

Overview of Single-Cell Assays and Platforms

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MGC Course on Single-Cell Analysis

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Why single-cell?

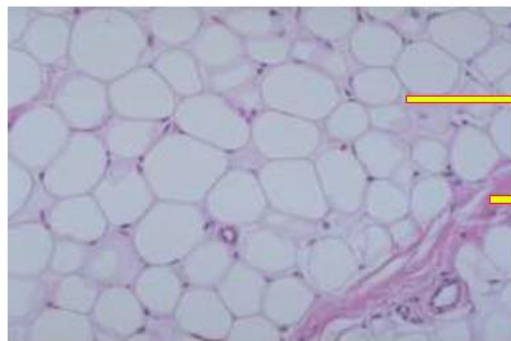
Bulk sample analysis is just like putting a fruit salad into a blender - the taste is an average of all ingredients.



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



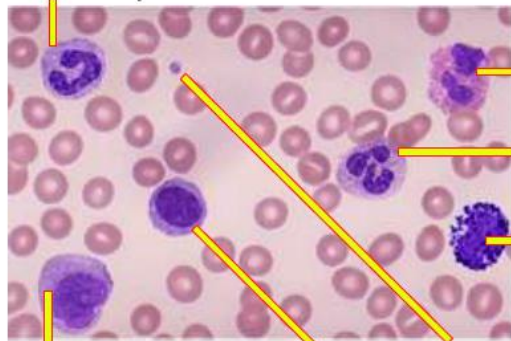
Adipose

Fat

Connective
tissue

Band
Neutrophil

Normal Peripheral Blood



Eosinophil

Segmented
Neutrophil

Basophil

Monocyte

Platelet
Lymphocyte

Small Intestine Mucosa



Epithelial cells

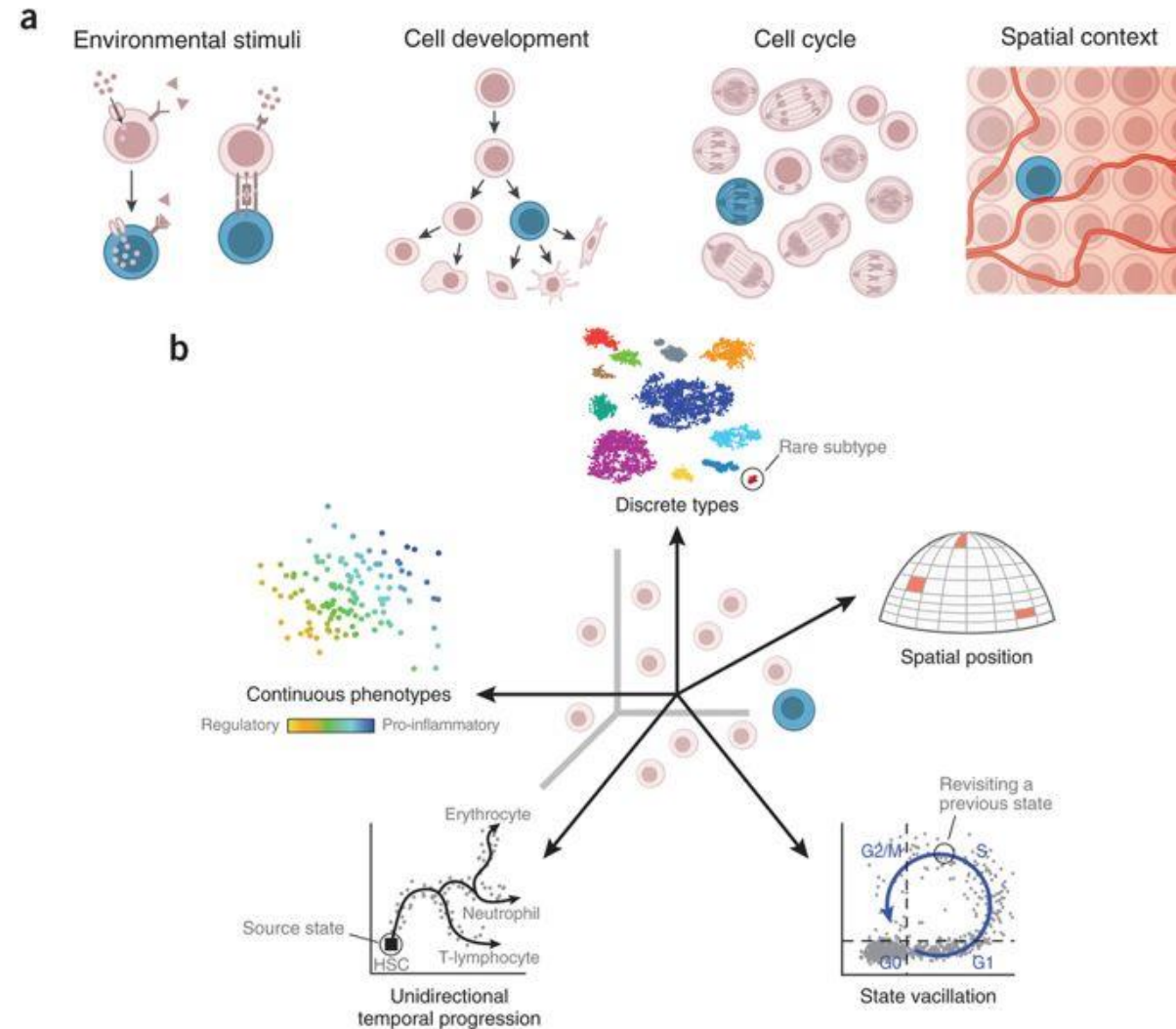
Goblet
cells

Lamina
propria

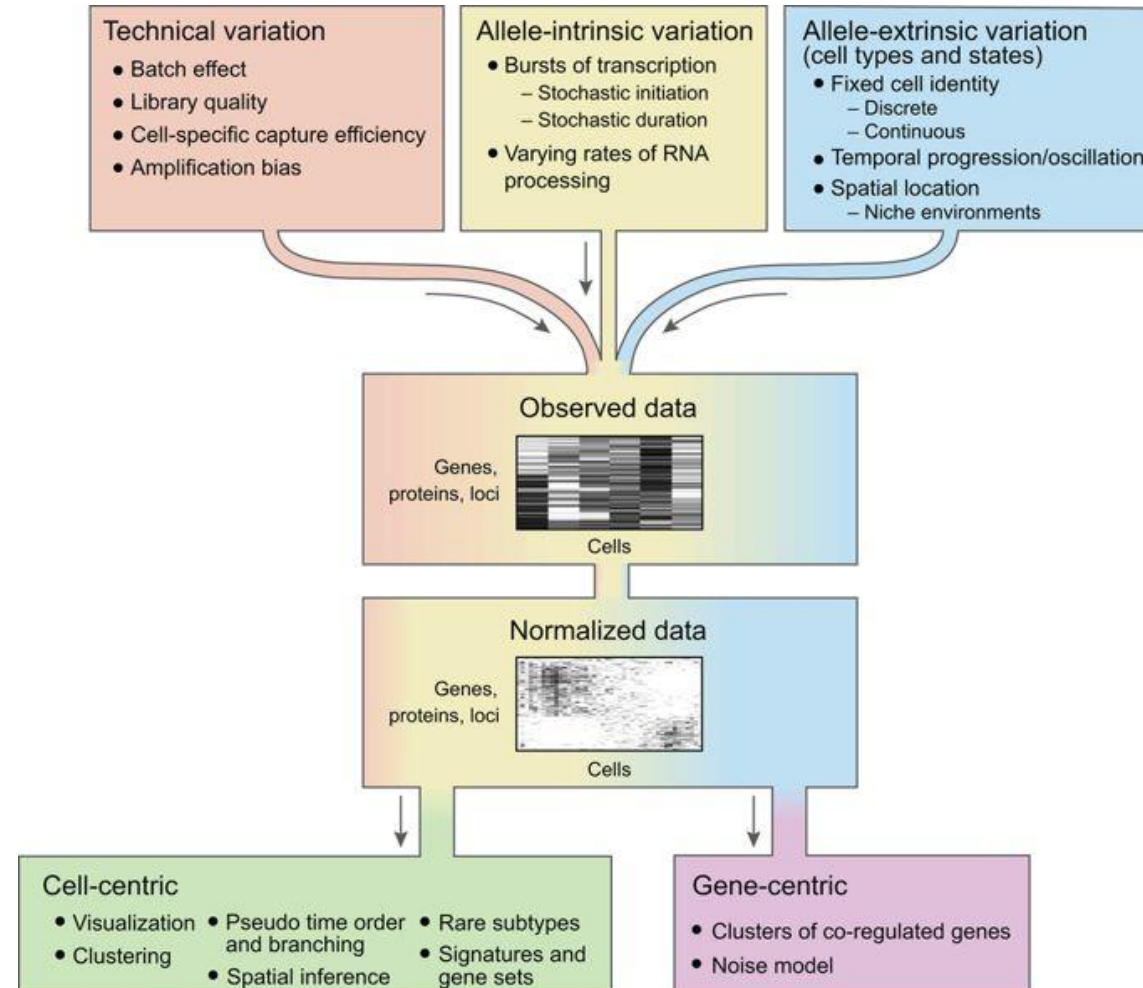
Muscularis mucosa
(smooth muscle)

