

BD Rhapsody™

单细胞简介及其在眼科学中的应用

翁云层 博士

BD Biosciences

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vincent.weng@bd.com

2022-09-20



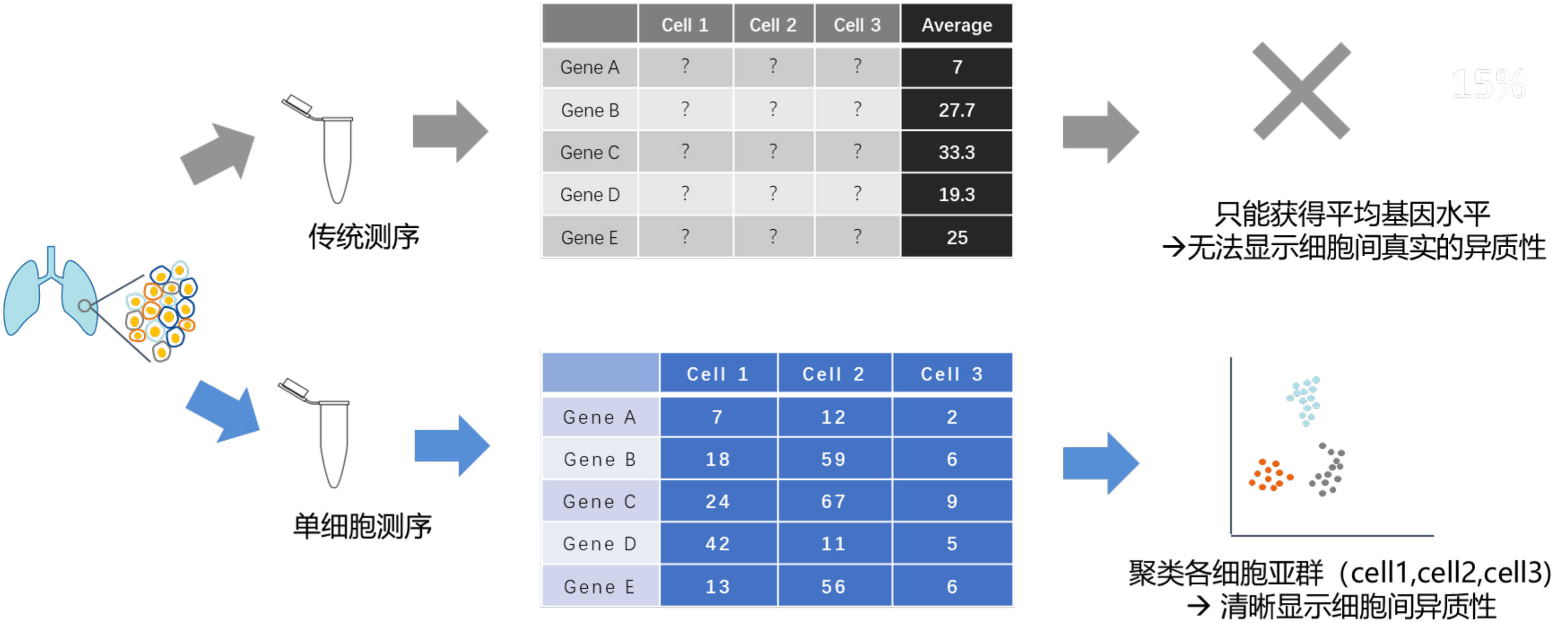
作为流式技术的先行者和领导者，BD提供集设备、试剂、软件和服务于一体的流式细胞领域整体解决方案，为生物医学前沿研究、药物开发、临床诊疗提供强大支持，满足客户需求。

自1994年进入中国市场以来，BD持续培训了众多专业流式细胞术实验操作人员，帮助提升科研工作者和临床医生的研发创新能力，助力中国生物科技和医疗事业的可持续发展。

生物科学



单细胞所解决的根本问题：细胞间的异质性

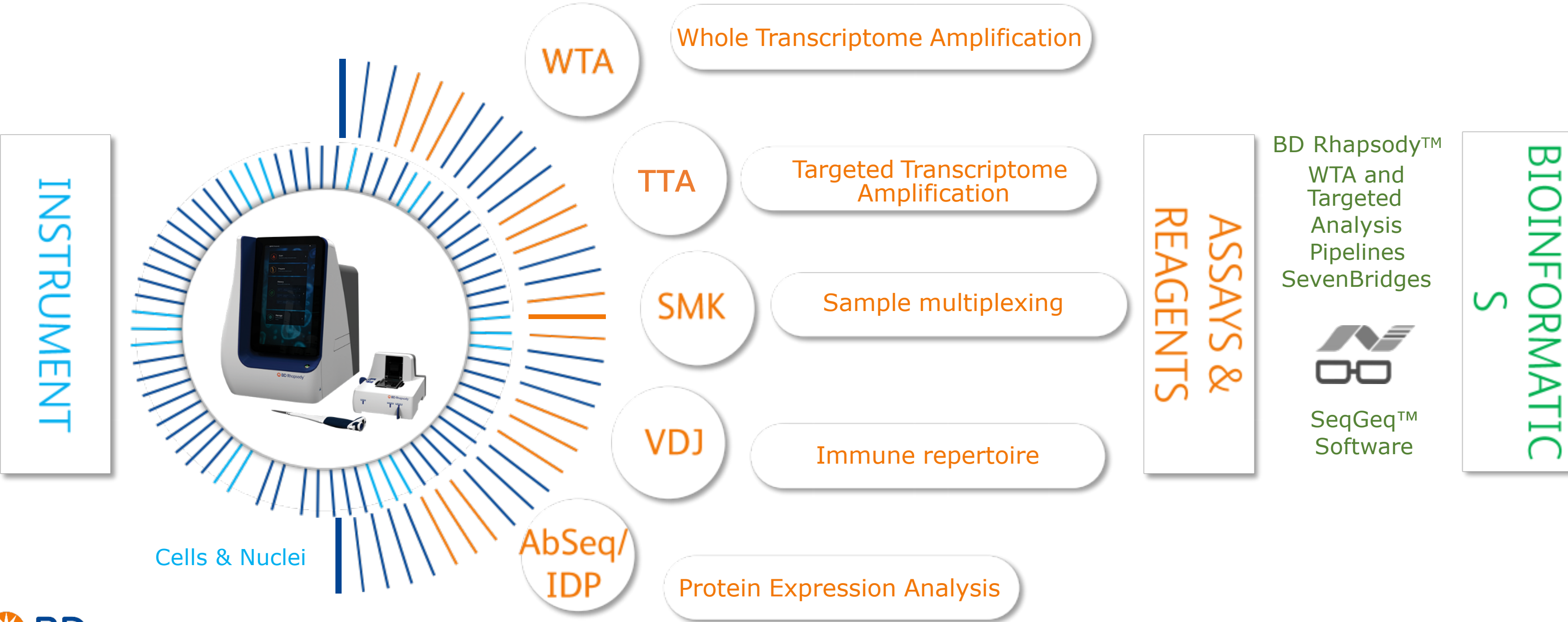


BD Rhapsody多组学

BD Rhapsody™
Single-Cell Analysis System

BD Rhapsody™
Assays & Reagents

BD Rhapsody™ Analysis Pipeline
& SeqGeq™ Software



内容

- **BD Rhapsody**原理简介
- **BD Rhapsody**工作流程
- 单细胞多组学在眼科学中的应用

BD Rhapsody多组学原理简介

单细胞分离的基本原理

Capture Bead设计原理

多组学-混样标签Sample Tag简介

多组学-表面蛋白标签AbSeq简介

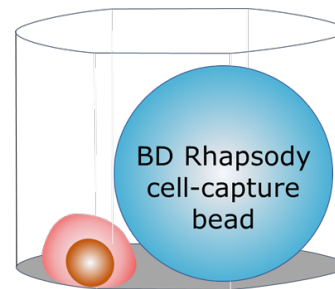
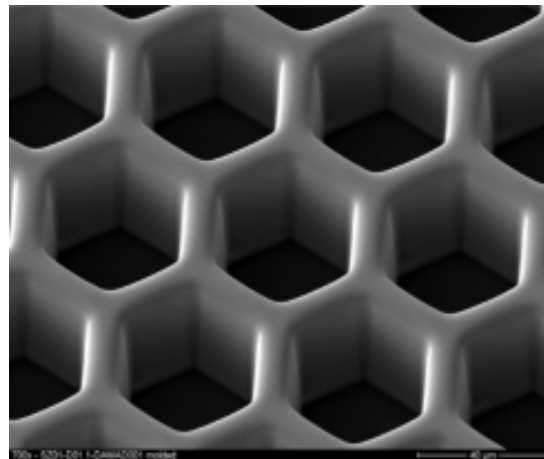
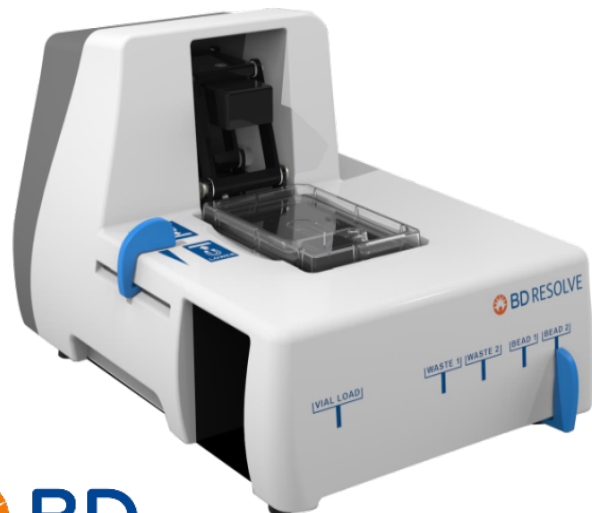
多组学-TCR/BCR VDJ的捕获简介



单细胞分离的基本原理

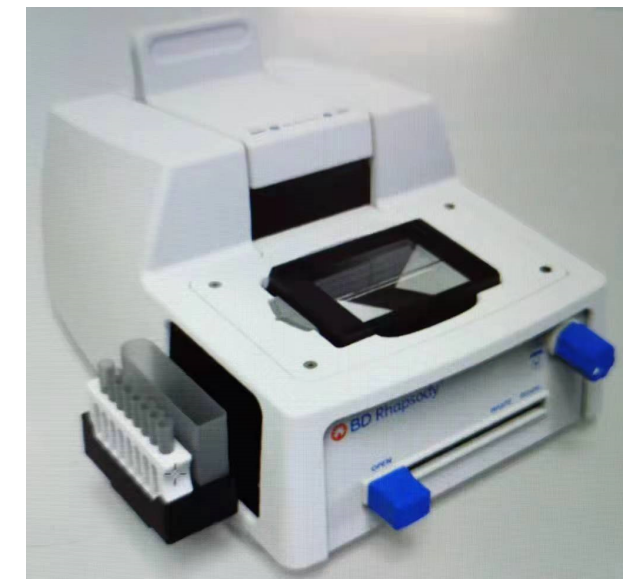
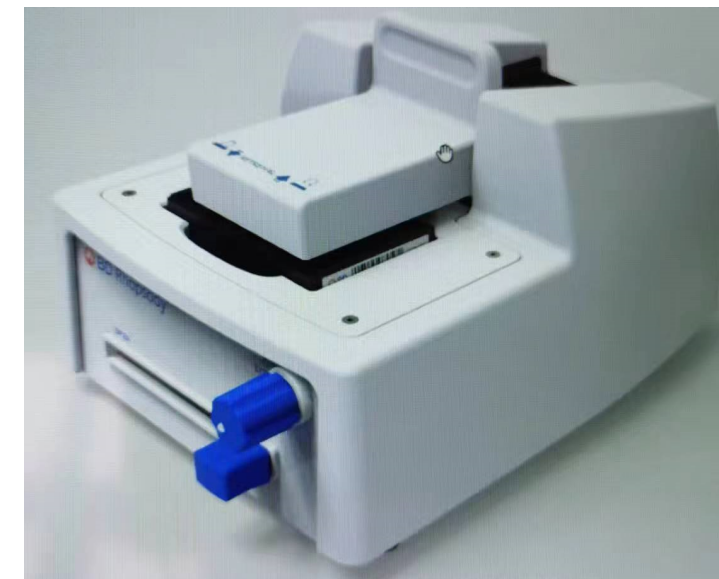
基于微孔板及自然沉降的单细胞分离

BD Rhapsody微孔板



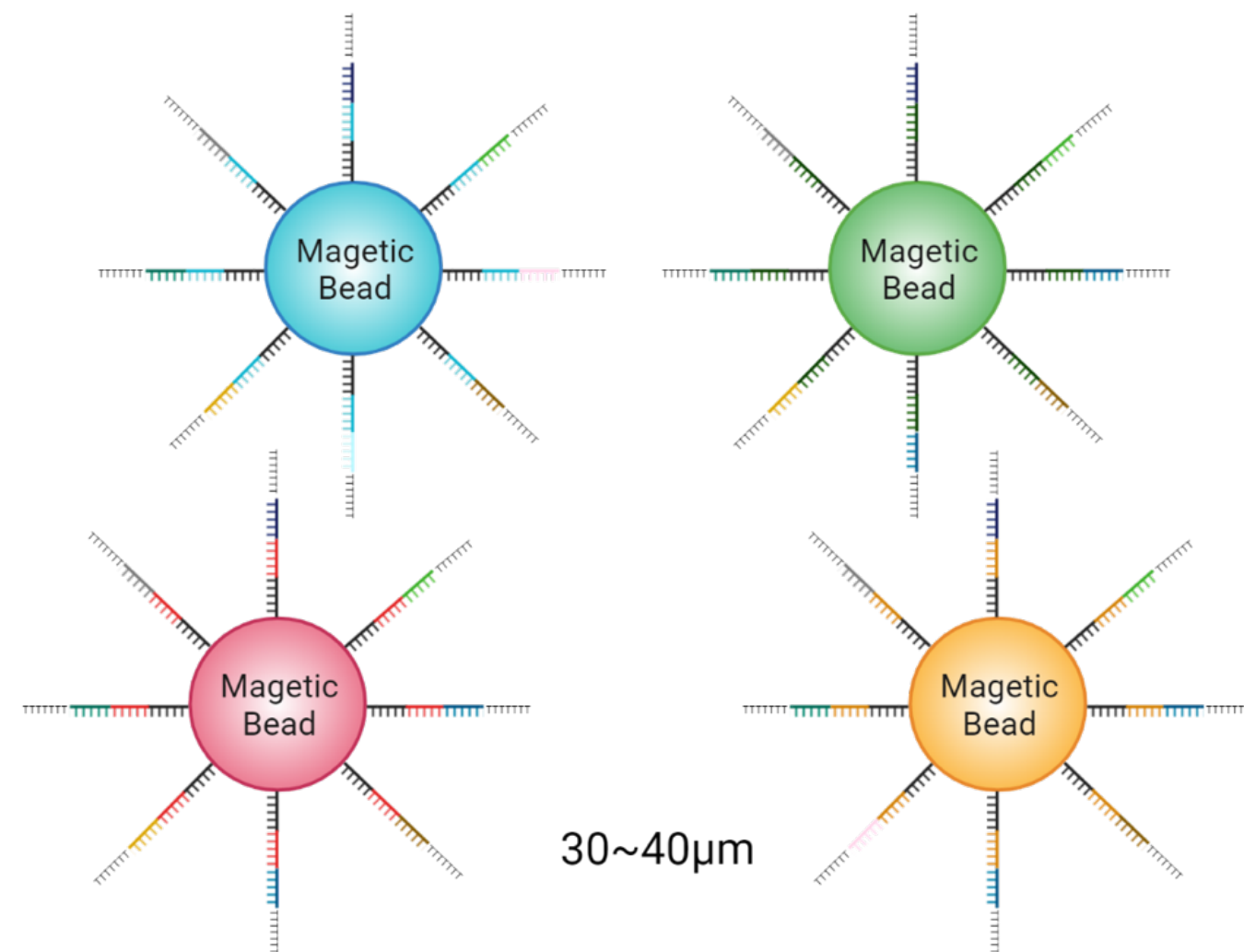
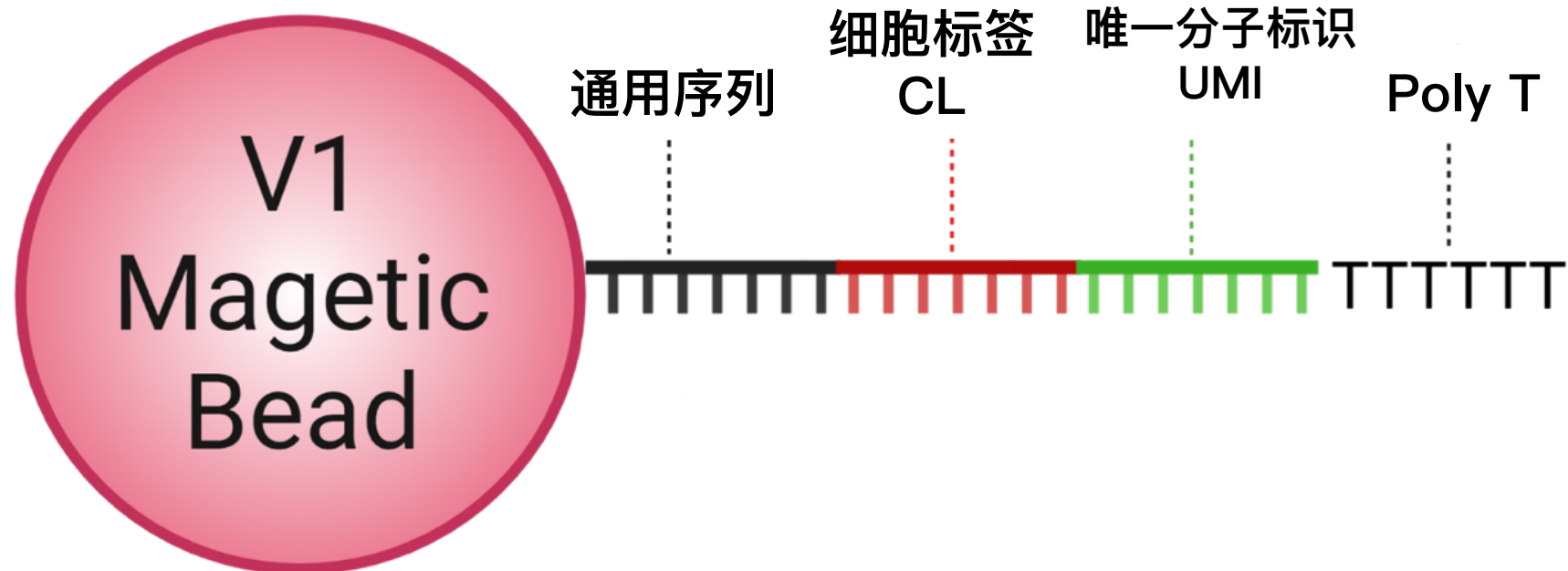
技术优势:

- 宽范的细胞上样量: 100~40000 cells/cartridge
- 多细胞率低
上样1万个细胞时, 多细胞率低仅为2%-3%
上样4万个细胞时, 多细胞率低仅为8%-10%
- 可捕获及操作脆弱细胞: 利用自然沉降, 特别适用于中性粒、干细胞等
- 上样体积大(575 μ L), 细胞浓度要求低, 兼容性强
- 细胞活率>50%即可满足上样要求
- Rhapsody Express 操作简便, 小巧便携



