Designing single cell experiments

Carla Jones
cjb@sanger.ac.uk
@carlajonesBio

wellcome sanger institute

Experimental design considerations

1- Single cell approaches

2- Sample preparation

3- Cell coverage

4- Deep atlasing and rare populations

5 - Multiplexing and proteomics

wellcome connecting science

Experimental design considerations

1- Single cell approaches

2- Sample preparation

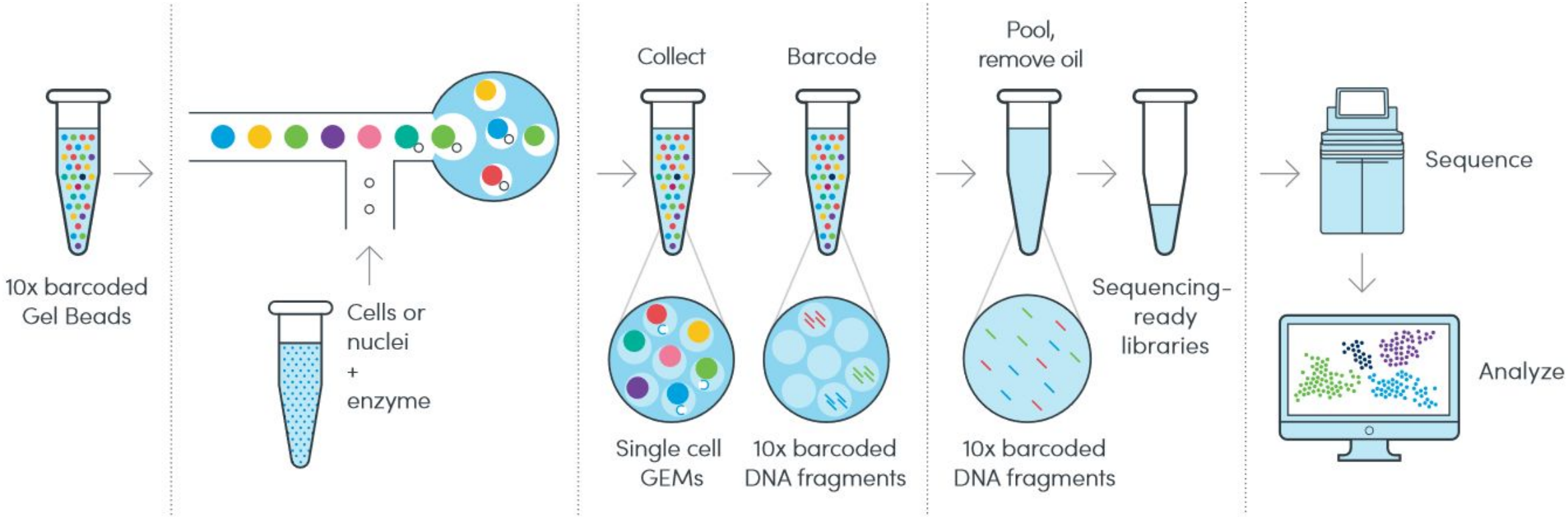
3-Cell coverage

4- Deep atlasing and rare populations

5 - Multiplexing and proteomics

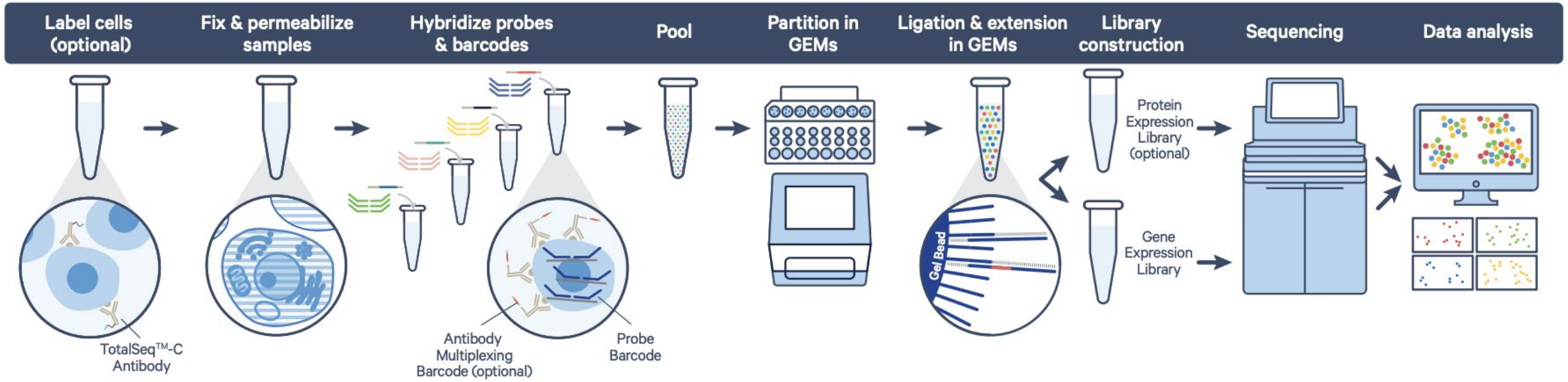
wellcome connecting science

Droplet versus split pool approaches



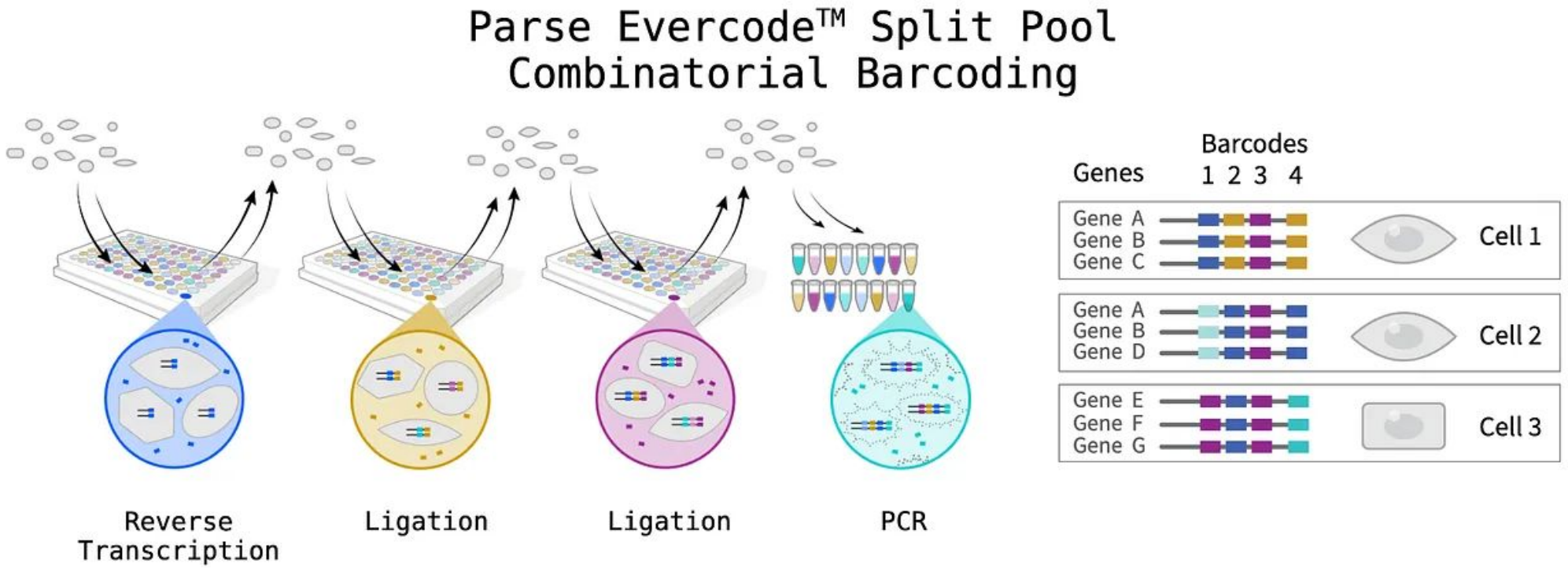
10x GENOMICS

Droplet versus split pool approaches



10x GENOMICS

Droplet versus split pool approaches



Parse BIOSCIENCES

Droplet versus split pool approaches

Superior molecular efficiency of Parse v2 compared to 10x Genomics v3.1

Downsampled Parse v2 data to same reads per cell as 10x Genomics v3.1

- E.g 10x Genomics 207M total reads / 16,181 cells = 12,817 reads per cell
- Accounts for effect of doublet rate and ambient RNA

Metric	Parse v2	10x Genomics v3.1
Cells	3,683 ($^1/_3$ 10k kit)	16,181 (1 capture)
Doublet rate (Heterotypic)	**0.73%**	**7%**
Reads per cell	16,587	12,817
Sequencing saturation	**5.5%**	**54.3%**
Reads in cells	**84.2%**	**50.8%**
Transcriptome mapping	72.8%	47.3%
Exonic mapping	22.4%	26.3%
Intronic mapping	?	47%

