

Flow cytometry analysis of hematopoietic stem cells in murine bone marrow and spleen

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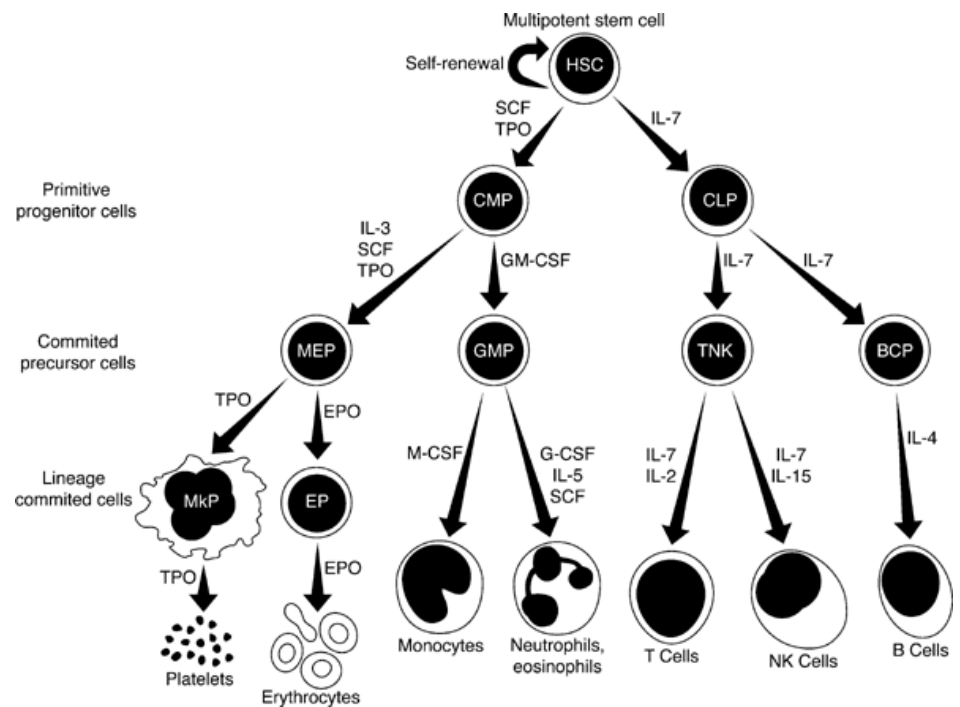


Murine hematopoiesis

- Bone marrow (95 % of multipotent progenitors) and spleen (5%)
- Hematopoietic stem cells (HSCs) – give rise to to all types of blood cells

– **Long-term repopulating cells** - “true” HSCs – self-renewal ability

– **Short-term repopulating cells** - progenitors

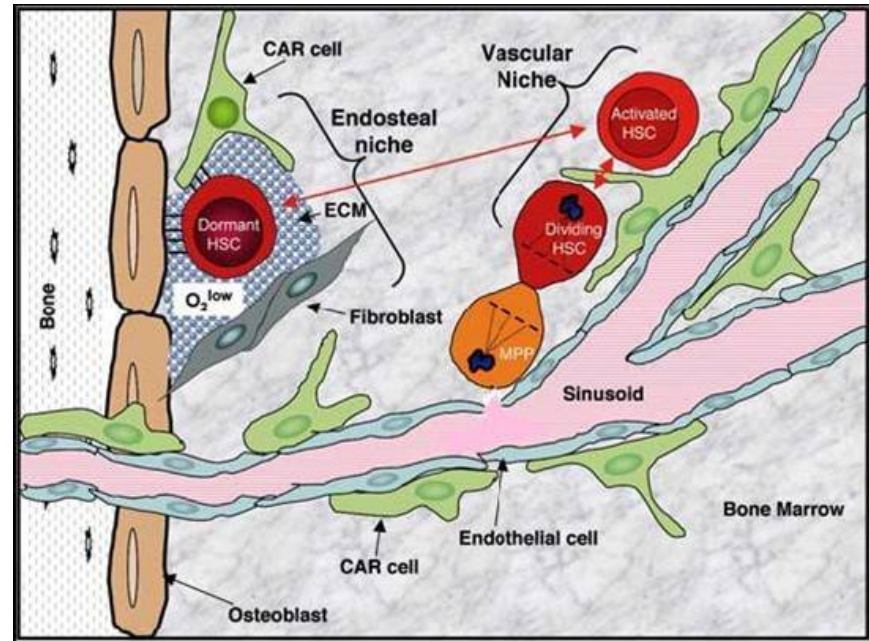


HSC niches

- Specialized micro-environments in BM
 - regulates maintenance and differentiation of HSCs

Endosteal niche

- Interface of bone and BM; formed by osteoblasts, fibroblasts and CAR cells
- Contains „dormant“ HSCs (LTRCs)
- Regulation of stem cells by adhesion, direct contact with stromal cells and by secretion of regulatory factors (angiopoietin, trombopoietin, CXCL12)



