Defining Diabetes Associated Defects in Stem/Progenitor Cell Populations Using Microfluidic Single Cell Analysis

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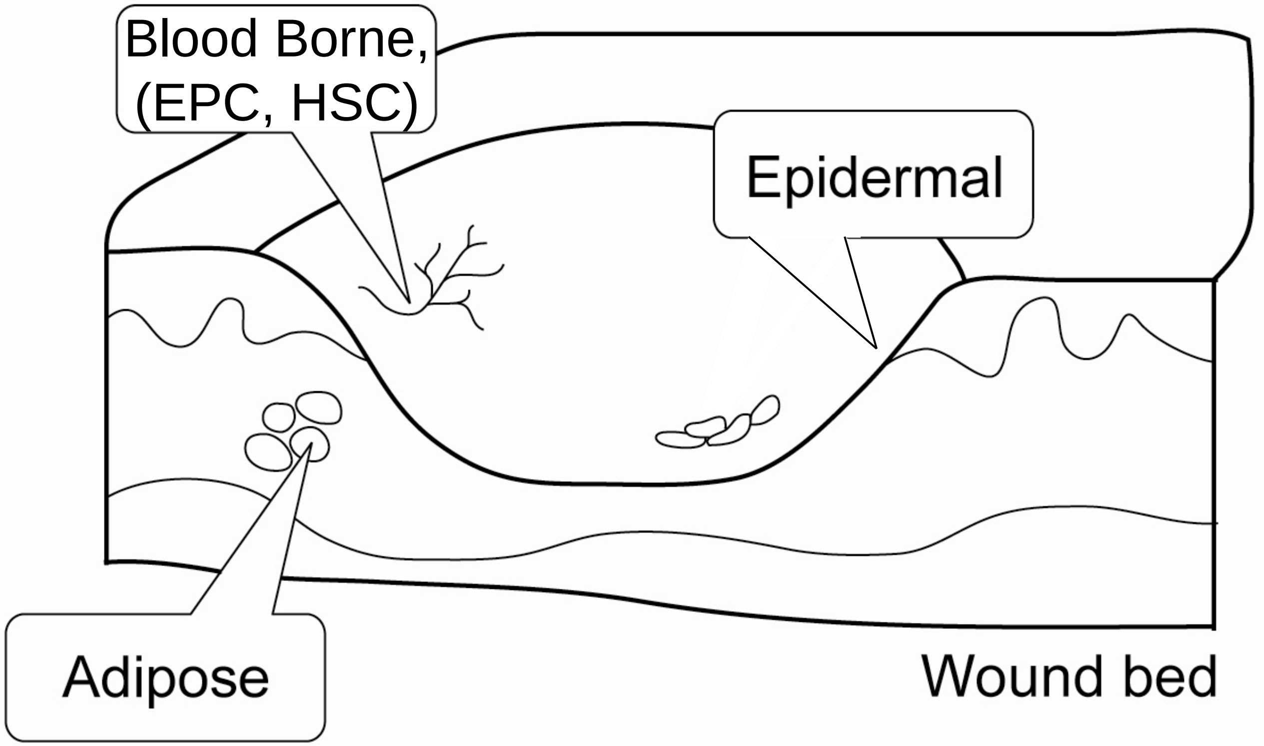
Potential Sources of Stem/Progenitor Cells During Wound Healing



EPCs Correlate with Cardiovascular Risk in Humans

Number and Migratory Activity of Circulating Endothelial Progenitor Cells Inversely Correlate With Risk Factors for Coronary Artery Disease

Mariuca Vasa, Stephan Fichtlscherer, Alexandra Aicher, Klaudia Adler, Carmen Urbich, Hans Martin, Andreas M. Zeiher, Stefanie Dimmeler

Circulation Research, 2001

Circulating Endothelial Progenitor Cells, Vascular Function, and Cardiovascular Risk

Jonathan M. Hill, M.R.C.P., Gloria Zalos, R.N., Julian P.J. Halcox, M.R.C.P., William H. Schenke, B.A., Myron A. Waclawiw, Ph.D., Arshed A. Quyyumi, M.D., and Toren Finkel, M.D., Ph.D.

NEJM, 2003

Human Endothelial Progenitor Cells From Type II Diabetics Exhibit Impaired Proliferation, Adhesion, and Incorporation Into Vascular Structures

Oren M. Tepper, BA; Robert D. Galiano, MD; Jennifer M. Capla, BA; Christoph Kalka, MD; Paul J. Gagne, MD; Glen R. Jacobowitz, MD; Jamie P. Levine, MD; Geoffrey C. Gurtner, MD

Circulation, 2002

What is an “EPC”?

