



VALT Summer School

Long-reads Transcriptomics

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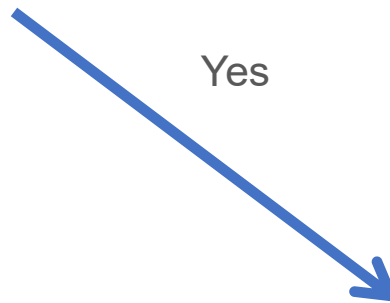
Section 2

Considerations for single-cell sequencing

Understanding the differences between bulk and single-cell approaches

- What is ***your question?***
- Can your question be answered using bulk sequencing?

Yes

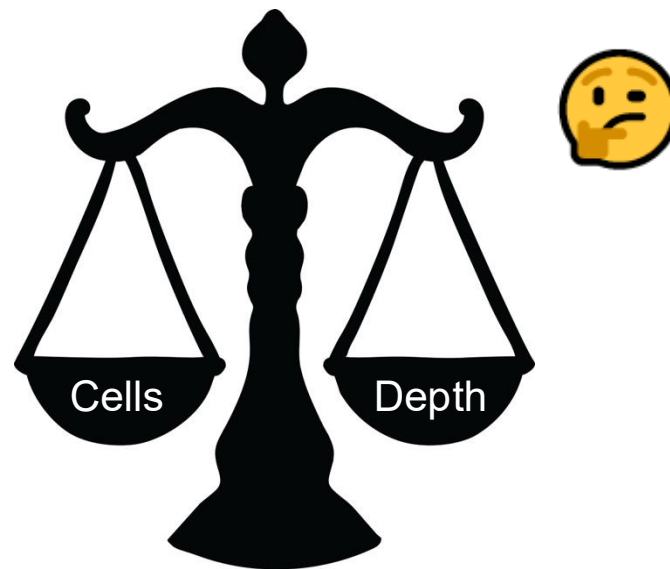


Proceed with bulk sequencing

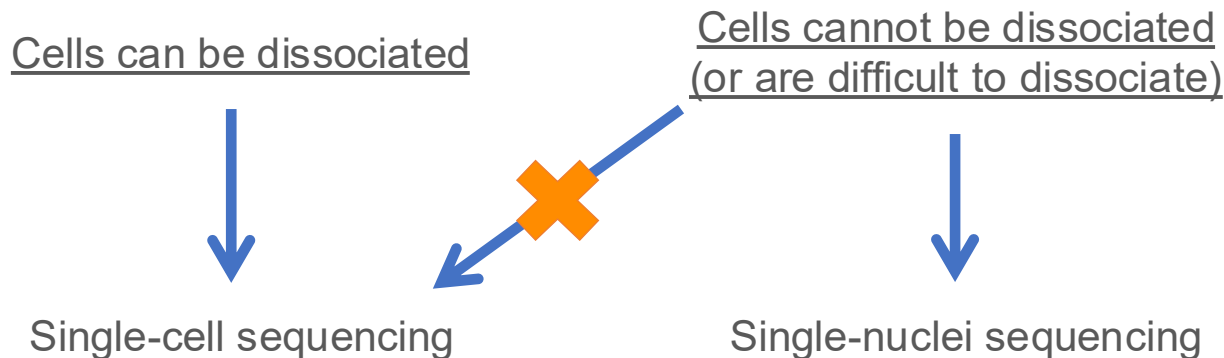
- What is ***your question?***
- Can your question be answered using bulk sequencing?

No

- Common vs rare cells
- Accessible?
- Rare events vs gene markers
- Full-length transcripts?



