流式细胞术应用介绍

贝克曼库尔特生命科学

流式细胞术的应用

临床研究

- ✓淋巴细胞亚群测定
- ✓血小板分析
- ✓网织红细胞分析
- ✓白血病/淋巴瘤免疫分析
- ✓HLA-B27分析，PNH诊断
- ✓人类同种异体器官移植中应用
- ✓细胞因子测定
- ✓AIDS诊断与治疗和疗效评价
- ✓Flow-FISH法测定端粒长度

基础研究

- ✓ 淋巴细胞功能
- ✓ 树突状细胞(DC)研究
- ✓ 造血干细胞研究
- ✓ 细胞周期分析
- ✓ 细胞功能研究
- ✓ 多药耐药基因研究(MDR)
- ✓ 肿瘤相关基因表达研究
- ✓ DNA，RNA测定
- ✓ 癌基因和抑癌基因表达产物测定
- ✓ 血管内皮细胞研究

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- 分选型流式的应用

分析型流式应用

- 多色--免疫分型
- 细胞周期/凋亡/增殖等
- 细胞因子检测—可溶性蛋白检测
- 细胞功能检测
- 微颗粒检测（微生物、细胞外囊泡）

- **分析型流式应用**

- ***多色流式--免疫分型***
- 细胞周期/凋亡/增殖等
- 细胞因子检测—可溶性蛋白检测
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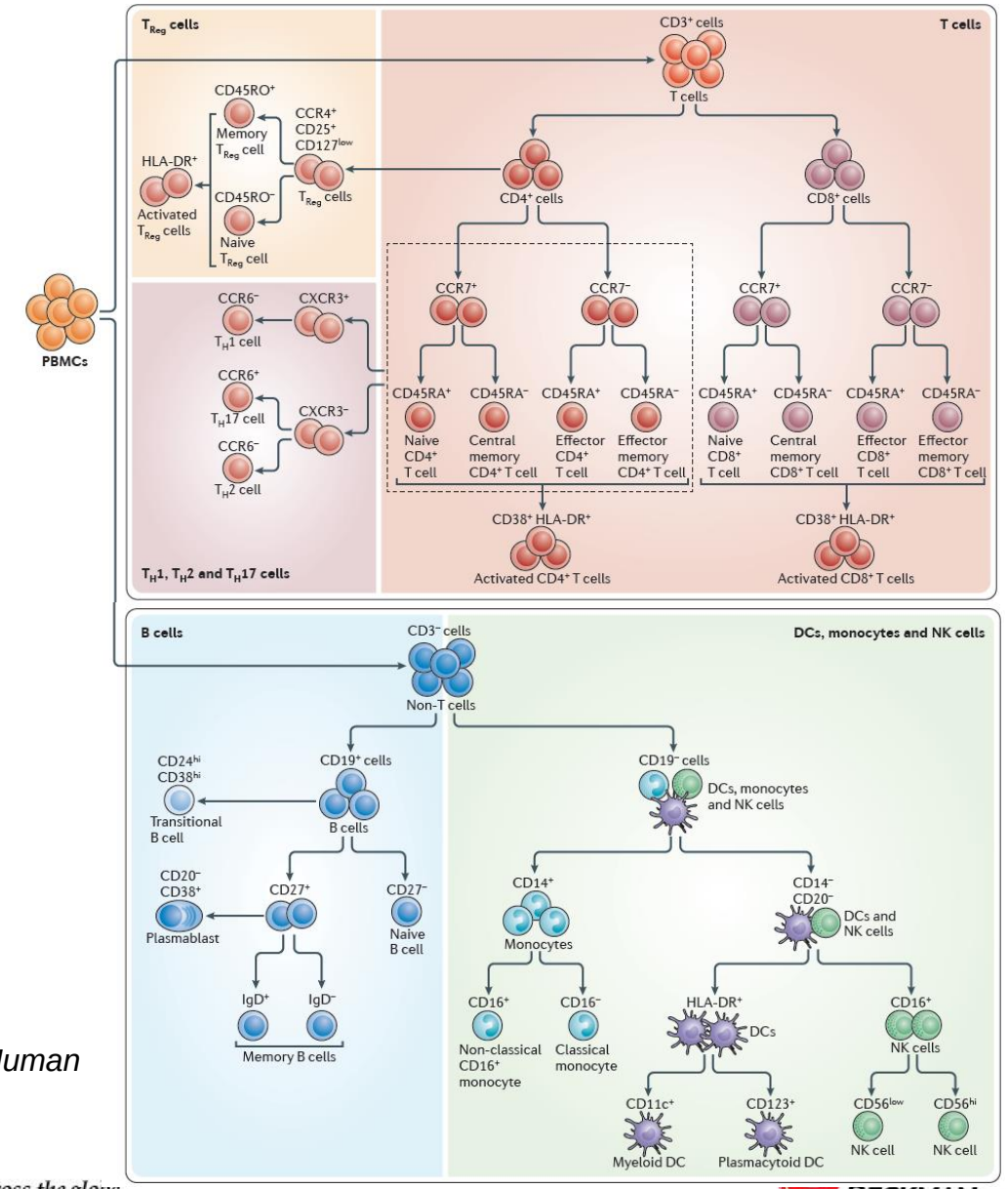
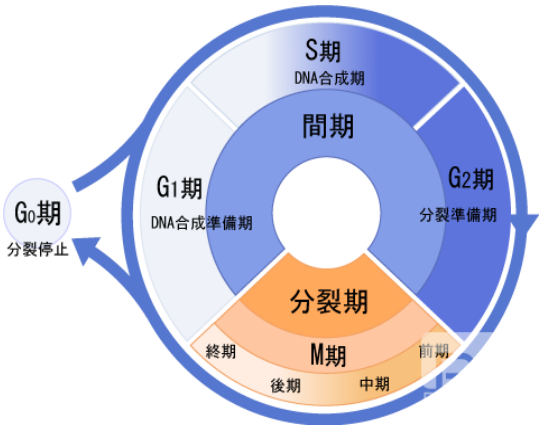
多色流式的应用



Standardizing immunophenotyping for the Human Immunology Project. Holden T ect. Nature Rev Immunol . ; 12(3): 191–200.

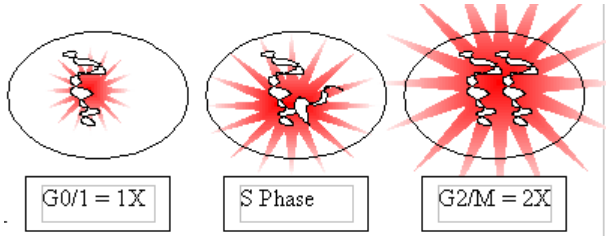
❑细胞周期检测

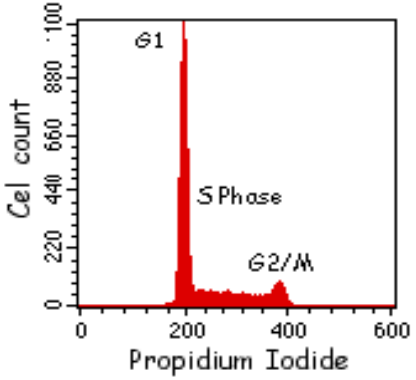


常用DNA染料

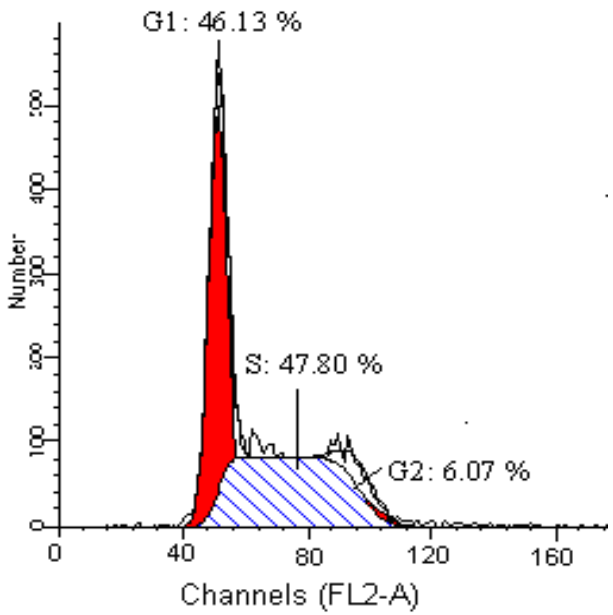
DAPI	}	
Hoechst	}	*UV*
Propidium iodide (PI)	}	
7-AAD	}	*488*
TOPRO-3	}	
DRAQ5	}	*633*

