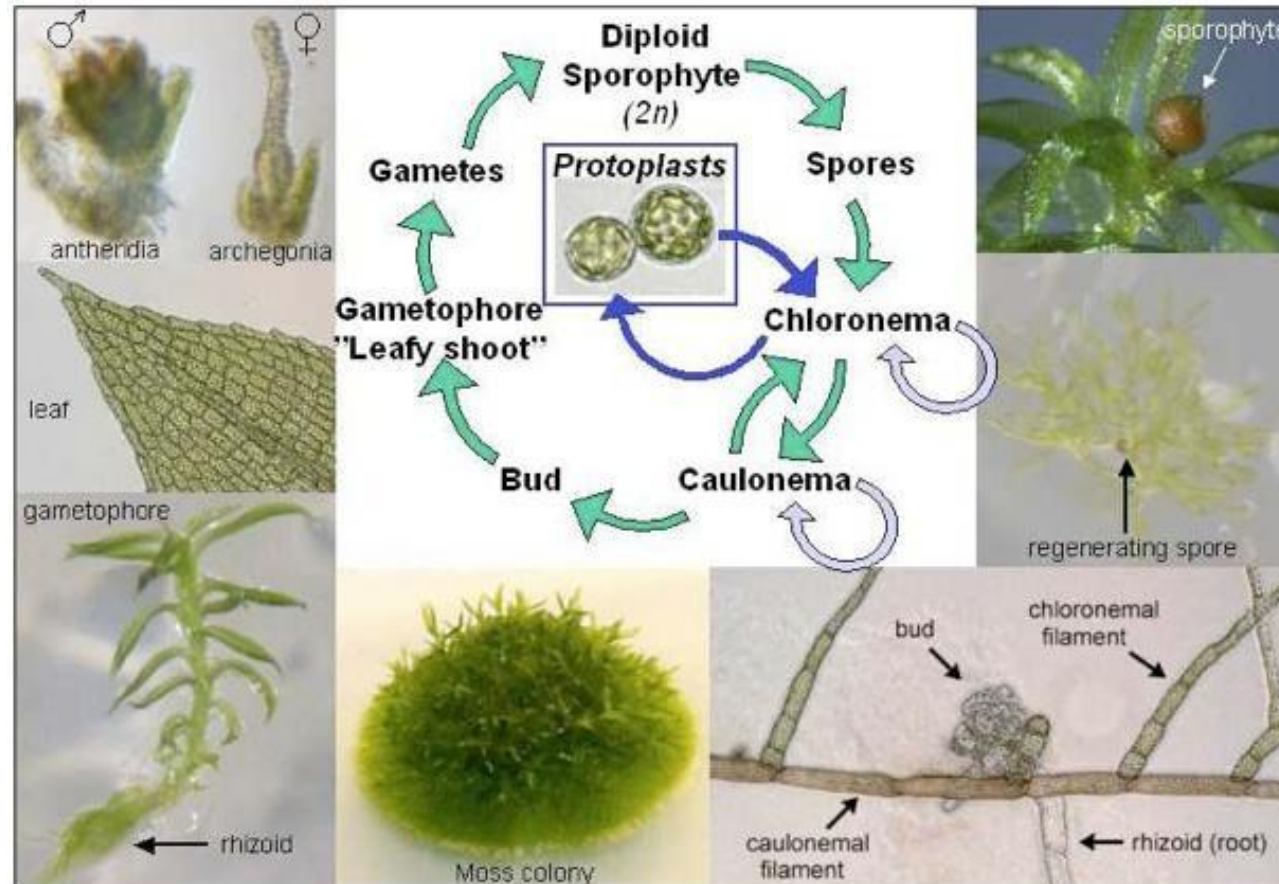
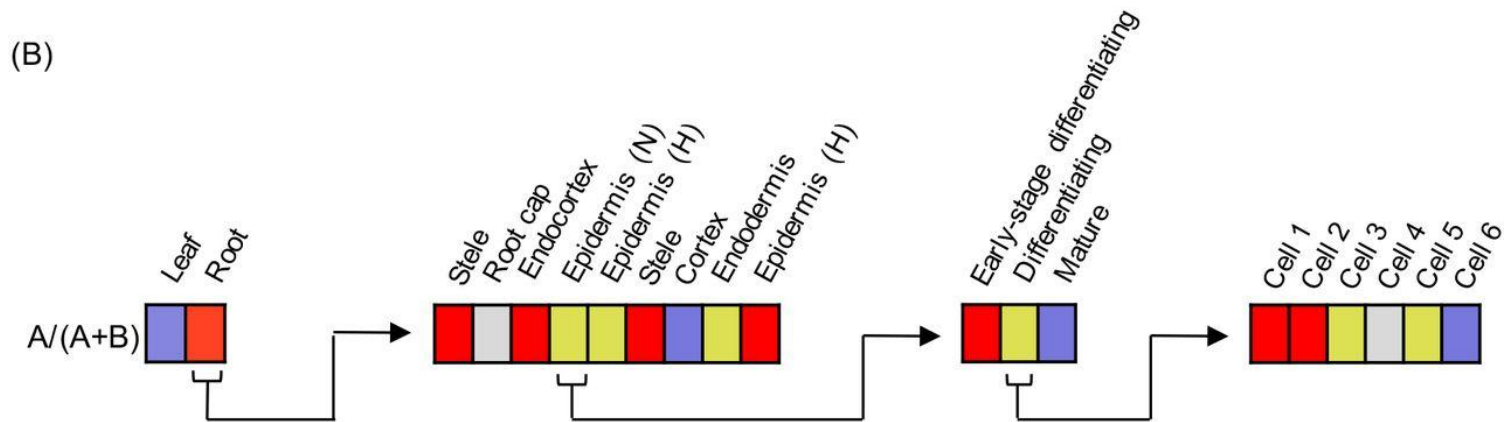
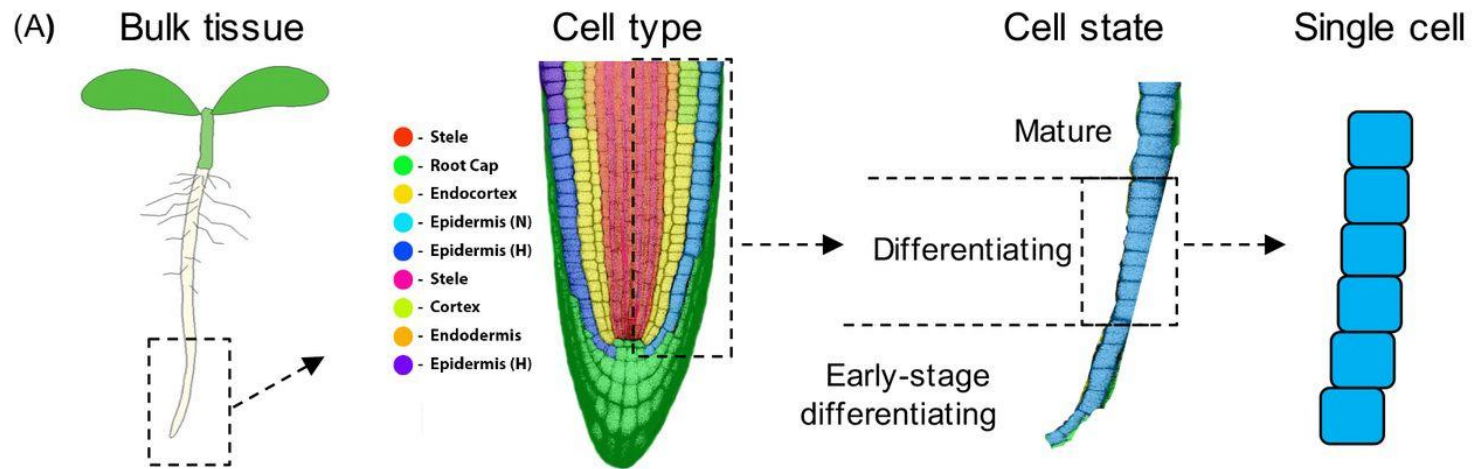