Created with figures from library.med.utah/WebPath/HISTHTML/HISTO.html

Cell identity is more than histopathology



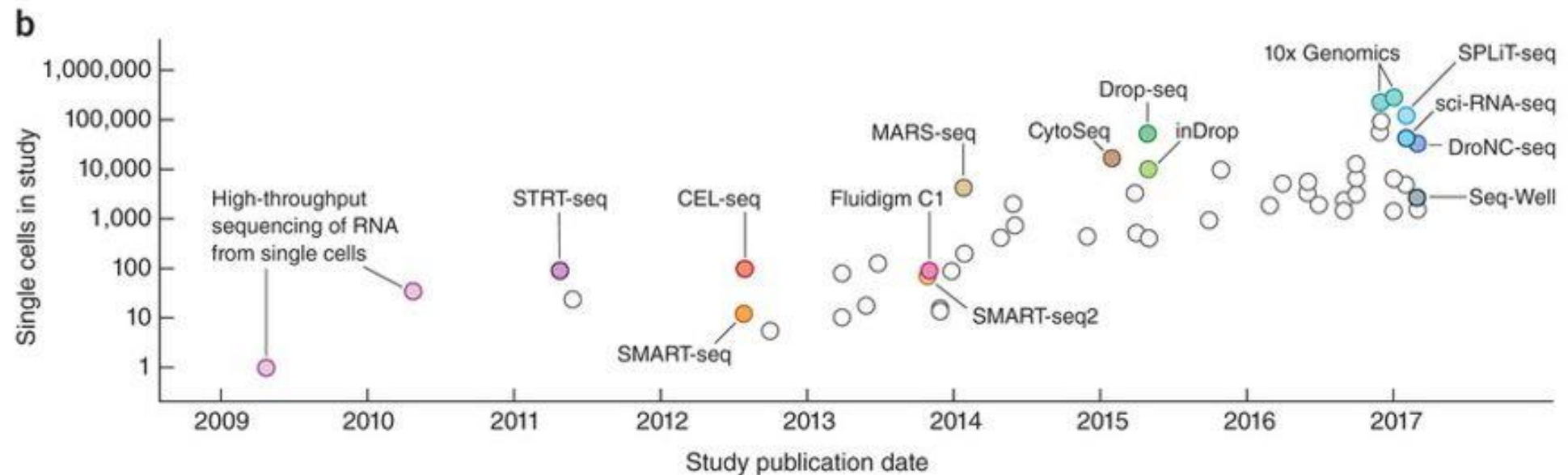
Biology is messy – computational methods help to clean this up



We will cover

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

Exponential scaling of single-cell throughput



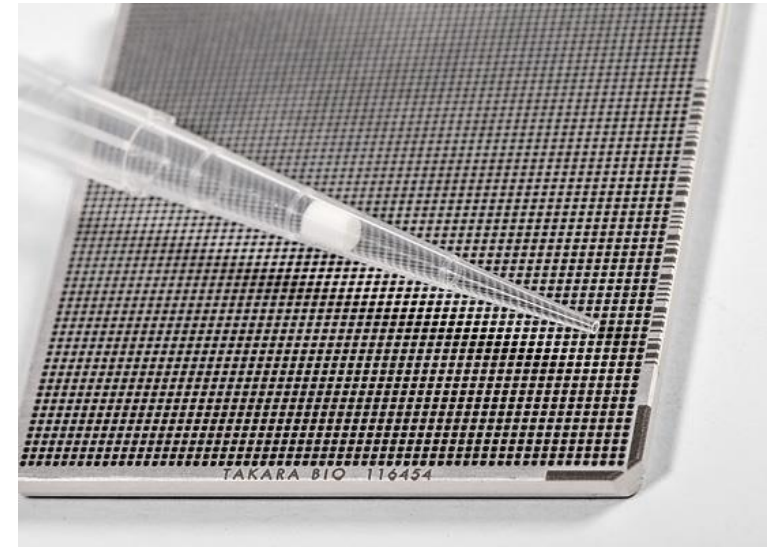
scRNA-seq

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

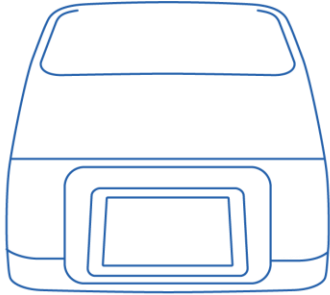


BD Rhapsody

- Works with targeted panels to reduce sequencing costs
- Up to 400 amplicons / sample
- Includes UMIs to reduce PCR amplification bias
- Increased flexibility
 - Archiving up to 3 months
 - Sub-sampling



