Why are we here?

- NYSCF is trying to determine how best to facilitate the work of NY stem cell biologists.
- One possibility is to set up a screening lab that would help develop and run stem cell assays.
- We want to give a general introduction to screening and begin to assess the number of interested scientists.

Can Small Molecule Screening Be Useful in an Academic Setting?

- Provides useful reagents.
- Probes molecular changes underlying important biological processes.
- May help in the discovery of new targets for important diseases, especially those that are “orphan”.

Stem Cell Assays (Small Molecule Biased)

- Regulate the state of differentiation
  - Reprogramming
  - Differentiation
  - Lineage reprogramming
- Studies on partially differentiated or fully differentiated cells
  - Proliferation
    - Stimulation of neural stem cells
    - Inhibition of tumor stem cells
  - Survival
    - Motor neurons derived from ALS iPS cells

Stem Cell Assays (Small Molecule Biased)

- Types of assays
  - Phenotypic (microscope)
  - Reporter gene or other enzymatic assays (plate reader)

Stem Cell Assays (Small Molecule Biased)

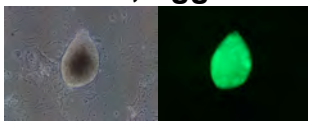
- Types of libraries
  - Diversity
    - Size and quality
    - Results from screens and need for follow-up chemistry
  - Annotated
    - Size and quality
    - Analysis of data
      - Biological clustering
      - Chemical clustering
        - » “Analogue by catalogue”

Stem Cell Assays (Small Molecule Biased)

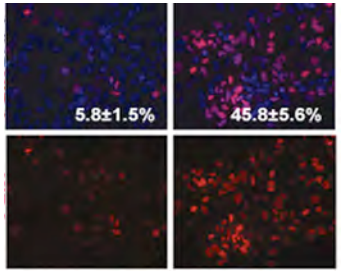
- Setting up a robotic screening assay
  - Bench top
  - Assay development to ensure sufficient robustness and reproducibility to run on a robotic liquid handling system
    - Positive control
  - Small-scale robotic screen
    - Positive control
  - Larger scale robotic screen
  - Data analysis
  - Hit confirmation and follow-up

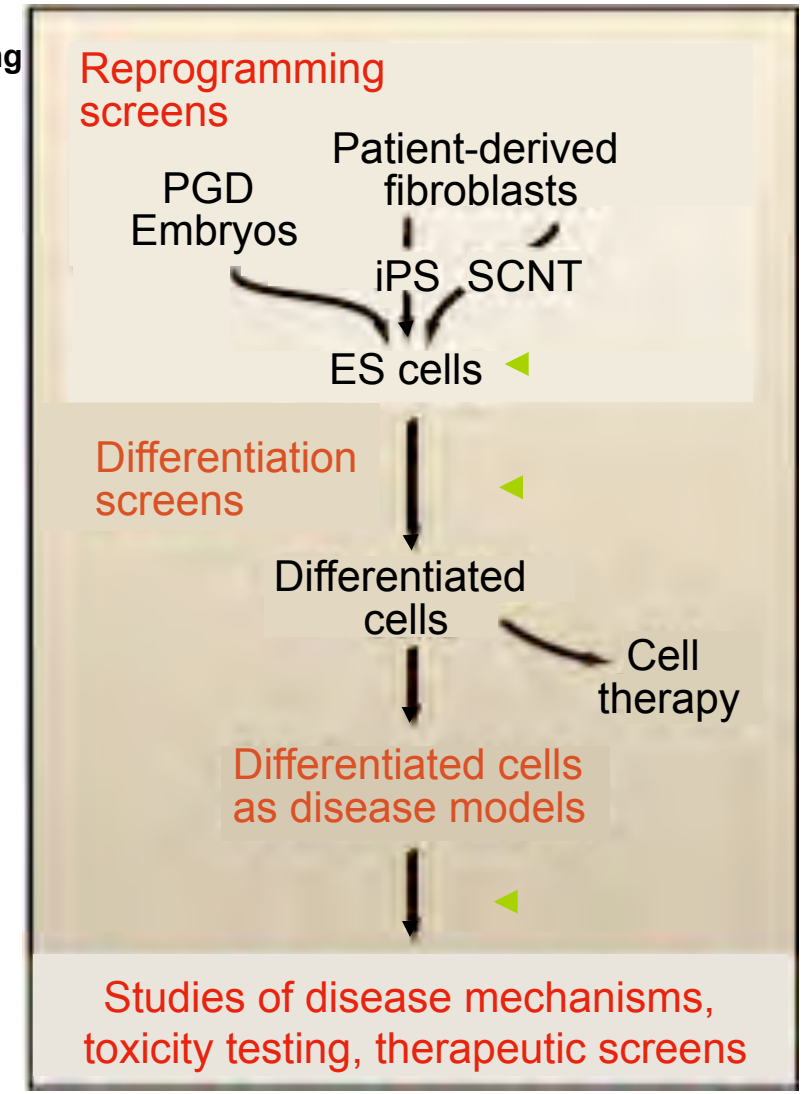
Various types of stem cell based screens can be used to discover useful reagents and (perhaps) therapeutics.

Chemical reprogramming
Ichida, Eggan



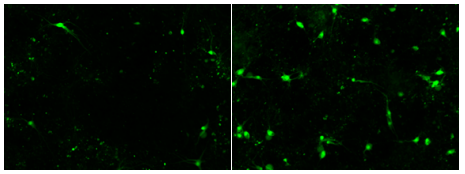
β-cell differentiation
Chen, Melton





Rubin, Cell, 2008

ES Cells and Drug Discovery/Validation

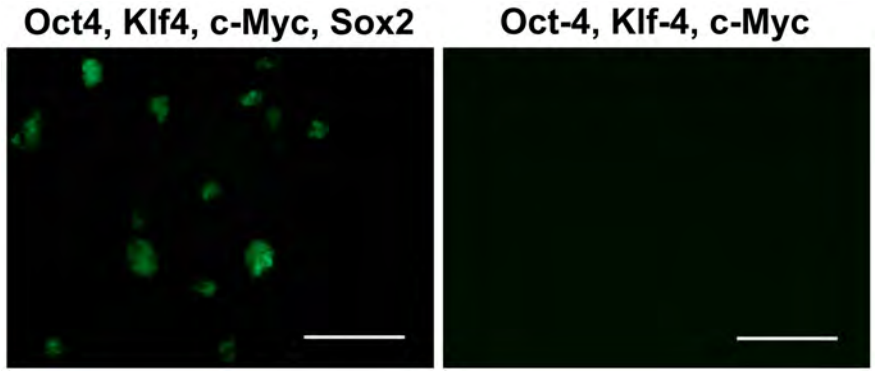


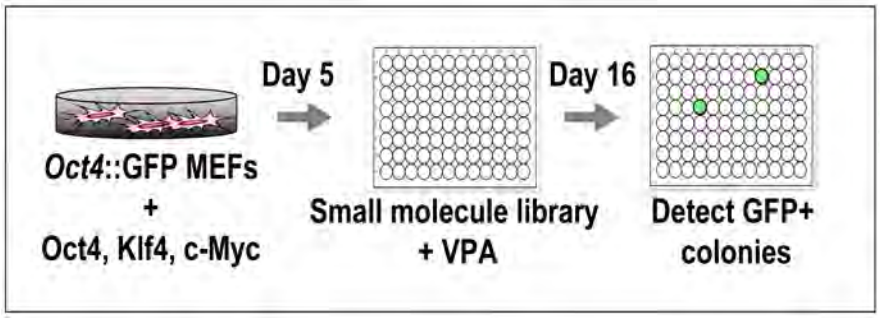
Motor neuron survival

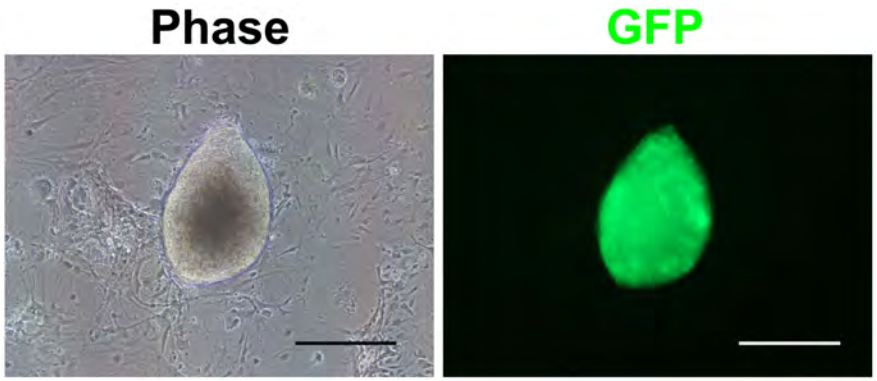
A Small Molecule Reprogramming Screen

- Adult cells of many different kinds can be reprogrammed to ES-like cells by techniques – mostly using viral vectors -- that allow for the expression of 4 genes (*c-Myc, Sox-2, Oct-4 and Klf-4*) that are highly expressed in ES cells.
- This observation has excited the entire stem cell community but has raised some concern because of the use of viruses and oncogenes in activating the dedifferentiation of the adult cells.

