Selecting iPSCs Starting Material for Regenerative Medicine Clinical Applications

Steve Geelhood, M.S. Ch.E.

pluristyx®

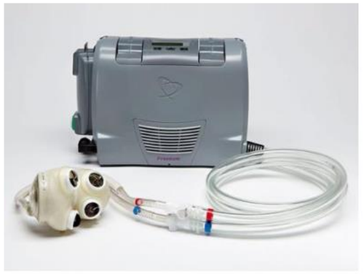
Tomorrow's Cell Therapies, Today®

Topics

1. The Stem Cell Opportunity
2. iPSC Source and Reprogramming
3. Standardized Cell Banking
4. Gene Editing
5. Common Differentiation Struggles

pluristyx®

Stem Cells in Cell Therapy

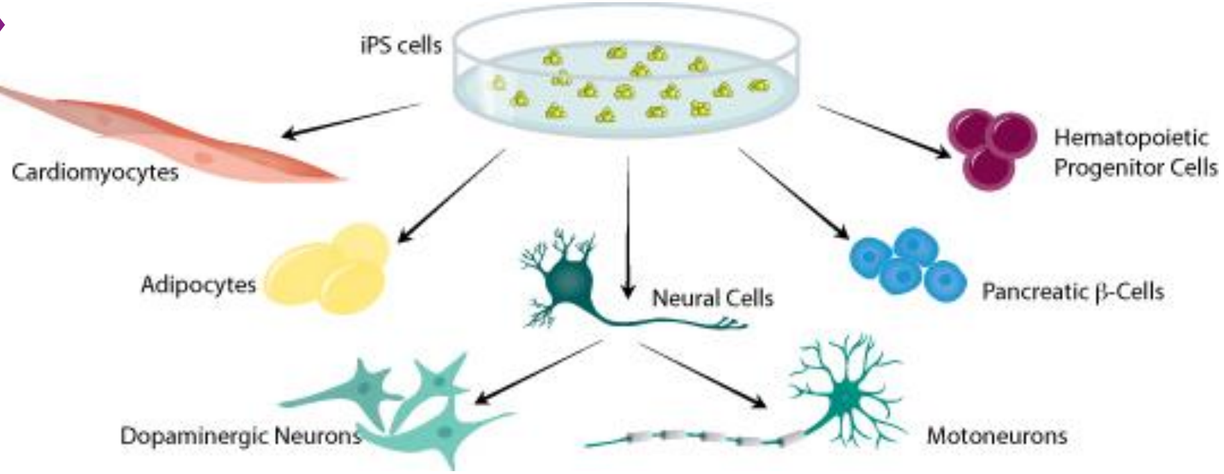


Conventional therapeutic approaches limited by:

- Cost and resource availability
- Efficacy
- Immune rejection

Stem Cells offer ability to make any cell or tissue in the body

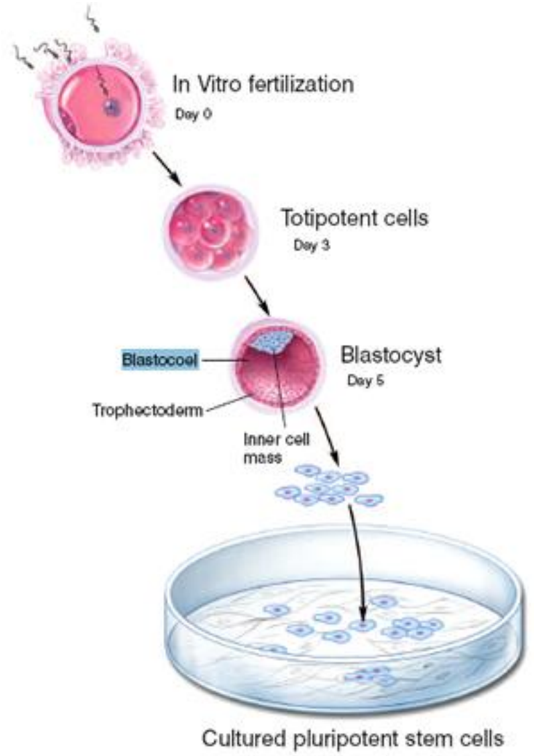
- Off the shelf
- Universal Starting Material
- Sustainable supply



