LongTREC

Funded by
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Bioinformatics Summer School

Long-reads Transcriptomics

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LongTREC - The Long-reads TRanscriptome European Consortium
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Section 2

Considerations for single-cell sequencing

Understanding the differences between bulk and single-cell approaches

Do you need single cells?

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

Yes

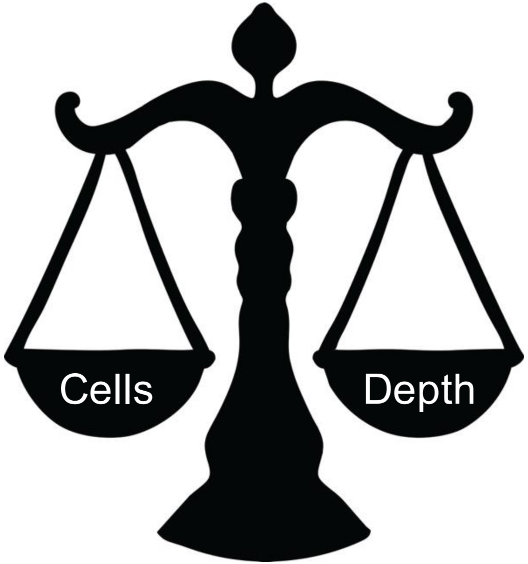
Proceed with bulk sequencing

Do you need single cells?

