

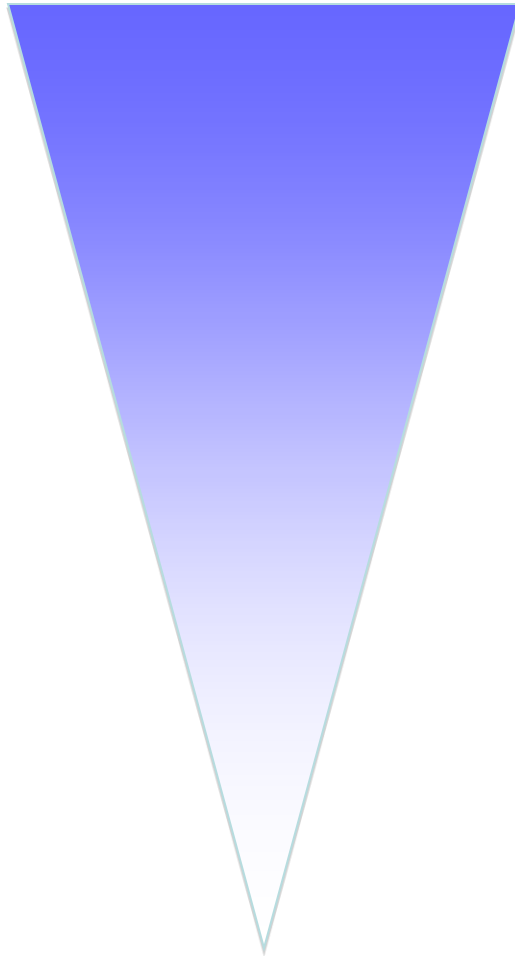
Stem Cell Therapies Research for Friedreich Ataxia

