Induced Pluripotent Stem Cells as Models of Human Disease: A Promising Approach for Studying Parkinson's Disease

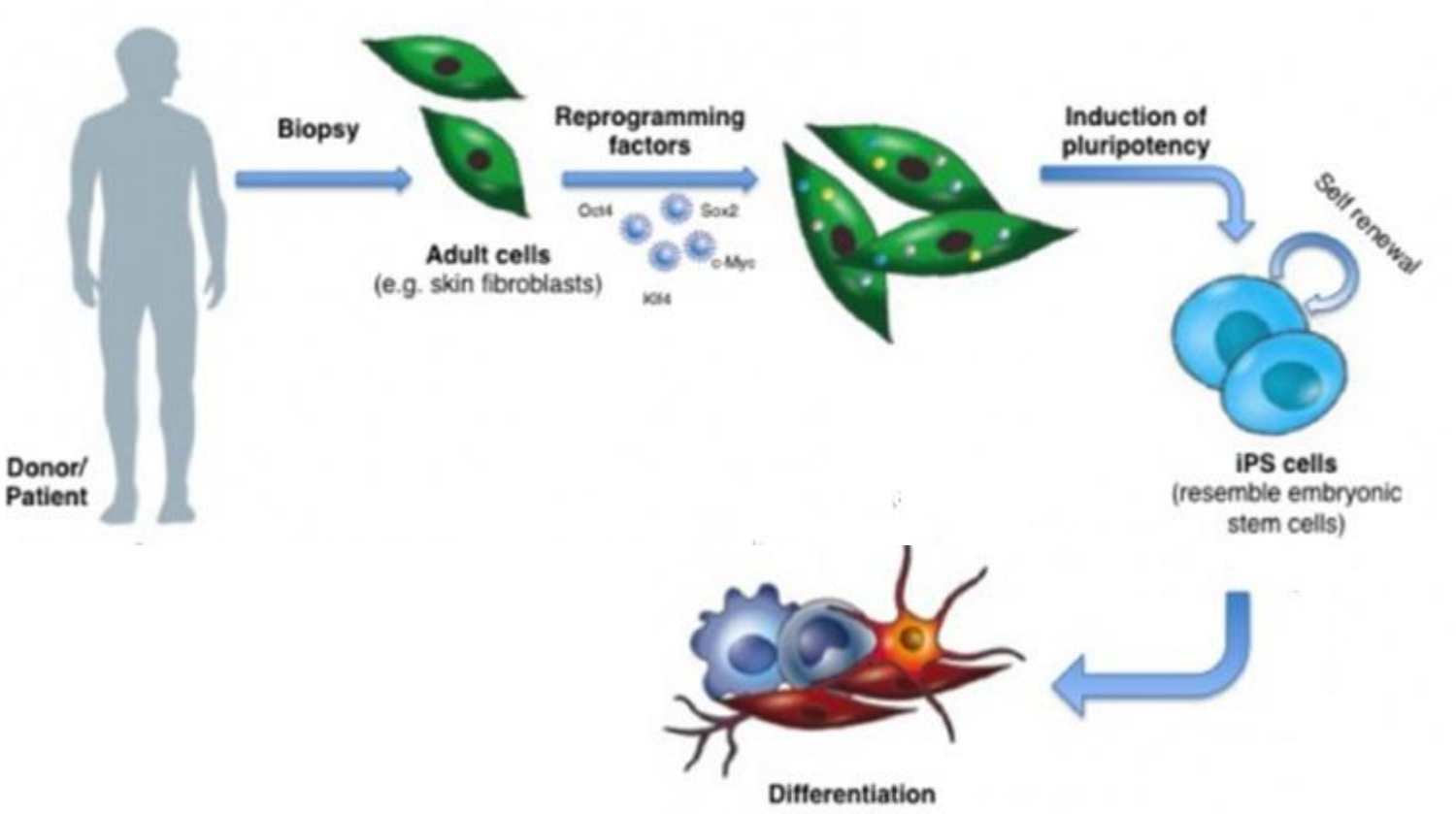
Stephen S. Lin, Ph.D.

Staff Scientist, Thermo Fisher Scientific

October 28, 2014

Proprietary & Confidential

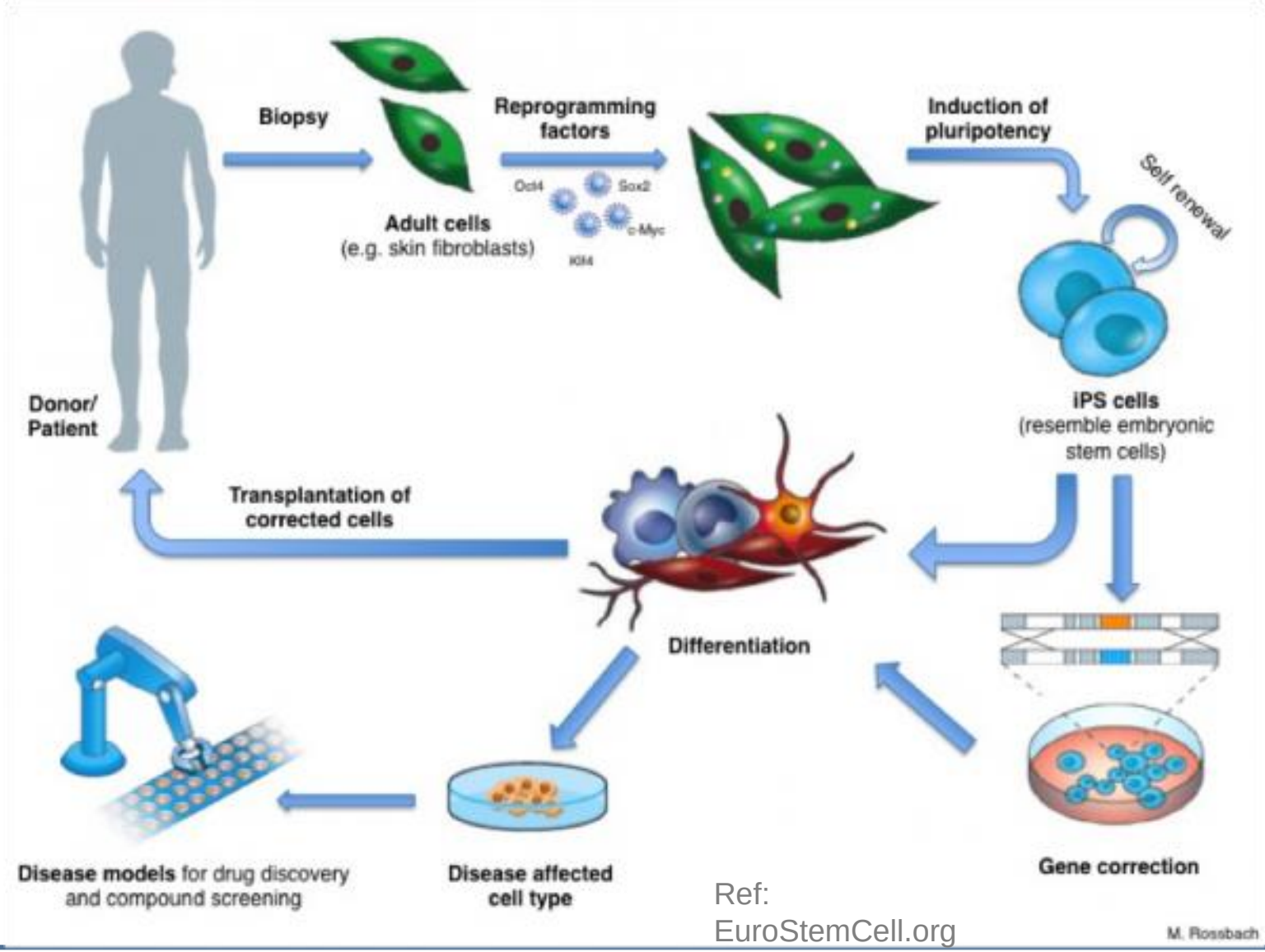
The Promise of iPSC Technology



Ref:
EuroStemCell.org

ThermoFisher SCIENTIFIC

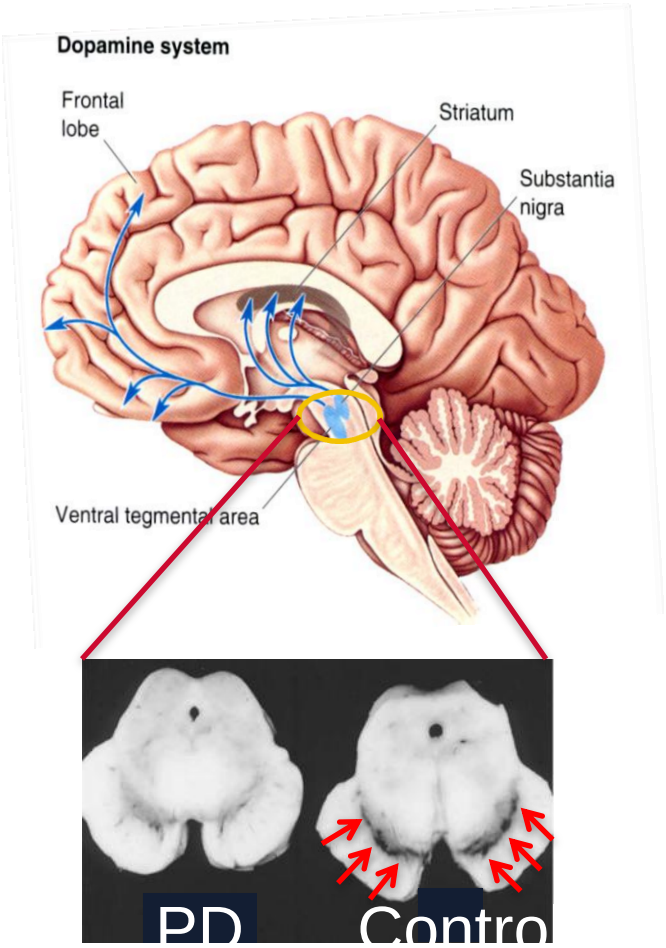
The Promise of iPSC Technology



Ref: EuroStemCell.org

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Parkinson's Disease



Section of SNPC from autopsy sample. Dark area in control sample shows healthy neurons.

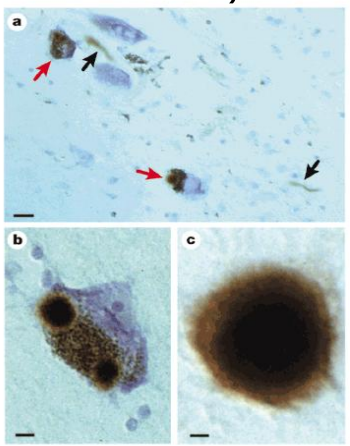
- Neurodegenerative disorder affecting 1-2 % of the population over age 65.
- Clinical symptoms include rest tremor, bradykinesia (slowness of movements) and muscle rigidity.



