Isolation and Analysis of Pluripotent, Neural, and Hematopoietic Stem Cells

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BD Biosciences

R&D Scientist

Stem Cell

23-10679-00

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Overview

- Introduction
  - Challenges in stem cell research
  - Antibody portfolio of stem cell markers
- Cell sorting of neural stem cells (NSCs) and neurons
  - BD Lyoplate™ CD screening panel
  - BD FACS™ CAP service
- Cell sorting of human embryonic stem cells (hESCs)
- Flow cytometry kits for pluripotent stem cell research
  - Compensation beads for larger cells and bright markers
- Mouse hematopoietic stem cell (mHSC) isolation
- Overview of stem cell flow kits

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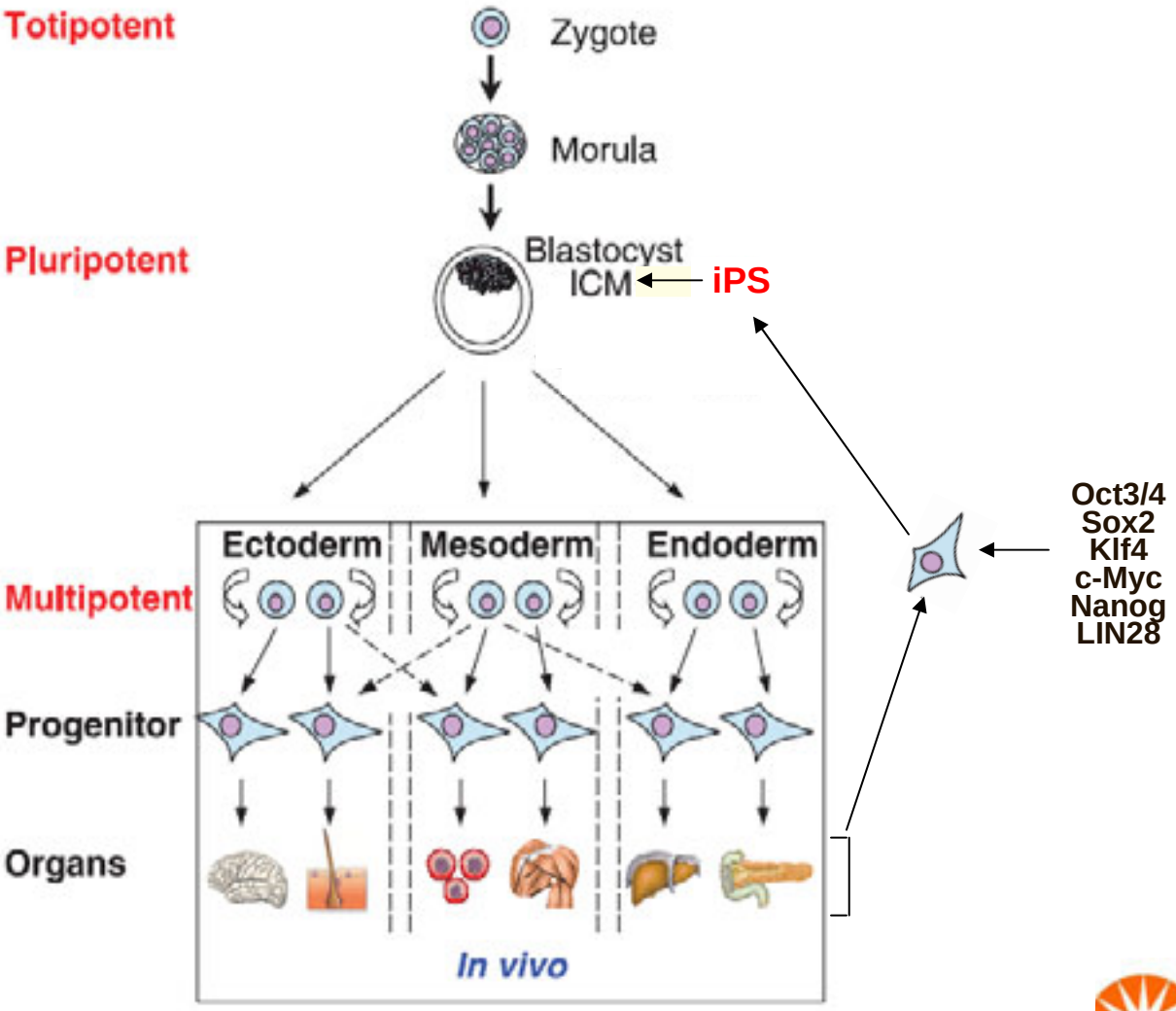
Stem Cell Background

Embryonic

Adult



Schematic adapted from Wobus AM. and Boheler, KR., *Physiol. Rev.2005;* 85:635-678.

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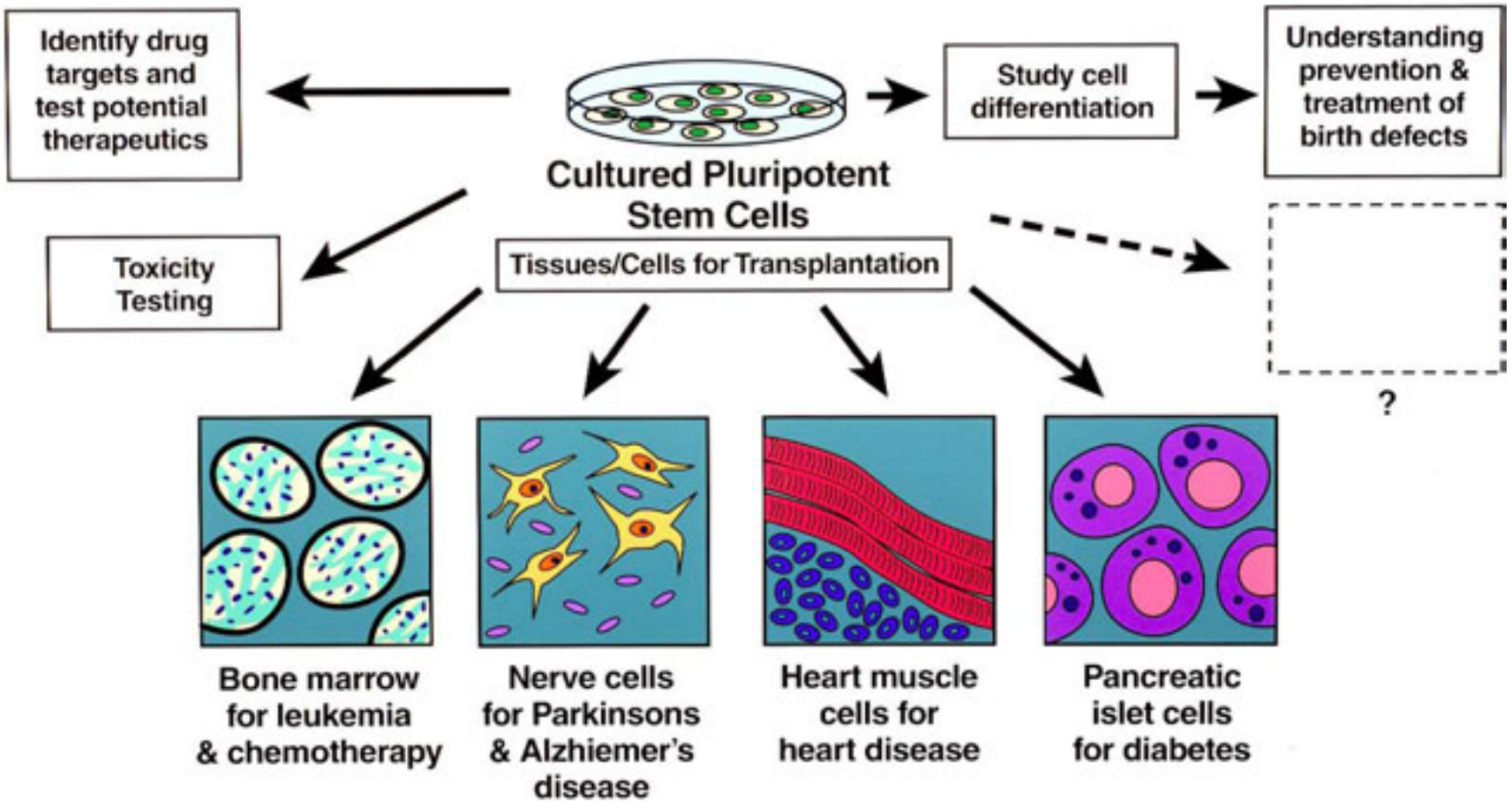
Stem Cell Background



Schematic adapted from http://stemcells.nih.gov/index.asp

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Challenges in Stem Cell Research

- Identify and isolate cells of interest from a heterogeneous pool
- Analyze cells for quality and purity
- Analyze cell function

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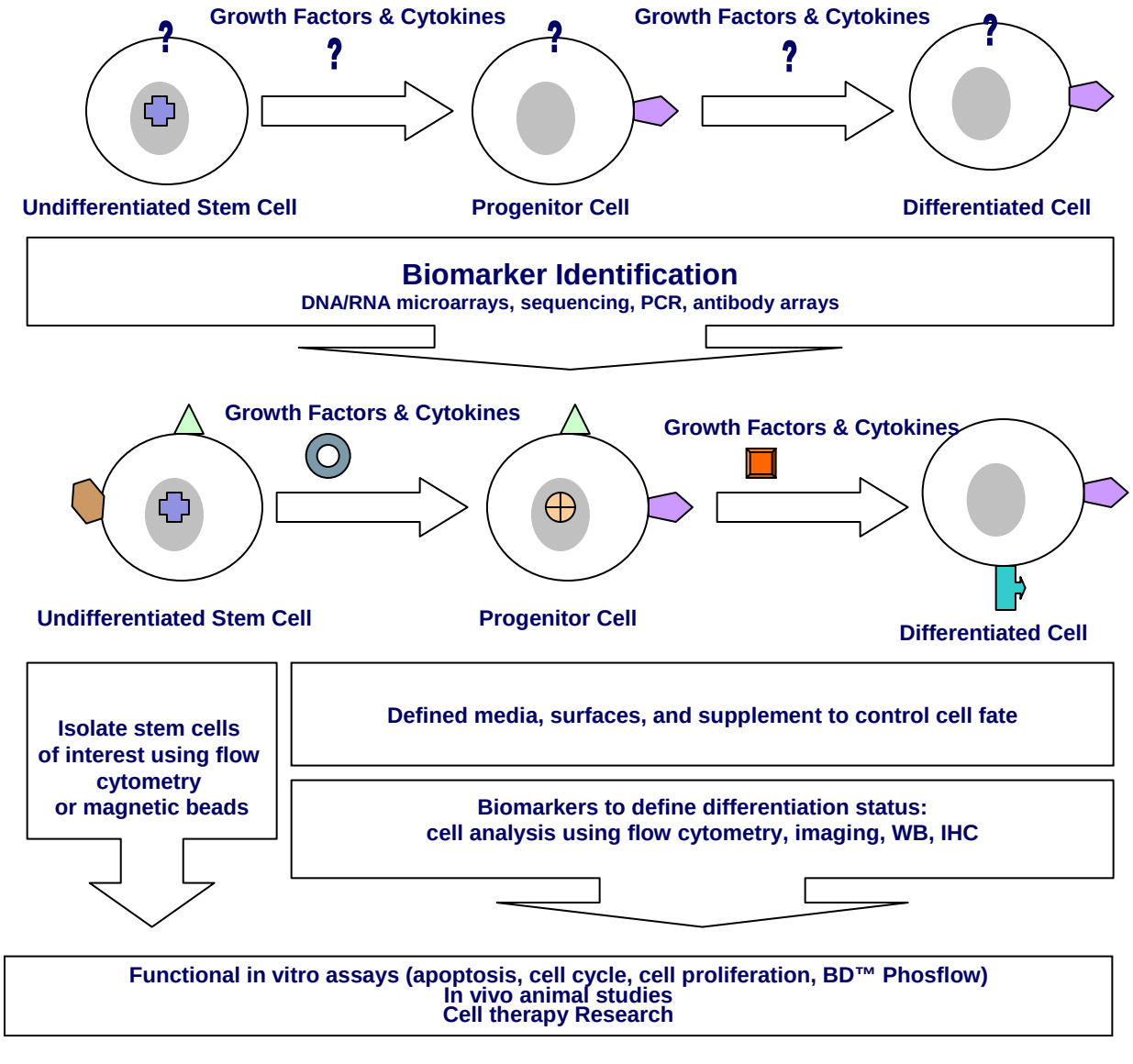
Biomarkers are Crucial for Stem Cell Analysis and Isolation



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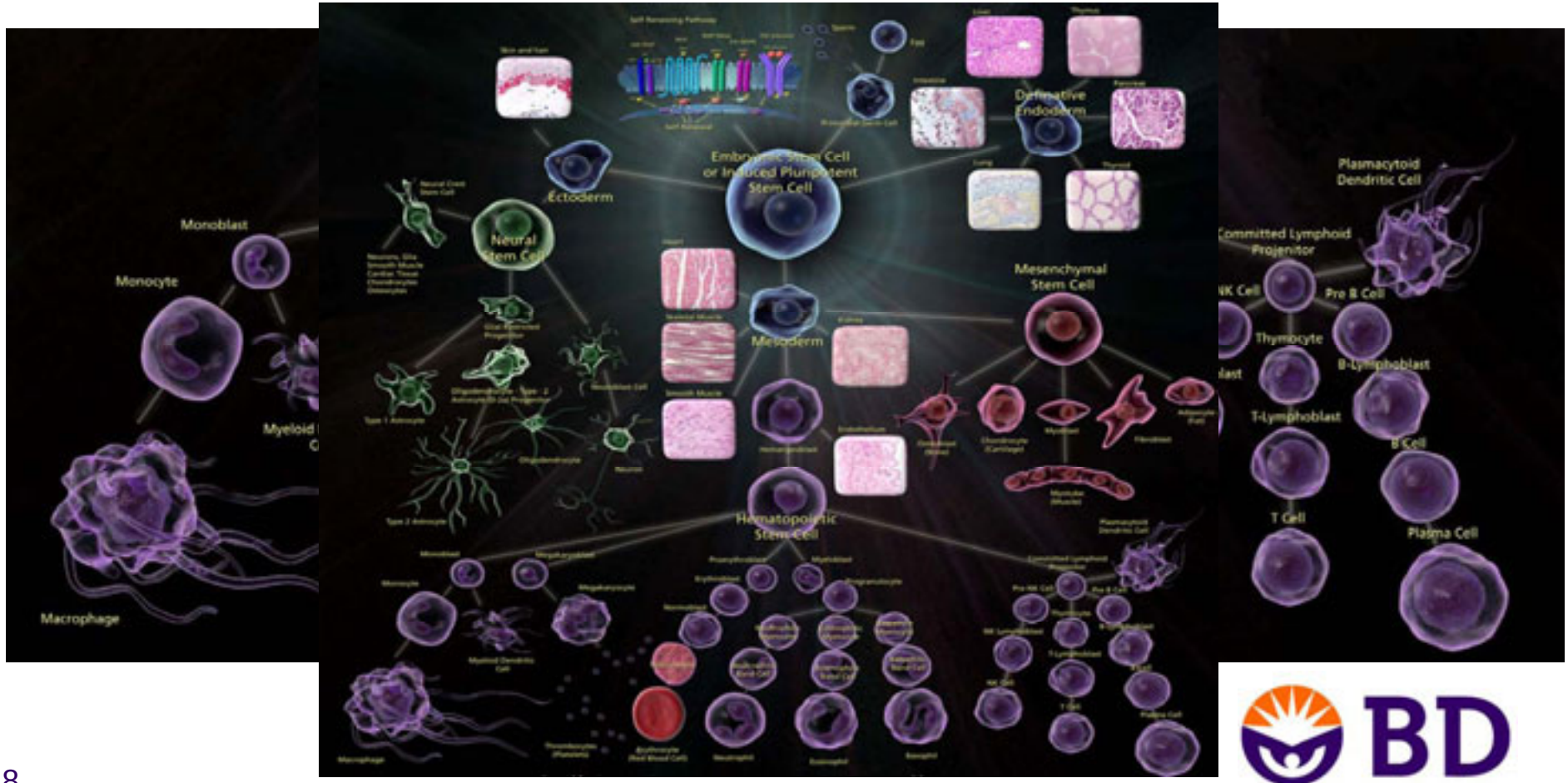
BD Biosciences

First to commercialize tools for isolating and analyzing hematopoietic stem cells, now bringing this expertise to the broader stem cell field