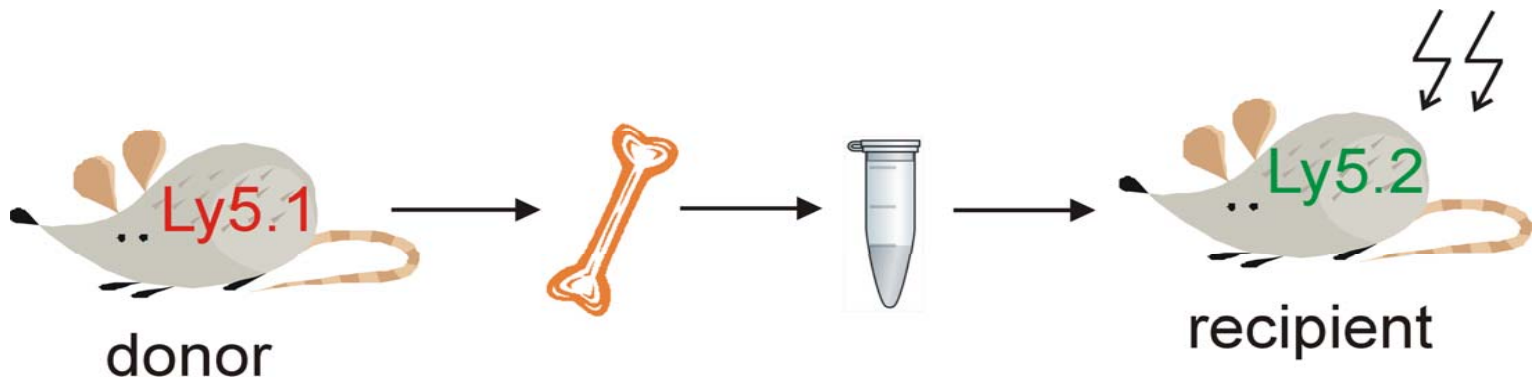
Vascular niche

- Around sinusoids
- More CAR cells (CXCL-12-abundant reticular cells)
- Contains progenitors (STRCs)
- Supports differentiation of the cells and entry to the circulation

Bone marrow transplantation

- emptying of the niches: total body irradiation (TBI, chemotherapy or combination of both)
- intravenous application of donor cells
- repopulation of recipients niches by donor HSCs => renewal of hematopoiesis
- homing – seeding – engraftment

Experimental arrangement



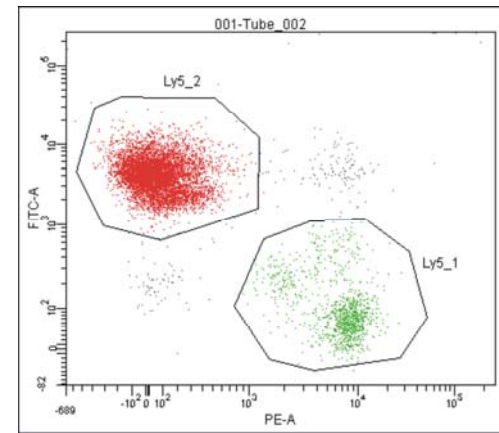
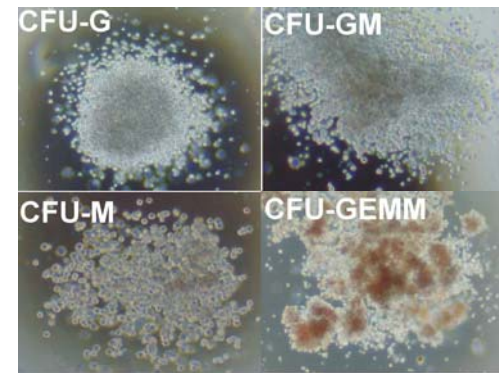
HSCs analysis

- **Functional**

- *In vitro* - cultivation in methyl- cellulose (colonies)
- *In vivo* – engraftment after BMT

- **Cytometric**

- Surface markers
 - Lack of lineage markers => Lin⁻
 - Unique cell surface molecules (e.g. Sca-1, cKit, CD150, CD34)
- Intracellular staining
 - Low staining with vital dyes (rhodamine 123, Hoechst 33342) => “side population” (SP)



HSCs analysis in our lab

- **LSK (Spangrude, 1989)**
- **SP (Goodell, 1997)**
- **SLAM (Kiel, 2005)**



FACS Aria II SORP, BD Bioscience

- LSK population – Lin-Sca-1⁺cKit⁺
 - Sca-1 – stem cell antigen 1
 - c-kit – stem cell factor receptor
- Side population (SP)
 - low staining with vital dyes (Hoechst 33342)
- SLAM population – CD150⁺CD48⁻ (CD34⁻)
 - SLAM – signaling lymphocyte activating molecule
 - CD34 – cell-cell adhesion factor

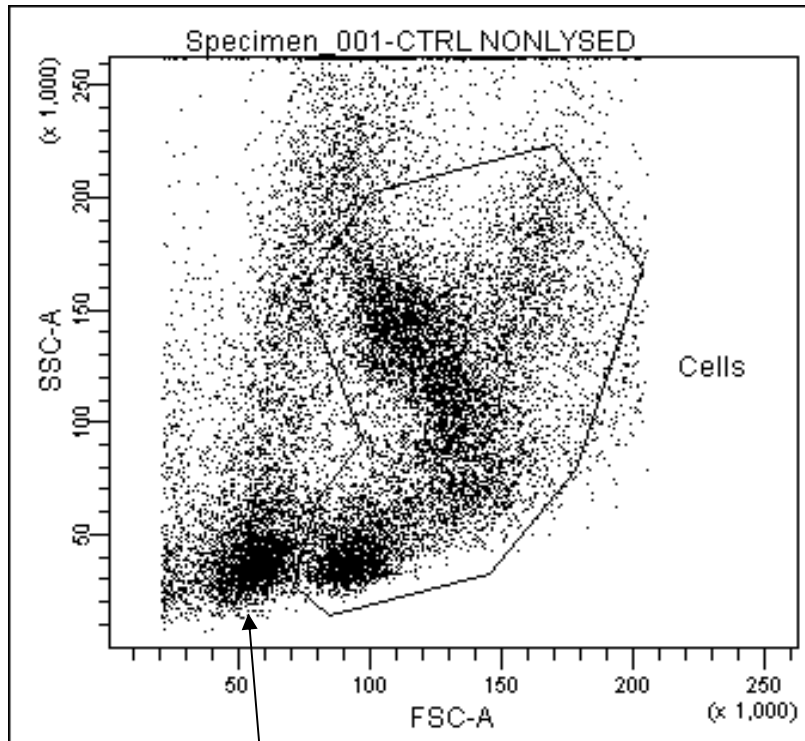
Part II:

