

The background features a dark blue field with various scientific illustrations. On the left, there are molecular structures with spheres and connecting lines. In the center, a DNA double helix is visible. On the right, there are antibody-like Y-shaped structures and a cell with a nucleus. The overall aesthetic is clean and scientific.

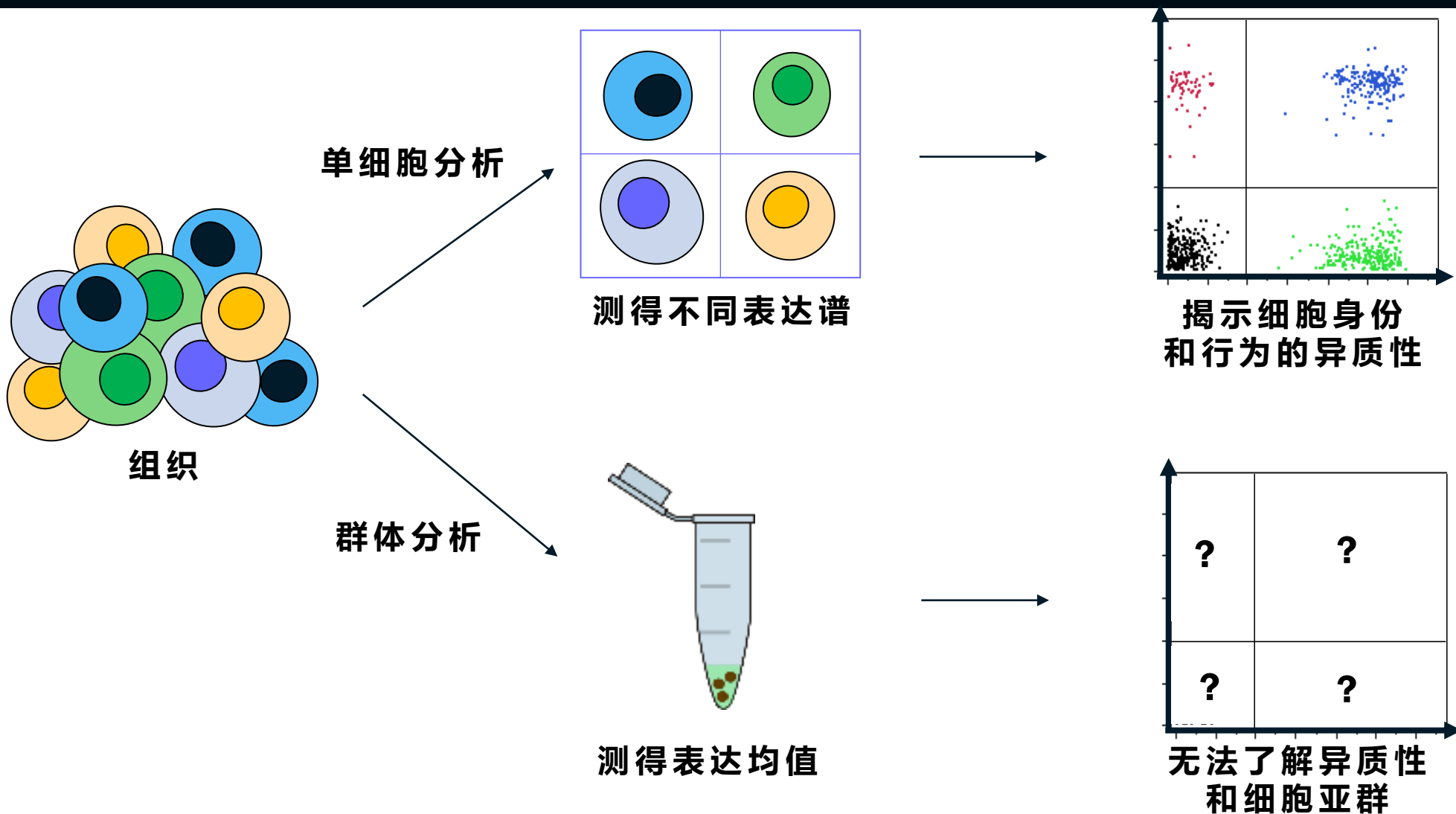
biotechne®

MILO 单细胞 WESTERN 技术原理及应用

杜颖颖

2022年9月21日 @ 浙江大学

揭示细胞异质性



单细胞工具助力全新生物学发现

nature

Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells

Devon A. Lawson, Nirav R. Bhakta, Kai Kessenbrock, Karin D. Prummel, Ying Yu, Ken Takai, Alicia Zhou, & Zena Werb

Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development

Marc A. Gavin^{*†}, Troy R. Torgerson^{*‡}, Evan Houston^{*}, Paul deRoos[§], William Y. Elizabeth L. Ocheltree[‡], Philip D. Greenberg^{*¶}, Hans D. Ochs^{*‡}, and Alexander

^{*}Department of Immunology and [§]Howard Hughes Medical Institute, University of Washington, Box 356160, Regional Medical Center, Seattle, WA 98105; [¶]Fred Hutchinson Cancer Research Center, Seattle, WA 98040; [‡]Rikshospitalet University Hospital, N-0027 Oslo, Norway

Edited by Richard A. Flavell, Yale University School of Medicine, New Haven, CT, and approved March 1, 2018

肿瘤学
免疫学
干细胞

.....

Cell Stem Cell



Volume 13, Issue 1, 3 July 2013, Pages 23–29

Brief Report

Single-Cell Analysis Reveals that Expression of Nanog Is Biallelic and Equally Variable as that of Other Pluripotency Factors in Mouse ESCs

Dina A. Faddah^{1,2}, Haoyi Wang¹, Albert Wu Cheng^{1,3}, Yarden Katz^{1,4}, Yosef Buganim¹, Rudolf Jaenisch^{1,2}

[Show more](#)

Cell

Molecular Architecture of the Mouse Nervous System

Amit Zeisel,^{1,3} Hannah Hochgerner,^{1,3} Peter Lönnerberg,¹ Anna Johnsson,¹ Fatima Memic,¹ Job van der Zwan,¹ Martin Häring,¹ Emelie Braun,¹ Lars E. Borm,¹ Gioele La Manno,¹ Simone Codeluppi,¹ Alessandro Furlan,^{1,4} Kawai Lee,¹ Nathan Skene,¹ Kenneth D. Harris,² Jens Hjerling-Leffler,¹ Ernest Arenas,¹ Patrik Ernfors,¹ Ulrika Marklund,¹ and Sten Linnarsson^{1,5,*}

¹Division of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-17177 Stockholm, Sweden

²UCL Institute of Neurology, London WC1N 3BG, UK

³Present address: Faculty of Biotechnology and Food Engineering, Technion, 3200003 Haifa, Israel

⁴Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

⁵Lead Contact

*Correspondence: sten.linnarsson@ki.se

<https://doi.org/10.1016/j.cell.2018.06.021>

MEET Milo™



全球第一款
单细胞 western 平台



- 单张芯片可进行 ~1000 个单细胞 western 检测；
- 单个细胞可进行 12 个靶蛋白检测；
- 可使用 western blot 抗体；
- 无需转膜，全程 4-6 小时；