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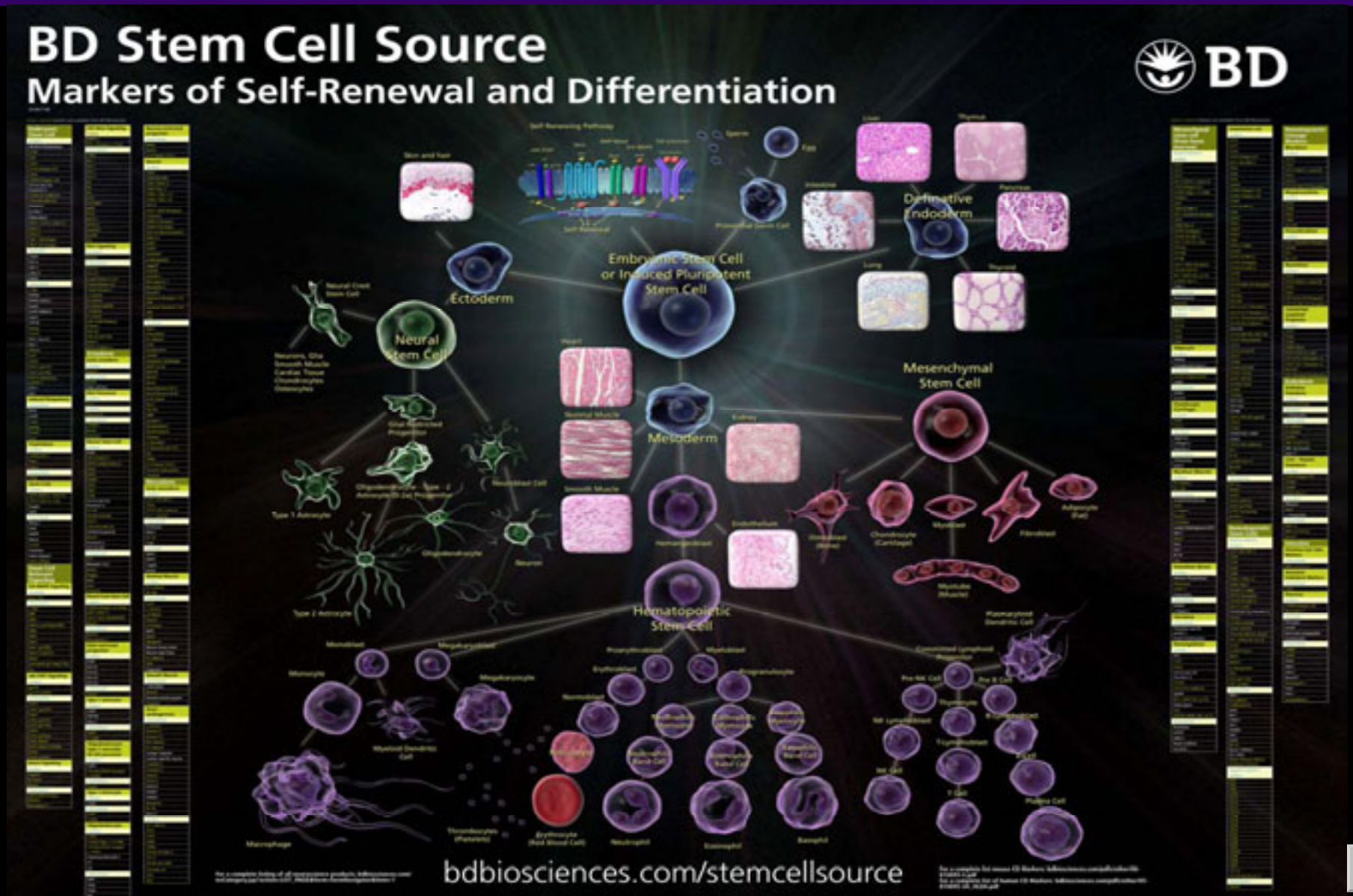
Portfolio of High Quality Antibodies



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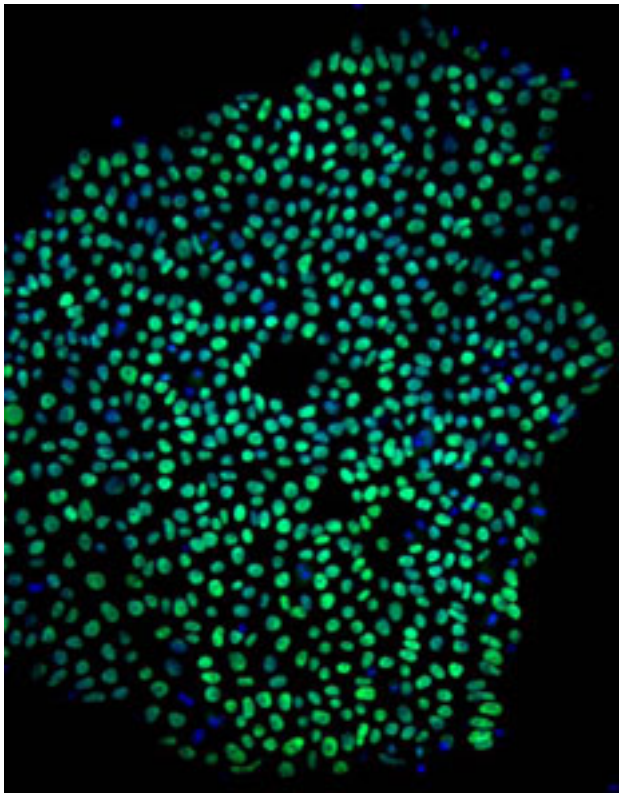
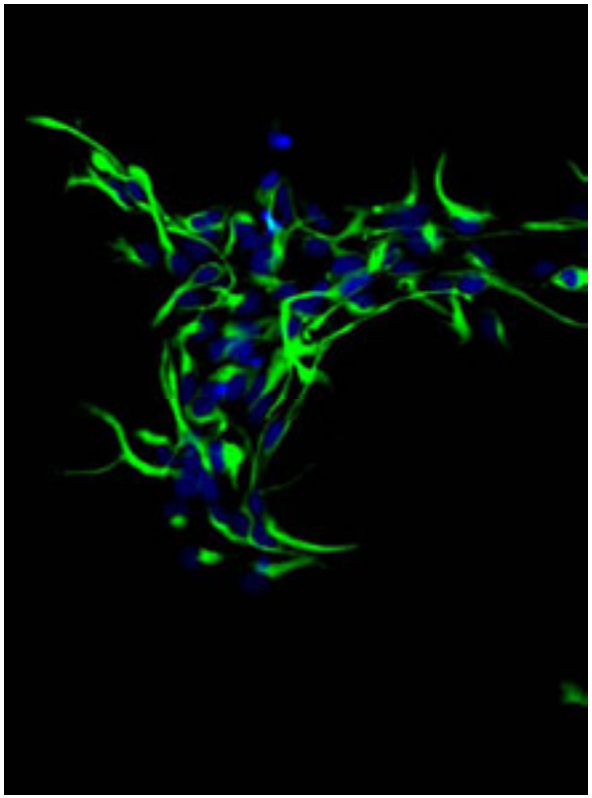
Purified mAbs for Stem Cell Research

human Nanog



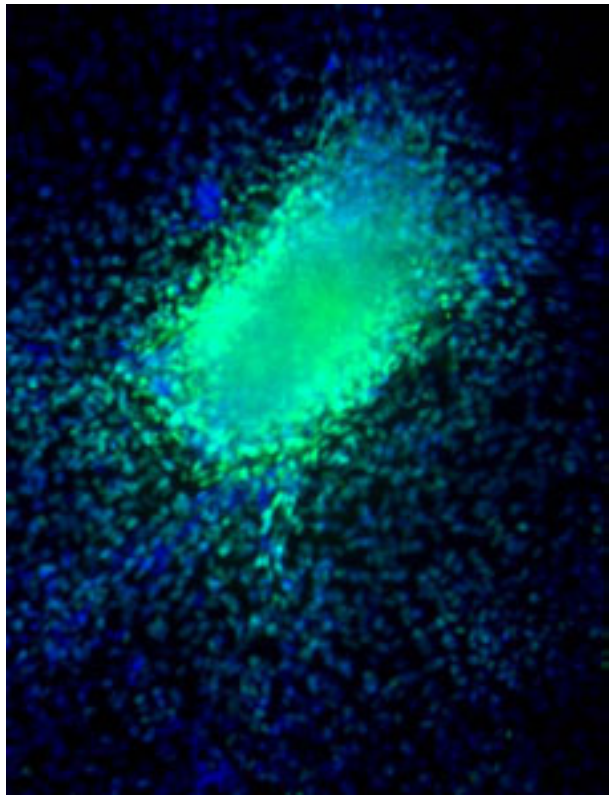
hESC

Nestin



hESC-derived neural stem cells

GATA4



hESC-derived cardiomyocytes

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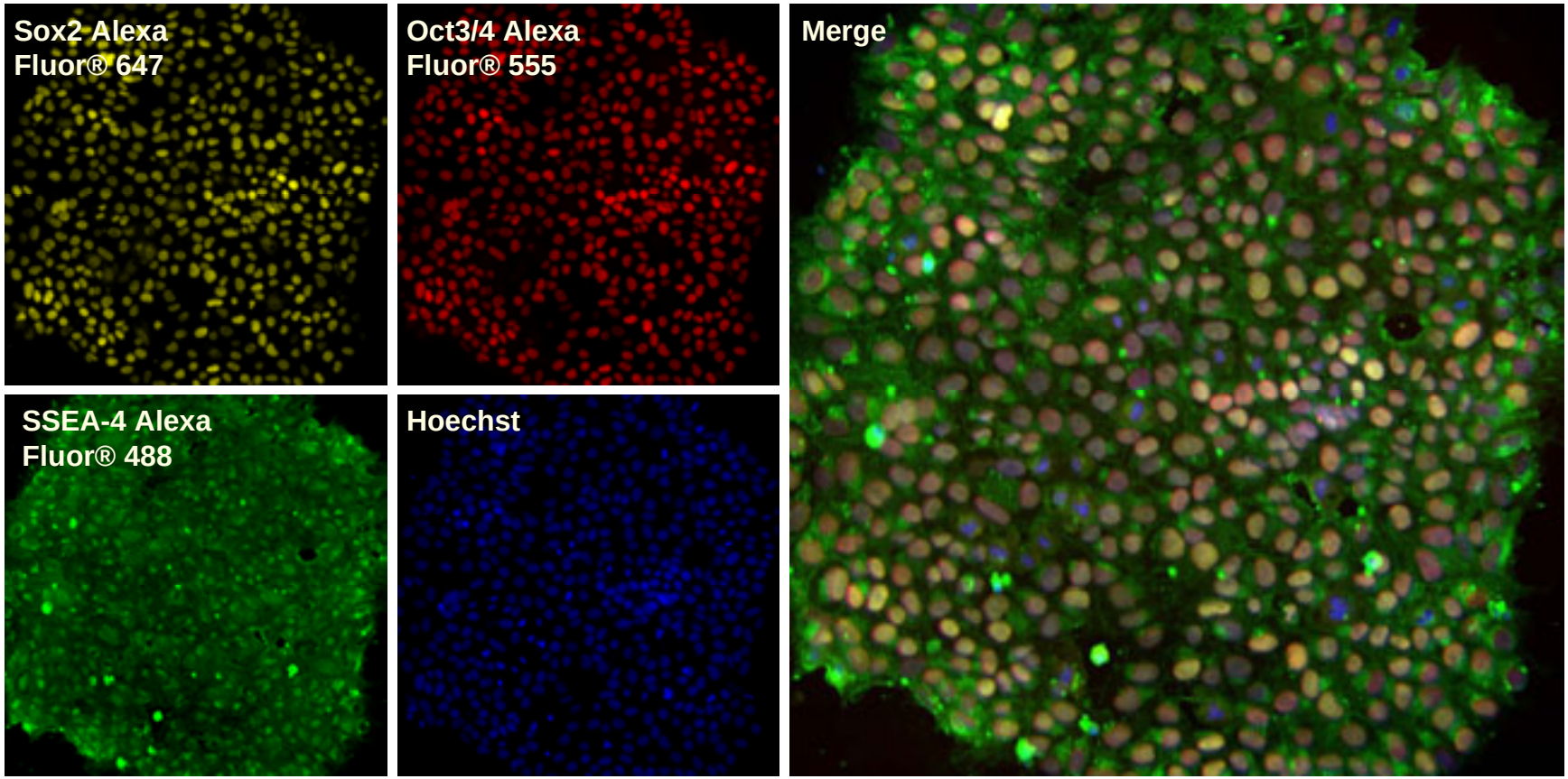
Fluorochrome-Conjugated mAbs for Imaging

H9 grown on BD Matrigel™ hESC-qualified matrix with mTeSR™1



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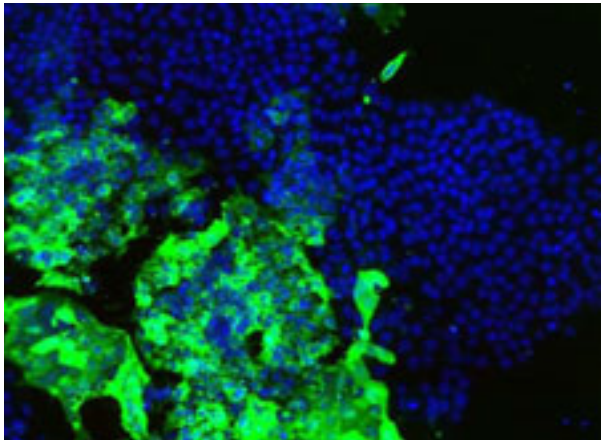
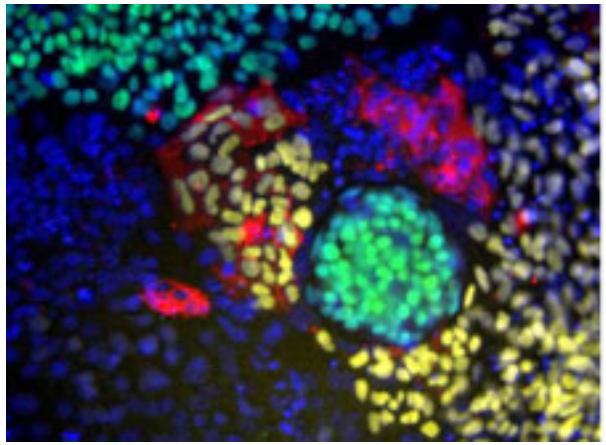
Fluorochrome-Conjugated mAbs for Imaging

Differentiated H9 hESCs

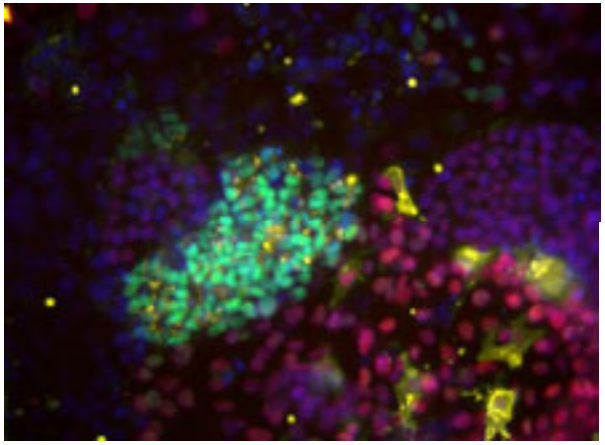
SSEA-1 Hoechst



Cdx2 SSEA-1 Oct3/4 Hoechst



SSEA-1 GATA4 Cdx2 Hoechst



SSEA-1 (CD15): Multi-lineage
Cdx2: Trophectoderm
Oct3/4: Pluripotency
GATA4: Endoderm

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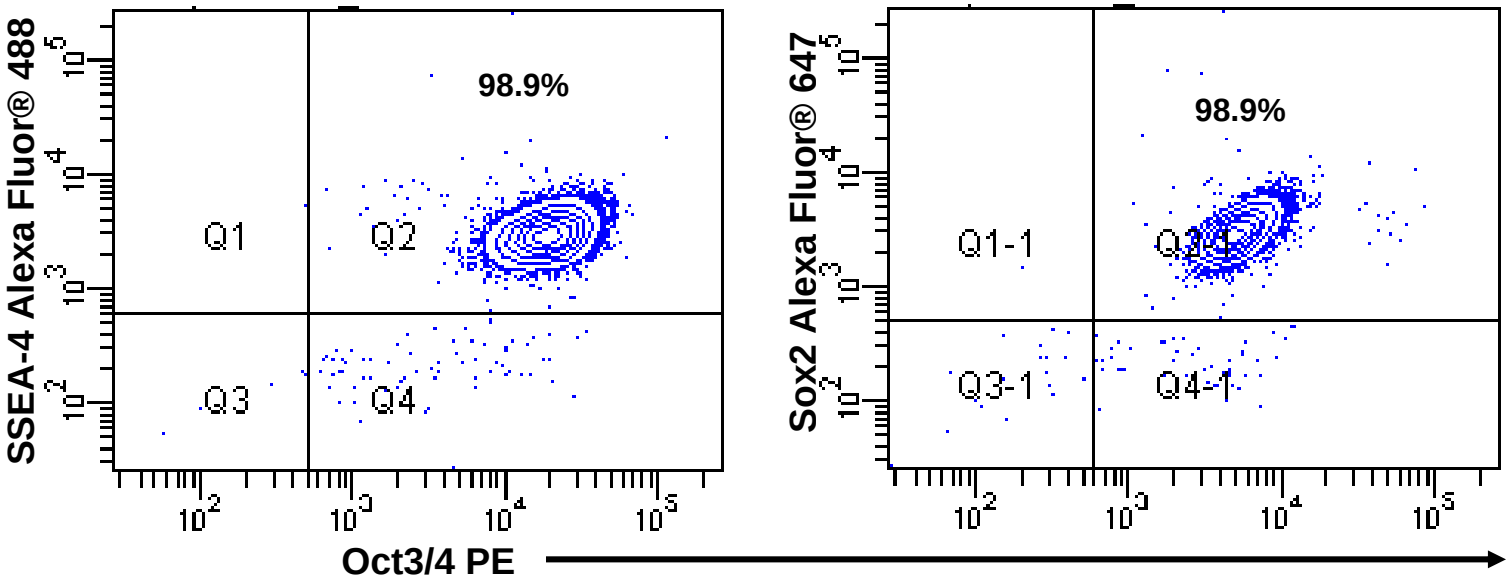
Fluorochrome-conjugated mAbs for Flow Cytometry

H9 hESCs grown on BD Matrigel™ hESC-qualified matrix with mTeSR™1



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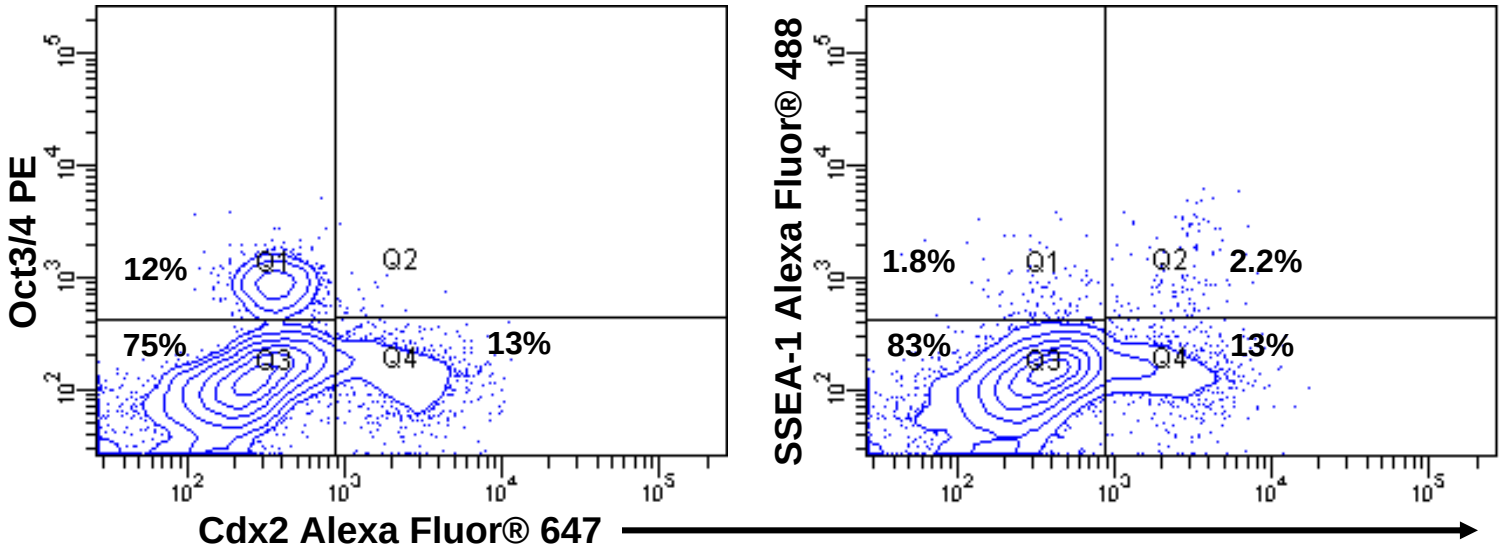
Fluorochrome-conjugated mAbs for Flow Cytometry