Parse BIOSCIENCES

Droplet versus split pool approaches

Combinatorial Indexing

Fixed cells or nuclei

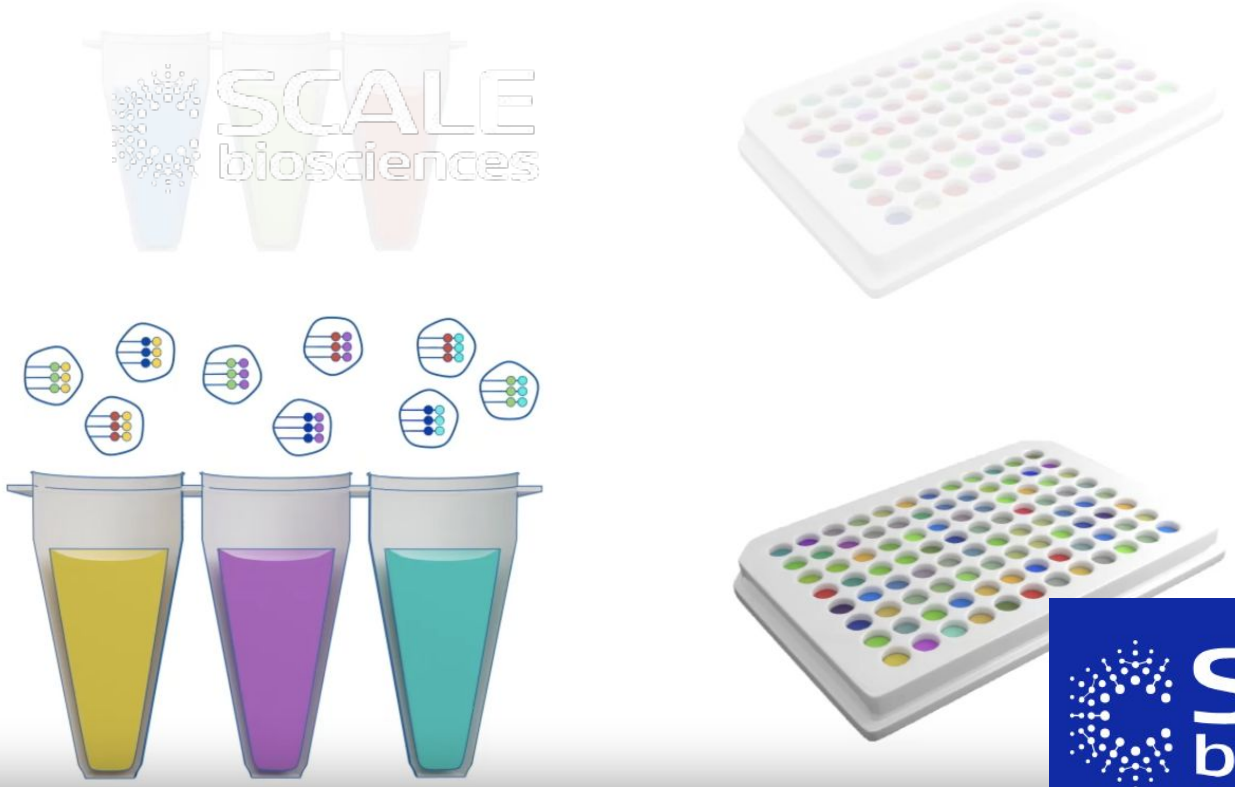
Distribute

First barcode

Pool

Distribute

Second barcode



Experimental design considerations

1- Single cell approaches

2- Sample preparation

3-Cell coverage

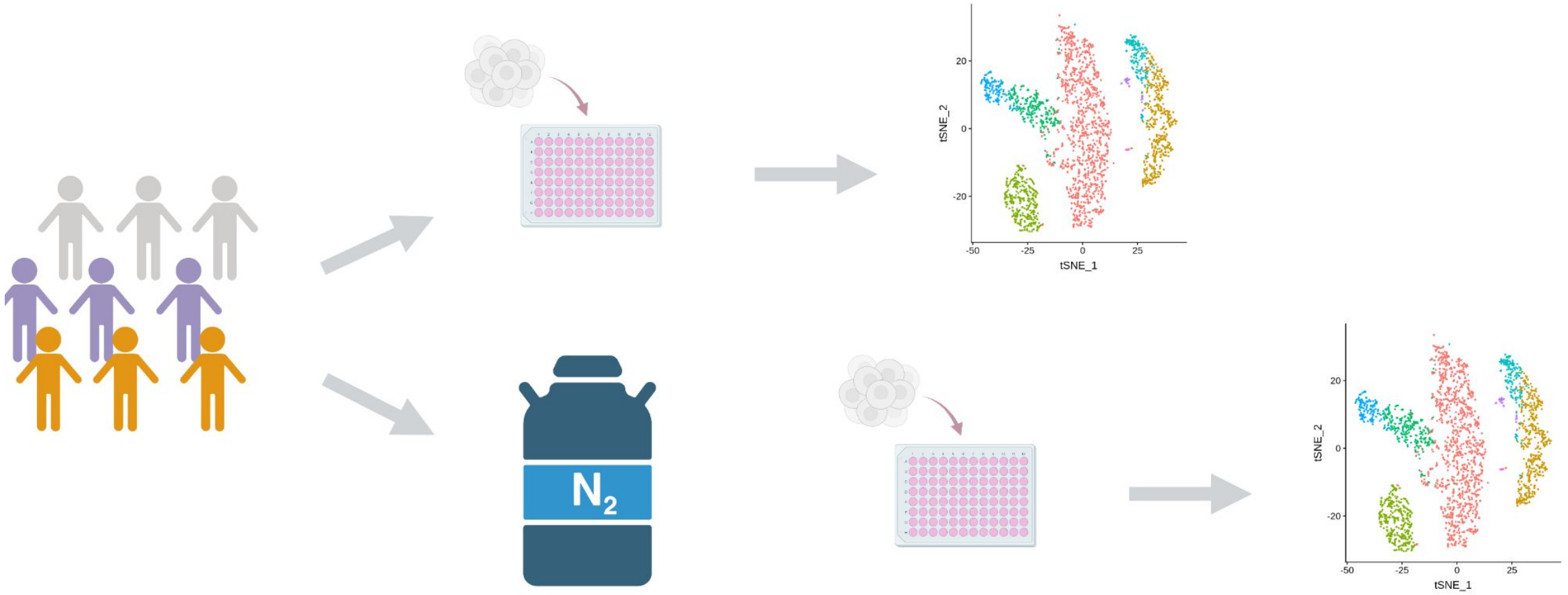
4- Deep atlasing and rare populations

5 - Multiplexing and proteomics

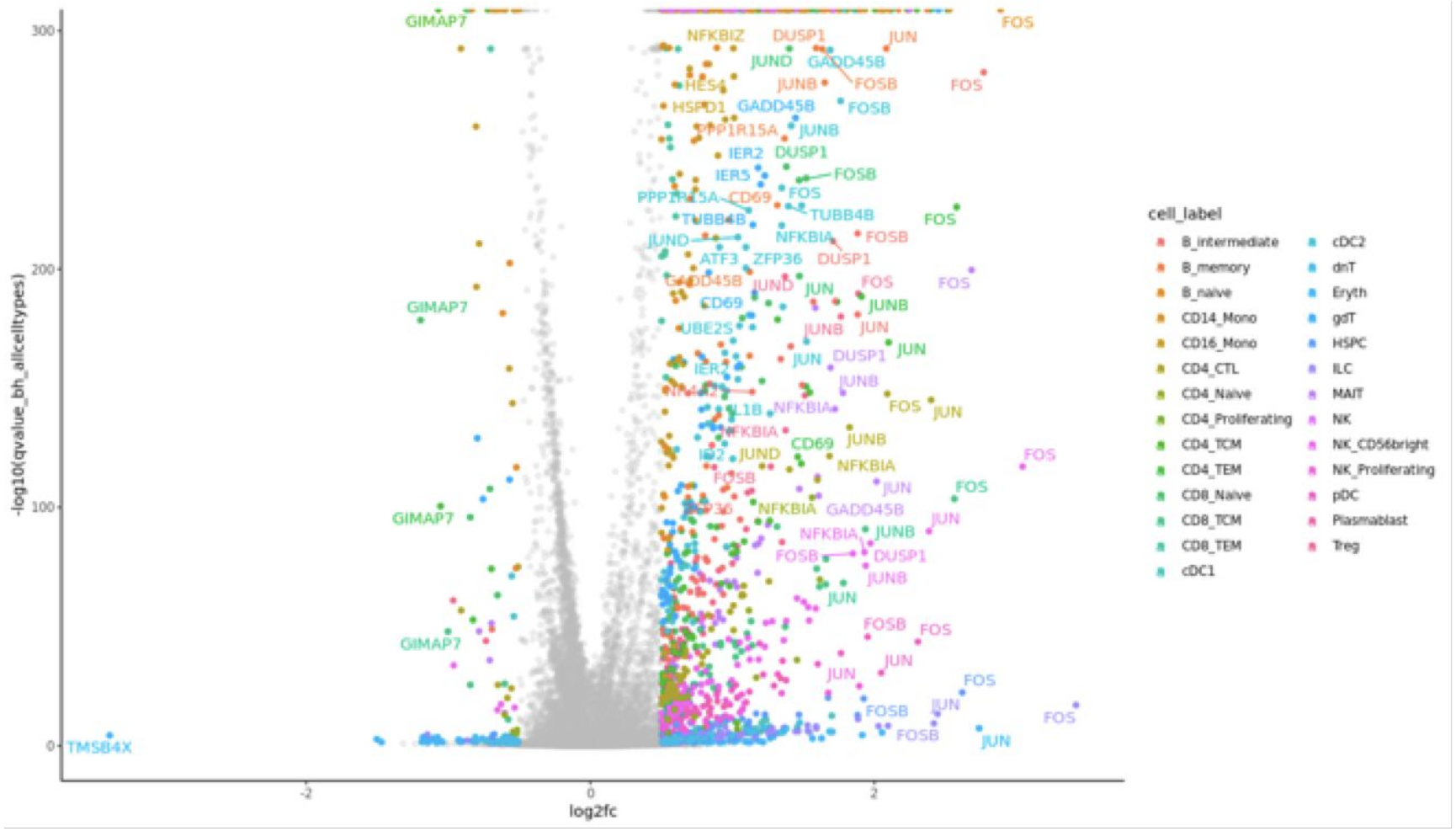
6- Batch controls

wellcome connecting science

Impact of freezing and resting on PBMC transcriptome