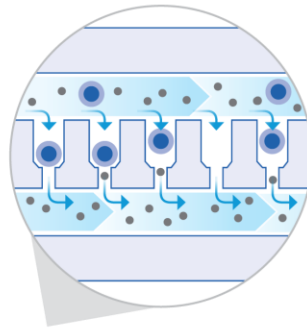
Celsee Genesis platform



Isolation



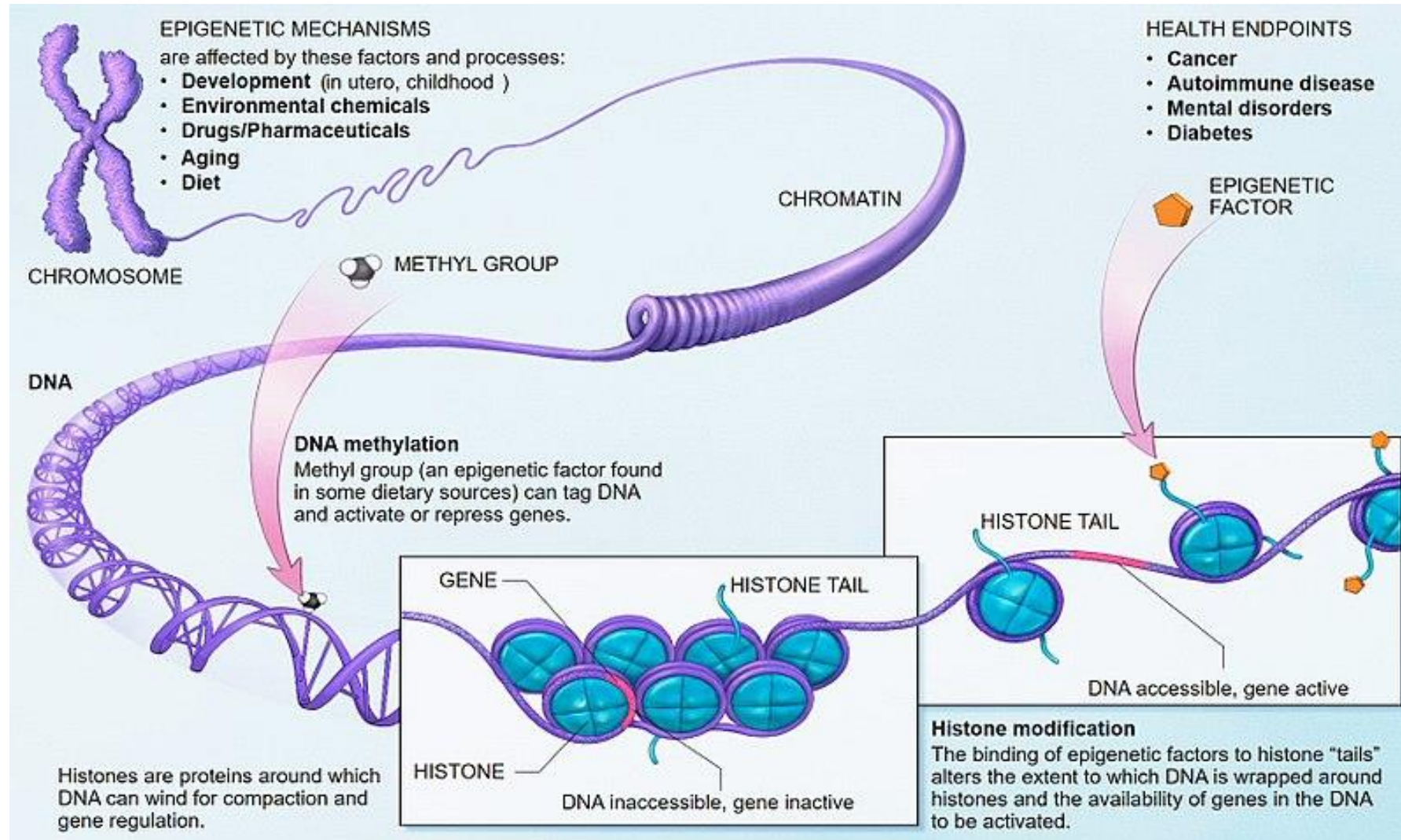
Celsingle™ Slide



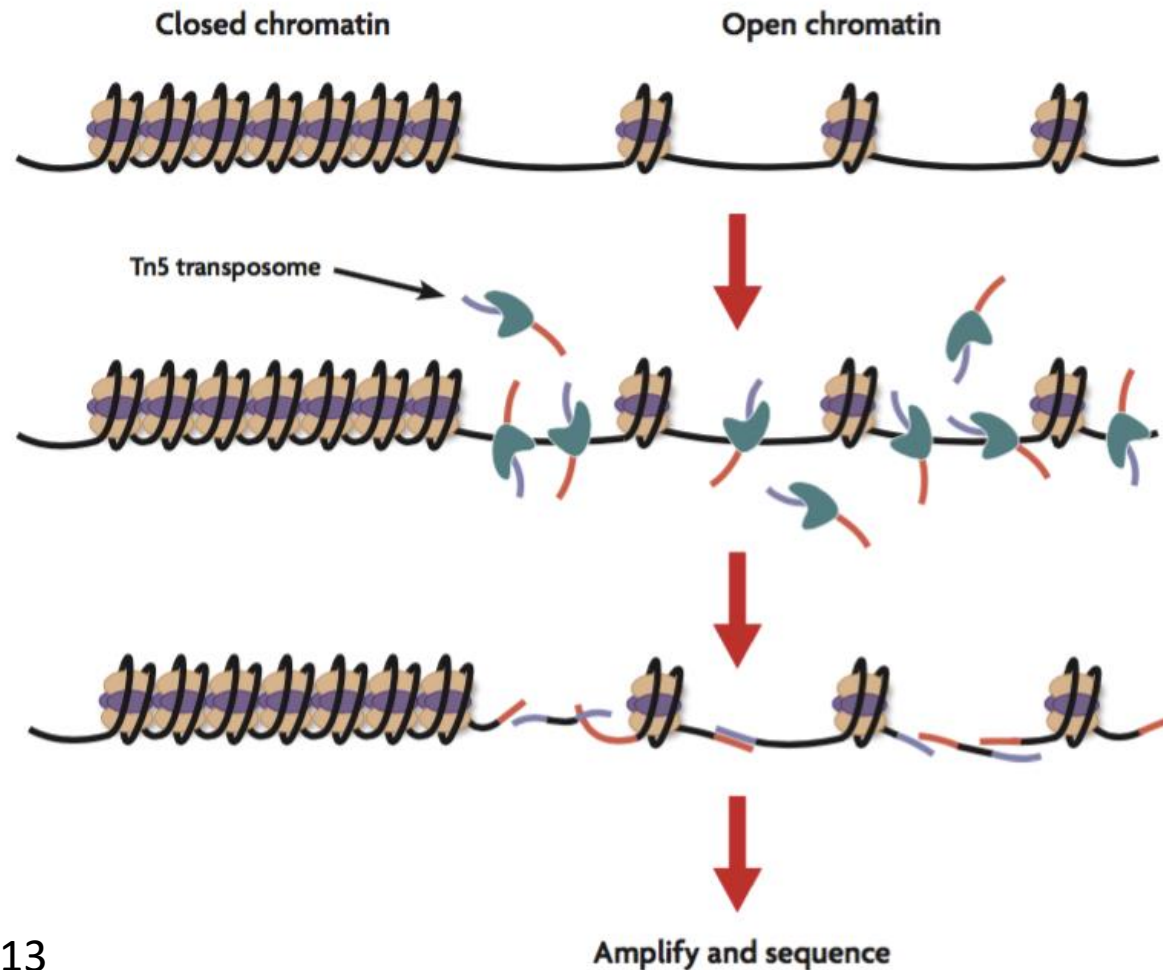
Celselect™ Slide



What is epigenetics?



Assay for transposase-accessible chromatin (ATAC-seq)



