

# Overview of Single-Cell Assays and Platforms

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MGC Course on Single-Cell Analysis

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# Why single-cell?

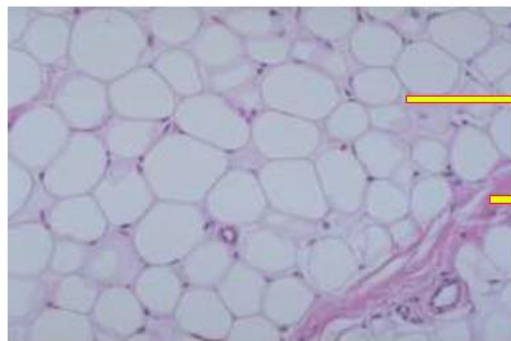
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



Analyzing single cells is like tasting each individual piece of fruit to gain a much more nuanced understanding of the composition of the fruit salad



# Tissues are heterogeneous



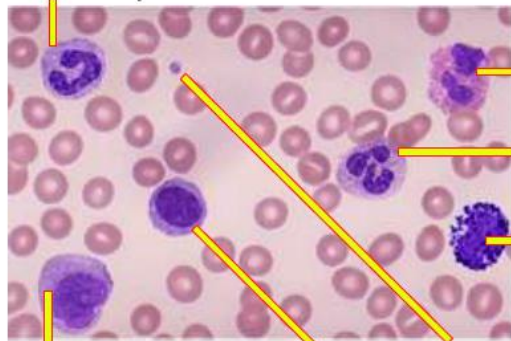
**Adipose**

Fat

Connective  
tissue

Band  
Neutrophil

**Normal Peripheral Blood**



Eosinophil

Segmented  
Neutrophil

Basophil

Monocyte

Platelet  
Lymphocyte

**Small Intestine Mucosa**



Epithelial cells

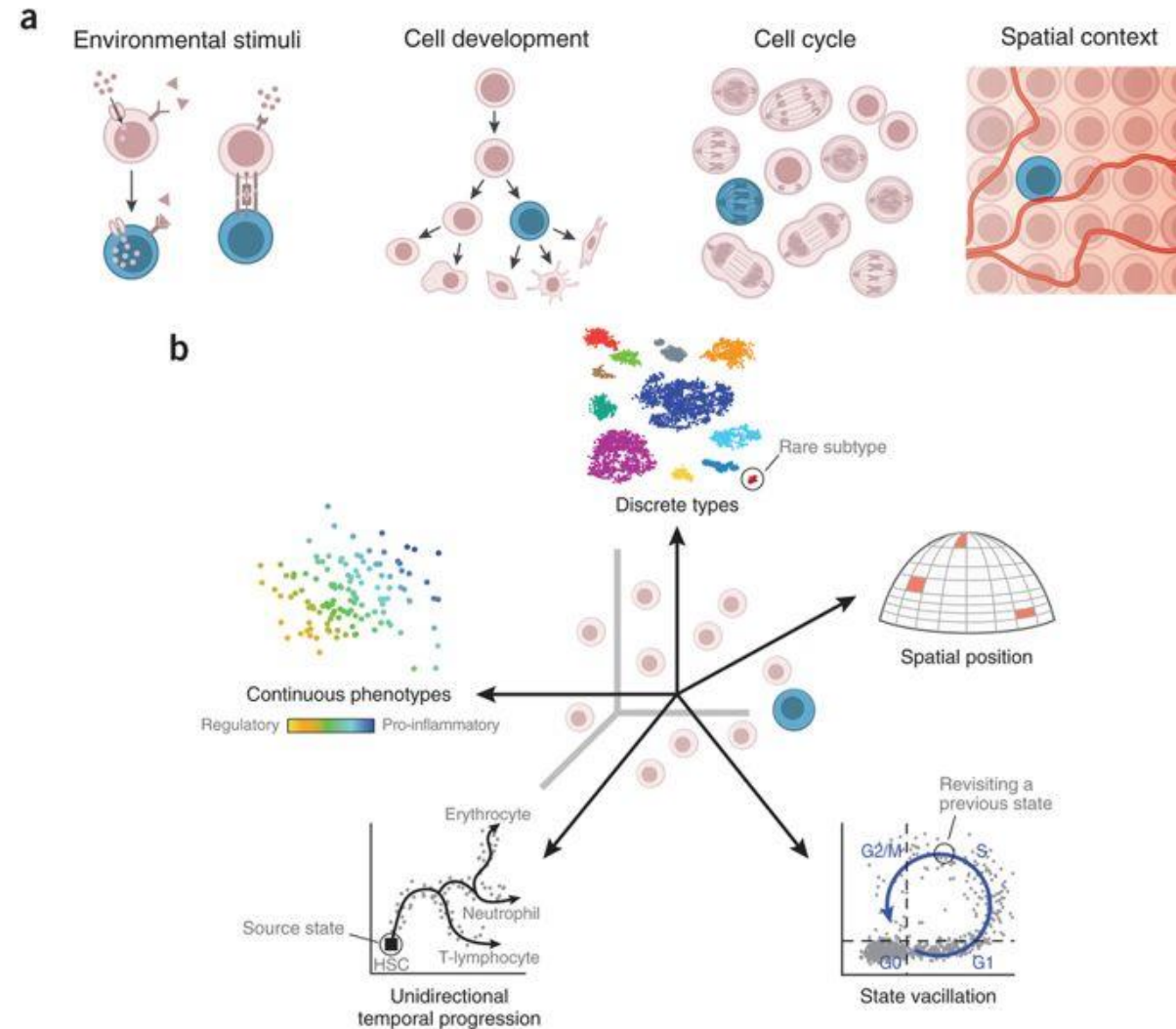
Goblet  
cells

Lamina  
propria

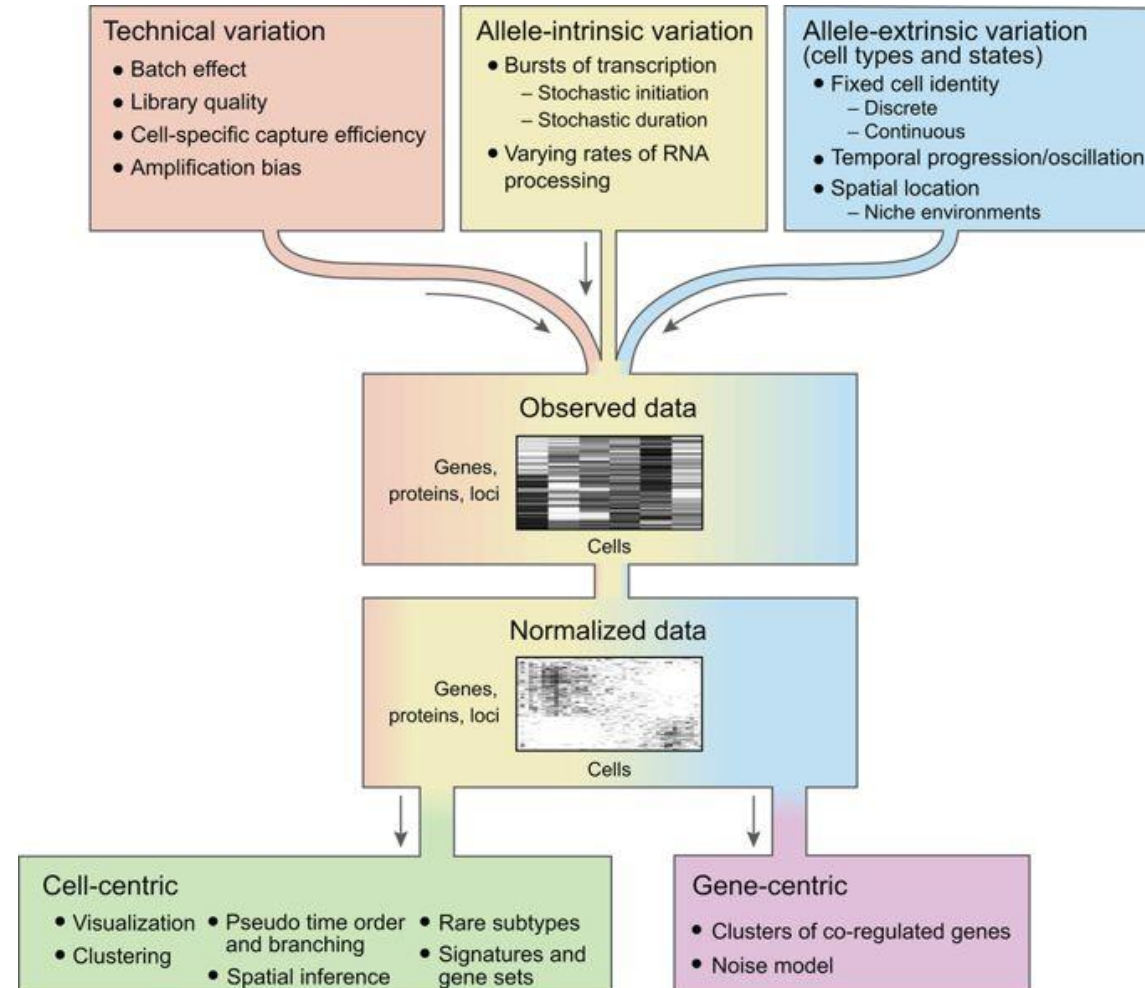
Muscularis mucosa  
(smooth muscle)

Created with figures from [library.med.utah/WebPath/HISTHTML/HISTO.html](http://library.med.utah/WebPath/HISTHTML/HISTO.html)