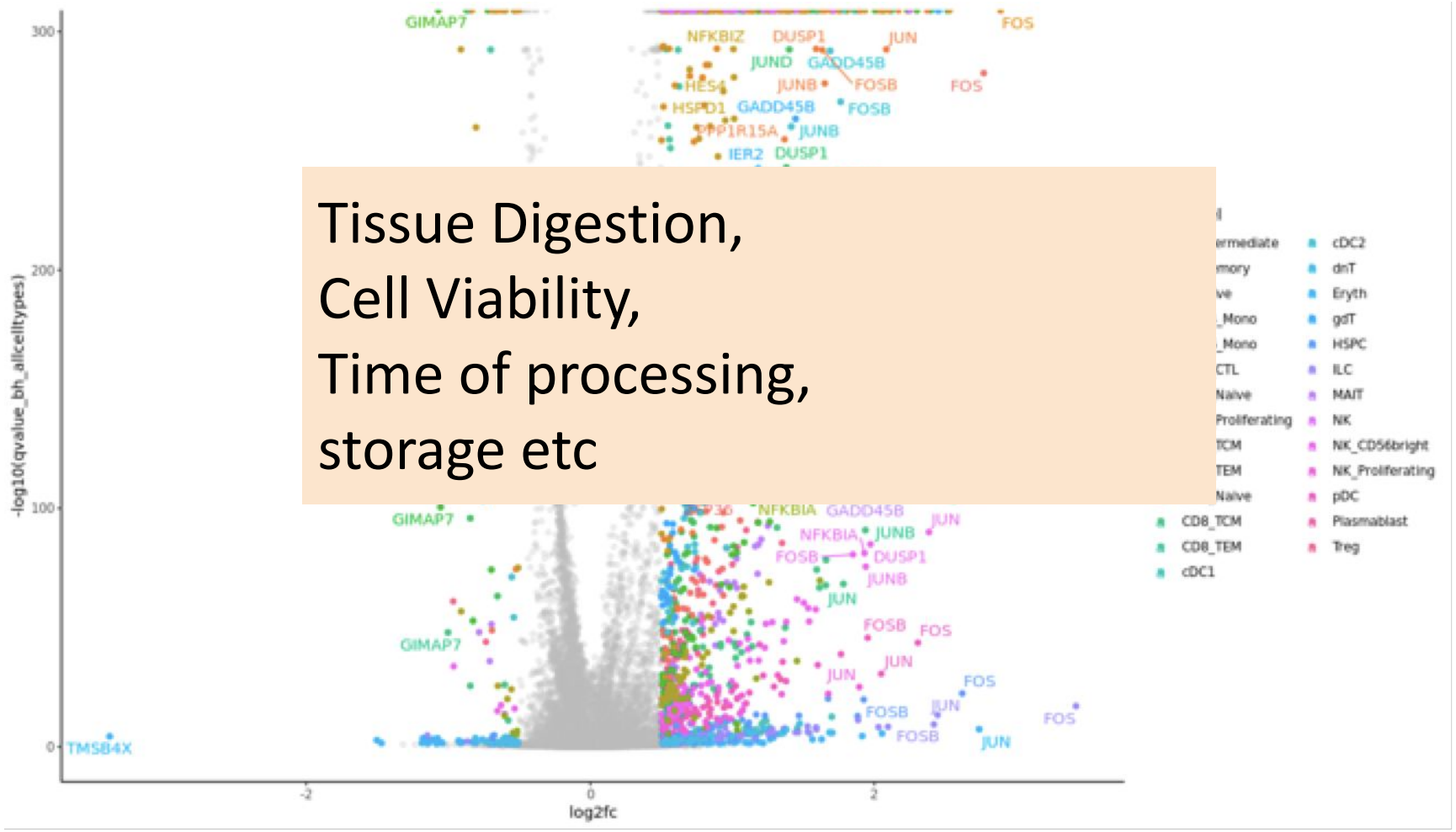


Frozen PBMCs upregulate stress response genes after thawing



Data from Cardinal & analysis from Tobi Alegbe, Carl Anderson group

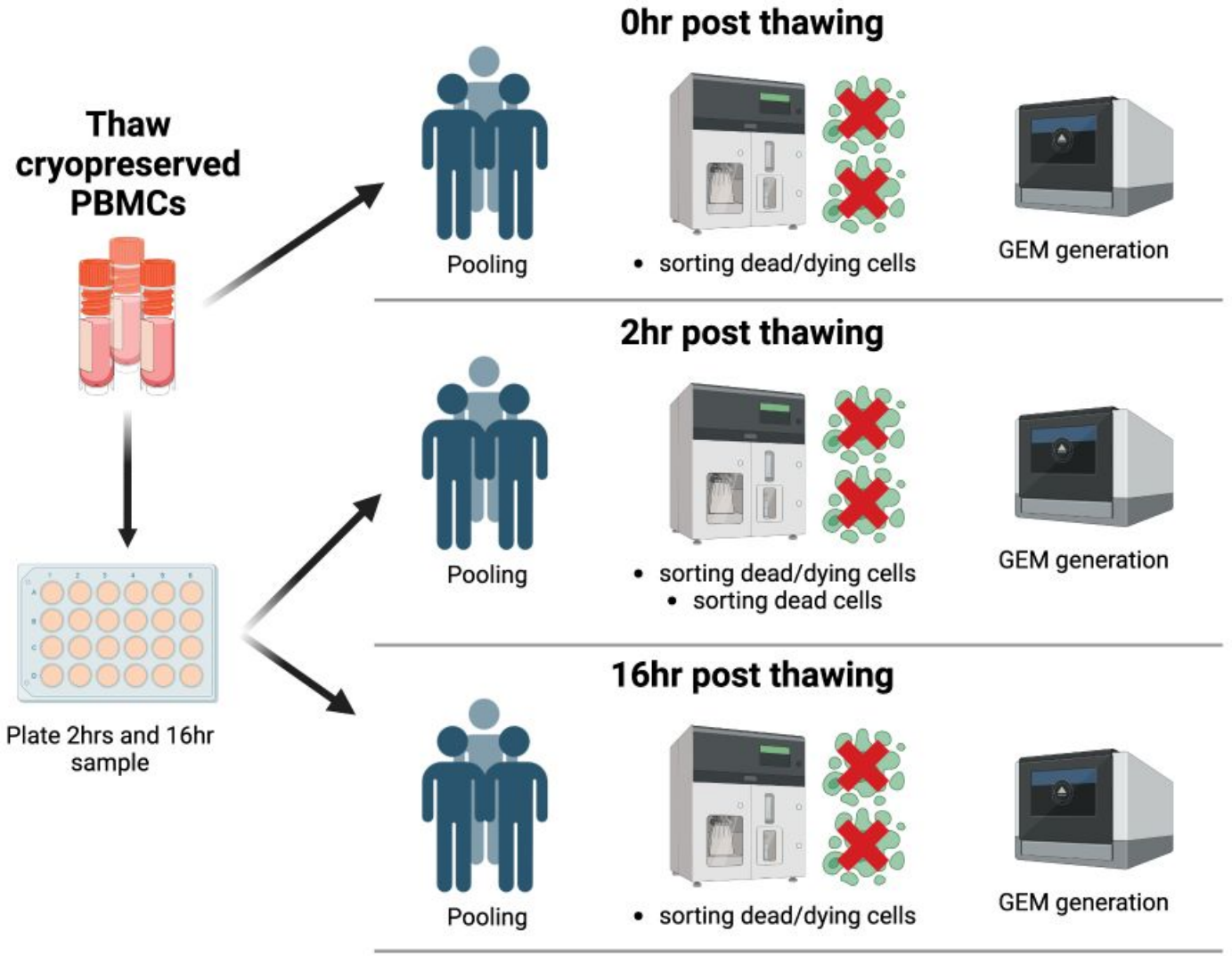
Frozen PBMCs upregulate stress response genes after thawing

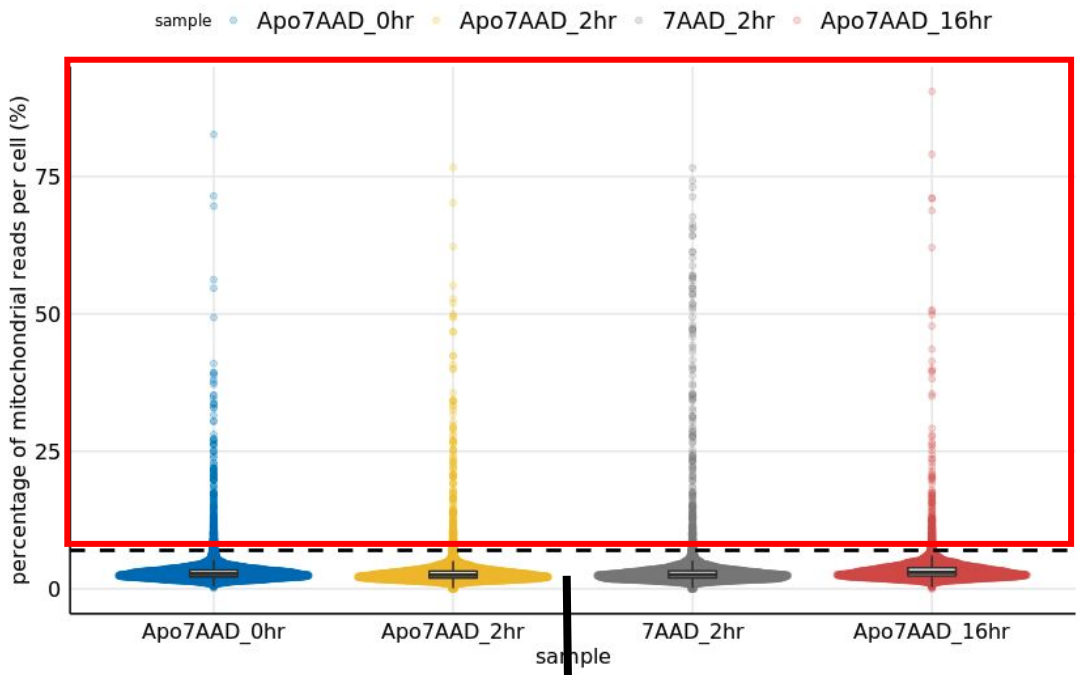


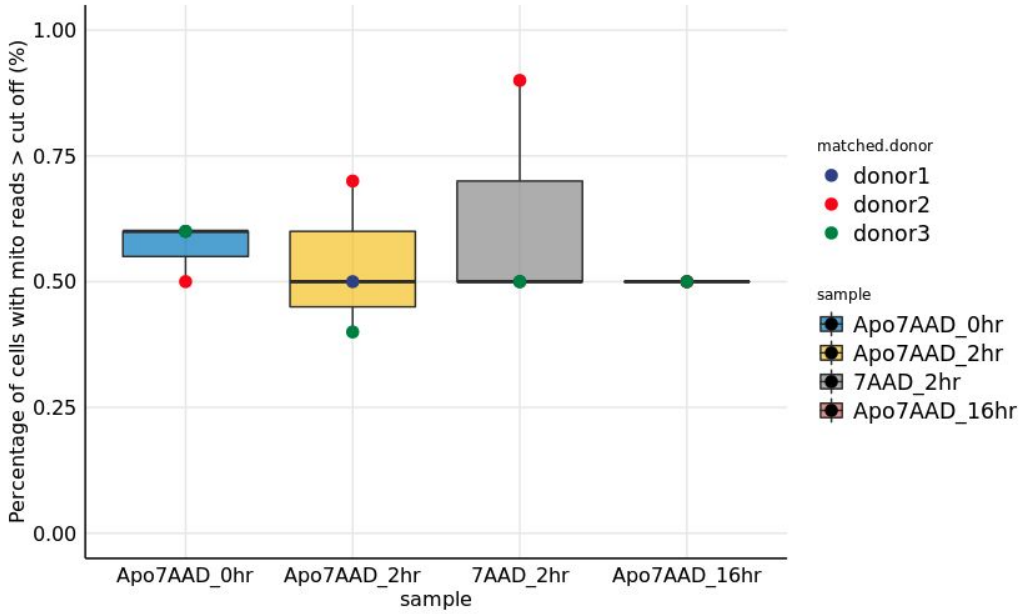
Tissue Digestion,
Cell Viability,
Time of processing,
storage etc

Data from Cardinal & analysis from Tobi Alegbe, Carl Anderson group

Would resting cells avoid cryopreservation stress response?



