellular development

Vol 439|19 January 2006|doi:10.1038/nature04367 nature

ARTICLES

A brain-specific microRNA regulates dendritic spine development

Gerhard M. Schratt[1,2,3], Fabian Tuebing[4], Elizabeth A. Nigh[1,2,3], Christina G. Kane[1,2,3], Mary E. Sabatini[3], Michael Kiebler[4] & Michael E. Greenberg[1,2,3]

Molecular Cancer

Research

Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues

E Bandrés*[1], E Cubedo[1], X Agirre[2], R Malumbres[1], R Zárate[1], N Ramirez[1] A Abajo[1], A Navarro[3], I Moreno[4], M Monzó[3] and J García-Foncillas[1]

Open Access

ARTICLE

Unique microRNA molecular profiles in lung cancer diagnosis and prognosis

Nozomu Yanaihara,[1] Natasha Caplen,[2] Elise Bowman,[1] Masahiro Seike,[1] Kensuke Kumamoto,[1] Ming Yi,[3] Robert M. Stephens,[3] Aikou Okamoto,[4] Jun Yokota,[5] Tadao Tanaka,[4] George Adrian Calin,[6] Chang-Gong Liu,[6] Carlo M. Croce,[6] and Curtis C. Harris[1,*]

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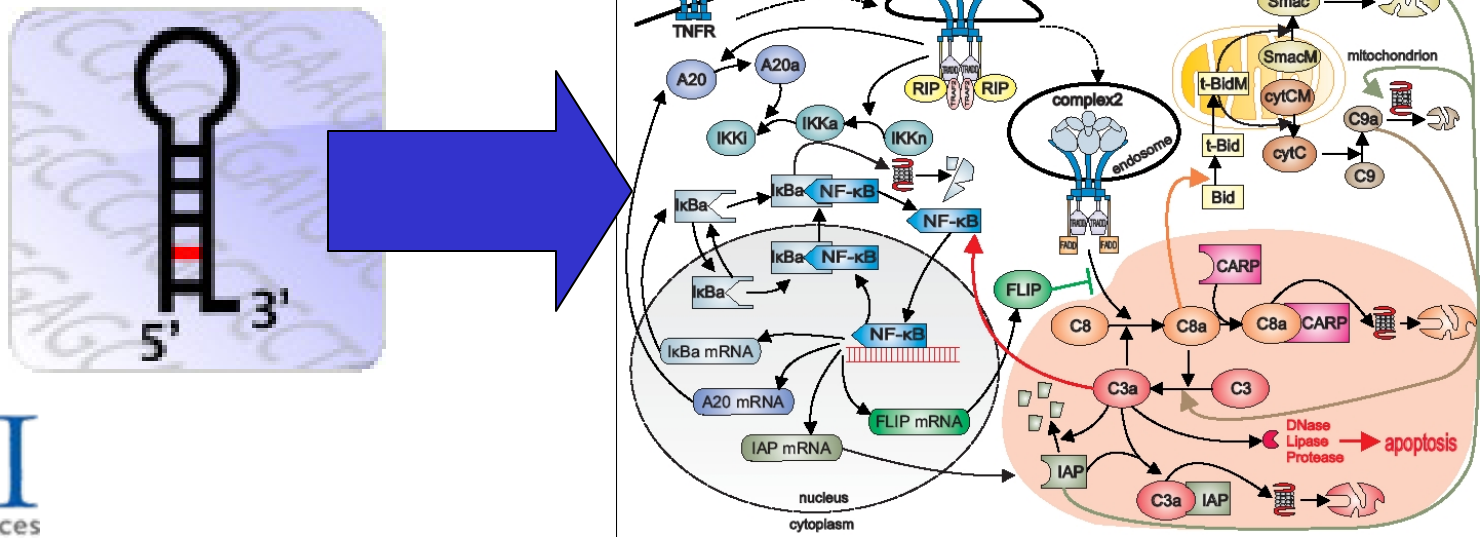
Why Profiling Matters

What can microRNA expression profiling tell us about the molecular pathways affected ?



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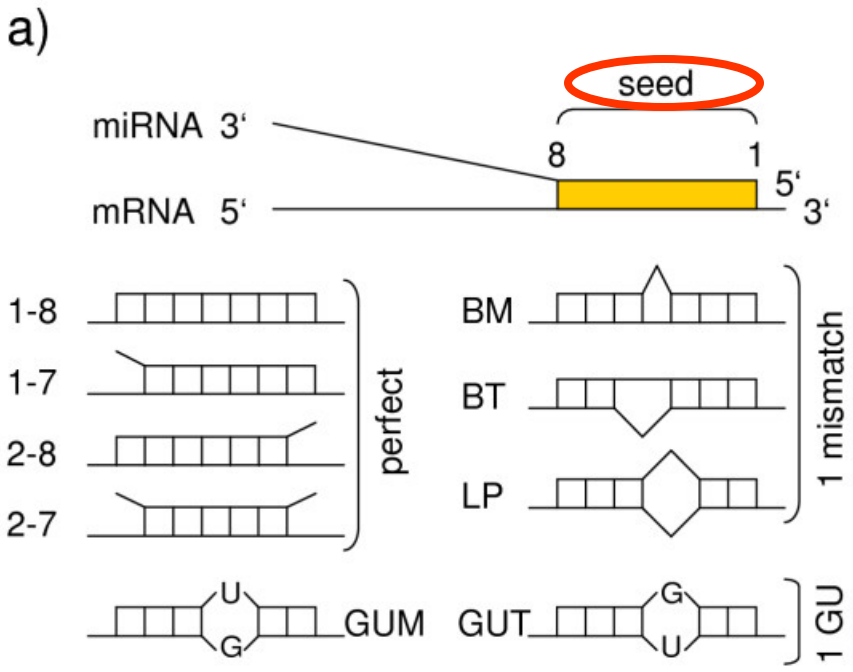
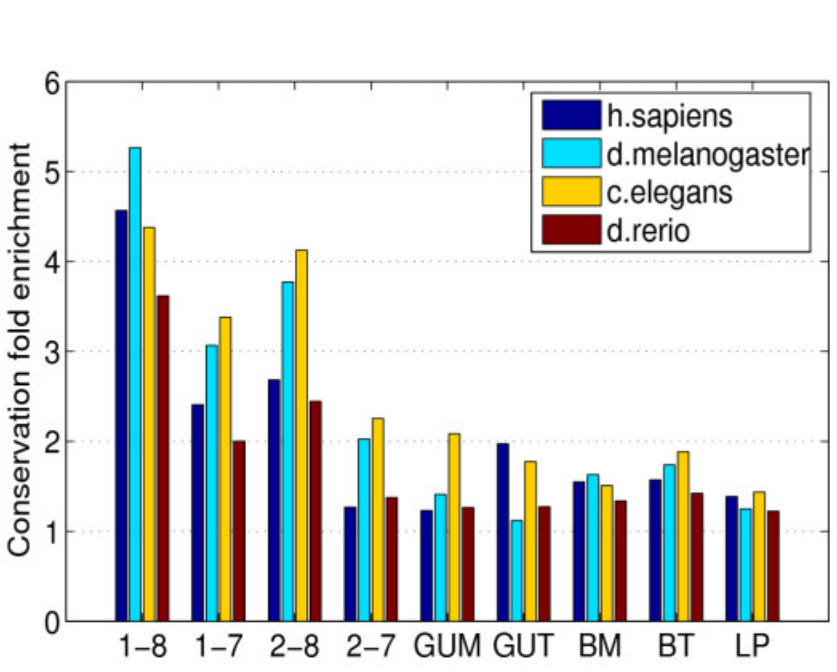
MicroRNA : mRNA Targeting

How do microRNAs recognize their mRNA targets?



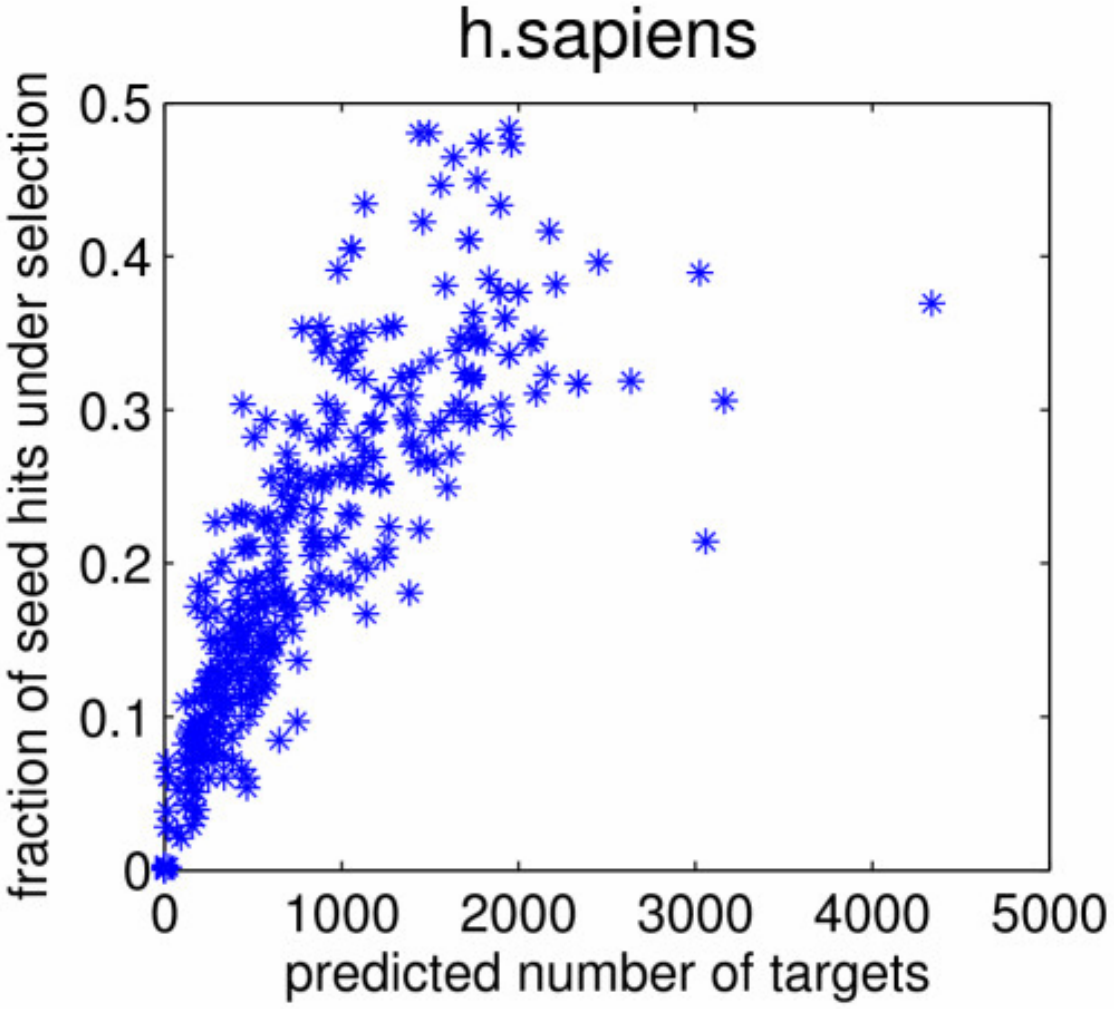


Inference of miRNA targets using evolutionary conservation and pathway analysis. Gaidatzis *et al. BMC Bioinformatics* 2007 **8:69.**

MicroRNA : mRNA Targeting

How many mRNAs are targeted by a given microRNA?

= One individual microRNA



Inference of miRNA targets using evolutionary conservation and pathway analysis. Gaidatzis *et al. BMC Bioinformatics* 2007 **8:69.**

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MicroRNA : mRNA Targeting

How many mRNAs are targeted by a given microRNA?

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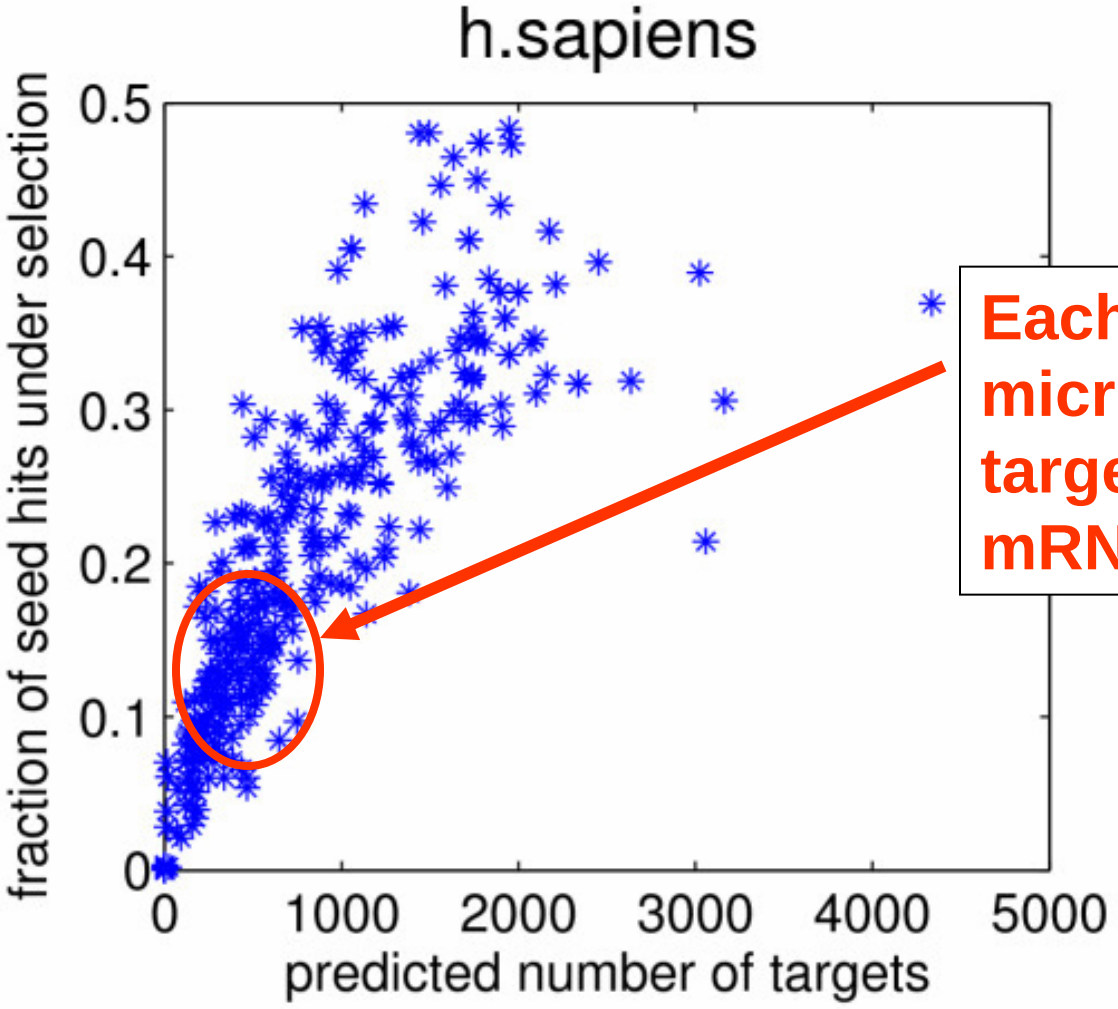

Each microRNA may target 300-500 mRNAs

Inference of miRNA targets using evolutionary conservation and pathway analysis. Gaidatzis *et al. BMC Bioinformatics* 2007 **8:69.**

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MicroRNA : mRNA Targeting

Search for predicted microRNA targets in mammals

[Go to TargetScanWorm]
[Go to TargetScanFly]

1. Select a species Human

AND

2. Enter an Entrez Gene symbol (e.g. "LIN28")

AND/OR

3. Do one of the following:

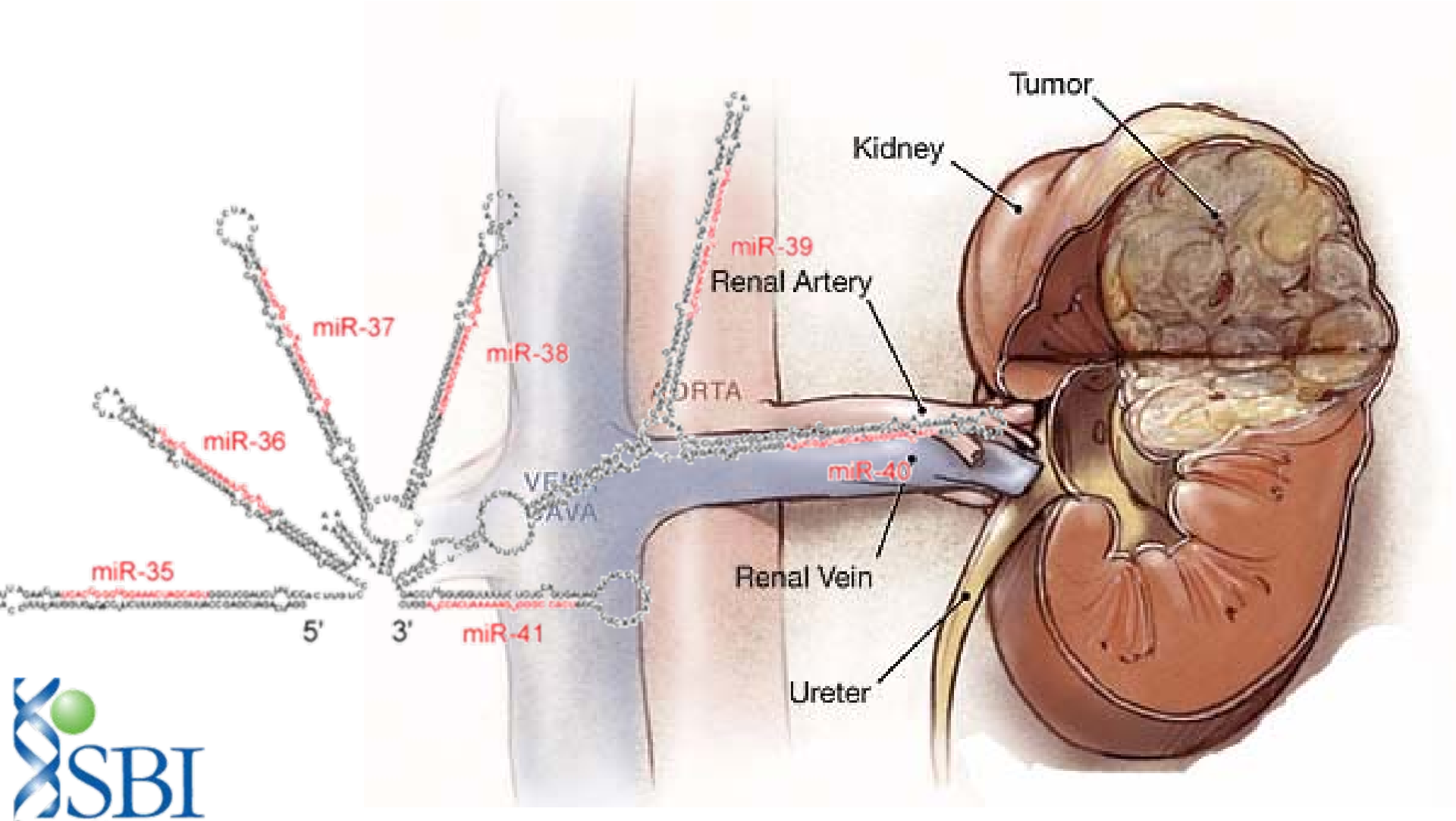
- Select a highly conserved* microRNA family: Highly conserved microRNA families
- Select a conserved* microRNA family: Conserved microRNA families
- Select a poorly conserved microRNA family: Poorly conserved microRNA families
- Enter a microRNA name (e.g. "mmu-miR-1")

Go to TargetScan Custom if your RNA is not included in the microRNA families listed above.

Submit Reset

http://www.targetscan.org/

MicroRNA Dysregulation in Kidney Cancer



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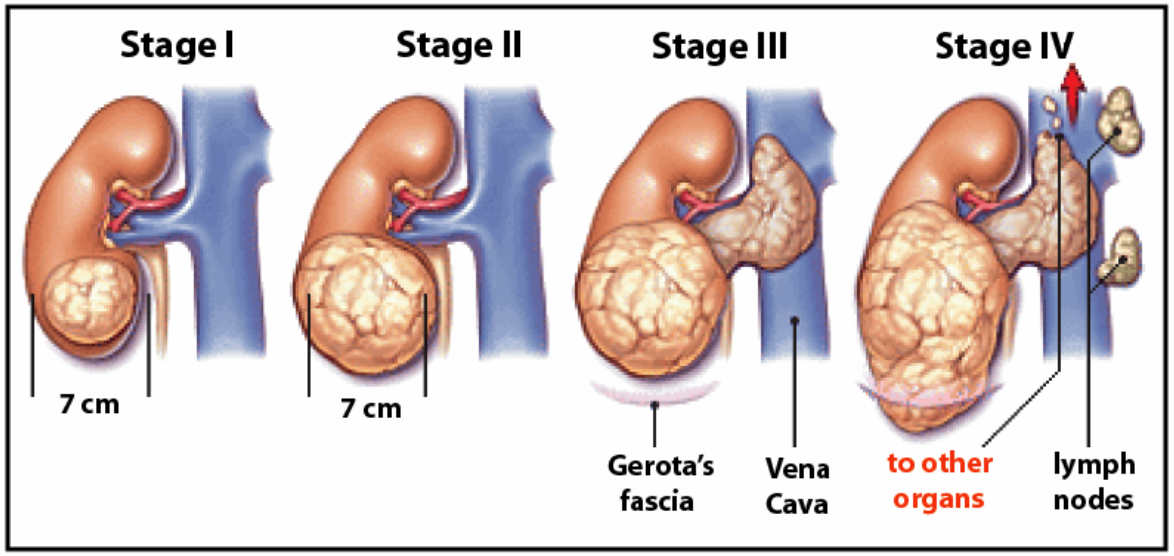
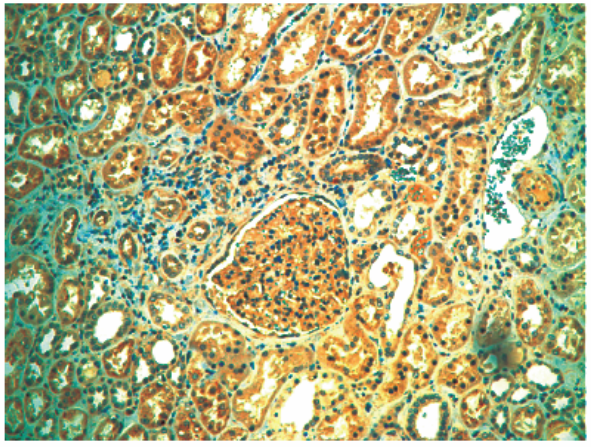
Kidney Cancer

Stages of Kidney Cancer (RCC)

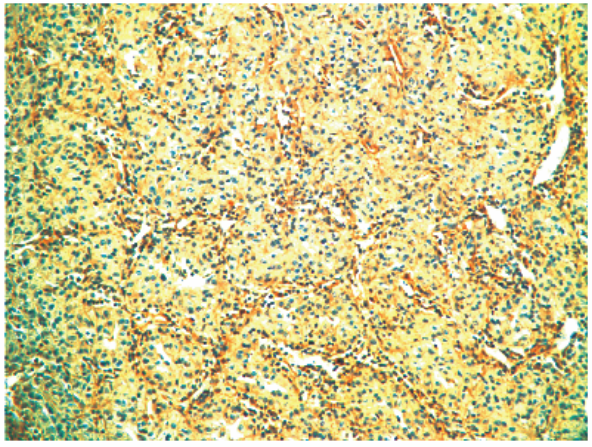


Histopathological Comparisons

Normal



Tumor (RCC)



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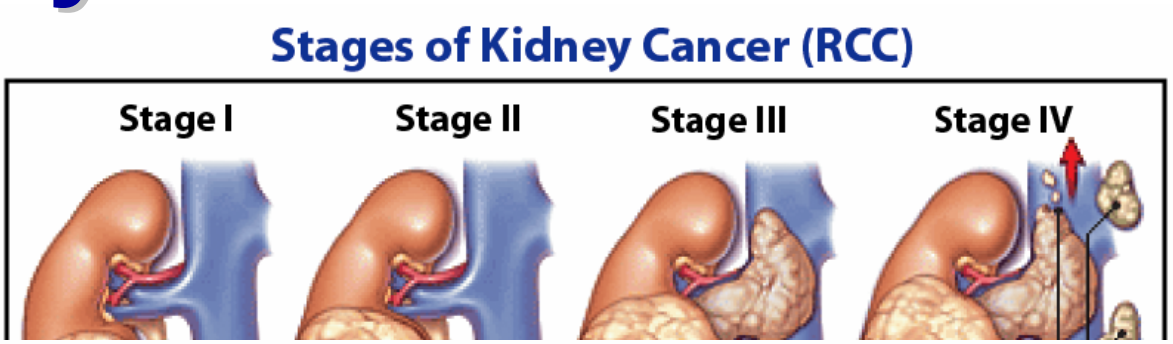
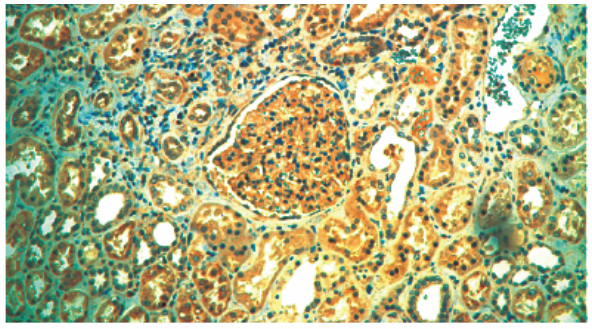
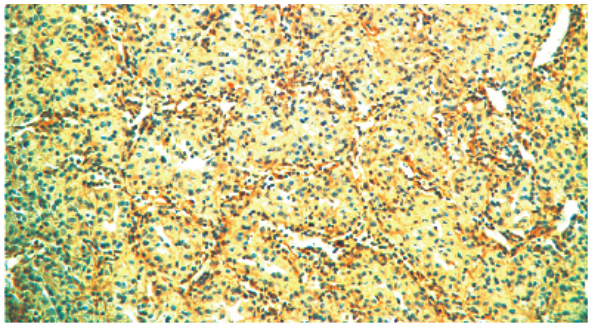
Kidney Cancer



"There are no good screening tools, and once the cancer spreads from the kidney, there are no good treatments."





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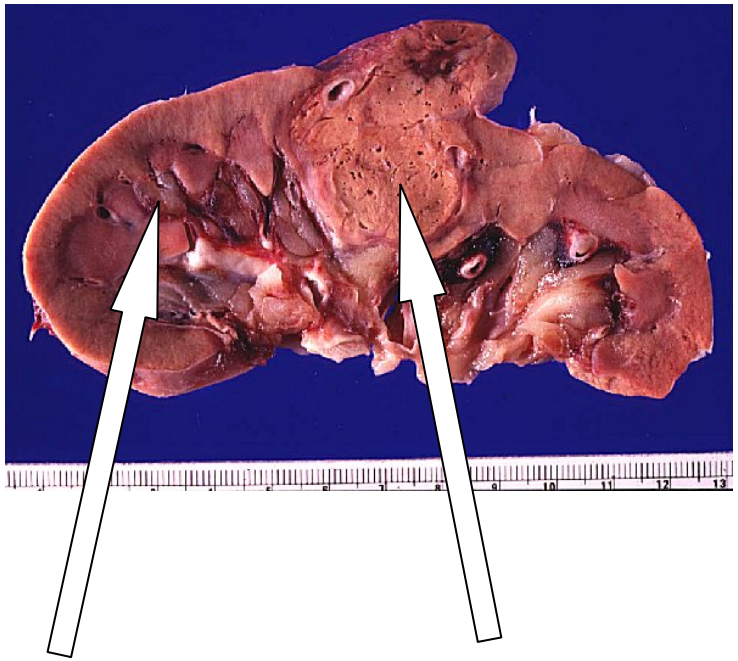
Kidney Cancer Samples

Boston University Kidney Cancer Patient Study

- 8 clear-cell RCC tissue specimens, along with their patient-matched normal kidney tissue, were obtained from patients at Boston Medical Center immediately after radical nephrectomy.
- Institutional Review Board-approved informed consent for the collection of specimens was obtained from all patients.
- Six of the tissue specimens were classified as high-grade RCC with a histological Fuhrman grade of three or four, while two of the specimens were classified as low-grade RCC with a Fuhrman grade of two.