Rhapsody Express HT

Capture Beads设计原理



偶联寡核苷酸的磁性微球

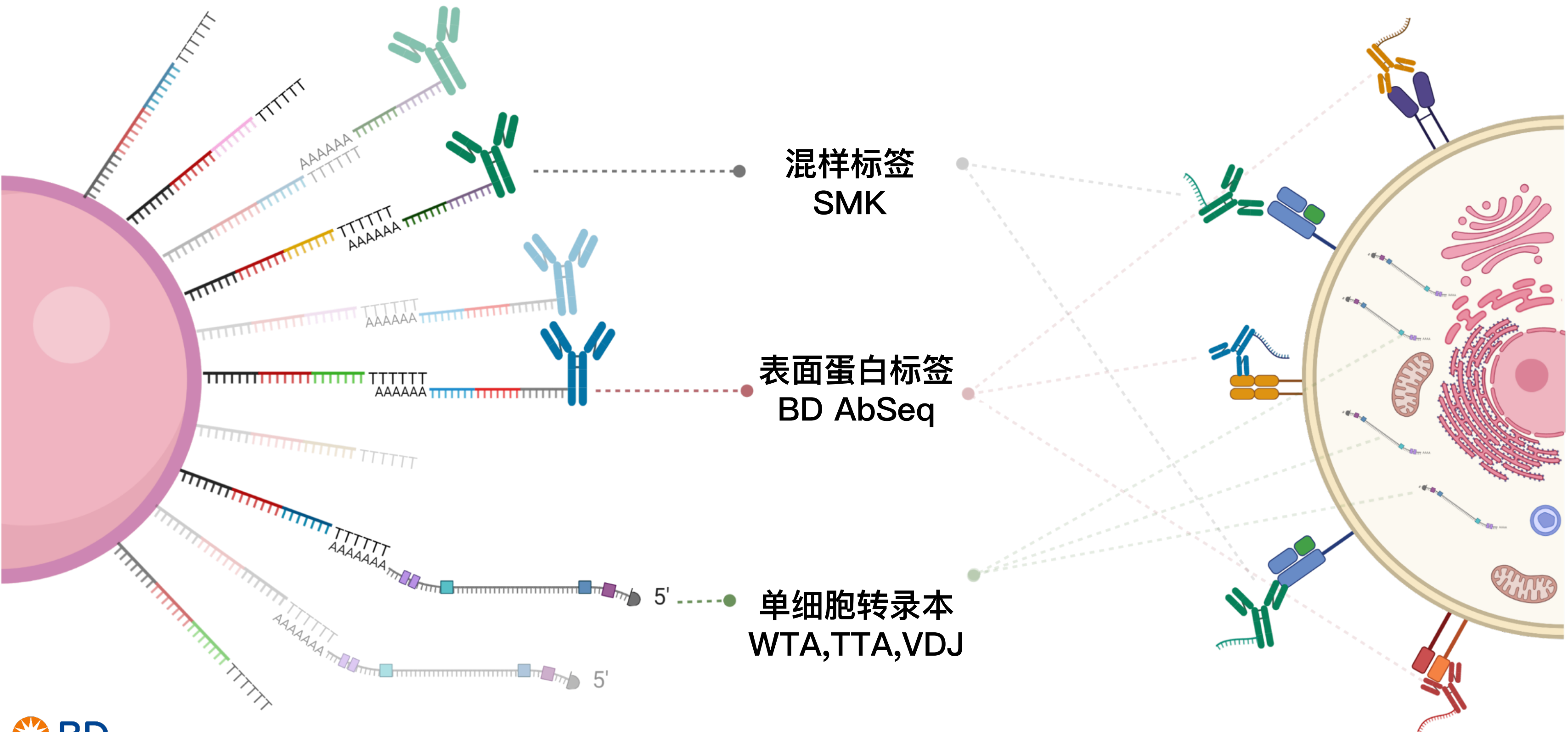
通用序列：用于PCR引物结合

细胞标签：用于标记单细胞

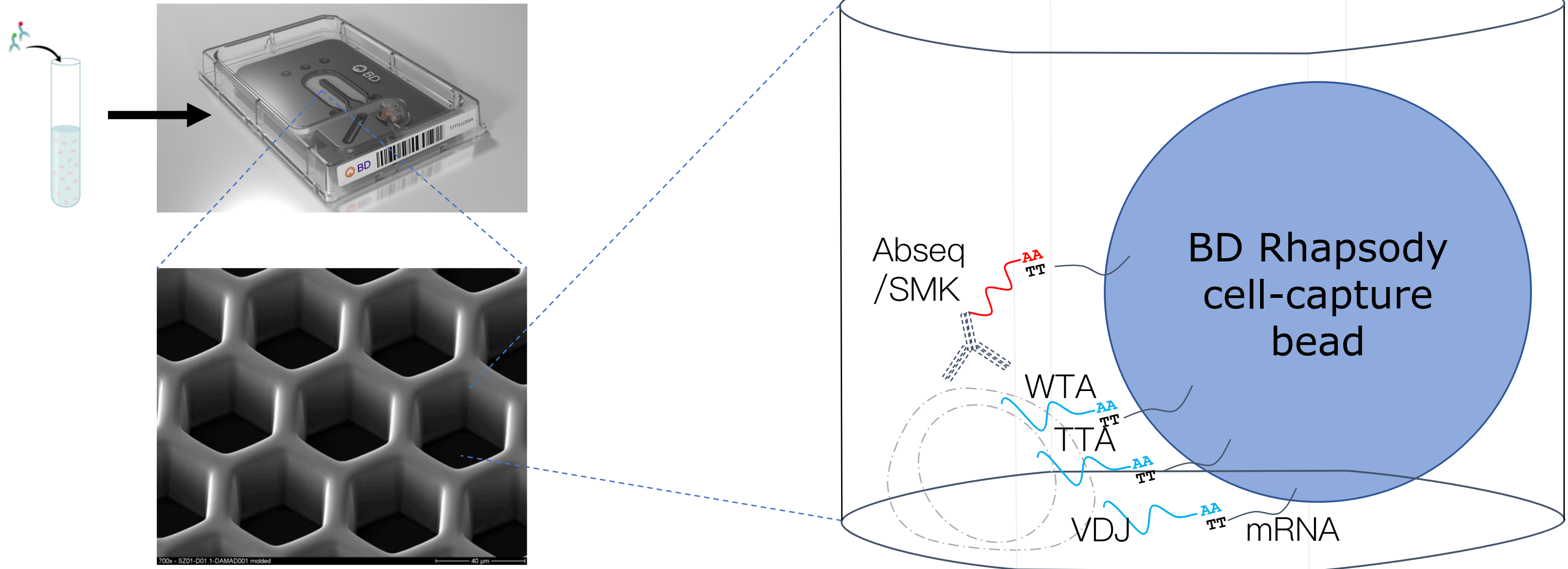
唯一分子标识：用于标记转录本或抗体标签

Poly T：与转录本mRNA或抗体标签 3'端的Poly A 结合

Capture Beads设计原理



单细胞水平同时检测mRNA及蛋白水平



多组学-混样标签Sample Tag简介

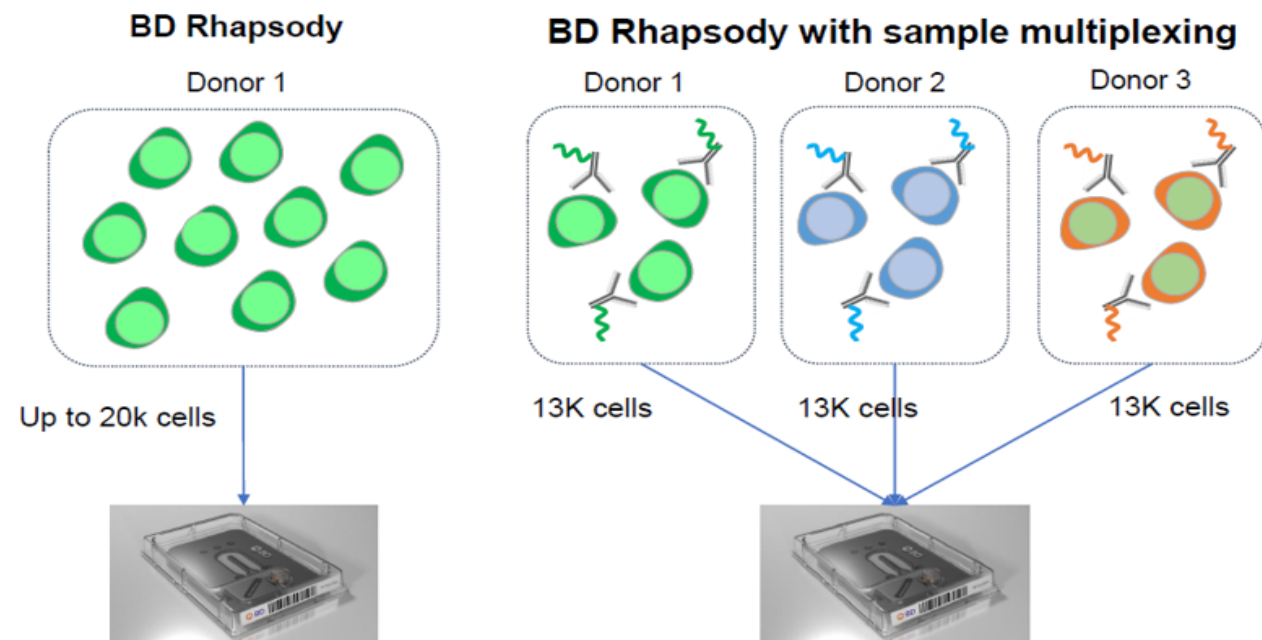


PCR
Handle

样本标签

PCR Handle: 用于PCR引物结合

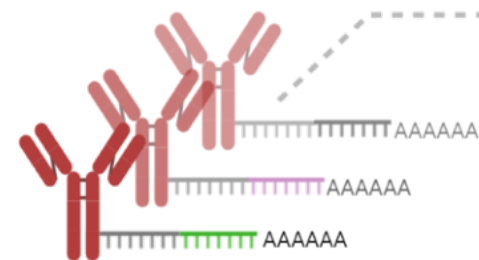
样本标签: 用于样本识别



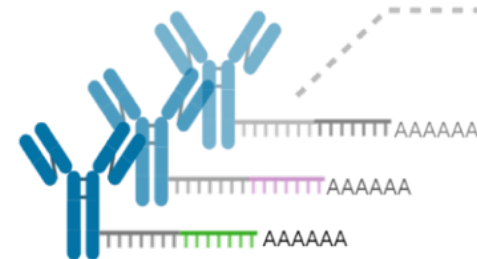
同一抗原特异性标签
可同时混样高达12个样本



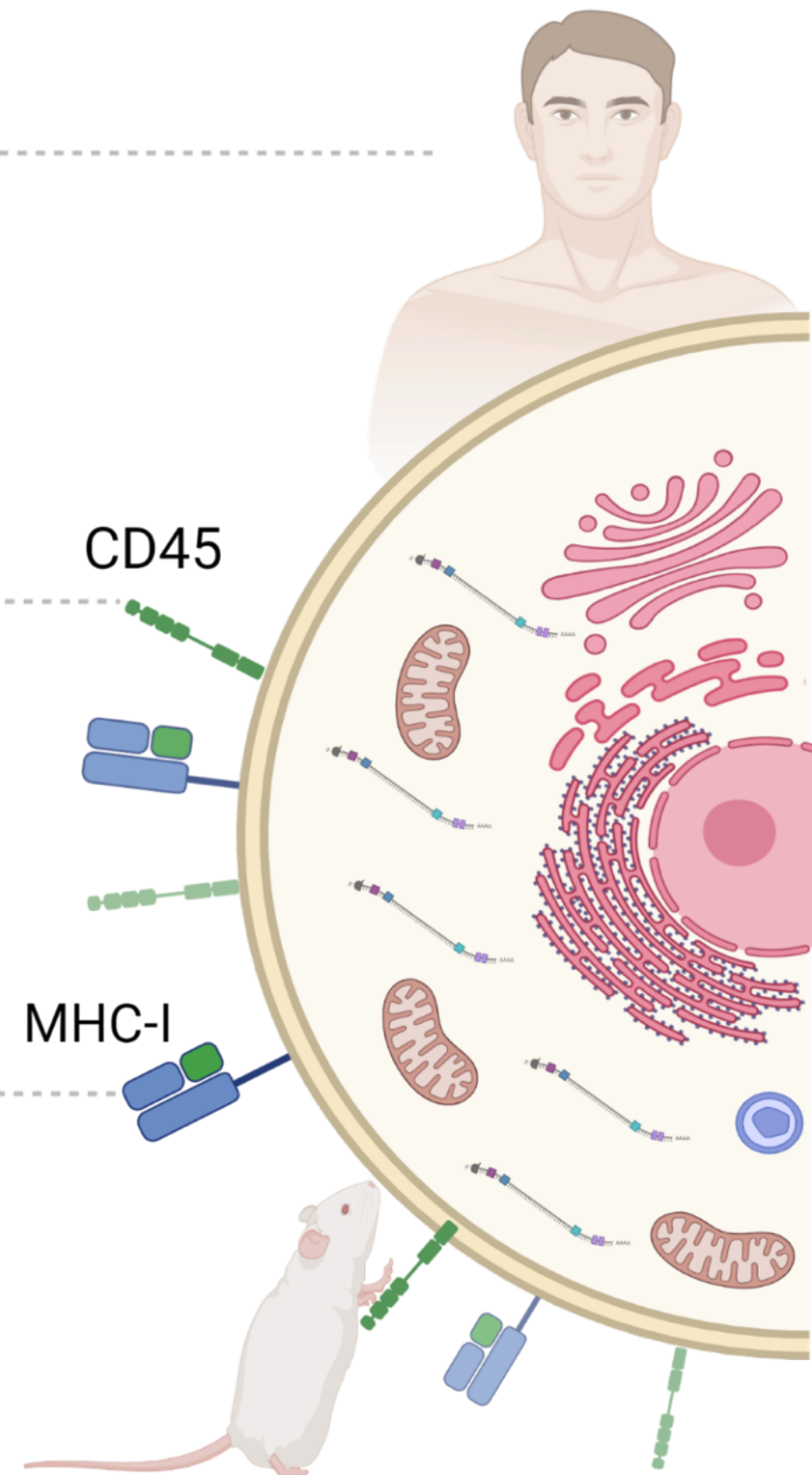
人源样本混样标签



小鼠免疫细胞混样标签
Anti-Mouse CD45

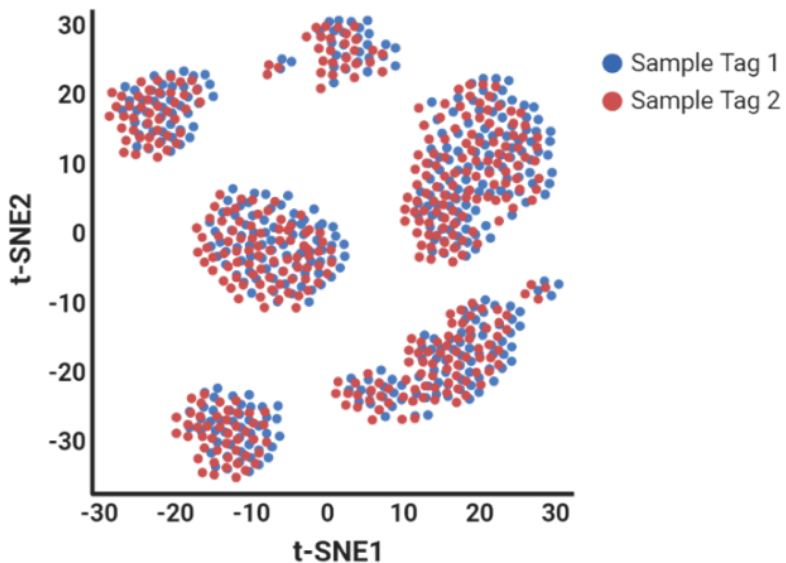


鼠源样本混样标签
Anti-Mouse MHC-I

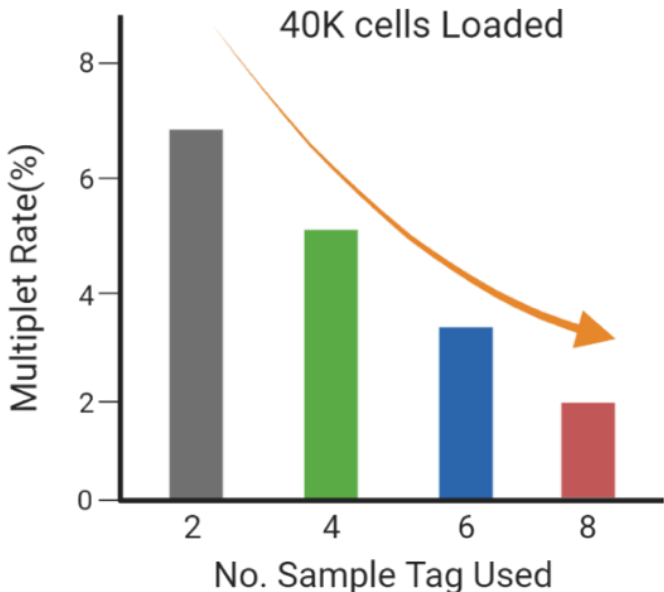


BD Sample Tag混样标签的应用

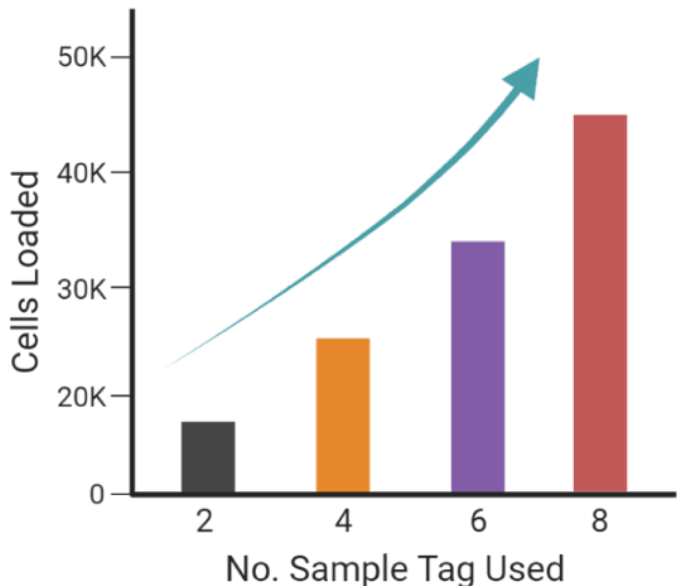
高通量



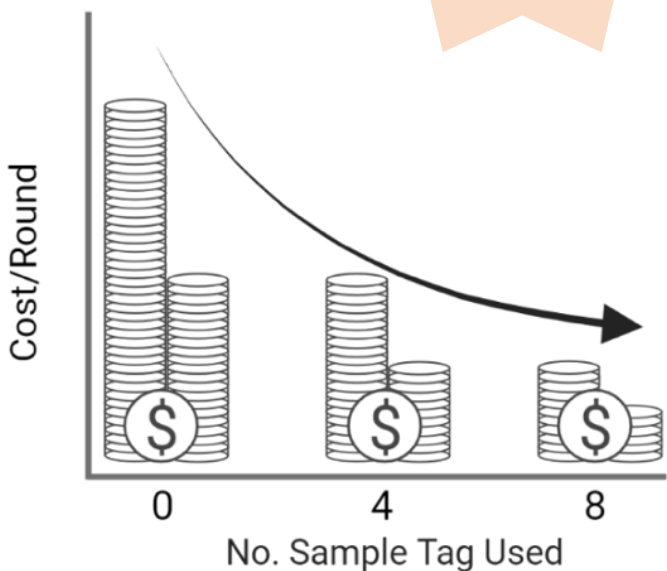
无批次效应



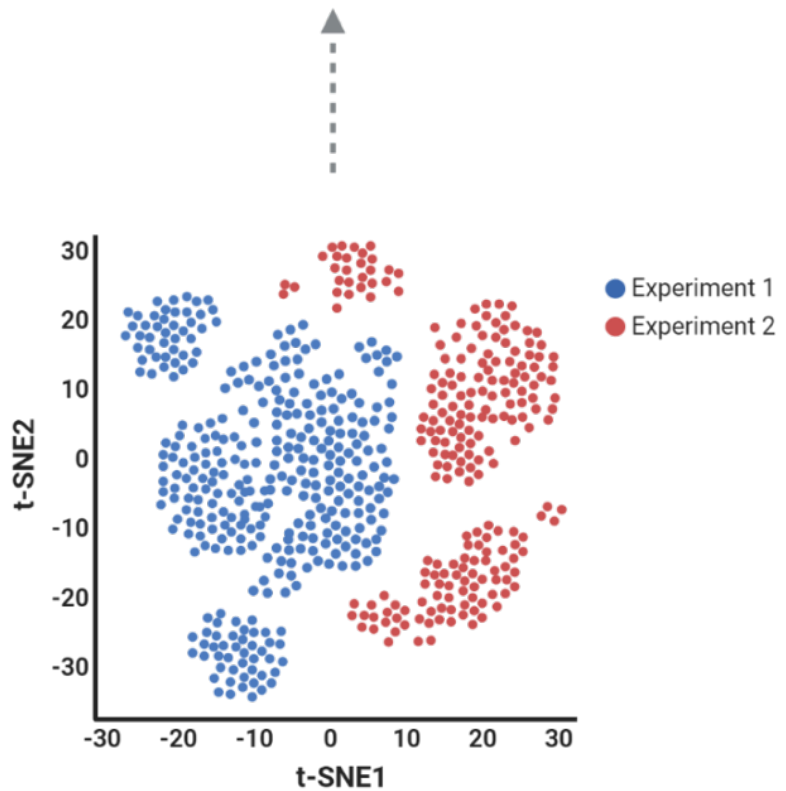
多细胞率低



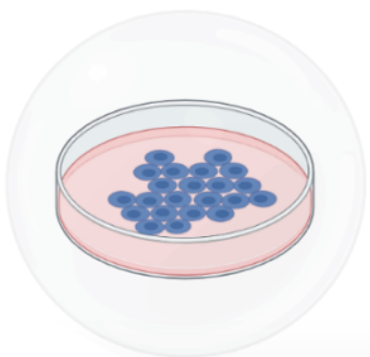
细胞上样量多



高效经济



批次效应



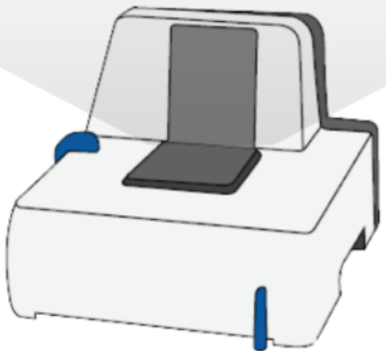
细胞系



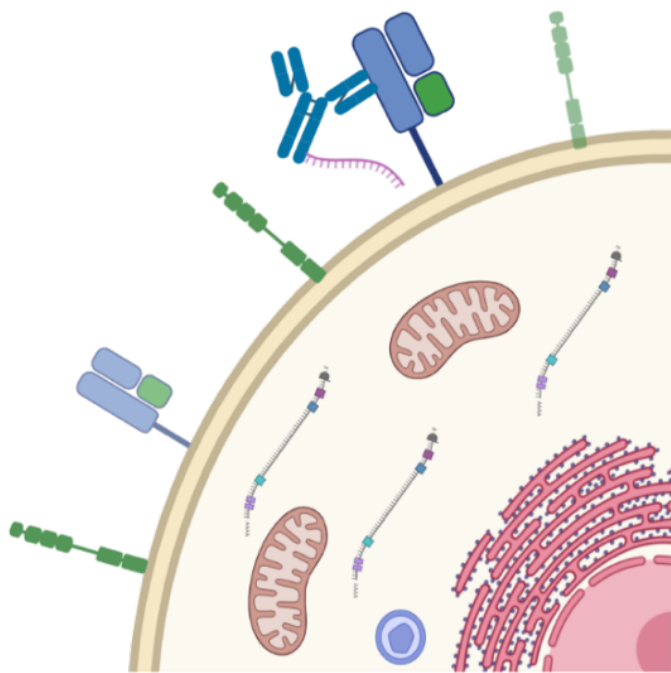
临床样本



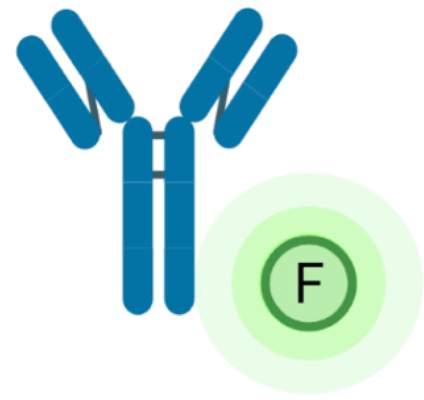
组织样本



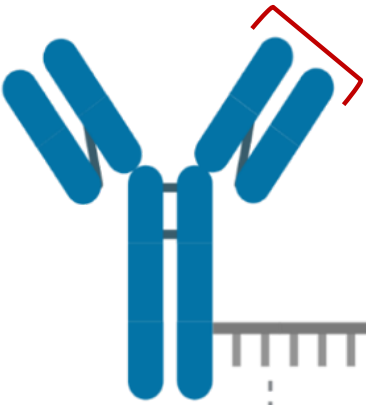
单次实验可混合
不同类型的细胞样本



多组学-表面蛋白标签AbSeq简介



偶联荧光素的抗体



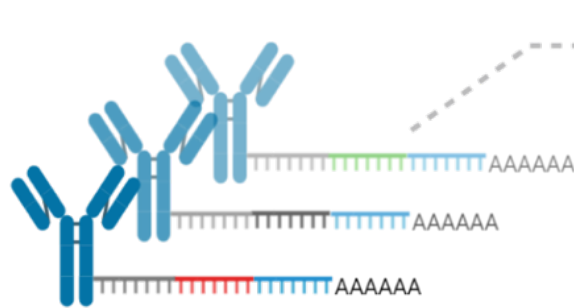
通过Fab段识别特定抗原

PCR Handle 分子标识 抗体标签

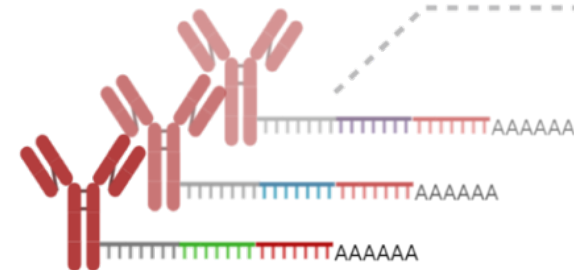
PCR Handle: 用于PCR引物结合

分子标识: 用于表面蛋白定量

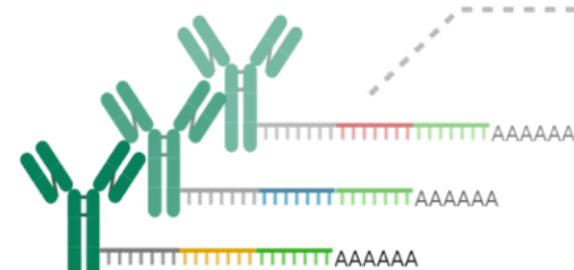
抗体标签: 用于抗体识别



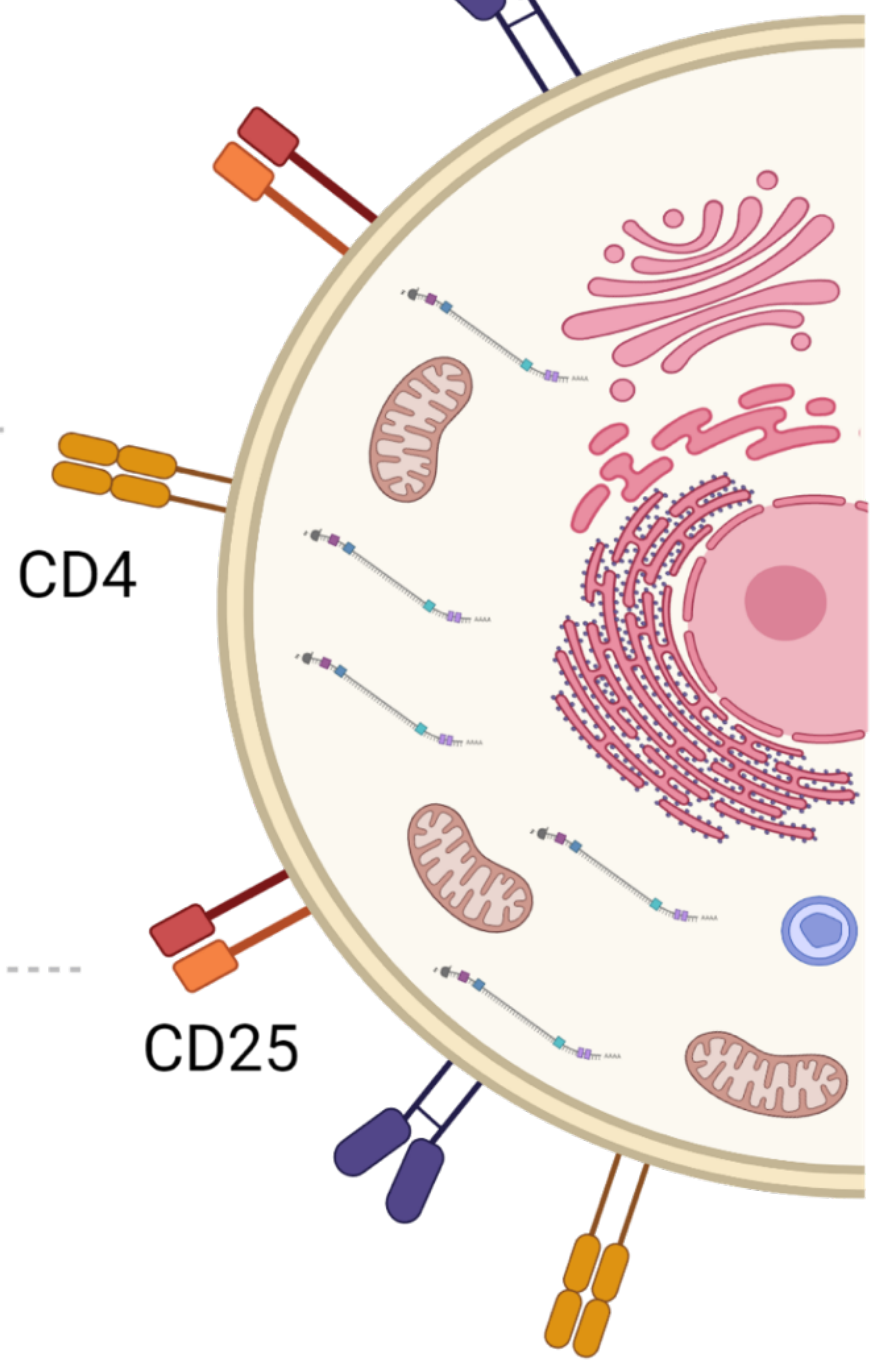
CD3



CD4

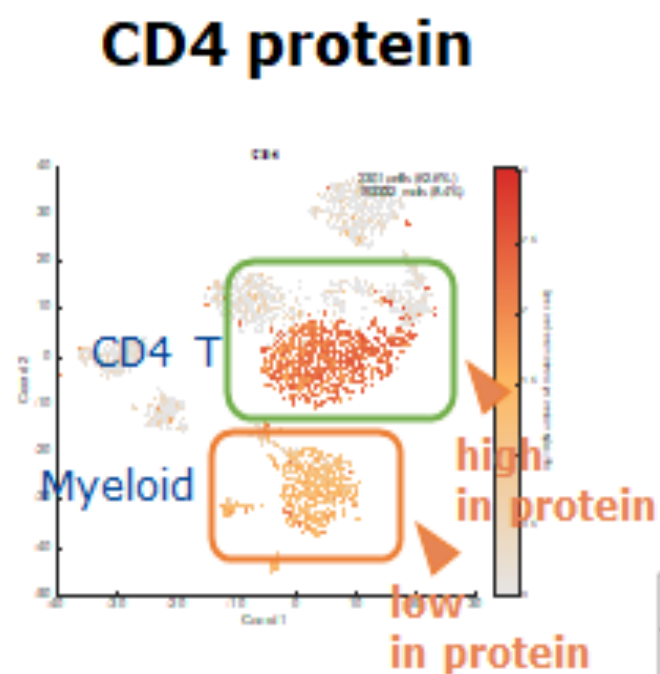
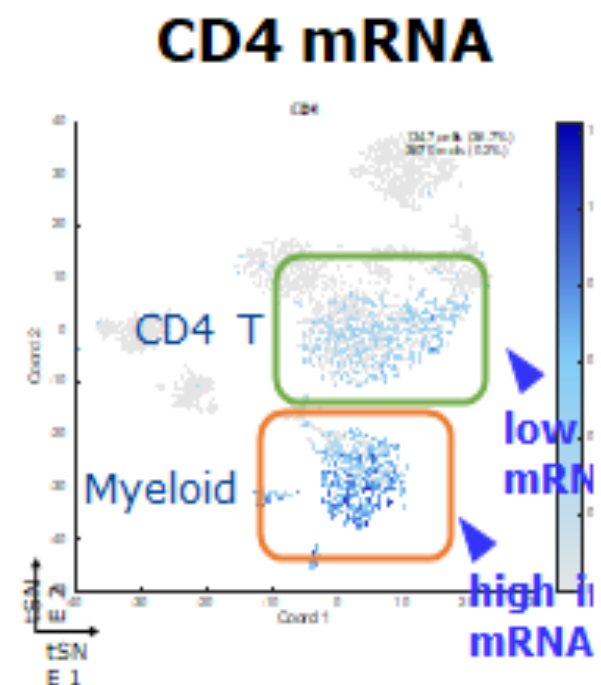


CD25

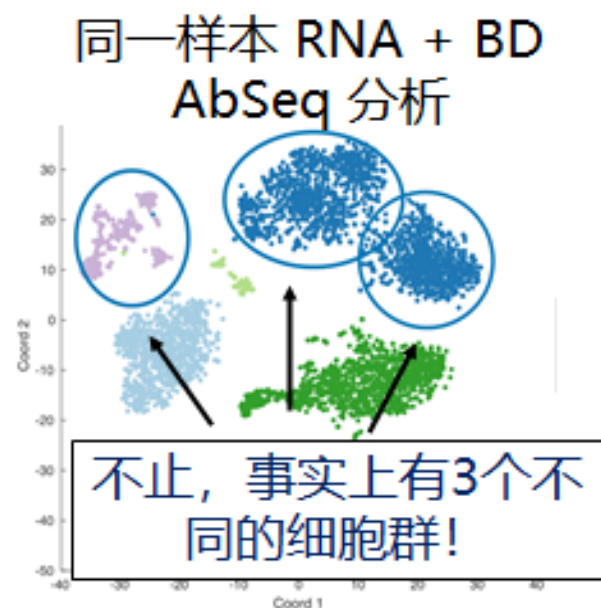
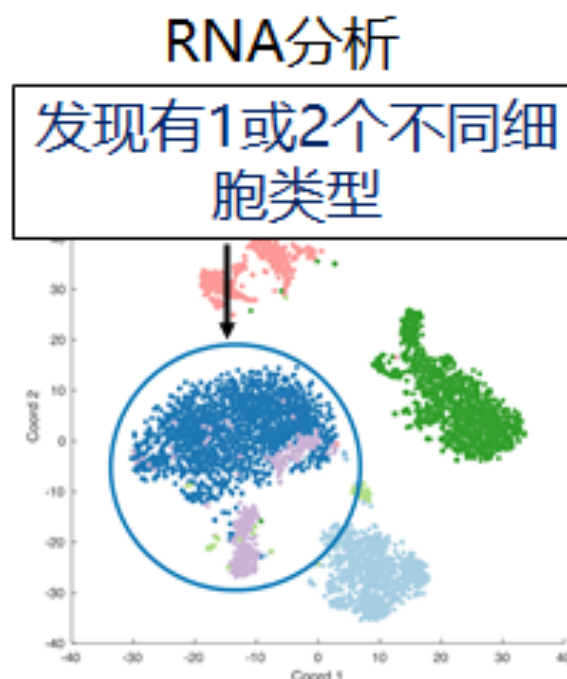


BD AbSeq表面蛋白标签的应用

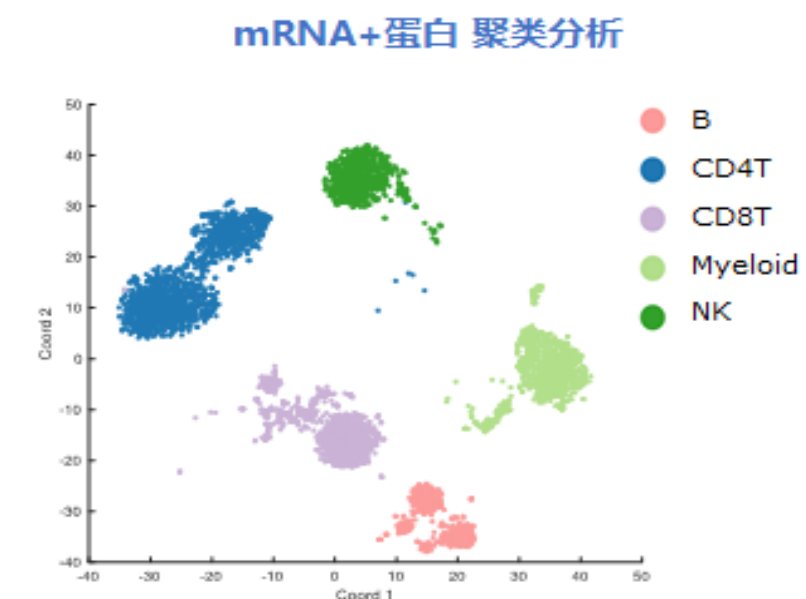
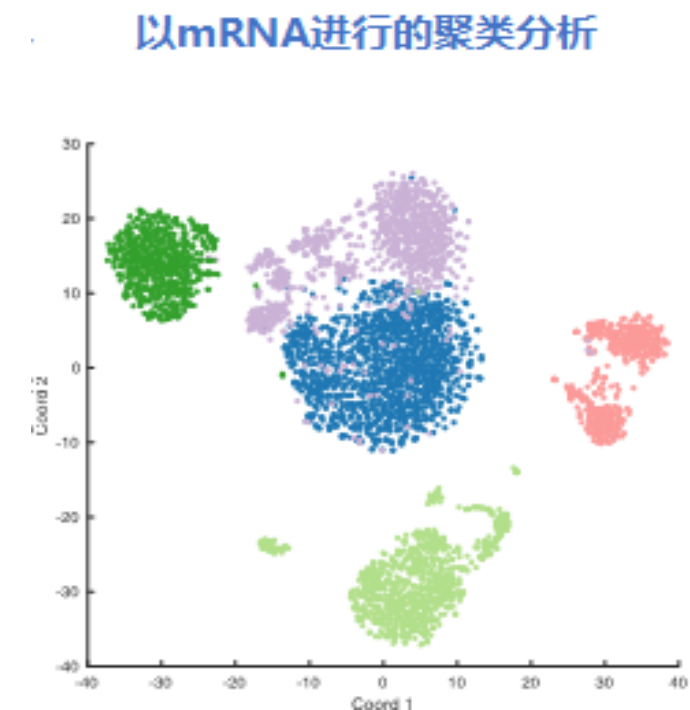
◆ 多组学分析帮助揭示内在生物机制



◆ 助力发现新的细胞群！



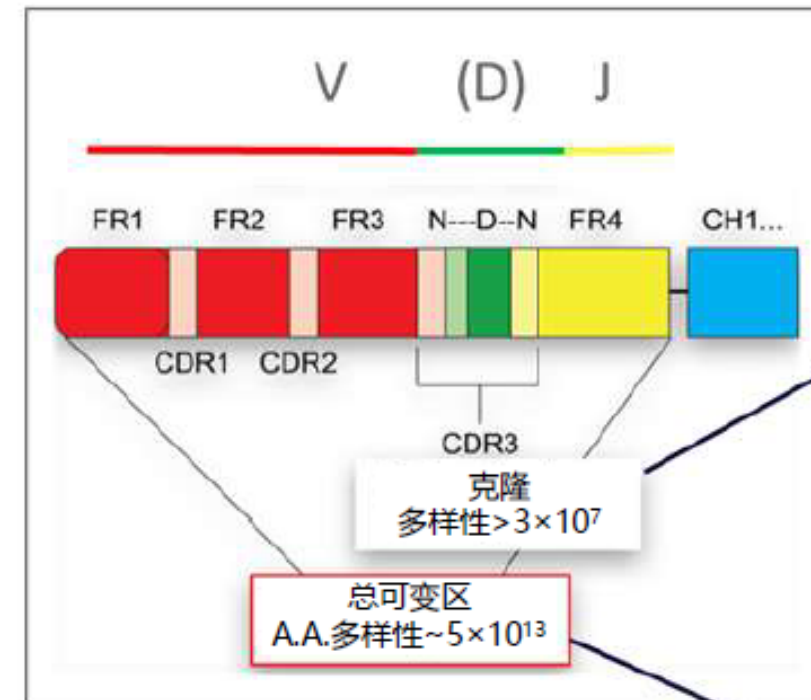
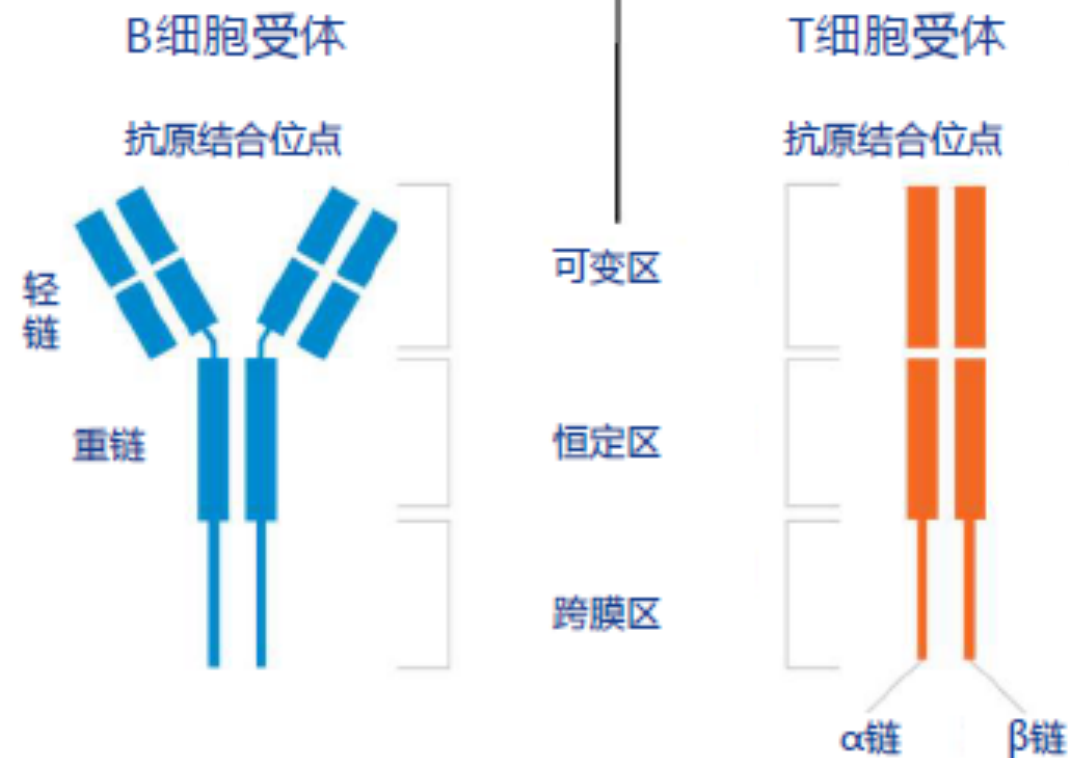
◆ 让聚类分析图谱更清晰！



多组学-TCR/BCR VDJ的捕获简介

研究TCR/BCR的两种方法

T细胞受体和B细胞受体的结构



CDR3

- 是受体的部分区域
- TCR/BCR分子的大部分可变区, 常用于识别克隆型

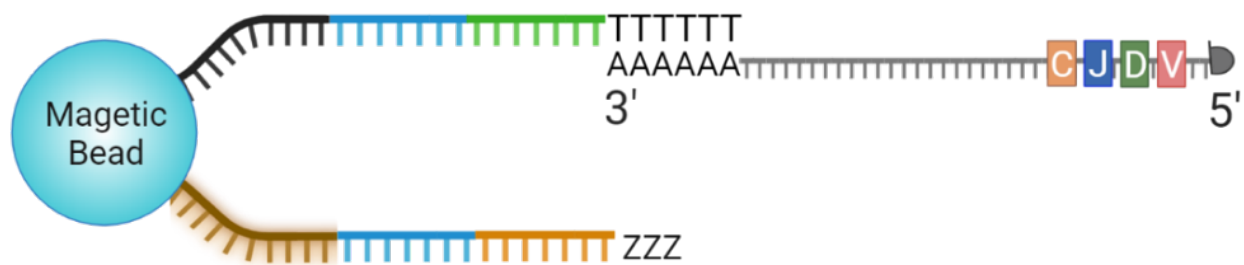
全长VDJ区域

- 包含CDR 1+2+3区
- CAR-T细胞疗法所需的广泛应用, BCR体细胞超突变分析

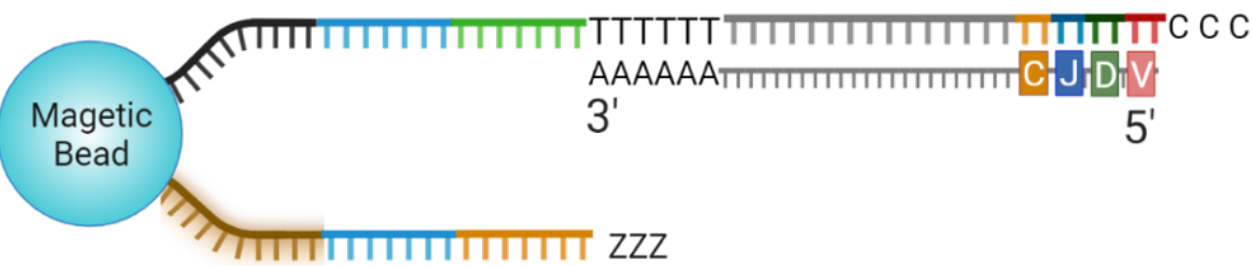
可检测alpha/beta链, 同时也检测gamma/delta链

全长 VDJ 扩增及文库构建

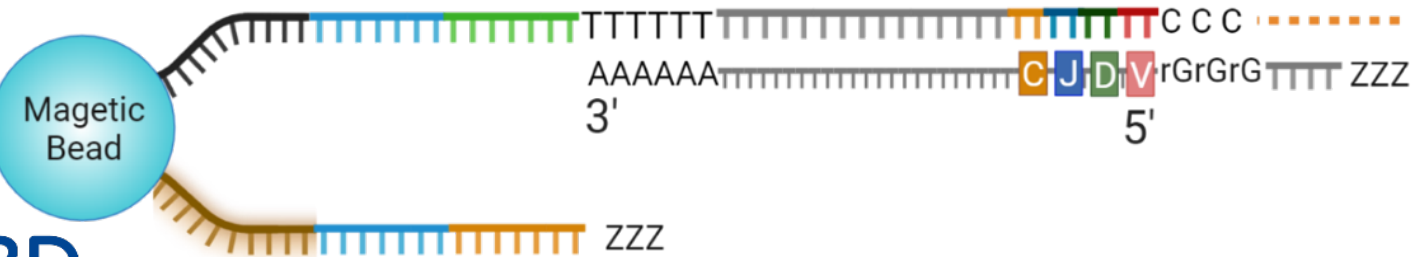
V2微球捕获VDJ转录本



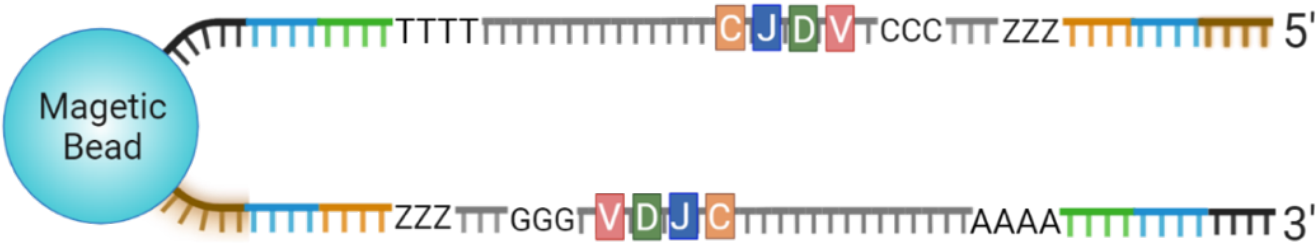
合成的cDNA含3个C



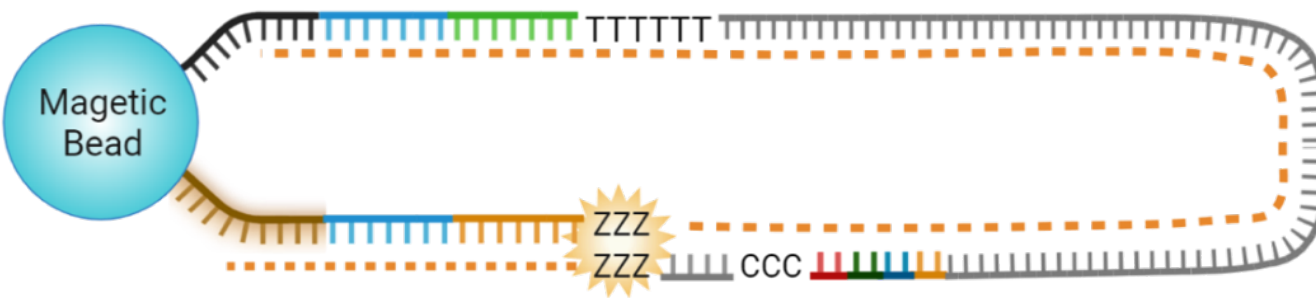
加入TSO完成模板转换



外切酶I处理



自我杂交及延申

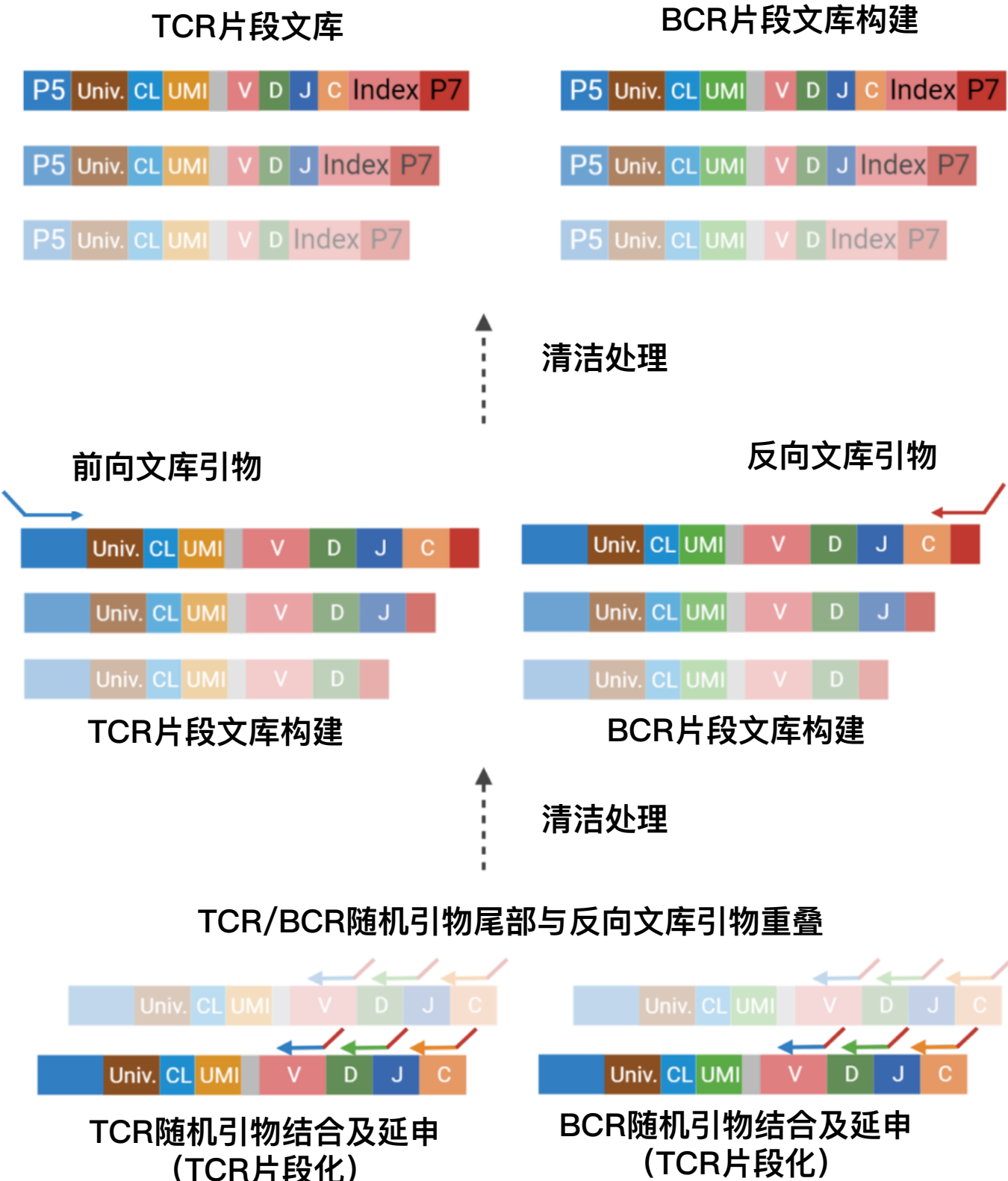
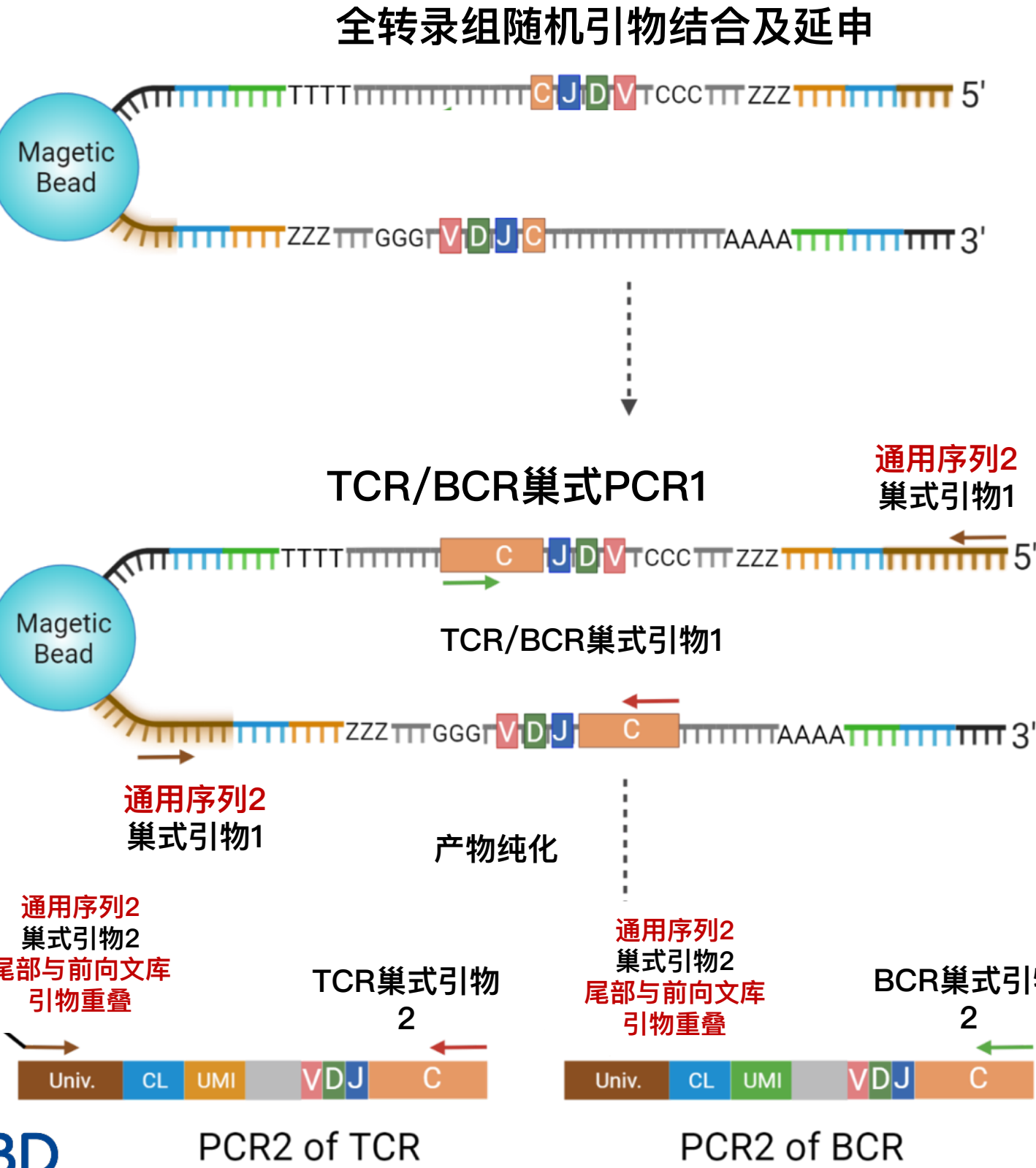