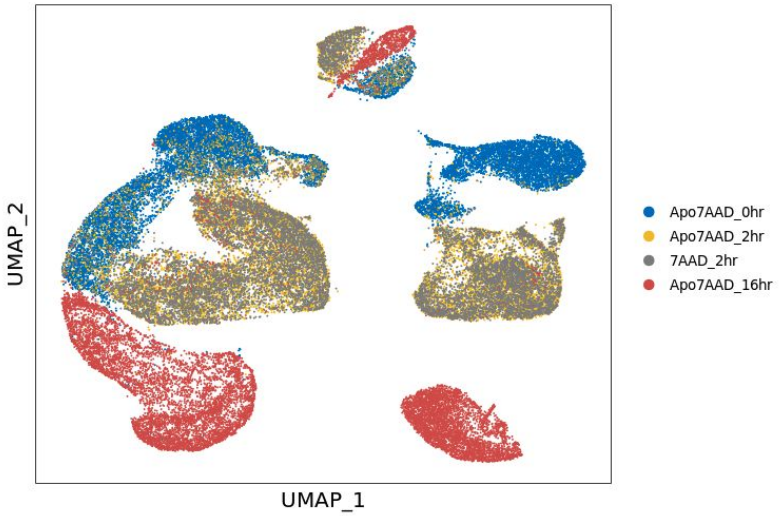


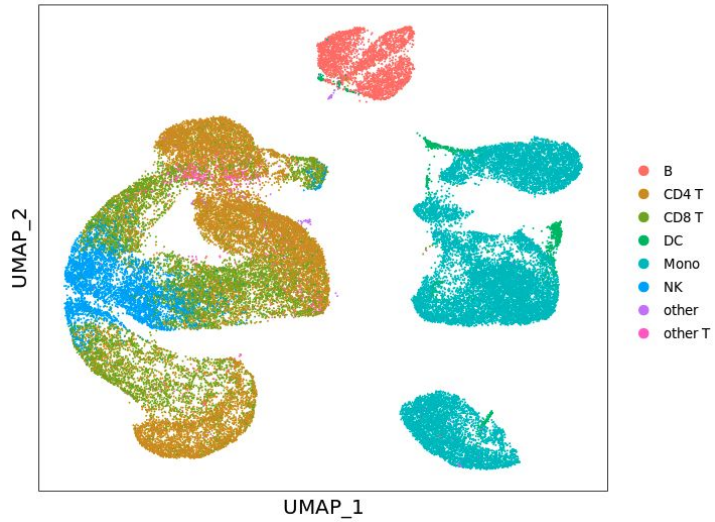


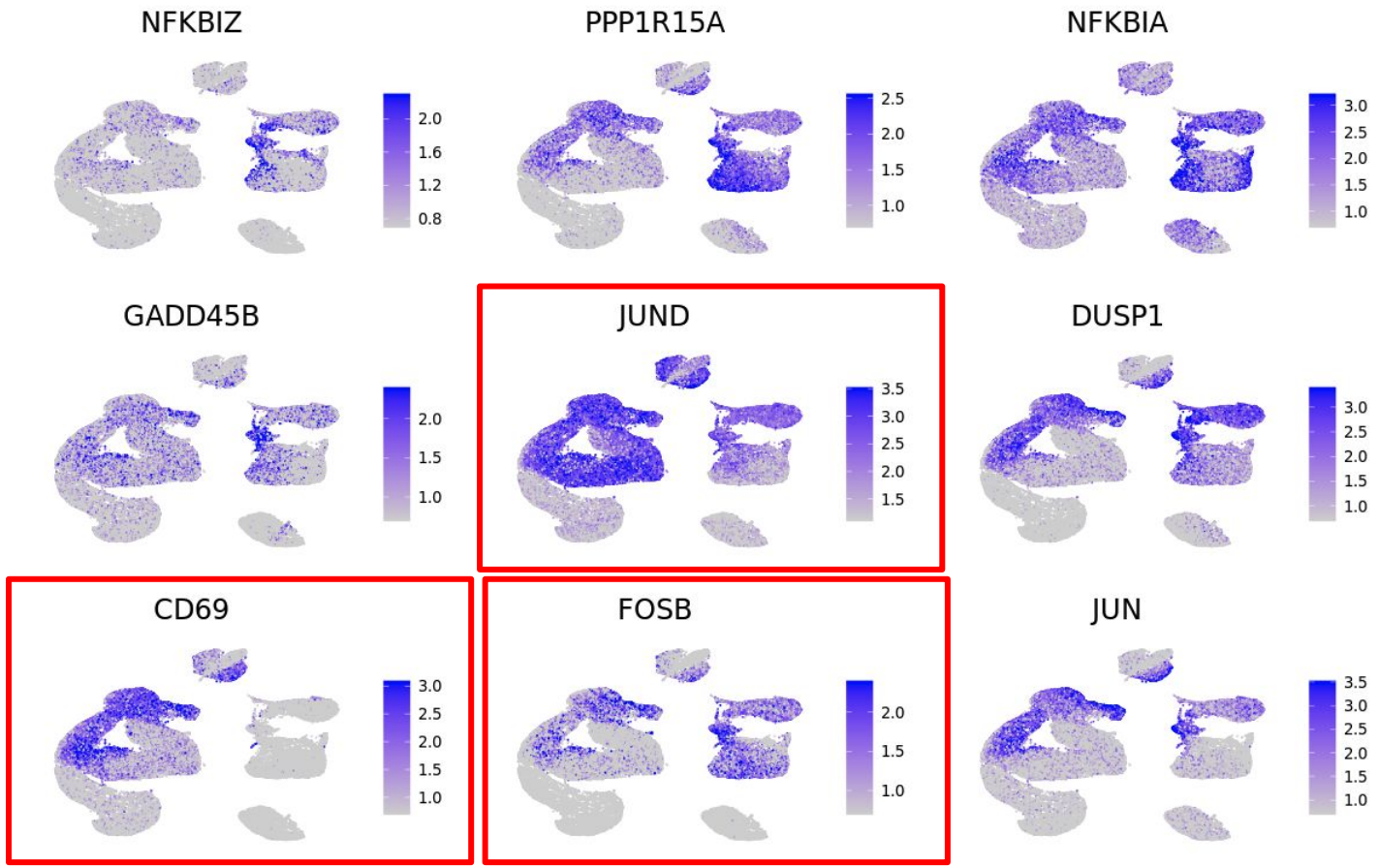
Are there higher levels of mitochondrial reads detected in cells at early time points?

No difference in % of cells expressing high mito reads across timepoints

High expression of stress response genes in multiple cell types at early time points







CAUTION: stress genes are not the only genes being modulated after resting. The choice of resting or not resting is experiment -dependent

Experimental design considerations

1- Single cell approaches

2- Sample preparation

3- Cell coverage

4- Deep atlasing and rare populations

5 - Multiplexing and proteomics

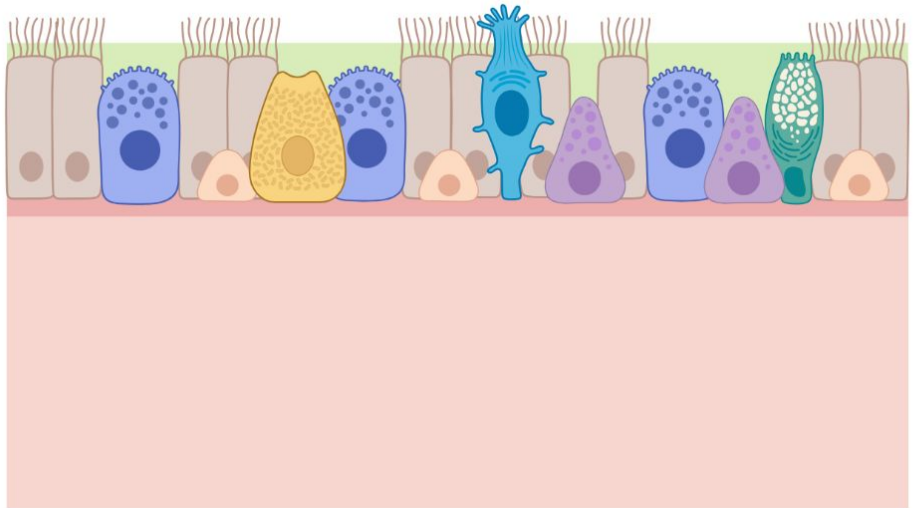
wellcome connecting science

How many cells do I need per sample

a minimum of ~ 100 cells are required for statistical analysis

Tissue heterogeneity

Distribution of populations of interest



Experimental design considerations

1- Single cell approaches

2- Sample preparation

3- Cell coverage

4- Deep atlasing and rare populations

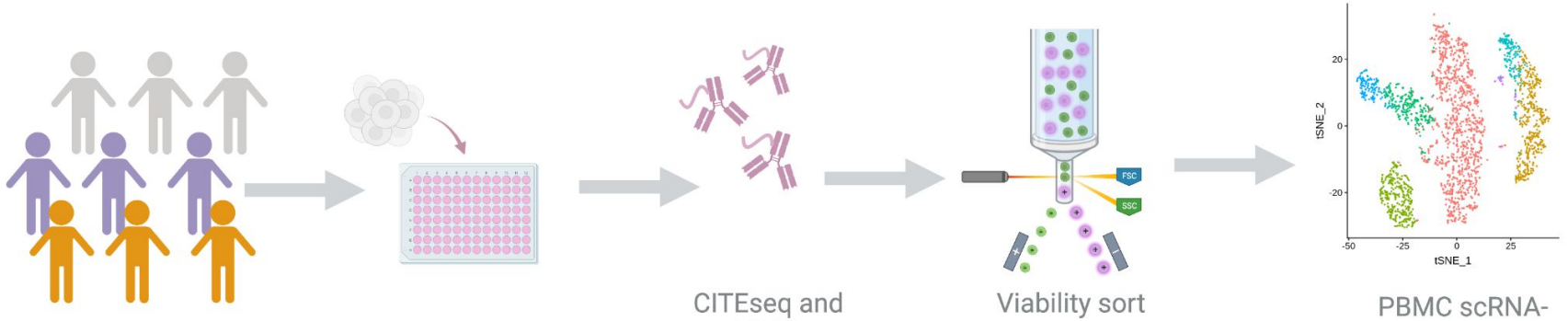
5 - Multiplexing and proteomics

6- Batch controls

wellcome connecting science


Oh no! I am investigating rare populations!!!

