Deciphering the Cell Surface Proteome of Stem Cells Using Antibody Libraries

Christian Carson, PhD
Associate Director
Research & Development
BD Biosciences

23-14255-00

BD

Outline

- Background
- Tools for cell surface marker antibody screening:
  - BD Lyoplate™ screening panels
  - BD FACS™ CAP
- Applications:
  - Identification of surface signatures of neural cell types
  - Multiplexing antibody screens with intracellular flow cytometry
  - Increasing throughput with fluorescent cell barcoding

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The Devil is in the Details – Heterogeneity

Heterogeneity of cell cultures is a major challenge

Potential solutions:

- Defined cell surface signatures for stem cells and their derivatives
- Robust methods for cell sorting
- Quantitative analysis tools for heterogeneous cell cultures

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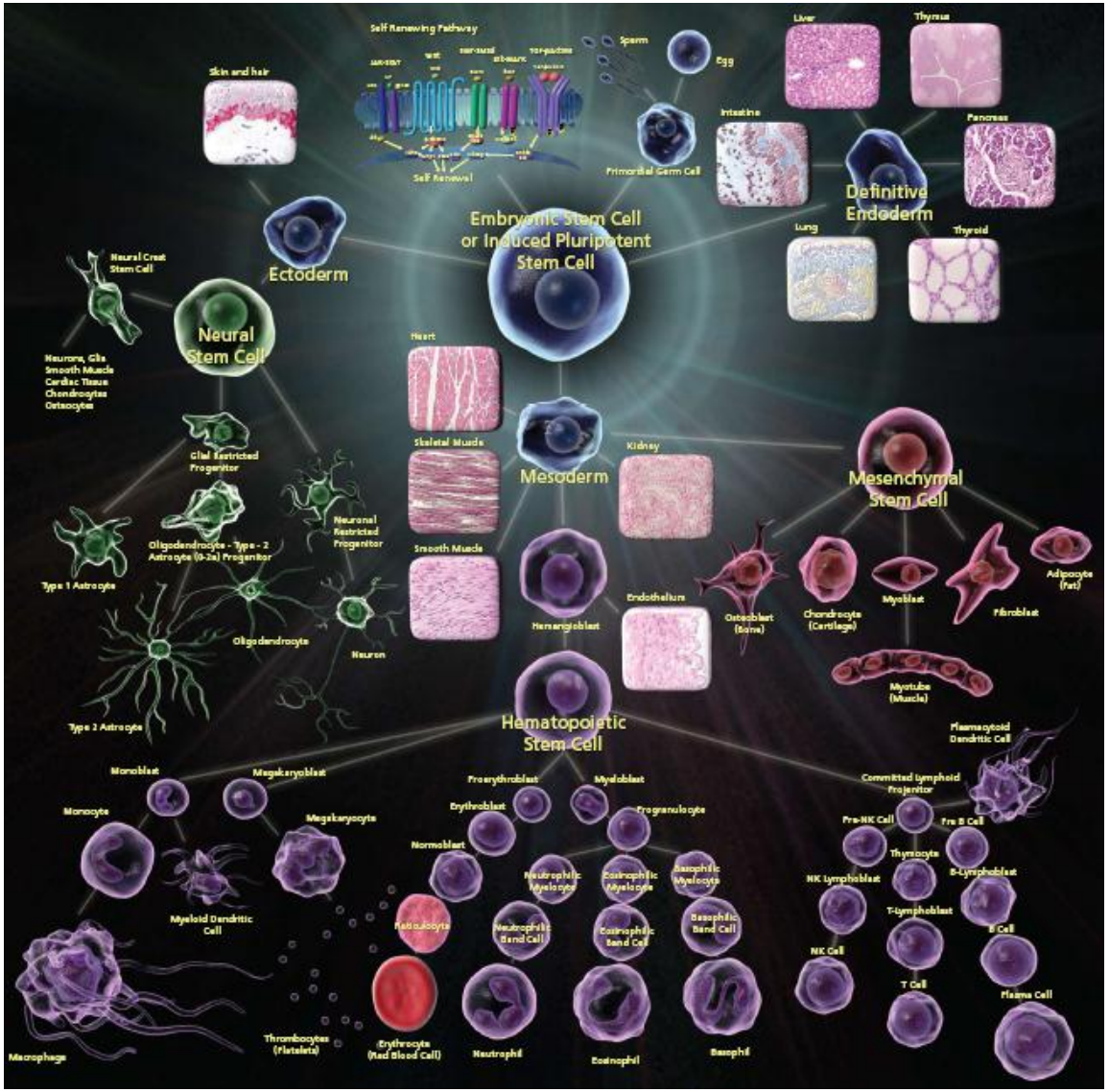
BD Biosciences Stem Cell Research



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BD Lyoplate Screening Panels

Enabling researchers to immunophenotype cell populations by flow cytometry or immunofluorescence microscopy using BD's portfolio of monoclonal antibodies

- Accelerate discovery of unique cell surface "signatures"
- A unique, cost-effective alternative to screening using hundreds of single-vial reagents

Product	Contents	Size
BD Lyoplate *Human* Cell Surface Marker Screening Panel Cat. No. 560747	• 242 CD Markers* • Isotype Controls • Alexa Fluor® 647 Second Step	5 tests
BD Lyoplate *Mouse* Cell Surface Marker Screening Panel Cat. No. 562208	• 176 CD Markers • Isotype Controls • Alexa Fluor® 647 Second Step	5 tests
*CD and other cell surface molecules. One marker per well.		

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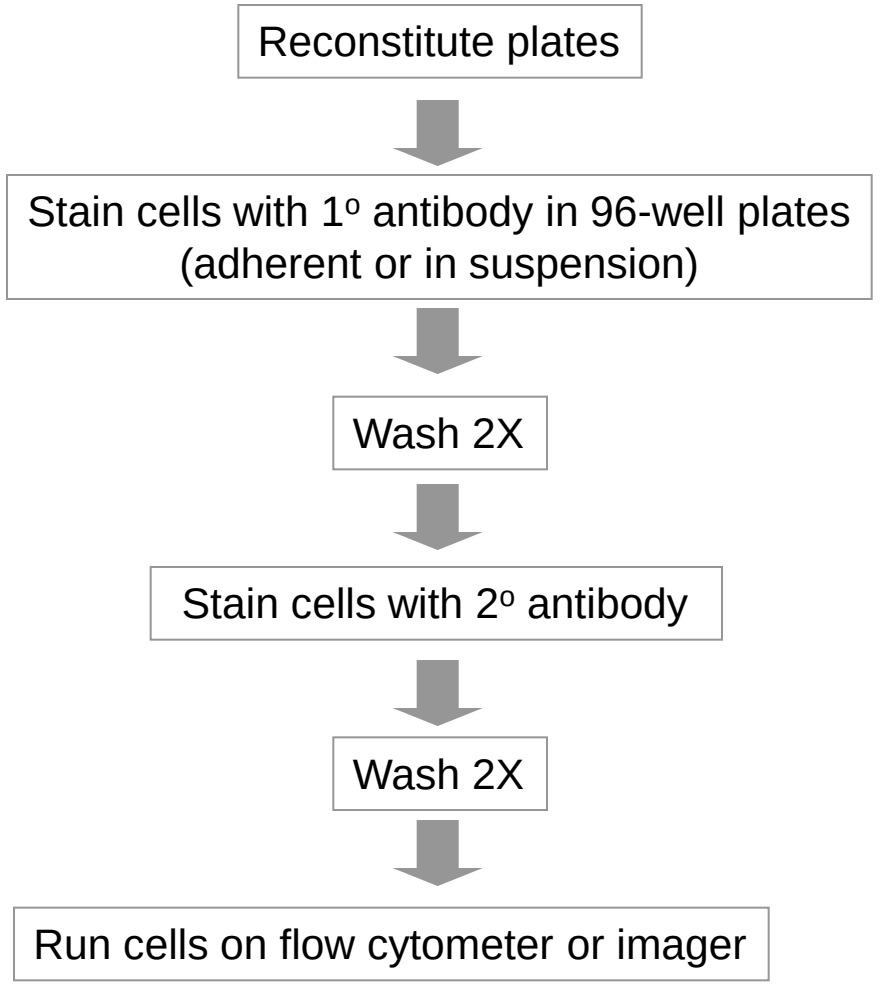
BD Lyoplate Screening Panels Overview

- The plate-based format is compatible with automation and multichannel pipetting.
- The proprietary lyophilized format allows for room-temperature storage.
- Open wells permit the use of additional markers of choice.
- May be combined with drop-in conjugates.
- Compatible with BFP, CFP, GFP, YFP, OFP, and RFP-expressing cells.
- All antibodies are available separately in conjugated formats or kits for building post-screen panels using markers of interest.
- Microsoft® Excel® data analysis templates are available.