Differentiated H9 hESCs



SSEA-1 (CD15): Multi-lineage
Cdx2: Trophectoderm
Oct3/4: Pluripotency

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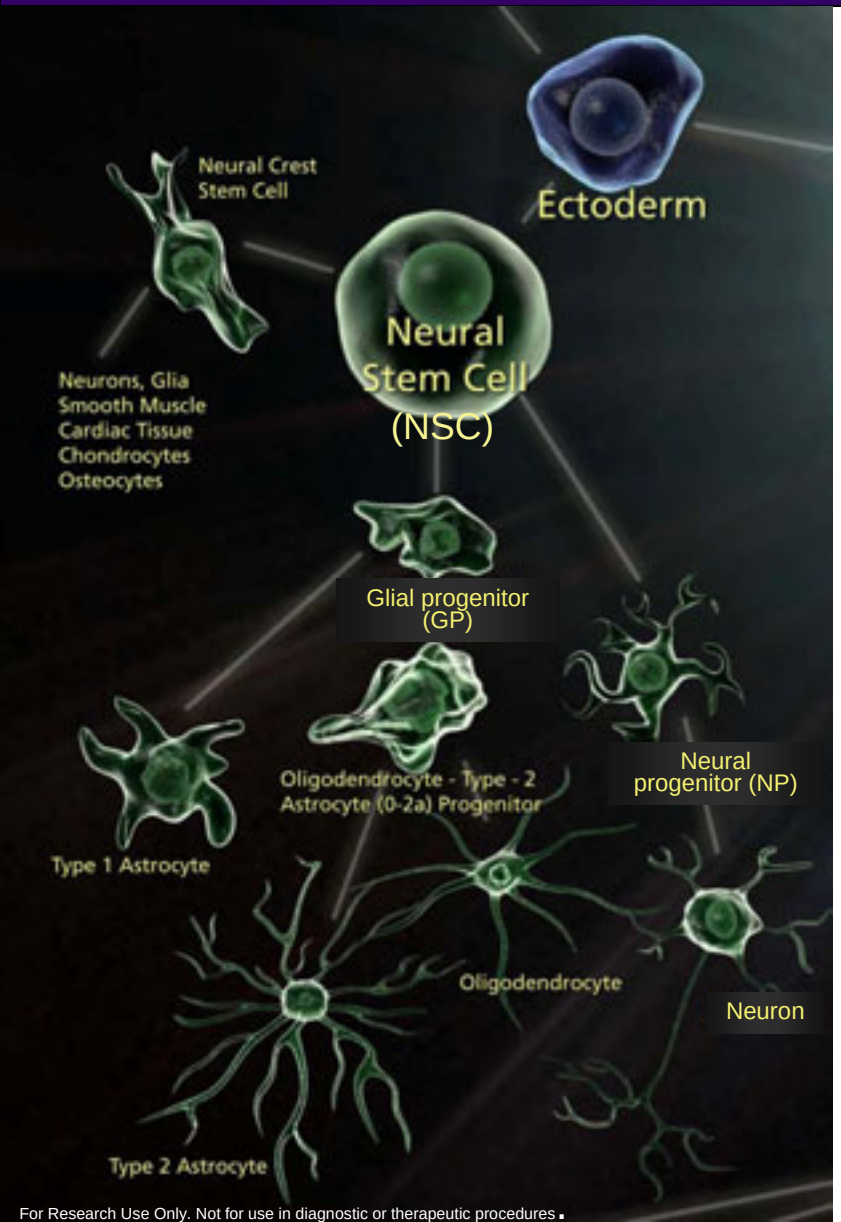
Neural Stem Cells: Background



- **Found during embryonic development and in restricted regions of the adult brain**
- **NSCs can be isolated and cultured in vitro**
  - Fetal and adult brain
  - Differentiated from hESCs
- **Promises of NSCs**
  - Transplantation therapy
  - In vitro models of human development
  - In vitro models of human diseases
    - Drug screening
    - Toxicology
    - Basic research

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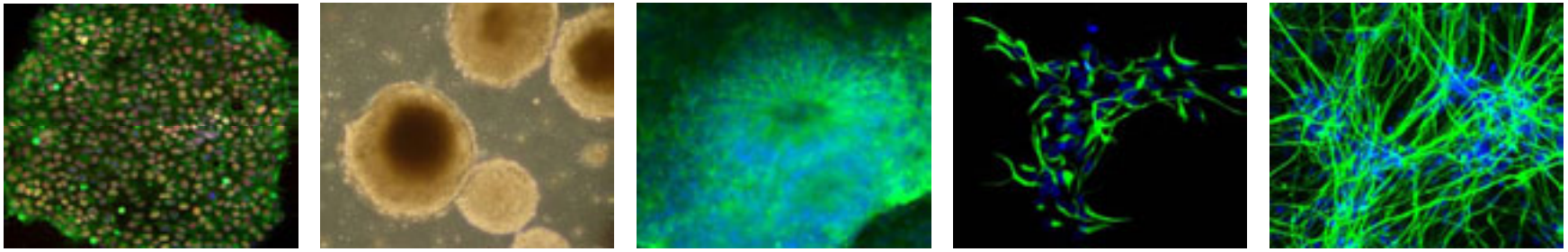
Generation of Neural Cells from hESCs

Day 0	7	17	20	35
hESCs	EBs	Rosettes	NSCs	Neurons, Glia
mTeSR™1	withdraw bFGF	N2	N2, B27, bFGF	N2, B27, dbcAMP



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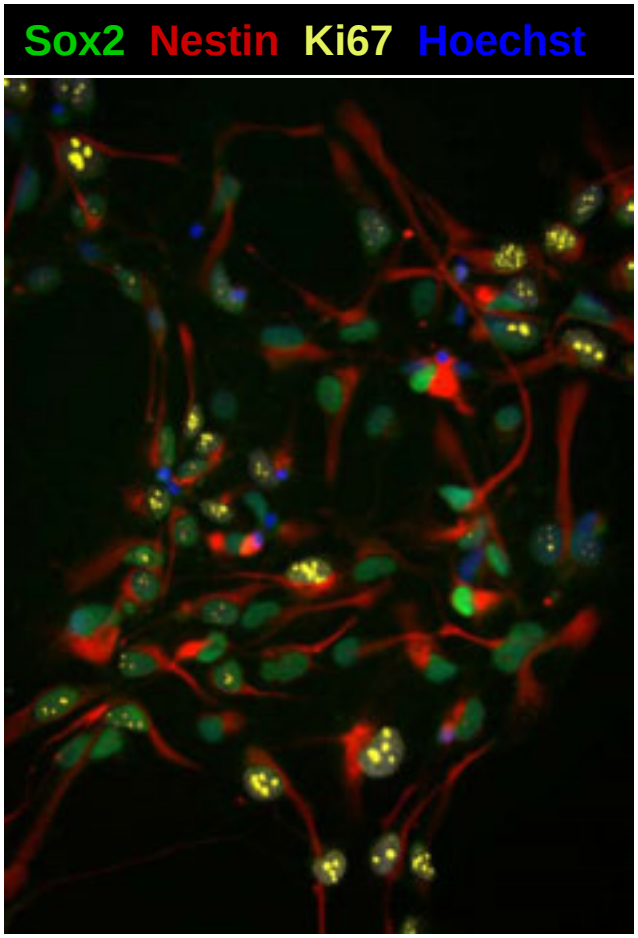
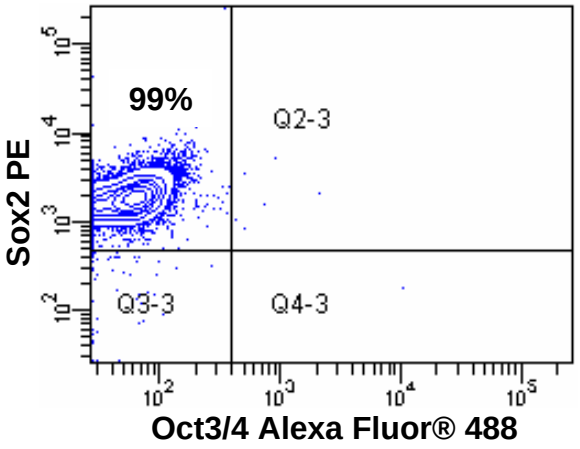
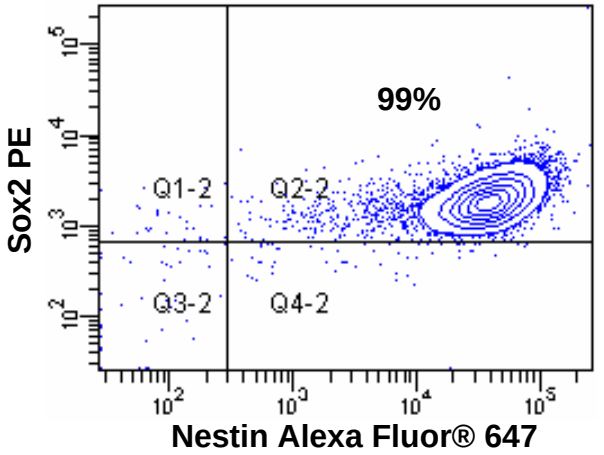
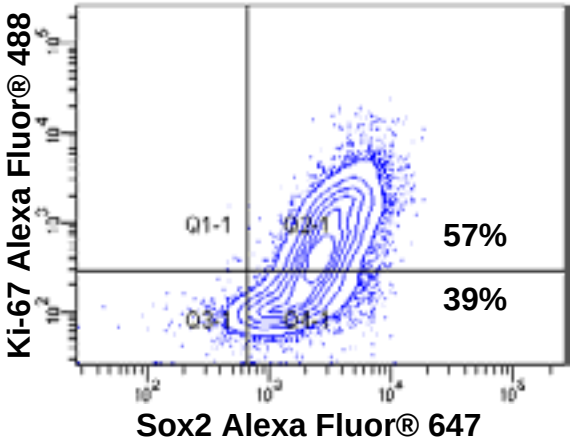
Closer Look at hESC-derived NSCs









Sox2: hESCs, NSCs
Nestin: NSCs
Oct3/4: hESCs
Ki67: proliferation

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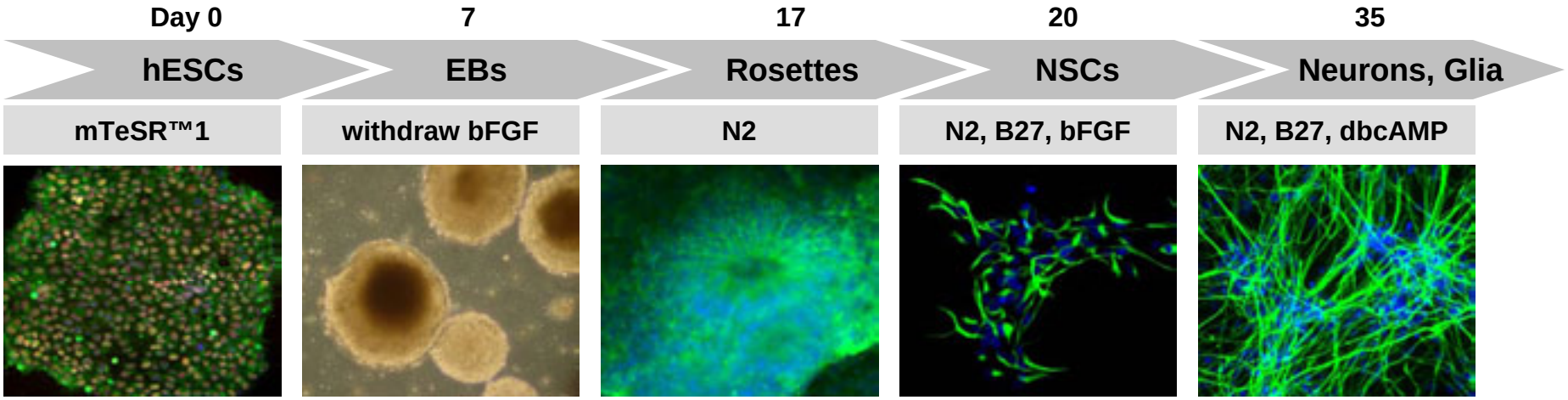
Generation of Neural Cells from hESC



- **Current Challenges**
  - Protocols are difficult
  - Batch-to-batch variability of NSCs
  - Neuronal differentiation is heterogeneous
    - Need pure populations for applications: transplantation, arrays/sequencing, in vitro disease models

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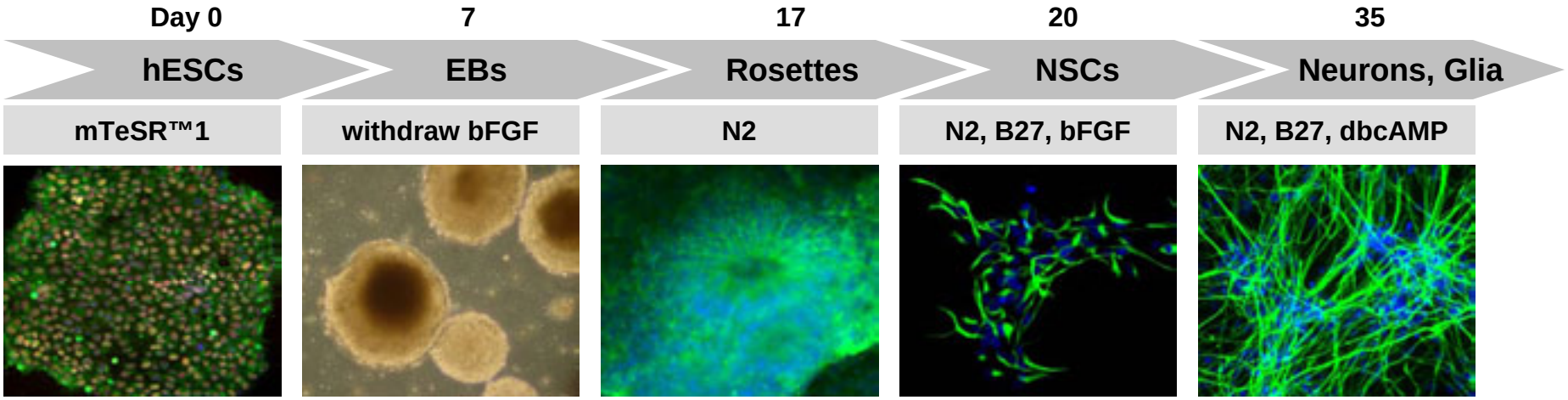
Generation of Neural Cells from hESCs



- **Performed a screen using 192 human CD markers by flow cytometry and bioimaging**
  - Define cell surface signatures of hESCs, NSCs, NPs (neural progenitors), neurons, and glia
  - Develop a method to isolate near-pure populations of NSCs, neurons, and glia

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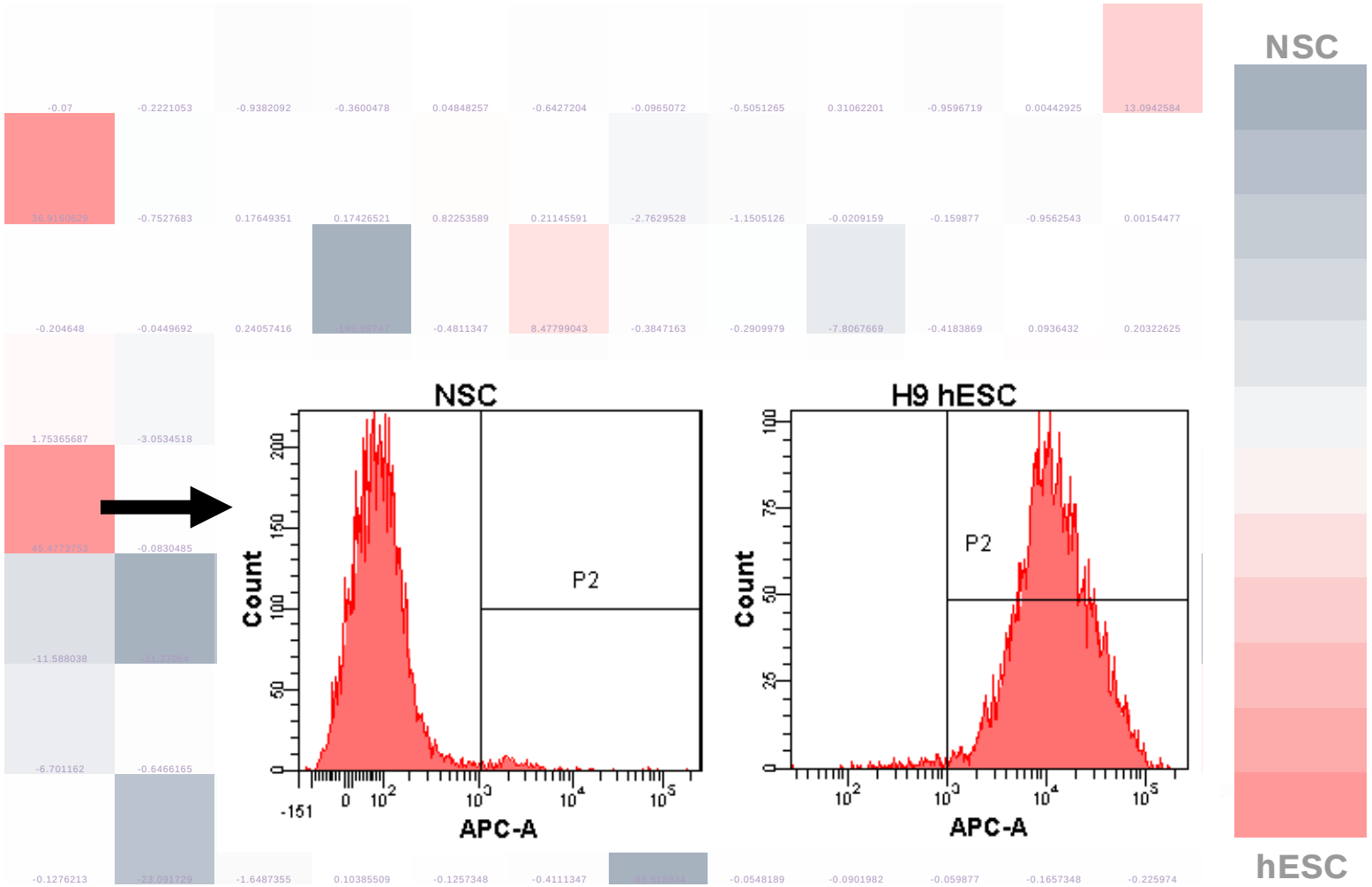
Cell Surface Marker Screening with BD Lyoplate™ Human CD Marker Panel