Markers and precise identification currently unclear

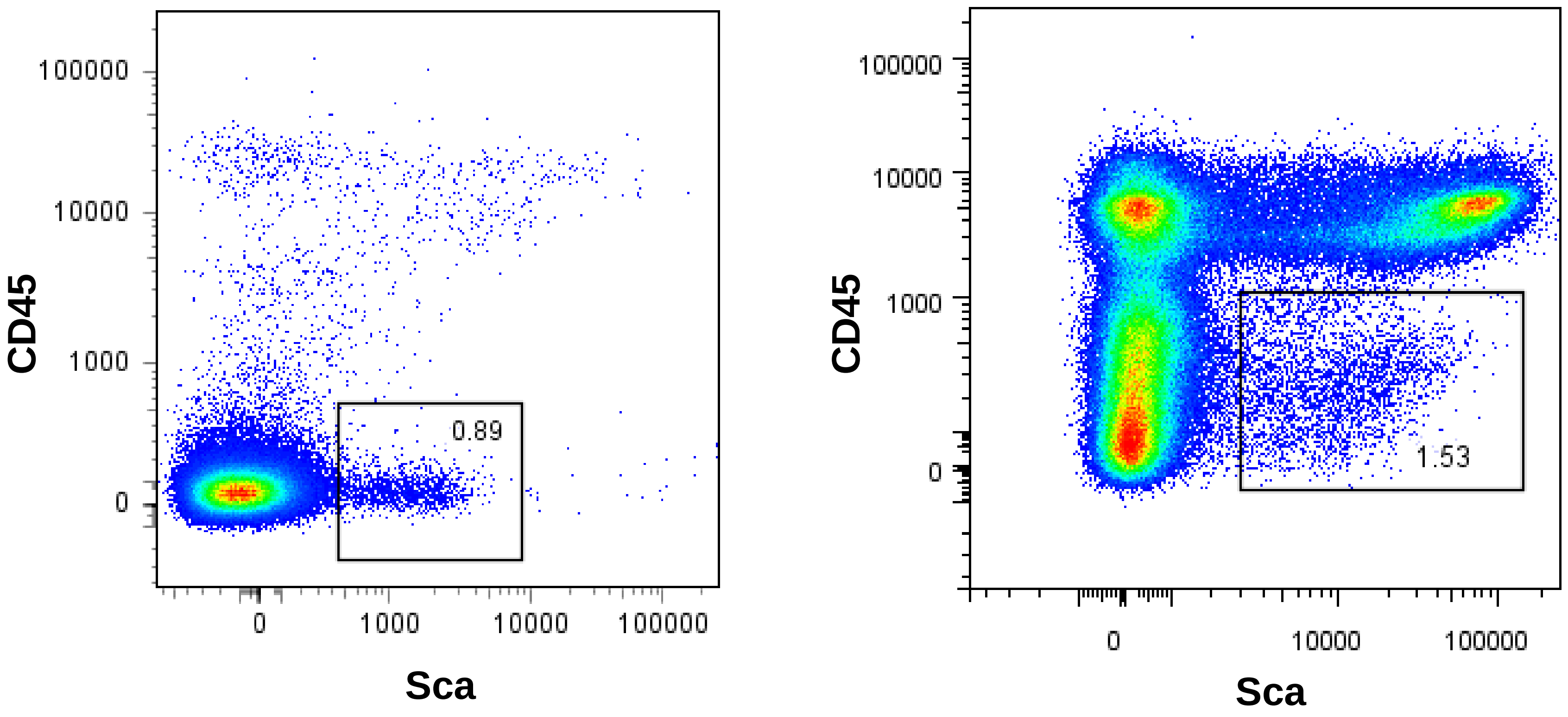
Nomenclature and identification in the literature inconsistent

Are circulating cells identical to bone marrow resident cells?

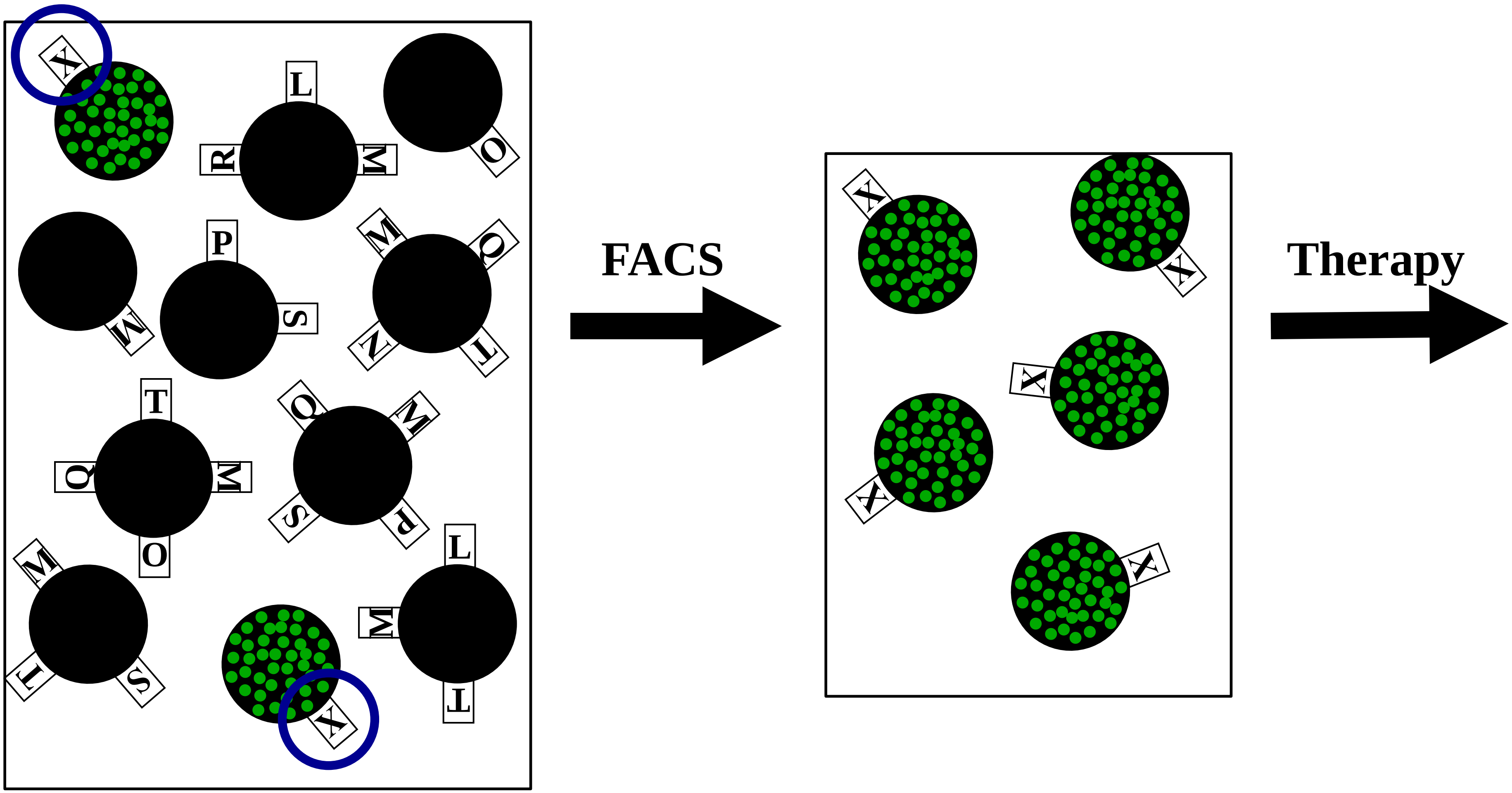
Are one or many different cells involved?