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BD Lyoplate Screening Panels Analysis Template

Plate 1 mean (MFI)

Buffer	CD1a	CD1b	CD1d	CD2	CD3	CD4	CD4V4	CD5	CD6	CD7	CD8a
CD8b	CD9	CD10	CD11a	CD11b	CD11c	CD13	CD14	CD15	CD15s	CD16	CD18
CD19	CD20	CD21	CD22	CD23	CD24	CD25	CD26	CD27	CD28	CD29	CD30
CD31	CD32	CD33	CD34	CD35	CD36	CD37	CD38	CD39	CD40	CD41a	CD41b
CD42a	CD42b	CD43	CD44	CD45	CD45RA	CD45RB	CD45RO	CD46	CD47	CD48	CD49a
CD49b	CD49c	CD49d	CD49e	CD50	CD51/61	CD53	CD54	CD55	CD56	CD57	CD58
CD59	CD61	CD62E	CD62L	CD62P	CD63	CD64	CD66 (a,c,d,e)	CD66b	CD66f	CD69	CD70
CD71	CD72	CD73	CD74	CD75	CD77	cd79B	CD80	CD81	CD83	CD84	CD85

- Microsoft Excel 2007
- Reorganizes data into corresponding 96-well format
- Performs normalization to isotype controls
- Analysis of % positive, median fluorescence intensity (MFI), bimodality, and normalized values
- Thresholding allows for analysis of specific cell populations
- Templates available for BD FACSDiva™ and FlowJo™ software
- http://www.bdbiosciences.com/support/resources/stemcell/index.jsp#stemtools

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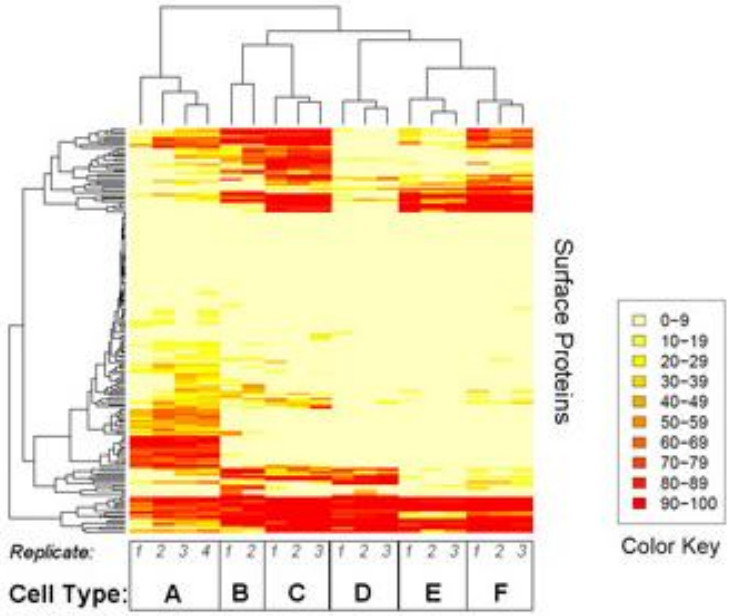
BD FACS CAP Service

BD FACS CAP (Combinatorial Antibody Profile) is a custom multicolor immunophenotyping and analysis service.



Graphical representation of BD FACS CAP screen showing relative expression of cell surface markers on multiple cell types from multiple donors. Color key represents percentage of positive cells.

The in-depth analysis service provides an inventory of markers present on the cell surface, yielding an information-rich “fingerprint.”

- 212 fluorescently-labeled anti-human antibodies
- Multicolor cocktails in each well of a 96-well plate
- Flexibility to integrate researchers’ specific markers
- Analysis supported by proprietary software

www.bdbiosciences.com/services

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Applications

OPEN ACCESS Freely available online

PLoS one

Cell-Surface Marker Signatures for the Isolation of Neural Stem Cells, Glia and Neurons Derived from Human Pluripotent Stem Cells

Shauna H. Yuan[1,2], Jody Martin[3], Jeanne Elia[3], Jessica Flippin[1], Rosanto I. Paramban[3], Mike P. Hefferan[4], Jason G. Vidal[3], Yangling Mu[5], Rhiannon L. Killian[1,6], Mason A. Israel[1,6], Nil Emre[3], Silvia Marsala[4], Martin Marsala[4,7], Fred H. Gage[5], Lawrence S. B. Goldstein[1], Christian T. Carson[3]*

1 Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, School of Medicine, University of California San Diego, La Jolla, California, United States of America, **2** Department of Neurosciences, School of Medicine, University of California San Diego, La Jolla, California, United States of America, **3** BD Biosciences, La Jolla, California, United States of America, **4** Anesthesiology Research Laboratory, Department of Anesthesiology, University of California San Diego, La Jolla, California, United States of America, **5** Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, California, United States of America, **6** Biomedical Sciences Graduate Program, University of California San Diego, La Jolla, California, United States of America, **7** Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovakia

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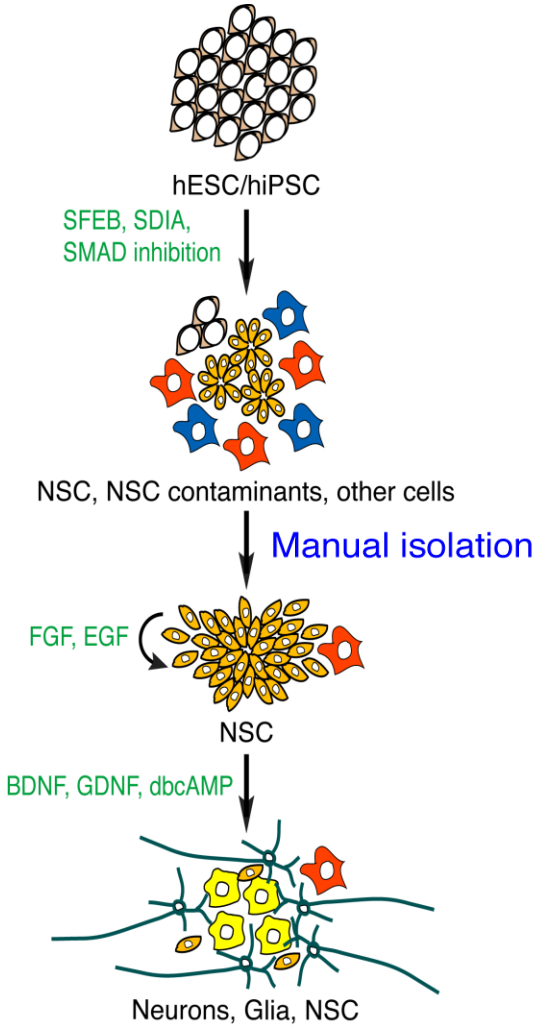
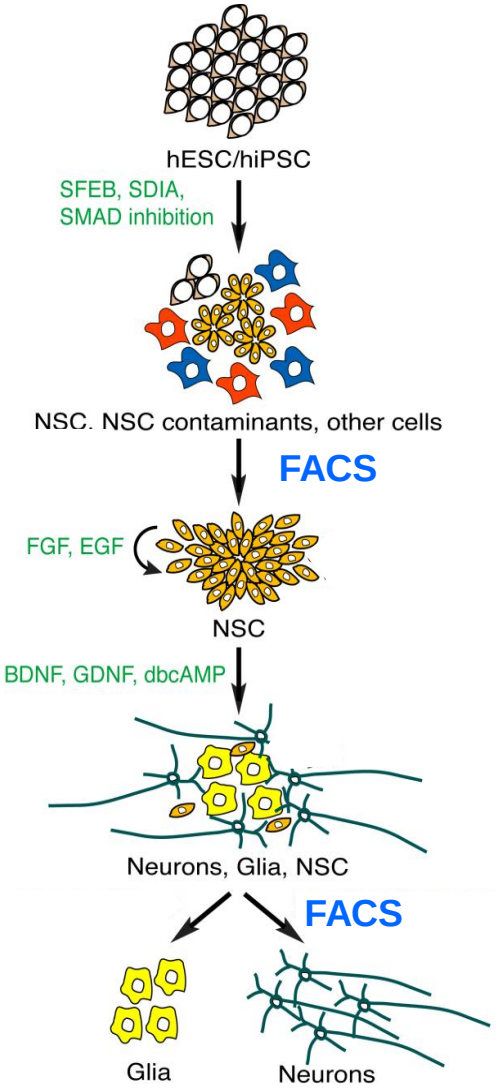
Neural Induction of Pluripotent Stem Cells