pluristyx®

Stem Cell Sources

Embryonic Stem Cells

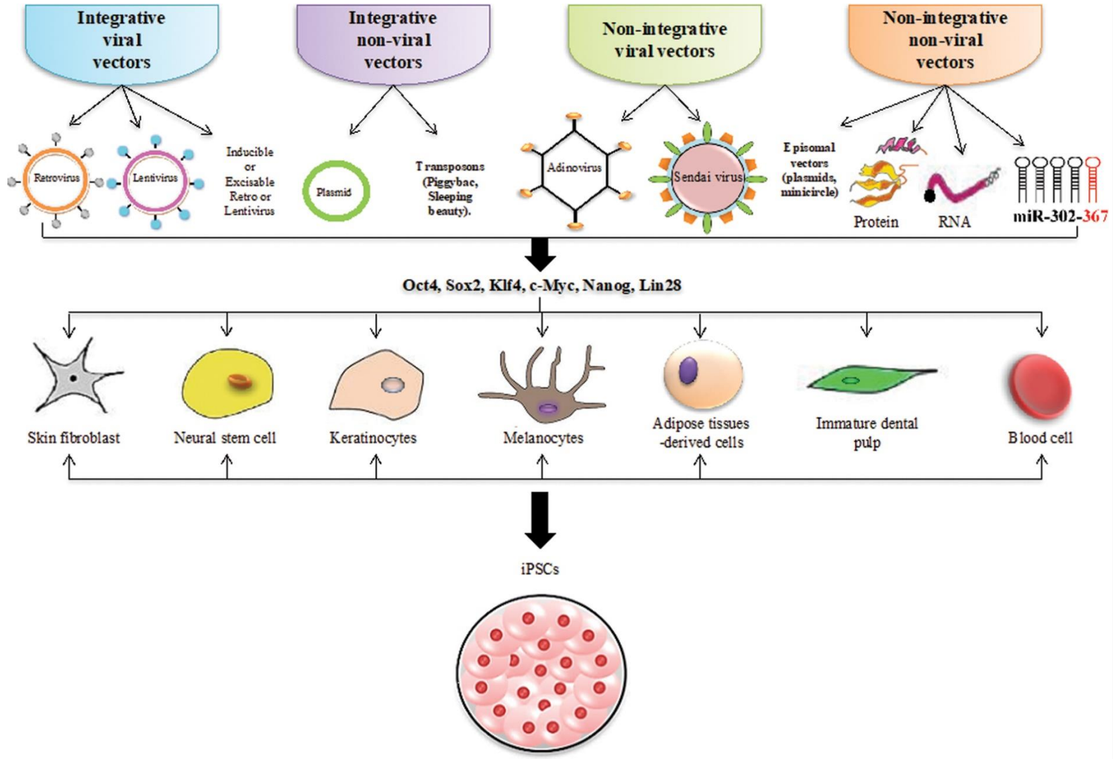


https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter1.htm

Polyclonal

Derivation:
< 10 passages
Or
40 population Doublings

Induced Pluripotent Stem Cells



https://www.liebertpub.com/doi/full/10.1089/biores.2019.0046

Clonal

Derivation:
≥ 20 passages
Or
80 Population Doublings

pluristyx®

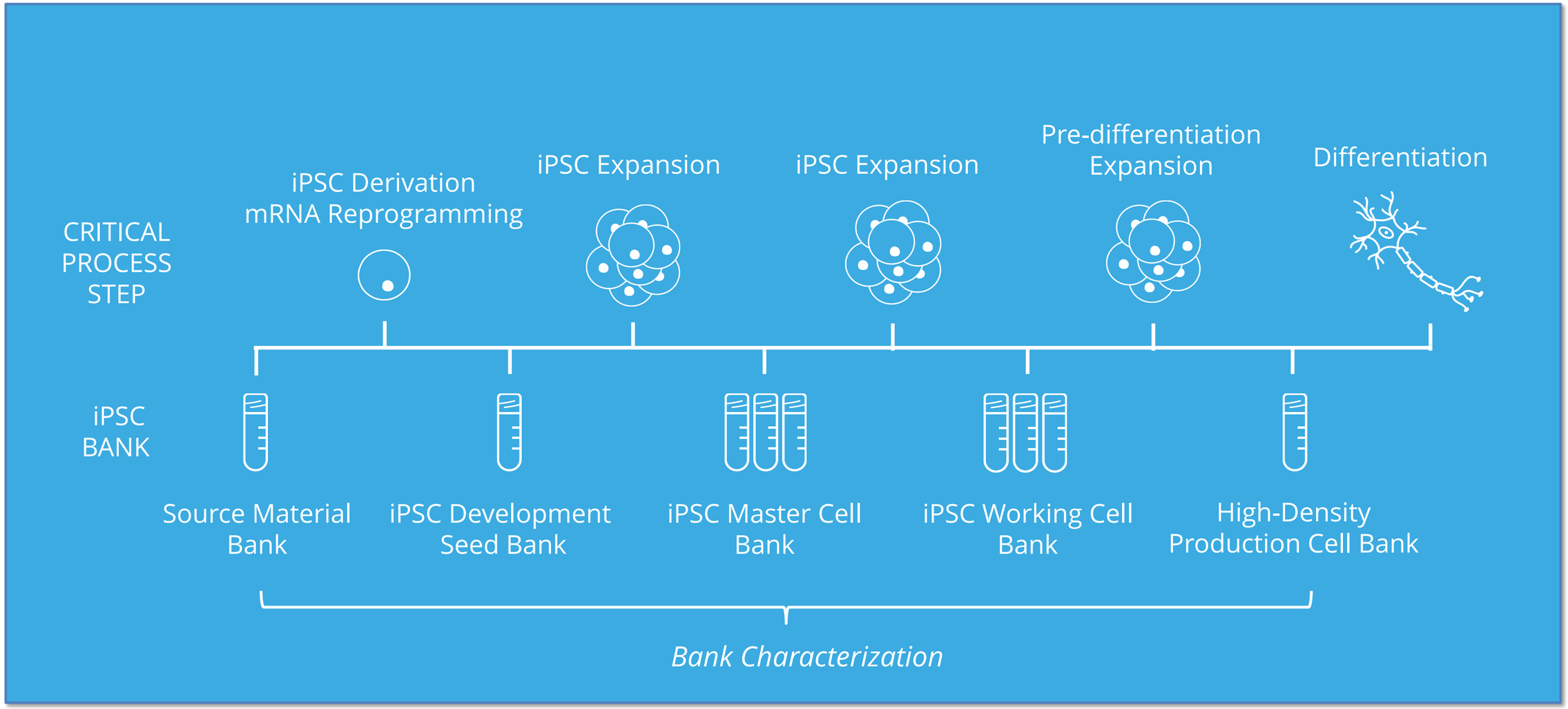
Pluristyx iPSCs

	Embryonic Stem Cells	Conventional iPSCs	Pluristyx iPSCs
Source Materials	Starting material ethical issues	**Informed Consented Donors**	**Informed Consented Donors**
Reprogramming Method	Blastocyst inner cell mass isolation	Lentivirus, DNA, Sendi, Episomal, RNA, Chemical	**Stemloop RNA**
Clonality	**Polyclonal**	Clonal	**Polyclonal**
Passage number	**< 10**	≥ 20	**10 for Master Cell Bank**

- ✓ Genetically Stabile
- ✓ Supported by Drug Master File
- ✓ Single License for Commercialization

