SINGLE CELL/NUCLEUS TECHNOLOGIES IN CANCER RESEARCH

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Overview of the course

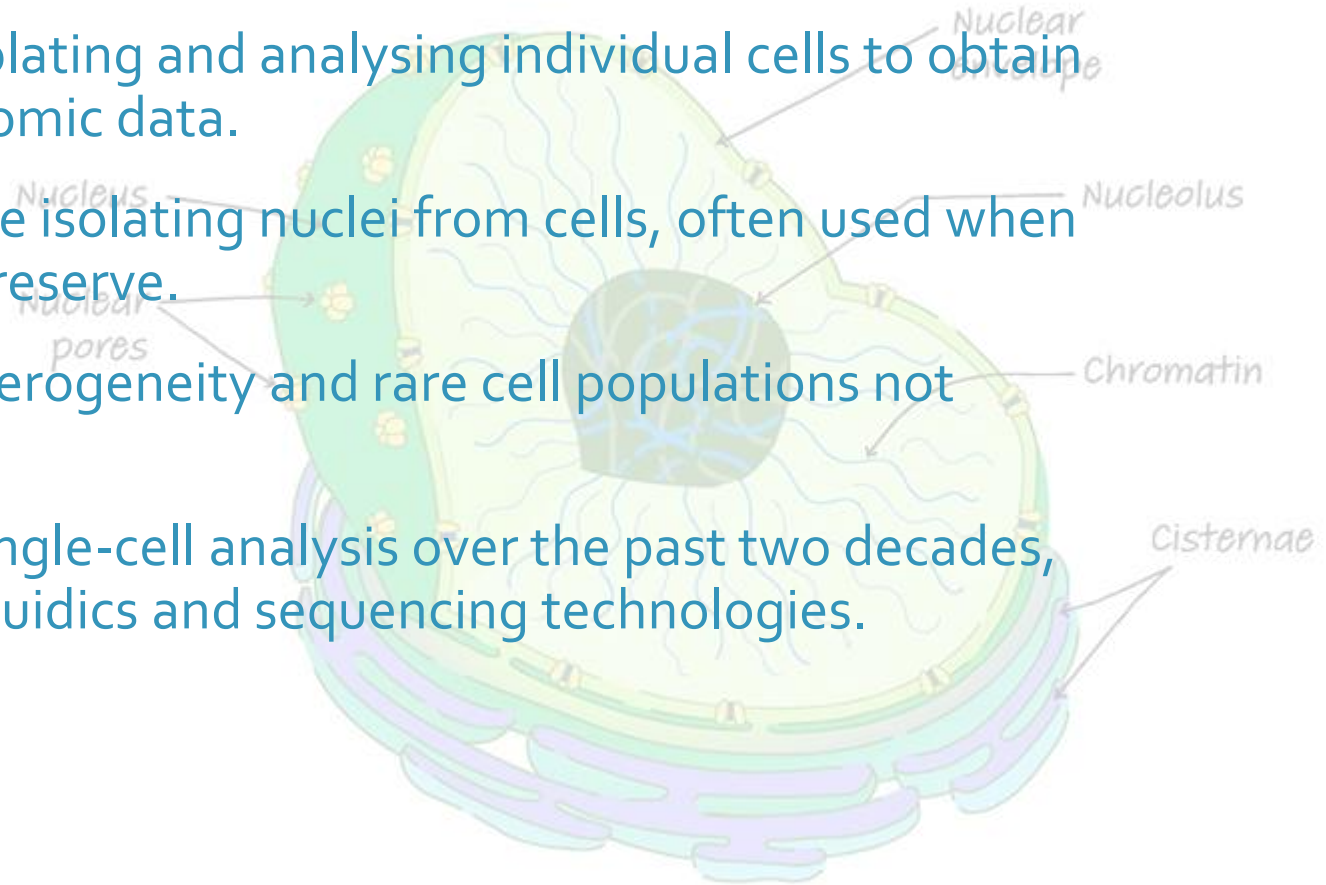
Date	1st Lecture	2nd Lecture
16th July	Icebreakers, ground rules and maybe and Intro to Molecular Biology and NGS	
17th July	Intro to Molecular Biology and NGS	Data analysis 1
18th July	Experiment: Nucleic acid extraction	Data analysis 2
19th July	Single-cell DNA, RNA and protein technologies	Proteomics, spatial technologies and epigenomics Data analysis 3
20th July	Data analysis 4	Experiment: Staining our own cells
21st July	Experiment: Gel electrophoresis	Data analysis 5 Data analysis 6
22nd July	Preparation for the final presentation	Final presentation and closing ceremony

Objectives

1. Understand the principles of single cell/nucleus technologies.
2. Compare single cell and single nucleus RNA sequencing.
3. Debate the impact of single-cell technologies versus bulk technologies.