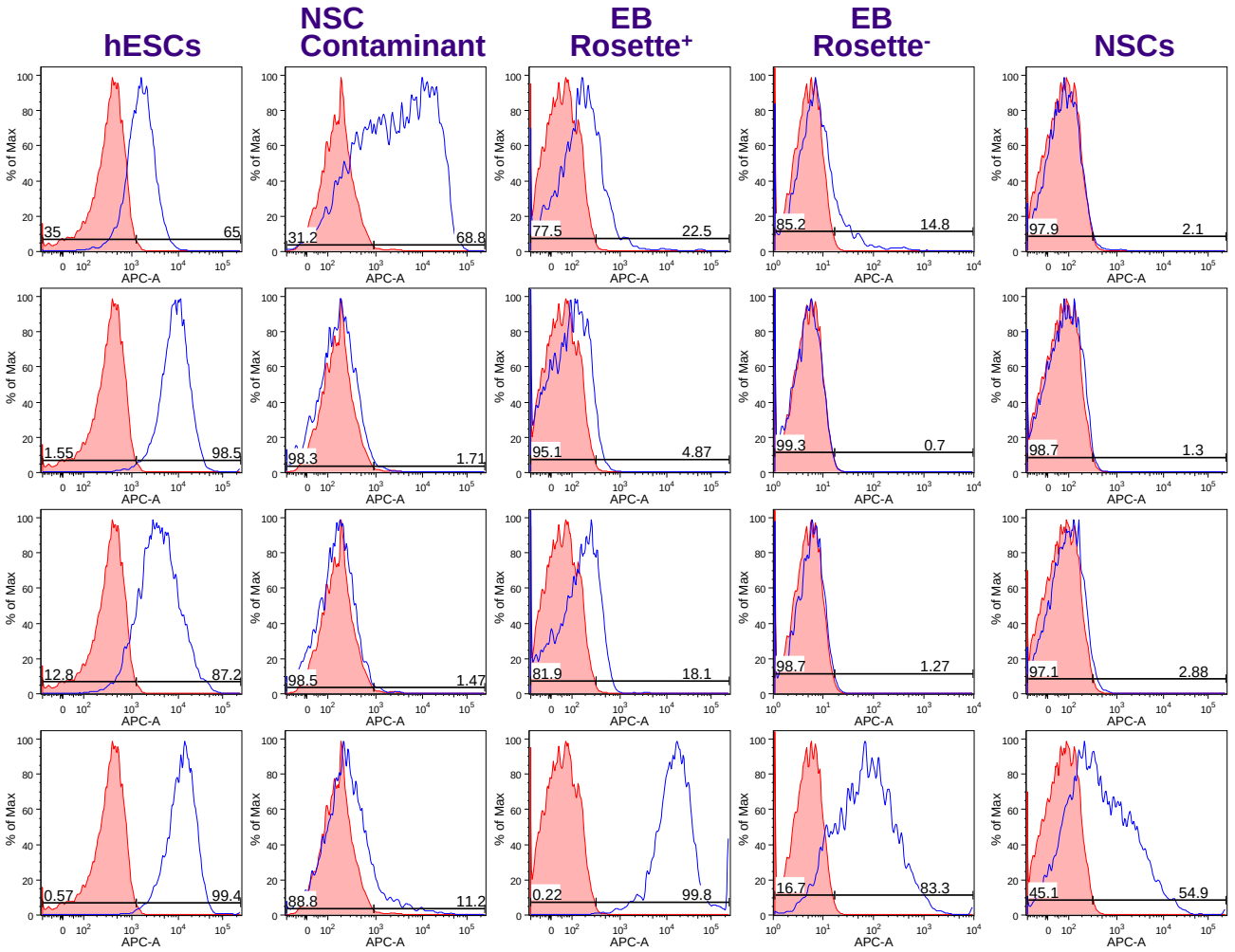


Cell sorting by flow cytometry using unique cell signatures of sub-populations provides:

- Efficient standardized method for isolating NSCs to eliminate batch-to-batch variability
- Robust standardized methods for isolating terminally differentiated neurons
- Identification of transplantable cell types that do not cause tumors

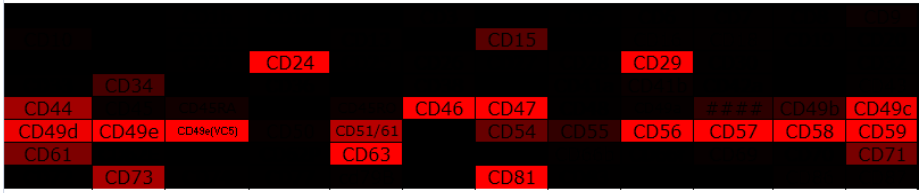


Cell Surface Marker Screen – Fluorescence Activated Cell Sorting (FACS)