# Single Cell Sequencing 2

Callum J. Bell, Ph.D.

# *Physcomitrella patens* life cycle





# Single cell RNA seq

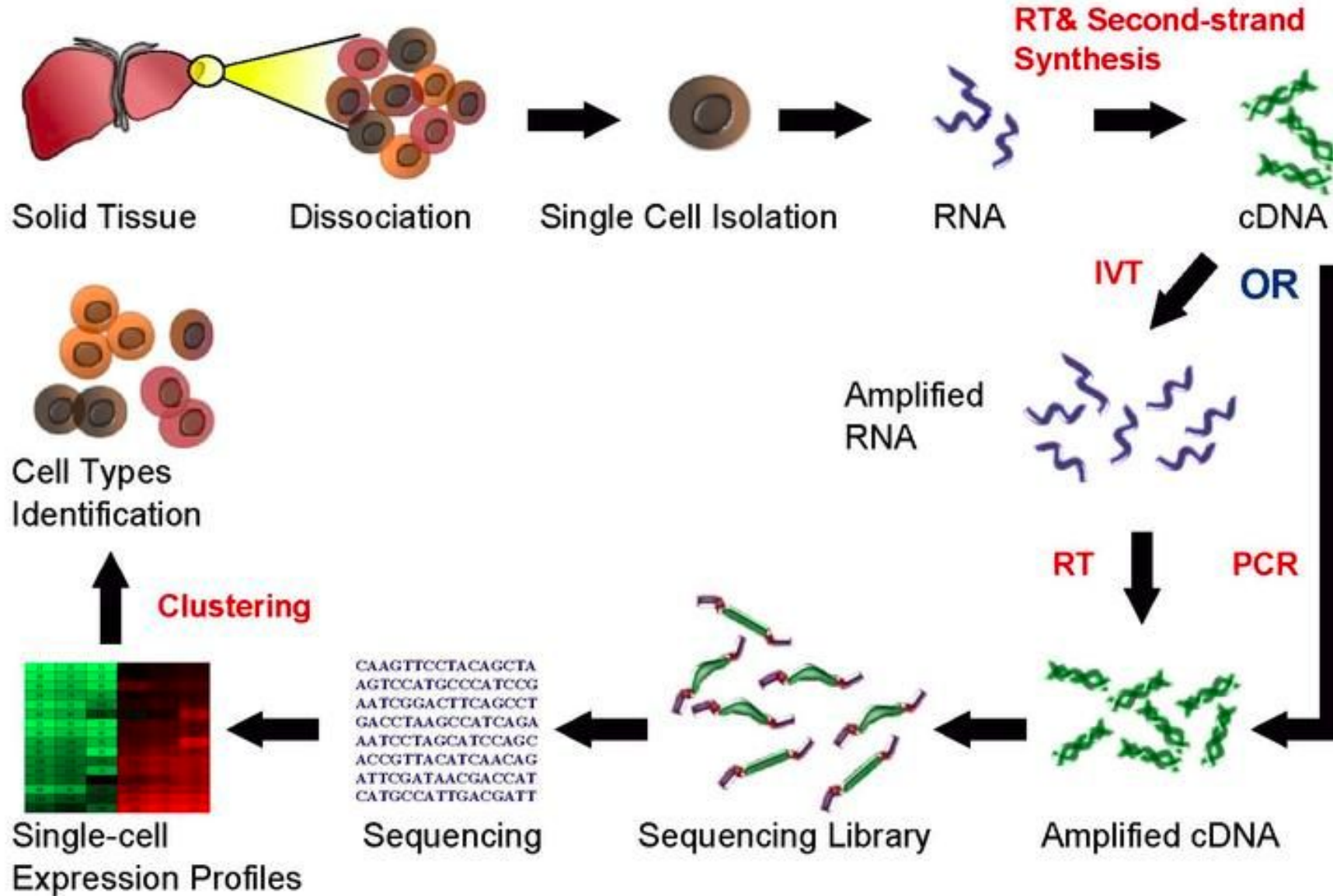
- Targeted

- Interrogate a known cell
- Precise
- Specific biological question
- Anatomical location known
- Histological identity known
- Very low throughput
- Technically demanding