CD marker screening in 96-well format by flow cytometry



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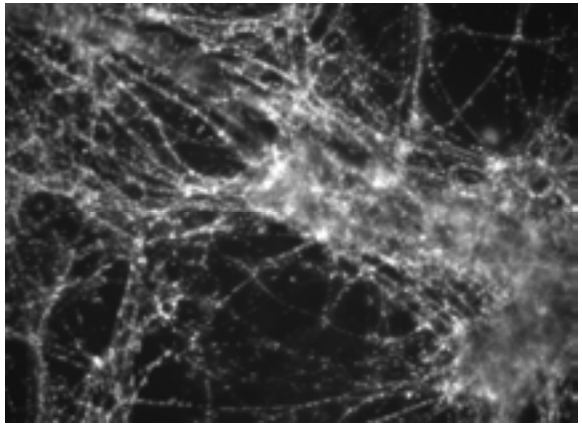
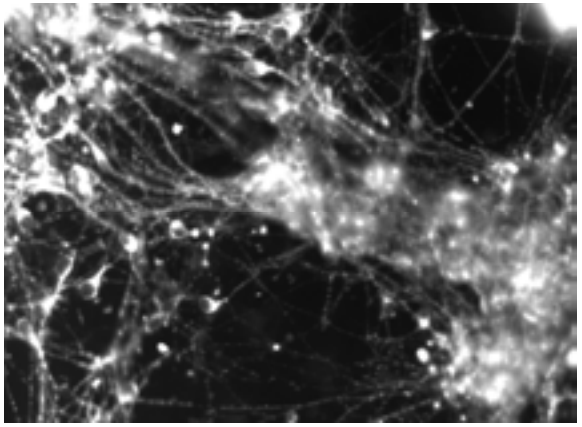
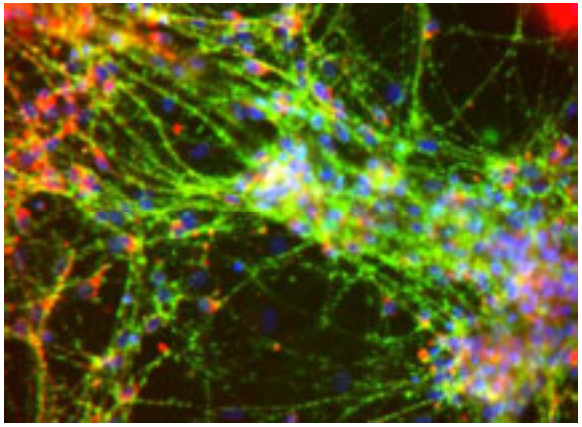
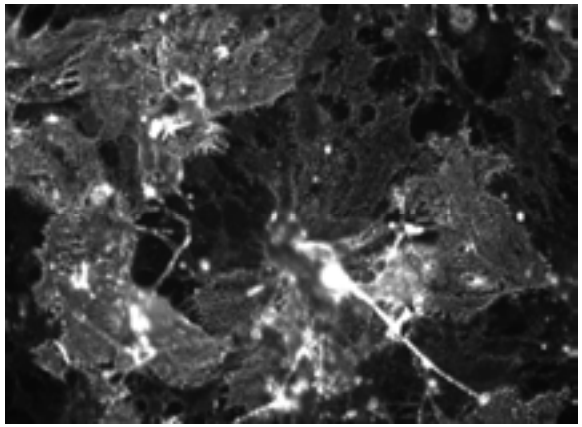
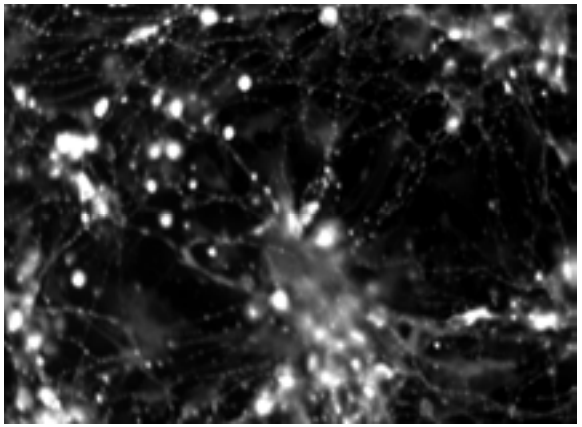
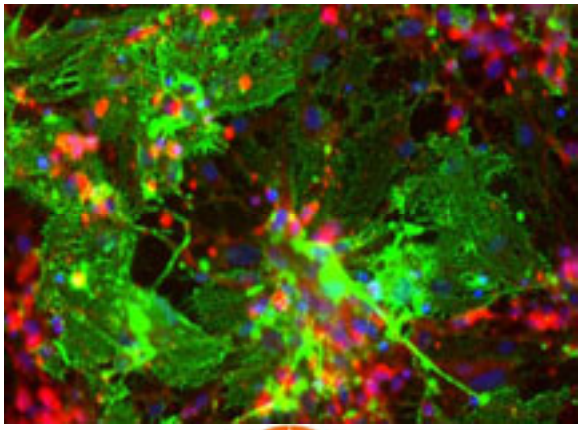
Cell Surface Marker Screening with BD Lyoplate™ Human CD Marker Panel

CD marker screening in 96-well format by imaging

CD Marker	Tuj1	Merge
		
		

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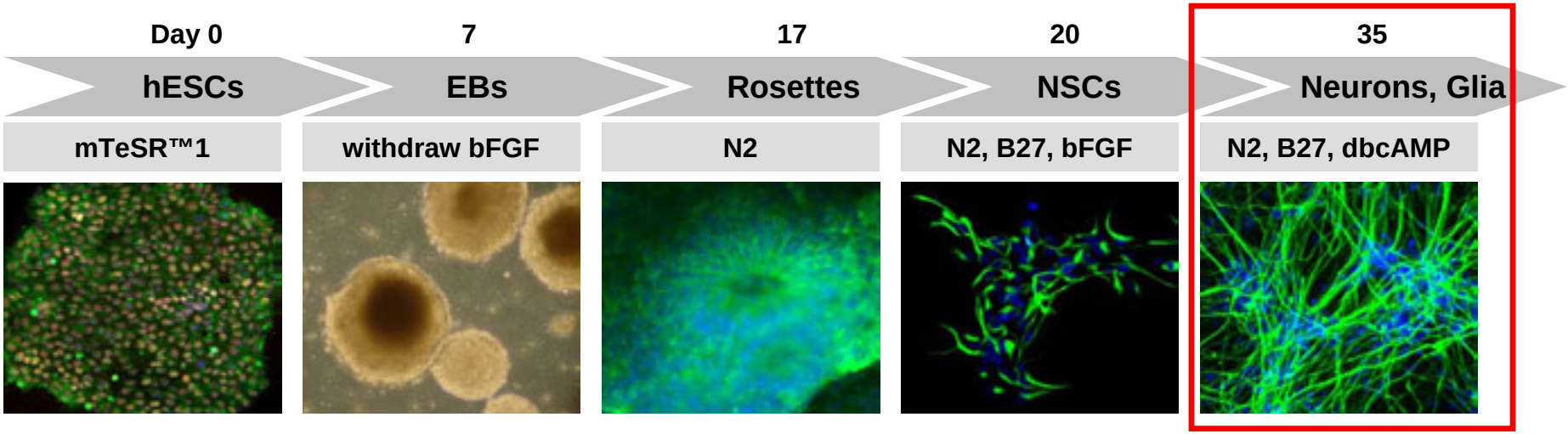
Generation of Neural Cells from hESCs



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  - Define cell surface signatures of hESCs, NSCs, NPs (neural progenitors), neurons, and glia
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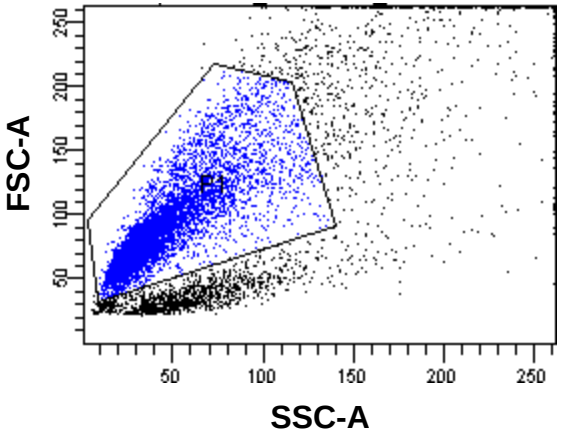
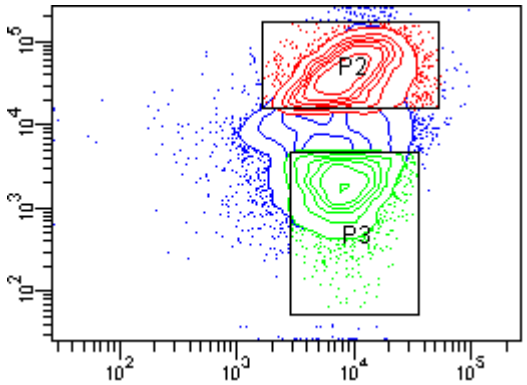
Isolation of Neural Subtypes from hESC-derived NSCs

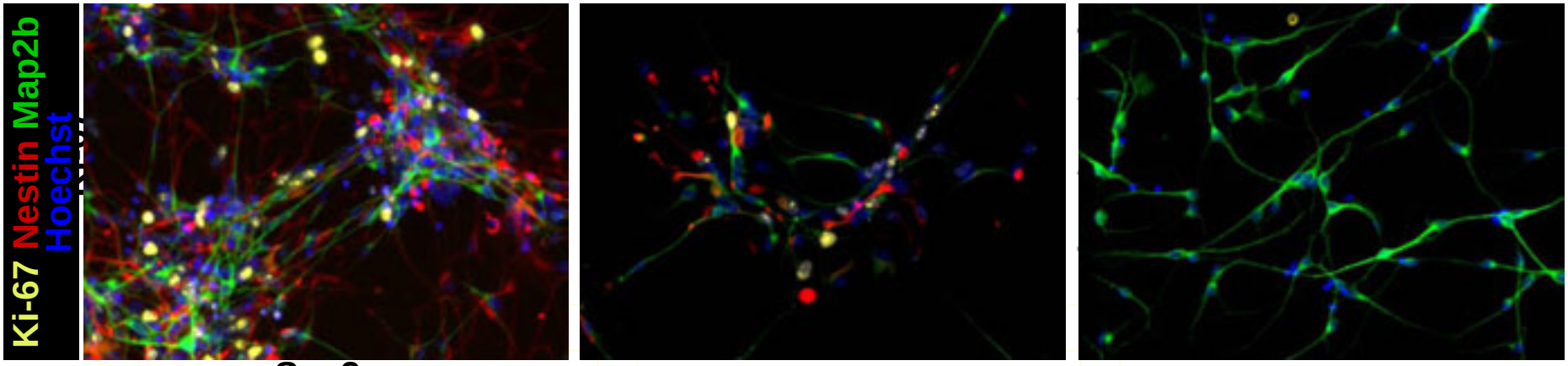




NSCs were differentiated 2 weeks prior to sorting

BD FACSAria™ II sorter
70 PSI, 70-µm nozzle

Presort | **Sorted: P2** | **Sorted: P3**



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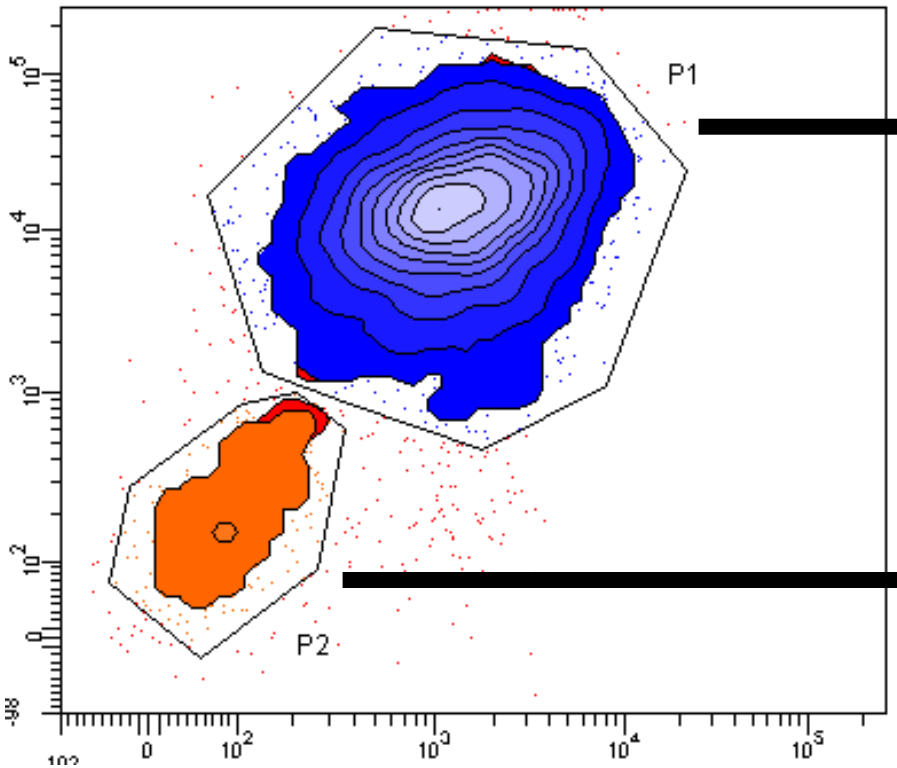
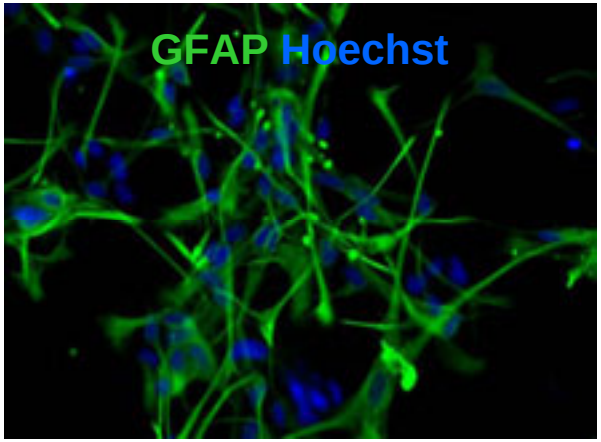
Isolation of Neural Subtypes from Differentiating hESC-derived NSCs

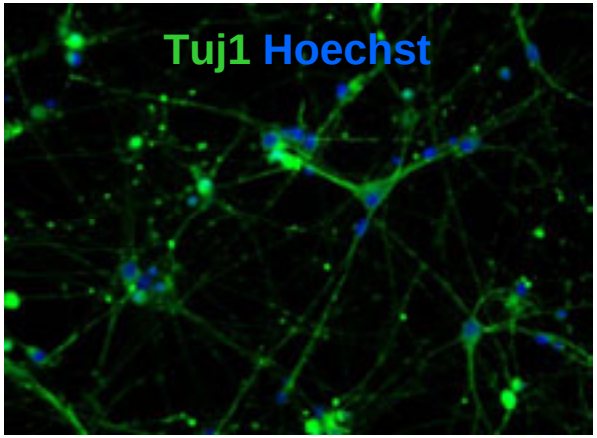
NSCs were differentiated 4 weeks prior to sorting

BD FACSAria II sorter, 25 PSI, 100-µm nozzle





Glia



Neurons

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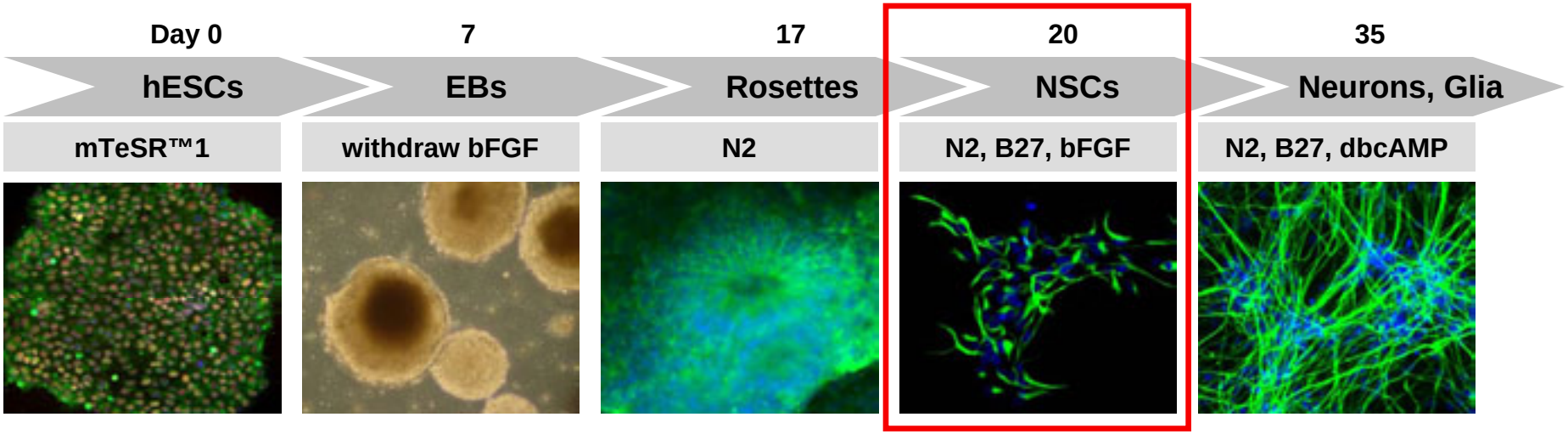
Generation of Neural Cells from hESCs