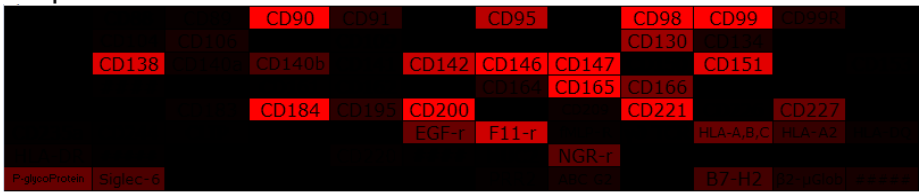


Analysis

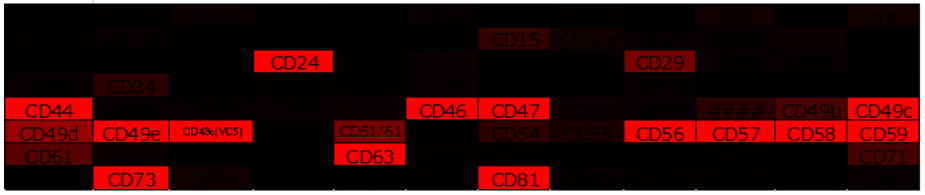
% positive Plate 1



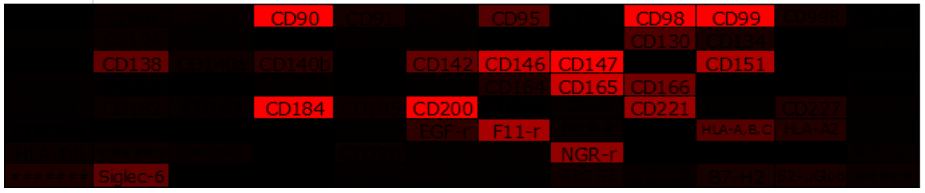
% positive Plate 2

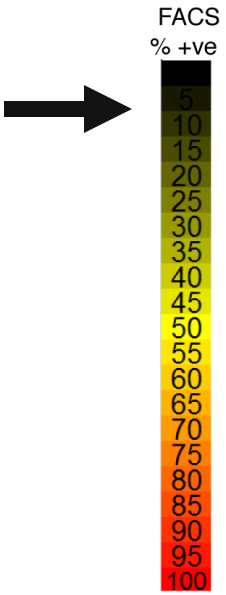


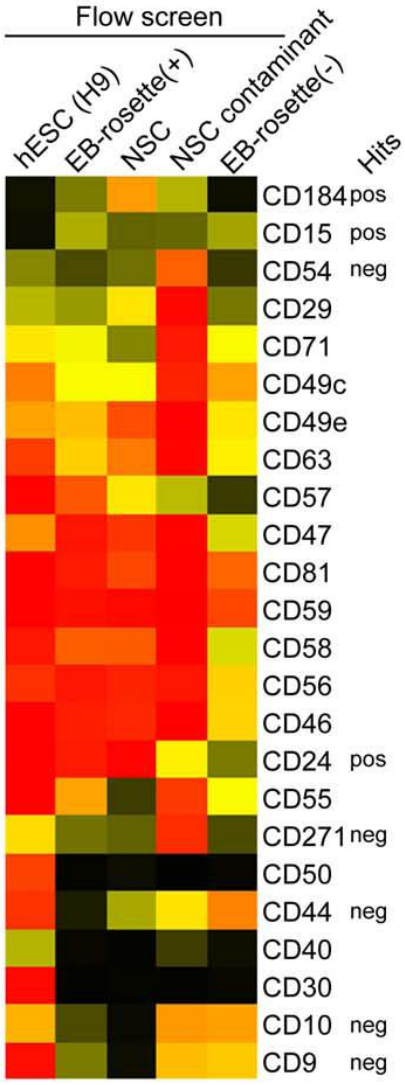
MFI Plate 1



MFI Plate 2

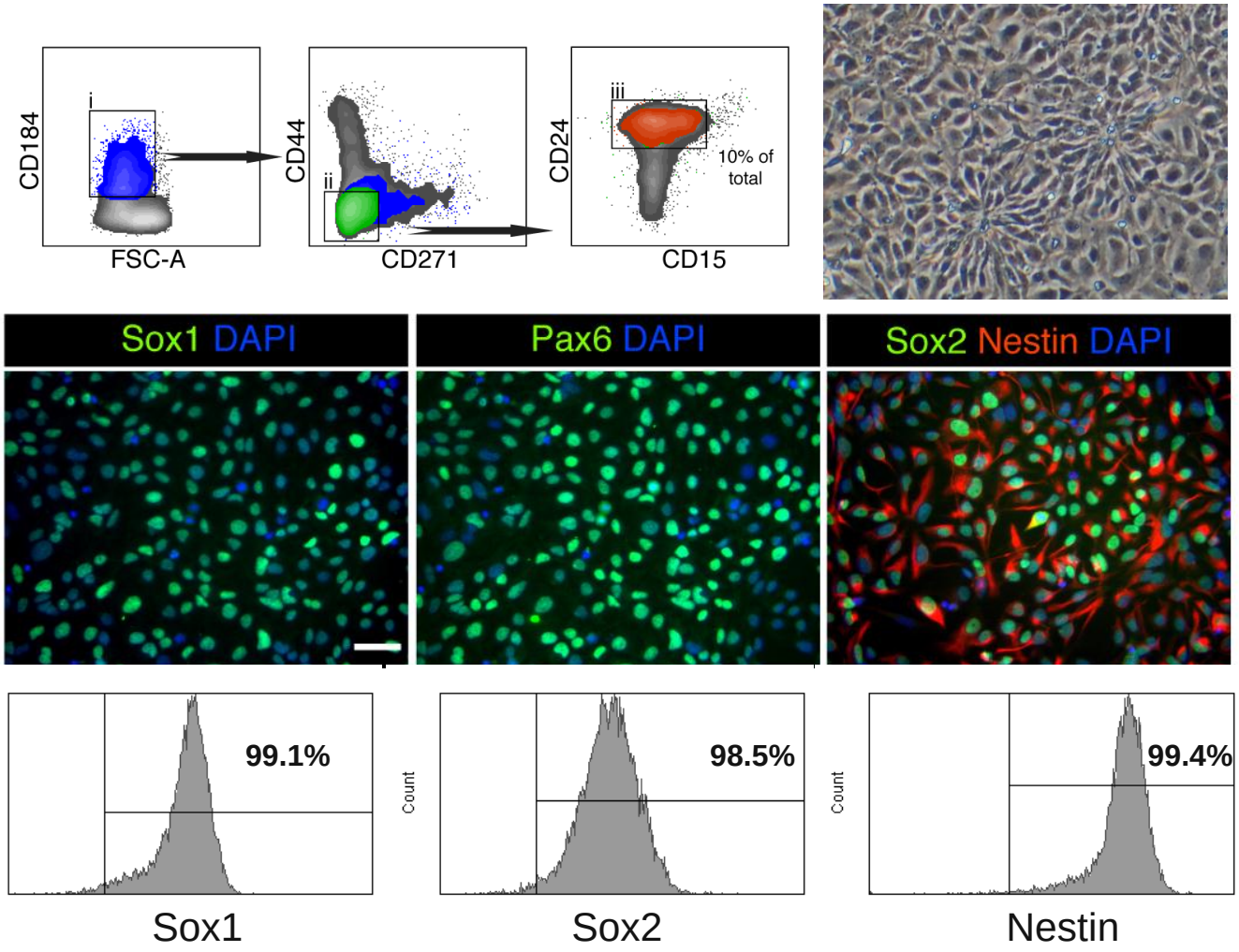






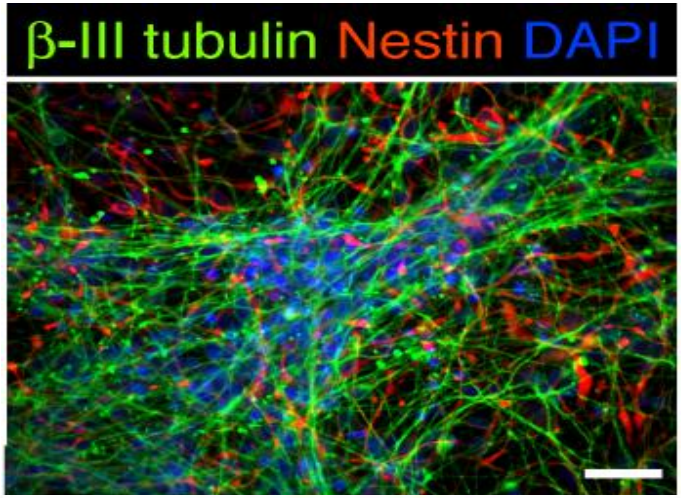
http://www.bdbiosciences.com/support/resources/stemcell/index.jsp#stemtools

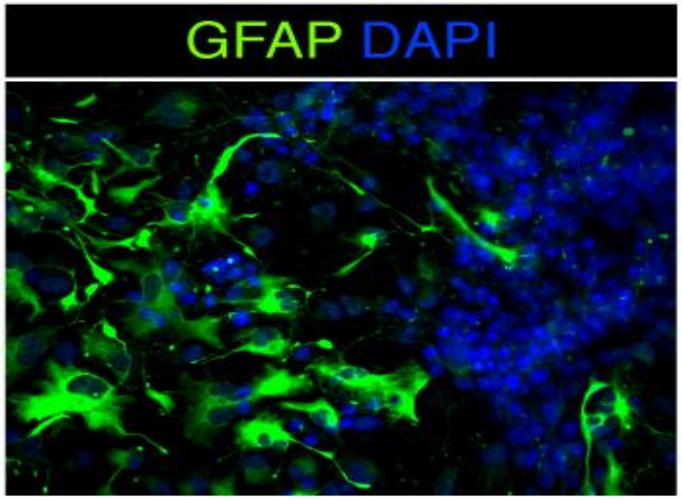
$CD184^{+}/CD44^{-}/CD271^{-}/CD24^{+}$ Define a Cell Surface Signature for the Isolation of NSCs



Sorted NSCs Differentiate to Mixed Cultures of Neurons and Glia and Differentiate to Neurons in vitro and in vivo

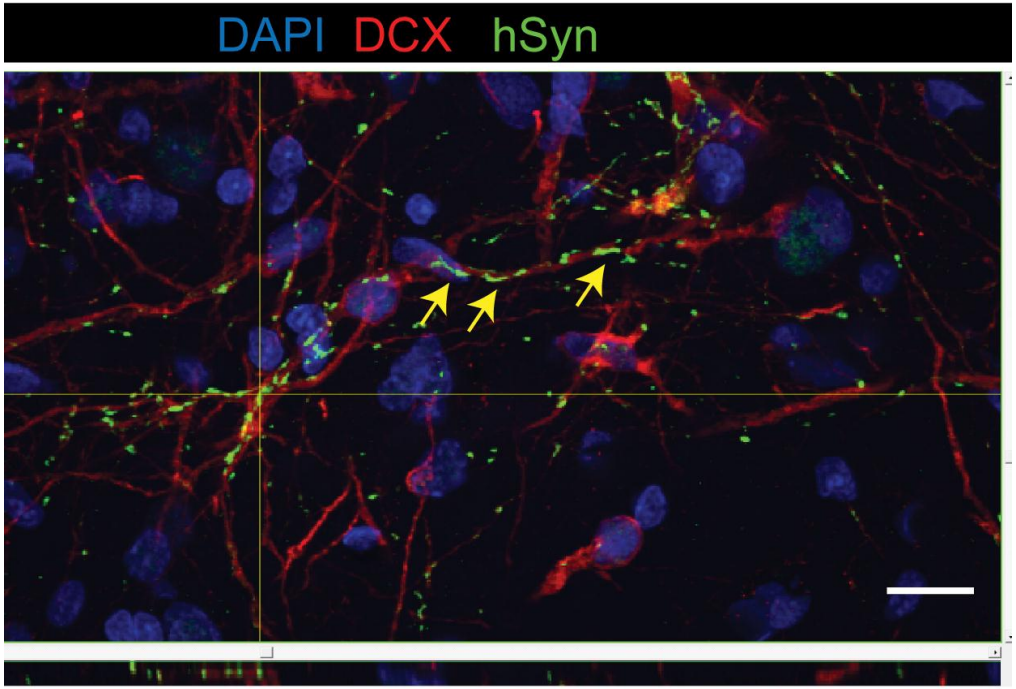
H9 NSCs differentiated for 3 weeks





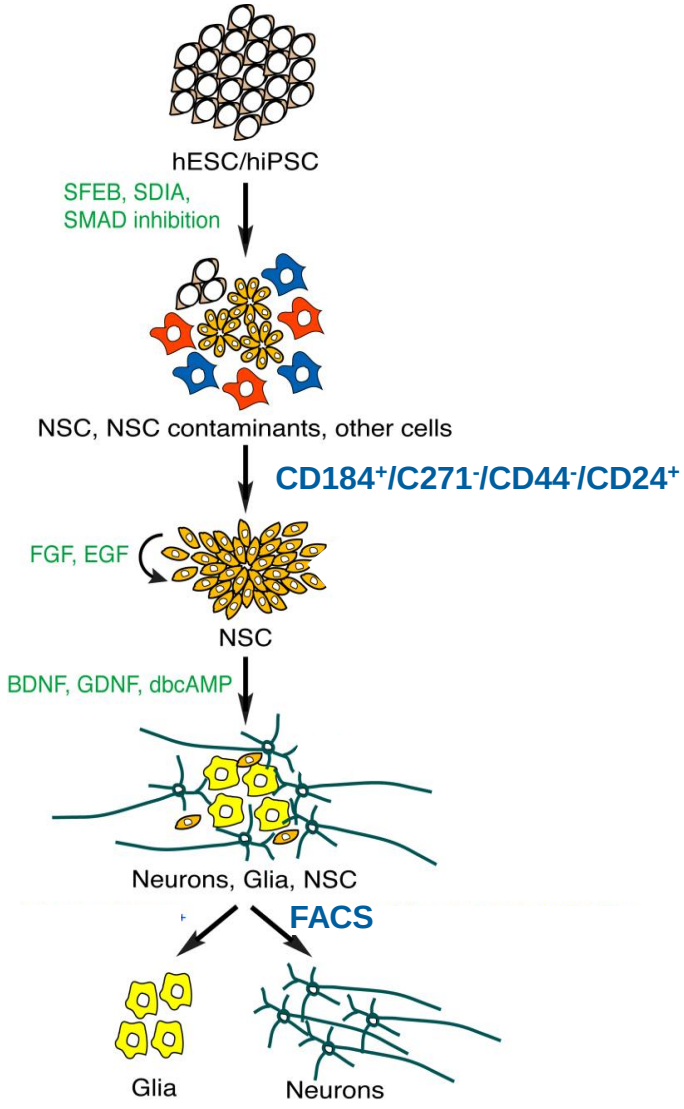
β-III tubulin = neurons, Nestin = NSCs, GFAP = astrocytes

HUES-9 NSCs 8 weeks post-engraftment in rat spinal cord



Images courtesy of Mike Hefferan and Martin Marsala, University of California, San Diego

Summary


hESC/hiPSC
SFEB, SDIA,
SMAD inhibition
NSC, NSC contaminants, other cells
CD184+/C271-/CD44-/CD24+
FGF, EGF
NSC
BDNF, GDNF, dbcAMP
Neurons, Glia, NSC
FACS
Glia
Neurons