Normal Kidney Tissue

Tumor Kidney Tissue

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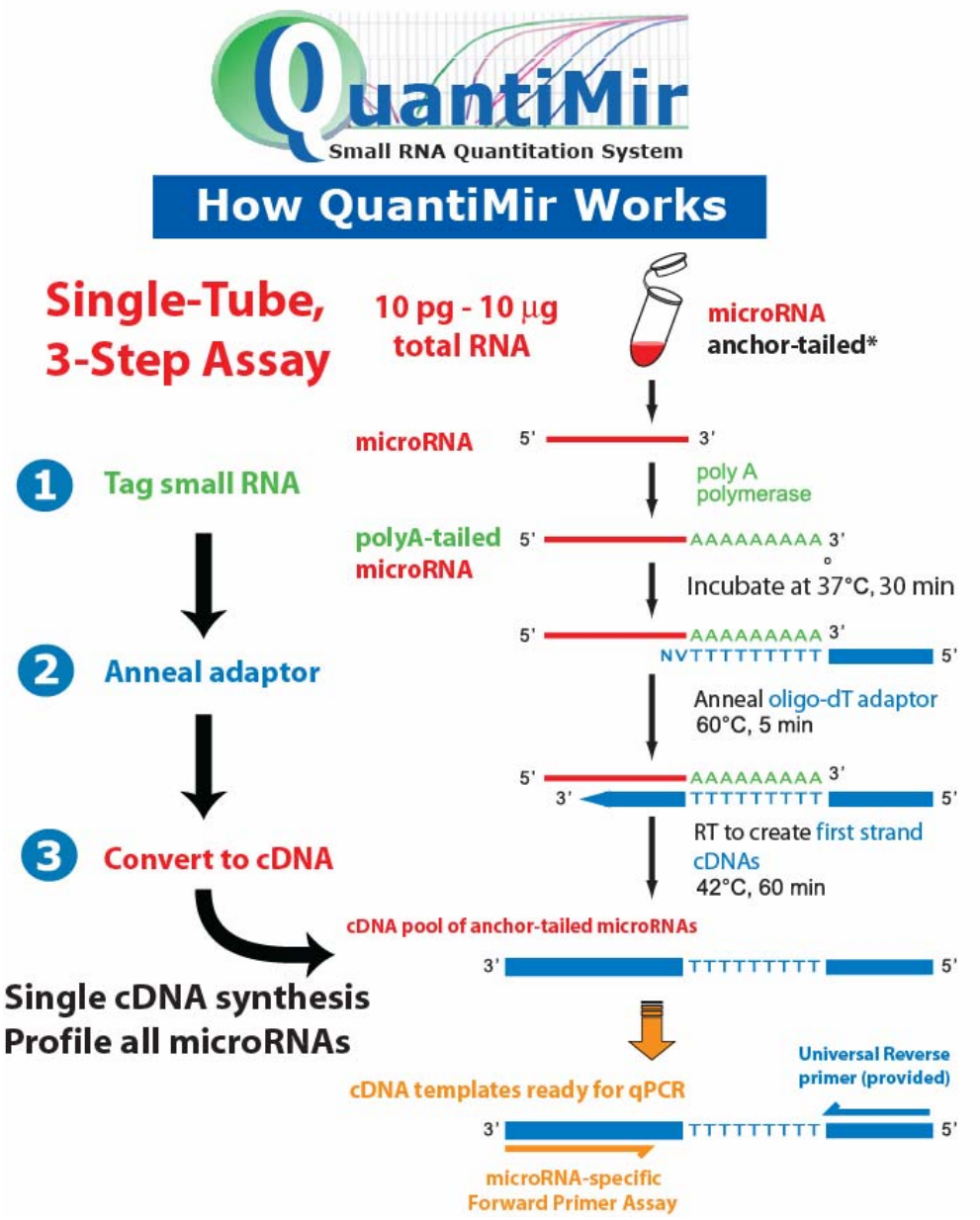
QuantiMir Technology



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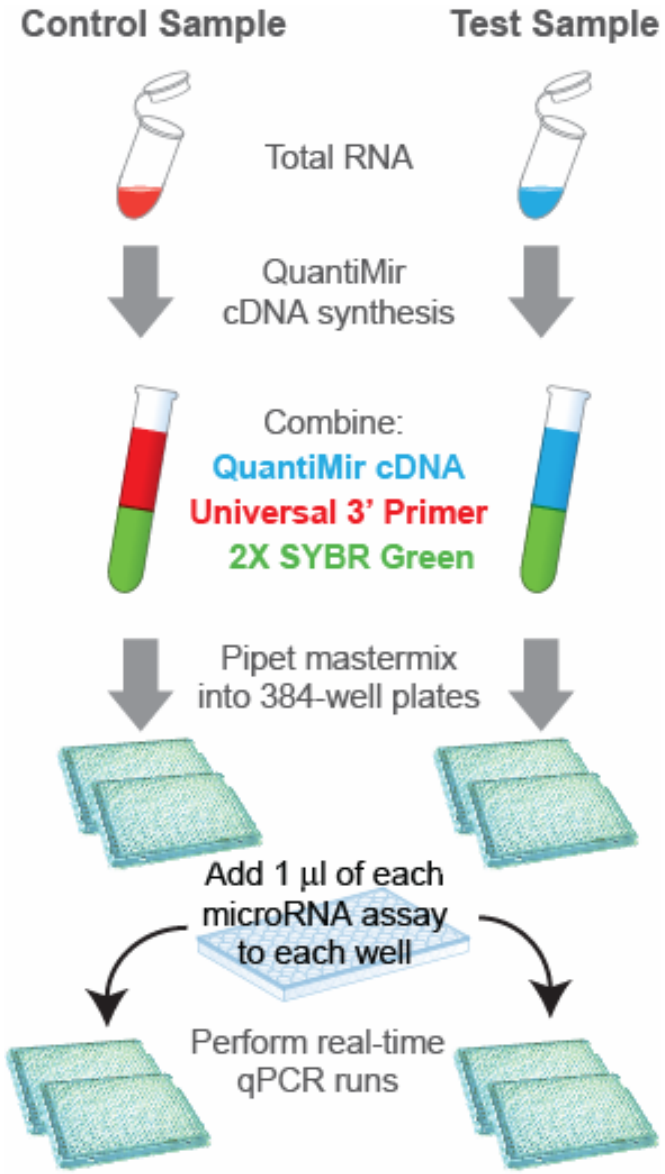
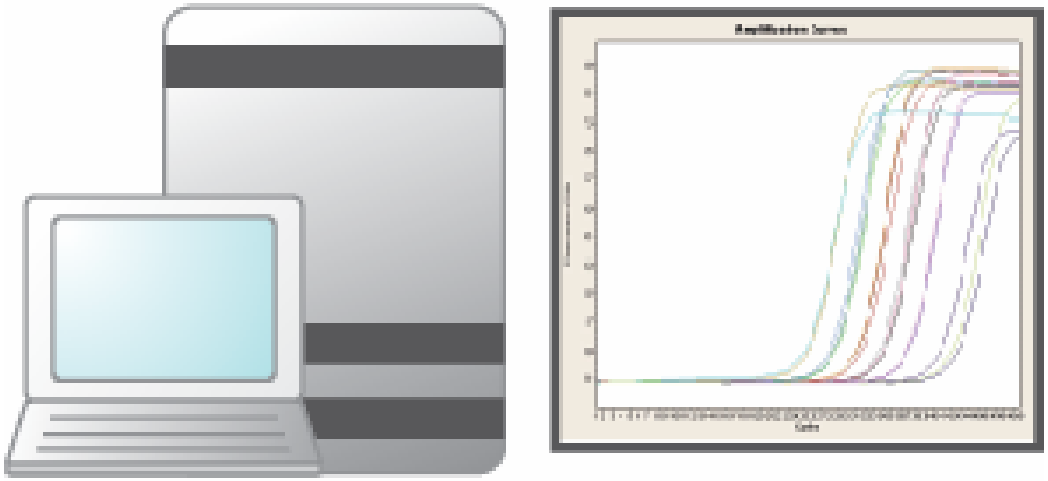
qPCR Array Setup

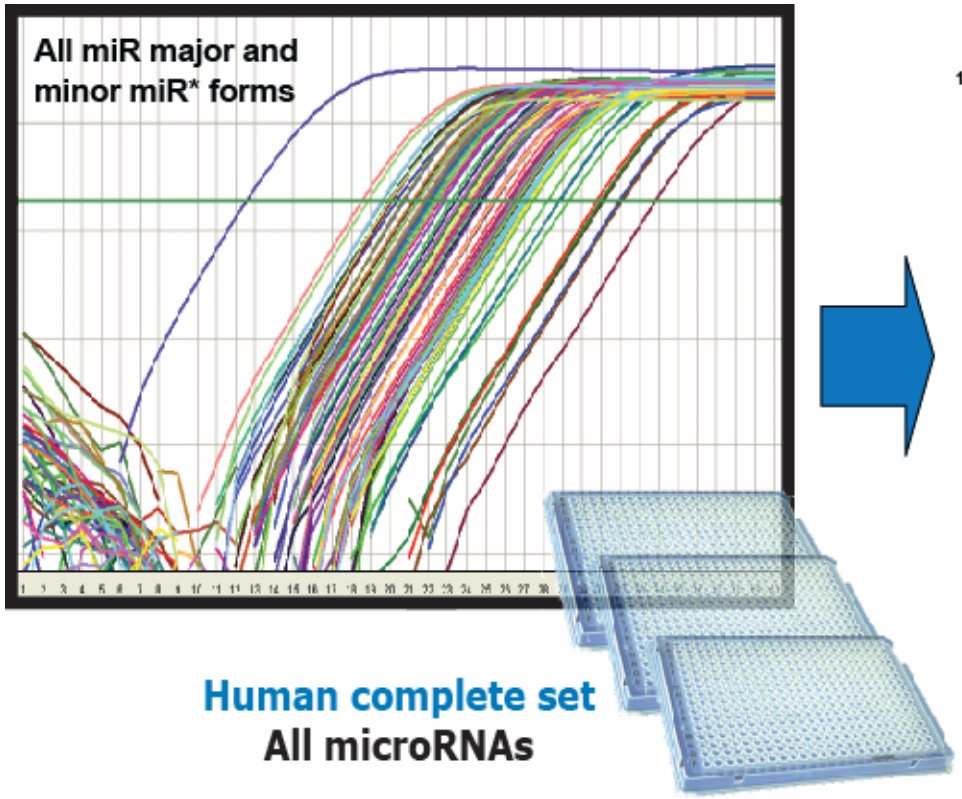


Perform real-time qPCR runs

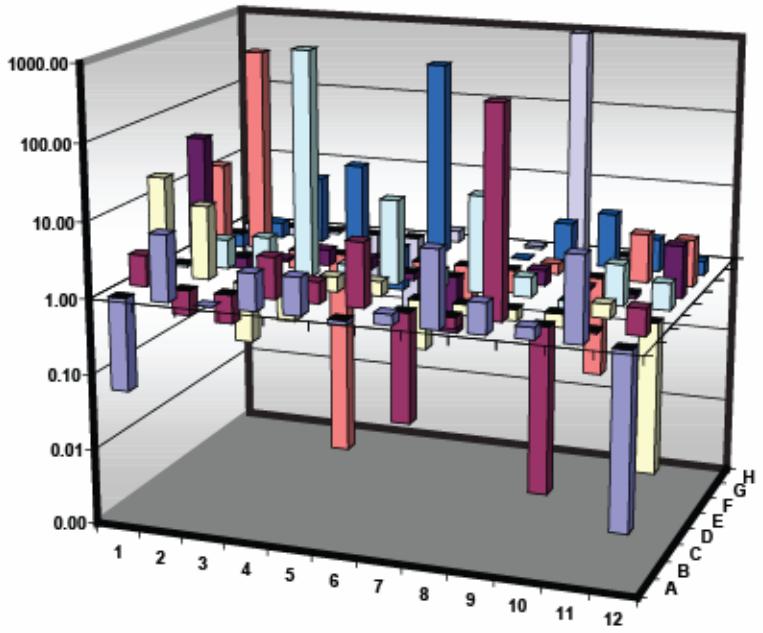


Cross-compare ΔΔCt measurements between Control and Tumor Samples

qPCR Runs and Data Analyses



Easy to use software included



- 8 Patients
- 2 samples
- Triplicates

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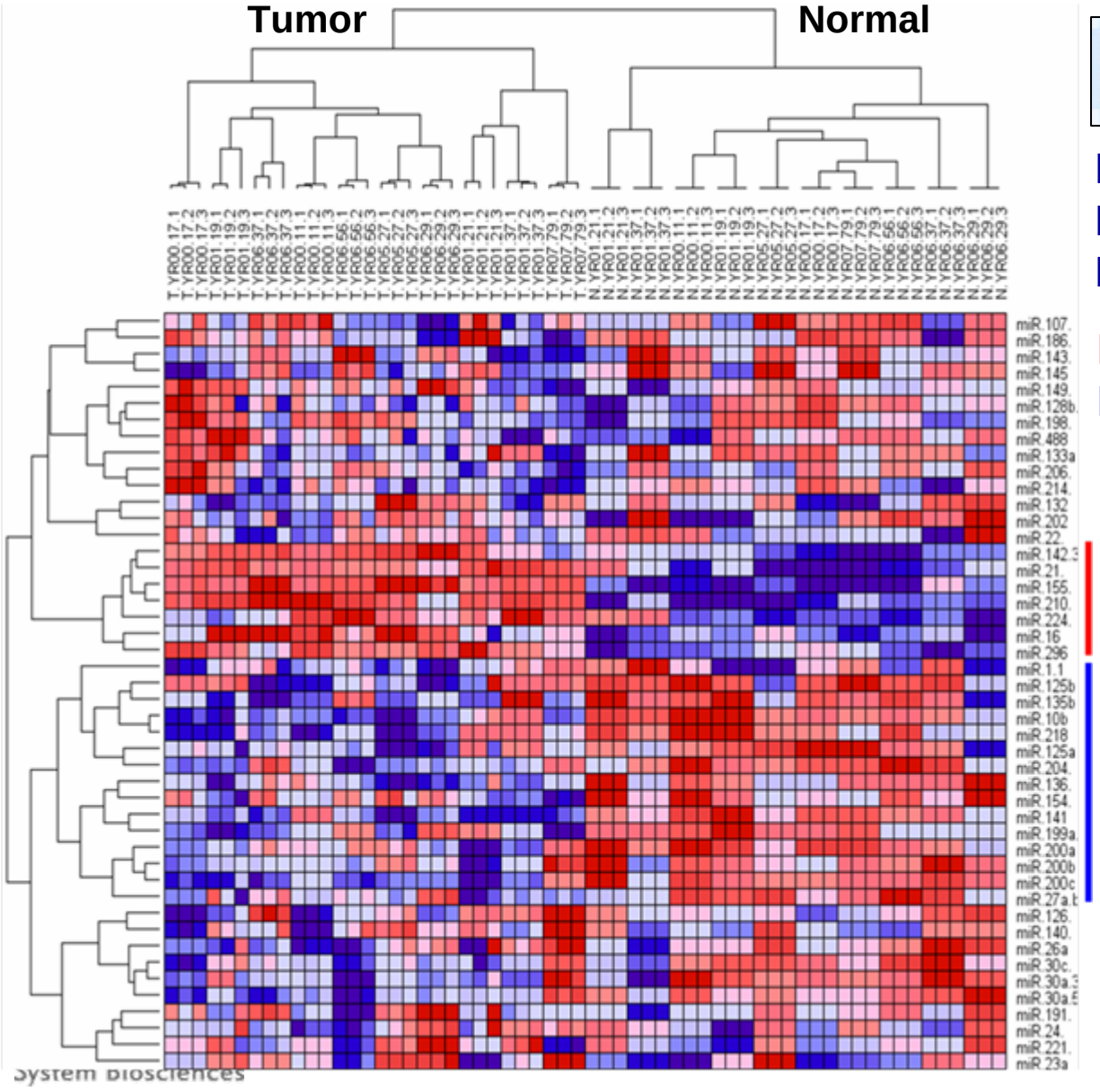
Heatmap View of Study Data



GenePattern

MicroRNA expression Is able to classify normal Kidney from Tumor

Red = high expression

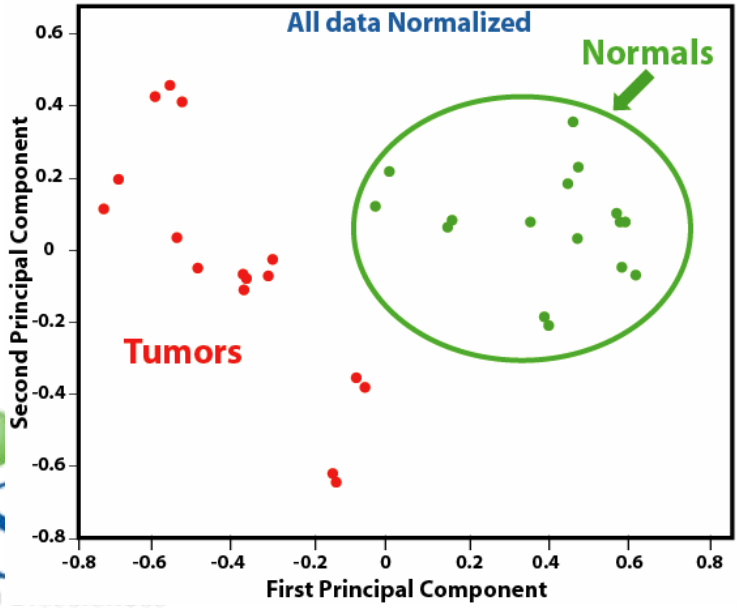
Blue= low expression

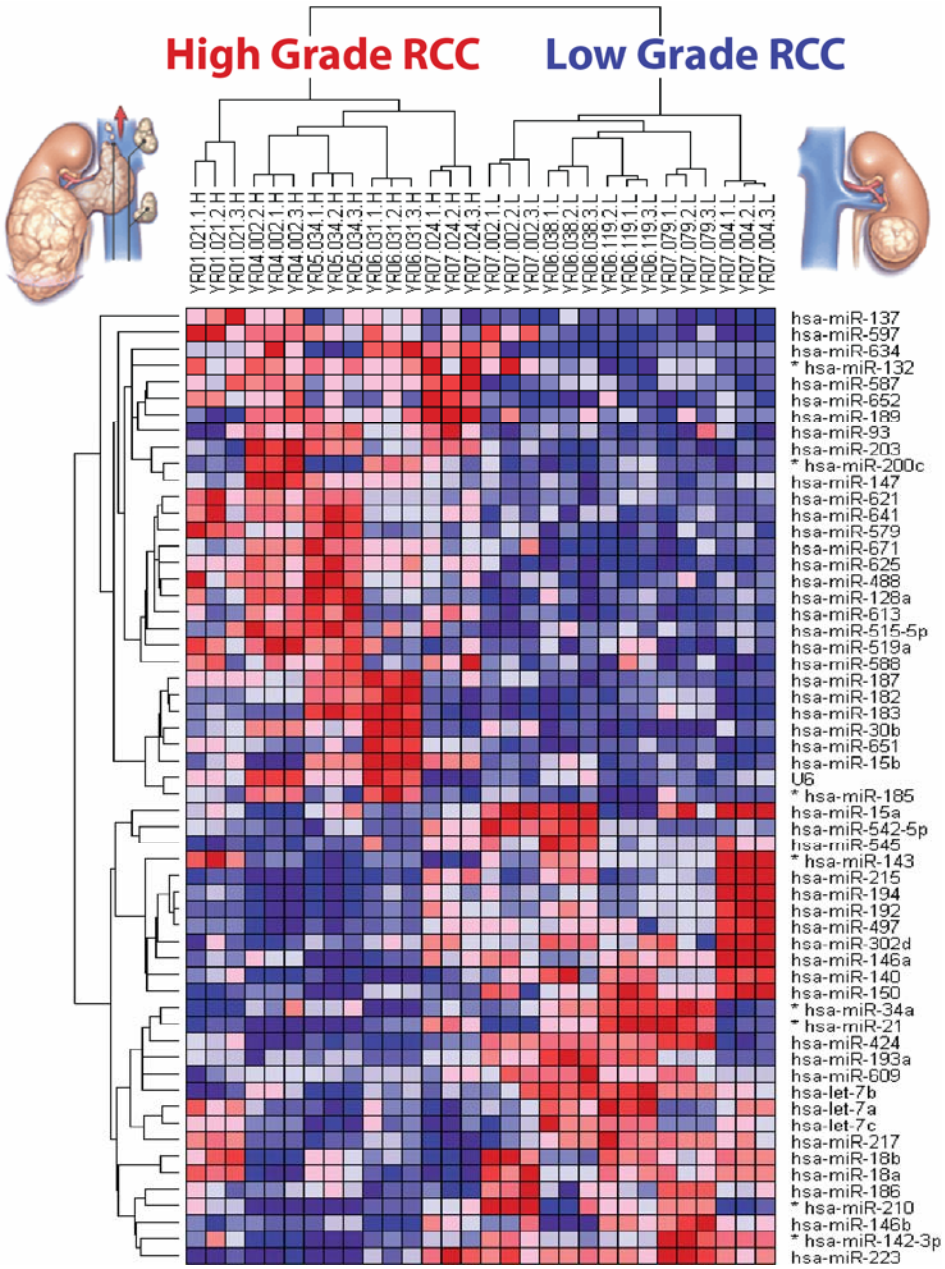
Heat map comparing ratios of expression in replicate tumor and normal samples using miR-106b as reference using the 38 down-regulated and the top 7 up-regulated microRNA. Note that the tumors seem to split into two clusters which have the samples **YR00.17**, **YR01.19**, YR06.37, **YR00.11**, YR06.56, **YR05.27, YR06.29** in the first group and the samples **YR01.21**, **YR01.37**,YR07.79 in the second group. The bold samples were scored as High Grade by pathological analysis. **Thus the natural clustering of the tumors by microRNA expression does not seem to assort them by pathological grade.** The normal samples on the other hand show no discernable organized sub-structure for these microRNA.

http://www.broad.mit.edu/cancer/software/genepattern/

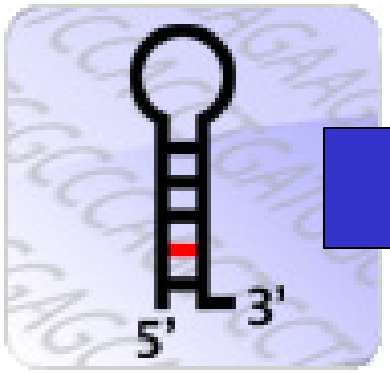
MicroRNA Signatures Stratify Stage of Kidney Cancer

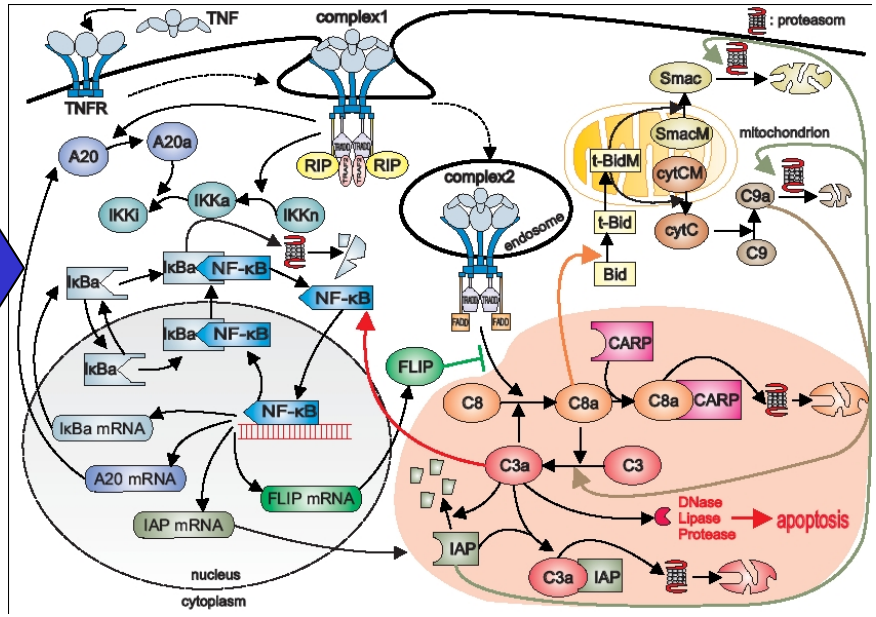
MicroRNA Signatures Stratify RCC samples from Normal samples with High Confidence





What can microRNA expression profiling tell us about the molecular pathways affected ?





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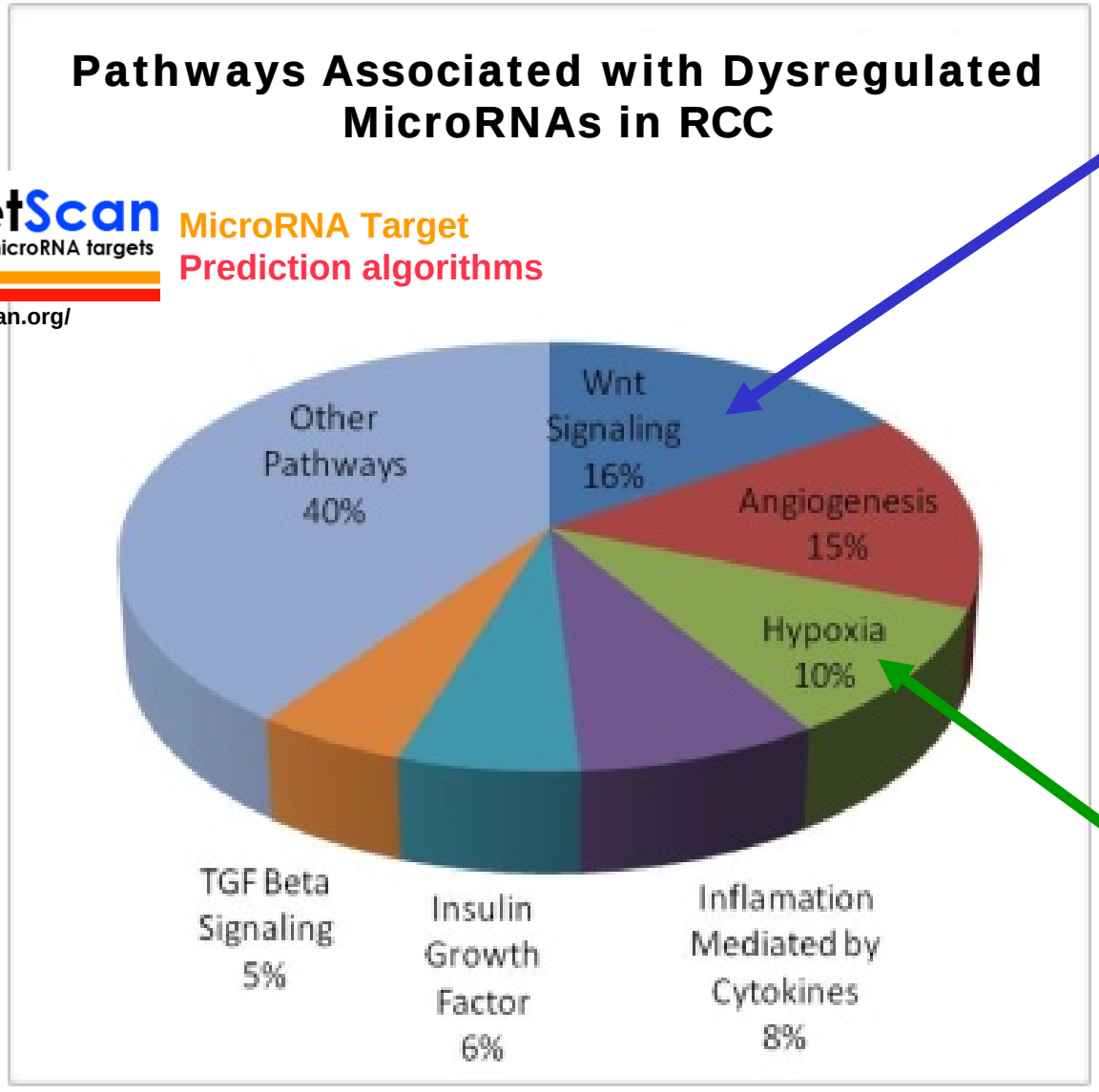
Potential Targets of Kidney Cancer MicroRNAs



TargetScan
Prediction of microRNA targets

MicroRNA Target Prediction algorithms

http://www.targetscan.org/

Already thought to be involved in kidney cancer

Ectopic Expression of Wnt-5a in Human Renal Cell Carcinoma Cells Suppresses in vitro Growth and Telomerase Activity.
Daniel J. Olsona, Mitsuo Oshimurab, Arie P. Ottec, Rakesh Kumard.
Tumor Biology 1998;19:244-252

Hypoxia observed in RCC kidneys

Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. George V Thomas Chris Tran Ingo K Mellinghoff Derek S Welsbie Emily Chan Barbara Fueger Johannes Czernin & Charles L Sawyers
Nature Medicine 12, 122 - 127 (2006).