A Small Molecule Reprogramming Screen with the Eggan Lab



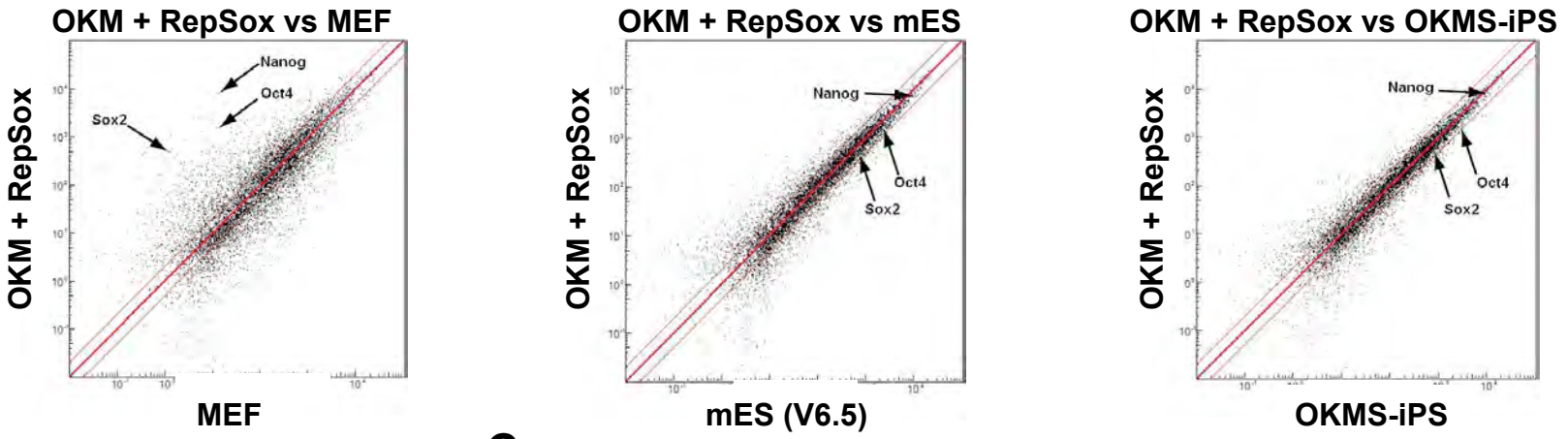




Goal: Chemical recipes that regulate the state of cell differentiation.

Chemically Reprogrammed Cells are Pluripotent

A



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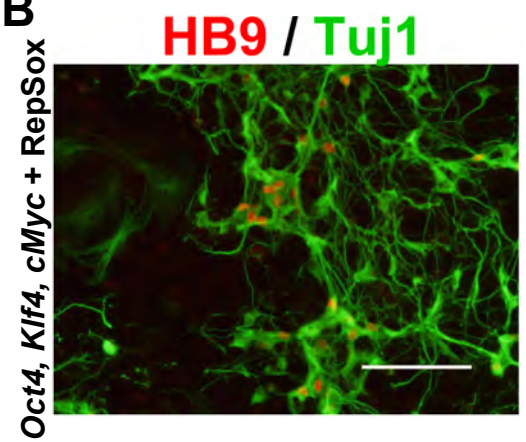
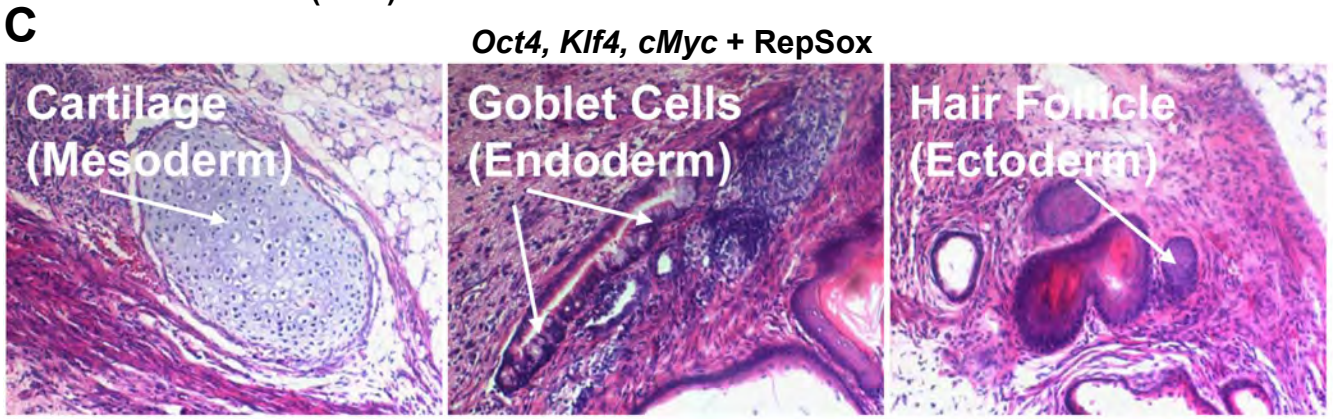
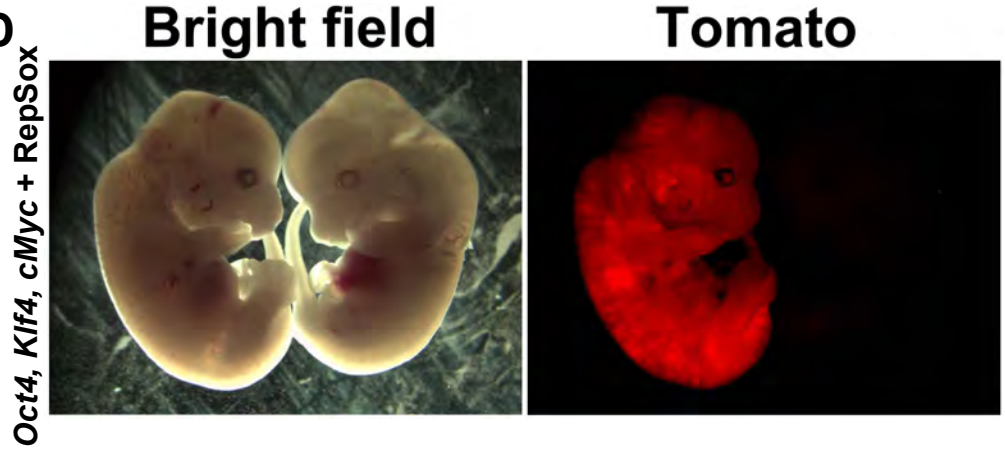


C



D



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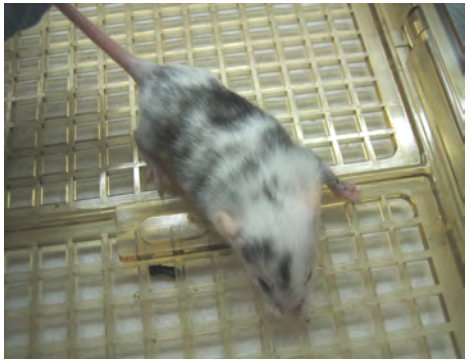
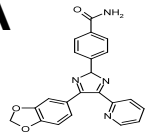


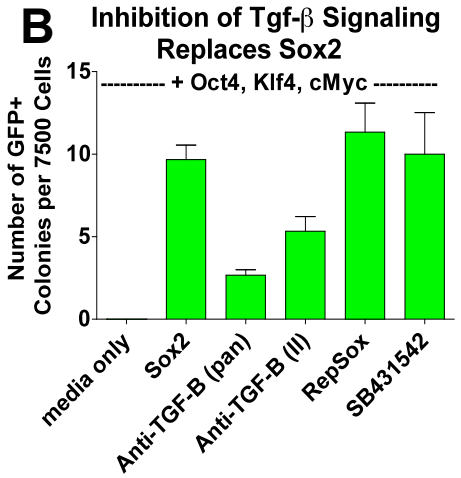
RepSox specifically replaces Sox2 by inhibiting TGF-β Signaling