Cell Surface Marker Screen – Imaging

Differentiate NSCs in 96-well plates for 3 weeks

↓

Stain cells with 1° and 2° antibodies live

↓

Fix and perm cells

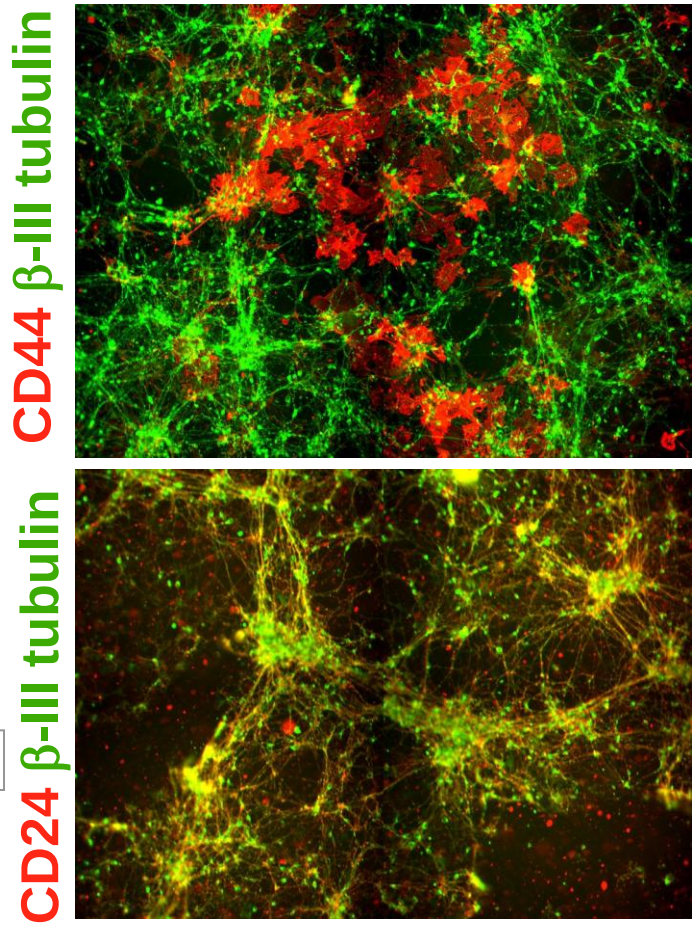
↓

Stain cells with anti-β-III tubulin

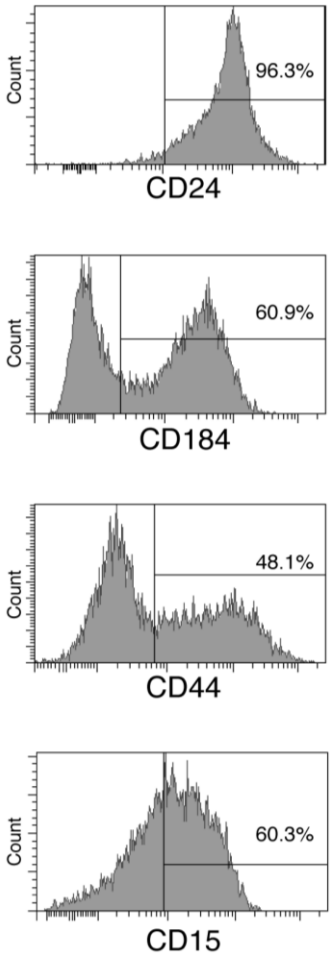
↓

Analyze cells on BD Pathway™ 435 bioimager

Examples from imaging screen



Hit verification from imaging screen



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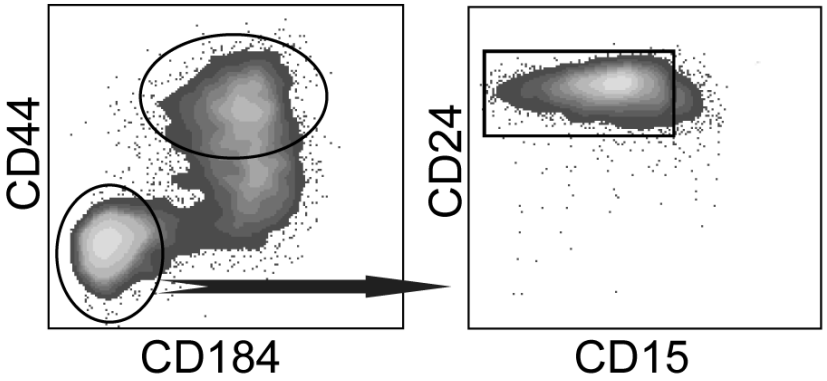
Isolation of Neurons and Glia by Sorting

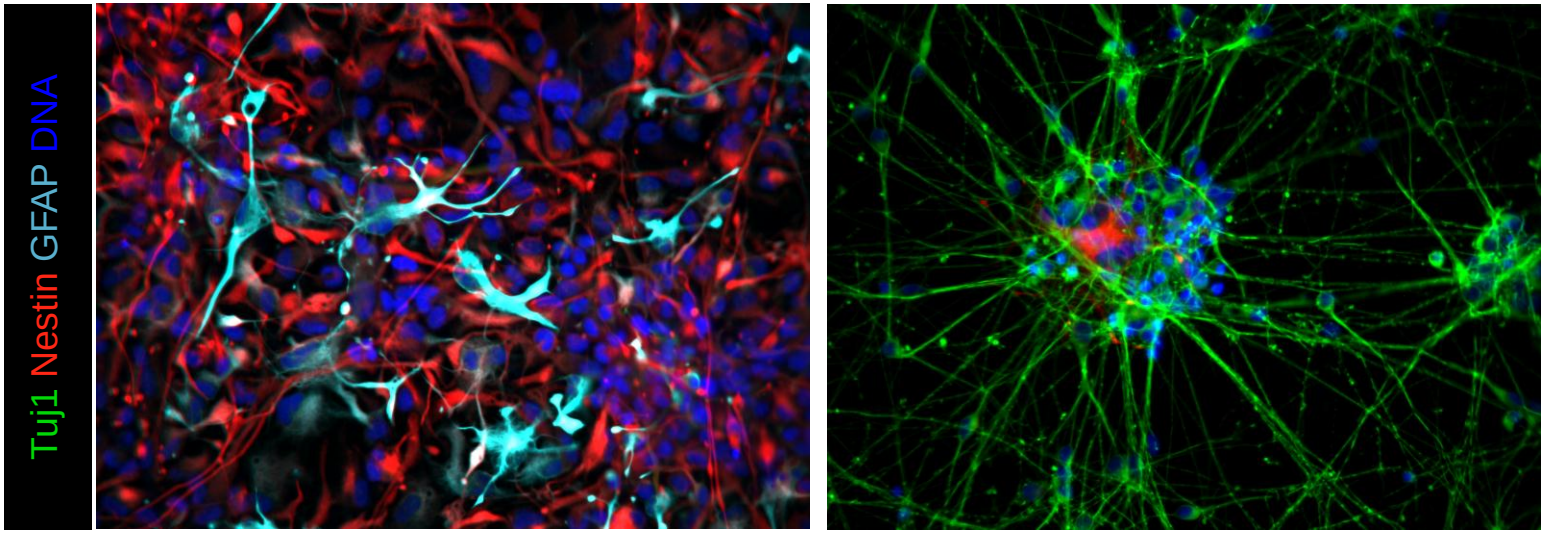


Glia

$CD184^{+}/CD44^{+}$

Neurons

$CD184^{-}/CD44^{-}/CD15^{LOW}/CD24^{+}$



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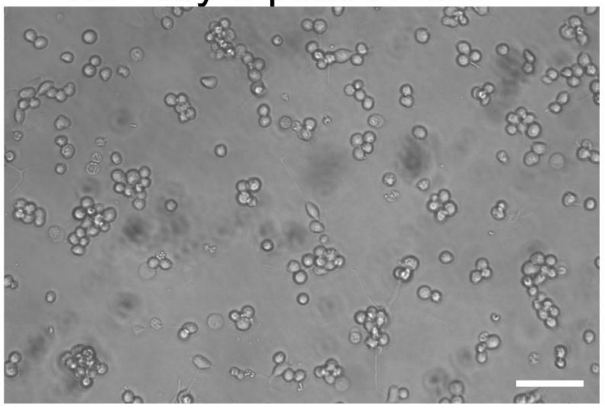
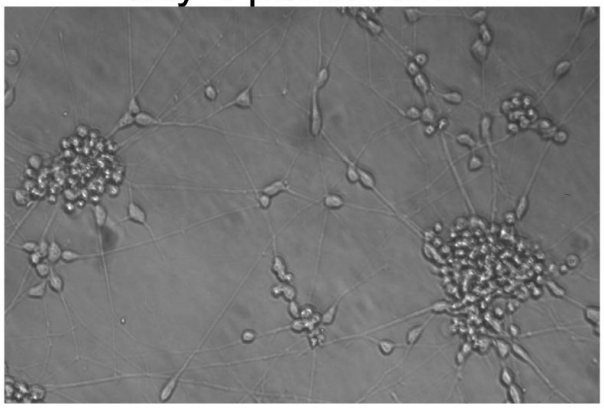
Sorted Neurons are Viable and Functional