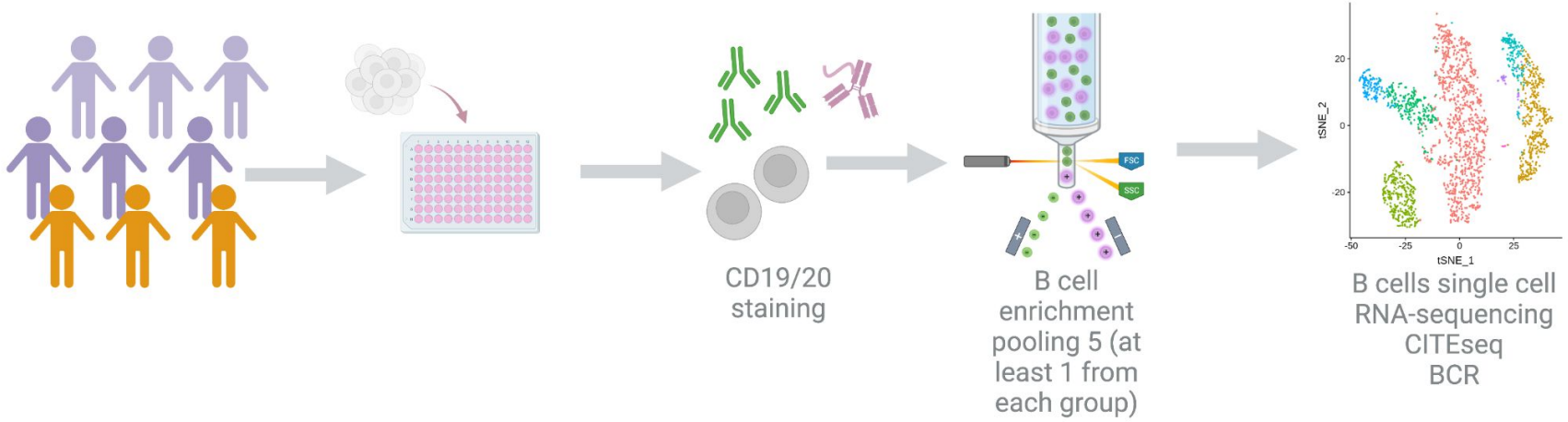
Enriching rare cell populations for sc-RNA-seq analysis



CITEseq and 7AAD

Viability sort

PBMC scRNA-sequencing
CITEseq
TCR
BCR

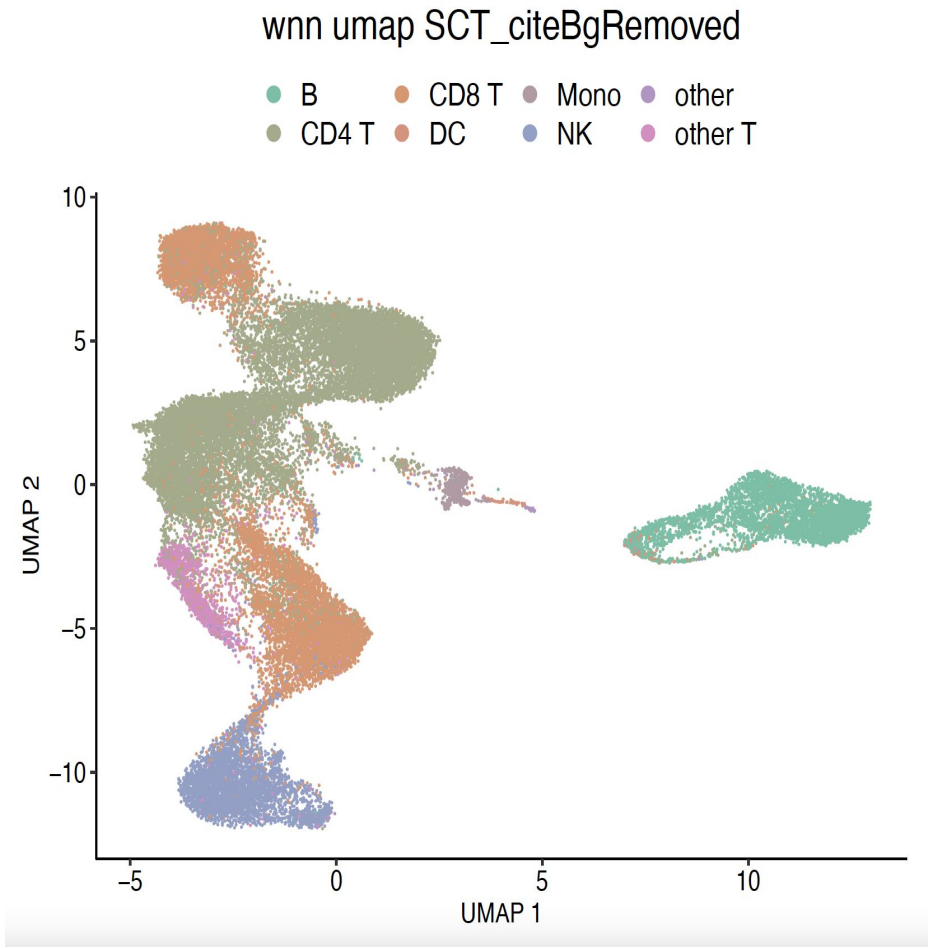


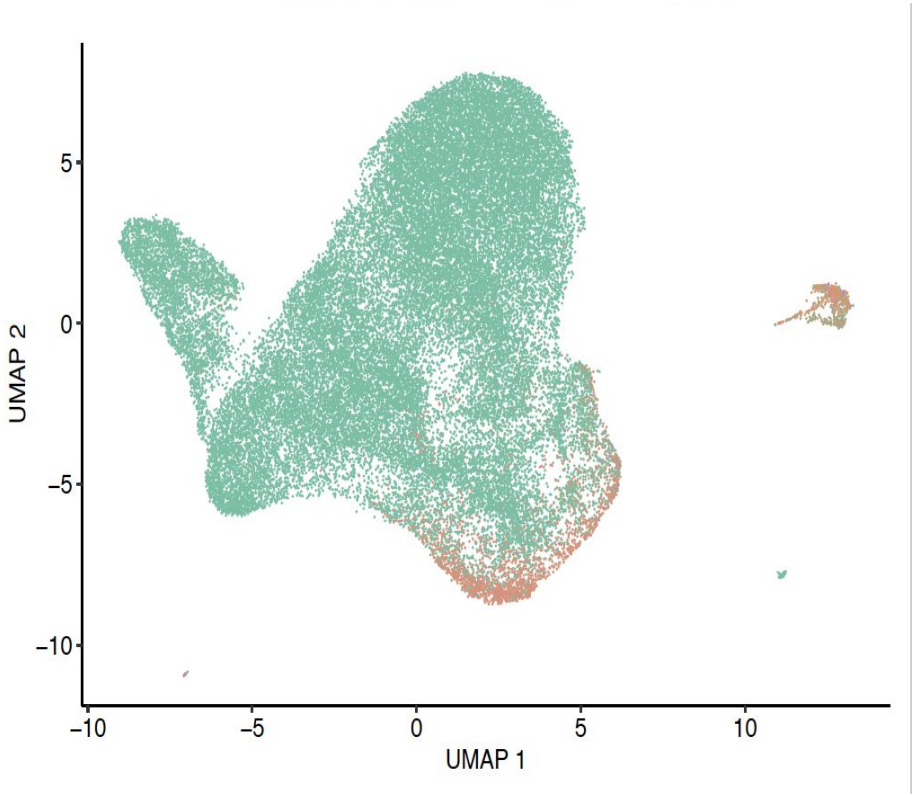
CD19/20 staining

B cell enrichment pooling 5 (at least 1 from each group)

B cells single cell RNA-sequencing
CITEseq
BCR

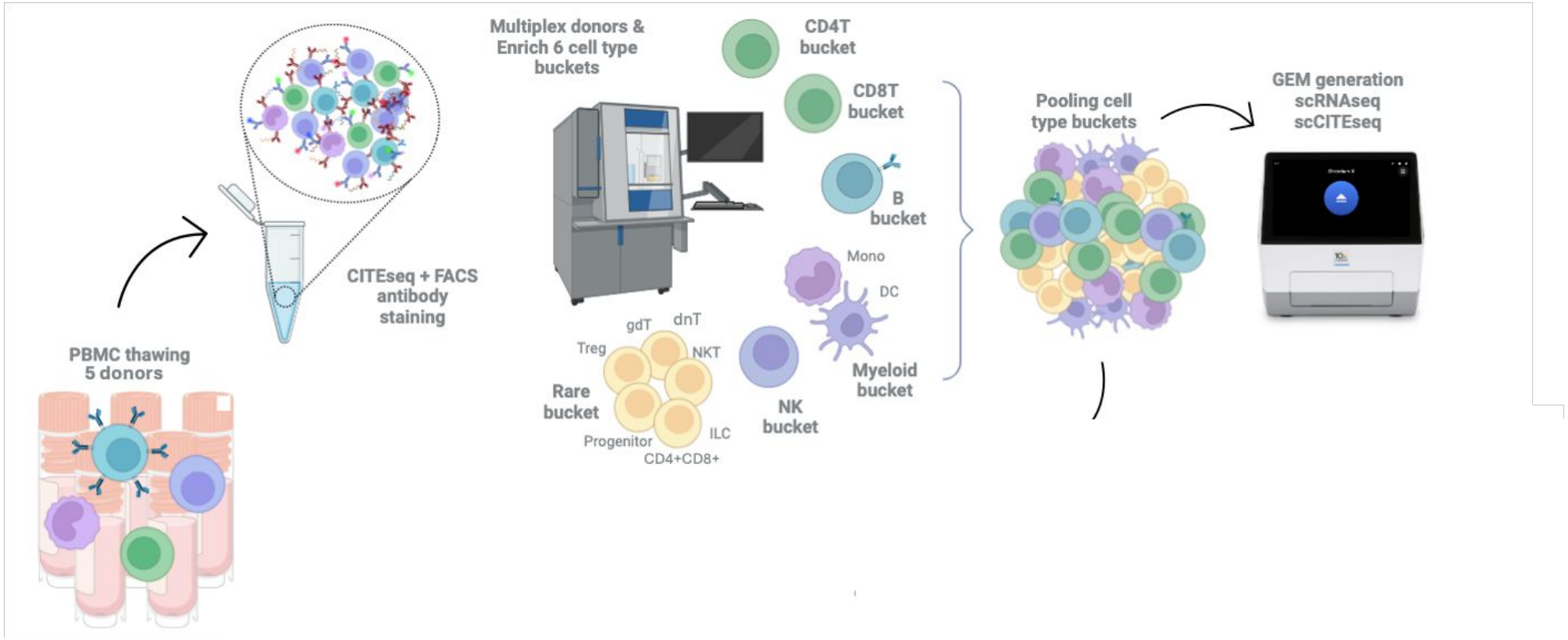
Enriching rare cell populations for sc-RNA-seq analysis