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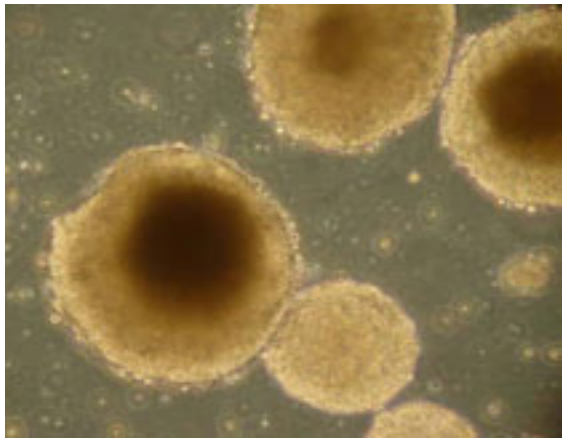
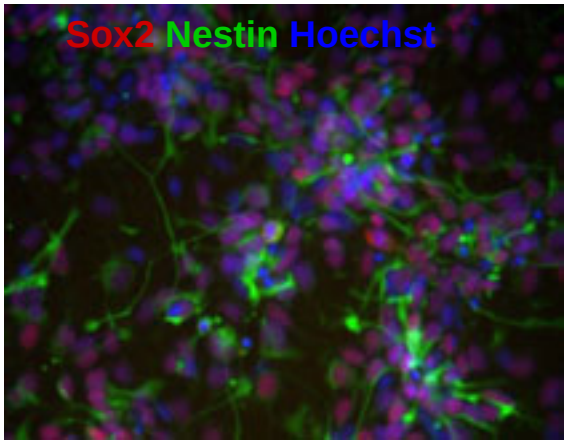
Isolation of NSCs from Embryoid Bodies by Flow Cytometry

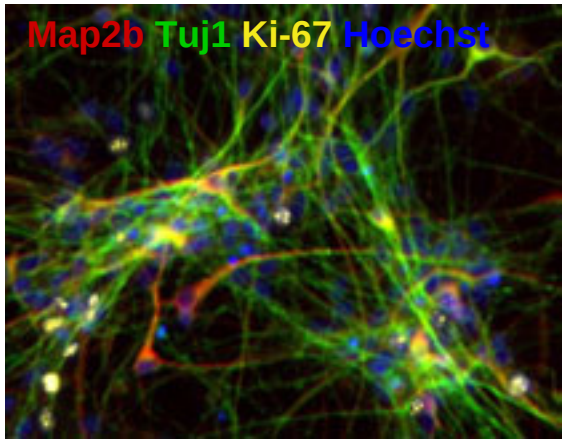


EB

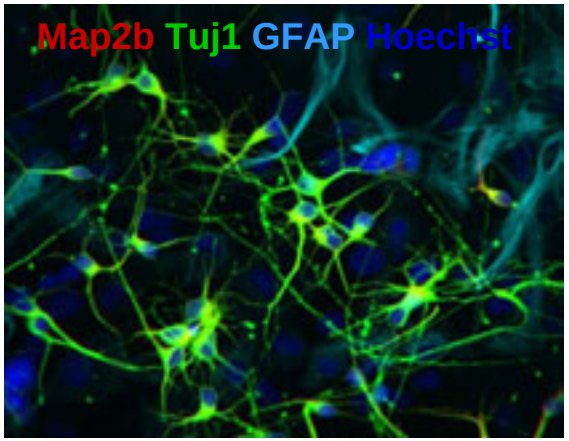
NSCs Sorted from EBs



Differentiation of NSCs



Neurons Sorted from Diff NSCs



Co-cultured on Astrocytes

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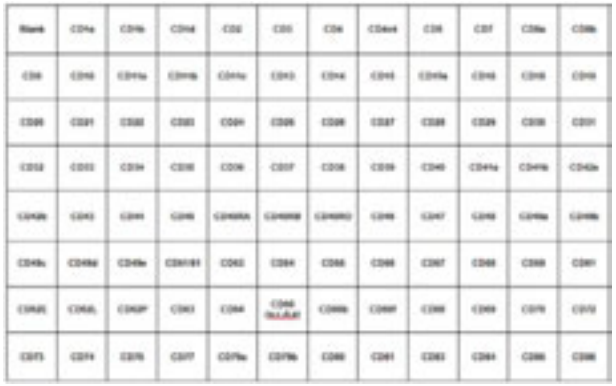
BD™ Lyoscreen Human Cell Surface Kit

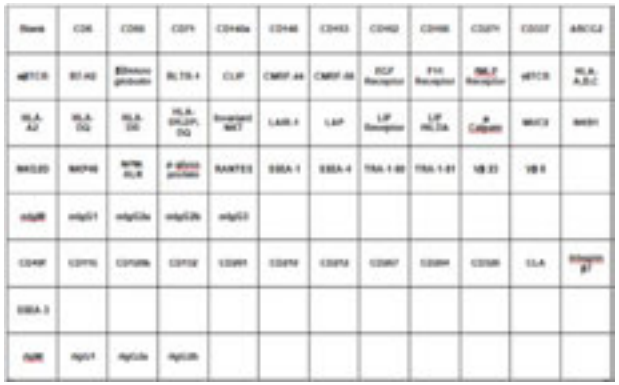
- Approximately 260 human cell surface markers in 96-well plates
- Each kit contains five tests of each antibody
- Customer dilutes antibodies to desired concentration into daughter plates
- Other kit components
  - Fluorchrome-conjugated secondary antibodies for flow cytometry and imaging
  - Isotype controls
  - Analysis template

1



2

3



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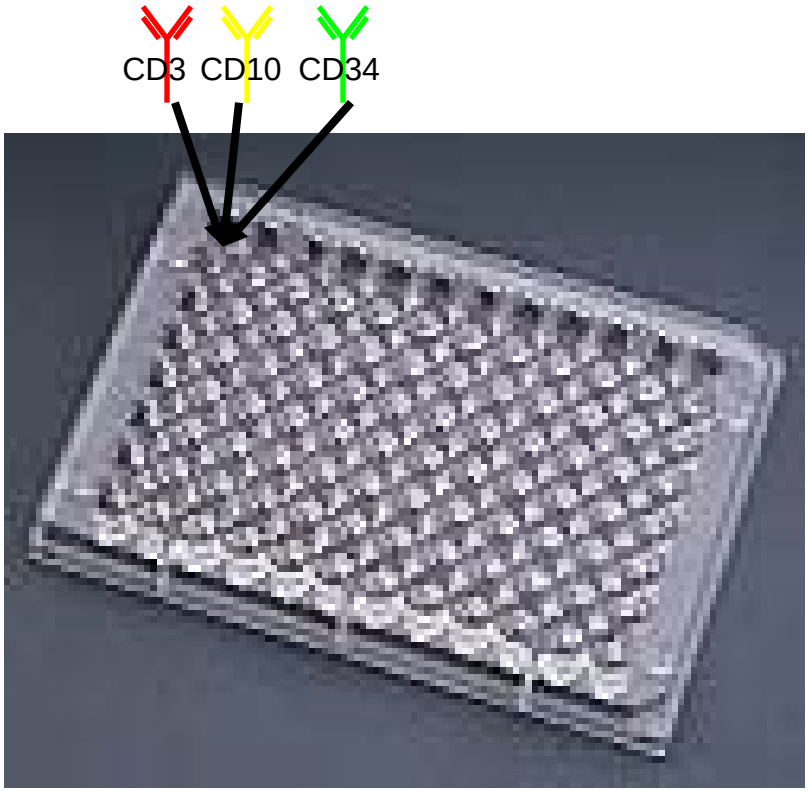
BD FACS™ CAP
Cell Surface Phenotyping Service

- Custom service leverages BD's expertise as the world leader in flow cytometry
- Flexible format can integrate customer's specific markers
- Continuous addition of new important monoclonal antibodies
- Delivers flow cytometry-based combinatorial antibody profile



CAP = Combinatorial Antibody Profile

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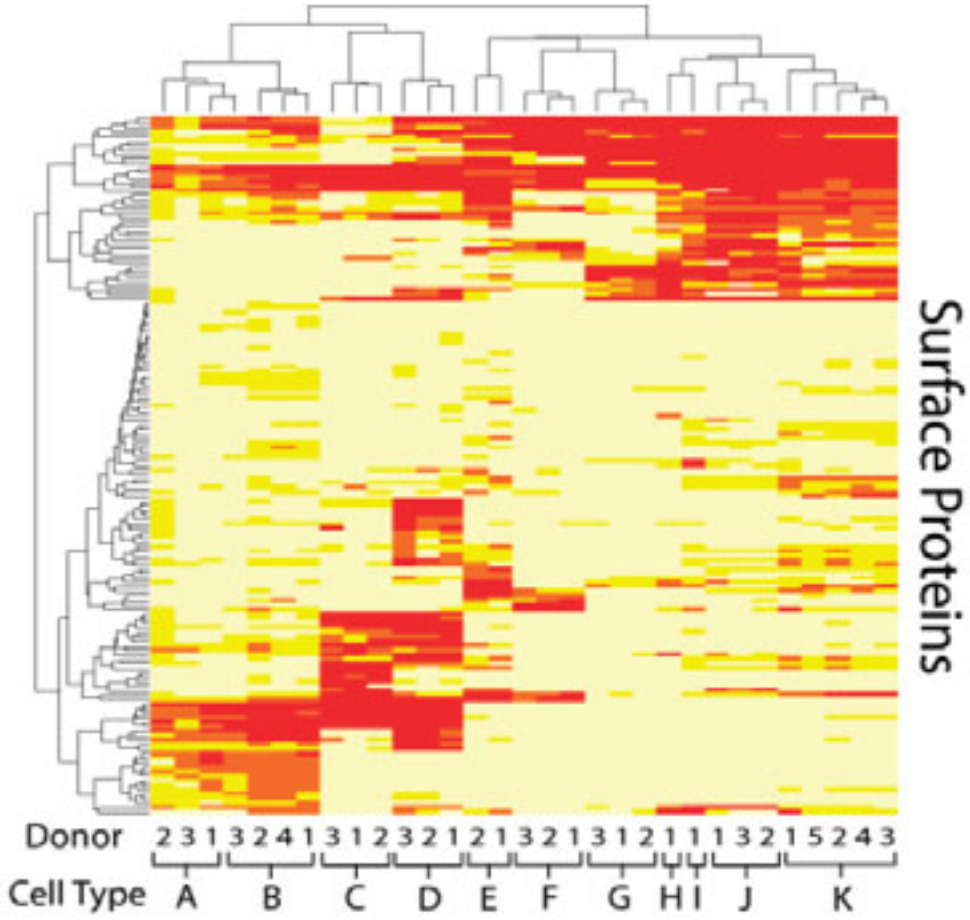
BD FACS™ CAP Service

Luminal-like cells and basal-like cells show distinct cell surface marker expression



Heat map showing relative expression of cell surface proteins of eleven different cell types. Color intensity indicates the percentage of cells positive for each marker.

BD FACS™ CAP technology may be used on a variety of human cell types

GO Category	Number of Proteins Annotated With This GO Term With Antibodies on FACS™CAP
Receptor activity	103
Protein binding	101
Immune response	80
Signal transduction	55
Cell adhesion	51
Inflammatory response	24
Chemotaxis	17
Apoptosis	15
Cell proliferation	12
Cell-cell signaling	12
Cell motility	12
Cell-cell adhesion	12

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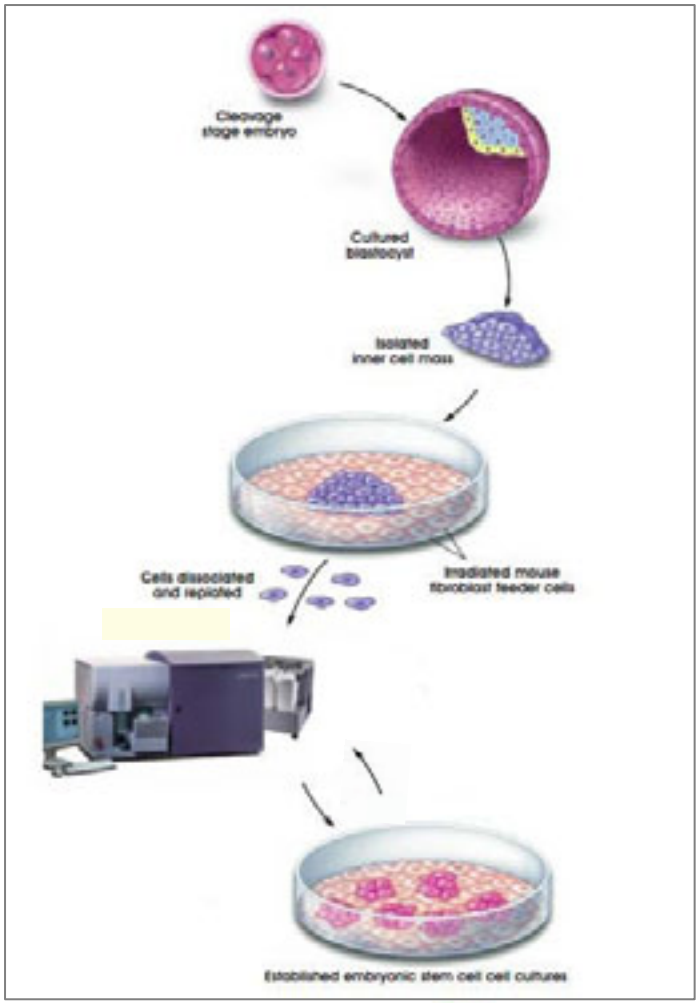
Pluripotent Stem Cell Research

23-10679-00

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

Sorting and Analysis of Pluripotent Stem Cells

- Are sorted hESCs viable?
  - Fong et al. *Stem Cell Rev.* 2009
  - Bajpai et al. *Mol Reprod Dev*. 2008
  - Nicholas et al. *Stem Cells Dev*. 2007
  - Sidhu et al. *Stem Cells Dev.* 2006
- Do sorted cells still express markers of pluripotency?
- Are sorted cells capable of further differentiation?
- No commercial, standardized methods for sorting or analysis by flow cytometry.



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Sorting Experimental Design

- Cell surface markers
  - SSEA-1 negative (differentiation)
  - SSEA-3 positive (pluripotency)
  - Tra-1-81 positive (pluripotency)
- Cell sorting with BD FACSAria II system
  - 25 PSI, 100-µm nozzle
- hESCs used:
  - H9 P48
    - Grown on BD Matrigel hESC-qualified Matrix with mTeSR™1
  - H9 P41
    - Cultured in KOSR on MEFs
  - HUES9
    - Cultured in HUES on MEFs (Goldstein Lab, UCSD)

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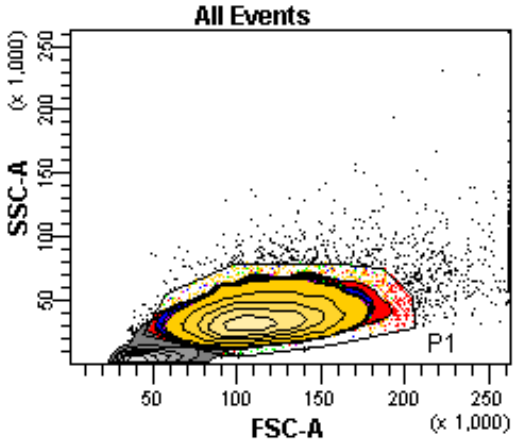
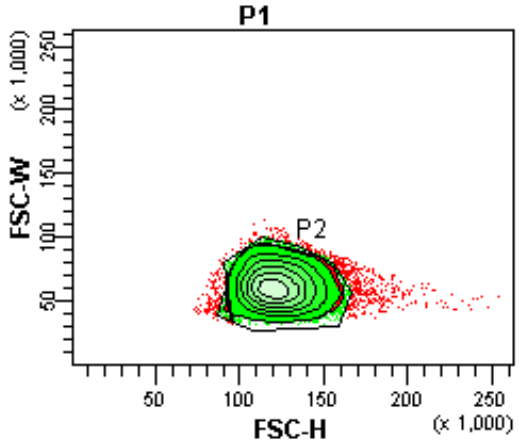
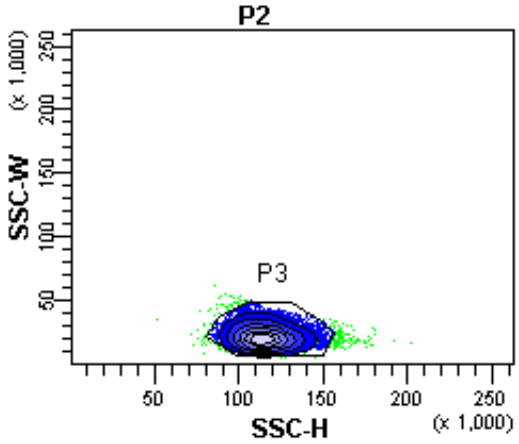
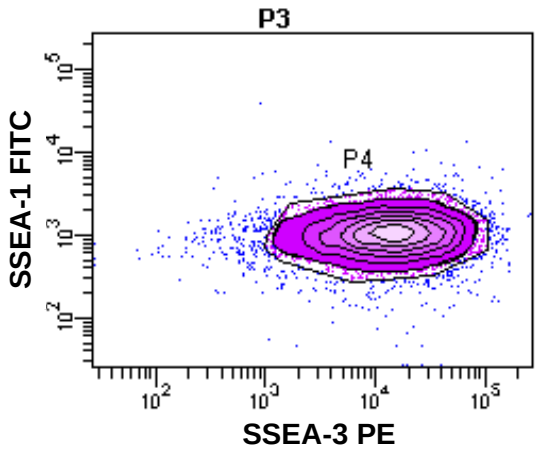
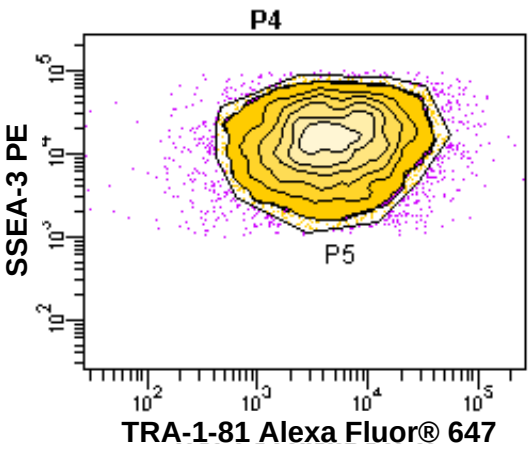
Sorting Based on Markers for Pluripotency and Differentiation