What are single cell and single nucleus technologies?

- Single cell technologies involve isolating and analysing individual cells to obtain genomic, transcriptomic, or proteomic data.
- Single nucleus technologies involve isolating nuclei from cells, often used when cells are difficult to dissociate or preserve.
- Allows for the study of cellular heterogeneity and rare cell populations not apparent in bulk.
- Evolution from bulk methods to single-cell analysis over the past two decades, driven by advancements in microfluidics and sequencing technologies.



What are single cell technologies?

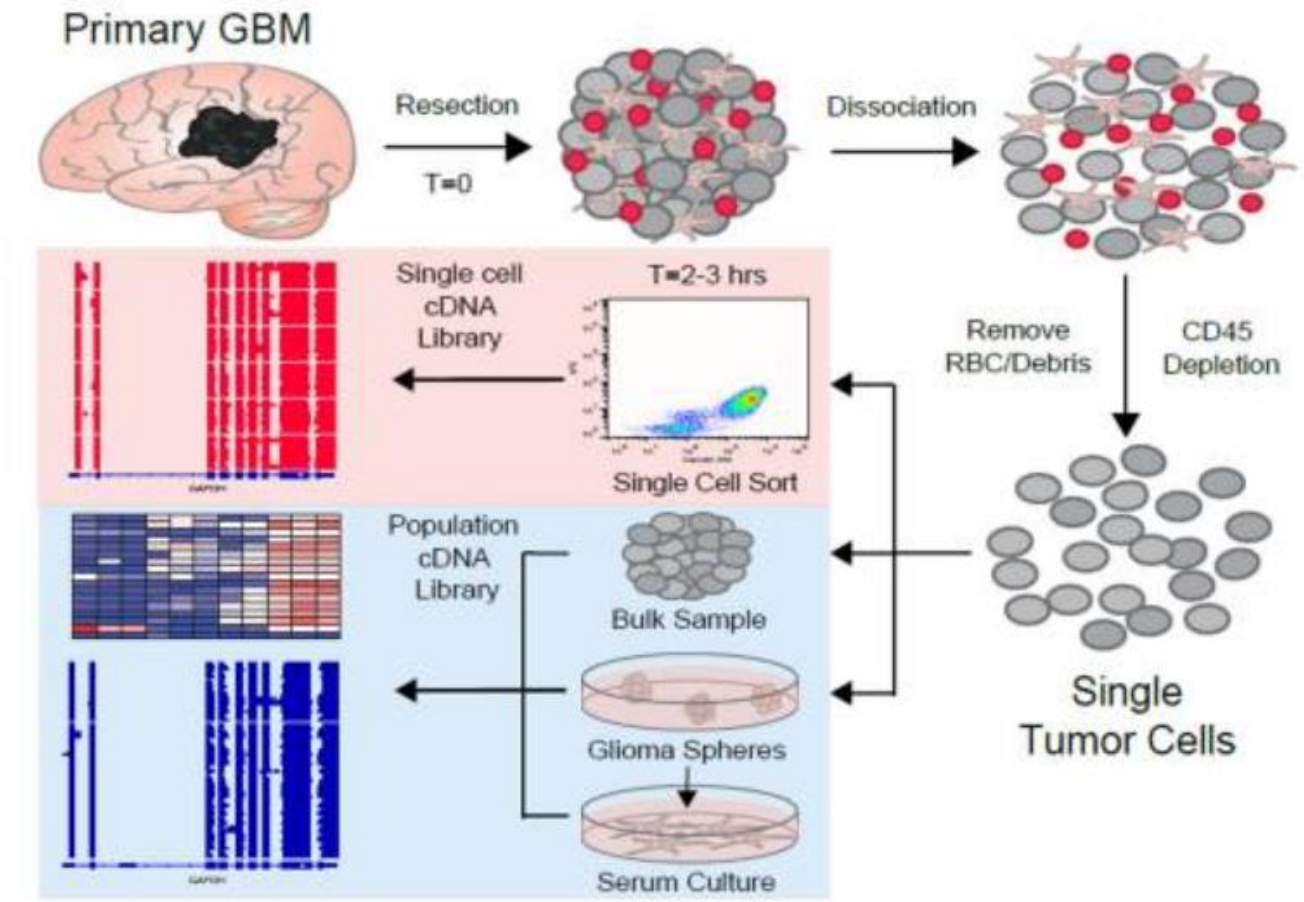
Why are single cell technologies needed?