pluristyx®

Pluristyx iPSC Banking Workflow

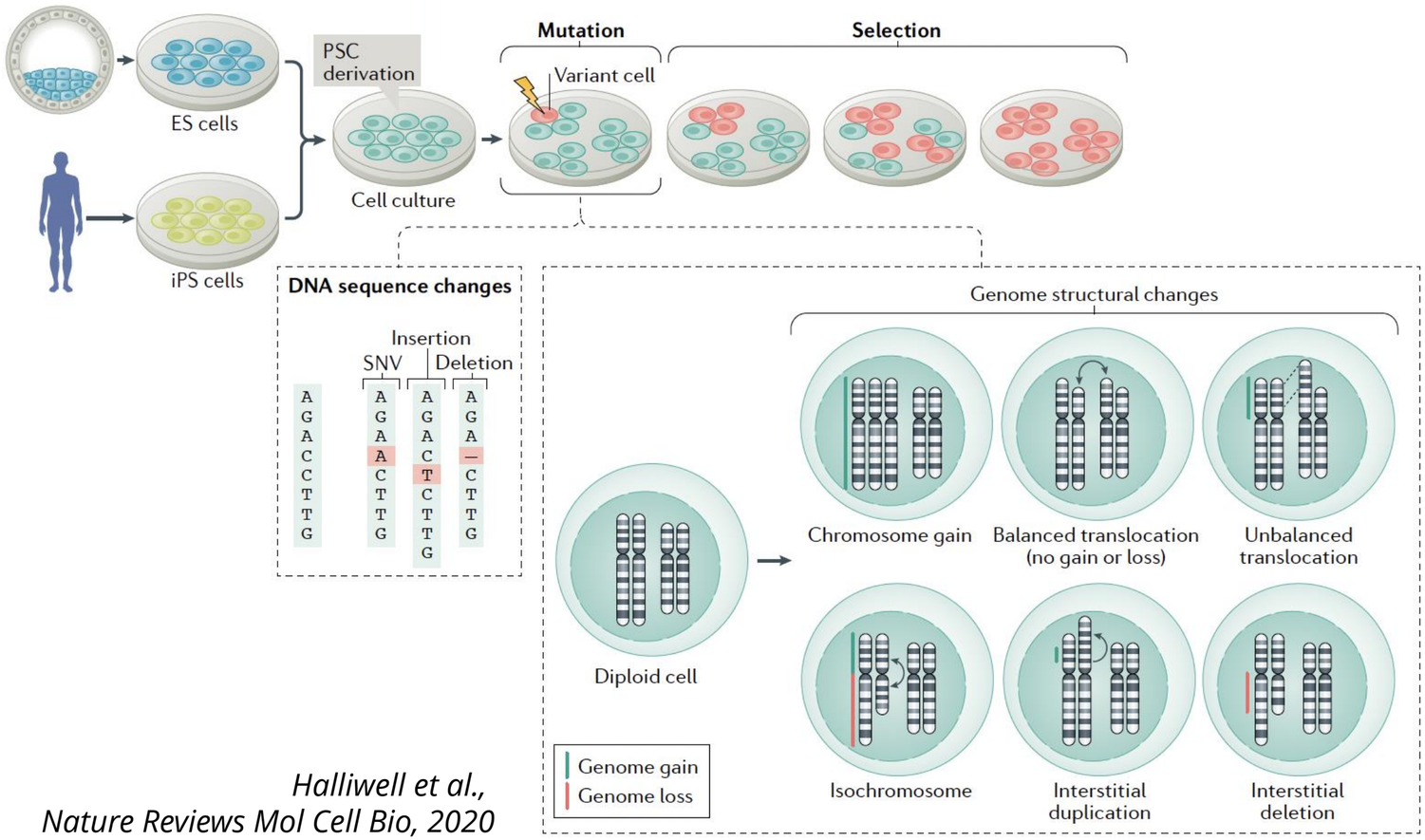


pluristyx®

Types of PSC-Acquired Mutations

- **DNA Sequence Changes**
  - Single-base changes (SNV)
  - Insertions
  - Deletions
- **Genome Structural Changes**
  - Chromosome gain/loss
  - Translocations
  - Interstitial duplications/deletions

