

STAT 35510

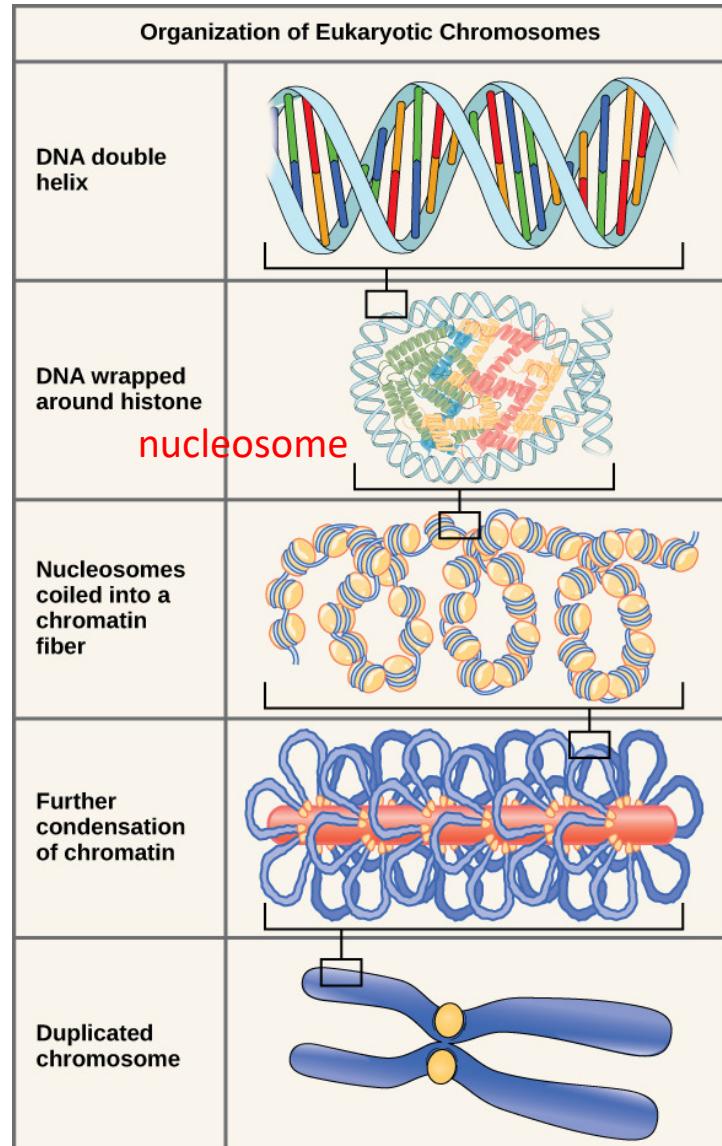
Lecture 10

Spring, 2024
Jingshu Wang

Outline

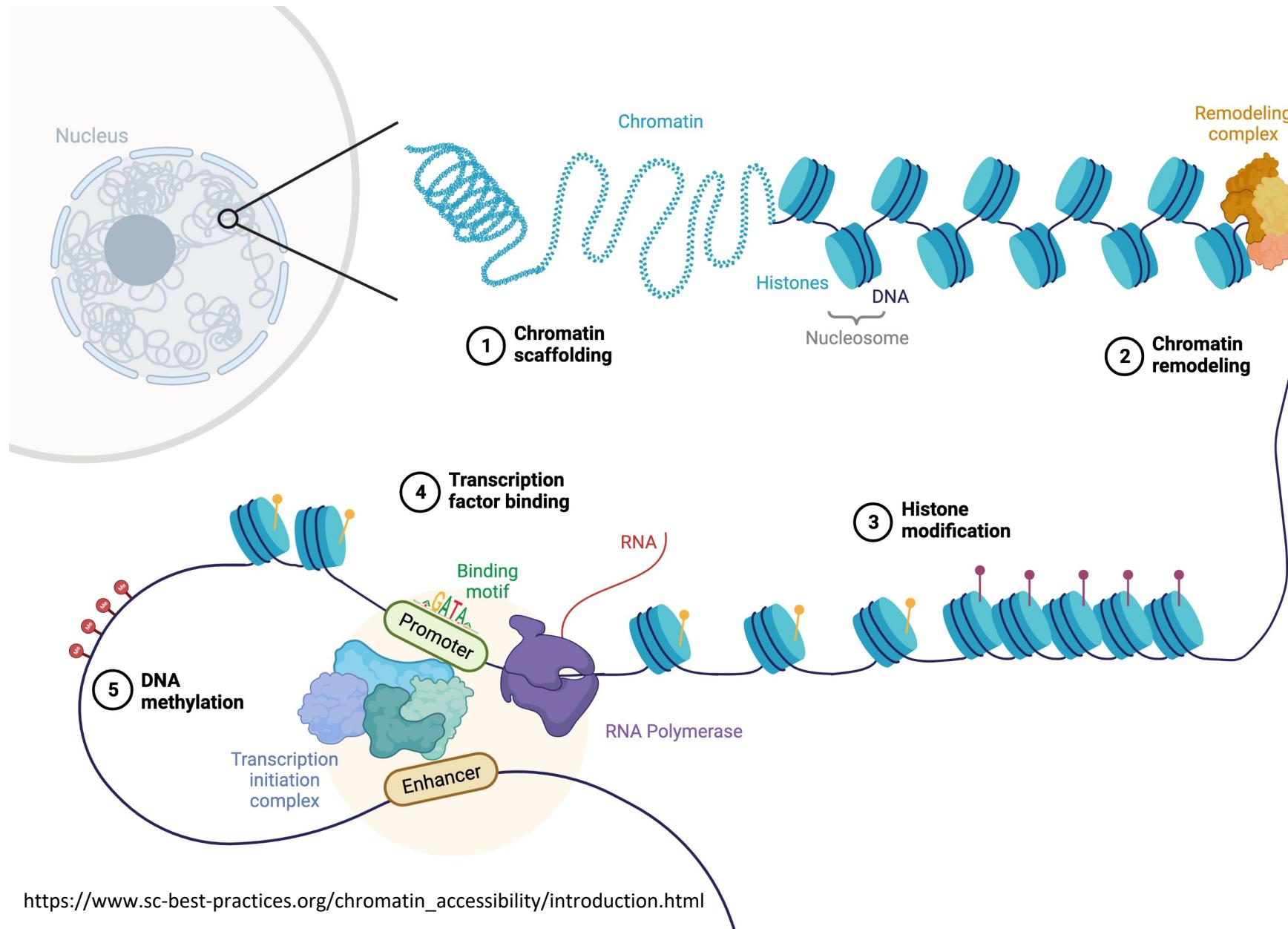
- scATAC-seq technology
- scATAC-seq preprocessing and quality control
 - Peak calling
 - Filtering low-quality cells
 - Doublet detection
 - Barcode multiplets
- Dimension reduction and feature transformations