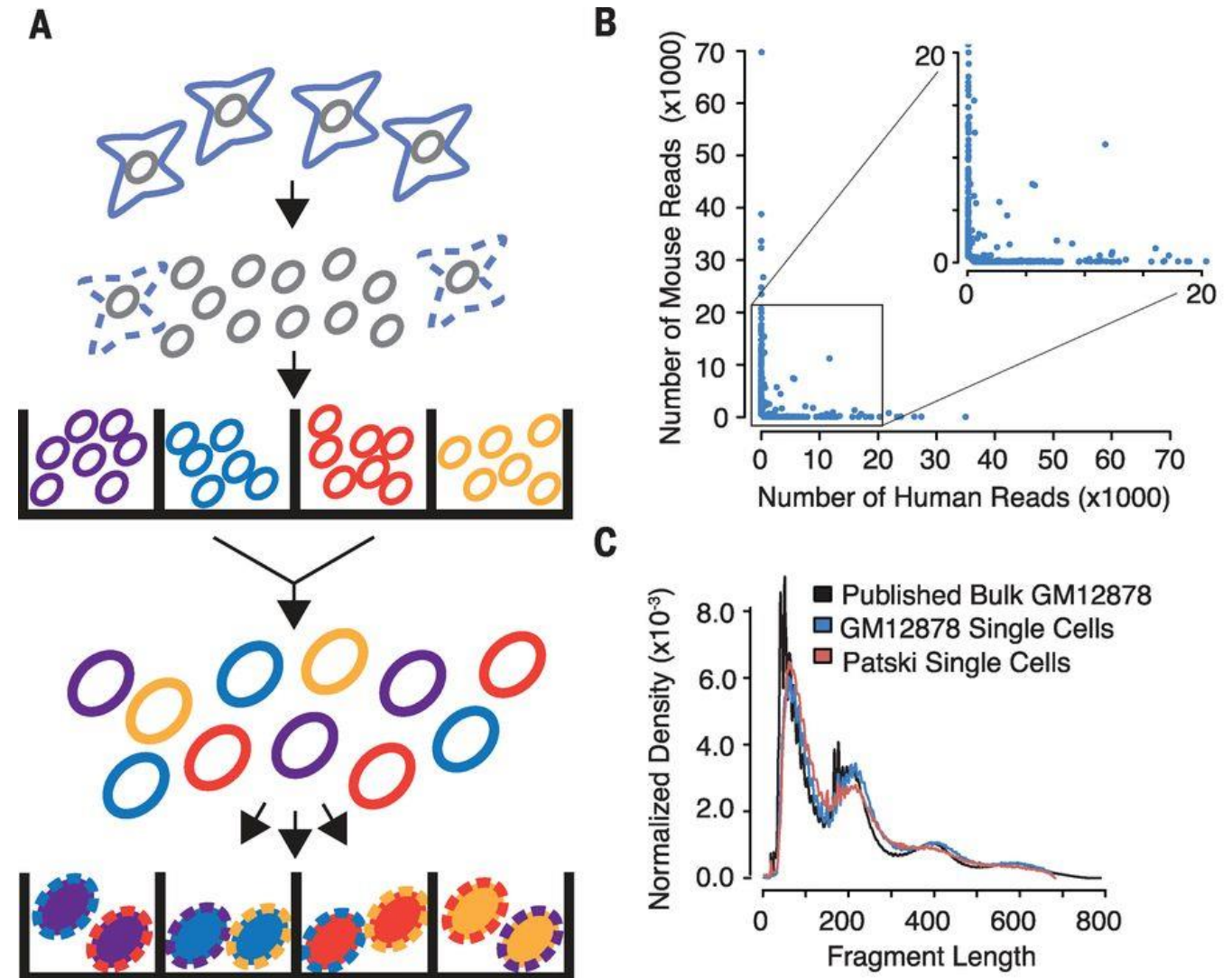
Single-cell ATAC-seq

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

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

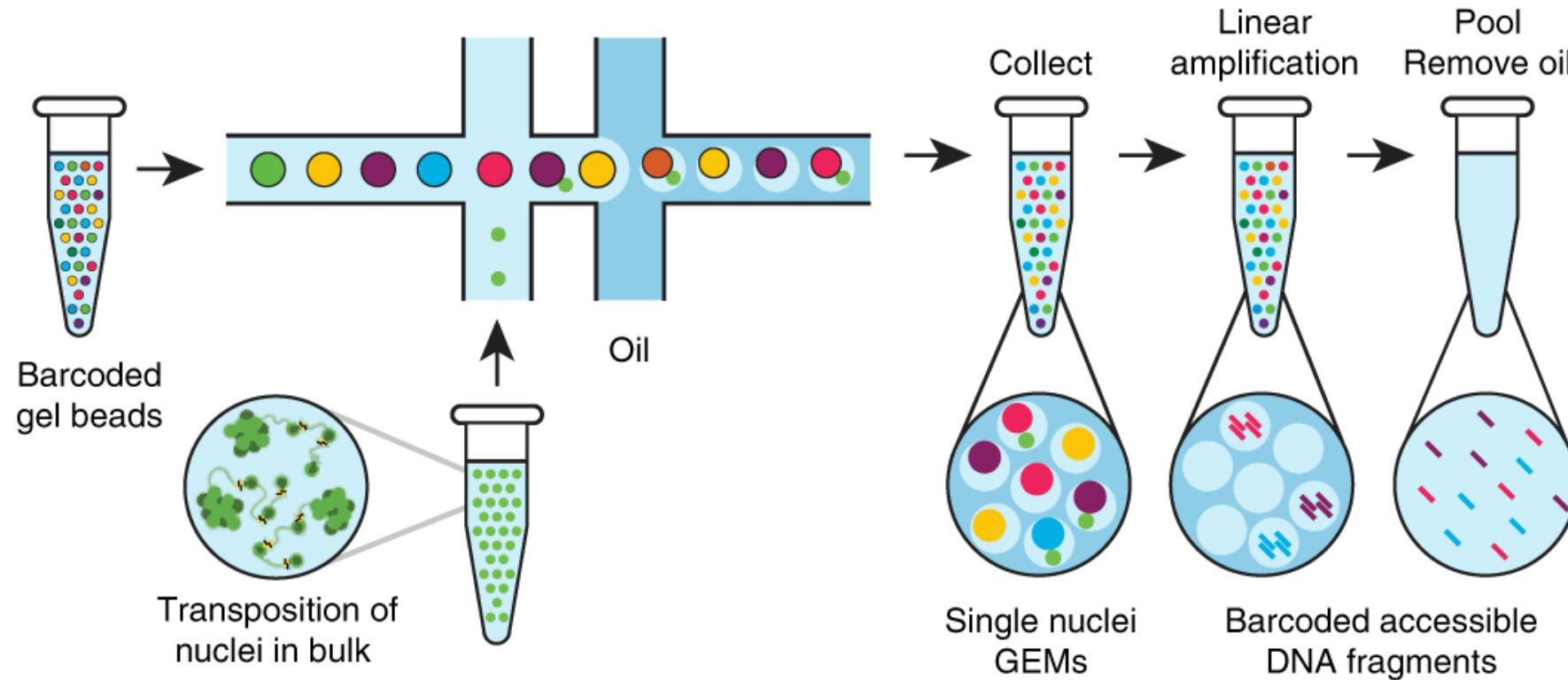
Drawbacks: low coverage,
max ~3000 unique reads/cell

Cusanovich et al Science 2015



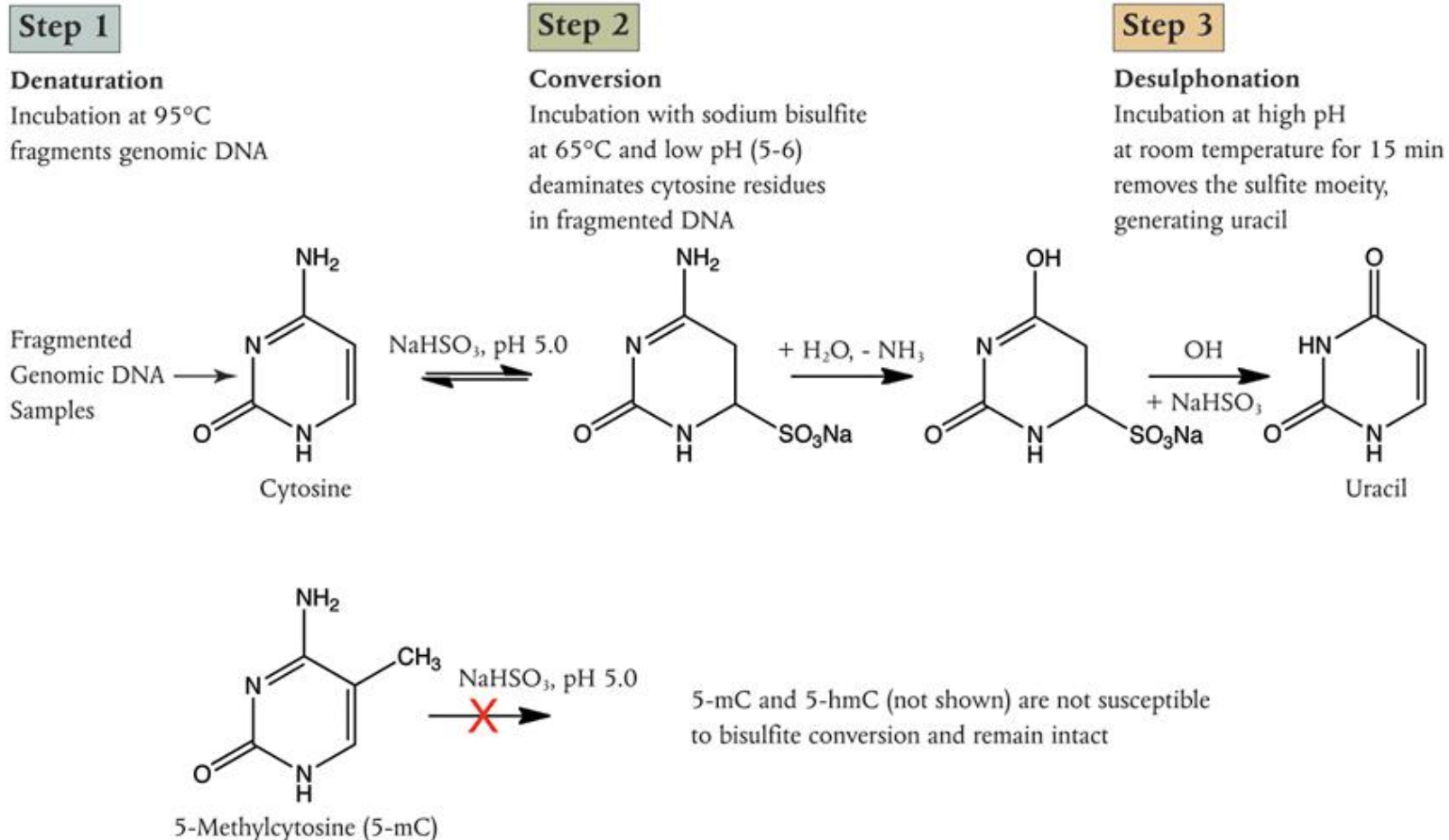
Single-cell ATAC-seq

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

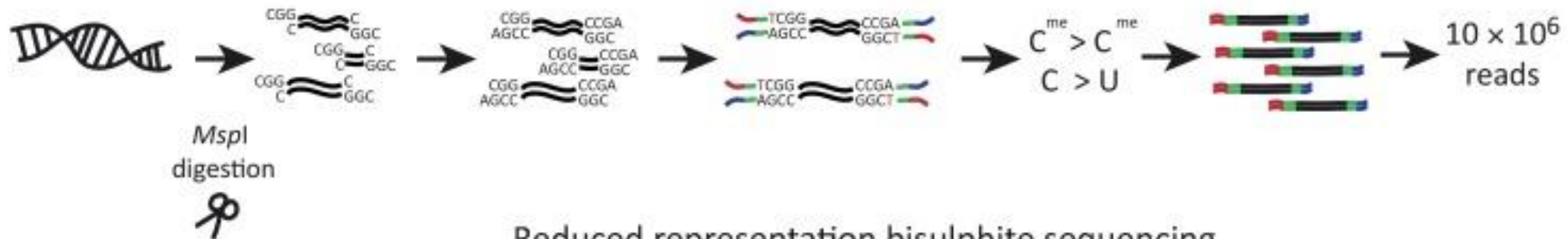
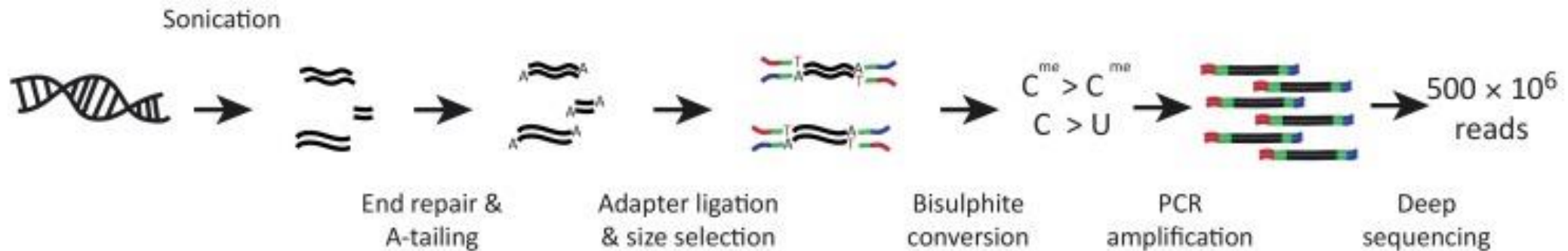
- Covers CpG and non-CpG methylation throughout the genome at single-base resolution.
- Covers 5mC in dense, less dense, and repeat regions