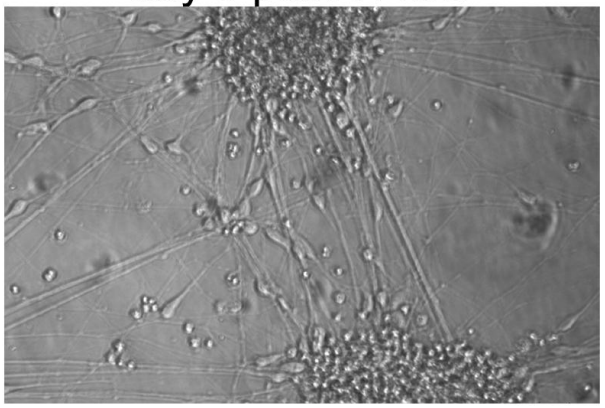
H9 day 0 post-FACS

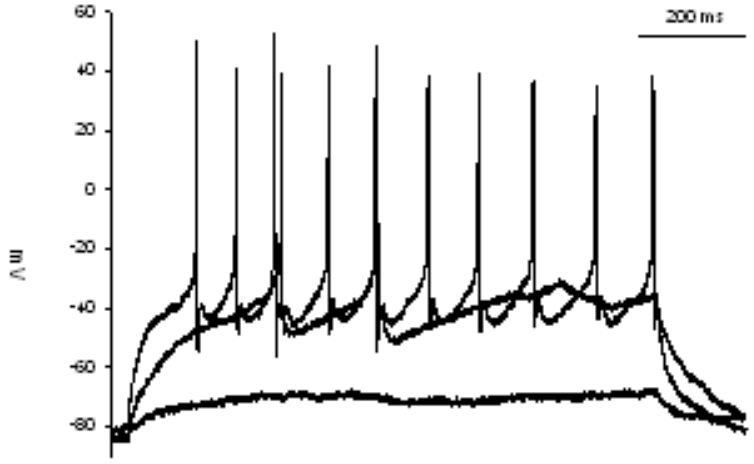


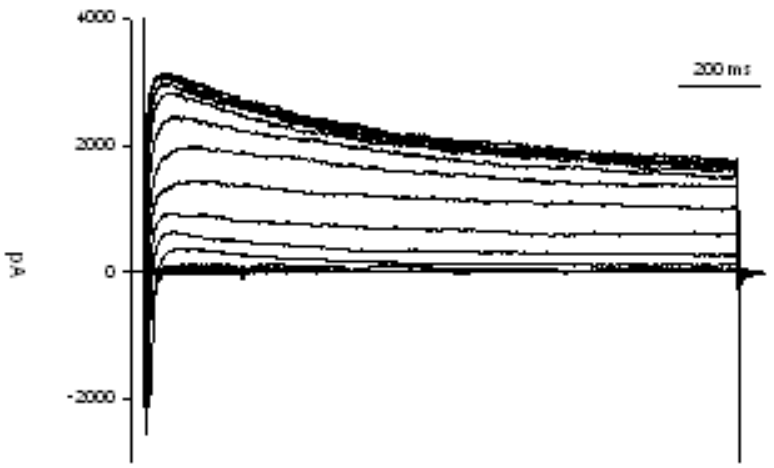
day 4 post-FACS



day 8 post-FACS







Data courtesy of Yangling Mu and Fred H. Gage, Salk Institute

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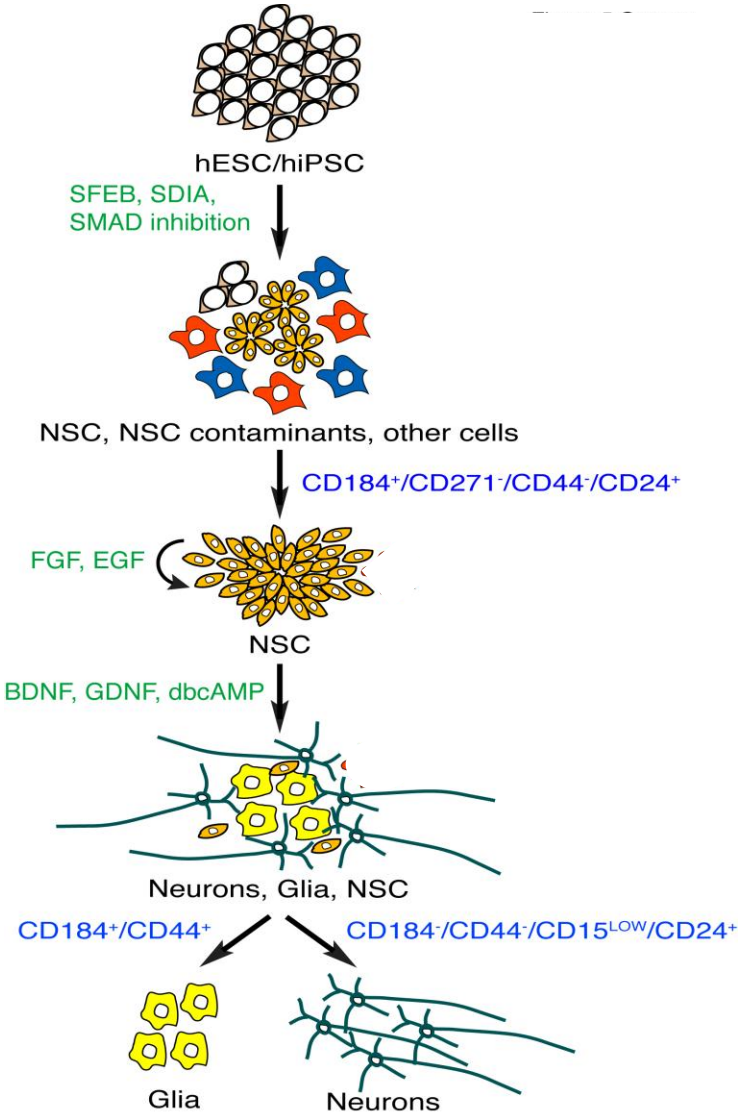
Summary



BD Stemflow™ Neural Cell Sorting kit

Specificity	*Isotype*	*Clone*	*Format*
	Antibody Conjugates		
CD24	mIgG$_1$	ML5	PE
CD271	mIgG$_1$	C40-1457	PerCP-Cy™5.5
CD44	mIgG$_1$	G44-26	PerCP-Cy5.5
CD15	mIgM	HI98	PE-Cy™7
CD184	mIgG$_1$	12G5	APC

Nature. 2012 Jan 25;482(7384):216-20. doi: 10.1038/nature10821.

Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells.

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LS.

Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California 92093, USA.

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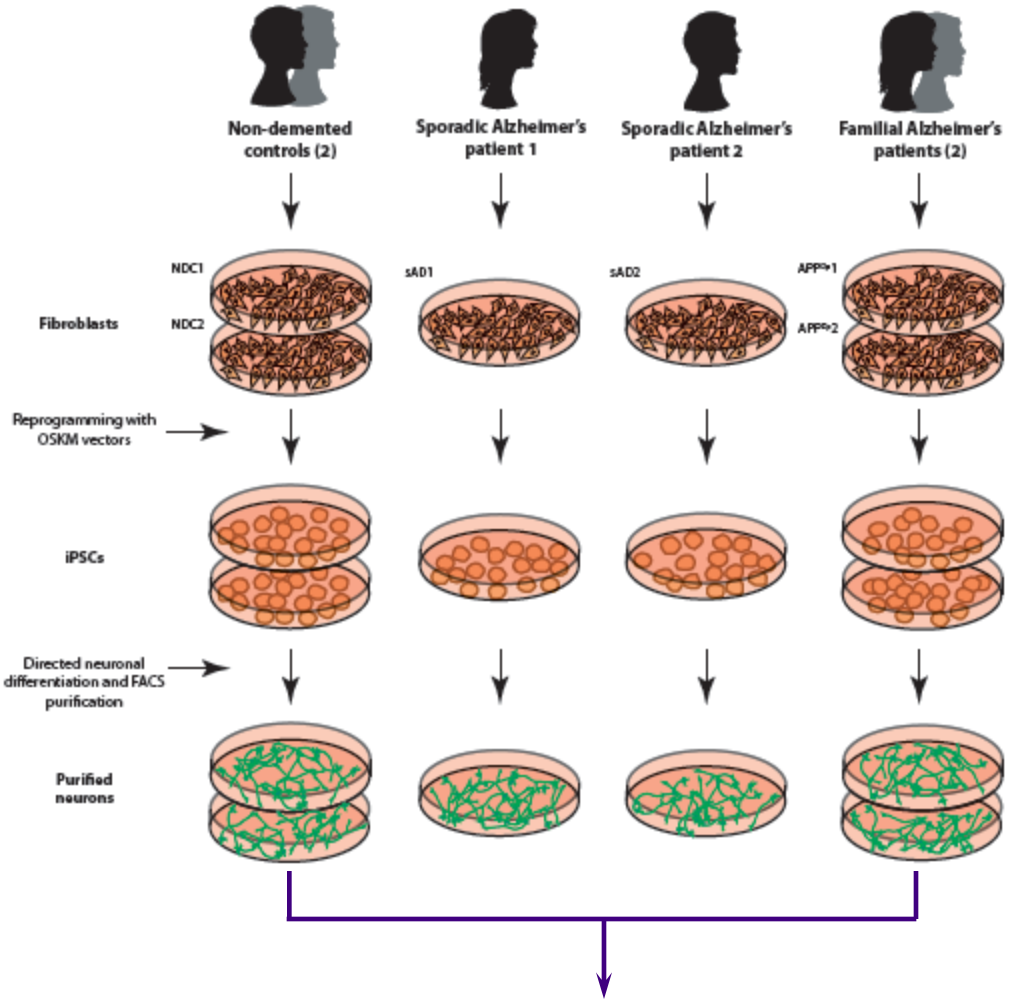
Nature doi:10.1038/nature10821