Authors		Marker Profile	Origin	Species
Asahara et al.	Science (1997)	**CD34+/ KDR (VEGF-R2)**	Blood	Mouse
Kalka and Asahara	PNAS (2000)	**CD31/VE-Cadherin KDR/LDL-R/UAE-1 R**	Blood	Mouse
Kawamoto et al.	Circ. (2001)	**CD31/UAE-1 binding**	Blood	Human
Walter et al.	Circ. (2002)	**Tie2?**	BM	Mouse, Rat Human
Takahashi et al.	Nat Med. (1999)	**CD31/Tie2/Sca1**	BM	Mouse
Dimmler et al.	Circ. Res. (2001)	**CD34/KDR**	Blood	Human
Rauscher et al.	Circ. (2003)	**CD31/CD-45$^-$**	BM	Mouse
Asahara and Isner	Stem Cells (2004)	**Flk-1/AC133/CD34 Tie2/c-Kit/Sca-1**	BM?	Human
		Tie2/VE-cadherin CD34/CD31	BM	Mouse
Chan and Verma	Transl. Physiol. (2005)	**CD34/VE-cadherin**	Blood	Human
Gurtner et al.	Nat Med. (2004)	**CD34+/Flk1+/CD133+ CD11b-**	Blood	Human
	Blood (2005)	**CD34+/Flk1+/sca-1+ lin-/CD11b-**	Blood, BM	Mouse

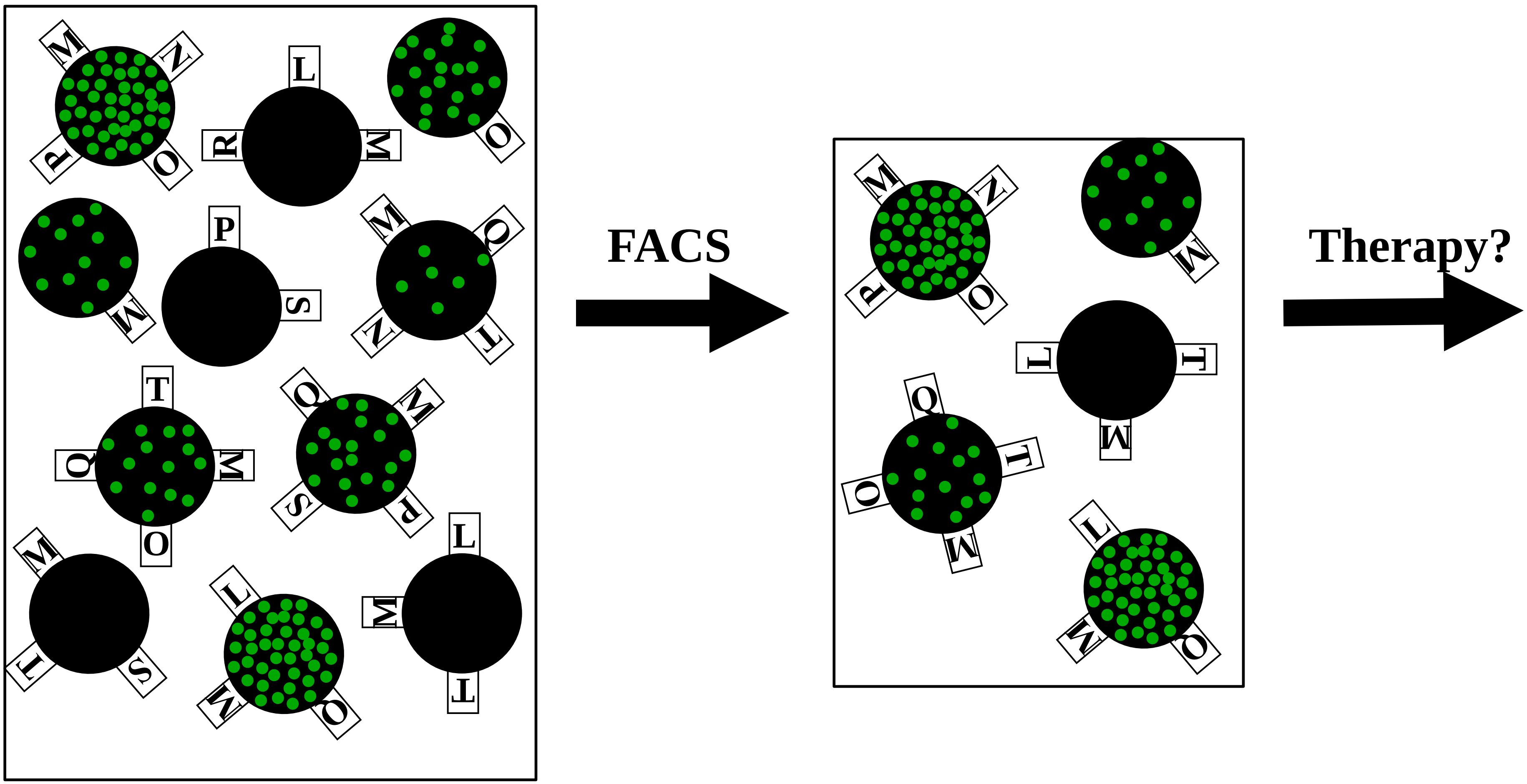
How Adult Stem Cell Population are Isolated: FACS Profiles



Ideal FACS Scenario:
Stem Cell Surface Marker “X”



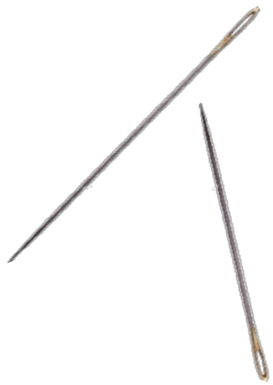
Heterogeneity in Adult Stem Cells



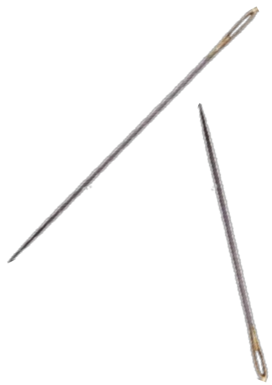
How can we isolate and understand rare stem cell subsets in the absence of discrete markers?