# Cell identity is more than histopathology



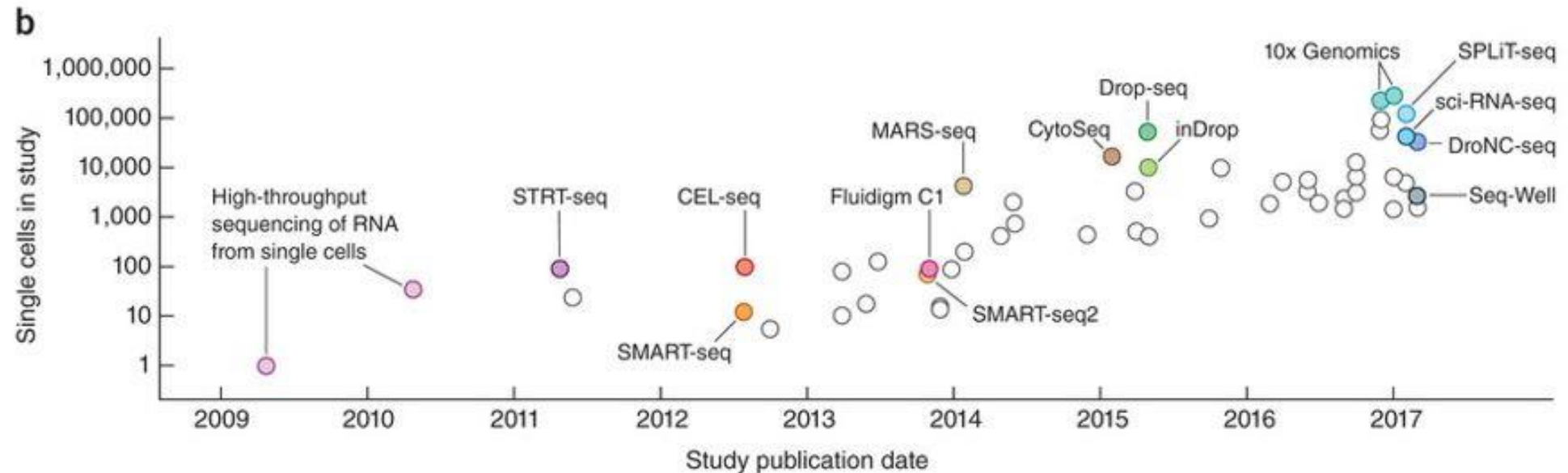
# Biology is messy – computational methods help to clean this up



# We will cover

- Description of single-cell assays/platforms/protocols
- Sample prep and experimental design concerns
- Gene and cell filtering
- Normalization
- Dimensionality reduction
- Data integration
- Trajectory inference
- Differential gene expression

# Exponential scaling of single-cell throughput





# scRNA-seq

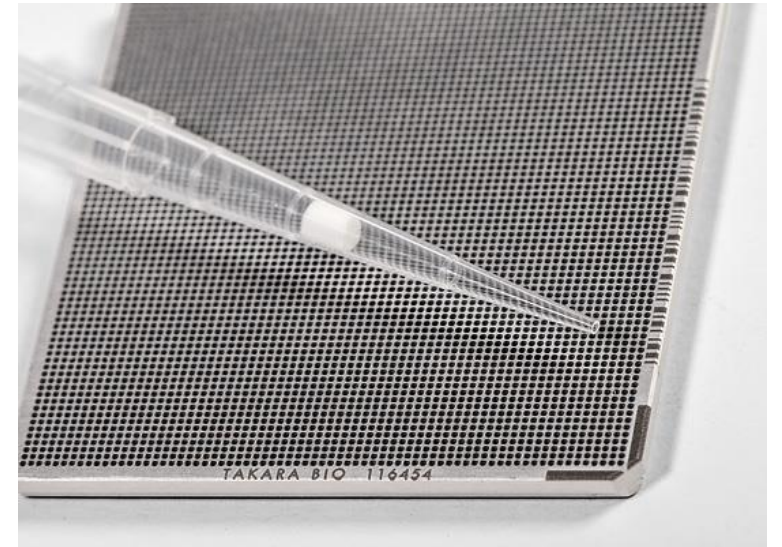
## **MANY** different assays

- Some commercial, some DIY
  - Full transcriptome vs 3' vs 5'
  - Automation varies
  - Throughput varies
  - Cost varies
- 
- Plate-based (Lecture from Miao)
  - Droplet-based (Lecture from Susan and Miao)
  - Microwell-based



# ICELL8 cx

- Available at ErasmusMC (Biomics facility)
- Uses 5184 nanowell chip, ~1800 cells loaded
- Compatible with immunofluorescence
- Protocols for single-cell
  - SMART-Seq full-length transcriptome analysis
  - Differential expression by 3' end counting
  - TCR profiling and 5' end differential expression
  - ATAC-seq

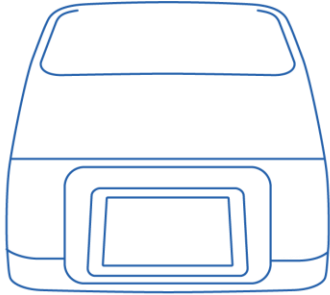


# BD Rhapsody

- Works with targeted panels to reduce sequencing costs
  - Immune response human/mouse
  - T-cell
  - Oncology – breast cancer
  - Custom panel add-ons
- Up to 400 amplicons / sample
- Includes UMIs to reduce PCR amplification bias
- Increased flexibility
  - Archiving up to 3 months
  - Sub-sampling