Milo™ 技术原理

ONE

Milo™ : 单细胞分离 + western

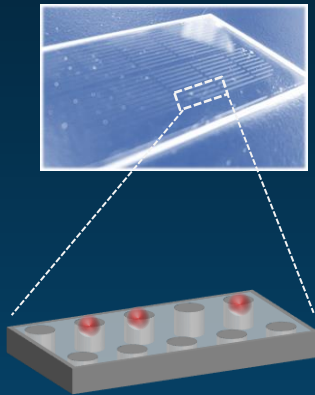
Step 1

单细胞悬液



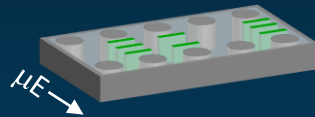
Step 2

单细胞捕获



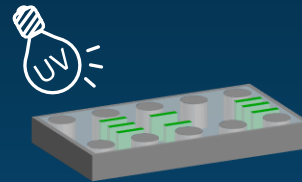
Step 3

裂解与电泳



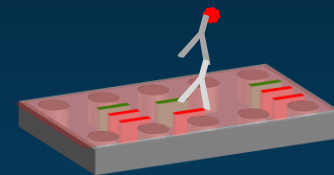
Step 4

原位固定



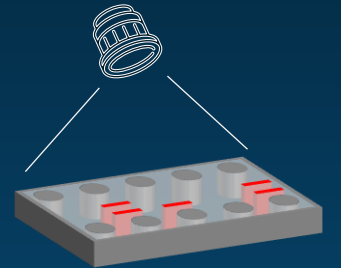
Step 5

杂交与洗涤

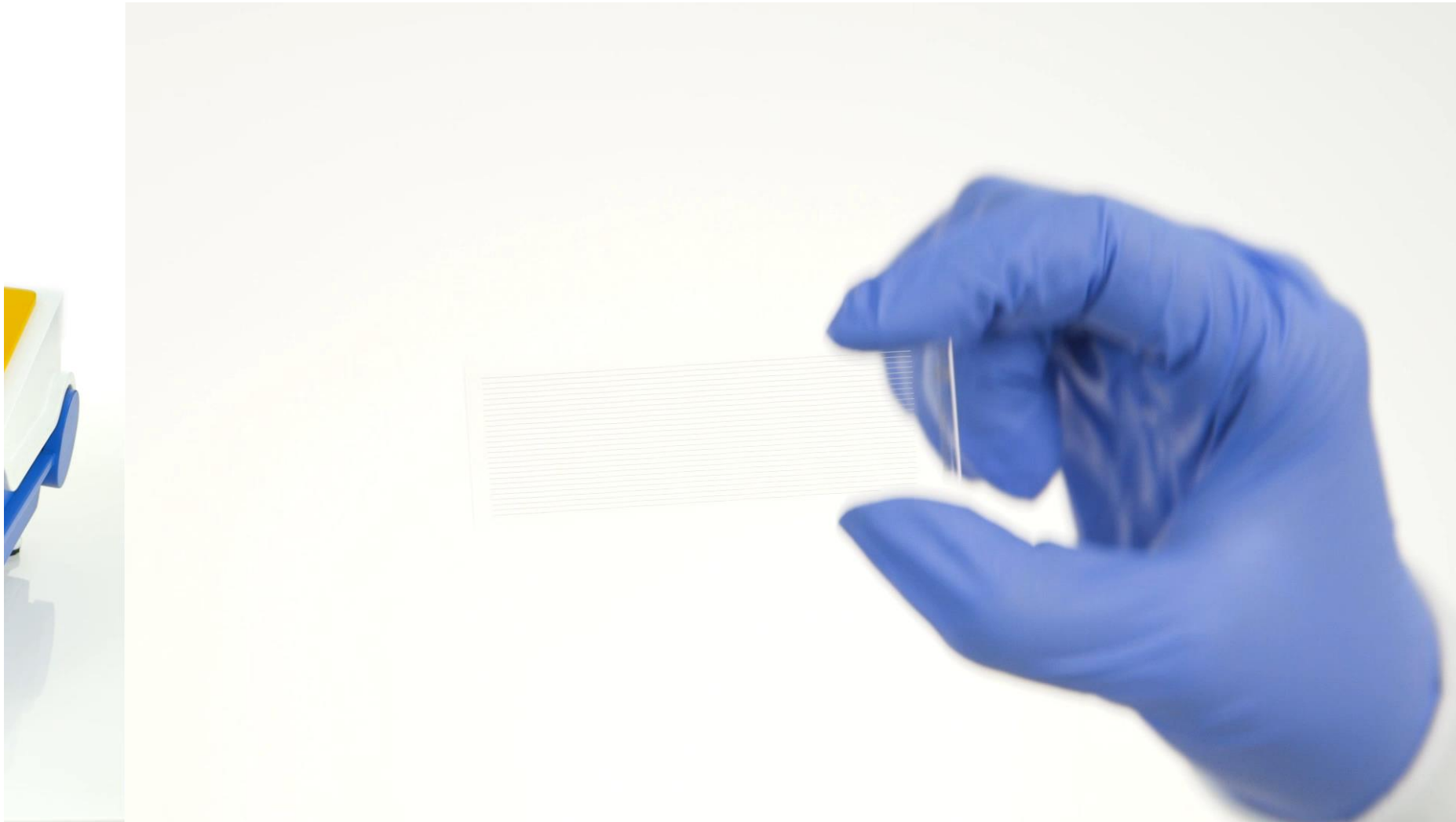


Step 6

荧光成像



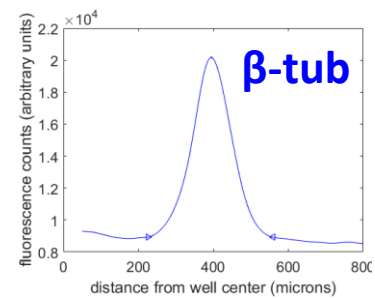
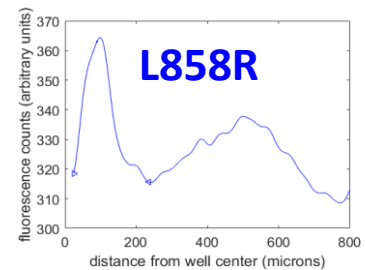
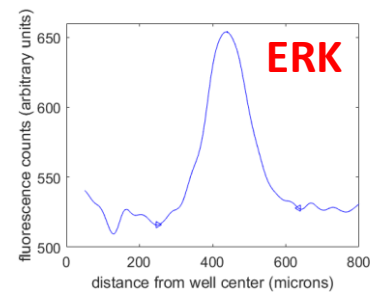
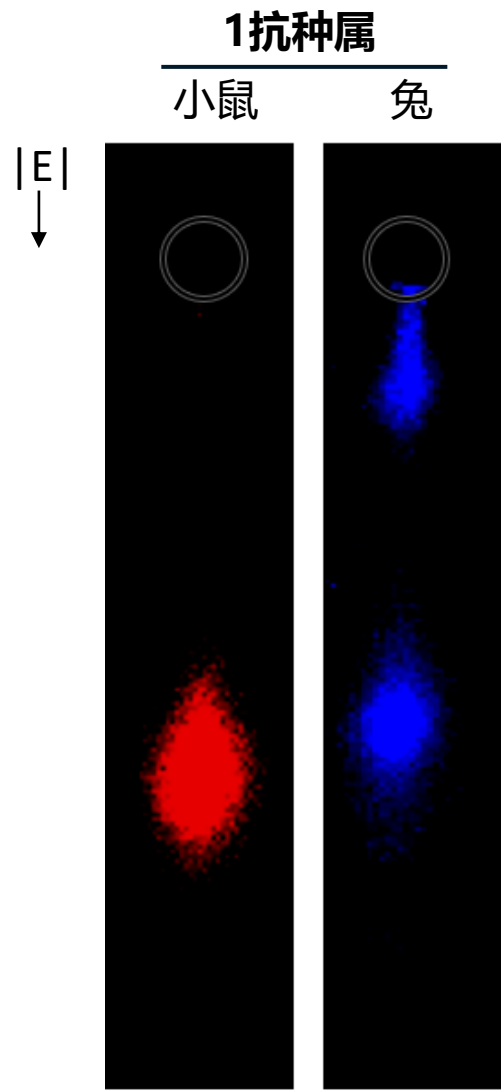
Milo™ : 单细胞分离 + western



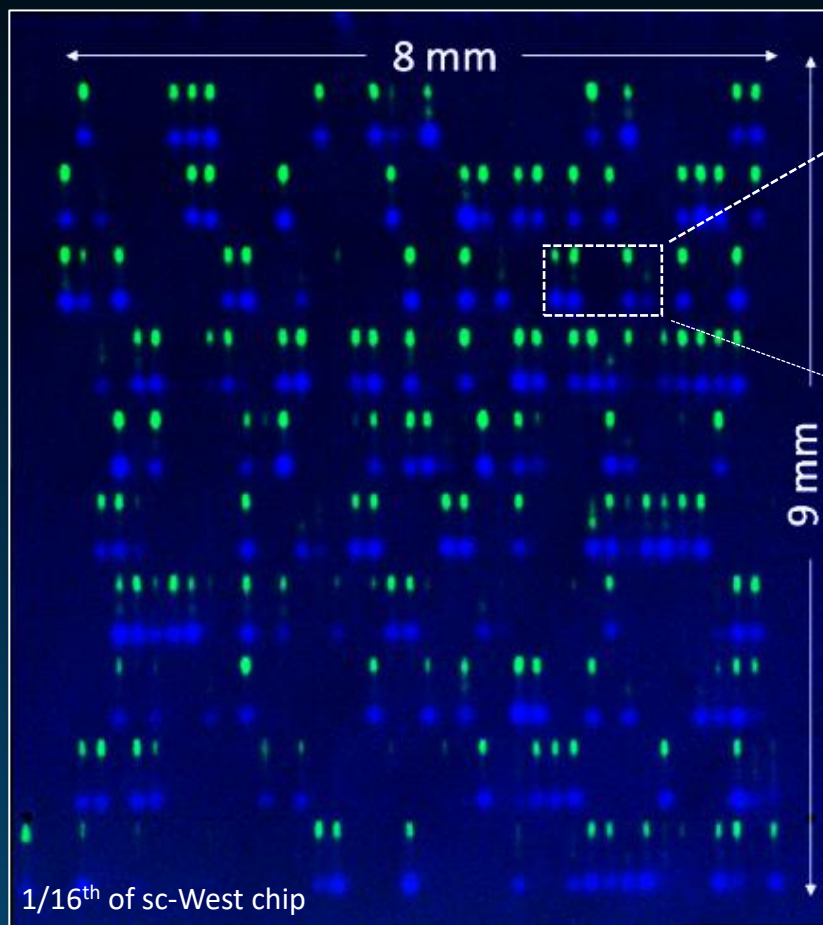
Milo™ 支持多靶点同时检测

1. 不同荧光通道
2. 分子量差异

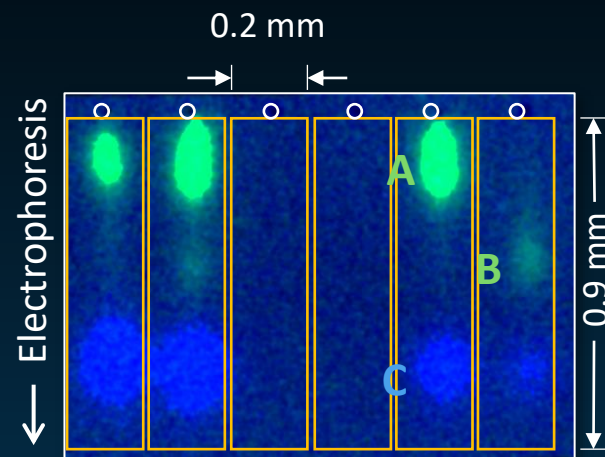
右图：1 个细胞同时
检测 3 个目的蛋白



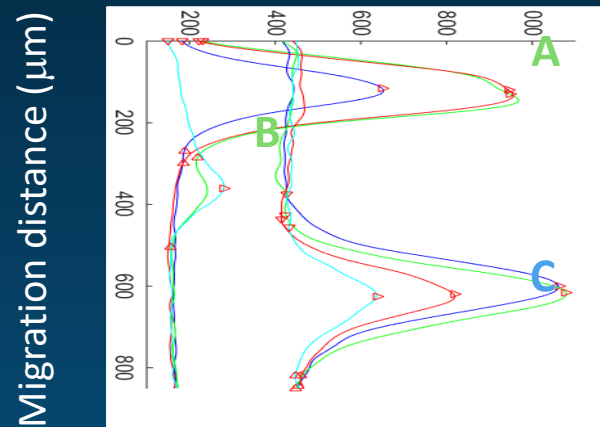
1000+ 单细胞 Western 结果



分子量分离 + 特异性抗体

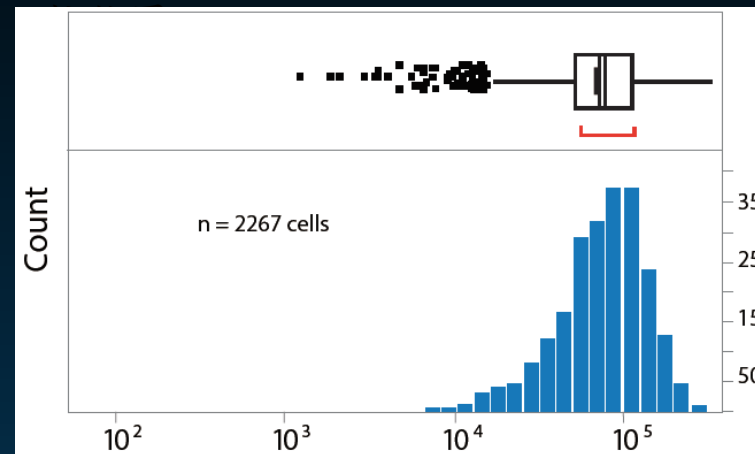
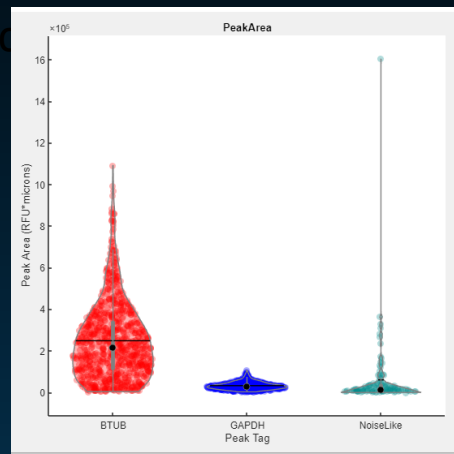