Dr Mirella Dottori

***Centre for Neuroscience
University of Melbourne***

Stem Cells

Potentiality



Totipotent

Fertilized Egg

Pluripotent

Embryonic Stem Cells
Induced-Pluripotent Stem Cells

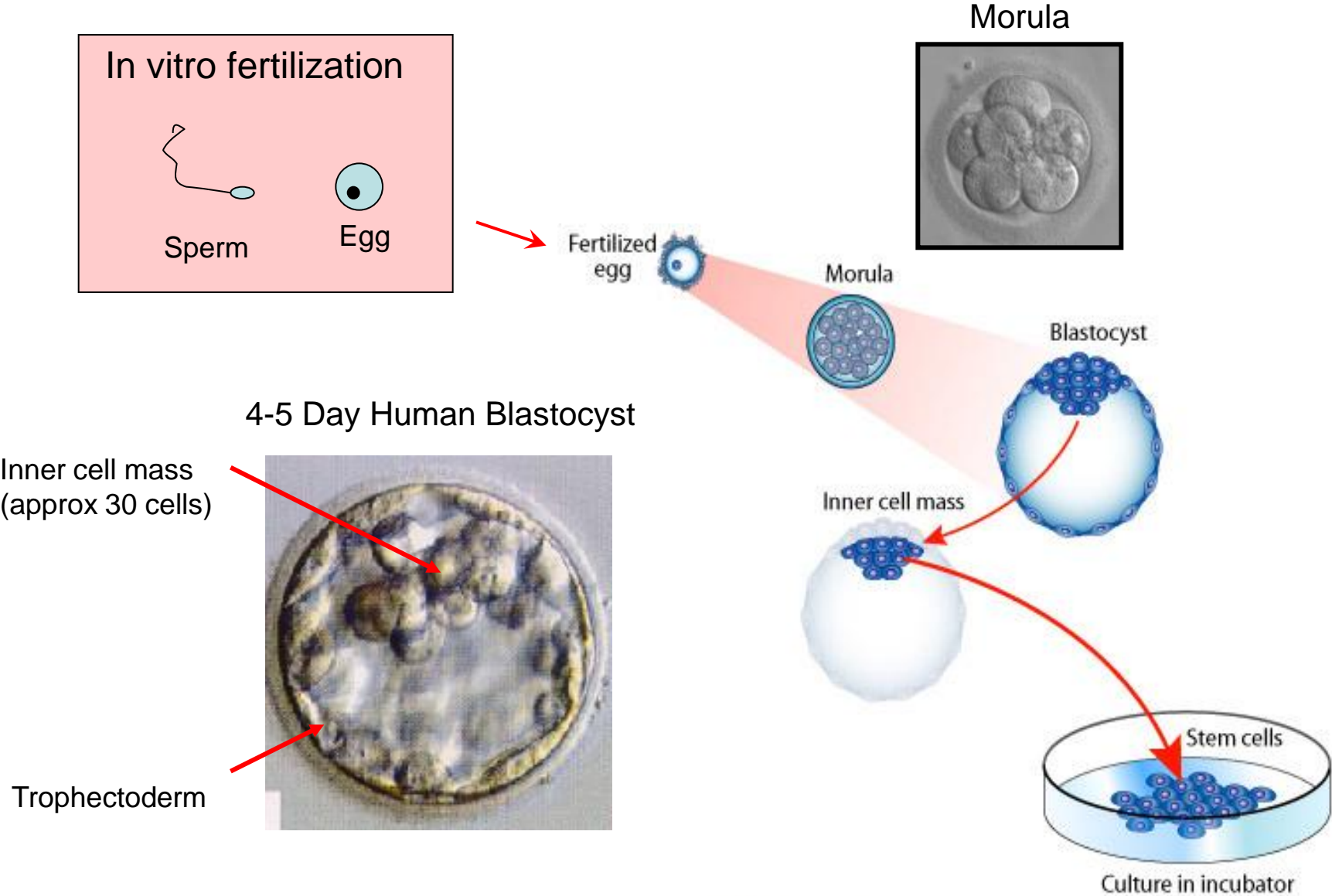
Multipotent

Fetal Stem Cells
Adult Stem Cells
Cord Blood Stem Cells

Unipotent

Fetal Stem Cells
Adult Stem Cells

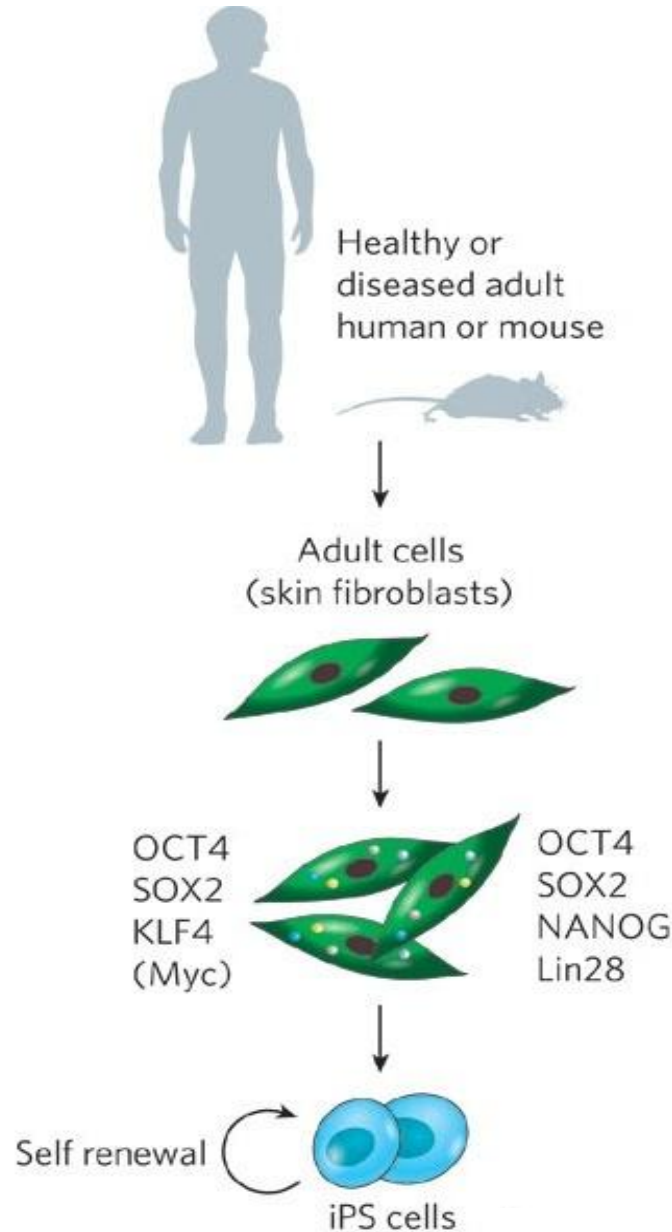
Derivation of Human Embryonic Stem Cell Lines



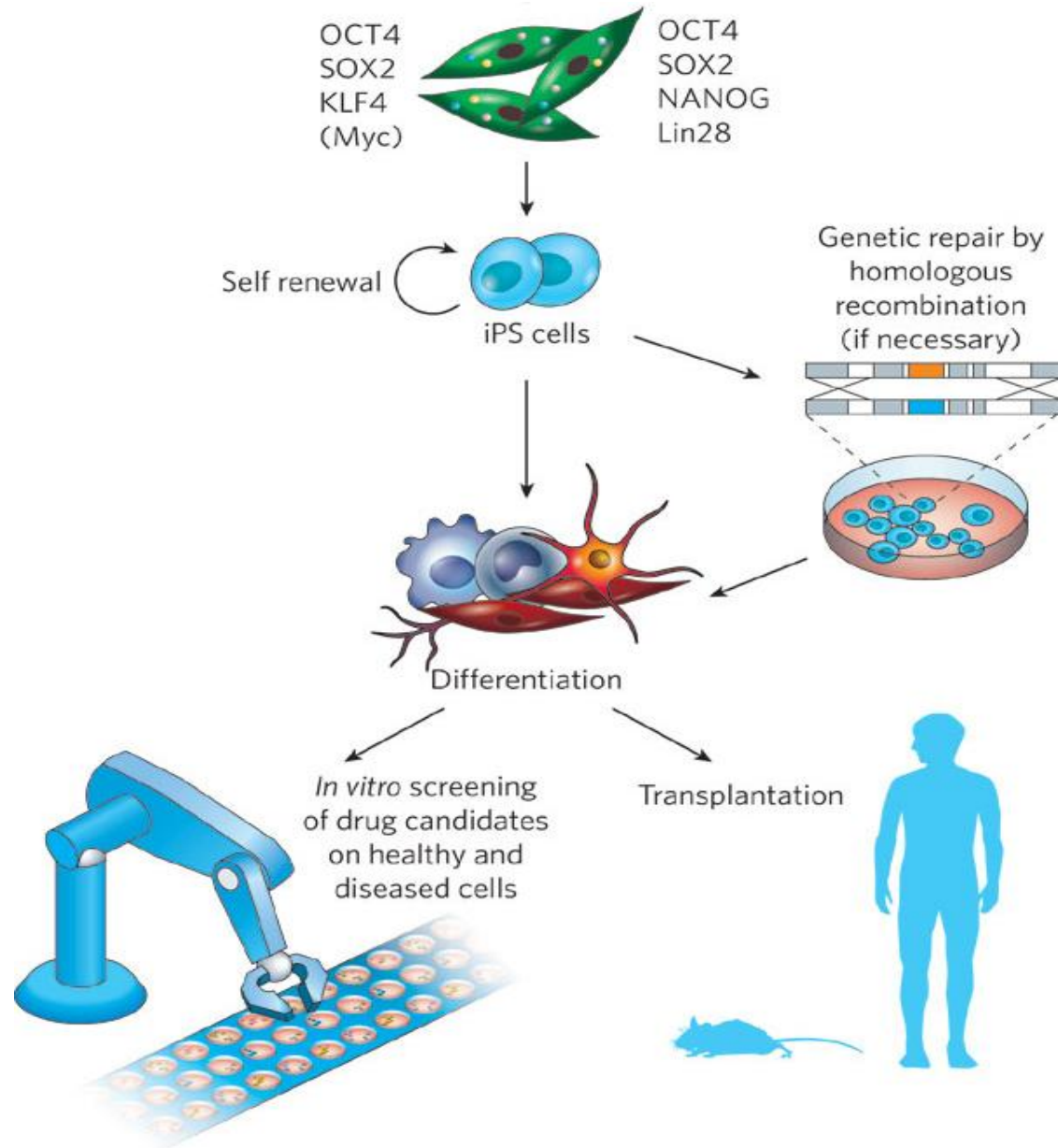
Induced Pluripotent Stem Cells

2006: Mouse iPS cells:
Takahashi & Yamanaka.