Cons

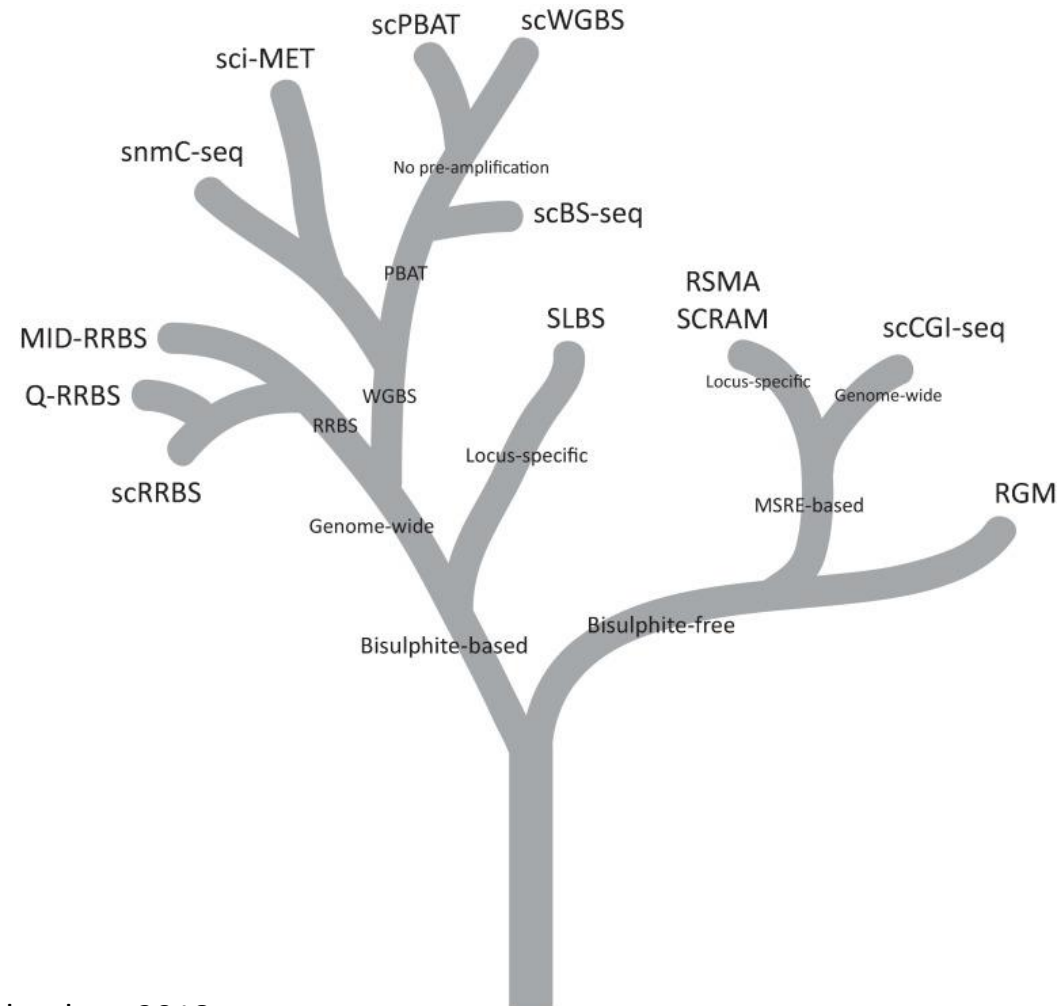
- Bisulfite converts unmethylated cytosines to thymidines, reducing sequence complexity, which can make it difficult to create alignments.
- SNPs where a cytosine is converted to thymidine will be missed upon bisulfite conversion.
- Bisulfite conversion does not distinguish between 5mC and 5hmC.

Single-cell methylation

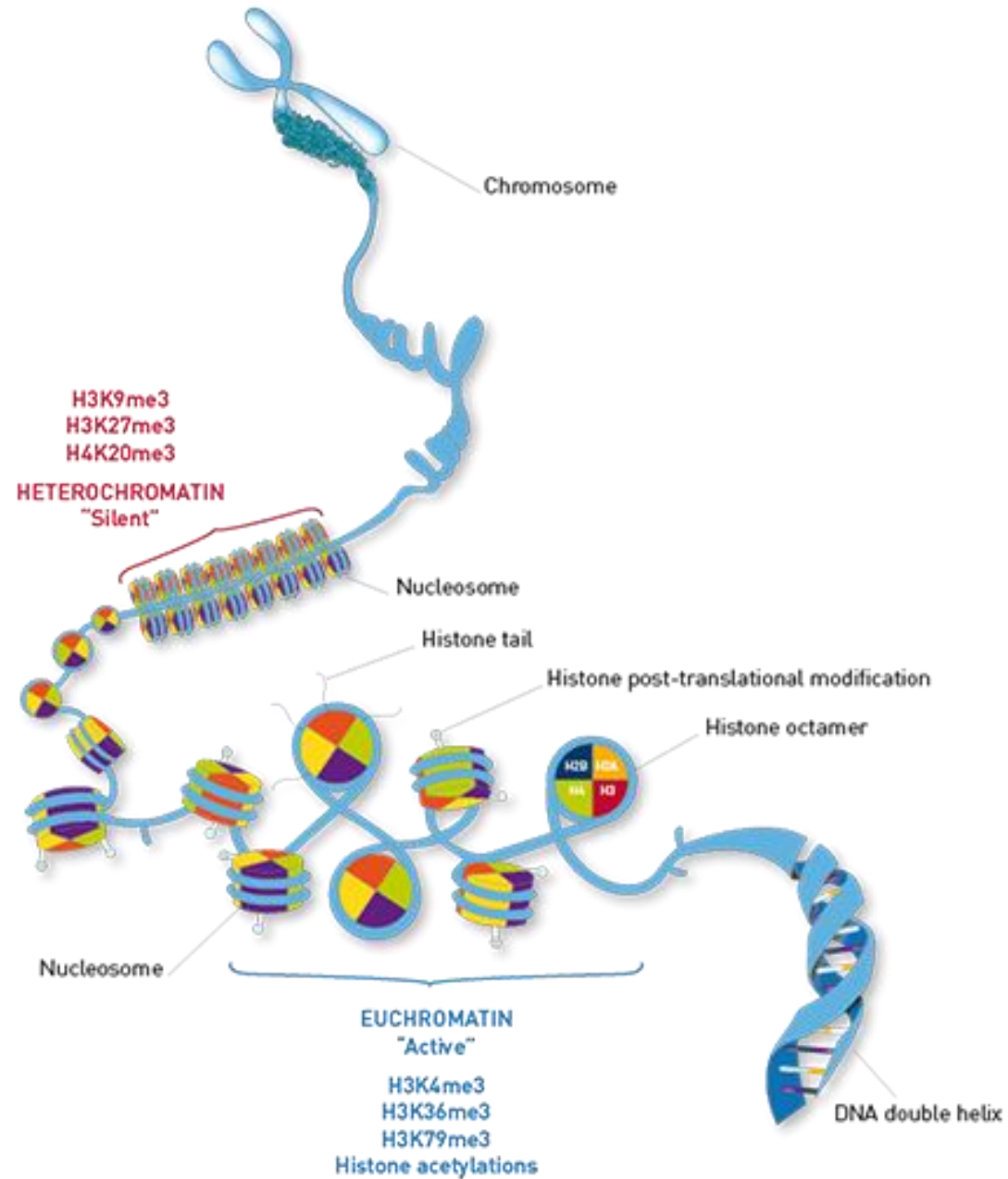
Whole-genome bisulfite sequencing



Single-cell methylation cont.