- You need to do single-cell sequencing: ***can you isolate your cells?***



Different biological insights

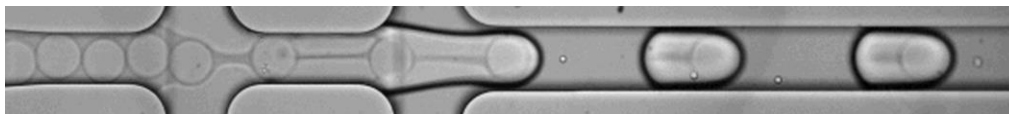
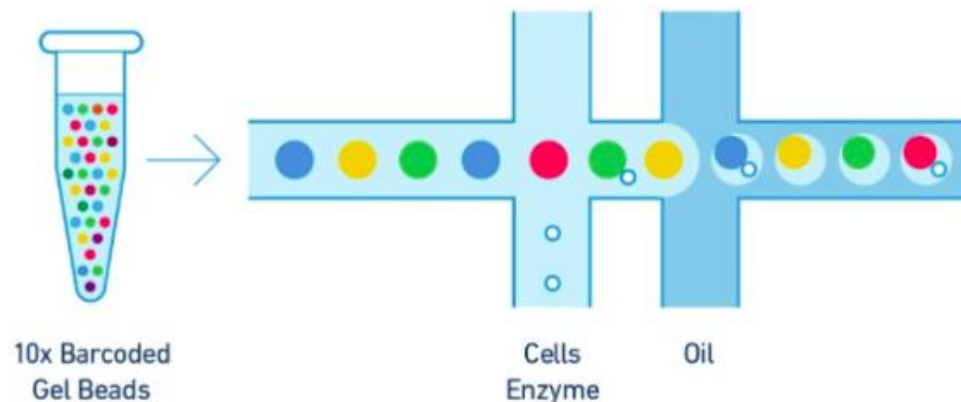
- How many samples you need?
- How many cells can you isolate?
- Do you have access to facilities/experts?
- What is your budget?



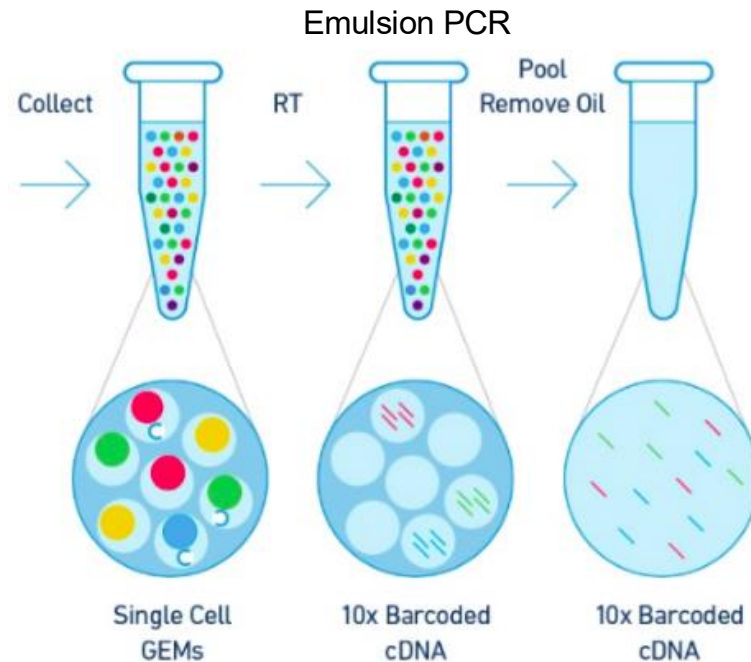
Berkshire Community College
Bioscience Image Library, Public
Domain Dedication (CC0)



PARSE
BIOSCIENCES



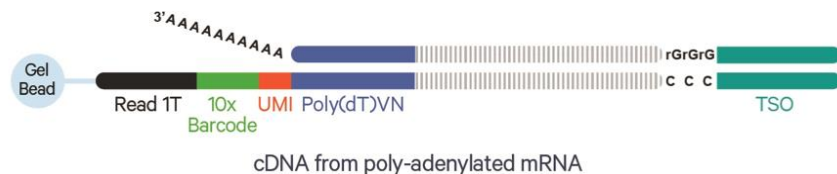
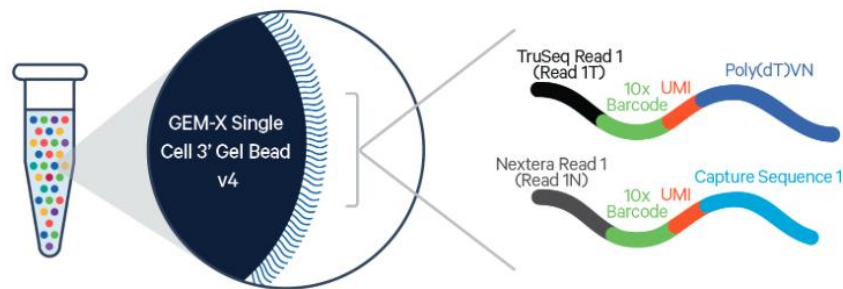
<https://www.10xgenomics.com/blog/the-next-generation-of-single-cell-rna-seq-an-introduction-to-gem-x-technology>