Alzheimer's disease biochemical marker comparative analysis

BD

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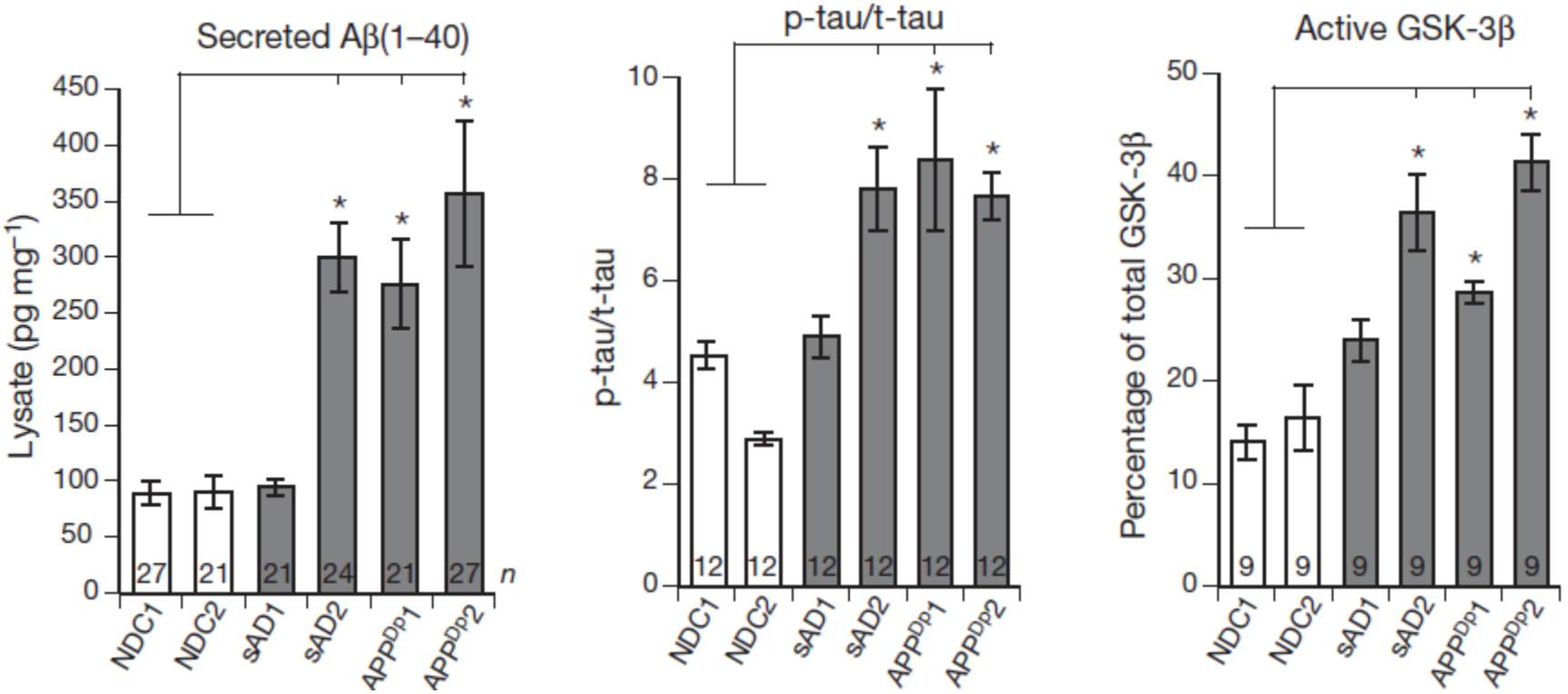

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BD Lyoplate Screen with Co-staining 3-week Neural Induction Cultures

Neural induction cultures

Disassociate cultures

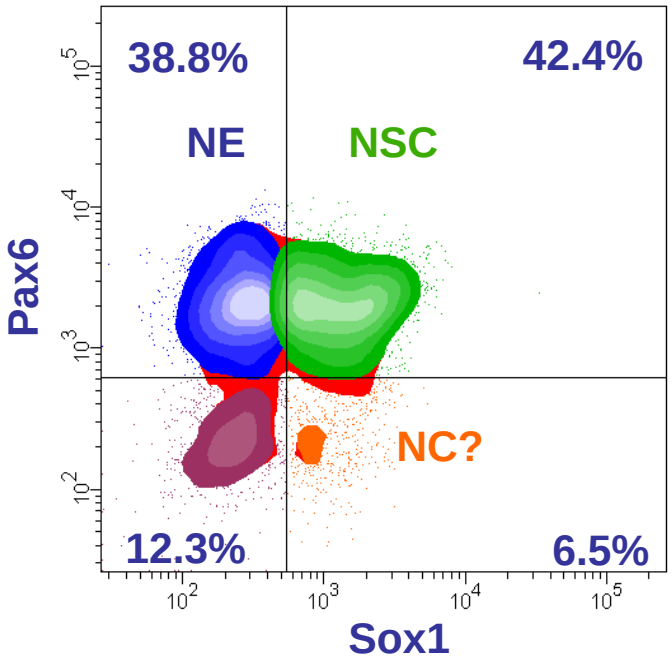
Stain with BD Lyoplate™ Human Cell Surface Marker Screening Panel

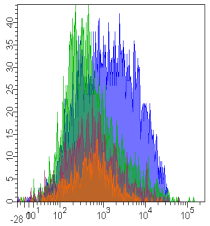
Fix Perm

Stain with Sox1, Sox2, Pax6, and DCX

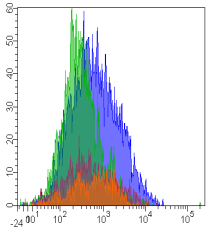
HTS Flow Cytometer

Analyze cell surface marker expression of populations defined by transcription factor expression

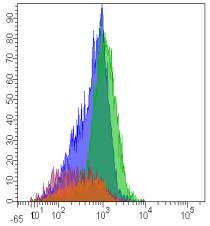




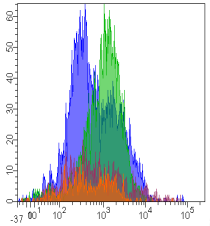
SSEA-4



CD166



CD49b

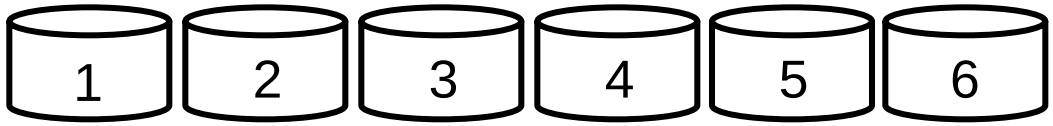


CD146

BD

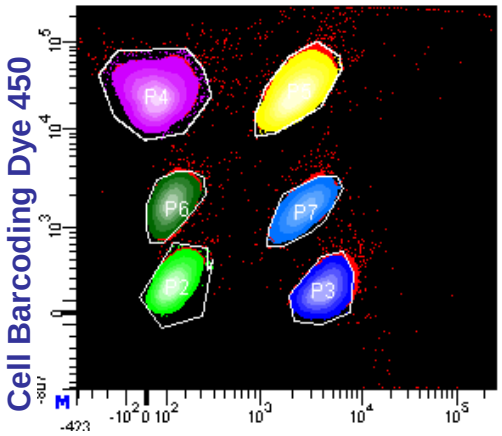
Fluorescent Cell Barcoding to Increase Throughput

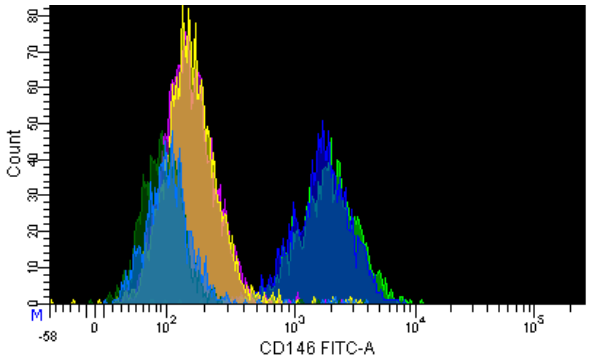
Label each sample with a different concentration of two dyes