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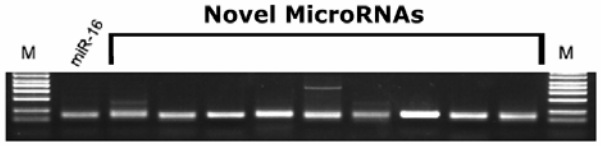
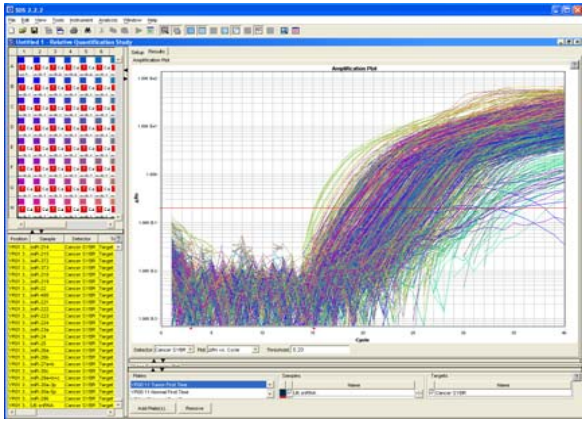
MicroRNA qPCR Arrays

QuantiMir
Small RNA Quantitation System

Design your own microRNA assays



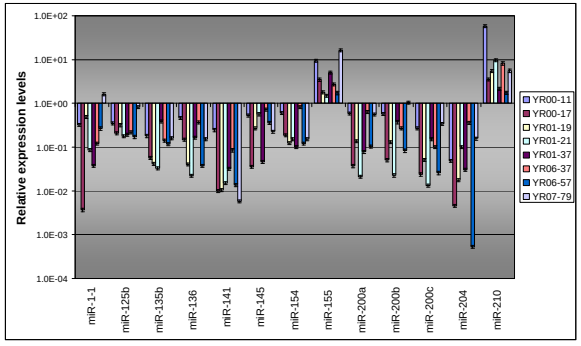


The OncoMir Collection

Preformatted Cancer microRNA assays –
most commonly found microRNAs in carcinogenesis

Plate Array Arrangement

	1	2	3	4	5	6	7	8	9	10	11	12
A	let-7 family	miR-7	miR-92	miR-93	miR-9-1	miR-101-1	miR-103	miR-106a	miR-106b	miR-107	miR-10b	miR-1-1
B	miR-122a	miR-125a	miR-125b	miR-126	miR-128b	miR-132	miR-133a	miR-134	miR-135b	miR-136	miR-137	miR-140
C	miR-141	miR-142-3p	miR-143	miR-145	miR-146a	miR-149	miR-150	miR-151	miR-153	miR-154	miR-155	miR-15a
D	miR-15b	miR-16	miR-17-5p	miR-17-5p	miR-181a	miR-181b	miR-181c	miR-181d	miR-183	miR-185	miR-186	miR-188
E	miR-18a	miR-190	miR-191	miR-192	miR-194	miR-195	miR-196a	miR-197	miR-198	miR-199a+b	miR-30b	miR-19a+b
F	miR-95	miR-20a	miR-200a	miR-200b	miR-200c	miR-202	miR-203	miR-204	miR-205	miR-206	miR-21	miR-210
G	miR-214	miR-215	miR-372	miR-373	miR-218	miR-219	miR-22	miR-488	miR-221	miR-222	miR-223	miR-224
H	miR-23a	miR-24	miR-25	miR-26a	miR-26b	miR-27a+b	miR-30c	miR-29a+b+c	miR-30a-3p	miR-30a-5p	miR-296	U6 snRNA



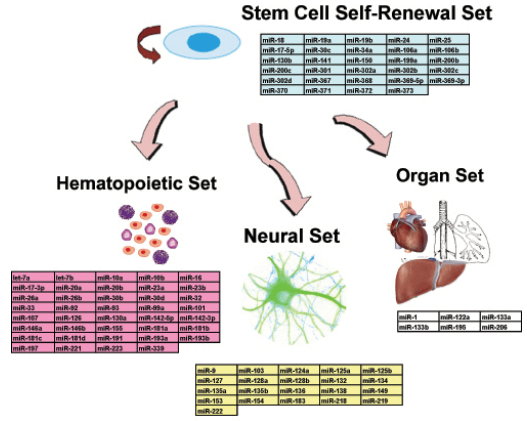
The Stem Cell Collection

Preformatted Differentiation microRNA assays –
Monitor renewal, hematopoiesis, neural progression and tissue-specific patterning

Convenient 96-well Plate Format, segmented into 4 separate Stem Cell Pathways

	1	2	3	4	5	6	7	8	9	10	11	12
A	miR-18	miR-19a	miR-19b	miR-24	miR-25	miR-17-5p	miR-30c	miR-34a	miR-106a	miR-106b	miR-130b	miR-141
B	miR-150	miR-199a	miR-200b	miR-200c	miR-301	miR-302a	miR-302b	miR-302c	miR-302d	miR-367	miR-368	miR-369-5
C	miR-369-3p	miR-370	miR-371	miR-372	miR-373	let-7a	let-7b	miR-19a	miR-19b	miR-16	miR-17-3p	miR-20a
D	miR-20b	miR-23a	miR-23b	miR-24a	miR-26b	miR-30b	miR-30d	miR-32	miR-33	miR-92	miR-93	miR-99a
E	miR-101	miR-107	miR-126	miR-130a	miR-142-5p	miR-142-3p	miR-146a	miR-146b	miR-155	miR-181a	miR-181b	miR-181c
F	miR-181d	miR-191	miR-192a	miR-193b	miR-197	miR-221	miR-222	miR-339	miR-9	miR-103	miR-124a	miR-125a
G	miR-125b	miR-127	miR-128a	miR-128b	miR-132	miR-134	miR-135a	miR-135b	miR-136	miR-138	miR-149	miR-153
H	miR-154	miR-183	miR-218	miR-219	miR-222	miR-1	miR-122a	miR-133a	miR-133b	miR-195	miR-206	U6

Stem Cell Signature Set
Neuronal Set
Hematopoietic Set
Organ Set



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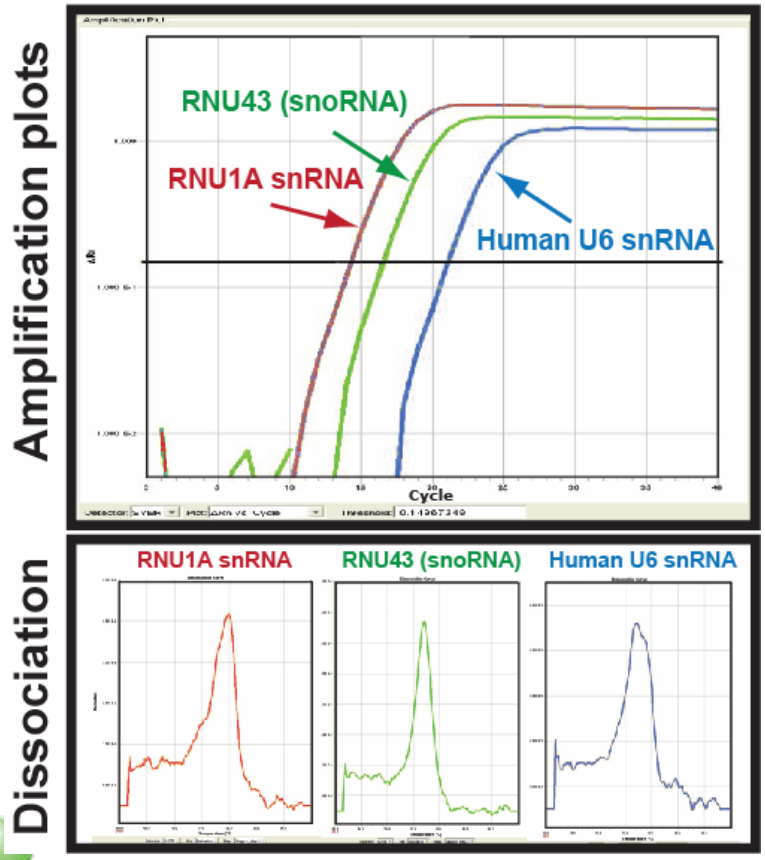
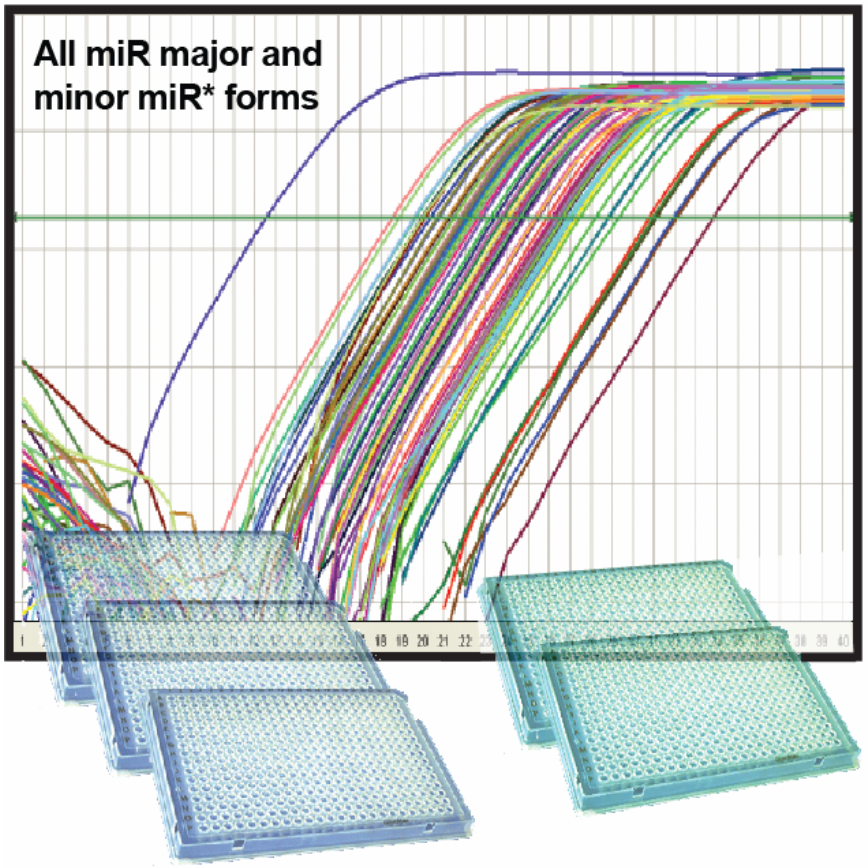
Pre-designed microRNA Profiling Panels

MicroRNA miRNome Profilers

Validated reference controls



100% miRBase updated



Human complete set
All microRNAs

Mouse complete set
All microRNAs

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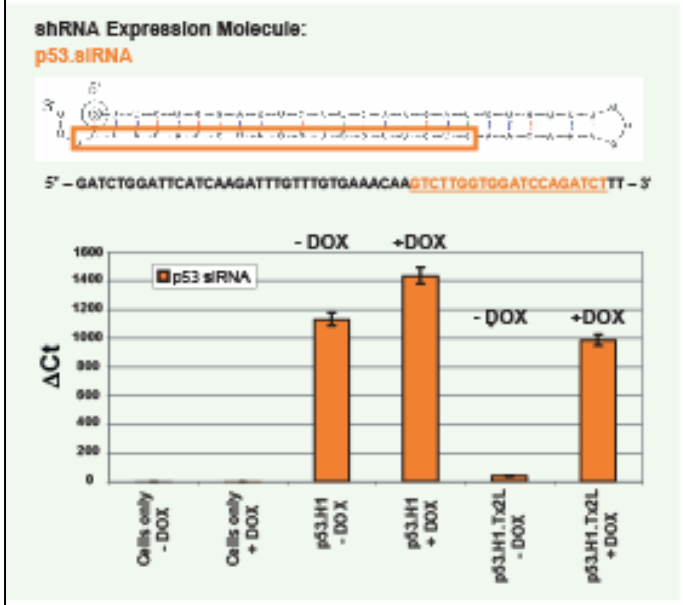
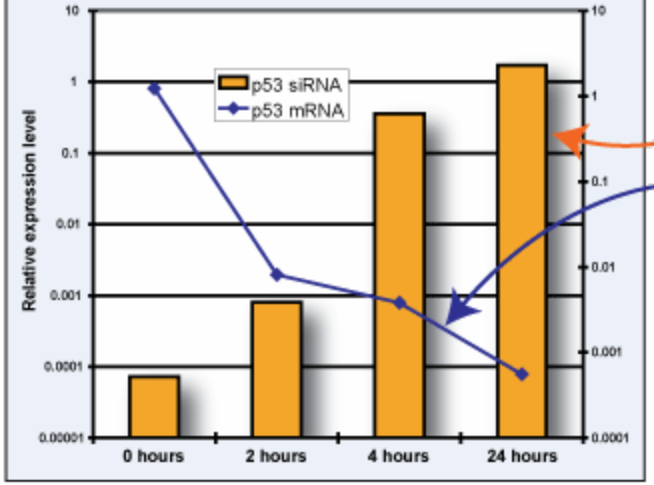
Other QuantiMir Applications

Measure siRNA expression from Knockdown experiments

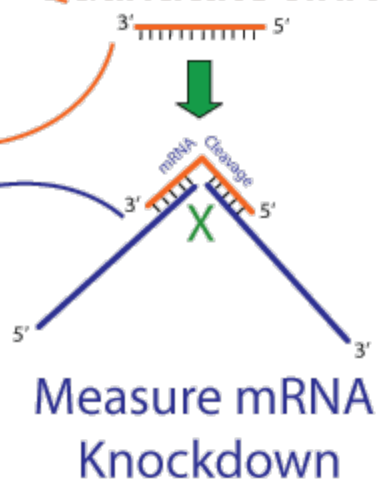


Example of siRNA detection and quantitation from short hairpin RNA expression constructs.

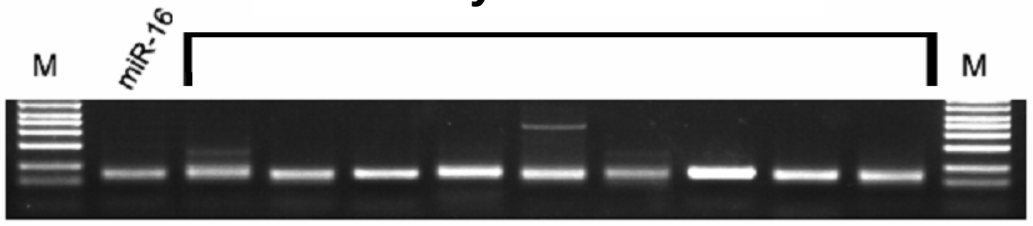
Quantitate Gene Knockdown and siRNA in same cDNA



From the same cDNA:



Gel analysis of siRNAs



QuantiMir
Small RNA Quantitation System

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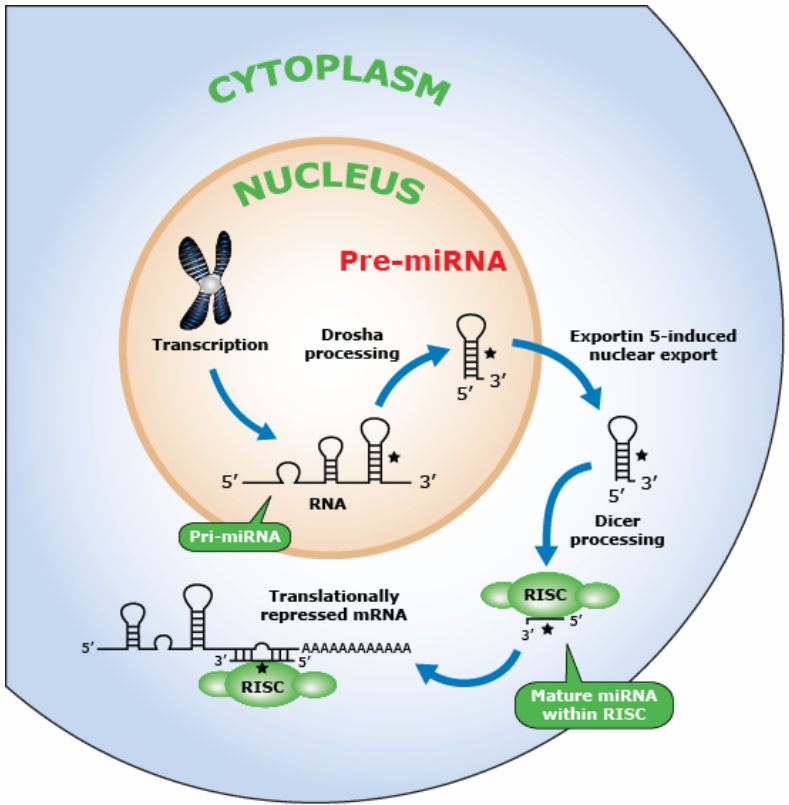
Discovery & Cloning

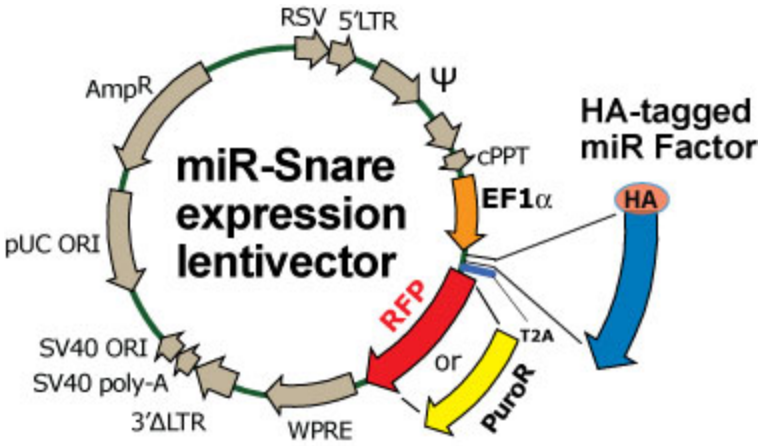


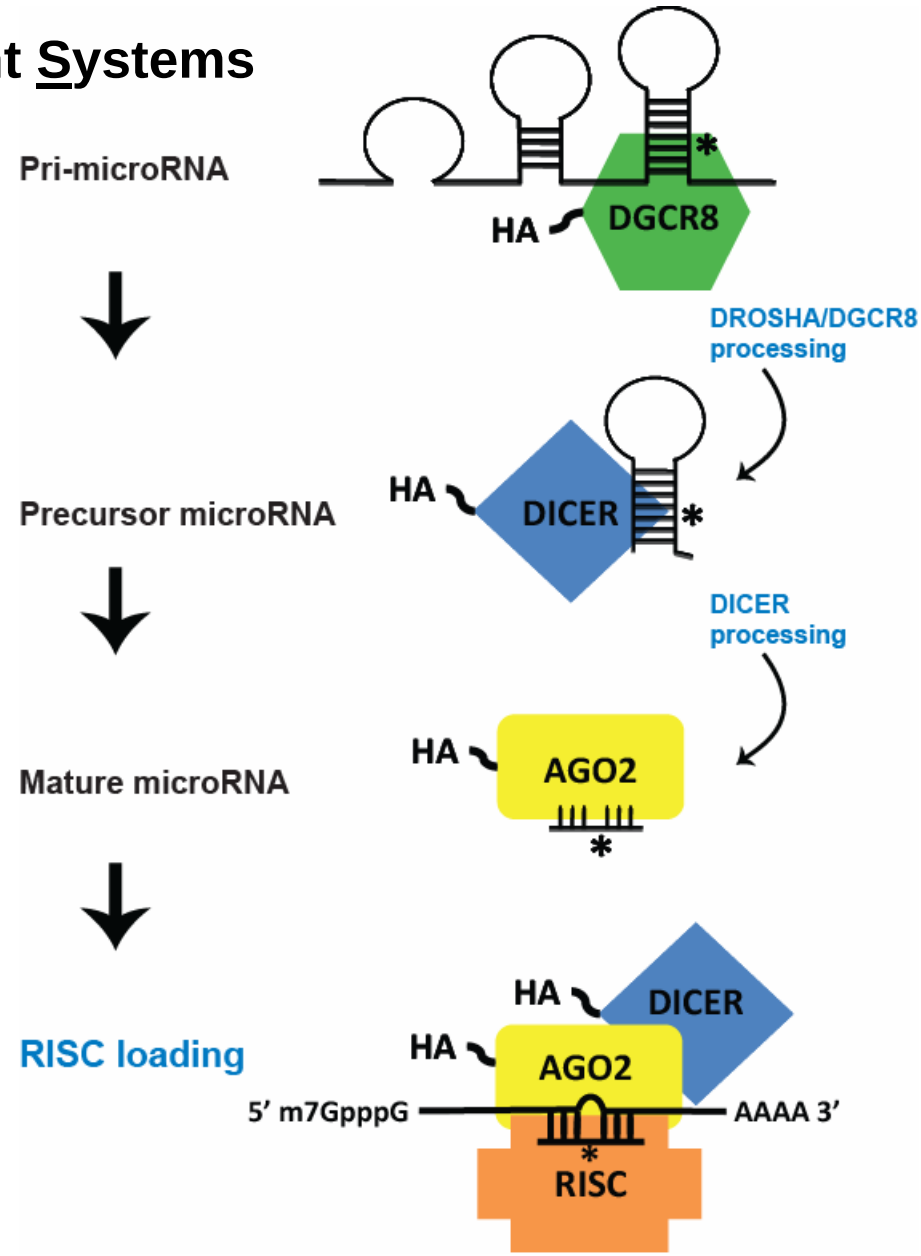
- **MicroRNA “Snares”**
- **Global Amplify & Clone**

miR-SNaRES

Small Non-coding RNA Enrichment Systems

Immunopurify microRNA complexes and their associated RNAs





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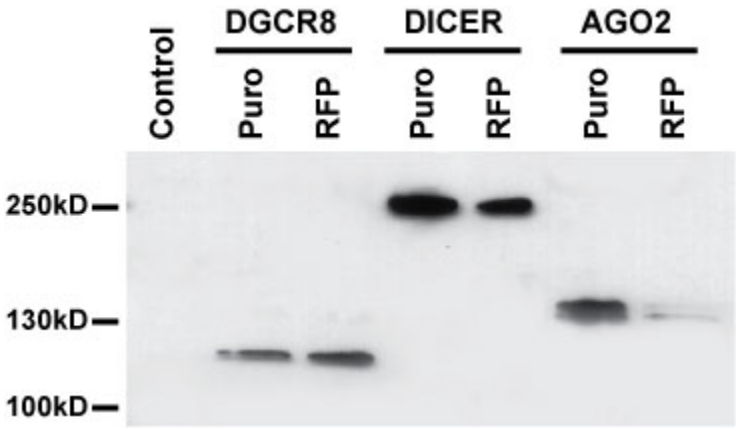
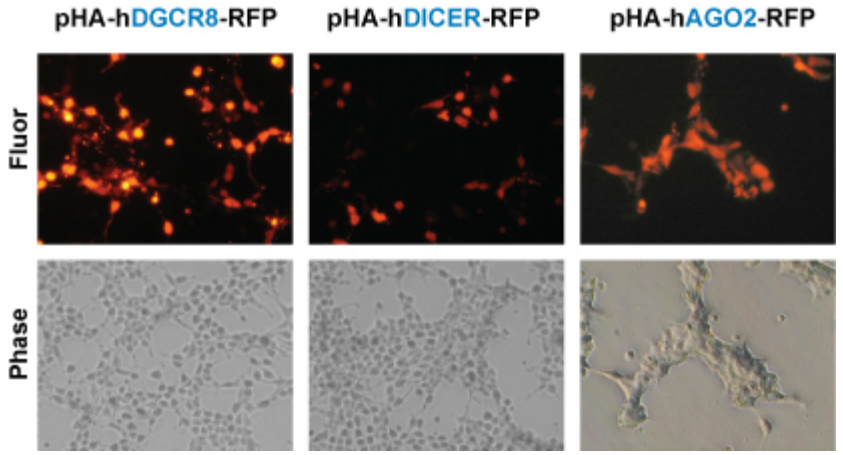
miR-SNaRES

Small Non-coding RNA Enrichment Systems

- **Create SNaRE Cell line**
- **IP pull-down MicroRNAs**
- **Enrich for RISC-associated mRNAs**
- **Discover low abundance microRNAs**
- **Identify new RISC protein factors**
- **Constructs fully sequence-verified and protein expression validated**