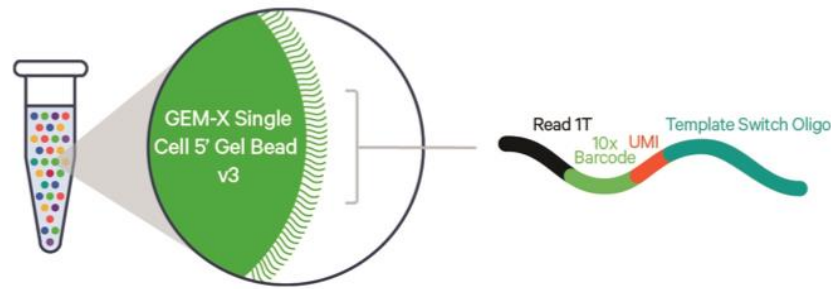
Gel Bead-In-Emulsion

Adapted from <https://bioinformatics.uconn.edu/single-cell-rna-sequencing-cell-ranger-2/>

3' and 5' 10x kits have different read designs



Adapted from https://cdn.10xgenomics.com/image/upload/v1710230393/support-documents/CG000731_ChromiumGEM-X_SingleCell3_ReagentKits_v4_UserGuide_RevA.pdf



Adapted from https://cdn.10xgenomics.com/image/upload/v1710231087/support-documents/CG000733_ChromiumGEM-X_SingleCell5_ReagentKitsv3_UserGuide_RevA.pdf

Multiple kit versions – CB whitelist, UMI length, ...

ONT does not use specific kits for single-cell experiments

Ligation Sequencing Kit V14 SQK-LSK114



<https://store.nanoporetech.com/uk/ligation-sequencing-kit-v14.html>

PCR Expansion EXP-PCA001



<https://store.nanoporetech.com/us/pcr-expansion.html>



Kinnex single-cell RNA kit

Kit advantage

Cell type-specific isoform discovery with the highest accuracy

Total throughput

30-40M (Sequel II)
50-60M (Vega)
100-120M (Revio SPRQ)