Gowers, 1893

Pathology:

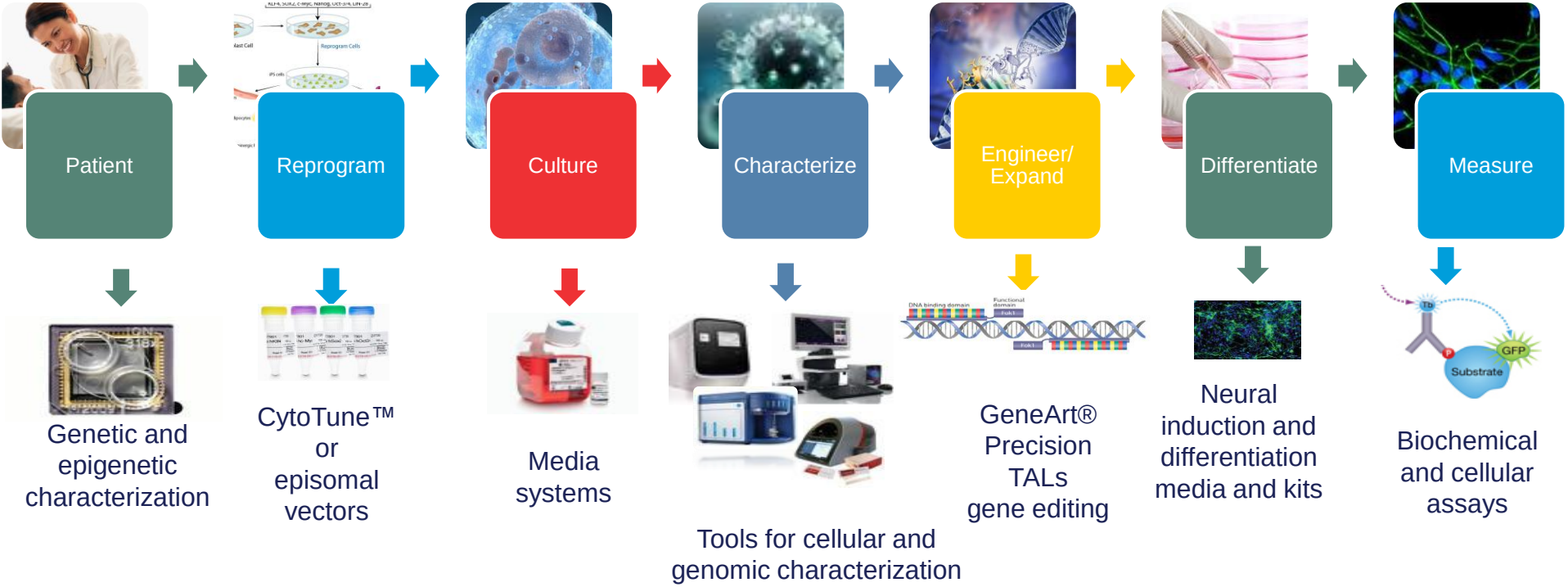
- Loss of dopaminergic neurons in the substantia nigra in the brainstem region.
- Characteristic protein inclusions known as Lewy Bodies.



Spillantini et al. 1997

ThermoFisher SCIENTIFIC

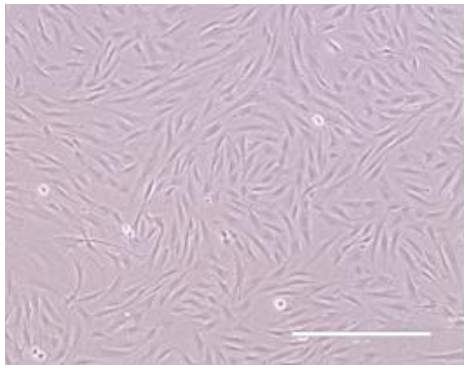
Many technologies are needed to utilize iPS cells



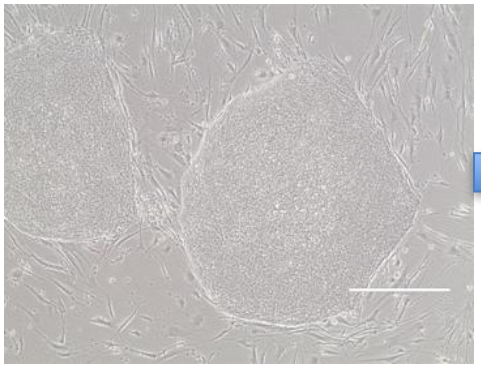
Proprietary & Confidential

Collaboration Overview With Parkinson's Institute

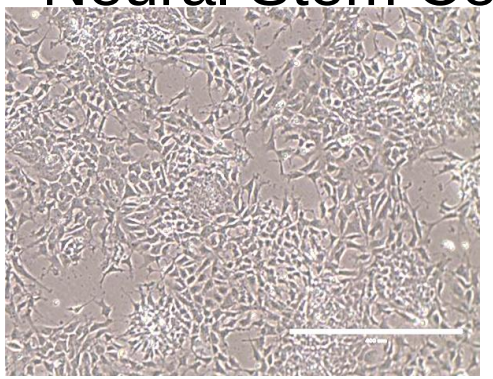
Fibroblasts



iPSCs



Neural Stem Cells



1

2

3

3

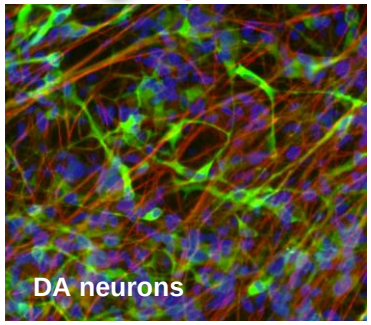
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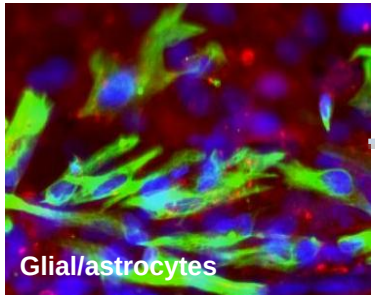
Functional Assays

ID	Age at biopsy	Disease	Mutation
PD-1	19	YOPD	PARKIN
Ctrl-1	25	control	
PD-2	74	PD	LRRK2
PD-3	61	PD	LRRK2/GBA
MSA	62	MSA	
Ctrl-2	69	control	

YOPD: Young onset Parkinson's disease
PD: Parkinson's disease
MSA: Multiple system atrophy

- Knock out α-synuclein in MSA iPSC line
- "Correct" LRRK2 and GBA mutations





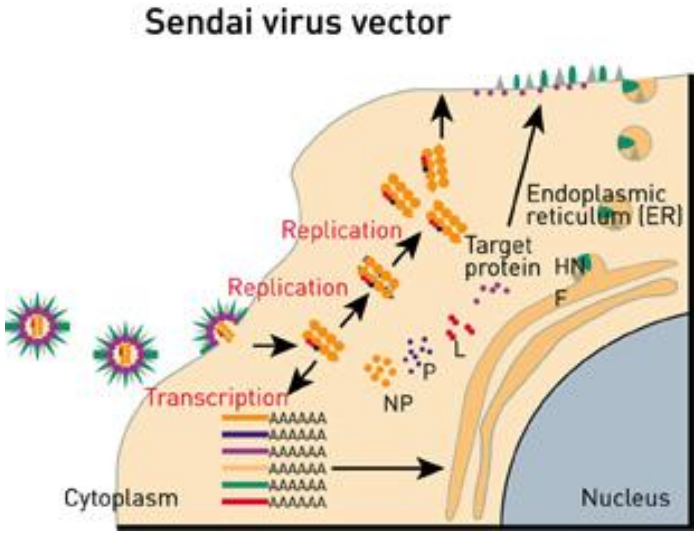
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CytoTune®-iPS Sendai Reprogramming Kit addresses major challenges in reprogramming

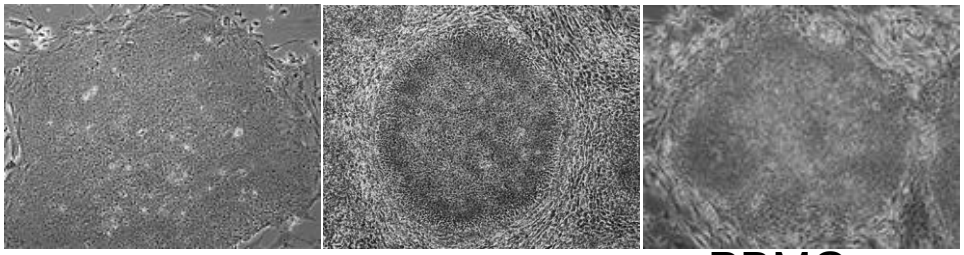
Reprogramming Challenges:

- **Integrating technologies** can affect cell function
- **Efficiency** in hard to reprogram cell types, or with limited samples
- **Ease-of-use**: certain technologies require multiple transfections



Sendai Virus:

- **RNA virus**. No DNA during replication, and replicates exclusively in the cytoplasm. No possibility of integration into host genome
- **Non-pathogenic**. Can delete the Fusion (F) gene so virus cannot produce infectious particles.
- **Broad tropism**. Capable of transducing a wide range of cells with a short contact time.