抗体标签

加入TSO完成模板转换



全长 VDJ 扩增及文库构建



内容

- BD Rhapsody原理简介
- **BD Rhapsody**工作流程
- 单细胞多组学在眼科学中的应用

BD Rhapsody™ – 完整的单细胞工作流程

✓ Rhapsody Scanner 可视化质控



■ 单细胞样本制备

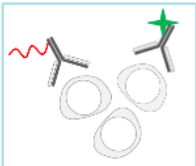
✓ AbSeq抗体



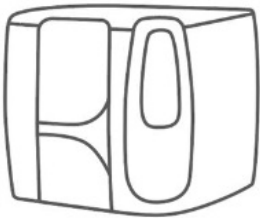
✓ 多样本标签



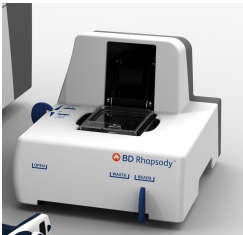
✓ 流式抗体+AbSeq抗体与细胞共染



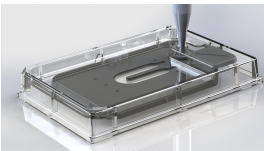
✓ 单细胞分选仪富集
(可选)



■ 单细胞分离



✓ Rhapsody样品上样
工作站



■ cDNA合成



✓ 单细胞分离捕获试剂&建库试剂

■ 文库构建



测序

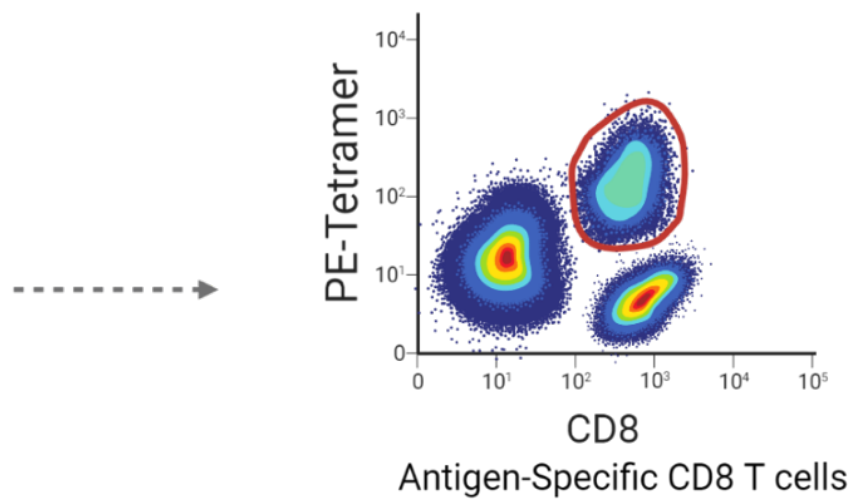
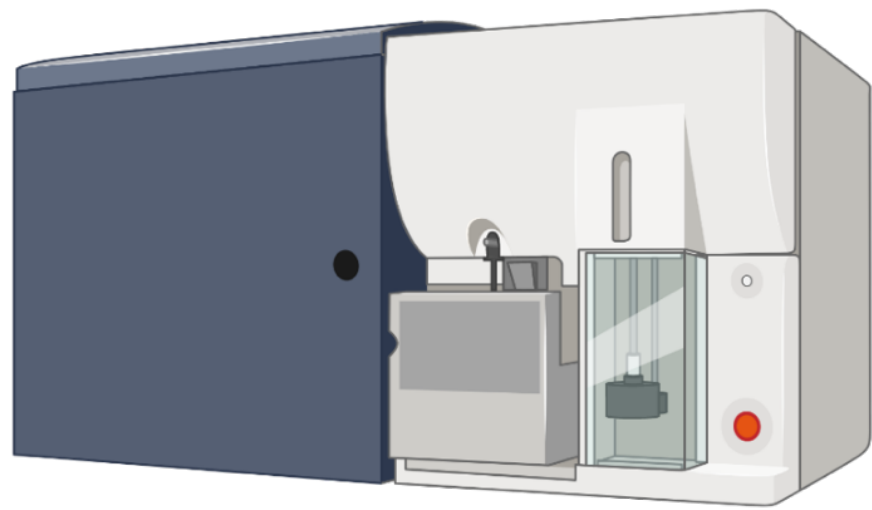
■ 数据分析

✓ 数据分析软件

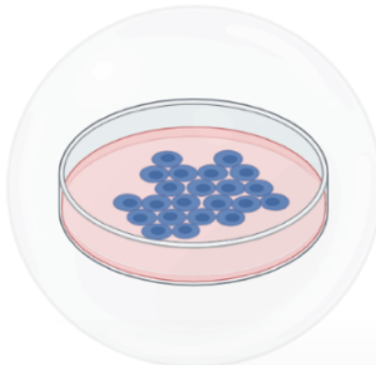


✓ 生信分析服务

Rhapsody工作流程：单细胞悬液的制备



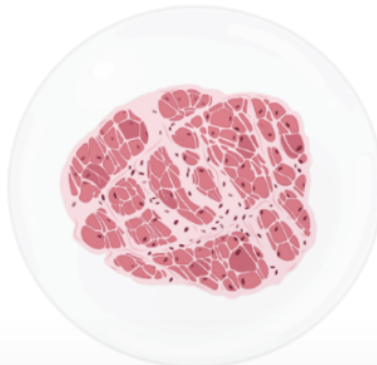
Sorting



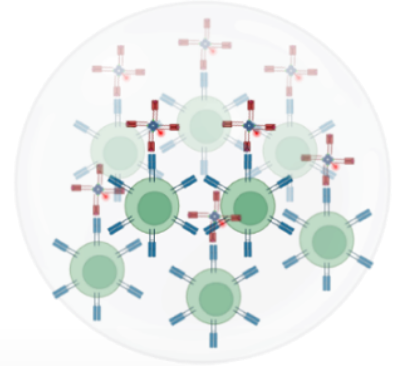
细胞系



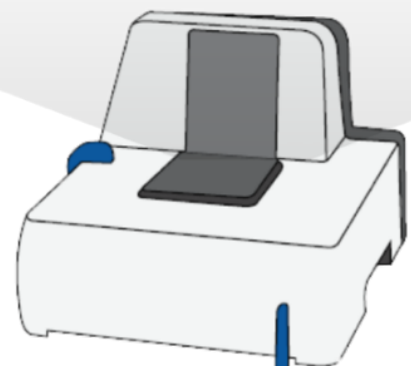
临床样本



解离的组织



流式分选富集

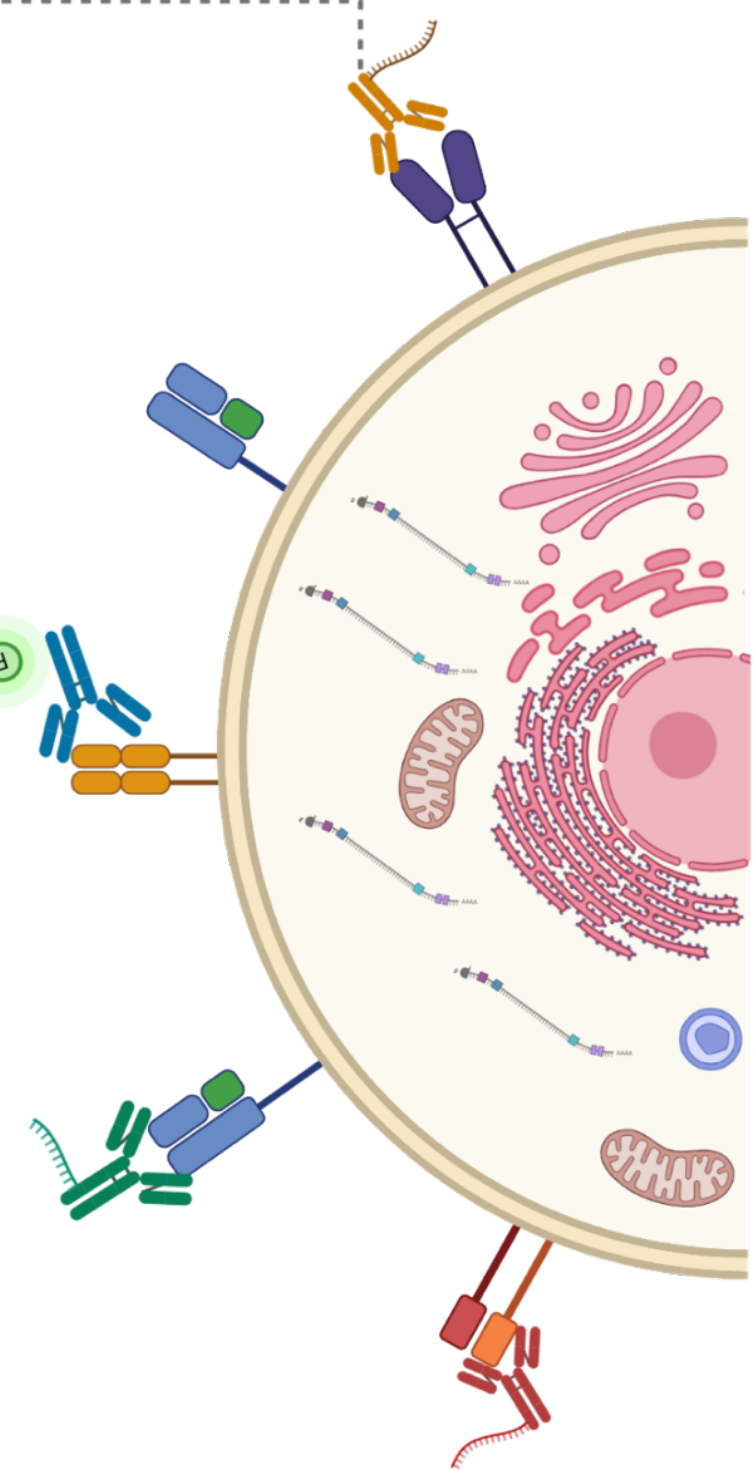


单次实验可混合多个不同类型的样本

表面蛋白标签
AbSeq标记靶蛋白

联用流式抗体
标记靶蛋白

混样标签SMK
标记不同的样本



BD Rhapsody™ – 完整的单细胞工作流程

✓ Rhapsody Scanner 可视化质控



■ 单细胞样本制备

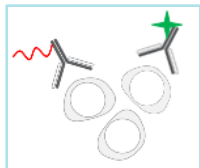
✓ AbSeq抗体



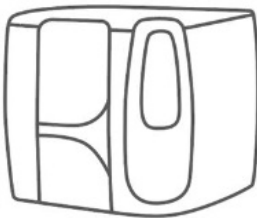
✓ 多样本标签



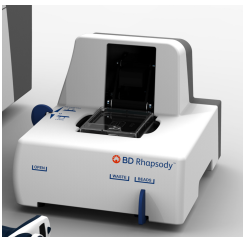
✓ 流式抗体+AbSeq抗体与细胞共染



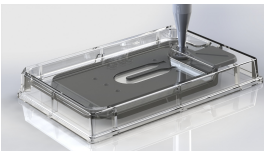
✓ 单细胞分选仪富集
(可选)



■ 单细胞分离



✓ Rhapsody样品上样
工作站



✓ 单细胞分离捕获试剂&建库试剂

■ cDNA合成



■ 文库构建



测序

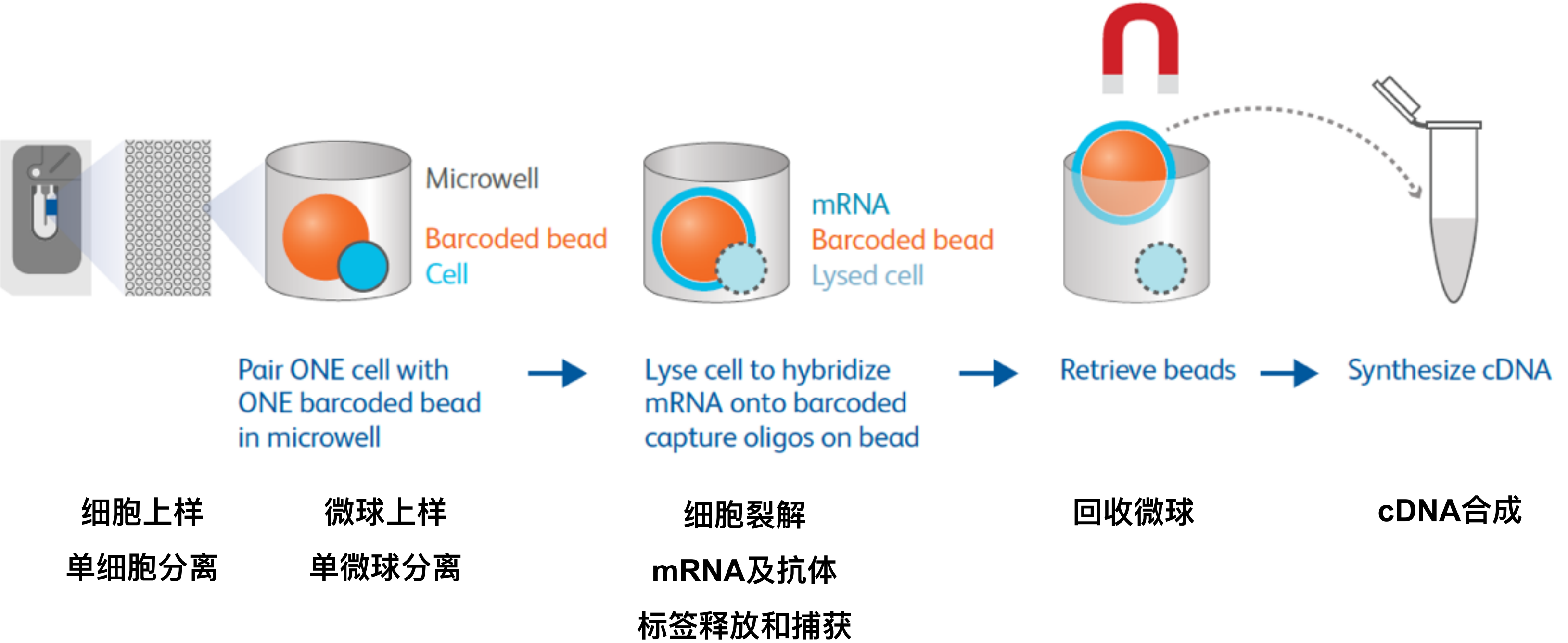
■ 数据分析

✓ 数据分析软件



✓ 生信分析服务

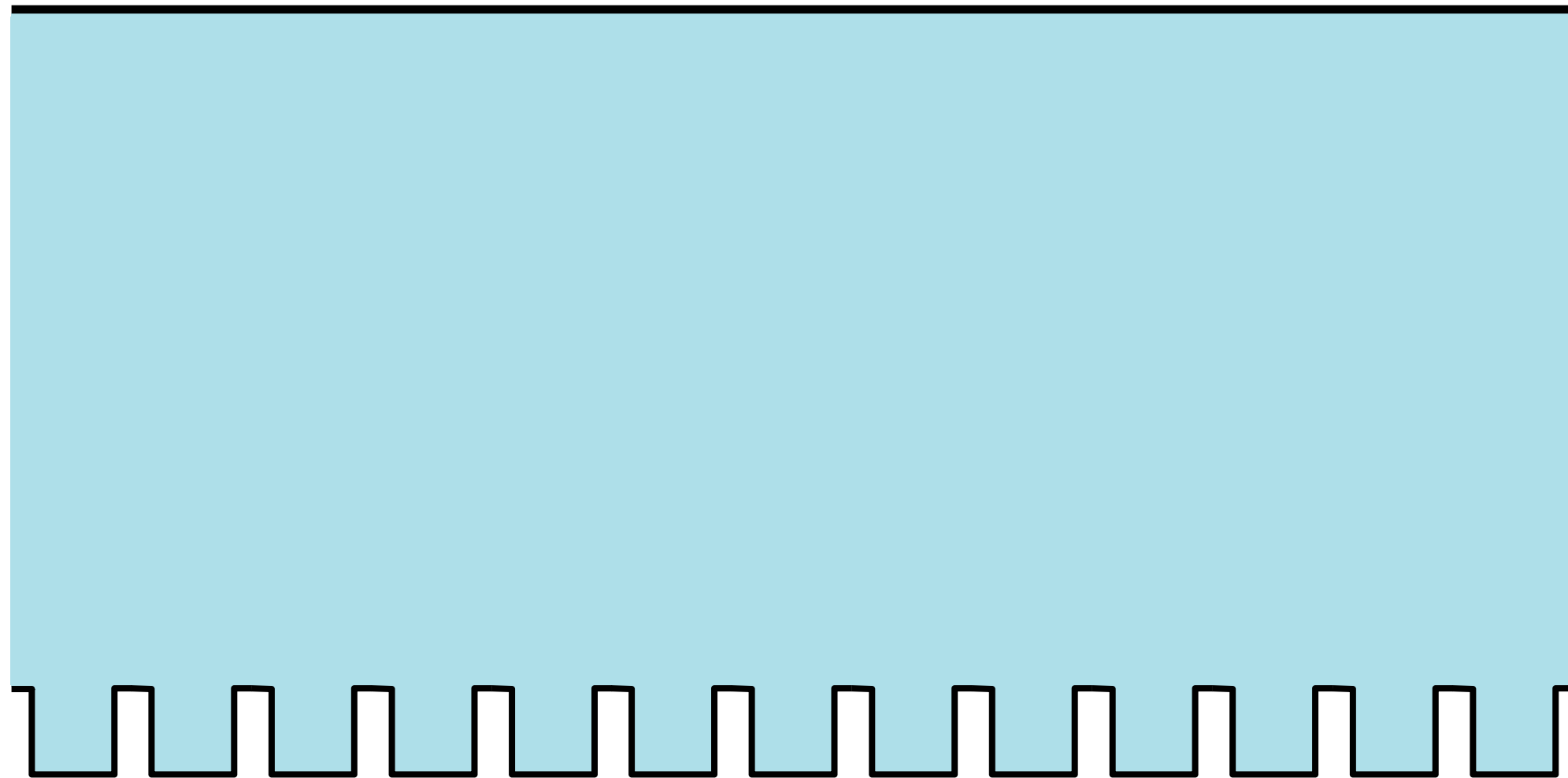
Rhapsody工作流程：单细胞分离



Rhapsody工作流程：单细胞分离、裂解、mRNA及抗体标签捕获

细胞上样

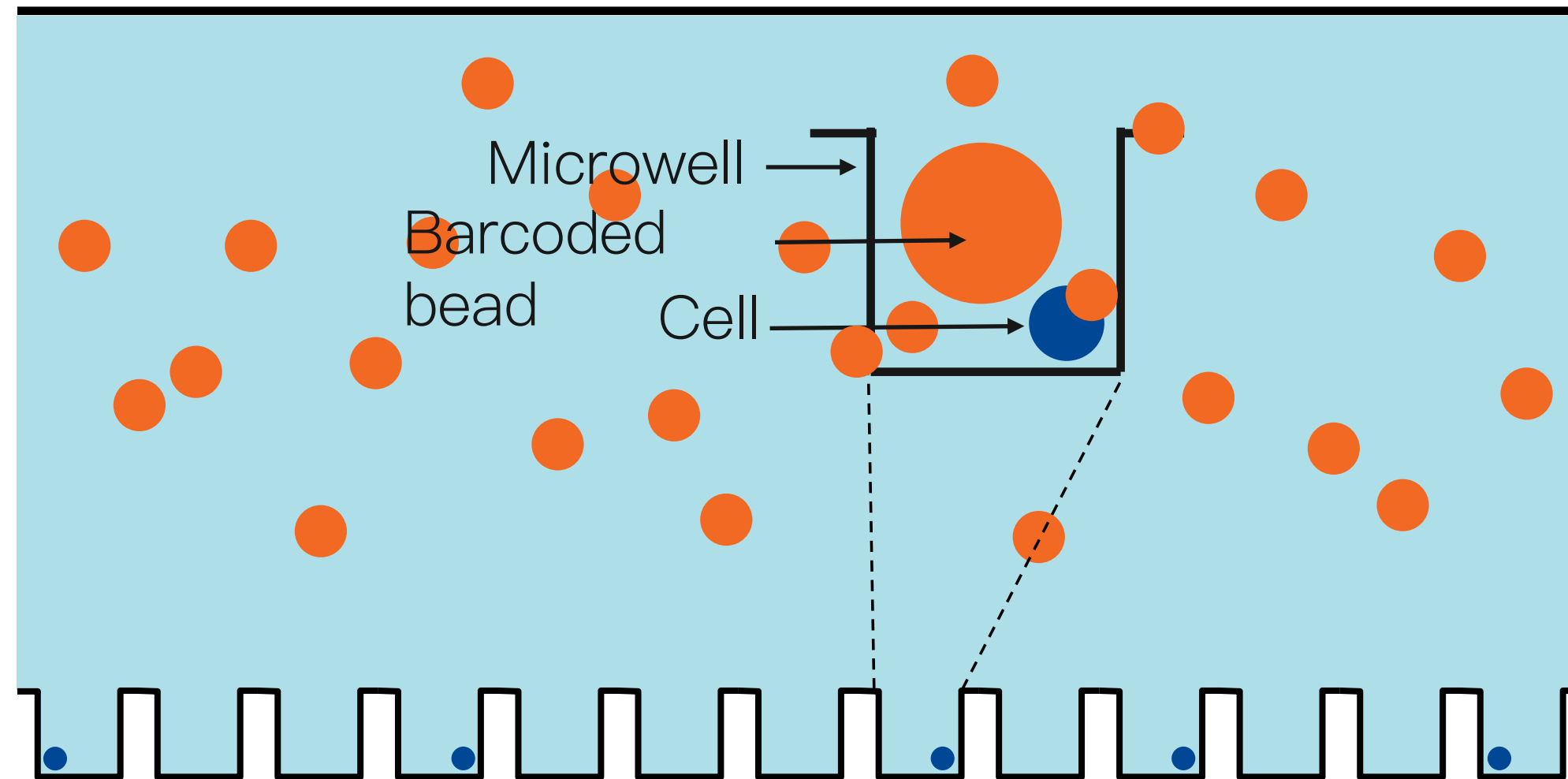
具体的细胞上样体积
可由BD Scanner提
供，无需手动计算



Rhapsody工作流程：单细胞分离、裂解、mRNA及抗体标签捕获

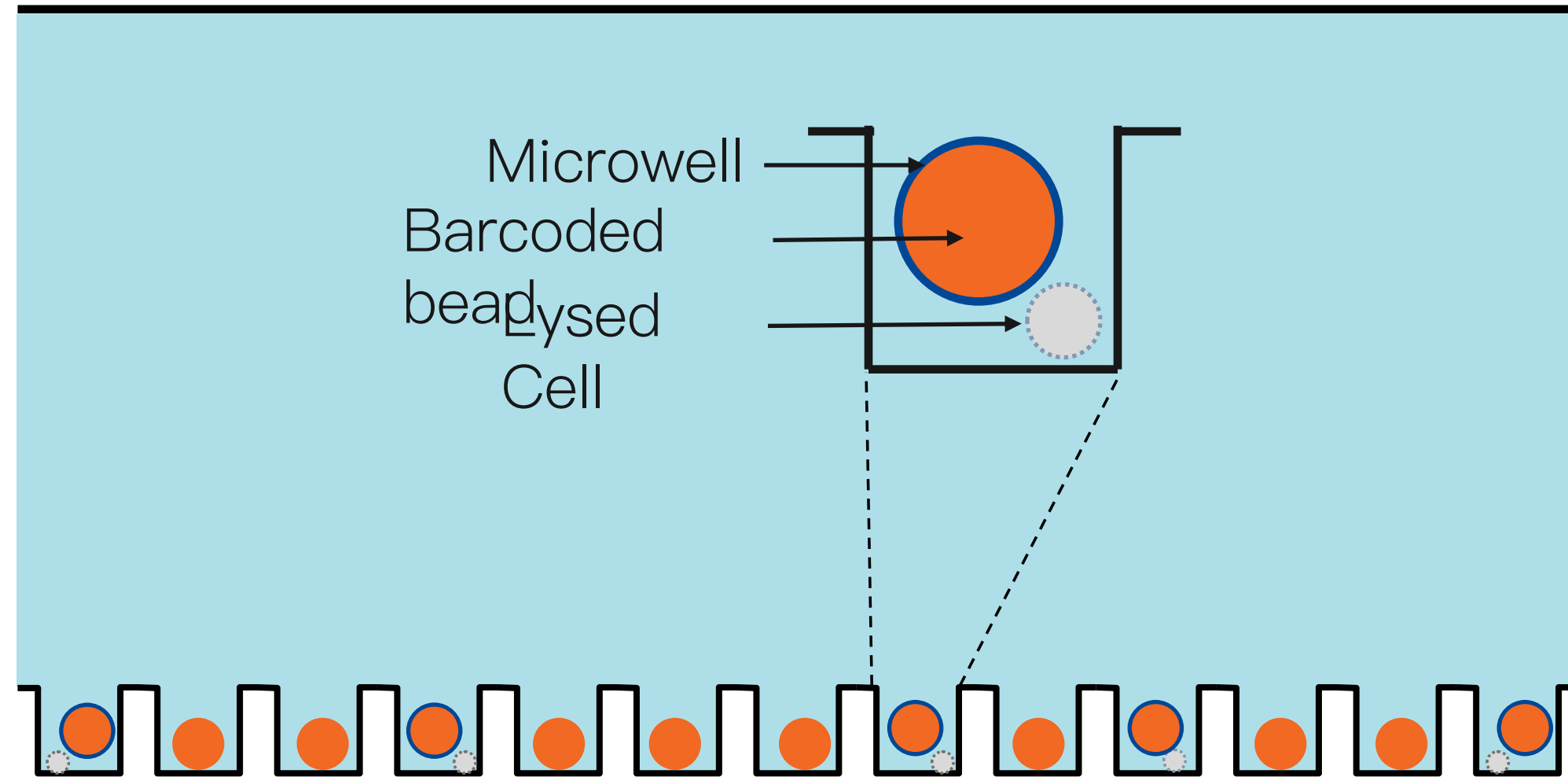
微球上样

加入过量的微球，
使绝大多数微孔
都含有1个微球



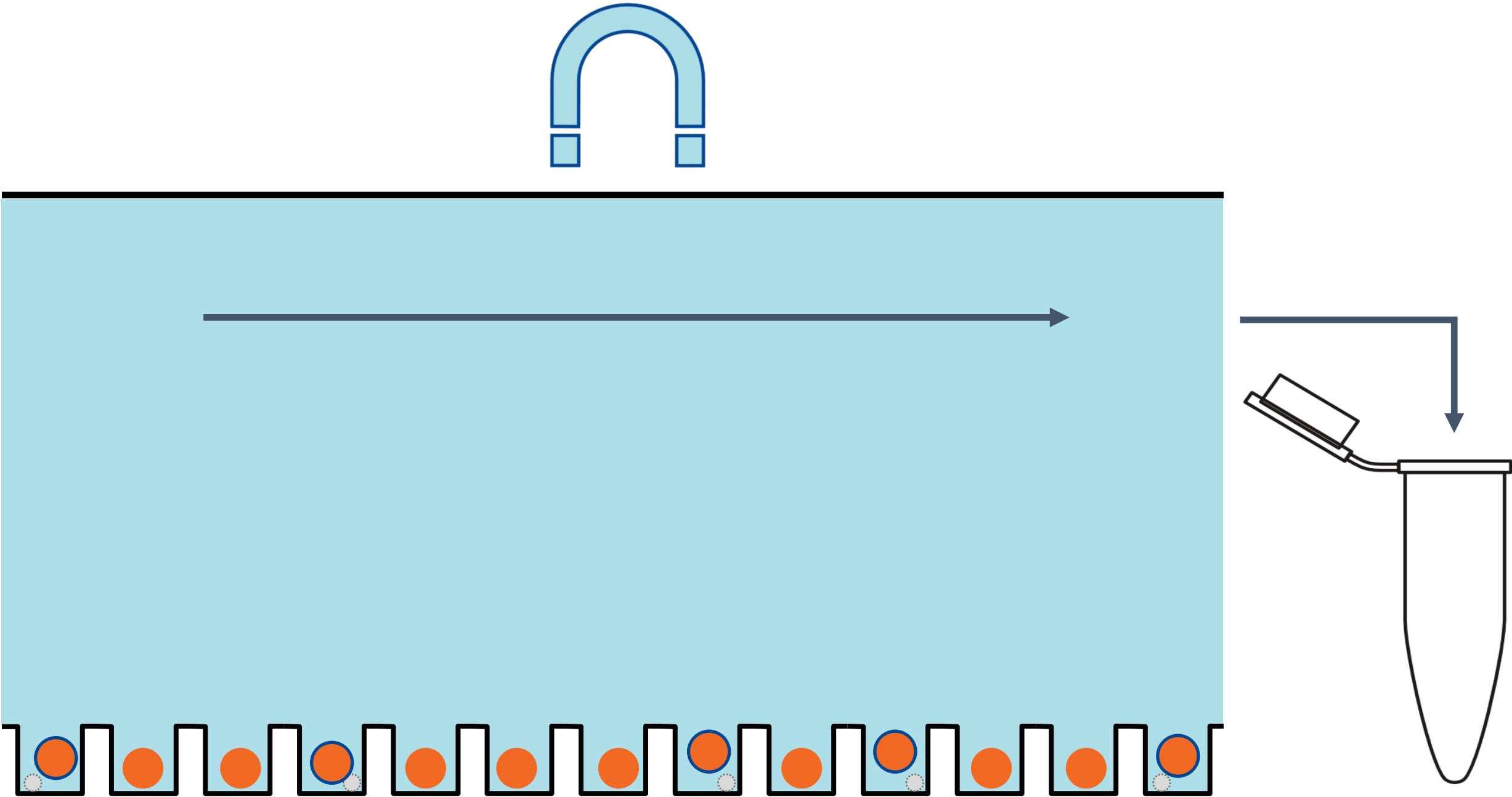
Rhapsody工作流程：单细胞分离、裂解、mRNA及抗体标签捕获

细胞裂解释放mRNA
及抗体标签，可被同
一孔内的微球捕获

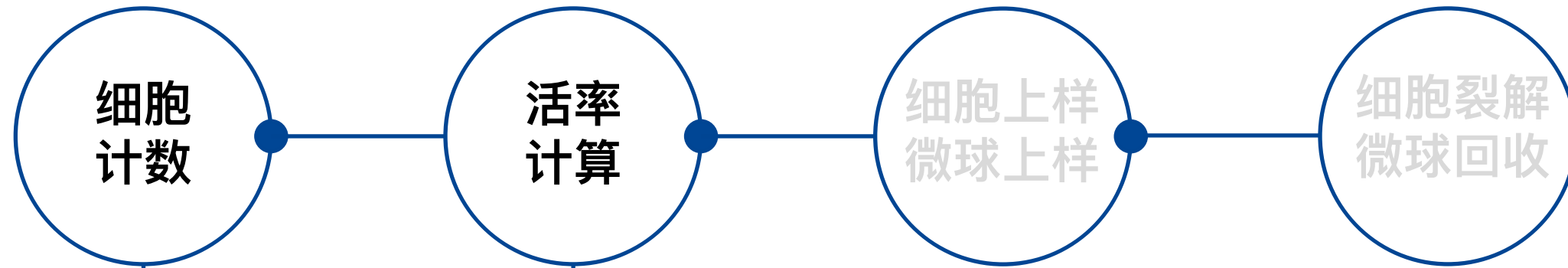


Rhapsody工作流程：单细胞分离、裂解、mRNA及抗体标签捕获

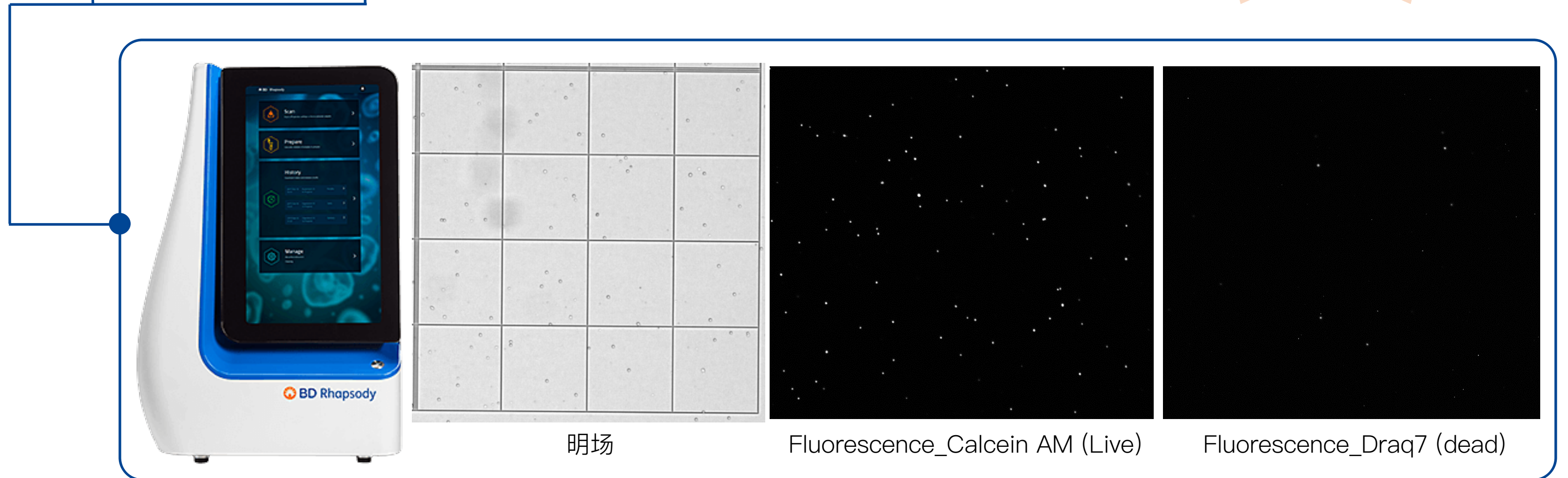
回收微球



Rhapsody工作流程

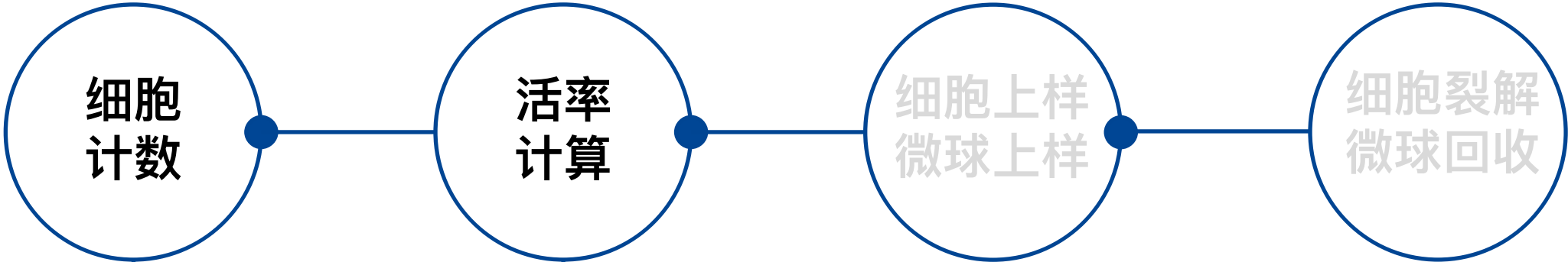


BD 独家技术
Rhapsody Scanner



使用BD Scanner进行细胞计数以及活率计算

Rhapsody工作流程



BD 独家技术
Rhapsody Scanner
智能化操作
减少操作力

捕获数：10000



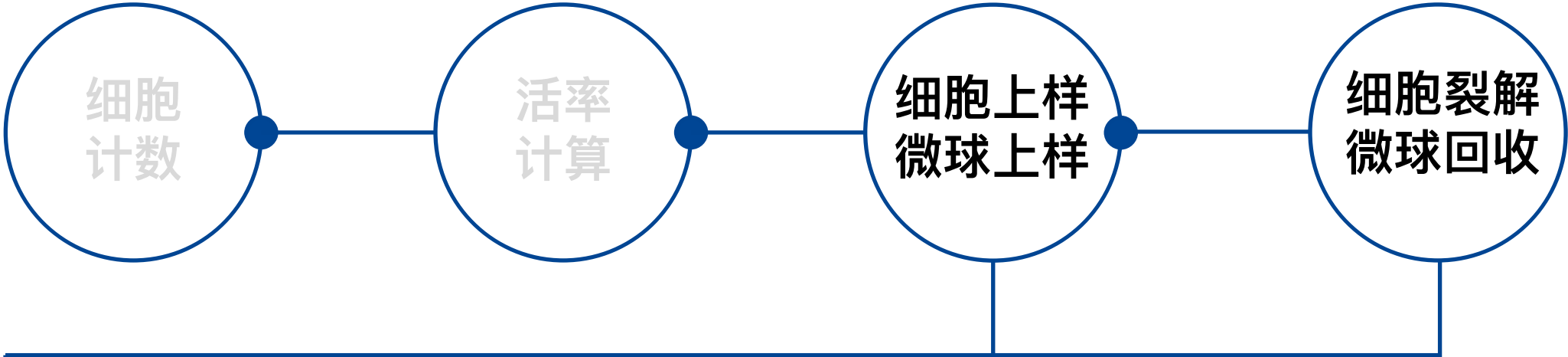
Jurkat

Date	Sample	Step	Analysis Status
2018-08-02	Jurkat	Side A	✓ Completed
Analysis			
Total cell concentration (live and dead) (cells/μl)			
387			
Cell viability (%)			

