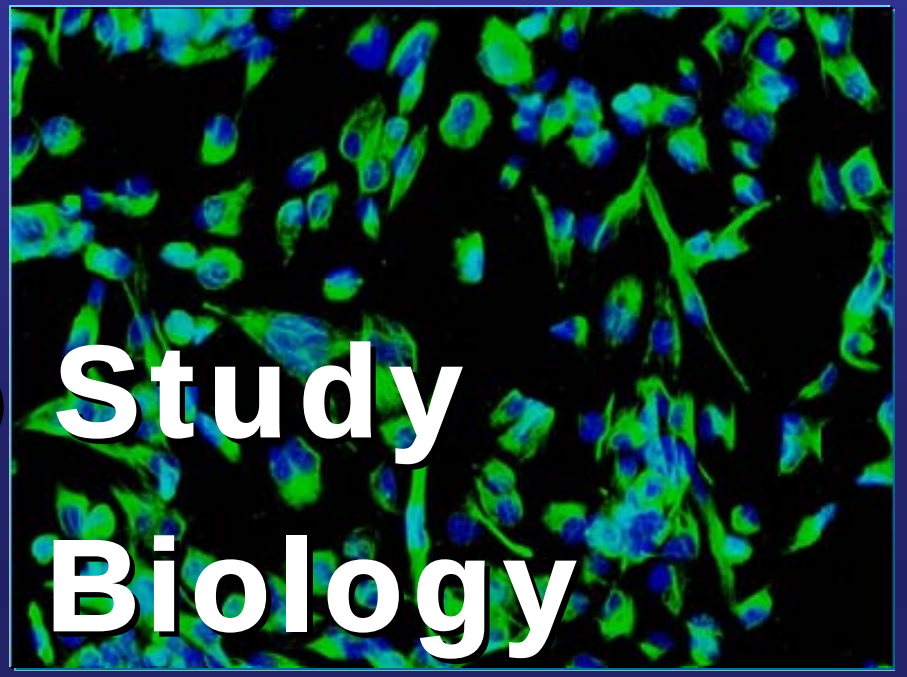
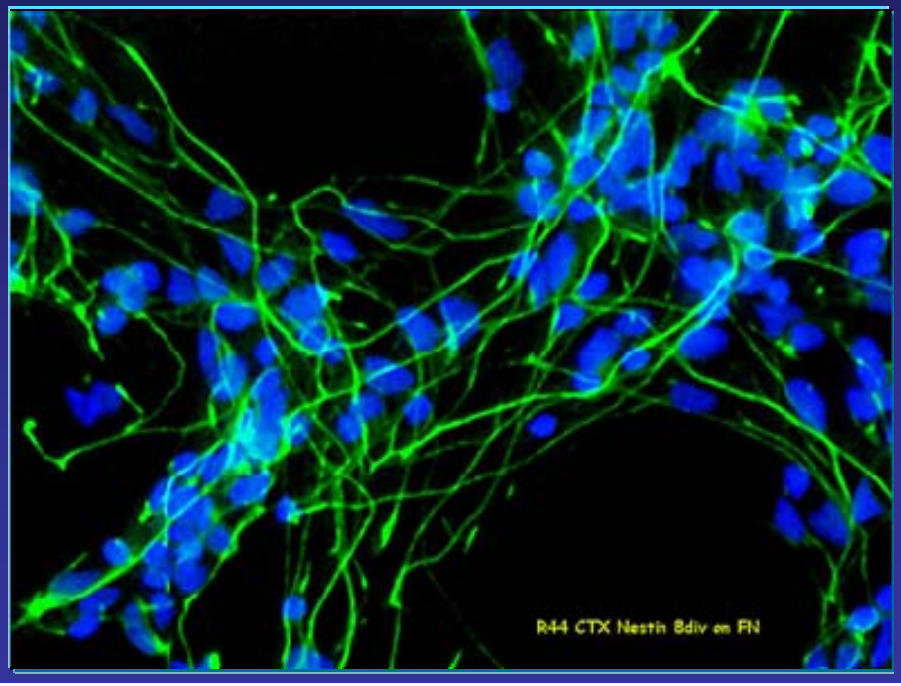
System Biosciences (SBI)



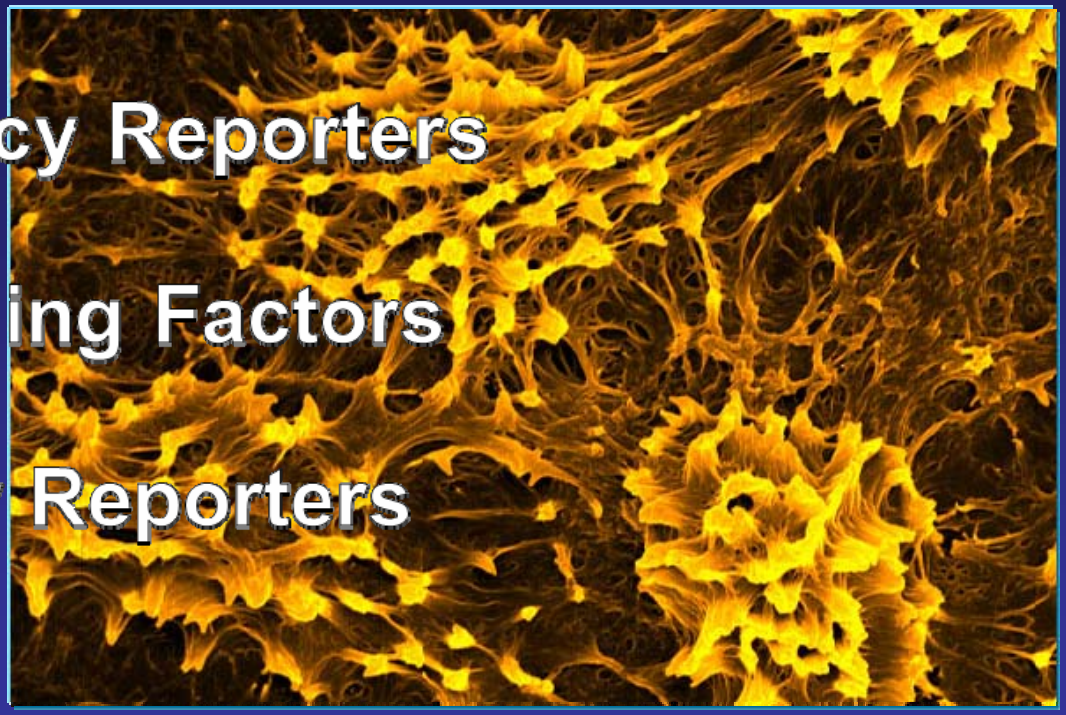
Tools to Study Stem Cell Biology



Jacob Lesnik
Assoc. Product Manager
System Biosciences

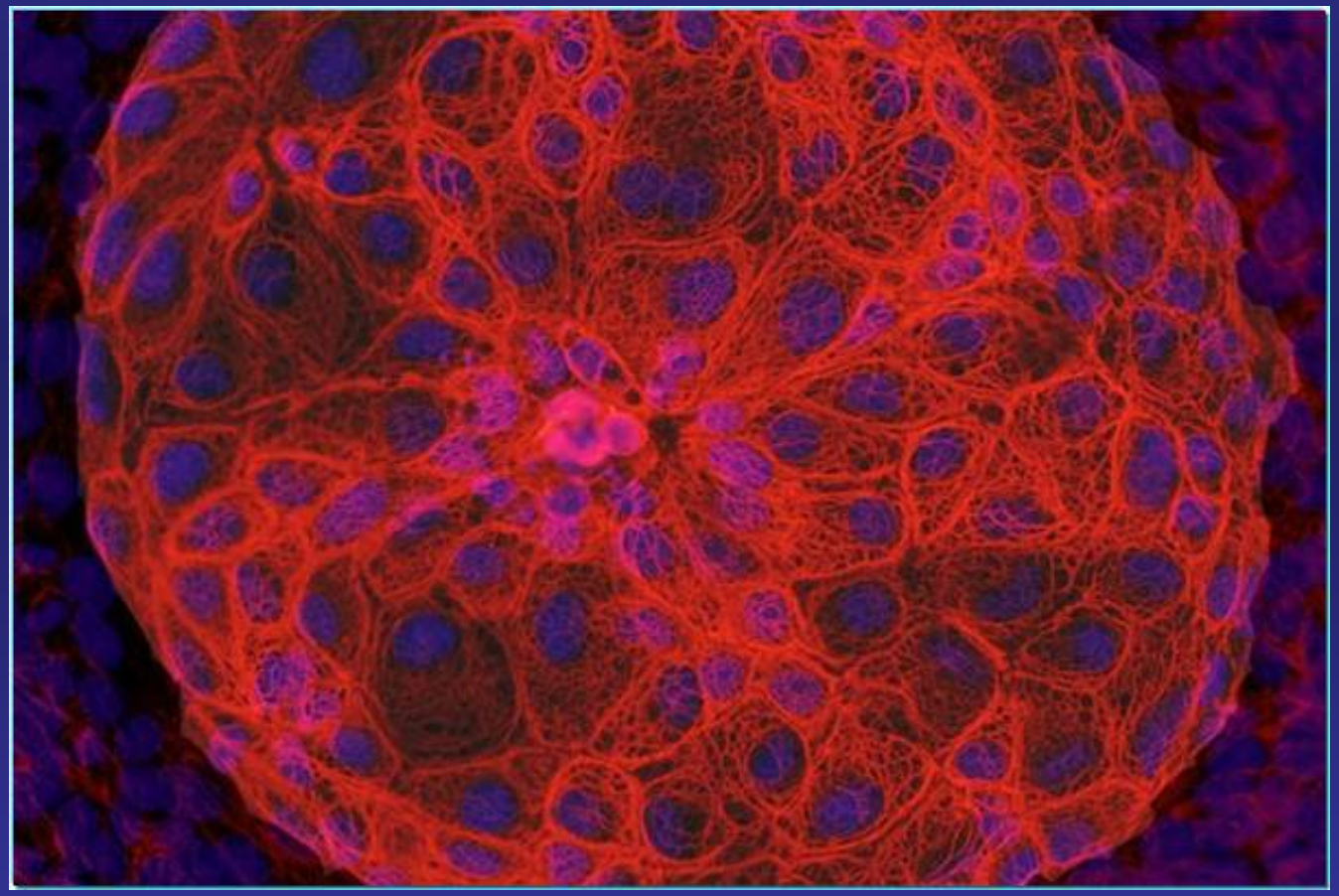
SBI's Suite of Tools for Studying Stem Cells