A



SB431542-
Tgfbr1 inhibitor
25 μM

B



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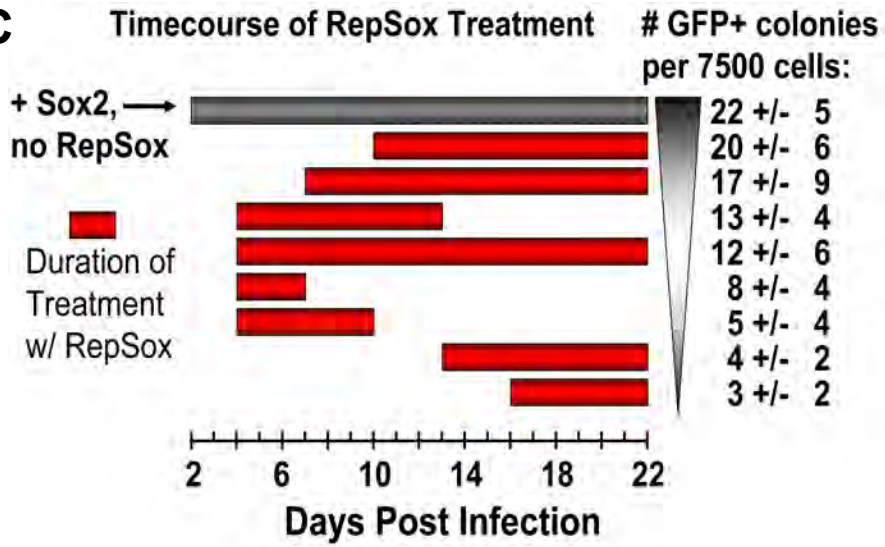

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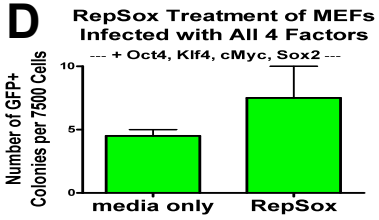

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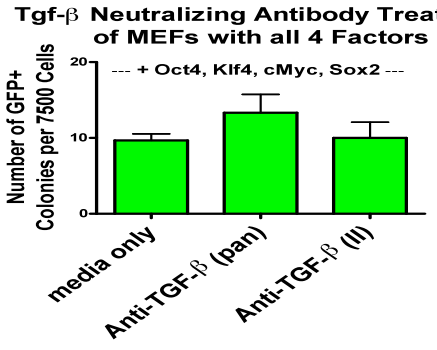
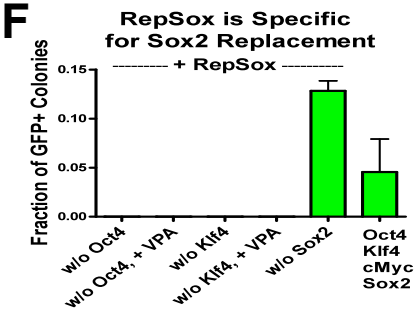
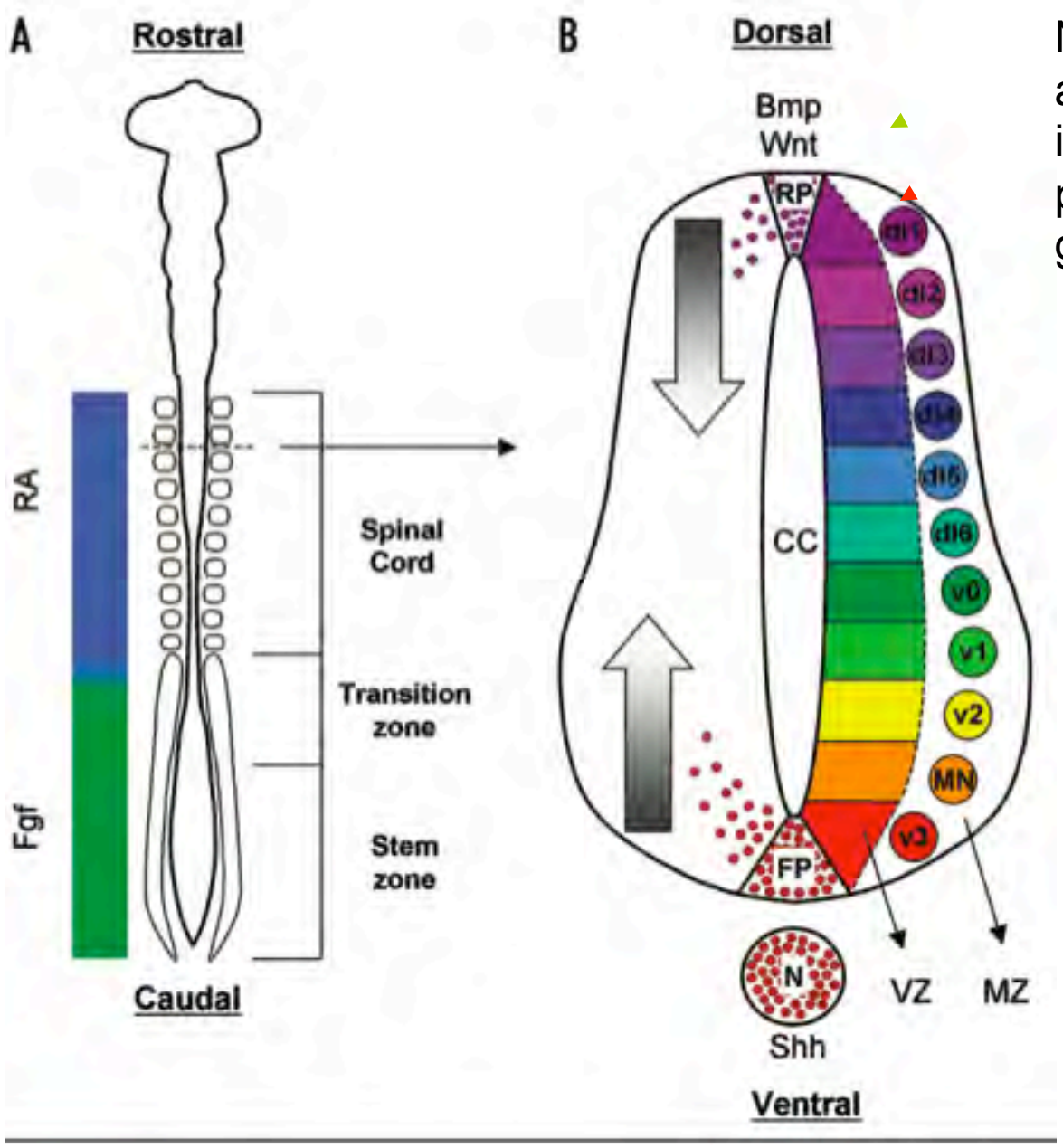


F



Reprogramming Screens

- We anticipate that it will be possible to come up with a reprogramming recipe that uses small molecules or biologicals alone.
  - Can we learn anything about the fundamentals of reprogramming?
- But just having iPS or ES cells is the beginning, not the end, of most projects.
- For example, how do you make a neuron from an ES or iPS cell?