These dyes are stoichiometric - number of bound molecules are equivalent to the number of DNA molecules present

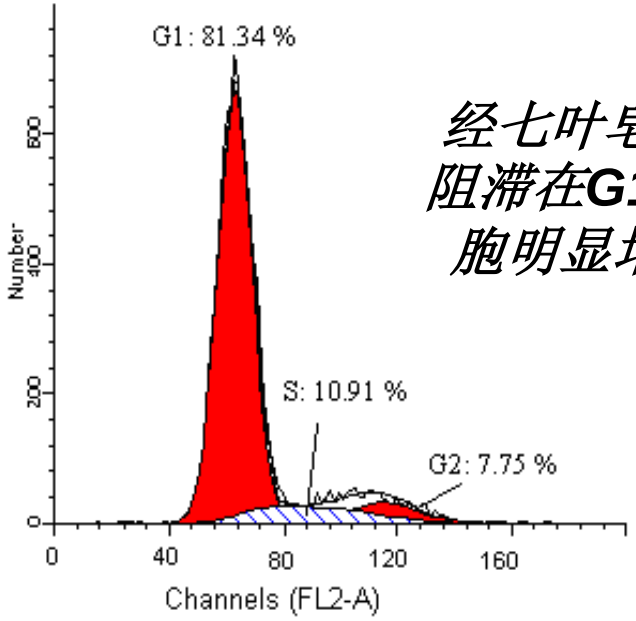




举例：药物对细胞周期的影响



正常细胞对照



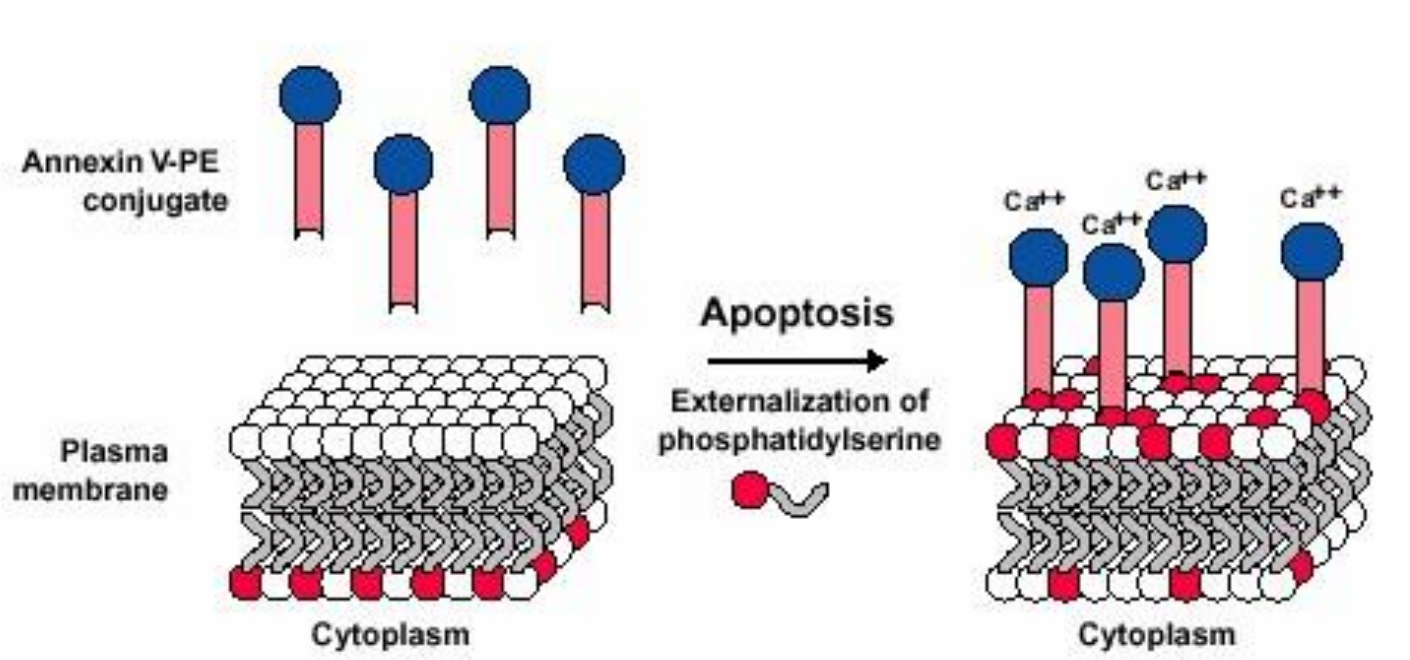
50ug/ml 七叶皂苷

经七叶皂苷诱导后，细胞被阻滞在G1期。表现在G1期细胞明显增多，S期明显减少

AV+PI双染法

- ***原理：***

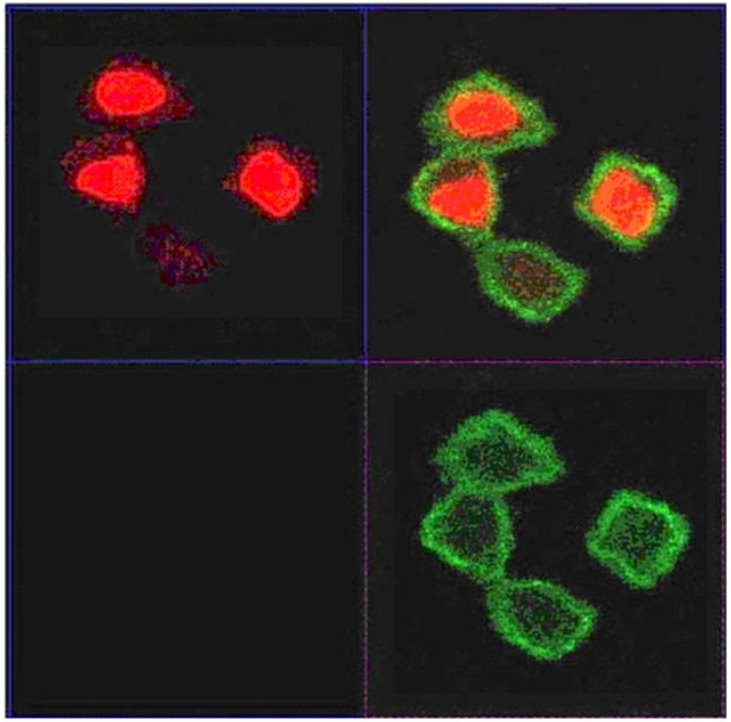
  凋亡启动后，膜内侧磷脂酰丝氨酸（PS）外翻到膜表面，胞膜结构仍然完整。

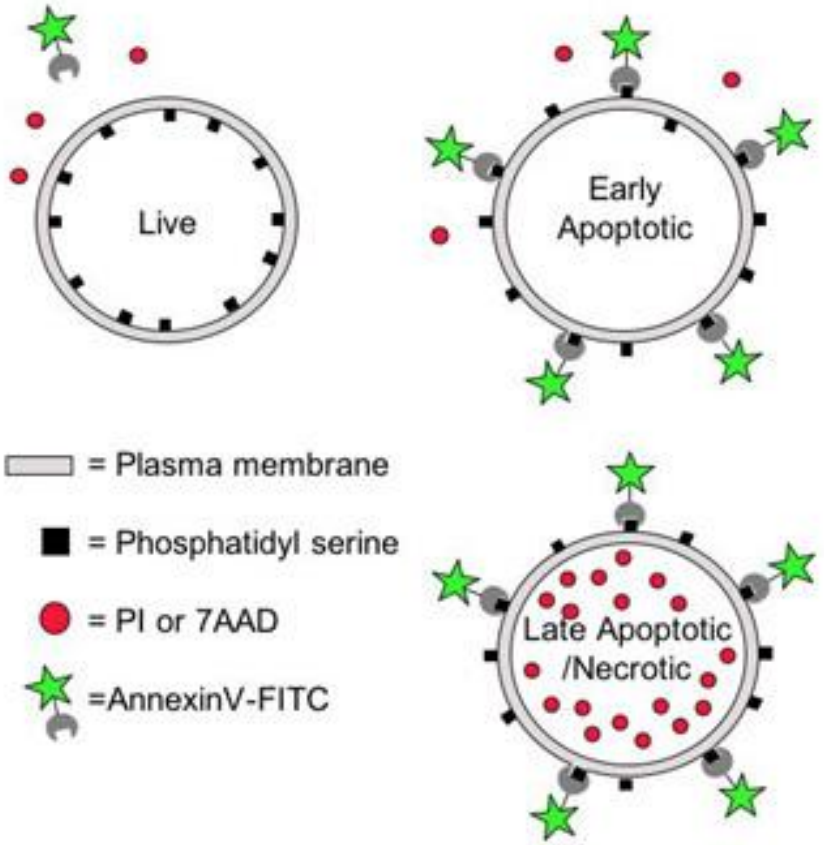


Schematic representation of the Annexin V assay.