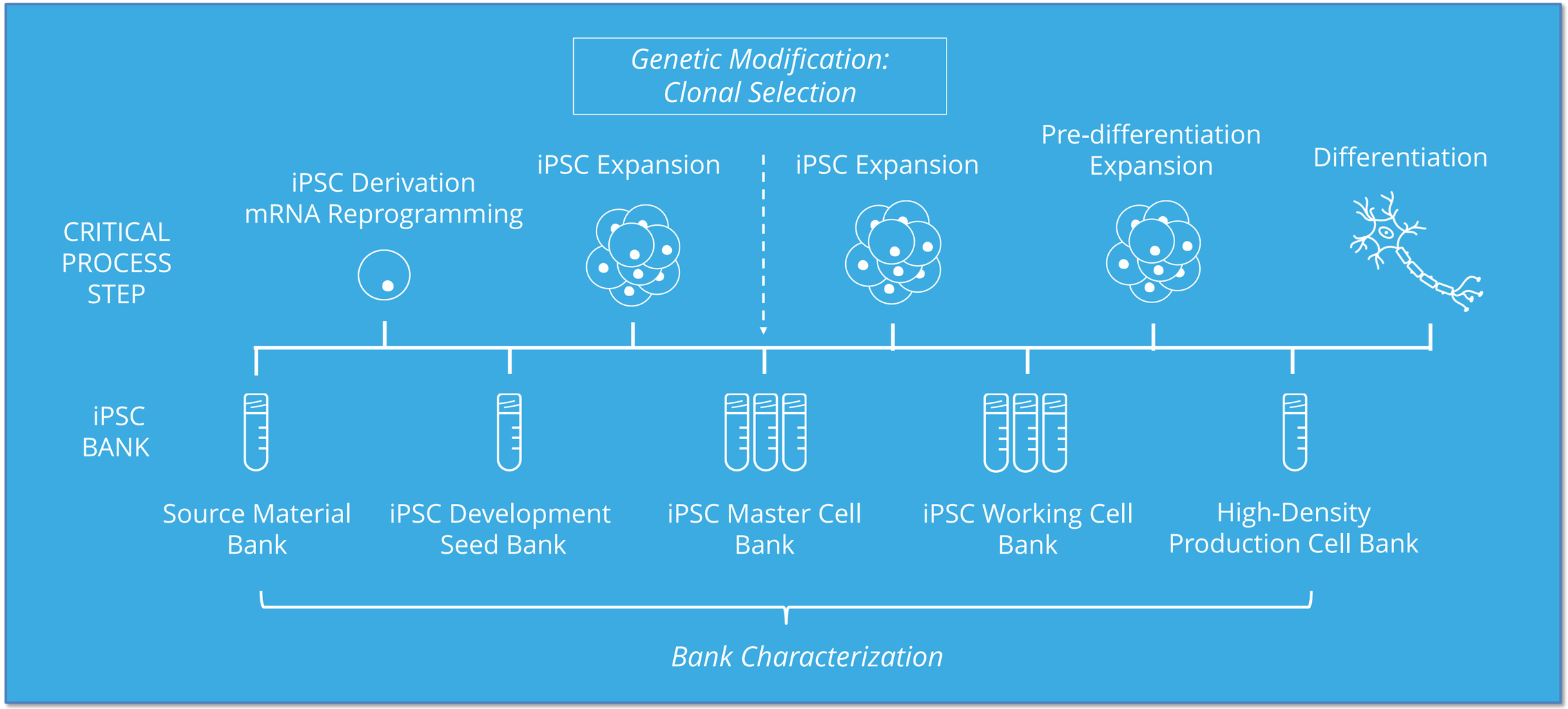
CNV & altered gene expression



Halliwell et al., Nature Reviews Mol Cell Bio, 2020

pluristyx®

Pluristyx iPSC Banking Workflow

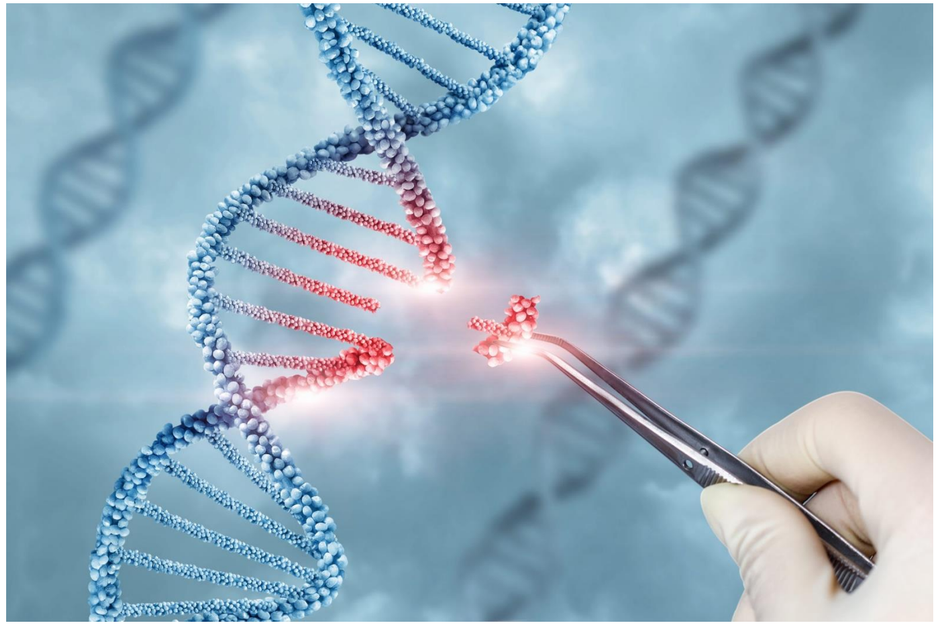


pluristyx®

Gene Editing Opportunity

Edit while cell number is limited then expand

- CRISPR CAS9
- MAD7
- Transposase



Pluristyx Platform Edits

- FailSafe®
- Immune Evasion
  - B2M/CIITA Knock out
  - iACT Stealth Cells™

pluristyx®

A Common Story of Pluripotent Stem Cell Differentiation

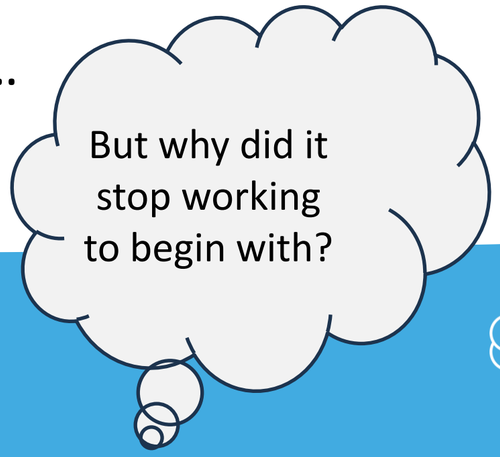
PSC Cell Line “X” differentiates into target cells with “80%” efficiency

Then…

Cell Line X one day stops differentiating as expected….