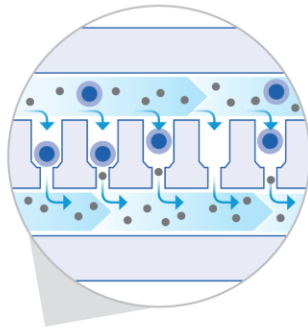
# Celsee Genesis platform



Isolation



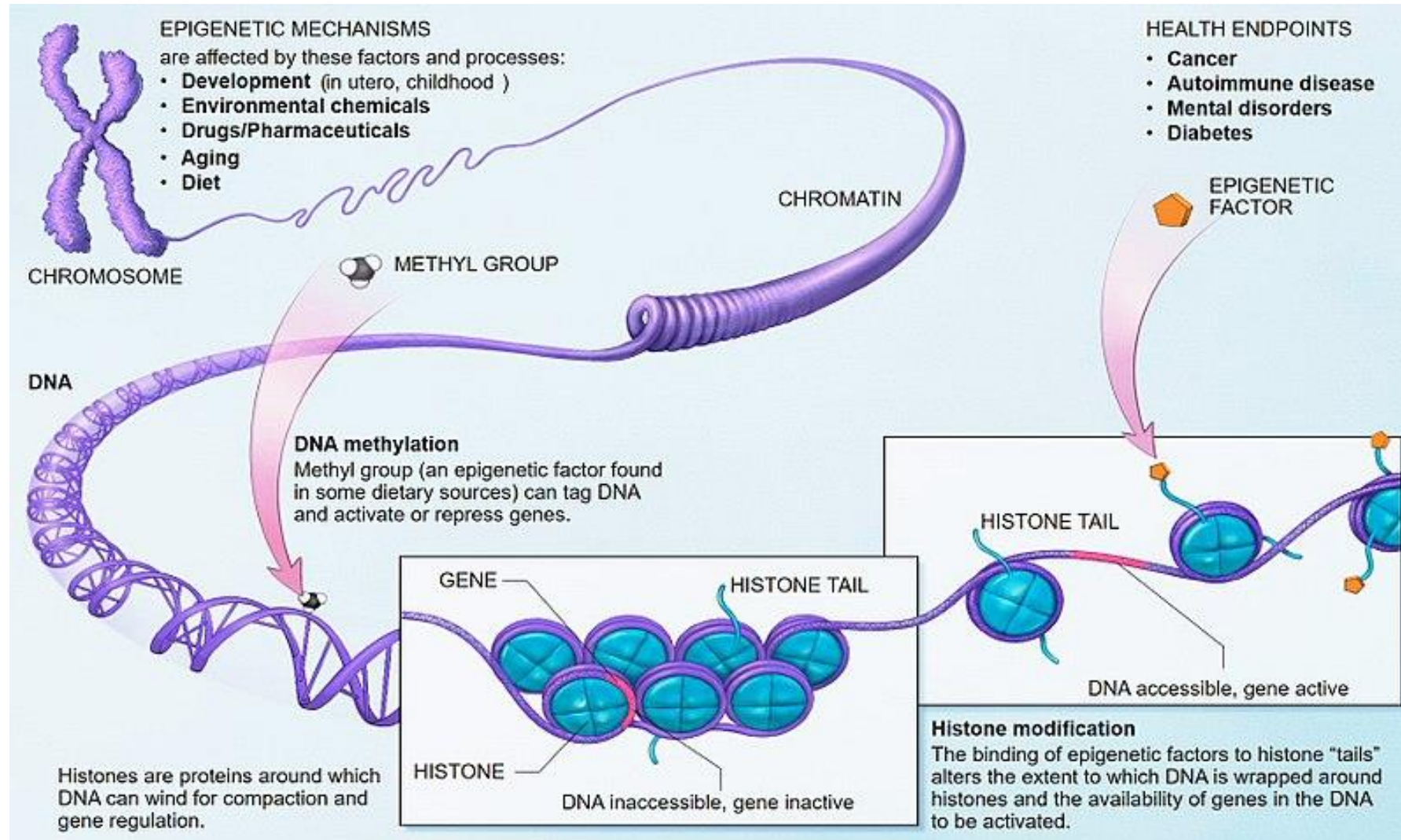
Celsingle™ Slide



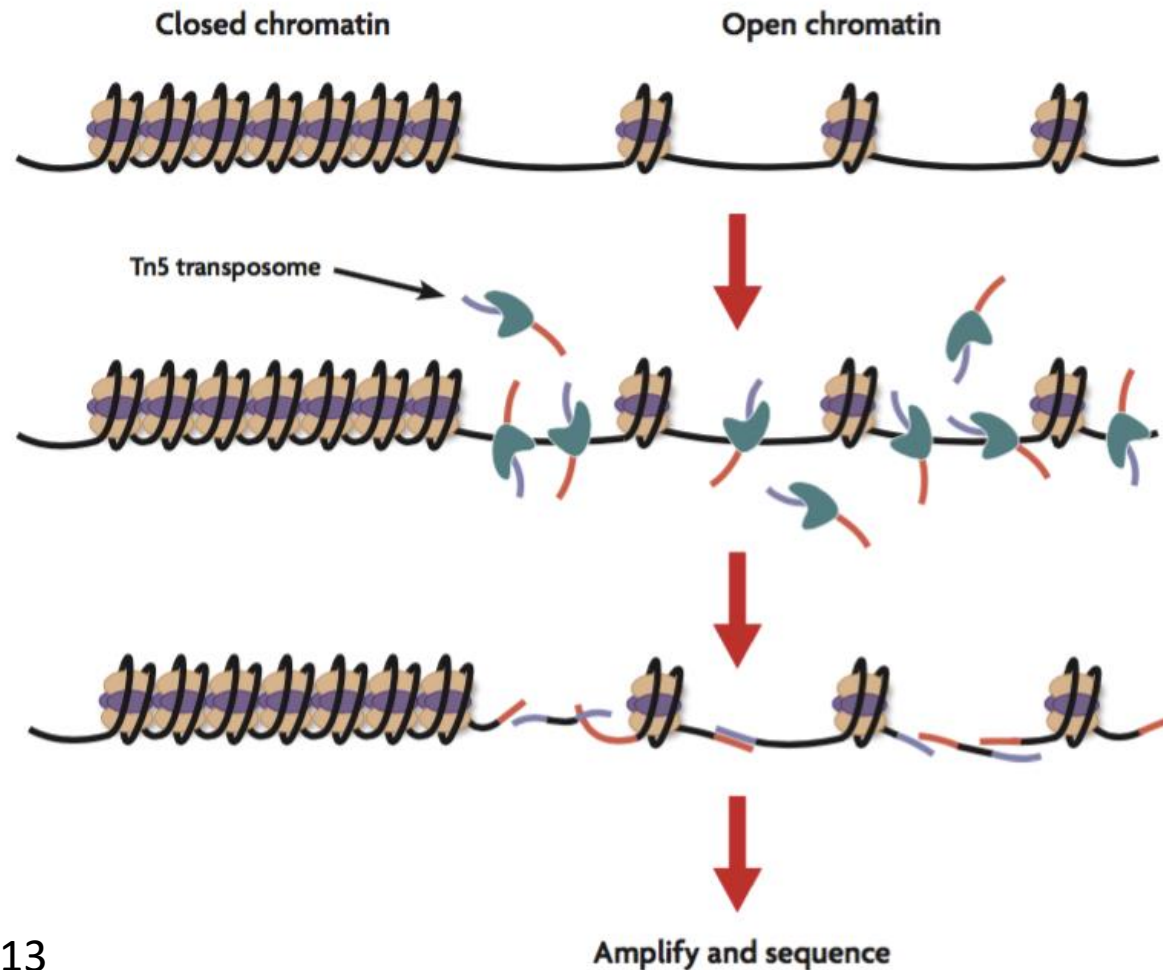
Celselect™ Slide



# What is epigenetics?



# Assay for transposase-accessible chromatin (ATAC-seq)





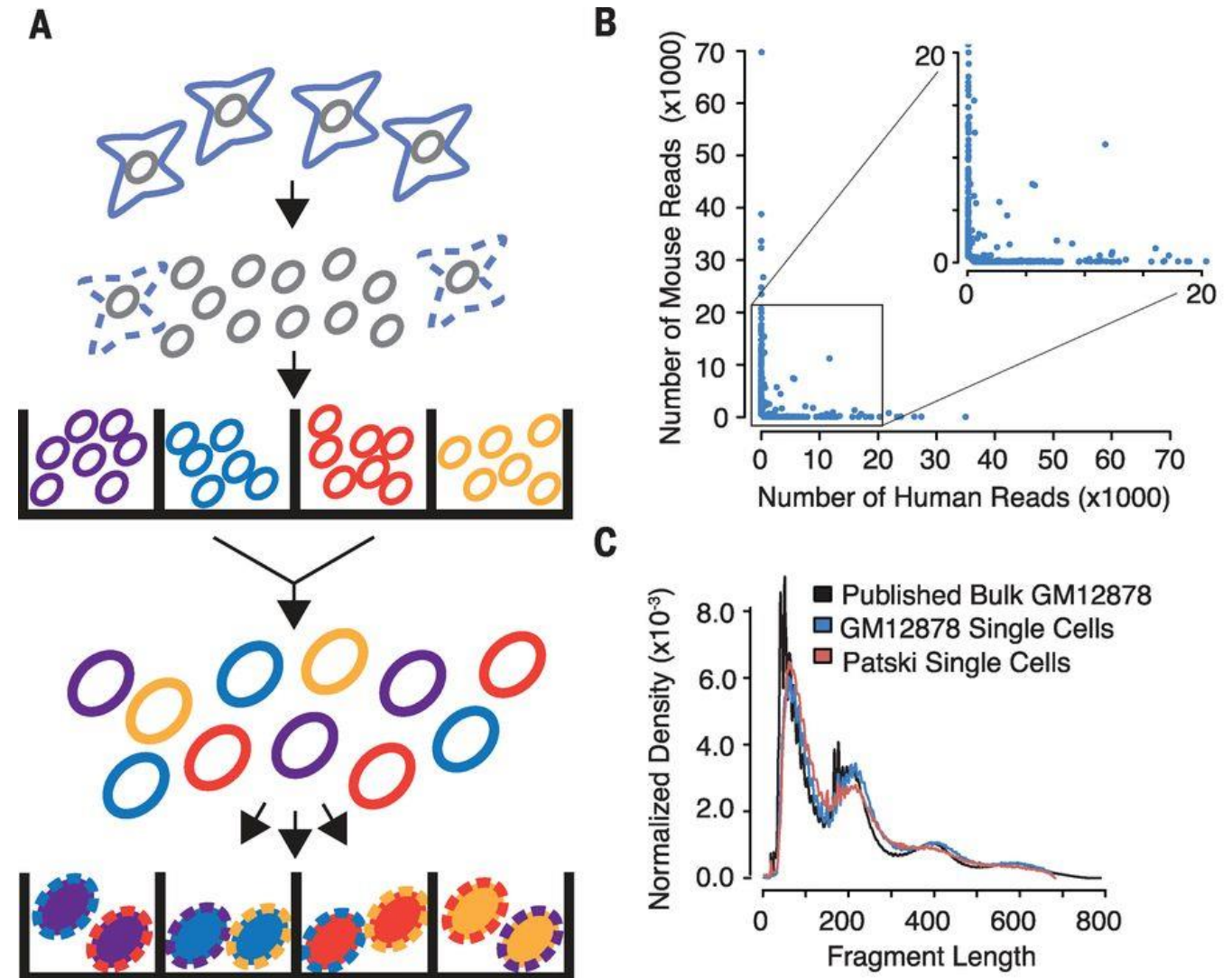
# Single-cell ATAC-seq

sci-ATAC-seq: single-cell  
combinatorial indexed  
sequencing

Potential throughput of  
17,280 cells/experiment if  
scaled to 384 well plates

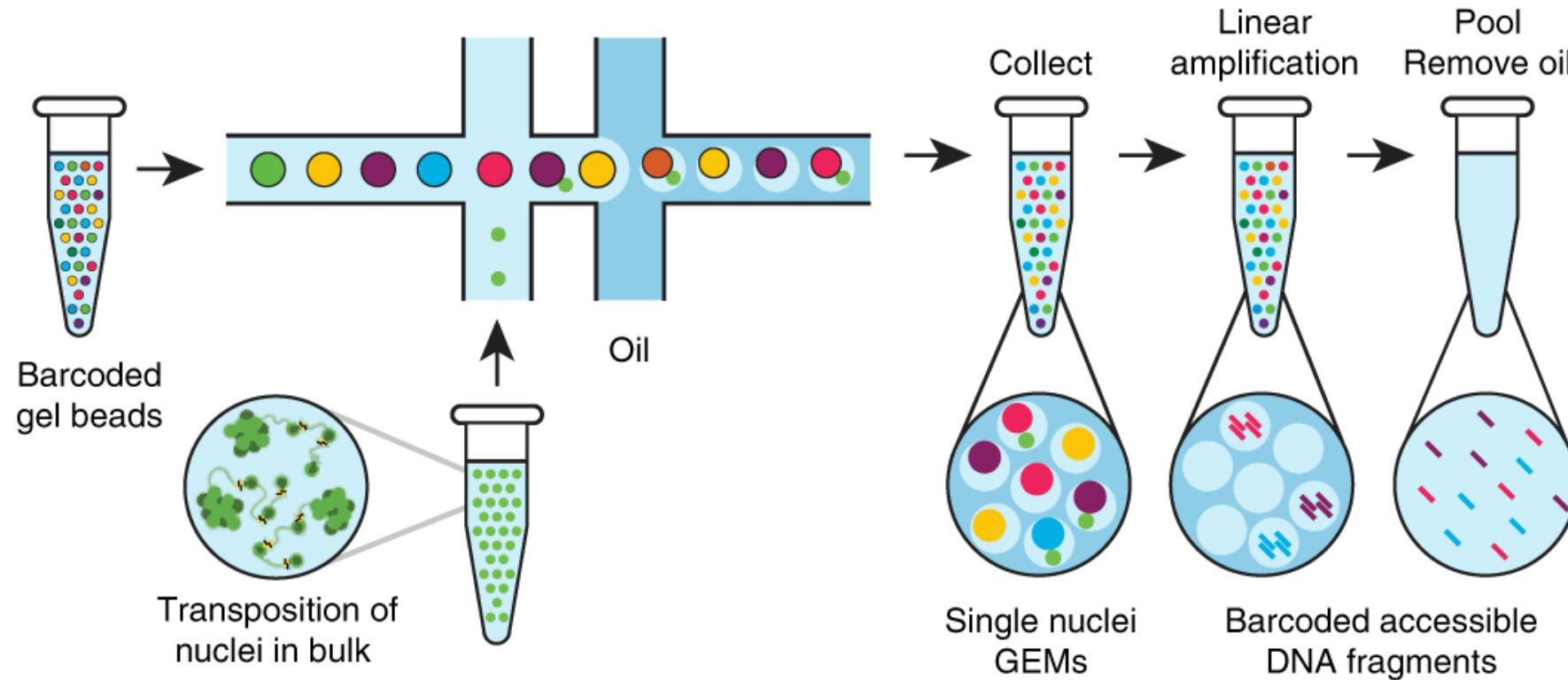