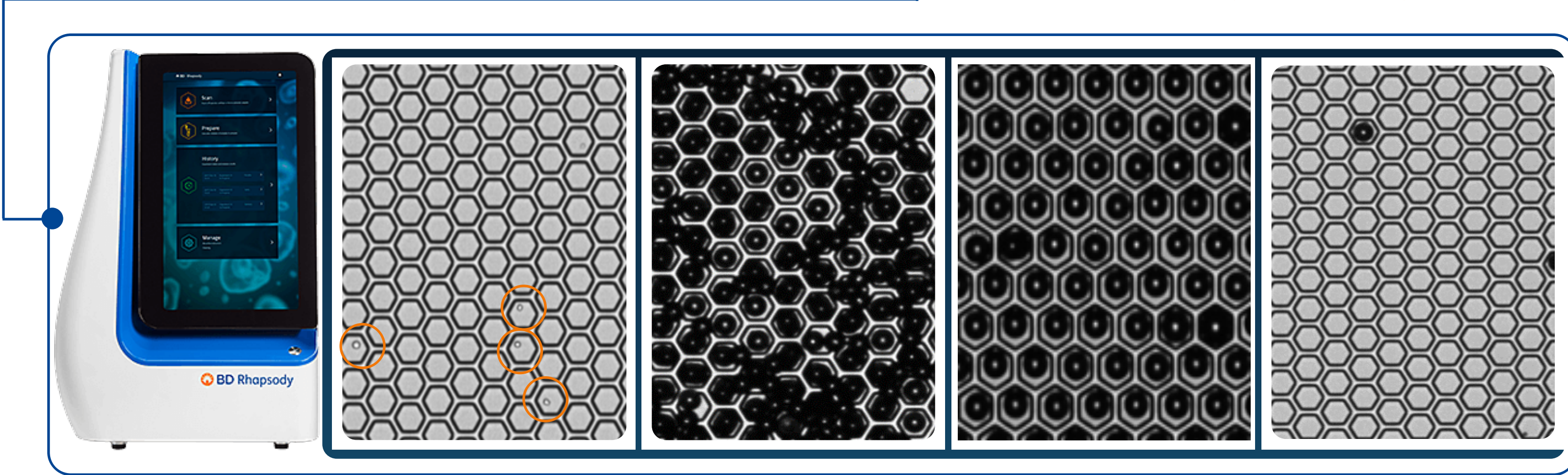
Jurkat	38.1 μl
Buffer volume	589.1 μl
Loading cell concentration	19.9 μl
Estimated cell doublet rate	2.4%

BD Scanner可提供细胞上样量以及相应的多细胞率估算

Rhapsody工作流程

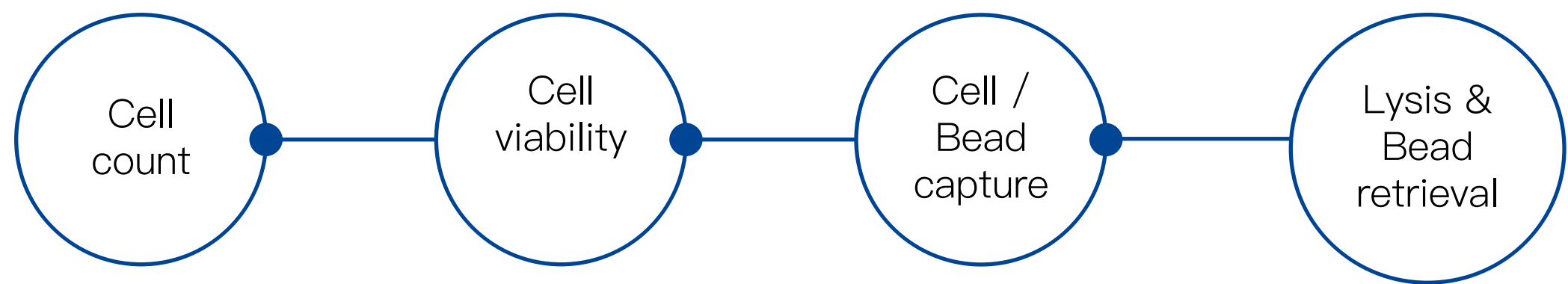


BD 独家技术
Rhapsody Scanner
可对整个实验流程
进行质控，及时止损



BD Scanner监测 “细胞上样”→“微球上样”→“细胞裂解”→“微球回收”

Rhapsody工作流程



BD 独家技术
Rhapsody Scanner
单细胞实验的
“定心丸”



Barcode,0109027202A
Experiment, trg
Sample, trg
Scan start date and time,2018/03/21
12:59:01
Operator, afg
Cell load analysis last updated,2018-03-21 13:19:11

Total number of tiles, 26
Cell load number of tiles processed, 26
Bead wash analysis last updated,2018-03-21 13:50:33
Bead wash number of tiles processed, 26

Number of viable cells captured with a bead, 6549
Cell multiplet rate, 1.58%
Bead loading efficiency, 94.85%
Bead loading acceptance PASS
Excess bead rate, 0.00%
Excess bead acceptance, PASS
Cell retention rate, 94.23%
Cell retention acceptance, PASS
Bead retrieval analysis last updated, 2018-03-21 13:57:59
Bead retrieval number of titles process, 26
Bead retrieval efficiency, 95.51%
Bead retrieval acceptance, PASS

BD Scanner可生成当次实验报告以及捕获的单细胞数

BD Rhapsody™ – 完整的单细胞工作流程

✓ Rhapsody Scanner 可视化质控



■ 单细胞样本制备

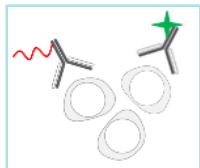
✓ AbSeq抗体



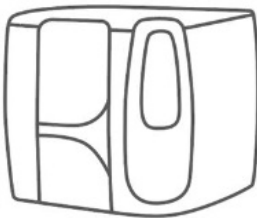
✓ 多样本标签



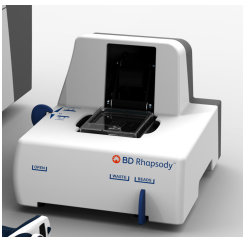
✓ 流式抗体+AbSeq抗体与细胞共染



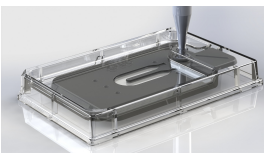
✓ 单细胞分选仪富集
(可选)



■ 单细胞分离



✓ Rhapsody样品上样
工作站



✓ 单细胞分离捕获试剂&建库试剂

■ cDNA合成



■ 文库构建



测序

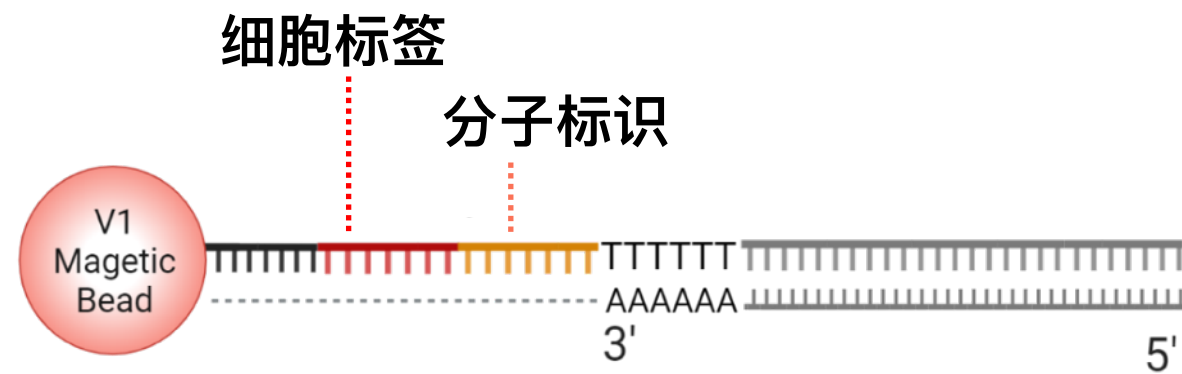
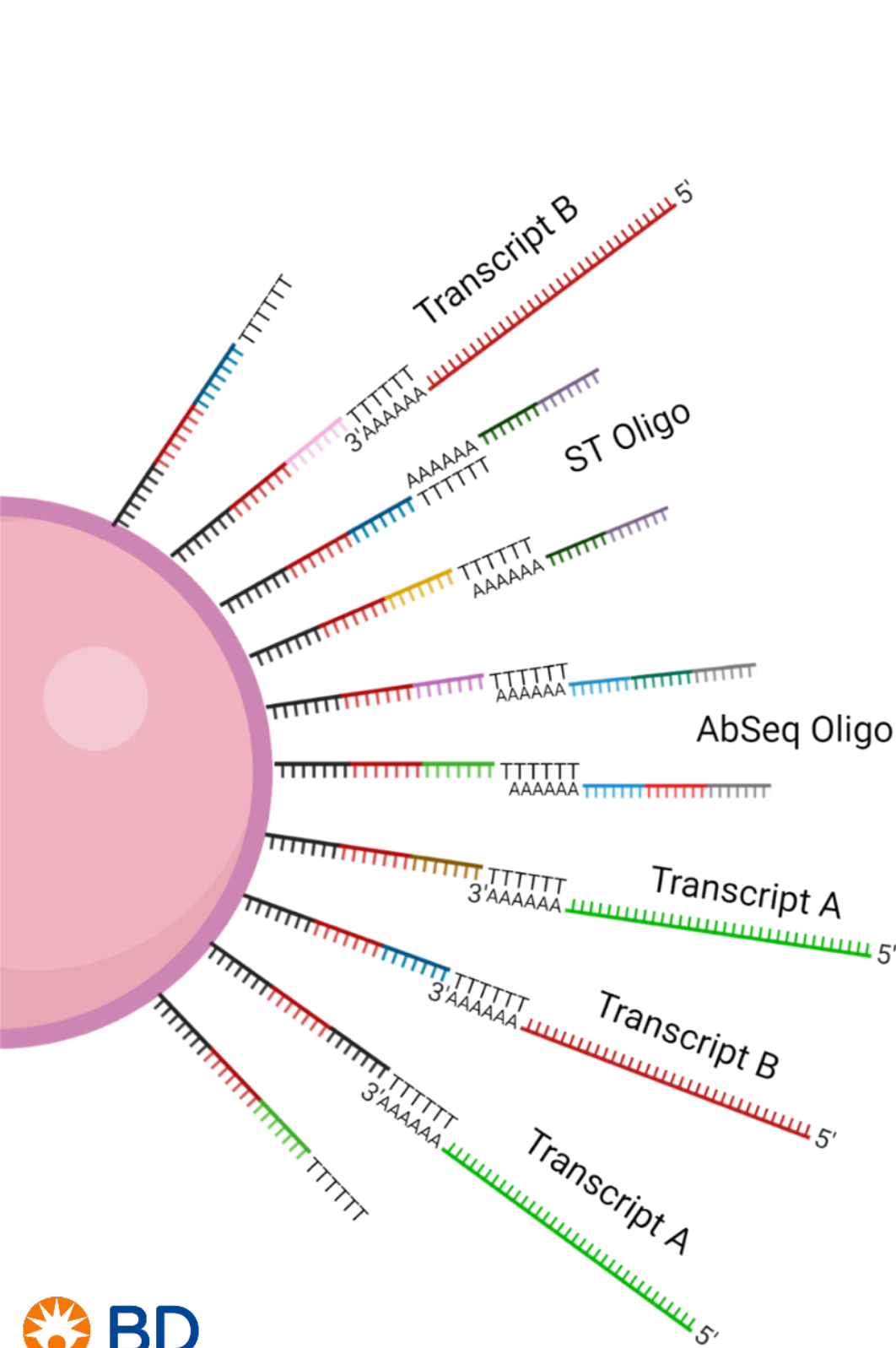
■ 数据分析

✓ 数据分析软件

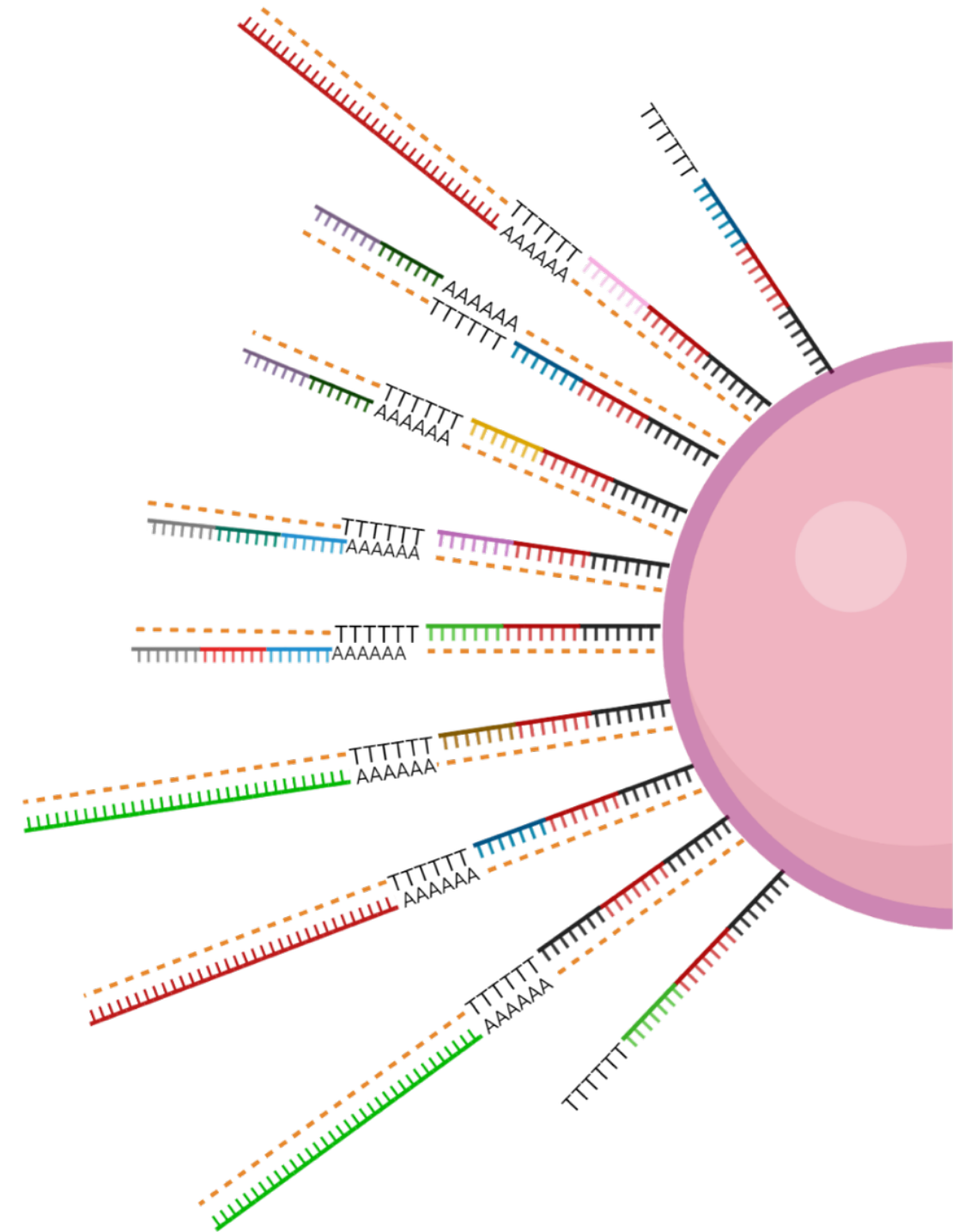


✓ 生信分析服务

Rhapsody工作流程：cDNA合成



- 来自同一个细胞的转录本以及抗体标签都带有**相同的细胞标签**
- 同一细胞内，所有的转录本以及抗体标签带有**不同的分子标识**
- **Cell Capture Beads**保存时间长，且性能稳定



BD Rhapsody™ – 完整的单细胞工作流程

✓ Rhapsody Scanner 可视化质控



■ 单细胞样本制备

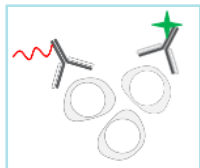
✓ AbSeq抗体



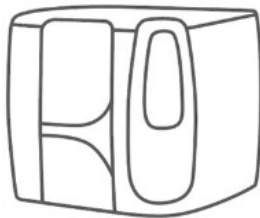
✓ 多样本标签



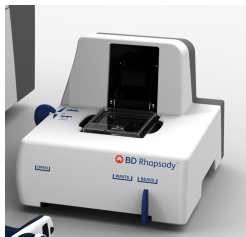
✓ 流式抗体+AbSeq抗体与细胞共染



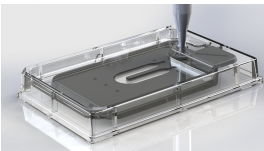
✓ 单细胞分选仪富集
(可选)



■ 单细胞分离



✓ Rhapsody样品上样
工作站



✓ 单细胞分离捕获试剂&建库试剂

■ cDNA合成



■ 文库构建



■ 测序

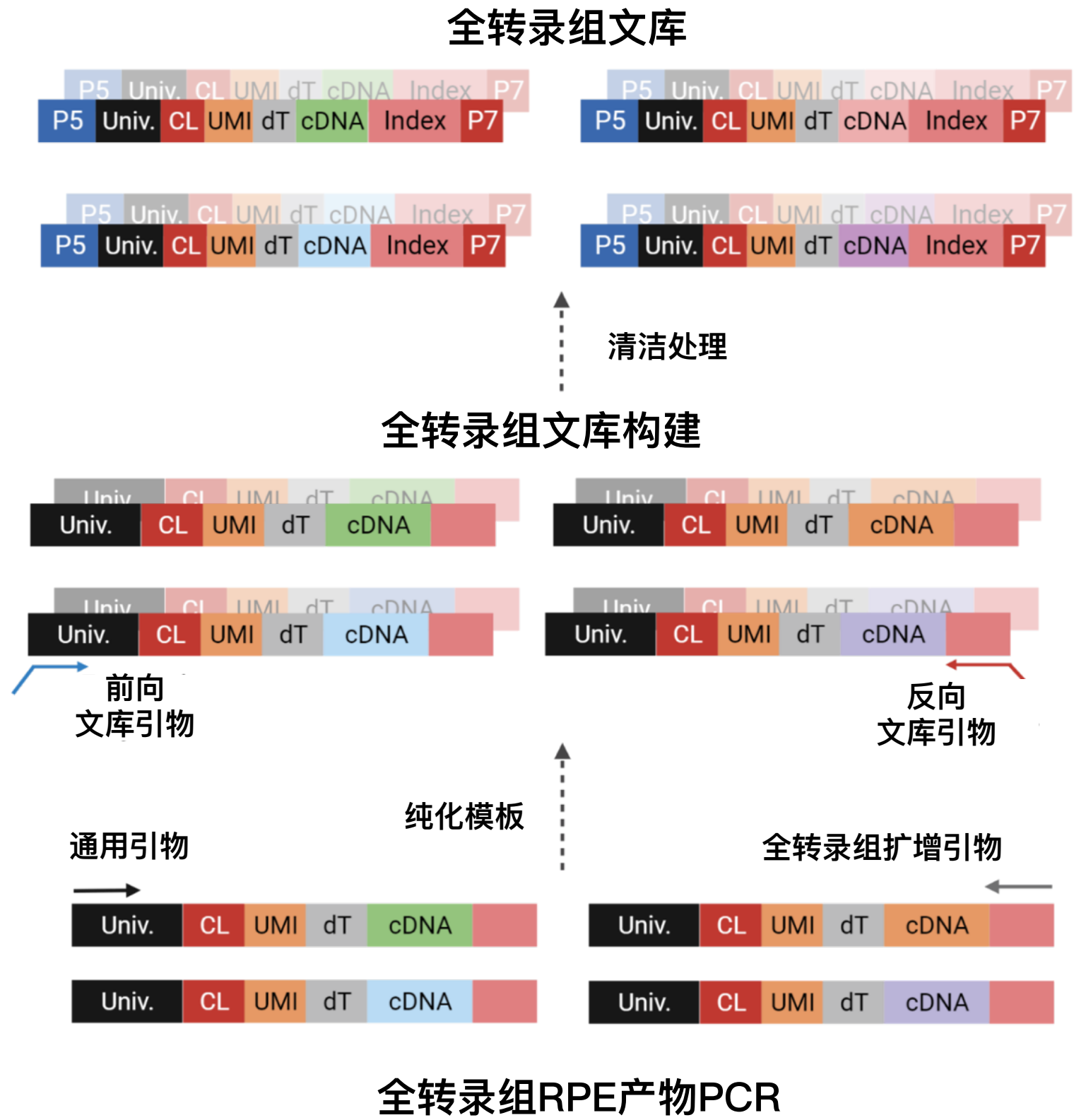
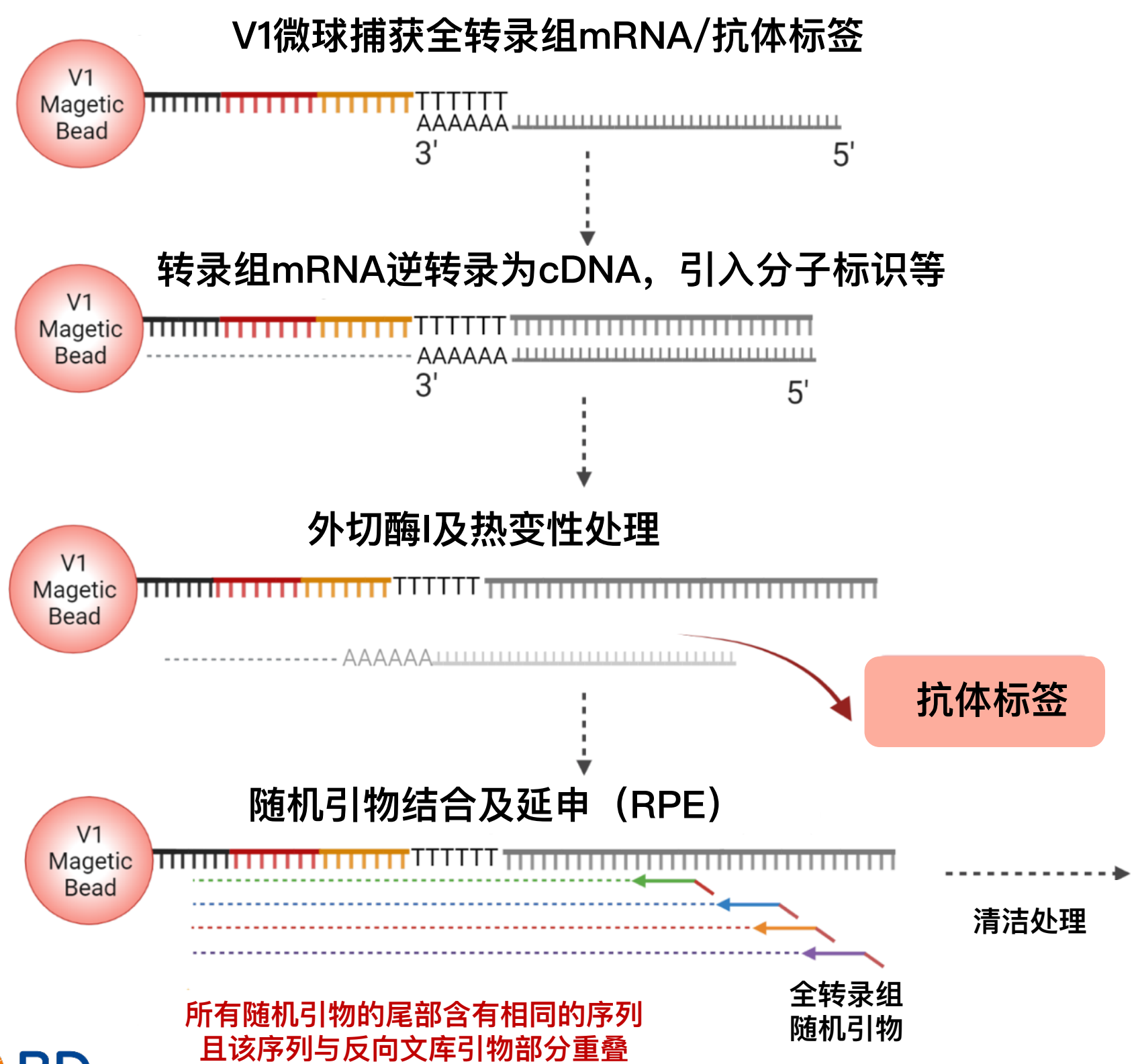
■ 数据分析

✓ 数据分析软件



✓ 生信分析服务

Rhapsody工作流程：全转录组基因扩增及文库构建



Rhapsody工作流程：全转录组基因扩增及文库构建

全转录组文库



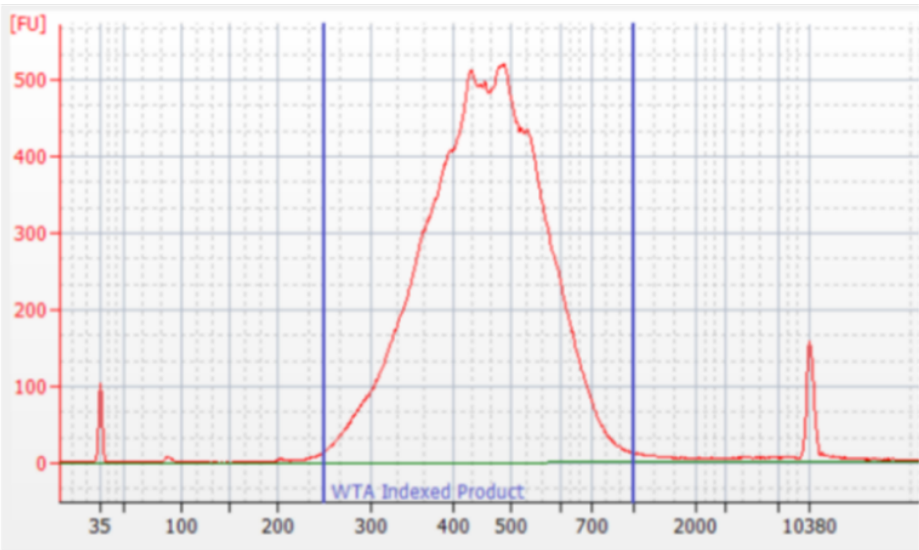
文库质检



Qubit 4.0



Bioanalyzer

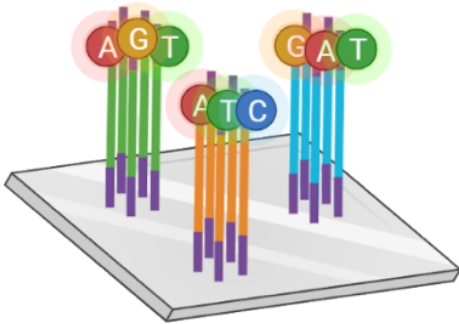


Sample Bioanalyzer high-sensitivity DNA trace

合格文库测序



生成FASTQ文件



Illumina

BD Rhapsody™ – 完整的单细胞工作流程

✓ Rhapsody Scanner 可视化质控



■ 单细胞样本制备

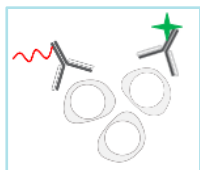
✓ AbSeq抗体



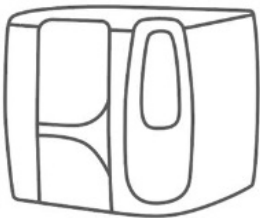
✓ 多样本标签



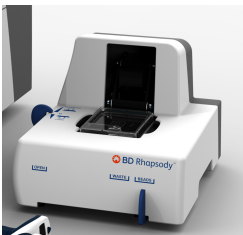
✓ 流式抗体+AbSeq抗体与细胞共染



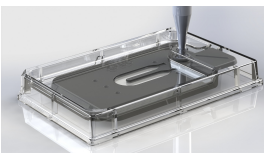
✓ 单细胞分选仪富集
(可选)



■ 单细胞分离



✓ Rhapsody样品上样
工作站



✓ 单细胞分离捕获试剂&建库试剂

■ cDNA合成



■ 文库构建



■ 测序

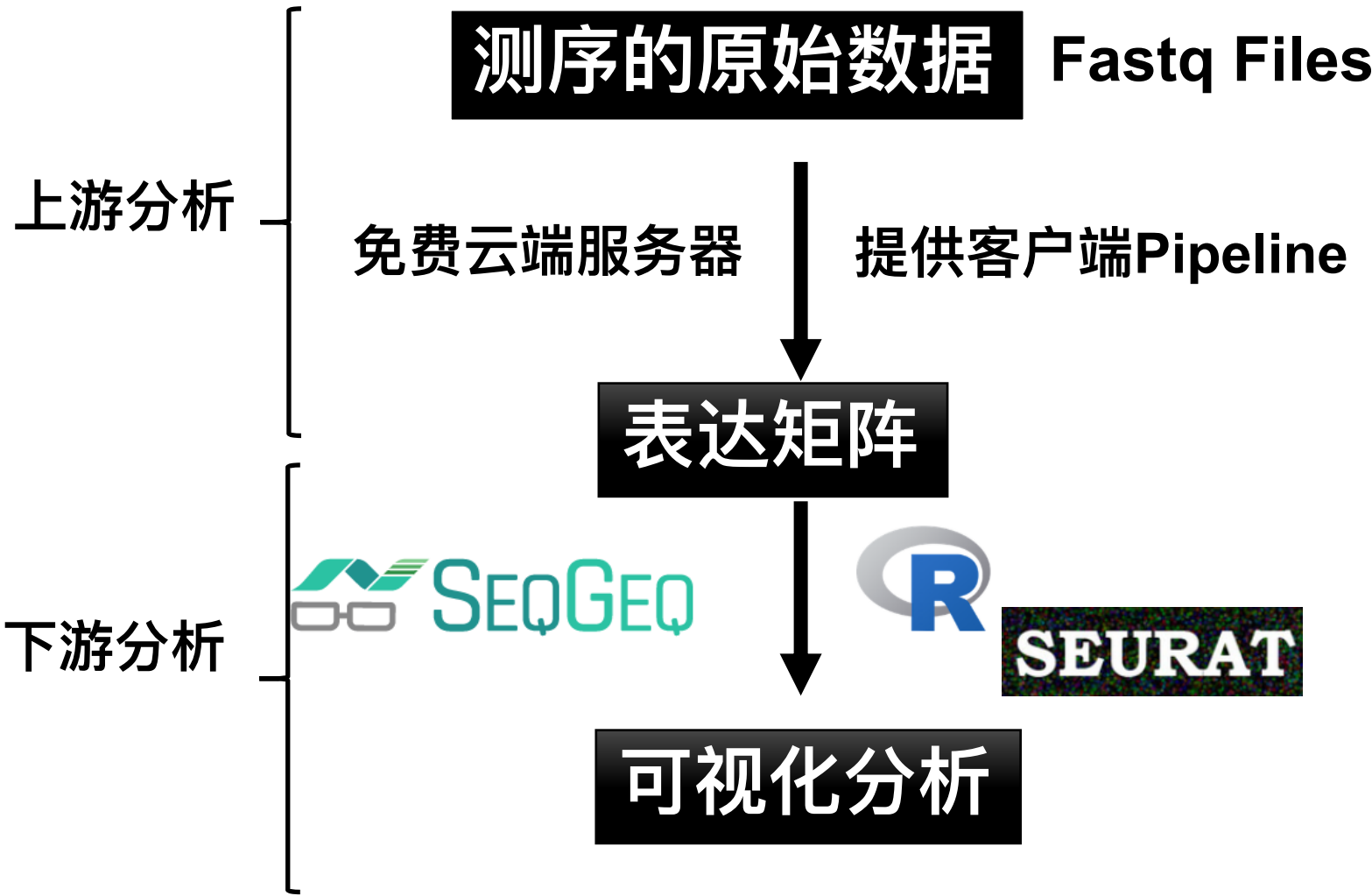
■ 数据分析

✓ 数据分析软件



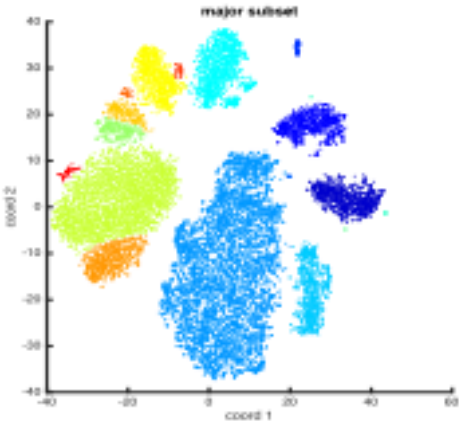
✓ 生信分析服务

Rhapsody 工作流程：原始数据处理及可视化分析



```
@SIM:1:FCX:1:15:6329:1045 1:N:0:2
TCGCACTCAACGCCCTGCATATGACAAGACAGAATC
+
<>;##=><9=AAAAAAAAAA9#:<#<;<<<????#=>
```

#####		
## BD Targeted Rhapsody Analysis Pipeline Version 1.0		
## Analysis Date: 2017-09-05 18:15:03		
## Sample: HumanImmResDemo		
## Reference: rhapsody_immune_response_panel_hs		
#####		
Cell_Index	ADA NM_000022.3 Reference_end	ADGRE1 NM_001974.4 Reference_end
465057	1	0
124703	2	0
468260	2	0
427285	0	0
738786	1	1
560306	5	3
575419	1	1
228010	1	0
195595	0	0



免费云端服务器-SevenBridge(国外)

Projects

Search

yunceng's Demo Project

Created by:yunceng · Sep 11, 2022, 12:08

+ Create Project

View all projects

<

>

BD Rhapsody Sequence Analysis PipelineEB-PBMC20k

Pipeline Version: 1.11
Fri Aug 26 2022 03:31:02 GMT+0000 (Coordinated Universal Time)

Summary

Graphs

Sequencing Quality

Library Quality

Alignment Categories

Reads and Molecules

Cells

Pipeline Inputs

Metric Alerts 0

Graphs

Select Graph: Single Bioproduct Expression (tSNE/histogram)

tSNE log10 expression: MT-CO1

Select Gene or AbSeq: MT-CO1

Total molecule count: 4,748,221

Log10 expression frequency: MT-CO1

Log10 Cell Expression

BD Rhapsody Sequence Analysis PipelineVDJ-FL-demo-singularity

Pipeline Version: 1.11
Thu Aug 25 2022 06:08:38 GMT+0000 (Coordinated Universal Time)

Summary

Graphs

Sequencing Quality

Library Quality

Alignment Categories

Reads and Molecules

Cells

Error Correction Level

Sample Multiplexing

VDJ

Pipeline Inputs

Metric Alerts 1

Molecules and Dominant Contigs

Chain Category	Mean Molecules Corrected per Putative Cell	Molecules VDJ Annotated	Molecules Corrected	Dominant Contigs Mean Nucleotide Length	Dominant Contigs Pct Full Length	Dominant Contigs Pct With CDR3
BCR	20.11	265,944	157,086	487	78.23%	99.43%
TCR	2.24	35,506	17,497	489	63.69%	99.72%

Cell Type VDJ Metrics: Paired Chains Pct

Cell Type	Number cells	BCR	TCR
B	3,390	78.58%	0.5%
Dendritic	91	0%	1.1%
Monocyte classical	1,293	1.86%	0.85%
Monocyte nonclassical	189	0.53%	0%
Natural killer	852	1.41%	0.23%
T CD4 memory	903	0%	47.62%
T CD4 naive	451	0.22%	34.15%
T CD8 memory	354	1.13%	31.92%
T CD8 naive	195	0%	39.49%
T gamma delta	92	0%	23.91%

Analysis

Search

Tasks

Data Studio

COMPLETED

BD AbSeq on Rhapsody™ Analysis Pipeline - Demo Task

Project: yunceng/yunceng-s-demo-project · Submitted by: yunceng · Sep 11, 2022 12:08

COMPLETED

Sample Multiplexing on Rhapsody™ Analysis Pipeline - Demo Task

Project: yunceng/yunceng-s-demo-project · Submitted by: yunceng · Sep 11, 2022 12:08

COMPLETED

BD Rhapsody™ WTA Analysis Pipeline - Demo Task

Project: yunceng/yunceng-s-demo-project · Submitted by: yunceng · Sep 11, 2022 12:08

COMPLETED

BD AbSeq and Sample Multiplexing on BD Rhapsody™ WTA Analysis Pipeline - Demo Task