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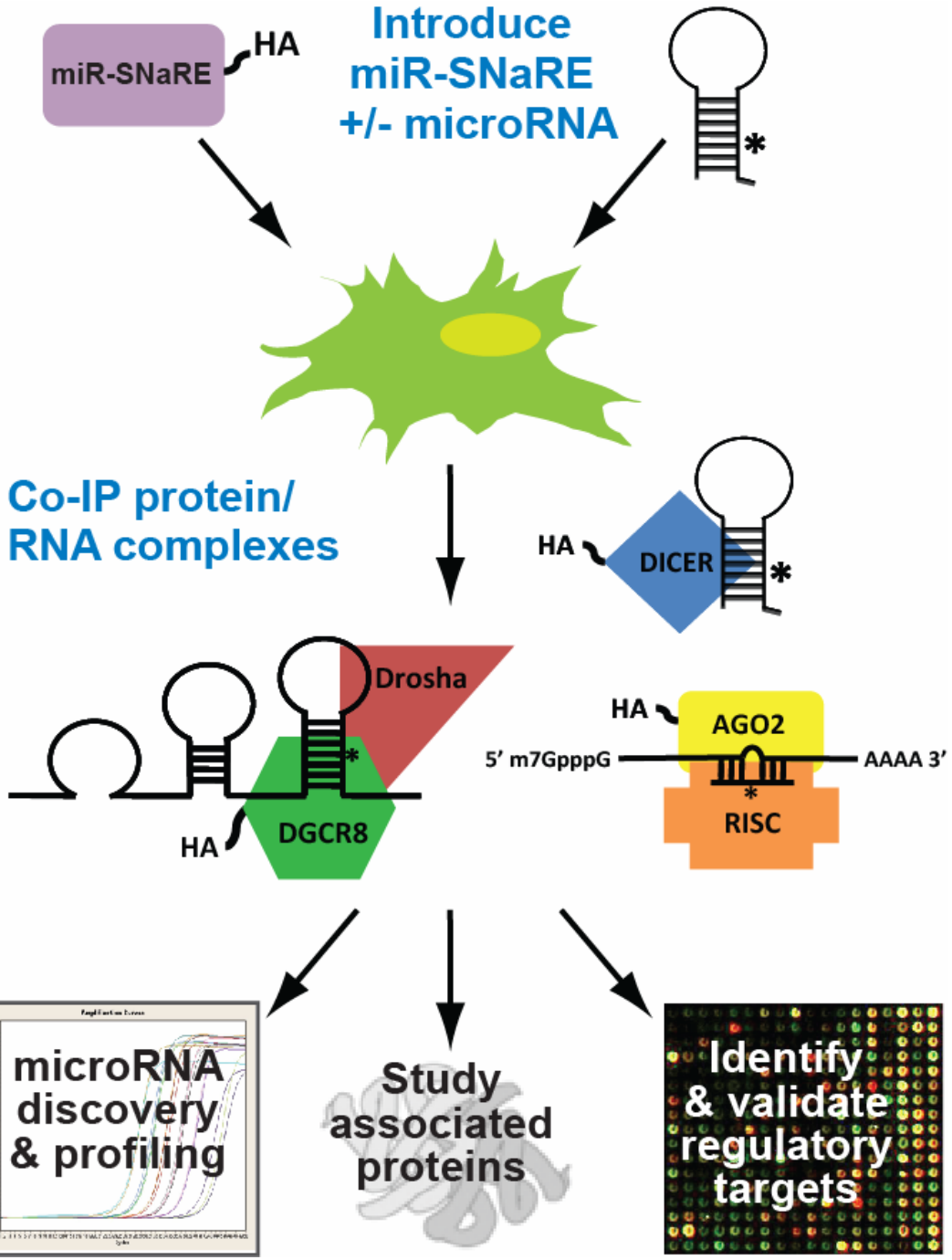
miR-SNaRE Applications



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miR-SNaRE Applications

OPEN ACCESS Freely available online

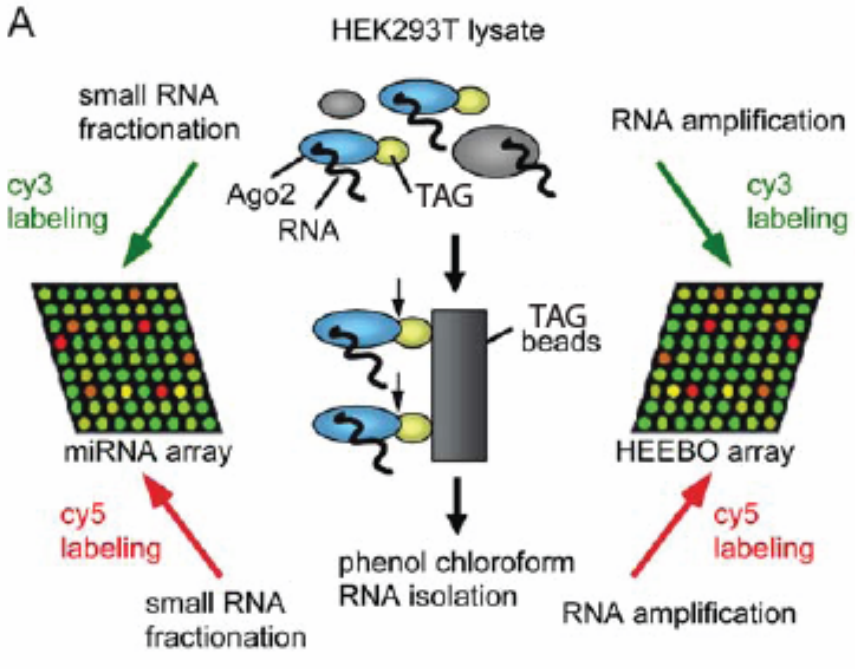
PLoS one

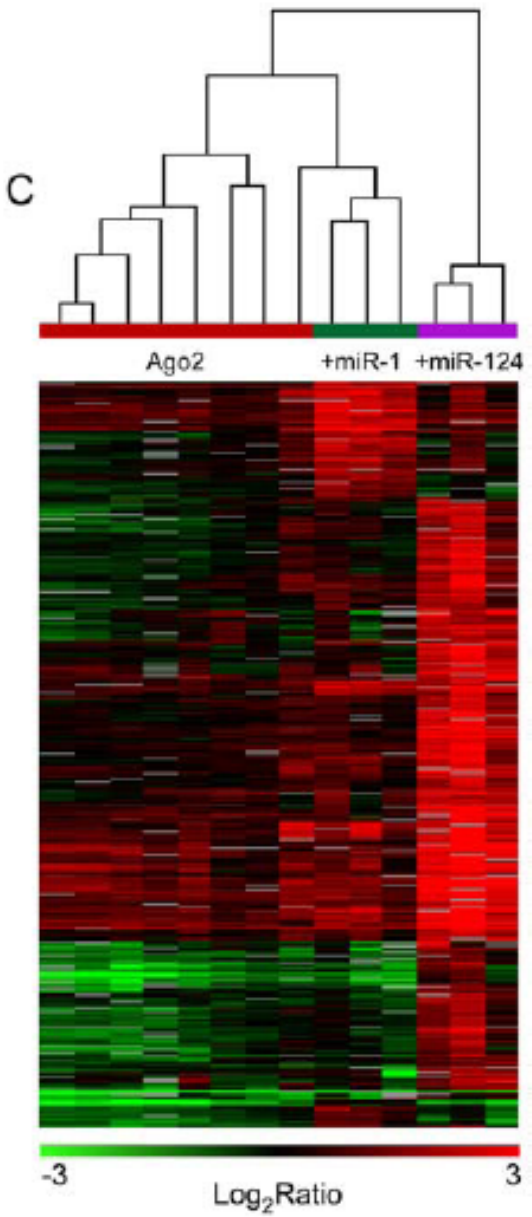
Systematic Identification of mRNAs Recruited to Argonaute 2 by Specific microRNAs and Corresponding Changes in Transcript Abundance

David G. Hendrickson[1☯], Daniel J. Hogan[2,3☯], Daniel Herschlag[2*], James E. Ferrell[1,2], Patrick O. Brown[2,3*]

1 Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California, United States of America, 2 Department of Biochemistry, Stanford University School of Medicine, Palo Alto, California, United States of America, 3 Howard Hughes Medical Institute, Stanford University School of Medicine, Palo Alto, California, United States of America

May 2008





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miR-SNaRE Applications

Look at all of these new miR-124 mRNA targets !

OPEN ACCESS Freely available online

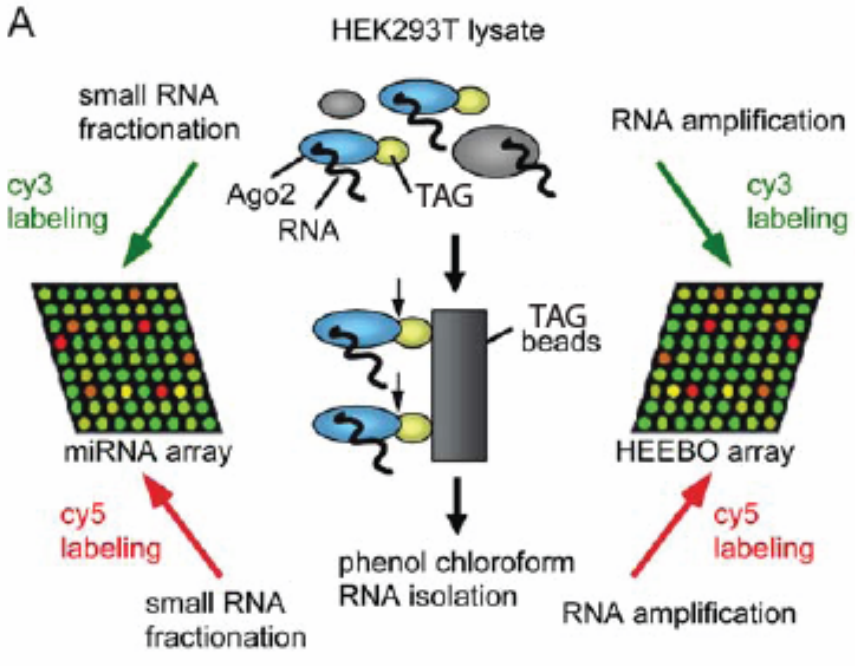
PLoS one

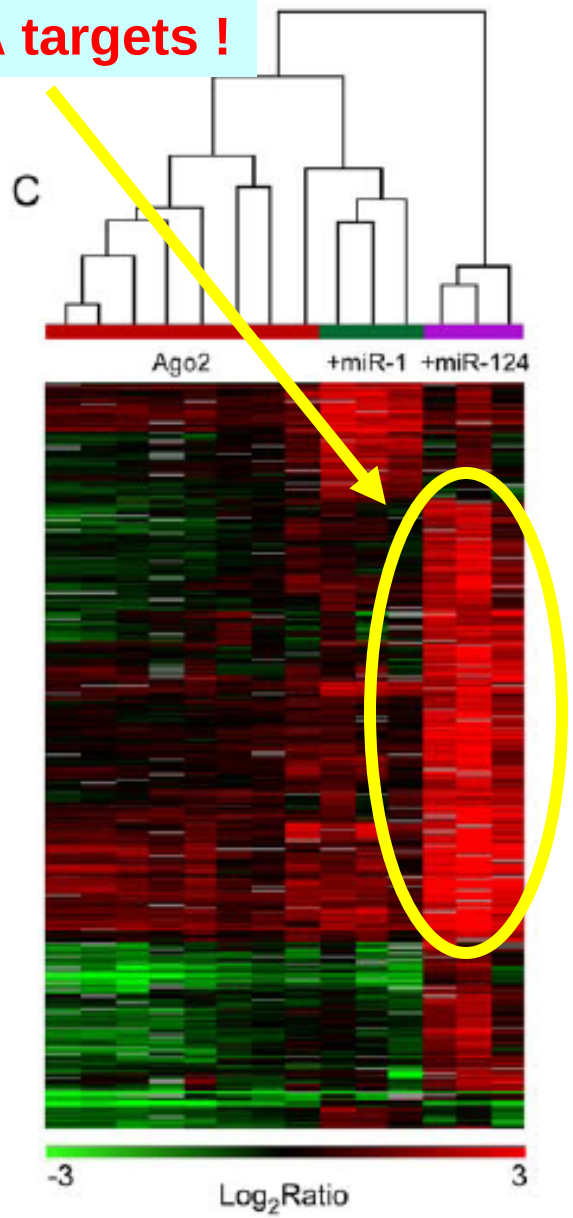
Systematic Identification of mRNAs Recruited to Argonaute 2 by Specific microRNAs and Corresponding Changes in Transcript Abundance

David G. Hendrickson[1☯], Daniel J. Hogan[2,3☯], Daniel Herschlag[2*], James E. Ferrell[1,2], Patrick O. Brown[2,3*]

1 Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California, United States of America, 2 Department of Biochemistry, Stanford University School of Medicine, Palo Alto, California, United States of America, 3 Howard Hughes Medical Institute, Stanford University School of Medicine, Palo Alto, California, United States of America

May 2008





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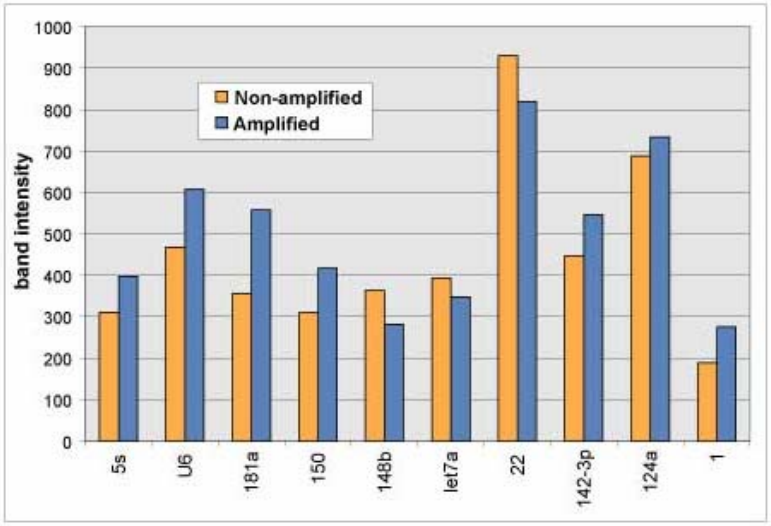
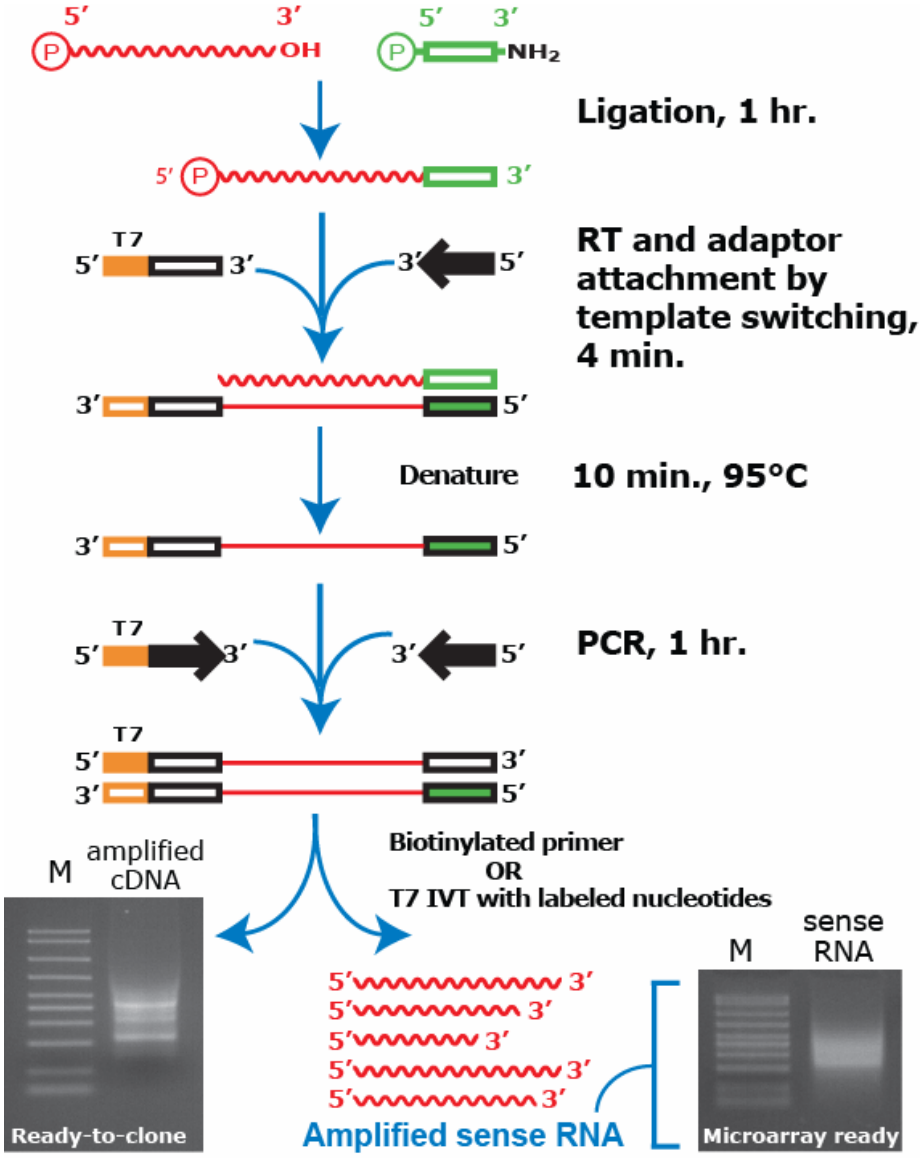
Global MicroRNA Amplification and Cloning





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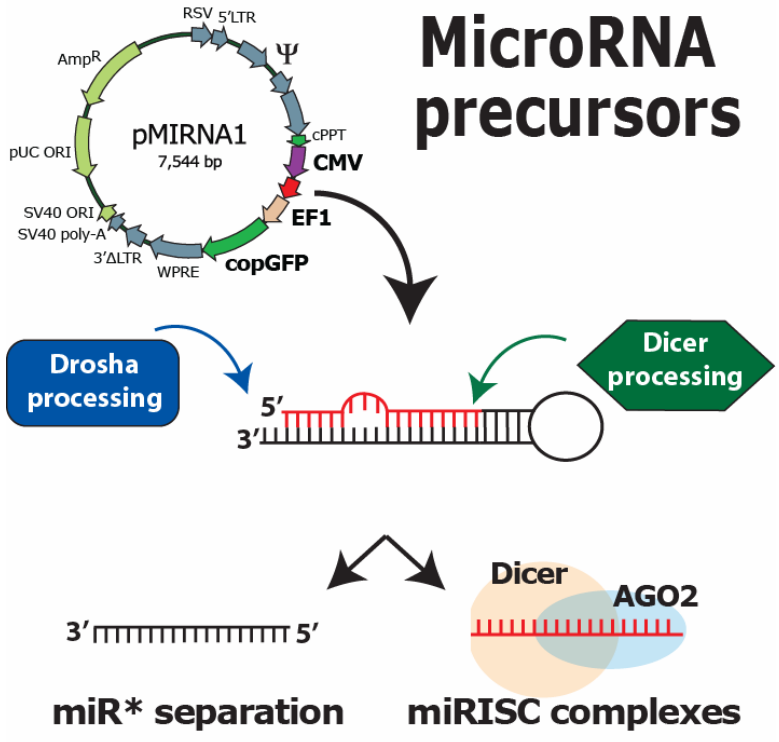
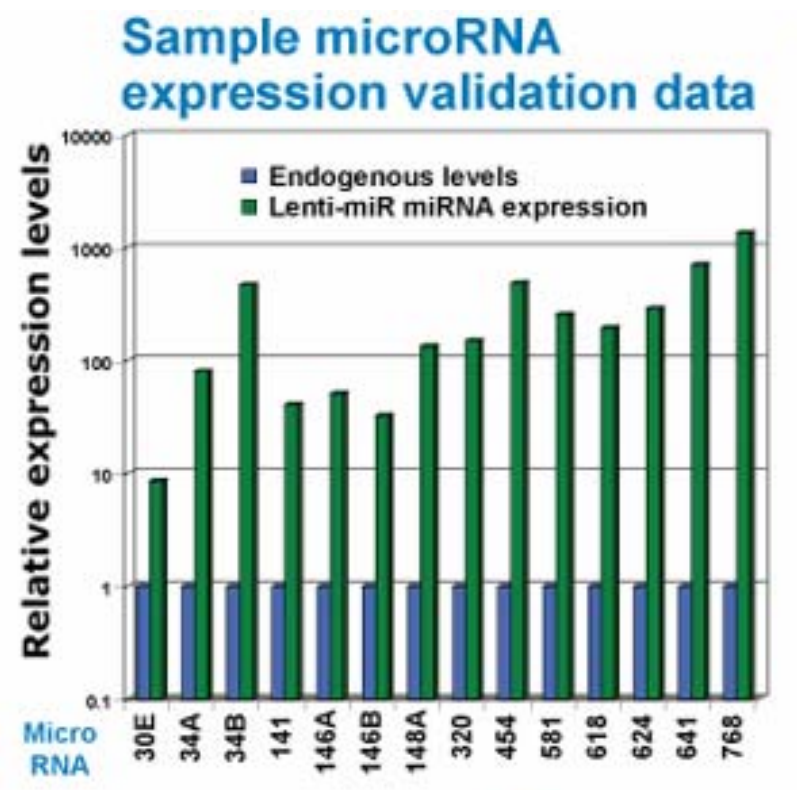
Overexpression Studies



- MicroRNA Precursor Clone Collection
- Multiplexed Precursor Virus Library

Lenti-miR Precursor Clones

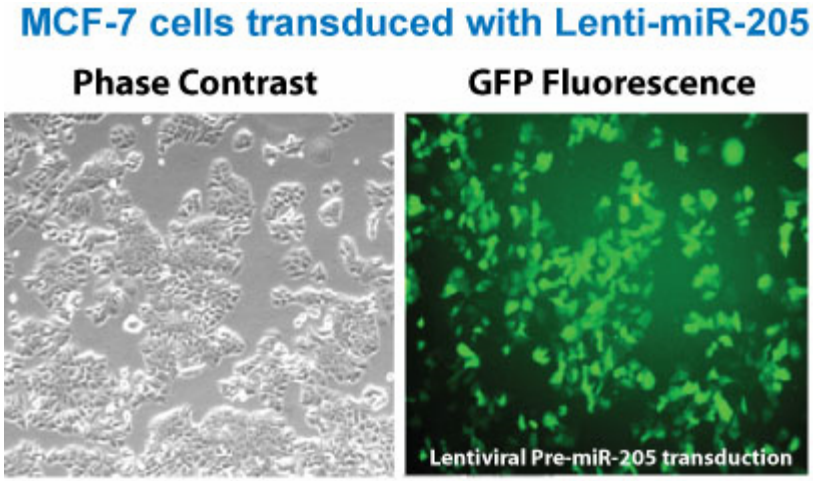
Transfection Overexpression

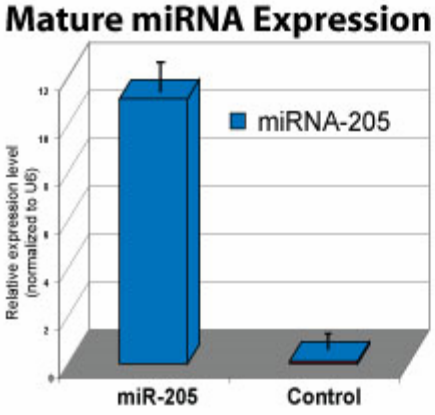


Lenti-miR constructs transfected into HEK 293 cells. MicroRNA expression measured using qPCR.

QuantiMir Small RNA Quantitation System

Stable Overexpression





MCF-7 Cells infected (MOI 5:1) with Lentivirus made from PMIRH205PA-1 (pre-miR-205) construct. Images taken after 48 hours. Expression of mature miR-205 was validated and quantitated using ABI TaqMan assays.

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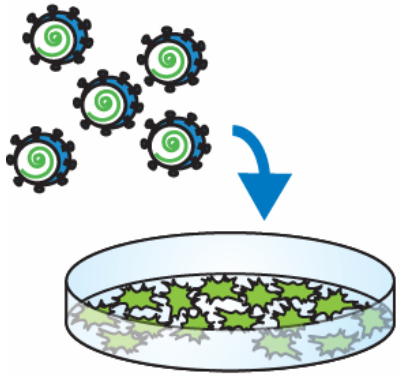
Lenti-miR Multiplexed Virus Library

HT Screens for MicroRNA Effectors

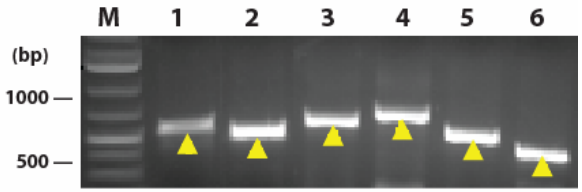
Transduce Lenti-miR Virus Library



Select for Phenotype

Tumor migration
Hematopoiesis
Metastatic potential
Apoptosis

Recover Precursors



Identify MicroRNA Effectors

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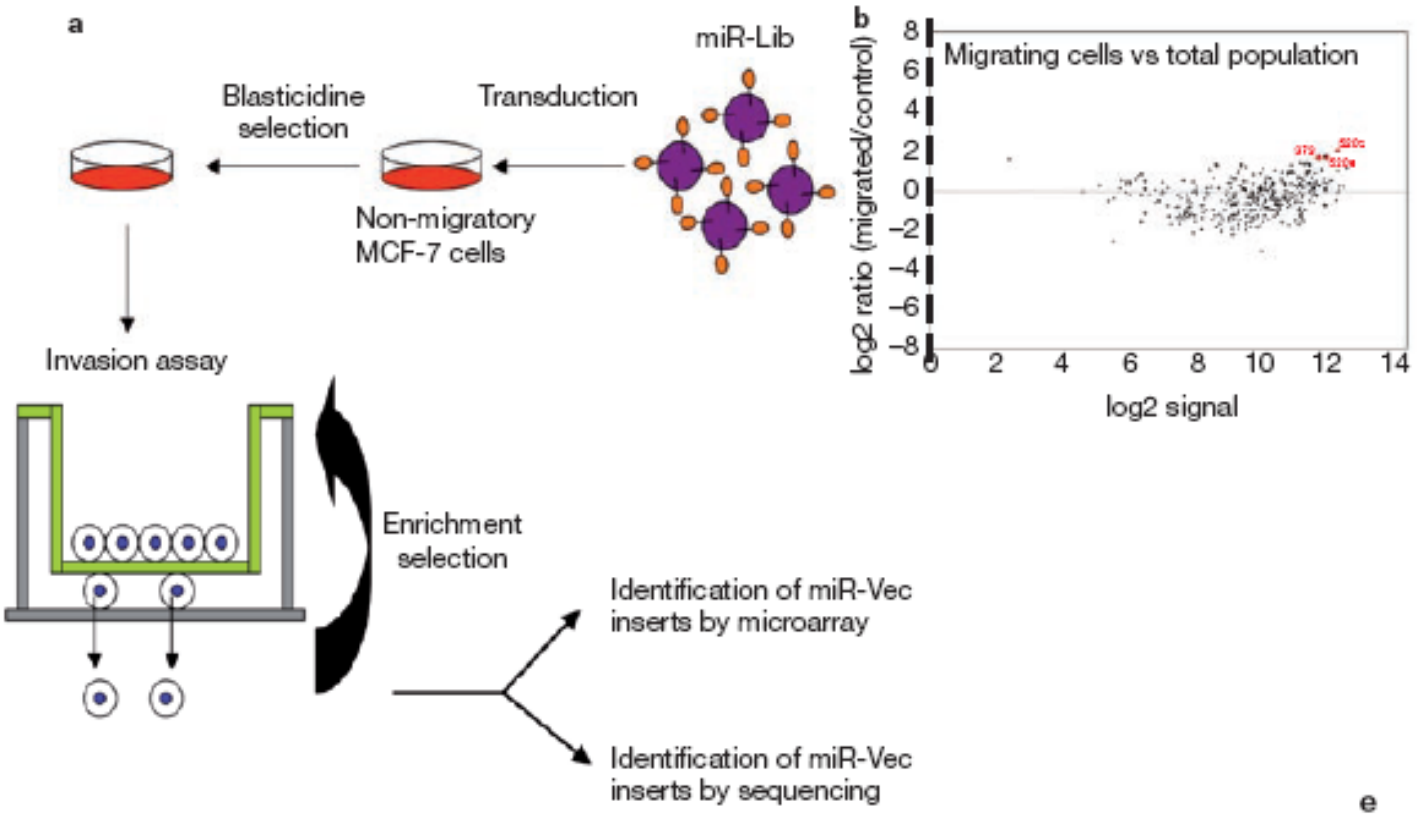
MicroRNA Virus Libraries

nature cell biology

The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis

Qihong Huang[1,10,11], Kiranmai Gumireddy[1,11], Mariette Schrier[2,11], Carlos le Sage[2], Remco Nagel[2], Suresh Nair[2], David A. Egan[3], Anping Li[1], Guanghua Huang[1], Andres J. Klein-Szanto[4], Phyllis A. Gimotty[5], Dionyssios Katsaros[6], George Coukos[7,8,9], Lin Zhang[7,8], Ellen Puré[1] and Reuven Agami[2,10]