Drawbacks: low coverage,  
max ~3000 unique reads/cell

Cusanovich et al Science 2015



# Single-cell ATAC-seq

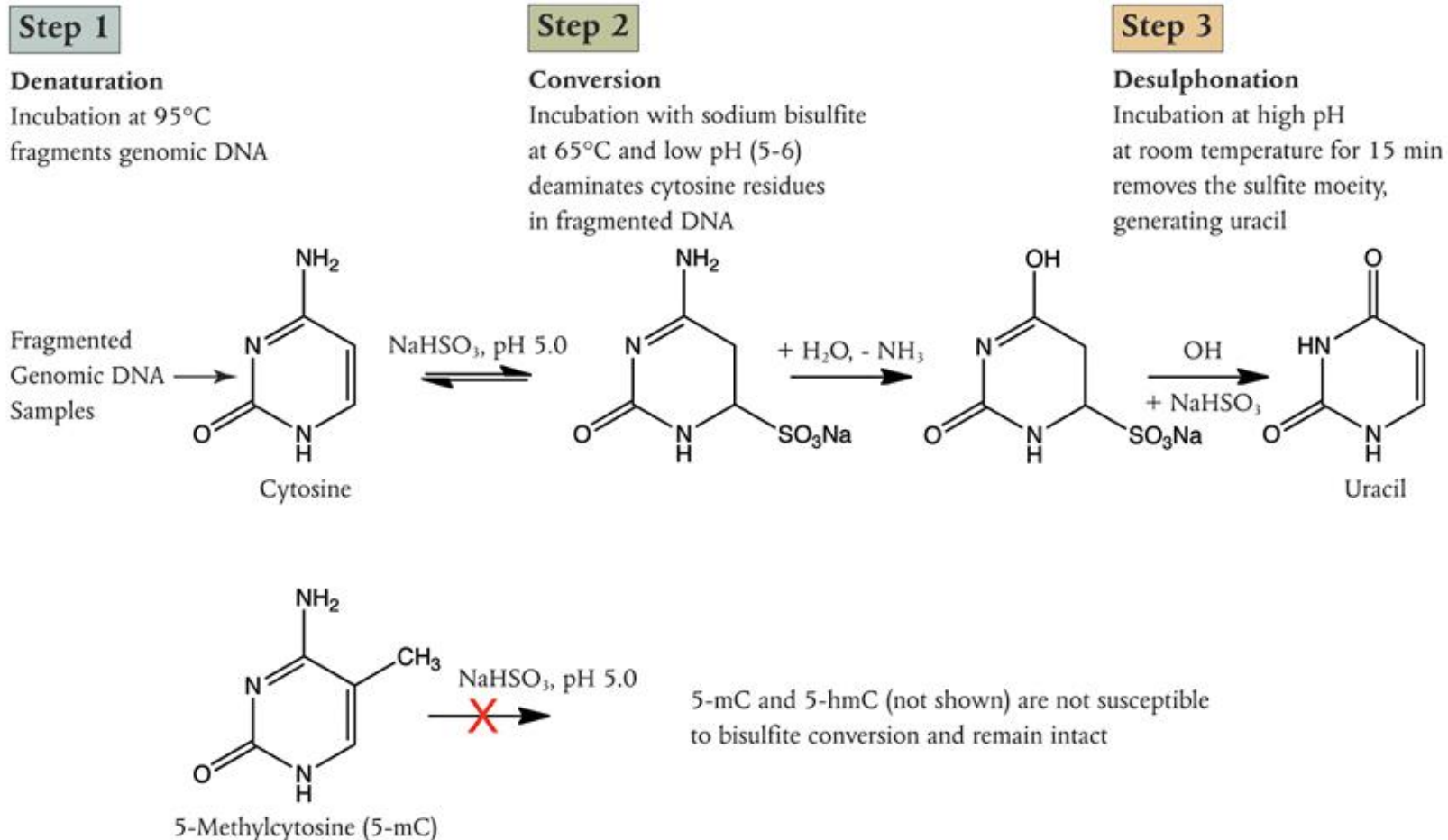
Droplet-based: Market leader is 10x Genomics





# Bisulfite conversion

Chemical treatment to distinguish C nucleotides from 5-mC and 5-hmC



# Pros and cons of bisulfite conversion

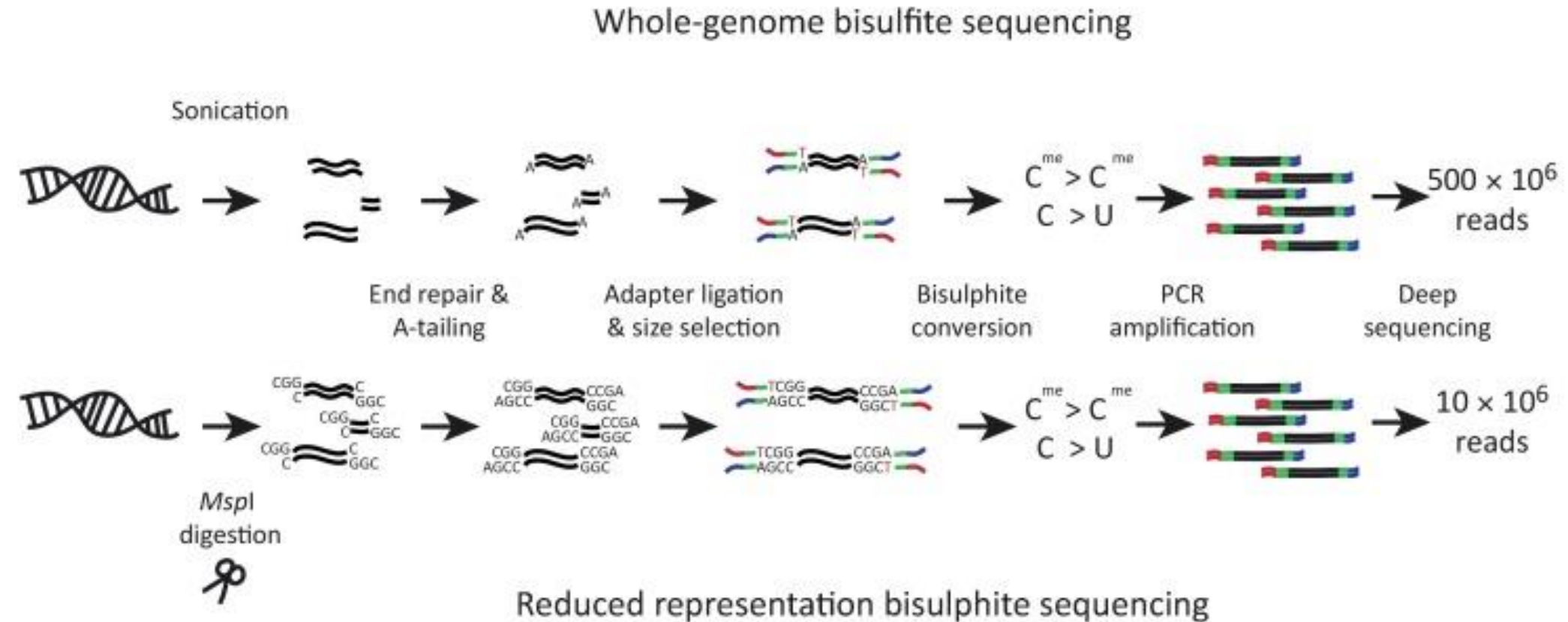
## Pros

- Covers CpG and non-CpG methylation throughout the genome at single-base resolution.
- Covers 5mC in dense, less dense, and repeat regions