Epigenomics and scATAC-seq



- DNA is packaged inside the nucleus
 - Make DNA fit into the nucleus and stable
 - Controls the activity of DNA: inactive if tightly packed
- The basic unit is called nucleosome: DNA wrapped around 8 histone proteins
- Epigenomics:
 - Modification of DNA / histones that does not alter the DNA sequence
 - Understand regulation of gene expression
- Single-cell ATAC-seq:
 - measure the open regions of DNA (chromatin accessibility)
 - Understand how nucleosome positioning regulates gene expression (transcriptional activation)

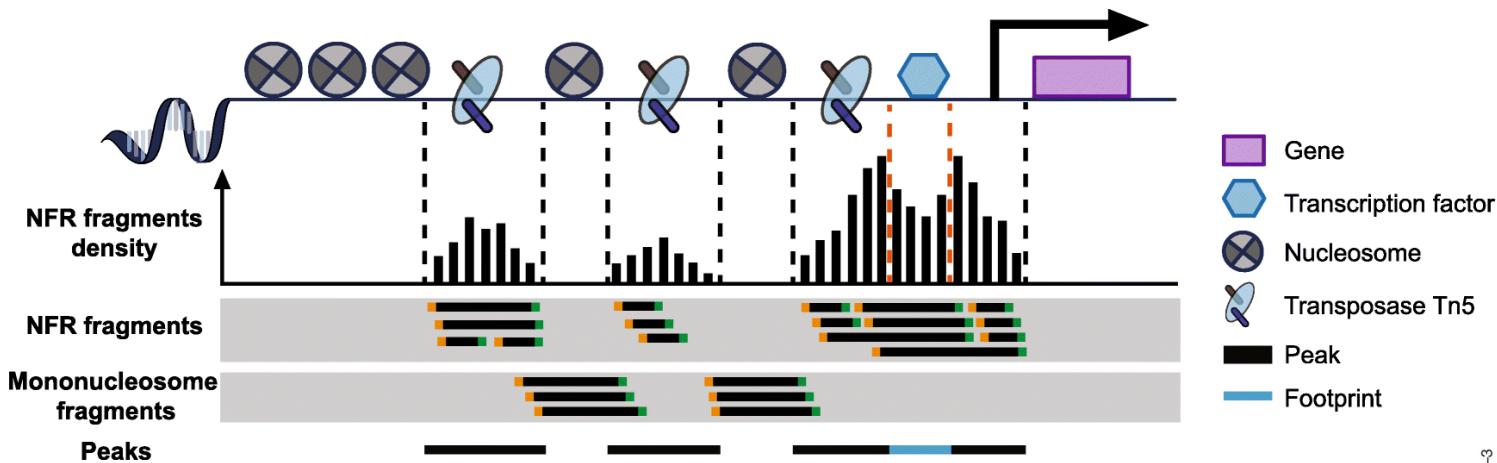
How is chromatin accessibility influenced?