Combine samples into one well or tube

Screen 242 mAbs by sorting and analyze data

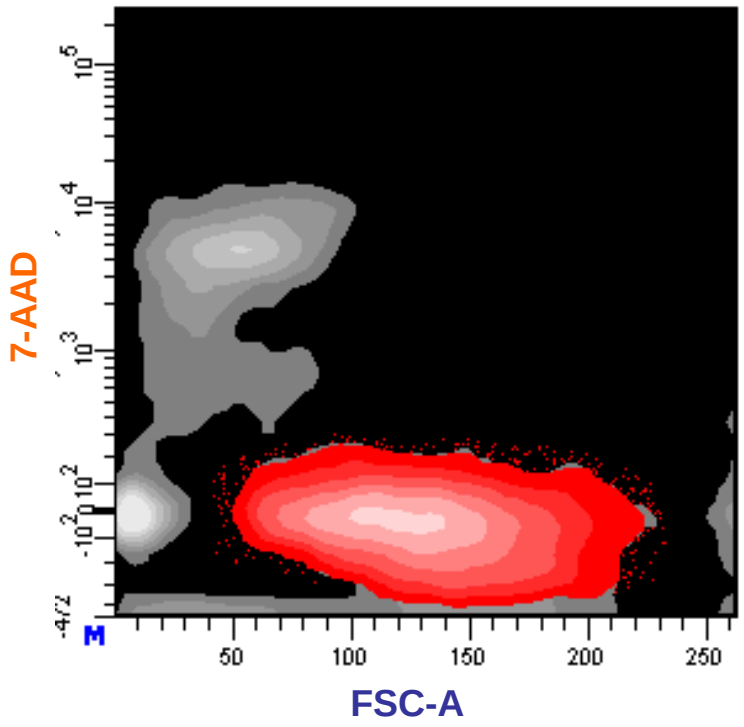


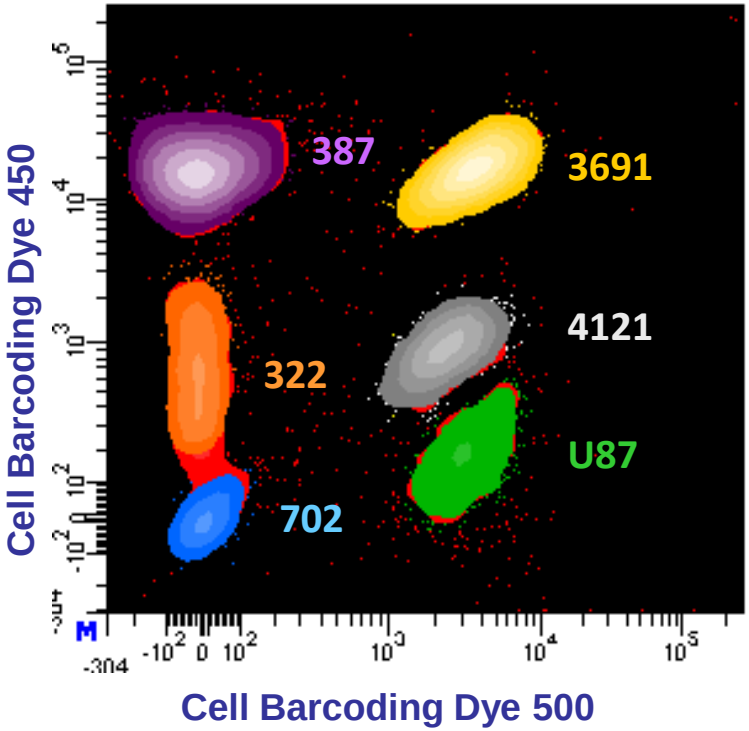


Analyze multiple samples from one tube

BD

Identification of Prospective Cell Surface Signatures of CSCs in Human Gliomas by Immunophenotyping





Images courtesy of Justin Lathia and Jeremy Rich, Cleveland Clinic

BD

Identification of Prospective Cell Surface Signatures of CSCs in Human Gliomas by Immunophenotyping

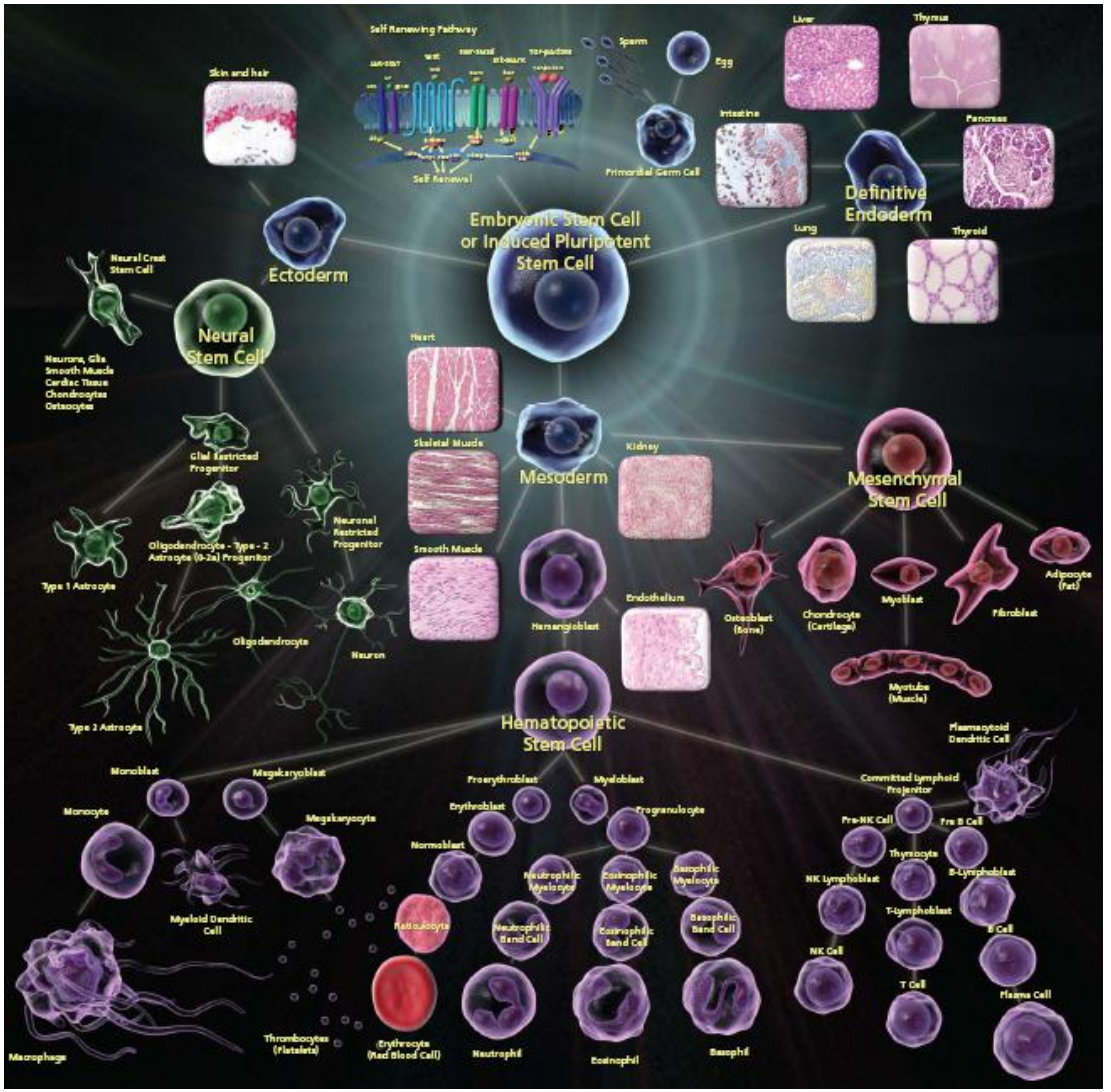
3691	4121	4302	322	387	619	702	U87
47.7	25.3	0.65	6	43	7.96	3.3	25.3
32.4	50.4	12.69	48.3	60.3	2.97	22.2	50.4
32.1	34.2	19.84	55.7	17.7	4.25	38.4	34.2
29.7	24.1	28.28	22.6	37.7	3.12	4.3	24.1
28.5	22.9	16.99	50.9	41.7	8.34	41.9	22.9
2	0.1	1.26	14.7	49.8	0.68	1.5	0.1
18.5	20.5	1.33	5.9	26.9	11.65	24.5	20.5
16.5	9.6	28.27	7.4	71.8	45.02	33.5	9.6
15.9	27.4	18.25	6.2	39.2	6.02	4.3	27.4
15.5	0.8	34.75	6.2	35.5	18.93	2.4	0.8
14.9	22	2.94	35	16.4	3.64	16.4	22
13	37.9	3.98	3	28.7	17.12	8.8	37.9
11.6	38.6	21.58	13.2	8.4	8.51	11.9	38.6
11.3	61.7	2.13	3.4	17.1	17.11	10.5	61.7
10.1	10.9	10.18	4.8	14.5	8.16	9	10.9
9.1	6.6	33.05	4.5	5.9	35.71	15	6.6
7.4	4.7	30.21	3.9	5	38.58	11.7	4.7
7.1	3.3	19.56	4	5.1	23.13	25.4	3.3
6.3	4.9	2.1	0.4	5.1	1.39	0.3	4.9
4.9	6.2	2.24	5.7	10	19.55	14.8	6.2
3.4	17.1	1.44	59.4	11.8	38.99	59.7	17.1
3.3	18	1.44	10.6	4.8	83.74	31.5	18
2.1	1.9	0.85	4.5	2.9	47.93	18.4	1.9
1.8	40.2	1.2	4.5	62.6	2.36	4.8	40.2
1.5	13.3	2.86	9.8	2.3	32.89	32.8	13.3
0.6	14.2	0.35	11.2	1.9	42.24	44.4	14.2
0.6	4.1	0.63	62.4	1.7	5.2	50.9	4.1
0.5	6	2.21	7.6	3.8	51.61	31.5	6
0.5	0.3	5.79	3.1	0.7	74.5	32	0.3
0	0	42.73	0.2	0.1	25.06	0.3	0

Data courtesy of Justin Lathia and Jeremy Rich, Cleveland Clinic

BD

Summary

- BD Lyoplate screening panels can be used to identify cell surface signatures of diverse cell populations
- Cell surface marker antibody screens can be combined with intracellular flow cytometry to facilitate identification of cell populations
- Fluorescent cell barcoding can be used to increase throughput of antibody screens by flow cytometry



Acknowledgments

BD Cell Analysis

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