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mRNA for Integration-free Cell Fate Manipulation

BRAD HAMILTON

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Presentation Outline

- mRNA Reprogramming System
- miRNA Enhanced mRNA Reprogramming
- mRNA for Differentiation

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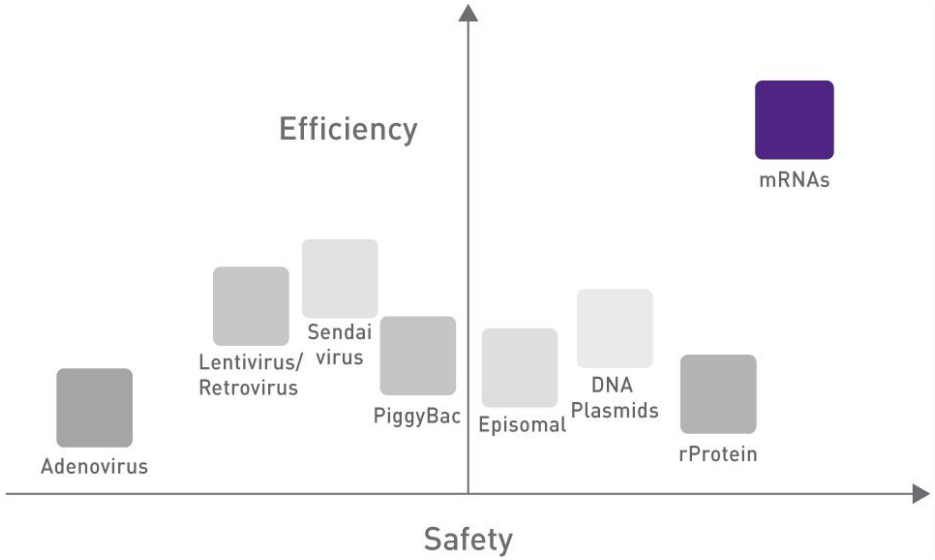
Advantages of mRNA Reprogramming

- **Fastest Method**
  - **Colonies emerge in 12 days**
- **Most Efficient (>1%)**
- **Non-integrating**
- **Non-viral/non-DNA**
  - No screening required
- **Safe**



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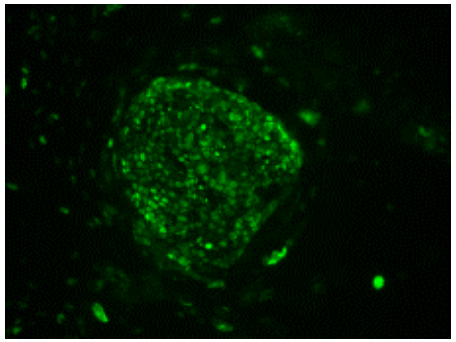
mRNA Reprogramming System

- **Functionally validated for iPS cell generation**
- **Kit composition**
  - mRNA Reprogramming Factors: hOSKML
  - Pluriton™ Reprogramming Medium
  - B18R, Recombinant Protein



Day 20 Primary iPS Colony from Parkinson's Disease fibroblasts, StainAlive™ Tra-1-81

- **Supporting Reagents**
  - Newborn Foreskin Fibroblasts, Irradiated (NuFFs-RQ)
  - StainAlive™ TRA-1-60/81 Antibodies

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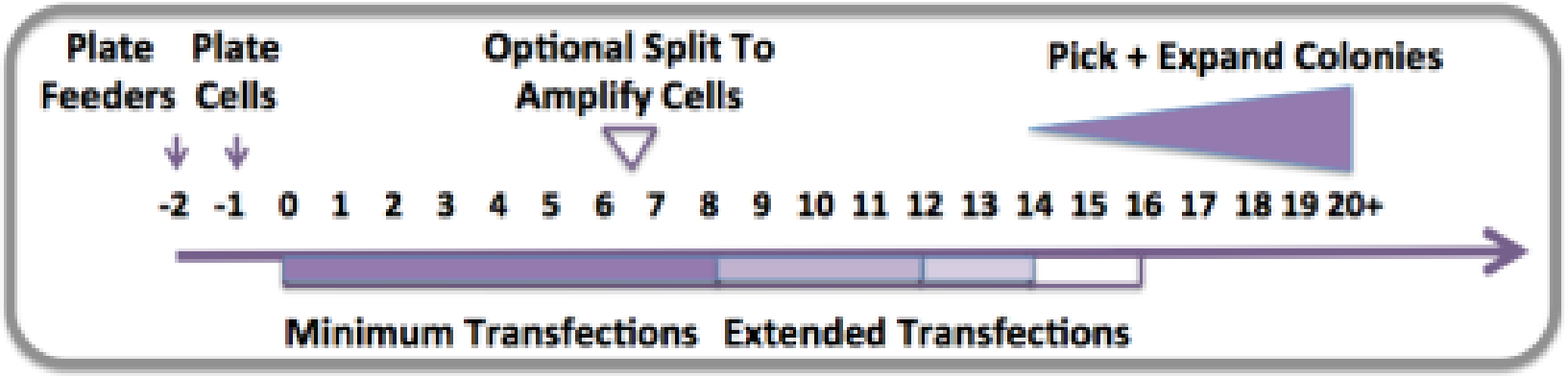
mRNA Reprogramming Timeline



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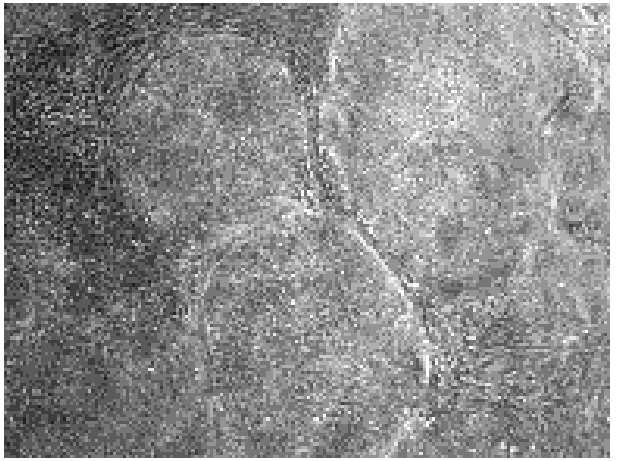
mRNA Reprogramming of Patient Fibroblasts