Fibroblasts | $CD34^+$ | PBMCs

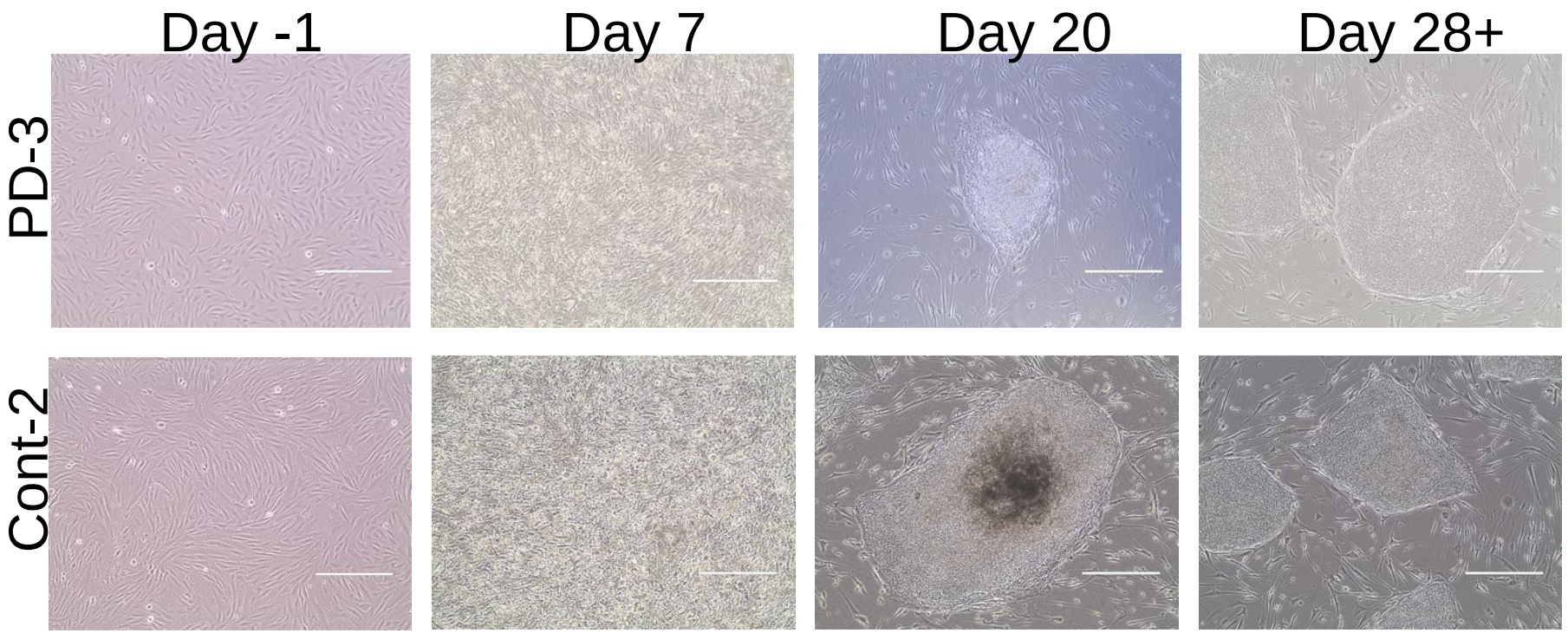
For Research Use Only. Not for use in diagnostic procedures.

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iPSC generation from PD donors and control fibroblasts





Fibroblasts cultured in DMEM / FBS

7 days post-transduction, passage to 10 cm MEF plates

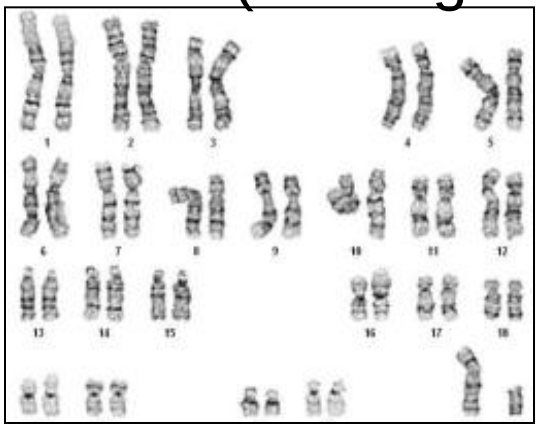
Pick colonies on day 20 (top) or 26 (bottom)

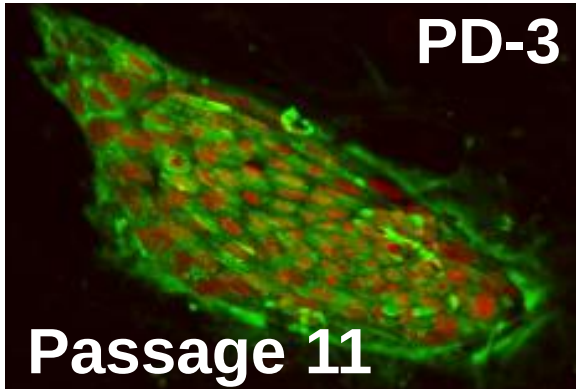
Expand individual colonies for cryopreservation and characterization

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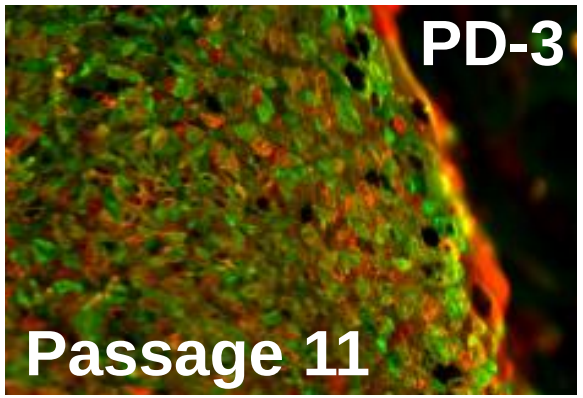
Characterization of iPSCs

PD-1 (Passage 14)

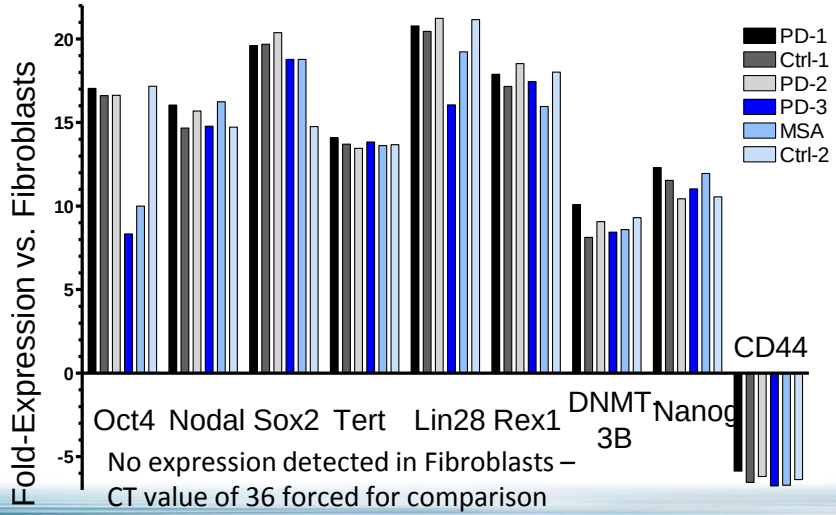


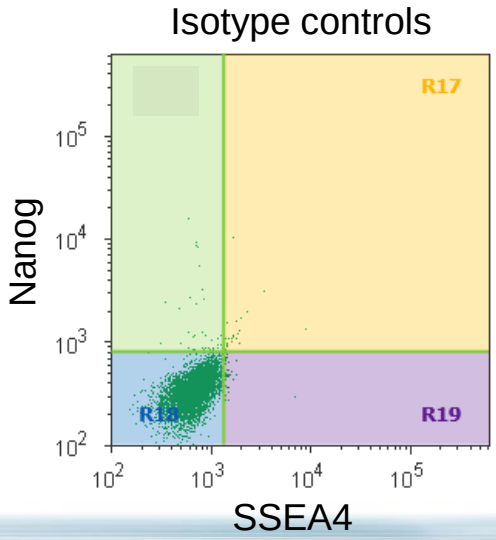


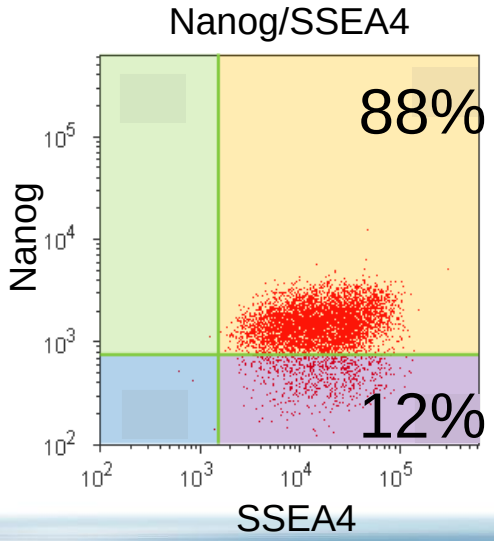
Oct4 / Tra1-81



SSEA4 / Tra1-60

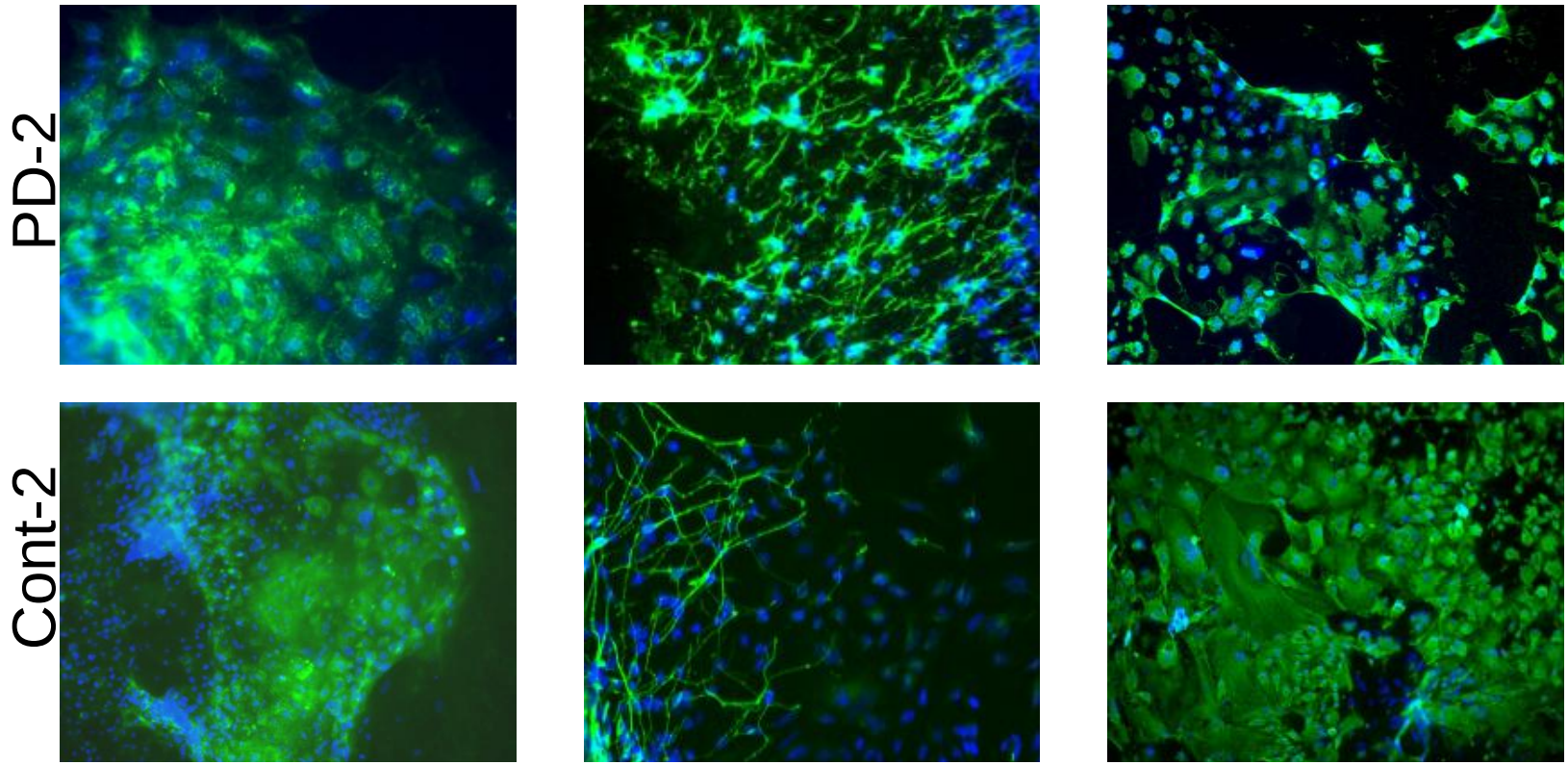






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Reprogrammed iPSCs form embryoid bodies containing cells of all three germ layers