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



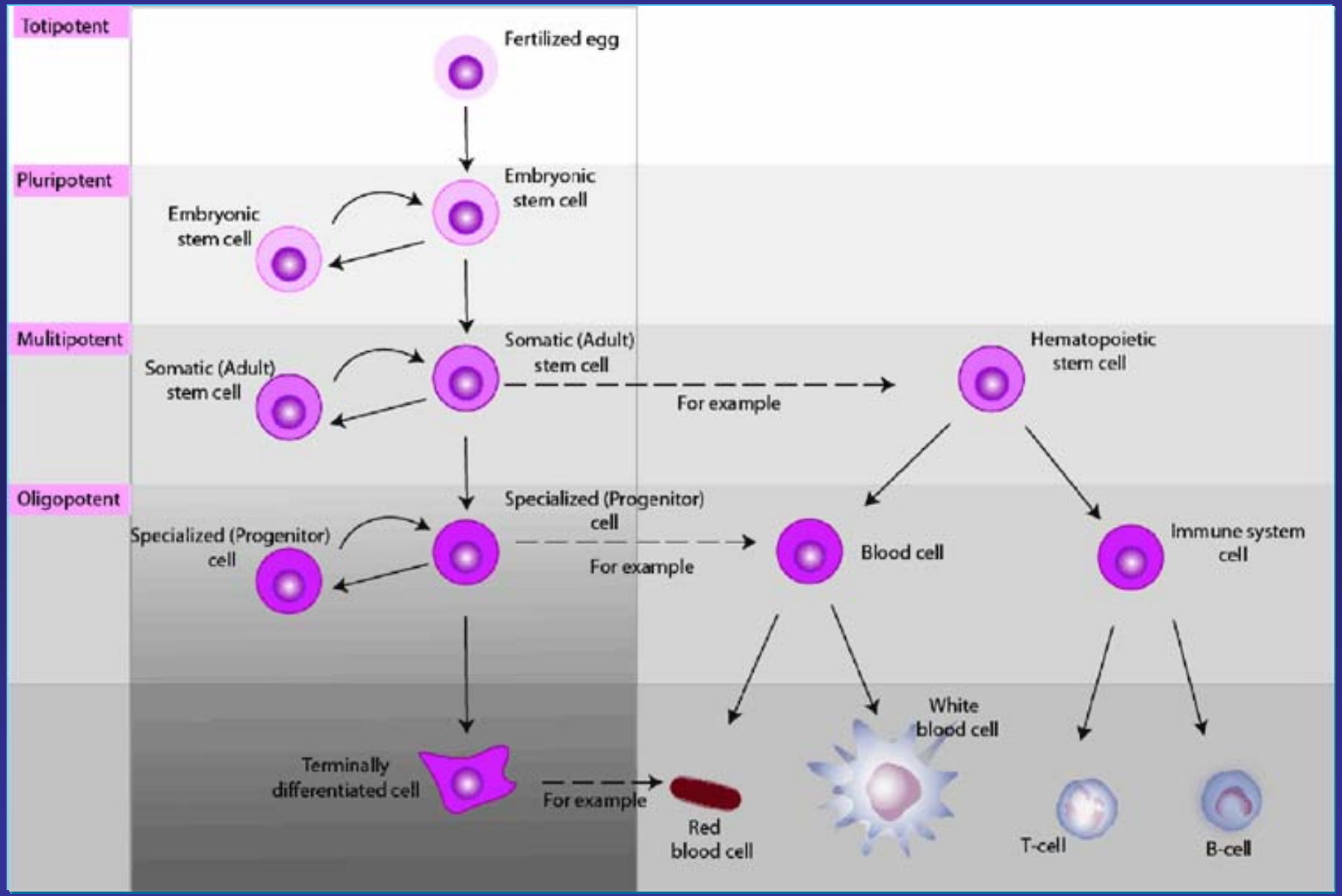
Lincon Stamp, *Monash Institute for Medical Research*

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

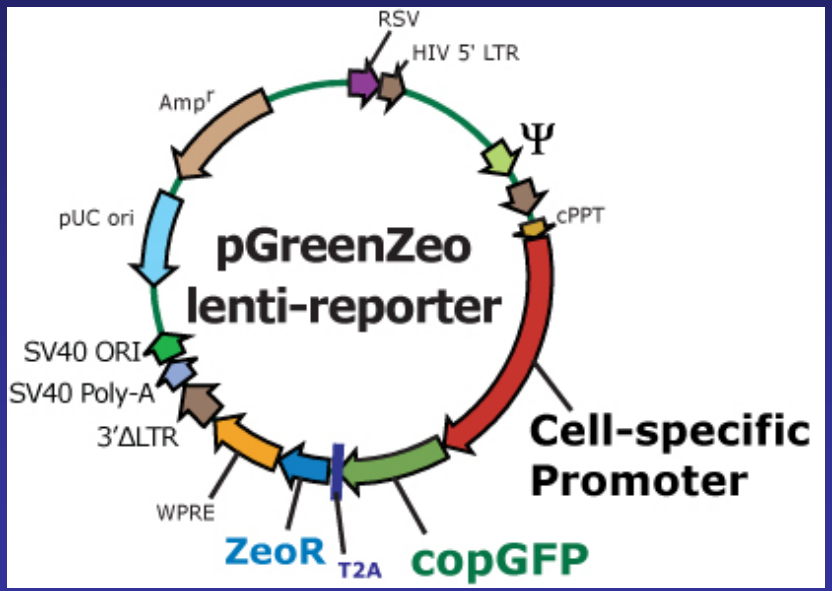


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

Stem Cell Differentiation Reporters

Sorting & Selection

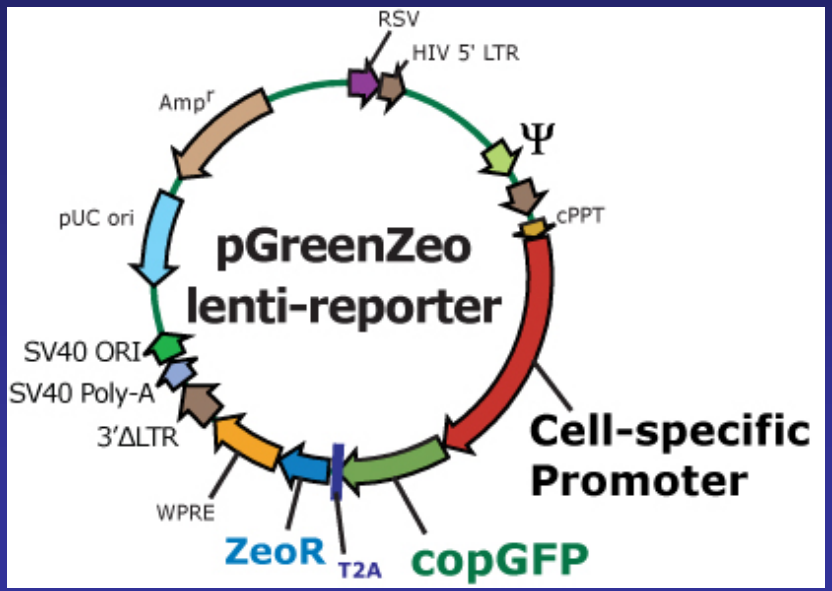


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

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

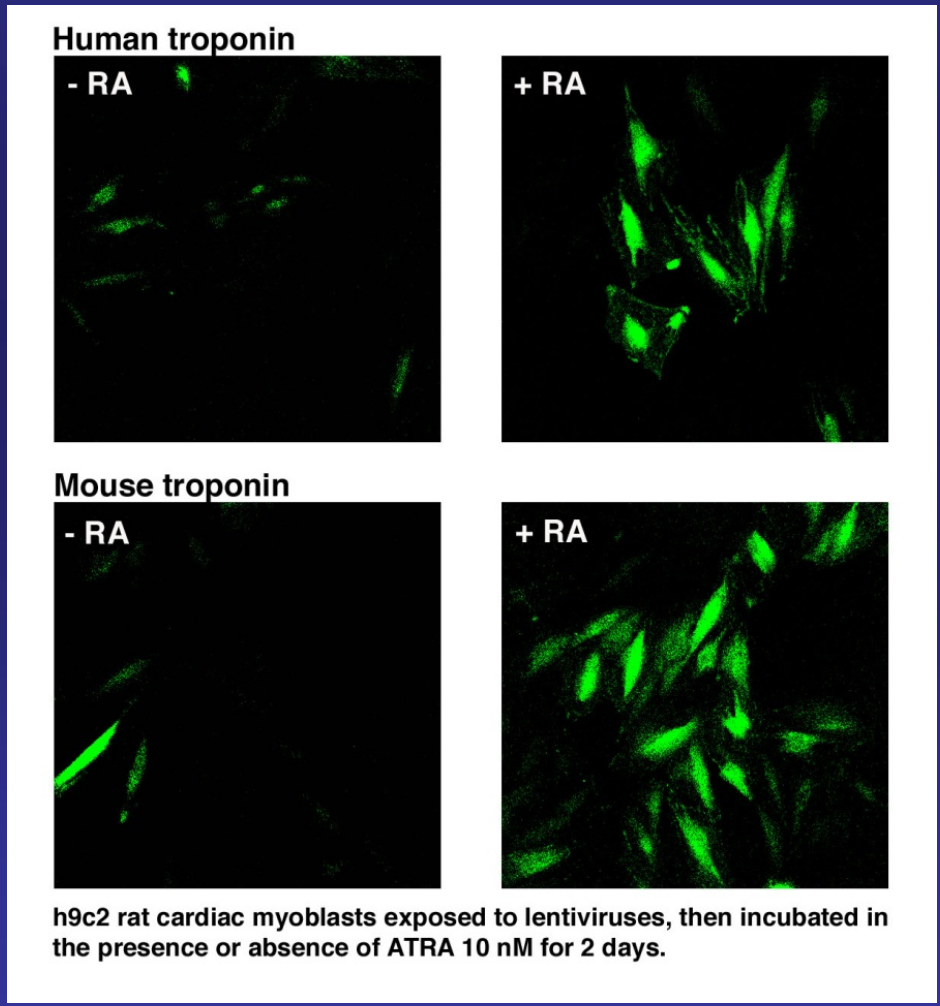
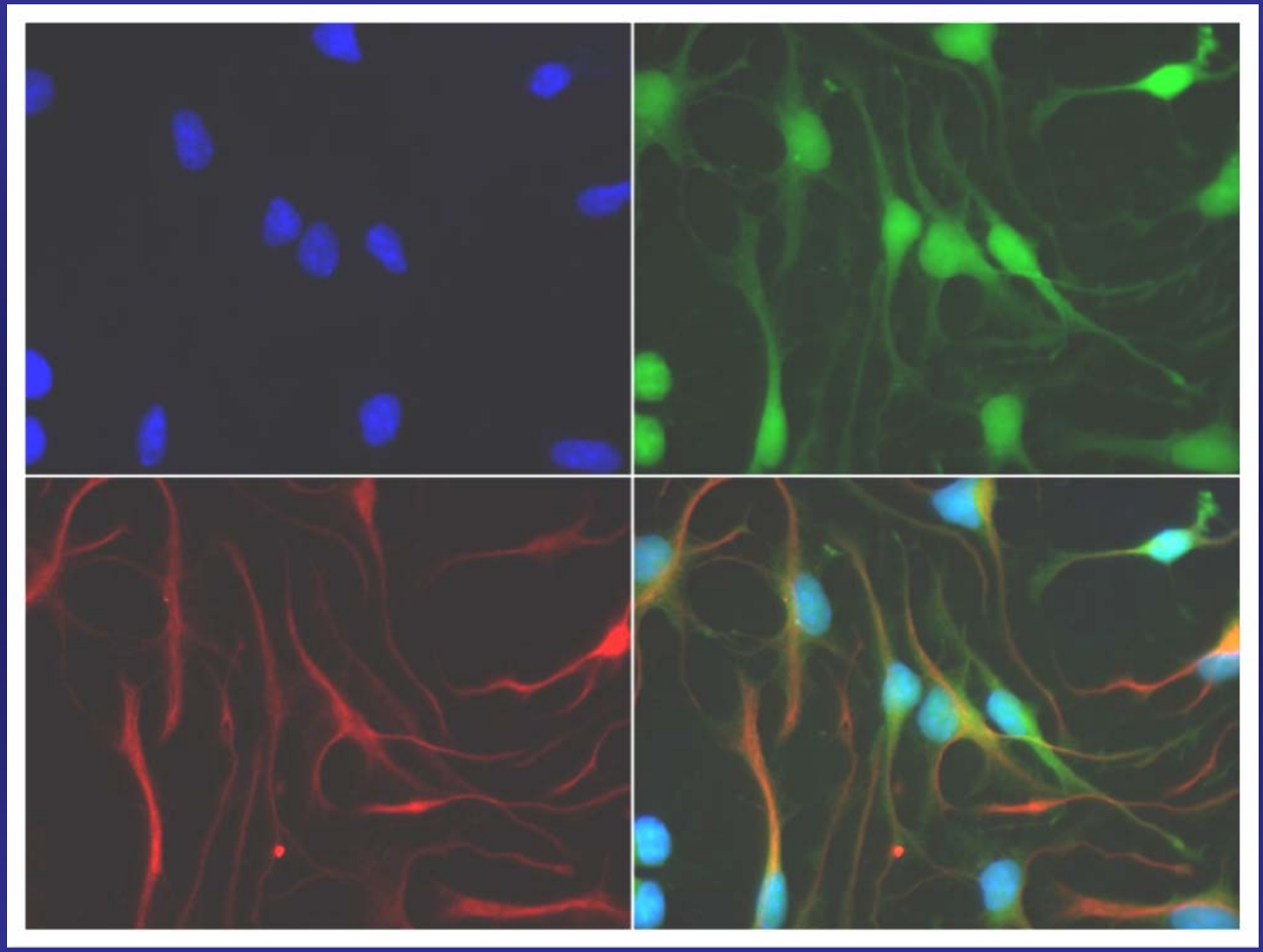