2007: Human iPS cells:
Takahashi & Yamanaka;
Thomson

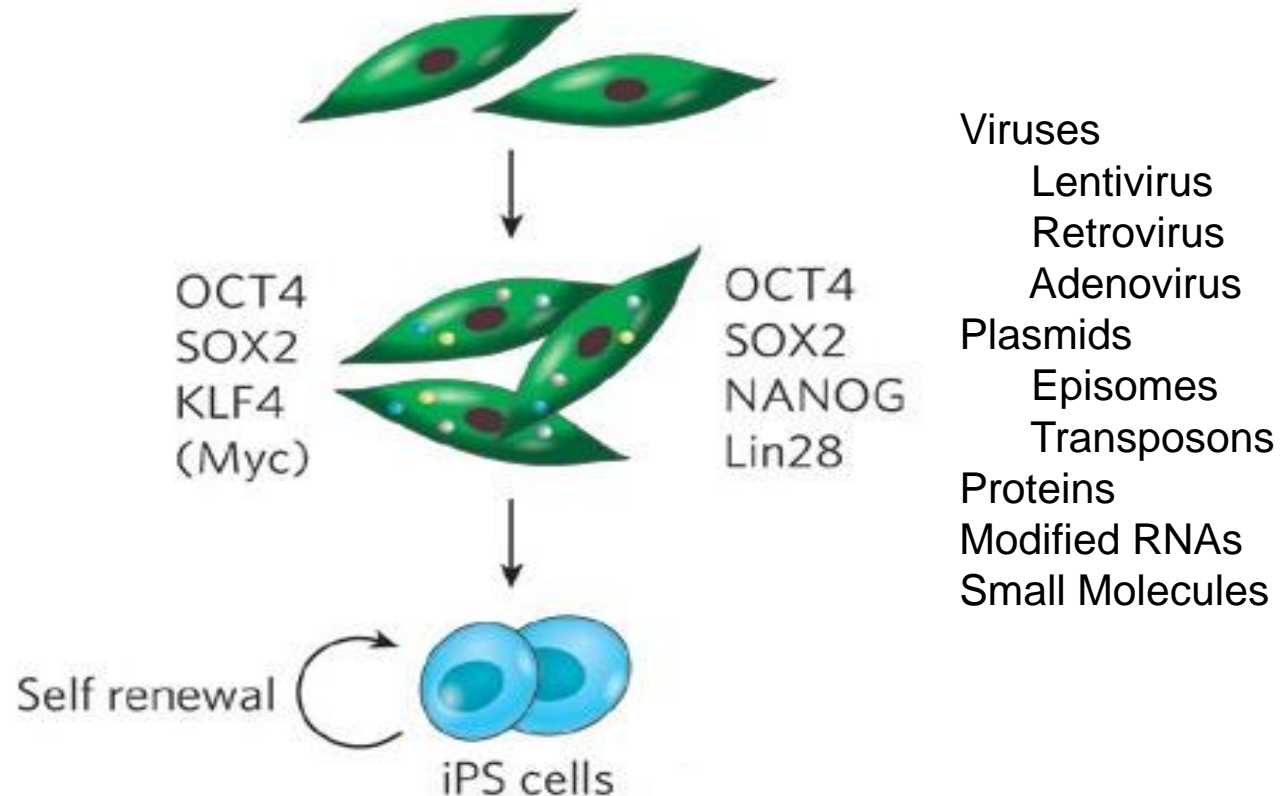


Induced Pluripotent Stem Cells



Improved Methods of Reprogramming

Fibroblasts, adipose cells, liver cells, stomach cells,
neural stem cells, germ cells...



iPS ≠ ES

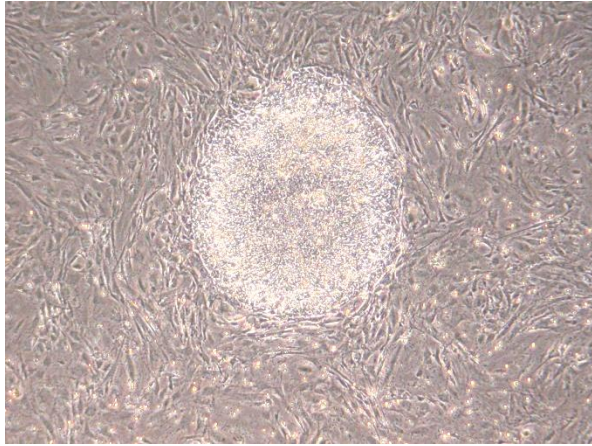
Generation of iPS Cell Lines from Adult FA and Control Skin Biopsies

Code	Age	Gender	FARS	GAA1	GAA2	Age of onset	Disease Duration
FA3	32	Male	63	527	1058	21	11.0833333
FA4	48	Female	92.5	751	1027	20	27.6666667
FAC4	20	Female					

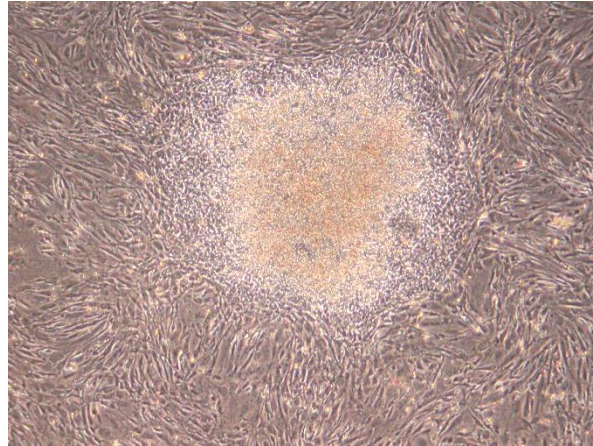
FARS: FRDA rating scale. GAA1: small allele repeats; GAA2: long allele repeats.