Tube: +DNase

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	7,616	76.2	76.2
P2	6,989	91.8	69.9
P3	6,760	96.7	67.6
P4	6,182	91.4	61.8
P5	5,629	91.1	56.3

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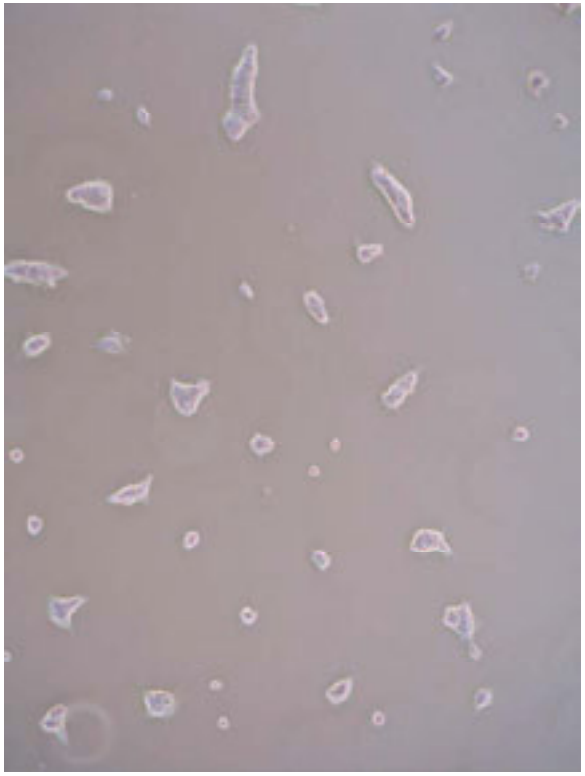
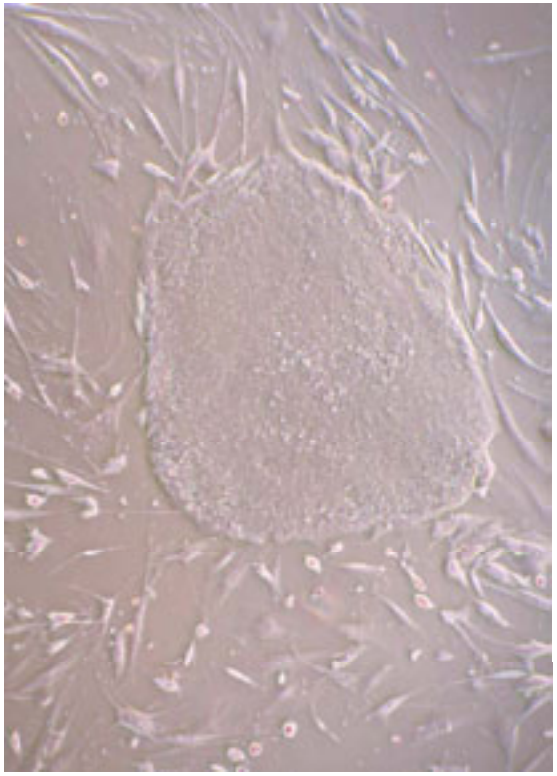
Sorting is Possible Under Feeder and Feeder-free Culturing Conditions

H9 P48 day 2 post-sort



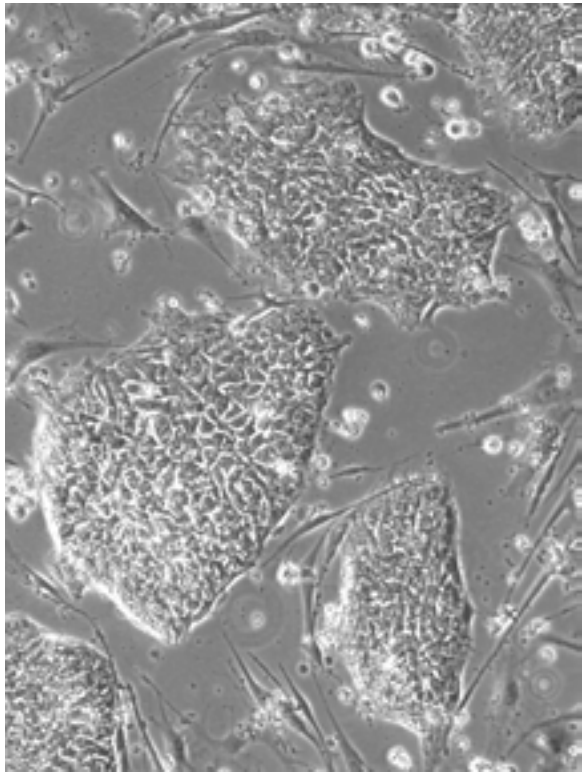
mTeSR™1, BD Matrigel, Accutase

H9 P41, P5 Day 3 post-sort



KOSR, MEF, Coll IV

HUES9 Day 4 post-sort



HUES, MEF, Trypsin
Goldstein Lab, UCSD

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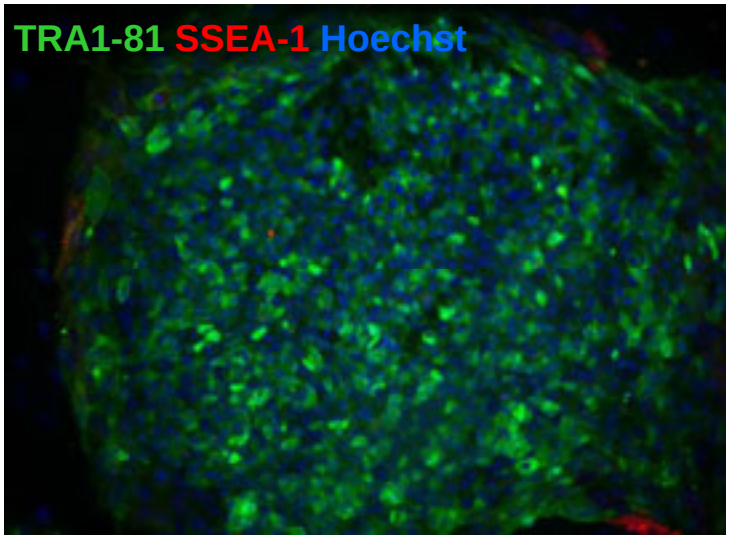
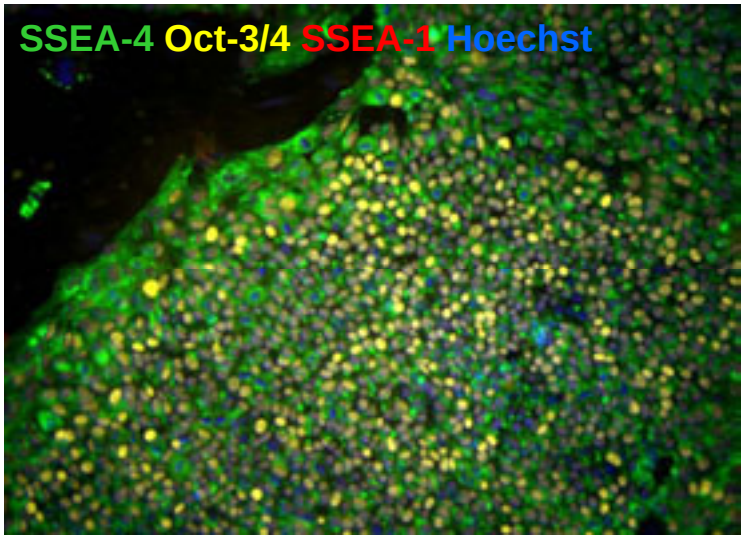
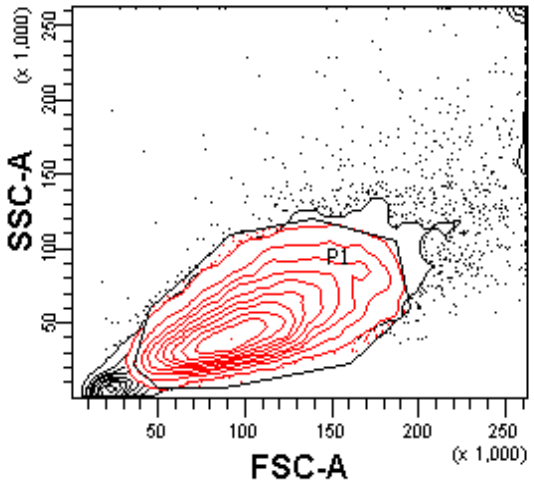
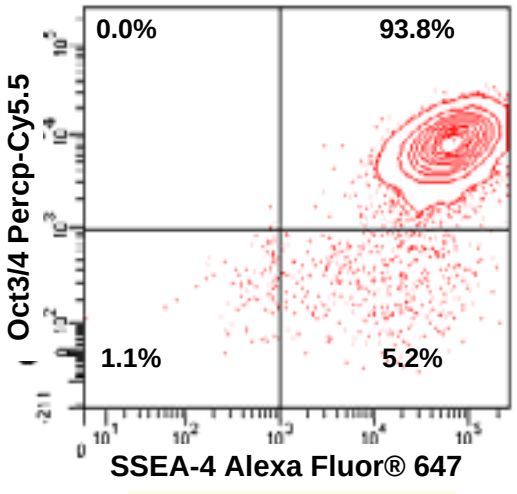
Sorted H9 hESCs Express Pluripotency Markers

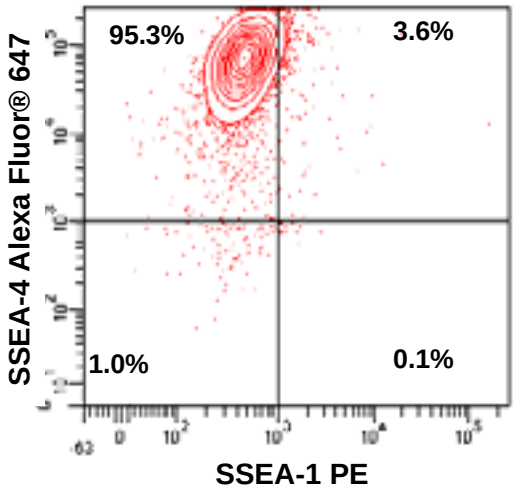
H9 P42 P6 post-sort











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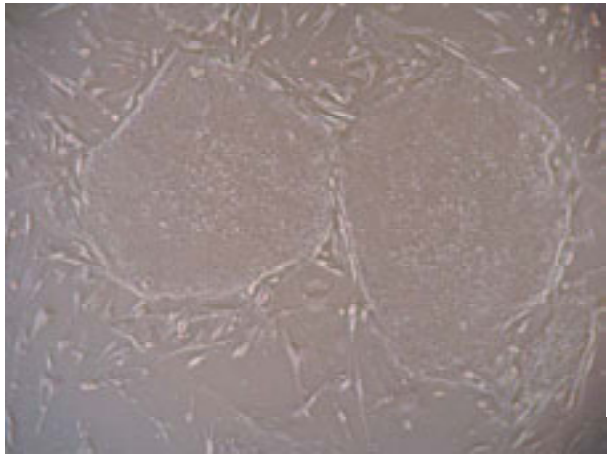
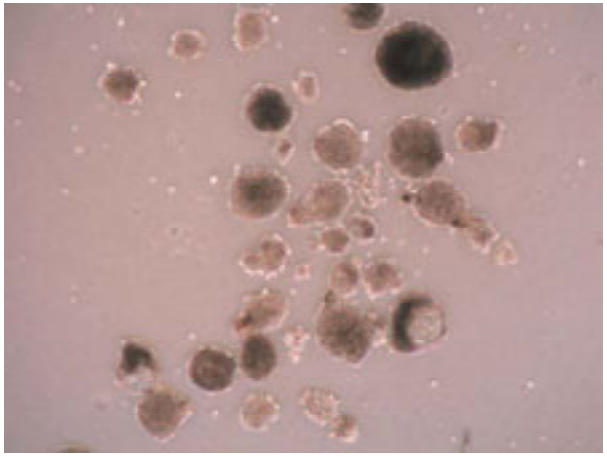
Sorted H9 hESCs Retain Differentiation Potential

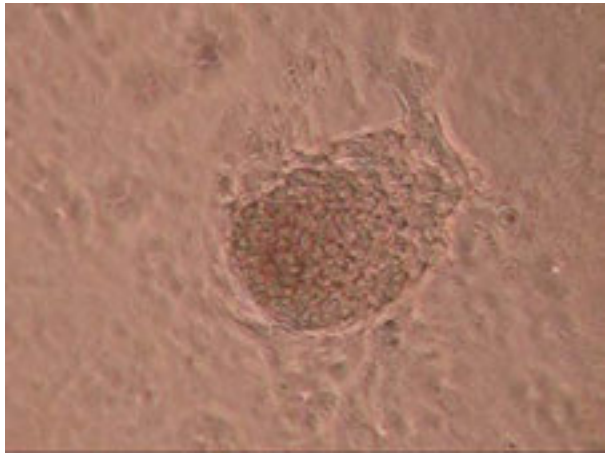
H9 P43, P7 sort



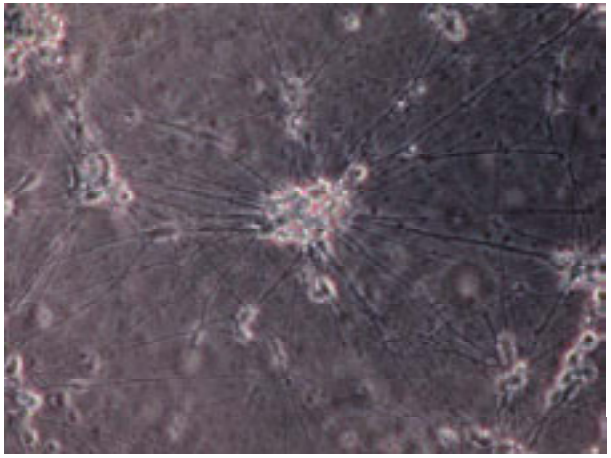
EBs



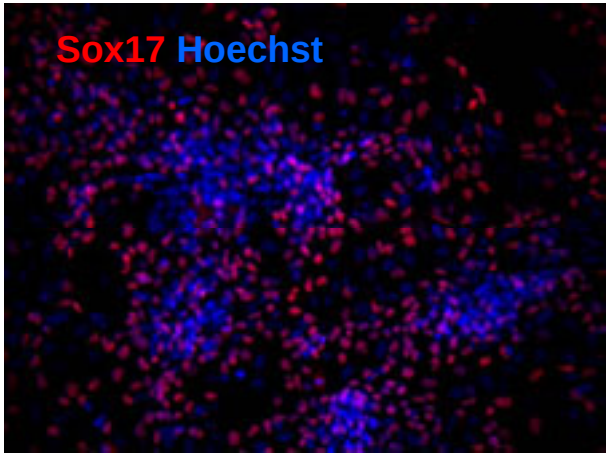
Mesoderm



Ectoderm



Endoderm



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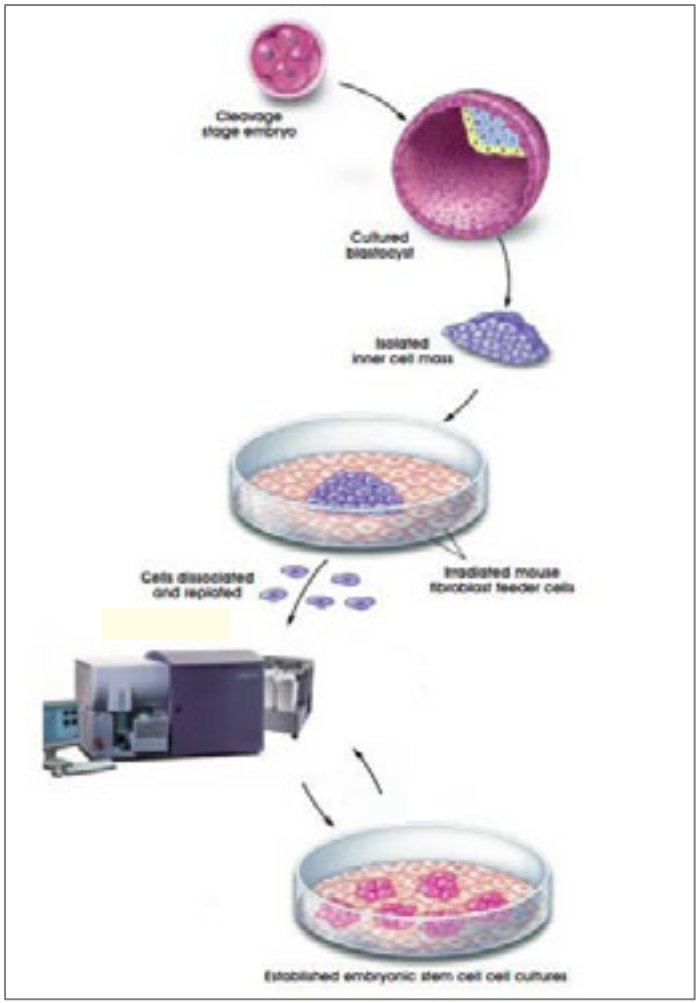

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Flow Cytometry Kits for Pluripotent Stem Cell Research