Fibroblast	TRA-1-60+ Colonies Day 16
Normal aHDF #1	68
Normal aHDF #2	190
Normal aHDF #3	128
Diseased aHDF #1	26
Diseased aHDF #2	38
Diseased aHDF #3	2
Diseased aHDF #4	619
BJ Fibroblast (Control)	674

- Reprogram in similar timeframe
- Range in productivity
- Reflection of proliferation rate



Primary mRNA iPS colonies
Day 16, Diseased aHDF #4

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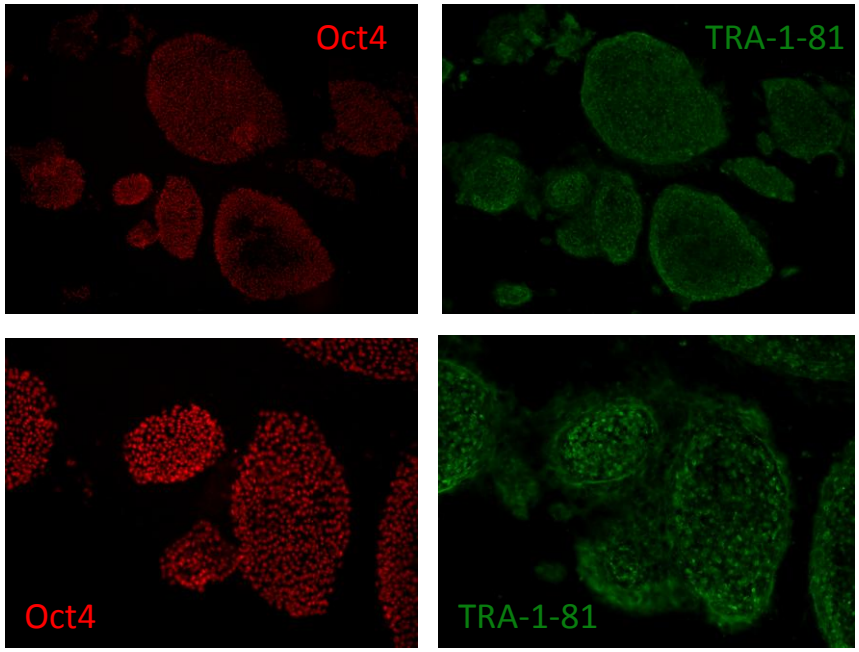
Homogeneous iPS Cell Colonies



p0 Primary Reprogramming Culture

- Uniform pluripotency
- Robust primary colony formation
- Efficient post-isolation line establishment
- No screening

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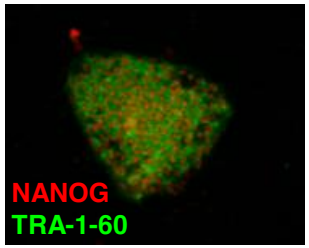
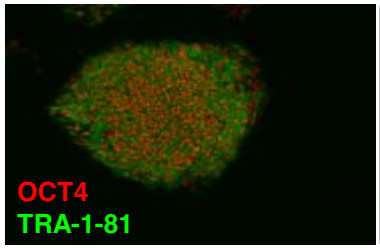
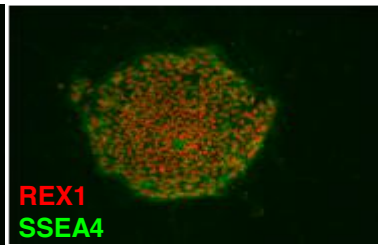
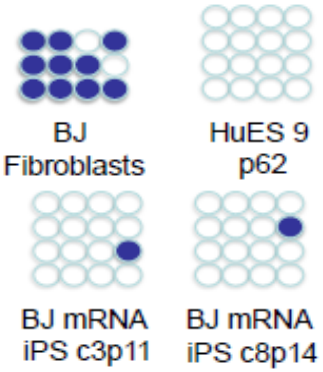
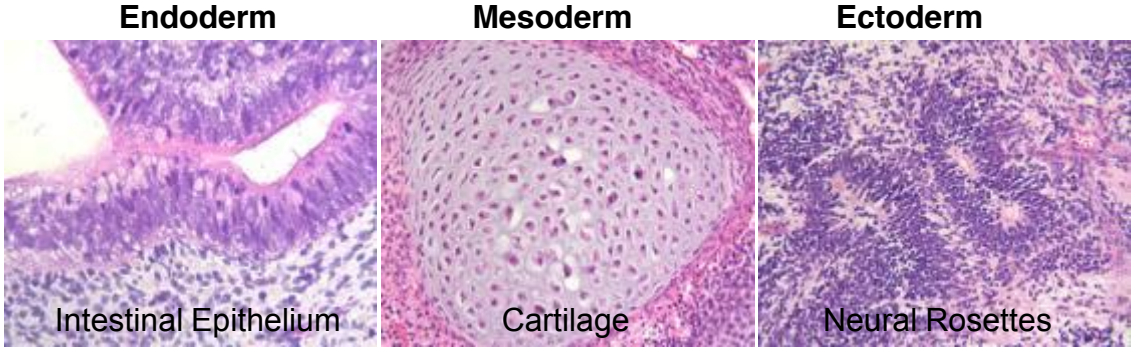
Stable, Pluripotent iPS Cell Lines











Courtesy of WIBR – Dirk Hockemeyer and Johanna Goldmann

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HSCI
HARVARD STEM CELL INSTITUTE
WHITEHEAD INSTITUTE
The University Of Sheffield.
IEO
European Institute of Oncology
imb
Institute of Medical Biology
STANFORD
SCHOOL OF MEDICINE
MONASH INSTITUTE OF MEDICAL RESEARCH
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- miRNA Enhanced mRNA Reprogramming
- mRNA for Differentiation

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miRNAs and Reprogramming

Embryonic stem cell–specific microRNAs promote induced pluripotency

Robert L Judson, Joshua E Babiarz, Monica Venere & Robert Blelloch

Highly Efficient miRNA-Mediated Reprogramming of Mouse and Human Somatic Cells to Pluripotency

Frederick Anokye-Danso,[1] Chinmay M. Trivedi,[2] Denise Juhr,[5] Mudit Gupta,[2] Zheng Cui,[1] Ying Tian,[1] Yuzhen Zhang,[1] Wenli Yang,[1,4] Peter J. Gruber,[3,4,5] Jonathan A. Epstein,[1,2,3,4] and Edward E. Morrisey[1,2,3,4,*]
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Reprogramming of Mouse and Human Cells to Pluripotency Using Mature MicroRNAs