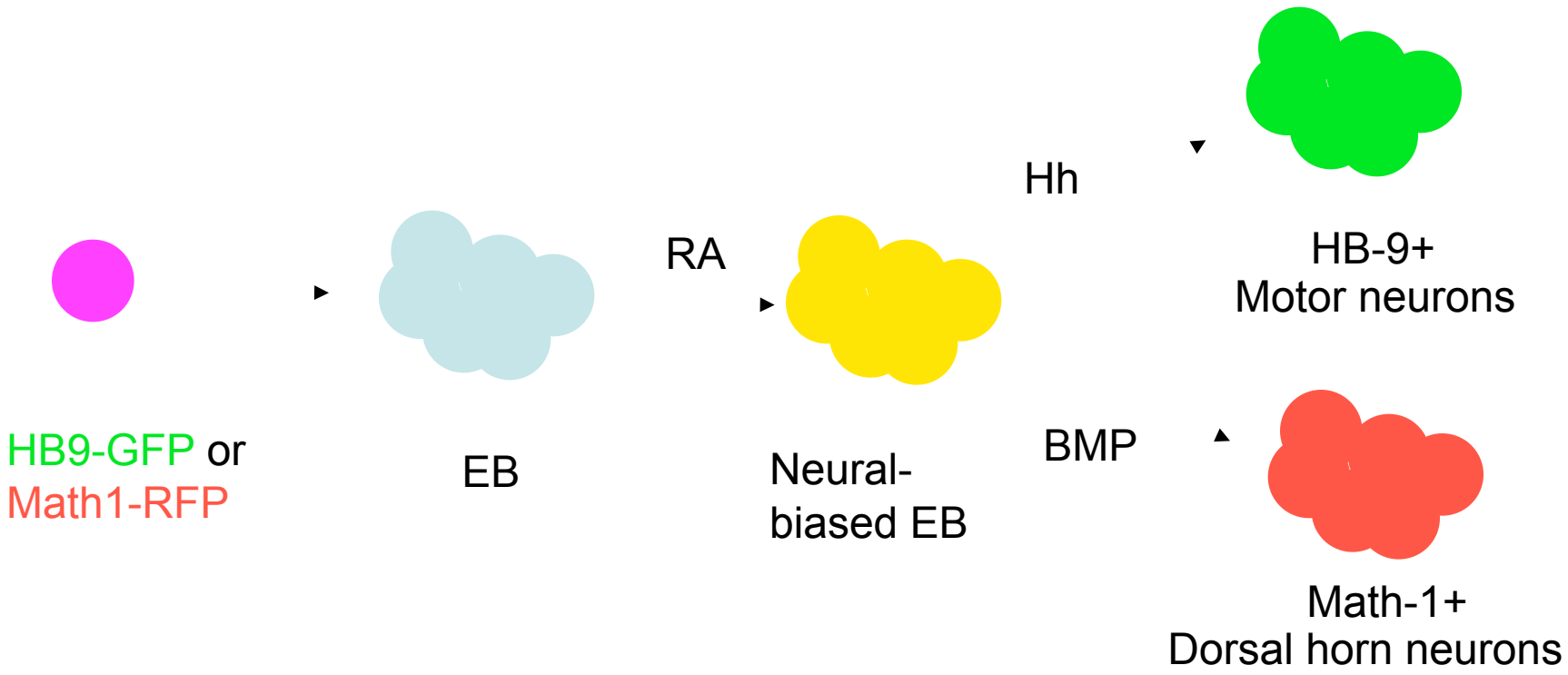


Nociceptive neurons (pain) and dorsal interneurons involved in the pain pathway are being generated here

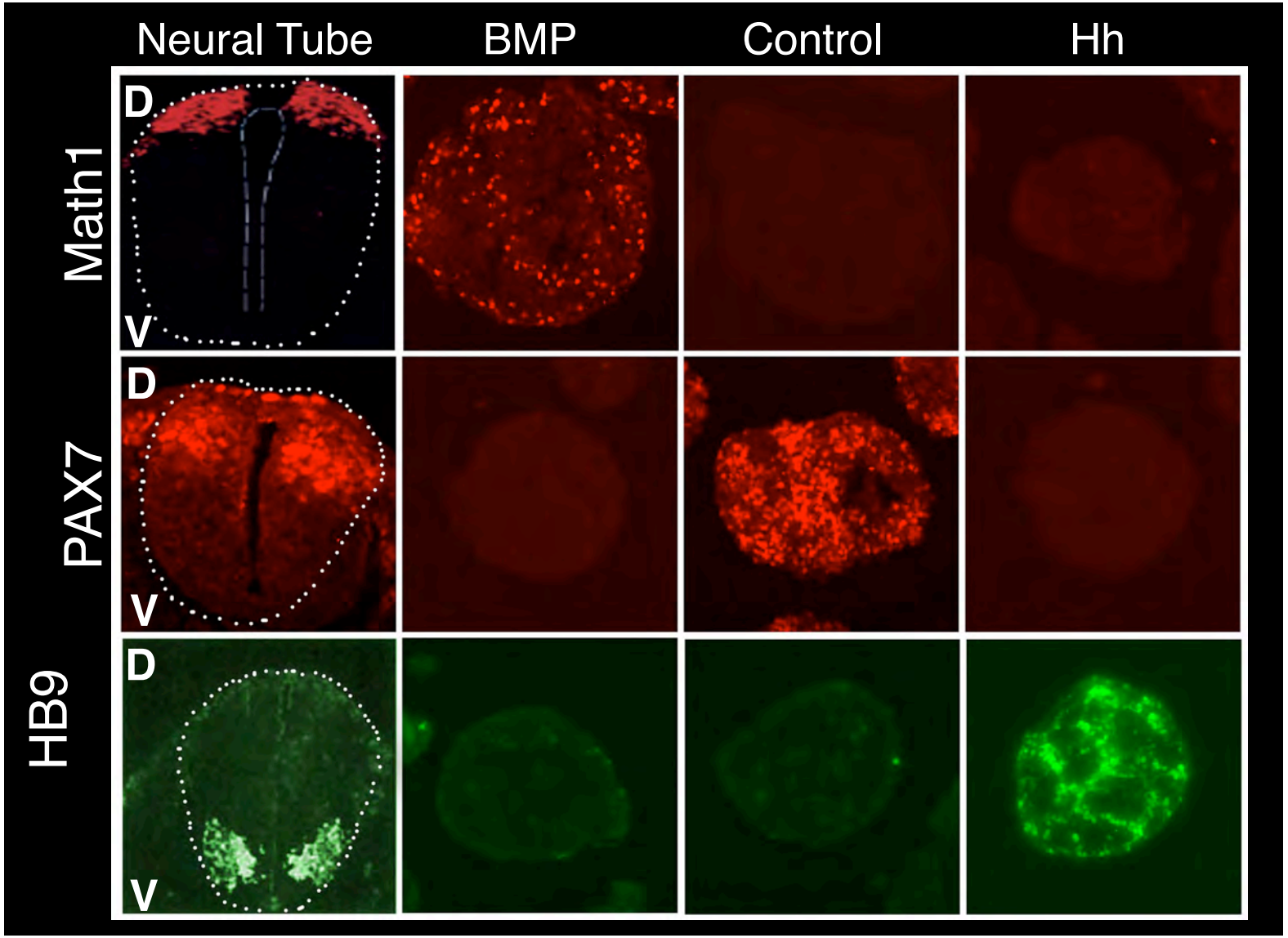
Morphogen gradients are also important in establishing neuronal cell identity in the spinal cord