Flow cytometric analysis of murine bone marrow hematopoietic stem cells

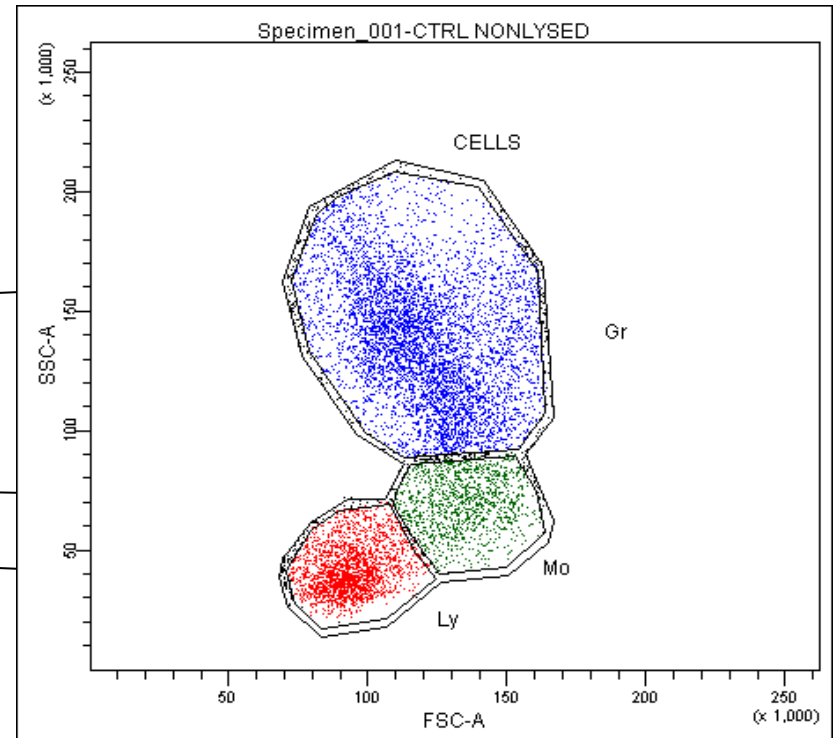
(BD FACSAria IIu Special Order System
equipped with UV laser and sorter unit,

BD FACSDiva software v.6.1.2)