Fluorescence counts (RFU)

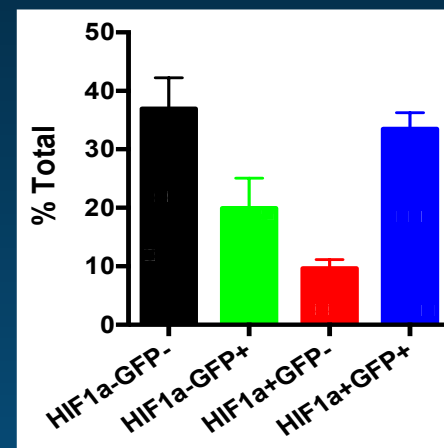
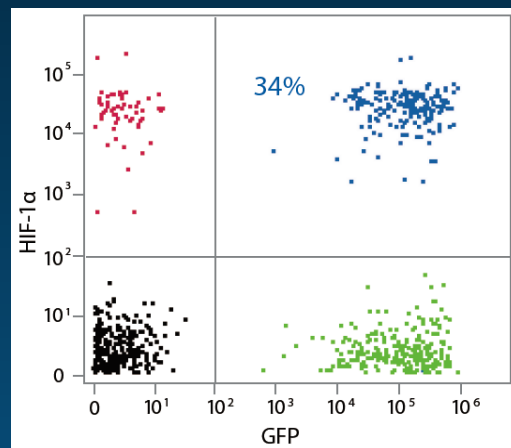


最终数据呈现

定量



占比



Milo™ 常见应用

TWOO

Milo™ 的常见应用



单细胞测序的蛋白验证



单细胞水平的蛋白共表达

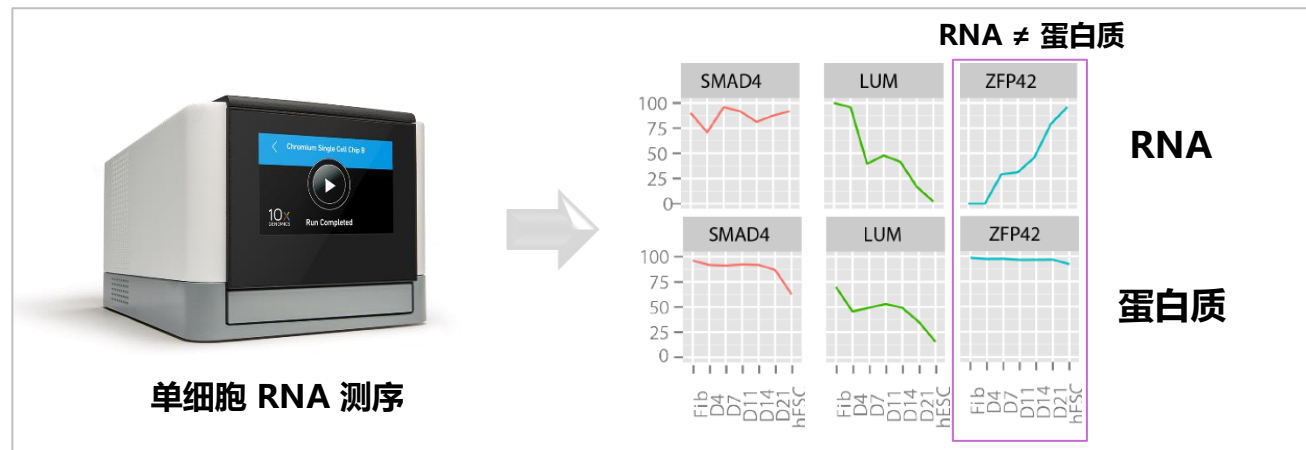


流式细胞术受限的蛋白检测

RNA 和蛋白质并不总是相关

- 蛋白质是构成细胞的基本有机物，是生命活动的主要执行者。
- 受翻译或翻译后修饰调节的影响，细胞内功能性蛋白质表达水平和 mRNA 转录水平有时并不匹配。
- microRNAs 可以抑制 mRNA 翻译成蛋白质。
- 蛋白酶和蛋白酶体可以降解蛋白质。
- 甲基化、磷酸化和其他翻译后修饰 (PTMs)