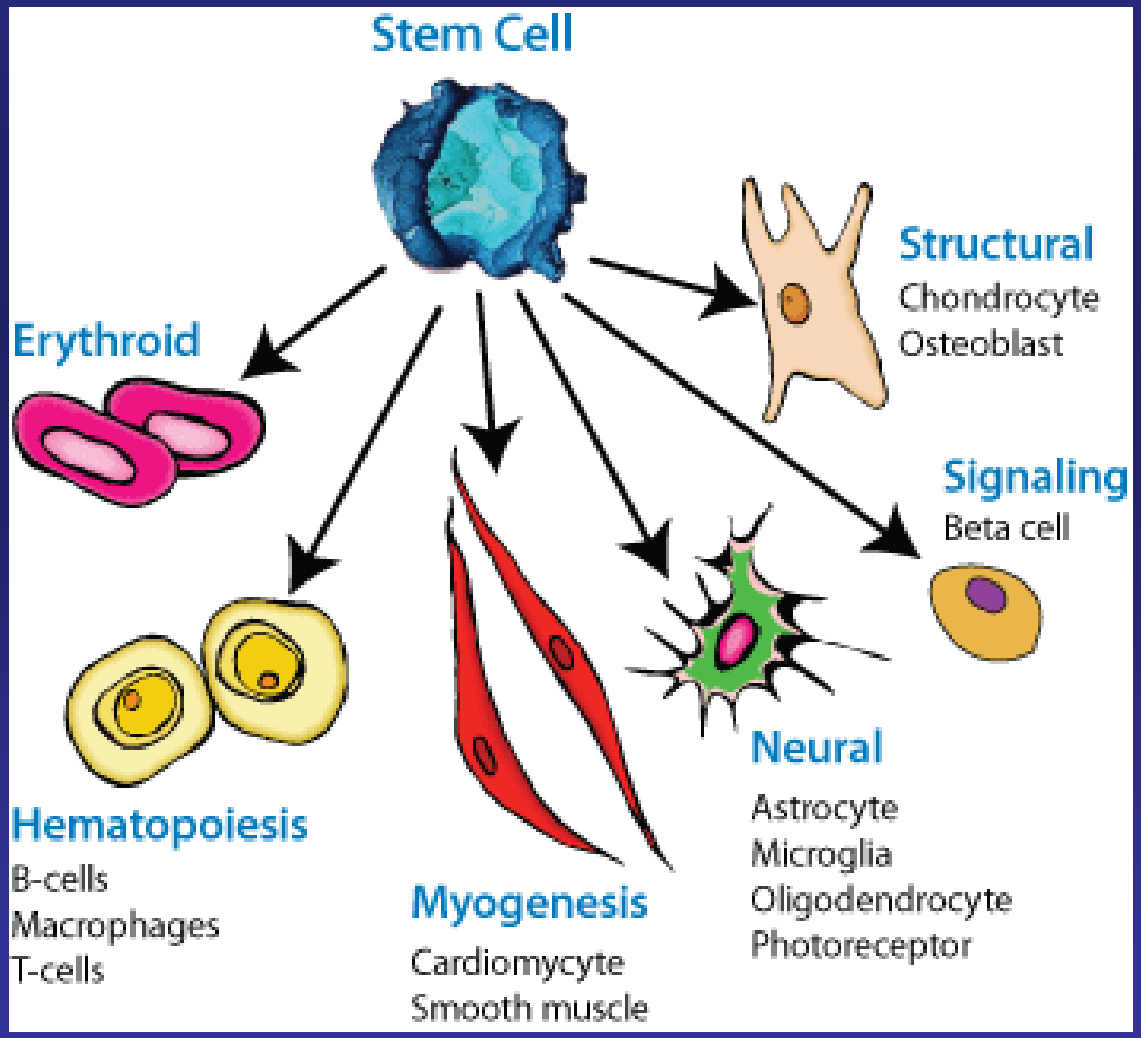
Figure: Cardiac myoblasts infected with TNNT2 reporter in presence or absence of ATRA

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,*]
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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

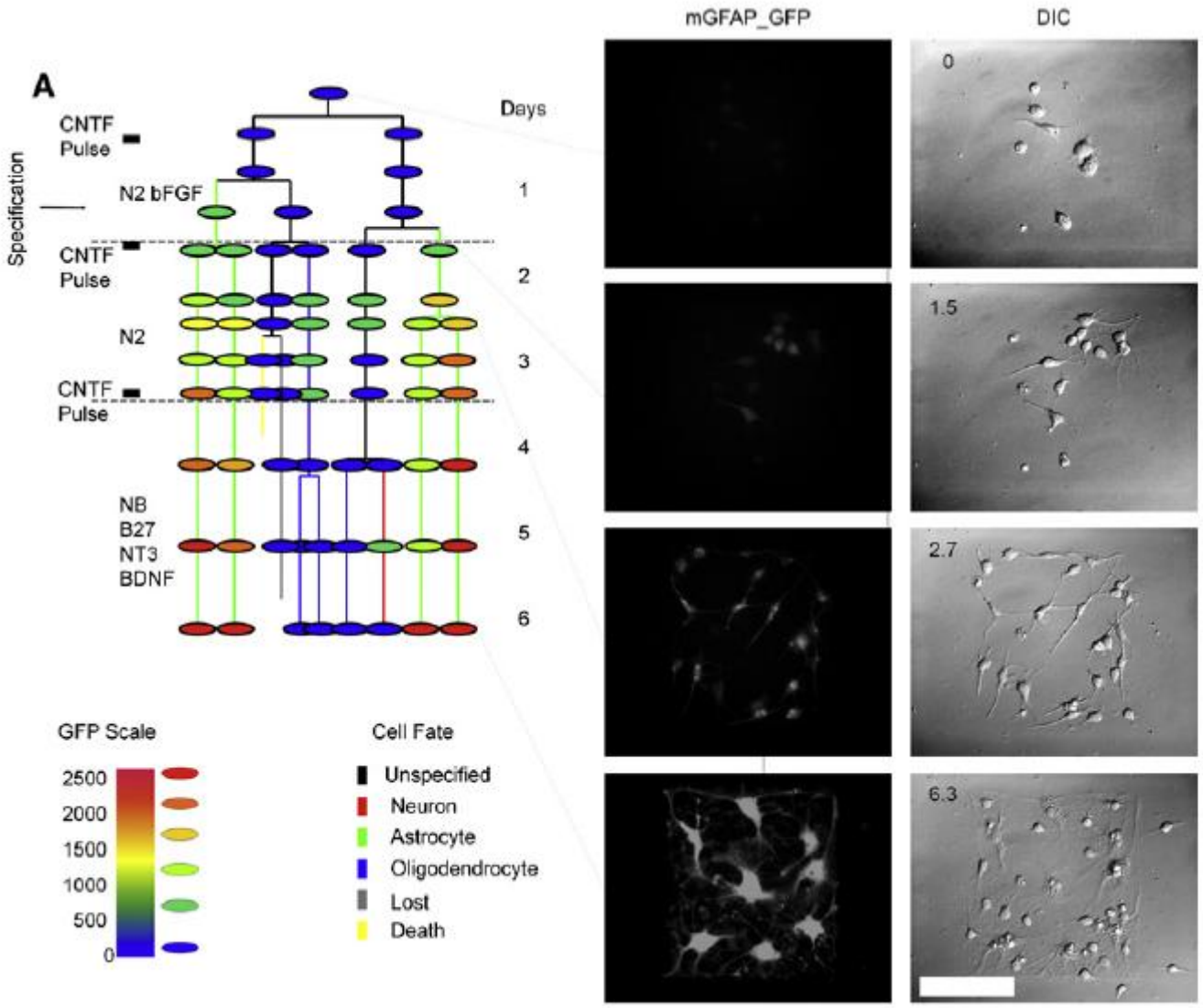


Figure 6. GFAP Reporter Expression in Neural Stem Cell Lineages Validates Early Astrocytic Fate Specification