- Non-targeted

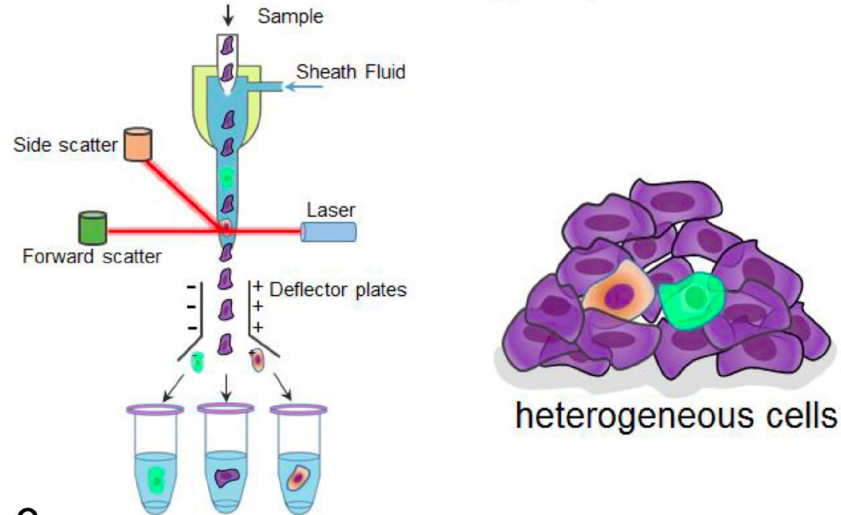
- Establish expression signatures for population of cells
- Discover novel cell types and functions
- High throughput
- Automated
- When you don't know the question to ask

# Single-cell RNA Sequencing Workflow

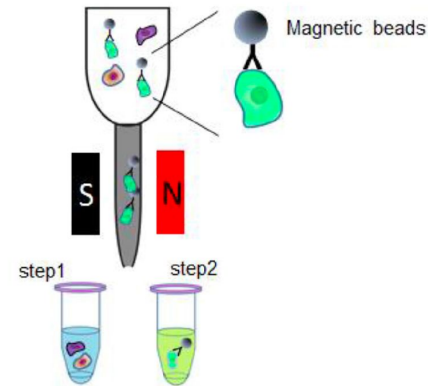


# Methods to Isolate Single Cells

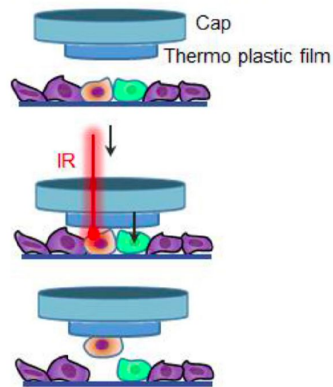
**A** Fluorescence activated cell sorting (FACS)



**B** Magnetic activated cell sorting (MACS)



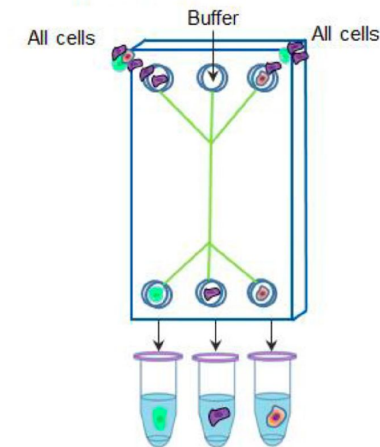
**C** Laser Capture Microdissection (LCM)