## Cons

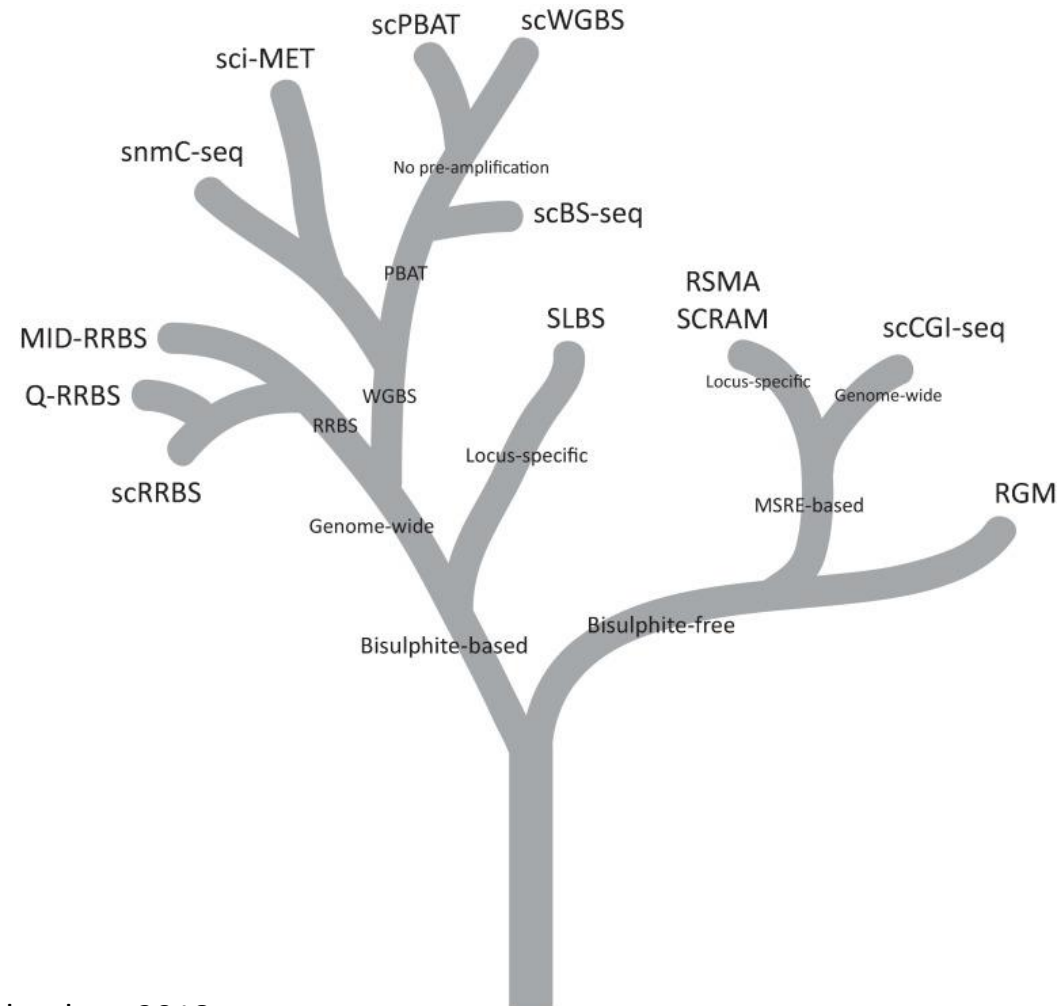
- Bisulfite converts unmethylated cytosines to thymidines, reducing sequence complexity, which can make it difficult to create alignments.
- SNPs where a cytosine is converted to thymidine will be missed upon bisulfite conversion.
- Bisulfite conversion does not distinguish between 5mC and 5hmC.