Norikatsu Miyoshi,[1] Hideshi Ishii,[1,2,4,*] Hiroaki Nagano,[1] Naotsugu Haraguchi,[1] Dyah Laksmi Dewi,[1] Yoshihiro Kano,[1] Shinpei Nishikawa,[1] Masahiro Tanemura,[1] Koshi Mimori,[2] Fumiaki Tanaka,[2] Toshiyuki Saito,[3] Junichi Nishimura,[1] Ichiro Takemasa,[1] Tsunekazu Mizushima,[1] Masataka Ikeda,[1] Hirofumi Yamamoto,[1] Mitsugu Sekimoto,[1] Yuichiro Doki,[1] and Masaki Mori[1,2,4,*]
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[4]These authors contributed equally to this work

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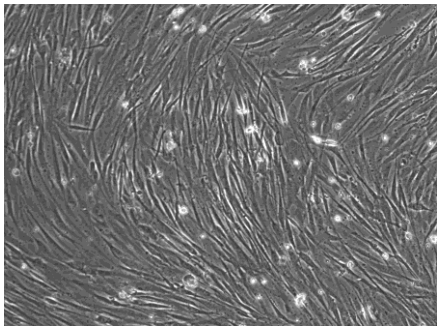
Refractory Target Cells: Options?

mRNA Only



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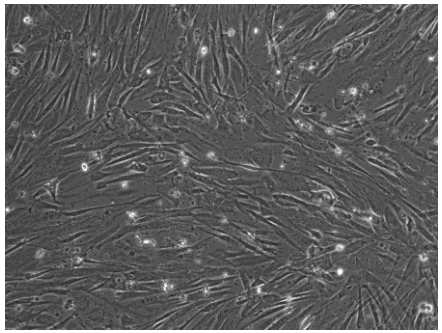

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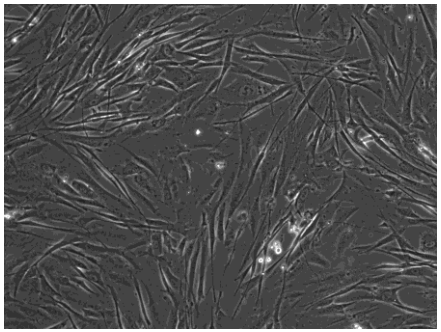

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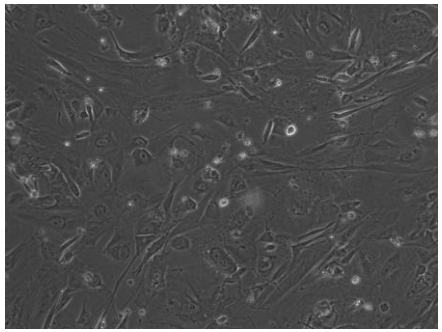
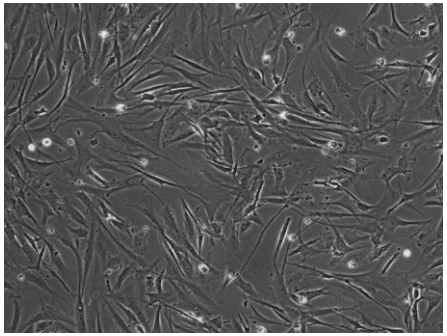


Day 18

mRNA + miRNA



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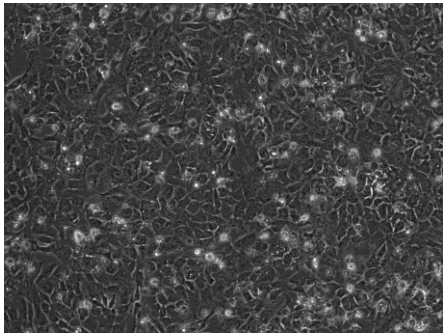

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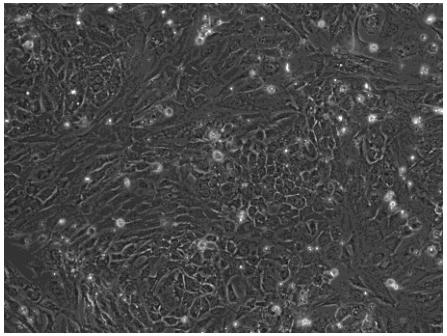

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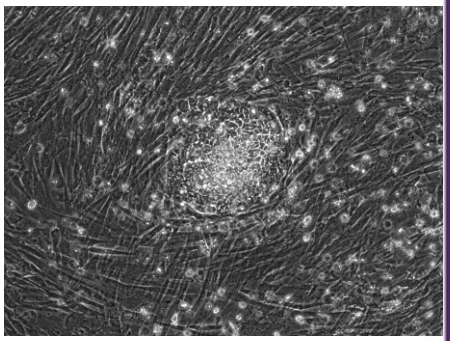

Day 13

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miRNA enhanced mRNA Reprogramming

- mRNA Reprogramming Factors: hOSKML
- Pluriton™ Reprogramming Medium
- B18R, Recombinant Protein
- Matrigel™ hES-qualified Matrix
- Stemfect™ RNA Transfection Kit
- Proprietary miRNA cocktail