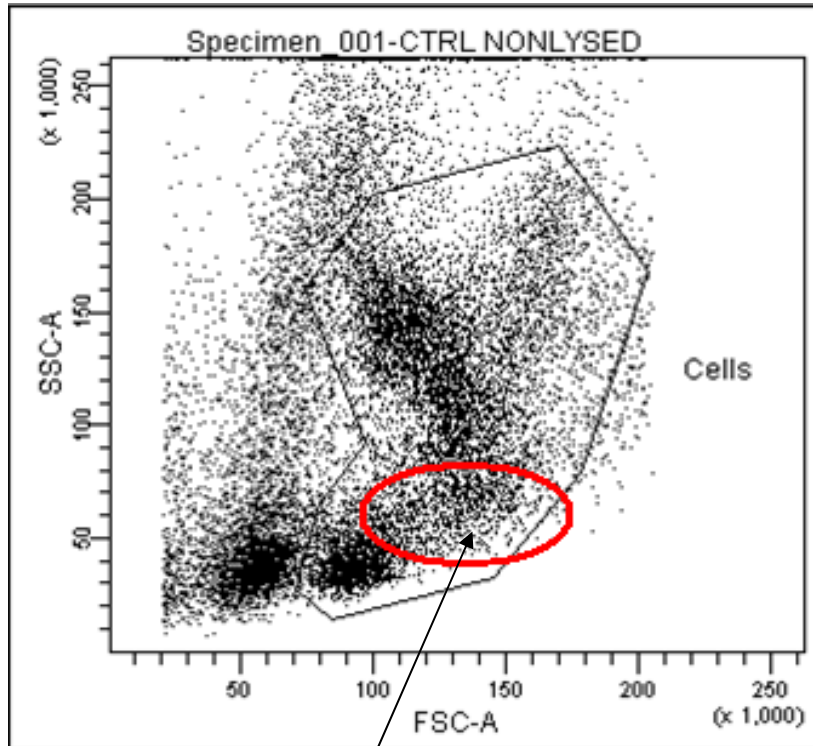
Cell subpopulations in normal murine BM



Erythrocytes



Size and granularity of murine BM HSC



HSC location on
SSC/FSC dotplot

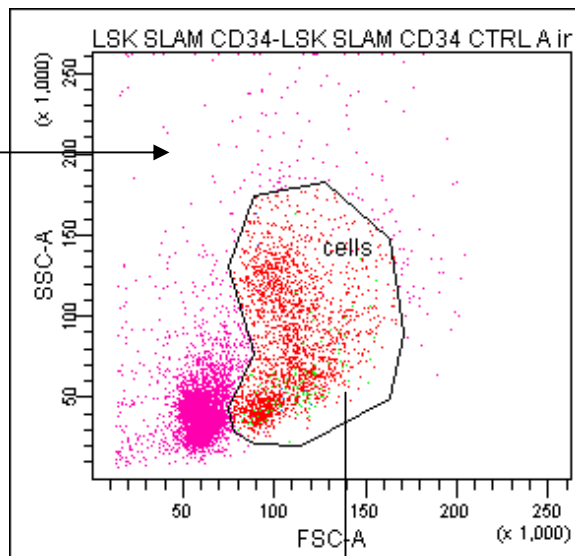
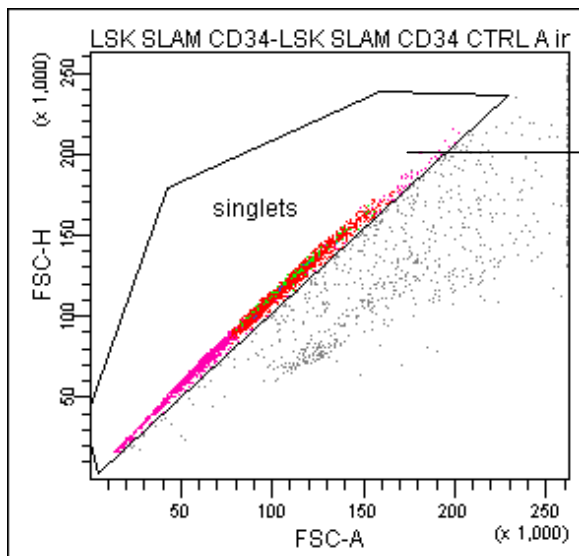
Markers of murine HSC

- 1) Lineage markers negative/low
- 2) cKit positive
- 3) Sca-1 positive
- 4) CD150 positive / CD48 negative (SLAM code)
- 5) CD34 negative
- 6) SP (Side Population, staining based on Hoechst 33342 exclusion)

Our routine combination for analysis of murine BM HSC:

L-S+K+ SLAM CD34- SP

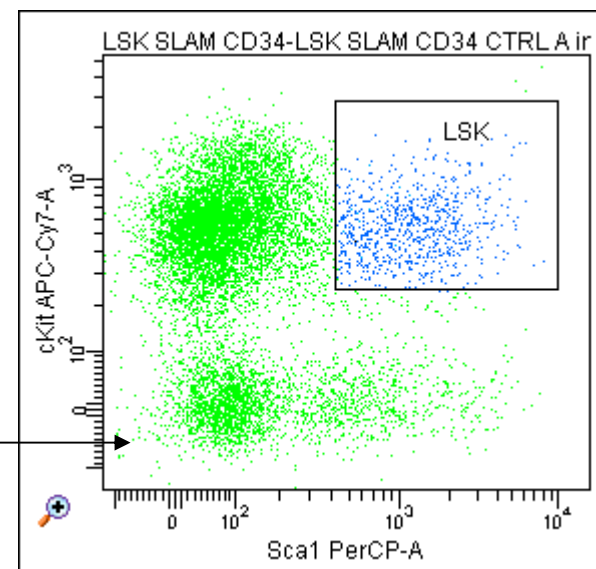
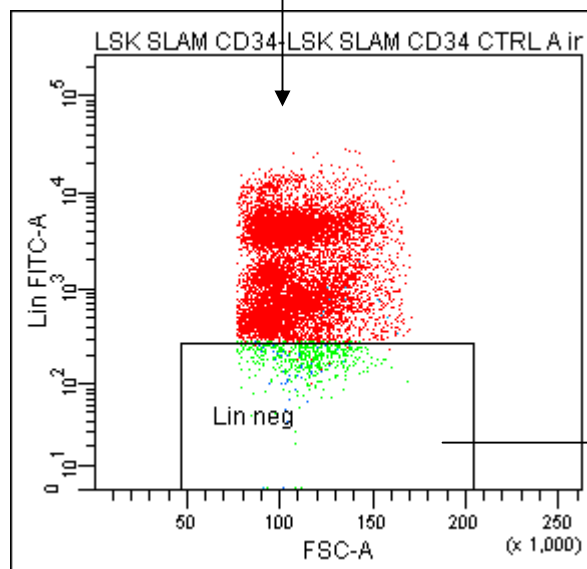
Multicolor gating hierarchy