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

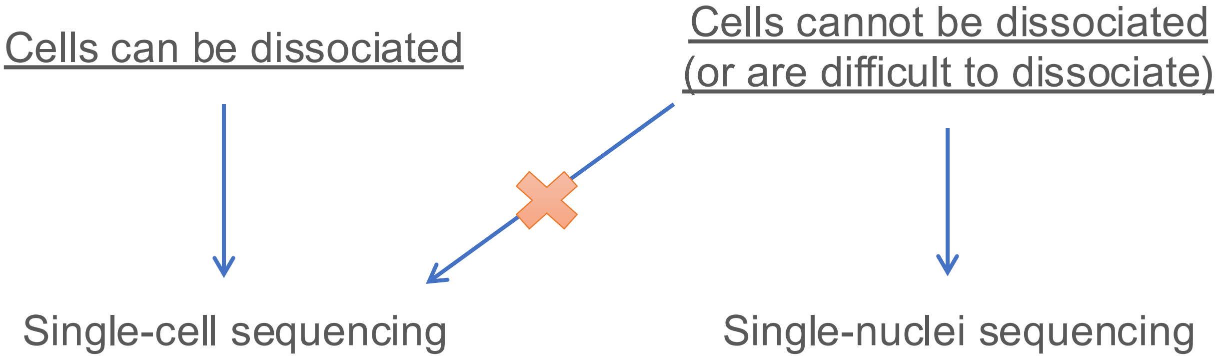
No

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



Getting your cells singled

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



<u>Cells can be dissociated</u>

<u>Cells cannot be dissociated
(or are difficult to dissociate)</u>

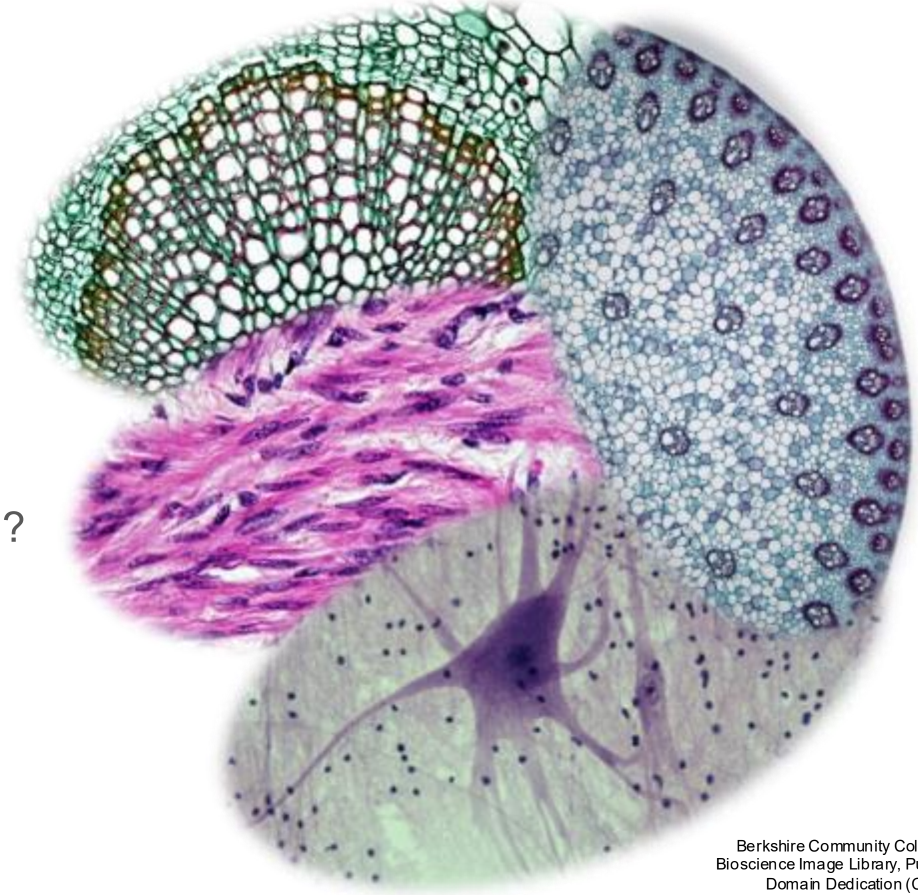
Single-cell sequencing

Single-nuclei sequencing

Different biological insights

Additional considerations

- 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)