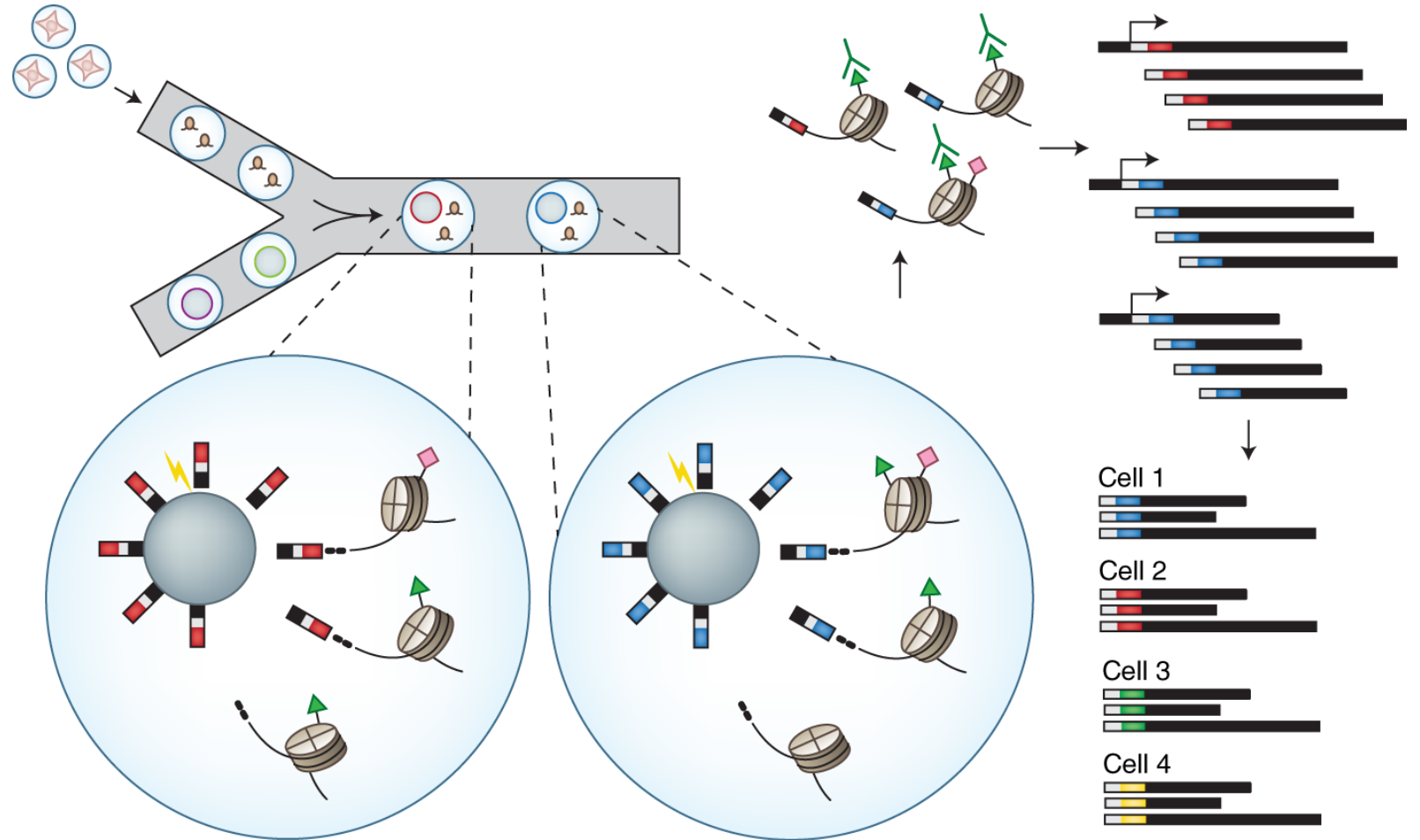
Histone code



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

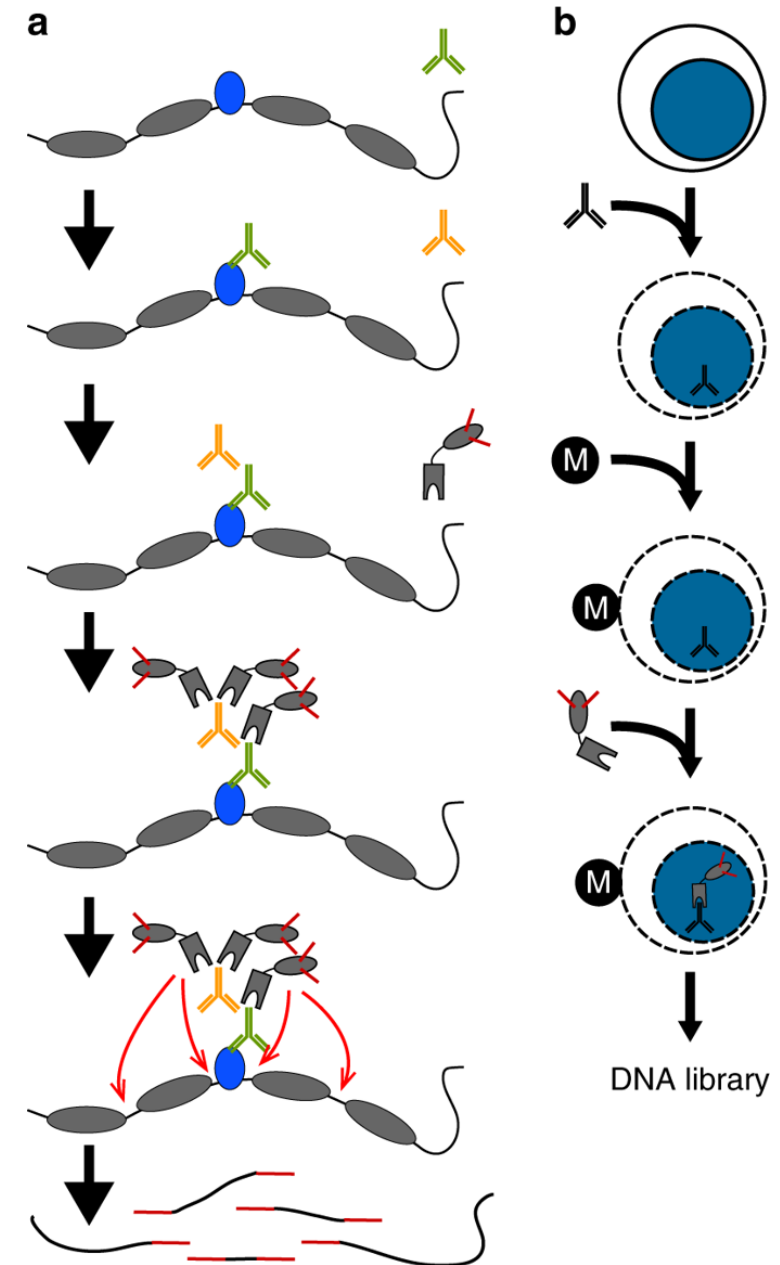
Microwell-based
CUT&Tag

Antibody binding in cell

Transposition in cell

Sort into iCELL8 microwells

Prep libraries + sequence

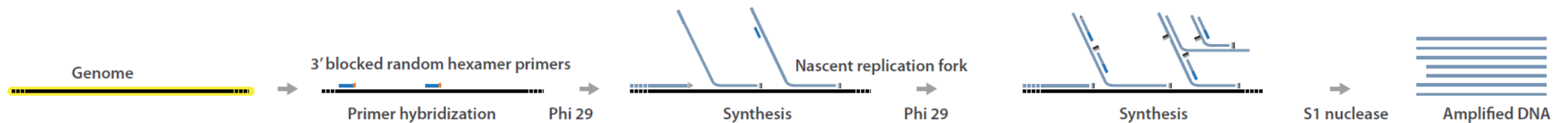


Single-cell whole genome - MDA

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%.²⁷³
- PCR biases can underrepresent GC-rich templates.
- Contaminated reagents can impact results.²⁷⁴