- ***染色方法：***

  活细胞同时染Annexin V+PI。（PI作用：标记碎片与死细胞）。

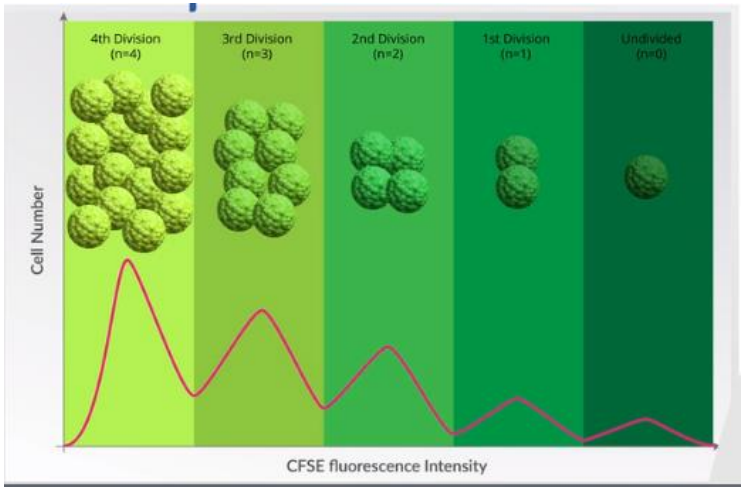


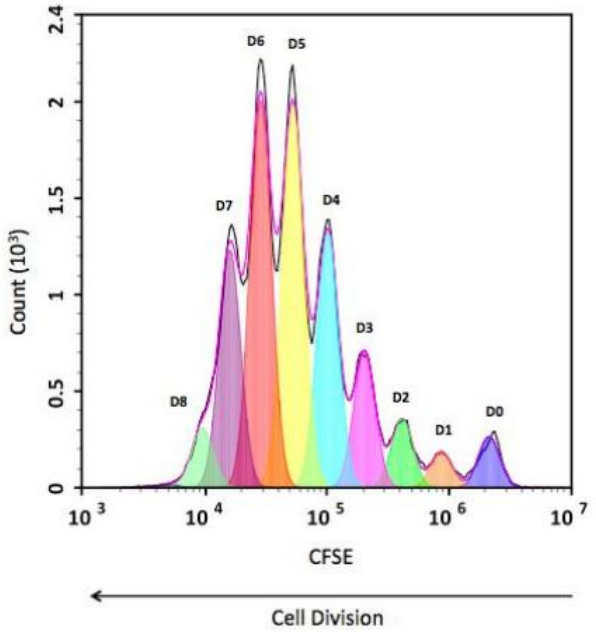


❑细胞增殖分析-CFSE法

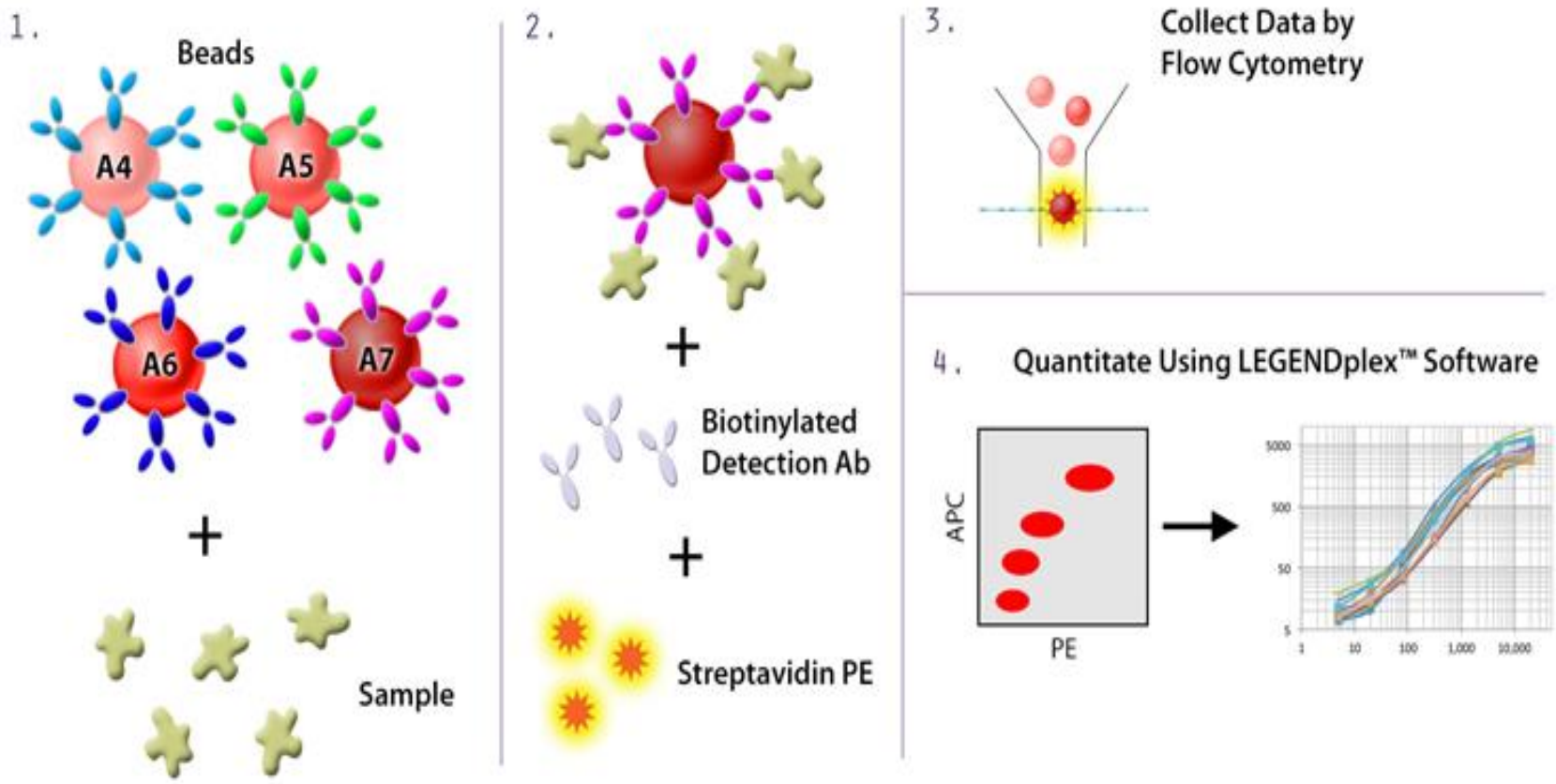
- 原理

荧光染料CFSE，也可称为CFDA-SE (5, 6- carboxyfluorescein diacetate, succinimidyl ester) 即羟基荧光素二醋酸盐琥珀酰亚胺脂，是一种可穿透细胞膜的荧光染料，在活细胞内与胞内蛋白共价结合，水解后释放出绿色荧光。当细胞进行分裂增殖时，具有荧光的胞质蛋白被平均分配到第二代细胞中，这样与第一代细胞相比，其荧光强度便会减弱至一半；以此类推，分裂得到的第三代细胞的荧光强度便会比第二代细胞再次减弱。