**D** Manual cell picking



**E** Microfluidic



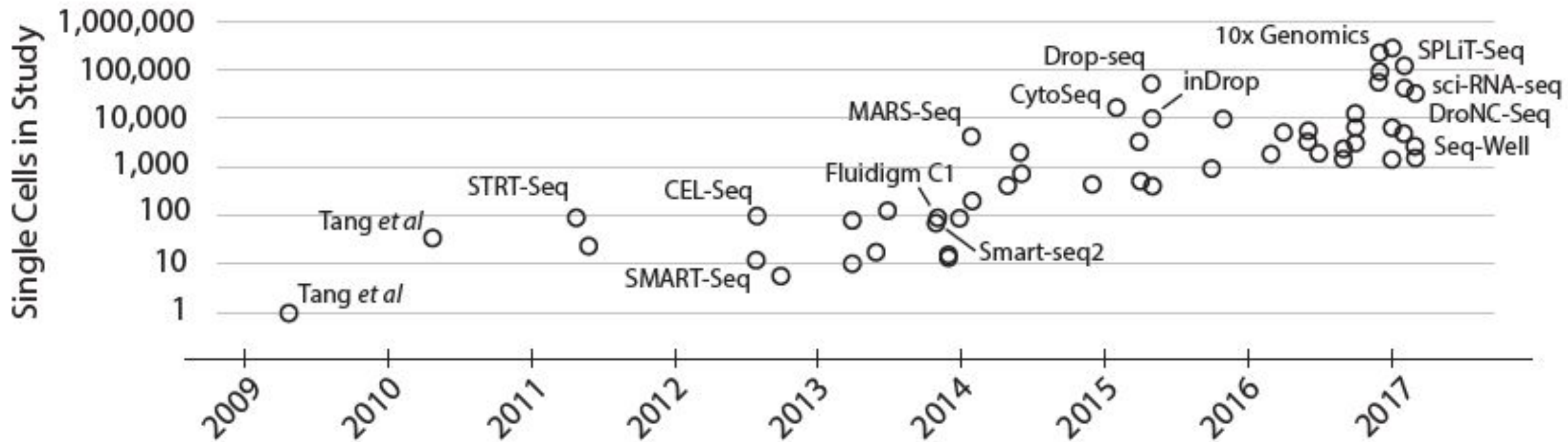
- Protoplasting
- Nuclear isolation

# Throughput of scRNA-Seq Methods

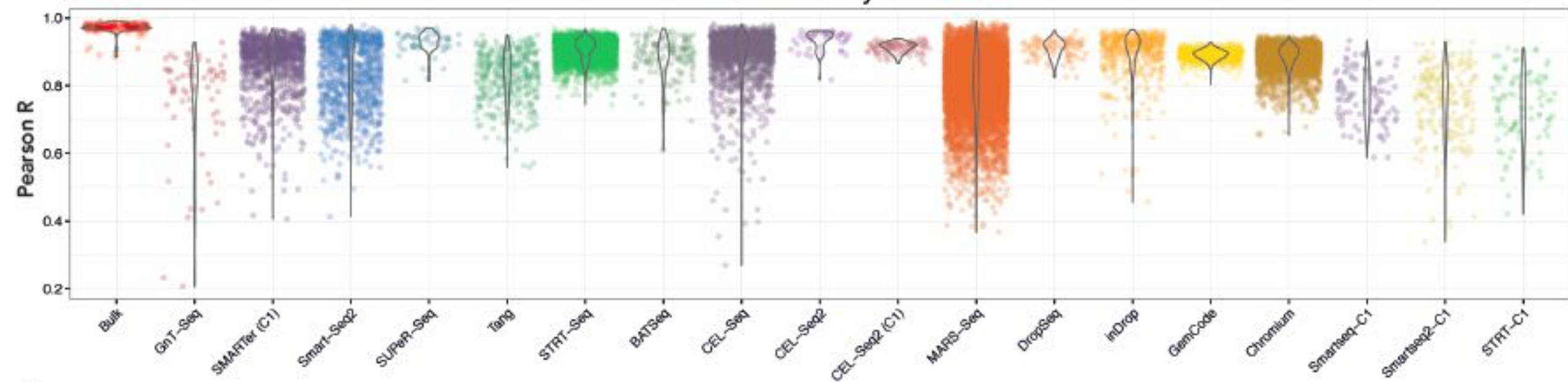
A



B



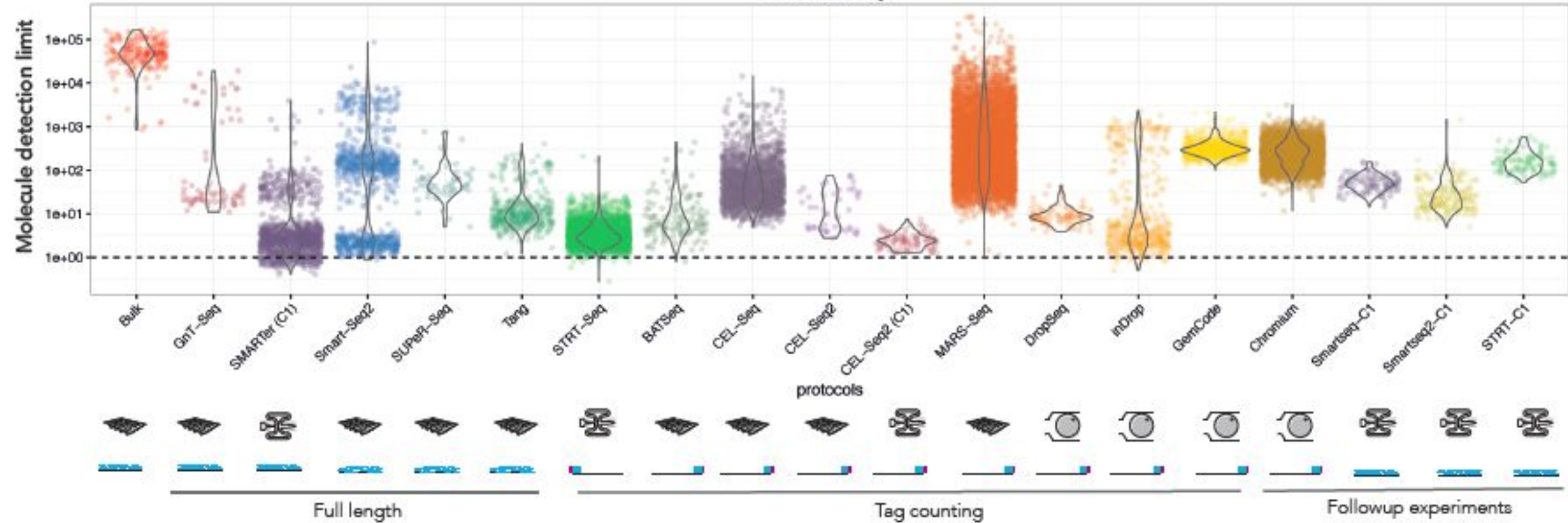
## Accuracy






Svensson et. al, *Nature Methods* doi: [10.1038/nmeth.4220](https://doi.org/10.1038/nmeth.4220)





# Sensitivity



## Protocol platform (volume order of magnitude)

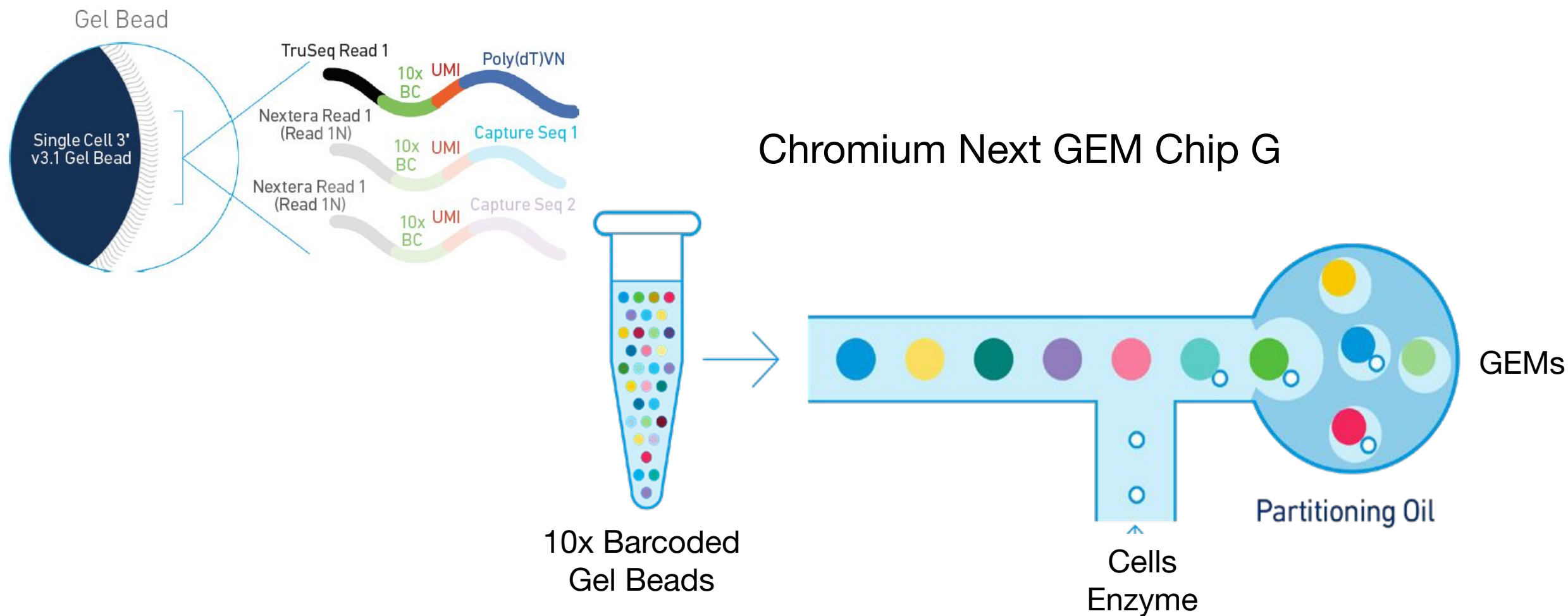
-  Microwell plates (~1  $\mu$ L)
-  Microfluidics chip (~10 nL)
-  Nanoliter droplets (~1 nL)