Ulloa and Briscoe, Cell Cycle, 2007

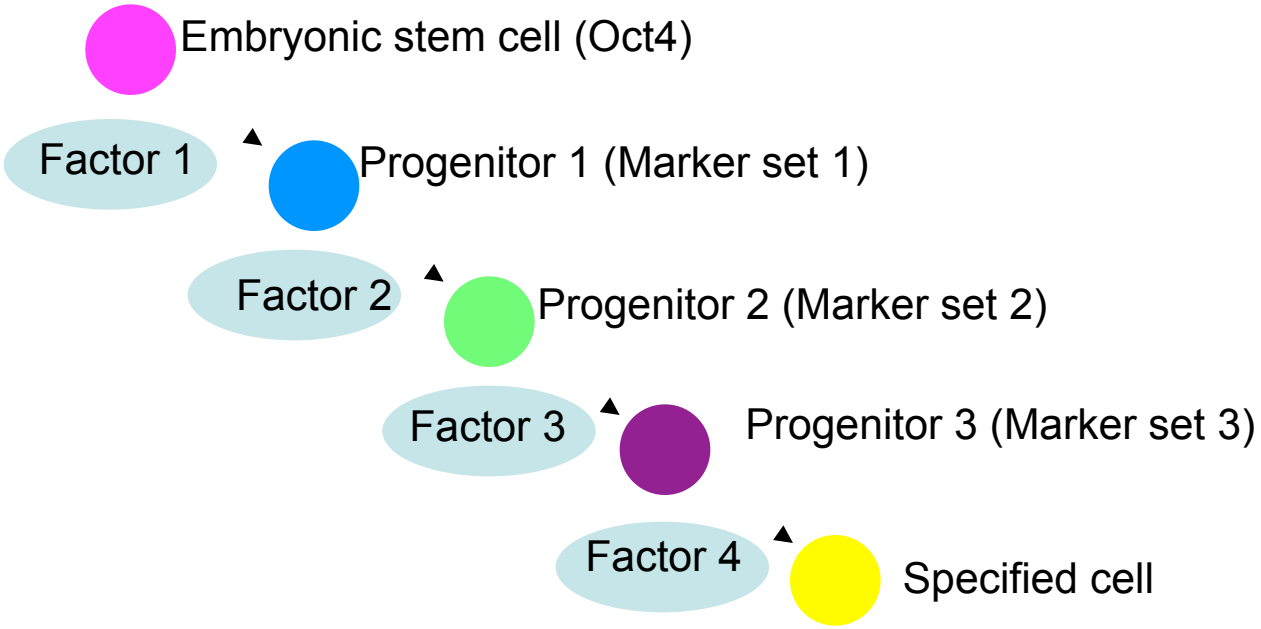
Generation of Neurons From ES Cells



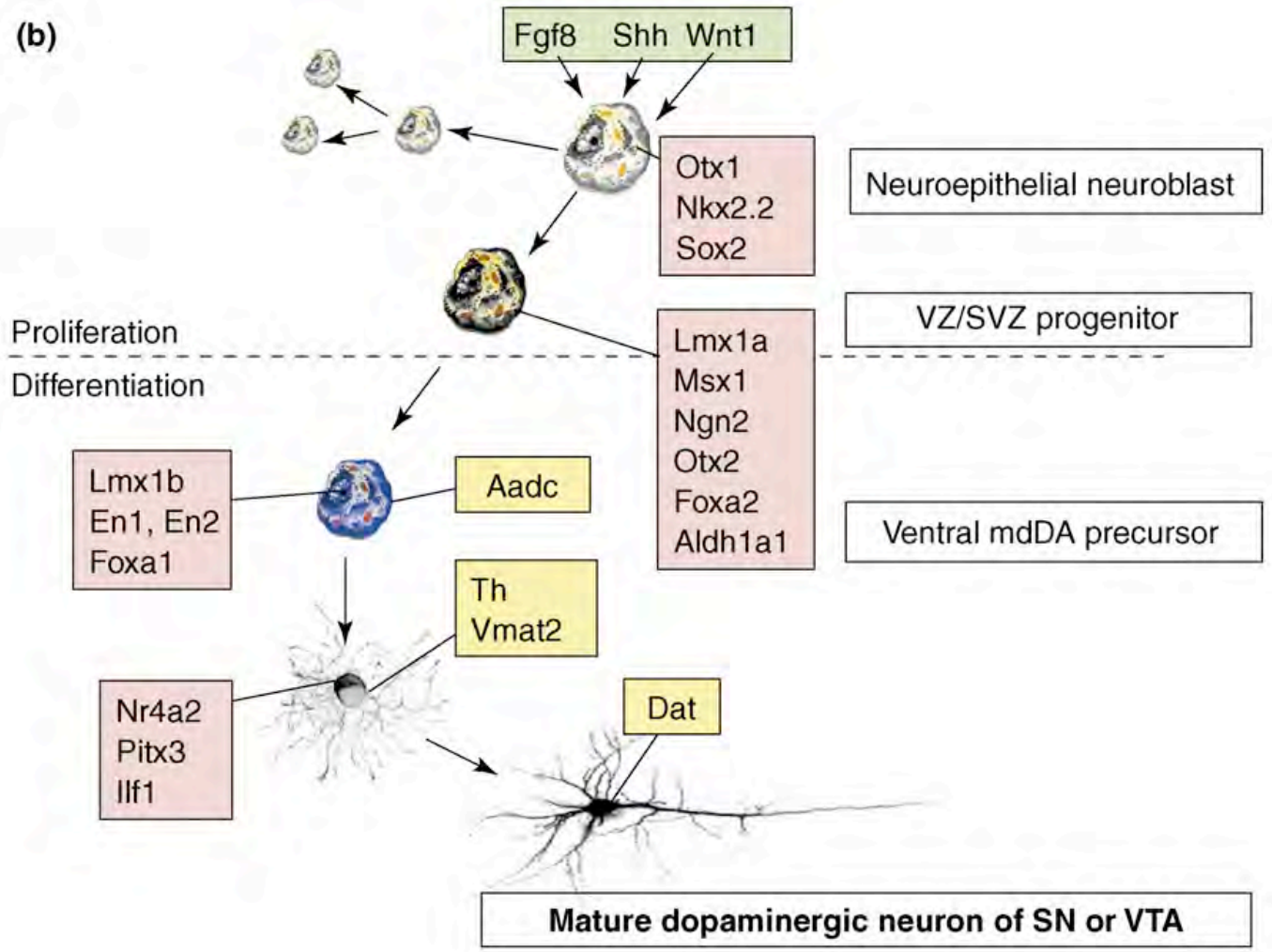
DV Patterning in Neural Tube and Mouse ES cells– See Wichterle and Jessell