Cell Line X is genetically modified and stops differentiating as expected…
Cell Line Y is derived and won’t differentiate like Cell Line X does…

The Normal response:

1. Throw everything out, buy fresh reagents, and run the differentiation protocol again
2. Re-optimize the culture conditions: Change matrix, growth medium, passaging agent
3. Give up and try a different cell line…



pluristyx®

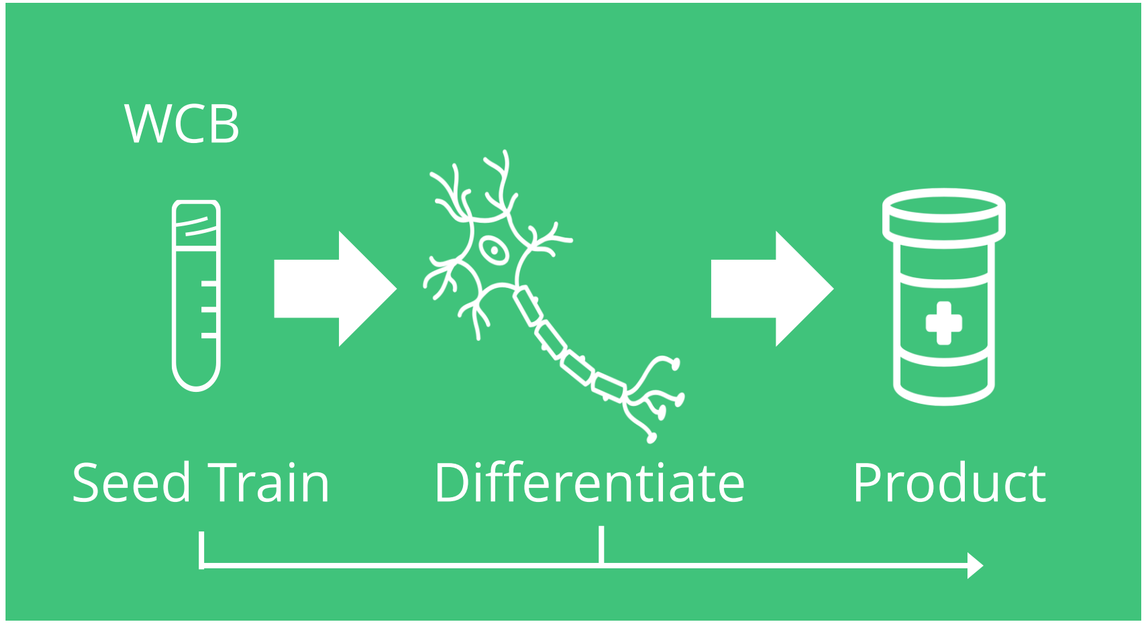
Standardized Differentiation Workflow

- Tight usage window – define passages

- Consistent Bank Process
  - Uniform Timings and Feeding Schedule

- High Quality and Consistent Reagents:
  - Media
  - Matrix
  - Passaging Reagent
  - Cryopreservation