Project: yunceng/yunceng-s-demo-project · Submitted by: yunceng · Sep 11, 2022 12:08

COMPLETED

BD Rhapsody™ Analysis Pipeline - Demo Task

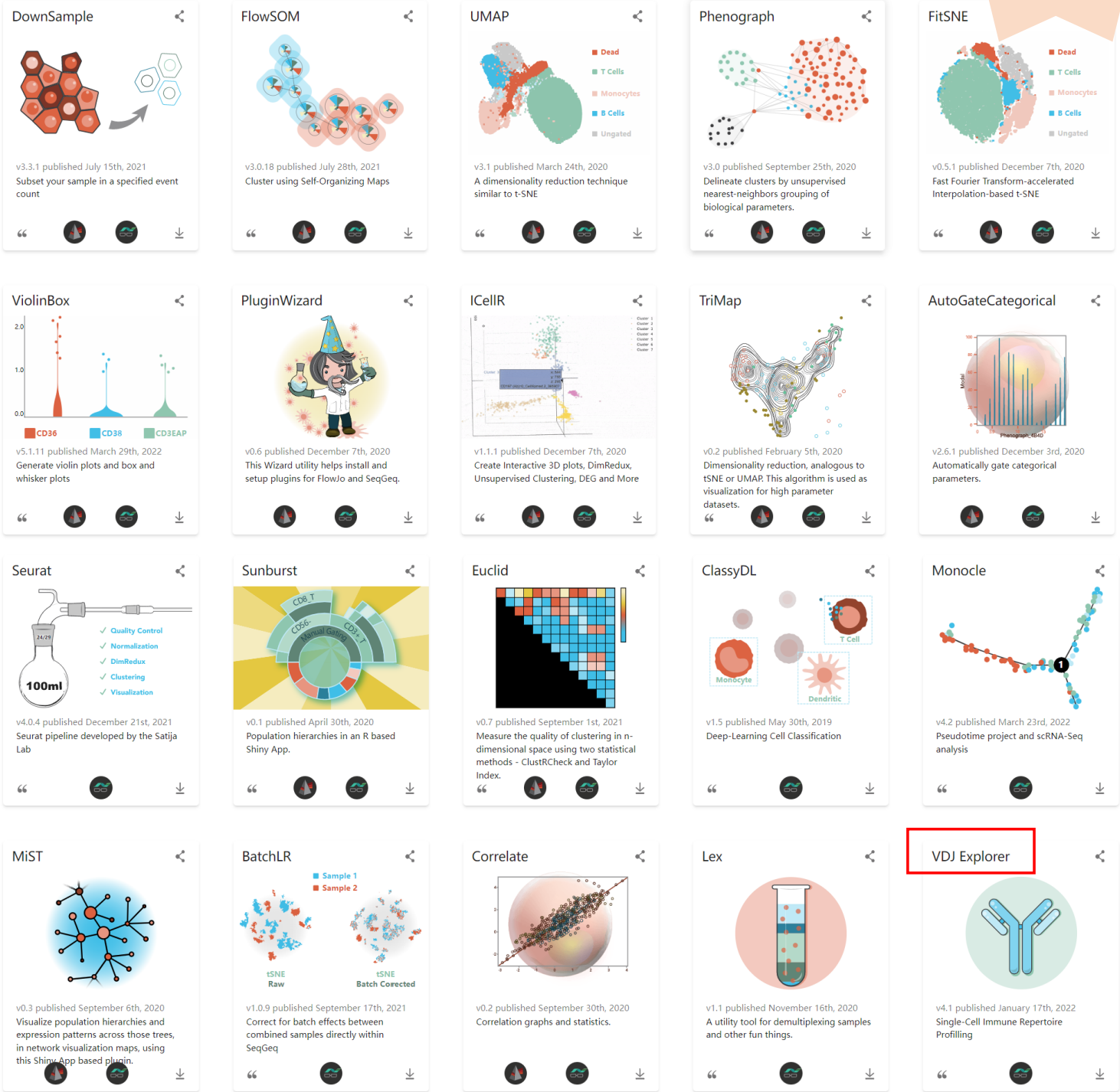
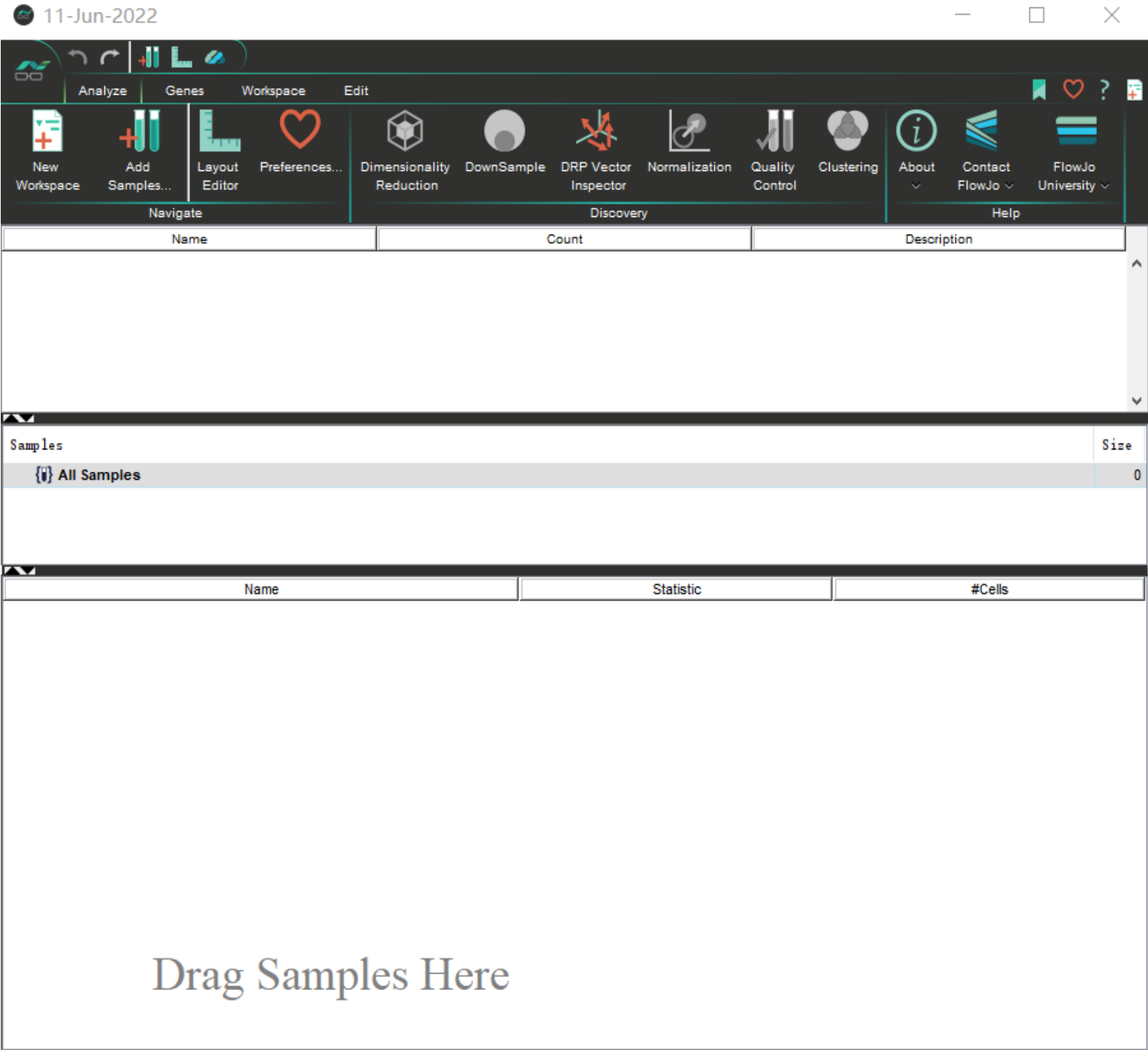
Project: yunceng/yunceng-s-demo-project · Submitted by: yunceng · Sep 11, 2022 12:08

<

>

Rhapsody 工作流程：BD 可视化分析软件 SeqGeq

免费试用
2个月



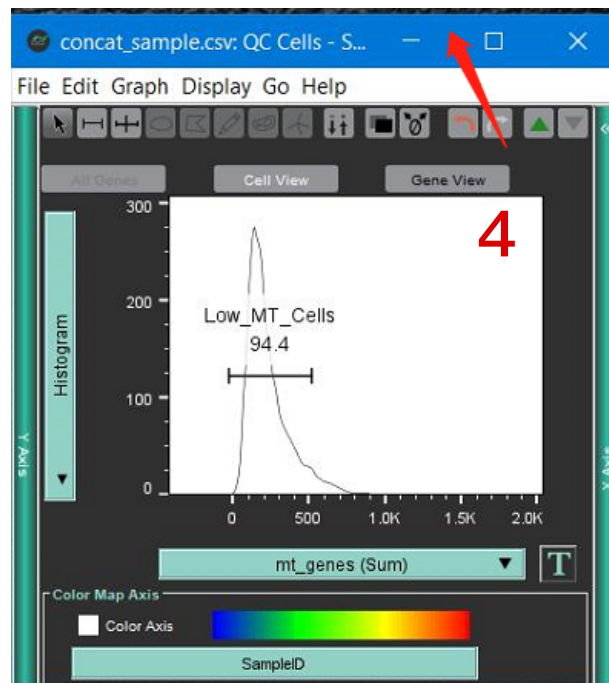
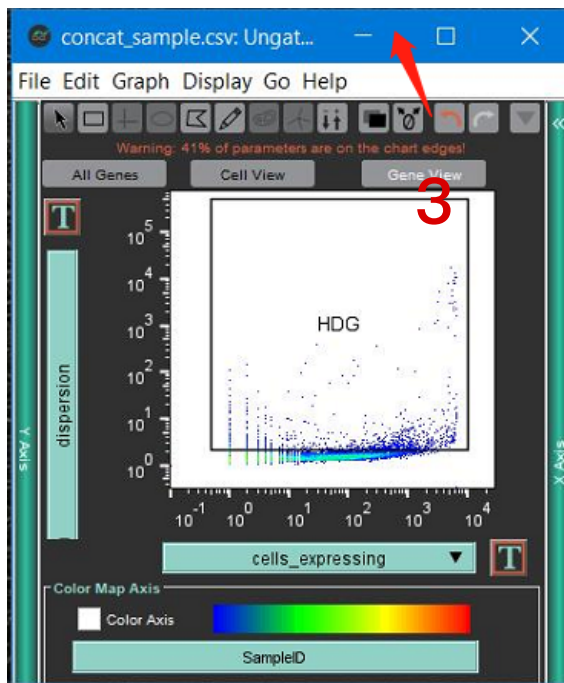
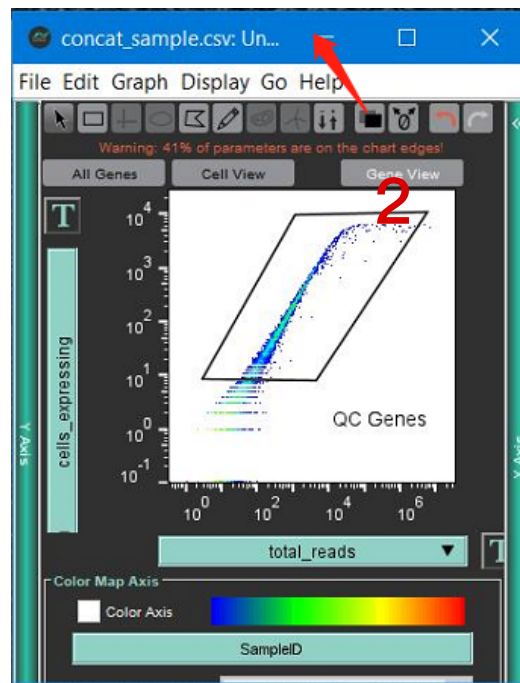
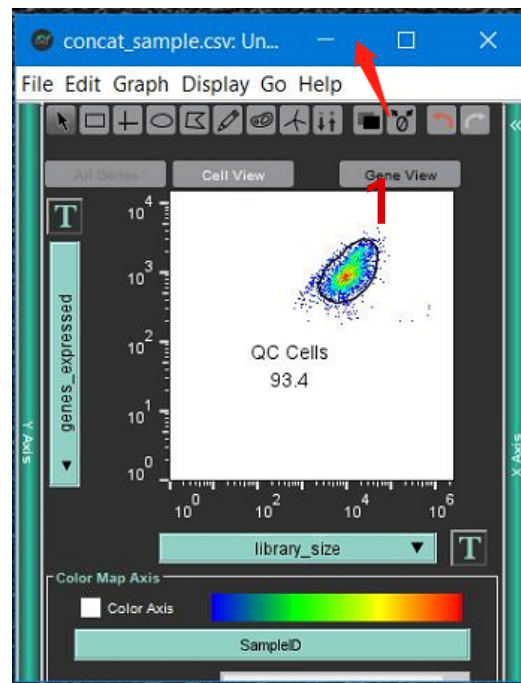
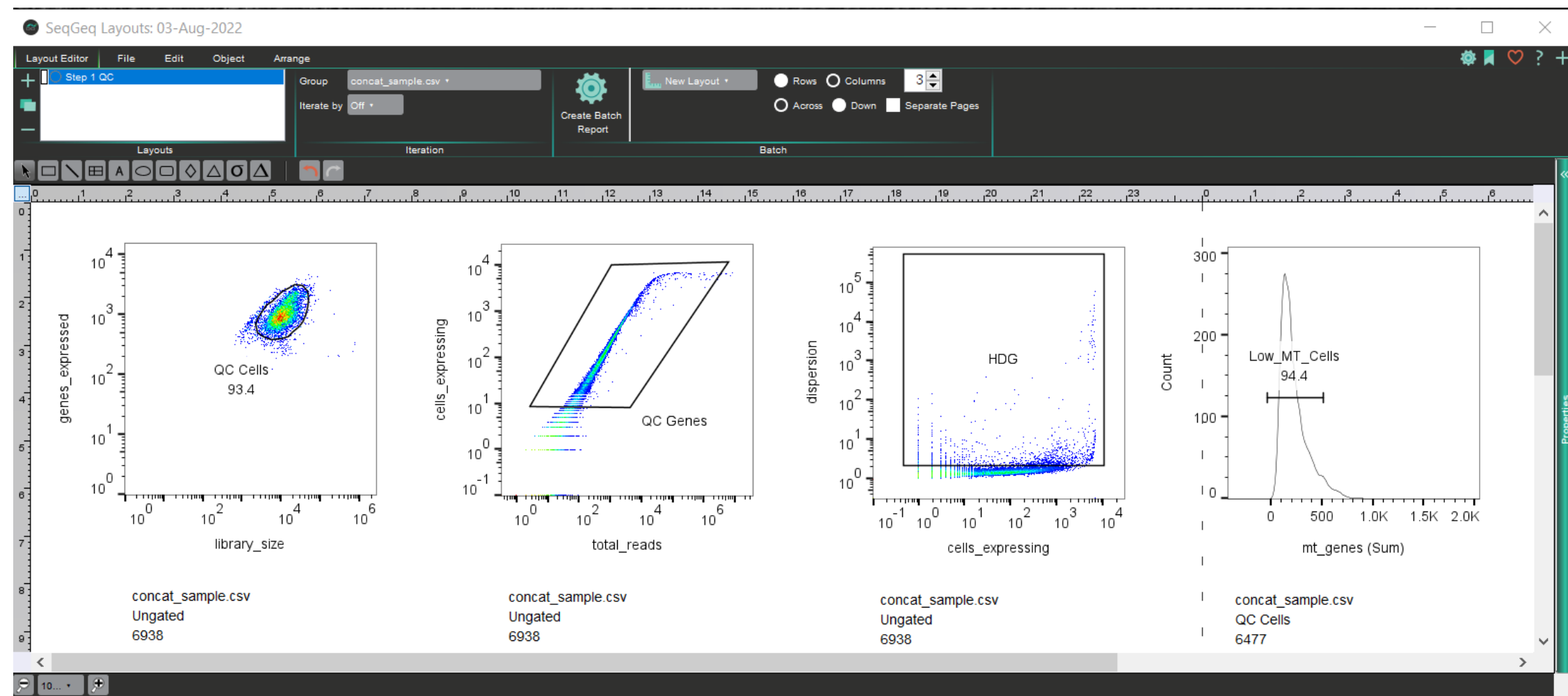
与FlowJo相似操作界面，无需编程操作

海量插件免费使用



SeqGeq对细胞和基因进行质控

- 1、选中“QC Cell”图（图中箭头位置变成蓝色表示选中），随后键盘Ctrl + L，将该图形拷贝到Layout Editor中
- 2、同样操作，将基因质控画门策略拷贝到Layout Editor中
- 3、同样操作，将高变基因画门策略拷贝到Layout Editor中
- 4、同样操作，将低线粒体基因细胞群拷贝到Layout Editor中

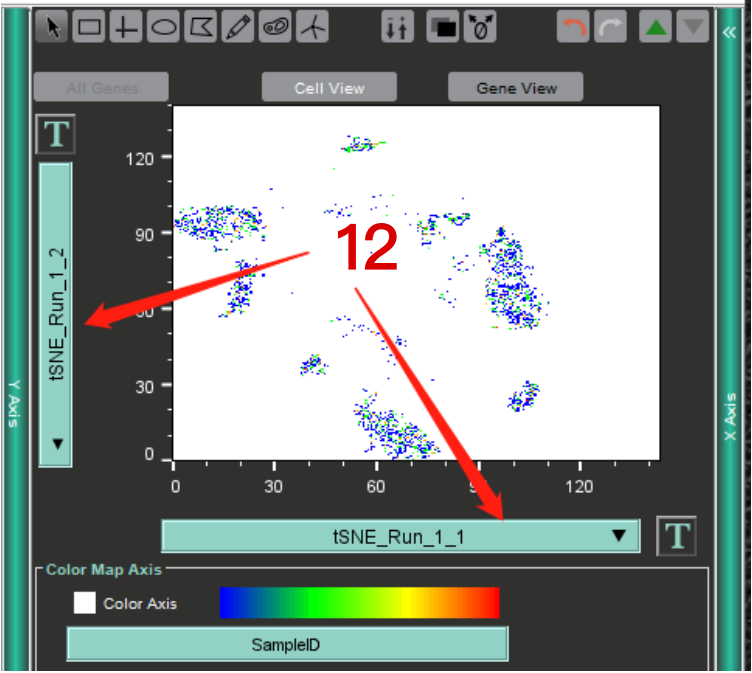


SeqGeq拆分混样数据

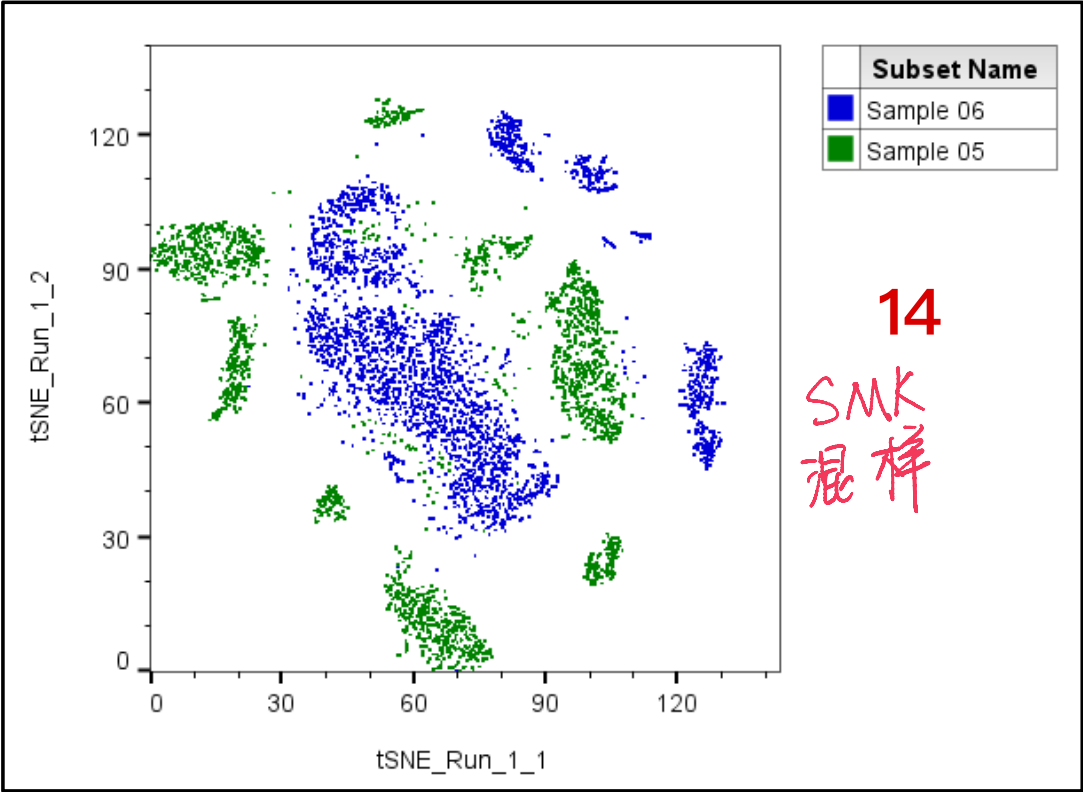
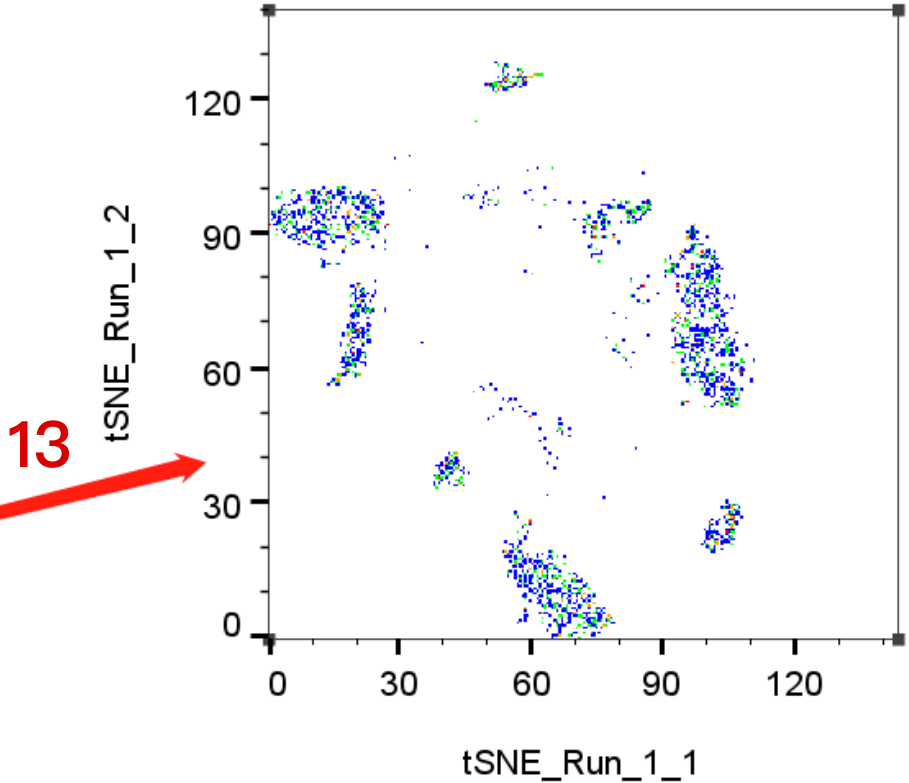
分组/分样品显示tSNE图

- 11、双击质控过的分组细胞亚群
- 12、弹出的窗口中，修改X轴和Y轴的参数为tSNE，随后Ctrl + L将tSNE图拷贝到Layout中
- 13、选中Sample 06，并将其拖入到tSNE图中重叠
- 14、改图为按照样本/组间分组显示的tSNE图

Name ▼	Statistic	#Cells
concat_sample.csv		6938
QC Cells	93.2	6466
Low_MT_Cells	94.4	6106
Autogate categorical parameters of Low_MT_Cells		
B cells 1	16.9	1031
B cells 2	25.3	1542
B cells 3	2.80	171
CD4 T cells 1	7.11	434
CD4 T cells 2	16.3	993
CD8 T cells	5.03	307
Monocytes	15.0	913
NK cells	11.7	715
Sample 05	45.1	2756
Sample 06	54.9	3350
Sample 05	47.4	3290
Sample 06	52.6	3648



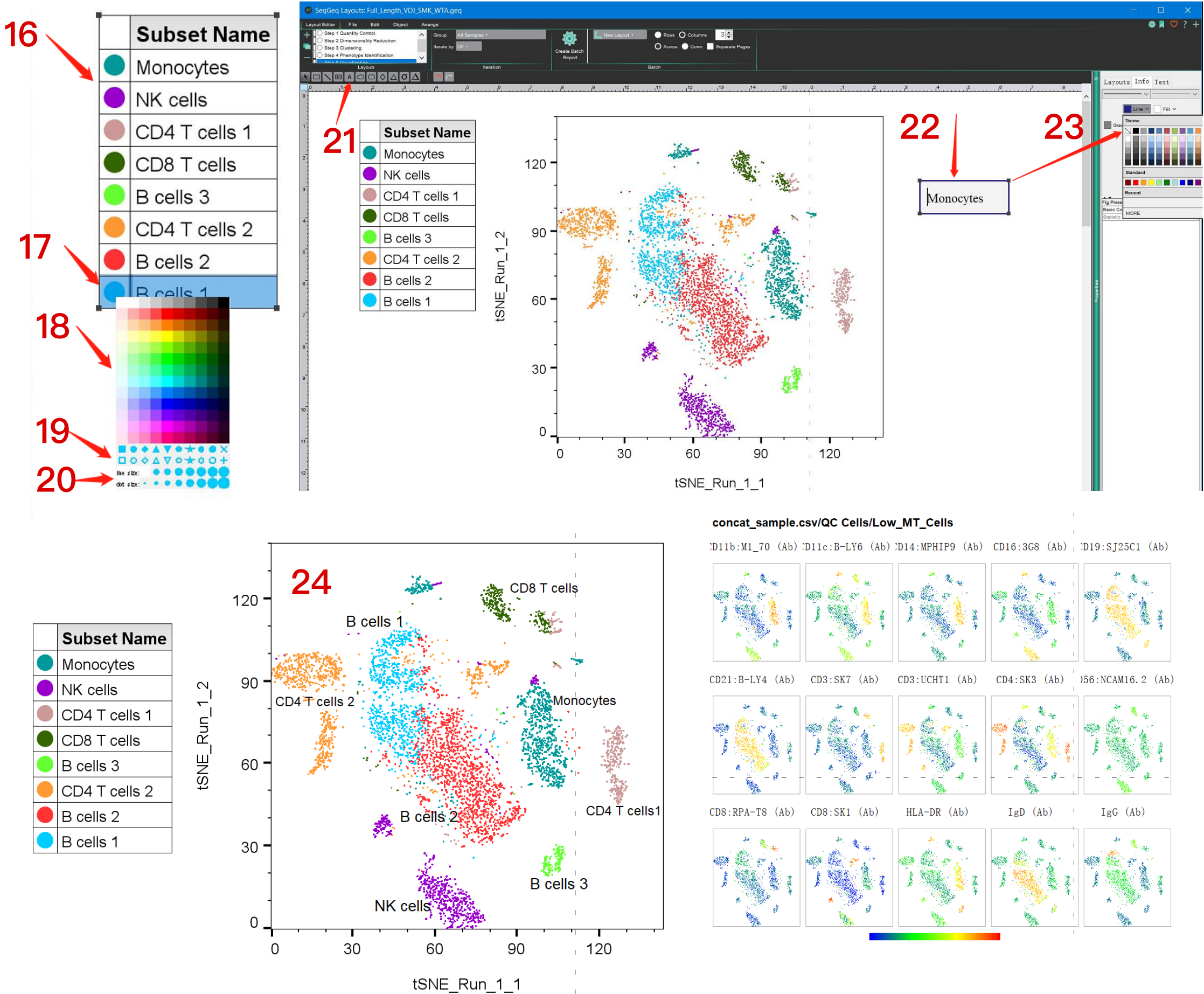
Name ▼	Statistic	#Cells
concat_sample.csv		6938
QC Cells	93.2	6466
Low_MT_Cells	94.4	6106
Autogate categorical parameters of Low_MT_Cells		
B cells 1	16.9	1031
B cells 2	25.3	1542
B cells 3	2.80	171
CD4 T cells 1	7.11	434
CD4 T cells 2	16.3	993
CD8 T cells	5.03	307
Monocytes	15.0	913
NK cells	11.7	715
Sample 05	45.1	2756
Sample 06	54.9	3350
Sample 05	47.4	3290
Sample 06	52.6	3648



SeqGeq对细胞进行亚群注释

对应Marker基因的表达热图，在主界面对KMeans子群进行重命名（相当于亚群注释）

- 16、选中Legend，边框出现4个“■”
- 17、鼠标点击圆形的图案弹出修改栏
- 18、点击相应颜色，可以修改“点”的颜色
- 19、点击相应图案，可以修改“点”的形状
- 20、点击相应大小，可以修改点的大小
- 21、点击左上方的“A”添加文本框
- 22、在空白处画出文本框并修改名字
- 23、点击右上方的“Line”可以修改文本框的框架，本次选择“\”去除框的颜色；同理，点击“Fill”，可以修改文本框的背景颜色，本次选择“\”去除文本框的背景颜色，
- 24、拖动没有框架和背景颜色的文本框到相对应的亚群上方，完成注释（可以参考Legend颜色，拖动到相应的位置），最后全选文本框和tSNE对其进行“Group”

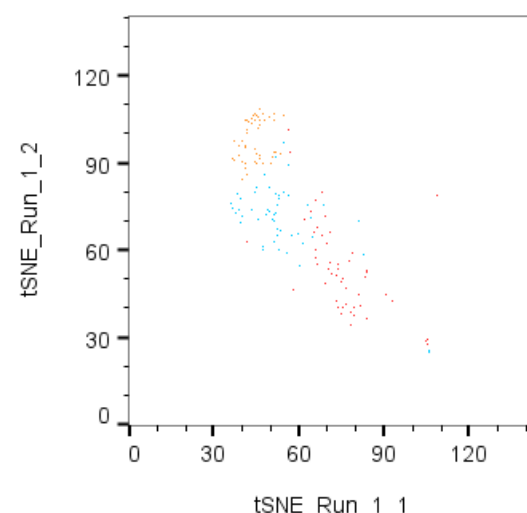


亚群特征基因热图展示

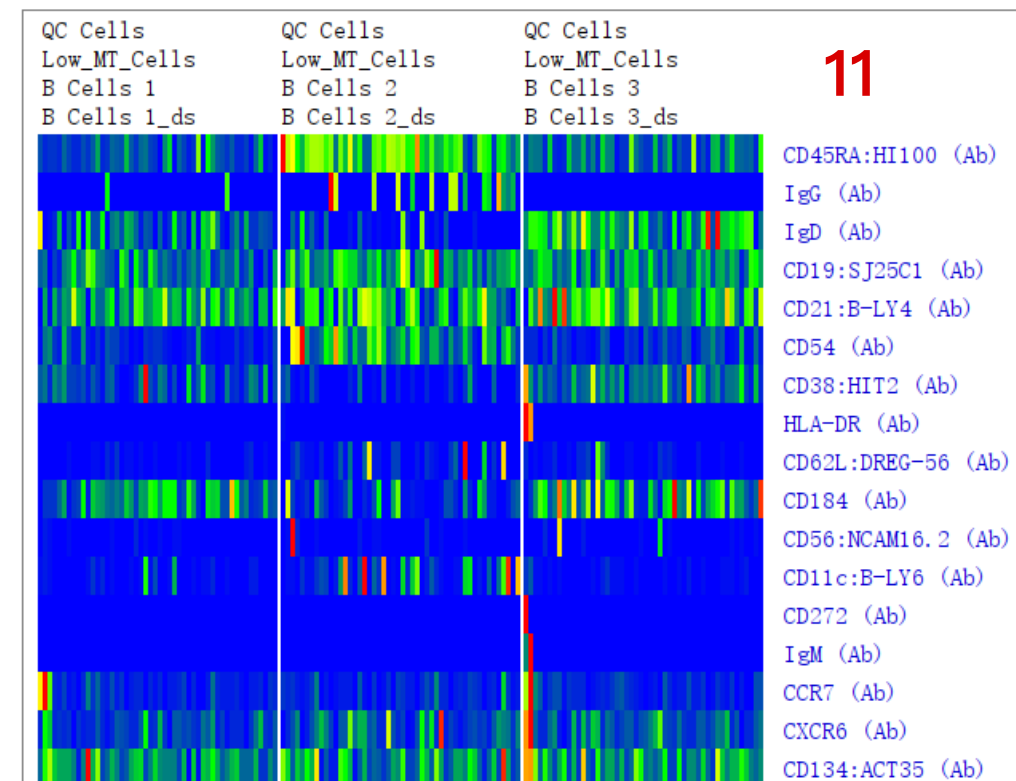
9、在弹出的窗口中，“Parameter Sets”选择感兴趣的基因集进行展示，本次选择“Canonical Genes”

10、此处无需再次挑选基因，否则热图中会重复显示，最后点击“OK”进行热图绘制

11、各降数后细胞群Canonical基因表达水平，只展示50个细胞的表达水平



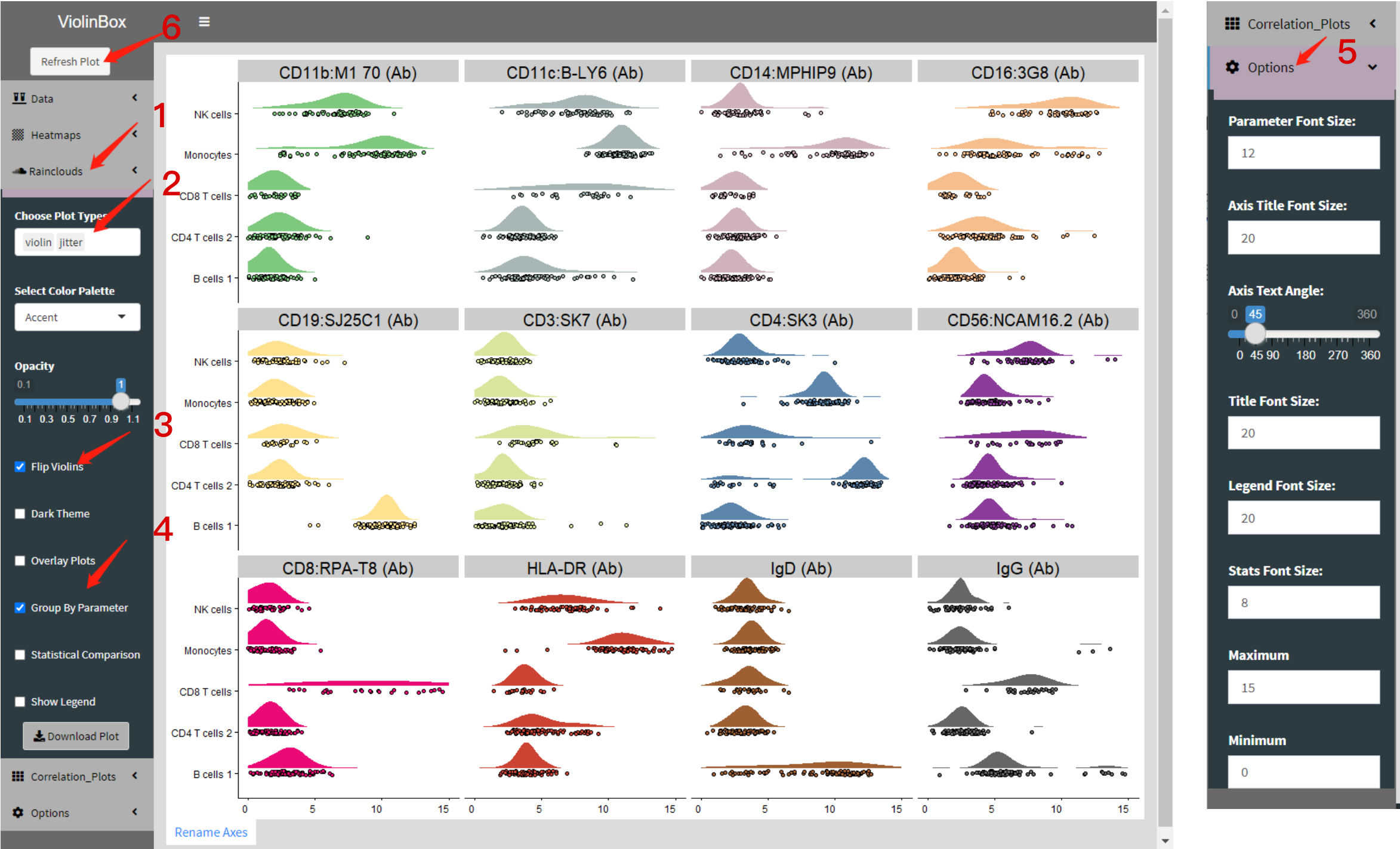
	Sample Name	Subset Name	Count
	concat_sample.csv	B Cells 1_ds	50.0
	concat_sample.csv	B Cells 2_ds	50.0
	concat_sample.csv	B Cells 3_ds	50.0