Tarran Rupall

Enriching rare cell populations for sc-RNA-seq analysis



PROJECT JAGUAR

Experimental design considerations

1- Single cell approaches

2- Sample preparation

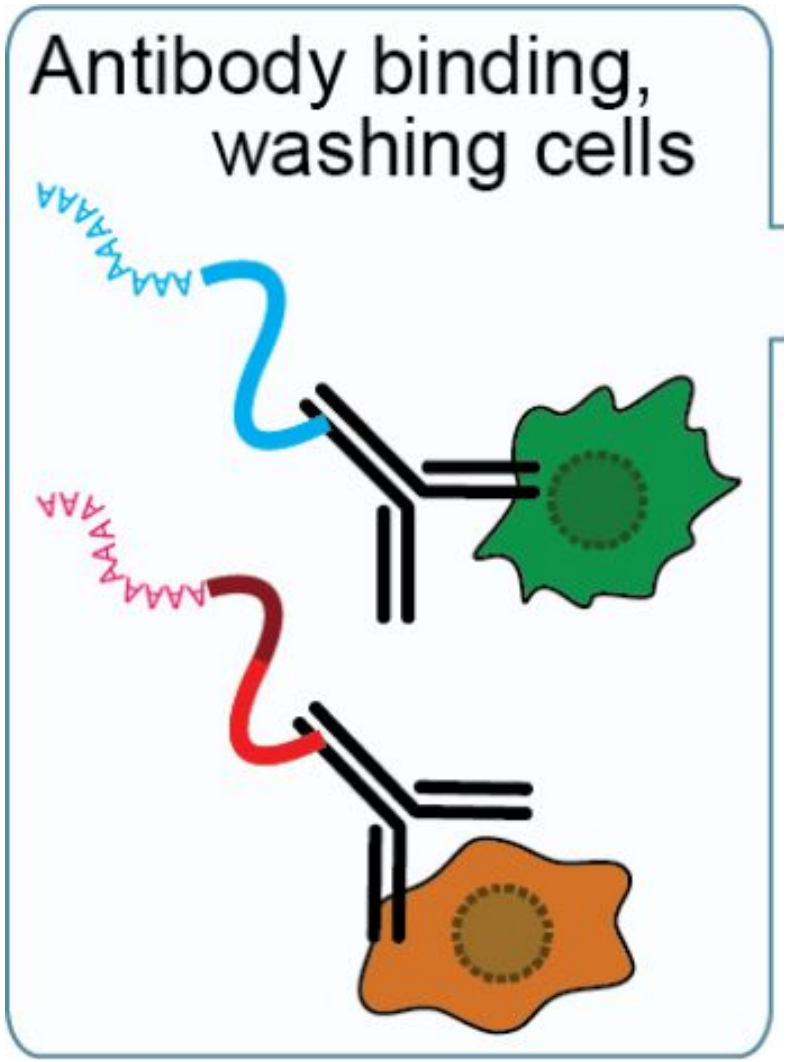
3- Cell coverage

4- Deep atlasing and rare populations

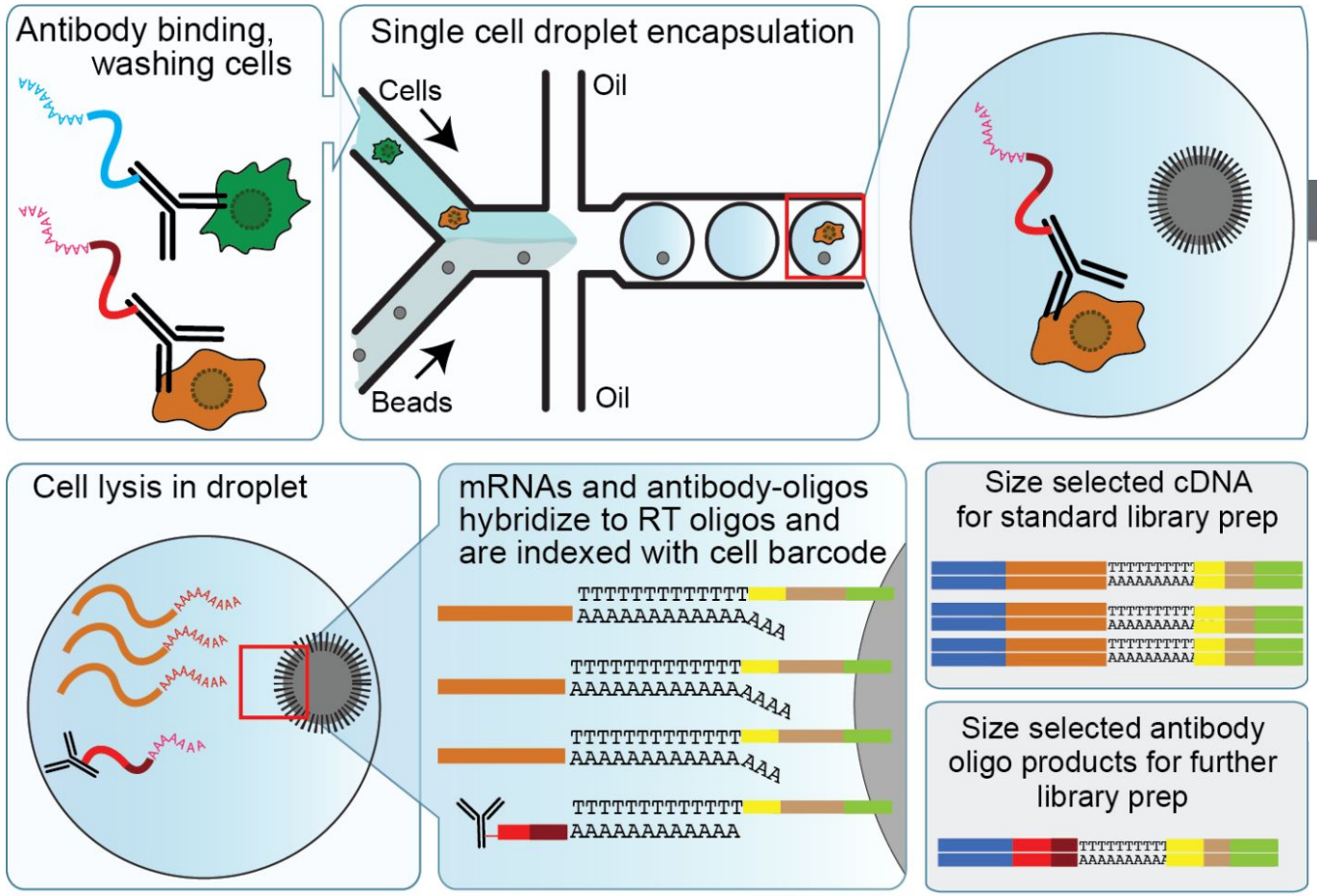
5 - Multiplexing and proteomics

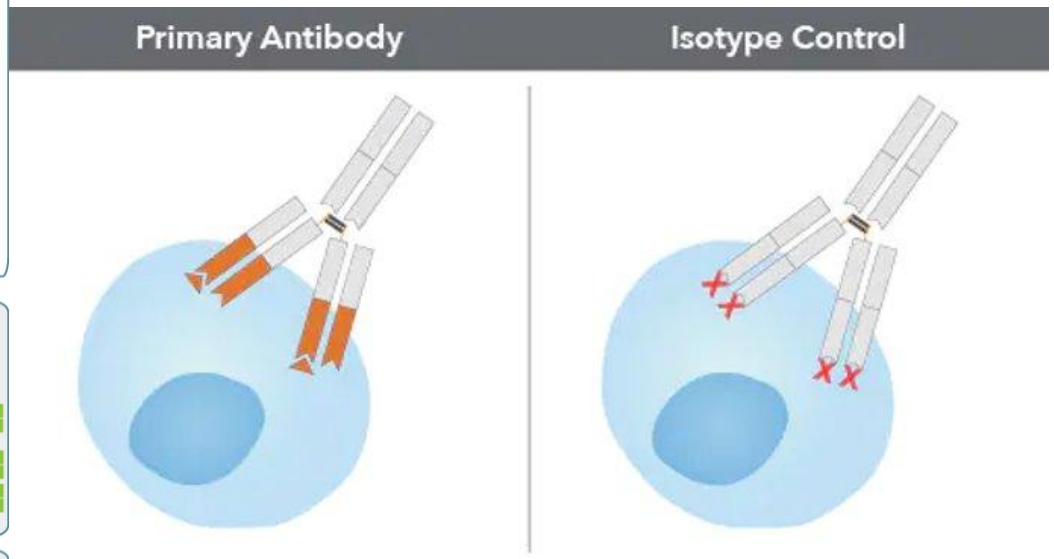
wellcome connecting science

What about protein analysis?