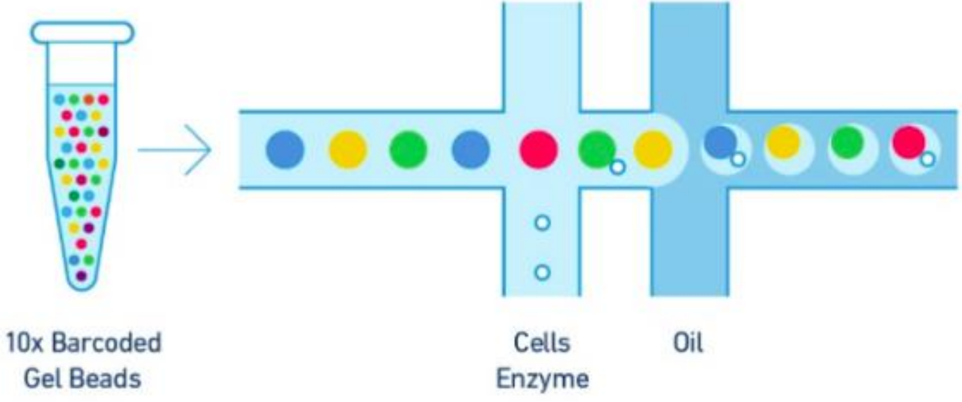
Most common single-cell barcoding kits

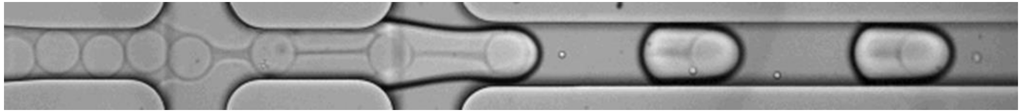
10x GENOMICS

PARSE
BIOSCIENCES

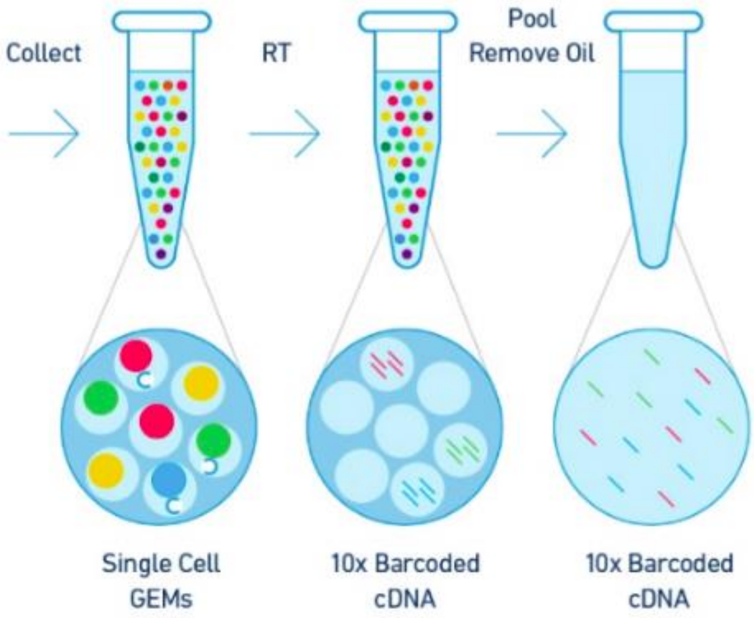
10x barcoded cDNA library overview

Emulsion PCR





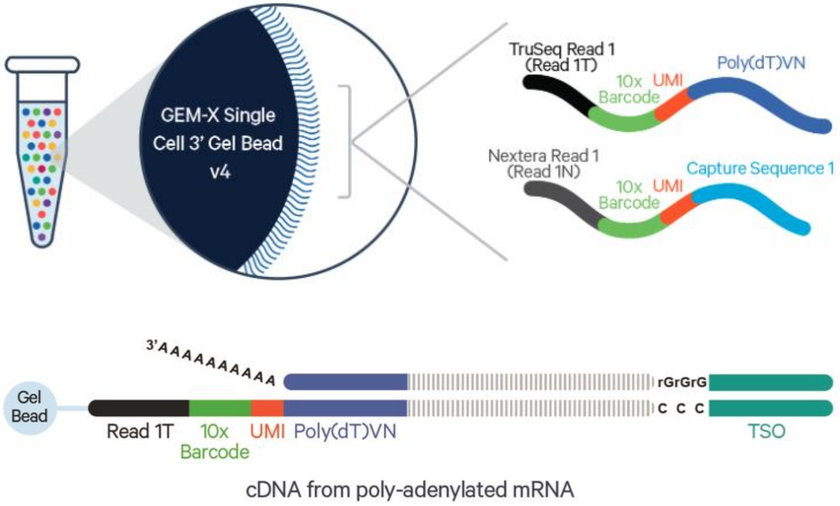
https://www.10xgenomics.com/blog/the-next-generation-of-single-cell-rna-seq-an-introduction-to-gem-x-technology



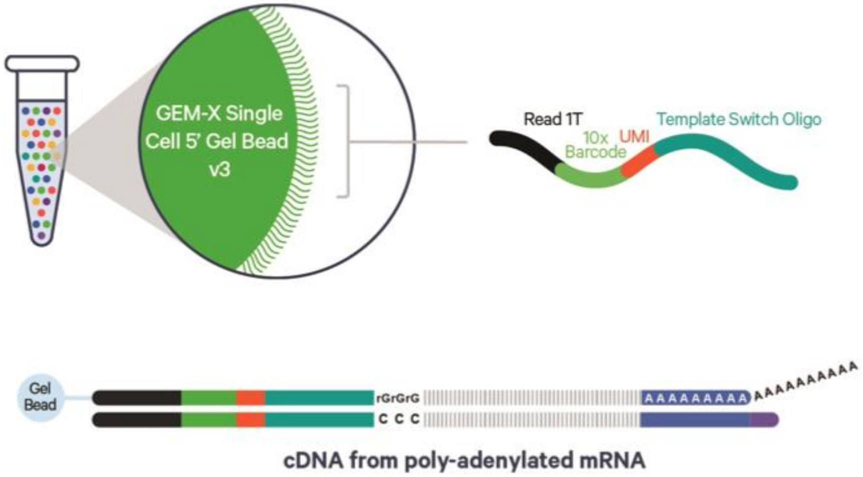
Gel Bead-In-EMulsion

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 kits for single-cell RNA-seq