Collaborators: Martin Delatycki (Murdoch Inst), Paul Verma (MIMR), Jun Liu (MIMR), Joe Sasero (Murdoch Inst), and Maggie Galea (Murdoch Inst)

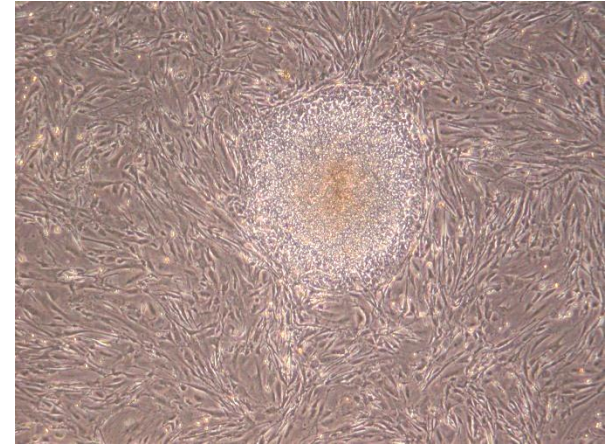
Generation of iPS Cell Lines from Adult Friedreich Ataxia Skin Biopsies



Human ES
Colony



Friedreich Ataxia iPS
Colony



Control iPS
Colony

Retroviral transduction of Oct4, Sox2, c-myc, and Klf4

Collaborators: Martin Delatycki (Murdoch Inst), Paul Verma (MIMR), Jun Liu (MIMR), Joe Sasero (Murdoch Inst), and Maggie Galea (Murdoch Inst)

GAA repeat expansion is retained in Friedreich Ataxia iPS cell lines

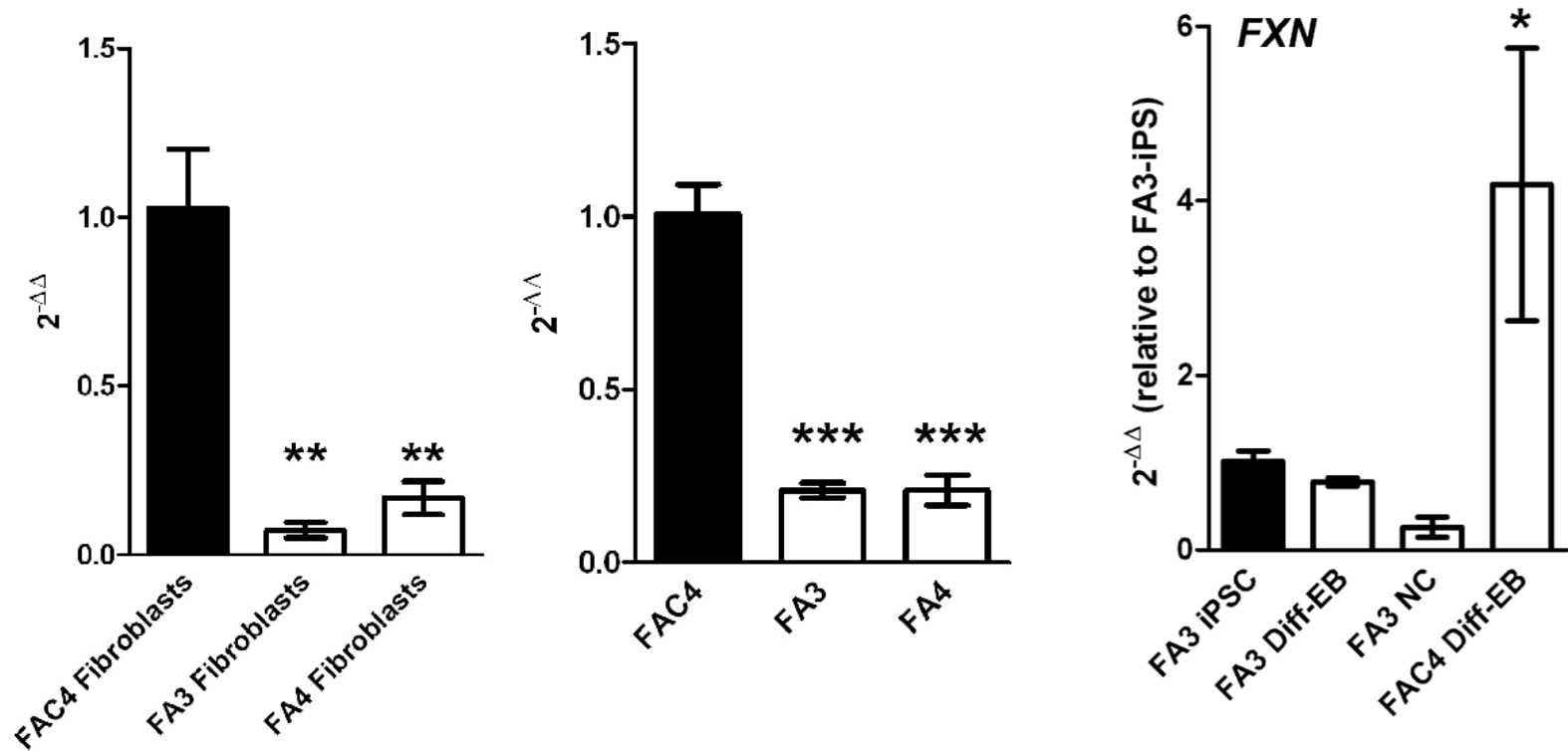
Fibroblasts

FAC4	<i>normal range</i>
FA3	1026, 532
FA4	718, 226

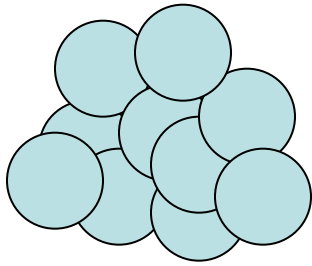
iPS cells

FAC4	<i>normal range</i>
FA3- <i>i</i>	649, 519, 224
FA3- <i>ii</i>	1010, 649, 519
FA4- <i>i</i>	819, 287
FA4- <i>ii</i>	758, 267
FA4- <i>iii</i>	758, 267

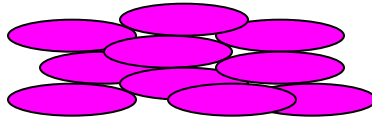
Friedreich Ataxia iPS cell lines show relatively low levels of Frataxin transcripts