- Comprehensive, easy-to-use
  - Compensation beads
  - Verified protocols and software analysis guidelines
- Analysis and sorting
- Multicolor
  - Pre-conjugated antibodies to markers for self-renewal and differentiation
- Open, modular
  - Compatible for “dropping-in” additional antibodies to cell surface markers, transcription factors, cytokines, and phosphorylated proteins
- Compatible with GFP-expressing cells

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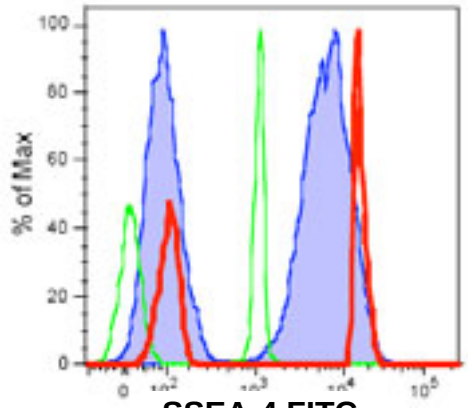
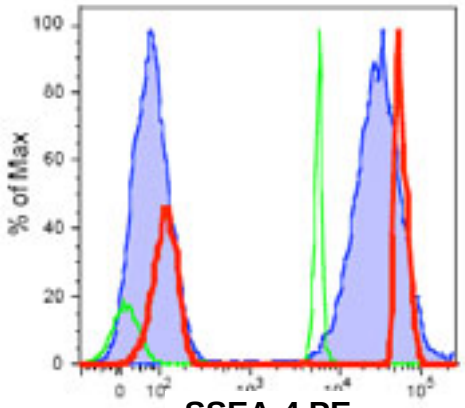
BD™ CompBead Plus

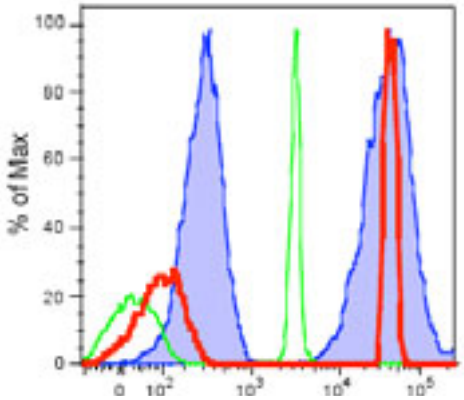
Overlays of unstained cells and cells and beads stained with SSEA-4 conjugates



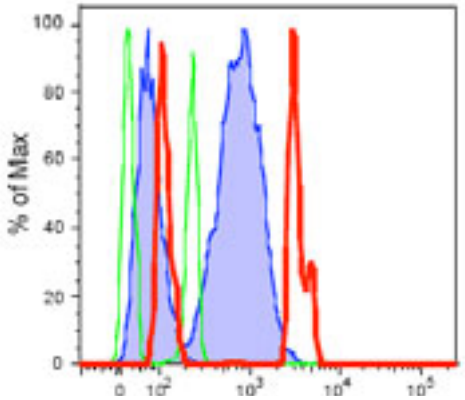
SSEA-4 FITC



SSEA-4 PE

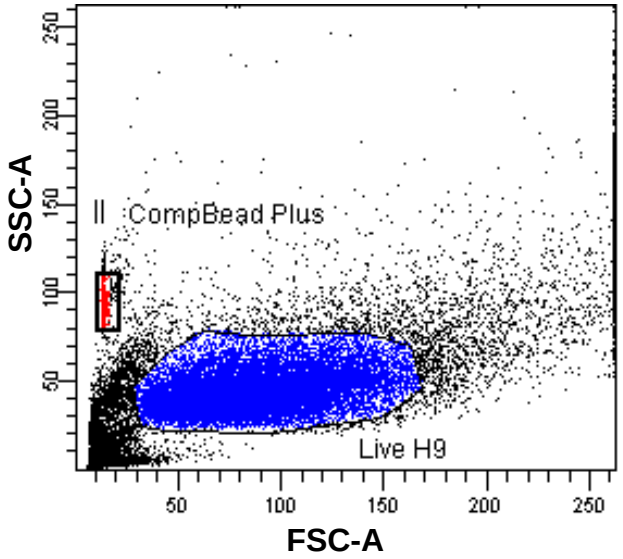


SSEA-4 AlexaFluor® 647



SSEA-4 BD Horizon™ V450

H9 hESC
CompBead
CompBead Plus



- Autofluorescence of beads tracks hESCs
- Facilitate scatter setup
- Compensation for any mouse or rat antibody

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Dropping in Additional Antibodies

- Drop-ins add to the number and type of markers to analyze per sample
- Enable detailed analysis of cell fate and function of single cells:
  - Correlation of marker expression (up regulation and down regulation)
  - Simultaneous analysis of transcription factors, cell surface markers, cellular processes (cell cycle, cell signaling, cell death)
- Example:
  - Mouse ES (E14) 5-day differentiation time-course (10 μM RA)
    - Analysis of mNanog, Oct3/4, Sox2 (Mouse Pluripotency Analysis Kit – TF) + GATA4- Alexa Fluor® 488 drop-in

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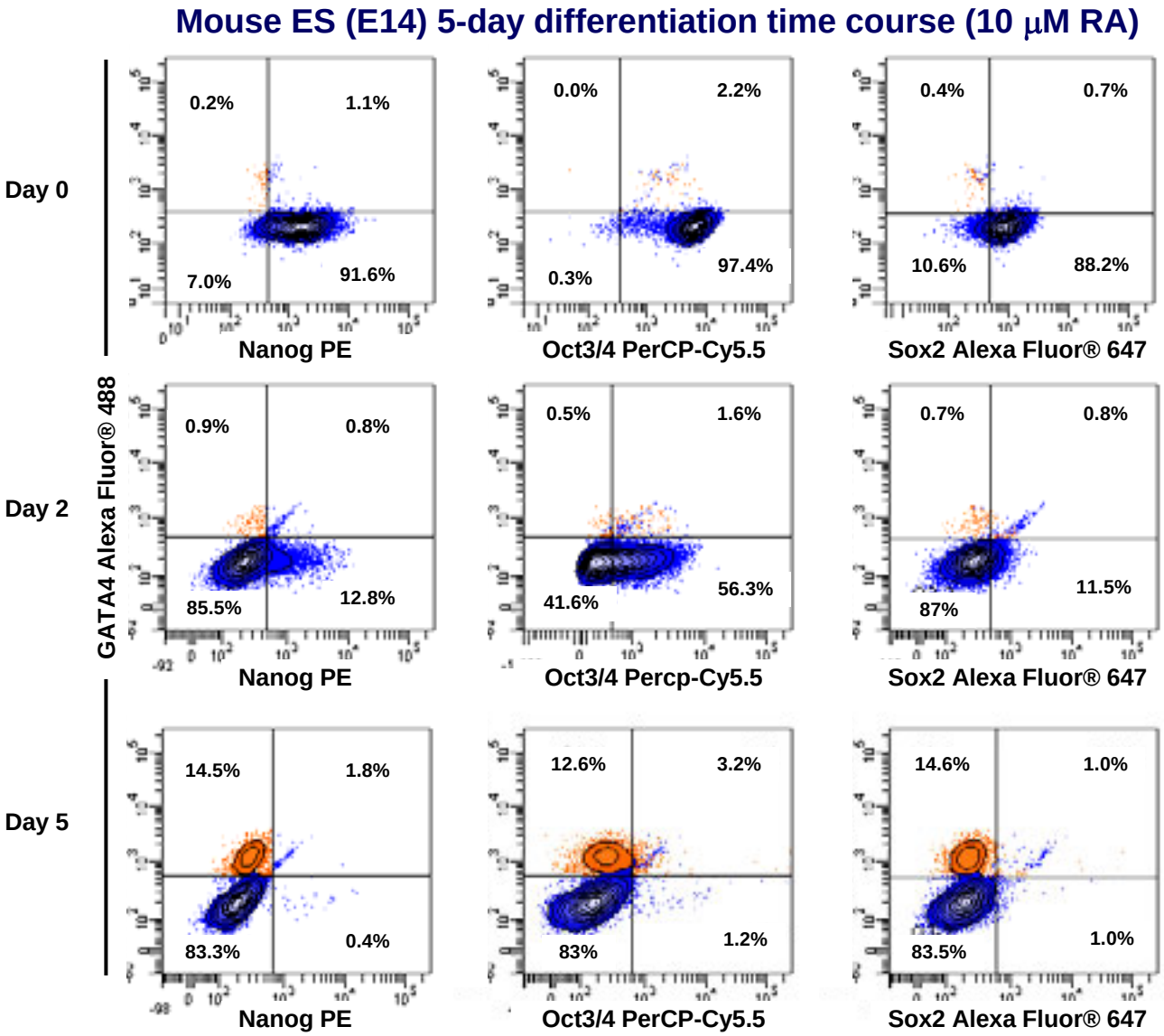
Dropping in Additional Antibodies



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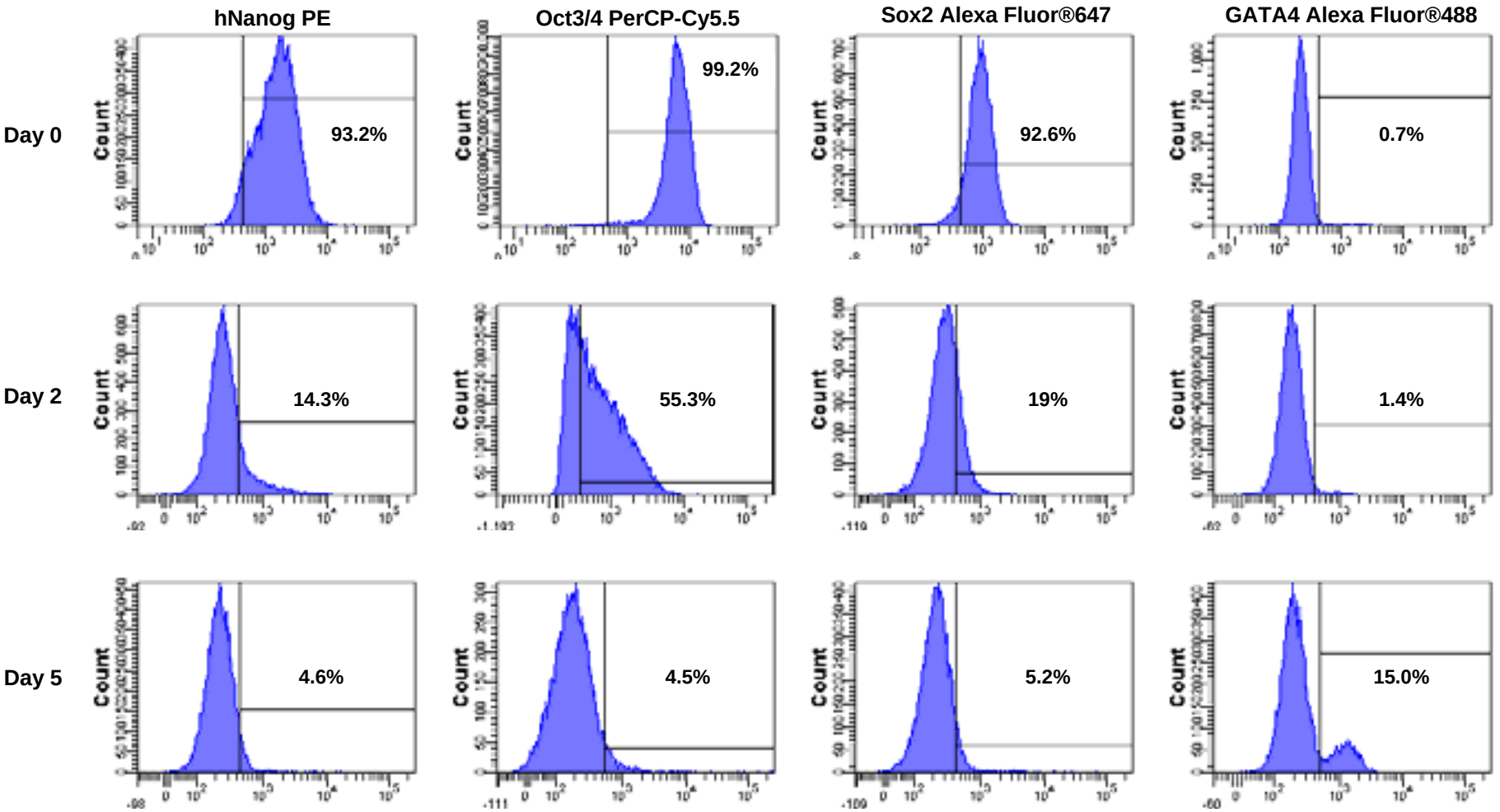
Dropping in Additional Antibodies

Mouse ES (E14) 5-day differentiation time course (10 μM RA)



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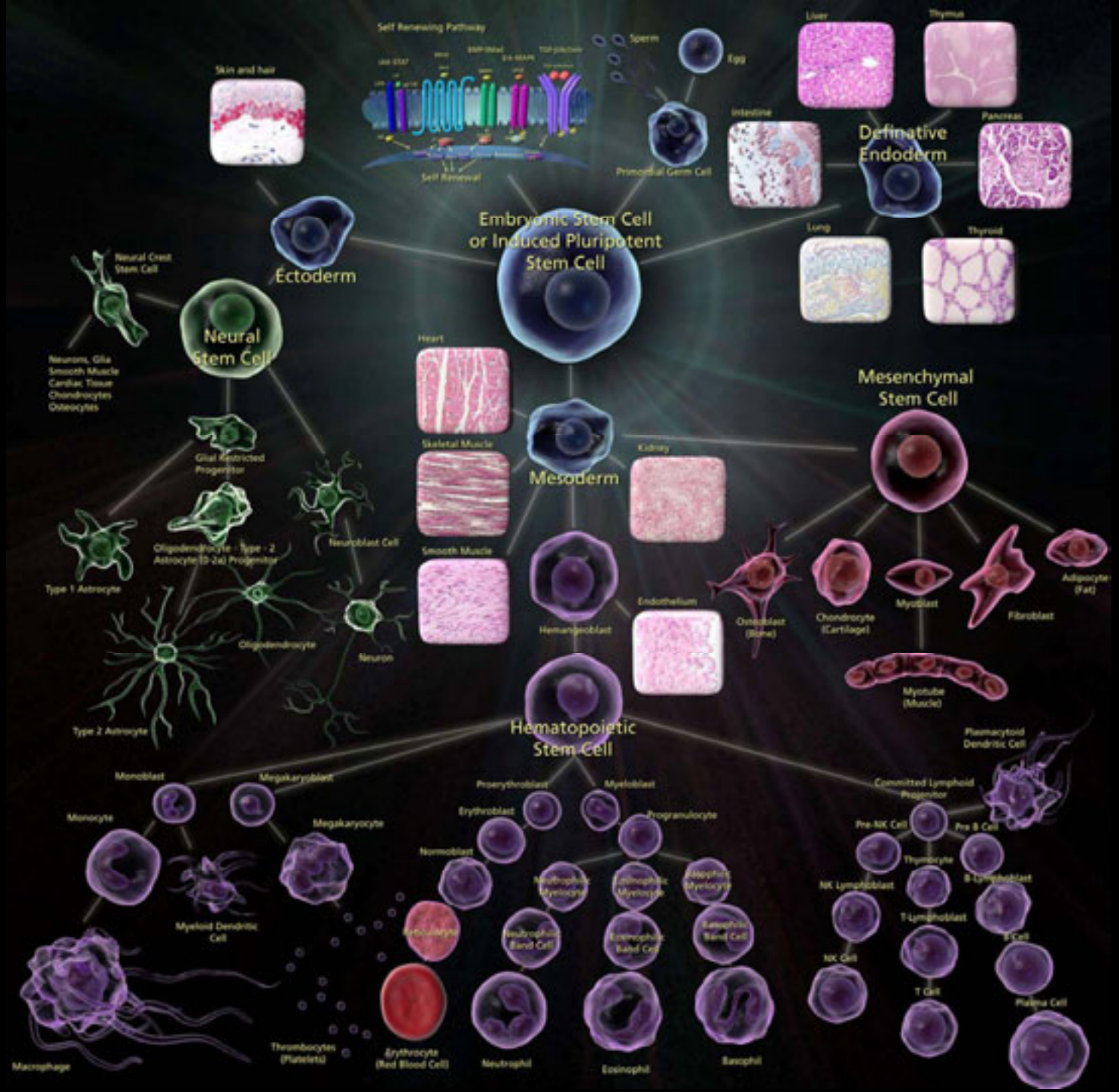
Self Renewing Pathway
Self Renewal
Sperm
Egg
Primordial Germ Cell
Skin and hair
Liver
Thymus
Intestine
Pancreas
Definative Endoderm
Lung
Thyroid
Embryonic Stem Cell or Induced Pluripotent Stem Cell
Ectoderm
Neural Crest Stem Cell
Neurons, Glia
Smooth Muscle
Cardiac Tissue
Chondrocytes
Osteocytes
Neural Stem Cell
Glial Restricted Progenitor
Oligodendrocyte - Type - 2 Astrocyte (O-2a) Progenitor
Neuroblast Cell
Type 1 Astrocyte
Oligodendrocyte
Neuron
Type 2 Astrocyte
Heart
Skeletal Muscle
Smooth Muscle
Mesoderm
Hemangioblast
Kidney
Endothelium
Mesenchymal Stem Cell
Osteoblast (Bone)
Chondrocyte (Cartilage)
Myoblast
Fibroblast
Adipocyte (Fat)
Myotube (Muscle)
Hematopoietic Stem Cell
Monoblast
Megakaryoblast
Monocyte
Megakaryocyte
Myeloid Dendritic Cell
Macrophage
Thrombocytes (Platelets)
Proerythroblast
Myeloblast
Erythroblast
Progranulocyte
Normoblast
Reticulocyte
Neutrophil Band Cell
Eosinophil Band Cell
Basophil Band Cell
Erythrocyte (Red Blood Cell)
Neutrophil
Eosinophil
Basophil
Plasmacytoid Dendritic Cell
Committed Lymphoid Progenitor
Pre-NK Cell
Pre B Cell
Thymocyte
NK Lymphoblast
B Lymphoblast
T-Lymphoblast
NK Cell
T Cell
B Cell
Plasma Cell