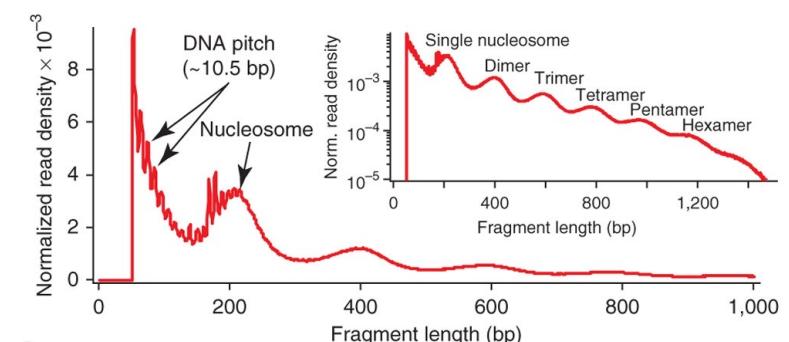
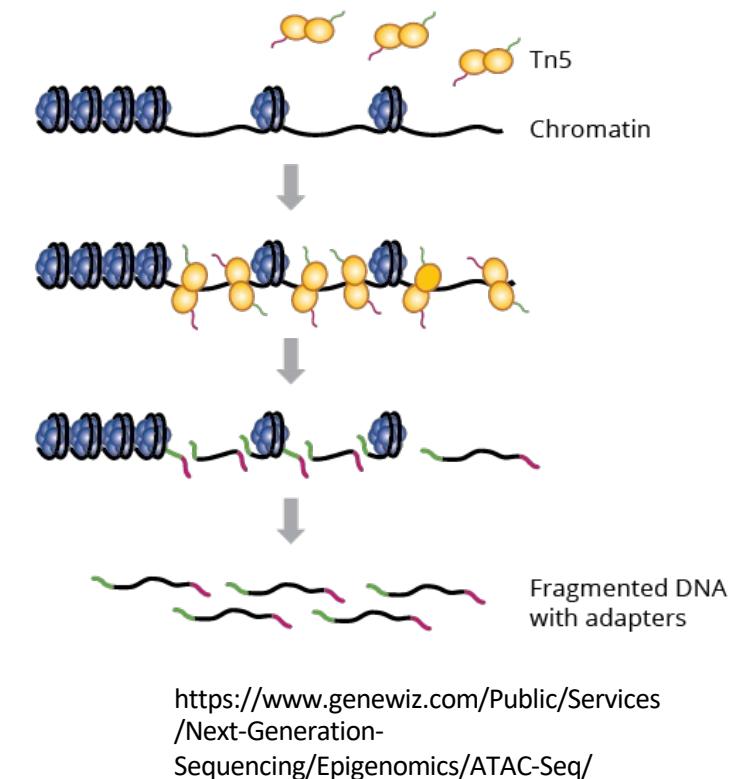
- **Transcriptional factor (TF):**
 - proteins that help turn specific genes "on" or "off" by binding to nearby DNA
- Promotor: a region of DNA upstream of a gene where relevant proteins such as TF bind to initiate transcription
- Enhancer: region of DNA that can be bound by proteins to increase likelihood of transcription

ATAC-seq for measuring chromatin accessibility

- ATAC-seq (Assay for Transposase-Accessible Chromatin with high-throughput sequencing) (Buenrostro et. al. Nature Methods 2015)
 - chromatin is fragmented and simultaneously tagmented with sequencing adapters using the Tn5 transposase
 - NFR fragments: represent the open chromatin nucleosome-bound fragments: reflect nucleosome position