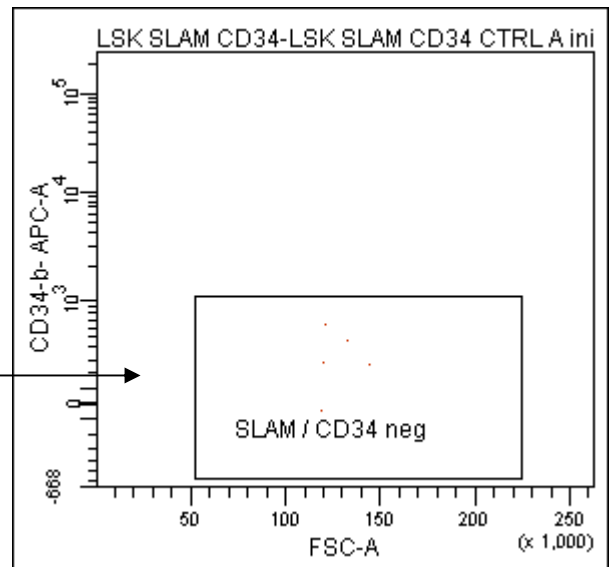
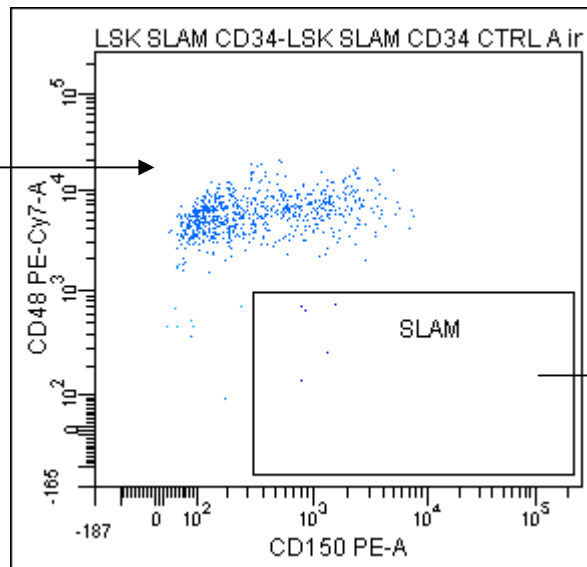
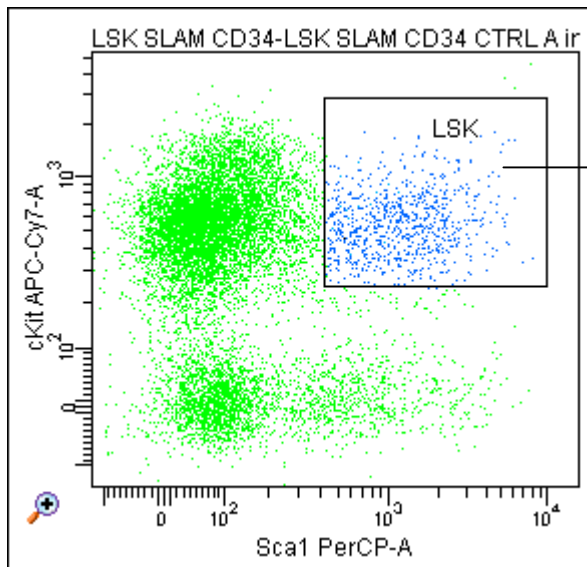


Population	#Events	%Parent	%Grand Par...	%Total
All Events	645,221	###	###	100.000
singlets	571,121	88.516	###	88.516
cells	204,641	35.831	31.716	31.716
Lin neg	10,721	5.239	1.877	1.662
LSK	793	7.397	0.388	0.123

Tube: LSK SLAM CD34 CTRL A init

Population	#Events	%Parent	%Total
All Events	645,221	###	100.0
singlets	571,121	88.5	88.5
cells	204,641	35.8	31.7
Lin neg	10,721	5.2	1.7
LSK	793	7.4	0.1
SLAM	5	0.6	0.0
SLAM / CD34	5	100.0	0.0
SP LSK	13	1.6	0.0
SP TOTAL	309	0.2	0.0