研究表明细胞内蛋白质水平只有
~40% 和 mRNA 浓度直接相关



RNA



假设

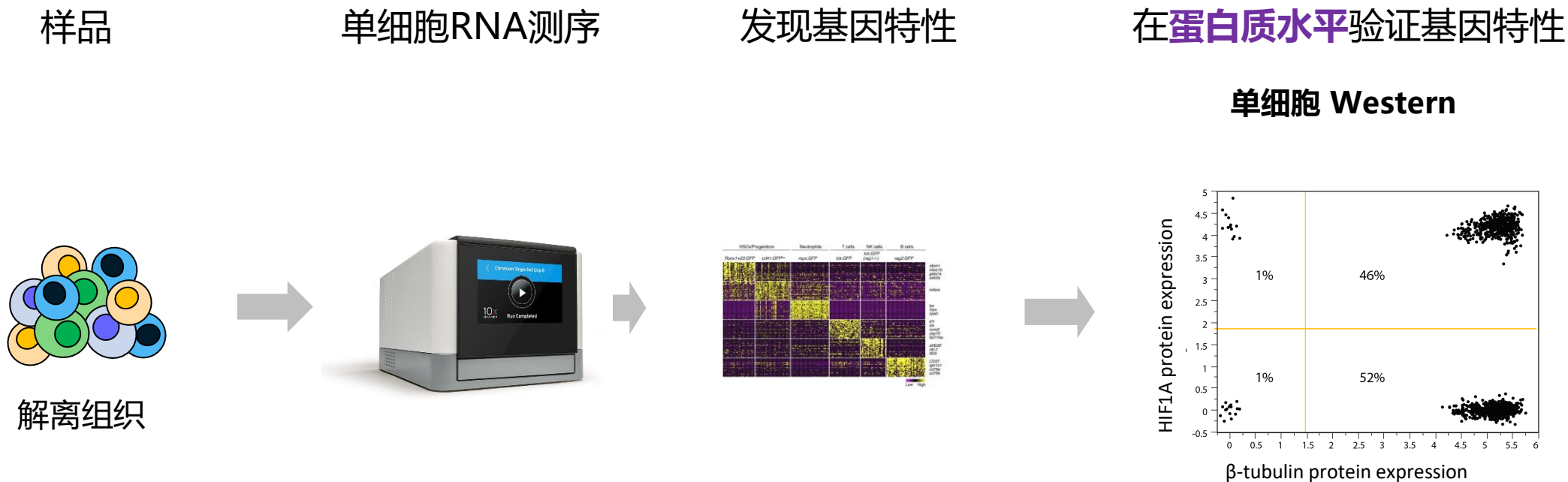
蛋白质



验证细胞功能和行为

想要准确、完整地描绘细胞功能，必须在蛋白质水平验证单细胞 RNA 测序的数据

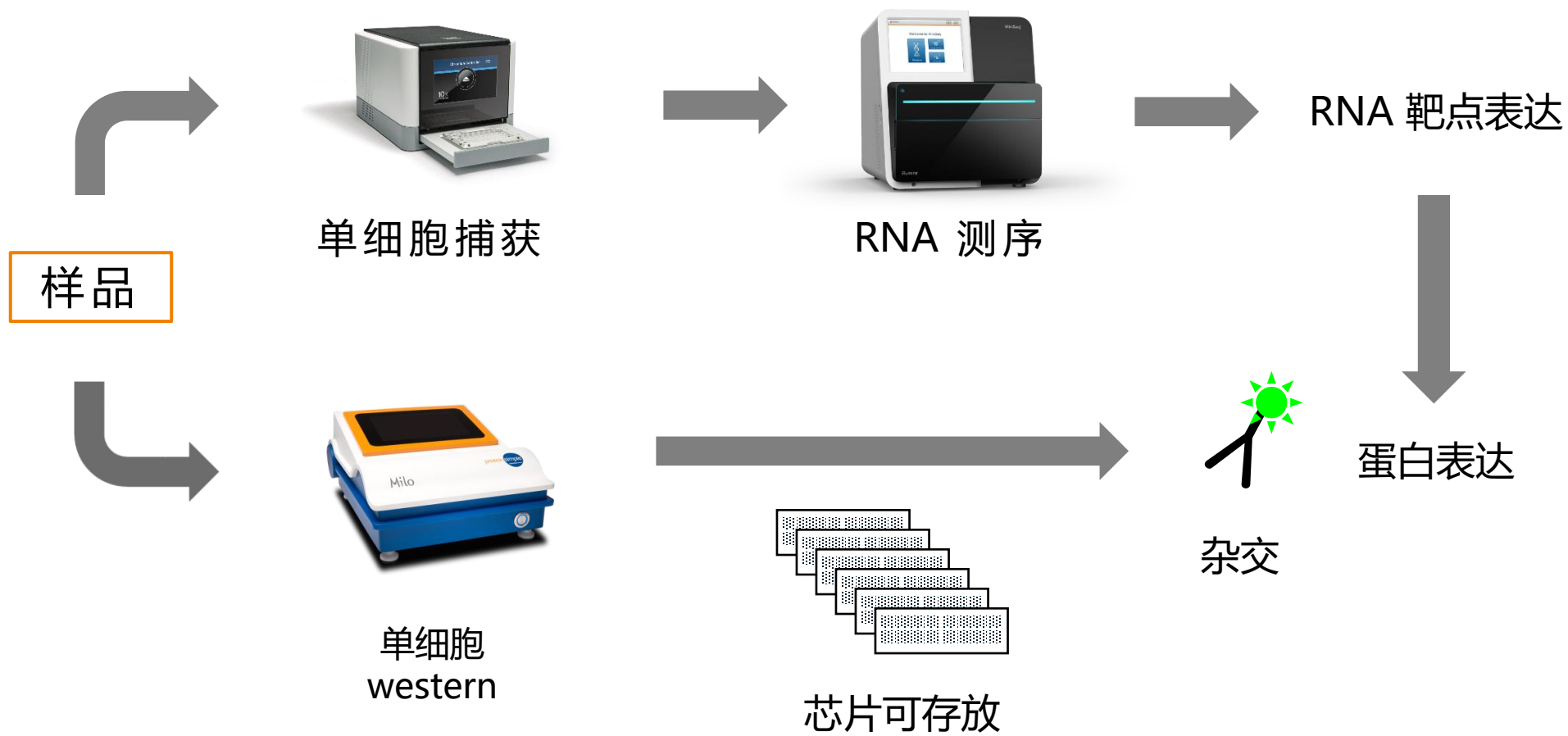
完整的单细胞分析流程



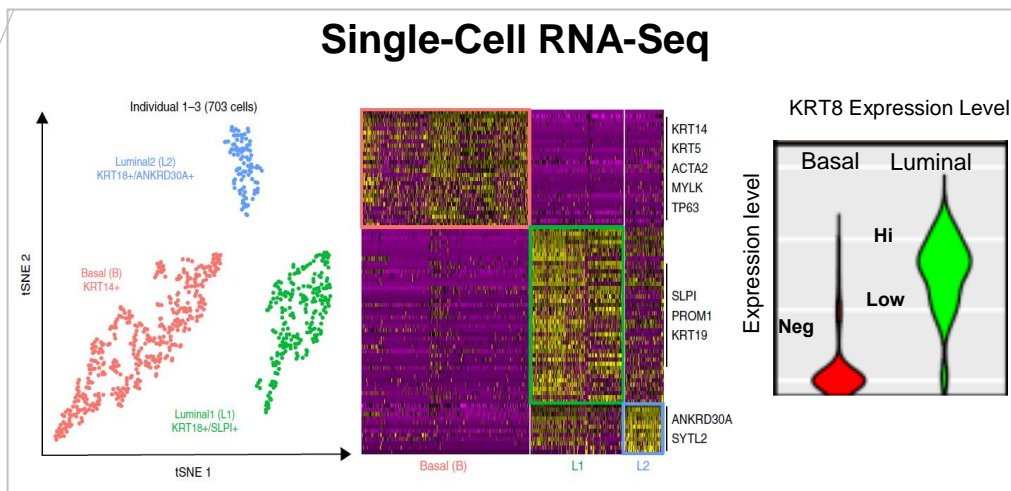
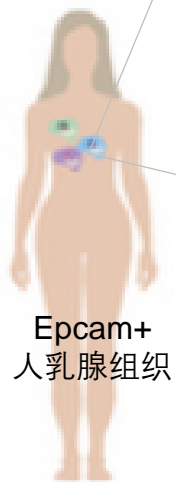
"Validating single-cell RNA sequencing by checking protein levels is quickly becoming standard practice."

- Alan Dove, Science (editorial) 2019

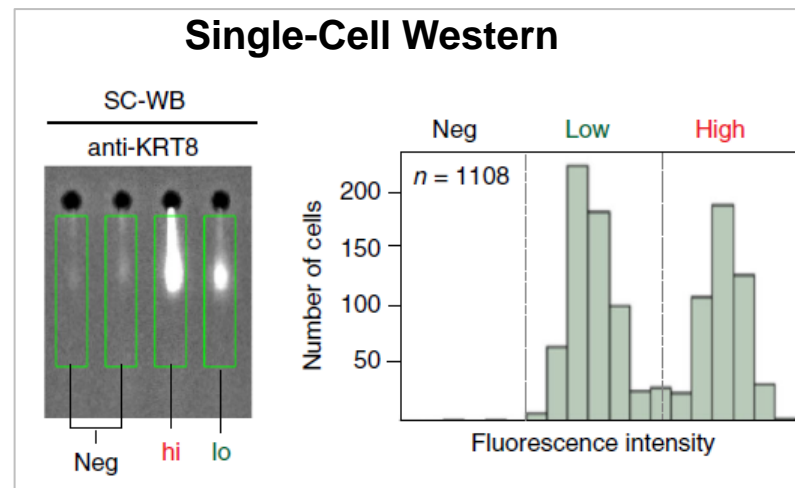
对同一份样品进行蛋白水平验证



验证乳腺癌异质性

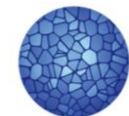


scRNA-seq identified 3 major epithelial cell types and their markers: Basal, Luminal L1 & Luminal L2. L1 & L2 types differ based on KRT8 expression levels



Single-Cell Westerns confirmed three cell states exist based on protein expression patterns: namely KRT8-negative, -low, and -high corresponding to Basal, L1 & L2 types.

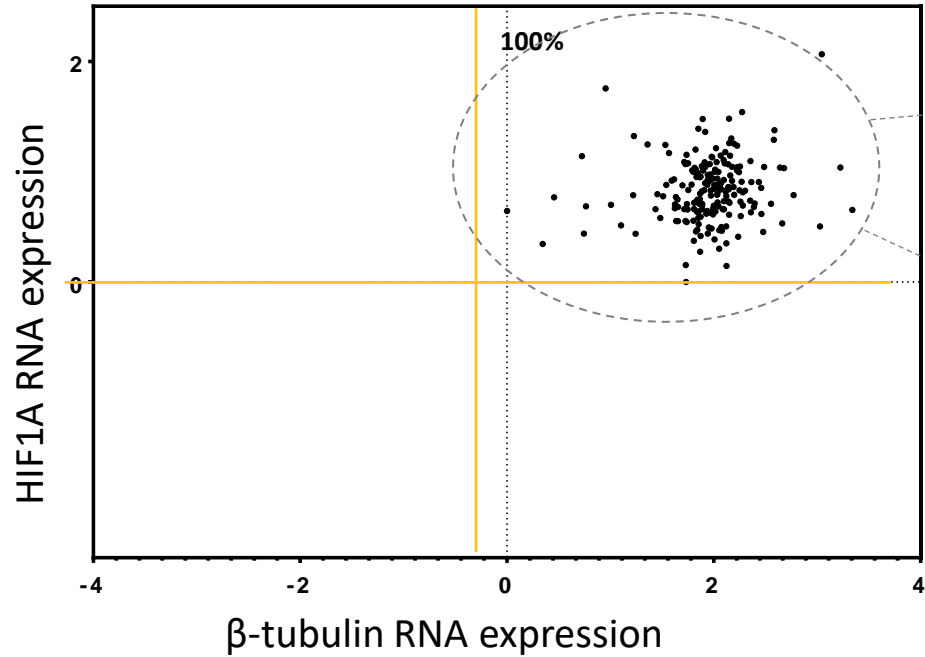
- Milo 定量了 KRT8 蛋白质表达水平的异质性，并且根据不表达、低表达和高表达 KRT8 蛋白确认了存在 3 种不同的细胞状态
- 功能验证了 RNA 水平观察到的 3 种细胞状态
- 对 3 种细胞类型精准的全面表征，为研究肿瘤发生和发展的系统性变化奠定了扎实的基础
- Milo 的定量能力是区分细胞类型的关键一环，而免疫荧光或免疫组化则不能



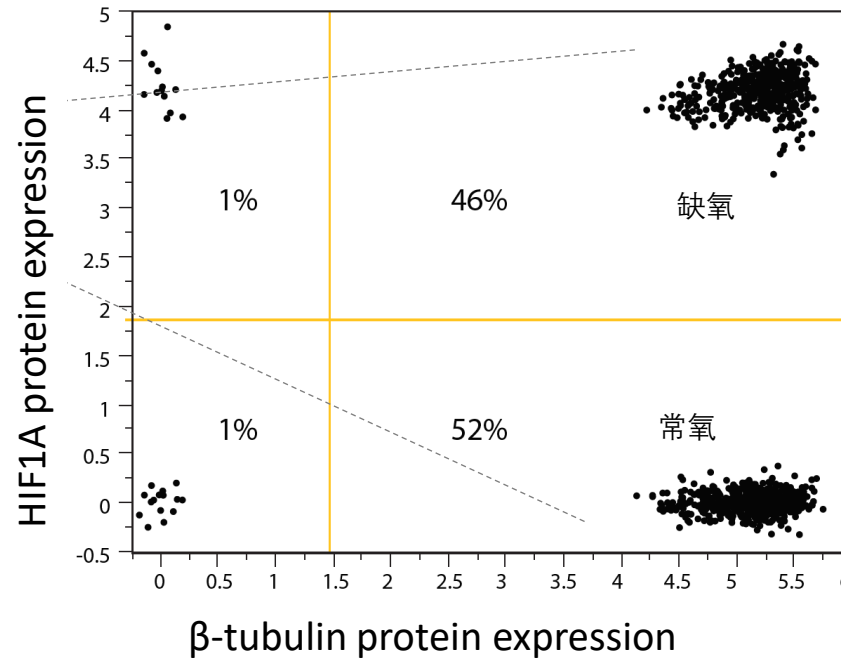
转录调节影响癌基因活性

1:1 缺氧: 常氧样品

Single-Cell RNA-Seq



Single-Cell Western



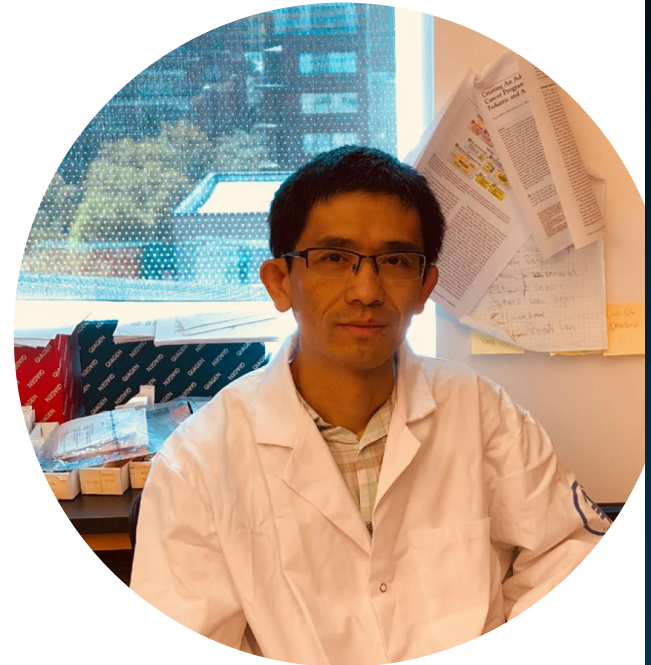
- Milo 揭示在同一个亚群中存在不同的转录调节
- Milo 可以看到常氧对蛋白转录和降解的影响, 但 RNA 水平则看不到这种影响