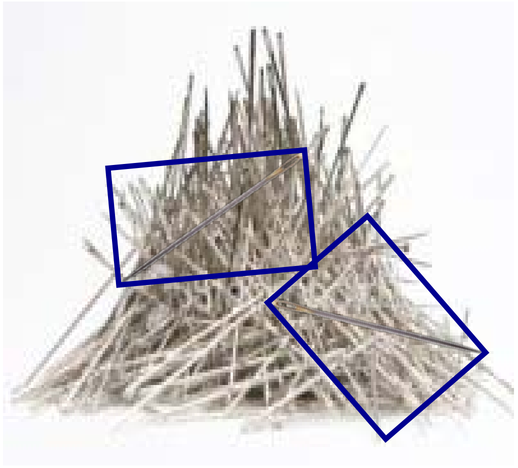
…need to understand stem cell heterogeneity

The Problem of Heterogeneity



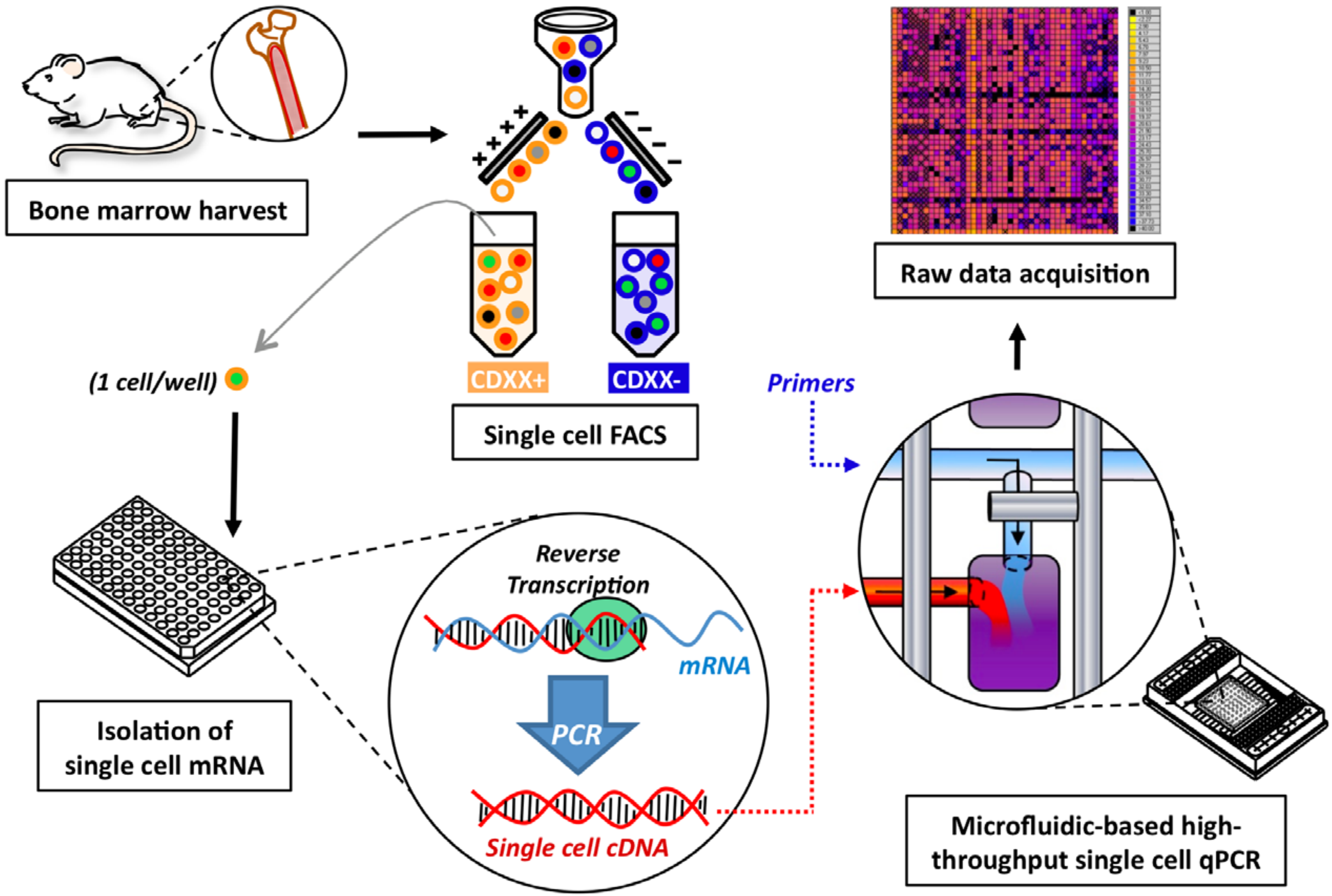
Tedious, but solvable



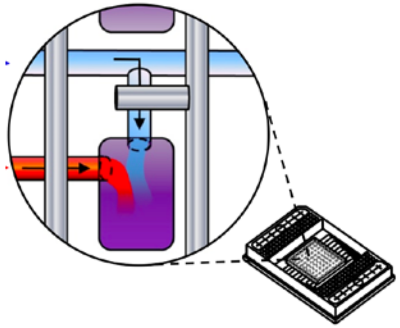


Unsolvable(?)