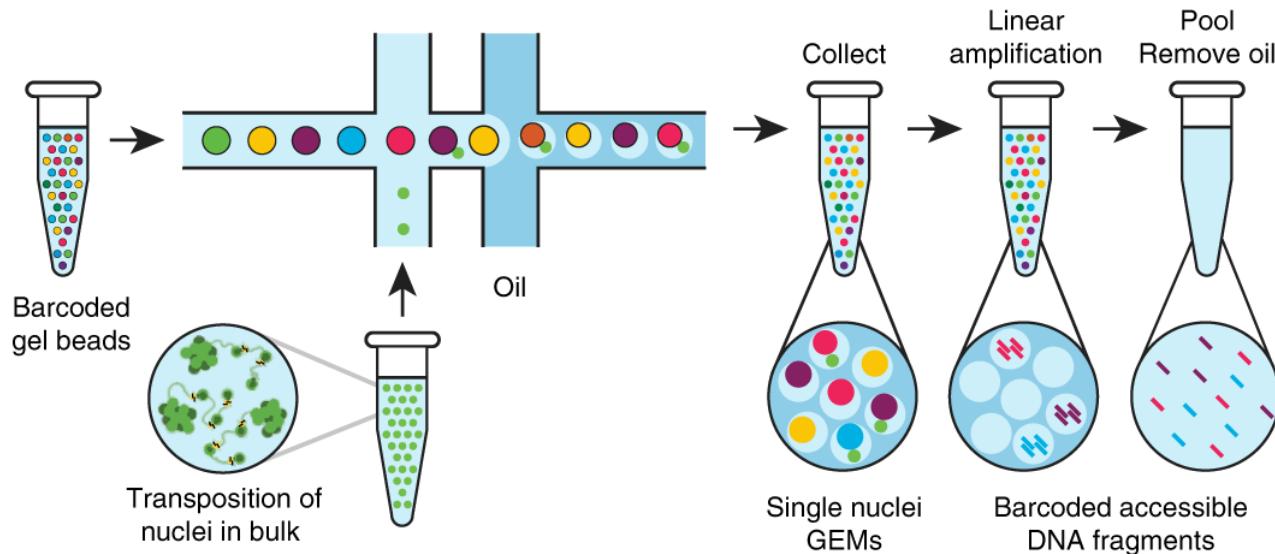


- Fragment length distribution
- Compared with other techniques, ATAC-seq requires few starting materials and less preparation time

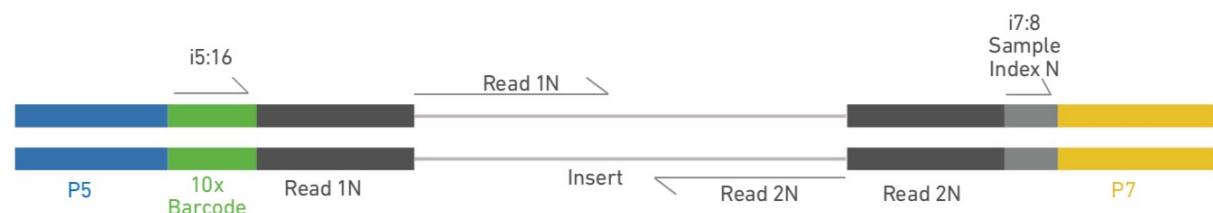


scATAC-seq by 10x Genomics (Satpathy et. al., 2019)

- Nuclei are transposed (chromatin fragmented and simultaneously tagmented) in bulk before isolated in a suspension
- Transposed DNA are amplified inside each nuclei first before PCR amplification