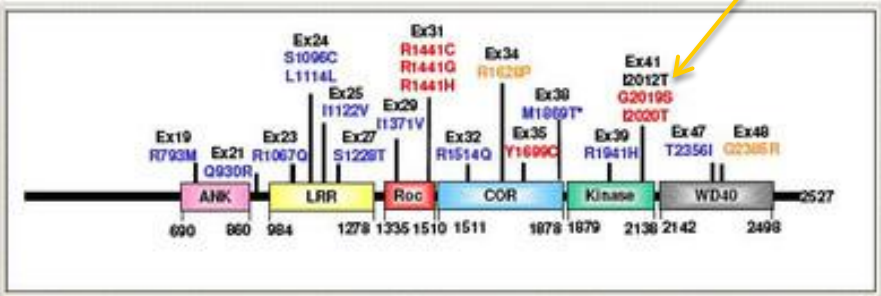
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Mutations in Parkinson's iPSC Lines

PD-3 donor info

61-year-old male, Parkinson's disease,
LRRK2 G2019S (confirmed by PGM™ sequencing)

LRRK2:
Leucine-rich repeat kinase 2

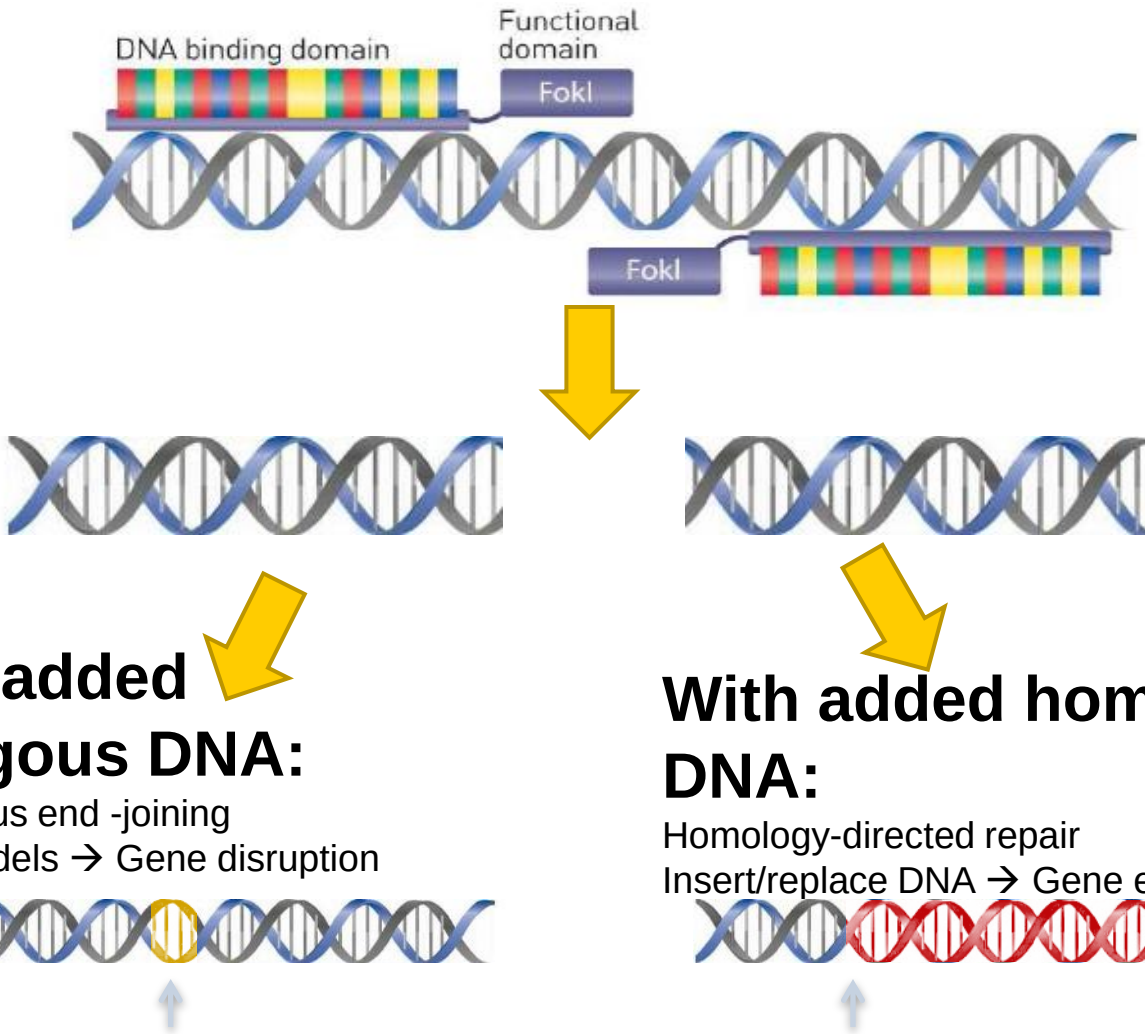


Schematic representation of LRRK2 domain structure
(© 2006 Elsevier Ltd)

LRRK2 G2019S:
Increased kinase activity
Linked to Parkinson's disease
Reinhardt et al, Cell Stem Cell, 2013

ThermoFisher SCIENTIFIC

Transcription activator-like (TAL) effector nucleases can correct mutations by homologous recombination



Without added homologous DNA:

Non homologous end -joining
Repairs with indels → Gene disruption

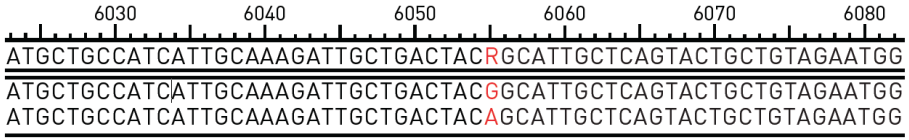
With added homologous DNA:

Homology-directed repair
Insert/replace DNA → Gene edit

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Editing via homologous recombination: LRRK2
Correcting mutation of LRRK2 G2019S to wild type

LRRK2 TAL pairs and donor fragment:



Forward LRRK2 TAL **Reverse LRRK2 TAL**

500bp…gctgactacGgcattgctcagtact…500bp

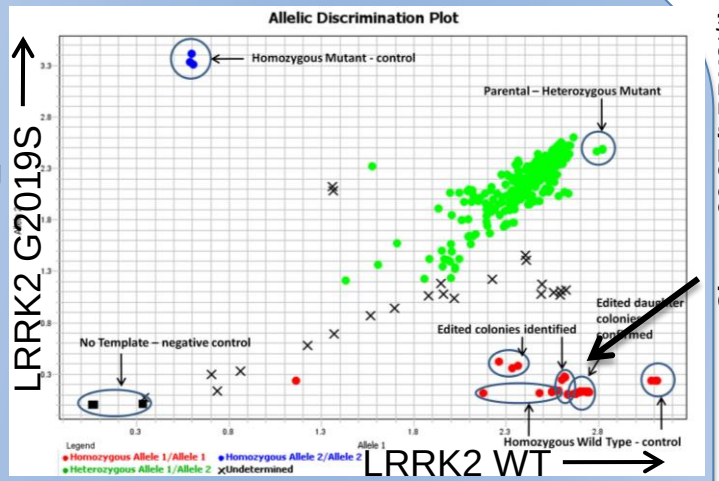
(~1kb donor fragment)

LRRK2 G2019S corrected to WT
Initial screen: 2 colonies out of 140

Original colony: 93% WT/WT
1st subcloning: 100% WT/WT

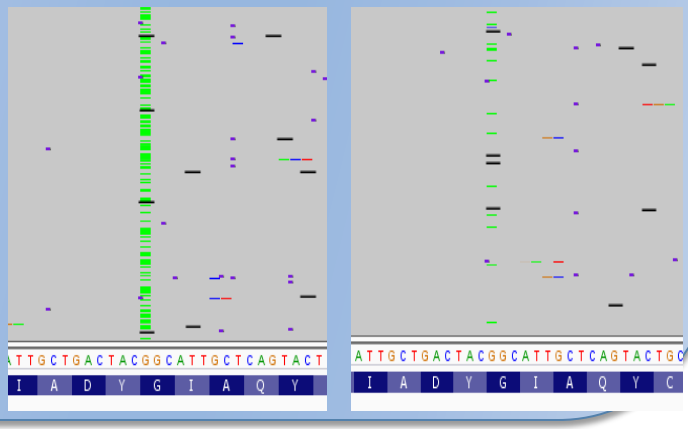
Screening colonies for LRRK2 genetic correction

TaqMan® SNP genotyping



Heterozygous Mutant Edited colony

PGM™ sequencing

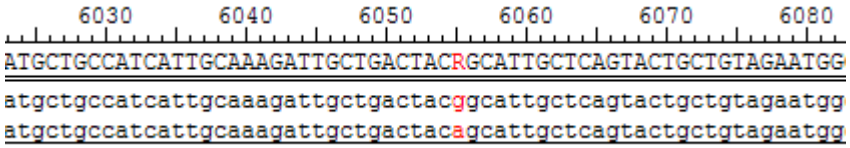


ThermoFisher SCIENTIFIC

Editing via homologous recombination

Correcting mutation of LRRK2 G2019S to wild type

LRRK2 TAL pairs and donor fragment:



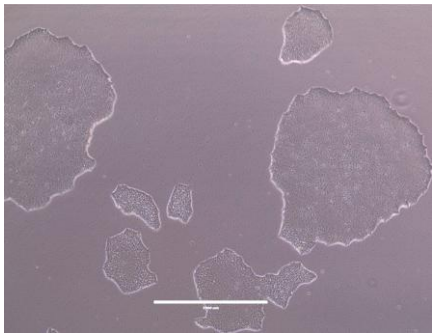
Forward LRRK2 TAL **Reverse LRRK2 TAL**

500 bp…gctgactacGgcattgctcagtact…500 bp

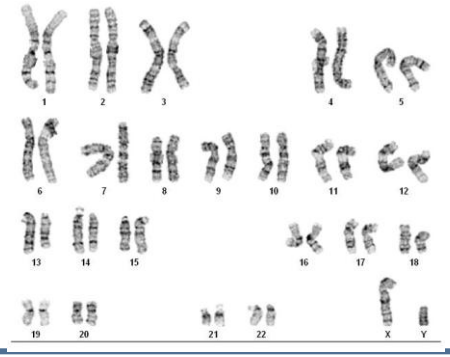
(~1 kb donor fragment)

LRRK2 G2019S corrected to WT
Intial screen: 2 out of 140 (1.4%)

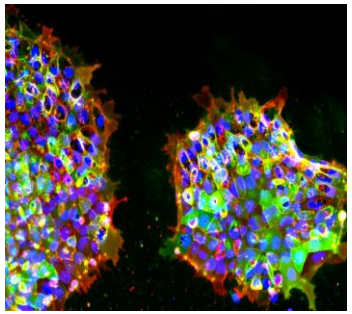
Clone 26 (LRRK2wt)



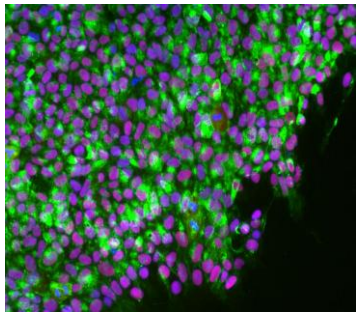
Clone 26 (LRRK2wt)
Karyotypically normal



Tra1-60/SSEA4/ Hoechst



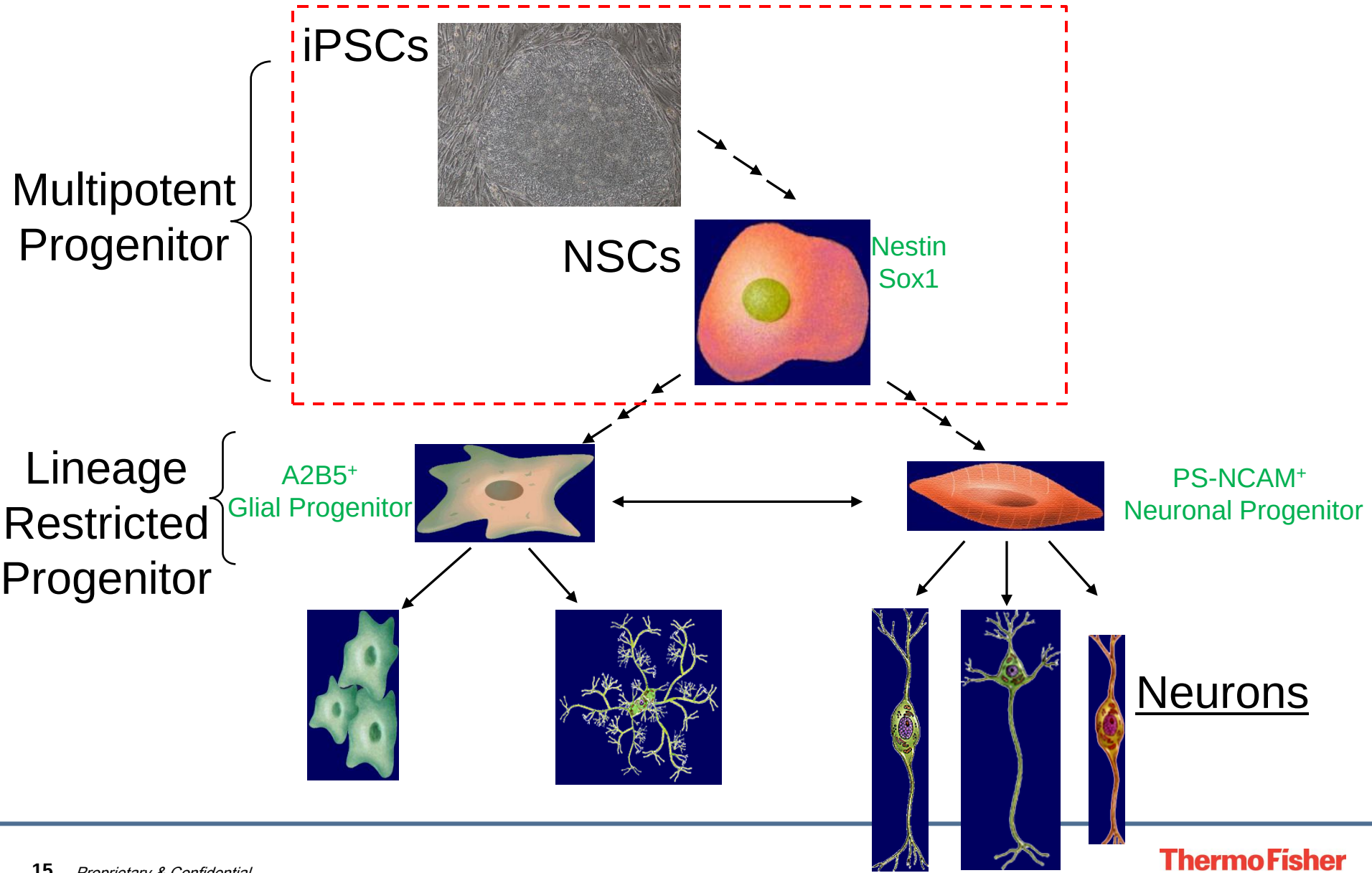
Tra1-81/Oct4/ Hoechst



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Neural stem cells overview

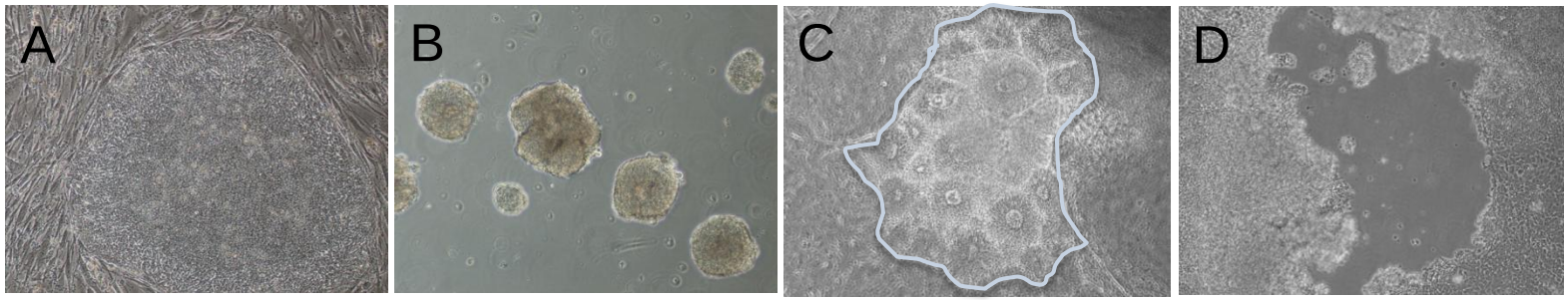
Lineages of neural cells



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Conventional Method of NSC Derivation via Embryoid Body Formation



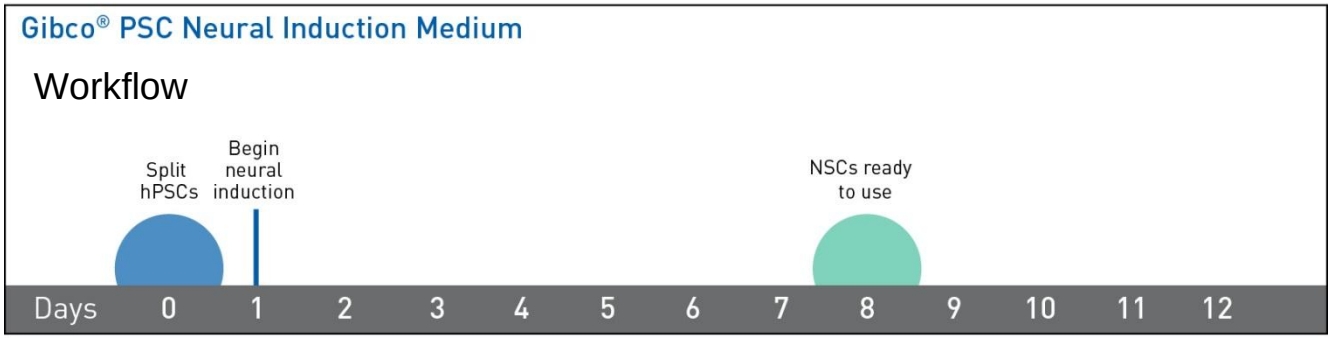
hPSCs → Embryoid body formation (4-6 days) → Rosette formation (6-8 days) → NSC isolation

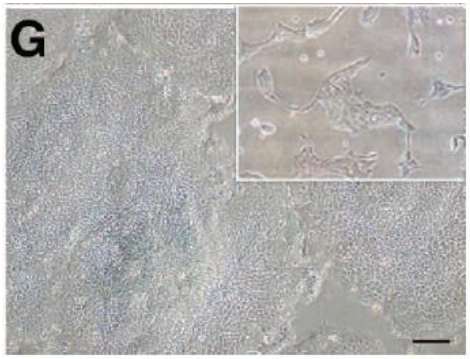
Disadvantages

- EB formation: Difficult to control the quality
- Duration: Long (14 days)
- Efficiency: Low
- Procedure: complicated

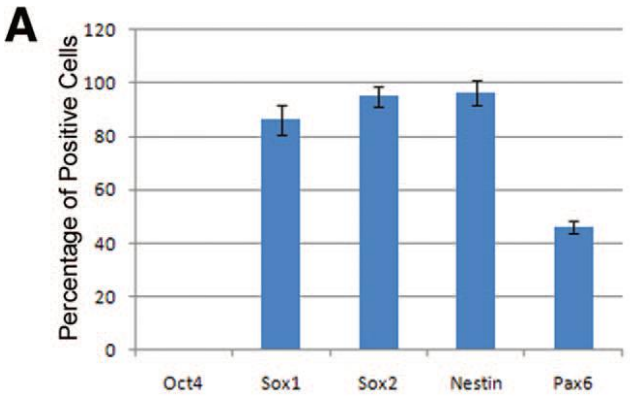
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Simple generation and expansion of primitive NSCs