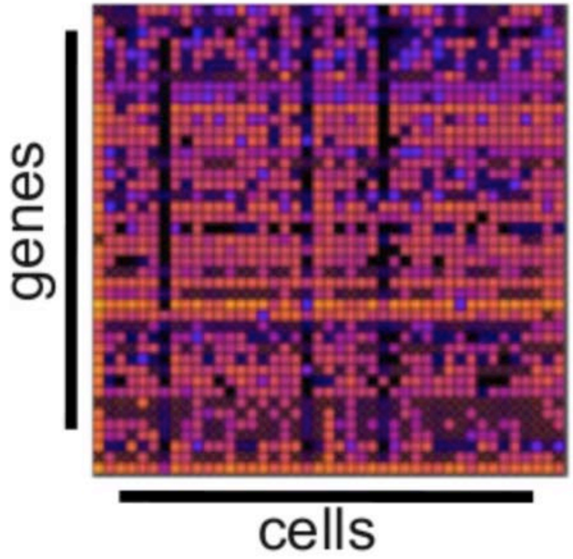
Single Cell Transcriptional Analysis



Single Cell Transcriptional Analysis: Microfluidic Technology



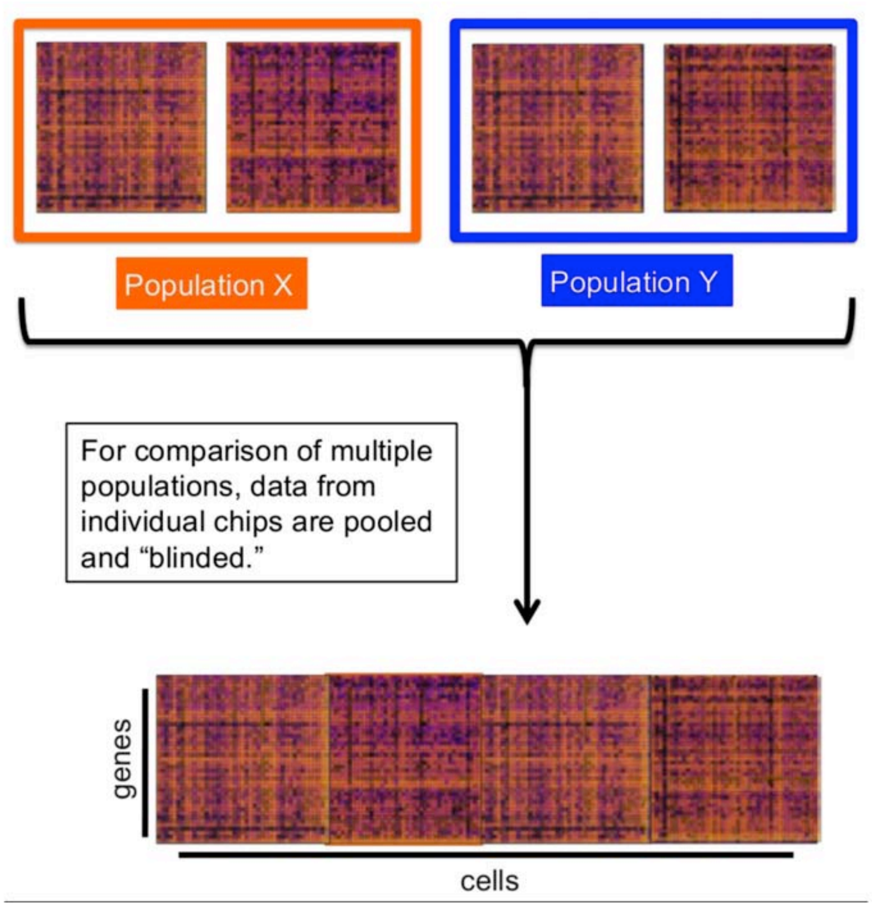
- Microfluidic chip accurately mixes reagents on the nanoliter scale



Raw Chip Data

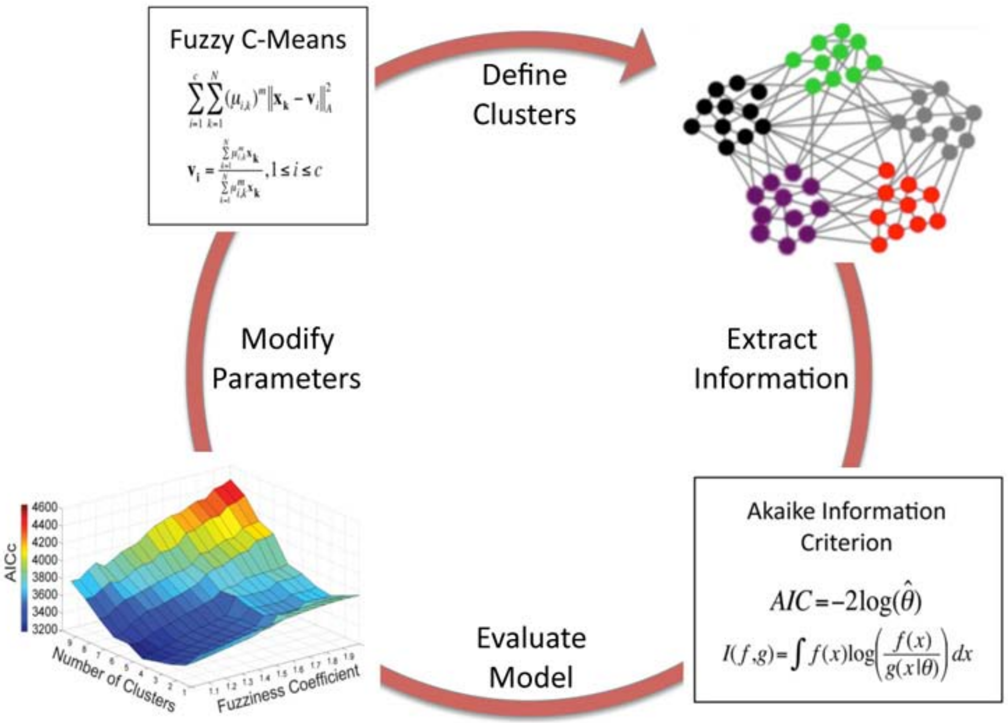
- Parallel processing of 2,304 qPCR reactions
- Simultaneously measure expression of 48 genes across 48 single cells

Single Cell Transcriptional Analysis: qPCR Data Processing



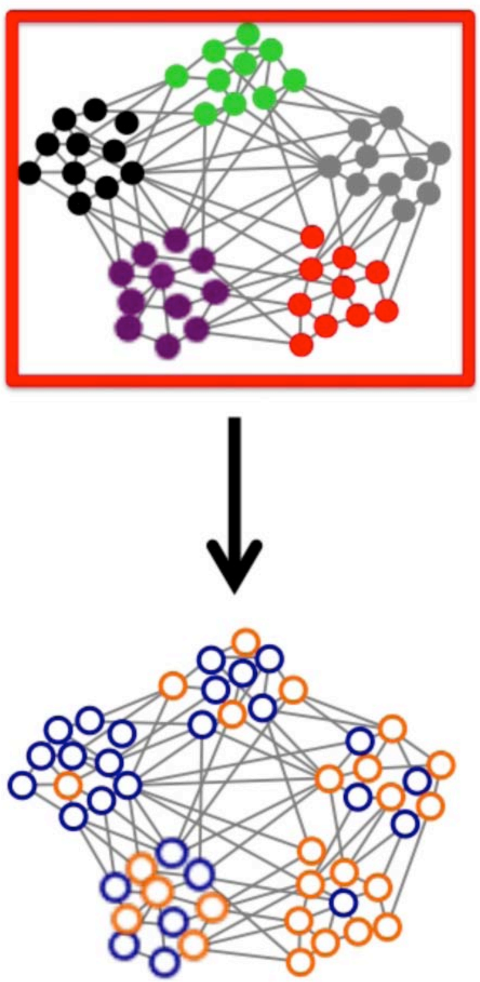
- Multiple chip runs for different cell populations
- Data is pooled and normalized to median expression of each gene
- Data blinded to population of origin for clustering