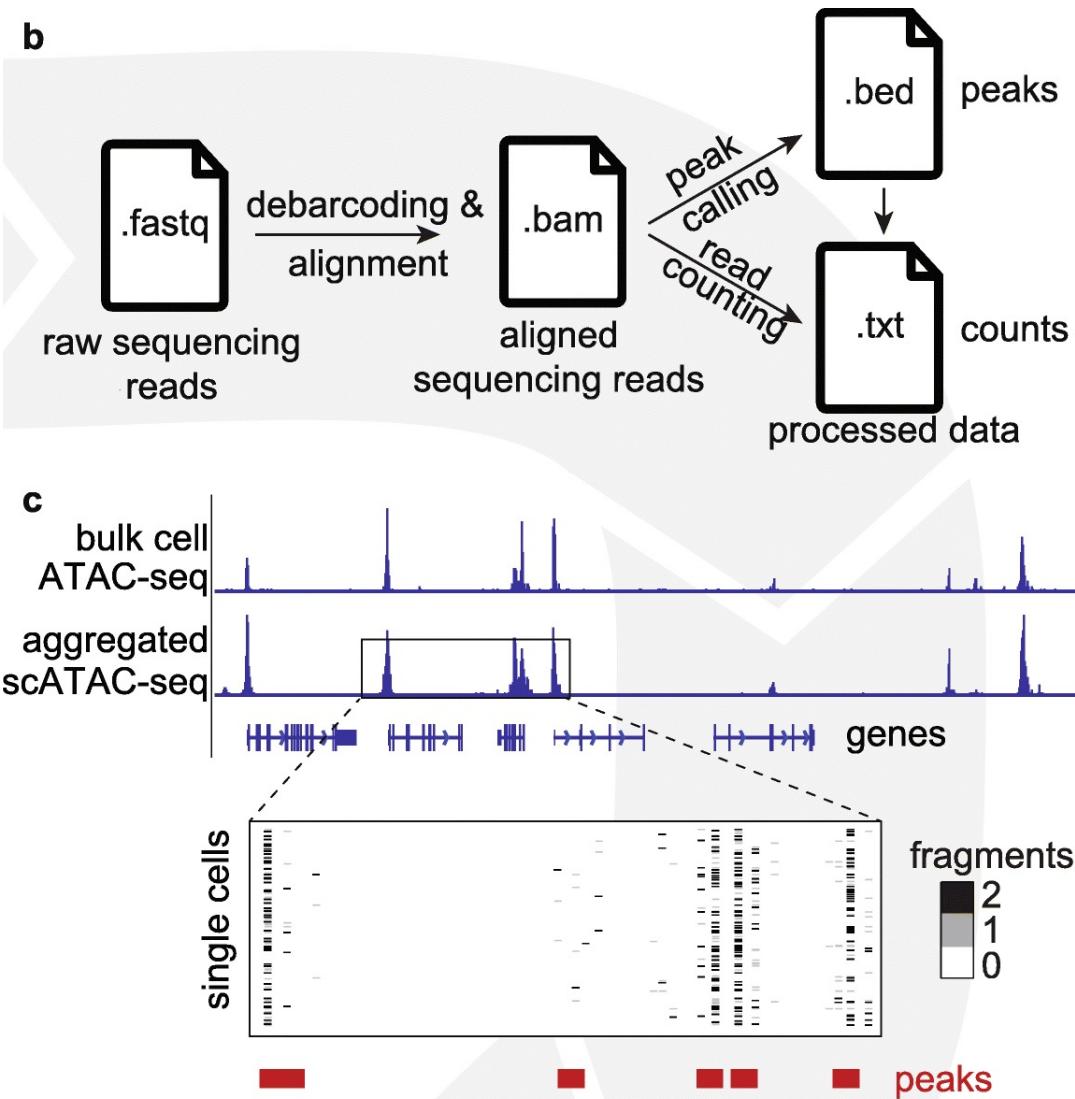
Chromium Single Cell ATAC Library



No UMI:

- One position on one chromatin can only be on/off
- One nuclei only have two copies of one chromosome
- Can easily remove duplicated fragments

How does scATAC-seq data look like?



- scATAC-seq preprocessing steps (Chen et. al. *Genome Biology* 2019)
- scATAC-seq peak by cell matrix is extremely sparse (much sparser than scRNA-seq)
 - DNA only have two copies per cell
 - 1-10% detection rate of accessible peaks
 - 10-20 times feature size than scRNA-seq
- Can have more than 2 fragments in a peak before amplification

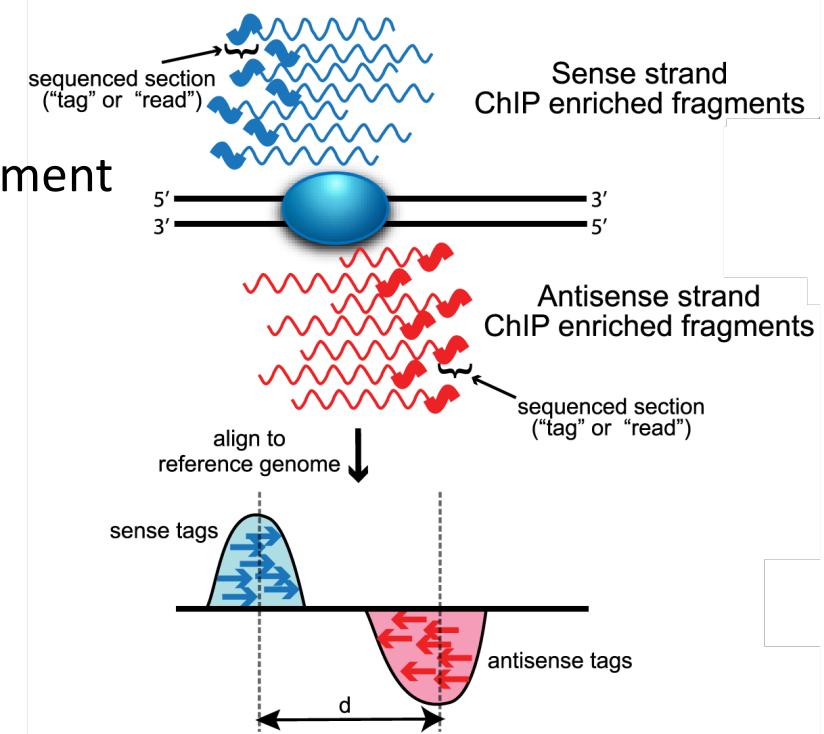
		cells			
		1	2	1	0
peaks	1	1	0	0	1
	0	1	0	0	0

Peak calling for scATAC-seq

- Peak calling methods have been developed for a long time for other types of epigenetic data
- Various ways to detect peaks
 - Detect peaks based on a reference bulk ATAC-seq data
 - Detect peaks based on pseudo-bulk ATAC-seq data (ignore cell barcode to create a “bulk” dataset)
 - Perform clustering first and perform calling for each cluster of cells (SnapATAC, Fang et. al., Nature communications, 2021)
 - Aim to identify small peaks that only appear in small cell types
 - To perform clustering without peaks
 - Create cell-by-bin count matrix
 - Segment the genome into bins (5kb size by default)
 - Count the number of read in each bin and binarize the matrix
 - One common method to detect peaks in MACS2