Adapted from <https://www.pacb.com/technology/kinnex/>

Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome

Double-stranded DNA



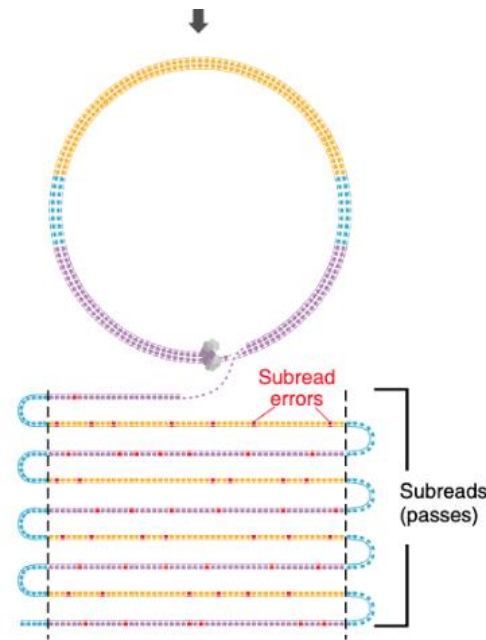
Ligate adapters



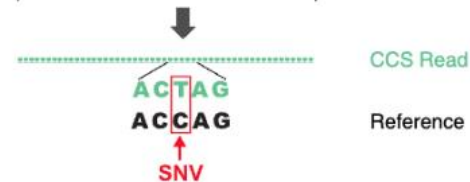
Anneal primer and
bind DNA polymerase



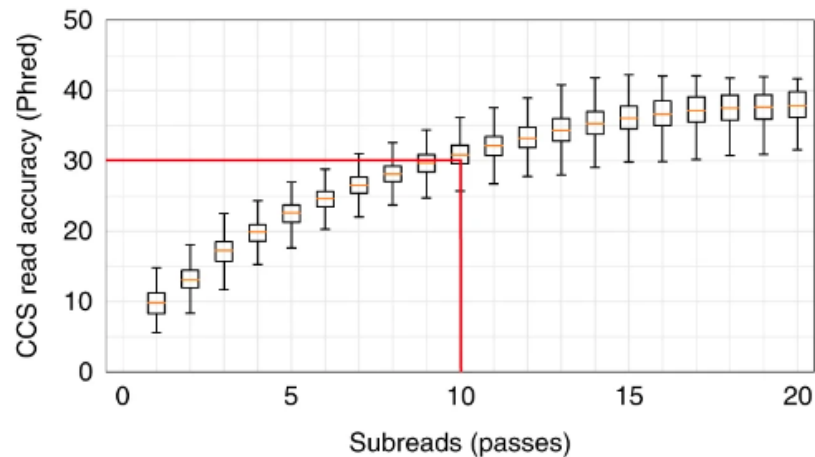
Sequence



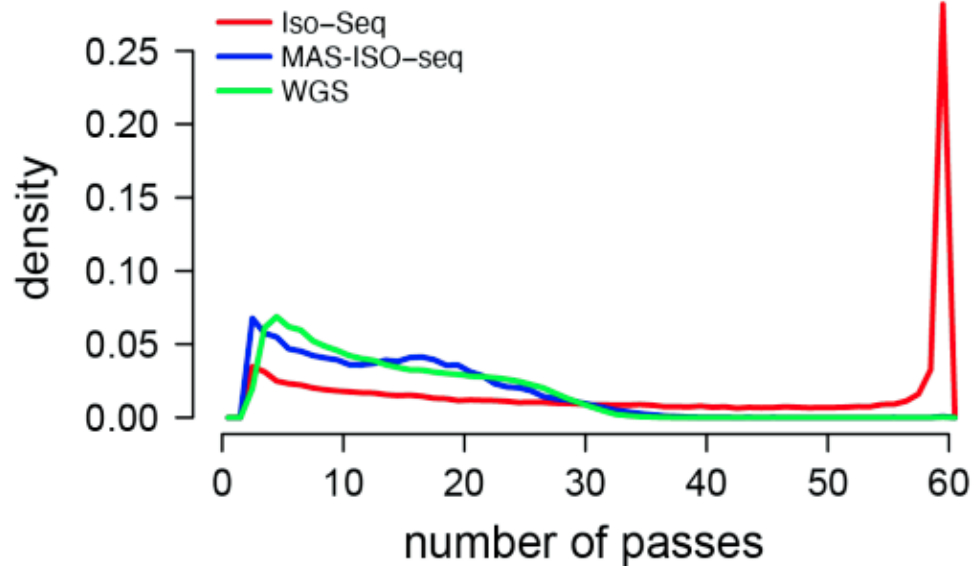
Generate
consensus read



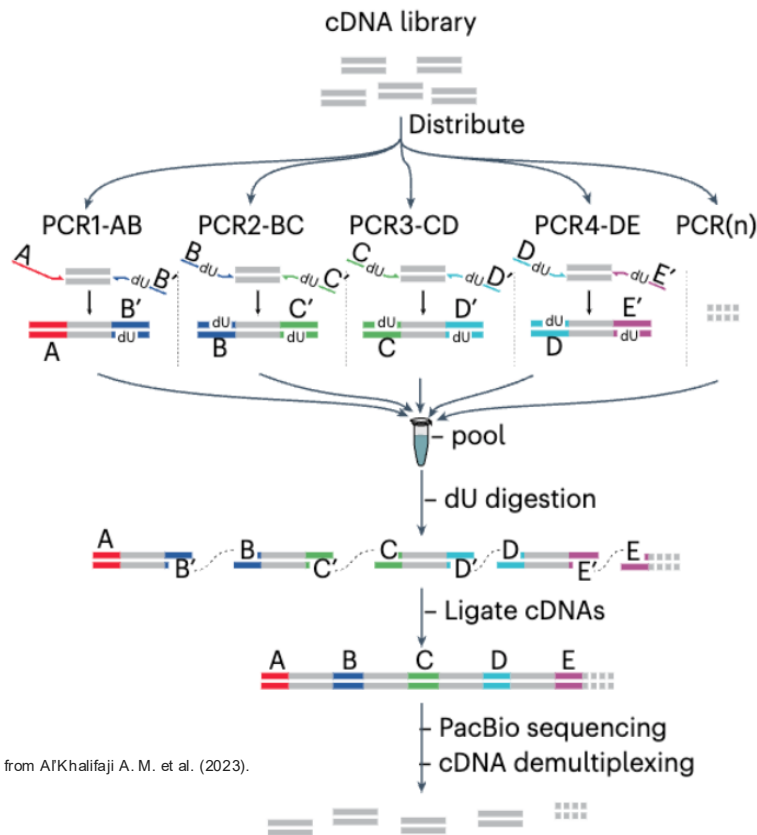
Adapted from Wenger, A.M. et al. (2019).