## Quantification strategy

-  Transcript coverage
-  Transcript 3' tag UMI counting

Svensson et. al, *Nature Methods* doi:  
[10.1038/nmeth.4220](https://doi.org/10.1038/nmeth.4220)

# Single-Cell RNA-Seq with 10X Chromium Next GEM Single Cell 3' KIT



# GEM Chip

Chromium Next  
GEM Chip



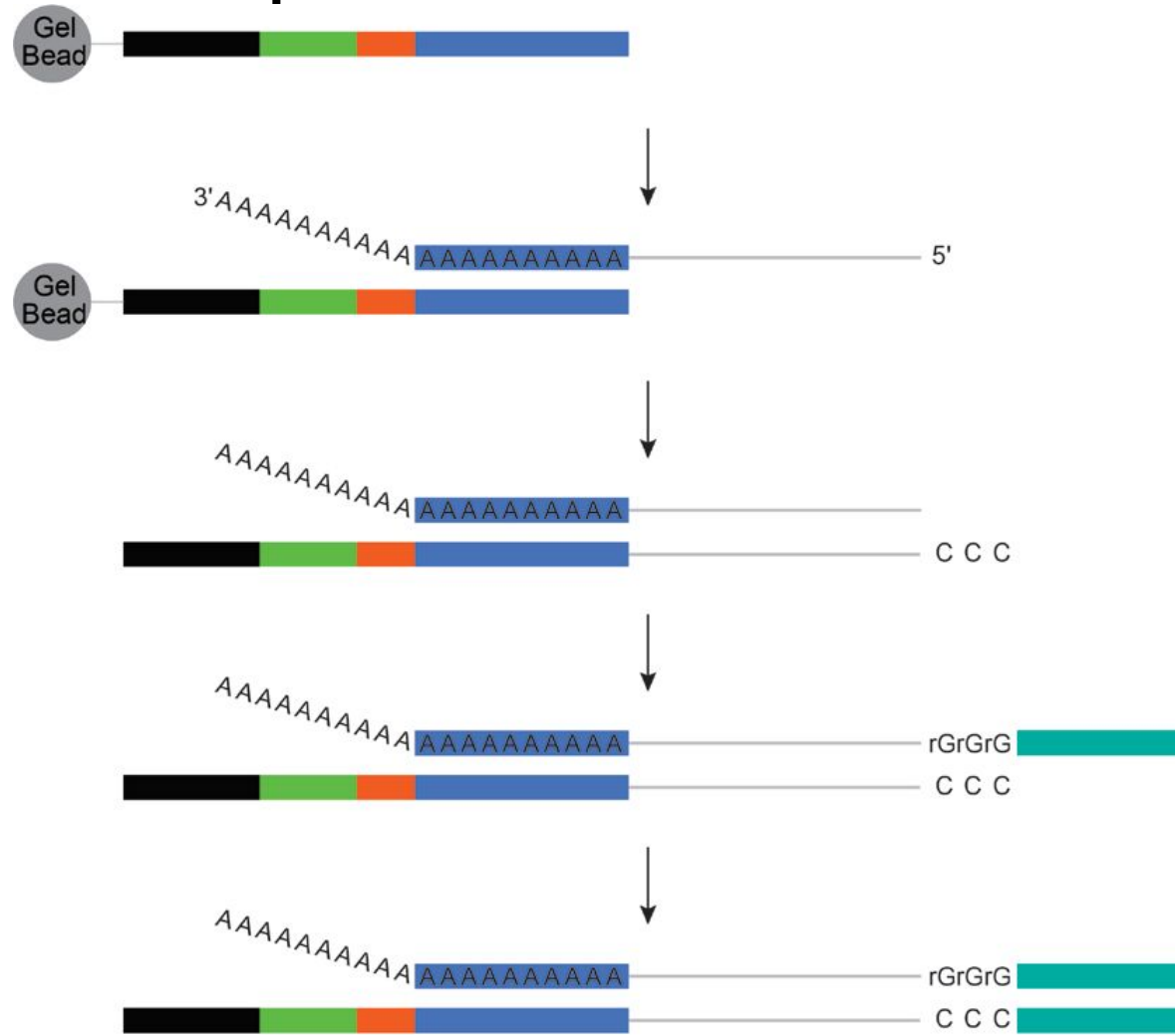