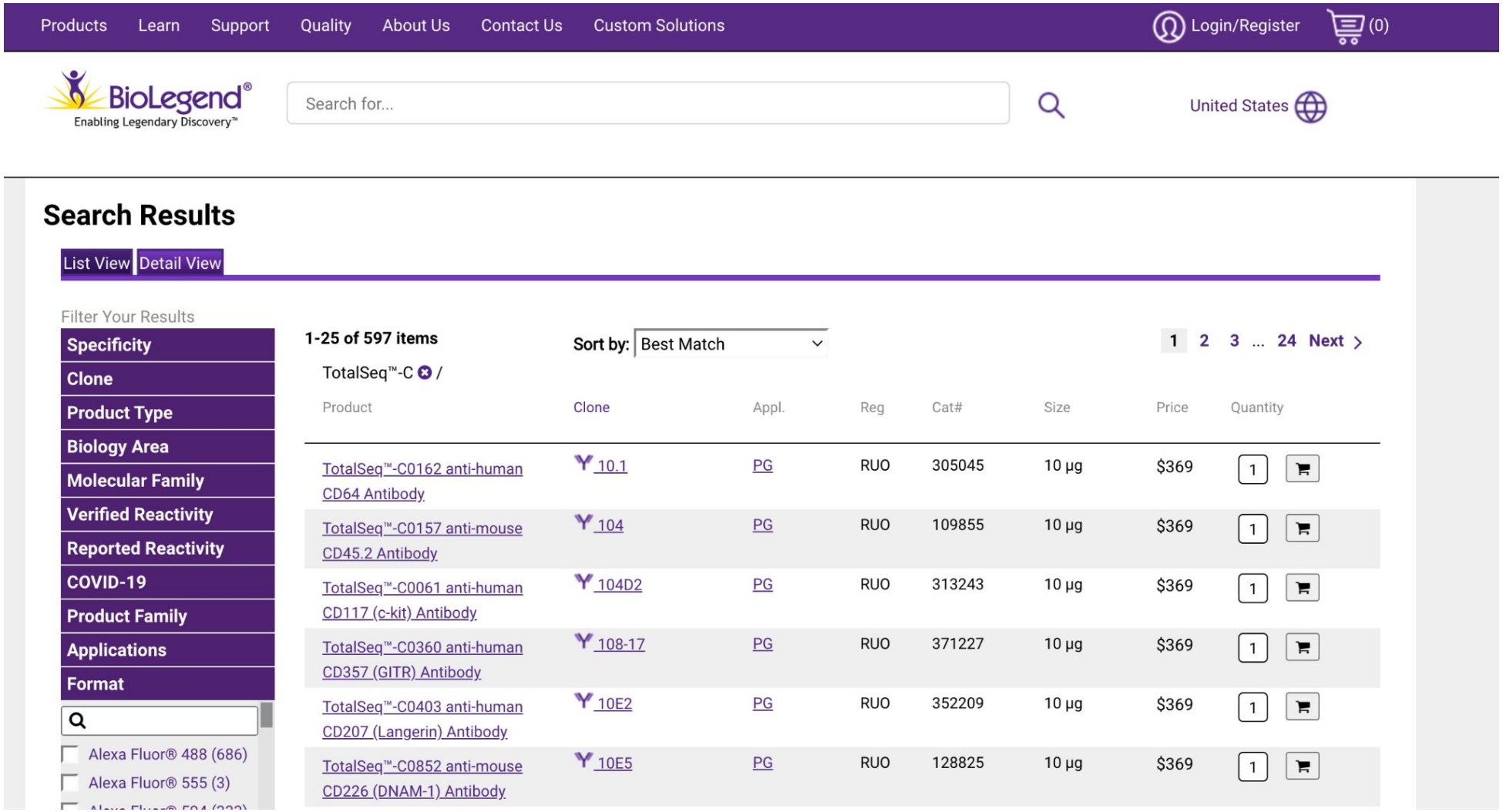


What about protein analysis?





Optimising CITEseq staining



Products Learn Support Quality About Us Contact Us Custom Solutions Login/Register (0)

BioLegend® Enabling Legendary Discovery™

Search for...

United States

Search Results

List View | Detail View

Filter Your Results

- Specificity
- Clone
- Product Type
- Biology Area
- Molecular Family
- Verified Reactivity
- Reported Reactivity
- COVID-19
- Product Family
- Applications
- Format

Alexa Fluor® 488 (686)
Alexa Fluor® 555 (3)

1-25 of 597 items Sort by: Best Match

1 2 3 ... 24 Next >

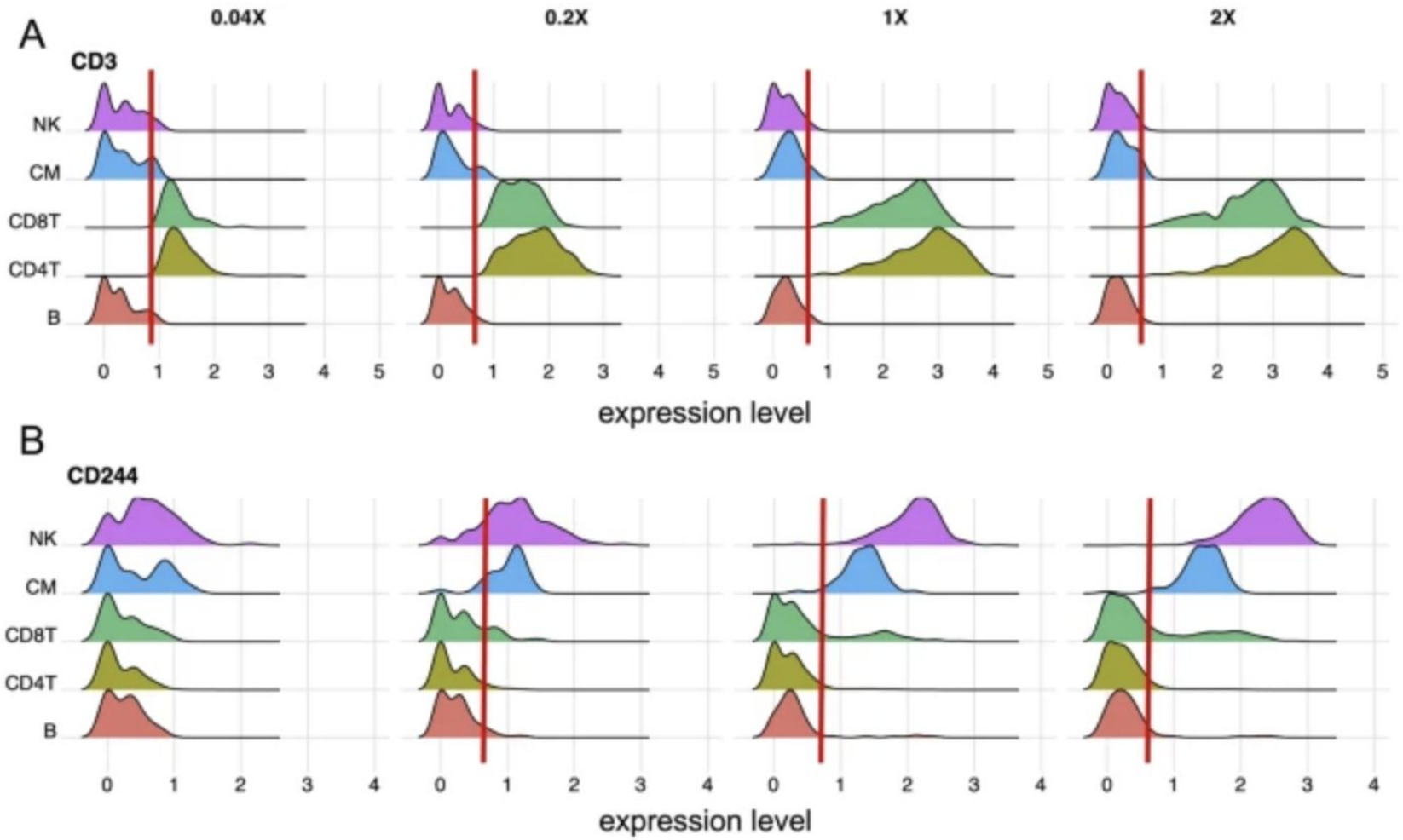
TotalSeq™-C /

Product	Clone	Appl.	Reg	Cat#	Size	Price	Quantity
TotalSeq™-C0162 anti-human CD64 Antibody	10.1	PG	RUO	305045	10 µg	$369	1
TotalSeq™-C0157 anti-mouse CD45.2 Antibody	104	PG	RUO	109855	10 µg	$369	1
TotalSeq™-C0061 anti-human CD117 (c-kit) Antibody	104D2	PG	RUO	313243	10 µg	$369	1
TotalSeq™-C0360 anti-human CD357 (GITR) Antibody	108-17	PG	RUO	371227	10 µg	$369	1
TotalSeq™-C0403 anti-human CD207 (Langerin) Antibody	10E2	PG	RUO	352209	10 µg	$369	1
TotalSeq™-C0852 anti-mouse CD226 (DNAM-1) Antibody	10E5	PG	RUO	128825	10 µg	$369	1

Optimising CITEseq staining

Optimisation for CITEseq experiments should start with FACS using PE

Followed by single cell sequencing



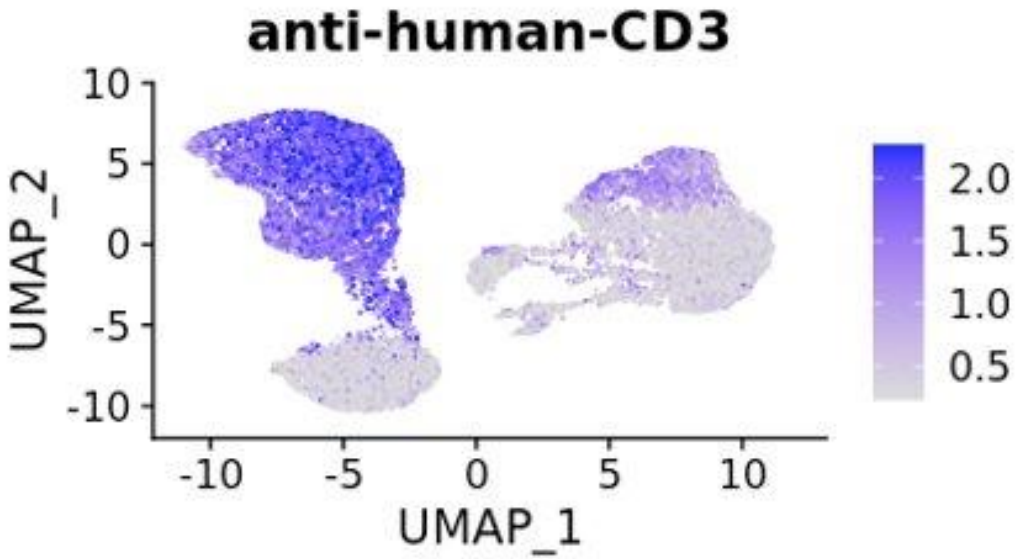
Scan me!