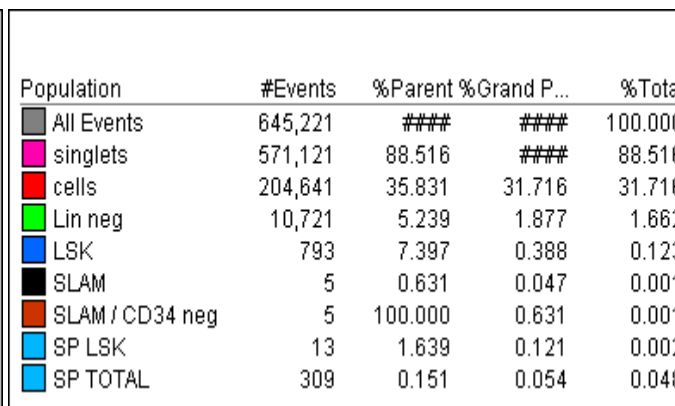
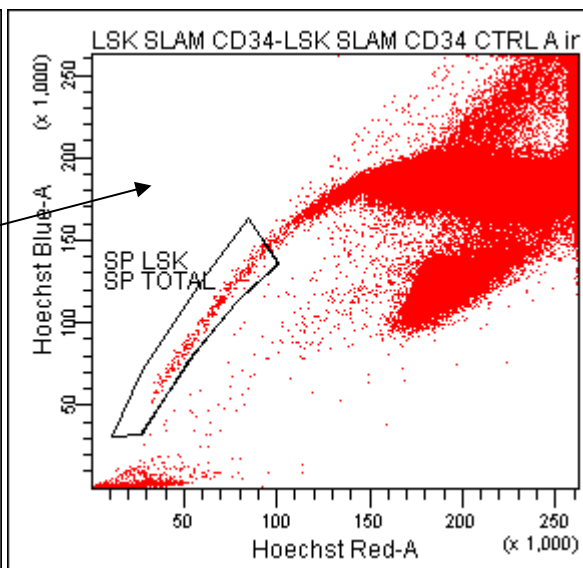
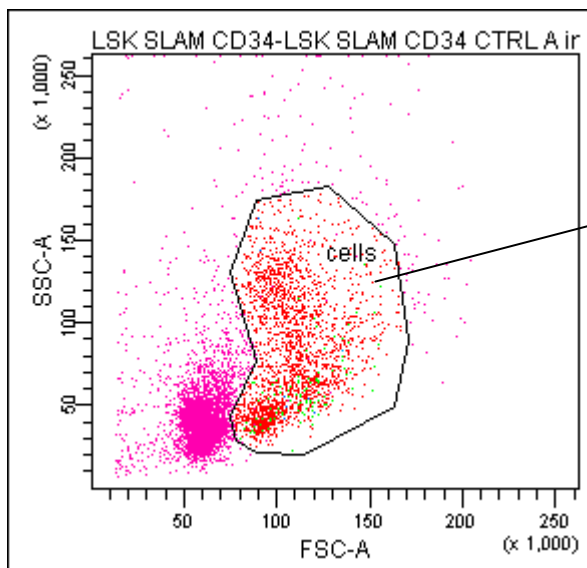
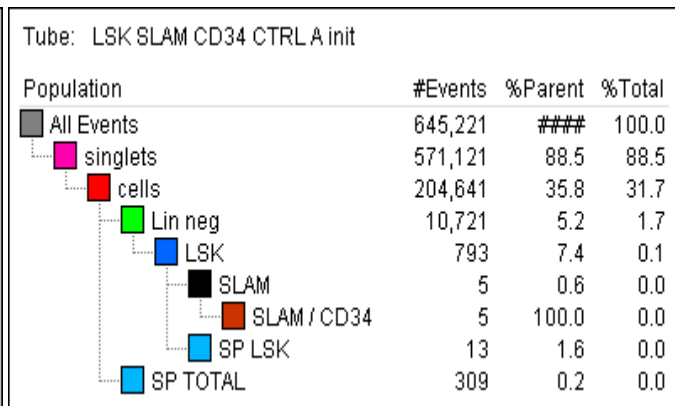
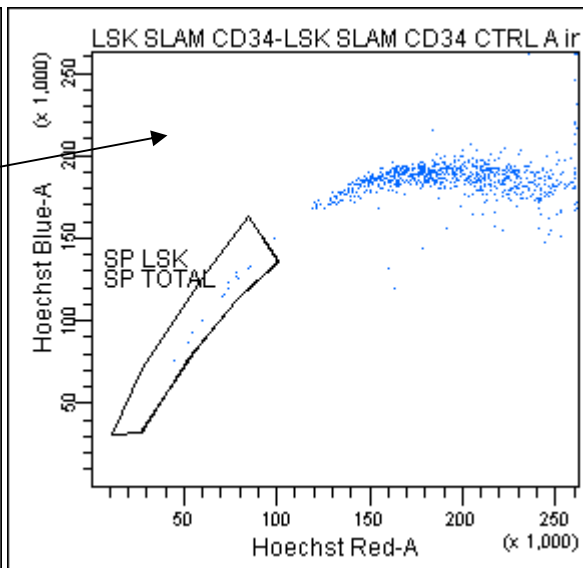
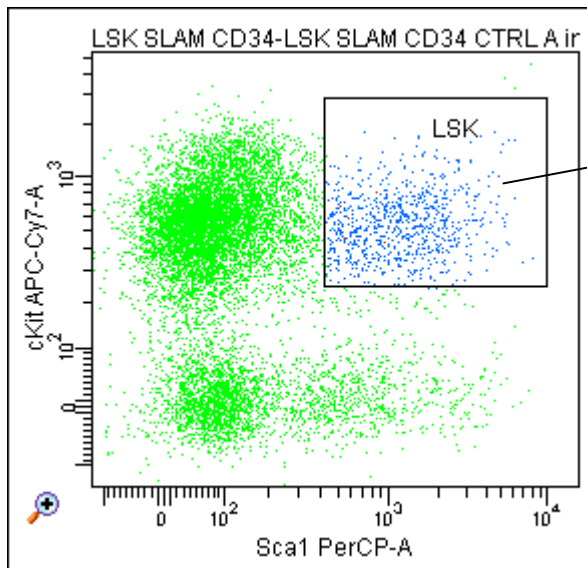




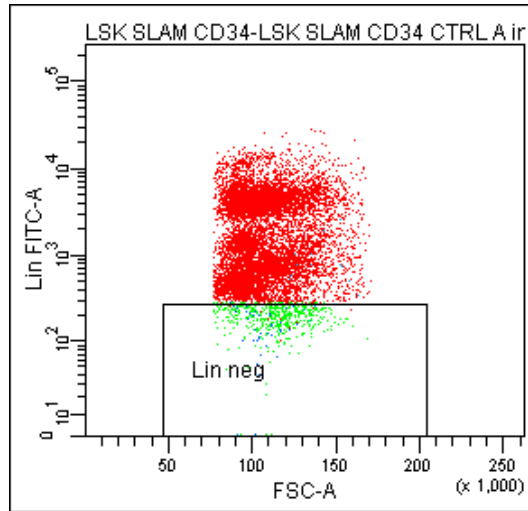
Tube: LSK SLAM CD34 CTRL A init

Population	#Events	%Parent	%Total
All Events	645,221	###	100.0
singlets	571,121	88.5	88.5
cells	204,641	35.8	31.7
Lin neg	10,721	5.2	1.7
LSK	793	7.4	0.1
SLAM	5	0.6	0.0
SLAM / CD34	5	100.0	0.0
SP LSK	13	1.6	0.0
SP TOTAL	309	0.2	0.0

Population	#Events	%Parent	%Grand P...	%Total
All Events	645,221	###	###	100.000
singlets	571,121	88.516	###	88.516
cells	204,641	35.831	31.716	31.716
Lin neg	10,721	5.239	1.877	1.662
LSK	793	7.397	0.388	0.123
SLAM	5	0.631	0.047	0.001
SLAM / CD34 neg	5	100.000	0.631	0.001