Stafford, et al. *Cold Spring Harbor Single-Cell Analysis Conference*

信号通路的异质性驱动转移的肿瘤细胞亚群

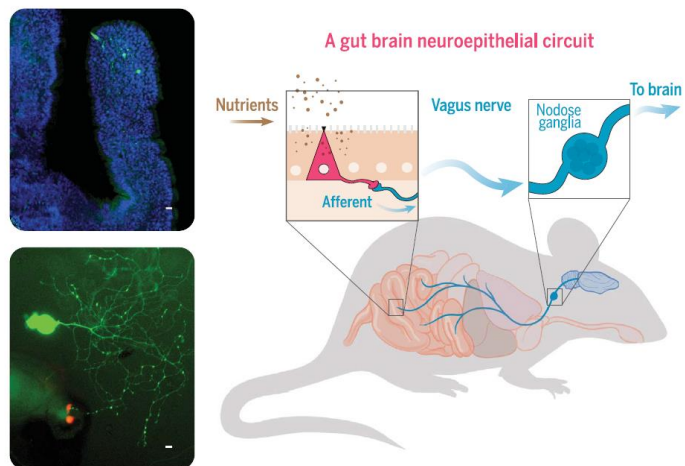
- TGFb 和 p53 信号通路失调与肿瘤肝肺转移显著相关
- 通过单细胞 RNA 测序对比原发和转移灶，可以确定转移相关的基因特性
- **因为 RNA 不是信号通路级联放大的指标**，所以我采用 Milo 来测定单细胞 RNA 测序分析所得到的信号通路上蛋白质的差异（例如磷酸化）
- Milo 能够详细解析转移的肿瘤细胞亚群中的信号通路
- 我的课题旨在“更详细地了解这些信号通路的差别，以期发现转移性胰腺癌中全新的治疗靶点”



“MILO PERMITS STUDY OF PROTEINS AT THE SINGLE-CELL LEVEL TO UNDERSTAND THE HETEROGENEITY AMONG METASTATIC CELLS WITHIN A POPULATION.”

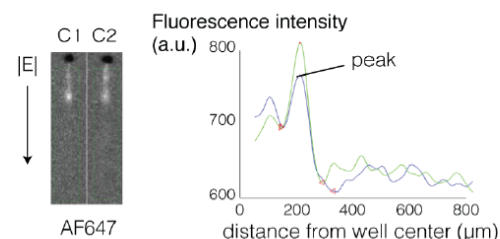
- YI ZHONG, M.D., PH.D., RESEARCH ASSOCIATE, MEMORIAL SLOAN KETTERING CANCER CENTER

发现新的连接胃脑的细胞类型

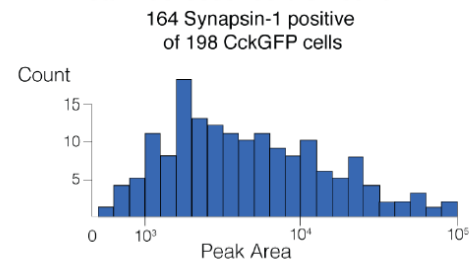


十二指肠肠内分泌细胞 Single-Cell Western

A Rabbit anti-Synapsin 1



B Peak fluorescence distribution



Milo 显示肠内分泌细胞中有一种新细胞亚群，能够表达突触蛋白（Synapsin1）并和末端迷走神经形成突触连接

- Milo 确认了表达突触前标志物的肠内分泌细胞亚型的存在，并且证实这群细胞能够表达形成突触的功能性蛋白质
- 因为突触前肠内分泌细胞的信号常常被样品中的其他细胞掩盖，所以无法通过传统 western 检测到差异

Milo 让基因表达的结果更可信

“In research, it is important to show results using different methods... We have been pairing Milo with single-cell real time qPCR data. We have the RNA and we know the transcripts are present. We used Milo to confirm our results and quantify the amount of protein that is in each individual cell.”



*- M. Maya Kaelberer, Ph.D.,
Postdoctoral Associate, Duke
University School of Medicine*

Milo™ 的常见应用



单细胞测序的蛋白验证

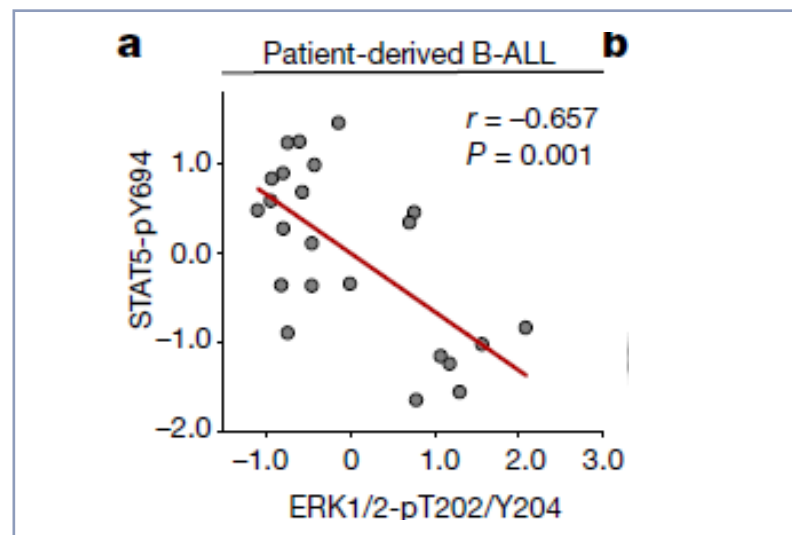
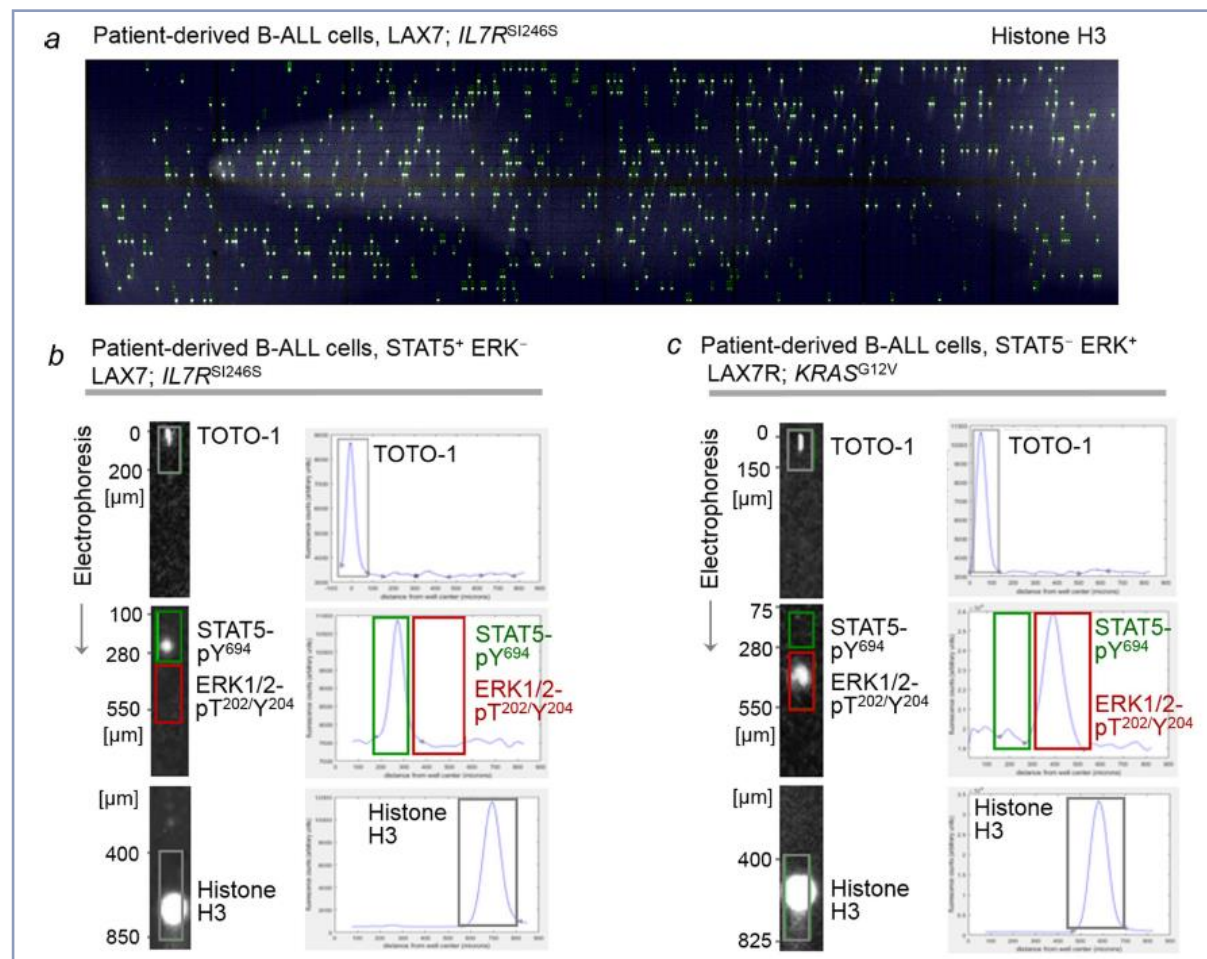