- **Tumour Heterogeneity:**
  - Cancer is not a single disease but a collection of diverse cell types with different genetic and phenotypic profiles.
- **Identifying Rare Cell Populations:**
  - Discovering cancer stem cells and treatment-resistant cell types that drive recurrence and metastasis.
- **Improved Diagnostics and Treatment:**
  - Better targeted therapies and personalized medicine strategies.

Tumour heterogeneity

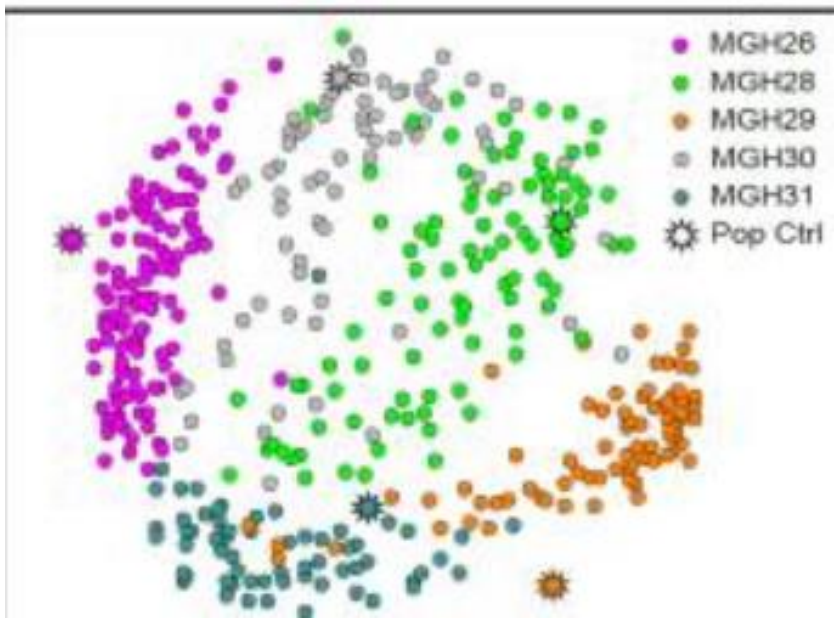
- Tumour heterogeneity: diverse cell populations within a tumour
- Patel et al. (2014)
  - Performed single-cell RNA sequencing on glioblastomas and quantified the extensive heterogeneity of these tumours
  - Cell types include macrophages, microglia, neurons and endothelial cells.
  - Identified potential targets for immunotherapy, such as and EGFR
  - Old study, methods since have advanced.

Tumour heterogeneity



Patel et al. (2014)