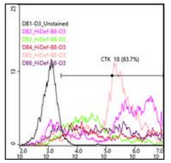
- **Consistent differentiation requires a stable and reproducible expansion process**



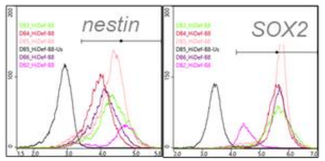
pluristyx®

Differentiation Capacity of Six iPSC Lines Grown in HiDef-B8 for 10 Passages

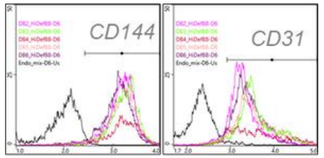
Differentiation



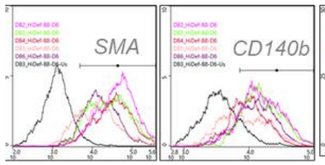
Epithelial



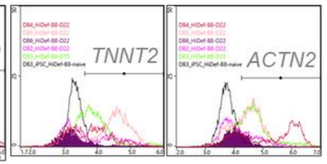
Neuronal



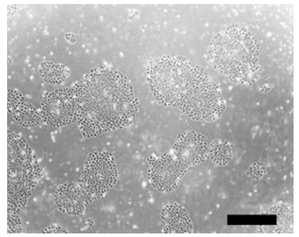
Endothelial

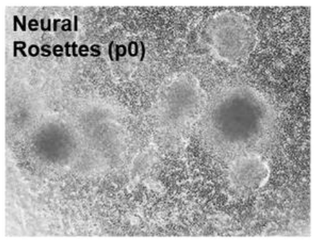


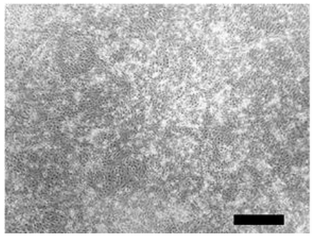
Smooth muscle

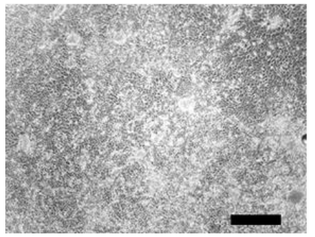


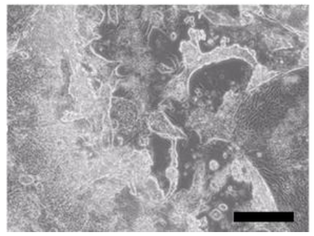
Cardiomyocyte



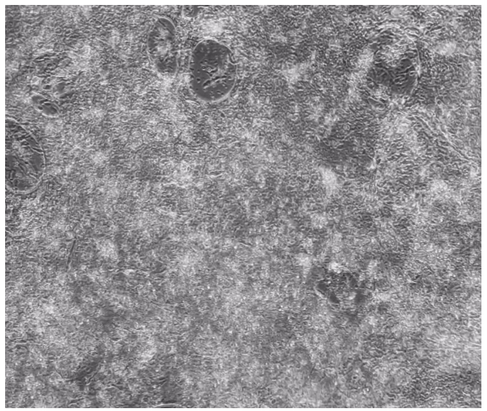




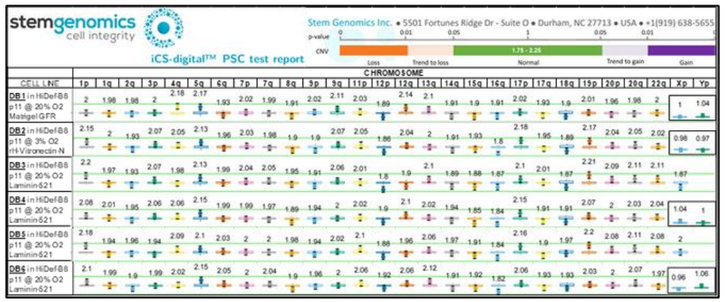




Functional Cardiomyocytes



Genomic Integrity:



Thank you

Email
steve.geelhood@pluristyx.com

Phone
888-588-9935

Website
www.pluristyx.com

LinkedIn
Pluristyx, Inc.

Address
201 Elliott Ave W,
Seattle, WA 98119

pluristyx®
Tomorrow's Cell Therapies, Today®