单细胞水平的蛋白共表达



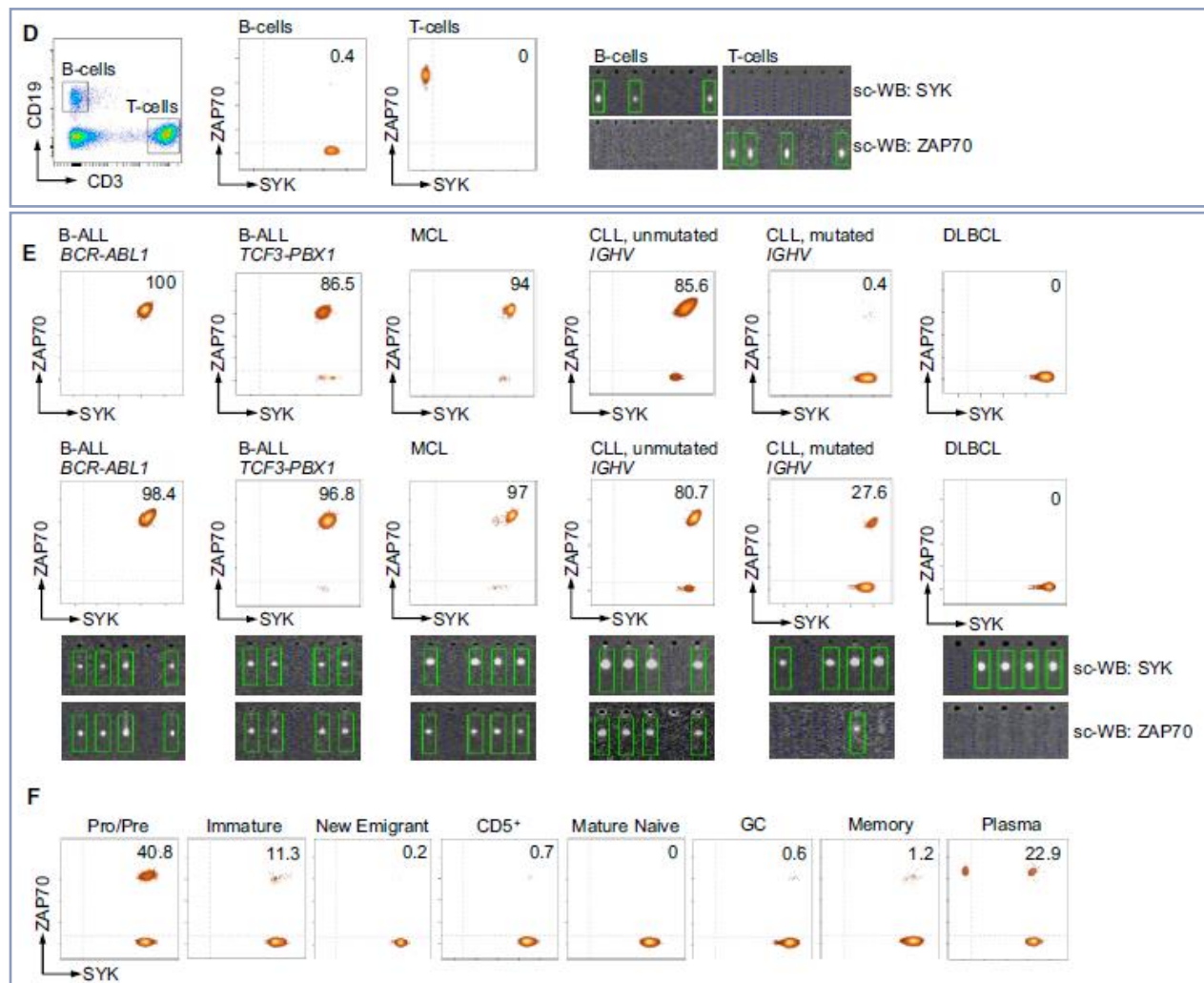
流式细胞术受限的蛋白检测

单细胞水平的蛋白不共/共表达

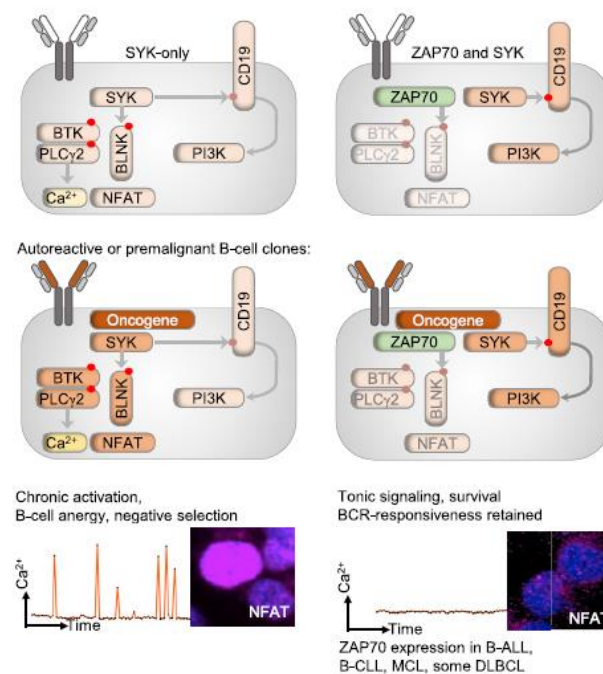


- Milo 确认了在患者来源的 B 细胞白血病样本中 STAT5 和 ERK 通路存在隔离，即两条通路不会同时被激活。
- 除了抑制主要激活通路，重新激活被抑制的通路有望降低耐药、增强疗效。

单细胞水平的蛋白不共/共表达



- Milo 确认了在正常细胞中 SYK 和 ZAP70 通路存在隔离，但在多种 B 细胞白血病样本中却存在共表达。
- ZAP70 除让 SYK 通路偏离了 NFAT 依赖性而转向 PI3K 依赖性，从而促进 B 细胞转化。



Milo™ 的常见应用



单细胞测序的蛋白验证

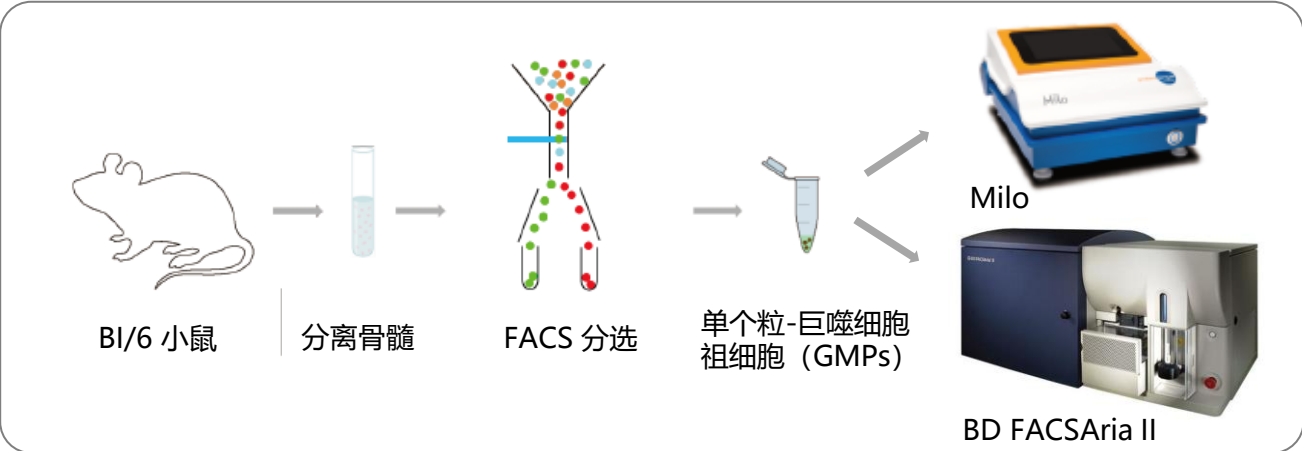


单细胞水平的蛋白共表达

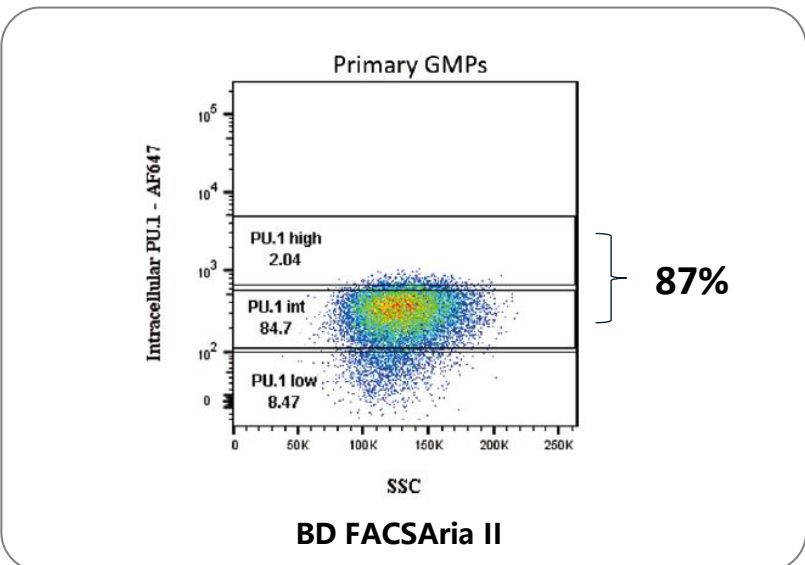
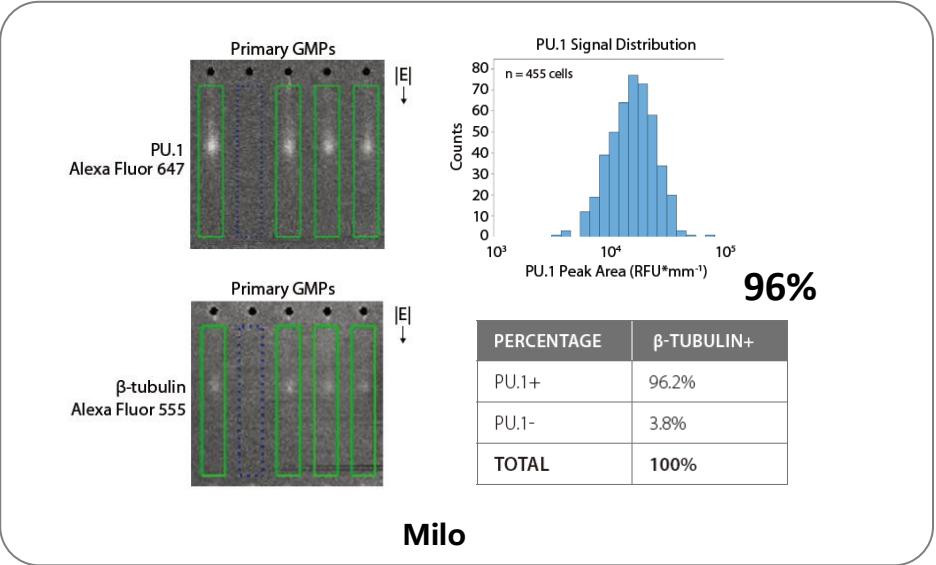