SeqGeq分析细胞基因

亚群特征基因云雨图

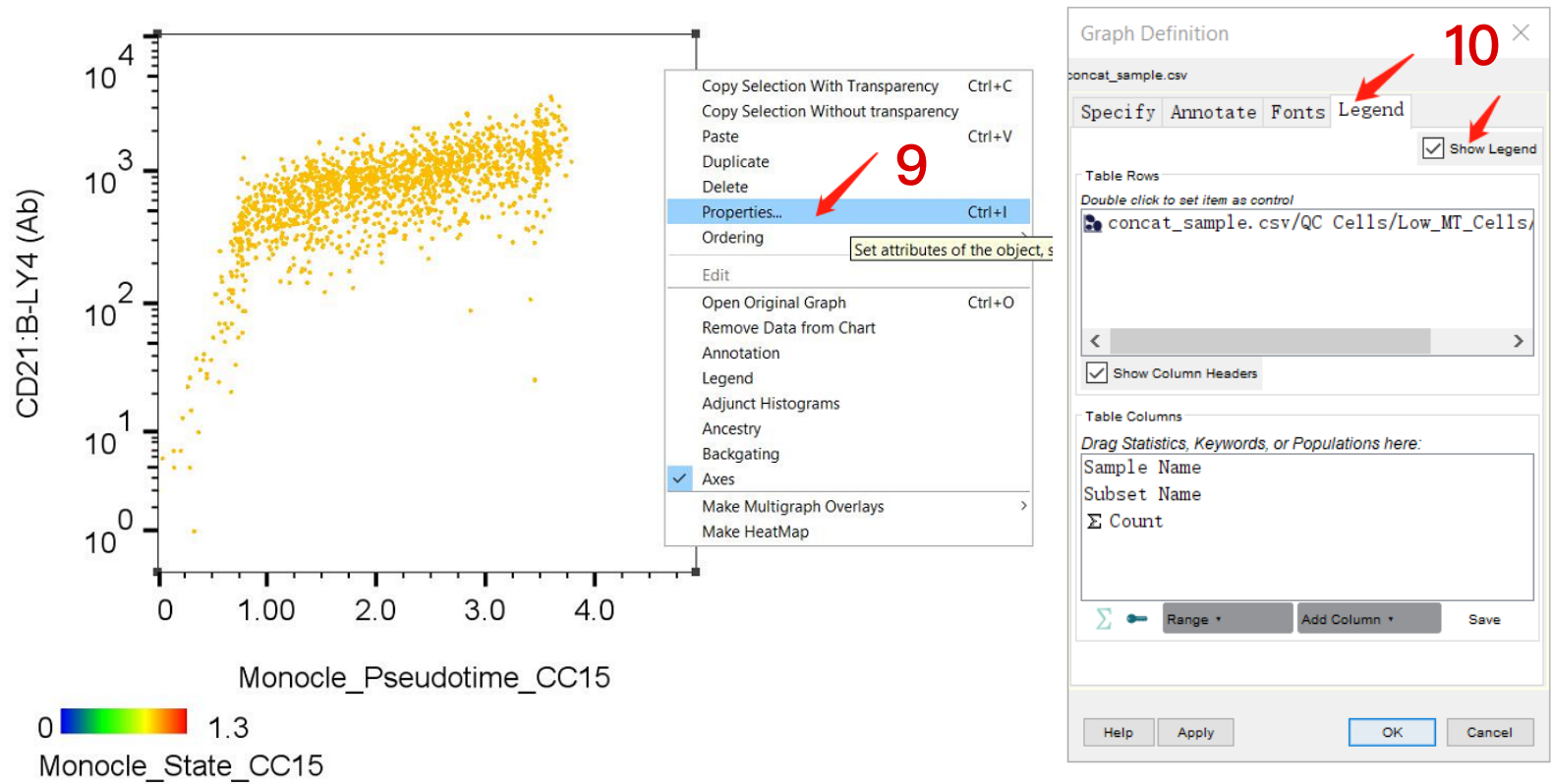
- 1、点击“Rainclouds”显示云雨图
 - 2、需要同时选中“violin”和“jitter”才可以展示云雨图
 - 3、必须勾选“Flip Violins”才可以展示云雨图
 - 4、按照“Parameter”进行分组显示云雨图
 - 5、点击“Options”可以修改图案
- Legend文字大小等



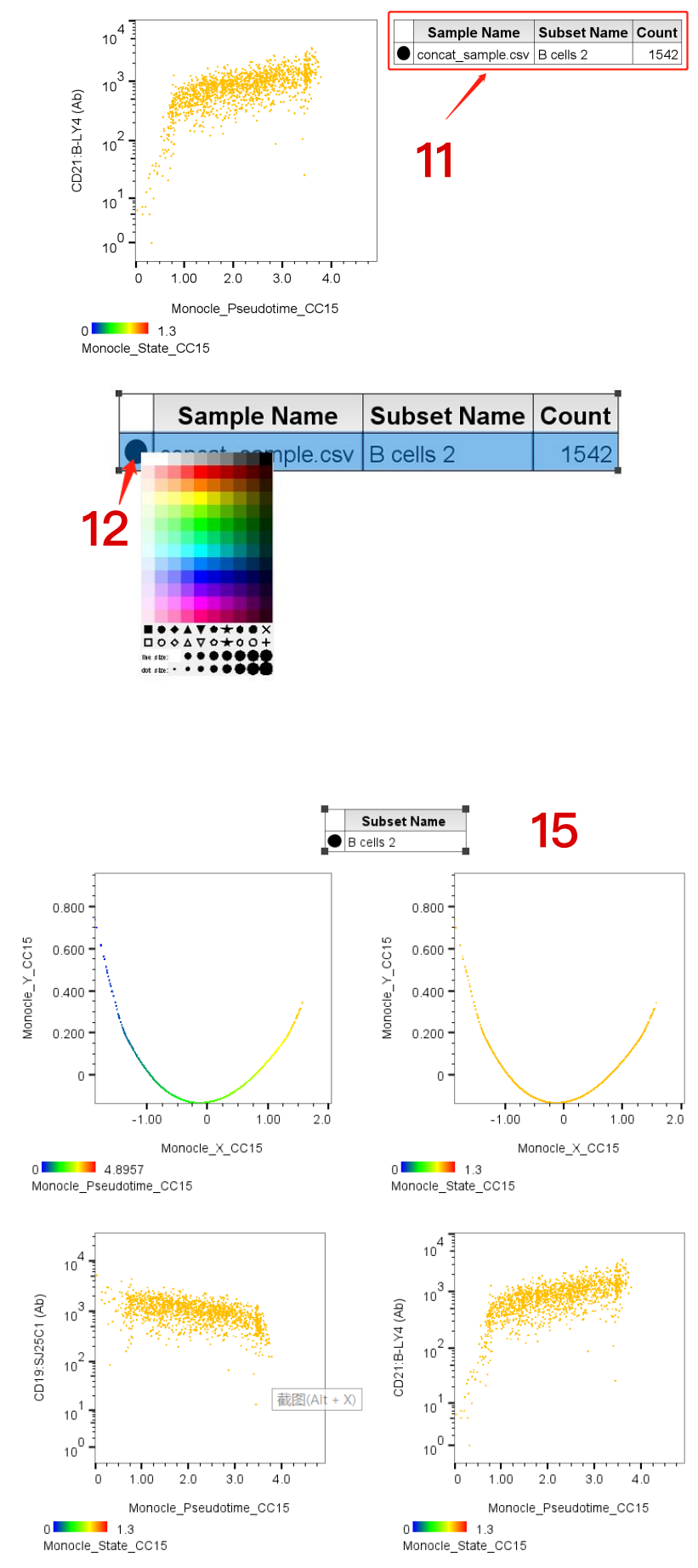
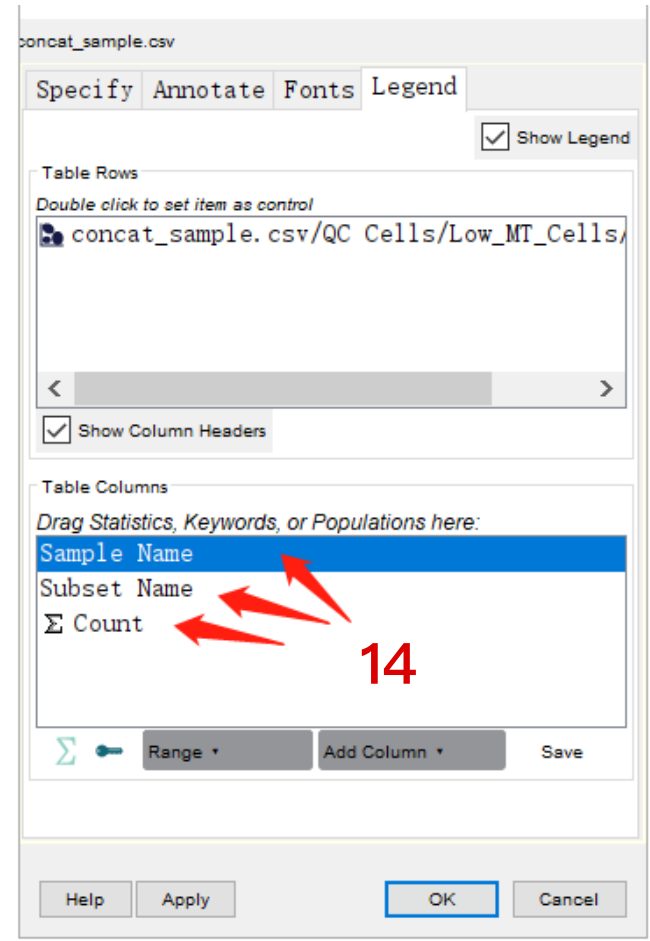
SeqGeq分析细胞基因

Monocle拟时序分析发育轨迹

- 9、选中拟时序图案，右键，下拉栏选择“Properties”
- 10、弹出的窗口中，点击“Legend”，随后勾选“Show Legend”
- 11、为弹出的”Legend”，可在此处修改点的形状，大小等
- 12、鼠标左键，点击黑色形状，在弹出的栏目中，可以修改点的形状，大小等
- 13、选中“Legend”，右键，再点击“Properties”
- 14、弹出的窗口中，可以对legend的“Columns”参数进行修改，选中名称后，直接按“Delete”则可删除不想要的Column
- 15、修改好参数后的拟时序分析结果图



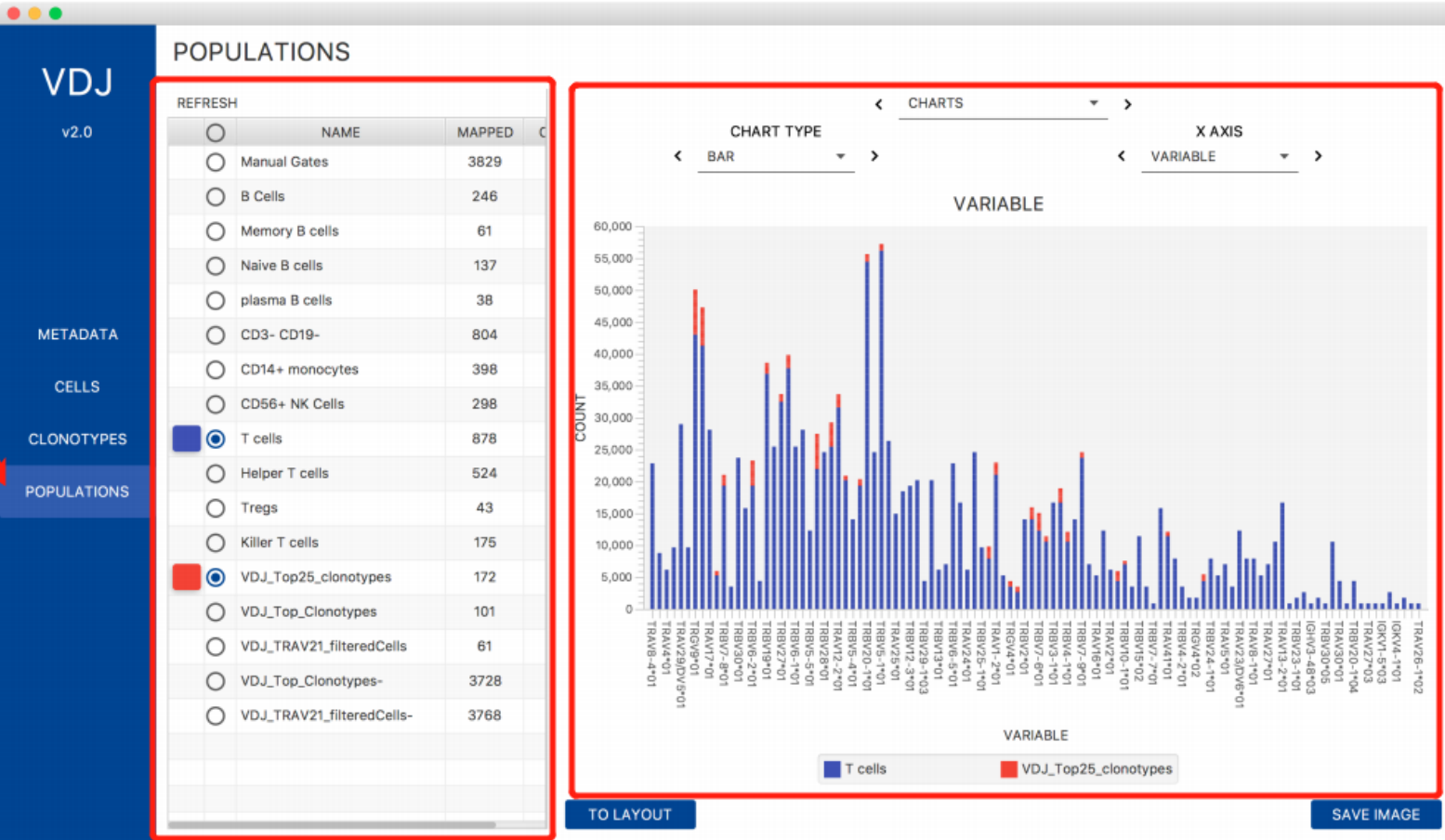
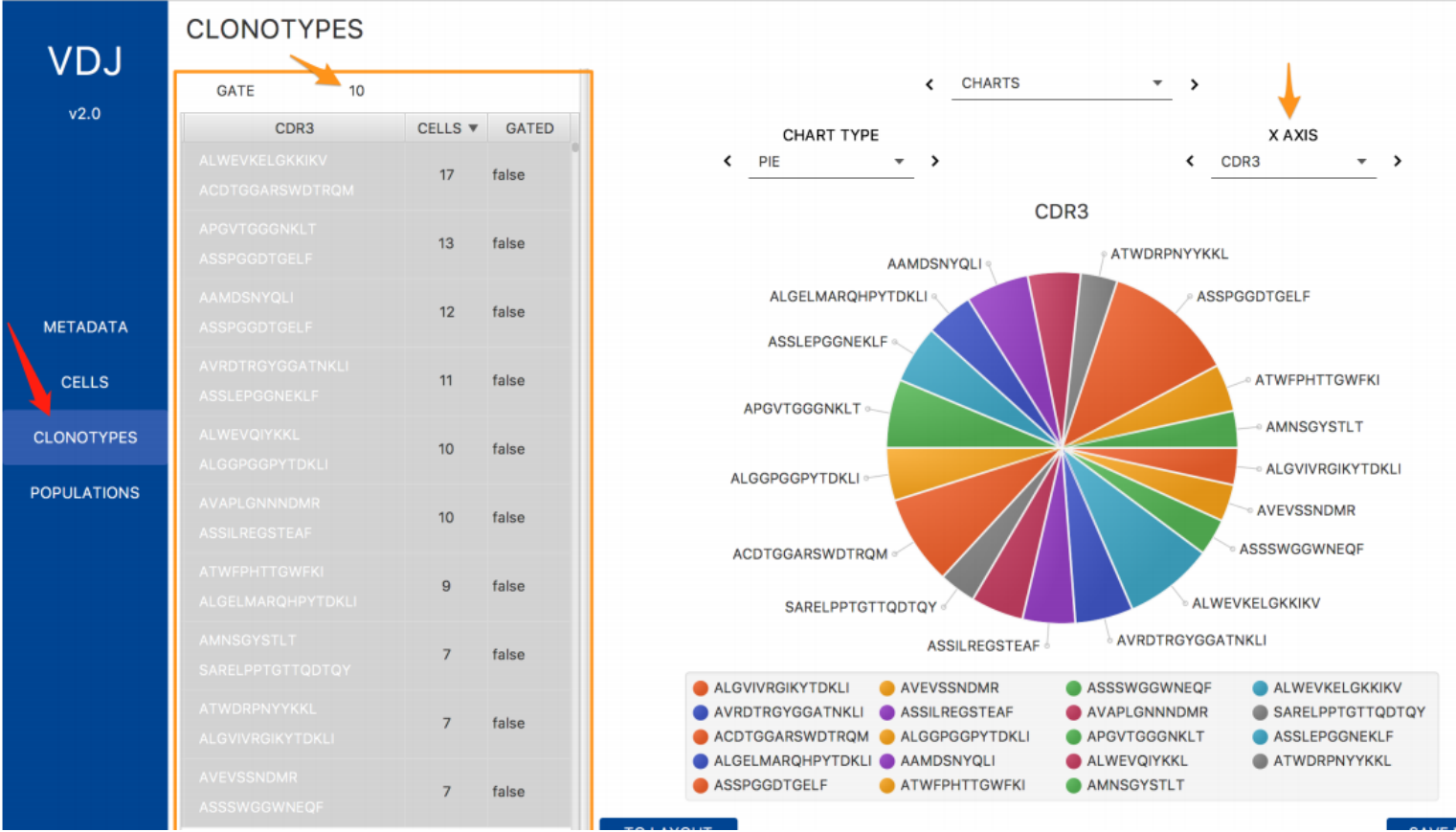
	Sample Name	Subset Name	Count
●	concat_sample.csv	B cells 2	1542



SeqGeq分析细胞基因

VDJ Explorer分析TCR/BCR多样性

- 11、点击“CLONOTYPES”进行克隆多样性分析
- 12、在此处可以选择想要比较分析的克隆
- 13、在此处可以修改图形的以及需要展示的内容

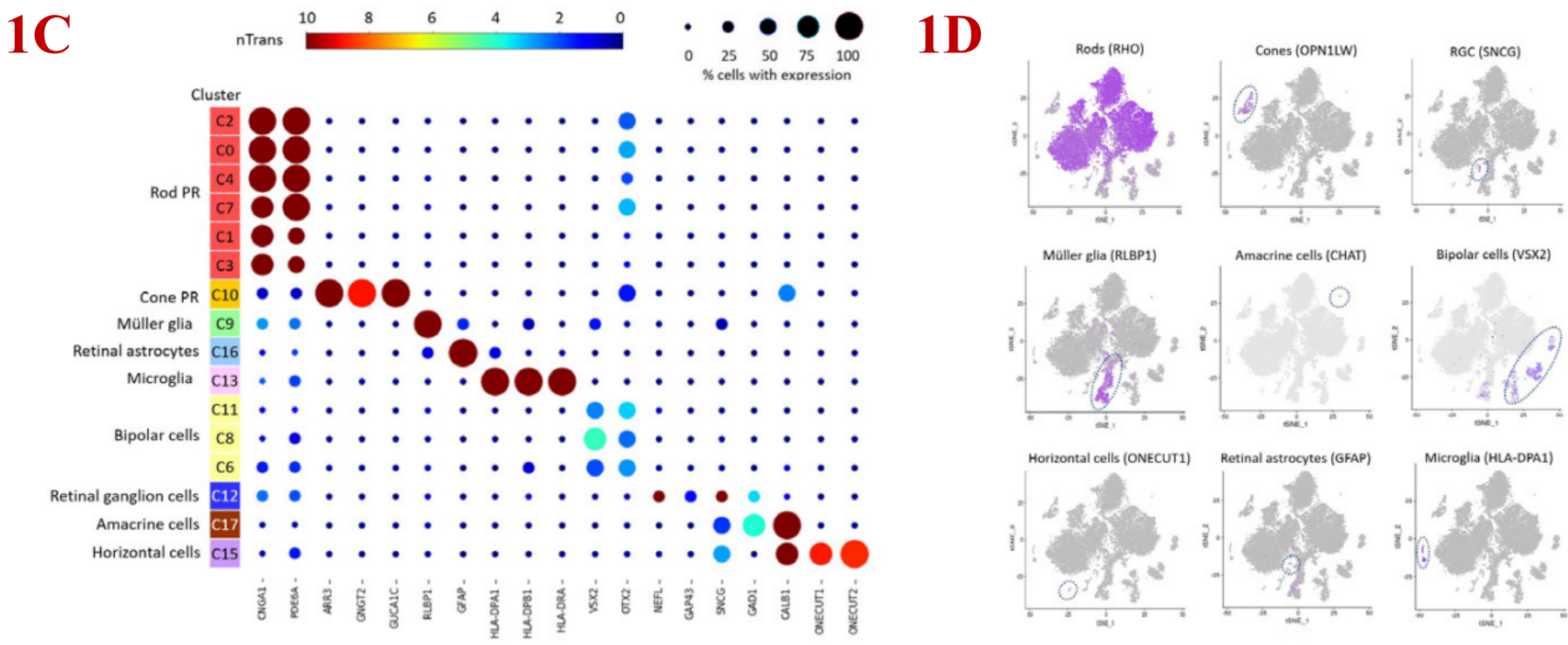
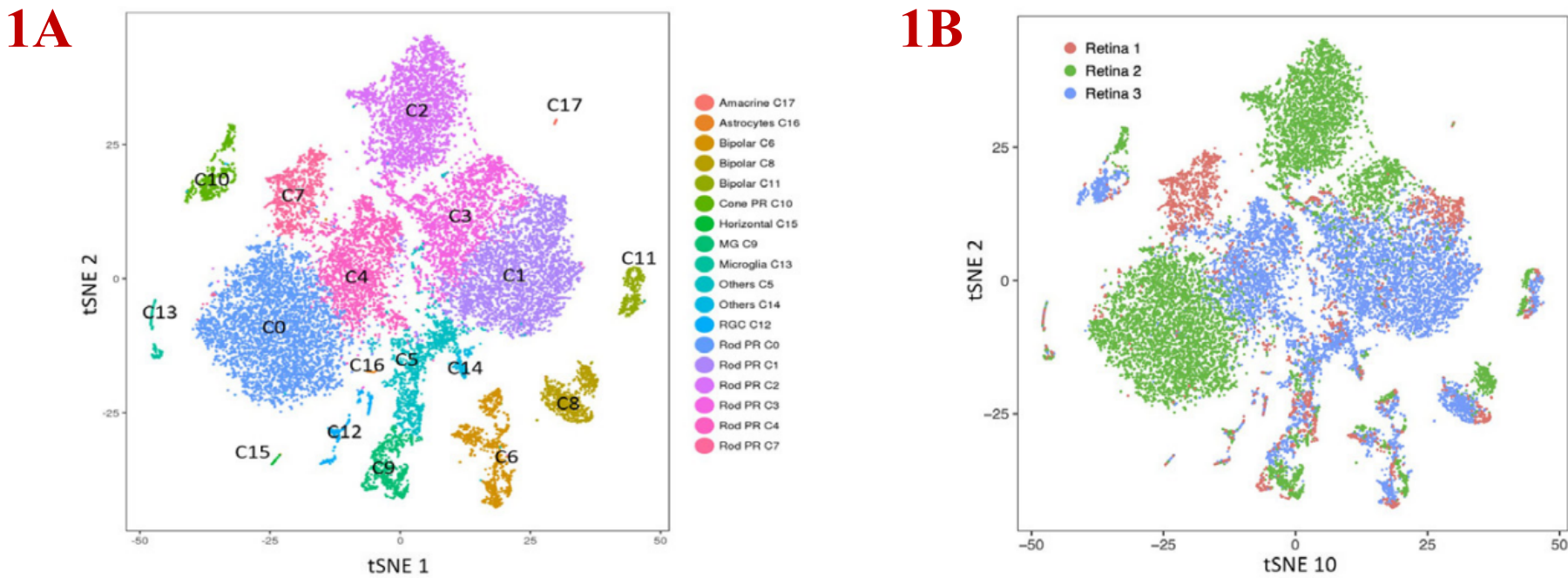


内容

- BD Rhapsody原理简介
- BD Rhapsody工作流程
- 单细胞多组学在眼科学中的应用

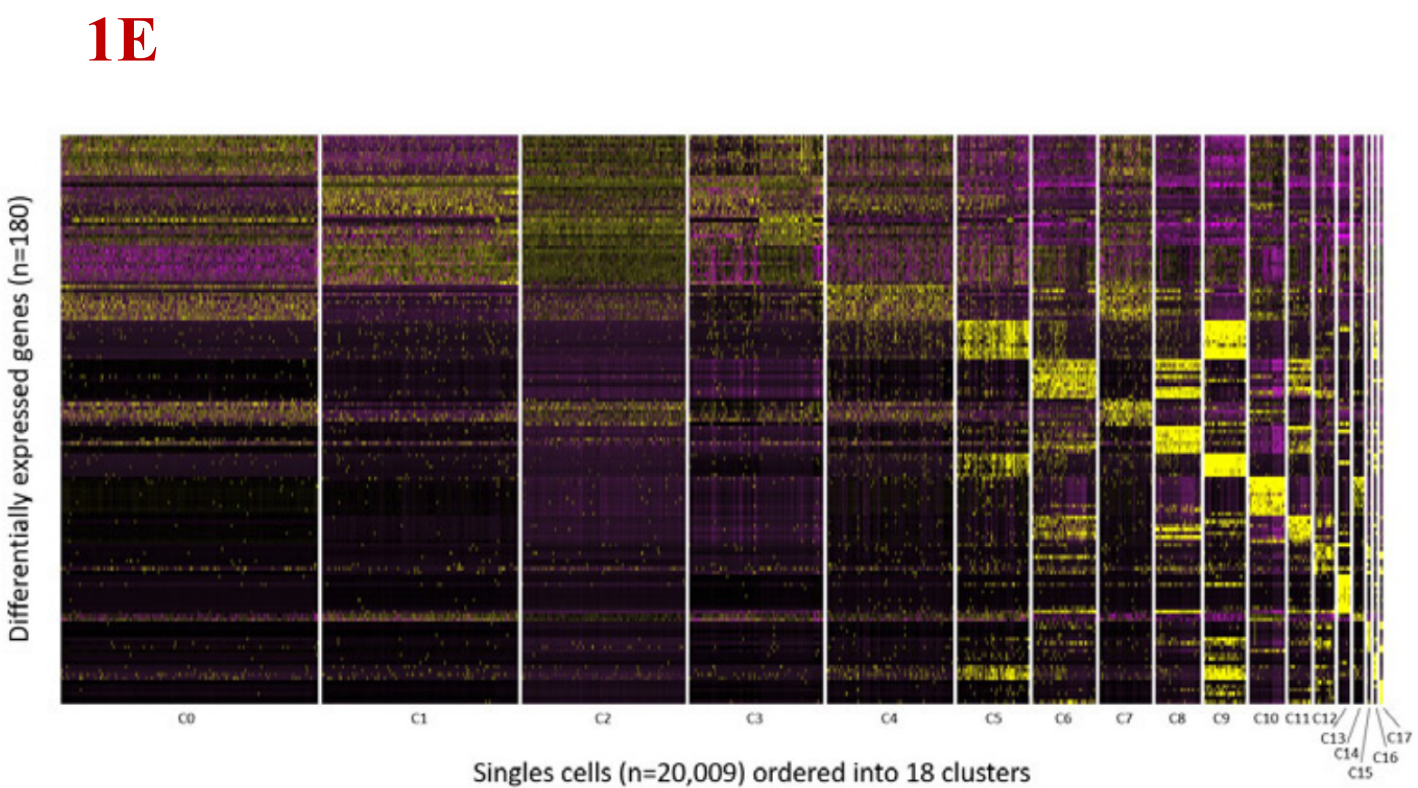
单细胞转录组用于视网膜异质性研究

A single-cell transcriptome atlas of the adult human retina



视网膜是一种特殊的神经组织，它可以感知光并启动图像处理。虽然某些视网膜细胞的功能已经得到了很好的研究，但在人类中仍有许多细胞类型的分子谱仍不清楚。

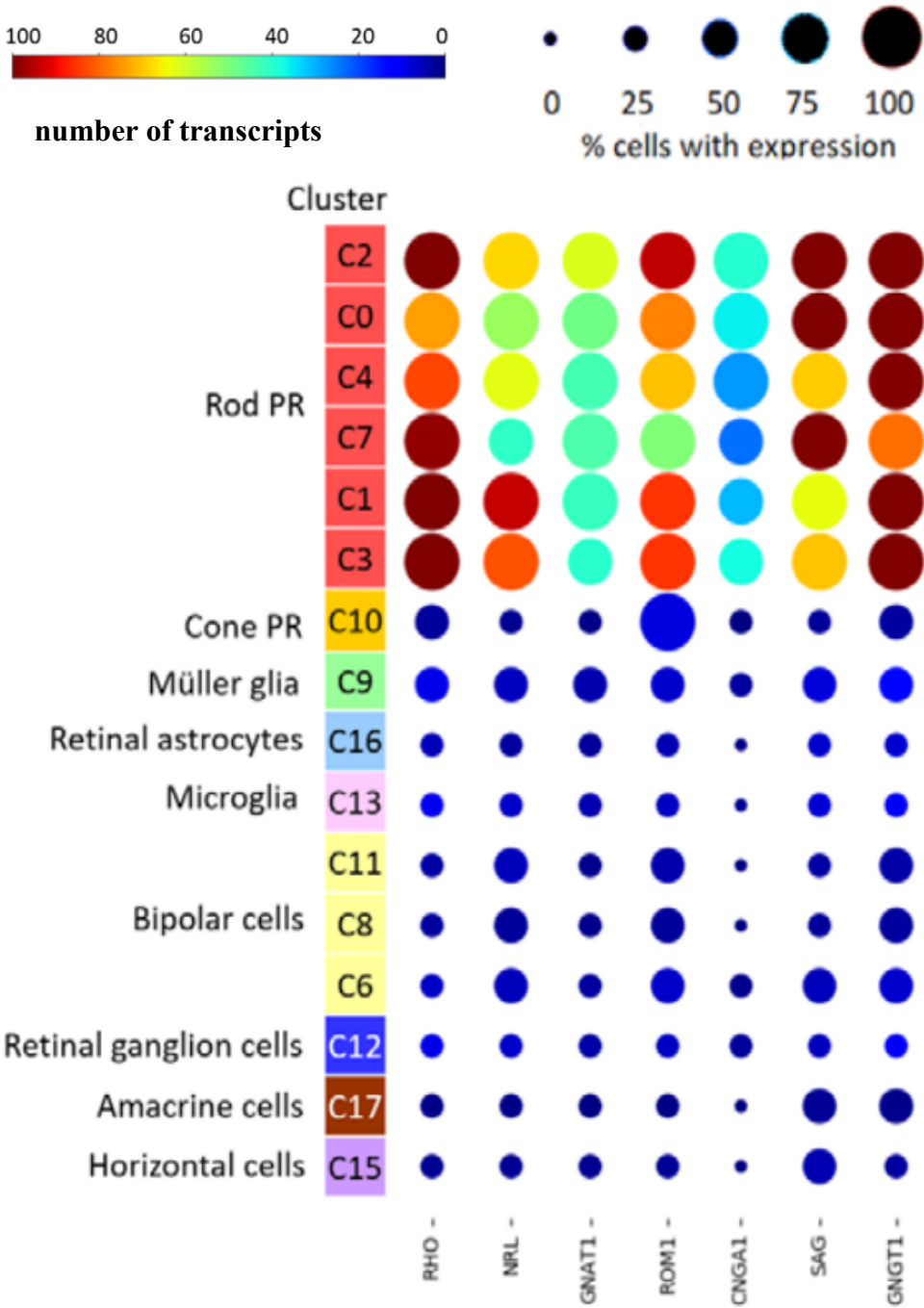
- 1A**
- 20009个单细胞可分为18个Clusters
 - C5和C14为多细胞亚群
- 1B**
- 20009个单细胞来自于3个志愿者在捐献眼角膜后的视网膜样本
 - 因此不包含视网膜色素上皮细胞
- 1C-1E**
- 各细胞亚群特征基因的表达水平
 - 其中，视杆细胞(Rod Photoreceptors)占比~74%
 - 胶质细胞(glial cells)占比10%



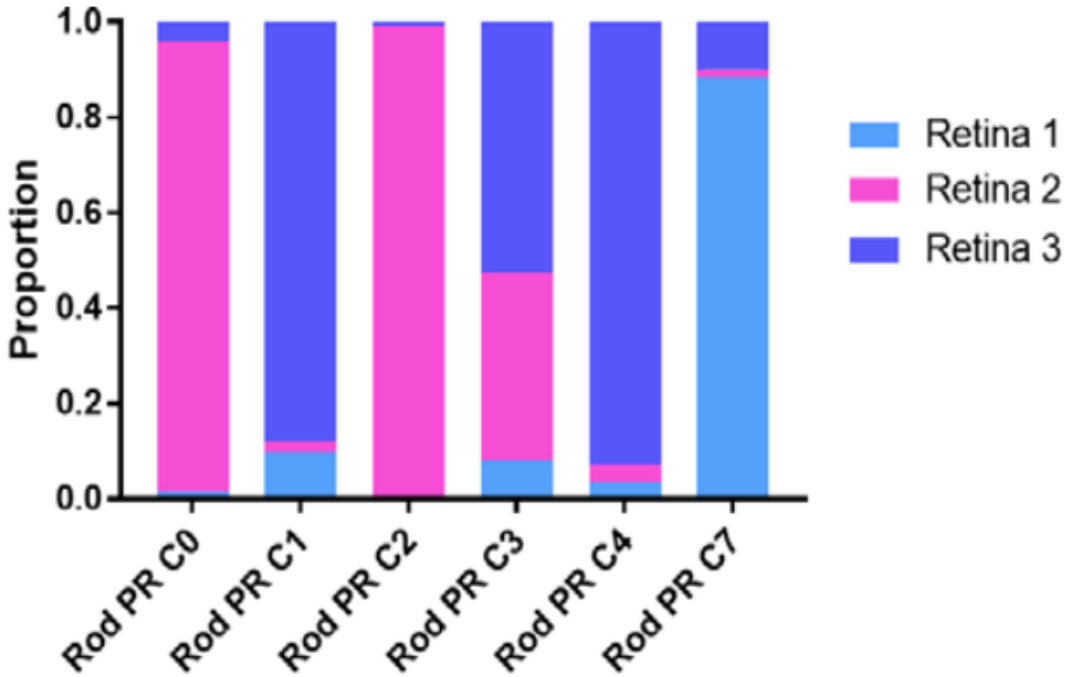
单细胞转录组用于视网膜异质性研究

A single-cell transcriptome atlas of the adult human retina

2A



2B



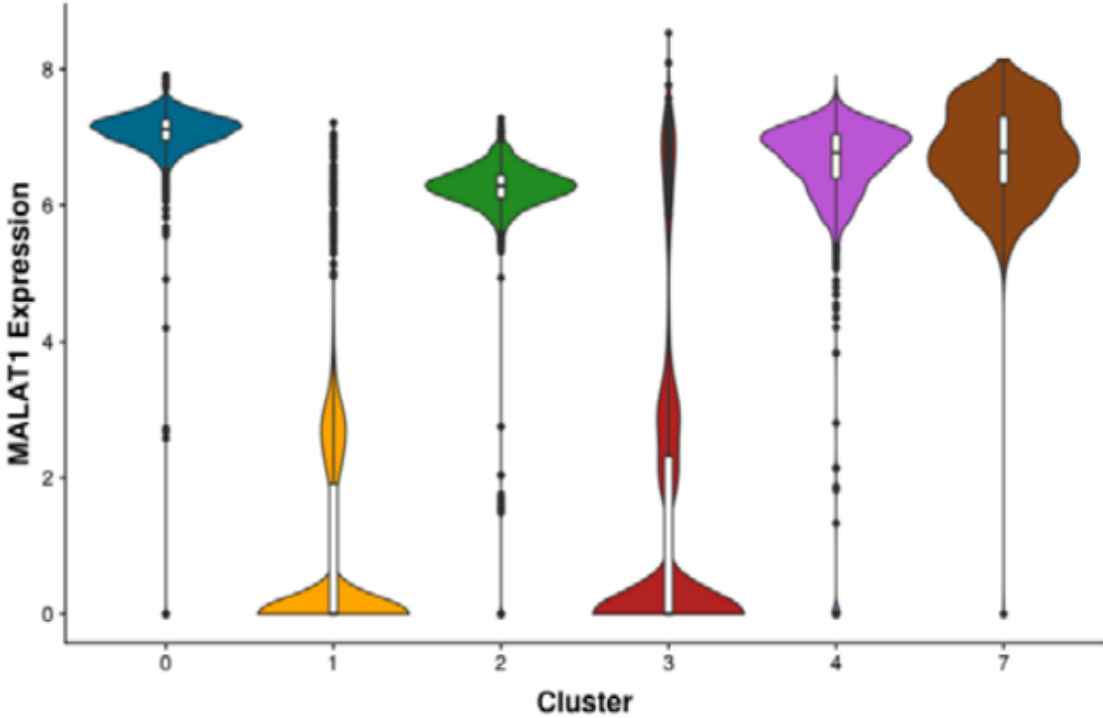
2A

- 14759个视杆细胞可分为6个Clusters
- 使用7个已知的视杆细胞特征基因 (RHO, GNAT1, SAG, NRL, ROM1, GNAT1, CNGA1), 可将这6个Clusters与其他的细胞亚群区分开