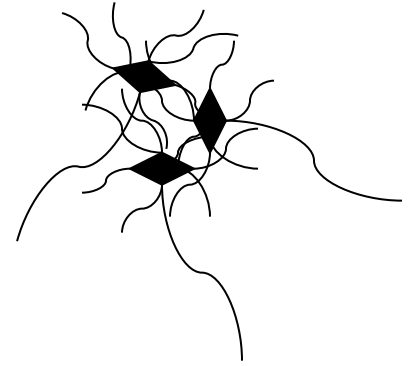
(Liu et al, Stem Cell Reviews and Reports 2010)



Pluripotent
Stem Cells



Multipotent
Neural Stem Cells



CNS and PNS
Neurons and Glia
Neural Crest

Neuronal Populations that Degenerate in Friedreich Ataxia

Central Nervous System

Cerebellar pathways

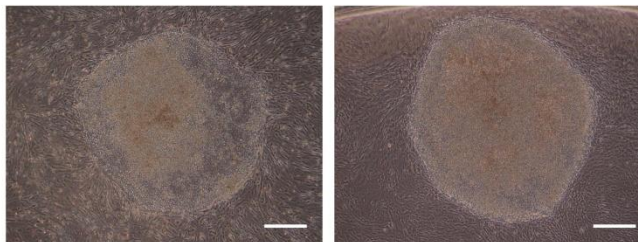
Corticospinal tract, spinocerebellar tracts

Other CNS pathways: impairments in the auditory system, visual system, dysarthria

Peripheral Nervous System (Neural crest derived)