ONT does not use specific kits for single-cell experiments

Ligation Sequencing Kit XL V14

SQK-LSK114-XL

https://store.nanoporetech.com/us/ligation-sequencing-kit-xl-v14.html

PCR Expansion

EXP-PCA001

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

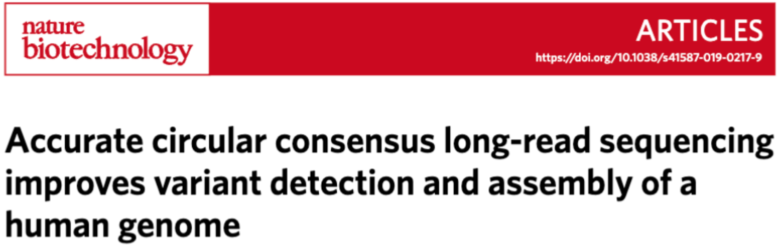
PacBio kit for single-cell RNA-seq

Kinnex single-cell RNA kit

Kit advantage	Cell type-specific isoform isoform discovery with the highest accuracy
Total throughput	30-40M (Sequel II) 80-100M (Revio)

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

CCS improves quality of the reads

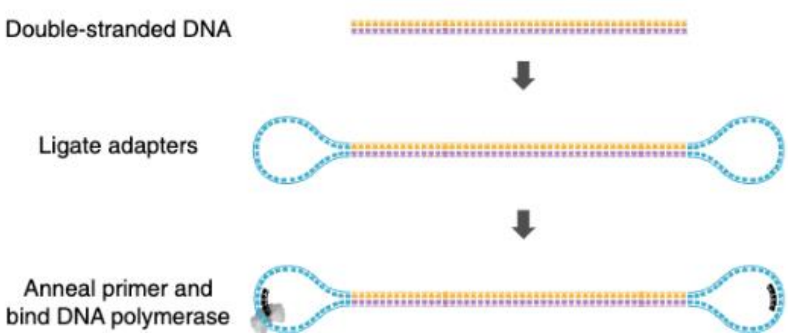


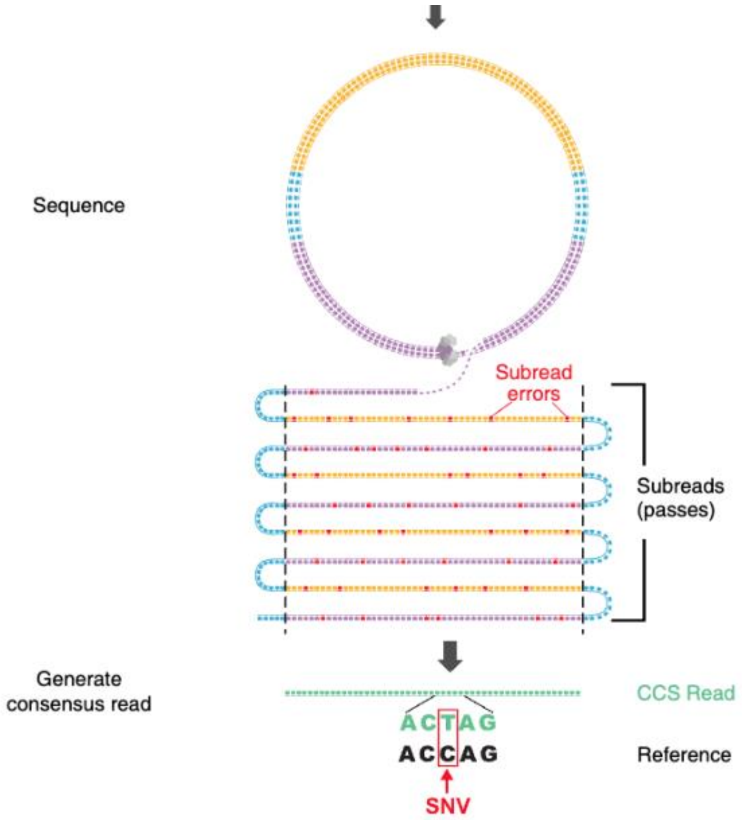
nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-019-0217-9