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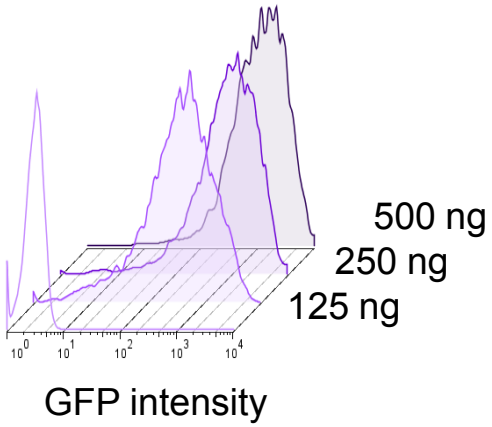
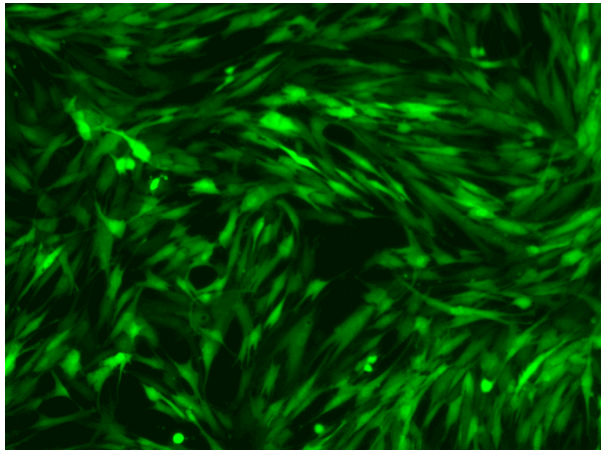
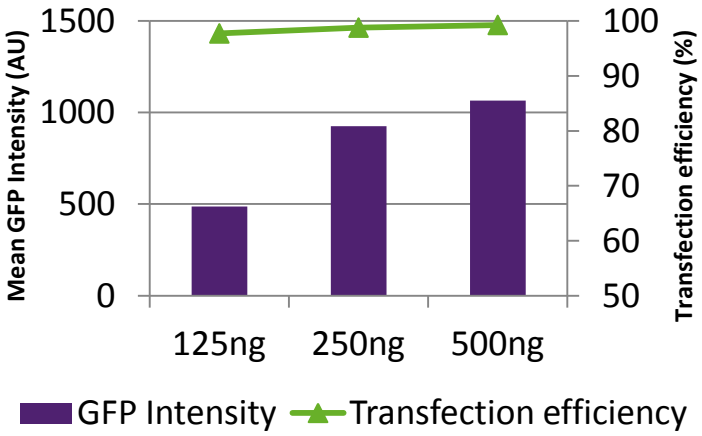
Stemfect™ RNA Transfection

- Tunable control of protein expression
- Higher average protein expression
- Uniform transfection efficiency
- Excellent cell viability supports overnight transfection







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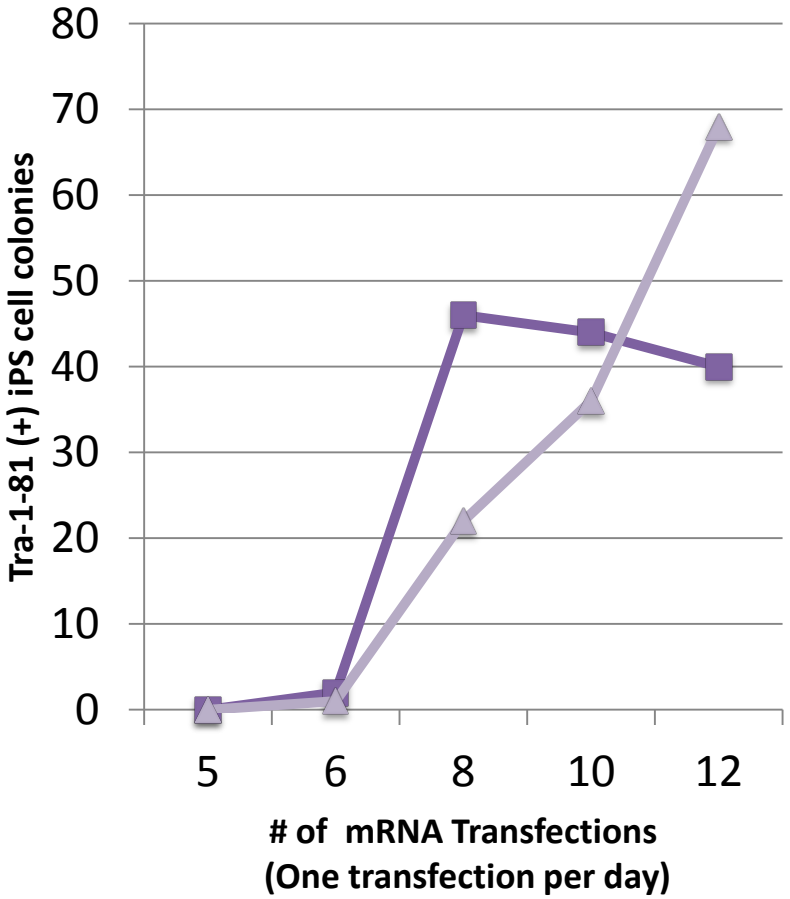
Reduced # of mRNA Transfections: Stemfect™



- Diseased patient dermal fibroblasts
- mRNA cocktail only
- Day 12: TRA-1-81 colony counts
- Colonies in wells with as few as 6 transfections
- Maximal iPS productivity between 8-12 transfections

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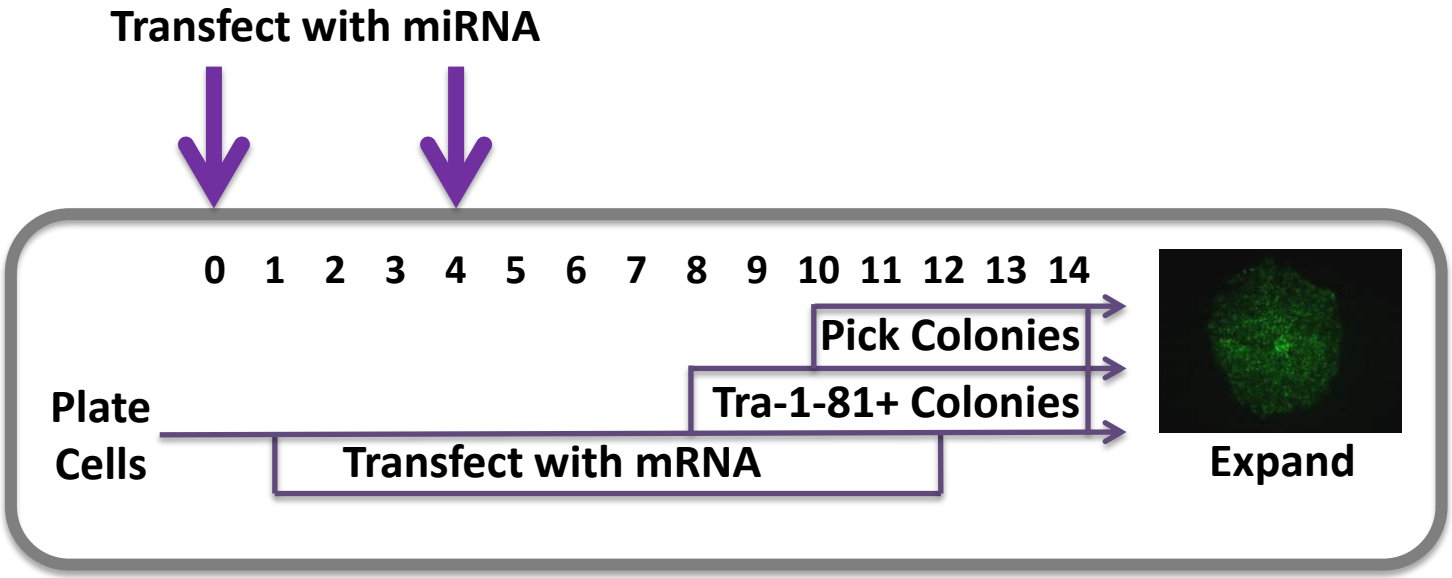
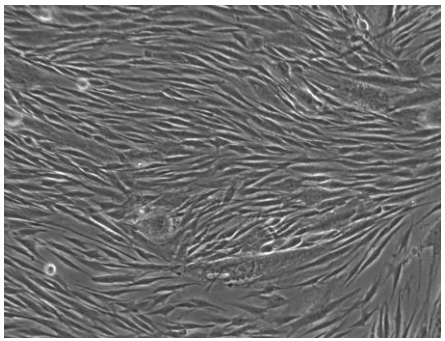
miRNA Enhanced Reprogramming: Timeline