Single Cell Transcriptional Analysis: Clustering Algorithm



- Mathematical algorithm based on fuzzy logic principles
- Use objective criteria to minimize information loss in the clustering process
- Each cell is assigned membership to a cluster based on similar transcriptional profiles

Single Cell Transcriptional Analysis: Cell Clustering

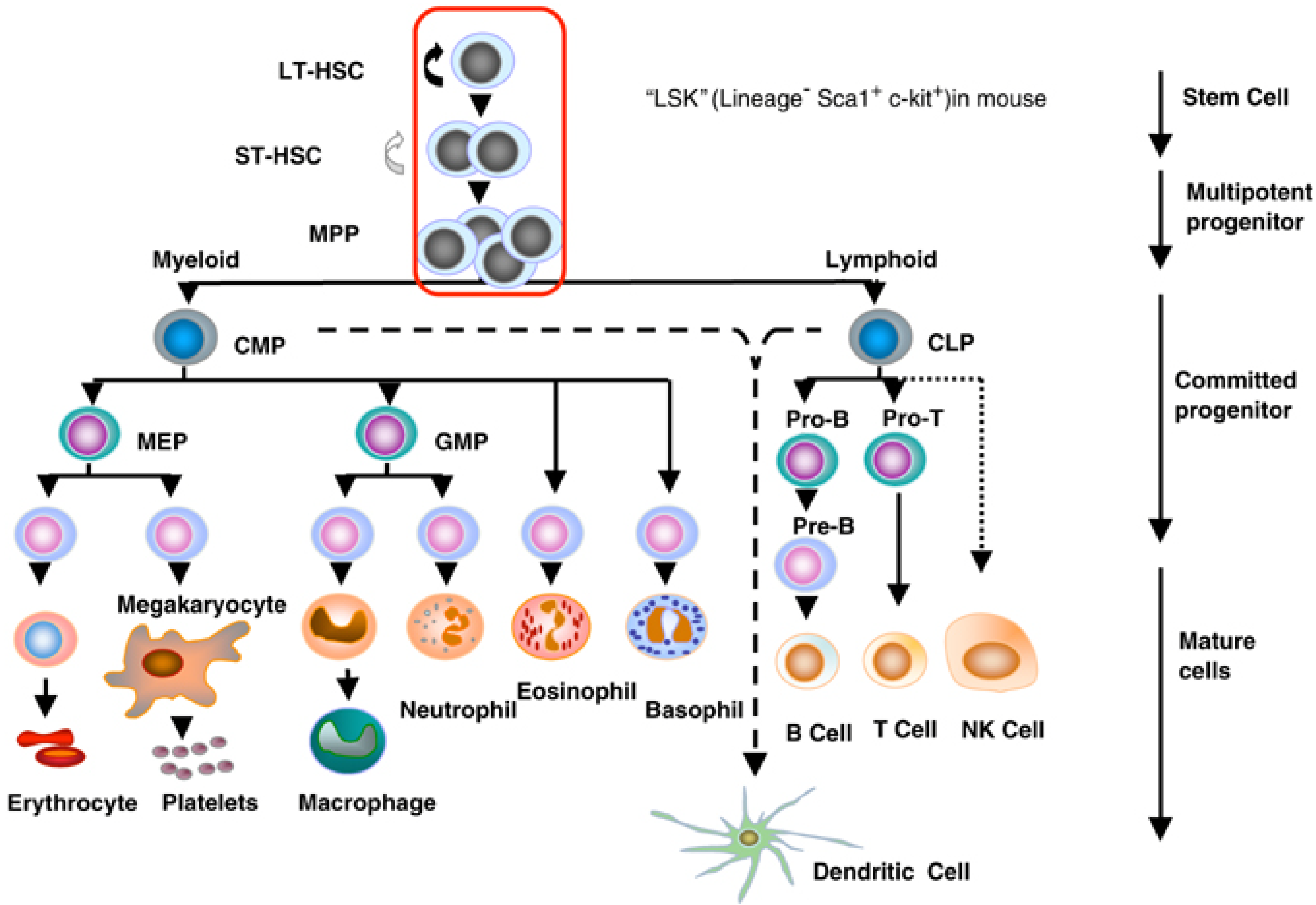


- Cells grouped into “clusters” based on similar transcriptional patterns
- Clusters unblinded to population of origin
- Data converted to visual display

Single Cell Transcriptional Analysis

- Need to validate new methodology
- Difficult to validate unproven methodology in ill-defined cell population
- Solution: validate methodology in well-defined cell type

Hematopoietic Stem Cells



Population of Interest

- Defined as KLS/CD150+/48-/135-/34-
  – Subset of SLAM cells split into CD34-/34+
  – CD34-: Cycle once every 145 days
  – CD34+: Cycle once every 20 days

Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal during Homeostasis and Repair

Anne Wilson,[3] Elisa Laurenti,[1,8] Gabriela Oser,[1,8] Richard C. van der Wath,[4,8] William Blanco-Bose,[1,8] Maike Jaworski,[1] Sandra Offner,[1] Cyrille F. Dunant,[6] Leonid Eshkind,[5] Ernesto Bockamp,[5] Pietro Lió,[4] H. Robson MacDonald,[3] and Andreas Trumpp[1,2,7,*]