Tumour heterogeneity: new and old



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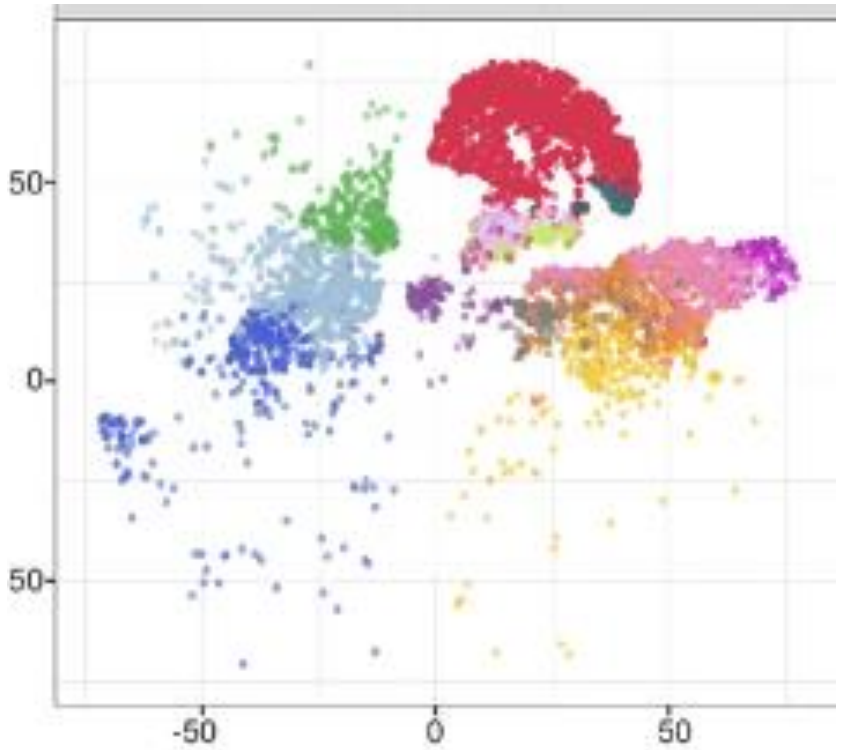
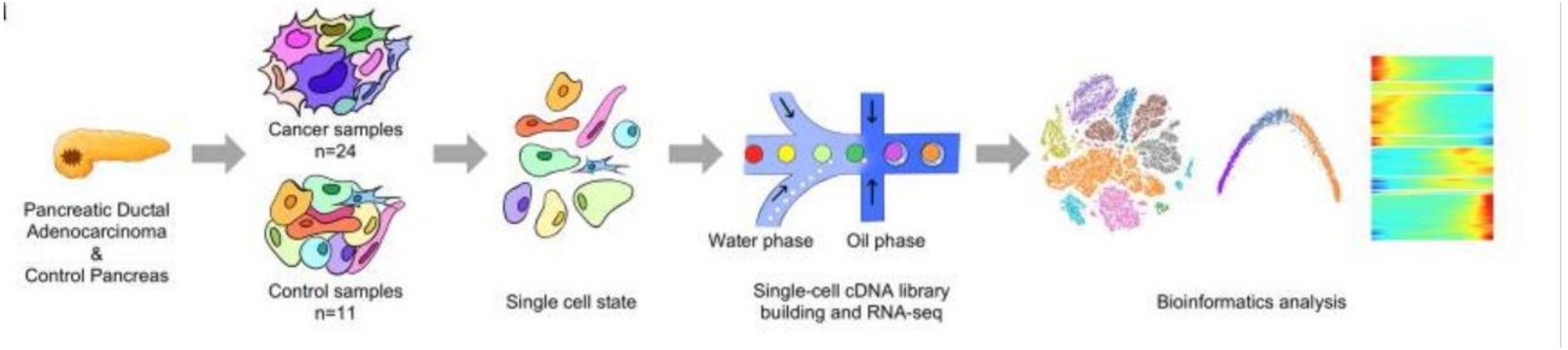


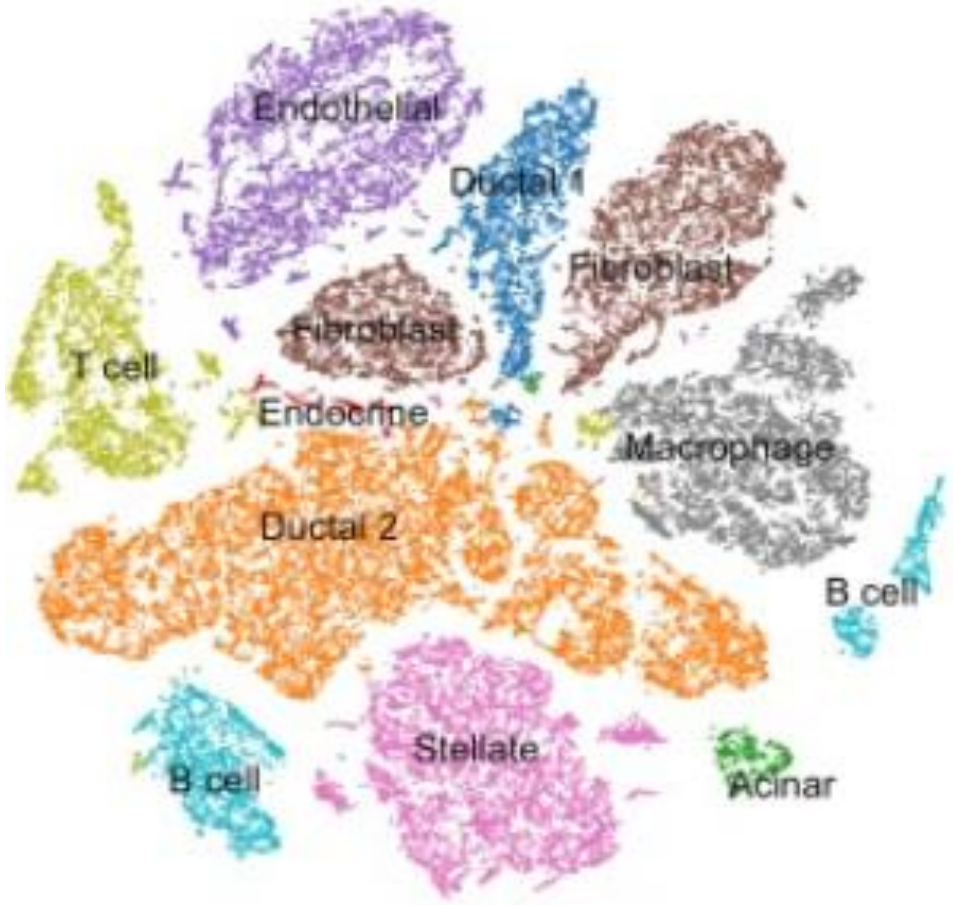
Schmassmann et al. (2023)