Mouse HSC Isolation Kit

- Contents:
  - APC Lineage Cocktail
  - PE c-Kit
  - FITC CD34
  - PE-Cy™7 Sca-1
  - Matched Isotype Controls
  - CD16/CD32 (Fc III/II Rec)
  - 7-AAD vital dye
  - BD CompBeads
  - Verified protocols

- Utility:
  - Sorting $CD34^{+/-}$ KLS from mouse bone marrow
  - 100 mice ~ 10 sorts
  - Compatible with magnetic enrichment
  - Compatible with side population KLS (SPKLS)

LT-HSC: $CD34^{-}$, $SCA\text{-}1^{+}$, $C\text{-}kit^{+}$

ST-HSC: $CD34^{+}$, $SCA\text{-}1^{+}$, $C\text{-}kit^{+}$

MPP: $CD34^{+}$, $SCA\text{-}1^{+}$, $C\text{-}kit^{+}$

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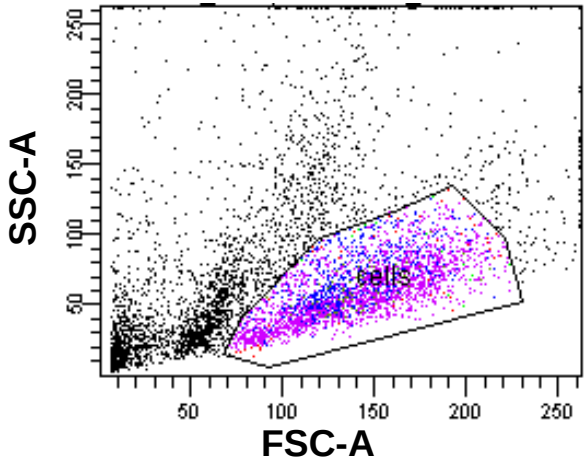
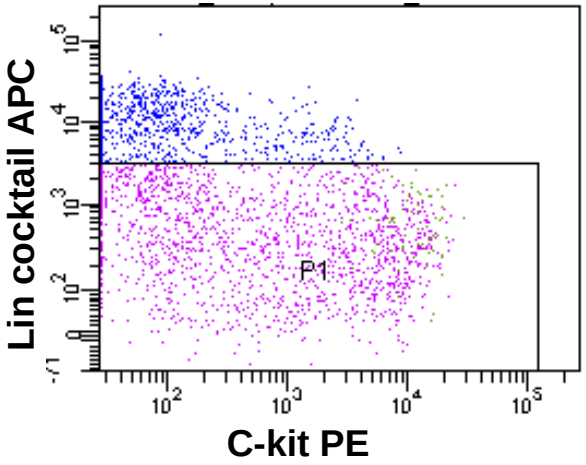
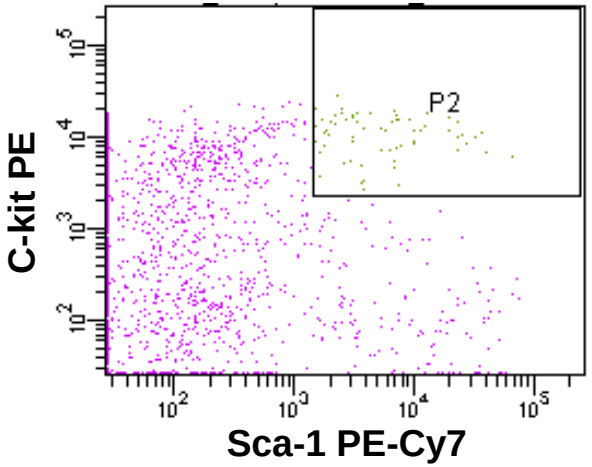
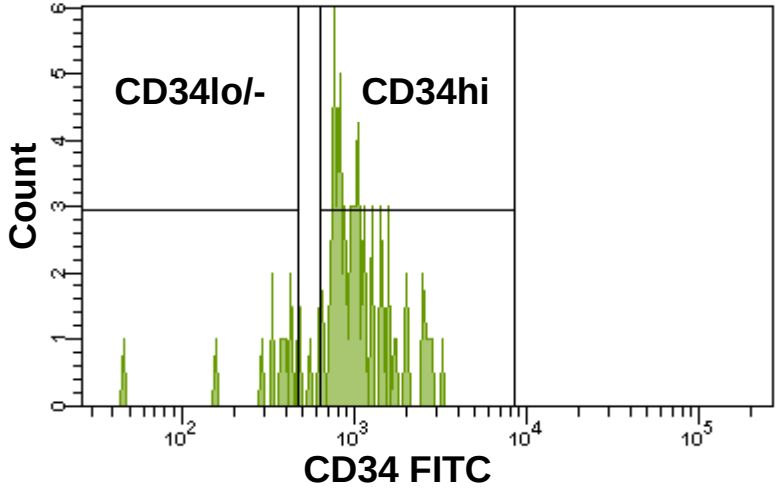
Mouse HSC Isolation Kit

C57 BM magnetic bead-enriched









Tube: pre sort BM_001

Population	#Events	%Parent	%Total
All Events	5,323	####	100.0
cells	2,608	49.0	49.0
SSC	2,548	97.7	47.9
FSC	2,527	99.2	47.5
P1	1,689	66.8	31.7
P2	66	3.9	1.2
P3	60	90.9	1.1
P4	6	9.1	0.1

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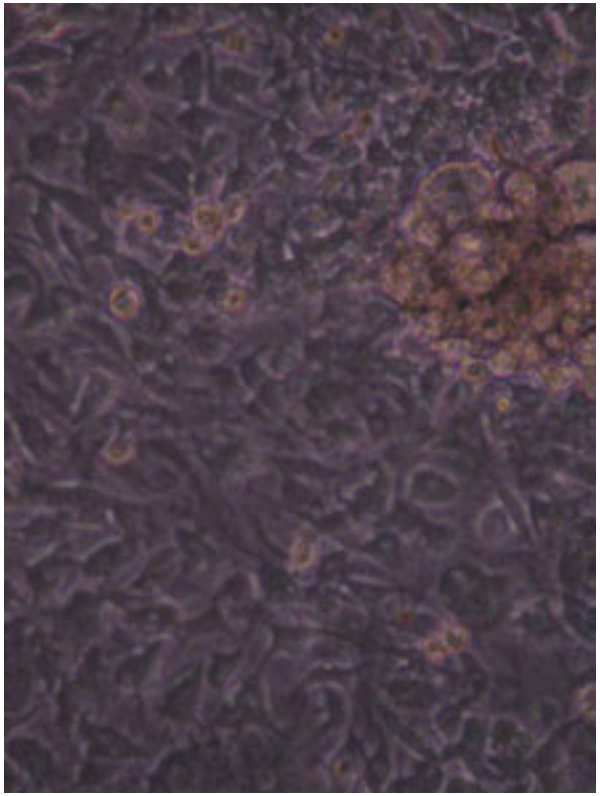
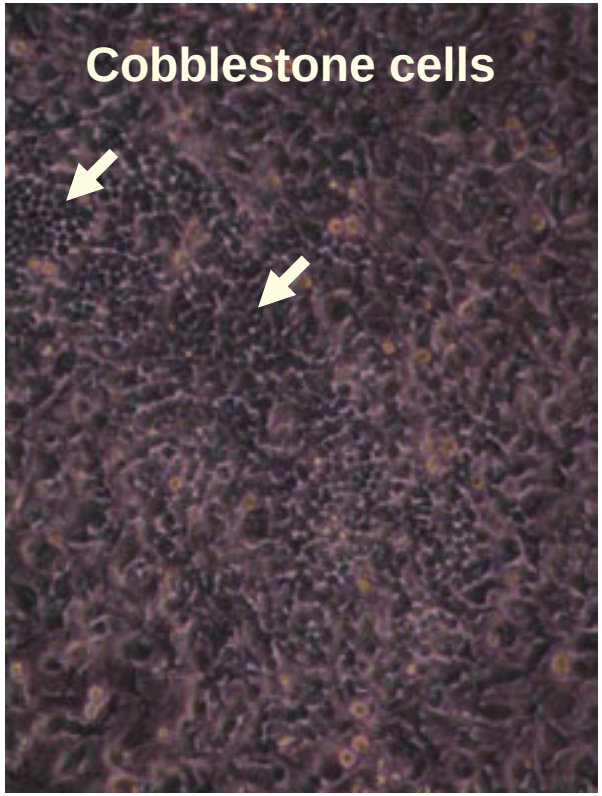
Mouse HSC Isolation Kit

Mitomycin-C treated M2-10B4 stromal cells

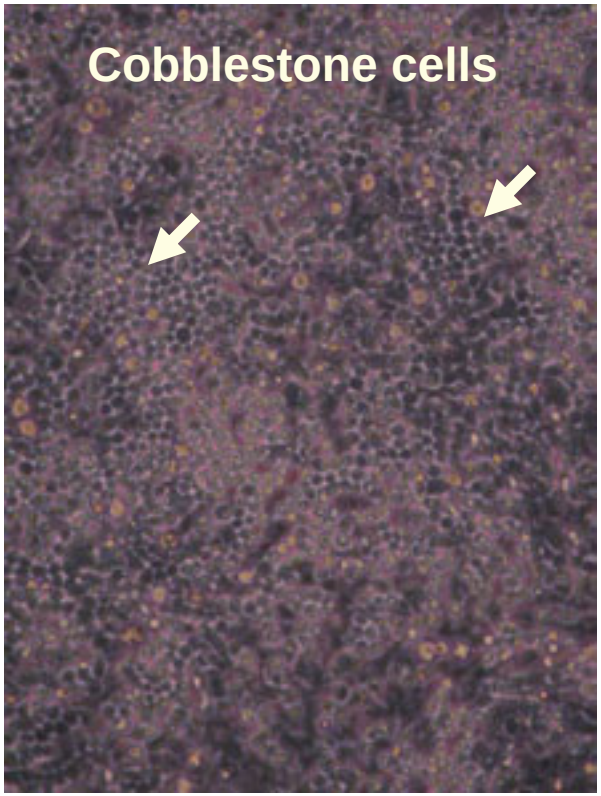
Unfractionated Bone Marrow



KLS CD34^{hi}



KLS CD34$^{lo/-}$



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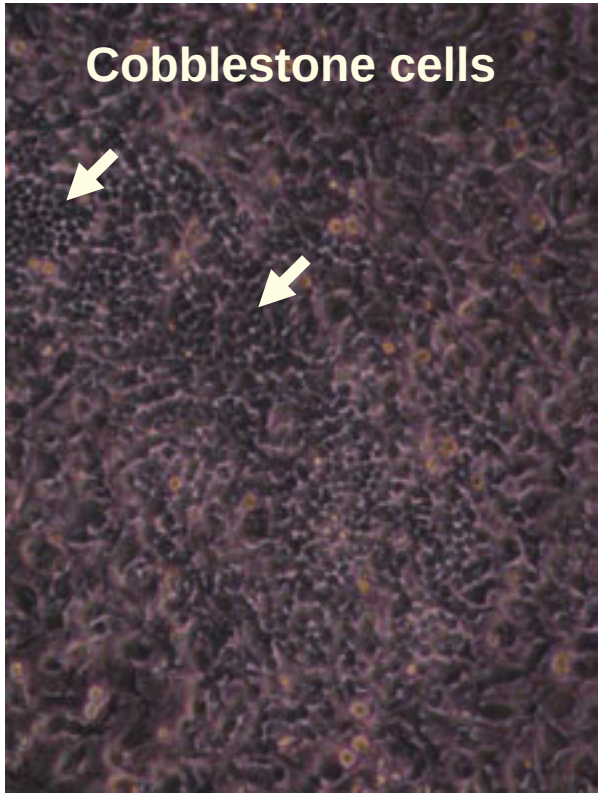
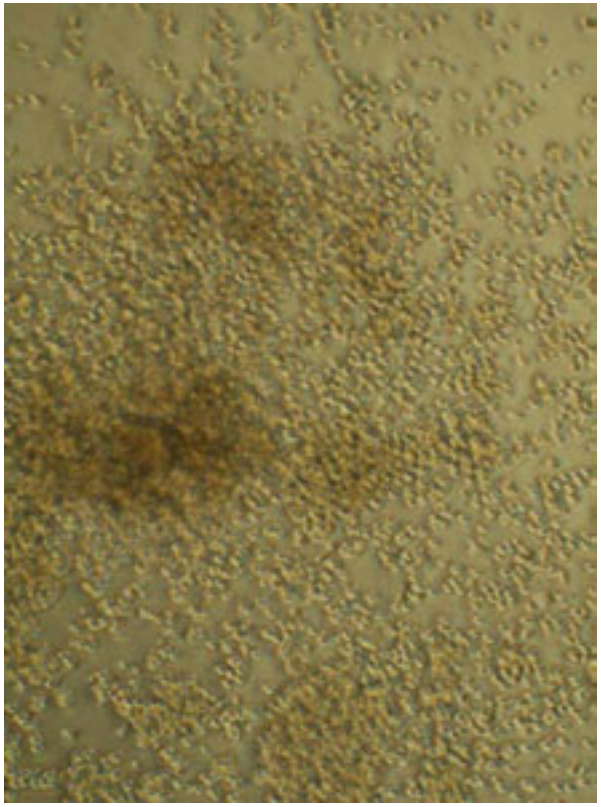
Mouse HSC Isolation Kit

Colony forming assay

KSL CD34^{hi}



CFU



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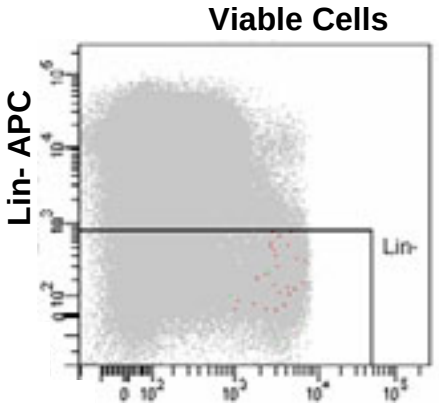
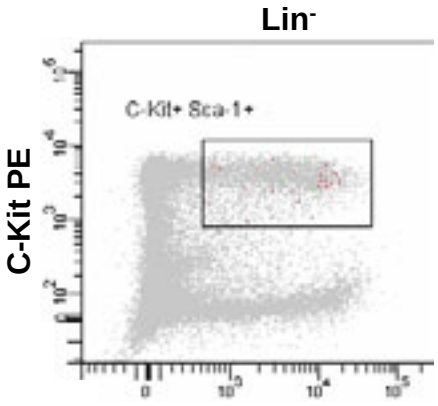
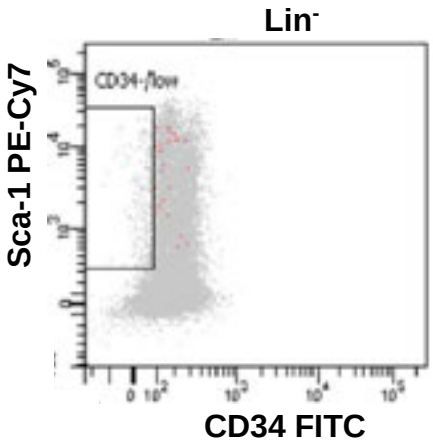
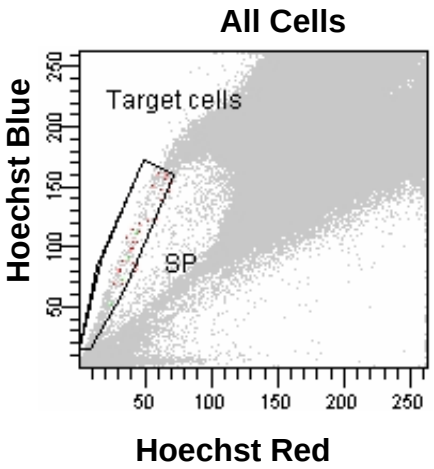
Mouse HSC Isolation Kit

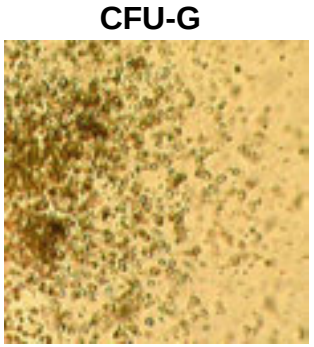
Side population, Kit$^+$, Sca-1$^+$, Lin$^-$, CD34$^-$ (SPKLS)

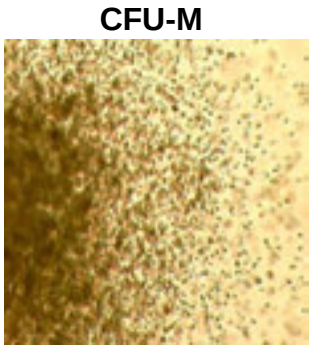


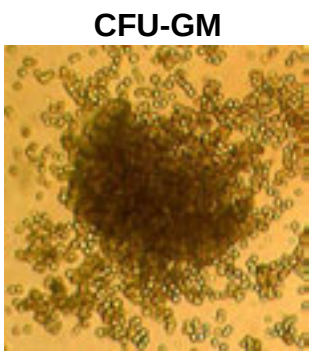












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Kits for Stem Cell Research

Kit	Sp	Antibodies	CS Analysis	IC Analysis	Sorting	Drop-ins	GFP
Human Pluripotent Stem Cell Sorting and Analysis Kit	Hu	SSEA-3 PE Tra-1-81 AlexaFluor® 647 SSEA-1 FITC	✓	-	✓	✓	-
Human and Mouse Pluripotency Analysis Kit	Hu Ms	Oct3/4 PerCP-Cy5.5 SSEA-4 AlexaFluor® 647 SSEA-1 PE	✓	✓	-	✓	✓
Human Pluripotency Analysis Kit-TF	Hu	Oct3/4 PerCP-Cy5.5 hNanog PE Sox2 AlexaFluor® 647	-	✓	-	✓	✓
Mouse Pluripotency Analysis Kit-TF	Ms	Oct3/4 PerCP-Cy5.5 mNanog PE Sox2 AlexaFluor® 647	-	✓	-	✓	✓
Mouse HSC Isolation Kit	Ms	c-Kit PE Sca-1 PE-Cy™7 CD34 FITC Lineage Cocktail APC	✓	-	✓	✓	-

- All kits contain BD™ CompBead Plus, matched isotype controls, and verified protocols
- IC Analysis kits contain fix and perm buffers

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Questions?

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