Large sensory neurons of dorsal root ganglia

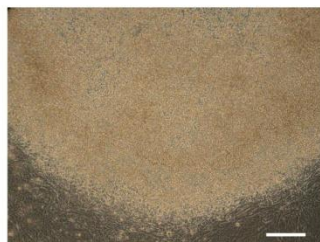
**Pluripotent Stem Cell
Expansion**



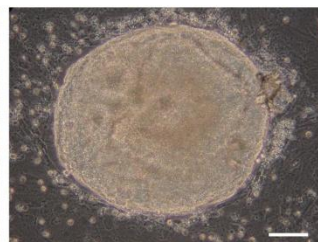
iPS

hES

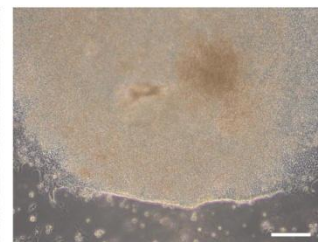
Neural Induction



Noggin Induction

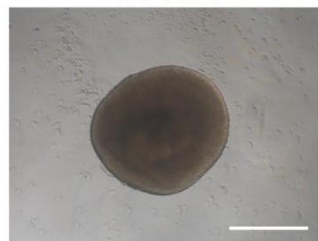


Co-Culture on PA6



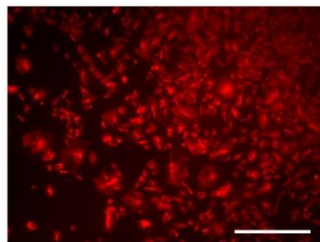
Defined Media Induction

**Neural Stem Cell
Expansion**

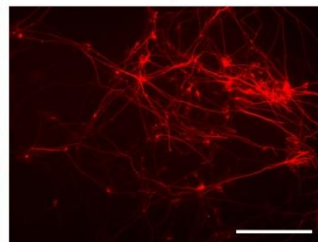


Neurosphere

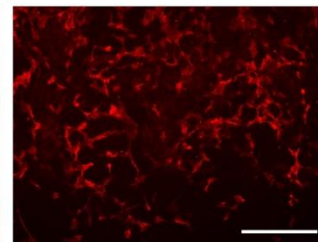
**Terminal
Differentiation**



Glial

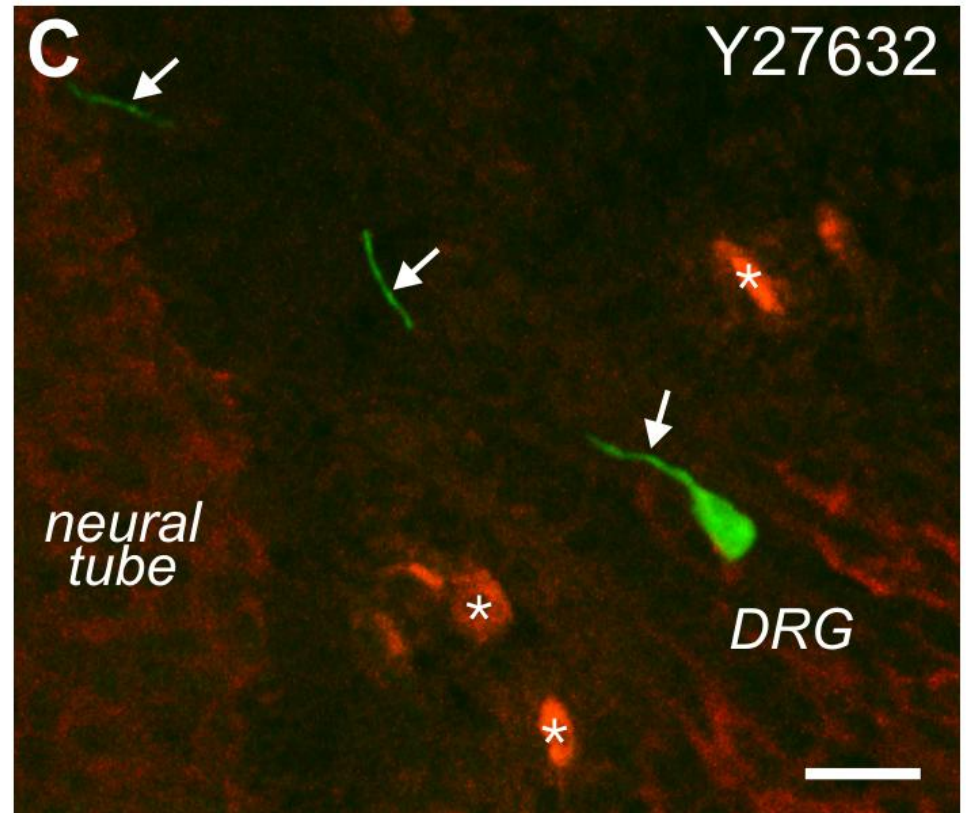
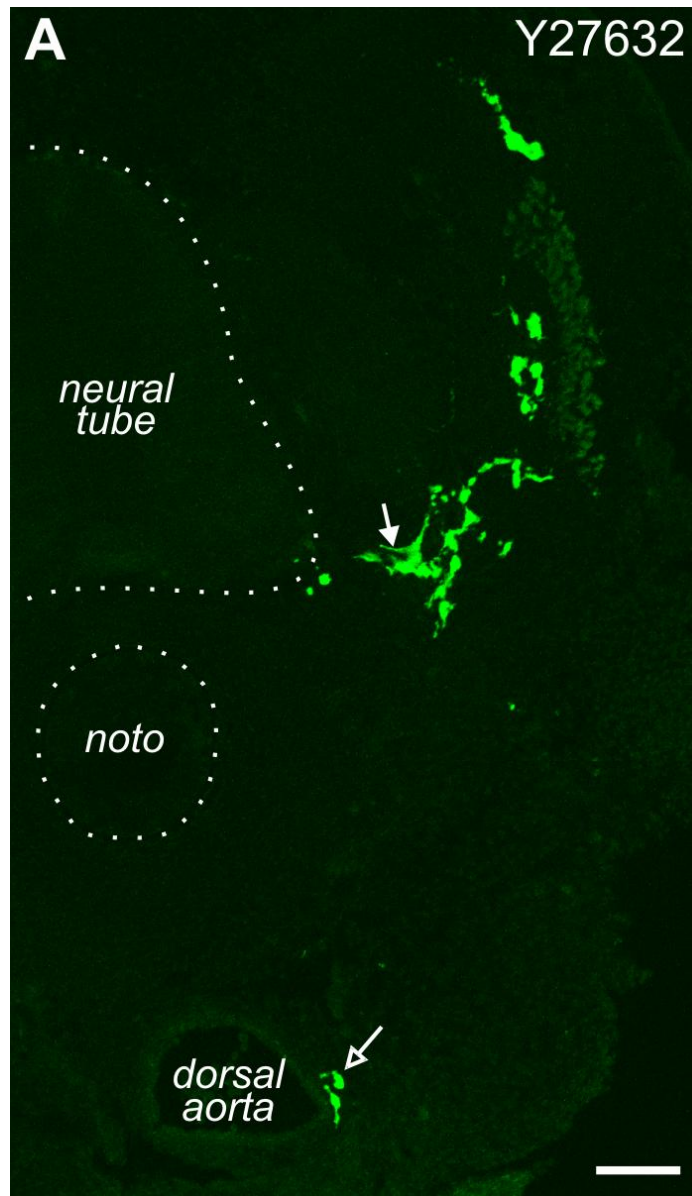


Neural



Neural Crest

Human ES-derived neural crest progenitors transplanted in quail embryos

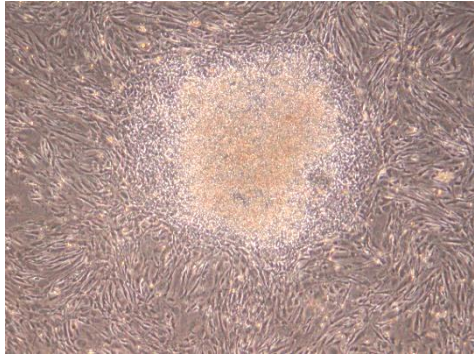


(Hotta et al., Stem Cells, 2009)

Generation of Neurons from Friedreich Ataxia iPS Cells...

Neural Differentiation of FA iPS Cell Lines

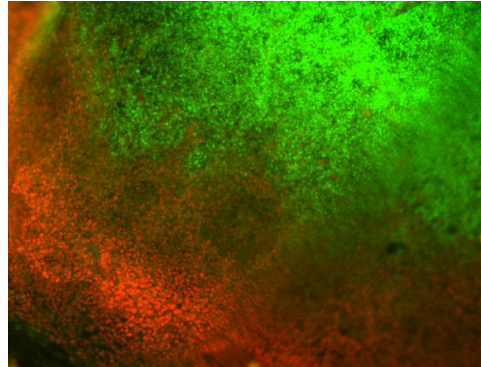
FA iPS



Noggin
14 days



Neural Induction



Oct4 Pax6

NBM
FGF & EGF



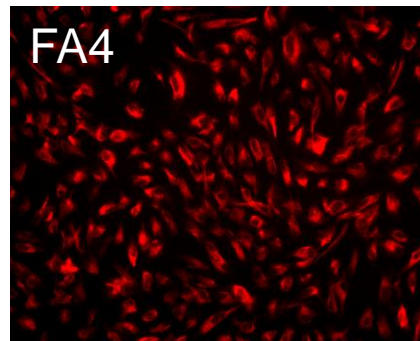
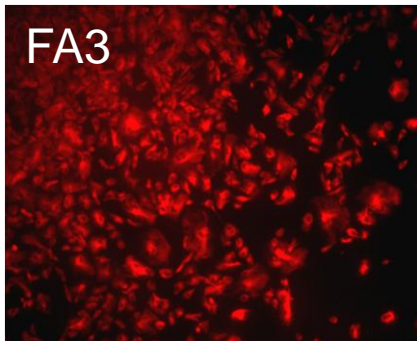
Neurosphere



Fibronectin



Glia

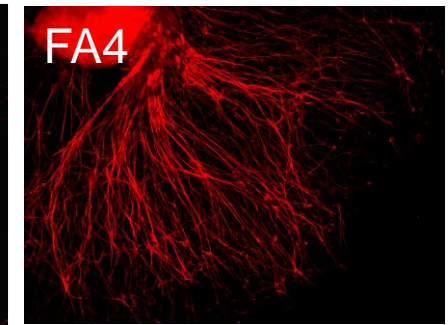
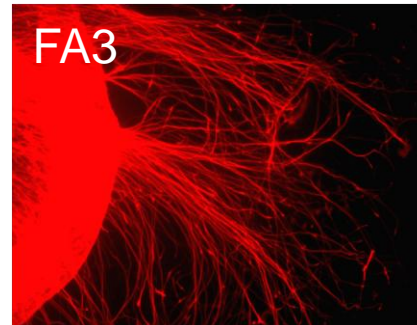