Detection of rare cell types

- Rare cell types can significantly influence disease progression and metastasis, e.g. cancer stem cells
- Single cell sequencing helps in identifying and characterizing these rare cells, which might be diluted in bulk analyses.
- Ting et al. (2021)



Rare cell types in predicting prognosis



Improved Diagnostics and Personalized Treatment

- Cancer has inter-patient and inter-tumour heterogeneity
- Tumour microenvironment (TME) influences response to treatment
- Personalised therapy will account for sensitivity and resistance of individual tumours
- Triosh et al. (2016)
  - Identified a cell state in a subpopulation that was linked to therapy resistance in metastatic melanoma

DNA technologies

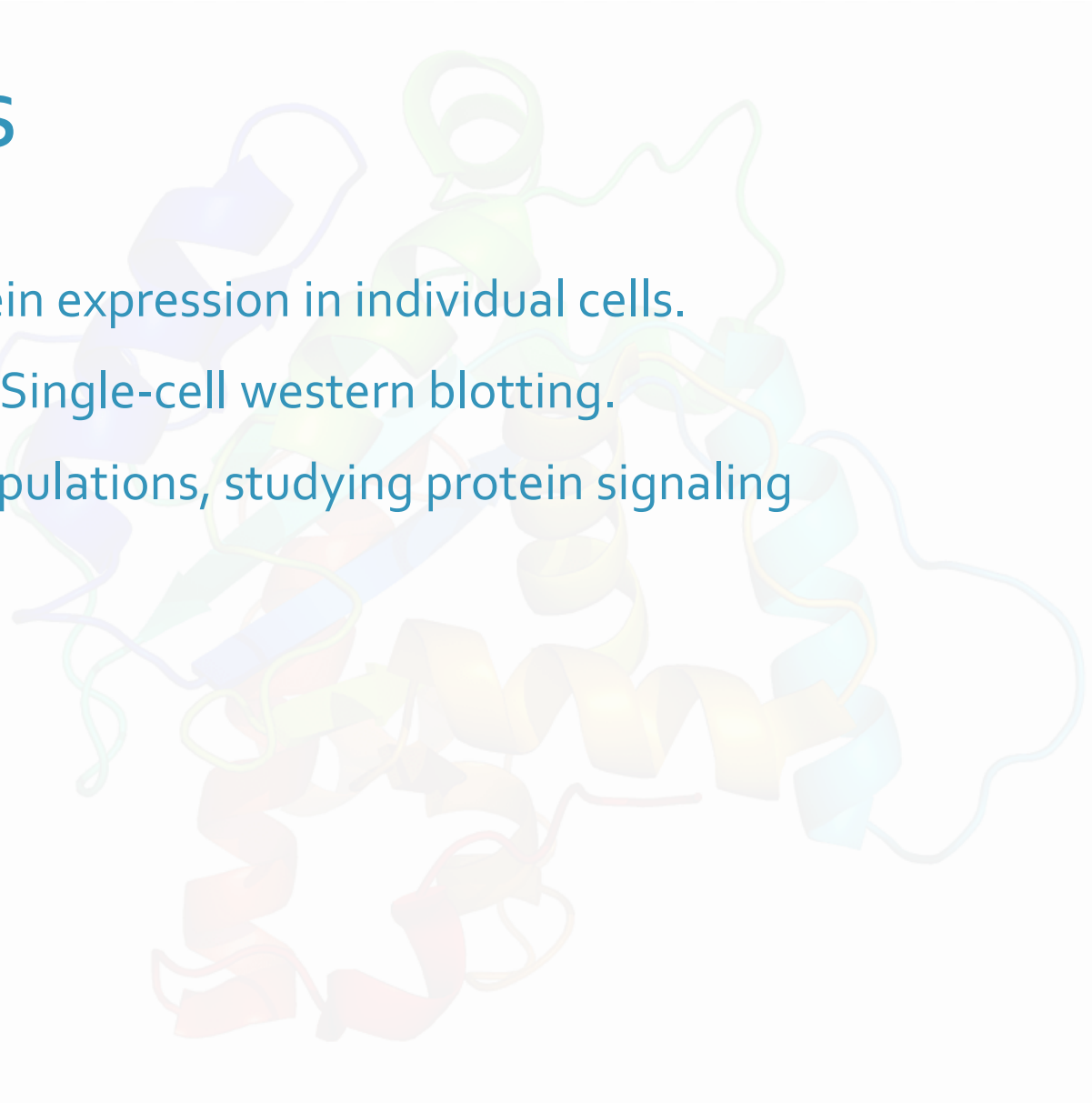
- Single-cell DNA sequencing: Technique to sequence the entire genome of individual cells.
- **Techniques:** Whole-genome amplification (WGA), single-cell exome sequencing.
- **Applications:** Identifying genetic mutations, studying clonal evolution in cancer.