Chromium Next GEM  
Secondary Holder



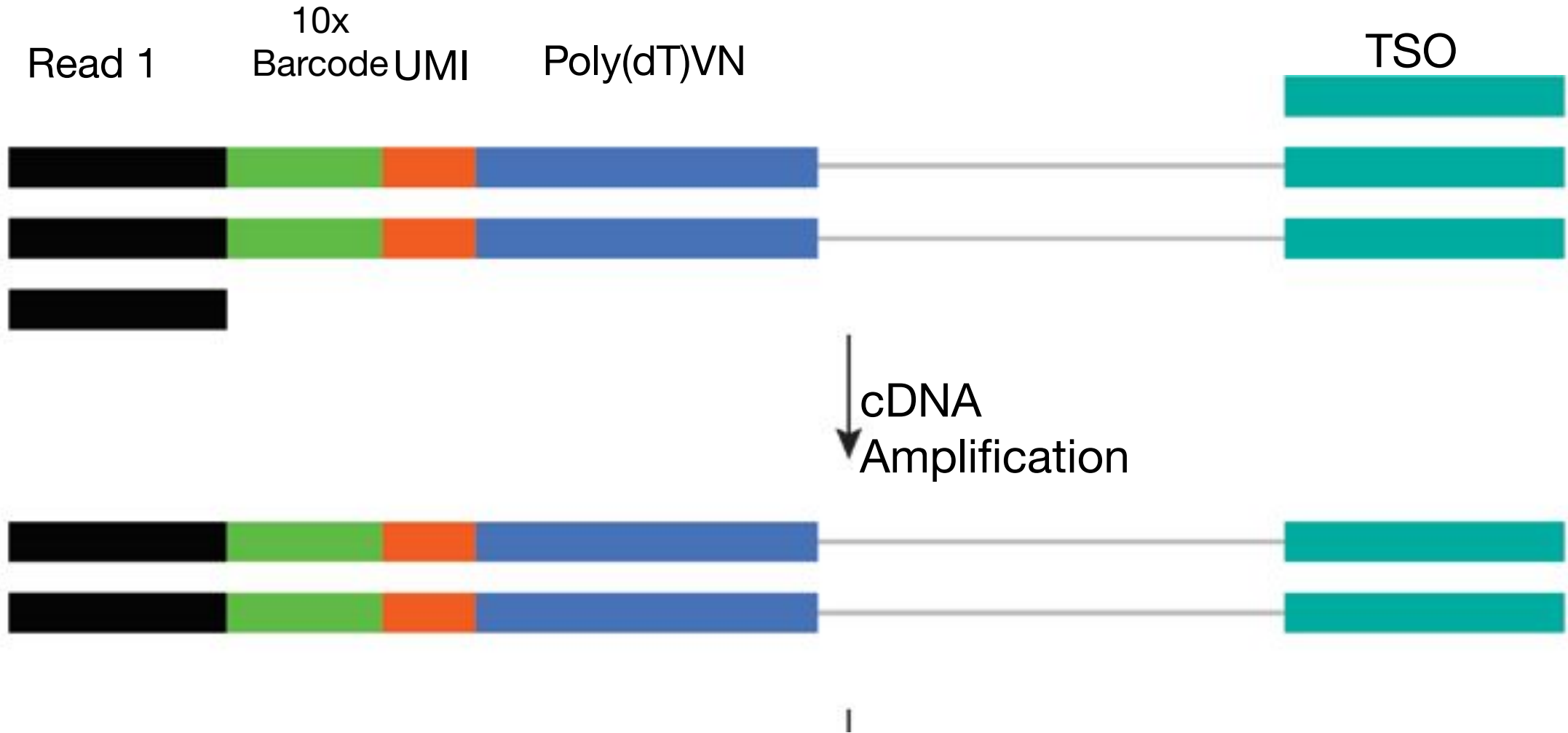
Assembled Next  
GEM Chip



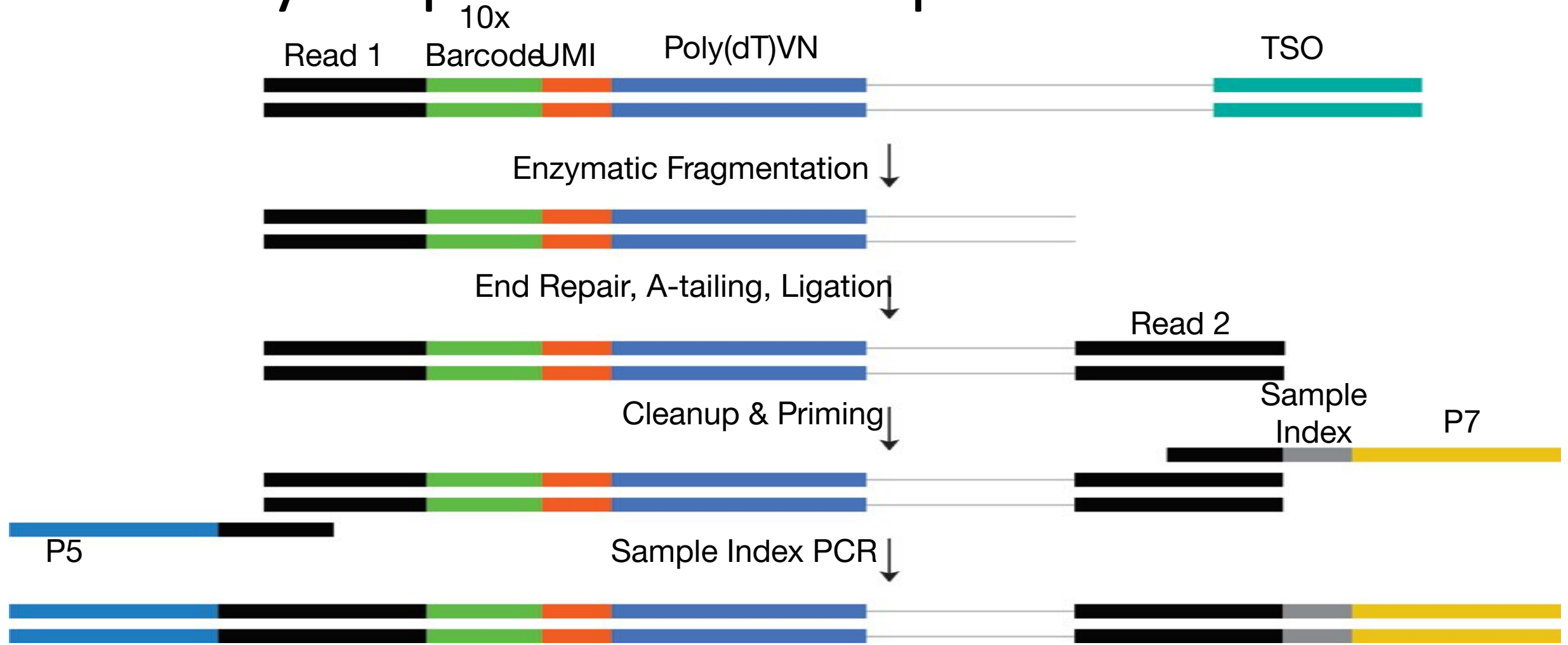
# Reverse Transcription Inside Individual GEMs



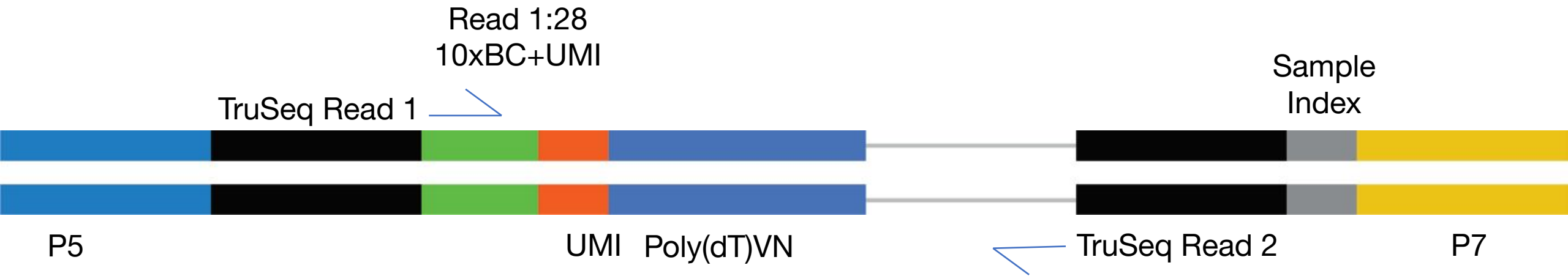
# 1<sup>st</sup> Strand cDNA Extracted, Pooled, and PCR Amplified