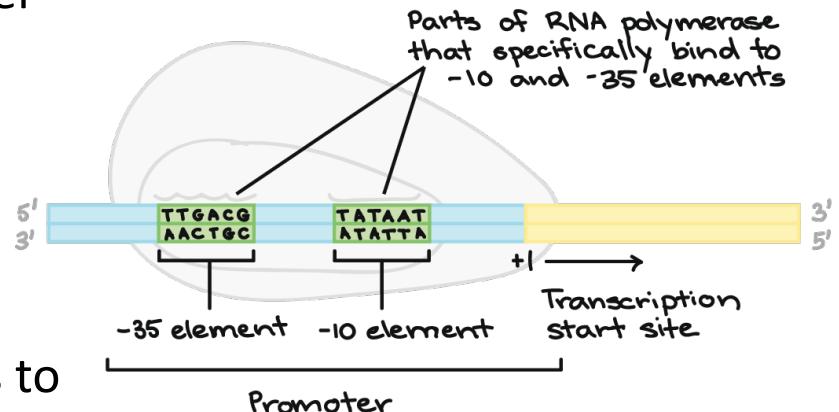
MACS2 (Zhang et. al. Genome Biology, 2008)

- Can work with both single-end reads and paired-end reads
 - scATAC-seq is paired-end, call peaks only use NFR fragments (fragment length less than 100bp)
 - Or use all reads and treat them as single-end
 - Need to recenter the reads
- Core steps when analyzing scATAC-seq
 - Remove duplicate reads: reads at the exact same location
 - Recenter the reads setting $d = 200$
 - Peak detection
 - Slide $2d$ window across the genome to find peaks
 - Given any window of the genome, assume number of reads follow a Poisson distribution with mean λ_{local} if there is no peak (null)
$$\lambda_{\text{local}} = \max(\lambda_{\text{BG}}, \lambda_{5k}, \lambda_{10k})$$
 - Compute a p-value for each window, selection all windows with small p-values (10^{-5})
 - Merge nearby peak regions and identify peak center as the “summit”
 - extend each read from its center by d so that reads can pile up



Quality control for scATAC-seq

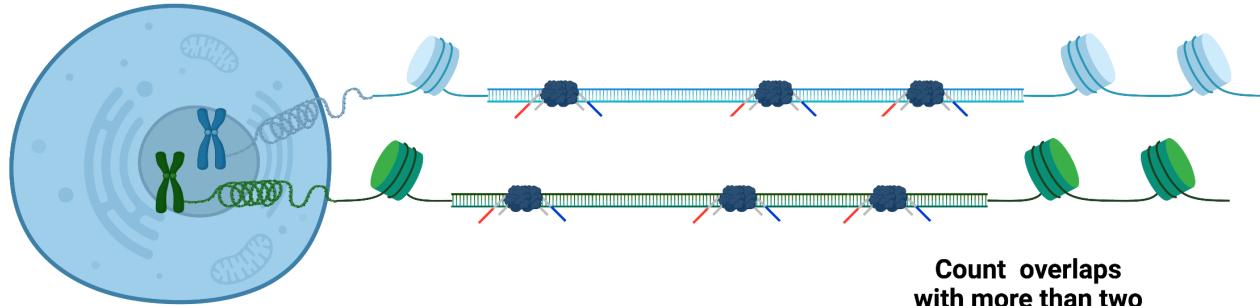
- Detect low-quality cells
 - General metrics: total fragment counts, number of features per cell
 - Transcription starting site (TSS) enrichment
 - scATAC-seq fragments should be enriched near the TSS
 - Select a random subset of TSS
 - For each TSS, compare number of overlapping fragments (+- 2000bp window) with nearby windows to calculate an enrichment score
- Doublet detection
 - Much more challenging as the scATAC-seq has much higher sparsity
 - If we use similar idea as in scRNA-seq, need to aggregate correlated features (a suggestion of number of features: 25, https://www.sc-best-practices.org/chromatin_accessibility/quality_control.html)
 - A different idea: At most two fragments detected per location in a single cell



AMULET (Thibodeau et. al., Genome Biology 2021)

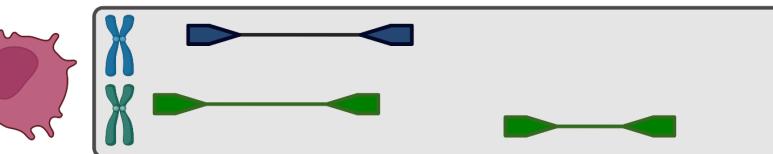
1

A regular cell with a diploid genome has two chromosomes that can be fragmented by Tn5 transposase