RNA technologies

- Single-cell RNA sequencing (scRNA-seq): Measures the transcriptome of individual cells.
- **Techniques:** Drop-seq, SMART-seq, 10x Genomics.
- **Applications**: Understanding cell type diversity, identifying cell states, and mapping developmental trajectories.

Protein technologies

- Single-cell proteomics: Measures protein expression in individual cells.
- **Techniques:** Mass cytometry (CyTOF), Single-cell western blotting.
- **Applications:** Profiling immune cell populations, studying protein signaling pathways and modifications.



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Single Cell RNA Sequencing Pipeline

- **Nuclei Isolation:** Mechanical dissociation, enzymatic digestion to release individual cells.
- **Library Preparation:** Techniques like SMART-seq (full-length transcripts), Drop-seq (droplet-based), and 10x Genomics (high-throughput).
- **Sequencing:** Platforms such as Illumina for high accuracy and 10x Genomics for high throughput.
- **Data Analysis:** Bioinformatics tools for alignment, and differential expression

Single Nucleus RNA Sequencing Pipeline

1. **Nuclei Isolation:** Dounce homogenization, detergent lysis to isolate nuclei, especially from frozen or difficult-to-dissociate tissues.
2. **Library Preparation:** Can use specific snRNA-seq protocols that handle nuclear RNA
3. **Sequencing:** Similar platforms as scRNA-seq, adapted for nuclear RNA.
4. **Data Analysis:** Adjusted bioinformatics pipelines to account for nuclear RNA data, typically using the same tools as scRNA-seq with some modifications.

ScRNA- vs SnRNA-seq Pros

Feature	scRNA-seq	snRNA-seq
Pros		
Cellular Detail	Captures entire cellular transcriptomes, including cytoplasmic RNA.	Focuses on nuclear RNA, useful for gene regulation insights.
Cell Viability	Works best with viable, live cells.	Can use non-viable or frozen cells, offering broader application.
Application	Ideal for fresh tissues and detailed cellular studies.	Good for hard-to-dissociate or archived tissues.

ScRNA- vs SnRNA-seq Cons

Feature	scRNA-seq	snRNA-seq
Cons		
Sample Limitation	Requires live cells; not suitable for archived tissues or solid tumours	May miss cytoplasmic mRNA transcripts.
Technical Complexity	Requires immediate processing and high-quality control.	Adjustments needed for handling nuclear material only.
Cost	Higher due to need for live cells and immediate processing.	Generally lower, allows use of preserved samples.

Cell vs Nuclei Isolation

- Single-cell RNA sequencing captures the transcriptome of intact, live cells.
- Single-nucleus RNA sequencing isolates nuclei from fixed or frozen tissues, capturing nuclear RNA.