Adapted from Wenger, A.M. et al. (2019).



Adapted from AlKhalifaji A. M. et al. (2023).



Adapted from AfKhalifaji A. M. et al. (2023).

nature biotechnology

Brief Communication

<https://doi.org/10.1038/s41587-023-01815-7>

High-throughput RNA isoform sequencing using programmed cDNA concatenation

- Input cDNA comes from 10X output
- Commercial PacBio's kit → **Kinnex**



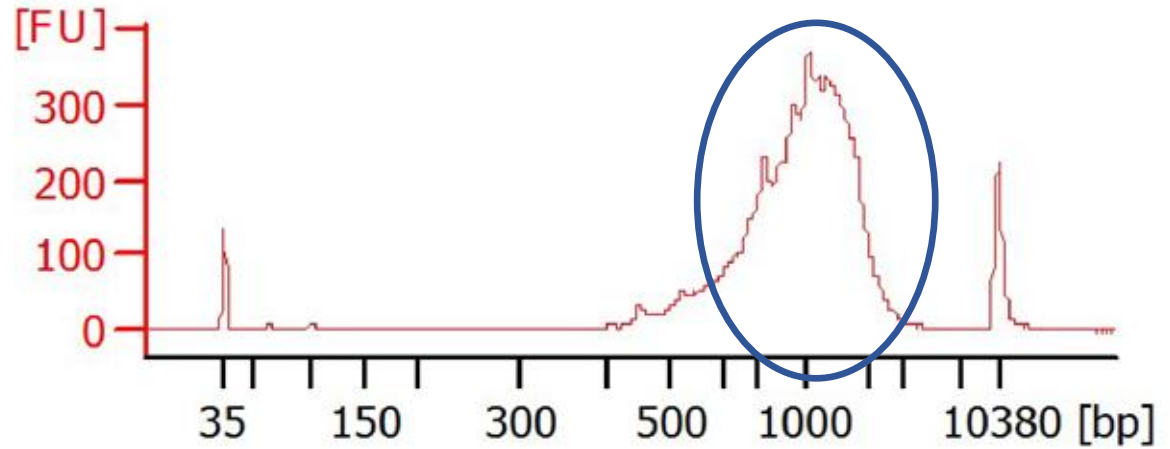
Kinnex single-cell RNA kit

- **Input cDNA** comes from 10x single-cell 3' or 5' kit
- **Number of samples** → 12
- **Working time** → 3 days for up to 12 samples
- **cDNA quantity input** → 15-75ng
- **Target number of cells** → 3,000 – 10,000 cells per library
- **cDNA quality input** → Check size distribution

<https://www.pacb.com/wp-content/uploads/Procedure-checklist-Preparing-Kinnex-libraries-using-Kinnex-single-cell-RNA-kit.pdf>



Kinnex single-cell RNA kit





Kinnex single-cell RNA kit

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- **cDNA quantity input** → 15-75ng
- **Target number of cells** → 3,000 – 10,000 cells per library
- **cDNA quality input** → Check size distribution
- **Number of concatenated transcripts** → 16

<https://www.pacb.com/wp-content/uploads/Procedure-checklist-Preparing-Kinnex-libraries-using-Kinnex-single-cell-RNA-kit.pdf>

- ***Why it is important to check 10x kit version?***

- 1 Focus on your question
- 2 Evaluate if single-cell sequencing is needed and can be performed
- 3 Take data analysis into consideration since the beginning
- 4 Choose technologies and kits that suit your question more

Thank You!



<https://longtrec.eu>