December 2007



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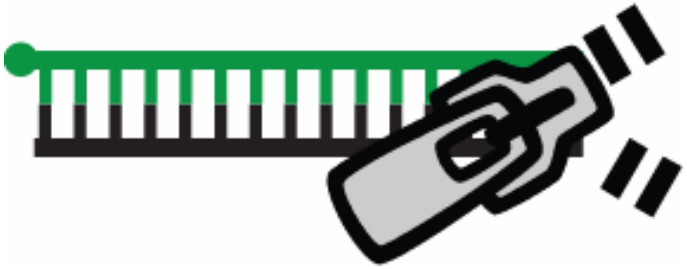
Knockdown Studies

miRZips™ permanent microRNA knockdown



- **Stable & permanent anti-microRNA expresssion**
- **Select for positive expressing cells with either GFP or Puro selection**
- **Uncover phenotypes using powerful anti-microRNA interference**

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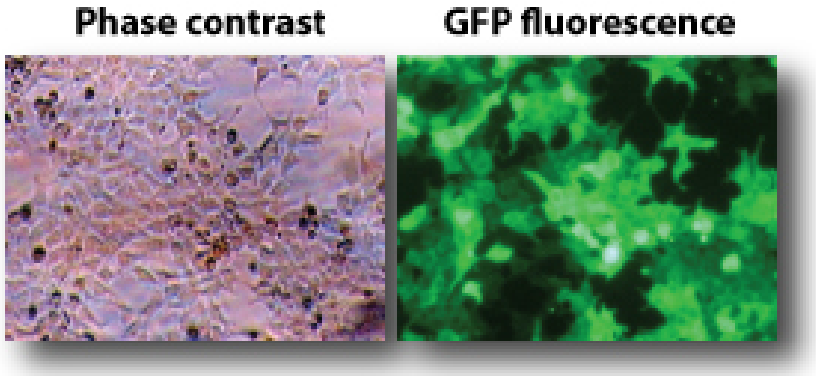
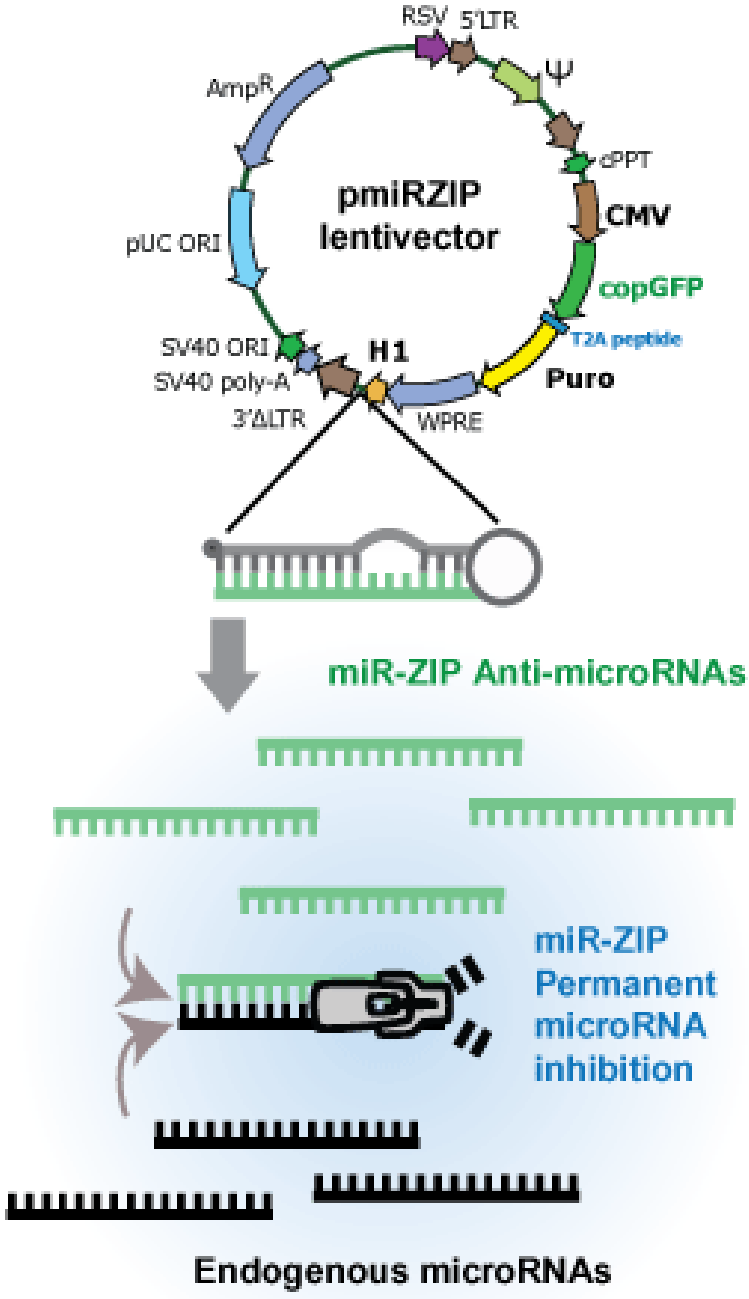
miRZips

miRZip lentivector constructs can be used for both GFP sorting and Puromycin selection for stable cell lines.





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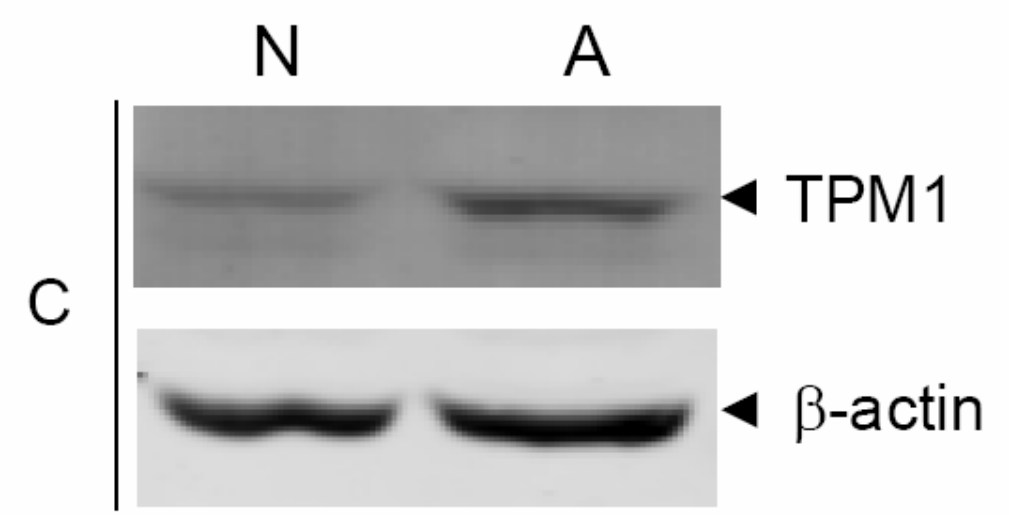
miRZips

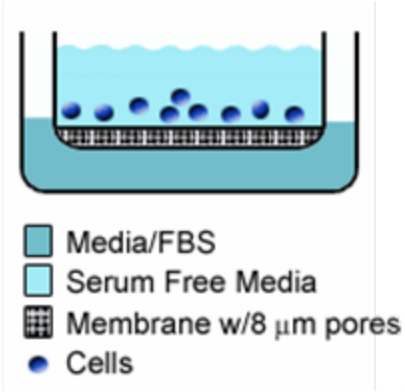
Uncover Novel Phenotypes using MicroRNA Interference

microRNA = target protein levels

TPM1 Identified as miR-21 Target

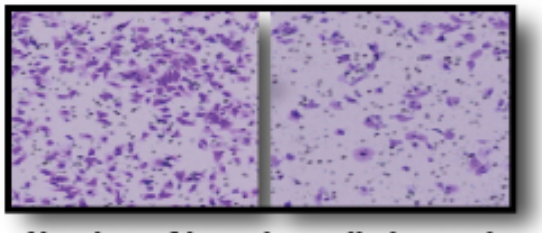


Tumor invasion assay with miRZip-21 virus

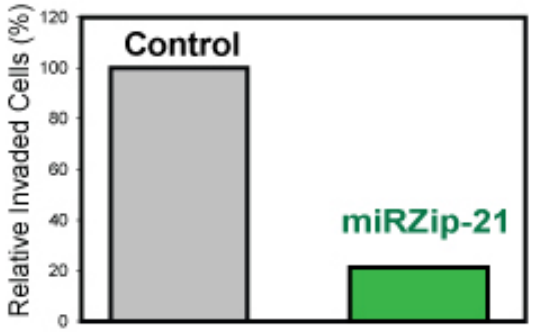


EMD

Control miRZip-21



Number of invasive cells imaged



Invasive cells reduced by 80% permanently by miRZip-21 lentivirus

MDA-MB-231 breast cancer cells

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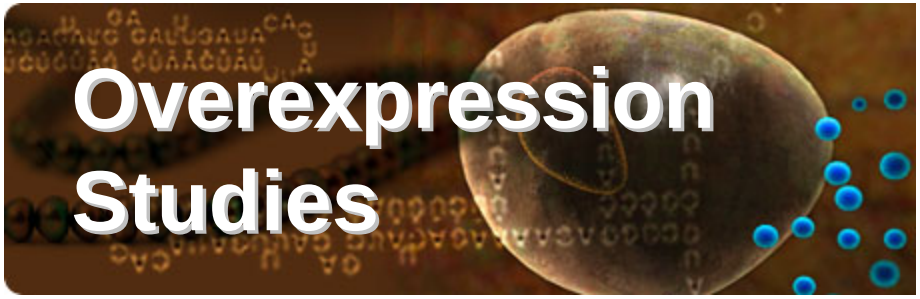
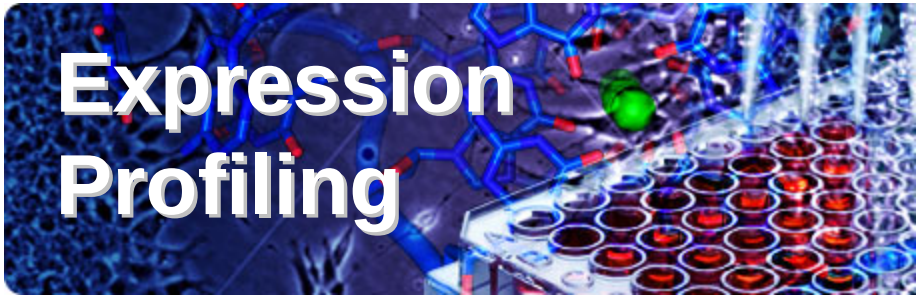
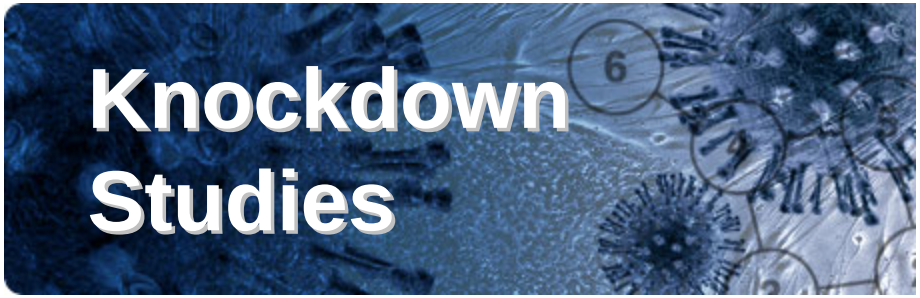
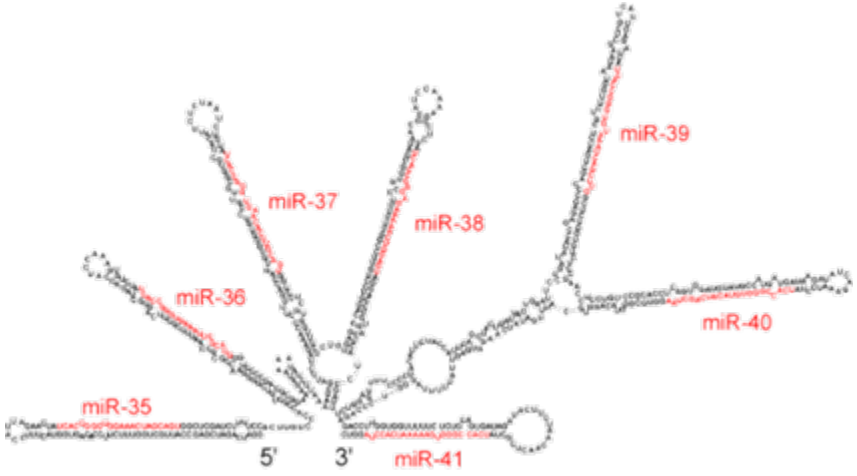
Areas of Investigation









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ASCB Booth # 516

Customer Service

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Mountain View, CA 94043

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Fax: 650-968-2277

Travis Antes, Ph.D.

Director,
Product Development
System Biosciences (SBI)

Tel: 650-968-2200 x108
Email: tantes@systembio.com

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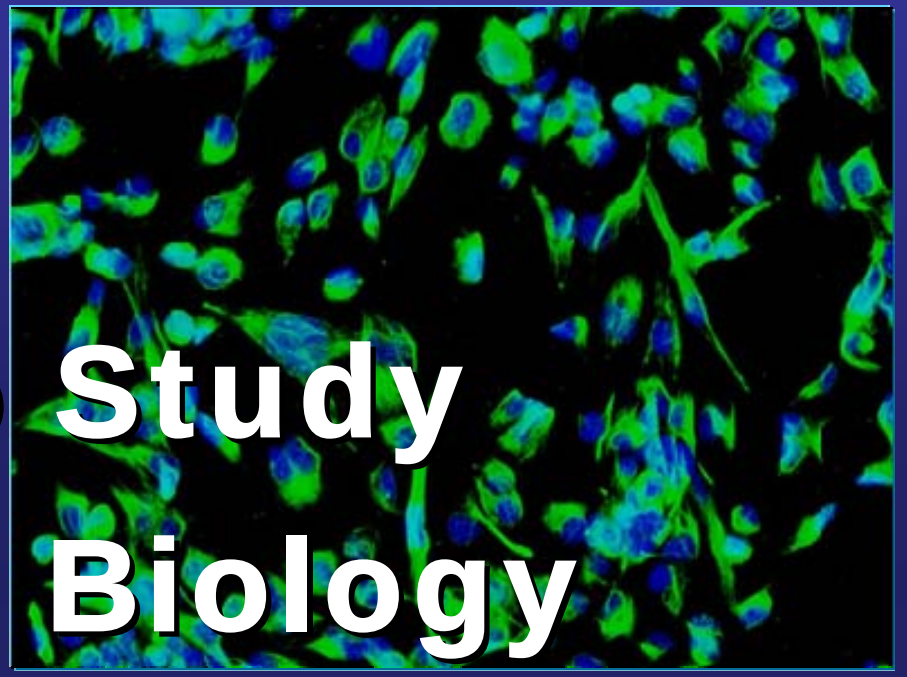
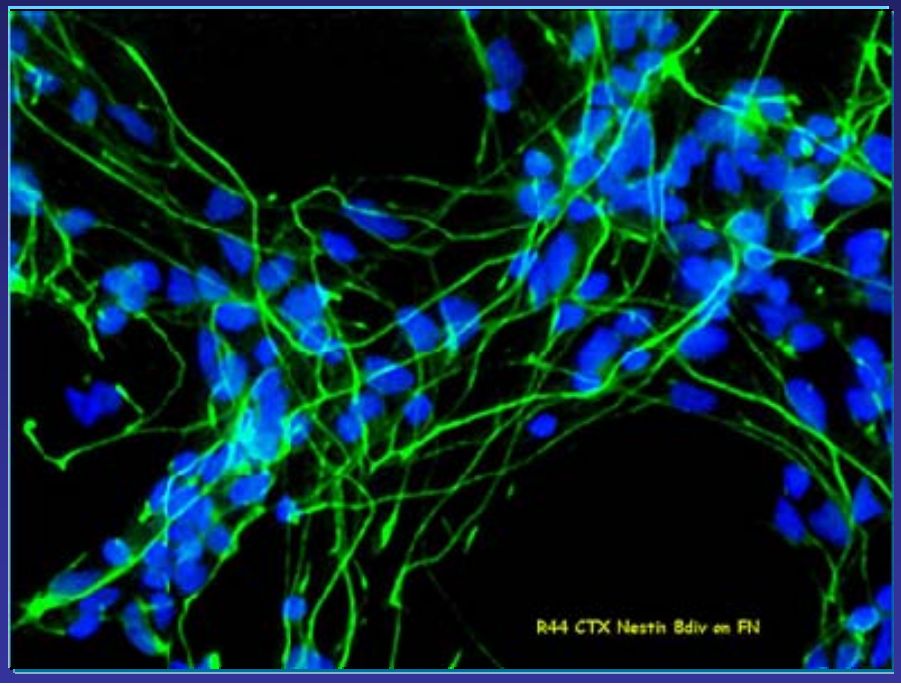


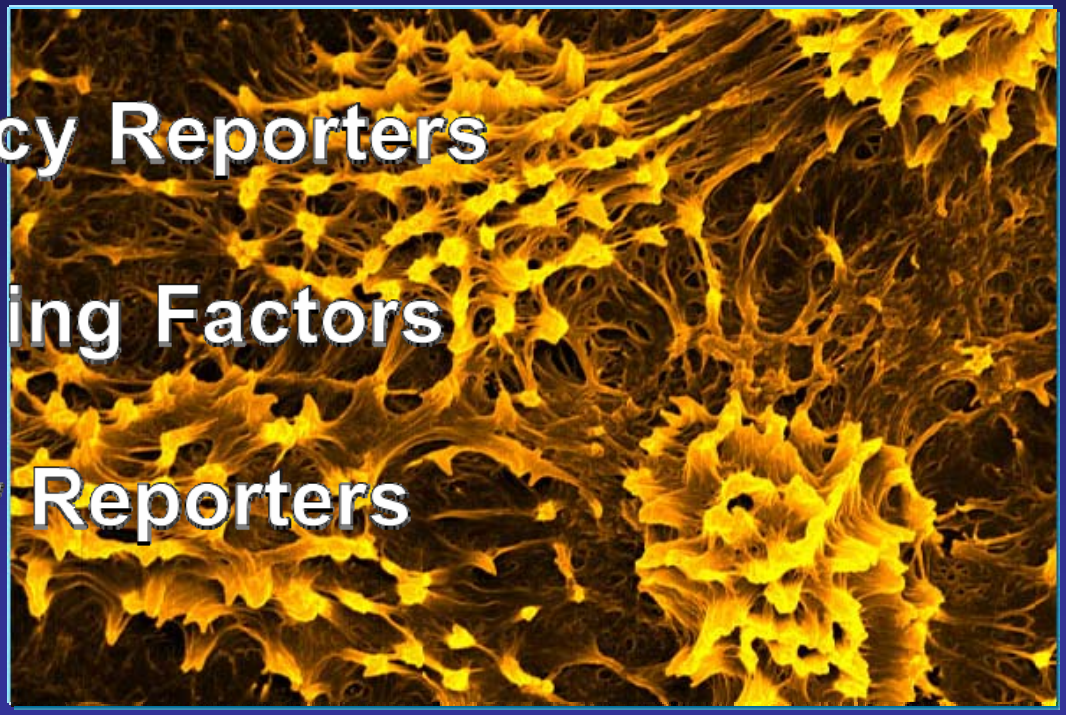
Tools to Study Stem Cell Biology



Jacob Lesnik
Assoc. Product Manager
System Biosciences

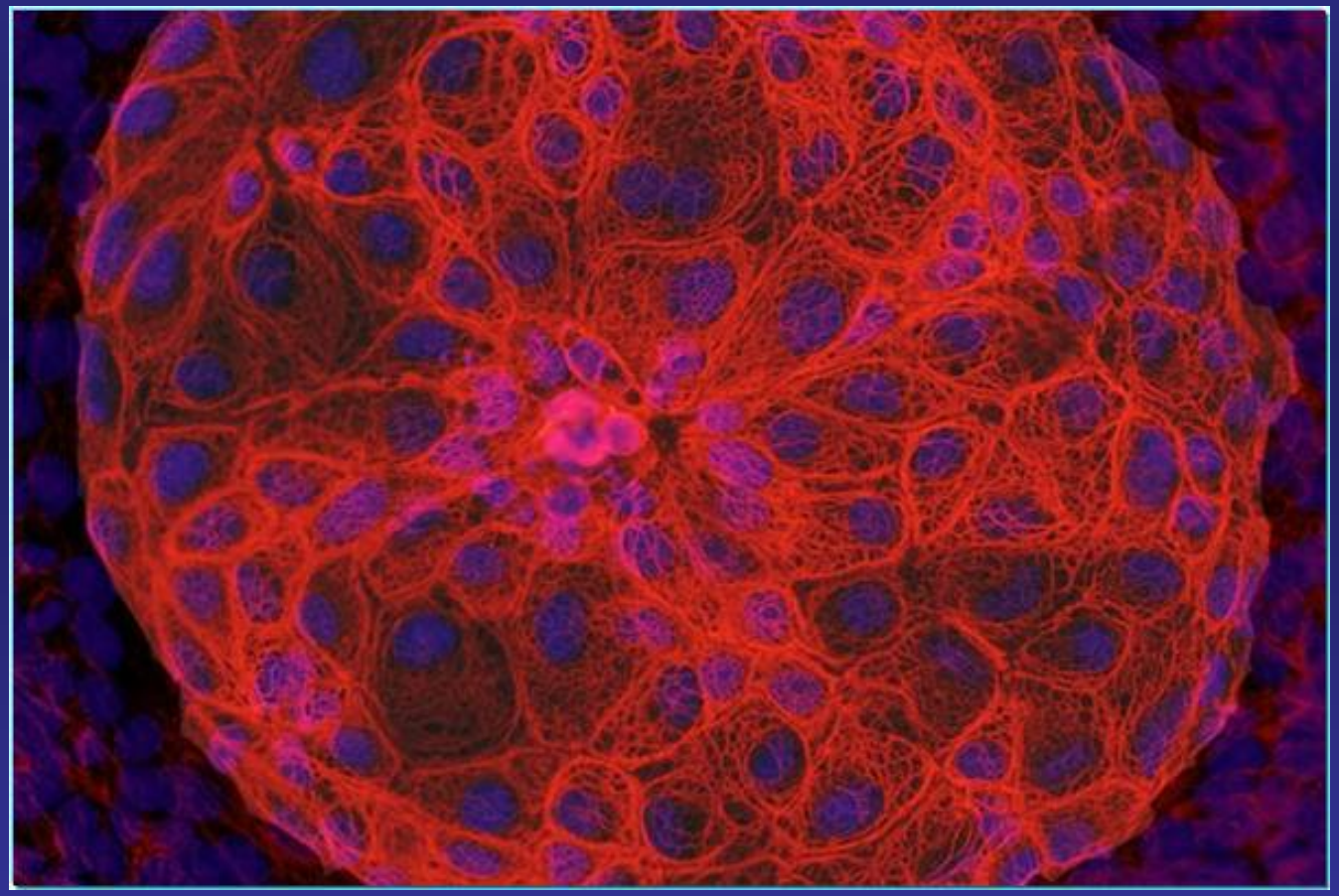
SBI's Suite of Tools for Studying Stem Cells

- Stem Cell Differentiation Reporters
- ES Cell Pluripotency Reporters
- iPSC Reprogramming Factors
- Signaling Pathway Reporters



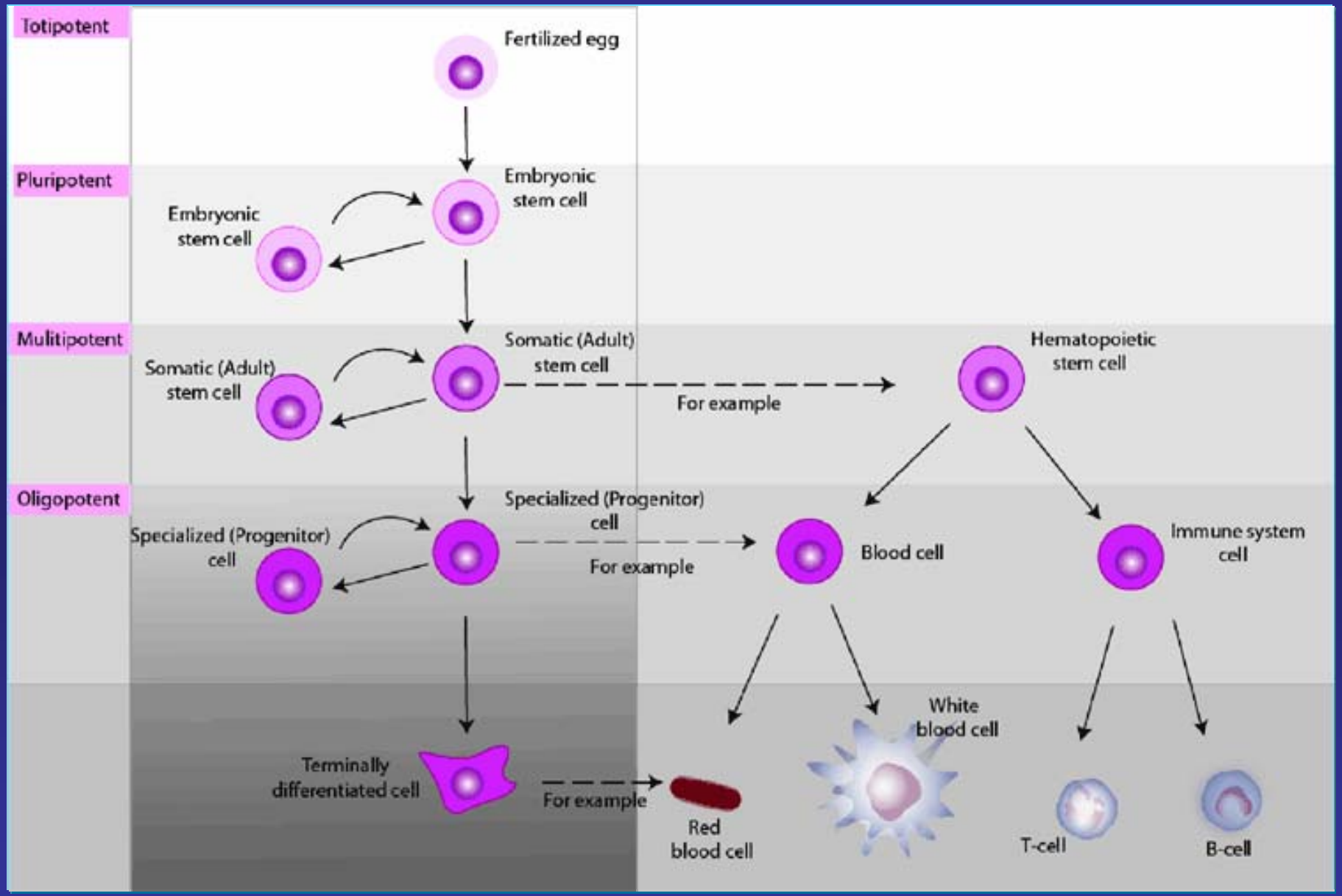
Lincon Stamp, *Monash Institute for Medical Research*

Types of Stem Cells



Cluster of epithelial progenitor cells grown from hES cells.
Ernst Wolvetang, Monash Institute for Medical Research

Tools for Stem Cell Biology



Stem Cell Differentiation Reporters

- Cell-specific promoters drive GFP and Zeocin selection in differentiated cells – **monitor differentiation in real time**

- Rapidly create transgenic lines and ES reporter cells

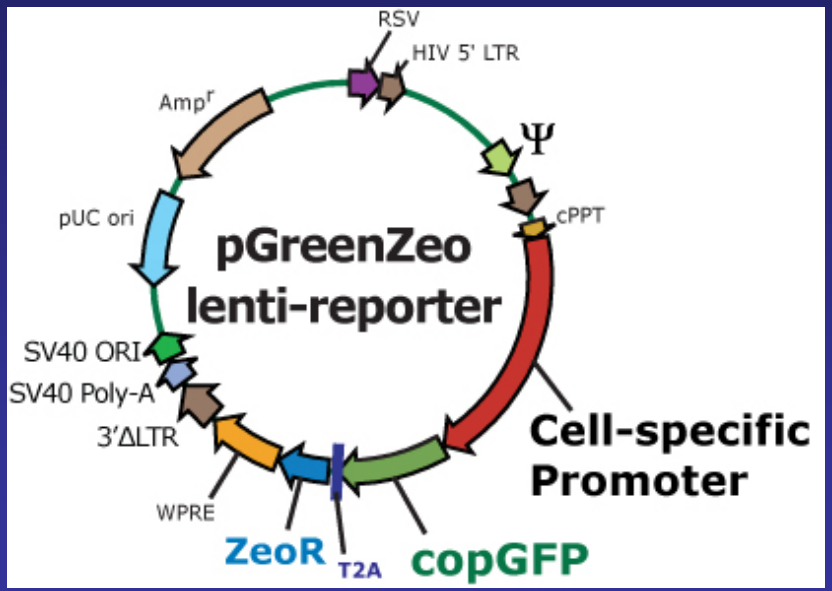


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

Stem Cell Differentiation Reporters

Sorting & Selection

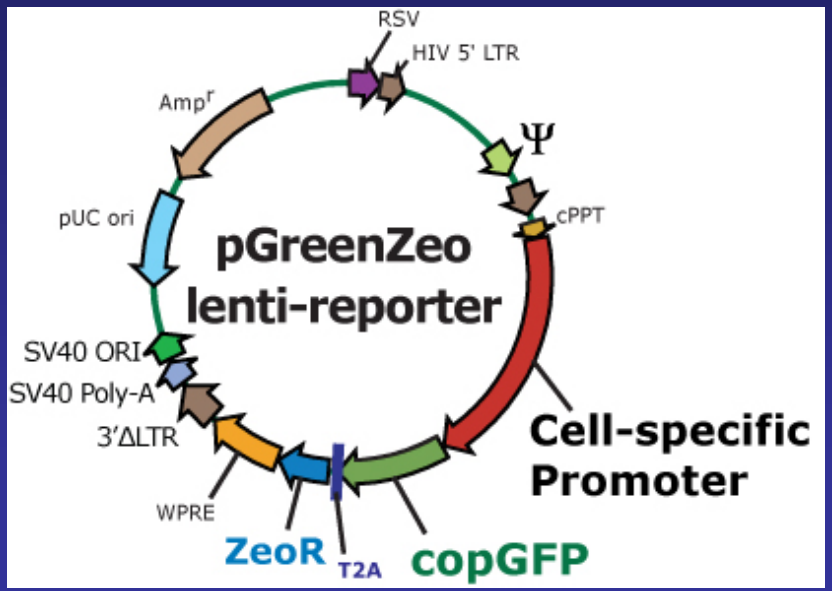


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

Stem Cell Differentiation Reporters

- Data from collaborator: Dr. Rouel Roque and TJ Bartosh

- Testing the effect of retinoic acid (RA) on cardiomyocyte differentiation.