# Single-cell methylation

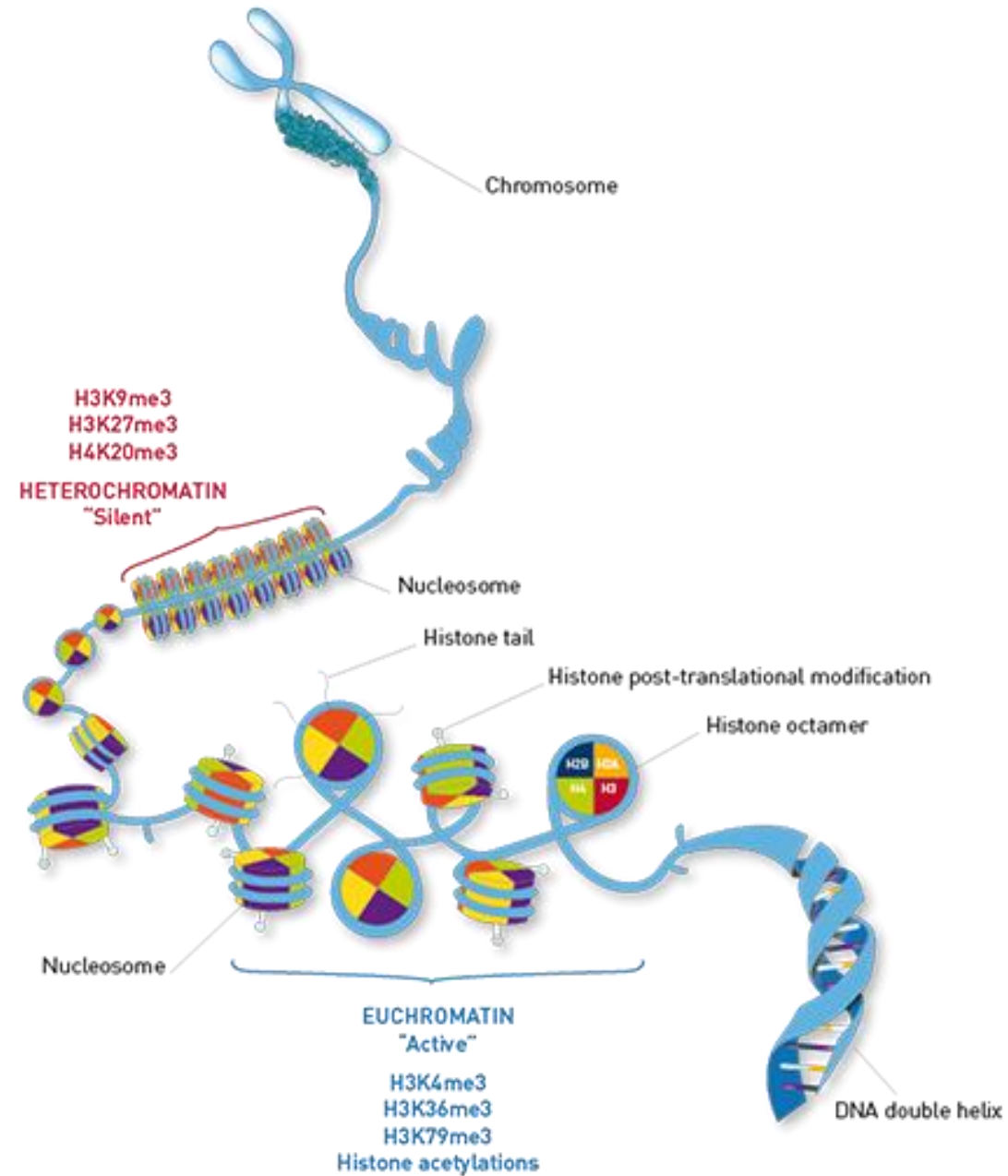


Trends in Biotechnology

# Single-cell methylation cont.



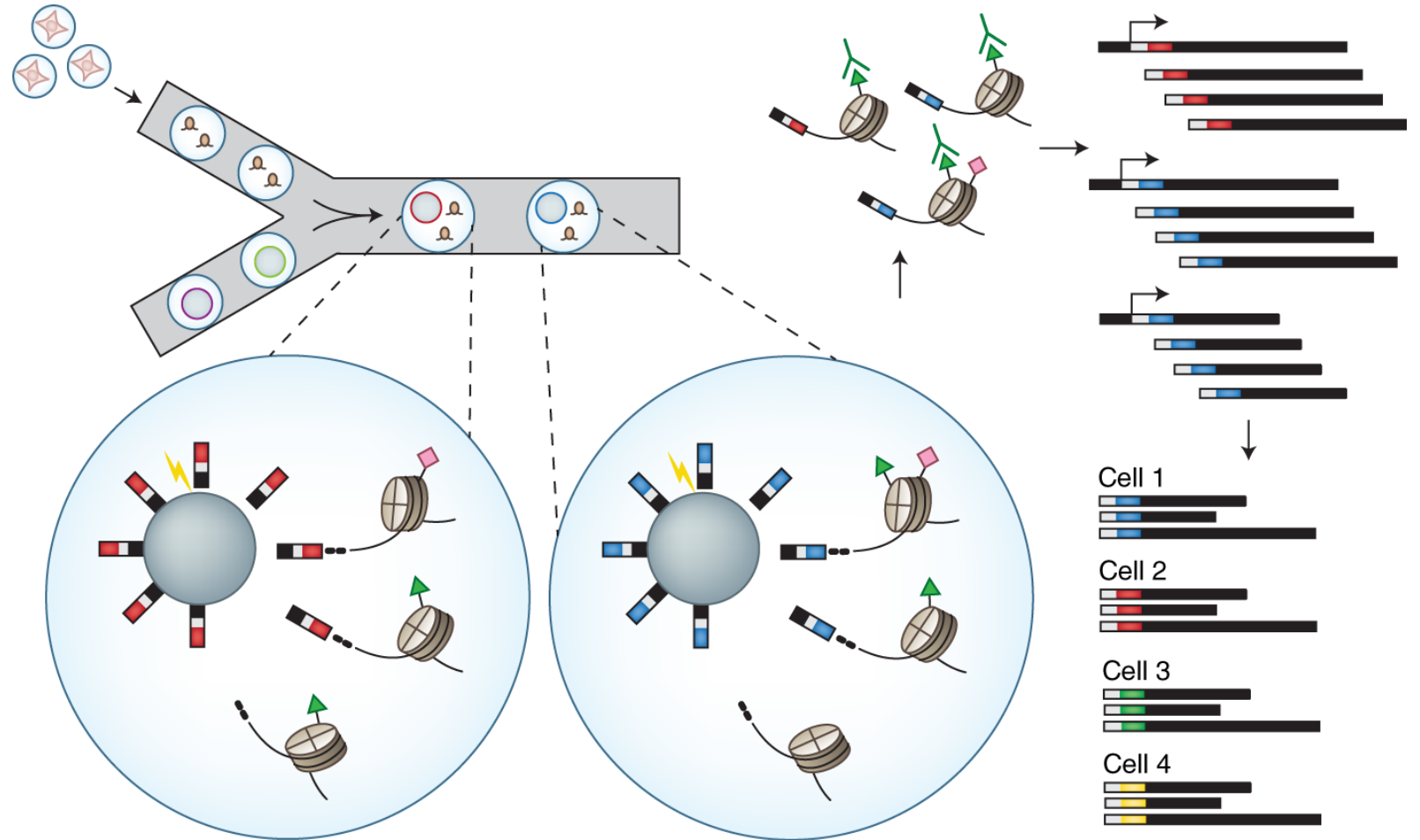
# Histone code



# Single-cell ChIP-seq

Droplet based:  
Drop-ChIP &  
scChIP-seq

MNase + gel bead  
Photocleavable barcodes  
Bulk IP  
Single-cell count table



# Single-cell ChIP-seq

Microwell-based  
CUT&Tag

Antibody binding in cell

Transposition in cell

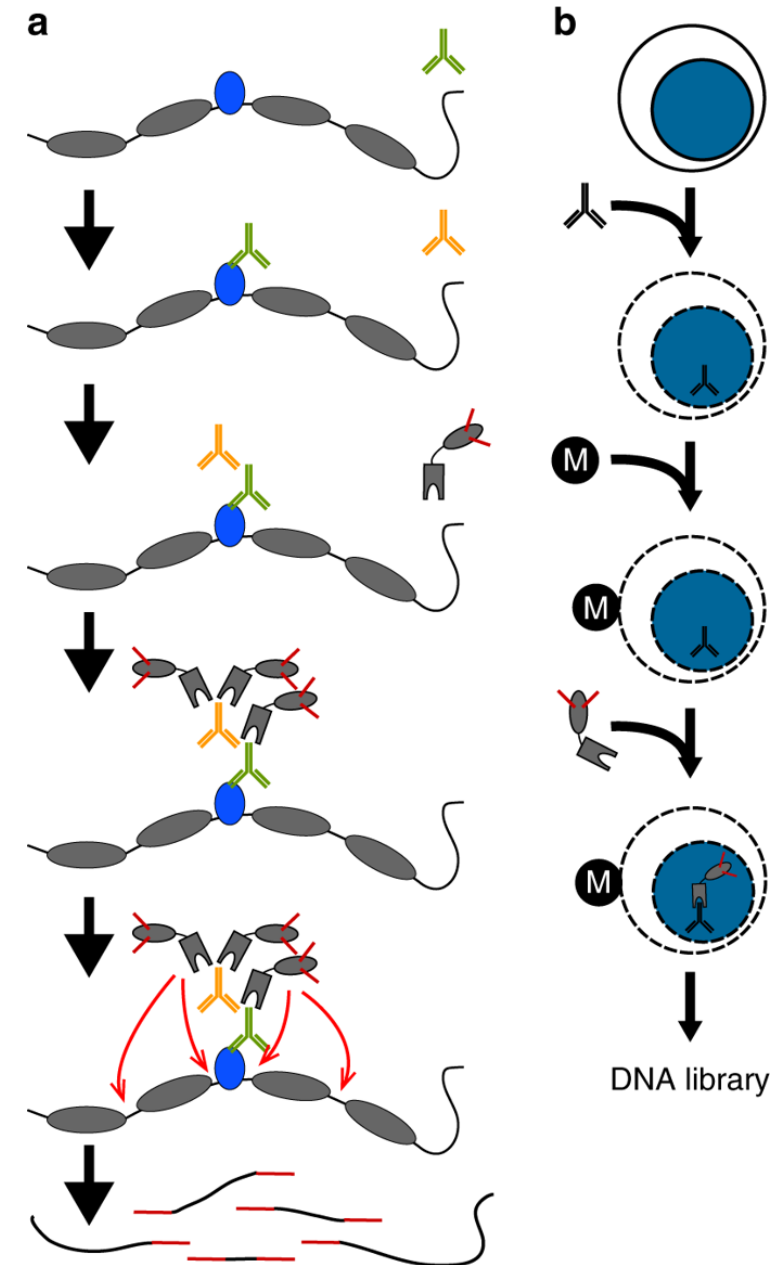
Sort into iCELL8 microwells

Prep libraries + sequence

[Kaya-Okur et al Nature Communications 2019](#)

Recently combined with 10x Genomics ATAC-seq gel beads:

[Bartosovic et al bioRxiv Sept 2020](#)



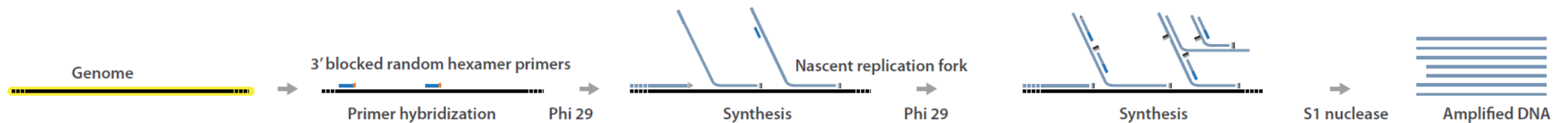


# Single-cell whole genome - MDA

A single human genome contains just 6.6pg DNA

⇒ Requires whole genome amplification (WGA)

Most common: Multiple strand displacement amplification (MDA)



## Advantages

- Template can be circular DNA (plasmids, bacterial DNA).
- Can sequence large templates.
- Can perform single-cell sequencing or sequencing for samples with very limited starting material.

## Disadvantages

- Strong amplification bias. Genome coverage as low as ~6%.<sup>273</sup>
- PCR biases can underrepresent GC-rich templates.
- Contaminated reagents can impact results.<sup>274</sup>

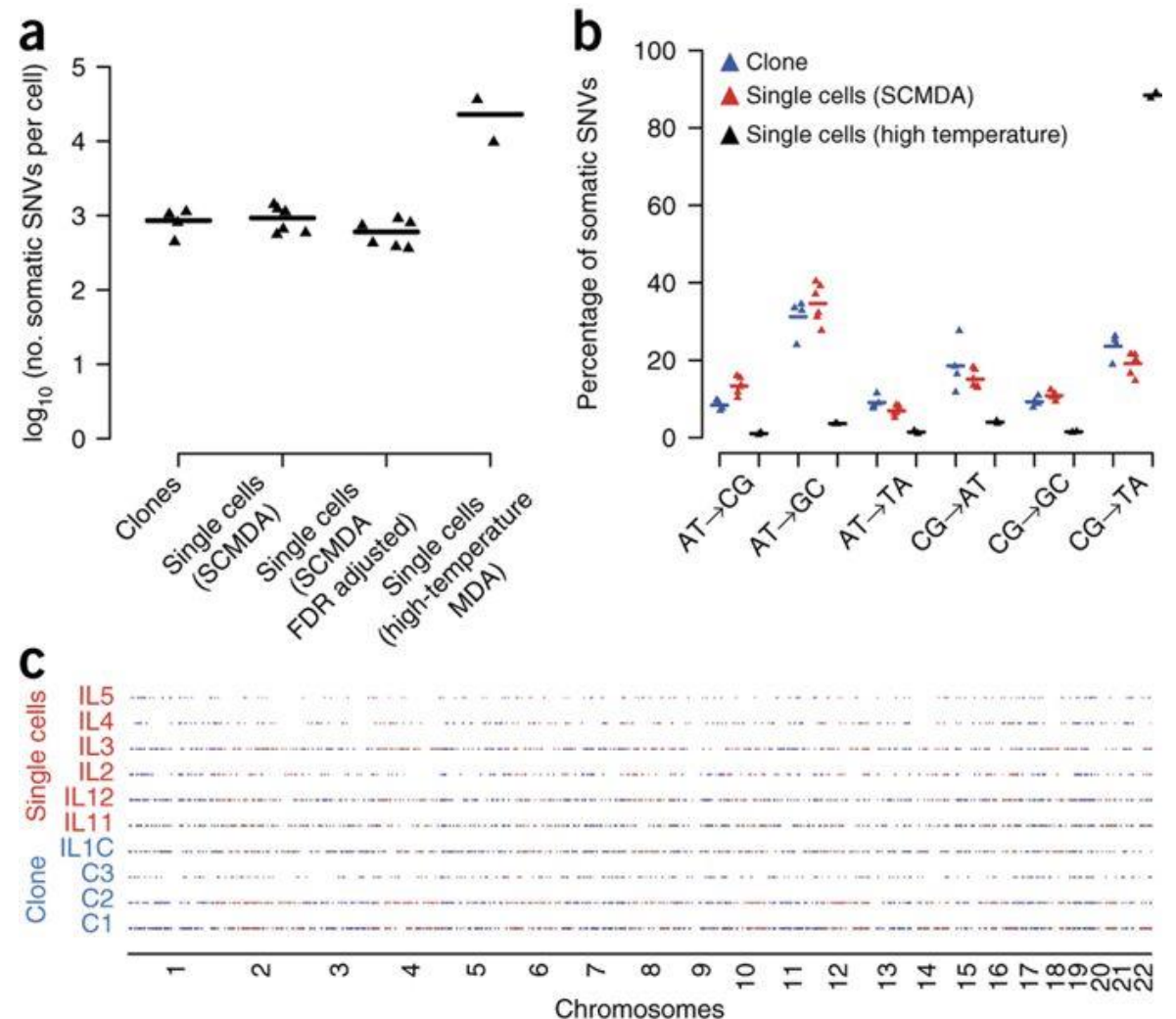
# Single-cell whole genome

## SCMDA protocol

- 85% of genome at >5x coverage
- SCMDA amplified cells are nearly identical to unamplified clones