miRNA Enhanced Reprogramming: Refractory Target Cells

mRNA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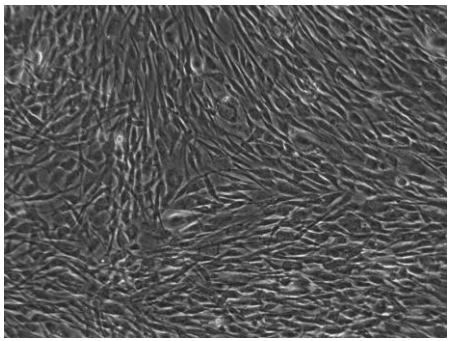
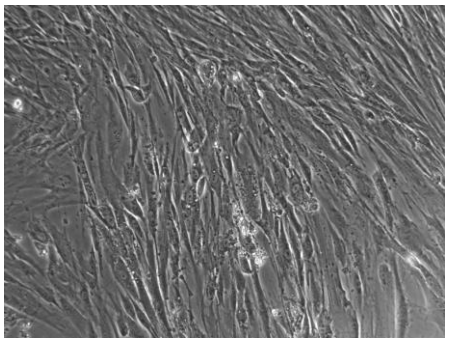
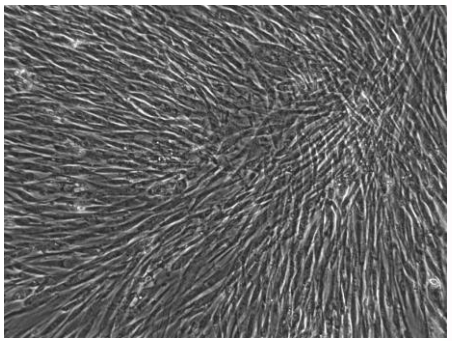

Day 5


Day 9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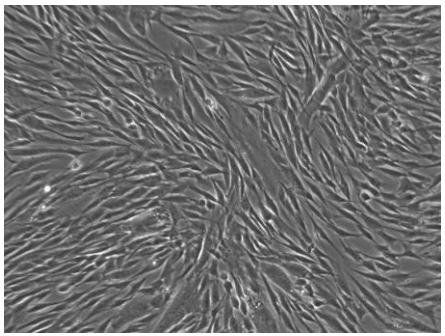

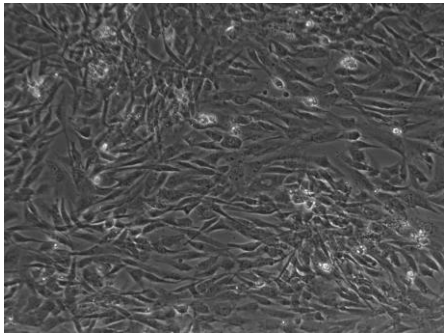

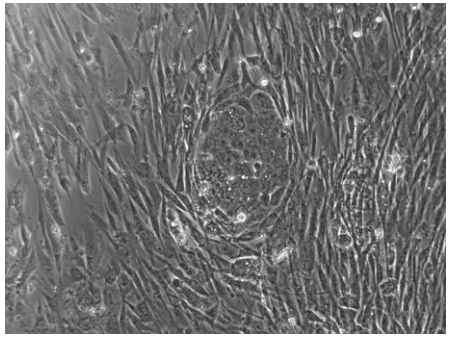

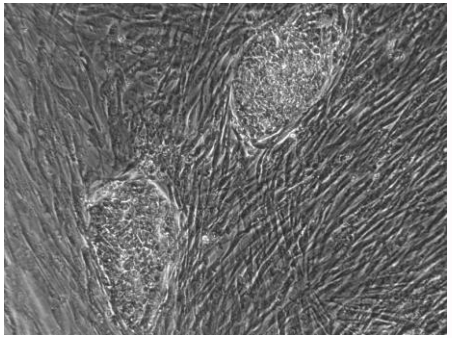

mRNA + miRNA

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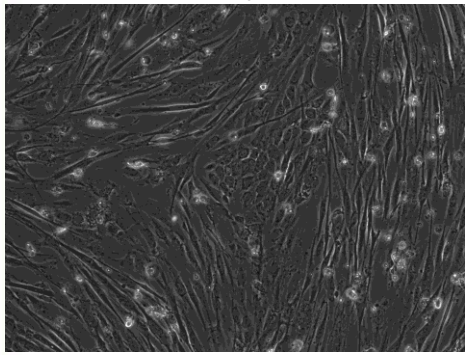
miRNA Enhanced Reprogramming - Faster

Feeder-based mRNA Protocol

Day 5



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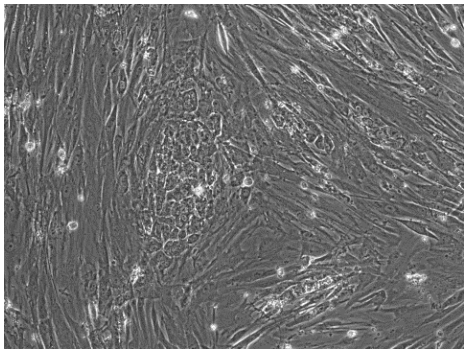