流式细胞术受限的蛋白检测

MILO 与流式细胞术结果可比

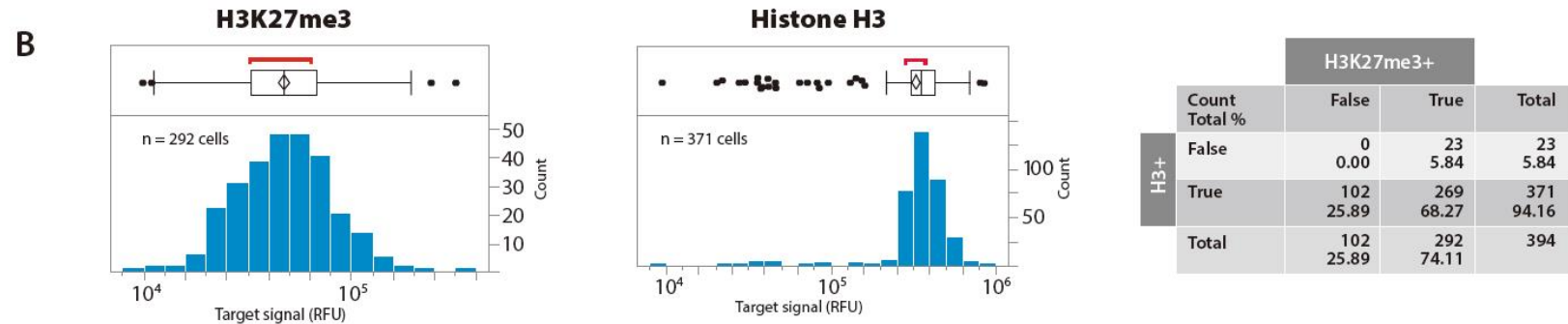
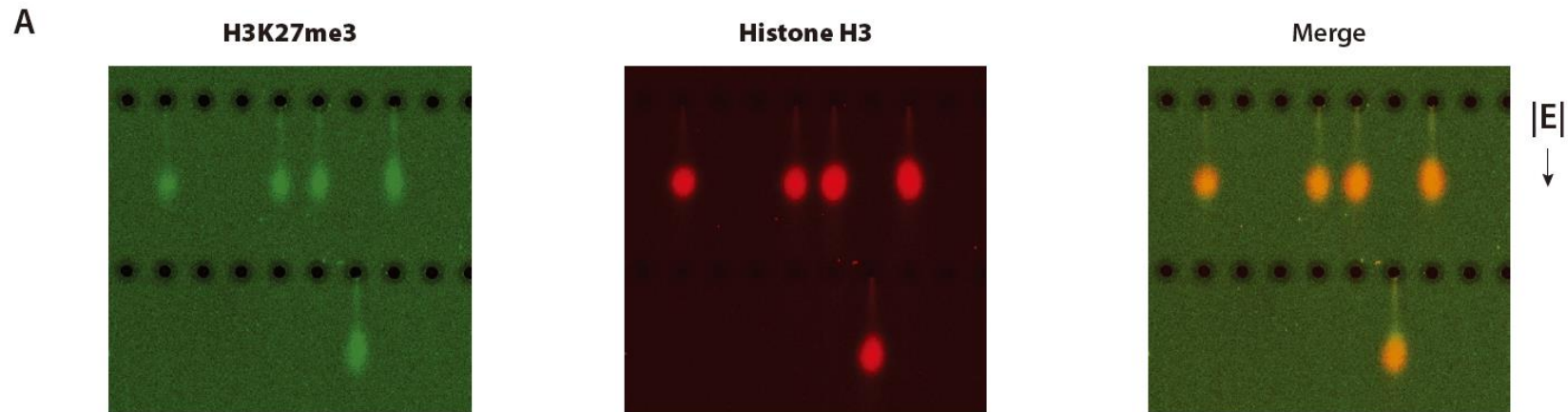
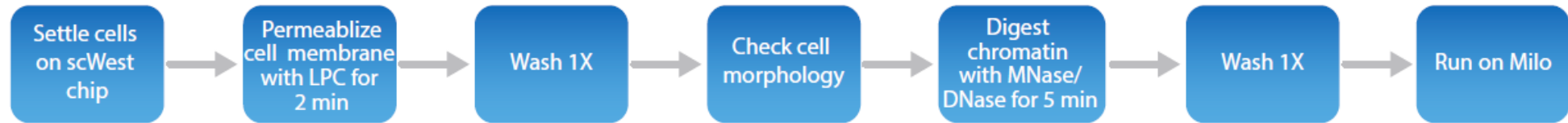


流式需要对GMPs 细胞进行固定和穿孔，PU-1抗体才能进入细胞内，而Milo则无需此操作



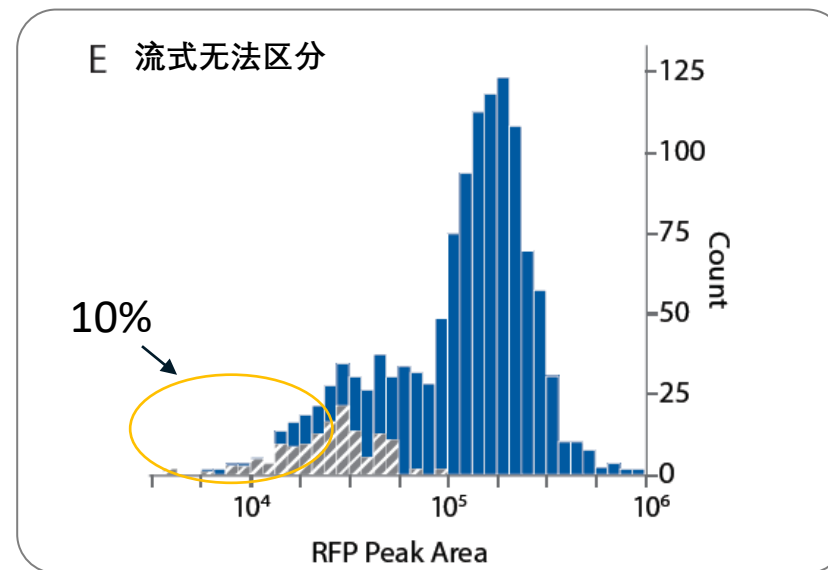
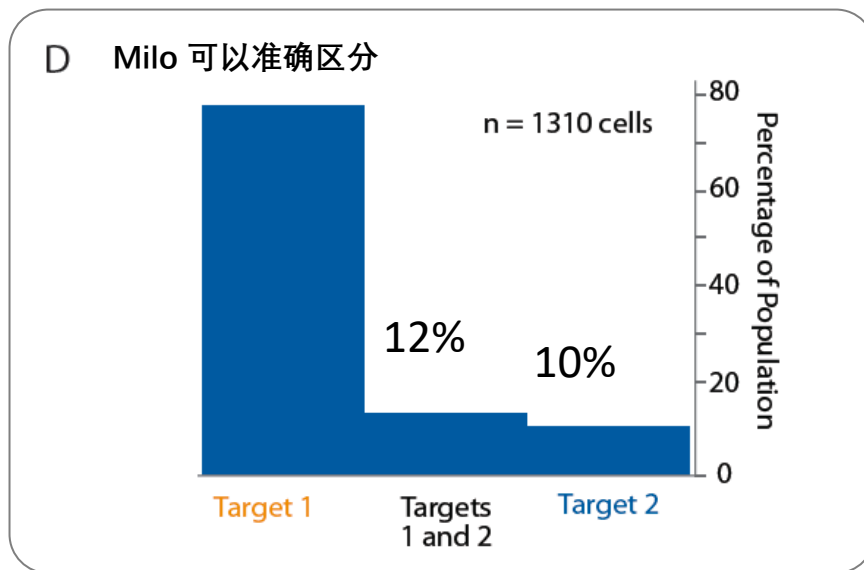
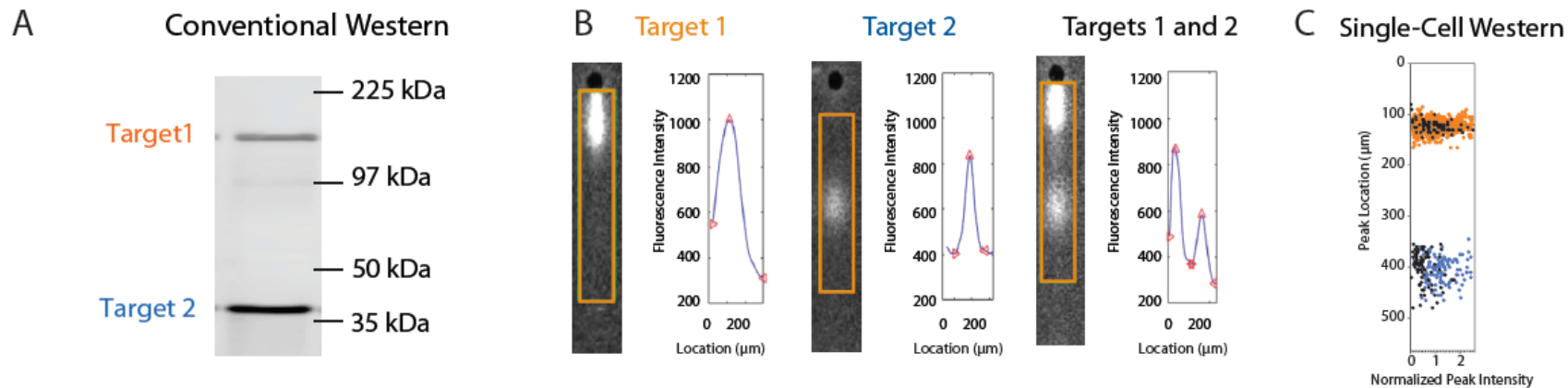
MILO 超越流式细胞术