S100β

Laminin

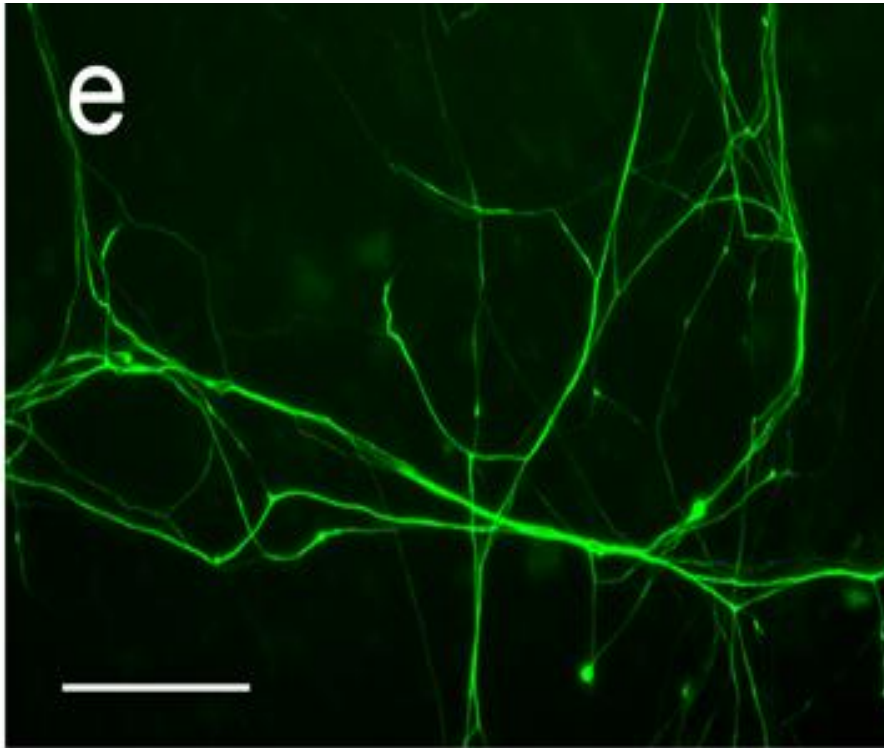


Neurons

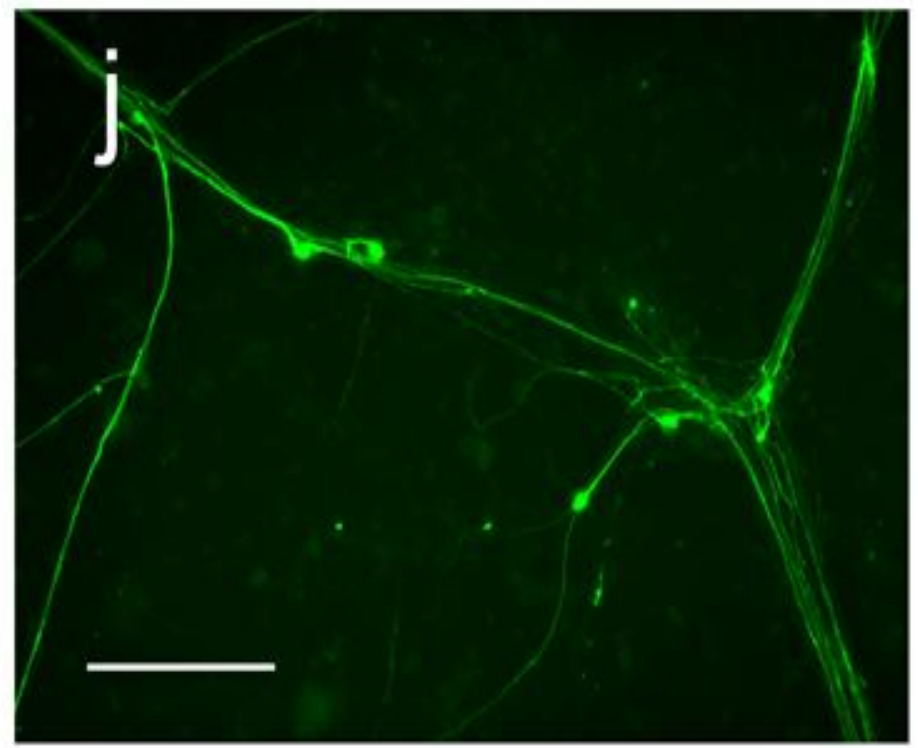


β III tubulin

Neurons derived from FA neural crest-like progenitors express Peripherin



FA3



FA4

Cellular Models of Friedreich Ataxia

WHY??

Understanding cellular mechanisms of pathogenesis
Developing treatments and drug discovery

WHAT??

Cells carrying FXN mutations
Patient-derived human cells
Cell types that specifically degenerate in FA
Cells that mimic pathogenic cellular processes

HOW???