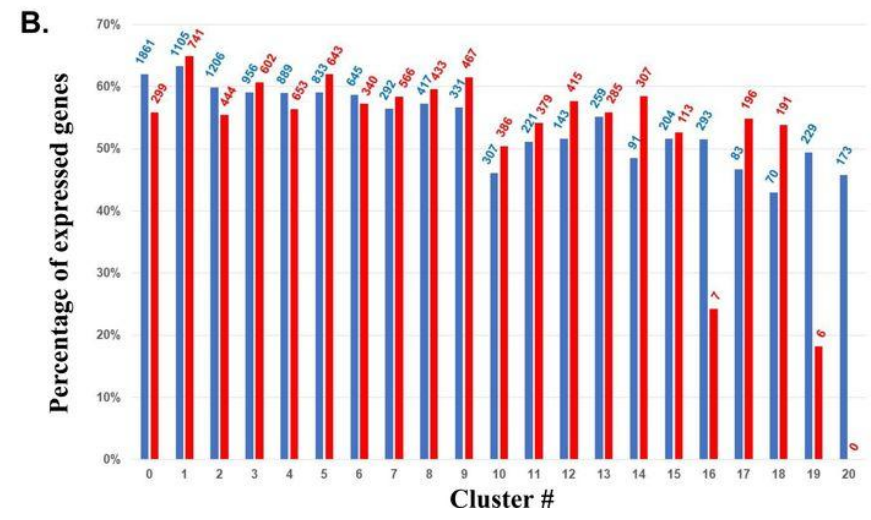
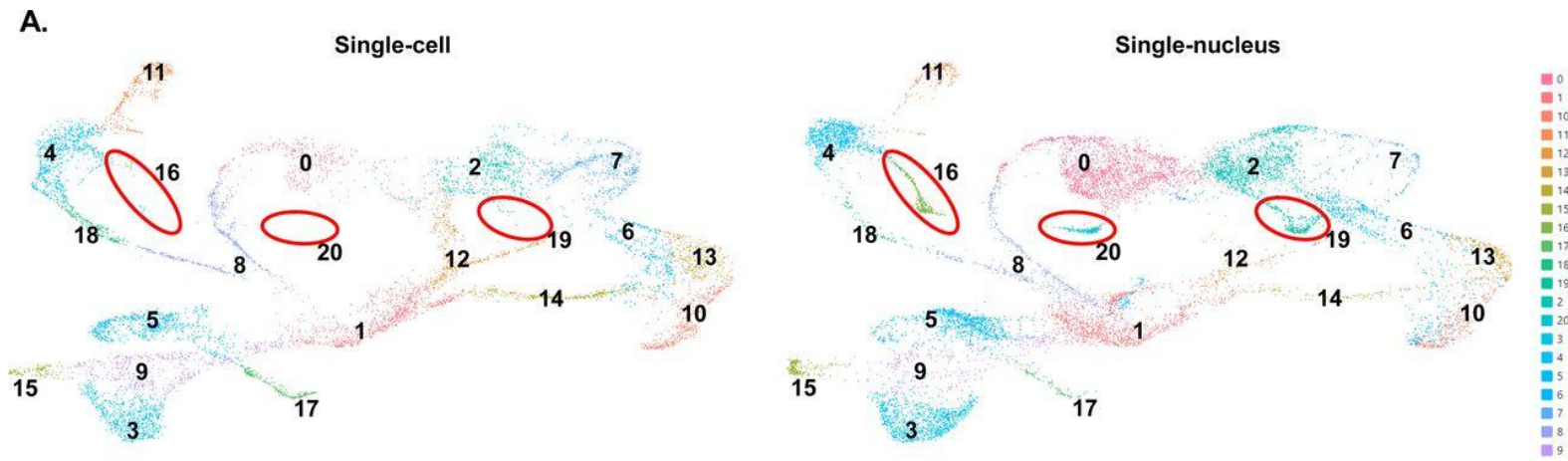