- Infected cells with human and murine TNNT2 reporter virus and differentiated with RA

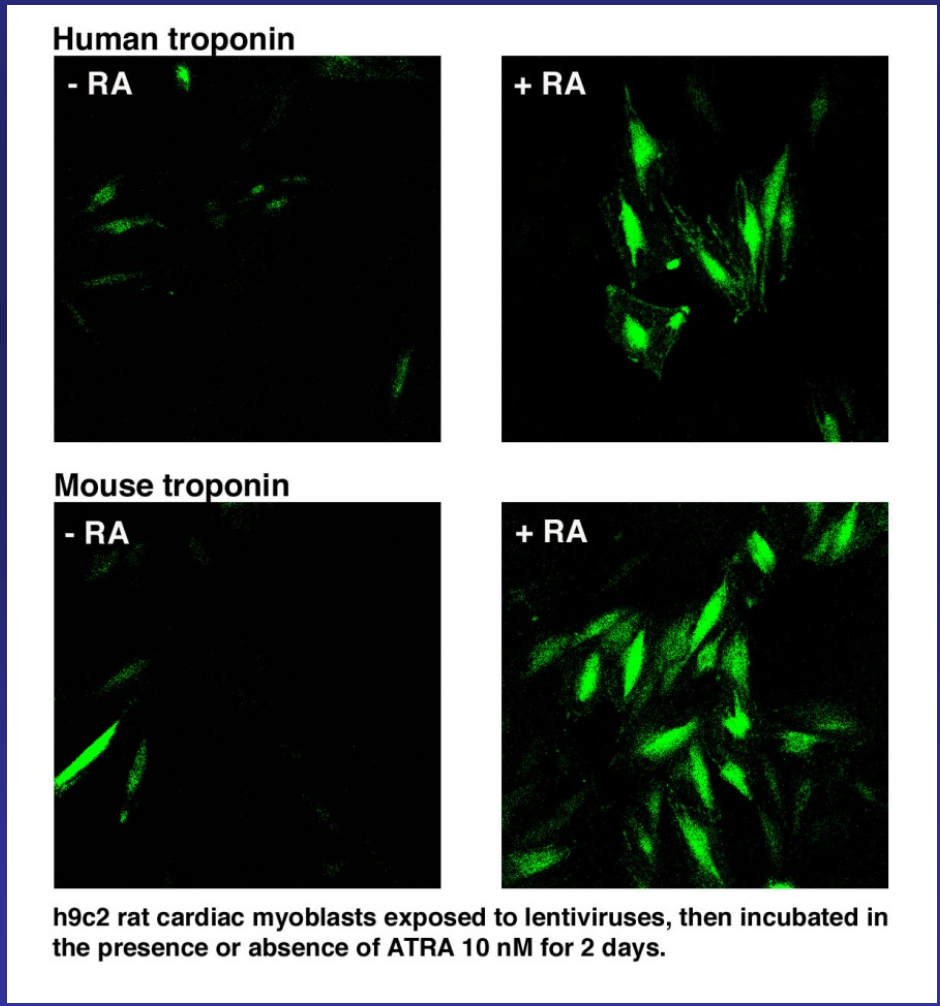
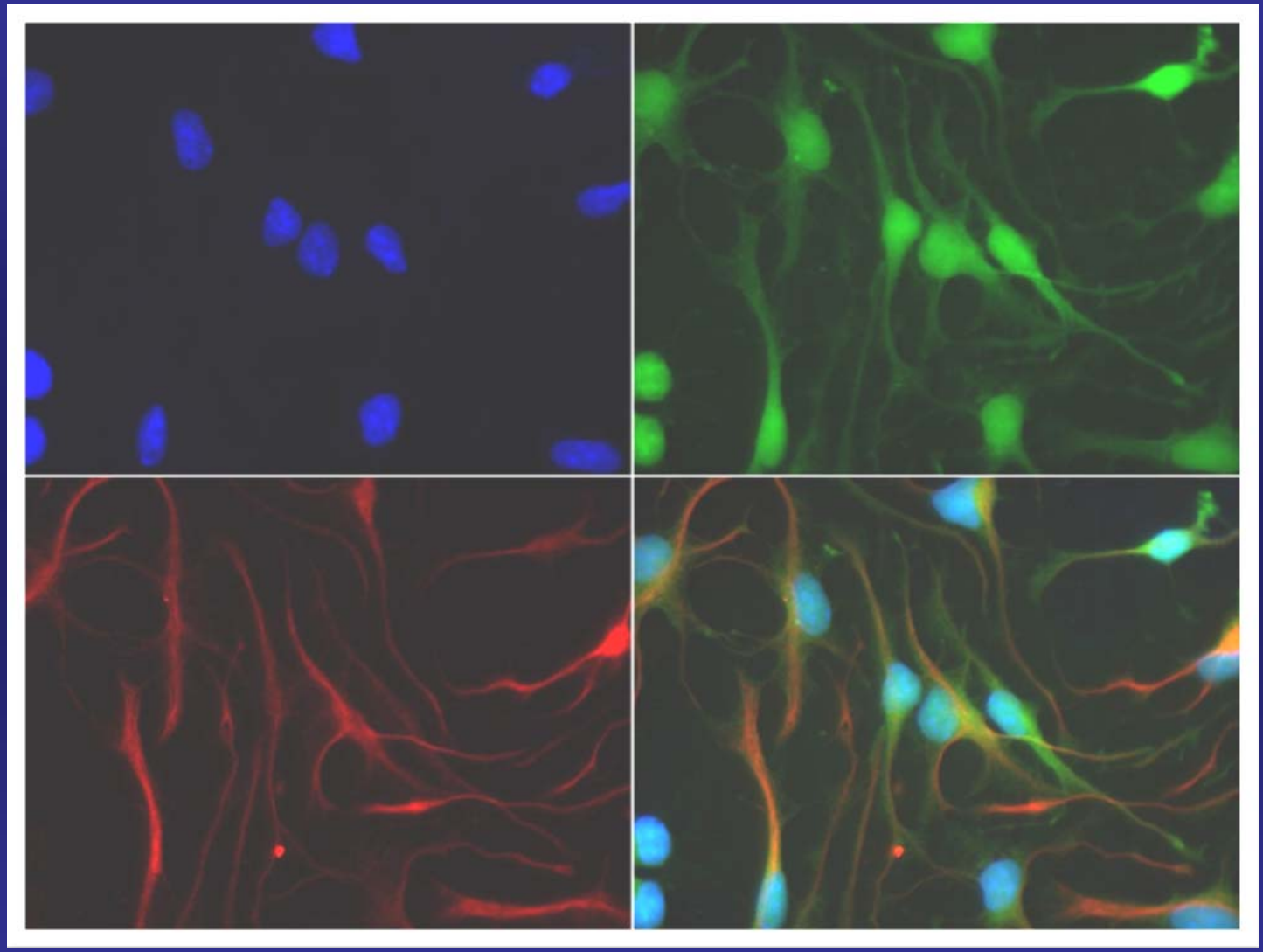


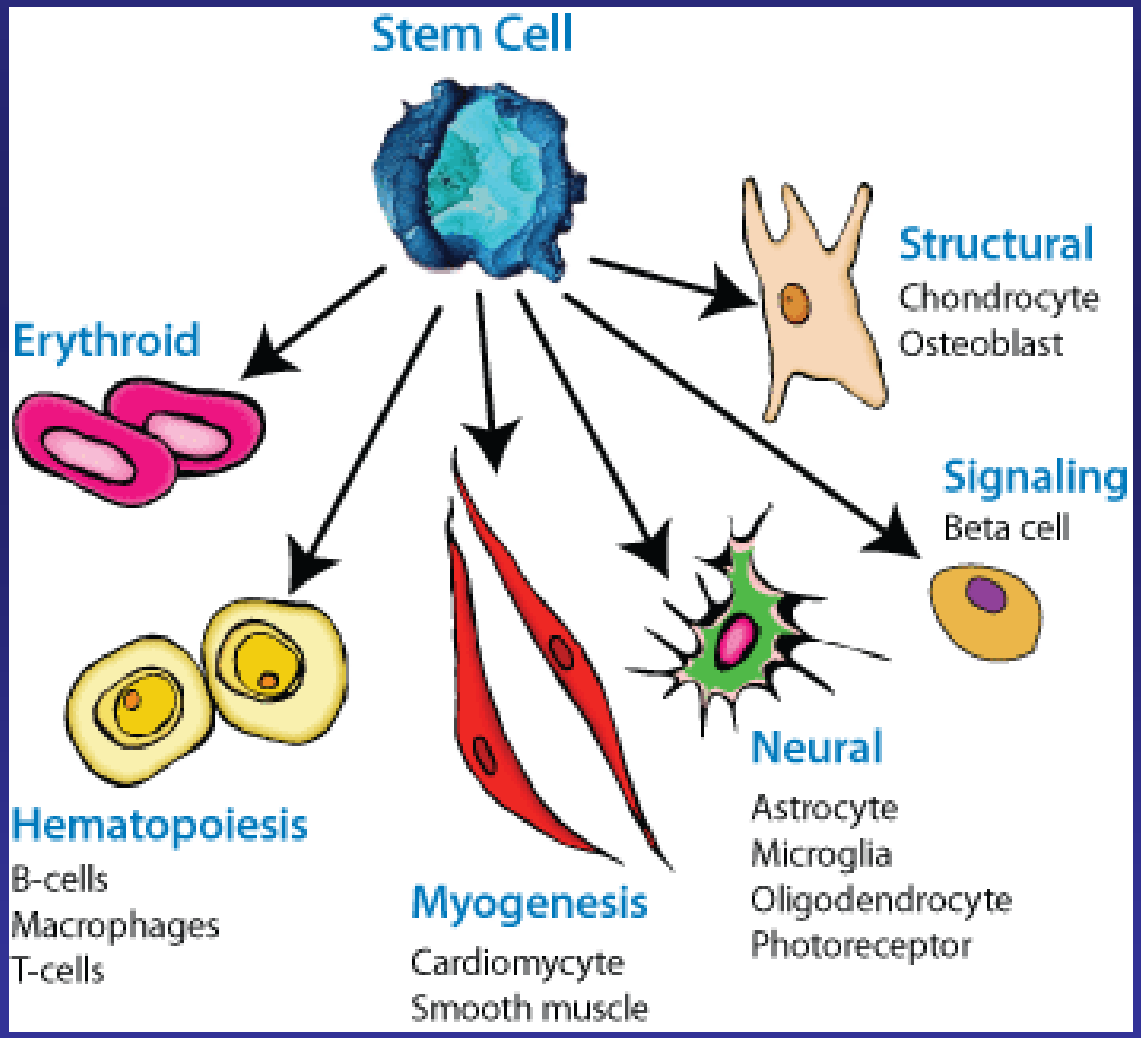
Figure: Cardiac myoblasts infected with TNNT2 reporter in presence or absence of ATRA

Tools for Stem Cell Biology



Astrocytes derived from neural stem cells co-express endogenous GFAP and GFP from a lentiviral mGFAP promoter. Clockwise from top left; DAPI (blue), mGFAP_GFP (green), merge, GFAP (red). Data provided courtesy of Dan Hoeppner, McKay Lab, NINDS.

Stem Cell Differentiation Reporters



Stem Cell Differentiation Reporters

- Available as prepackaged virus or plasmid off the shelf

- Custom construction for any Reporter Gene also offered

Panel	Target Cell type	Species	Reporter Gene
Structural	Chondrocyte	Mouse	col2a1
Structural	Osteoblast	Human	SPP1
Structural	Osteoblast	Human	Osteocalcin
Hematopoetic	B-cell	Human	B29
Hematopoetic	B-cell	Mouse	B29
Hematopoetic	CD8 T-cell	Mouse	CD8
Hematopoetic	Erythroid	Human	HLA-DRa
Hematopoetic	Macrophage, microglia	Mouse	CD68
Hematopoetic	Pan T-cell	Human	CD2 (ciii)
Myogenesis	Cardiomyocyte	Mouse	ACTC
Myogenesis	Cardiomyocyte	Human	MLC-2v
Myogenesis	Cardiomyocyte	Human	Tnnt2
Myogenesis	Cardiomyocyte	Mouse	Tnnt2
Myogenesis	Smooth muscle myocyte	Mouse	SM22a
Neural	Astrocyte	Human	GFAP
Neural	Astrocyte	Mouse	GFAP
Neural	Microglia	Human	CD11b
Neural	Microglia	Mouse	EMR1
Neural	Microglia	Mouse	iba-1
Neural	Muller glia	Mouse	CD44
Neural	Neuron	Human	BM88
Neural	Neuron	Mouse	CAMK2a
Neural	Neuron	Mouse	GAD67
Neural	Neuron	Rat	NSE
Neural	Neuron	Mouse	Tα1 α-tubulin
Neural	Oligodendrocyte	Mouse	MBP
Neural	Photoreceptor	Human	Opsin
Secretory	Beta cell	Human	Insulin
Undifferentiated	ES cell	Mouse	Oct4
Undifferentiated	ES cell	Human	Nanog
Undifferentiated	ES cell	Mouse	Nanog

Stem Cell Differentiation Reporters

Astrocyte Reporter

Beta cell Reporter

Panel	Target Cell type	Species	Reporter Gene
Structural	Chondrocyte	Mouse	col2a1
Structural	Osteoblast	Human	SPP1
Structural	Osteoblast	Human	Osteocalcin
Hematopoetic	B-cell	Human	B29
Hematopoetic	B-cell	Mouse	B29
Hematopoetic	CD8 T-cell	Mouse	CD8
Hematopoetic	Erythroid	Human	HLA-DRa
Hematopoetic	Macrophage, microglia	Mouse	CD68
Hematopoetic	Pan T-cell	Human	CD2 (ciii)
Myogenesis	Cardiomyocyte	Mouse	ACTC
Myogenesis	Cardiomyocyte	Human	MLC-2v
Myogenesis	Cardiomyocyte	Human	Tnnt2
Myogenesis	Cardiomyocyte	Mouse	Tnnt2
Myogenesis	Smooth muscle myocyte	Mouse	SM22a
Neural	Astrocyte	Human	GFAP
Neural	Astrocyte	Mouse	GFAP
Neural	Microglia	Human	CD11b
Neural	Microglia	Mouse	EMR1
Neural	Microglia	Mouse	iba-1
Neural	Muller glia	Mouse	CD44
Neural	Neuron	Human	BM88
Neural	Neuron	Mouse	CAMK2a
Neural	Neuron	Mouse	GAD67
Neural	Neuron	Rat	NSE
Neural	Neuron	Mouse	Tα1 α-tubulin
Neural	Oligodendrocyte	Mouse	MBP
Neural	Photoreceptor	Human	Opsin
Secretory	Beta cell	Human	Insulin
Undifferentiated	ES cell	Mouse	Oct4
Undifferentiated	ES cell	Human	Nanog
Undifferentiated	ES cell	Mouse	Nanog

Cell Stem Cell
Article

Potency and Fate Specification in CNS Stem Cell Populations In Vitro

Rea Ravin,[1,5] Daniel J. Hoeppner,[1,5] David M. Munno,[1] Liran Carmel,[2] Jim Sullivan,[1] David L. Levitt,[1] Jennifer L. Miller,[1] Christopher Athaide,[3] David M. Panchision,[4] and Ronald D.G. McKay[1,*]
[1]Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA
[2]National Library of Medicine, National Institutes of Health, Bethesda MD 20894, USA
[3]EYE Biomachines, Houston, TX 77005, USA
[4]Center for Neuroscience Research, Children's Research Institute, Children's National Medical Center, Washington, DC 20010, USA
[5]These authors contributed equally to this work
*Correspondence: mckay@codon.nih.gov
DOI 10.1016/j.stem.2008.09.012

SUMMARY

A complete stem cell lineage remains to be determined for the

mGFAP Reporter used to trace Astrocyte Differentiation

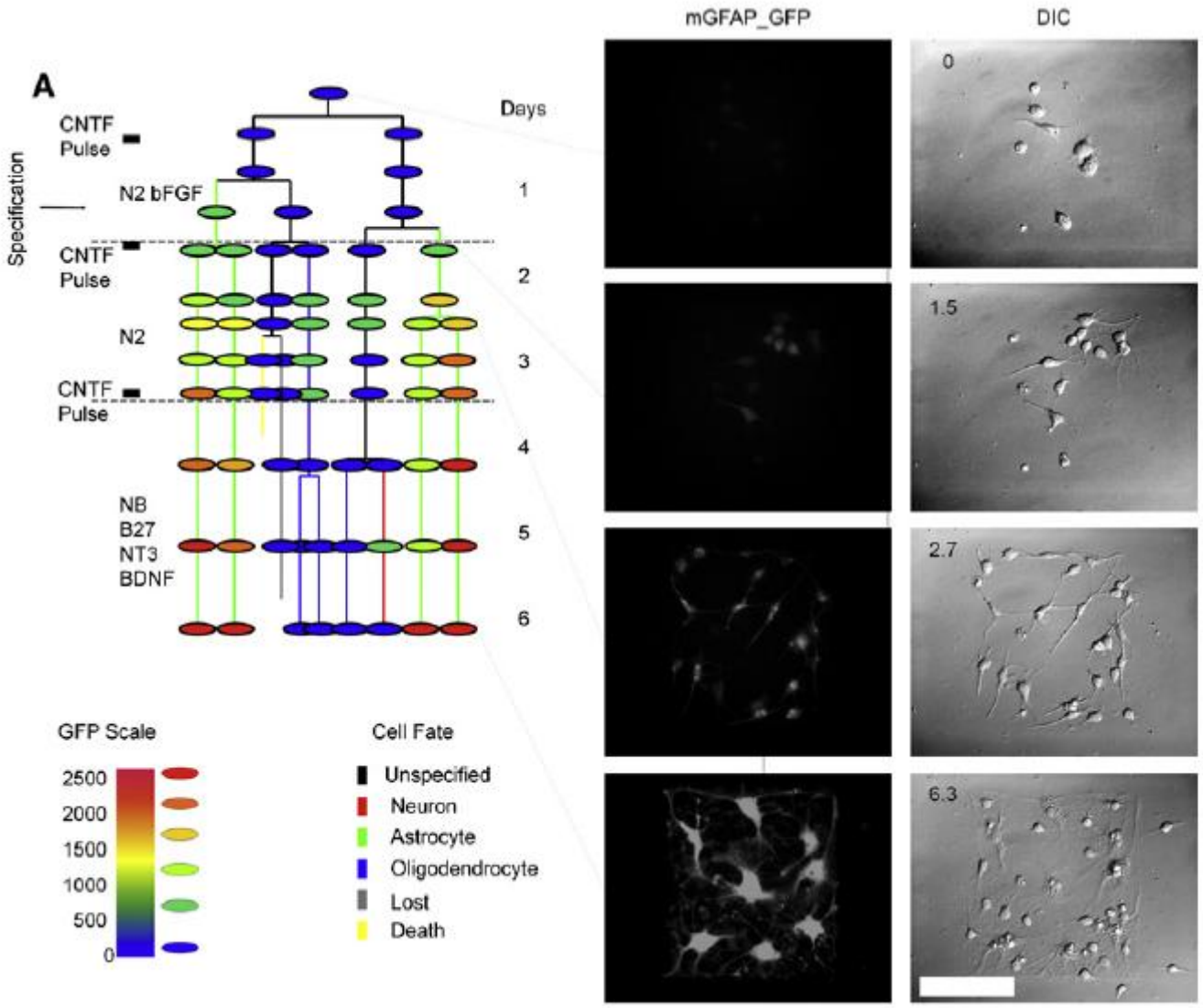


Figure 6. GFAP Reporter Expression in Neural Stem Cell Lineages Validates Early Astrocytic Fate Specification

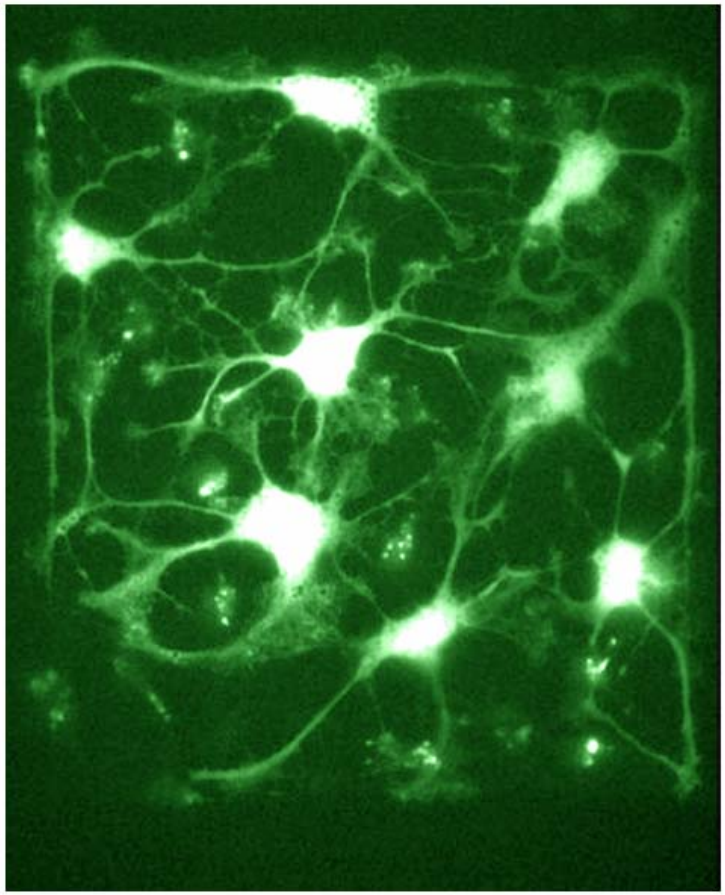
(A) Lineage fate map from a single infected founder cell. The vertical colored lines represent fate. The colored ovals indicate GFP expression level at each point of fluorescence imaging. The GFP scale represents mean fluorescence, in arbitrary units, as discrete colors. Example fluorescence micrographs and corresponding DIC micrographs demonstrate the background-subtracted signal for each cell. Scale bar, 100 μm. Media conditions and CNTF pulse chase are shown to the left (black boxes represent the duration of CNTF pulse).