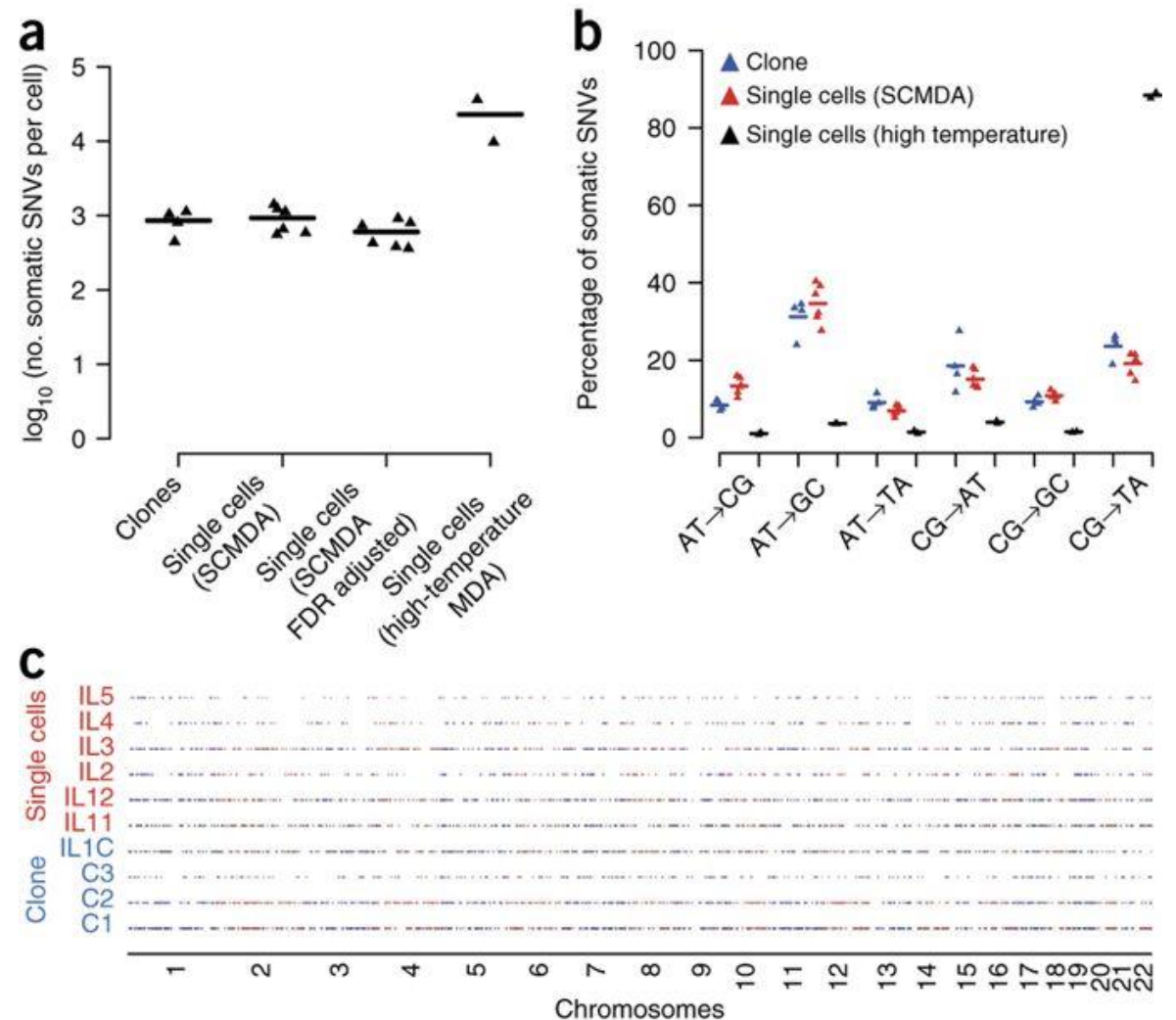
Single-cell whole genome

SCMDA protocol

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

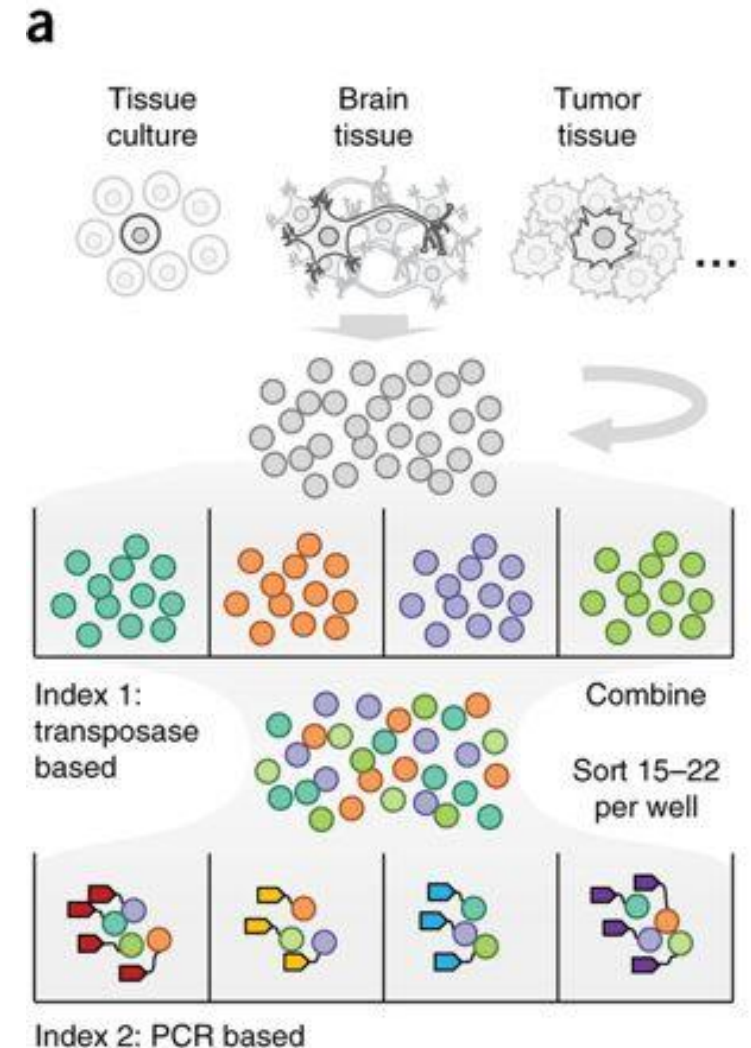
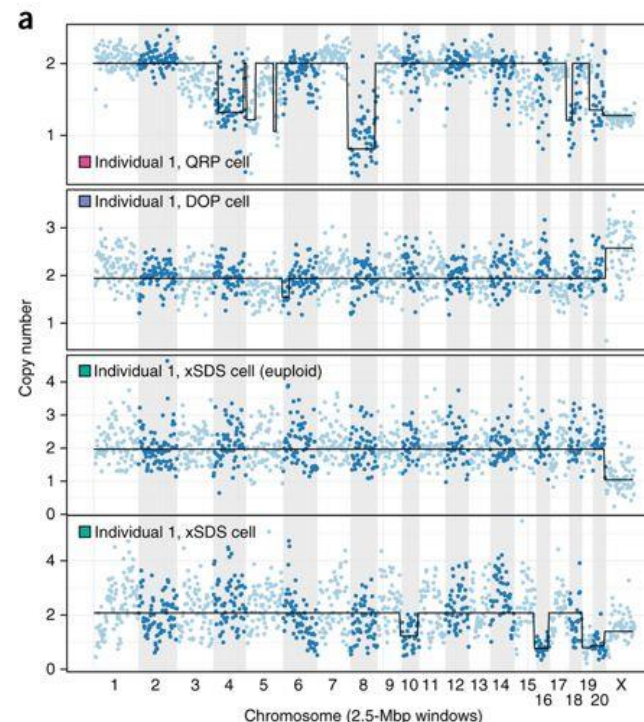
SCcaller software

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%

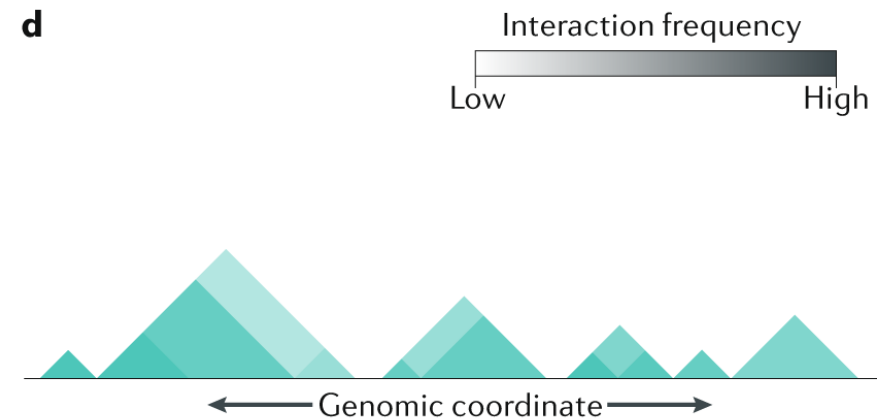
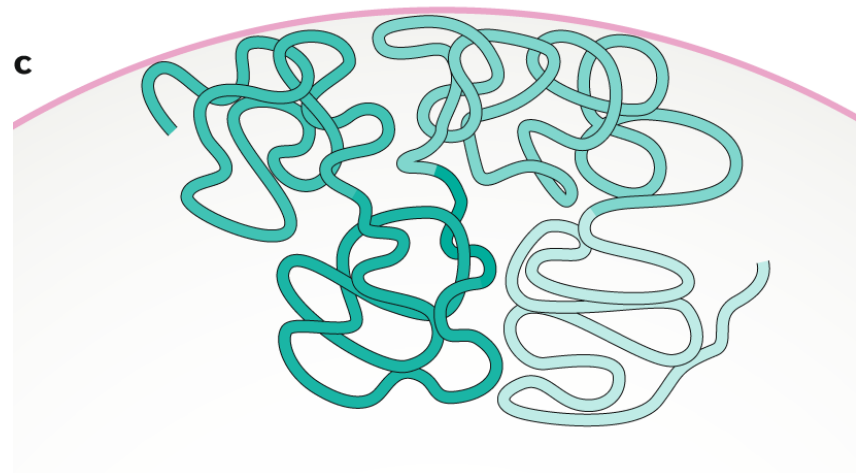
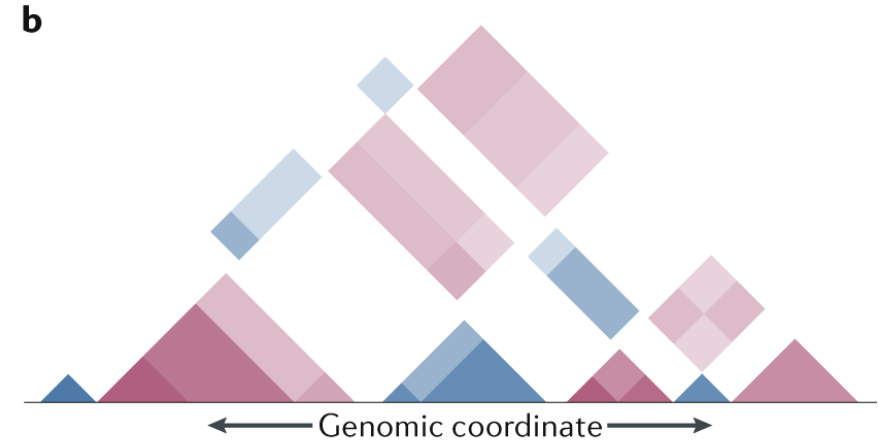
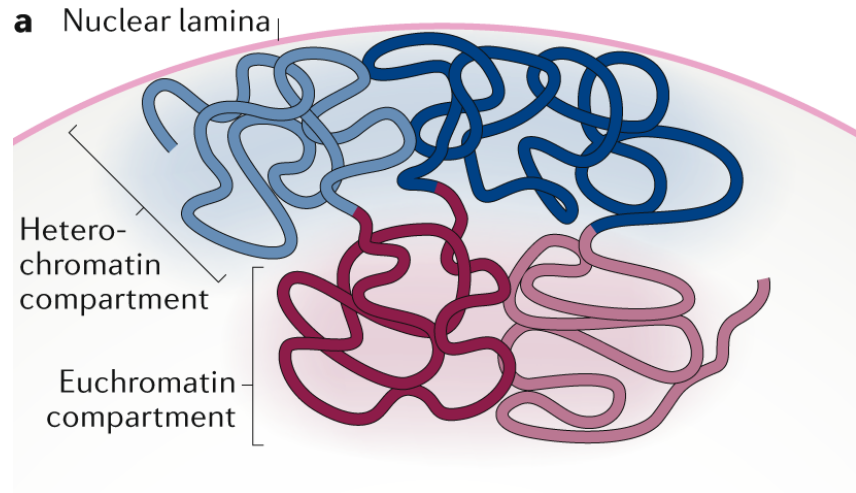