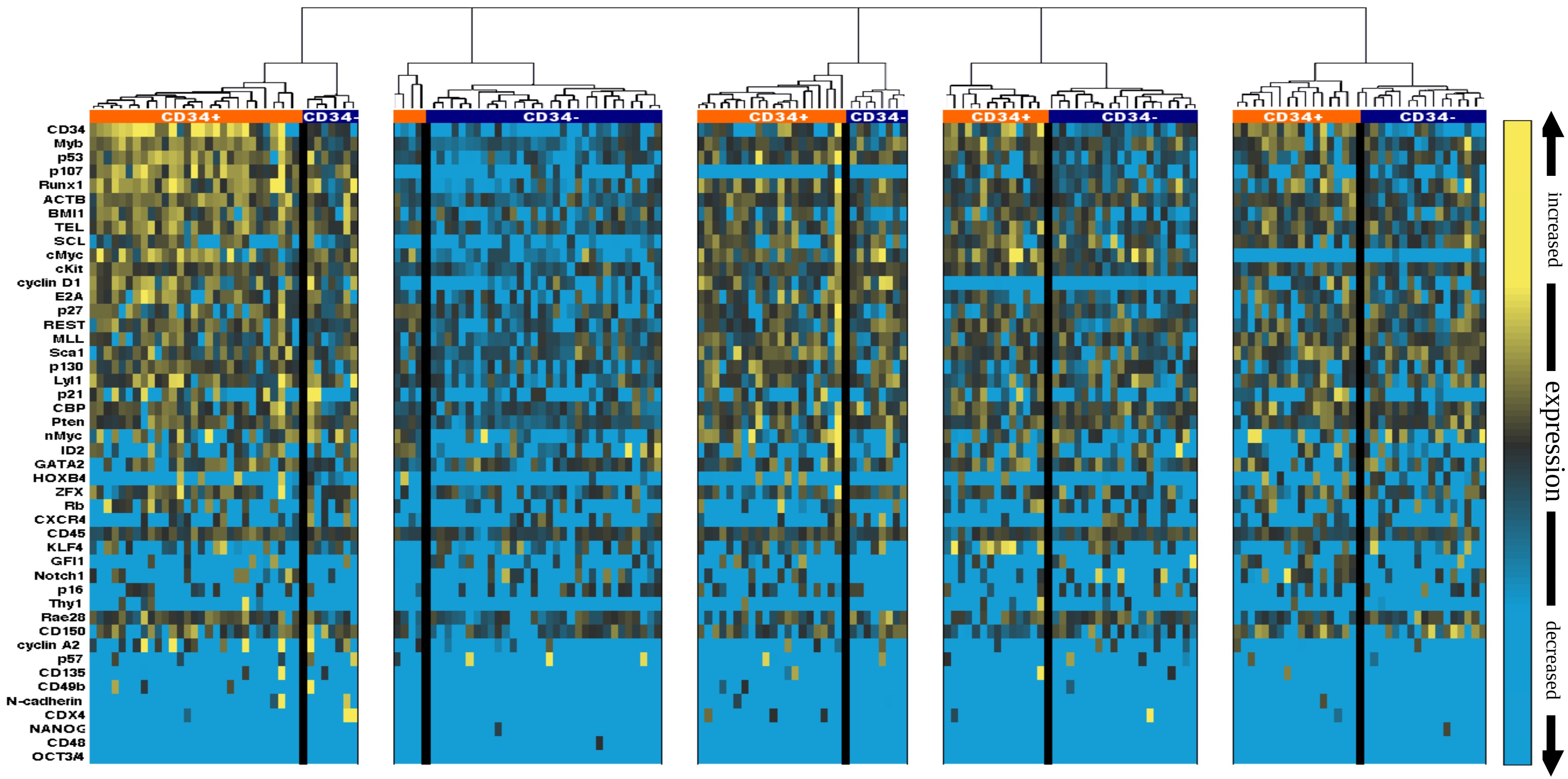
– Editorial Mentions:

pate in homeostasis and therefore examined the dormant cells under the stress conditions generated by destruction of dividing cells. The data must be interpreted with caution given that only populations and not individual cells could be examined, but the results are consistent with recruitment of this population into cycle (though death was not excluded).

How Do We Define a Stem Cell?
Construction of Microfluidic Chip

Housekeeping	Transcription Factors	Surface Markers
ACTB	TEL (ETV6)	CD150
Cell Cycle	BMI-1	CD48
p57	GFI-1	CD34
Rb	Myb	CD135 (Flt3)
p107	GATA2	cKit (CD117)
p130	HOXB4	Sca-1
cyclin D1	Rae28	Thy1 (CD90)
cyclin A2	E2A	CD45
p16	CDX4	CD49b
p21	ZFX	**General Stem Cell**
p27	MLL	Oct 3/4
pten	CBP	KLF4
p53	SCL	TERT
HSC Niche-Related	Lyl-1	NANOG
N-cadherin	Notch1	REST
CXCR4	Foxo3a	cMyc
ID2	Runx1	nMyc

Clustered Data



Cluster 1

- **High transcriptional activity**
- **Likely cycling**
- **Highest expression of p107, myb, p53, runx1**
- **Predominantly $CD34^{HI}$**