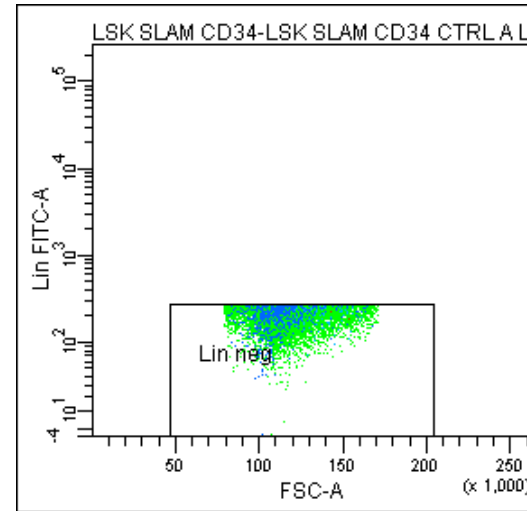
Recording hint: use DIVA “storage gate” option



Population	#Events	%Parent	%Grand Par...	%Total
All Events	645,221	####	####	100.000
singlets	571,121	88.516	####	88.516
cells	204,641	35.831	31.716	31.716
Lin neg	10,721	5.239	1.877	1.662

Size: 34.4 MB (36,135,356 bytes)

Only 0.6 million events processed,
all events stored

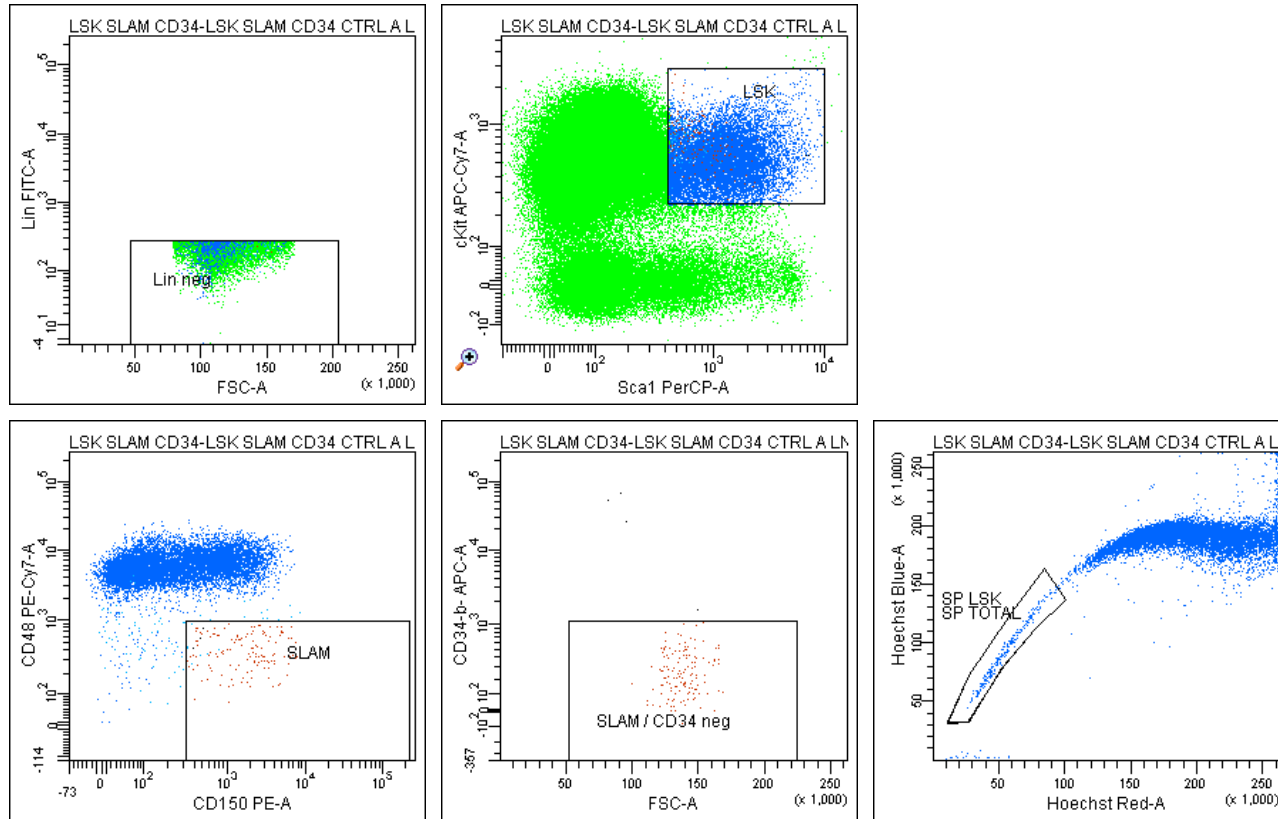


Population	#Events	%Parent	%Grand Par...	%Total
All Events	156,504	####	####	100.000
singlets	156,504	100.000	####	100.000
cells	156,504	100.000	100.000	100.000
Lin neg	156,504	100.000	100.000	100.000

Size: 8.36 MB (8,767,196 bytes)