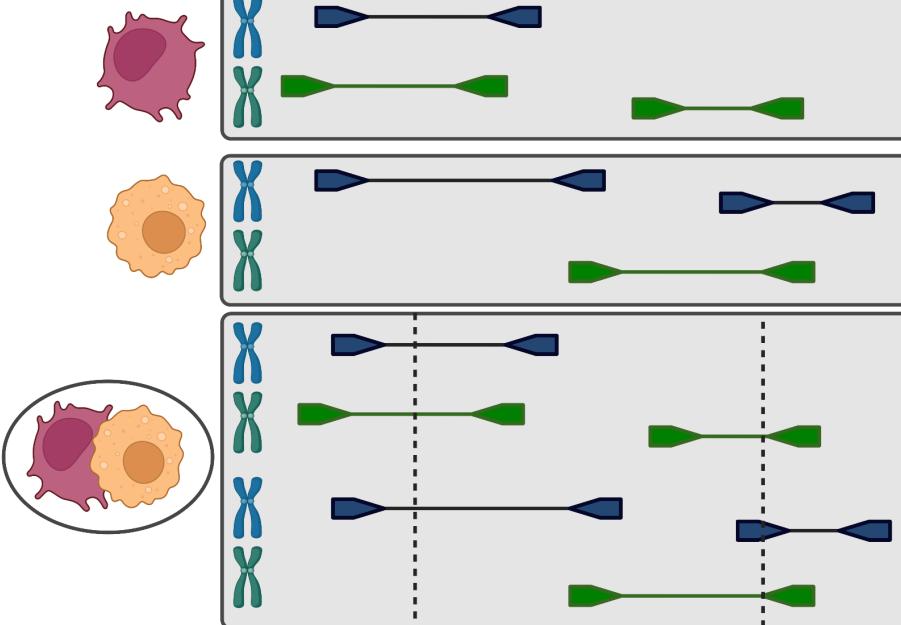
2

A regular fragment pattern. There can be maximally two fragments at the same position



3

Doublots can result in an irregular fragment pattern. We observe more than two fragments at the same position.