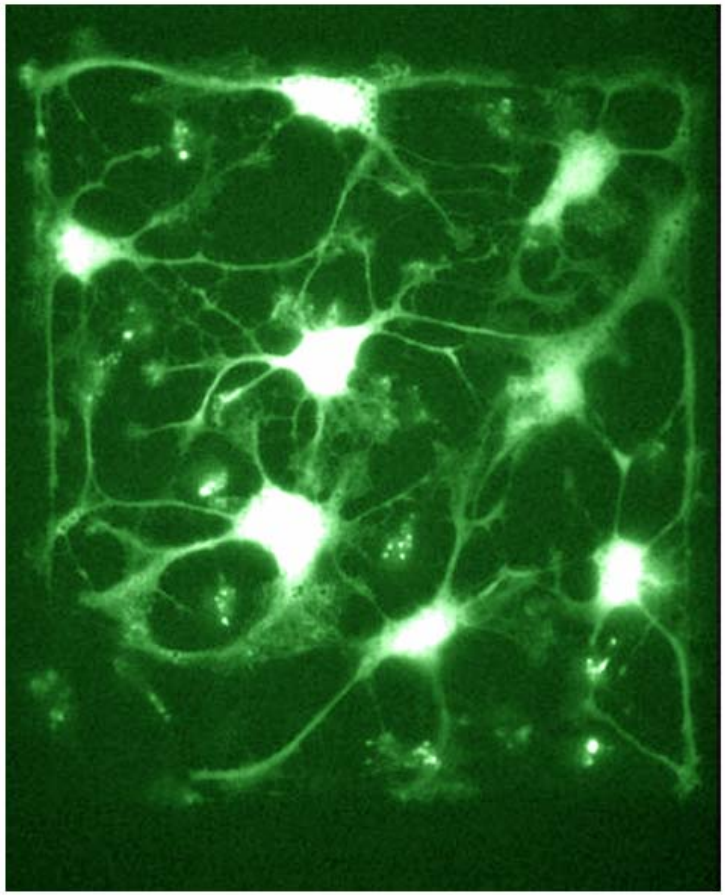
(A) Lineage fate map from a single infected founder cell. The vertical colored lines represent fate. The colored ovals indicate GFP expression level at each point of fluorescence imaging. The GFP scale represents mean fluorescence, in arbitrary units, as discrete colors. Example fluorescence micrographs 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