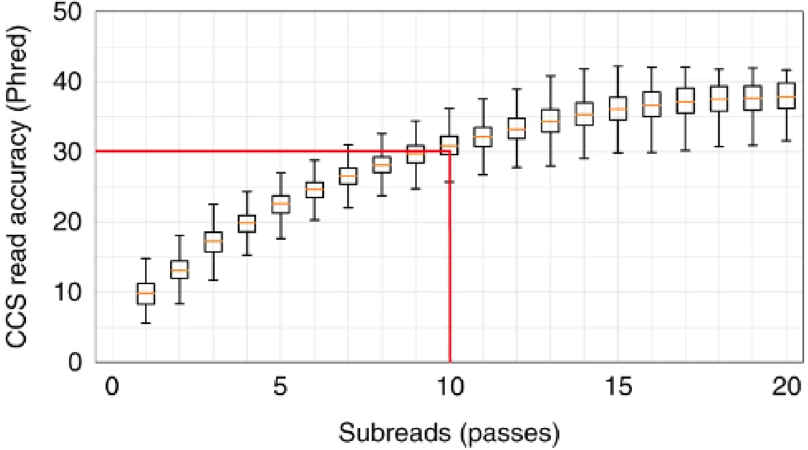
Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome





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

But low complexity results…



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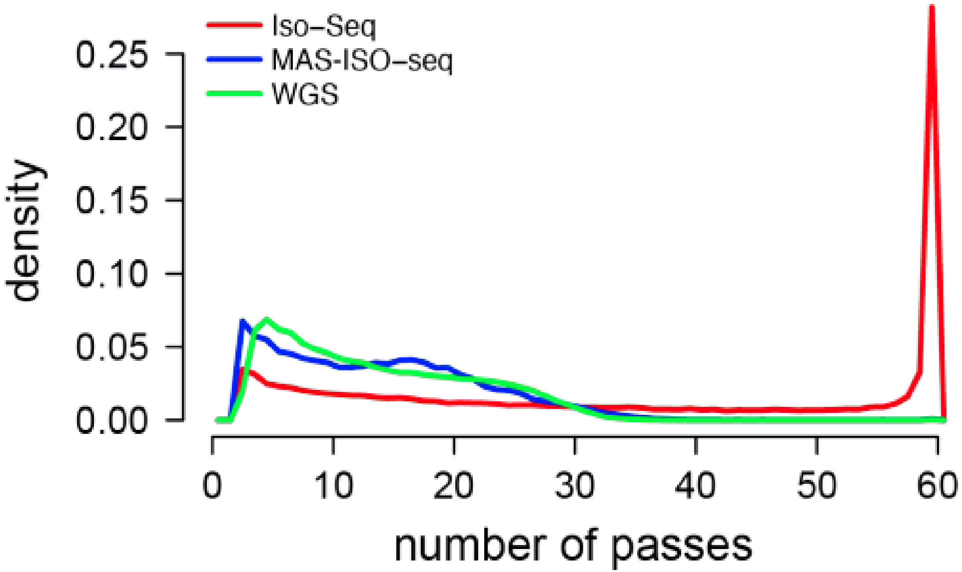
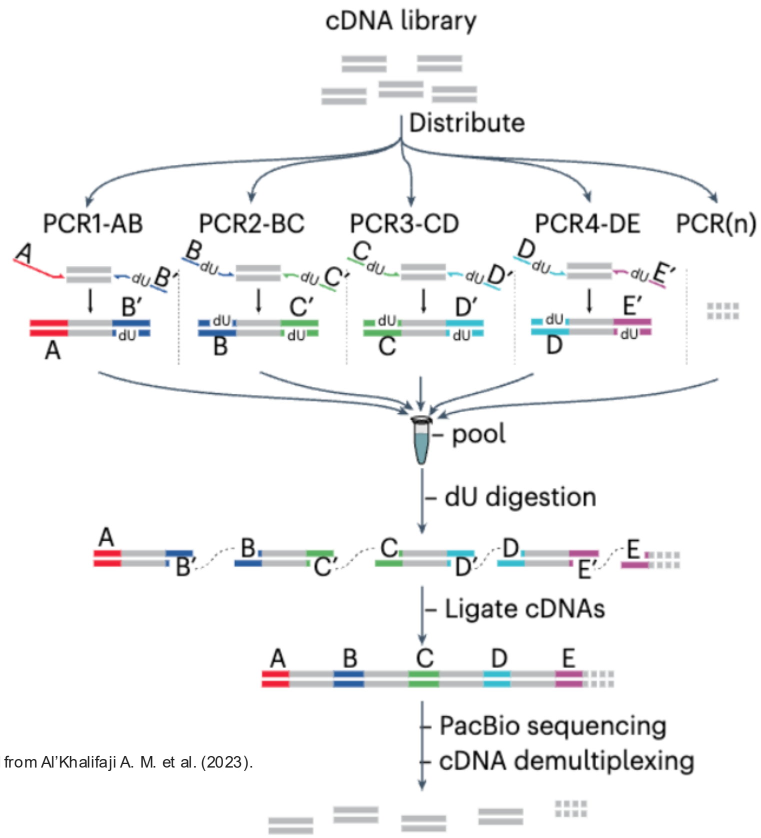