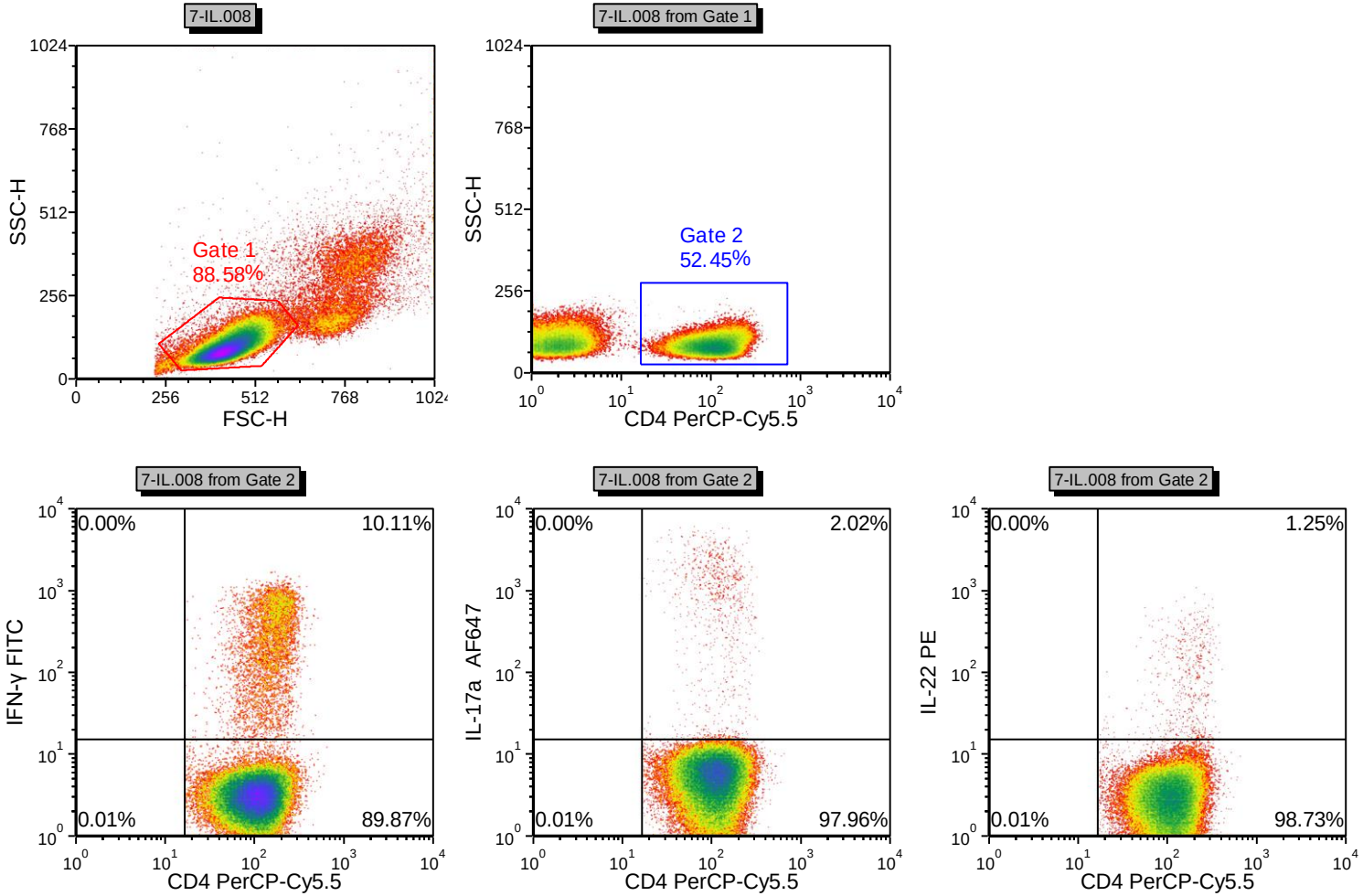


❑血清血浆中可溶性蛋白的检测



以微球为反应载体，以达到实现多种蛋白分子同时定量的目的

❑细胞功能-细胞因子的产生



微颗粒检测（微生物、细胞外囊泡）

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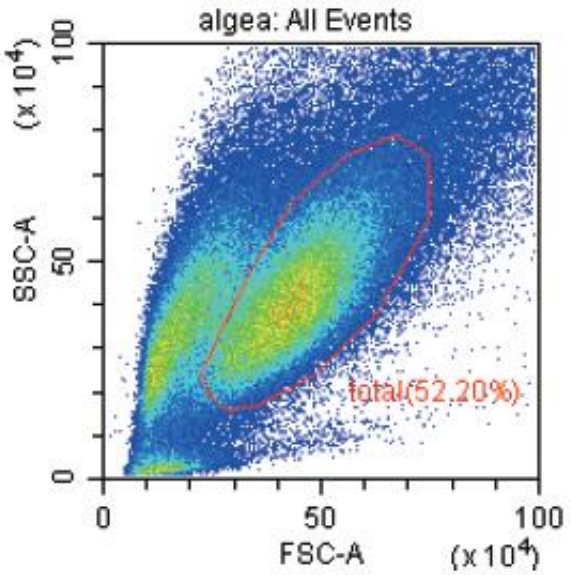
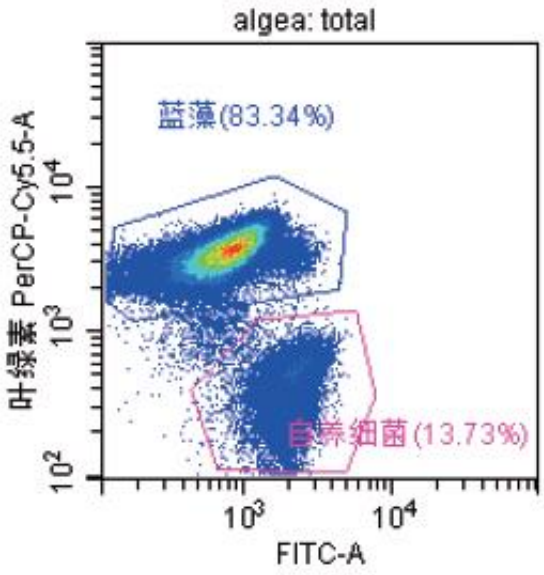
❑蓝藻计数

实验结果





Tube Name: algea

Sample ID:

Volume(μL): 3.7

Population	Events	% Parent	Events/μL(V)
蓝藻	77090	83.34%	20704.57
自养细菌	12697	13.73%	3410.12

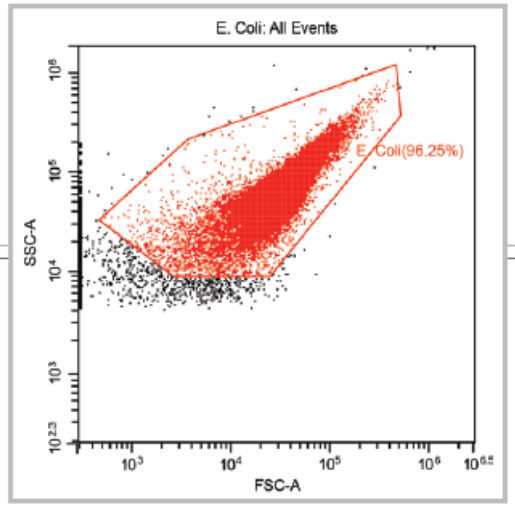
大肠杆菌的检测

Materials and tools

E coli sample

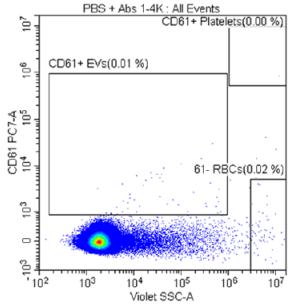
Tube Name: E. Coli
Sample ID:

Population	Events	% Total	% Parent	Events/µL(V)
E. Coli	46645	96.25%	96.25%	27344.41