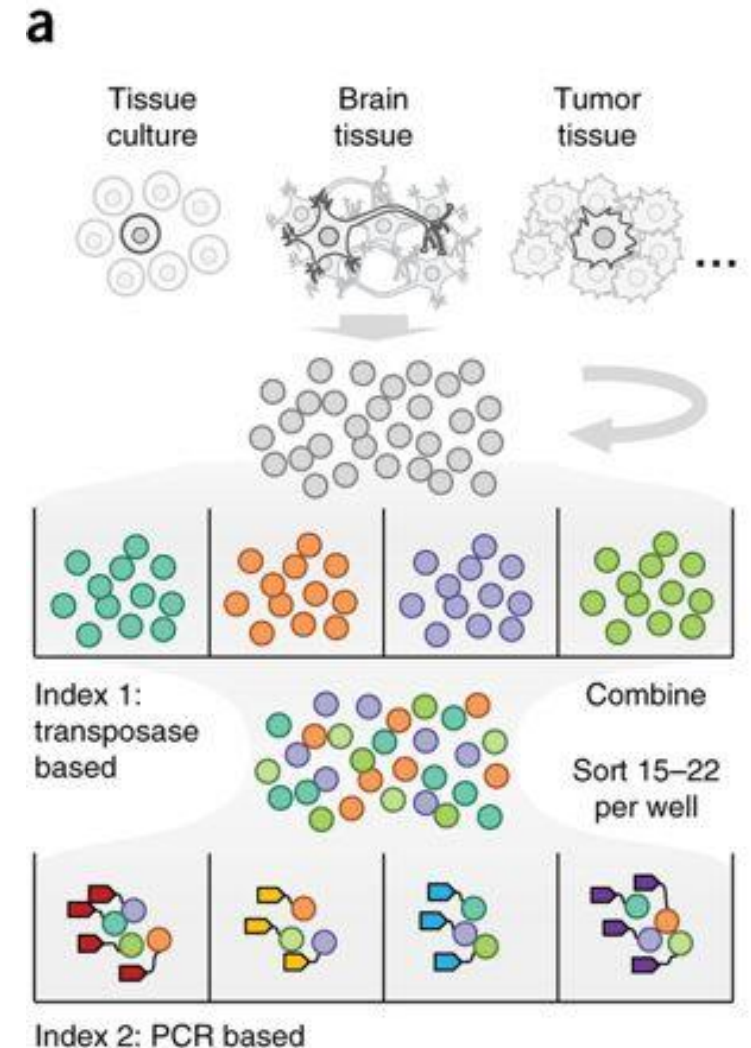
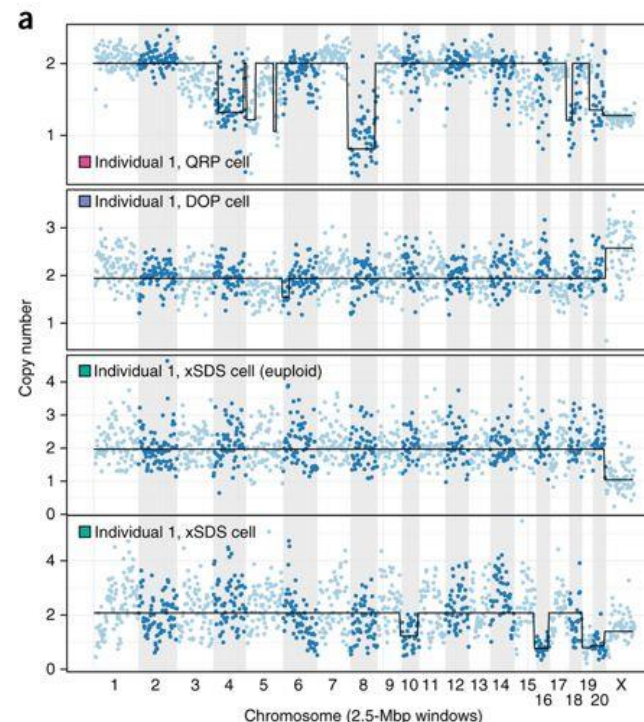
## SCcaller software

## General purpose single-cell variant-caller



# scDNA-seq – Copy number variation

- Plate-based
  - SCI-seq = single-cell combinatorial indexed sequencing
    - CNV calling on >10k cells from cancer and Rhesus macaque brain



# Copy number variation – Droplet based

- Mission Bio Tapestri
  - Uses proteases to break down chromatin
  - Panel-based PCR (up to 400 targets)
  - Can call both CNVs and SNVs in target regions
- Up to 10k cells
- Rare subclone detection, down to ~0.1%

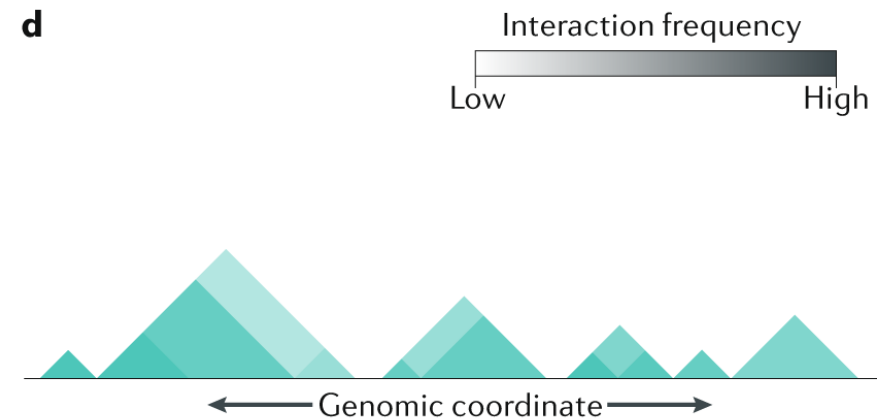
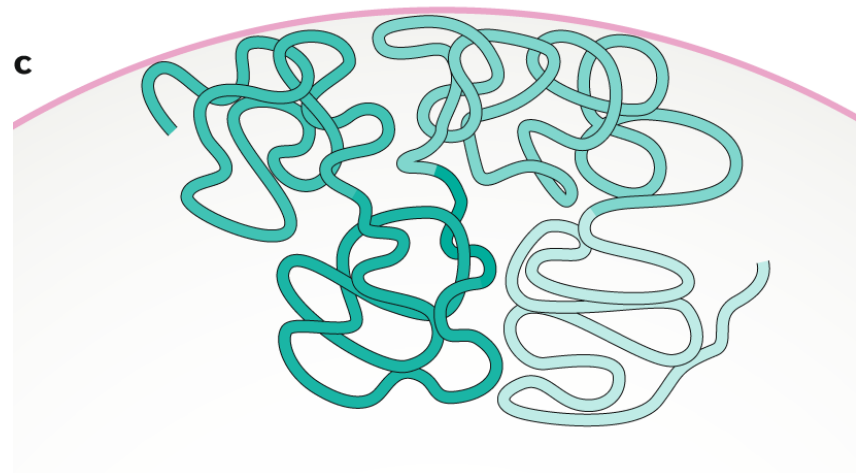
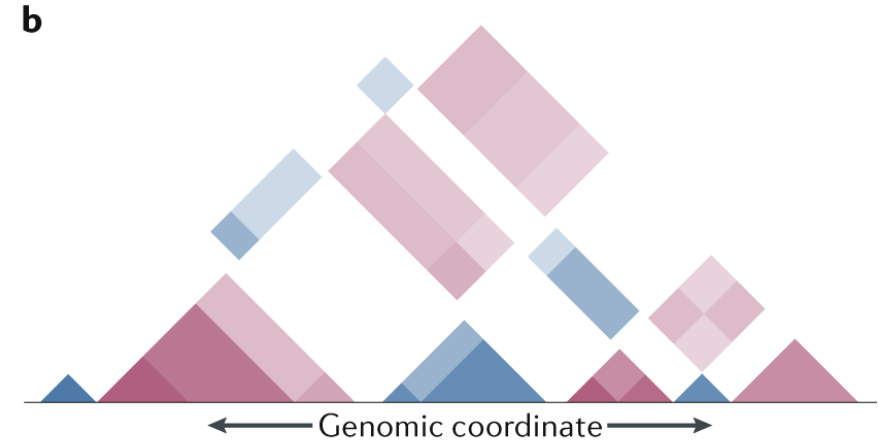
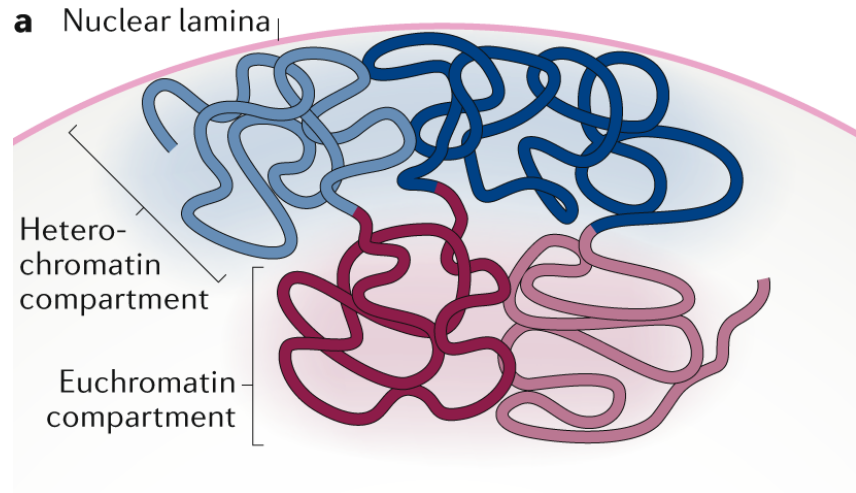