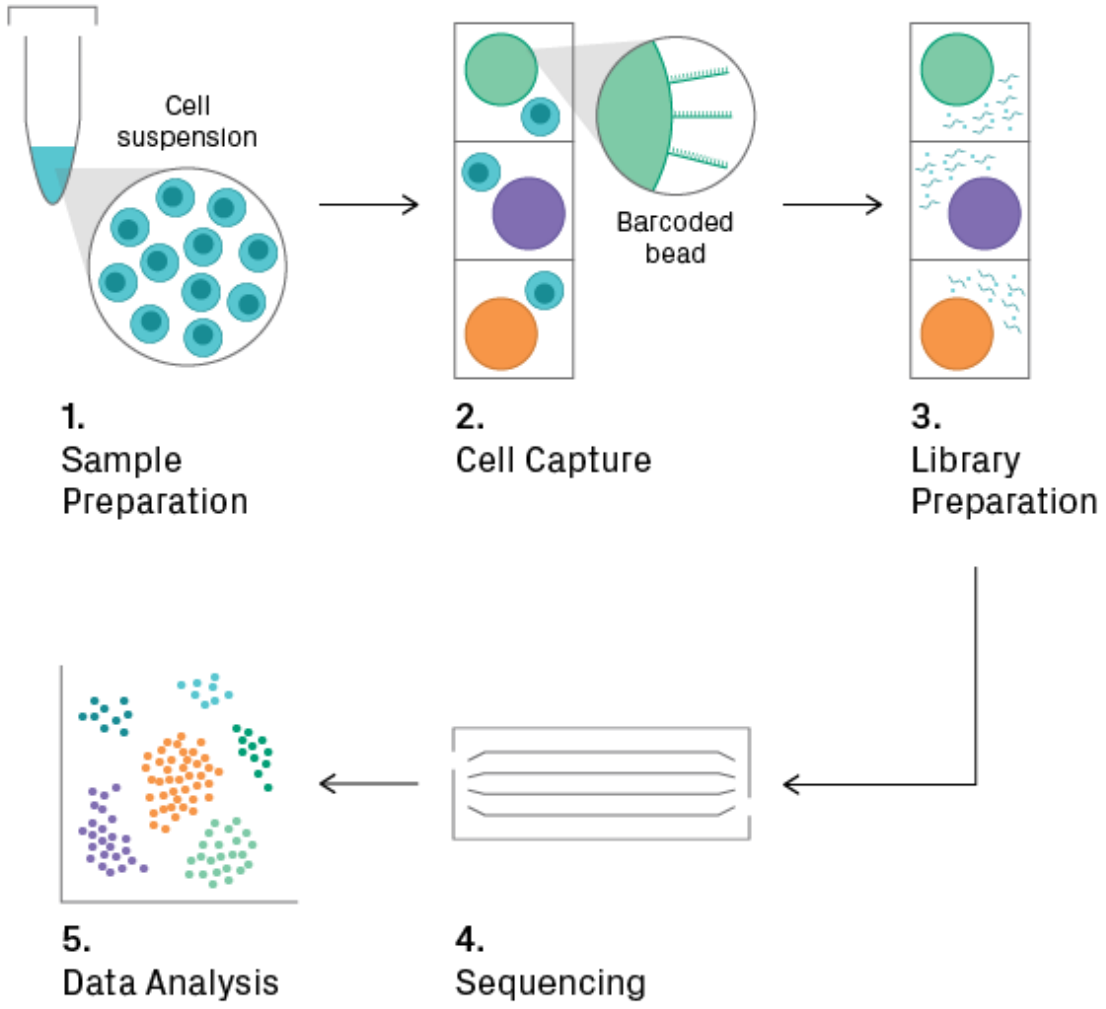
Cell vs Nuclei Isolation

Single Cell	**Single Nucleus**
Enzymatic dissociation of tissues to obtain a suspension of live cells.	Lysis of cells to release nuclei.
Sorting cells using microfluidics	Sorting cells using microfluidics