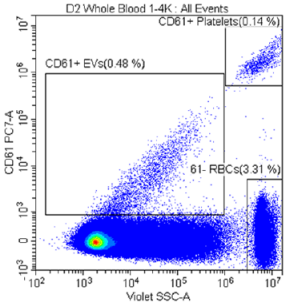


细胞外囊泡

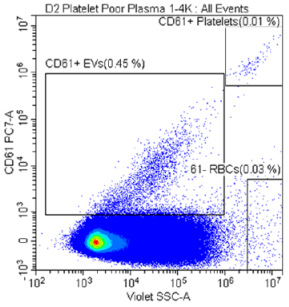
A. PBS + Abs



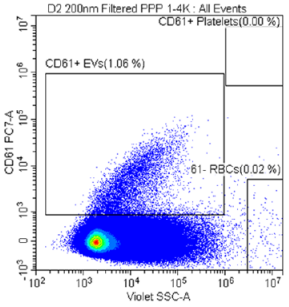
B. Whole Blood



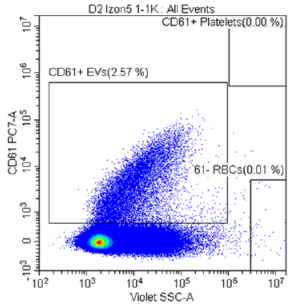
C. Plasma



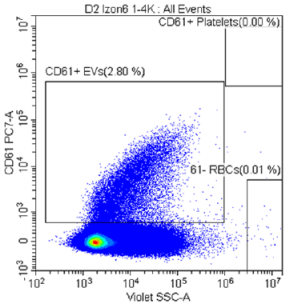
D. Filtered Plasma



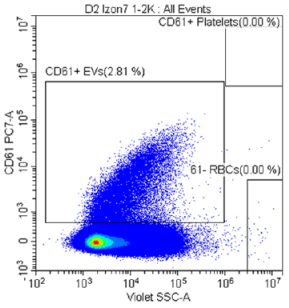
E. Izon Fraction 5



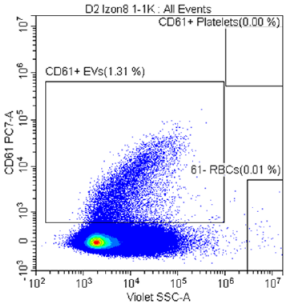
F. Izon Fraction 6

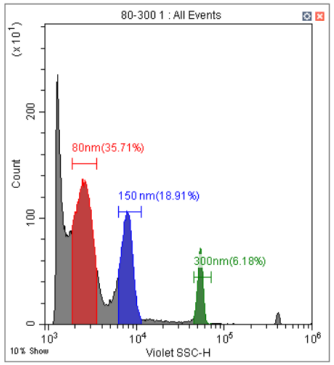


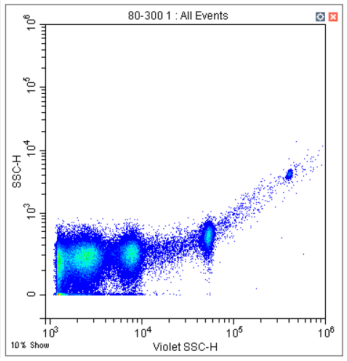
G. Izon Fraction 7



H. Izon Fraction 8







Brittain, George C., et al. "A novel Semiconductor-Based flow cytometer with enhanced Light-Scatter Sensitivity for the Analysis of Biological nanoparticles." Scientific reports 9.1 (2019): 1-13.

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分选型流式应用

- 分选特定群体；
- 干细胞分选;
- 单克隆筛选/单细胞分选；
- 微生物分选；

- ***几种必须使用流式分选的情况***

（1）需要根据多个细胞参数（多色荧光标记）来分选细胞;

（2）需要根据表面蛋白的密度差异（或即同一信号的强弱差异）来分类分离细胞;

（3）要求目标细胞群有极高的纯度(95%-100%);

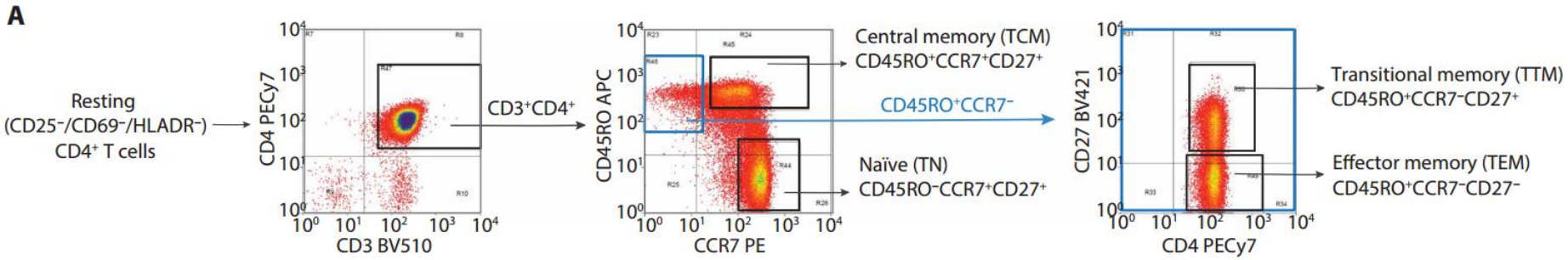
（4）根据细胞功能活动及状态特征（胞吞、凋亡等，非抗体标记）来分选细胞；

（5）多孔板分选；如单克隆分选，在96、384 每个孔中精确的分入1个细胞；

（6）需要分选极低含量的稀有细胞（如0.001%或更低）；

（7）分选感兴趣的未知特征的细胞亚群。

- **分选特定细胞亚群-T Naïve /Memory**

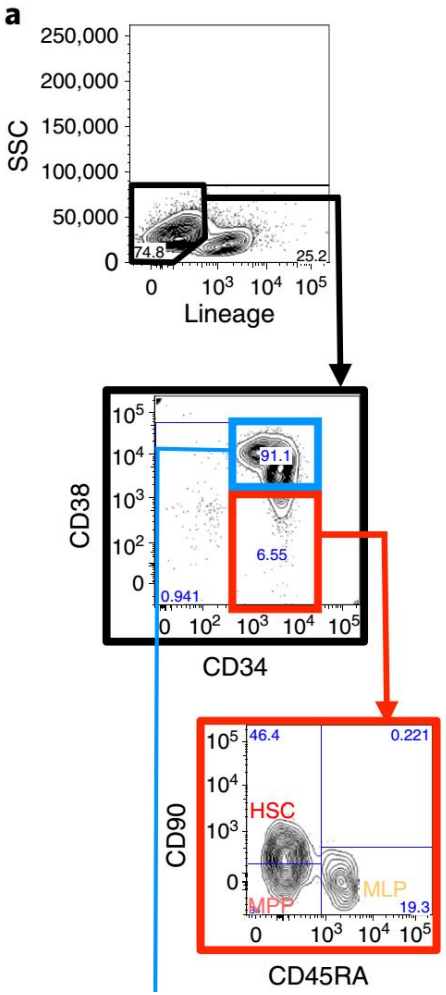


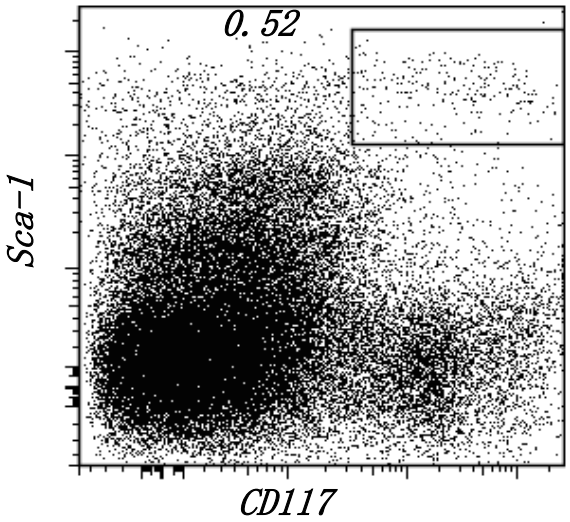
TN: CD45RO−/CCR7+/CD27+;
TCM: CD45RO+/CCR7+/CD27+;
TTM: CD45RO+/CCR7−/CD27+;
TEM: CD45RO+/CCR7−/CD27−.