Directed Differentiation Screens: Progression from ES Cell to Target Cell

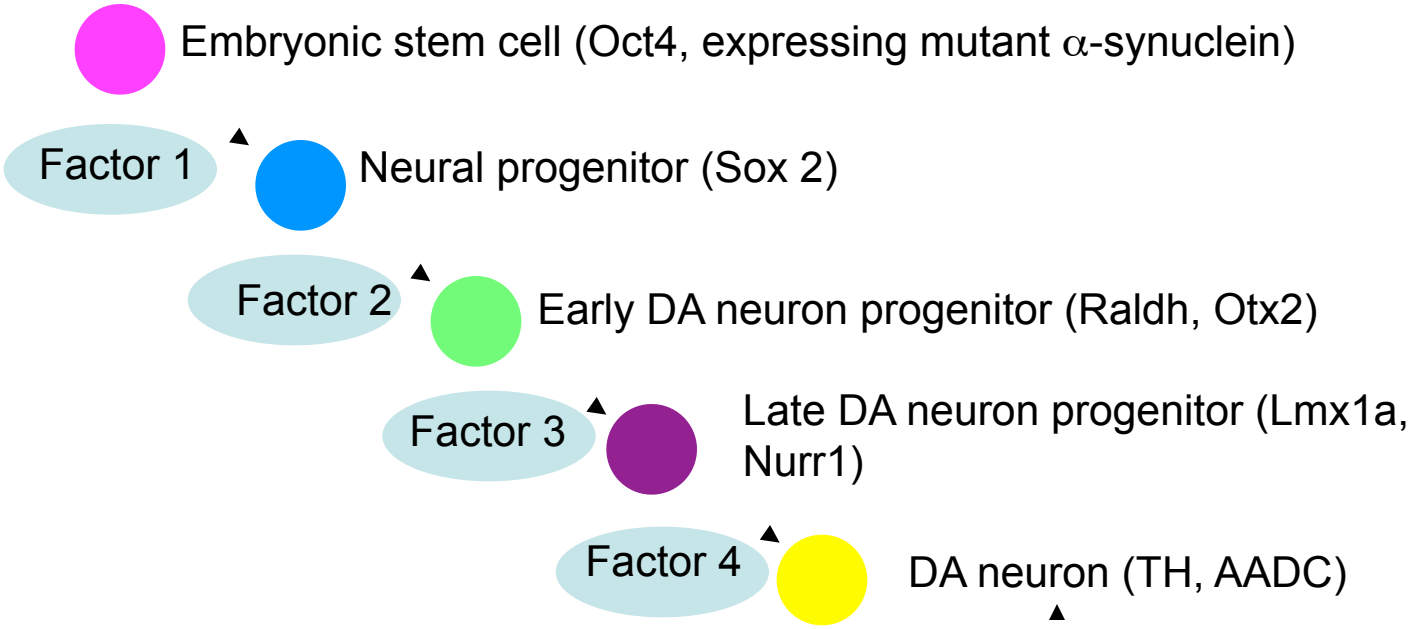


Molecular Coding for the Specification of midbrain DA neurons



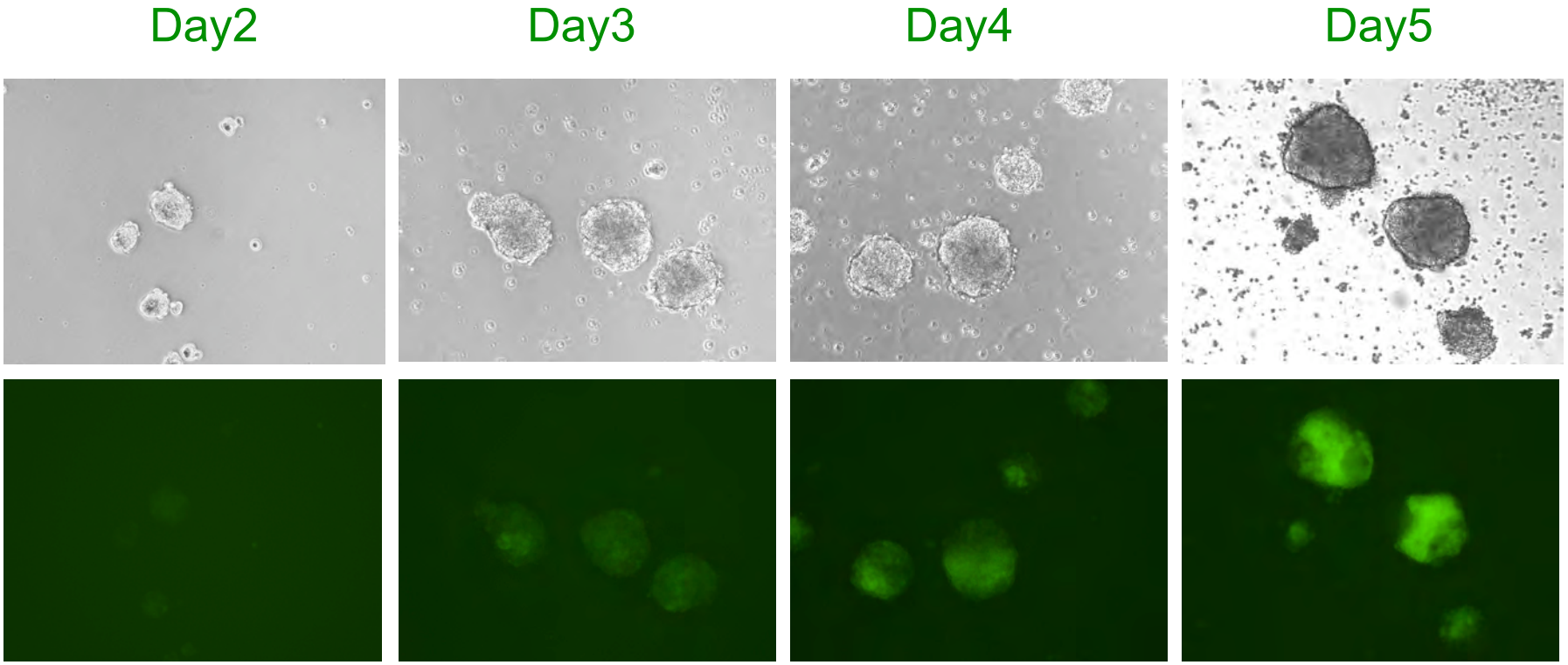
Burbach and Smidts *Trends Neurosci* 2006

Progressive Differentiation from ES Cell to Dopaminergic Neuron (Parkinson's Disease)

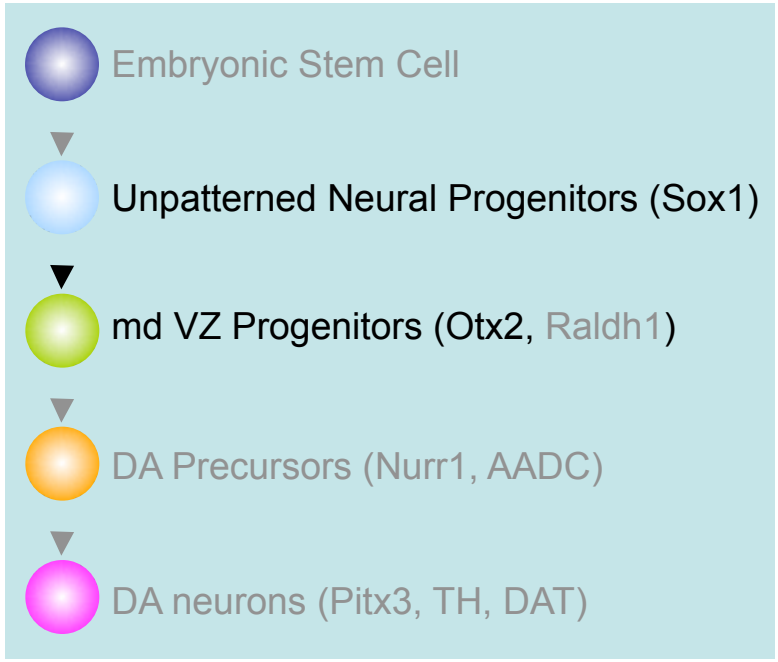


Survival, aggregation screens

Differentiation of Mouse ES cells to Neural Stem Cells in Serum-Free Medium

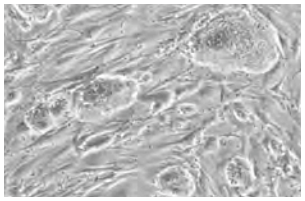


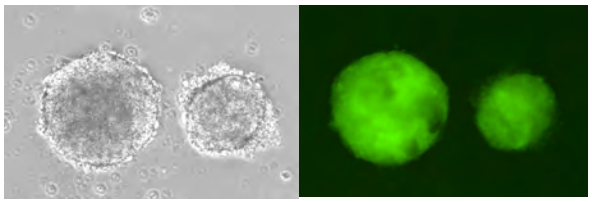
Stage 2 Differentiation Screen

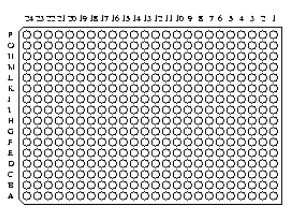


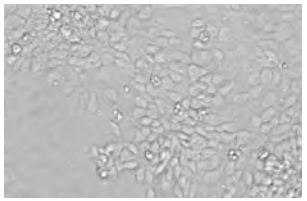
1. Generation of neuronal progenitors from ES cells.
2. **Run differentiation screen from neural progenitors to DA neural progenitor.**
3. Run differentiation screen from early DA neural progenitors to DA precursors.
4. Run differentiation screen from DA precursors to DA neurons.