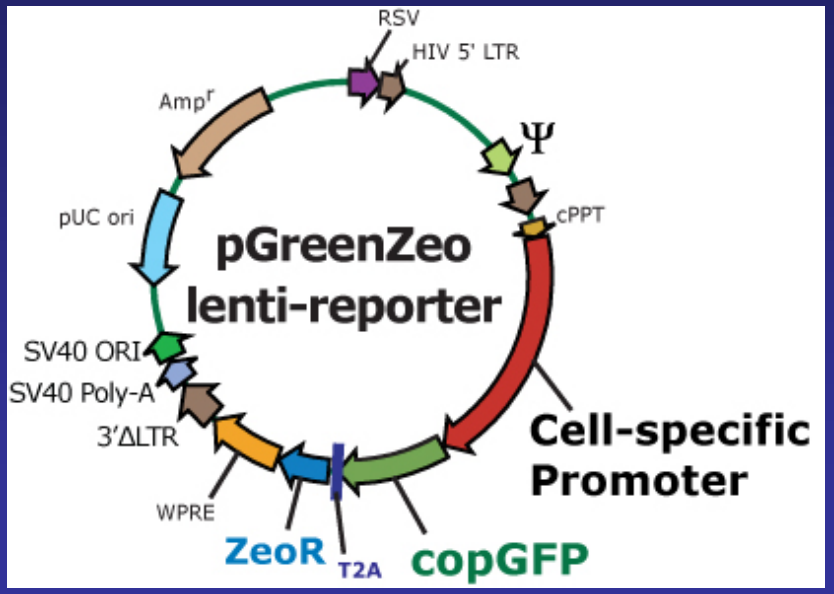


Figure: New generation of dual reporter vector uses both GFP and ZeoR reporters

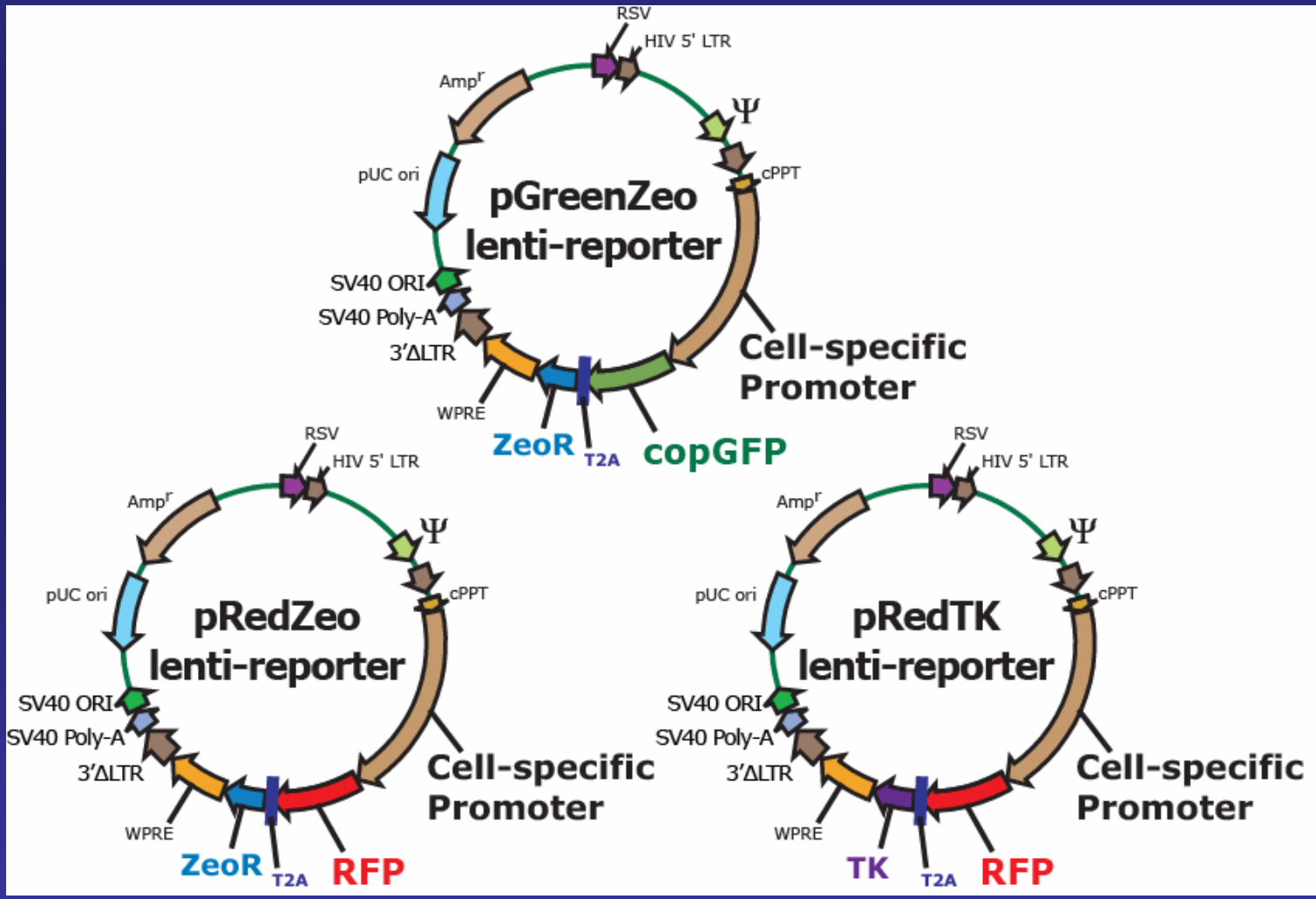
Stem Cell Pluripotency Reporters

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

pGreenZeo

pRedZeo

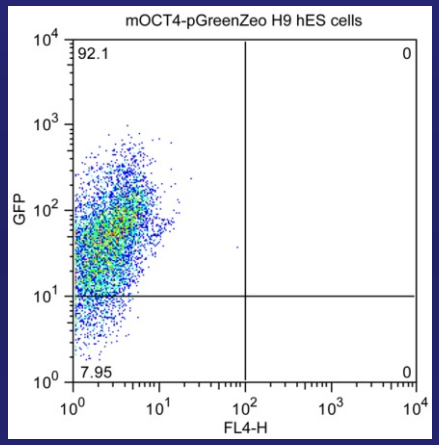
pRedTK

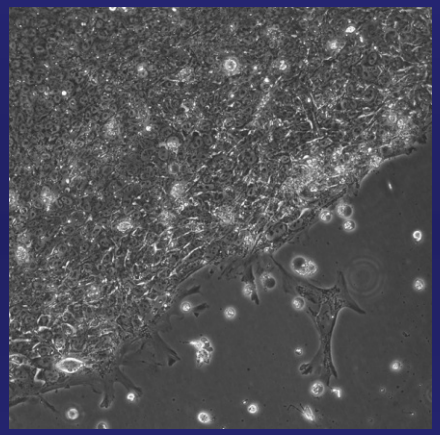


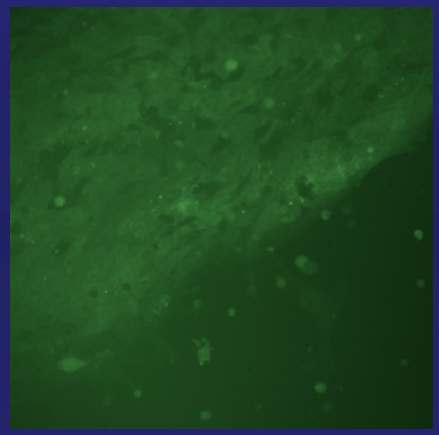
Stem Cell Pluripotency Reporters

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

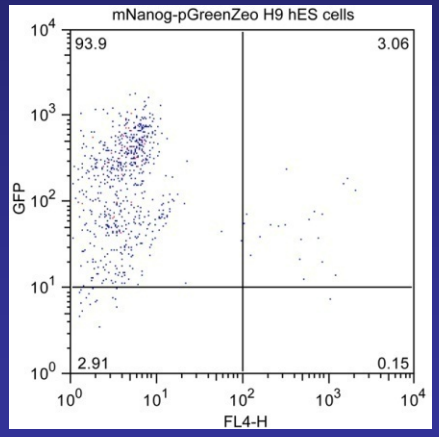
pGreenZeo-mOct4

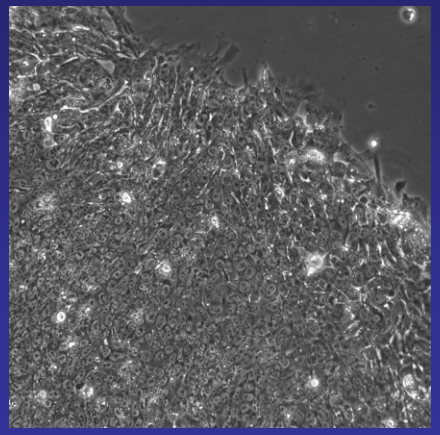






pGreenZeo-mNanog





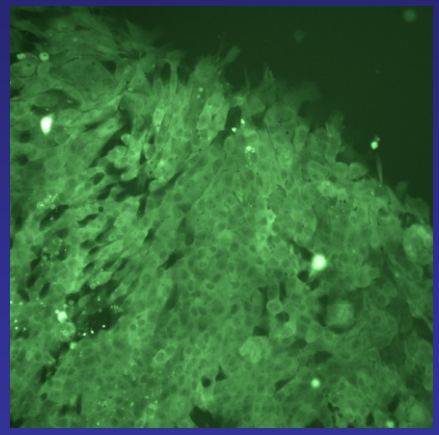
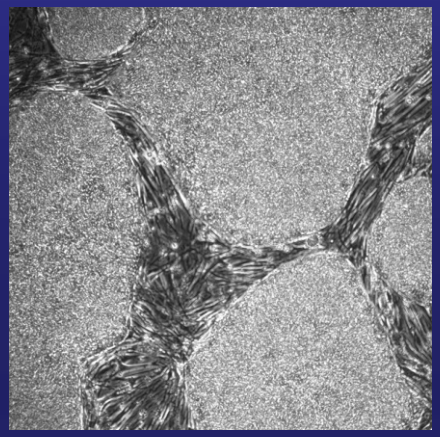
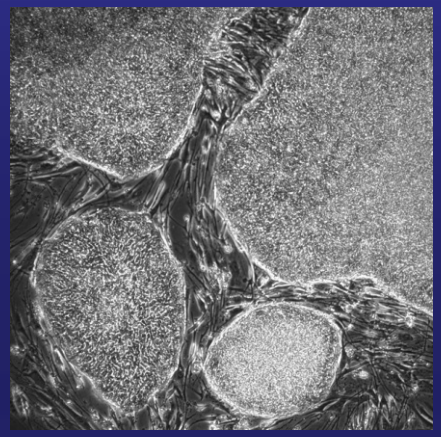
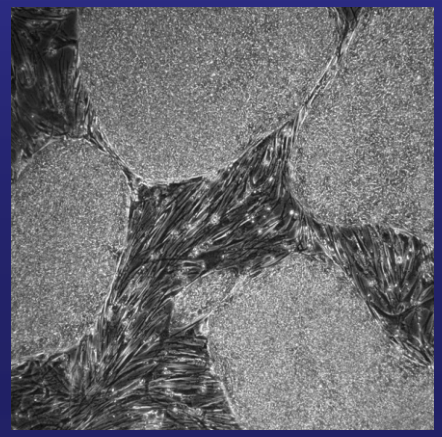
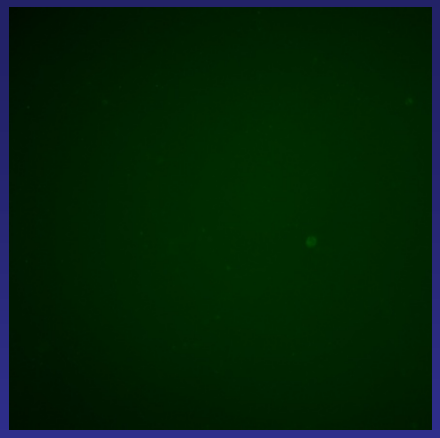
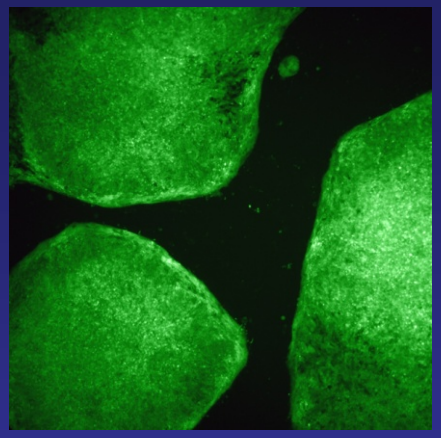
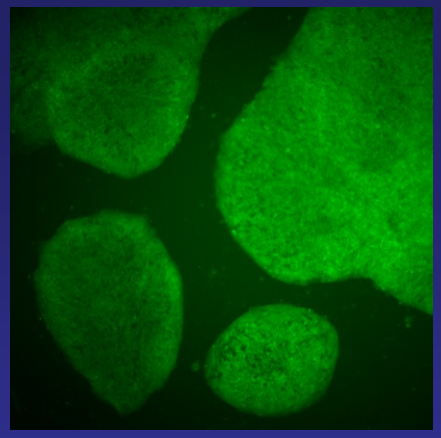
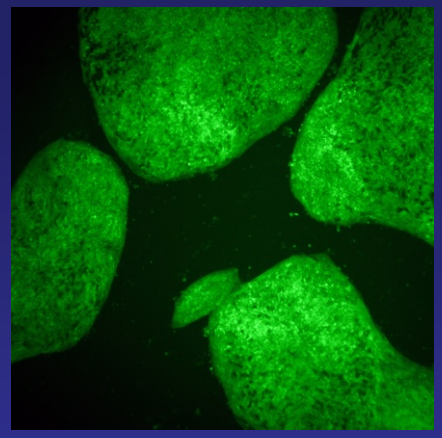


Figure: Transduced H9 hES cells showing Oct4 and Nanog expression

Tools for Stem Cell Biology

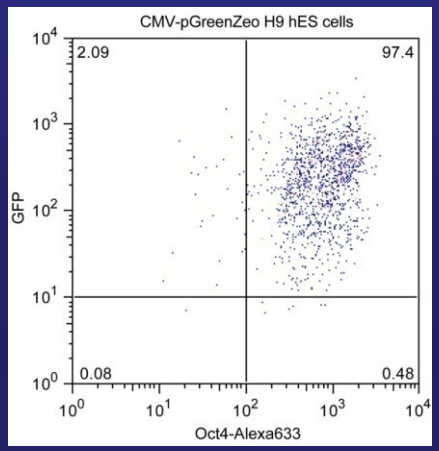
	H9 hES cells	pGreenZeo-CMV Transduced H9 hES cells	pGreenZeo-mOct4 Transduced H9 hES cells	pGreenZeo-mNanog Transduced H9 hES cells
Phase Contrast				
GFP				

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

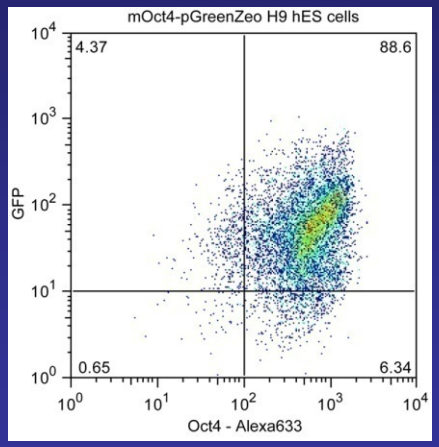


Figure: GFP-Reporter and Oct4-Immunostained Double Positive Cells

Stem Cell Pluripotency Reporters

- Data from collaborator: Dr. Tim Kamp

pGreenZeo-CMV

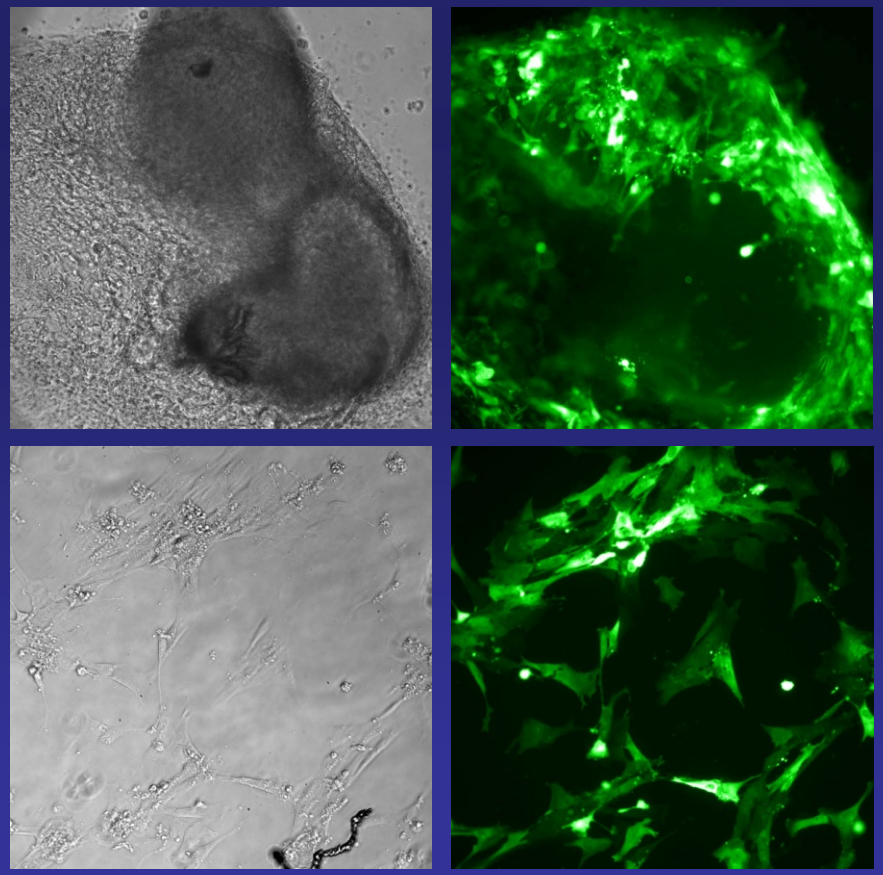
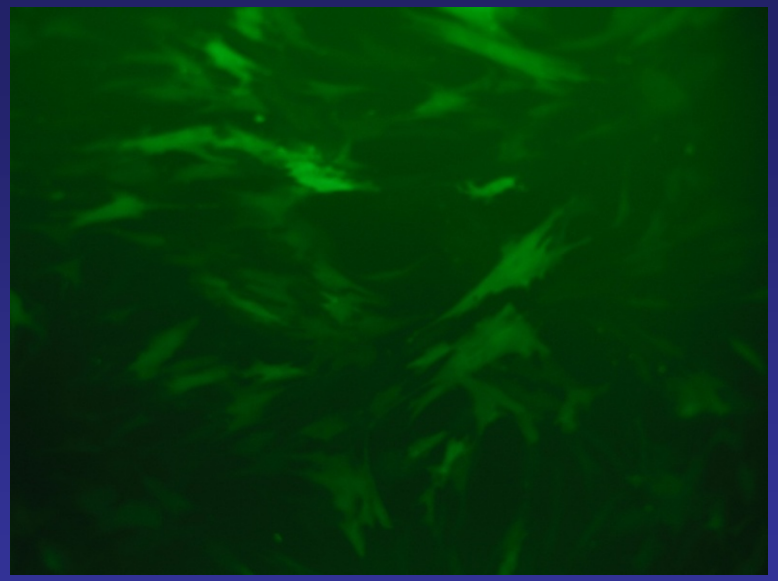