Copy number variation – Droplet based

- 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

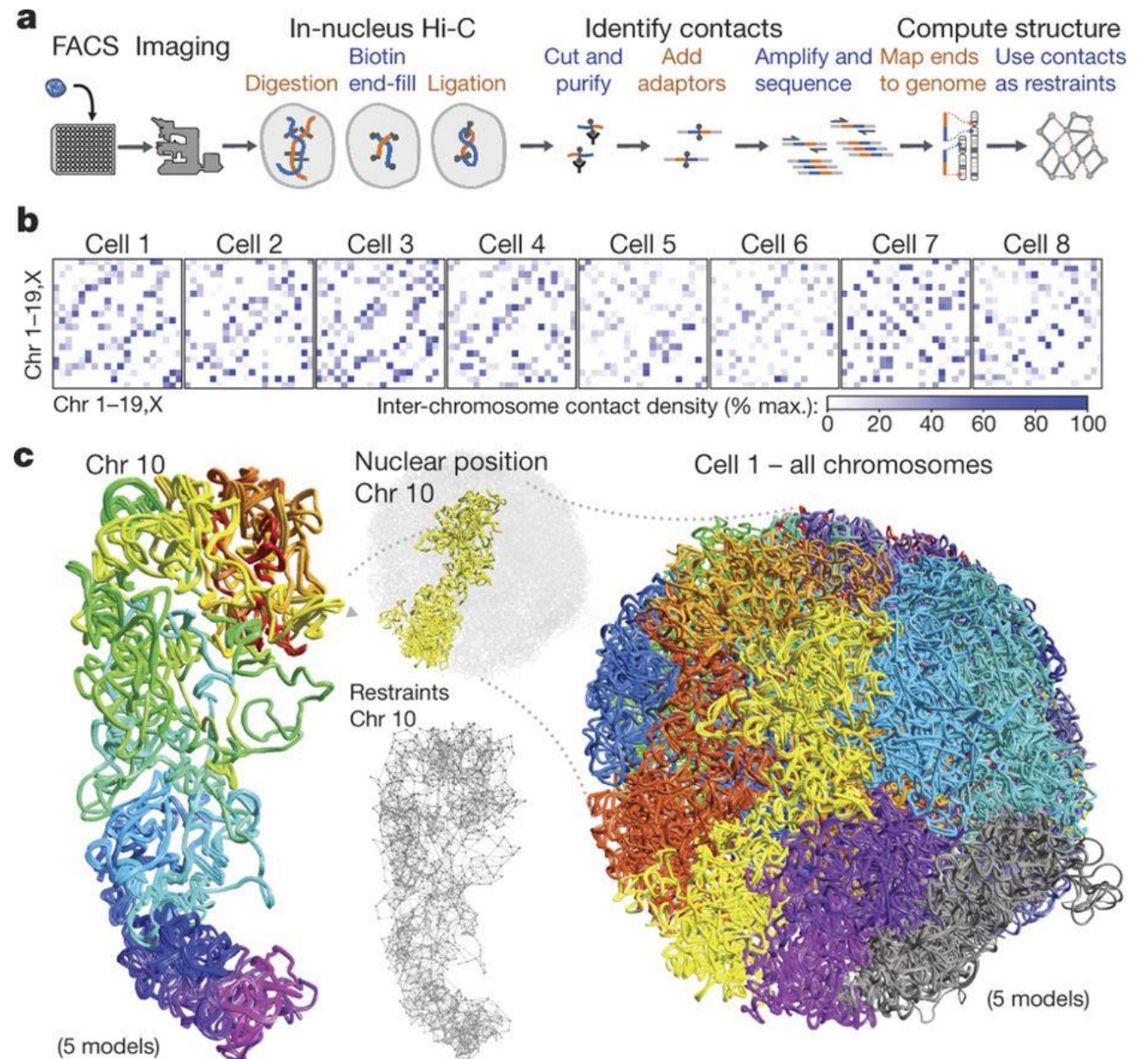
Individual haploid G1 phase mESCs

Super-resolution microscopy +
single-cell Hi-C

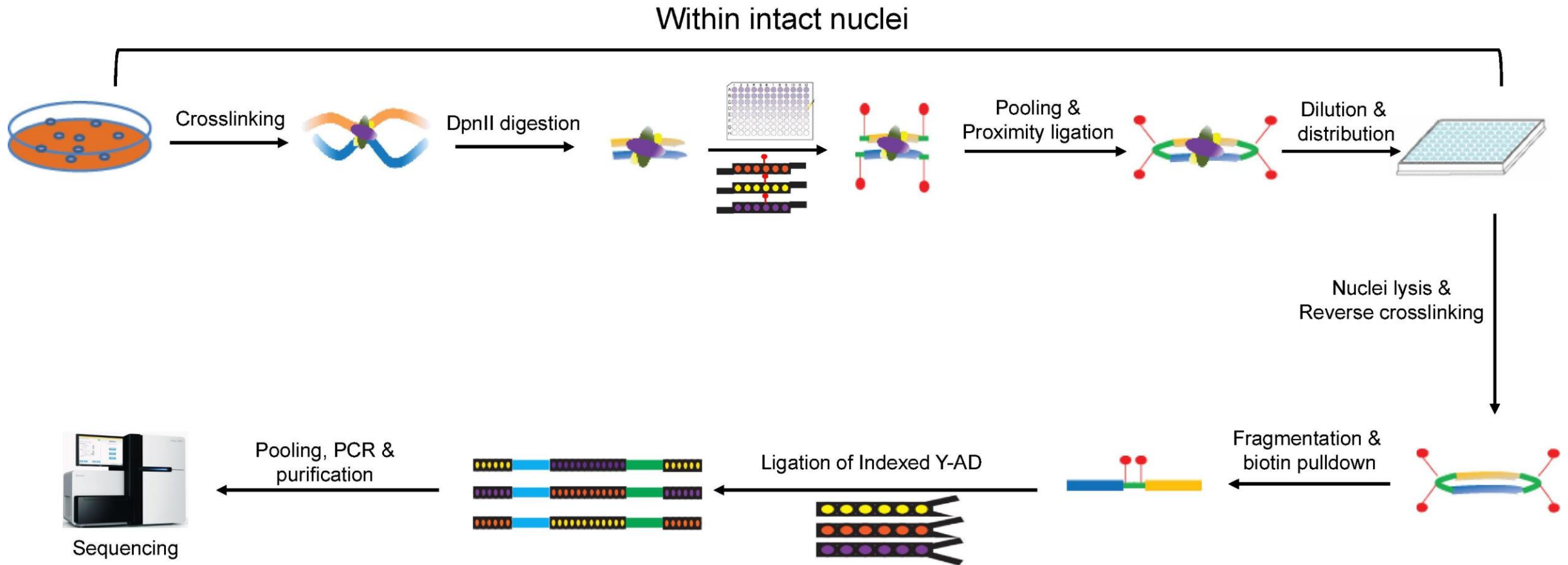
Examine topological domains +
looping at <100kb scale

Can validate sequencing data with
imaging data

Stevens et al Nature 2017



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



Single-cell proteomics

- CyTOF lecture tomorrow (Natasja de Vries)
- Proteogenomic lecture Thursday (Miao Chien)
- sc-Western blots
 - High throughput (1000 cells)
 - Low resolution (12 antibodies)
- SCoPE-MS
 - Low throughput (dozens of cells)
 - High resolution (1000 proteins)