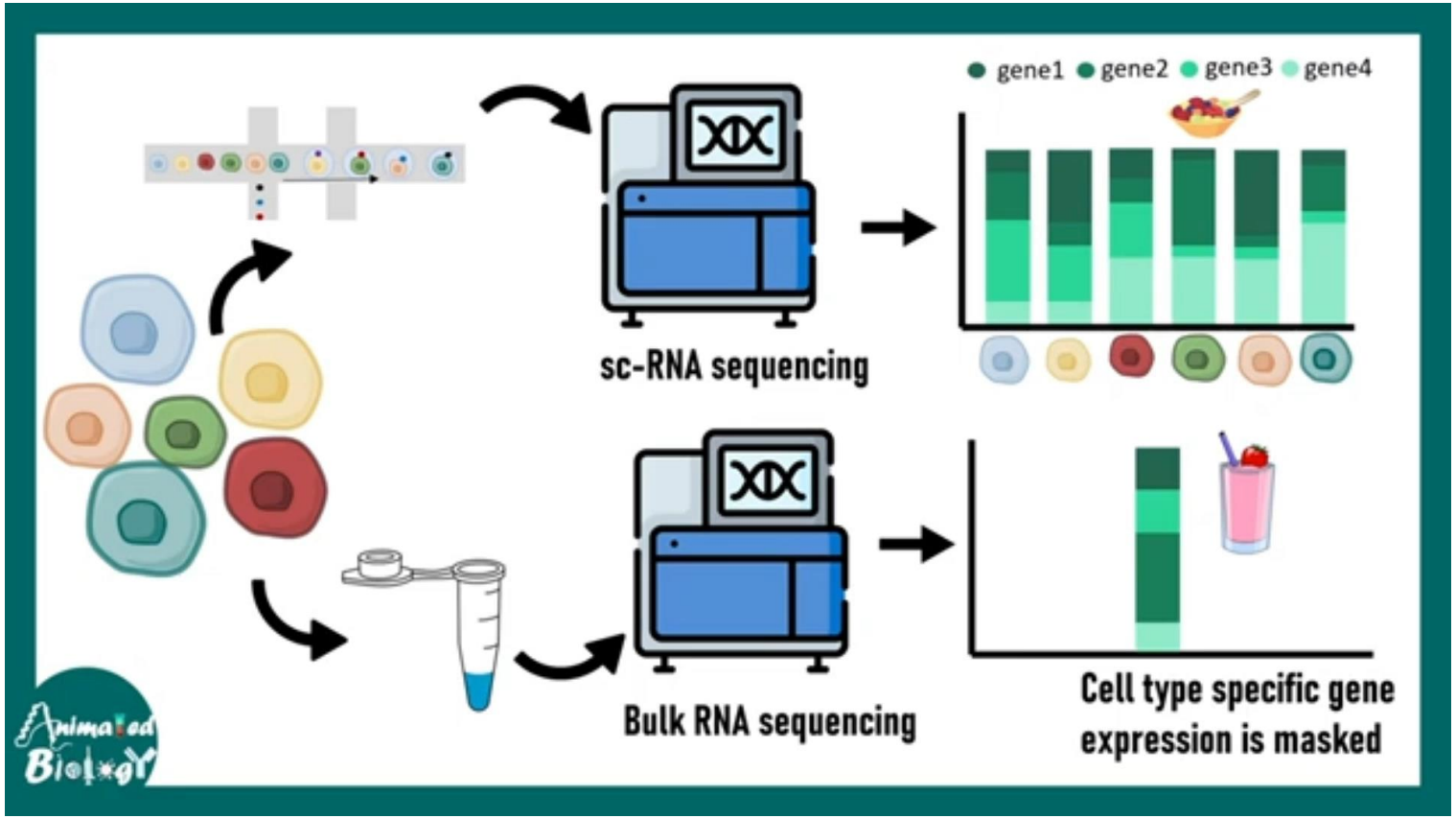
Library preparation

- RNA is converted into a library of cDNA fragments which can be sequenced
- Types of library prep methods for sc-RNA-seq
  - Plate-based methods
  - Microfluidics
  - Droplet-based methods

Single-cell RNA sequencing workflow



Single-cell RNA sequencing workflow



Quiz!

https://create.kahoot.it/share/enter-kahoot-title/bfbf7632-d3b7-4ccd-8bdf-76d460286d2f

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Any questions?

Debate

"With the emergence of single-cell technologies, there is no longer a place for bulk RNA sequencing. Discuss."

- Up to 40 minutes to prepare
- Up to 25 minutes to debate

Structure of debate

1. Both sides introduce their stance
2. Main arguments (at least 1 main + 1 support)
3. Rebuttals (at least 1 counterargument)
4. Closing statements

Provide case studies to support your arguments.

Teams

1. Team 1 will argue that bulk RNA sequencing is no longer needed with the advent of single cell sequencing.
2. Team 2 will argue that bulk RNA sequencing is still necessary and can be used with single cell sequencing (or instead of).

Resources to start

For applications of single cell and some comments on future perspectives:

Application of single-cell sequencing to the research of tumor microenvironment.pdf

sequencing in cancer- Applications vadvances and emerging challenges.pdf

Single-cell RNA sequencing technologies and applications A brief overview.pdf

For applications both bulk and single cell:

RNA sequencing new technologies.pdf

RNA sequencing new technologies and applications in cancer research.pdf

Changing Technologies of RNA Sequencing and Their Applications in Clinical Oncology.pdf

From bulk, single-cell to spatial RNA sequencing.pdf

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References

- https://elifesciences.org/articles/92678
- https://www.science.org/doi/10.1126/science.1254257?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
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- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4944528/