Pluripotent stem cells derived from FA patients

Use of Friedreich Ataxia iPS cells for Drug Discovery

Cell types

Neurospheres

Differentiated neurons in culture

In vivo transplanted human GFP+ neurons

Assay Read-out

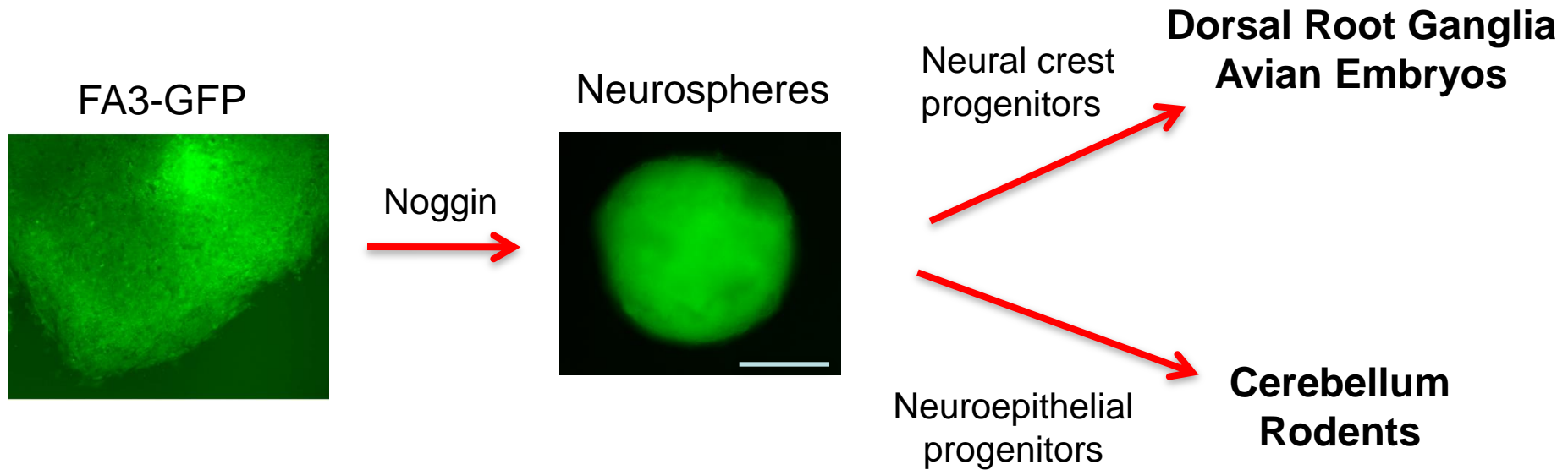
- increase in *FXN* expression (Q-PCR and Westerns)
- improvement of mitochondrial function (ATP production)
- decrease in cellular stress (ROS production)
- increased cell survival
- improvement of cellular function (eg. electrophysiology)

Dosage levels

Exposure times

Frequency of Dose

In Vivo Differentiation of FRDA Neural Progenitors

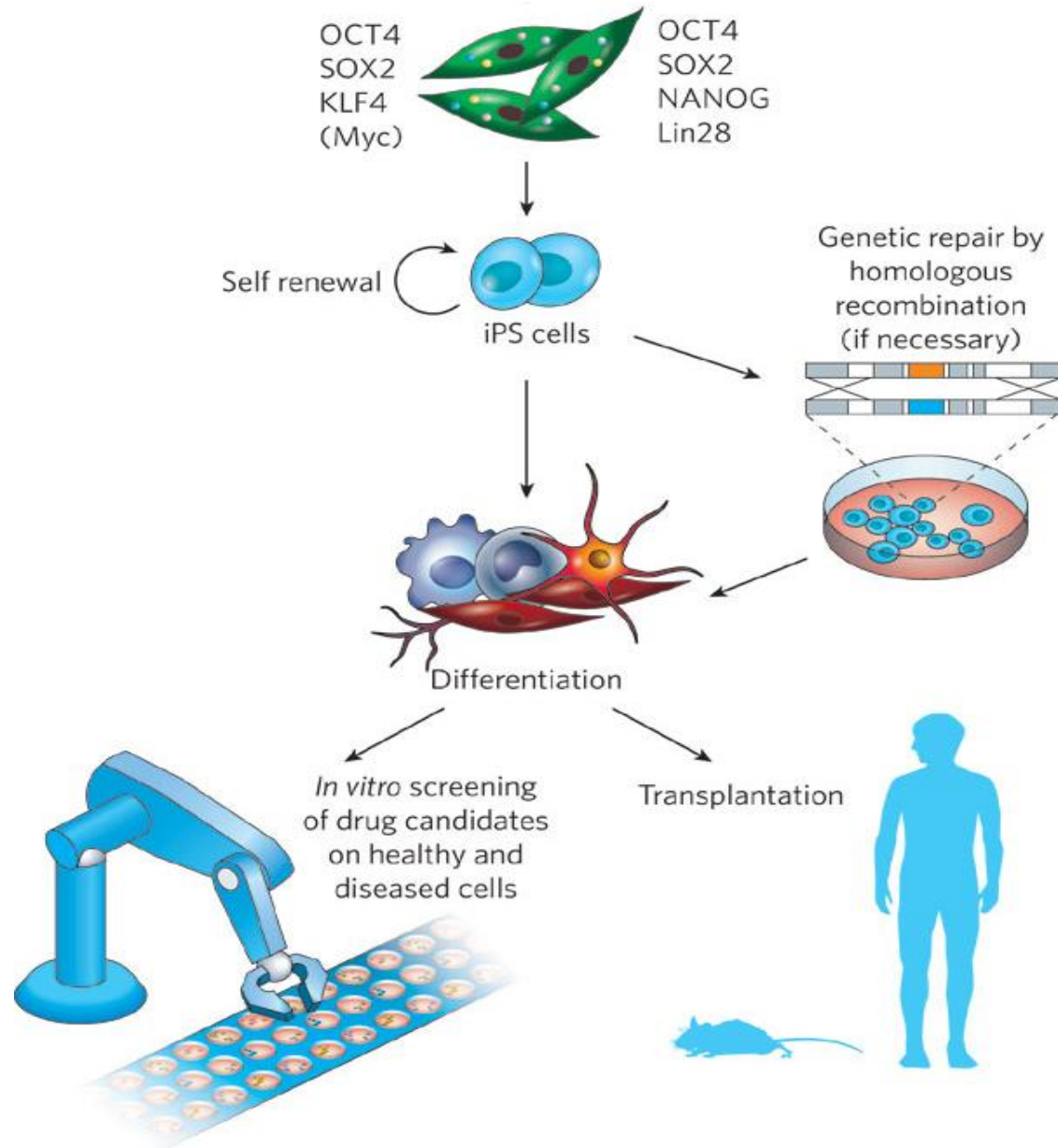


Collaborators:

Donald Newgreen (Murdoch Institute)

Lachlan Thompson (Florey Neurosci Institute), Giampero Leanza (University of Trieste, Italy)

Induced Pluripotent Stem Cells





THE UNIVERSITY OF
MELBOURNE

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Dept of Pharmacology*

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Karina Needham (*Dept of Otolaryngology*)
Heather Young (*Dept of Anatomy and Cell Biology*)
Ryo Hotta (*Dept of Anatomy and Cell Biology*)

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Florey Neuroscience Institute

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Joel Gottesfeld

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