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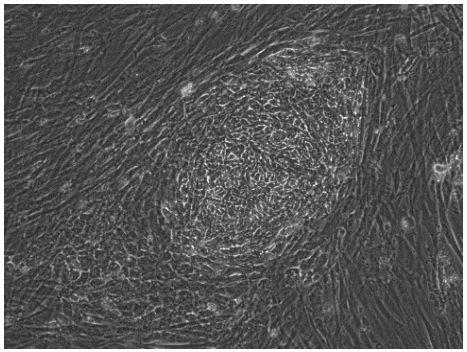
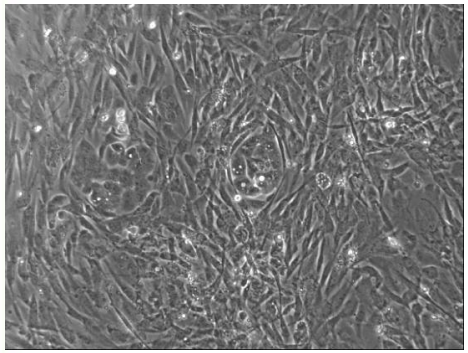
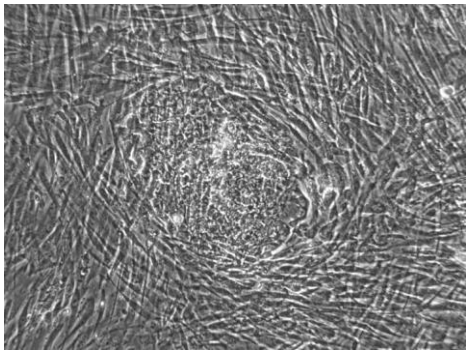




Day 5



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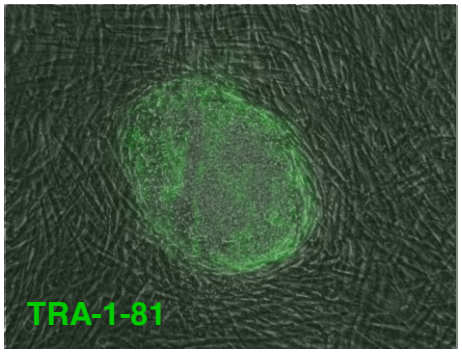

Day 12

mRNA + miRNA Protocol

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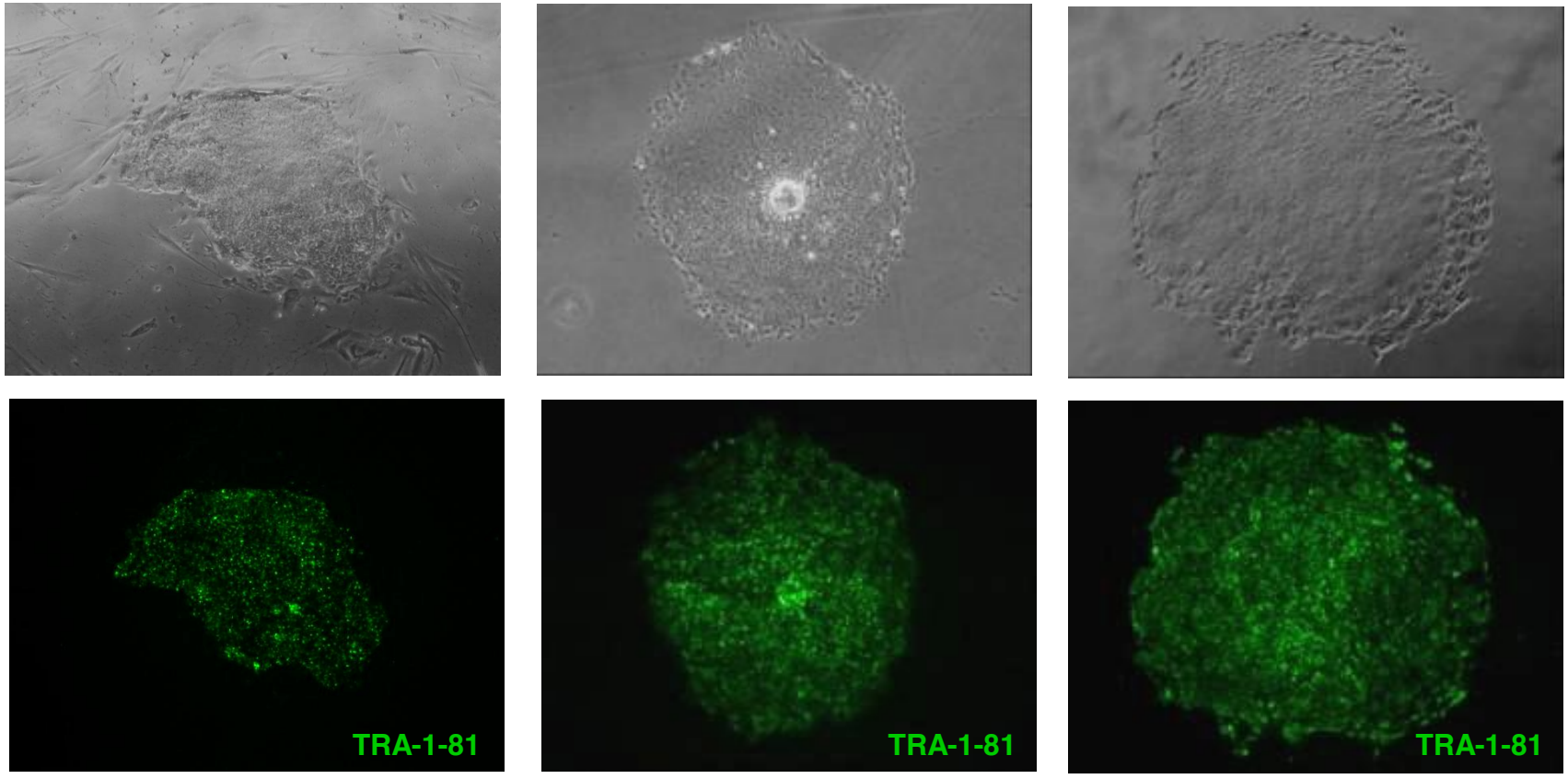
Primary Colony Expansion