Figure: Embryoid bodies transduced with lentivirus

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

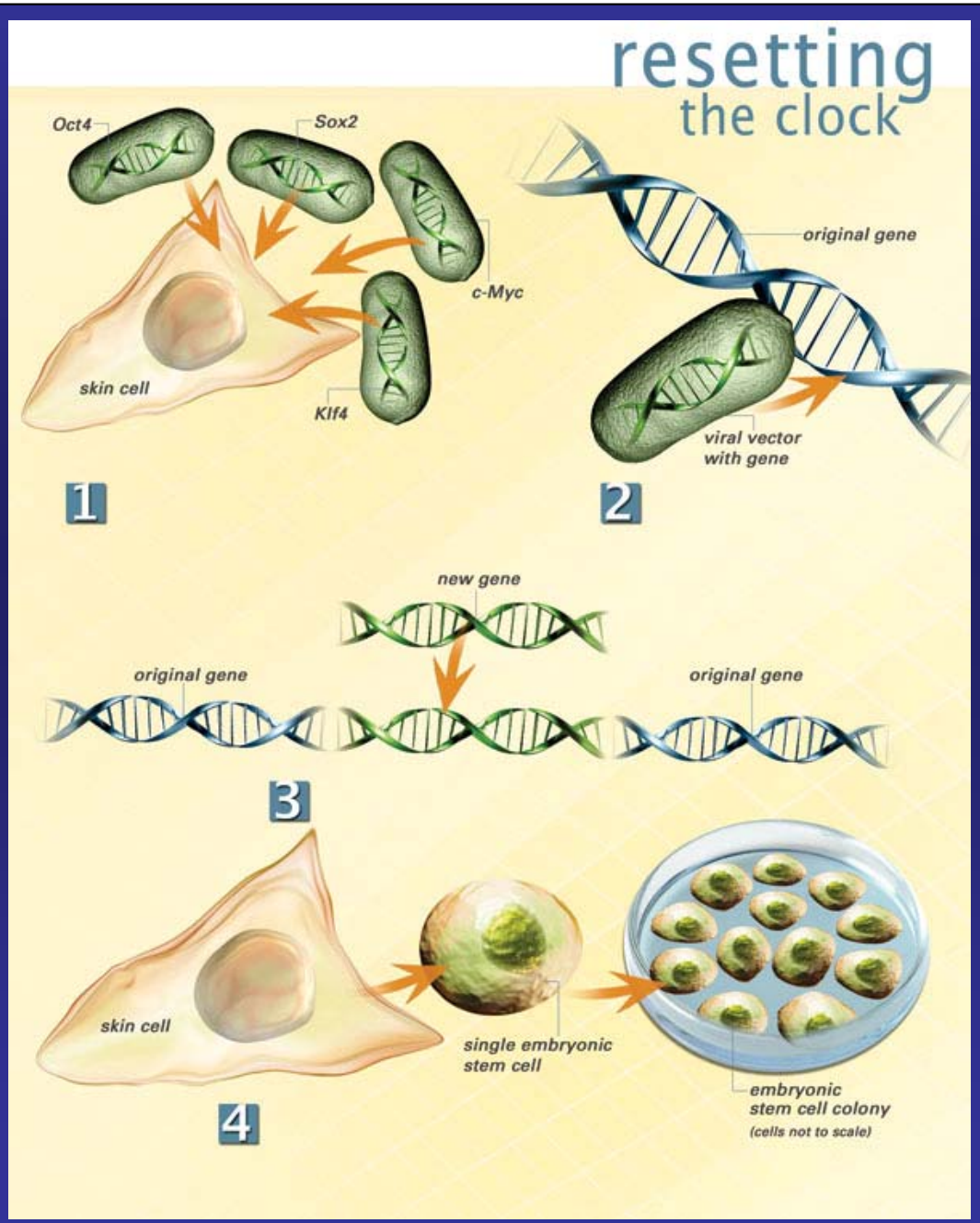
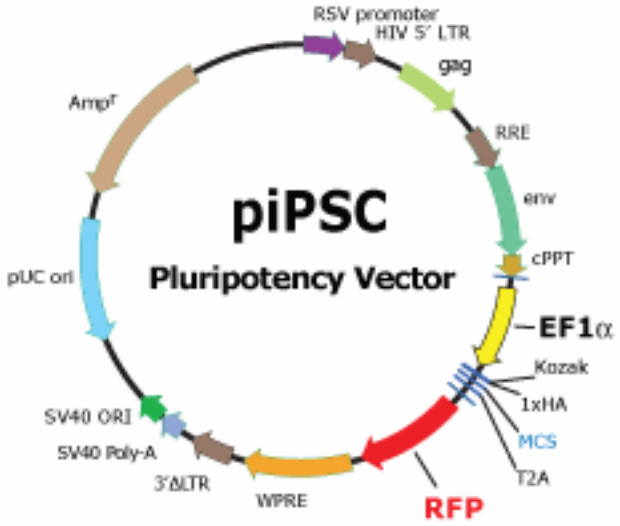