(B) ΔF/F at the indicated time points. The inset magnifies the low values centered at day 2.5. Error bars reflect SEM.

ablation discussion above). There is significant interest in the mechanism of reprogramming somatic cells to a pluripotent state after viral transduction (Takahashi and Yamanaka, 2006). It has also been demonstrated that it is harder to reprogram fully differentiated B cells in

Tools for Stem Cell Biology

mGFAP_GFP



all cells

Stem Cell Pluripotency Reporters

- Reporters for Human and Mouse Nanog & Oct4 allow easy monitoring of undifferentiated ES cells

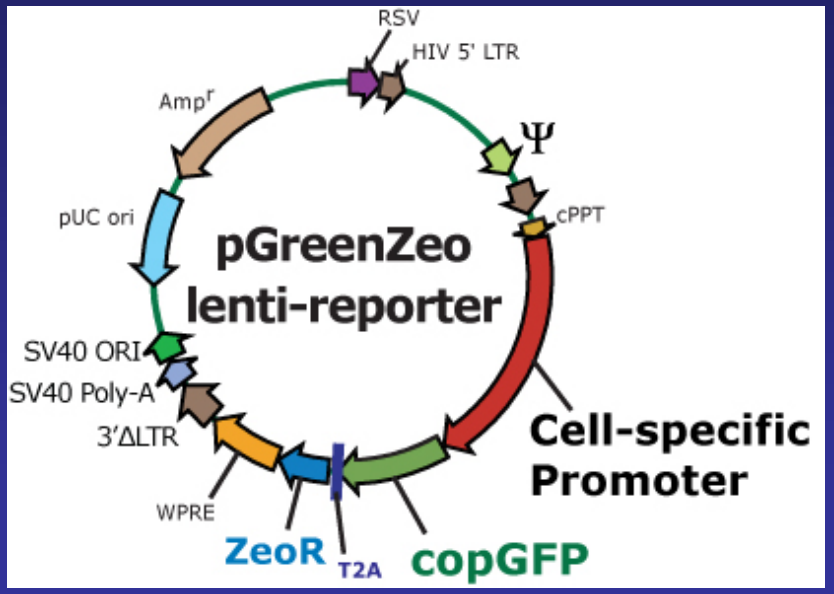


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

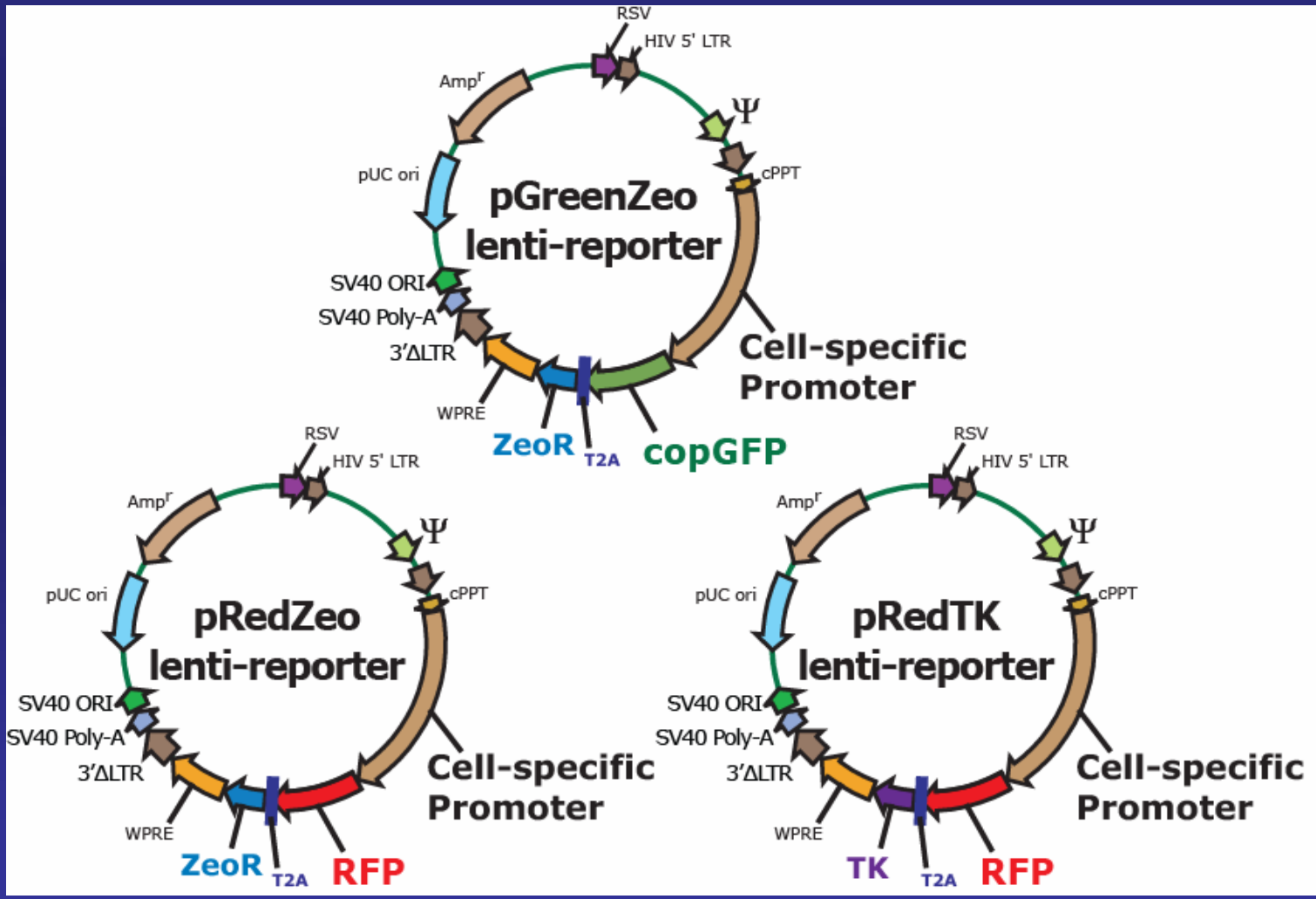
Stem Cell Pluripotency Reporters

Nanog & **Oct4** Reporters available in 3 different backbones:

pGreenZeo

pRedZeo

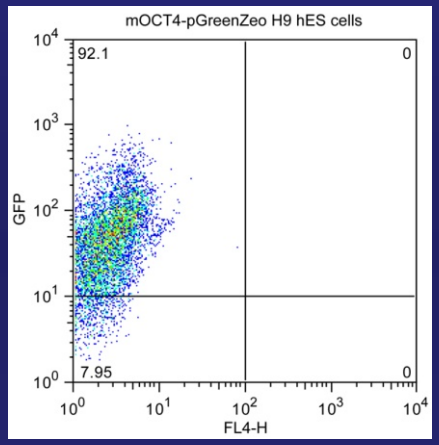
pRedTK

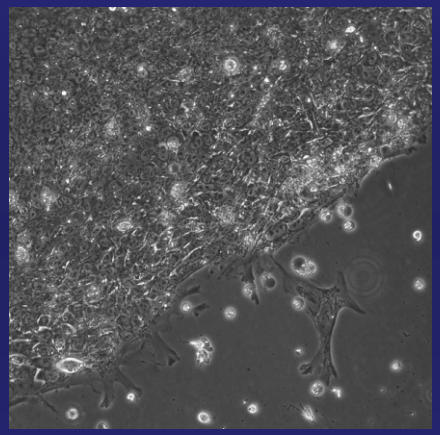


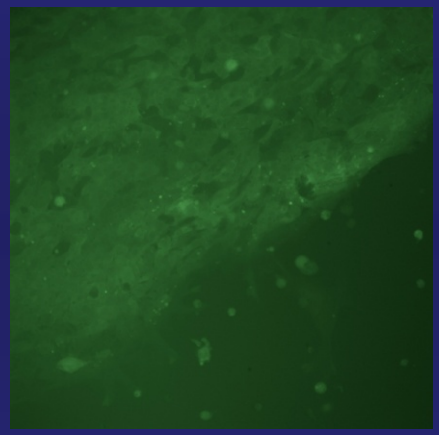
Stem Cell Pluripotency Reporters

- Data from collaborator: Dr. Tim Kamp and Chad Koonce

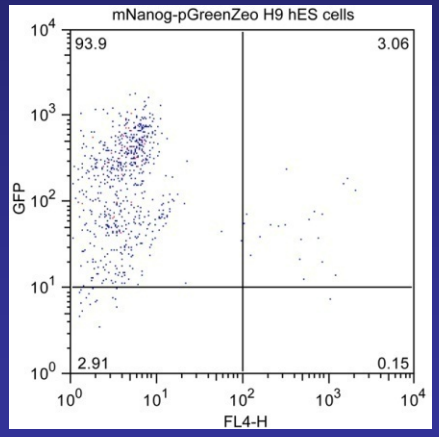
pGreenZeo-mOct4

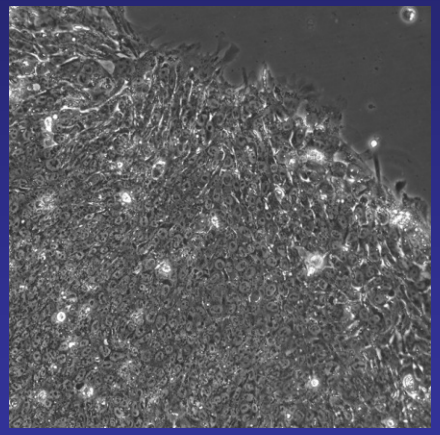






pGreenZeo-mNanog





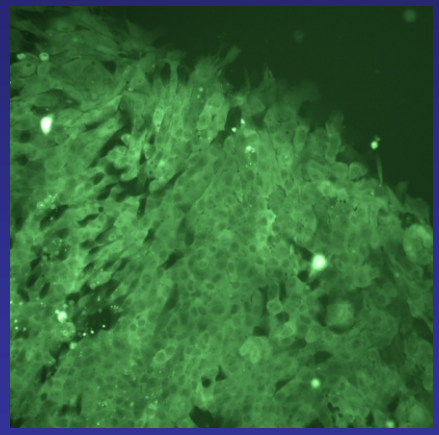
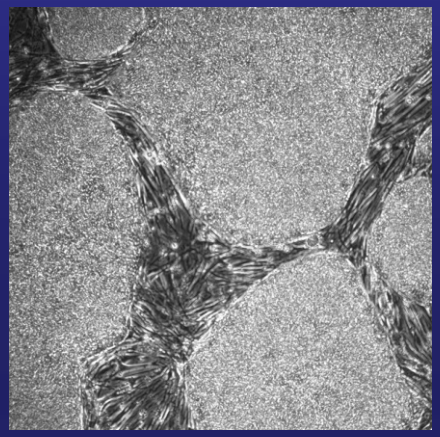
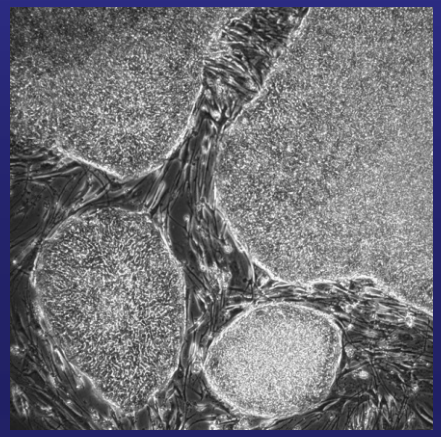
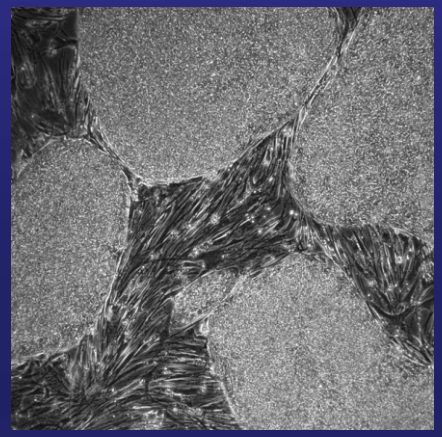
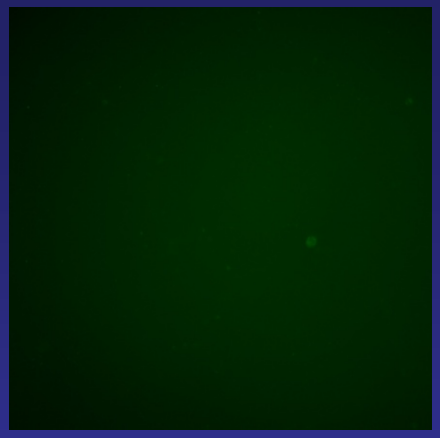
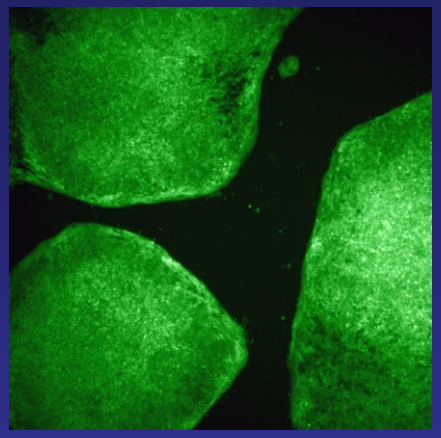
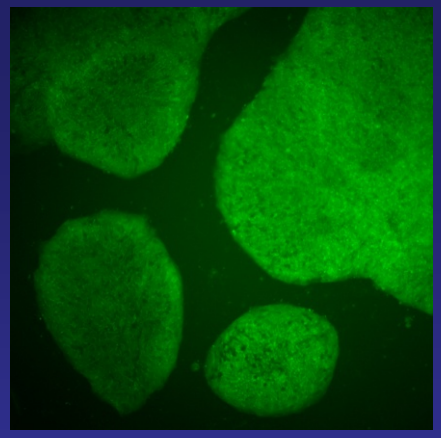
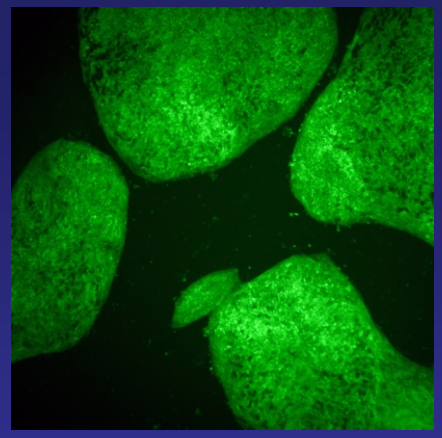


Figure: Transduced H9 hES cells showing Oct4 and Nanog expression

Tools for Stem Cell Biology

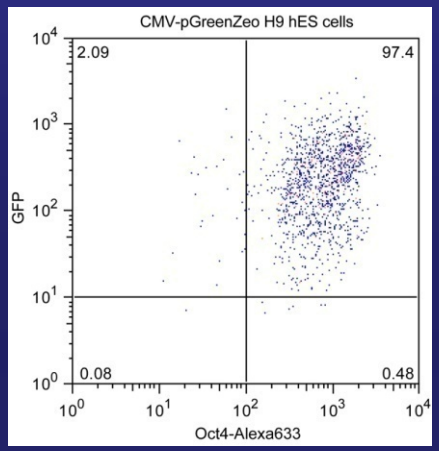
	H9 hES cells	pGreenZeo-CMV Transduced H9 hES cells	pGreenZeo-mOct4 Transduced H9 hES cells	pGreenZeo-mNanog Transduced H9 hES cells
Phase Contrast				
GFP				

H9 hES cells were transduced with pGreenZeo reporter constructs containing specific promoters for CMV, mOCT4, or mNANOG. Cells were cultured for 8 weeks on Matrigel coated plates with MEF conditioned medium containing 1 ug/ml Zeocin. Cells photographed here were split and grown on MEF feeders layer for four days. Data courtesy of Dr. Timothy Kamp and Chad H. Koonce, UW-Madison Medical School & WiCell Research Institute.

Stem Cell Pluripotency Reporters

- Data from collaborator: Dr. Tim Kamp

pGreenZeo-CMV transduced cells



pGreenZeo-mOct4 transduced cells

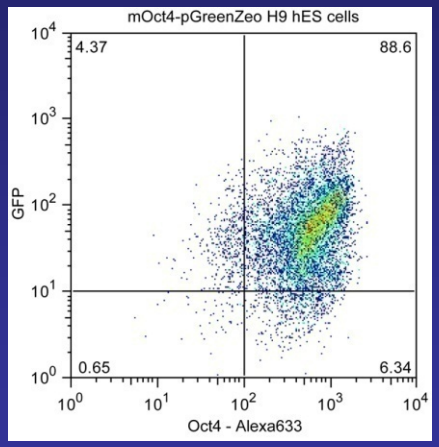


Figure: GFP-Reporter and Oct4-Immunostained Double Positive Cells

Stem Cell Pluripotency Reporters

- Data from collaborator: Dr. Tim Kamp

pGreenZeo-CMV

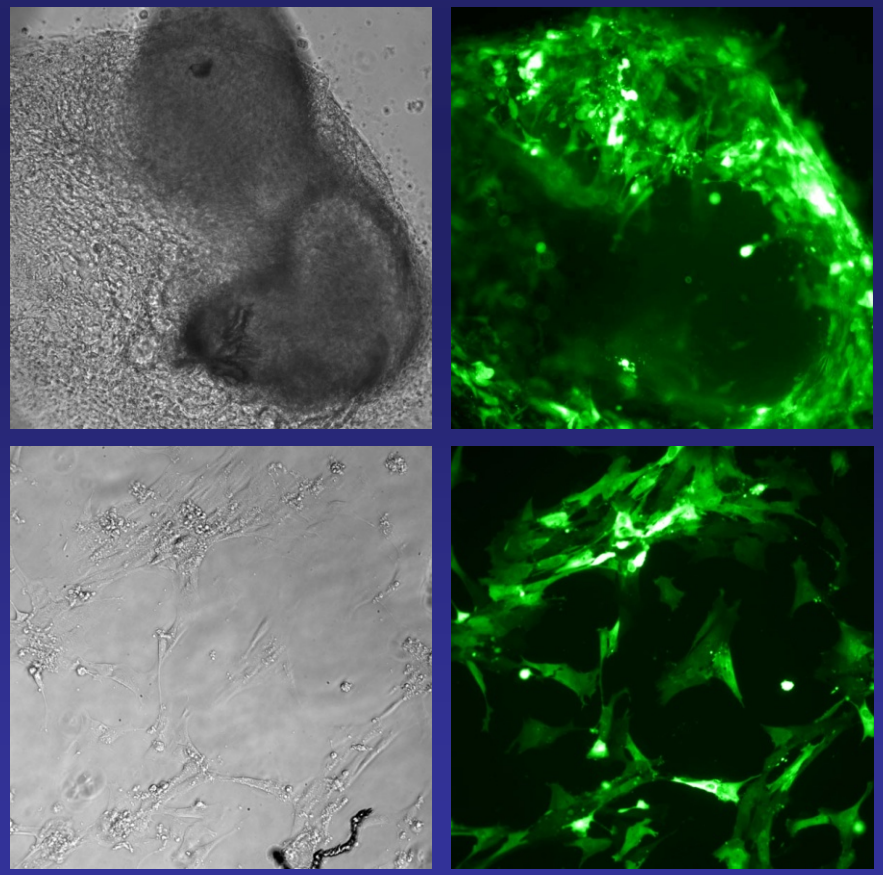
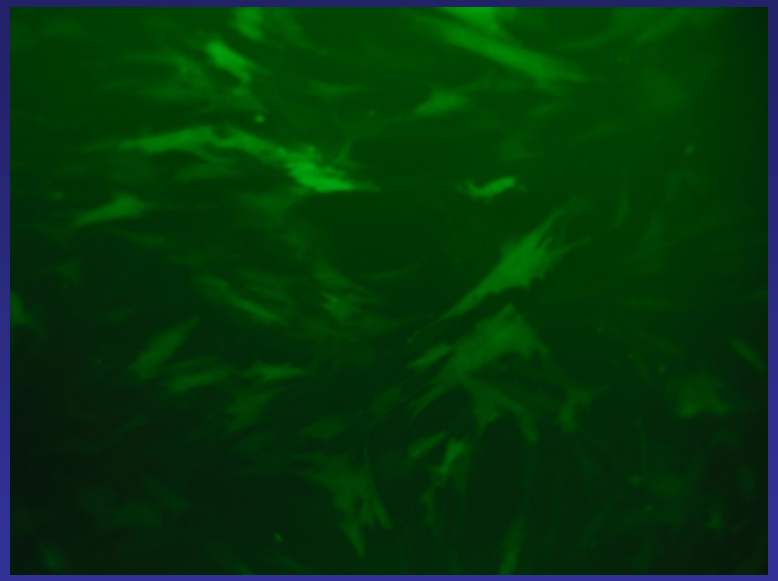


Figure: Embryoid bodies transduced with lentivirus

Stem Cell Reporters

- Also can use positive control (CMV promoter) to create Tracer Lines for tracing cell lineage



pGZ-CMV

Figure: Human Mesenchymal Stem Cell Reporter Line

Tools for Stem Cell Biology

Induce Pluripotency with iPSC Factors

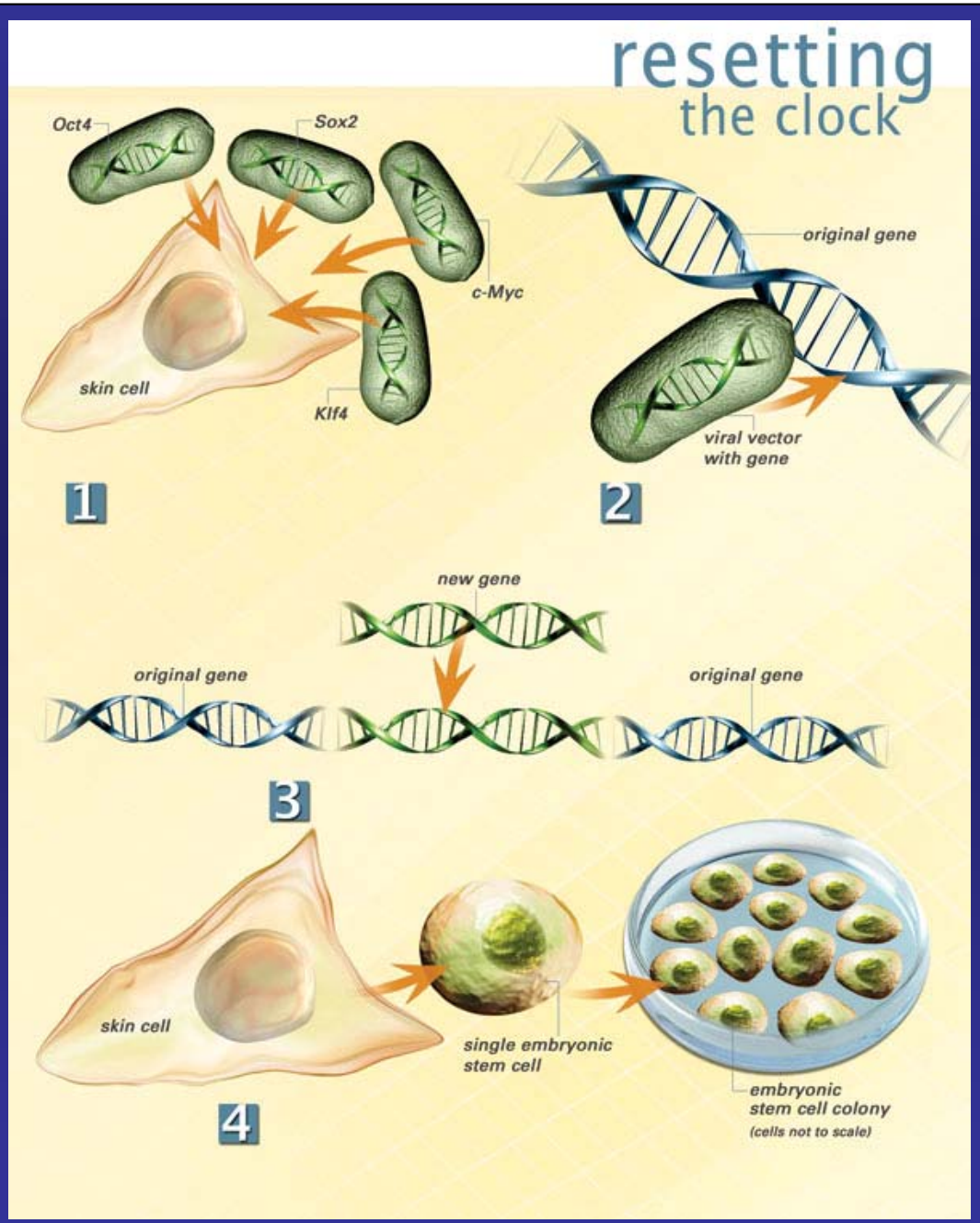
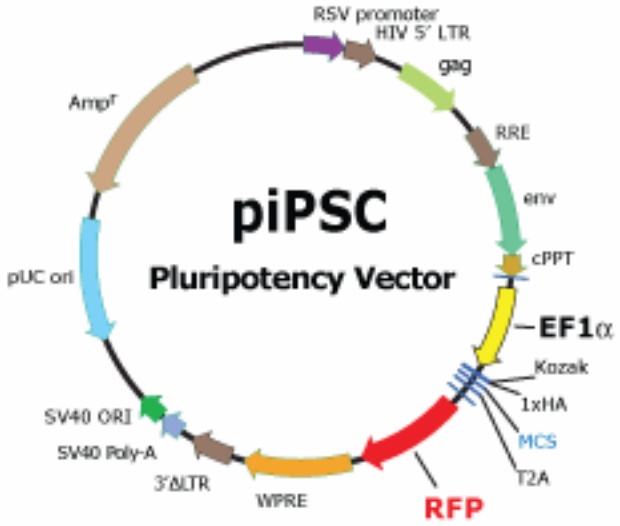


Image: Christina Ullman

Induce Pluripotency with iPSC Factors

Reprogram adult cells to create iPS cells with SBI's pluripotency factor constructs