Different human resting memory CD4+ T cell subsets show similar low inducibility of latent HIV-1 proviruses. Kwon KJ et al. Sci Transl Med. (2020)

- **分选造血干细胞**

流式分选是目前富集纯化造血干细胞最重要的方法。目前认为人的造血干细胞的标志为Lin-CD34+CD38-，人造血干细胞 的分离纯化可以选择骨髓，胚胎和脐带血的单细胞悬液。

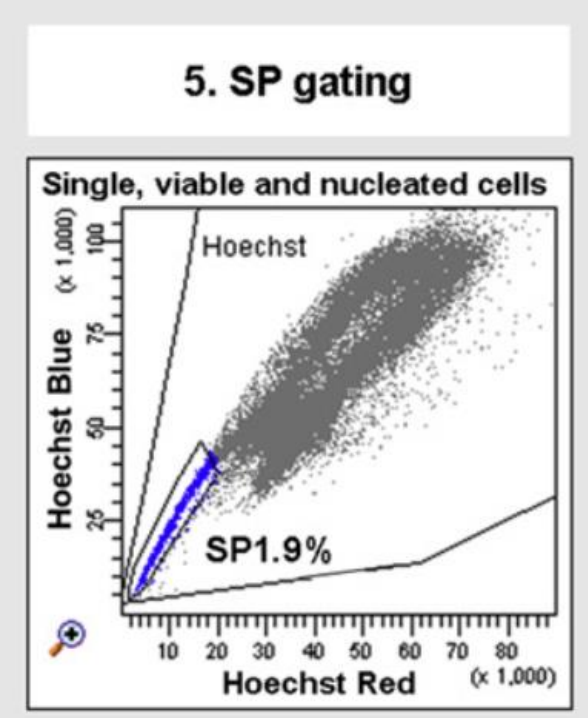




小鼠的造血干细胞的标志为Lin-Sca-1+CD117+

Dynamics of genetically engineered hematopoietic stem and progenitor cells after autologous transplantation in humans. Scala S et al. Nat Med. (2018)

- **分选侧群细胞（Side population cells）**



Hoechst33342 是一种亲脂性 *DNA* 荧光染料，可以自由通过活细胞的细胞膜进入细胞内，与 *DNA* 结合，所以可以直接标记活细胞的 *DNA*。干细胞能够通过 *ABC* （*ATP Bind Cassette*）运输蛋白 *Bcrp1/ABCG2* 将细胞内的 *Hoechst33342* 泵出细胞外，而非干细胞没有这种能力，所以用该染料标记时，干细胞染色较低，在流式图上能够明显区分与其他细胞。

Critical appraisal of the side population assay in stem cell and cancer stem cell research. Golebiewska A et al. Cell Stem Cell. (2011)

- **分选肿瘤干细胞**

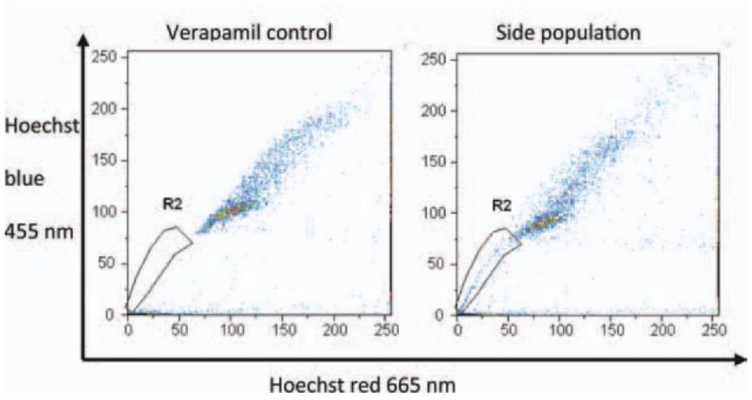


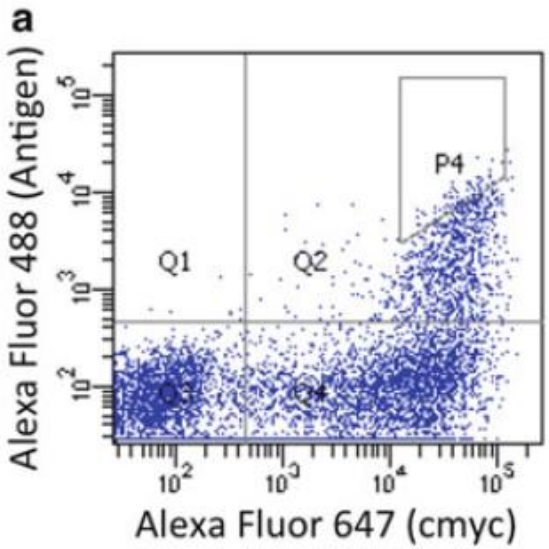
Table 1. Overview of cancer stem cell markers suitable for flow cytometric analysis

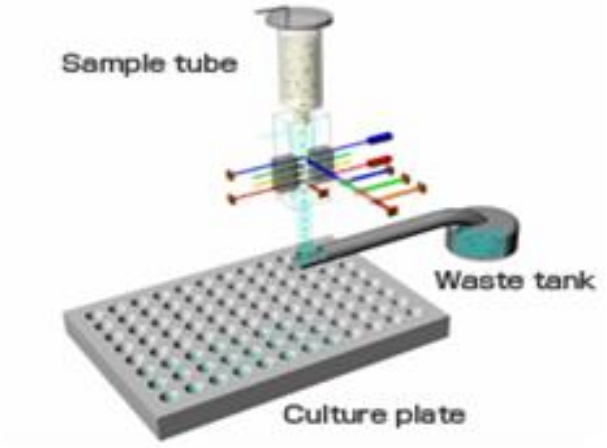
	PUTATIVE MARKER PATTERNS FOR CANCER STEM CELLS (CSCS)	REFERENCES	ANNOTATION
Acute myelogenic leukemia (AML)	$CD34^+/CD38^-$	(53,54)	
	$CD90^+$	(55)	High risk AML
Acute lymphoblastic leukemia	$CD34^+/CD38^-/CD19^+$	(56)	
Bone sarcoma	$Stro\text{-}1^+/CD105^+/CD44^+$	(57)	
Brain tumor	$CD133^+$	(50,58)	
Breast cancer	$ESA^+/CD44^+/CD24^{-/low}/lin^{-a}$)	(18)	Further enrichment by selection of ESA^+ cells
	$CD90^{low}/CD44^+$	(59)	Cells localized at the invasive front
	$CD44^+/CD24^{-/low}/ALDH1^{high}$	(51)	
Colon cancer	$CD133^+$	(48,60)	
	$ESA^{high}/CD44^+/(CD166^+)$	(61)	
	$CD133^+/CD44^+$	(62,63)	
	$CD133^+/CD24^+$	(9)	
Colon cancer (metastatic)	$CD133^+/CD44^{low}/CD24^+$	(64)	Two subsets of tumor-initiating cells identified
	$CD133^-/CD44^+/CD24^-$		
Endometrial cancer	$CD133^+$	(65)	
	SP^+	(66)	
Gall bladder cancer	$CD133^+/CD44^+$	(67)	
Gastric cancer	$CD44^+$	(68)	
Liver cancer	$CD133^+/CD44^+$	(69)	
	$CD90^+$	(70)	
	$EpCAM^+$	(71)	
	$CD133^+$	(24,72)	
Metastatic melanoma	$CD20^+$	(34)	
Ovarian cancer	$CD133^+/ALDH^+$	(73)	
	$CD44^+/CD117^+$	(74)	
Pancreatic cancer	$CD44^+/CD24^+/ESA^+$	(75)	
Prostate cancer	$CD44^+/\alpha_2\beta_1^{hi}/CD133^+$	(20)	
	$CD44^+/CD24^-$	(76)	
	SP^+	(77)	
Renal cell carcinoma	$CD105^+/(CD133^-/CD24^-)$	(78)	
Head and neck cancer	$CD44^+$	(79)	No further enrichment by endothelial-specific antigen or CD24

[a] lin = lineage marker CD3, CD14, CD16, CD19, CD20, and CD56.