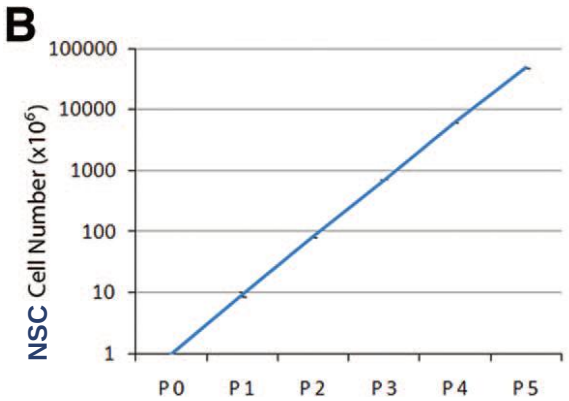




NSC Morphology



NSC Marker Expression



NSC Expansion



Karyotypically normal

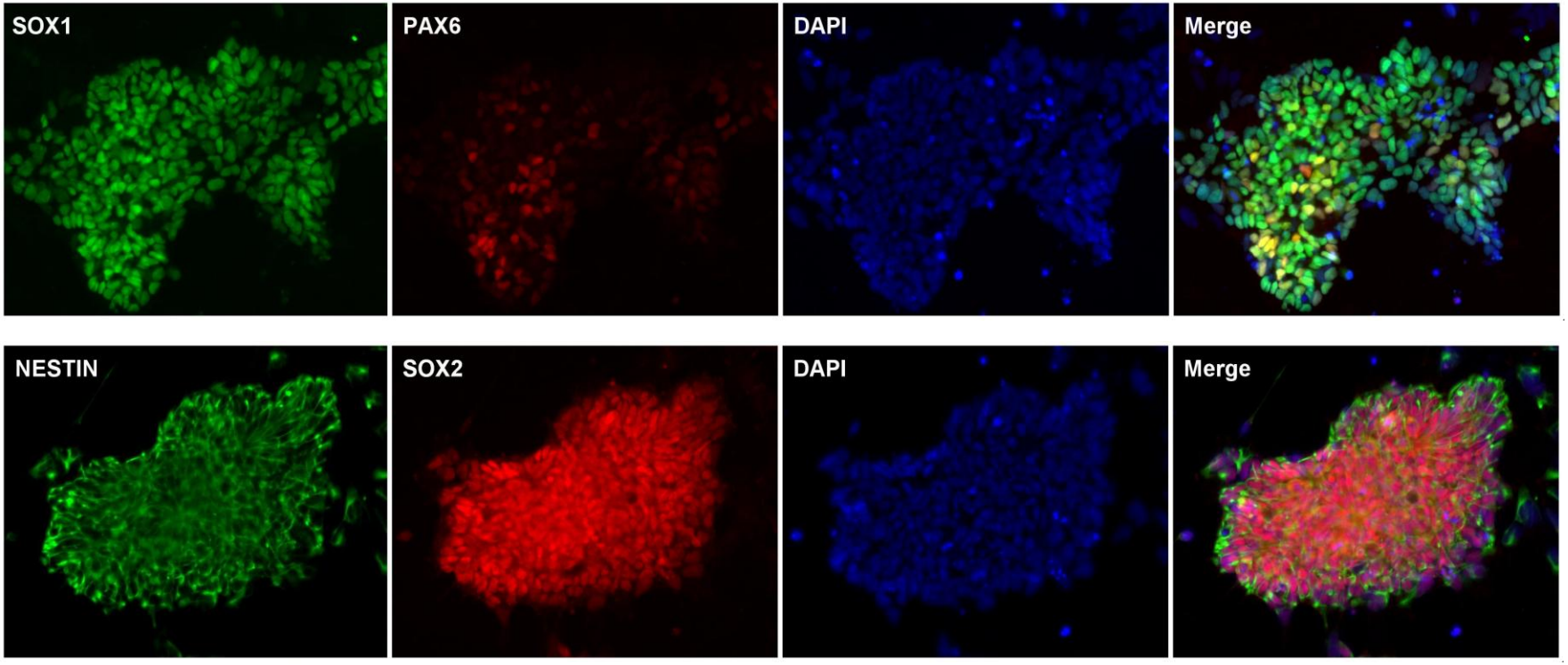
- Method avoids differentiation via EBs and neural rosettes
- NSCs can be differentiated towards fore-, mid-, and hind-brain neuronal subtypes

Yan, Y. et.al. *Stem Cells Translational Medicine* 2013;2(11):862-70.

ThermoFisher SCIENTIFIC

NSCs express expected markers by ICC

PD-3 NSCs p1





Staining using Human Neural Stem Cell Immunocytochemistry Kit

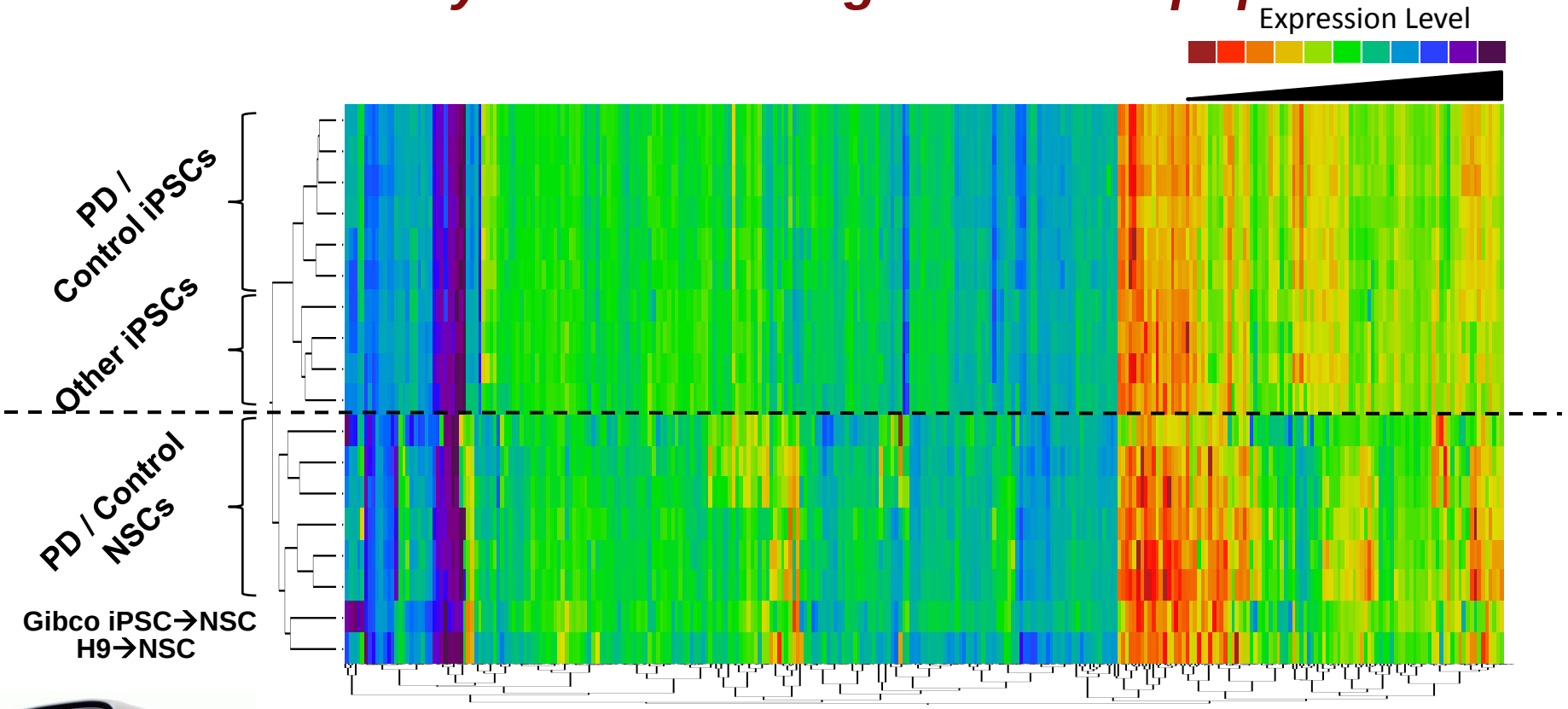


Images obtained using Evos® FLoid® imaging station

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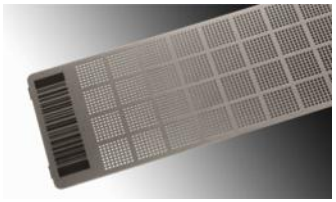
Gene express profiles cluster NSCs distinctly from iPSCs