2B

- 6个视杆细胞的Clusters在3个捐赠者视网膜中的比例
- 6个视杆细胞的Clusters存在个体差异

2C

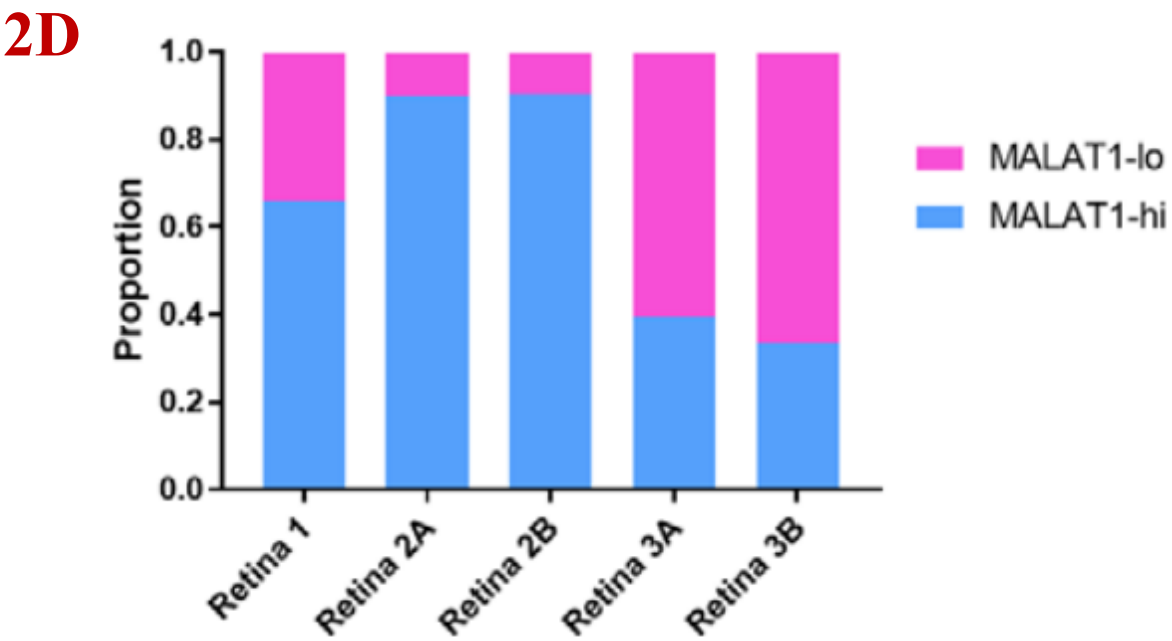
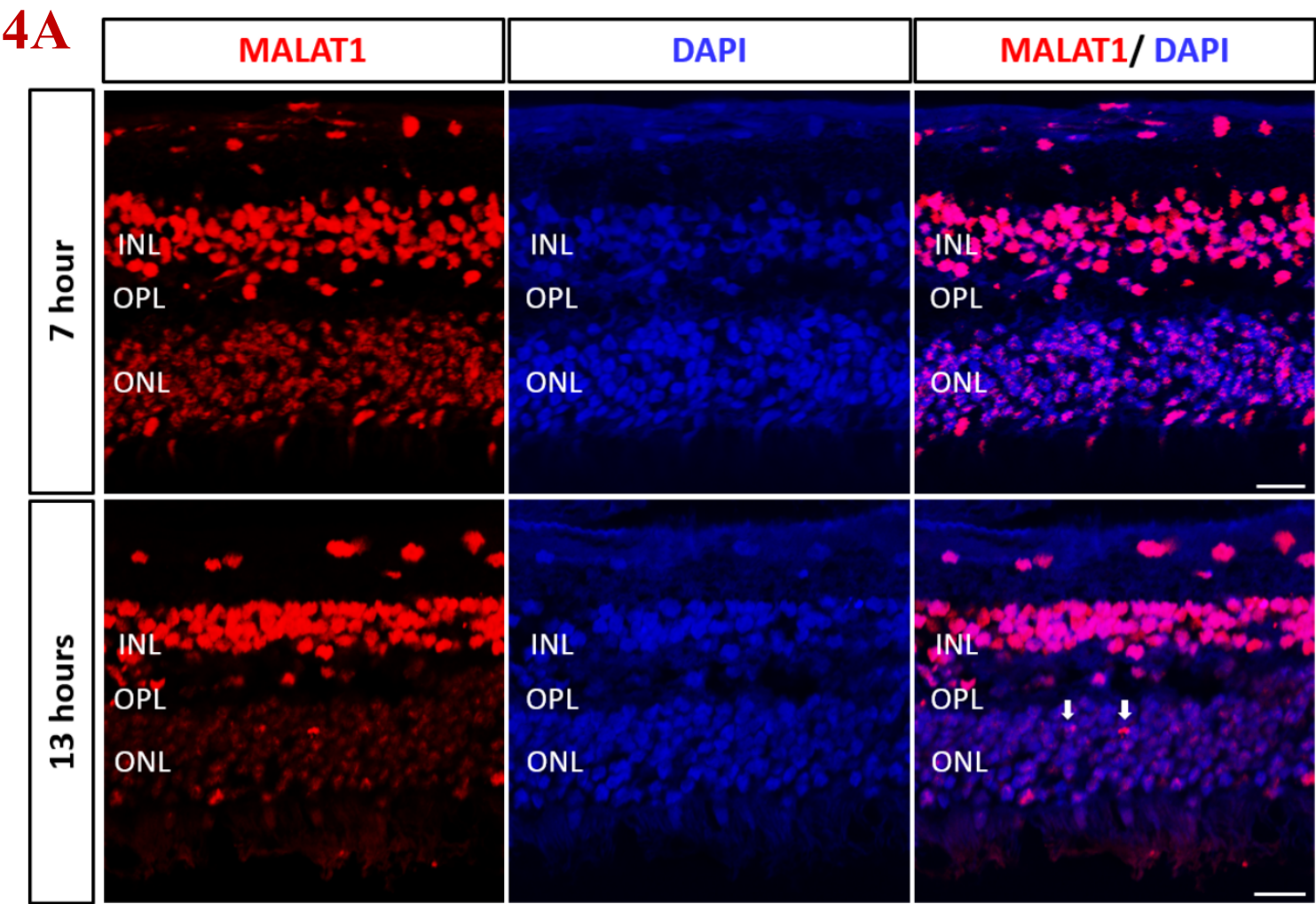
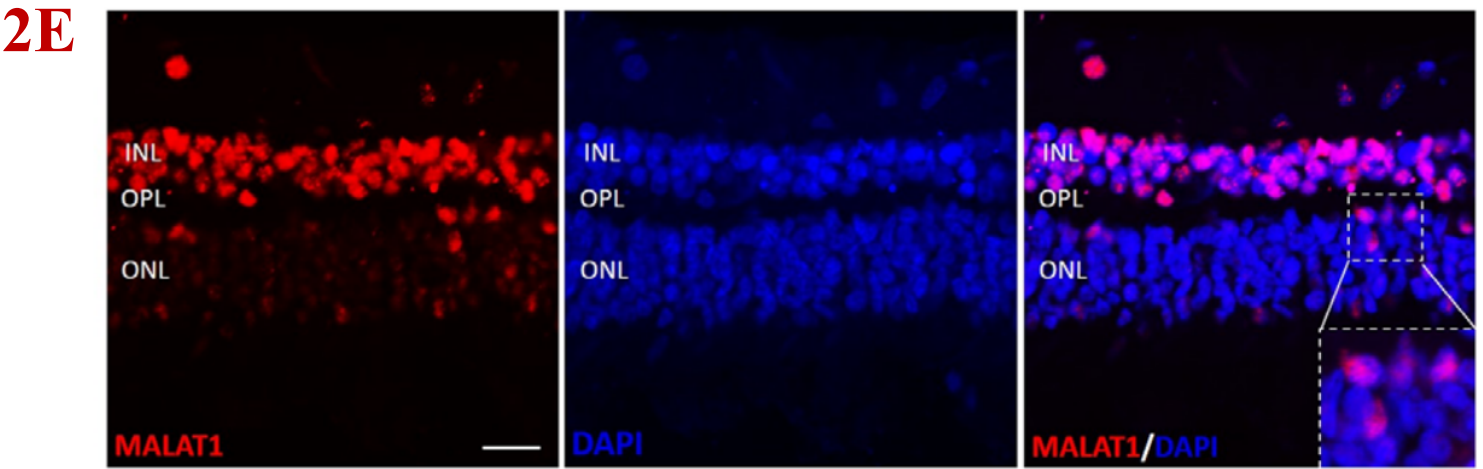


2C

- 已知MALAT1(长链非编码RNA)在视网膜稳态和疾病中发挥重要作用
- 查看其在6个视杆细胞的Clusters的表达水平发现, C0, C2, C4和C7高表达MALAT1, 而C1和C3则低表达MALAT1

单细胞转录组用于视网膜异质性研究

A single-cell transcriptome atlas of the adult human retina



2D

- MALAT1-hi和MALAT1-lo视杆细胞在每个捐赠者的视网膜中均被发现
- MALAT1-hi在Retina1占比约为66%；
- 在Retina2占比约为90%；
- 在Retina3占比约为36%

2E

- 为了进一步验证这一发现，对另外三个捐赠者的视网膜样本进行了RNA原位杂交
- MALAT1-hi和MALAT1-lo的视杆细胞在视网膜确实存在

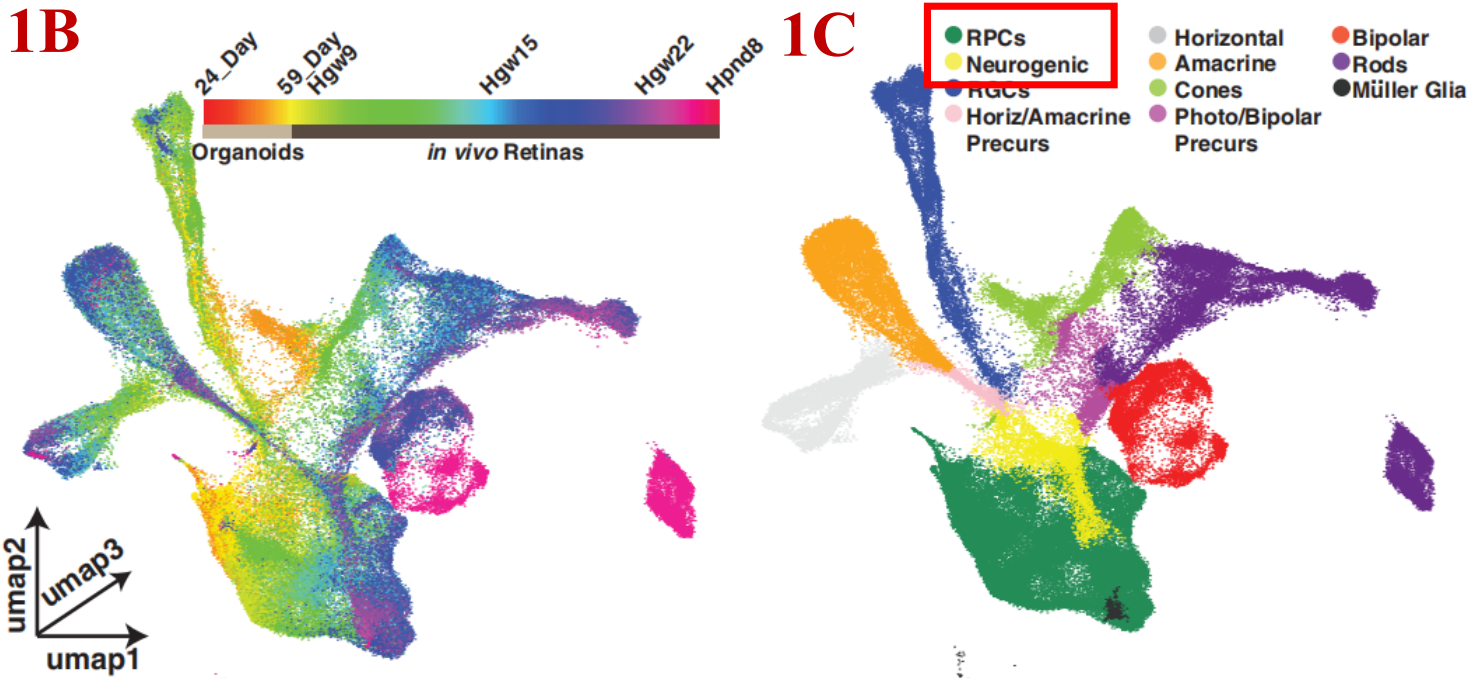
4A

- 由于本研究使用了捐赠者死后的视网膜样本，因此推断MALAT1-lo亚群可能反映了视杆细胞在死后的早期退行性病变
- 捐赠者死后7小时的视网膜样本，其MALAT1仍处于较高的表达水平
- 捐赠者死后13小时的视网膜样本，其MALAT1表达急剧下降

以上结果表明，MALAT1是健康感光细胞的一种新的标记物，可在细胞退行性病变时开始表达缺失

单细胞转录组用于视网膜发育的研究

Single-Cell Analysis of Human Retina Identifies Evolutionarily Conserved and Species-Specific Mechanisms Controlling Development



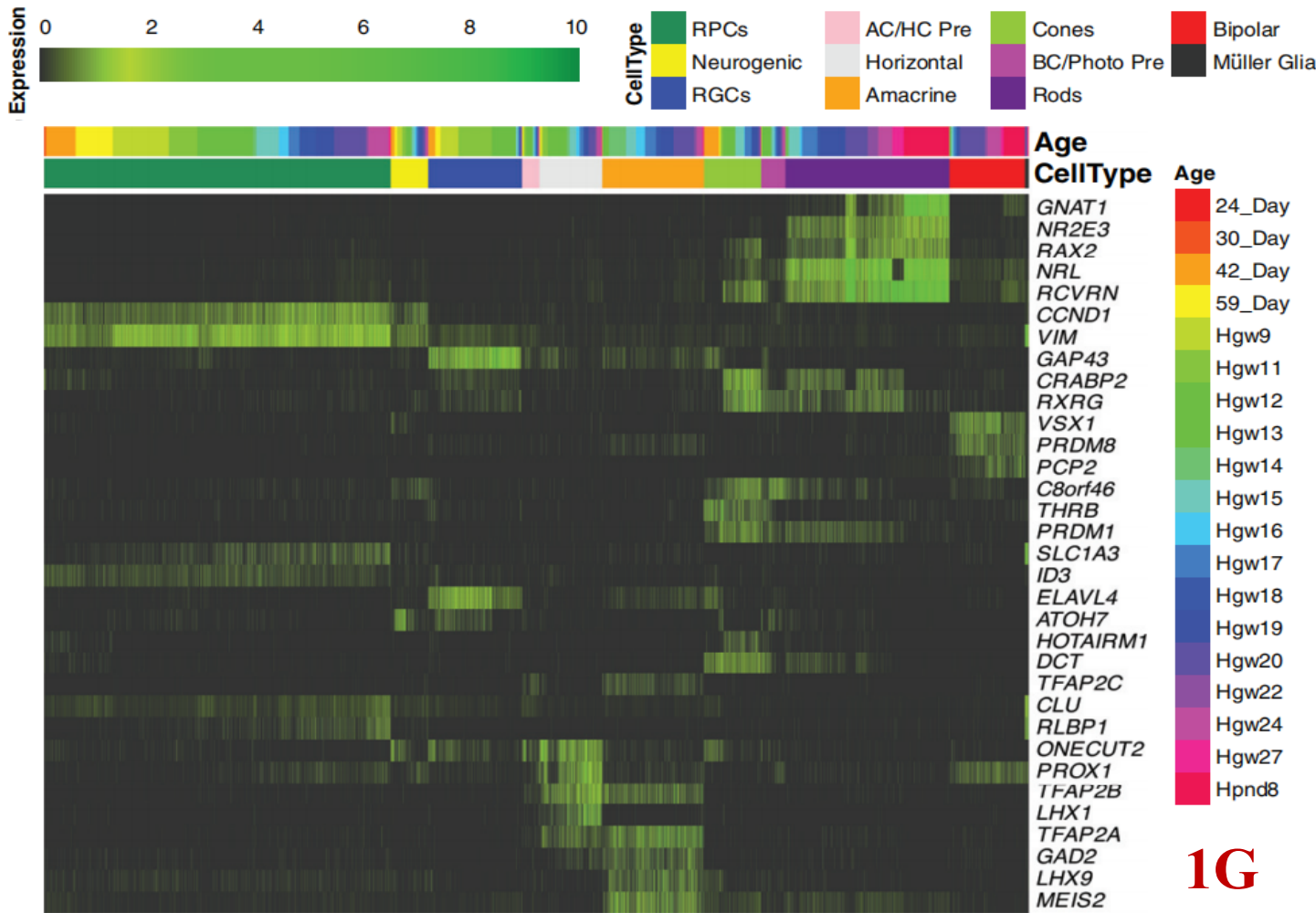
为了确定控制人类视网膜发育的转录网络，作者对视网膜发育的16个时间点以及视网膜类器官分化的4个早期阶段进行了scRNA-seq分析。

- 1B**
- 4份类器官单细胞样本（模拟早期视网膜）+ 13份视网膜原代单细胞样本（孕周9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22, 24, 27）+ 2份黄斑和周围组织原代单细胞样本（孕周20+产后8天），共118,555个单细胞

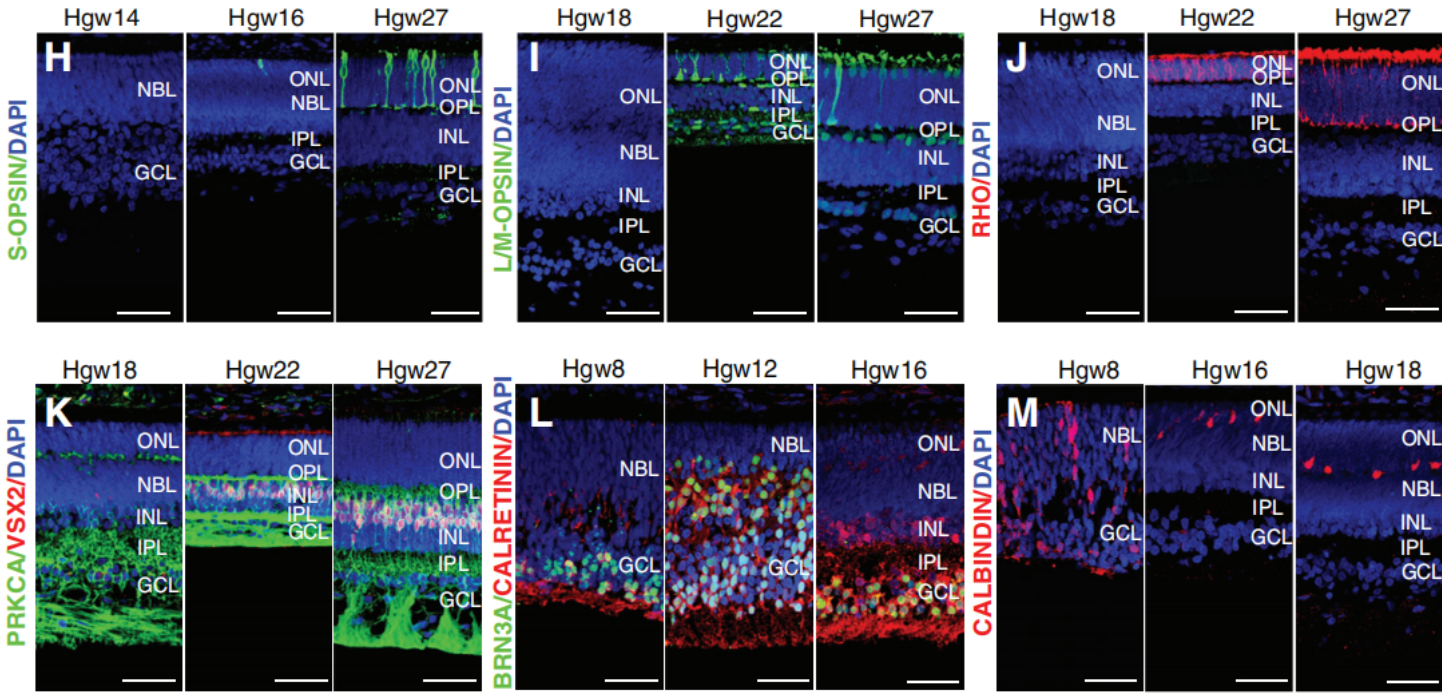
- 1C**
- 单细胞亚群鉴定

- 1G**
- 热图显示各细胞亚群Marker基因的表达水平

- 1H-1M**
- 免疫组化验证Marker基因在细胞亚群中的表达

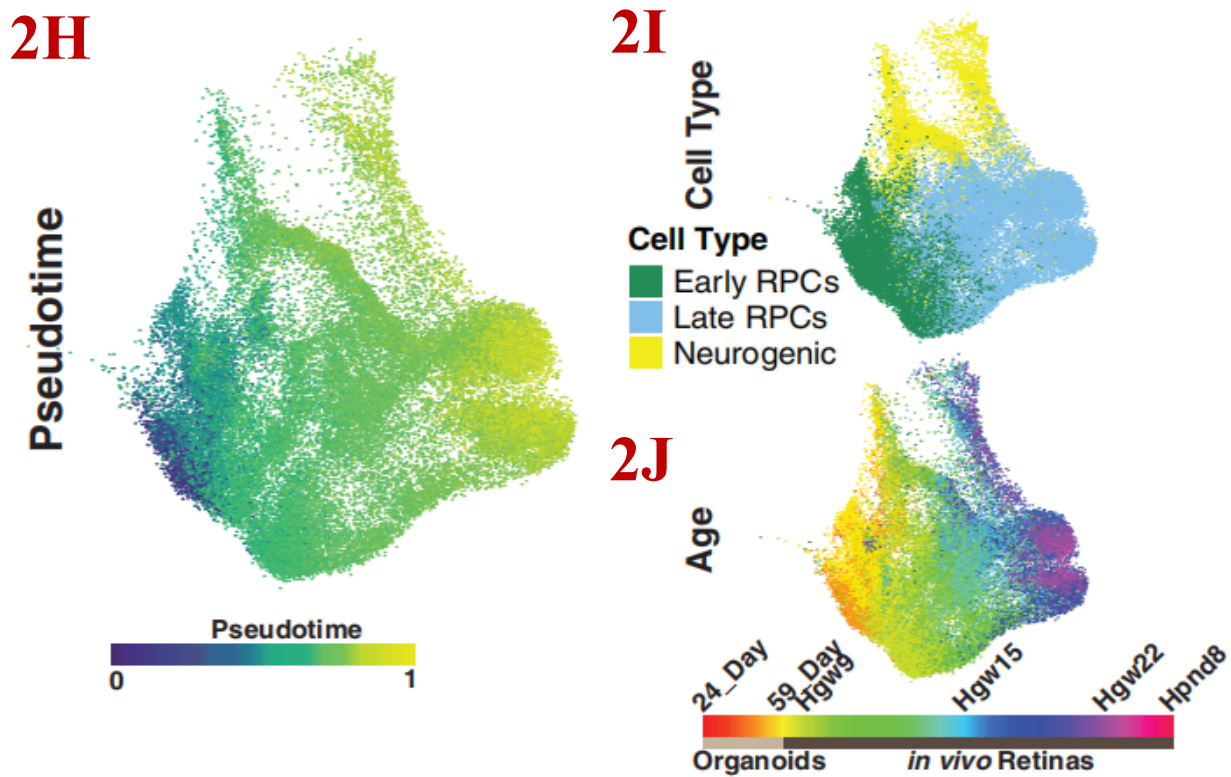


1H-1M



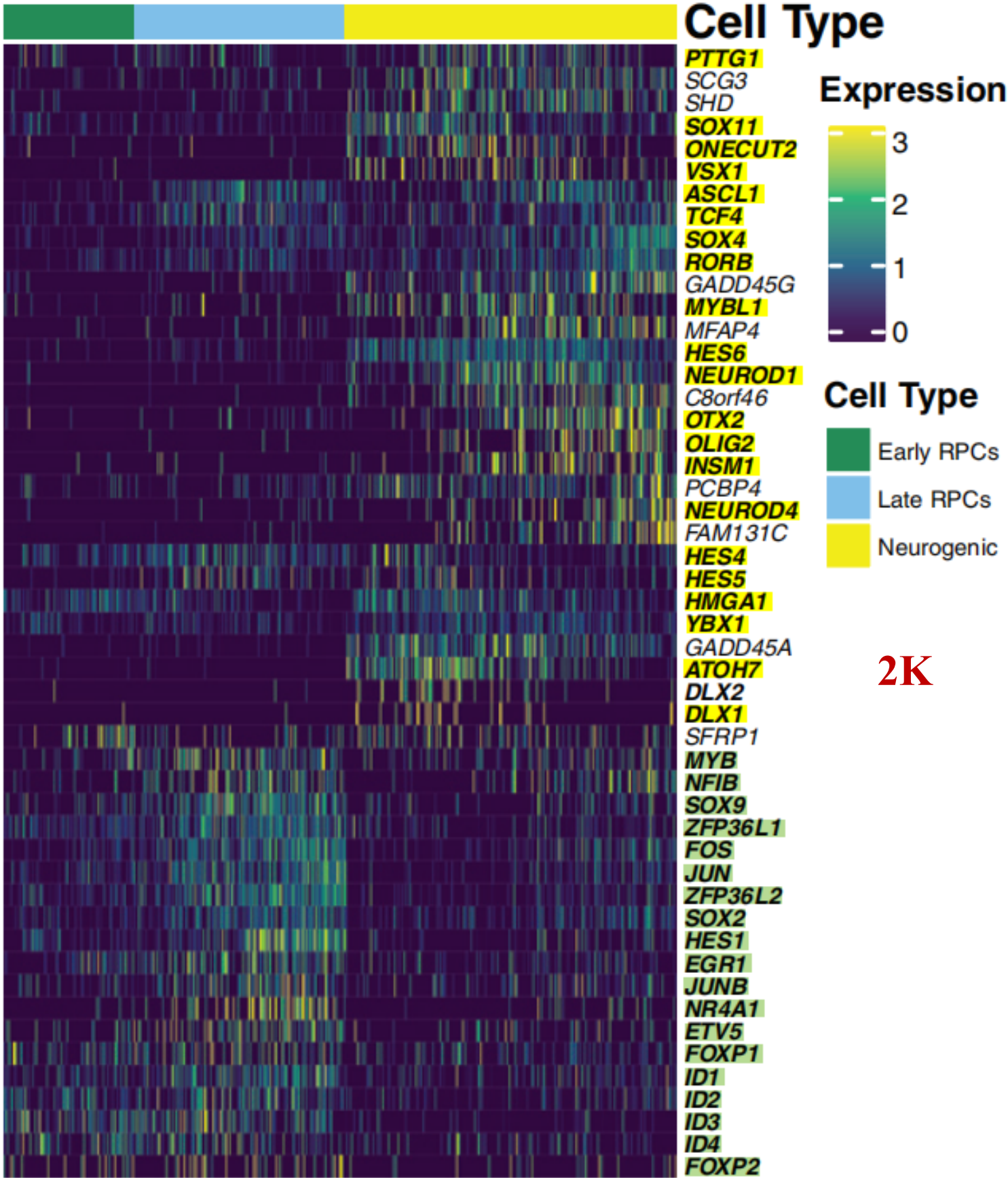
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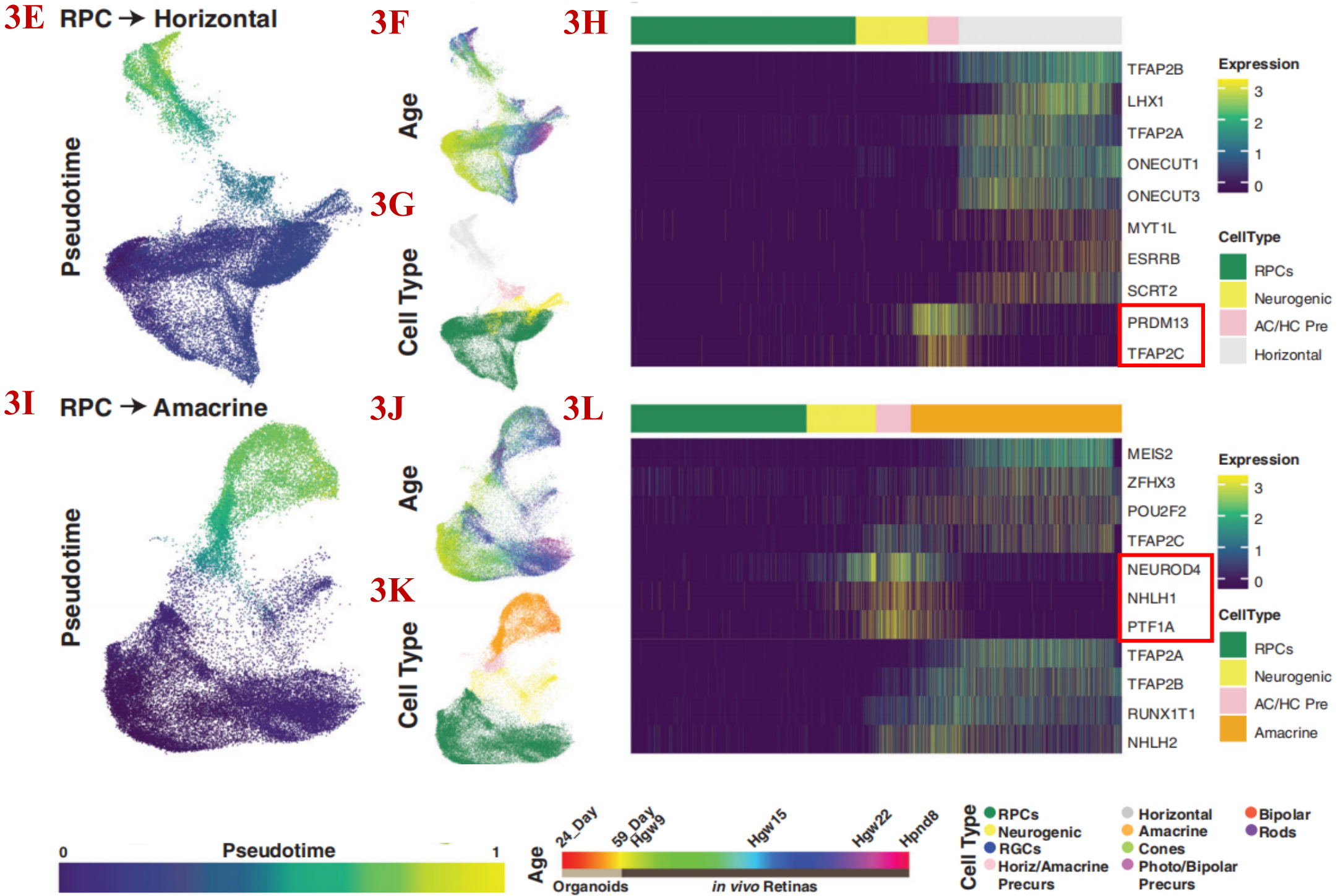
已有scRNA-seq研究小鼠视网膜的发育，并确定了早期和晚期原代RPCs的转录特征，其中包含控制增殖、神经发生和细胞命运决定基因的差异表达。同样的，为了确定人的神经元性RPCs在时间模式中的进化保守程度，作者对原代RPCs和神经元性RPCs的基因表达变化进行了拟时序分析。

- 2H
● Monocle拟时间分析RPCs（视网膜前体细胞）和神经元性RPCs
- 2I
● 按Pseudotime数值分布，神经元性RPCs含部分Early RPCs和Late RPCs
- 2J
● 神经元性RPCs可来源于任何孕周时期（类器官Day24和产后Day8除外）
- 2K
● 神经元性RPCs与RPCs的主要差异集中在转录因子的表达水平



单细胞转录组用于视网膜发育的研究

Single-Cell Analysis of Human Retina Identifies Evolutionarily Conserved and Species-Specific Mechanisms Controlling Development



为了明确控制视网膜细胞分化的基因调控网络，作者沿着从RPCs到每种主要视网膜细胞类型的发育轨迹进行了独立的拟时间分析

- 3E-3H**
- Monocle拟时间分析原代RPCs（视网膜前体细胞）、神经元性RPCs、Horiz/Amacrine前体细胞和Horizontal细胞
 - 发育轨迹为：
原代RPCs→神经元性RPCs→
Horiz/Amacrine前体细胞→Horizontal细胞
 - Horiz/Amacrine前体细胞表达PRDM13和TFAP2C转录因子时，则向Horizontal细胞分化

- 3I-3L**
- Monocle拟时间分析原代RPCs（视网膜前体细胞）、神经元性RPCs、Horiz/Amacrine前体细胞和Amacrine细胞
 - 发育轨迹为：
原代RPCs→神经元性RPCs→
Horiz/Amacrine前体细胞→Amacrine细胞
 - Horiz/Amacrine前体细胞表达NEUROD4\NHLH1\PTF1A转录因子时，则向Amacrine细胞分化

不同转录因子的活化，决定了细胞的发育方向

单细胞转录组用于视网膜发育的研究

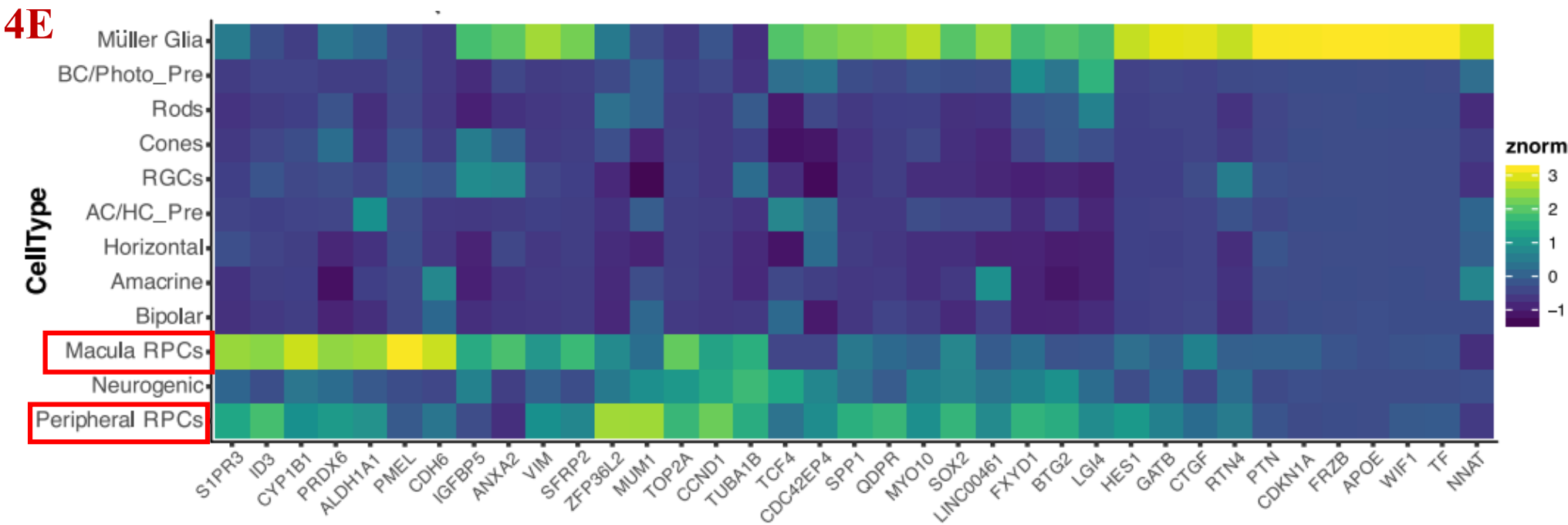
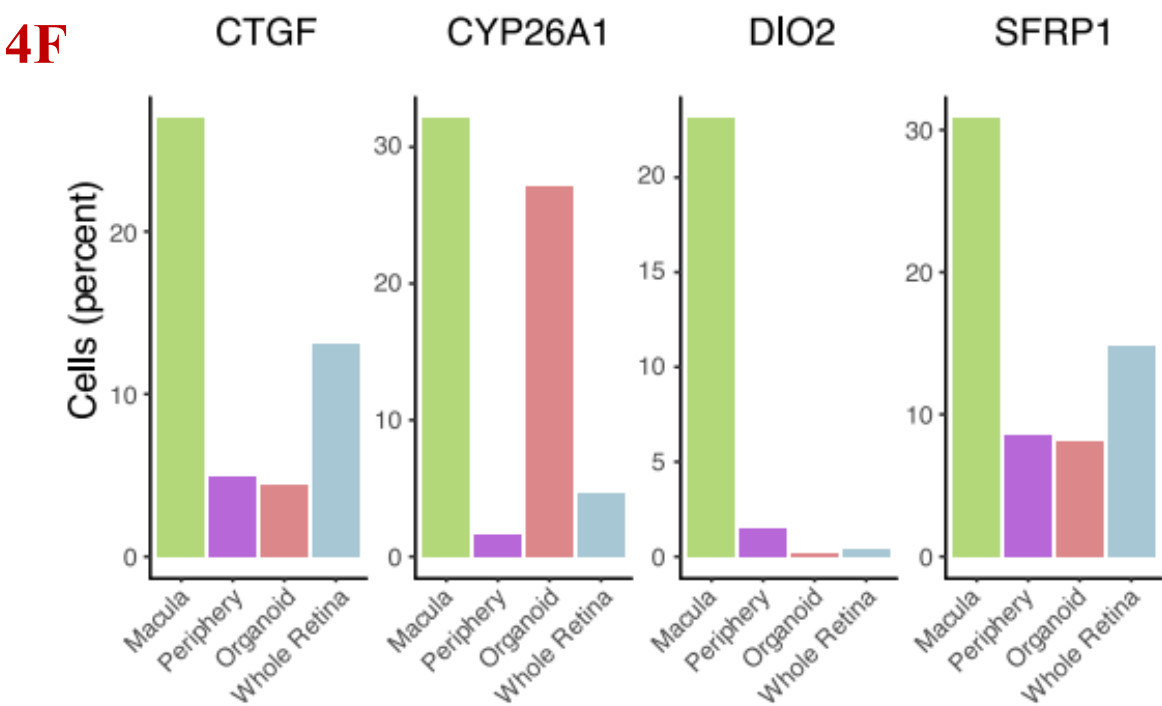
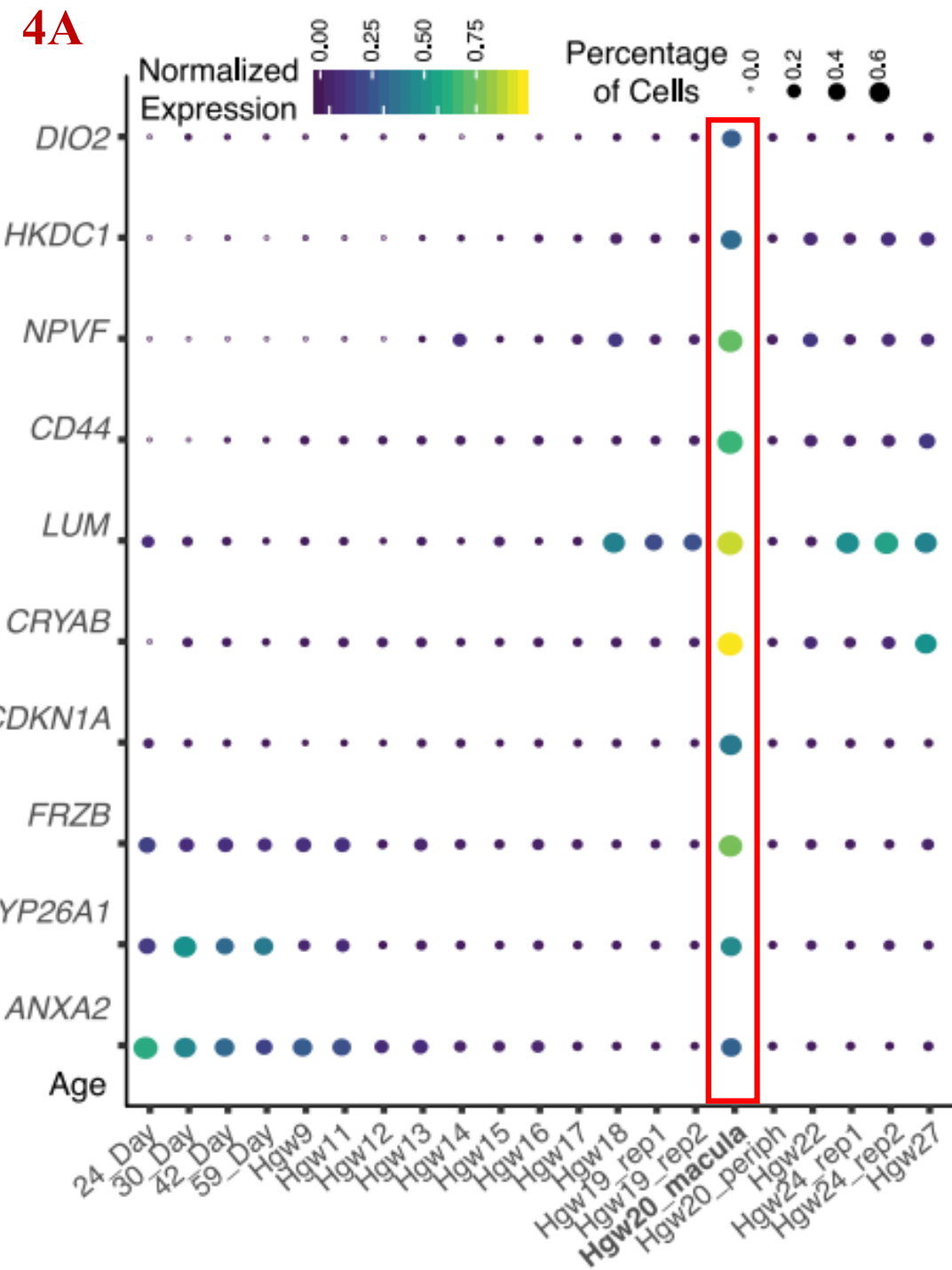
Single-Cell Analysis of Human Retina Identifies Evolutionarily Conserved and Species-Specific Mechanisms Controlling Development