p0 p1 p2

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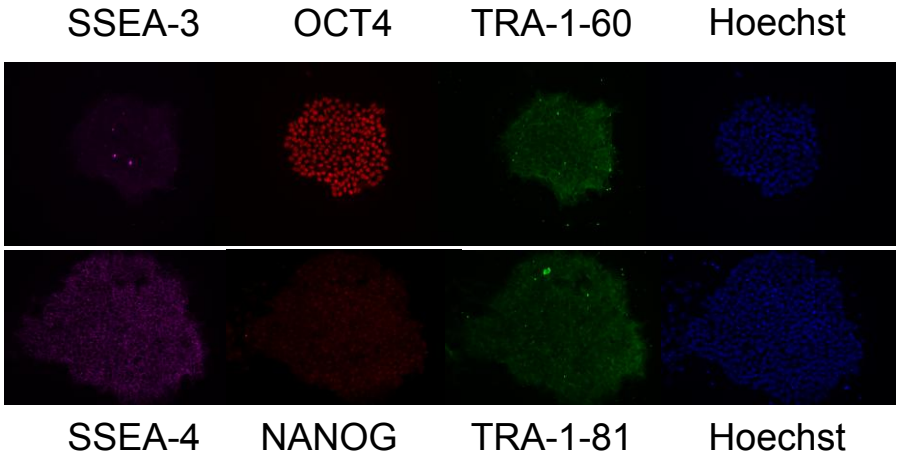
mRNA + miRNA iPS Cell Line Characterization



Courtesy of CHB – Thorsten Schlaeger and Andrew Ettenger

- Diseased patient dermal fibroblast
- Pluripotent
- Stable Karyotype
- No screening
- No subcloning

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Collaborative Results: mRNA + miRNA

	Cell Lines Tested	Lines Established
MSSM	Control + 3 HDF	4/4
HSCI	1 HDF	1/1
Children's Hospital Boston	1 HDF	1/1
NIH	Control + 3 HDF	3/3
NYSCF	3 HDF	3/3

- **5 independent labs**
- **11/11: patient iPS lines**
  - No dropouts
- **Testing format for all:**
  - miRNA cocktail
  - Stemfect™ RNA Transfection Kit
  - No feeder layer in primary well
  - 6-well format

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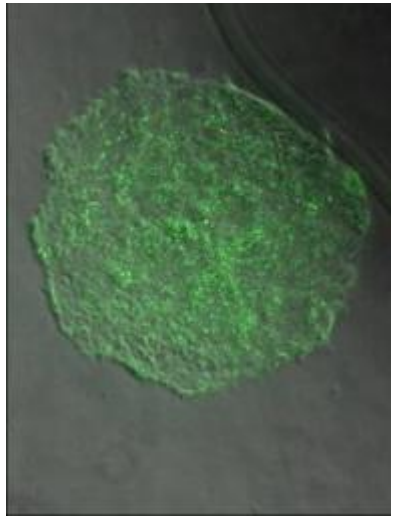
Benefits of miRNA Enhanced mRNA Reprogramming

- **Faster than existing fastest mRNA method**
  - Colonies in as few as 8 days
  - Colony isolation in 10-12 days
- **Captures refractory lines**
- **Simplified protocol**
  - Reduced # of transfections
  - Overnight transfections
  - 30 minutes of work per day



BJ mRNA iPS P1, Day 4
Without MEFs in Nutristem

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- miRNA Enhanced mRNA Reprogramming
- mRNA for Differentiation

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Programming Cell Fate by Gene Delivery

Lineage	Factors	Reference
Muscle	*MyoD*	Davis *et al*. Cell 1987 Weintraub *et al*. PNAS 1989
Neural	*Ascl1, Brn2, Myt1l, NeuroD1*	Vierbuchen *et al*. Nature 2010 Pang *et al*. Nature 2011
Cardiac	*Gata4, Mef2C, Tbx5*	Leda *et al*. Cell 2010
Blood	*Oct4*	Szabo *et al*. Nature 2010
Pancreatic	*Pdx1, MafA, Ngn3*	Zhou *et al*. Nature 2009

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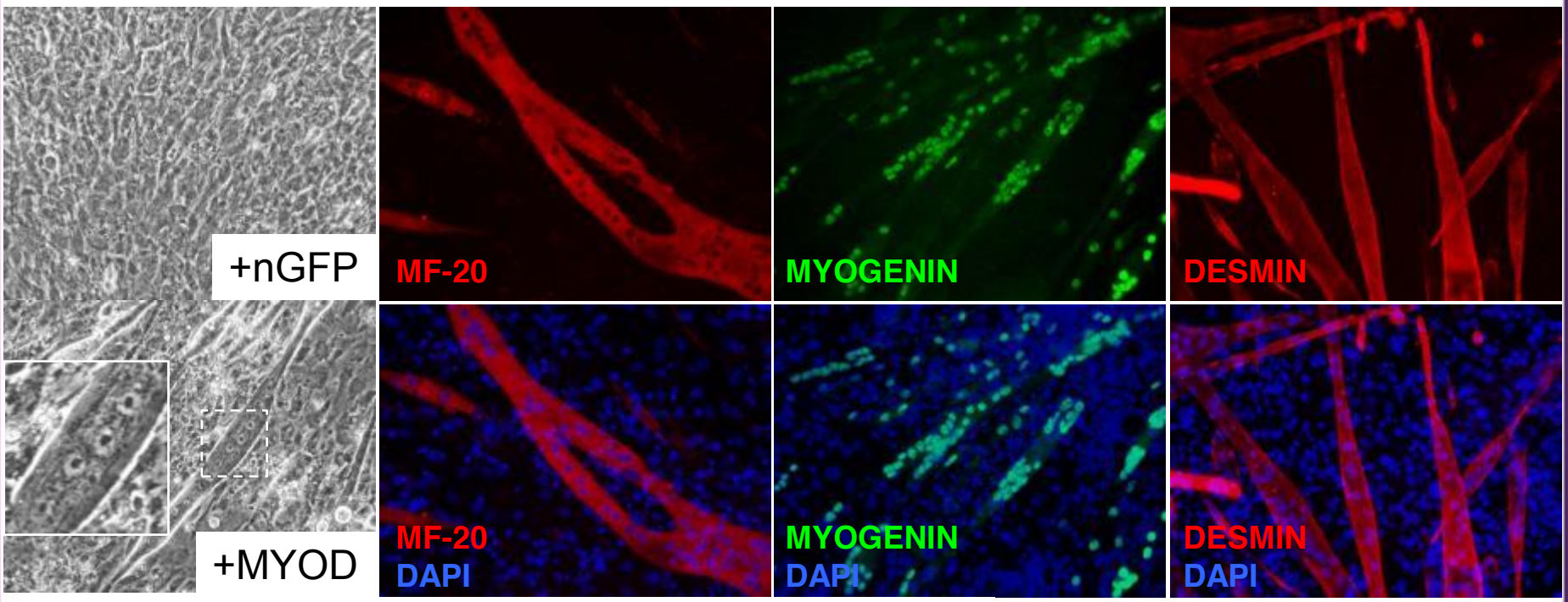
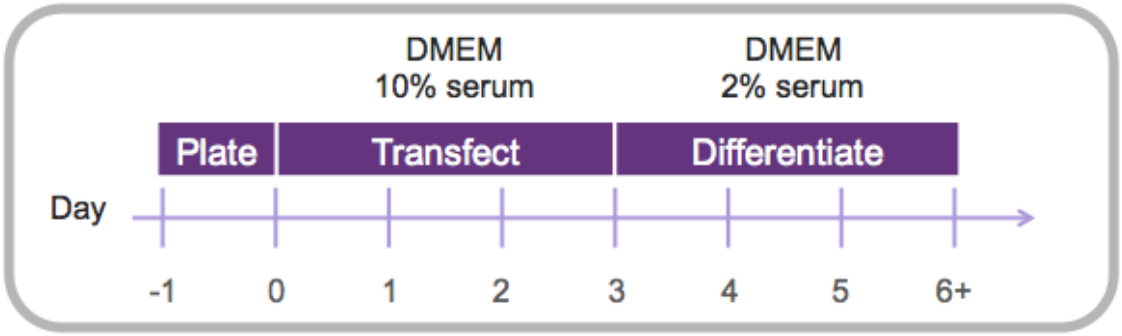
MyoD mRNA-derived Mouse Myotubes Express Muscle-specific Markers