Adapted from Al'Khalifaji A. M. et al. (2023).

MAS-ISO-seq improves sequencing throughput



Adapted from Al'Khalifaji 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***

PacBio kit for single-cell RNA-seq

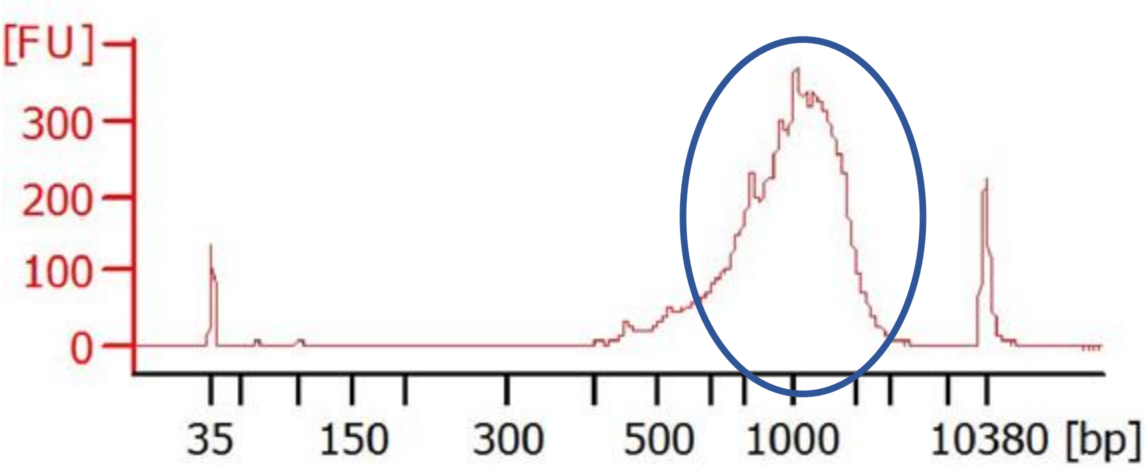
Kinnex single-cell RNA kit

- **Input cDNA** comes from 10x single-cell 3’ or 5’ kit
- ***Number of samples*** → 12
- ***Working time*** → 3 days for up to 12 samples (without QC)
- ***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

PacBio kit for single-cell RNA-seq

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

Question

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

Take home points

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!

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For more information about the LongTREC Summer School:

https://longtrec.eu