About 10 million events processed,
Lin negative events only stored

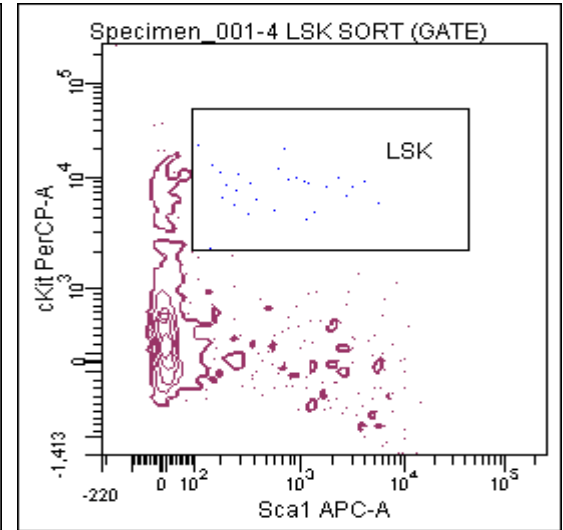
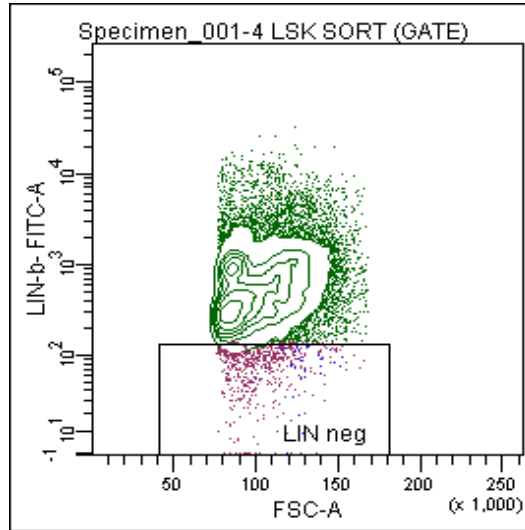
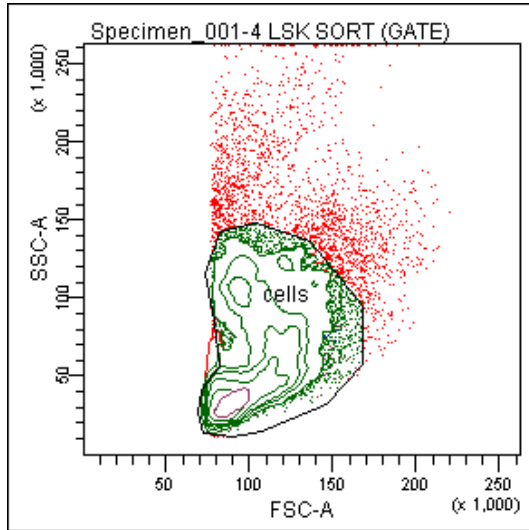
Storage gate “Lin negative”:



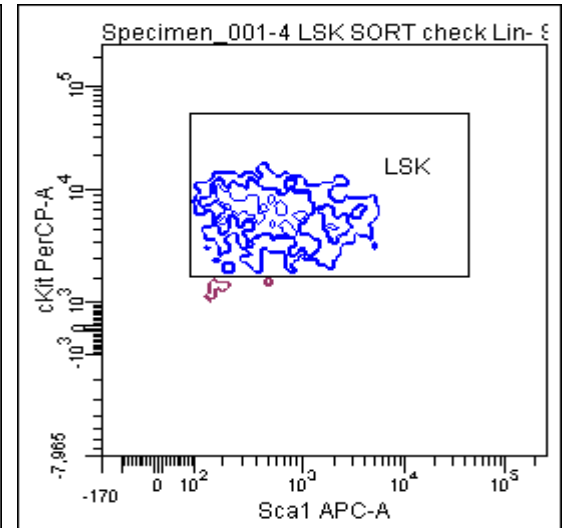
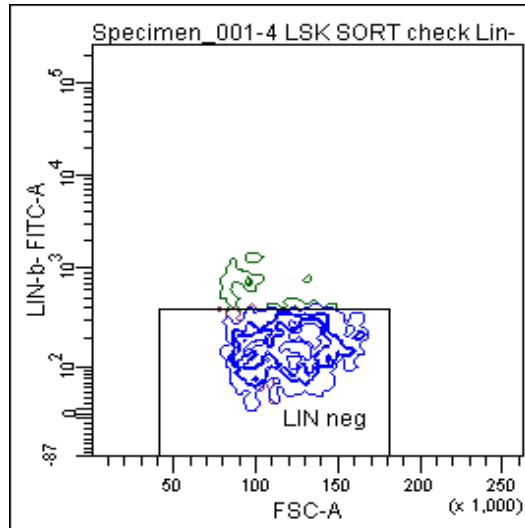
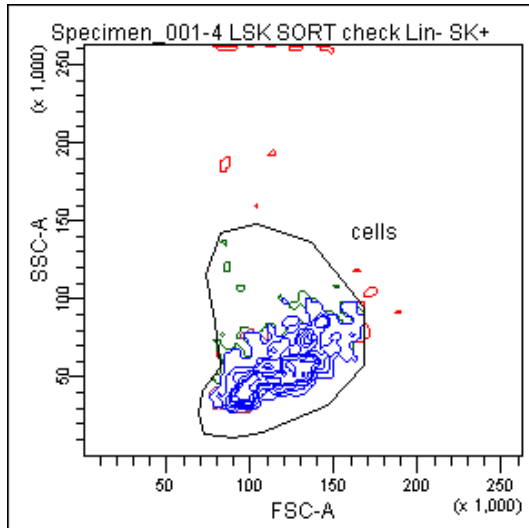
Population	#Events
Lin neg	156,504
LSK	12,015
SLAM	147
SLAM / CD34 neg	142
SP LSK	224
SP TOTAL	1,262

FACSria Sorting unit (70-130 micron nozzle options with diff. pressures; 4-way sorting in 3 ml tubes or 2-way sorting in 15-50 ml tubes or 1-way sorting in 6-96 wells plates, slides; few sort precision modes (purity, yield, single cell); semi-sterile mode; usual purity of sorted populations is about **95% or more**)

N/S



Sorted
for LSK



Some key-points:

- 1) For determination of murine HSC we use LSK SLAM CD34 SP panel
- 2) HSC are very rare; special software option need to be applied for accurate HSC analysis

Thank you for your attention.