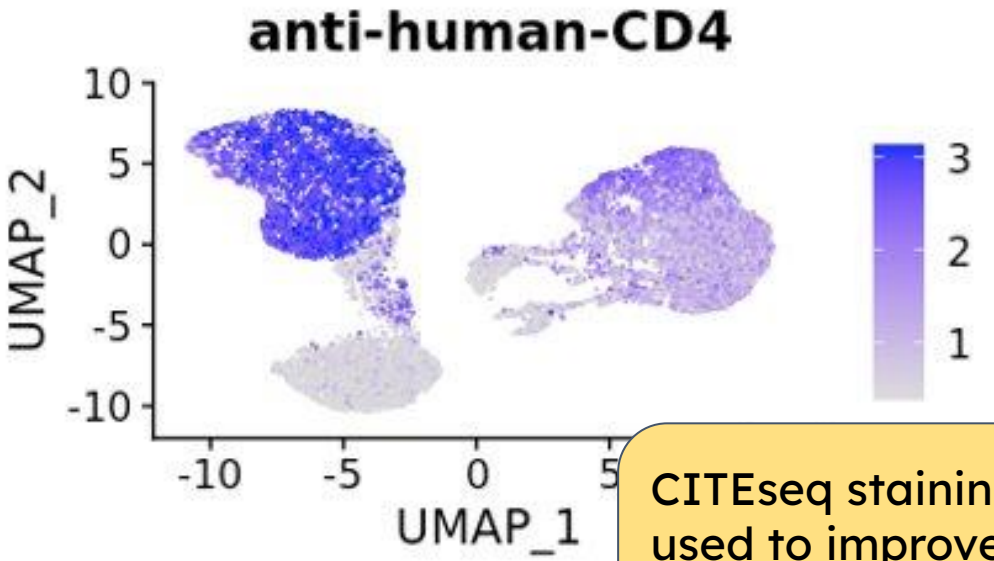
Article | Open access | Published: 02 December 2022

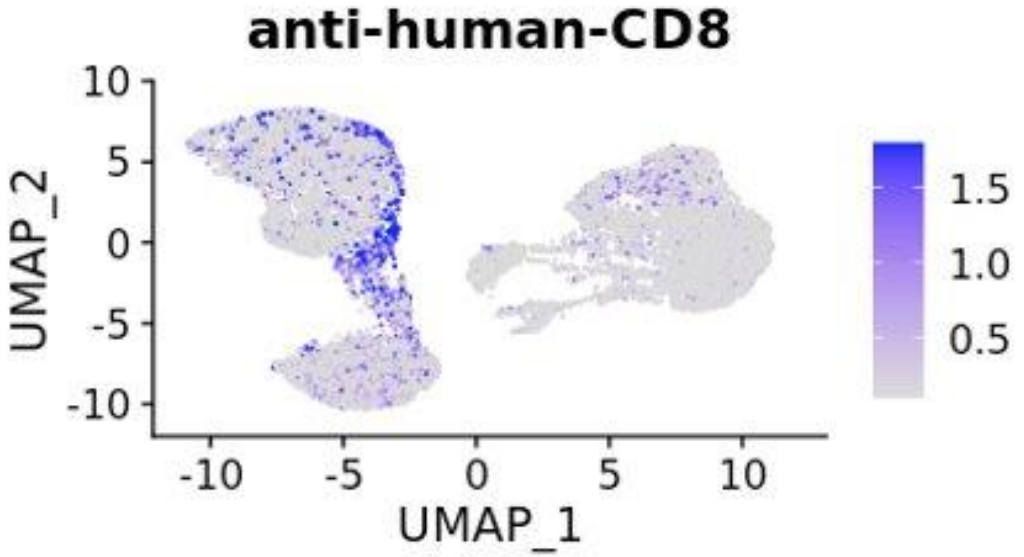
Titration of 124 antibodies using CITE-Seq on human PBMCs

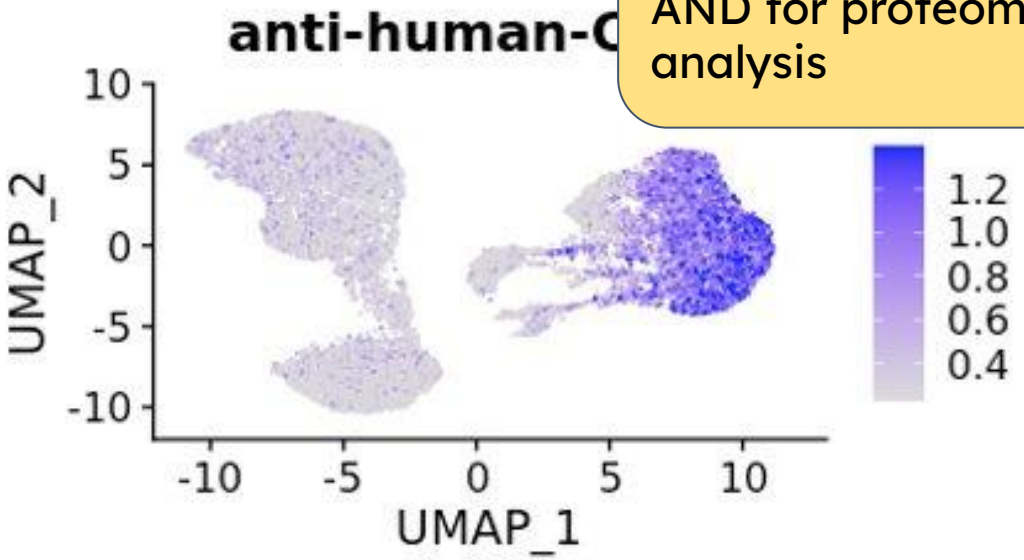
Felix Sebastian Nettersheim, Sujit Silas Armstrong, Christopher Durant, Rafael Blanco-Dominguez, Payel Roy, Marco Orecchioni, Vasantika Suryawanshi & Klaus Ley

Scientific Reports **12**, Article number: 20817 (2022) | Cite this article









CITEseq staining can be used to improve clustering AND for proteomics analysis

wellcome sanger institute

Multiplexing Samples

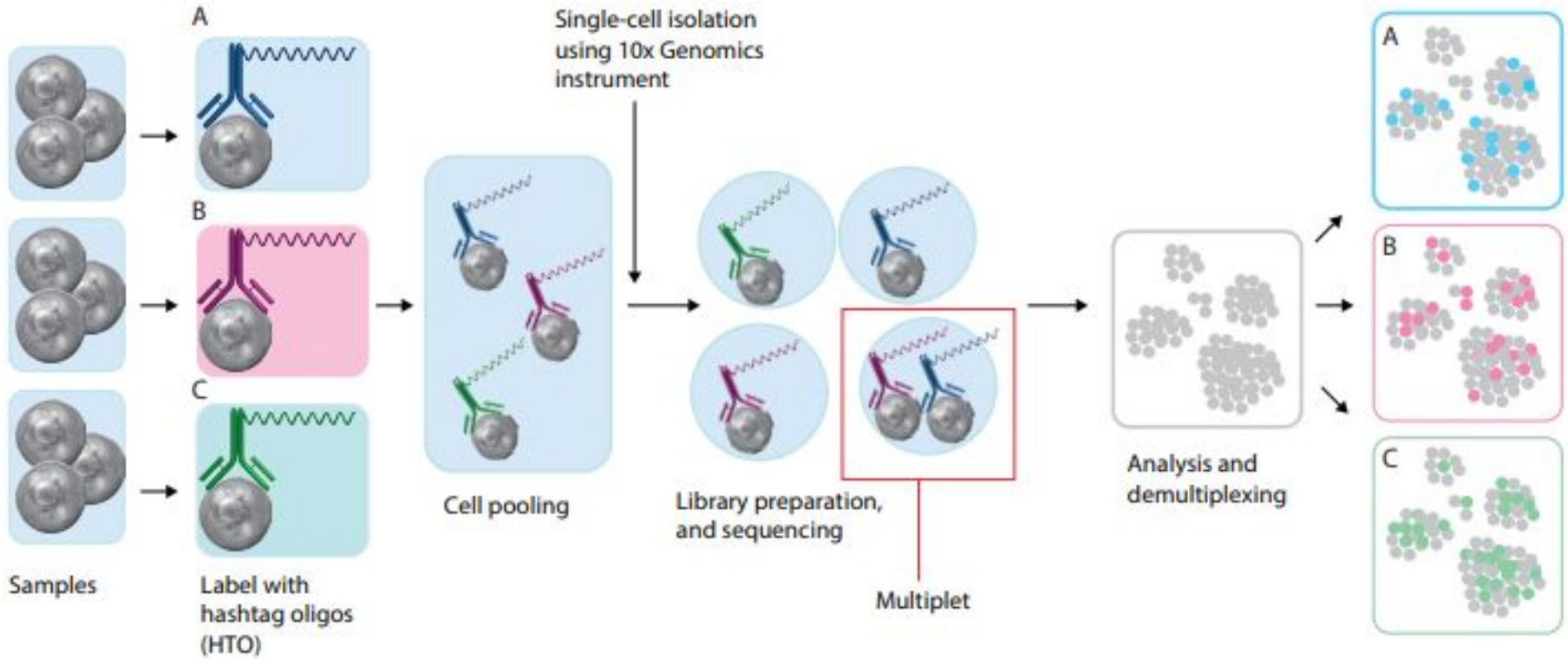


figure from biolegend.com

nature reviews genetics

Expert Recommendation | Published: 31 March 2023

Best practices for single-cell analysis across modalities

Lukas Heumos, Anna C. Schaar, Christopher Lance, Anastasia Litinetskaya, Felix Drost, Luke Zappia, Malte D. Lücken, Daniel C. Strobl, Juan Henao, Fabiola Curion, Single-cell Best Practices Consortium, Herbert B. Schiller & Fabian J. Theis ✉

Nature Reviews Genetics **24**, 550–572 (2023) | Cite this article

Scan me!

wellcome sanger institute

Questions

cjb@sanger.ac.uk
@carlajonesBio

TRYNKA GROUP

wellcome sanger institute