# Library Prep of Pooled Samples



# Sequencing



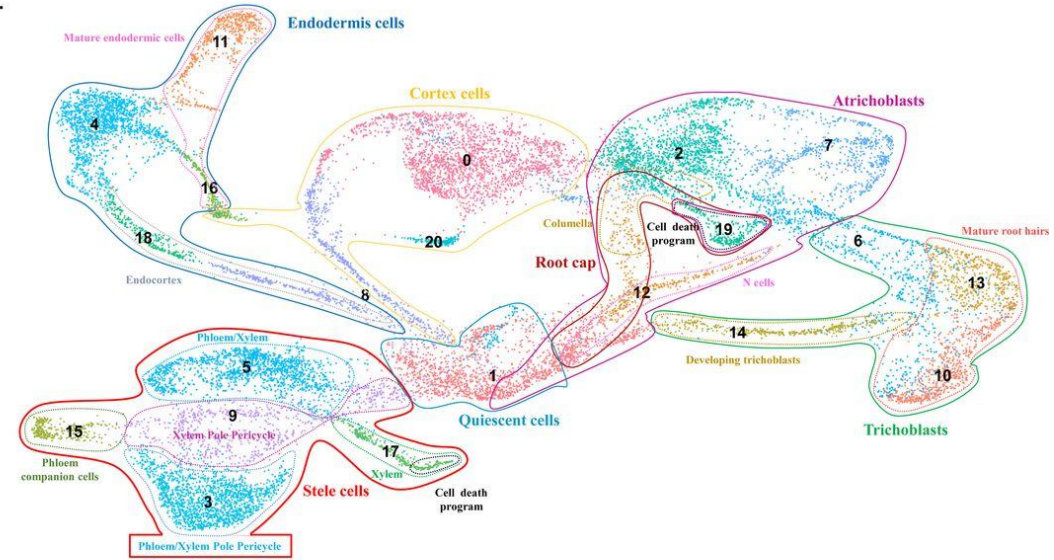


**The impact of chromatin remodeling on gene expression at the single cell level in *Arabidopsis thaliana***

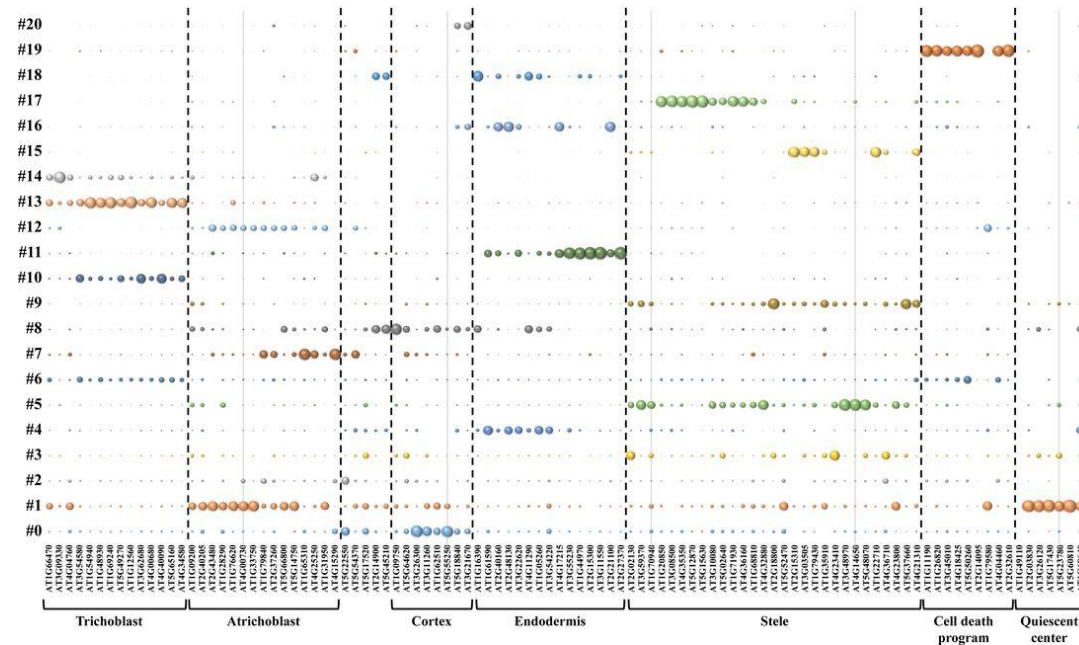
Andrew Farmer, Sandra Thibivilliers, Kook Hui Ryu, John Schiefelbein, Marc Libault  
 doi: <https://doi.org/10.1101/2020.07.27.223156>



A.






B.



ARTICLE | [VOLUME 73, ISSUE 1, P130-142.E5, JANUARY 03, 2019](#)

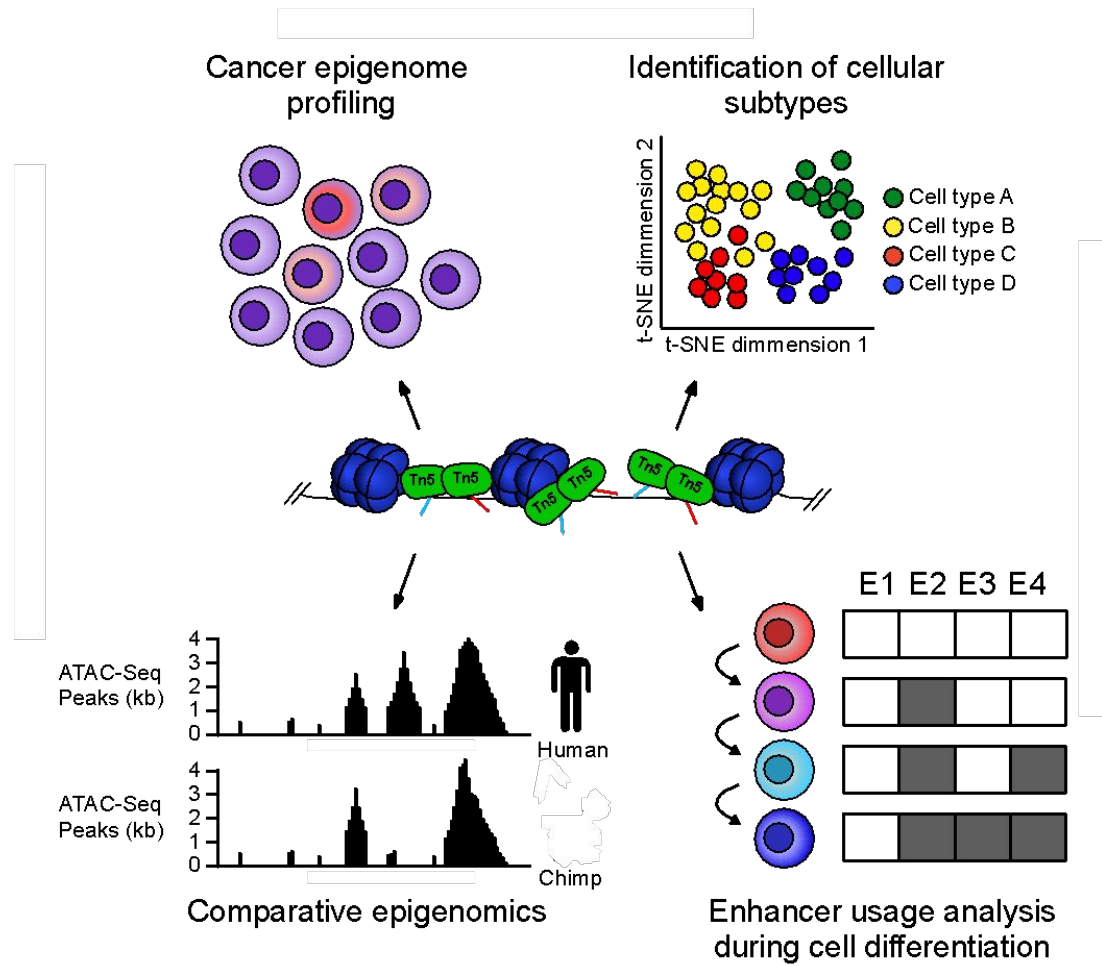
# Comparative Analysis of Droplet-Based Ultra-High-Throughput Single-Cell RNA-Seq Systems

[Xiannian Zhang](#) <sup>6</sup> • [Tianqi Li](#) <sup>6</sup> • [Feng Liu](#) <sup>6</sup> • ... [Zeyao Li](#) • [Yanyi Huang](#)   • [Jianbin Wang](#)  <sup>7</sup> 

[Show all authors](#) • [Show footnotes](#)

[Open Archive](#) • Published: November 21, 2018 • DOI: <https://doi.org/10.1016/j.molcel.2018.10.020>

# ATAC (Assay for Transposase-Accessible Chromatin using sequencing)



The bioinformatics workshop is supported by New Mexico INBRE through an Institutional Development Award (IDeA) from National Institute of General Medical Sciences of the NIH grant number P20GM103451