Gene cluster analysis also distinguishes cell populations





QuantStudio 12k Flex qPCR System

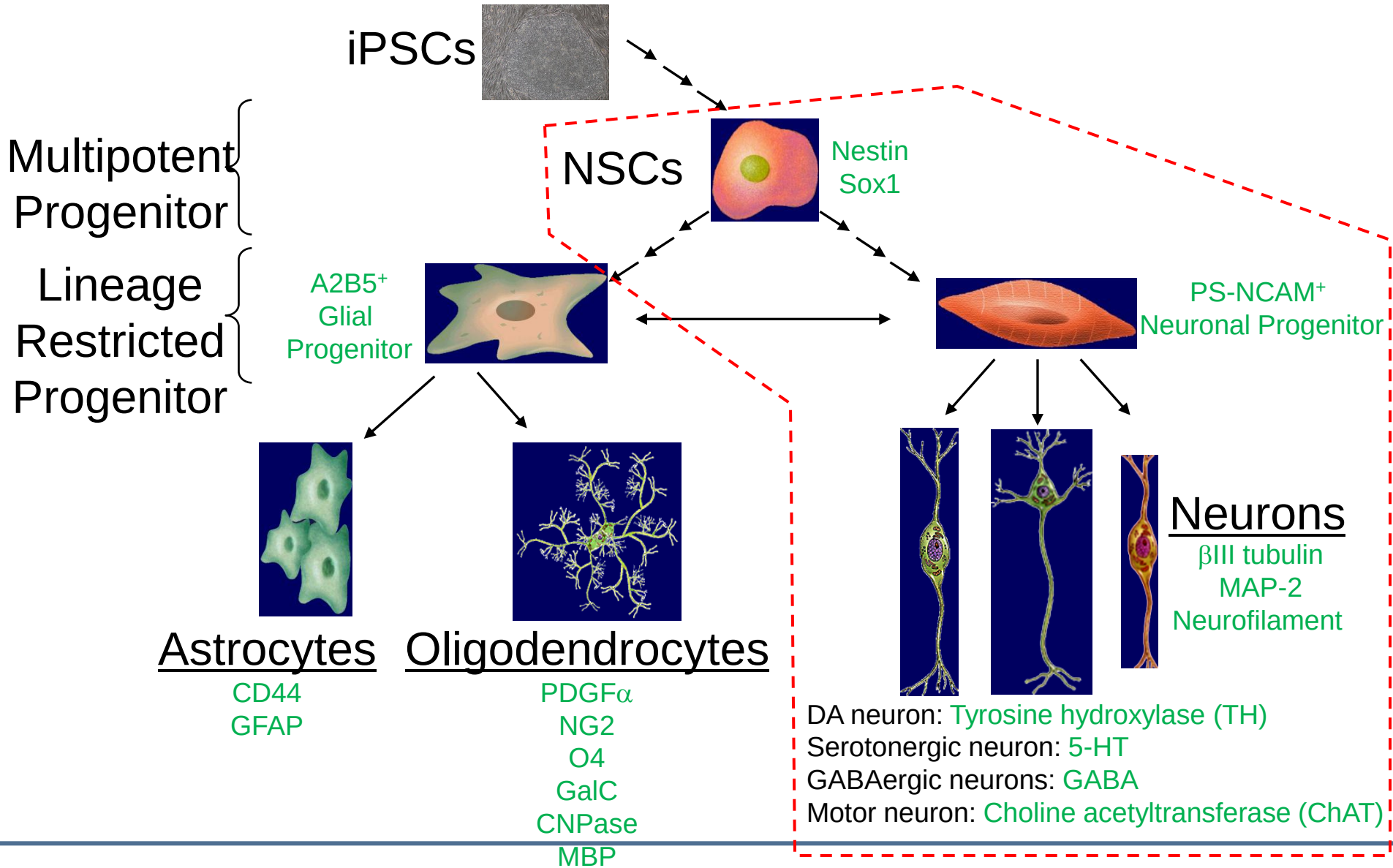


TaqMan® OpenArray® Human Stem Cell Panel:
631 assays comprised of pluripotency and differentiation genes

ThermoFisher SCIENTIFIC

Neural stem cells overview

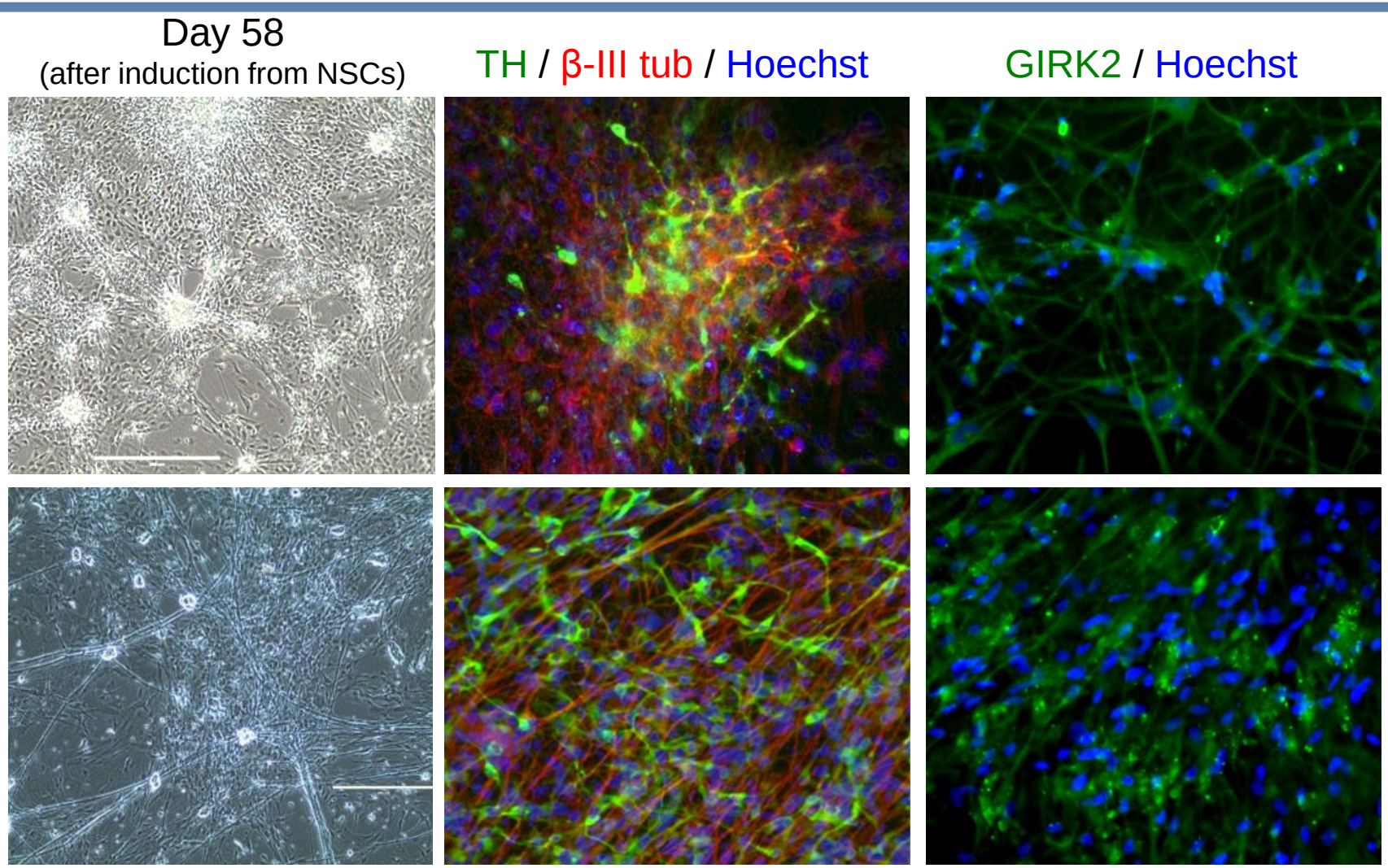
Lineages of neural cells



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NSC Differentiation into Dopaminergic (DA) Neurons



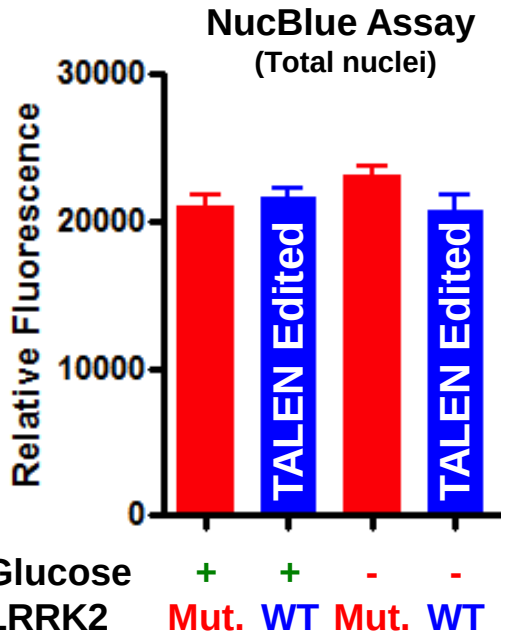
Cells are positive for DA marker TH and A9 subtype DA neuron marker GIRK2.

DA differentiation protocol based on Swistowski et. al. *PLoS One.* 2009 Jul 14;4(7)

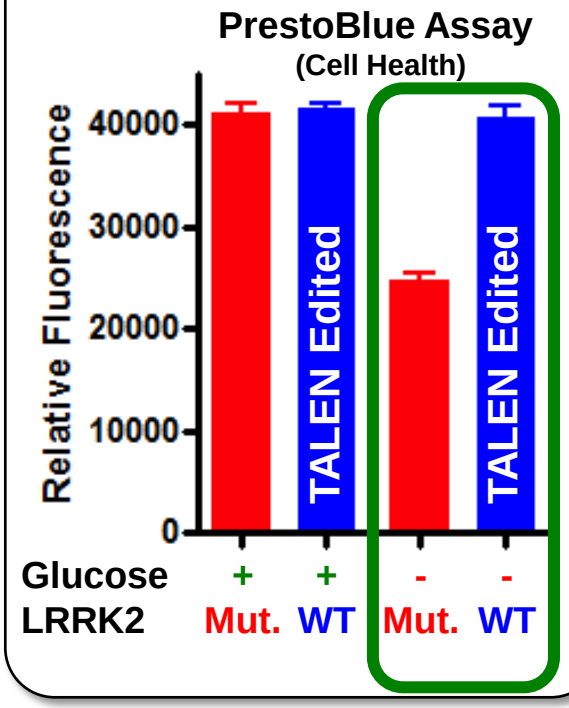
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Observing “Disease in a Dish” Function – First Glimpse!