Design of Otx2 Screen









46C
ESC

EB Day0

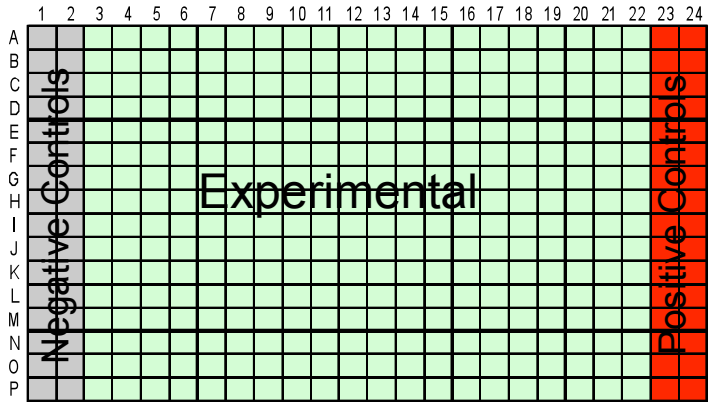
EB Day6
Dissociate/
384 well plate

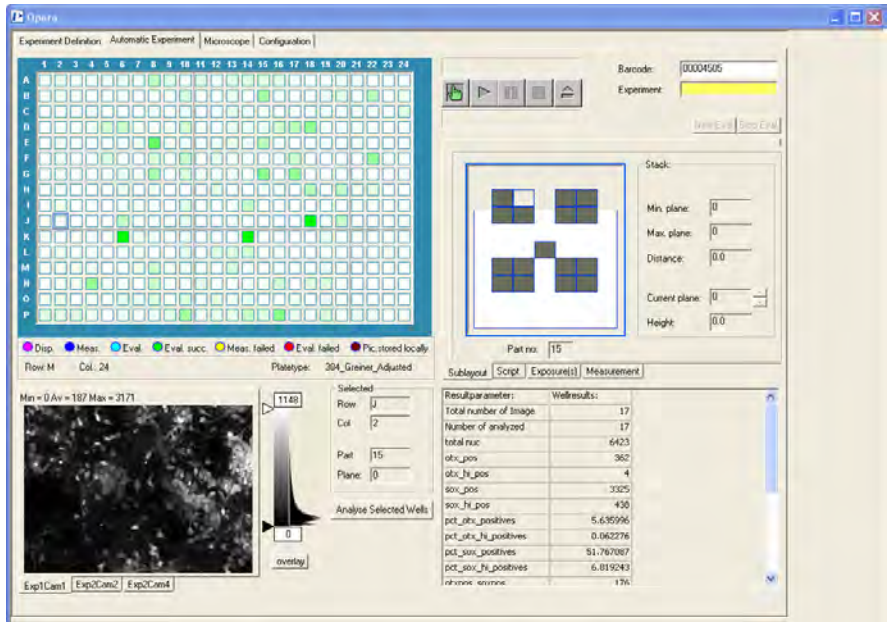
Day1
Compound
Addition

Day X
Fix &
Stain

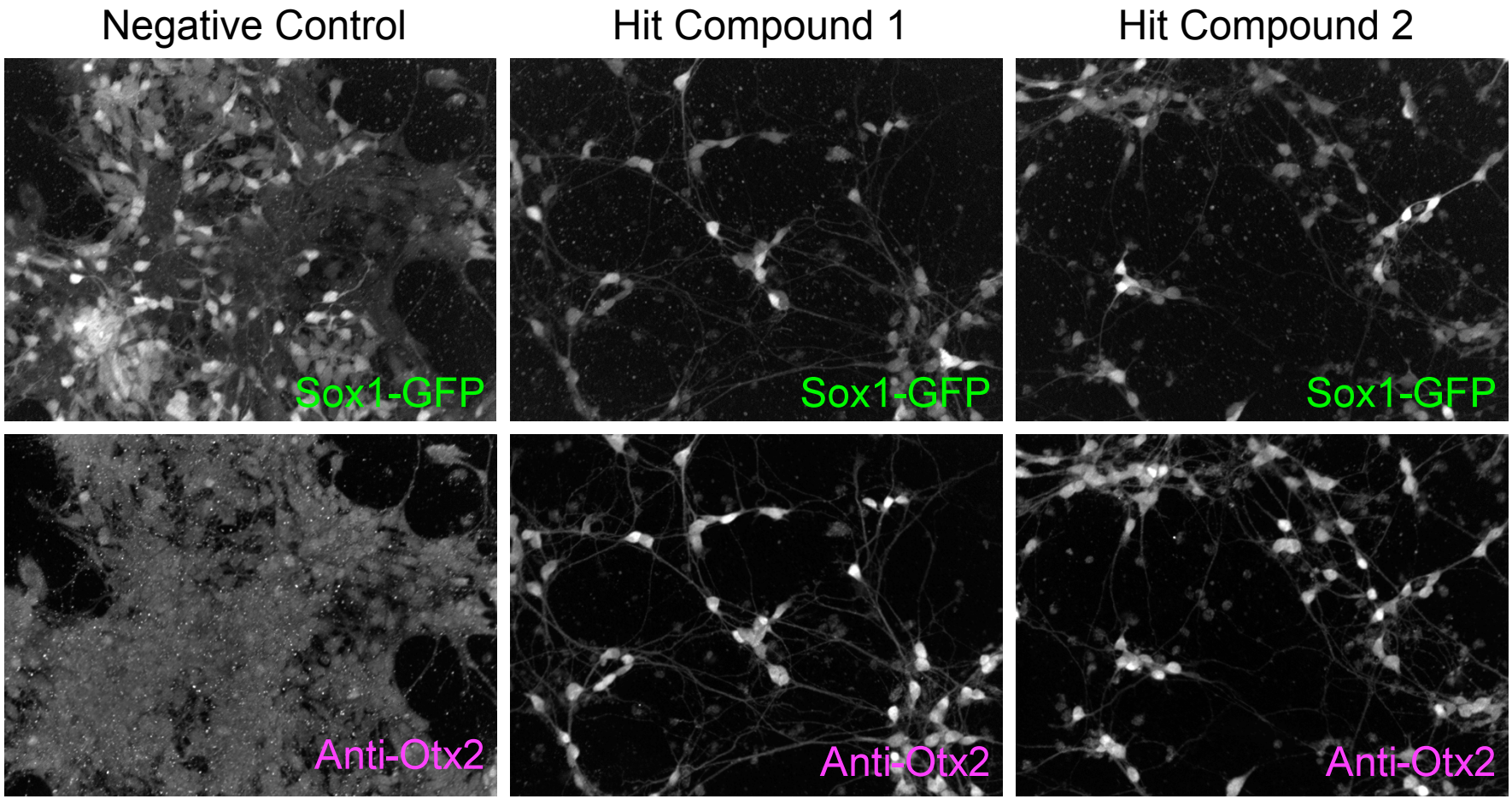
Evotec
Opera
Scan &
Analysis

Screening Flow





Otx2 Primary Screening Results: What Are Hits?

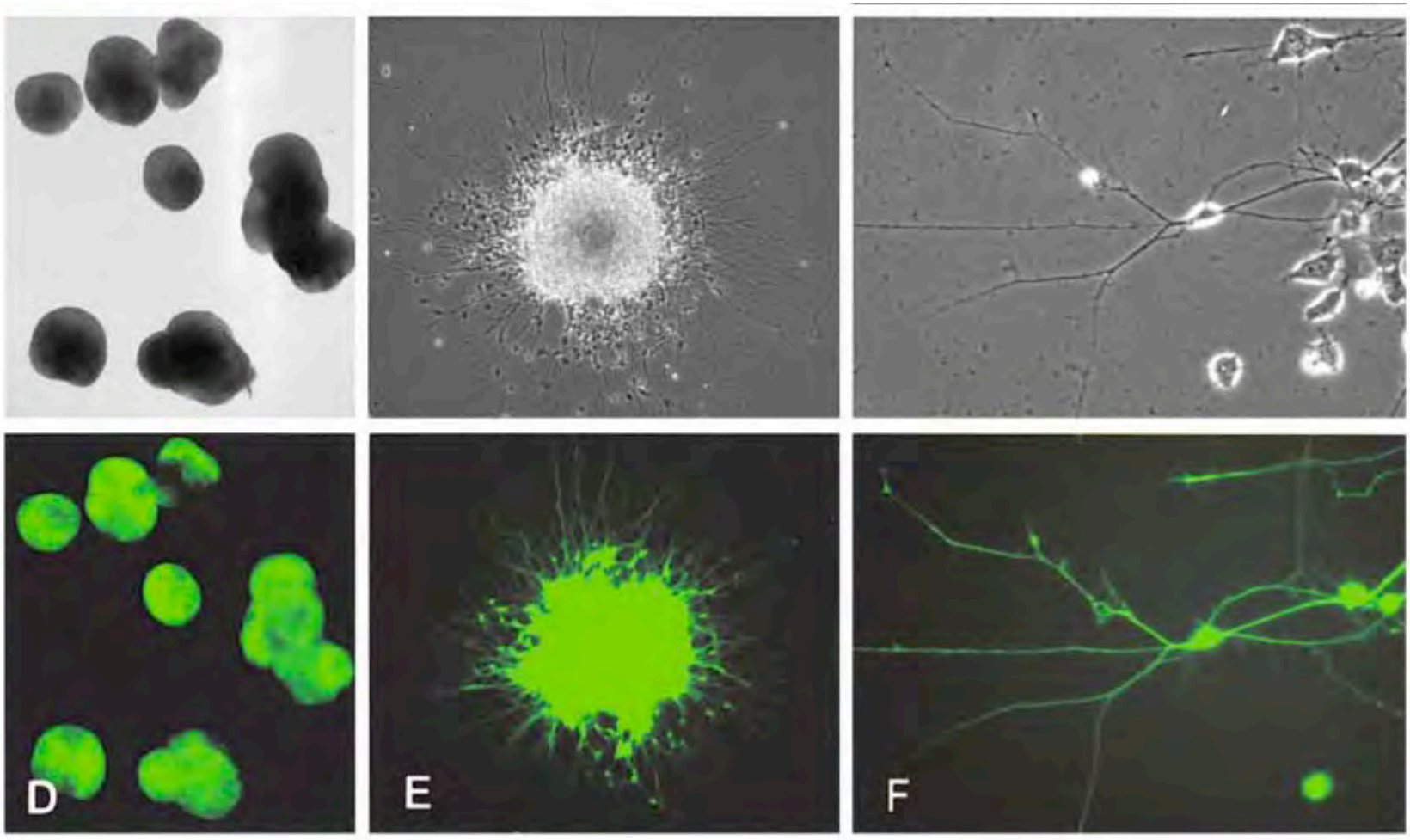


Starting with ES cells, it should be possible to make large numbers of differentiated cells.