# Copy number variation – Droplet based

- 10x Genomics
  - Whole genome CNV
  - Resolution down to 2 Mb at single-cell level
  - 750k reads pairs / cell = <1x coverage
  - Sensitivity increases with >10 cells / phenotype
    - 100-200kb events
- Max of 5000 cells/sample
- Sequencing costs remain high (2000 cells = ~5000 EUR)
  - This is ~15x more expensive than scRNA-seq sequencing



# Nuclear architecture - Hi-C assay





# Single-cell Hi-C

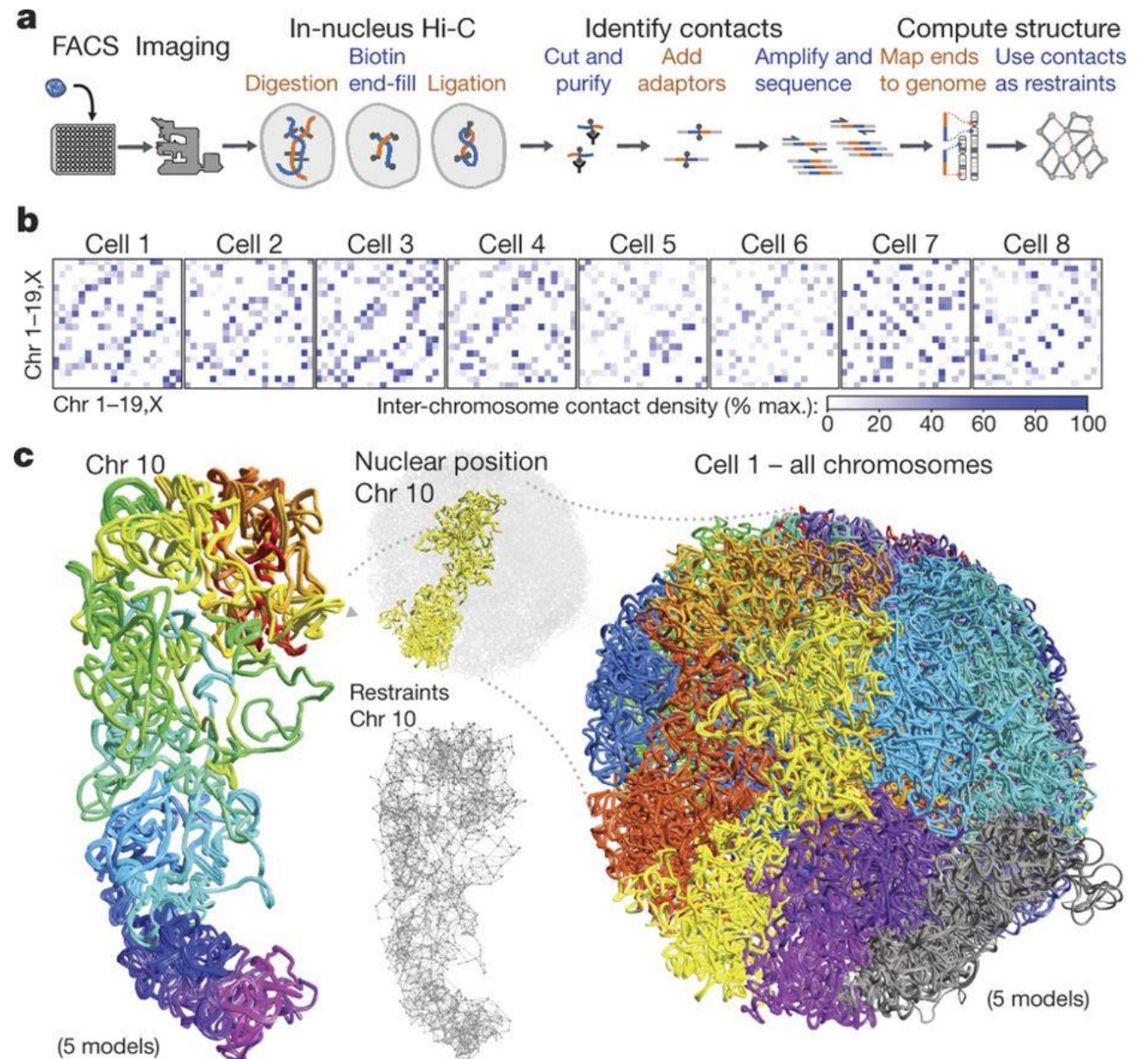
Individual haploid G1 phase mESCs

Super-resolution microscopy +  
single-cell Hi-C

Examine topological domains +  
looping at <100kb scale

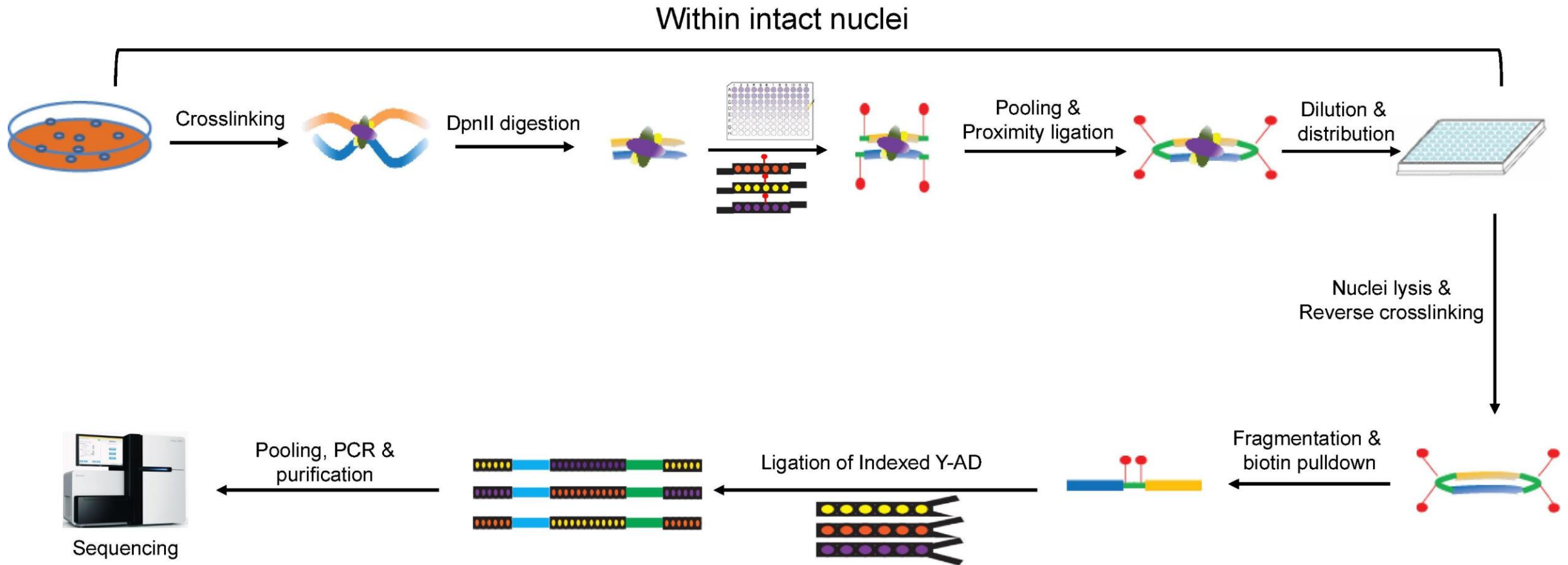
Can validate sequencing data with  
imaging data

Stevens et al Nature 2017





# Single-cell Hi-C, sci-Hi-C



# Single-cell proteomics

- CyTOF
- Proteogenomics (lecture from Miao)
- [sc-Western blots](#)
  - High throughput (1000 cells)
  - Low resolution (12 antibodies)
- [SCoPE-MS](#)
  - Low throughput (dozens of cells)
  - High resolution (1000 proteins)