不受流氏抗体限制：核内蛋白



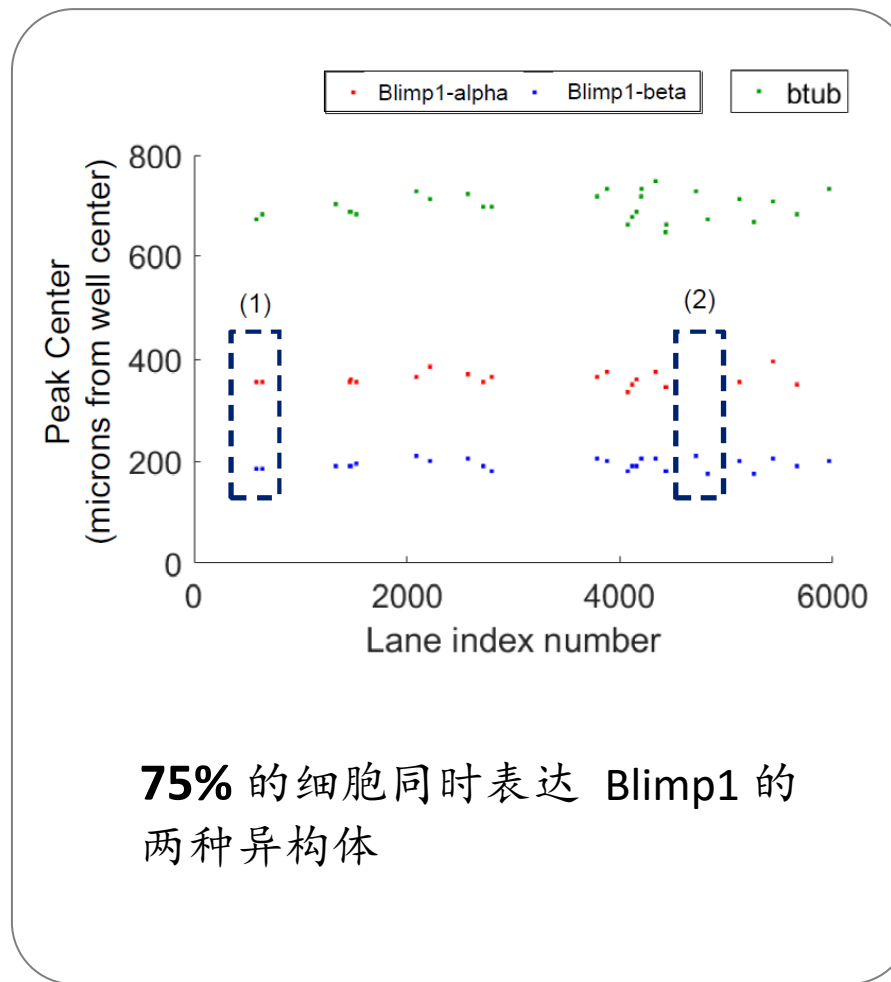
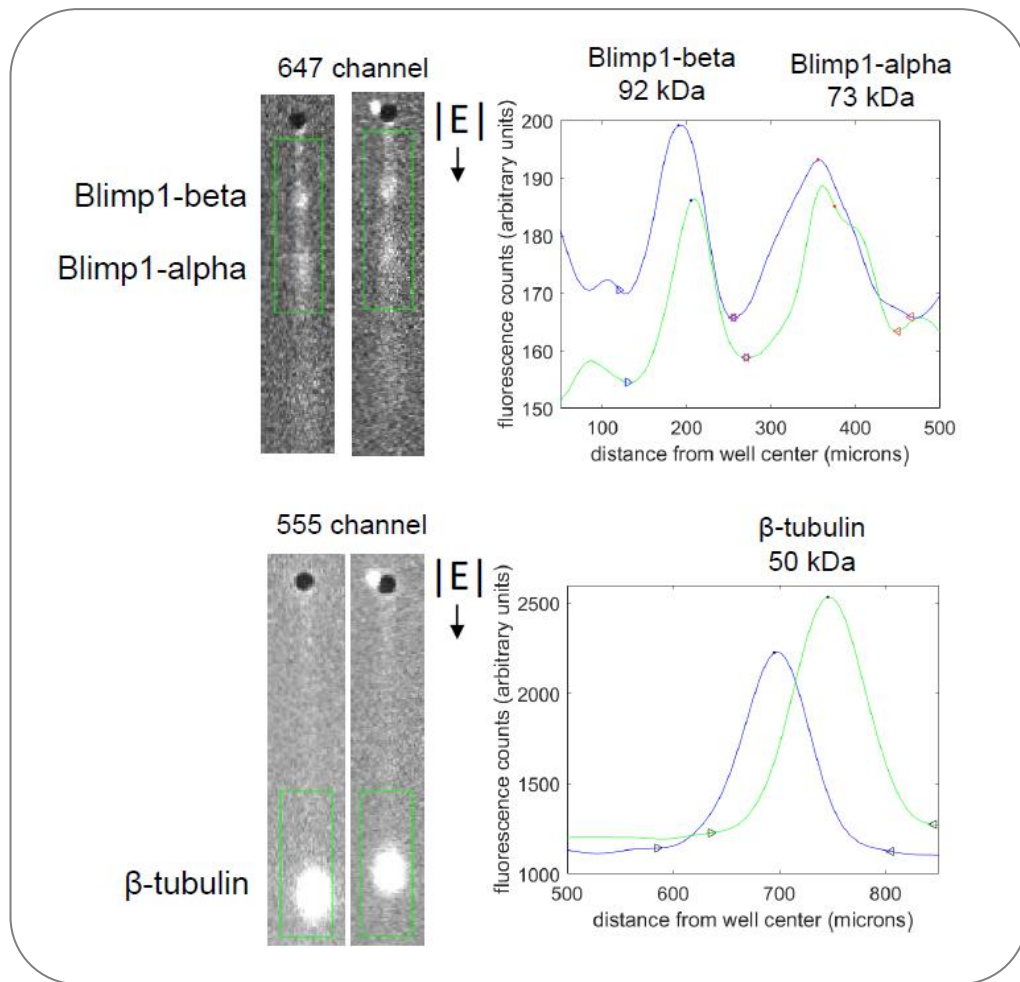
MILO 超越流式细胞术

可区分分子量大小变异体



MILO 超越流式细胞术

可区分分子量大小变异体



真实客户数据

单细胞
蛋白分析

固定后芯片
可存放

Western
抗体适用



洗脱
重杂交

单次
多靶点

Milo™ 操作

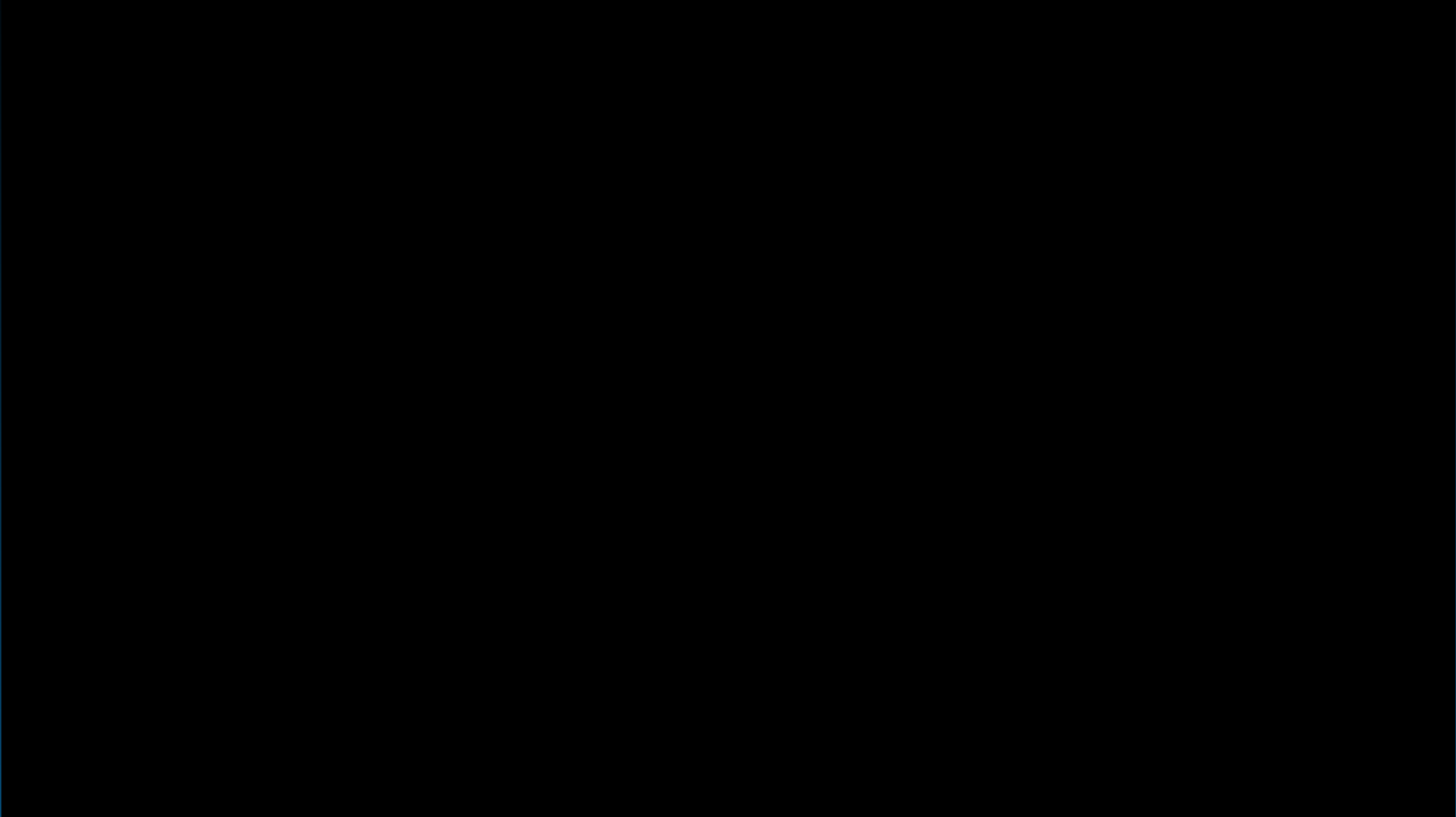
THREE

实验设计

1. 样品准备：制备单细胞悬液
2. 选择合适的 scWest 芯片
3. 选择合适的靶蛋白抗体
4. 选择合适的内参蛋白
5. 设置阳性和阴性对照

实验操作

1. 单细胞悬液的沉降 (已水化的芯片)
2. 清洗芯片, 评估细胞占有率
3. 运行 Milo (裂解, 分离, 捕获蛋白)
4. 抗体孵育和清洗
5. 使用芯片扫描仪扫描 scWest 芯片



QUESTIONS