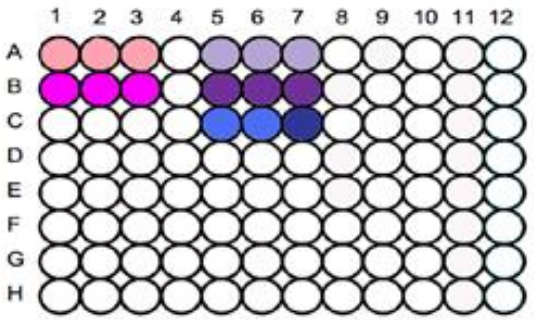
Flow cytometry in cancer stem cell analysis and separation. Greve B et al. Cytometry A. (2012)

单克隆细胞株的筛选

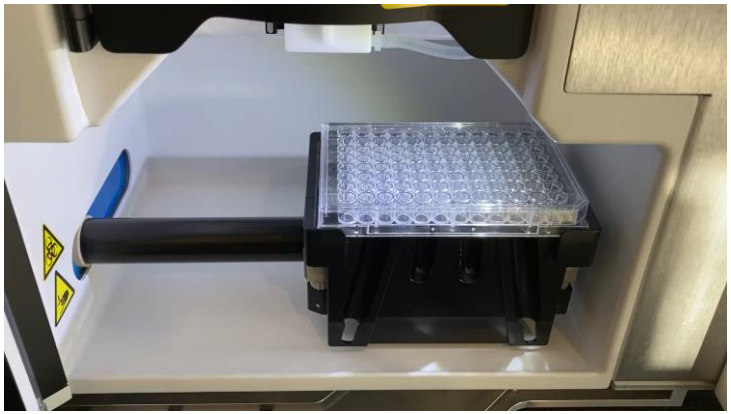


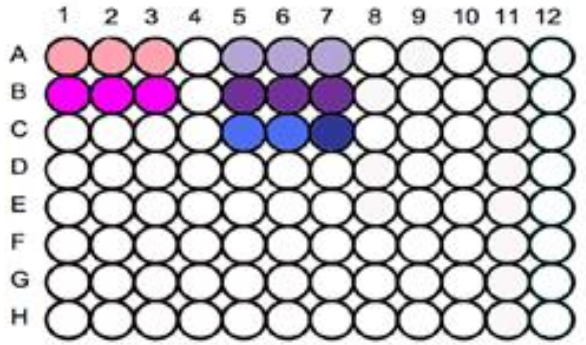


流式细胞技术筛选法的组件和工作流程图，可同时检测抗体的分泌，细胞数量和细胞存活率，筛选出合适的细胞构建细胞株。



单克隆筛选





流式单克隆分选优势

- 省时省力
- 更高的精准度（不会出现一个孔两个克隆的情况）

流式分选VS传统有限稀释法

单细胞分选

- 分选B细胞/中和抗体筛选

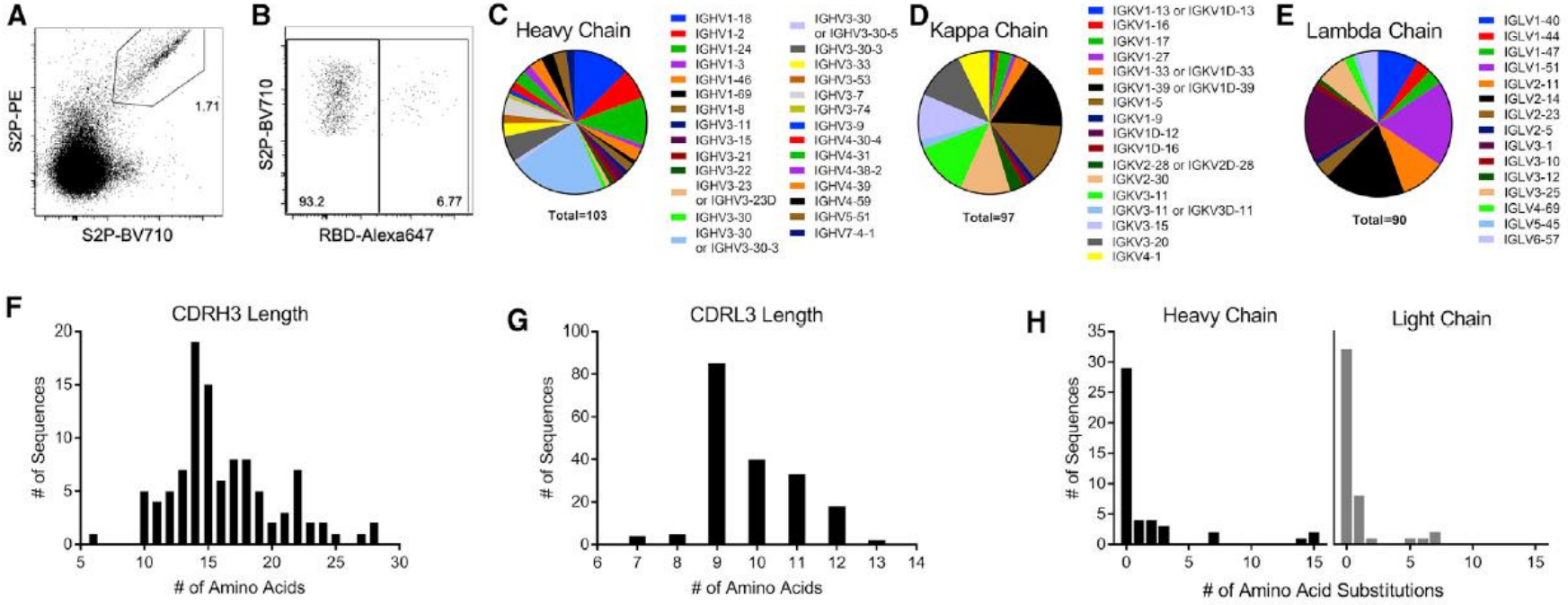


Figure 2. Early B Cell Response to SARS-CoV-2 Is Diverse and Largely Unmutated

Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent Neutralizing Antibodies with Limited Somatic Mutation, Immunity (2020)

流式分选应用：酵母表面展示

Rosowski et al. Microb Cell Fact (2018) 17:3
https://doi.org/10.1186/s12934-017-0853-z

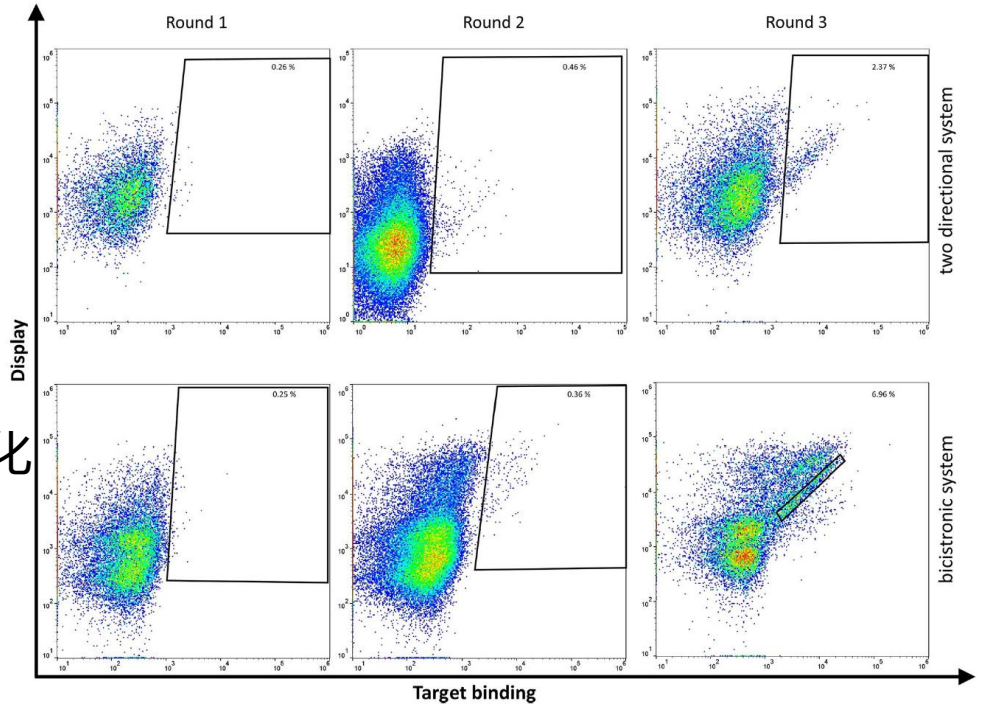
Microbial Cell Factories

RESEARCH Open Access

A novel one-step approach for the construction of yeast surface display Fab antibody libraries