iPSC Factor Expression Vector



Click here to download piPSC vector sequence

Six iPSC Factors for Stem Cell Reprogramming

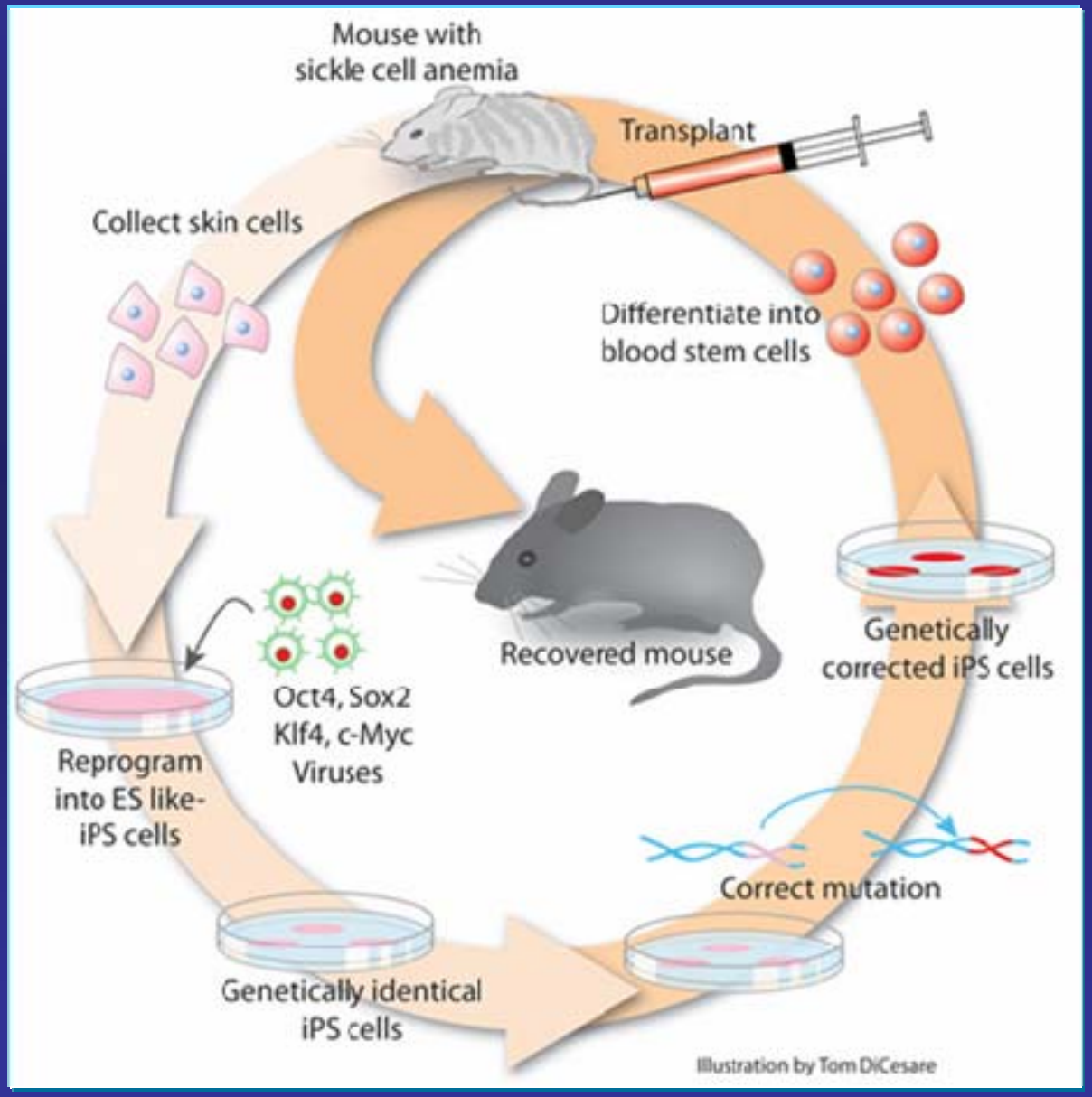
Oct4 • SOX2

Nanog • KLF4

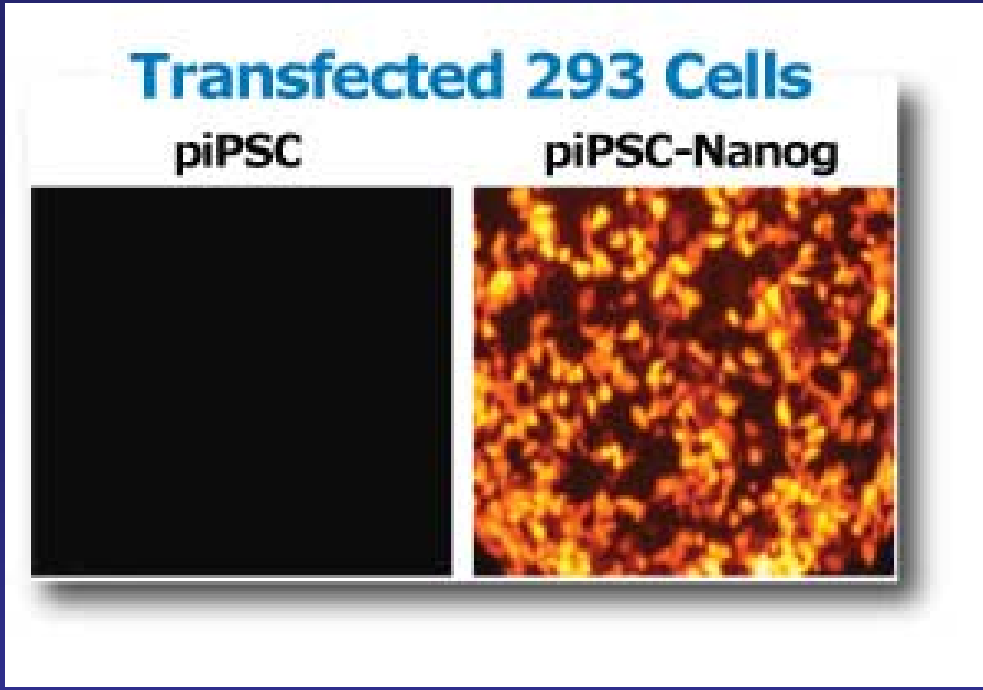
Lin-28 • c-Myc

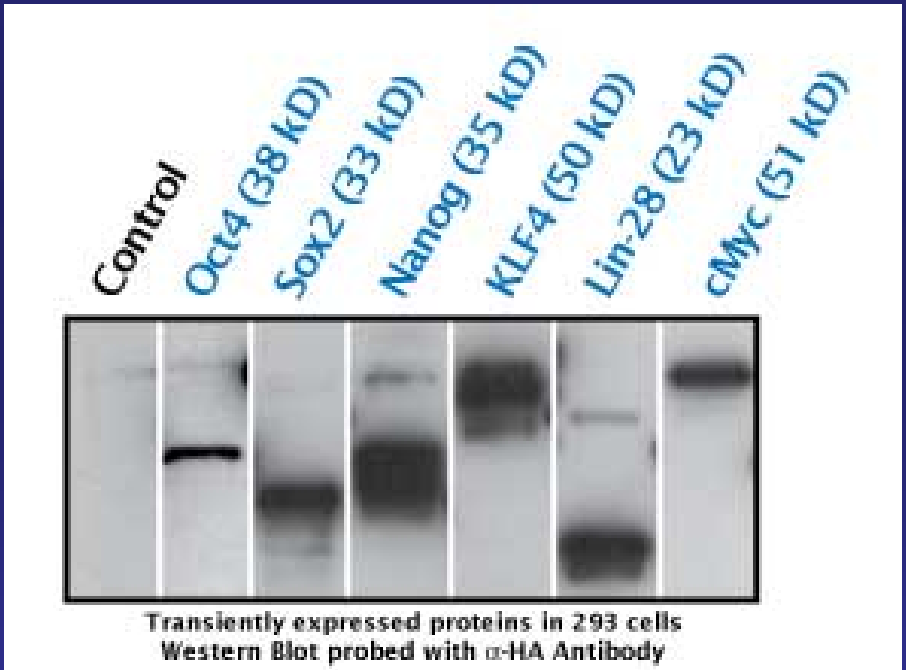
"The Magic Brew" - Rossant, 2007

Tools for Stem Cell Biology

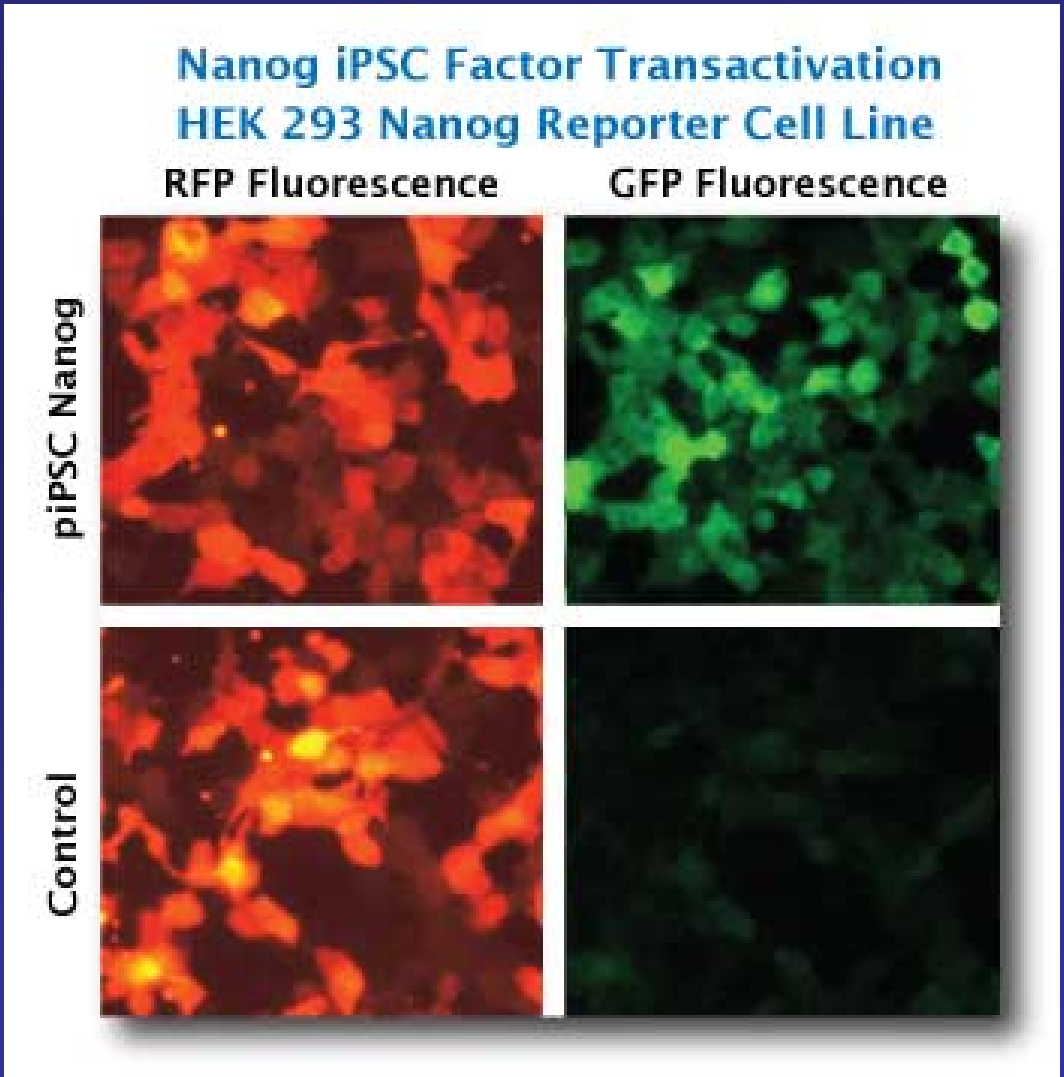


Induce Pluripotency with iPSC Factors





Induce Pluripotency with iPSC Factors



pGreenFire Pathway Reporters

- Accurately monitor activity of transcriptional factors natively

- Easily construct stable reporter cell lines

- pGreenFire: New generation of dual reporter vector uses both GFP and Luciferase reporters

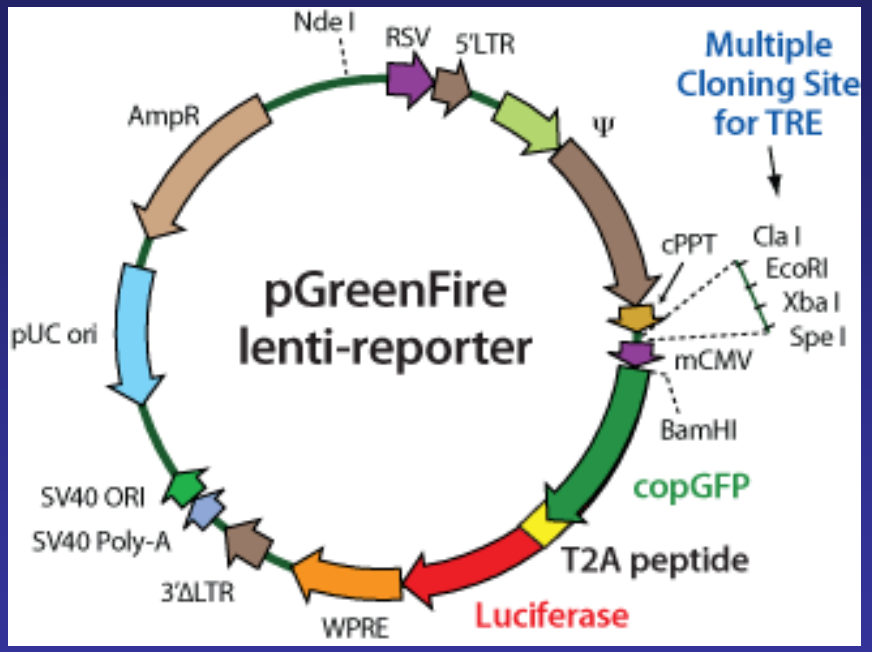


Figure: New generation of dual reporter vector uses both GFP and Luciferase reporters

pGreenFire Pathway Reporters

Sorting & Quantitation

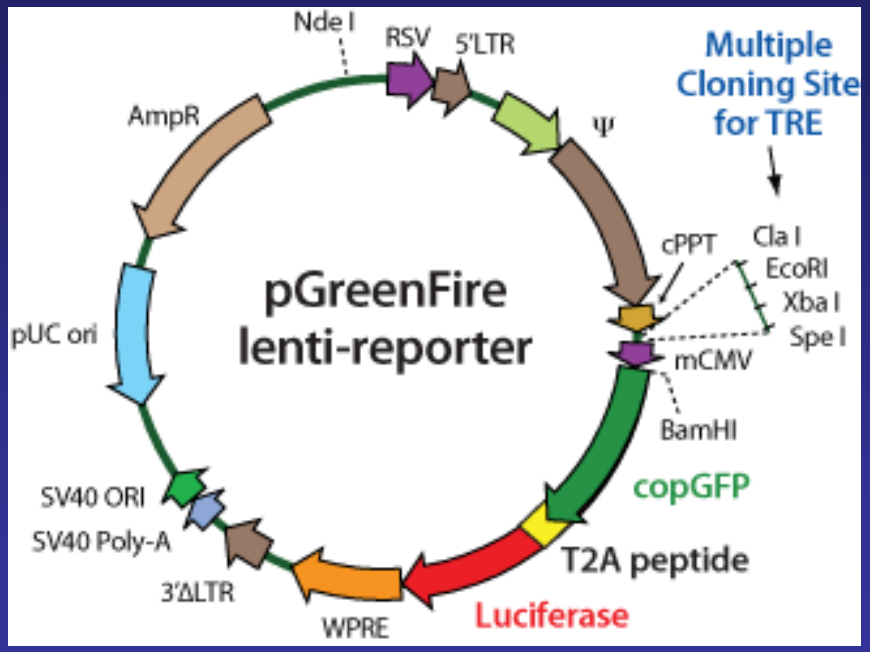


Figure: New generation of dual reporter vector uses both GFP and Luciferase reporters

pGreenFire Pathway Reporters

Example of NFkB Reporter Structure used to create Stable Cell Lines:

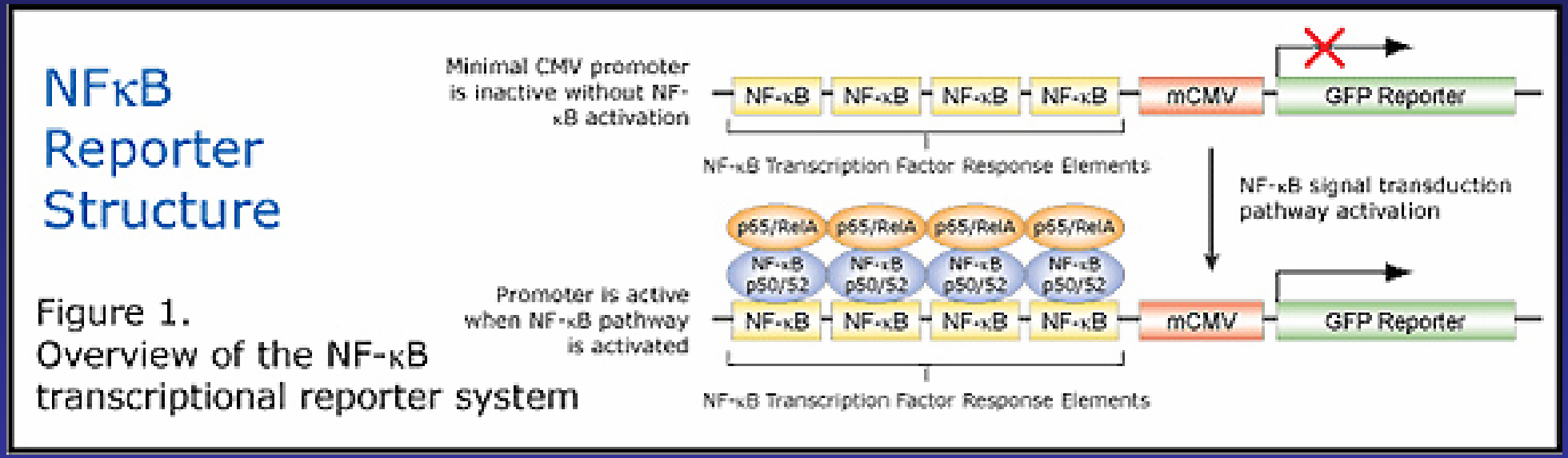


Figure 1.
Overview of the NF-κB transcriptional reporter system

pGreenFire Pathway Reporters

Easily Sort cells based on Transcriptional Activation:

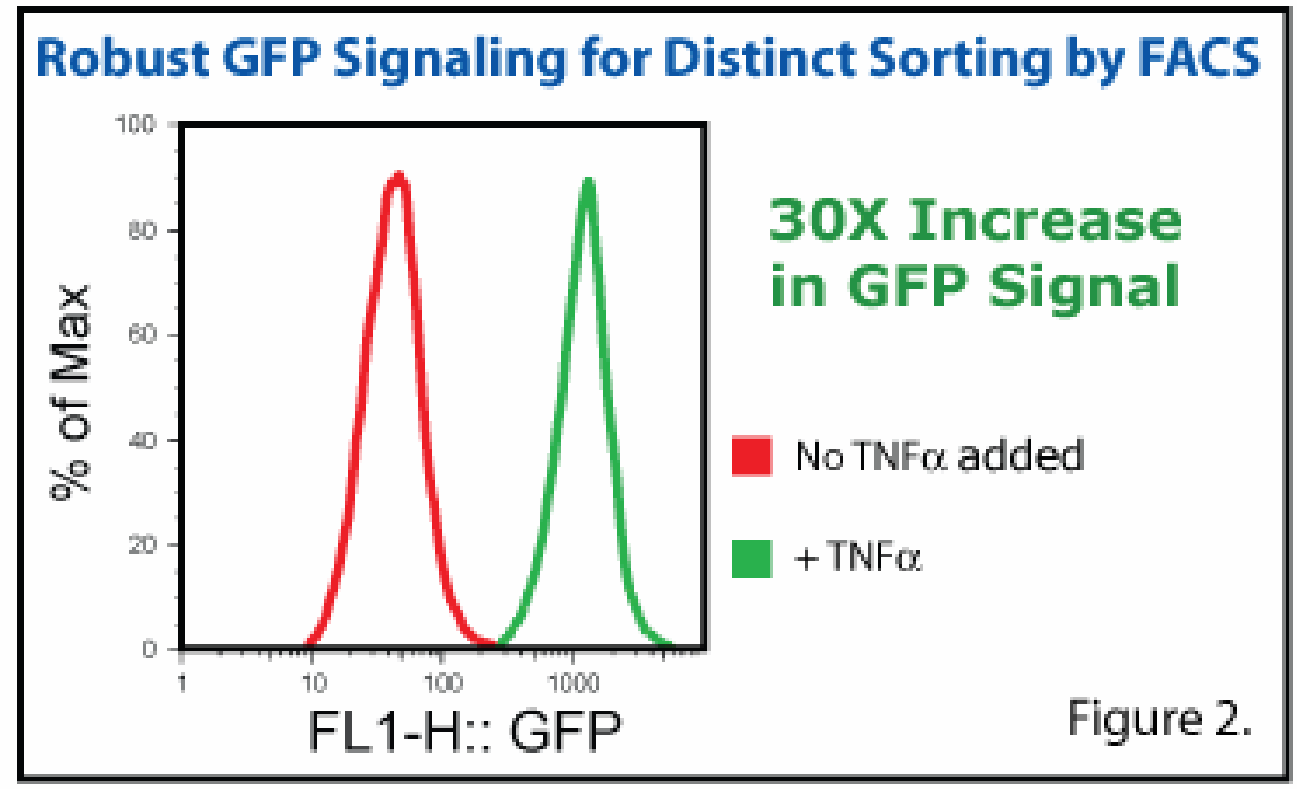
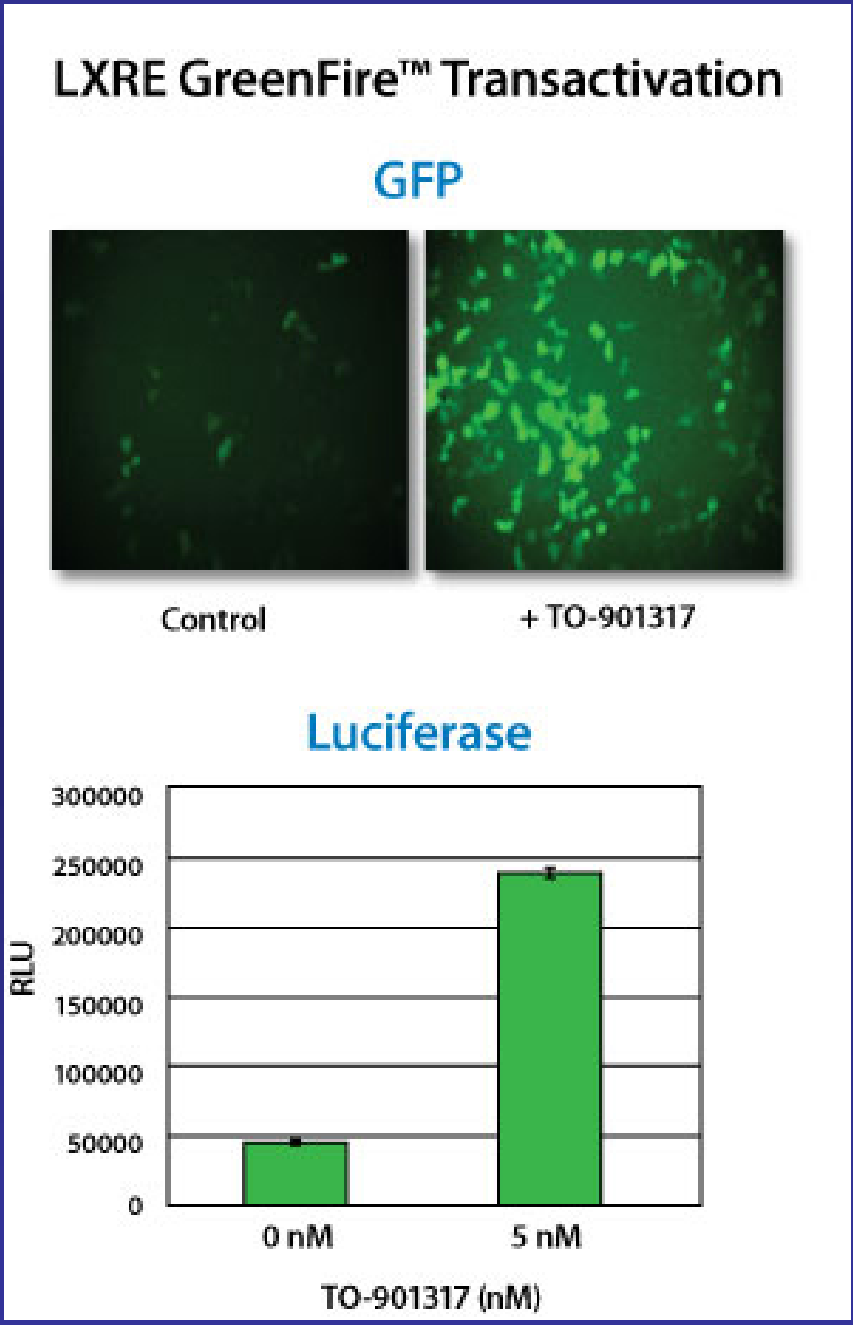


Figure: Jurkat/NFkB/GFP reporter cells sorted after treatment with TNF-alpha

Tools for Stem Cell Biology

pGreenFire-LXRE Sample Data



GeneNet™ Genome-wide shRNA Libraries

Figure: Example of high-throughput screen using an siRNA library

PNAS PNAS PNAS

A high-throughput siRNA library screen identifies osteogenic suppressors in human mesenchymal stem cells

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Communicated by Steven P. Briggs, University of California at San Diego, La Jolla, CA, April 19, 2007 (received for review August 22, 2006)

Tissue-specific (or adult) stem/progenitor cells are regarded as the source for normal tissue homeostasis and tissue repair. They also provide tremendous promise for regenerative medicine because of their capacity to proliferate and differentiate into a variety of mature cell types. Human mesenchymal stem cells (hMSCs) can differentiate into osteocytes, adipocytes, chondrocytes, muscle cells, and neurons. However, the molecular mechanisms underlying these differentiation processes are poorly understood. We screened a synthetic siRNA library targeting 5,000 human genes to identify the endogenous repressors of osteogenic specification, which when silenced could initiate differentiation of hMSCs into osteoblasts. This screen yielded 53 candidate suppressors, and 12 of those were further confirmed for their dynamic roles in suppressing osteogenic specification in hMSCs. Furthermore, cAMP was identified to play opposing roles in osteogenesis vs. adipogenesis. This study provides a basis for further elucidation of the genetic network controlling osteogenesis and, potentially, the molecular rationale for treating bone diseases.

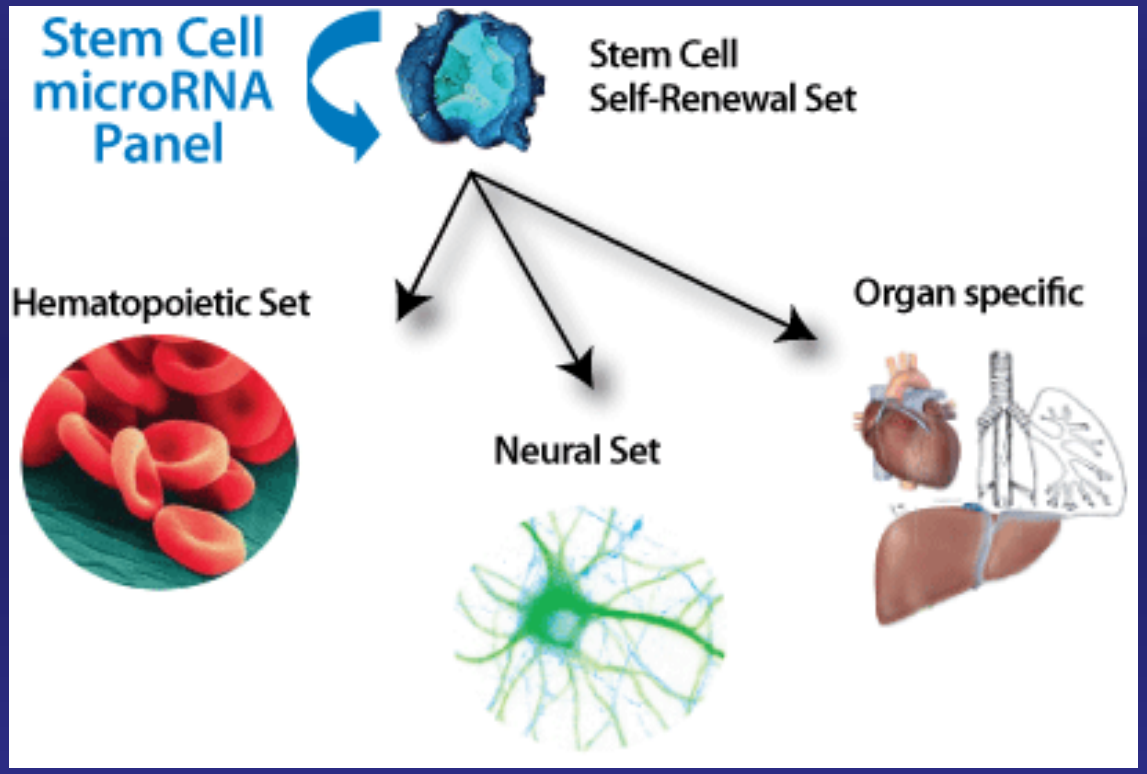
adipogenic differentiation | osteogenic differentiation | high-throughput RNAi screen

RNAi is a highly conserved gene-silencing mechanism functioning through targeted destruction of individual mRNA by a homologous double-stranded siRNA (1). siRNAs generated by both chemical synthesis and *in vitro* or *in vivo* transcription through vector-based expression systems have been proven very useful tools in studying gene loss-of-function in mammalian cells (2–10). Although high-throughput screens using genome-scale siRNA libraries have been successfully carried out in mammalian cells (11–13), effective application of arrayed synthetic siRNA library in stem cells has not been reported. Human mesenchymal stem cells (hMSCs) can be easily isolated from adults and expanded rapidly *in vitro*.

transfection efficiency and minimum cellular toxicity in hMSCs [supporting information (SI) Fig. 4] (also see *Materials and Methods* for details). This highly effective siRNA transfection method was then implemented into a high-throughput screen that was based on enzymatic assay of alkaline phosphatase (ALP), an early marker for osteogenic differentiation (26). Fifty-five hits that gave rise to a significant increase of ALP activity on day 7 after siRNA transfection in hMSCs were identified and confirmed (Fig. 1*a* and SI Table 1). Each image was taken from a representative field of the whole well (and the same applies to all other cell culture images thereafter).

Among the primary siRNA hits, the corresponding genes encode proteases, kinases, ion channels, protein receptors, ligands, transcription factors, extracellular matrix proteins, hypothetical proteins, etc., some of which are members of the same gene family (integrin family, angiopoietin family, adenylate cyclase family, and olfactory receptor family) (SI Table 1). Although the majority of the identified genes have not been implicated in bone development, two genes, *TBX3* (T-box 3) and *GNAS*, have been found to cause skeletal abnormalities when mutated in mouse and human, respectively (24, 25, 27–29). To verify the screen, we picked 12 targeted genes (SI Fig. 5), including *GNAS* (human GNAS complex locus, transcript variant 2, isoform b of the alpha subunit of G_s; NM_080426), *ADCY8* (adenylate cyclase 8; NM_001115), *ADK* (adenosine kinase; NM_001123), *P2RY11* (purinergic receptor P2R, G protein coupled, 11; NM_002566), *TBX3* (T-box 3 or ulnar mammary syndrome; NM_005996), *BIRC4* (baculoviral IAP repeat-containing 4; NM_001167), *BCL2l2* (BCL2-like 2; NM_004050), *SLC12A2* (solute carrier family 12, member 2; NM_001046), *KCNT1* (potassium channel, subfamily T, member 1; XM_029962.2), *GDBR1* (putative glial blastoma cell differentiation-related; NM_016172), *DUSP6* (dual specificity

MicroRNA Profiling



qPCR Array for 95 miRNAs involved in self-renewal and development.

Tools for Stem Cell Biology

SBI Launches New Website!

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Spend less time making your tools and more time making discoveries.