10T1/2 mouse embryonic fibroblasts

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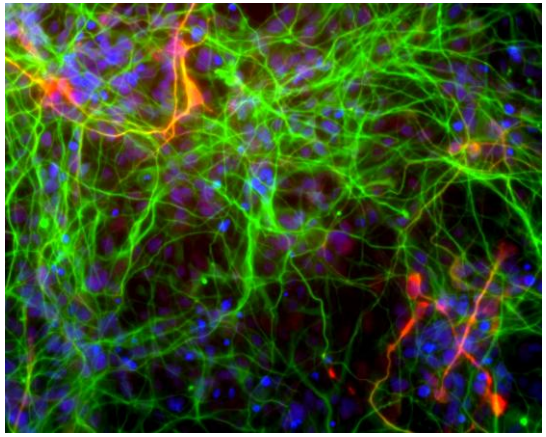
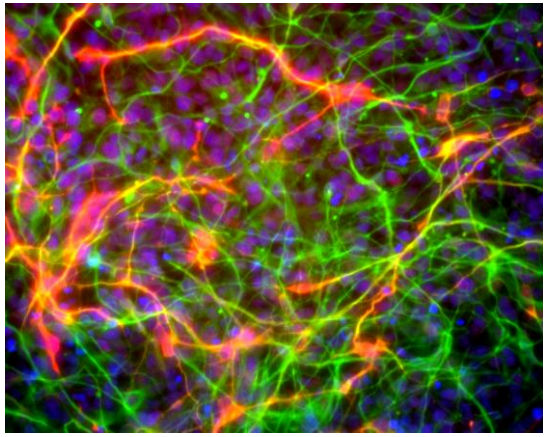
Directed Differentiation: NPCs to Dopaminergic Neurons



No mRNA Control

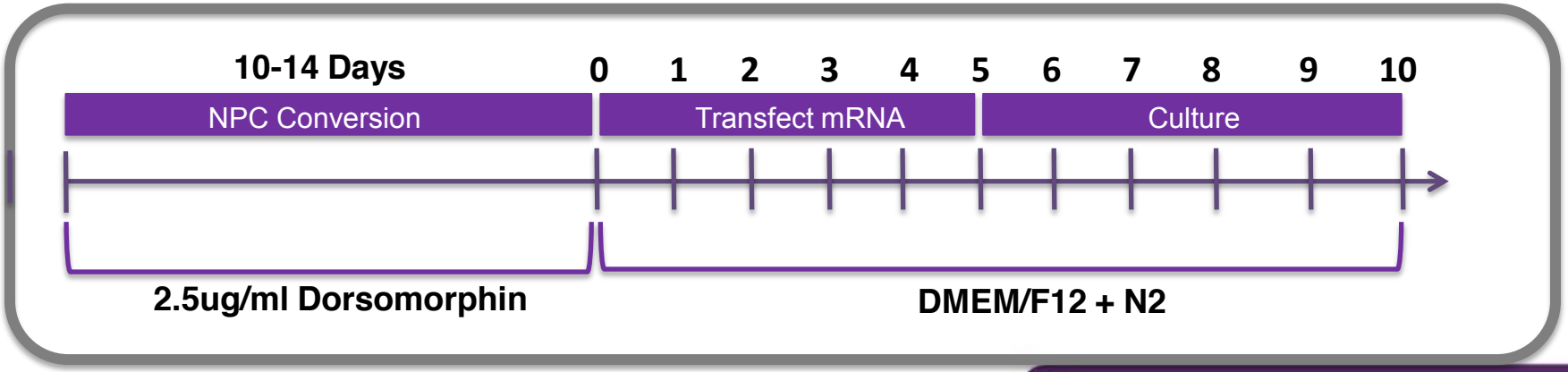
Tuj1

TH+



2ug mRNA Nurr1/Pitx3

hiPS **NPCs** **Dopaminergic**



Courtesy of WIBR – Julien Muffat

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Summary of mRNA Developments

- **mRNA Reprogramming**
  - Fast, efficient, safe, non-viral/non-integrating iPS derivation
  - No screening or subcloning required
- **miRNA Enhanced Reprogramming**
  - Faster derivation and isolation of iPS cell colonies (<2 weeks)
  - Generates iPS cell lines from refractory target cells
  - Non-toxic, overnight transfections (Stemfect)
  - Amenable to defined culture environment
- **mRNA for Differentiation**
  - Compatible with directed and transdifferentiation manipulations

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mRNA REPROGRAMMING COURSE

Stemgent mRNA Reprogramming Courses in collaboration with

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