Simon Rosowski[1], Stefan Becker[2], Lars Toleikis[2], Bernhard Valldorf[3], Julius Grzeschik[1], Deniz Demir[2], Iris Willenbücher[2], Ramona Gaa[2], Harald Kolmar[1], Stefan Zielonka[2*] and Simon Krah[2*]

- 酵母表面展示
  - 技术成熟，广泛应用于蛋白定向进化
  - 流式分选可大大简化筛选流程

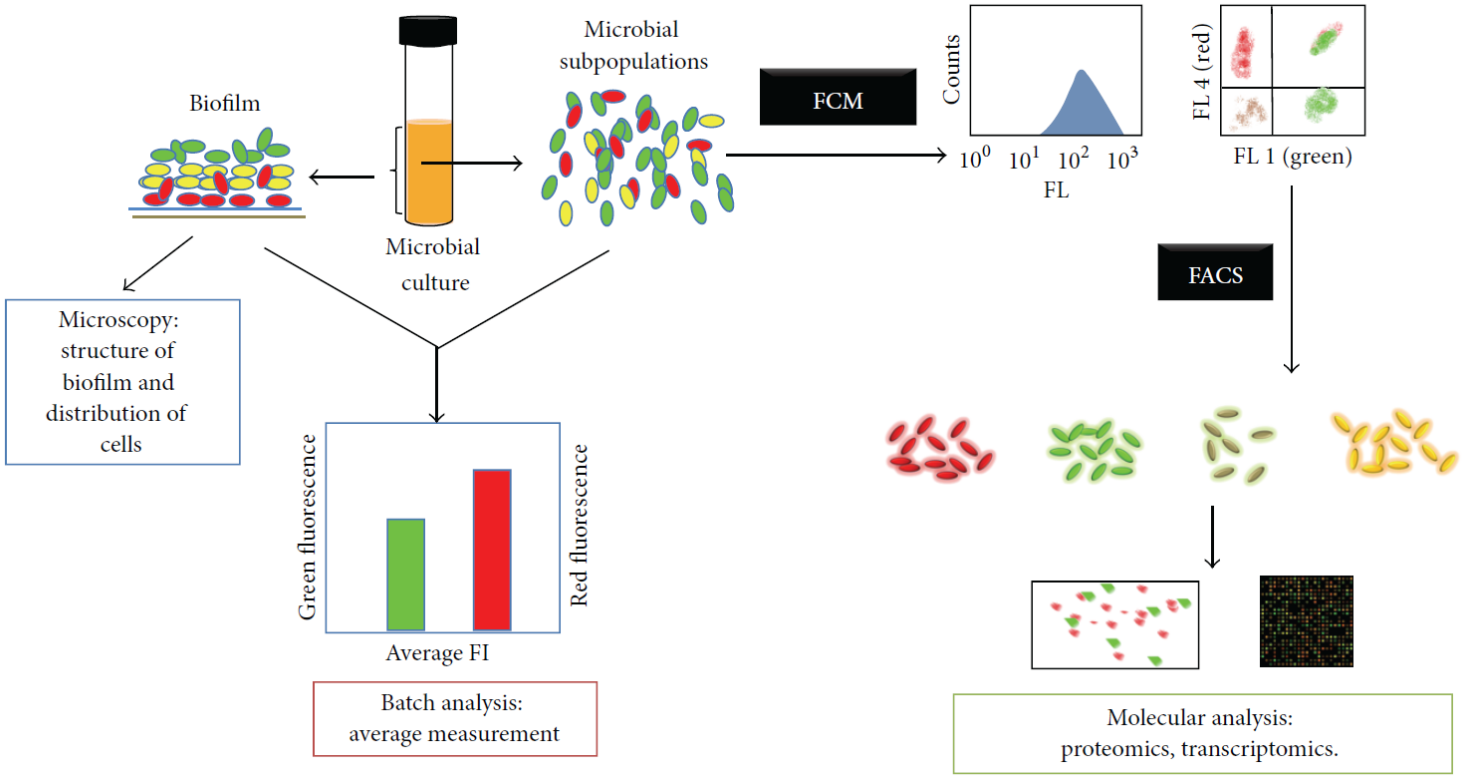


--------Rosowski, S., et al, 2018, ***Microbial cell factories***

BECKMAN COULTER Life Sciences

流式分选应用：培养菌群分选

FCM allows analyzing subpopulations of cultured bacterial cells with different physiological states



--------*Ambriz-Avina, V., et al, 2014,* ***BioMed research international***

流式分选应用：环境菌群分选鉴定

SCIENTIFIC REPORTS

OPEN

Single cell genomics yields a wide diversity of small planktonic protists across major ocean ecosystems

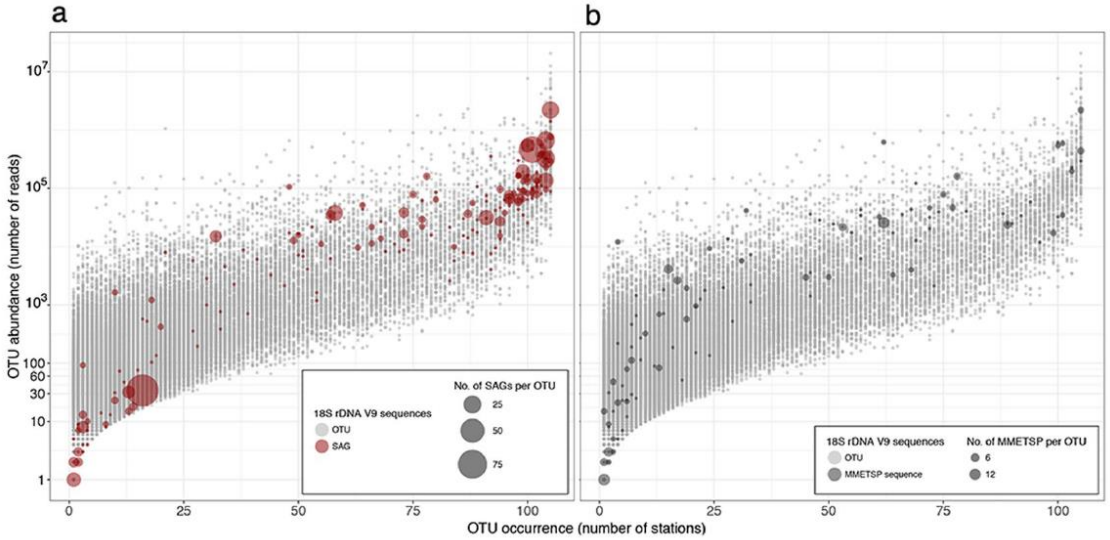
- 环境菌群分选
  - 单细胞分选及测序
  - 菌株鉴定

海洋生物样本

SYBR Green I

流式分选单个细胞

单细胞基因组学-海洋生物多样性



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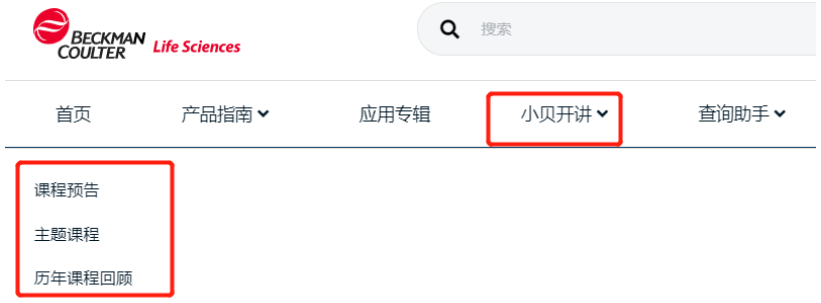
感谢聆听



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