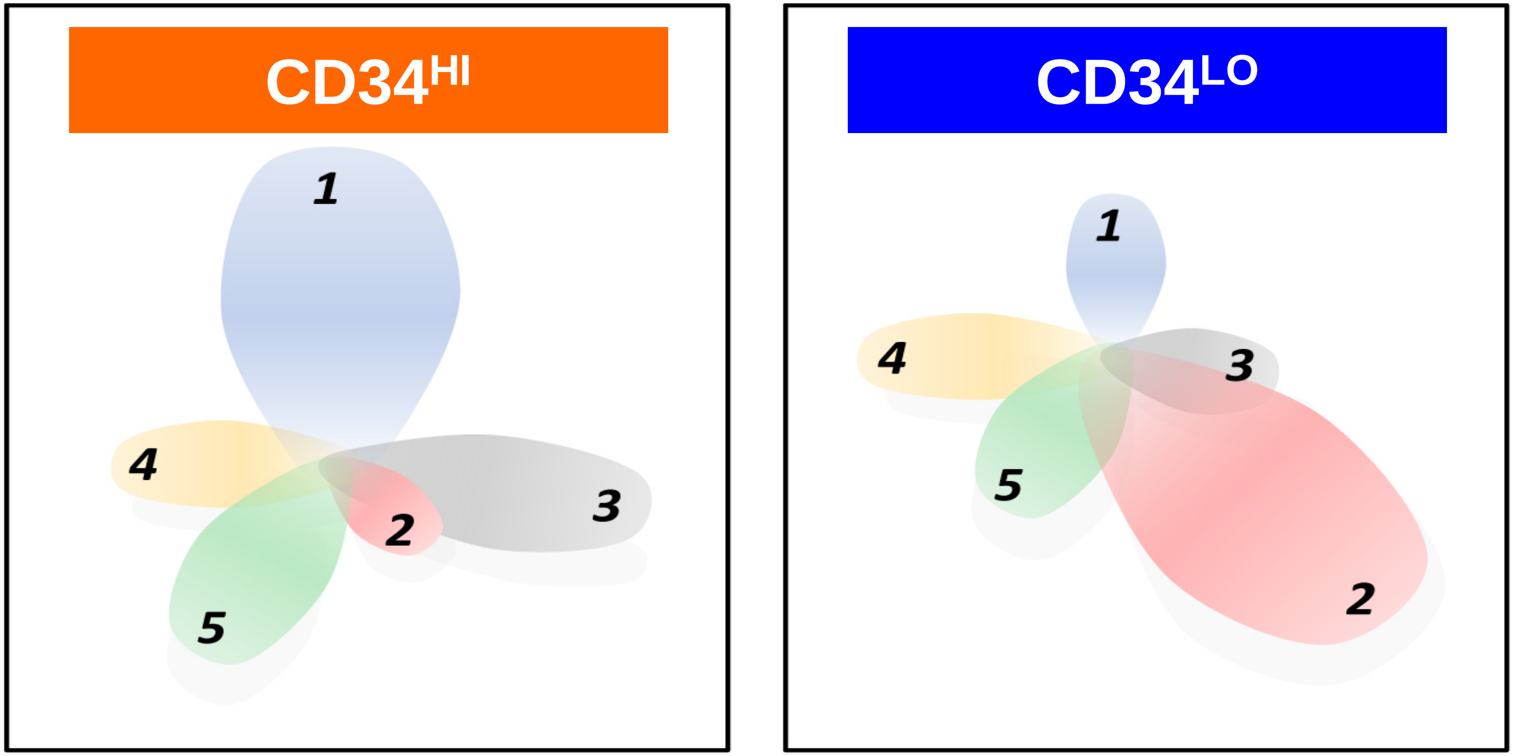
Cluster 2

- **Low transcriptional activity**
- **Largely quiescent**
- **Low expression of CD34, p107, myb, scl, cyclin D1**

Clusters 3, 4, 5

- Intermediate transcriptional activity
- Each cluster primarily defined by minimal expression of a single gene (e.g., p107 [3], cyclin D1 [4], cmyc [5])
- Roughly equal membership between $CD34^{HI}$ and $CD34^{Lo}$

Cluster Membership



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Summary

1. Can group individual cells based on similarities in gene expression profiles

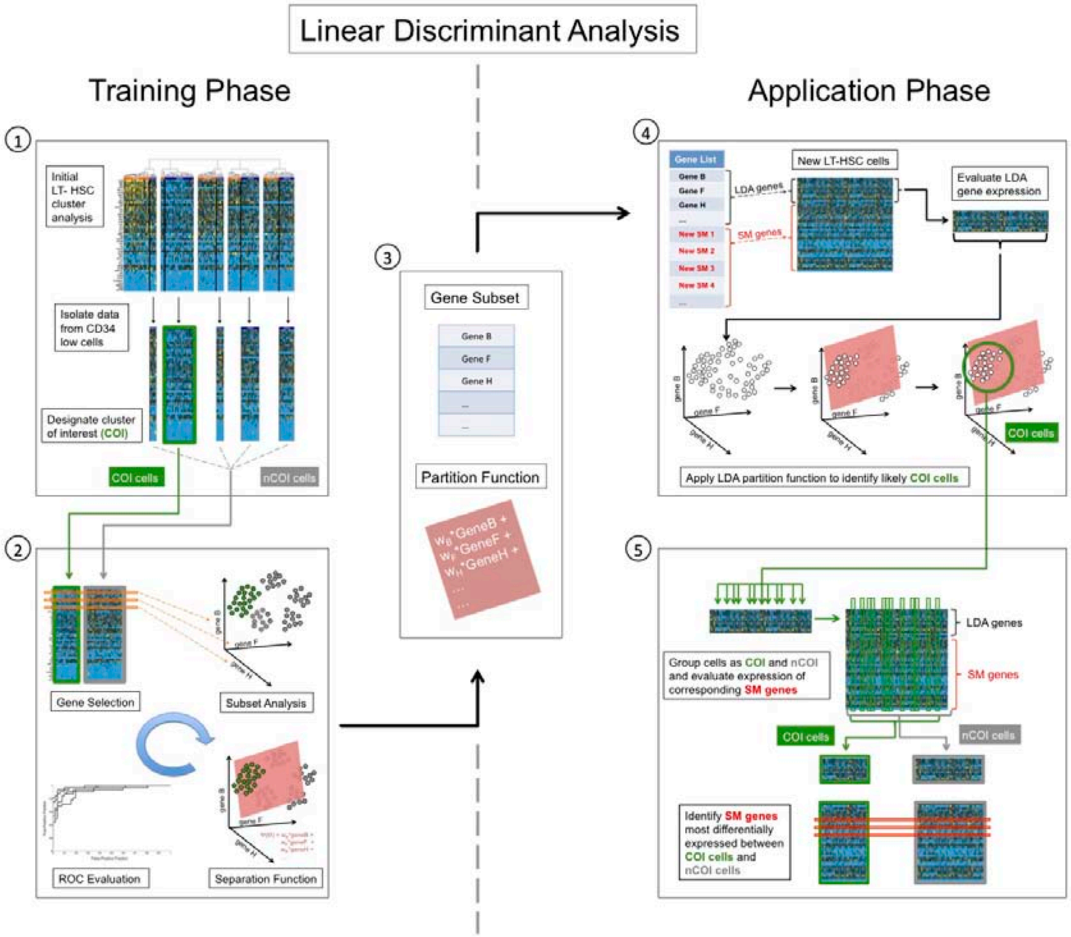
2. Considerable transcriptional heterogeneity exists within both CD34+ and CD34- HSC populations

How can we leverage this single-cell data to permit <u>prospective</u> isolation of rare stem cell populations?

…need a method to identify novel combinations of robust surface markers

Linear Discriminant Analysis

- Identify subset of genes capable of identifying specific subpopulations (“fingerprints”)
- Effectively “opens up” space on chip for other genes → allows us to screen for expression of surface antigen genes.
- Achieved through recursive application of fuzzy c-means clustering and linear discriminant analysis (LDA)



Conclusions

1. Pluripotent stem and progenitor cells are minority populations following isolation using existing techniques

2. Since pluripotency is defined by gene expression (i.e., iPS cells), isolated cells can be partitioned into sub-populations using single cell microfluidic qPCR

3. Linear discriminant analysis can prospectively identify new and better combinations of existing surface markers to more precisely isolate pluripotent populations for therapeutic or investigational purposes

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