Why might this be useful?

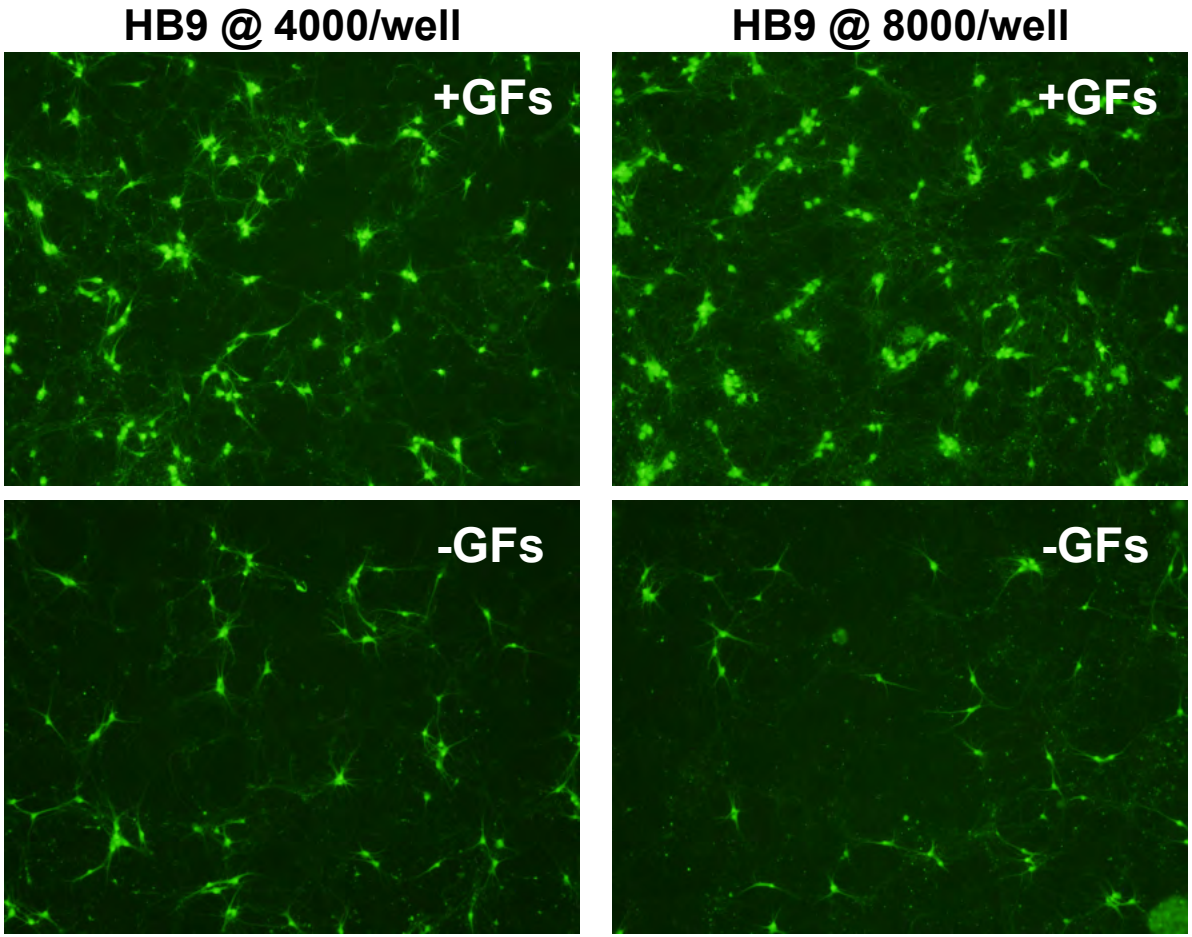
- ES cells can be derived from mice that model different human diseases (or by reprogramming from humans with motor neuron disease)
- For example, motor neurons produced from ES cells can be:
  - Studied to provide added insight into the causes of motor neuron diseases.
  - Used to set up screens to look for therapeutics.

Small Molecule Hh Agonists Induce Motor Neuron Differentiation from Mouse ES Cells

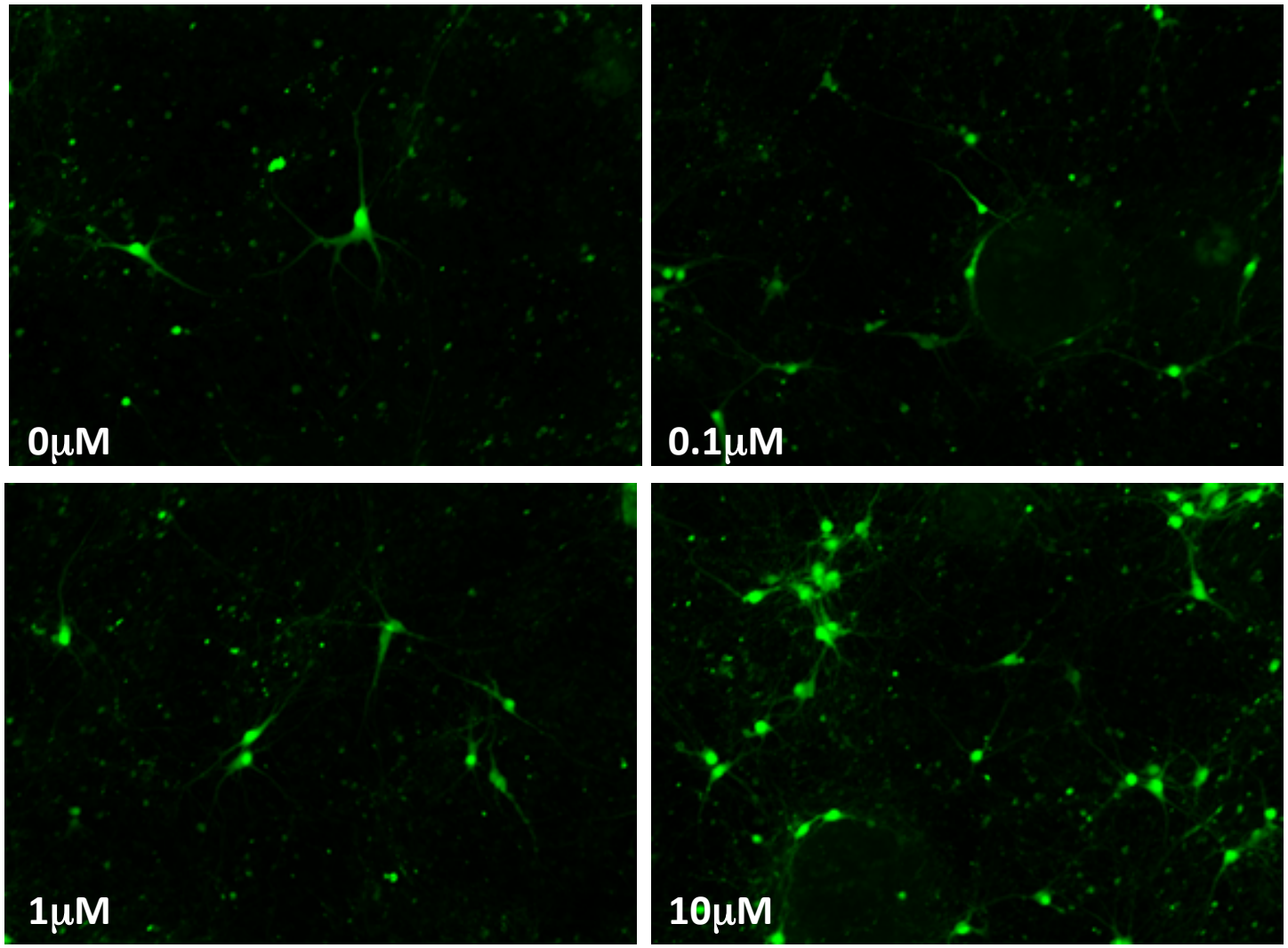


Wichterle et al., Cell, 2002

Motor Neuron Survival Assay



A Cannabinoid Receptor Agonist Inhibits the Death of Motor Neurons



M. Yang, H. Ngo, K. Rosowski

Diphenyleneiodonium Chloride, A Nitric Oxide Synthase (NOS) Inhibitor, Inhibits the Death of Both Types of Motor Neurons