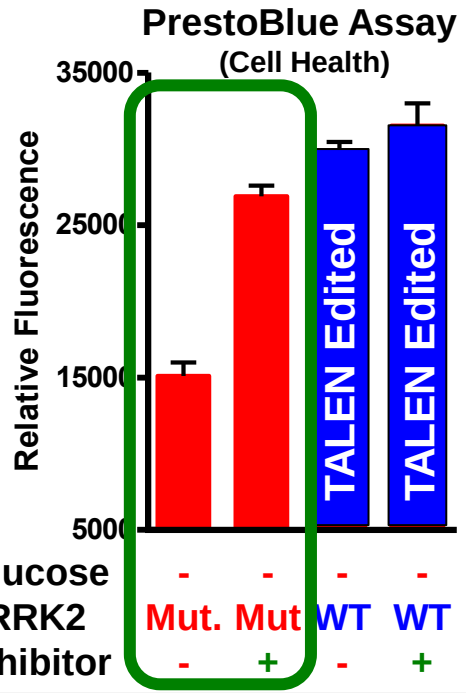
Control



Disease-Relevant Phenotype



Pharmacological Rescue



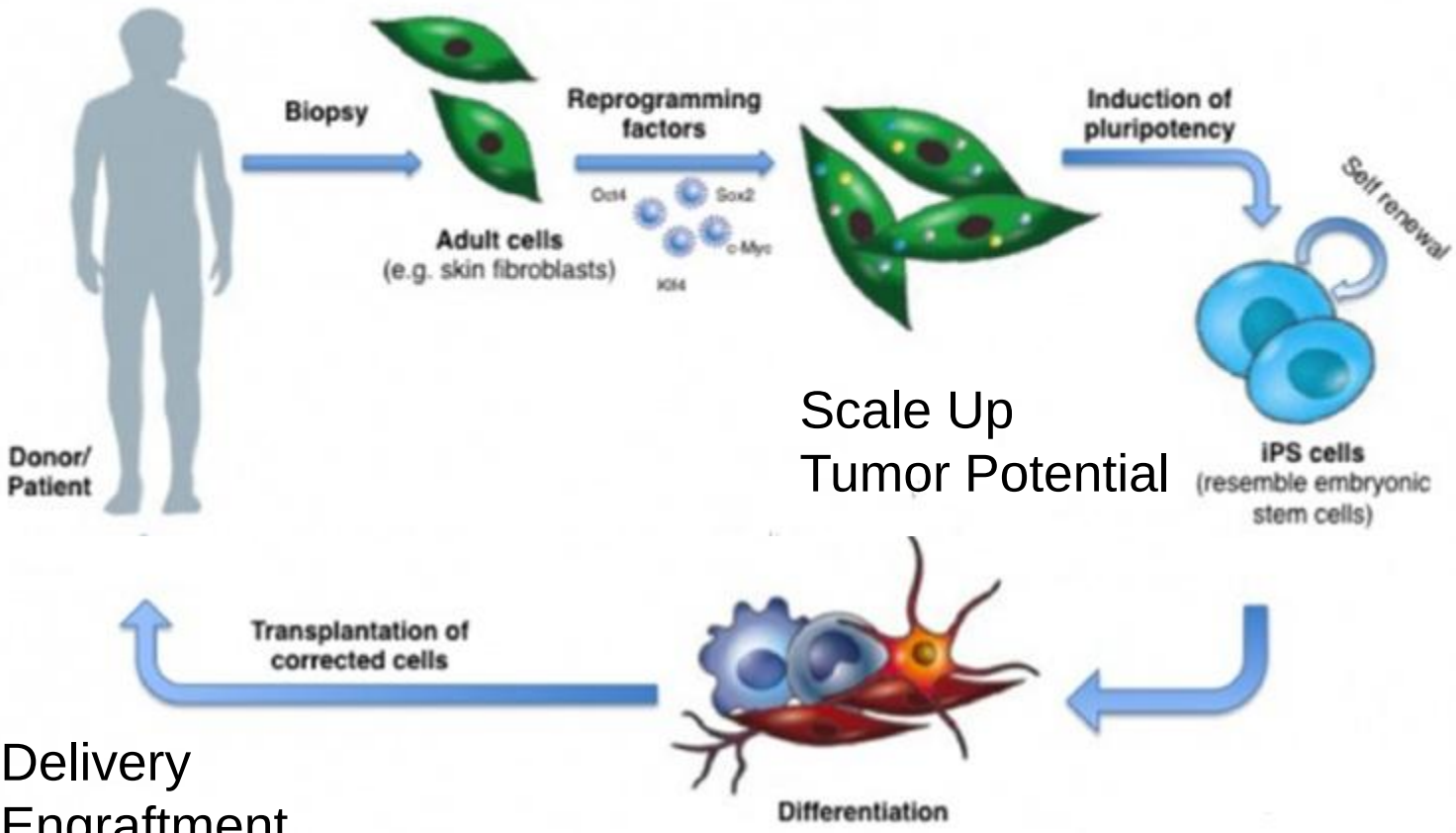
Same # of cells in all experiments

TALEN correction of mutant protein reverses glucose sensitivity

Drug Inhibition of mutant protein reverses glucose sensitivity

ThermoFisher SCIENTIFIC

The Promise and Challenge of iPSC Therapy



Scale Up
Tumor Potential

Delivery
Engraftment
Migration
Immunotolerance
Function

How complete is conversion?

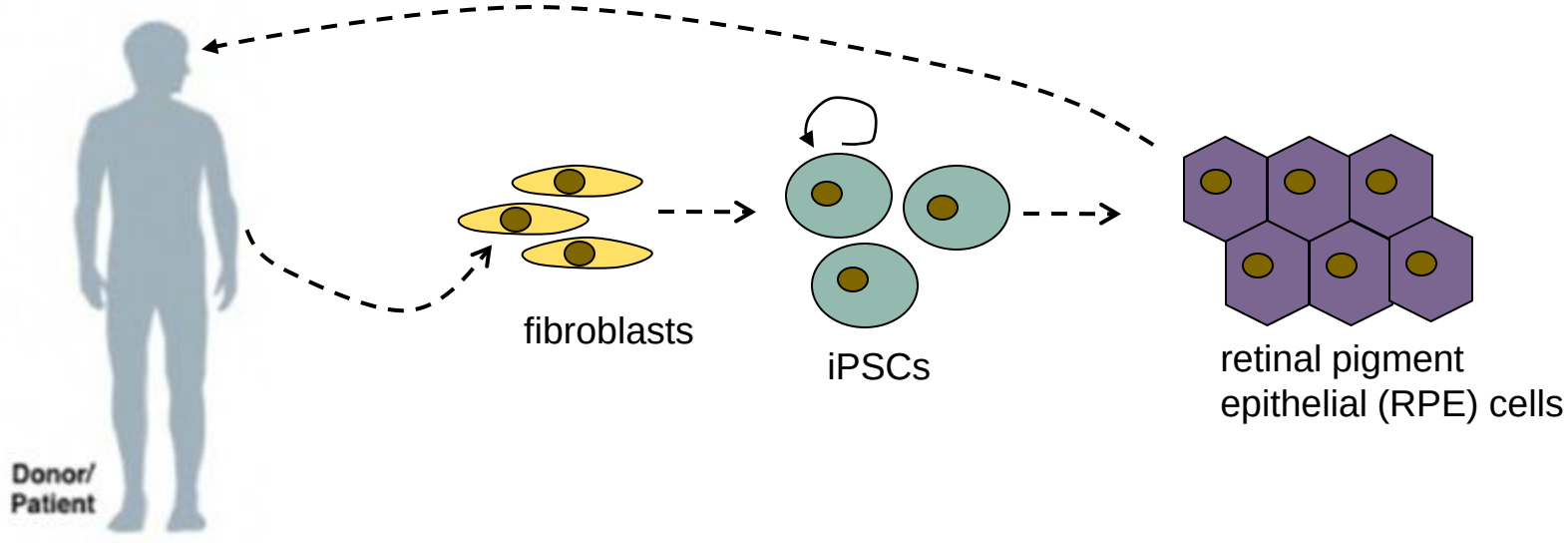
Heart,
Liver,
Pancreas,
CNS, etc

Ref:
EuroStemCell.org

ThermoFisher SCIENTIFIC

First iPSC human clinical trial underway

For age-related macular degeneration.
Conducted at RIKEN Center For Development Biology in Japan.
First patient transplanted September 2014

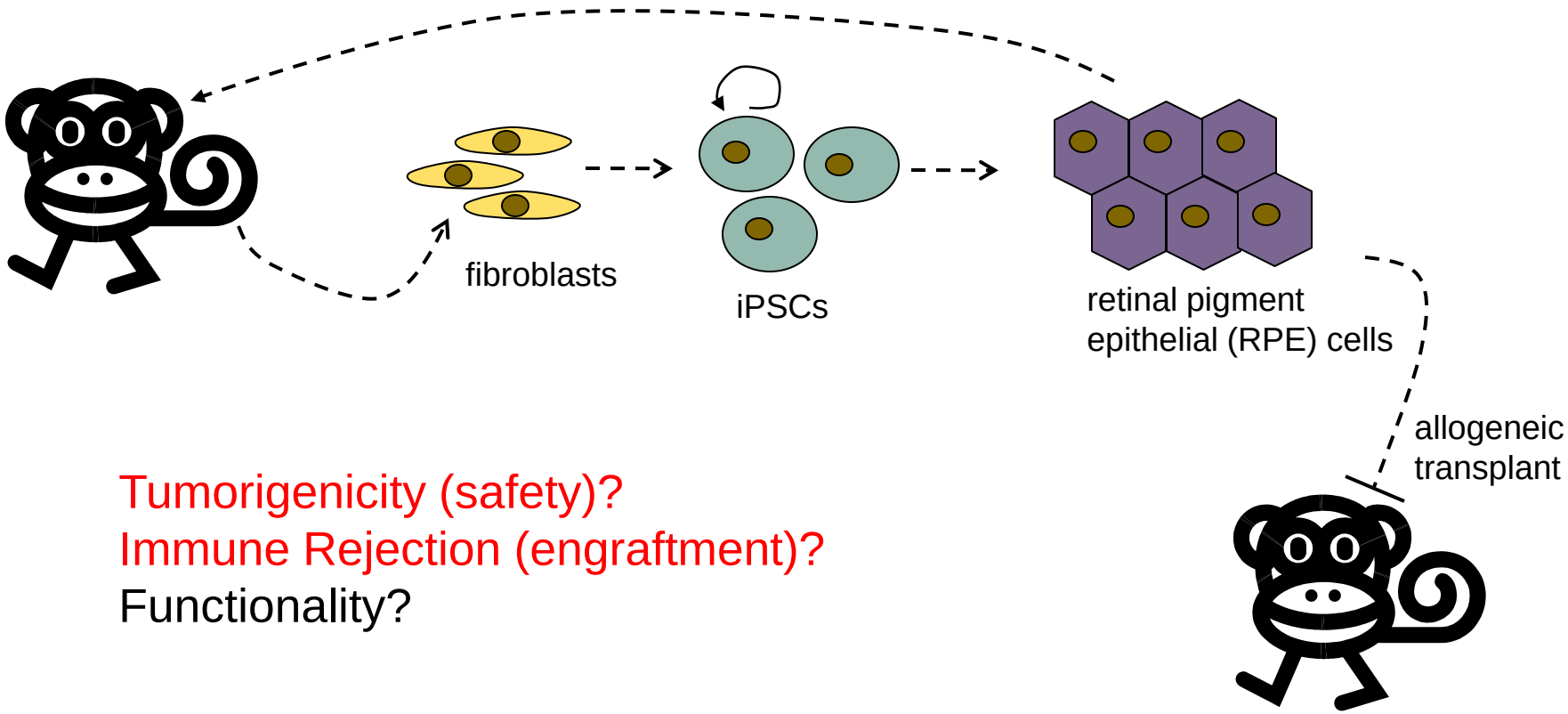


Tumorigenicity (safety)?
Immune Rejection (engraftment)?
Functionality?

ThermoFisher SCIENTIFIC

First iPSC human clinical trial underway

For age-related macular degeneration.
Conducted at RIKEN Center For Development Biology in Japan.
First patient transplanted September 2014



Tumorigenicity (safety)?
Immune Rejection (engraftment)?
Functionality?

Kamao et al., Stem Cell Reports 2, 205-218 (2014).

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