Image: Christina Ullman

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

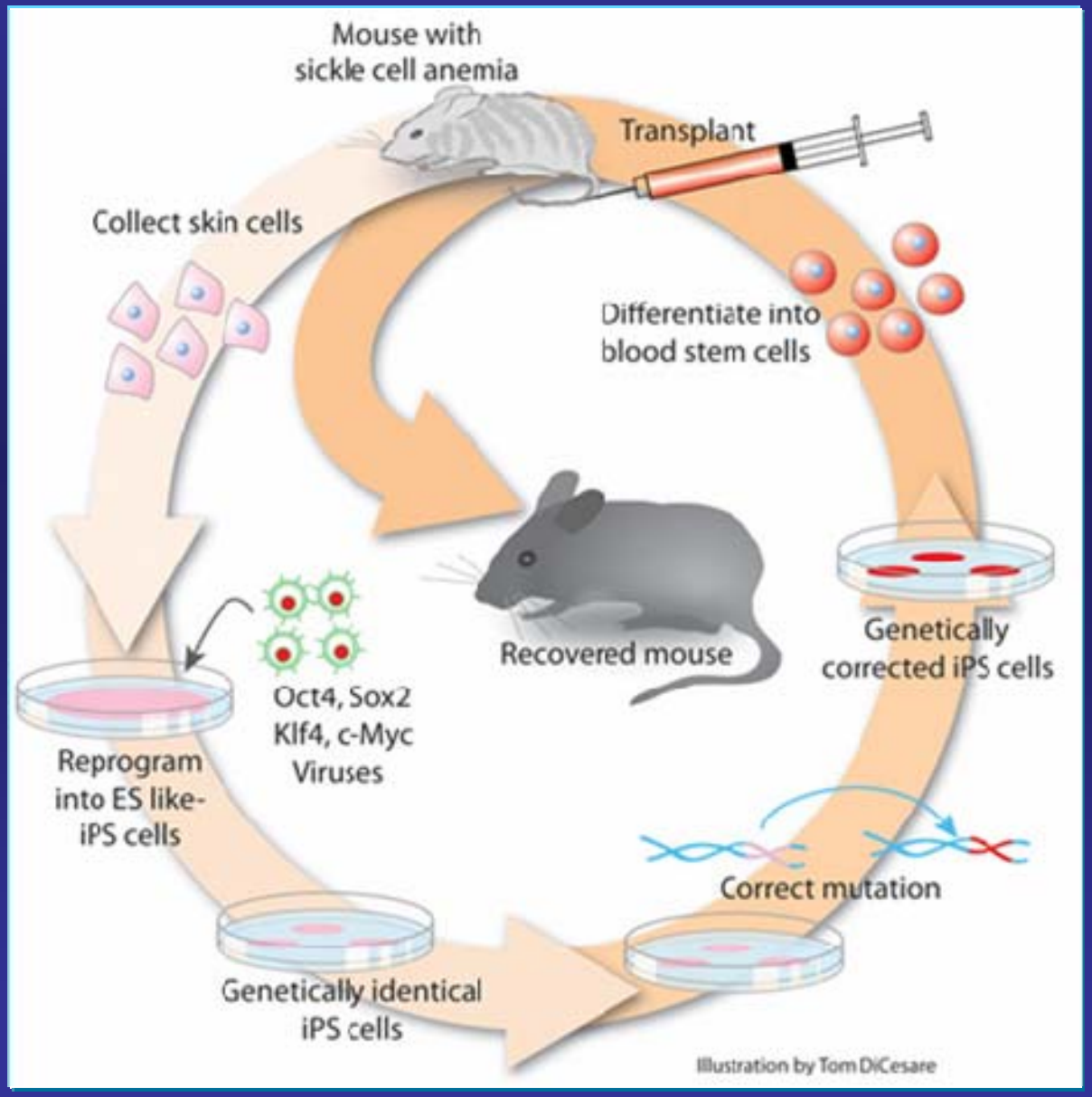
Oct4 • SOX2

Nanog • KLF4

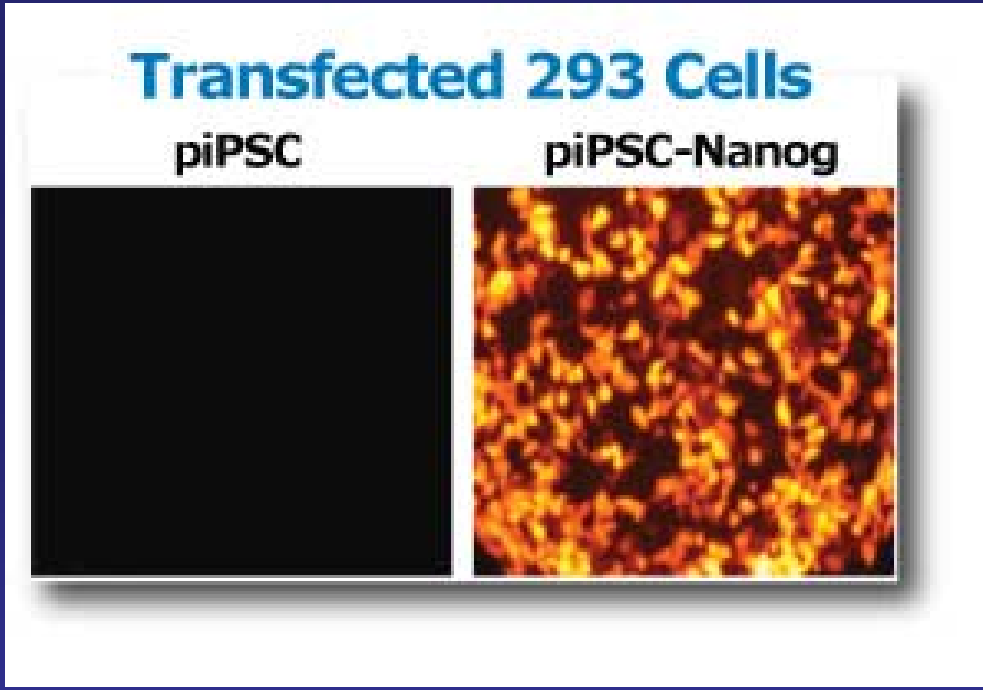
Lin-28 • c-Myc

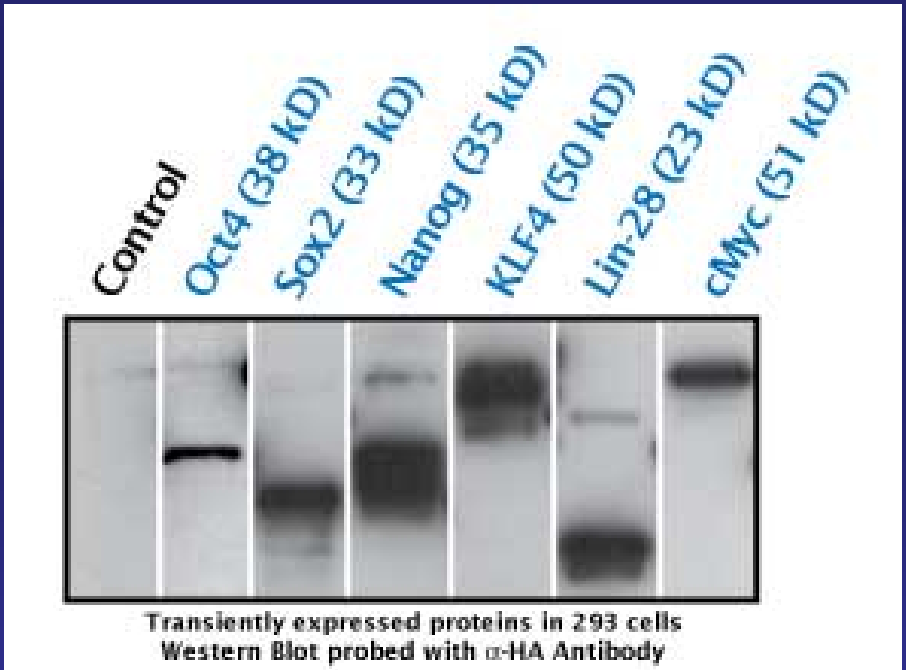
"The Magic Brew" - Rossant, 2007

Tools for Stem Cell Biology

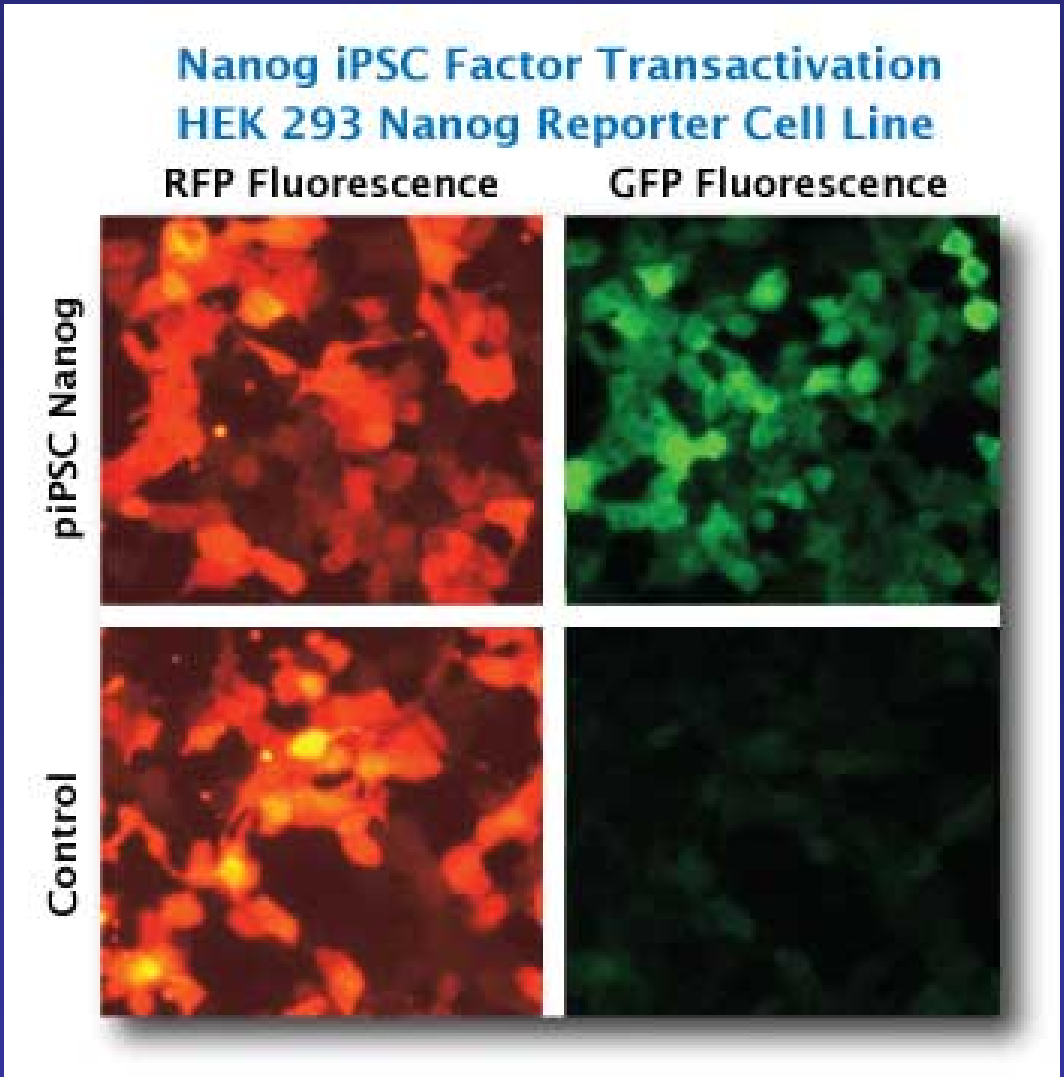


Induce Pluripotency with iPSC Factors





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

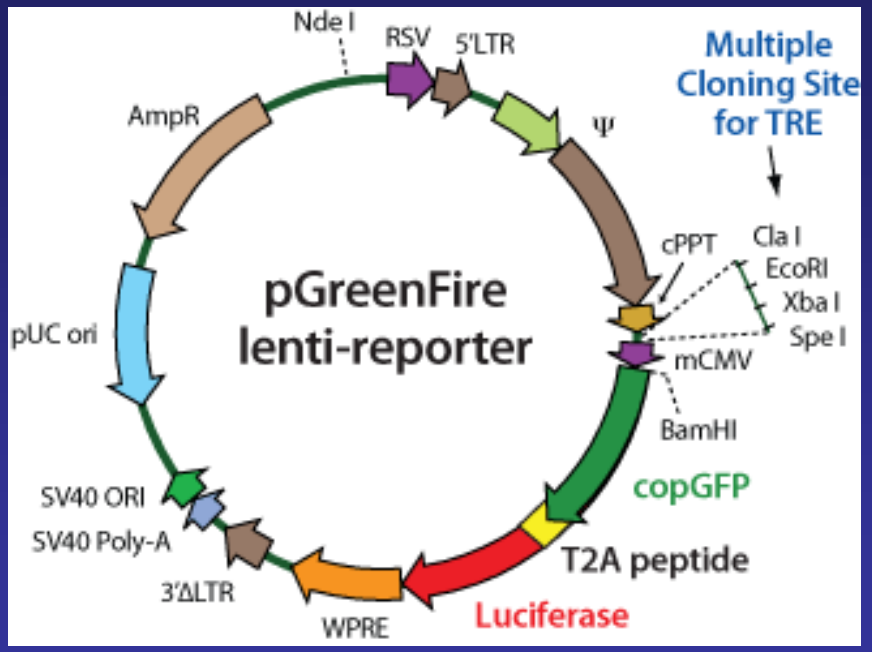


Figure: New generation of dual reporter vector uses both GFP and Luciferase reporters

pGreenFire Pathway Reporters

Sorting & Quantitation

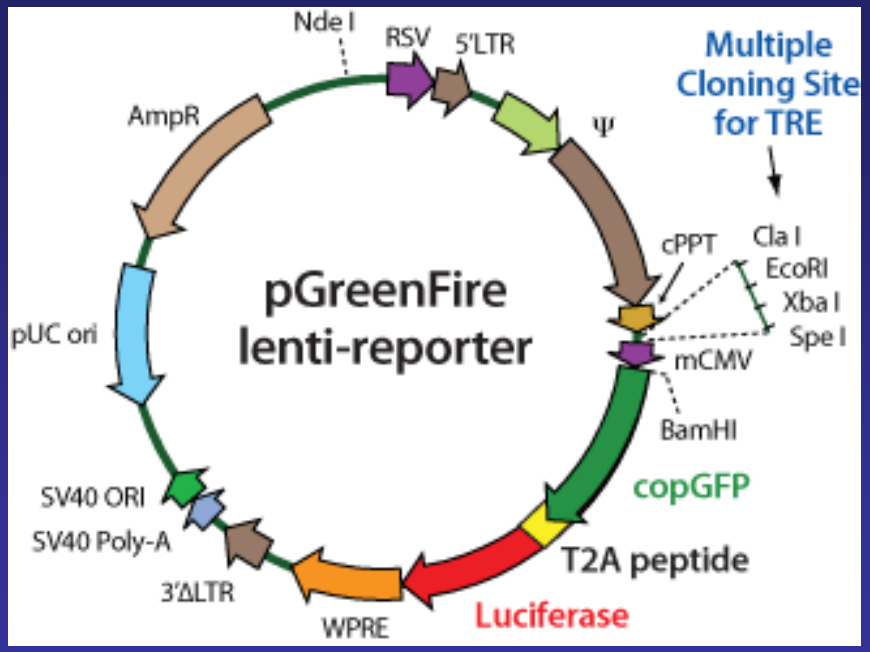


Figure: New generation of dual reporter vector uses both GFP and Luciferase reporters

pGreenFire Pathway Reporters

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

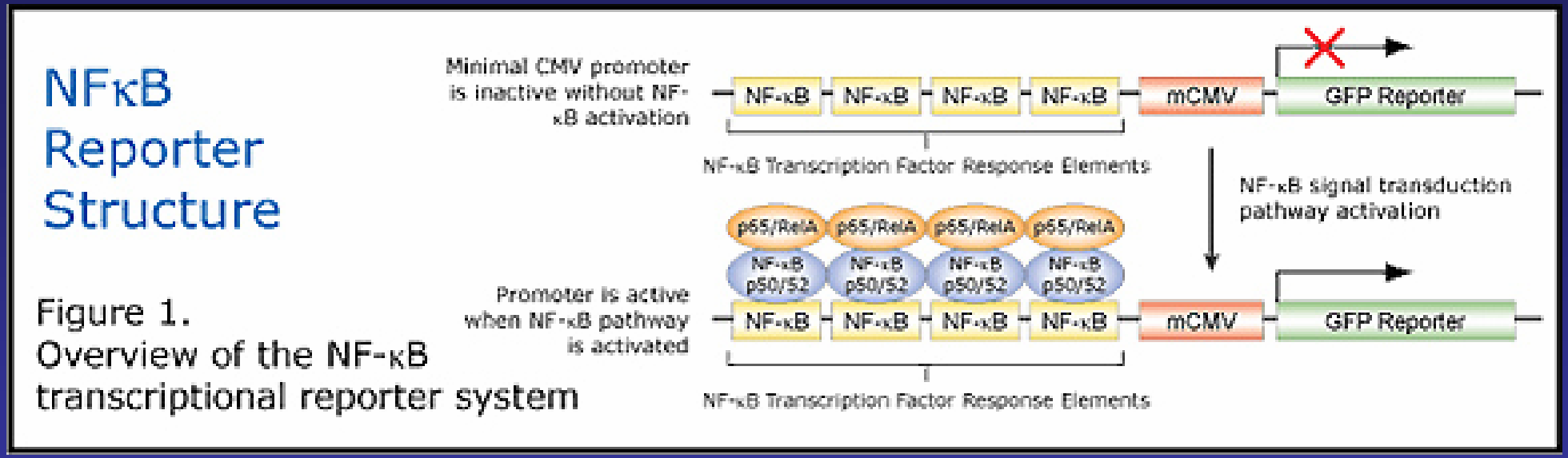


Figure 1.
Overview of the NF-κB transcriptional reporter system

pGreenFire Pathway Reporters

Easily Sort cells based on Transcriptional Activation:

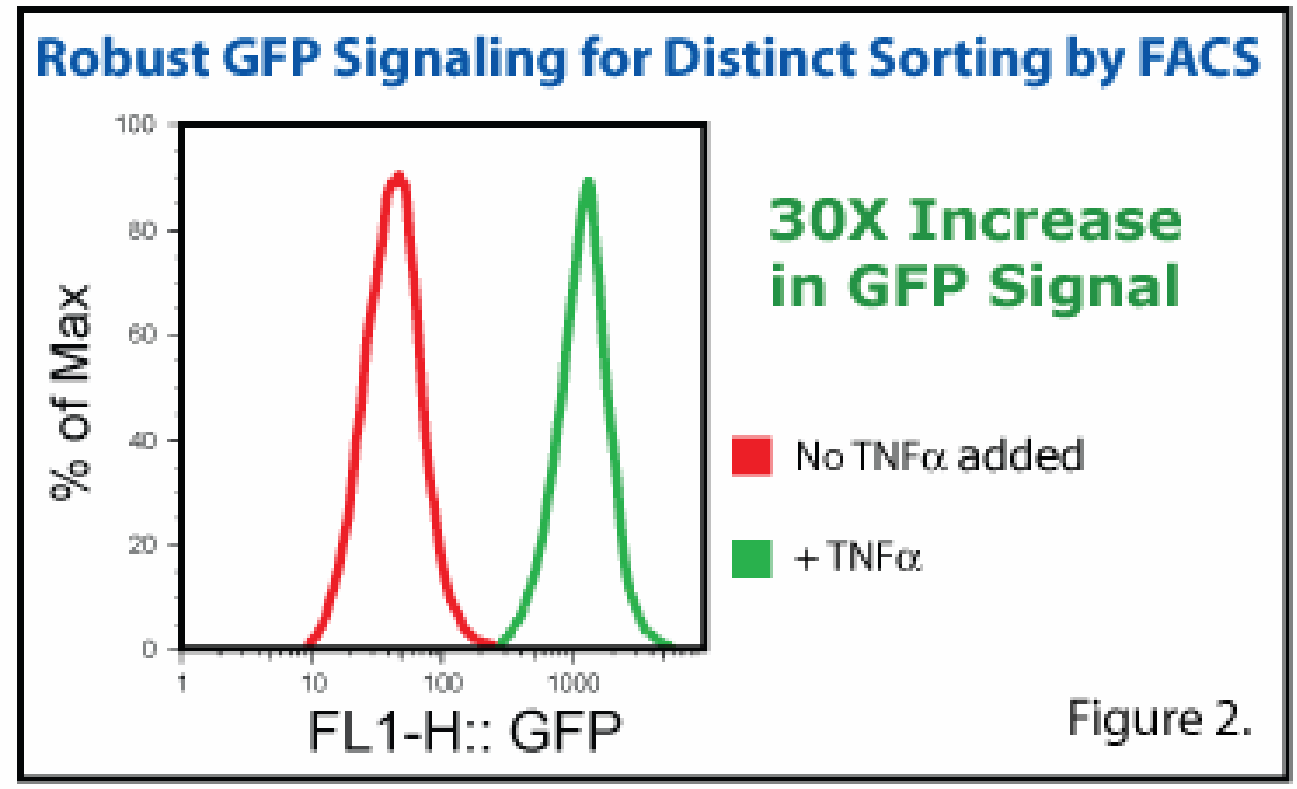
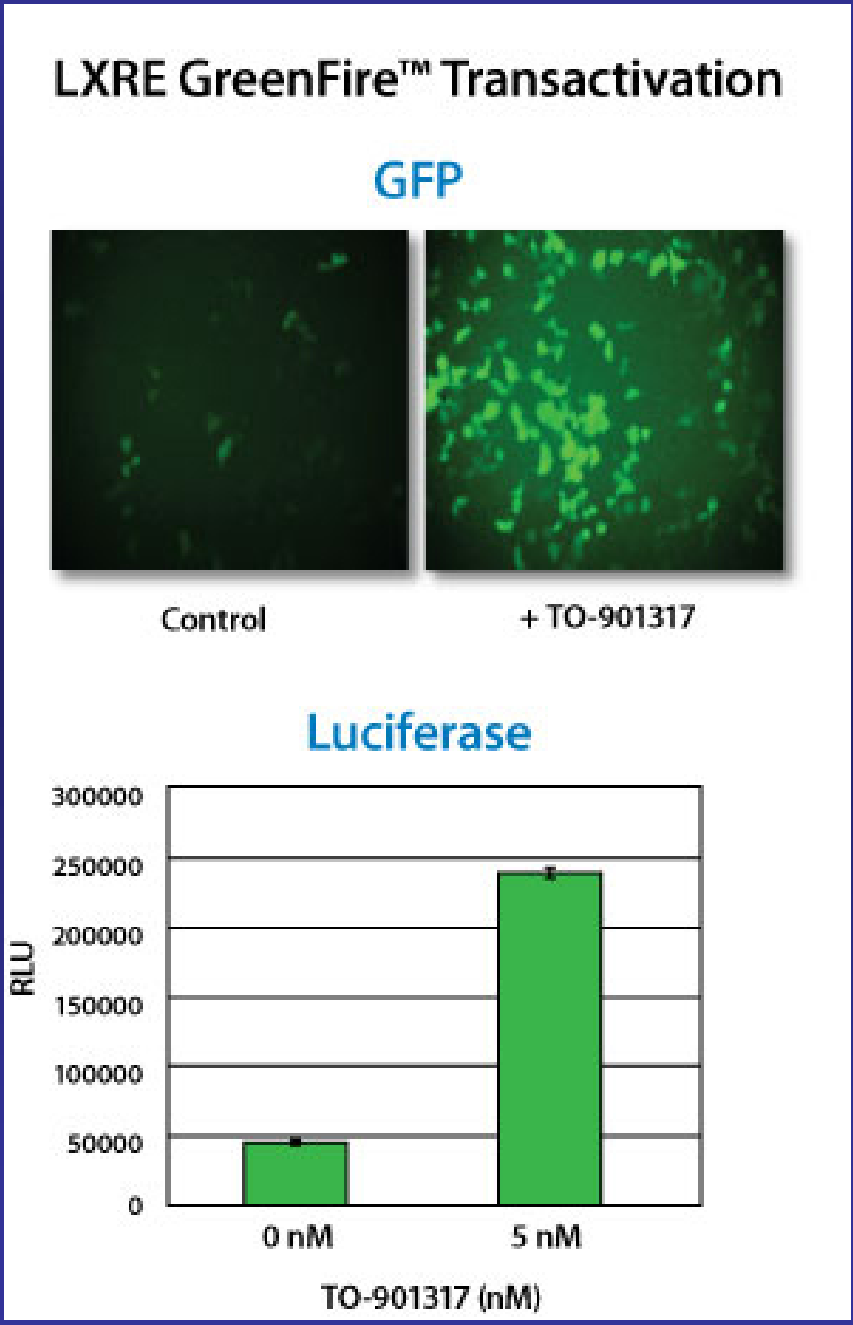


Figure: Jurkat/NFkB/GFP reporter cells sorted after treatment with TNF-alpha

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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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.

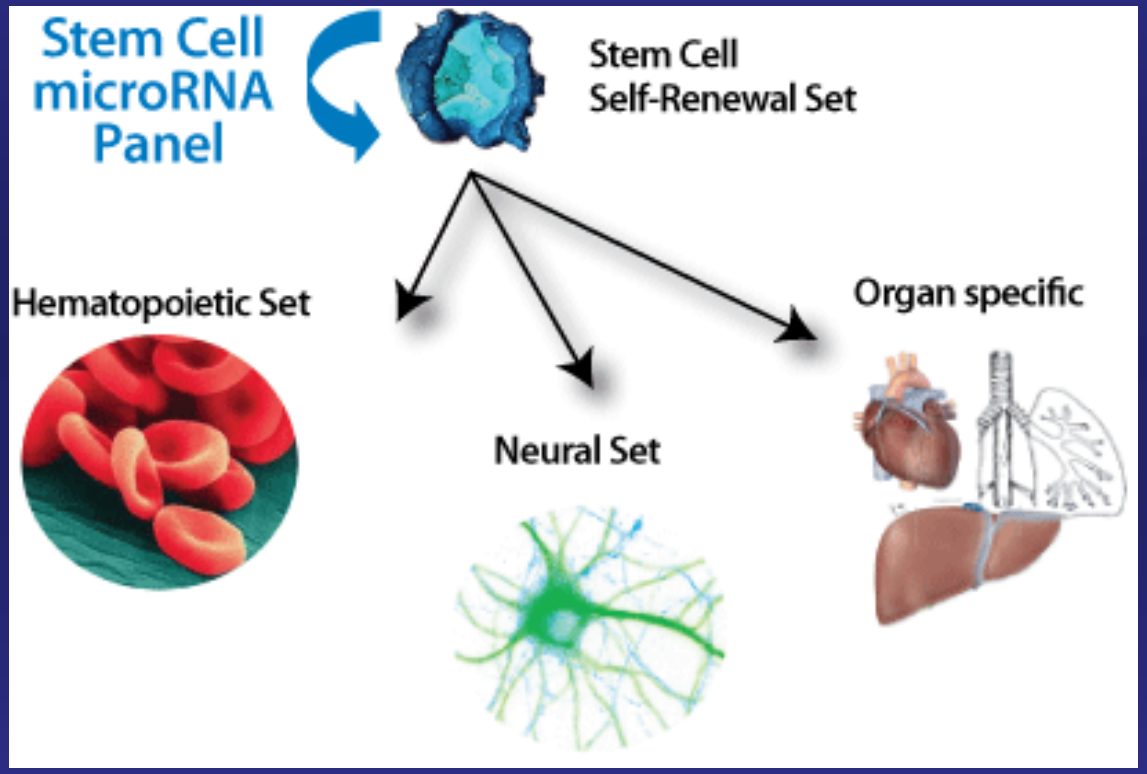
adipogenic differentiation | osteogenic differentiation | high-throughput RNAi screen

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.

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SBI Launches New Website!