为了识别黄斑视网膜与周围视网膜发育相关基因的区域表达差异

- 4A
- 不同时间点样本中，黄斑和外周视网膜RPCs差异基因表达和细胞百分比的点图
 - 其中，来自孕周22周的黄斑样本，其RPCs的含量较外周视网膜RPCs高

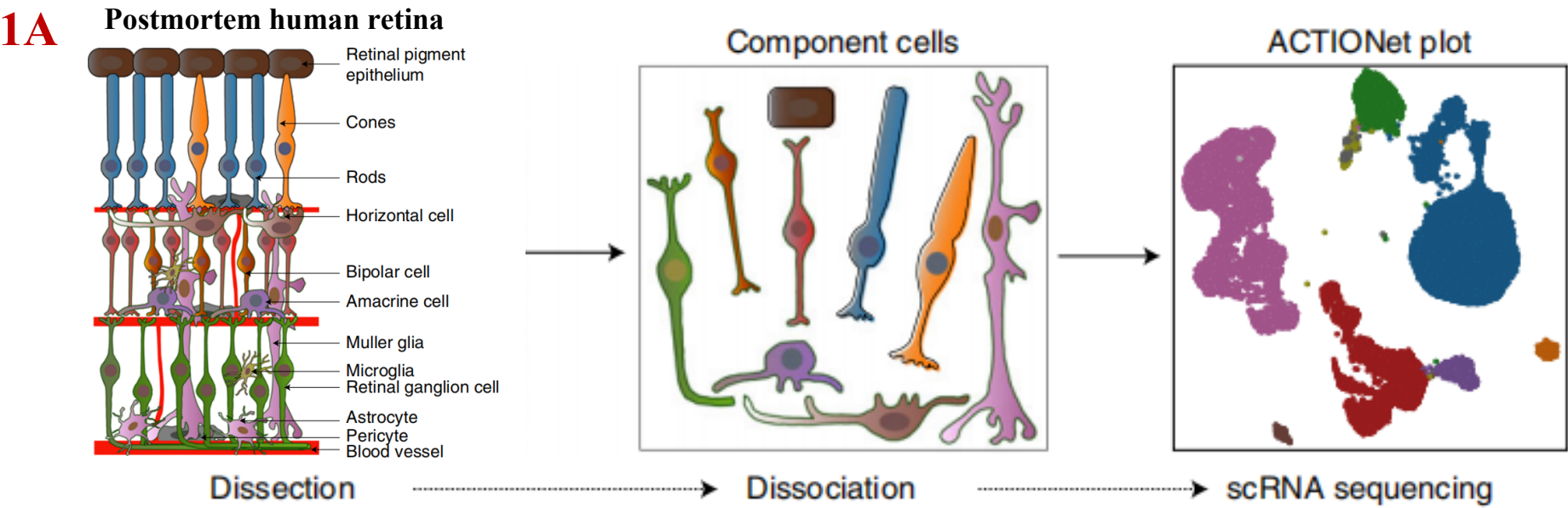
- 4E-4F
- 黄斑和外周视网膜RPCs与视网膜其他类型的细胞，其转录组存在明显的差异

视网膜发育相关基因的表达存在区域差异



单细胞转录组用于视网膜疾病相关性研究

Single-cell transcriptomic atlas of the human retina identifies cell types associated with age-related macular degeneration



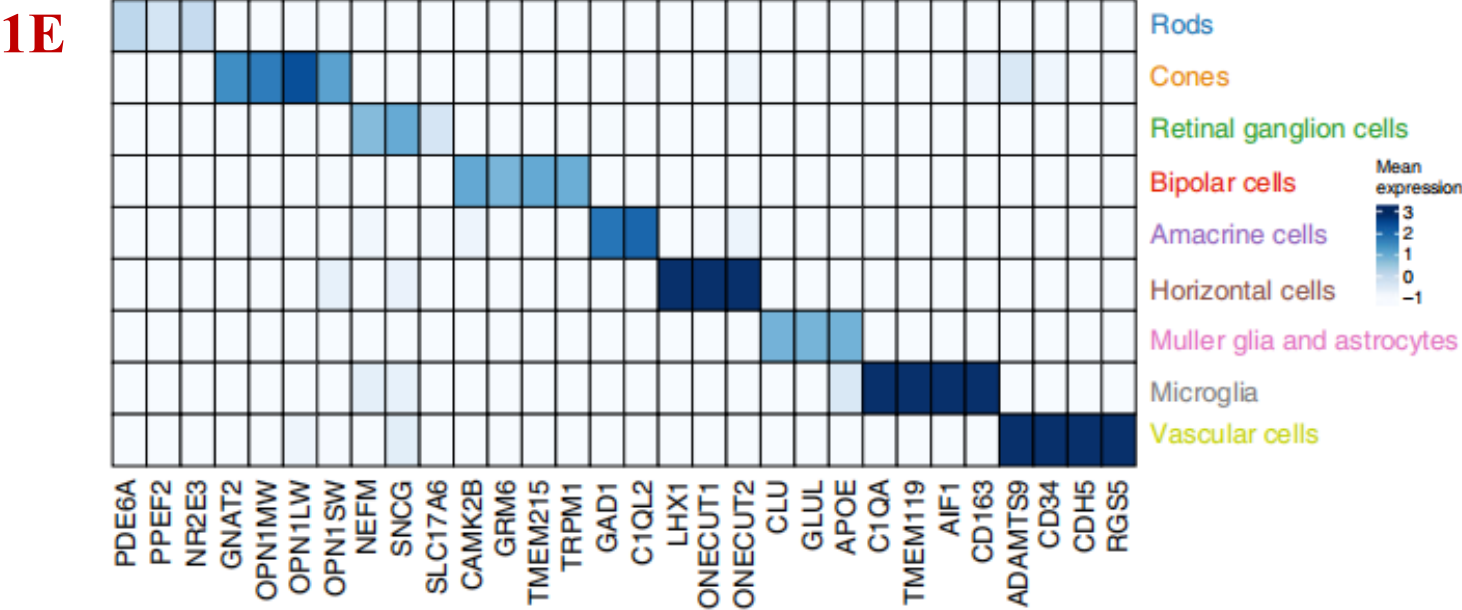
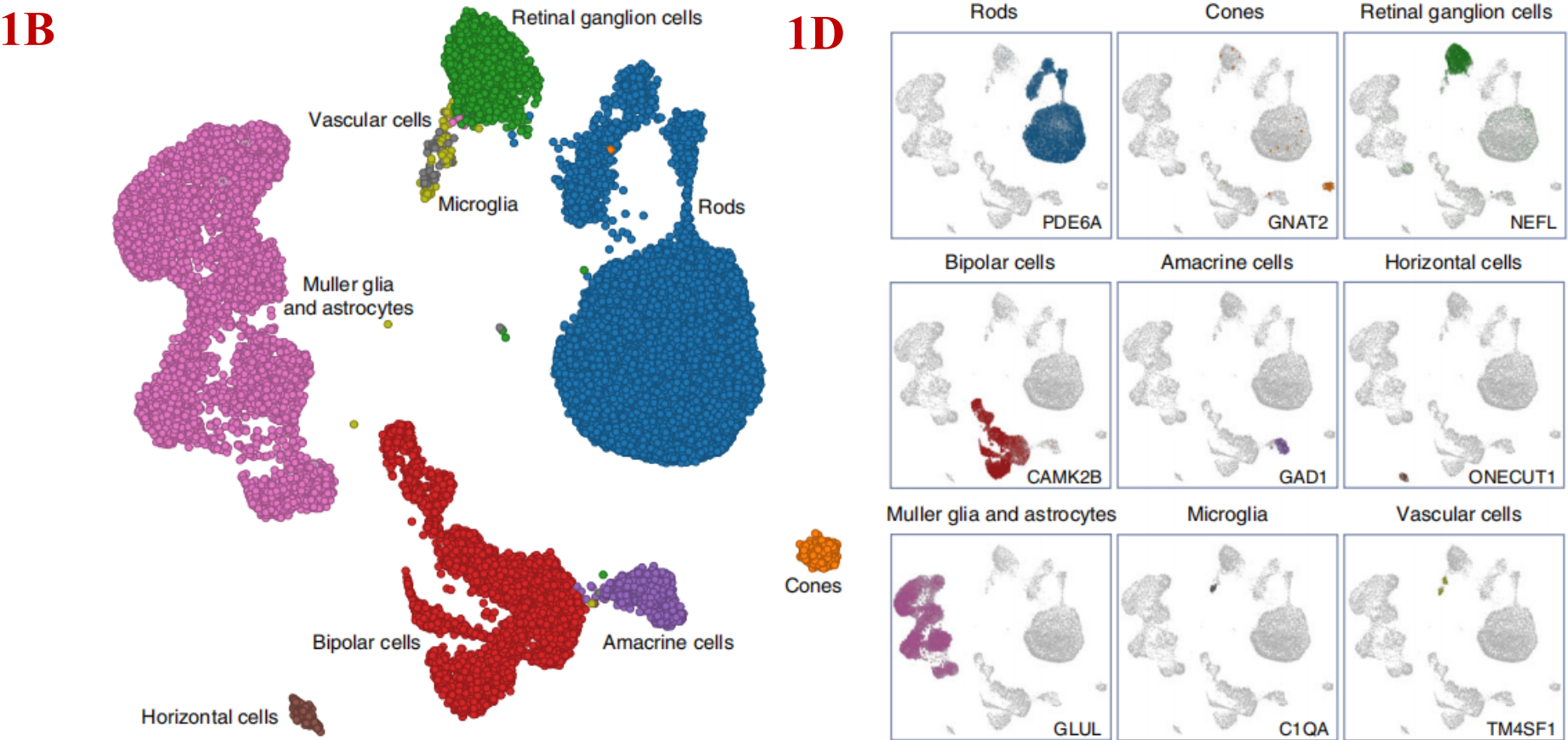
年龄相关性黄斑病变是导致老年人失明的主要原因之一。然而，鉴于AMD疾病的遗传复杂性，因此识别与AMD相关的细胞亚型和基因对理解AMD发病机制具有重要意义

作者通过视网膜的单细胞转录组研究发现，胶质细胞、血管细胞以及视锥细胞与AMD的发生最为相关

- 1A**
- 6例捐赠者视网膜单细胞样本（黄斑和周围视网膜）

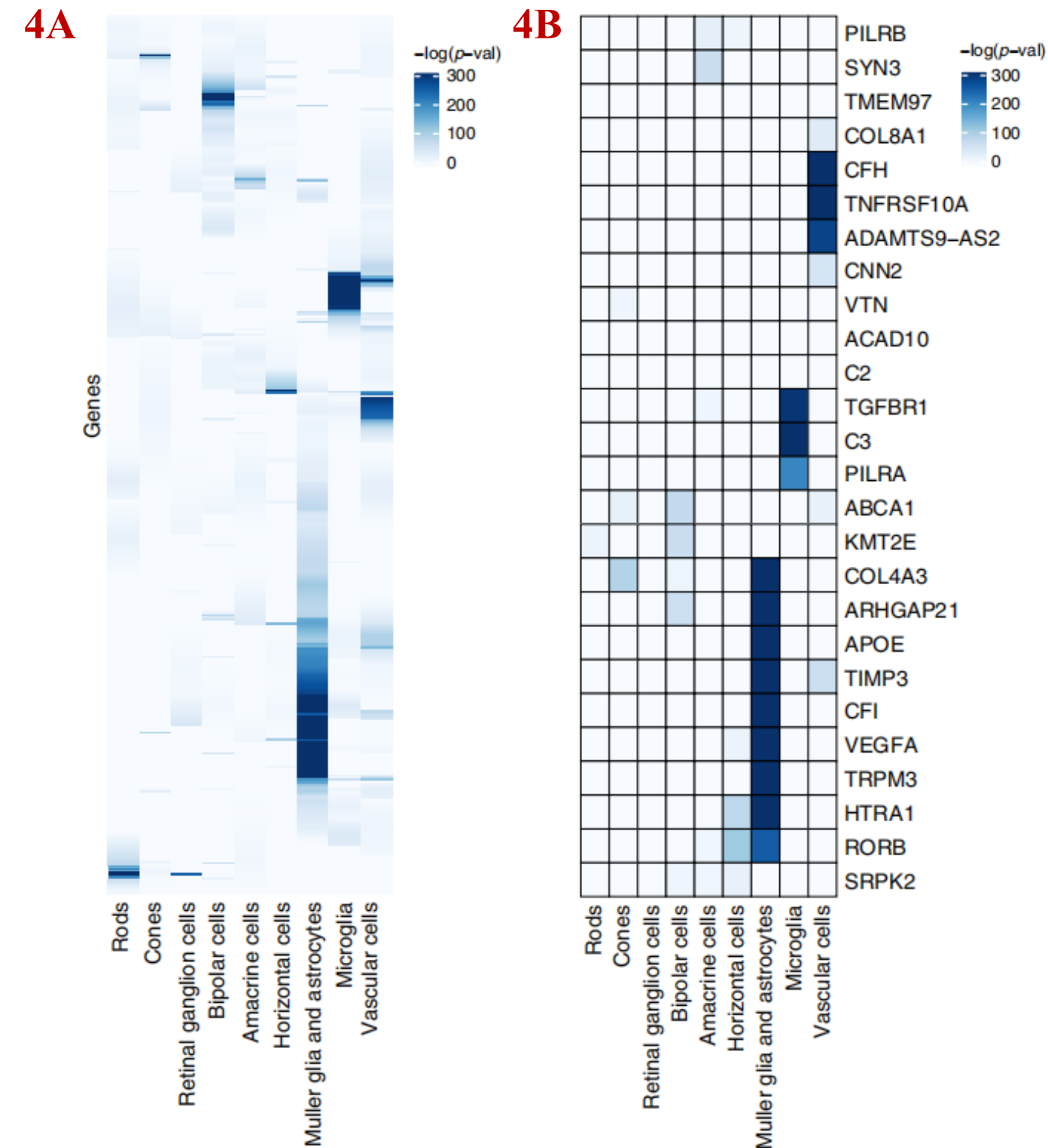
- 1B**
- 共捕获20091个单细胞
 - 非线性降维分析将其分为9个Clusters(UMAP)

- 1D-1E**
- 各细胞亚群特征基因表达



单细胞转录组用于视网膜疾病相关性研究

Single-cell transcriptomic atlas of the human retina identifies cell types associated with age-related macular degeneration



A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants

Advanced age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, with limited therapeutic options. Here we report on a study of >12 million variants, including 163,714 directly genotyped, mostly rare, protein-altering variants. Analyzing 16,144 patients and 17,832 controls, we identify 52 independently associated common and rare variants ($P < 5 \times 10^{-8}$) distributed across 34 loci. Although wet and dry AMD subtypes exhibit predominantly shared genetics, we identify the first genetic association signal specific to wet AMD, near *MMP9* (difference P value = 4.1×10^{-10}). Very rare coding variants (frequency <0.1%) in *CFH*, *CFI* and *TIMP3* suggest causal roles for these genes, as does a splice variant in *SIC16A8*. Our results support the hypothesis that rare coding variants can pinpoint causal genes within known genetic loci and illustrate that applying the approach systematically to detect new loci requires extremely large sample sizes.

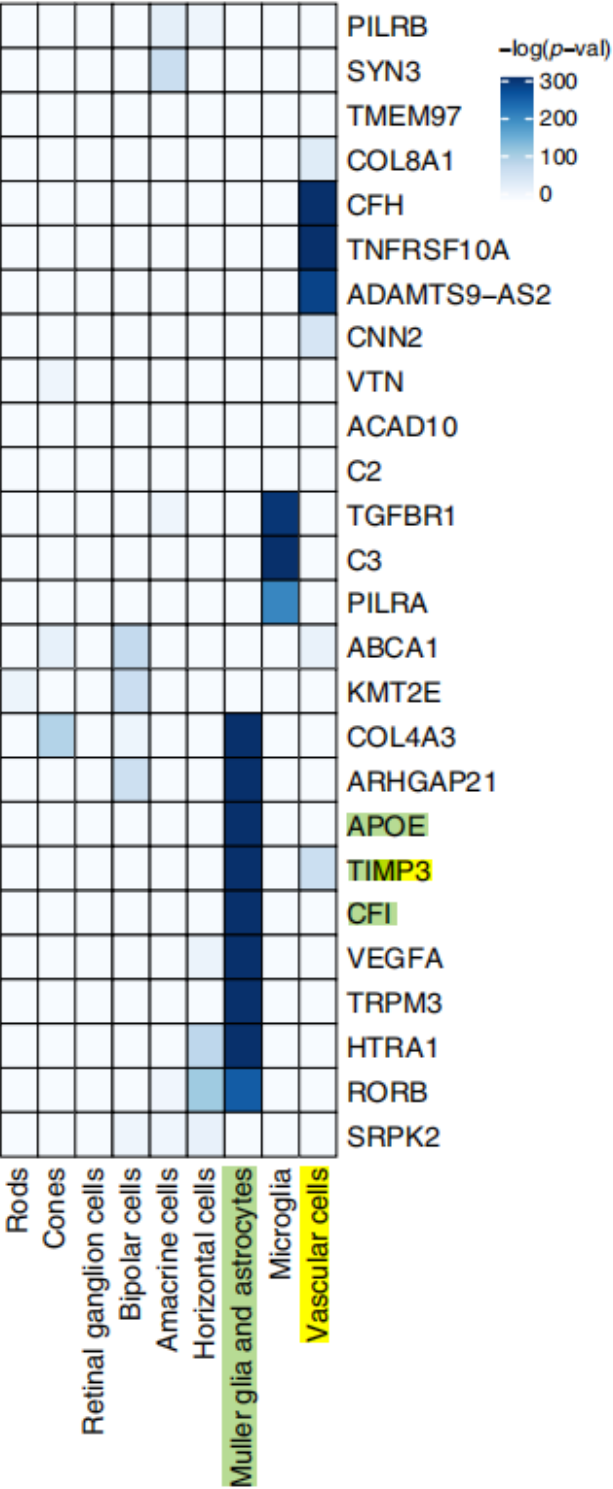
2016年，一项大型全基因组相关研究WGAS(样本量>15000)确定了34个与AMD相关的风险位点(含585基因)

- 4A
 - 585个AMD相关基因在各细胞亚群的表达水平(单细胞转录组)
- 4B
 - AMD高度相关的基因在各细胞亚群的表达水平(单细胞转录组)

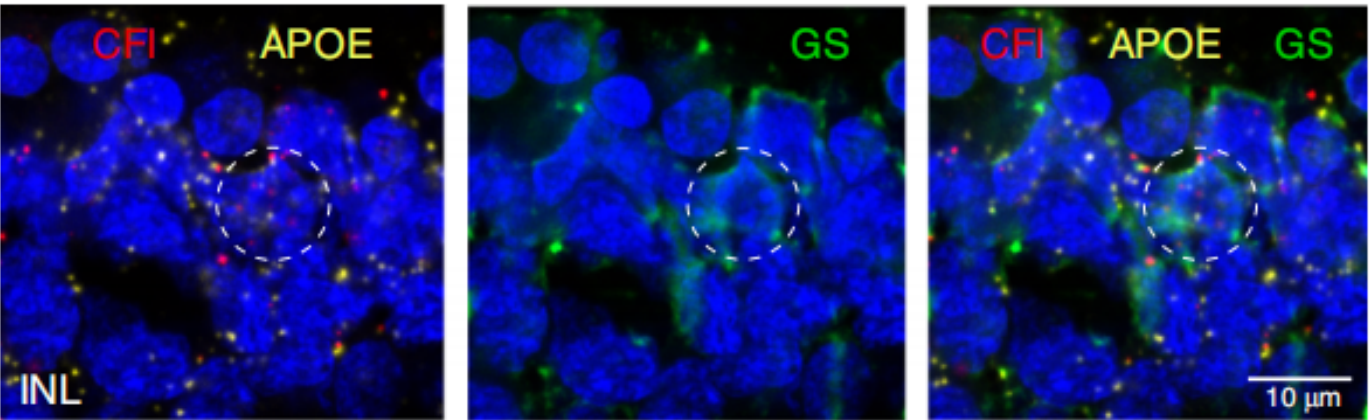
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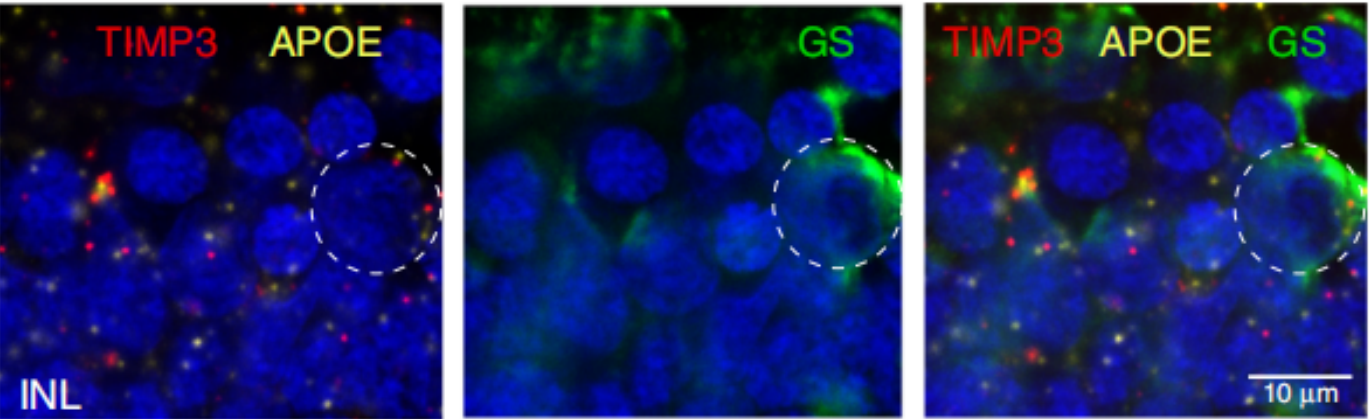
4B



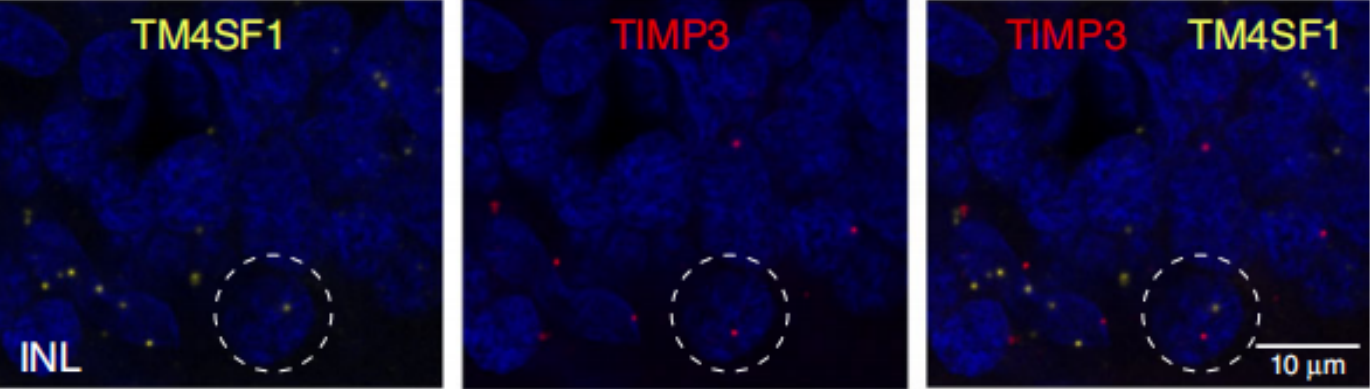
4C



4D



4E



4B

- AMD高度相关的基因在胶质细胞和血管细胞的表达水平(单细胞转录组)

4C

- 免疫荧光原位杂交显示CFI和GS(谷氨酰胺合成酶)在Muller胶质细胞(APOE)的表达

4D

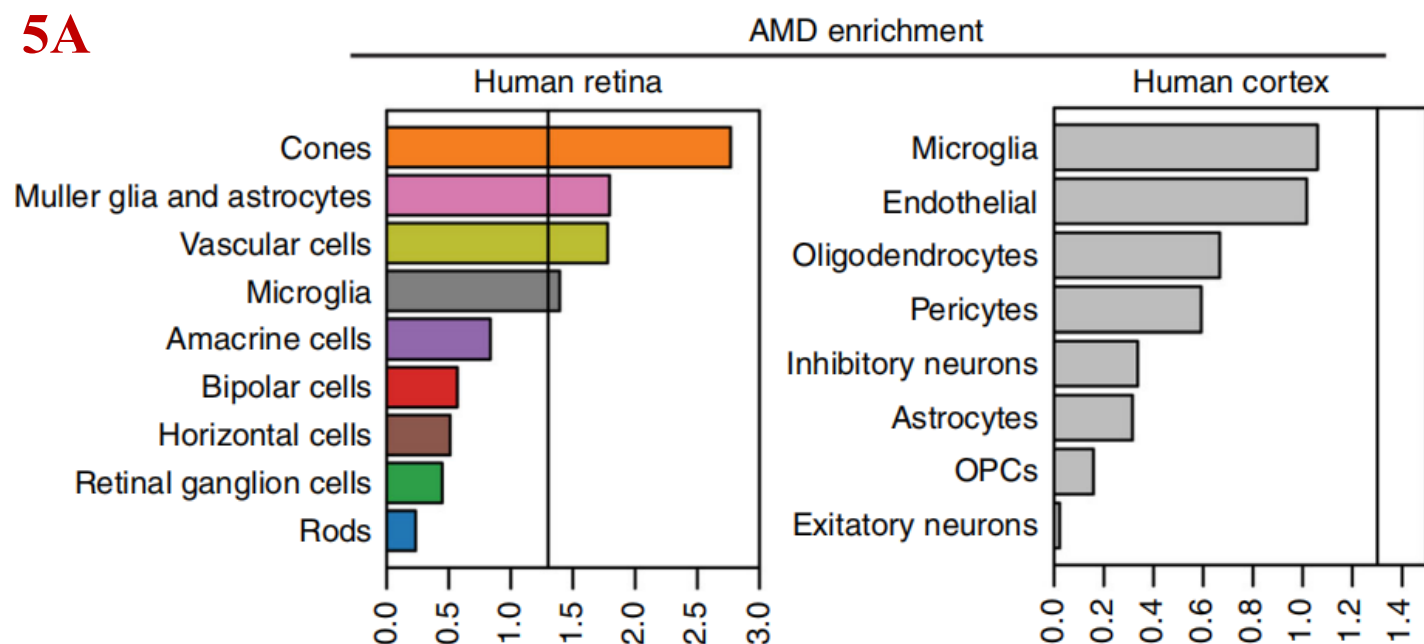
- 免疫荧光原位杂交显示TIMP3和GS(谷氨酰胺合成酶)在Muller胶质细胞(APOE)的表达

4E

- 免疫荧光原位杂交显示TIMP3在血管细胞(TM4SF1)的表达

单细胞转录组用于视网膜疾病相关性研究

Single-cell transcriptomic atlas of the human retina identifies cell types associated with age-related macular degeneration

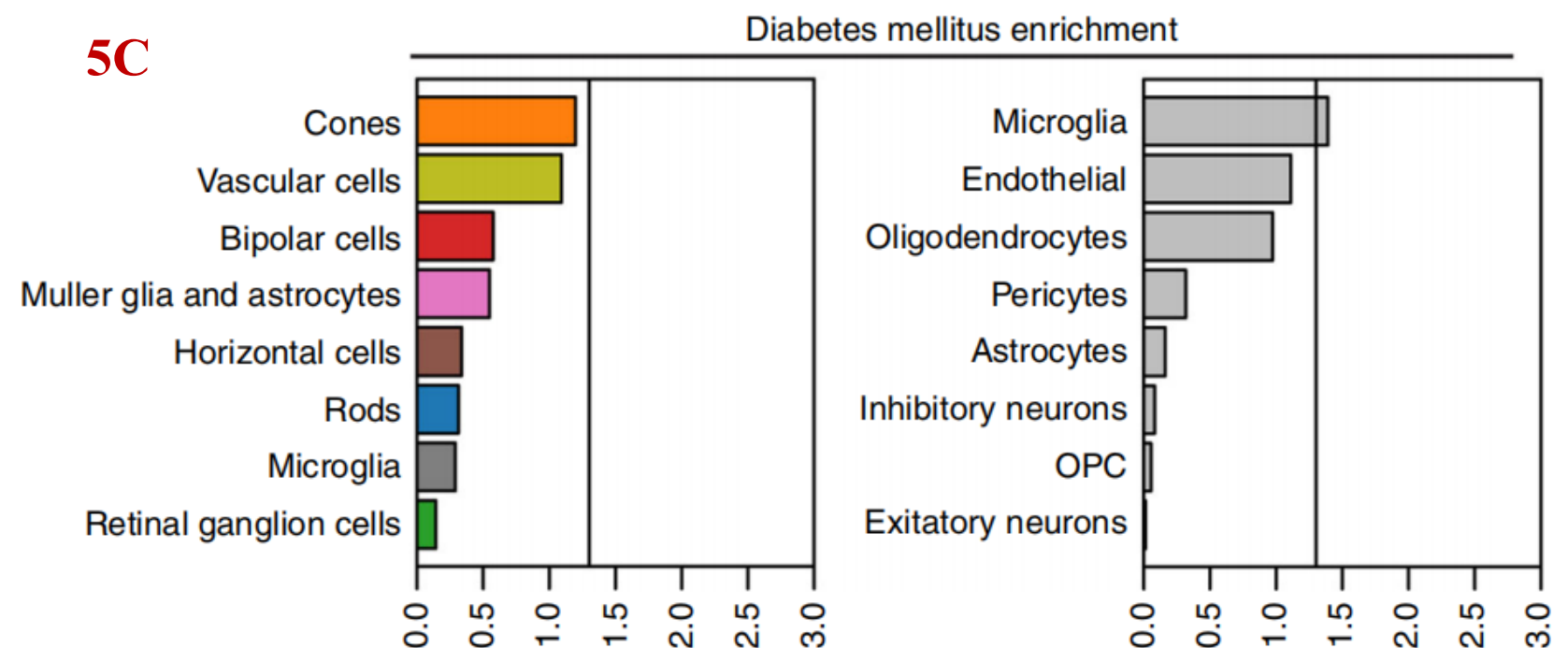
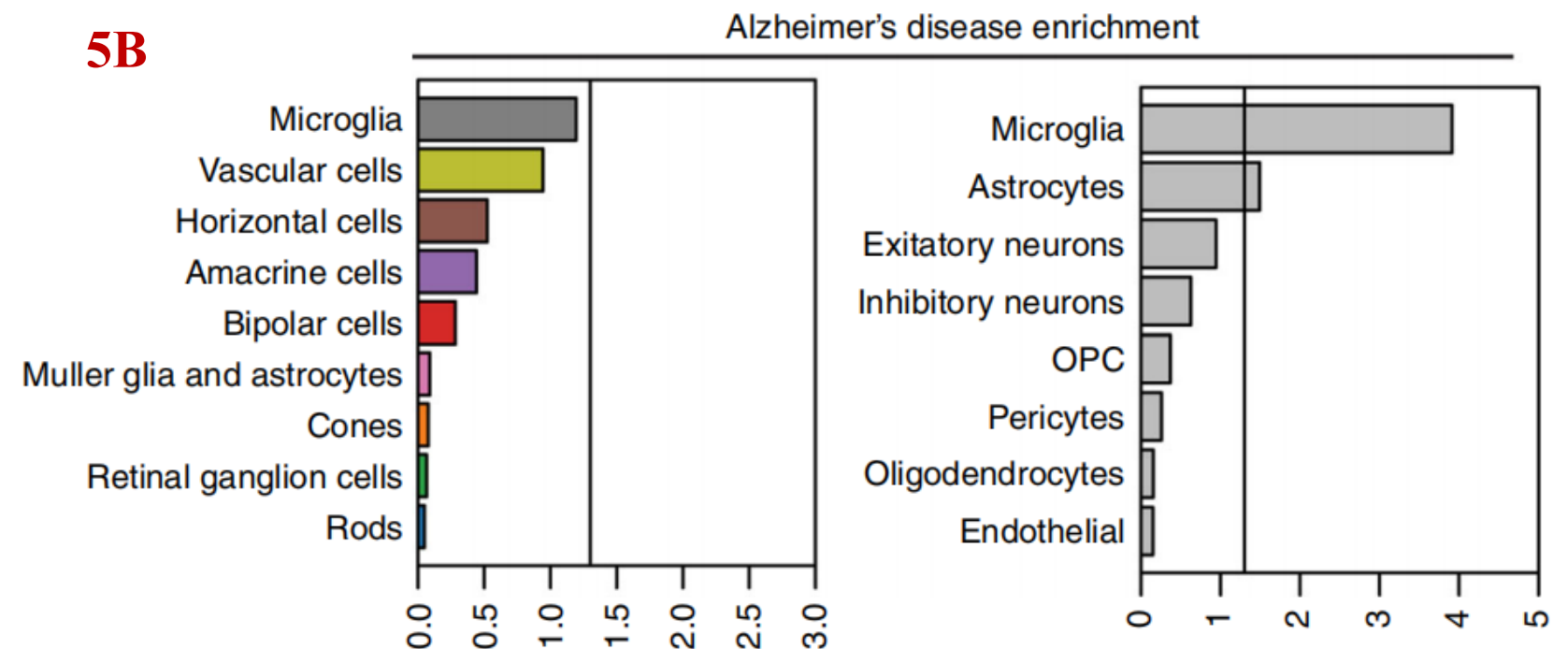


为了验证特异基因在细胞亚群上的偏好表达与罹患AMD的风险相关性，作者使用MAGMA(Multi-marker Analysis of GenoMic Annotation)的分析方法

5A-5C

- 视网膜视锥细胞、胶质细胞和血管细胞表达的基因最能用于预测AMD的罹患风险
- 但这些细胞表达的基因与阿尔兹海默病和糖尿病的罹患风险不相关
- 来自于大脑皮层的相似细胞亚群的基因表达，却与AMD的罹患风险不相关

来自于视网膜的特定细胞亚群与老年性黄斑病变相关，可作为后续研究疾病发生机制和治疗的靶点



THANK YOU!