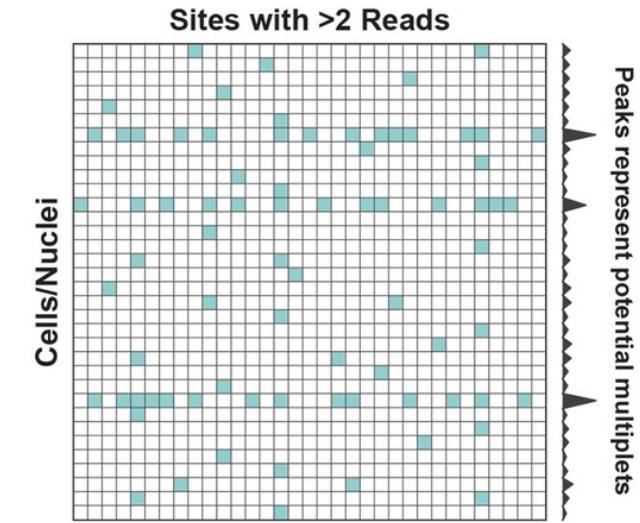


Count overlaps
with more than two
fragments

0 ✓

0 ✓

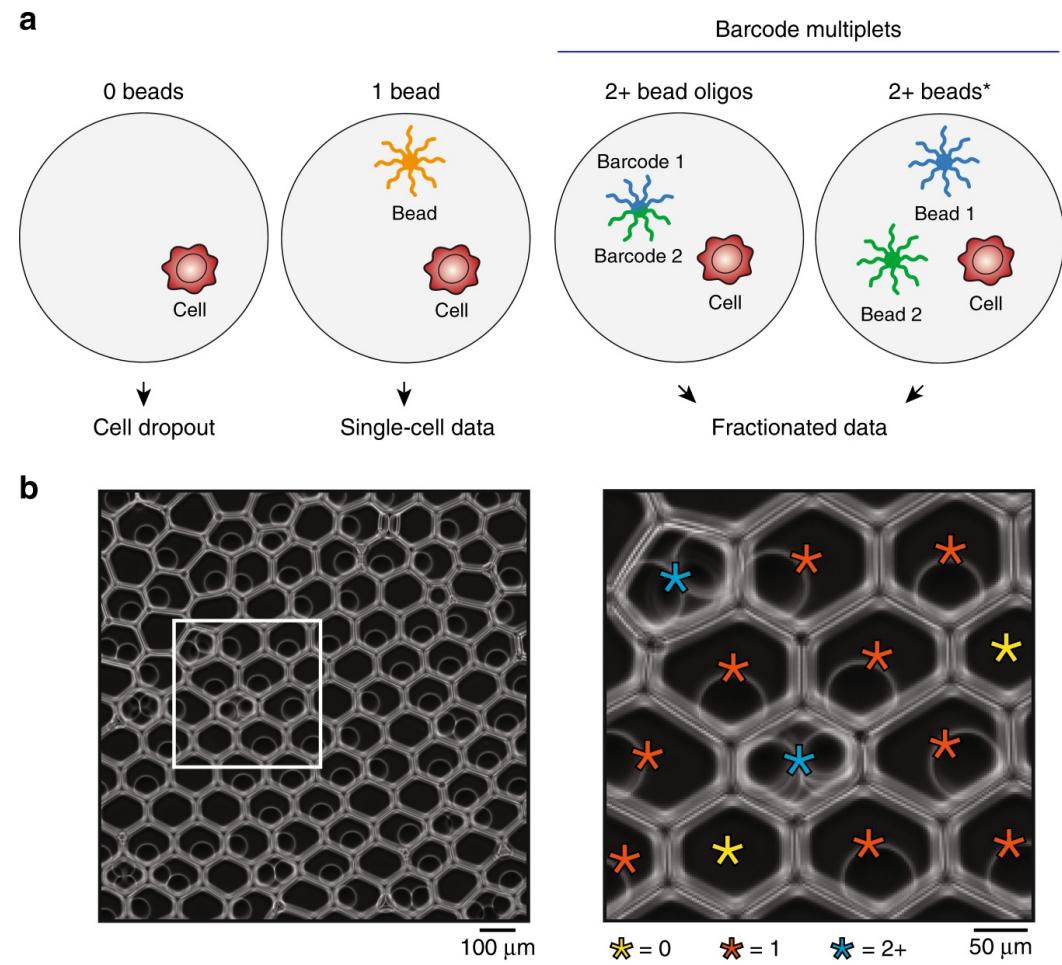
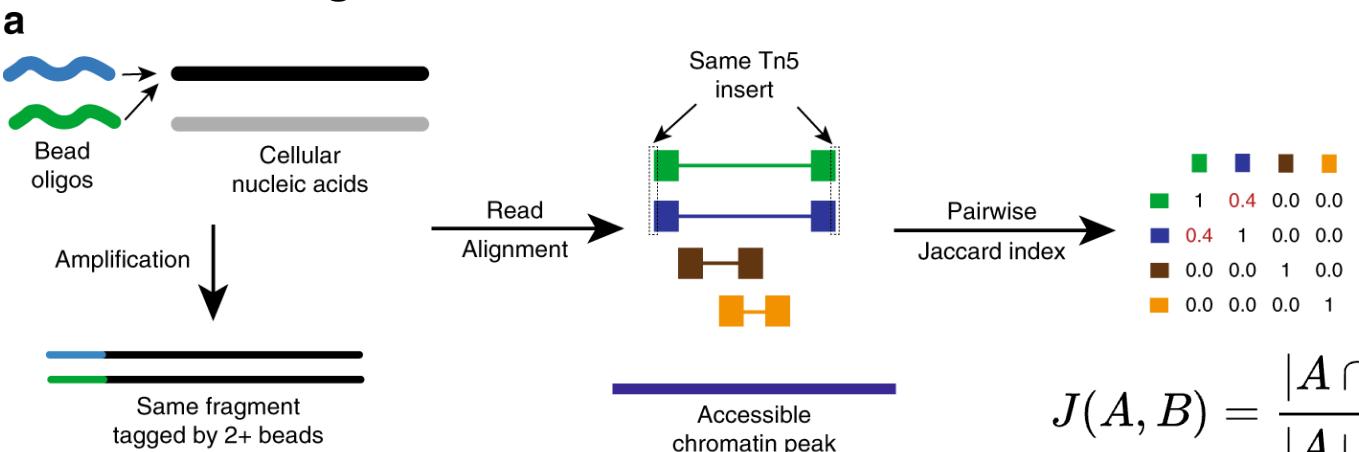
2 ✗



- Requires a relatively large library size
- Can identify homotypic doublets

Barcode multiplets (Lareau et. al., Nature Communications 2019)

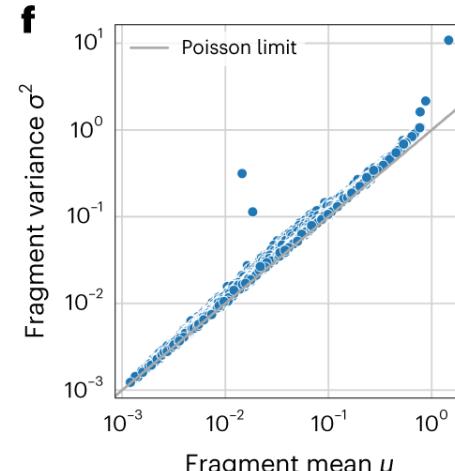
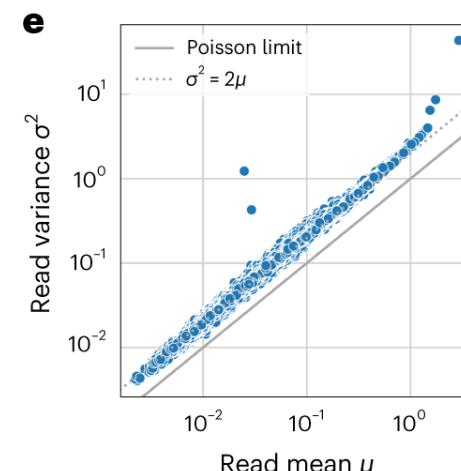
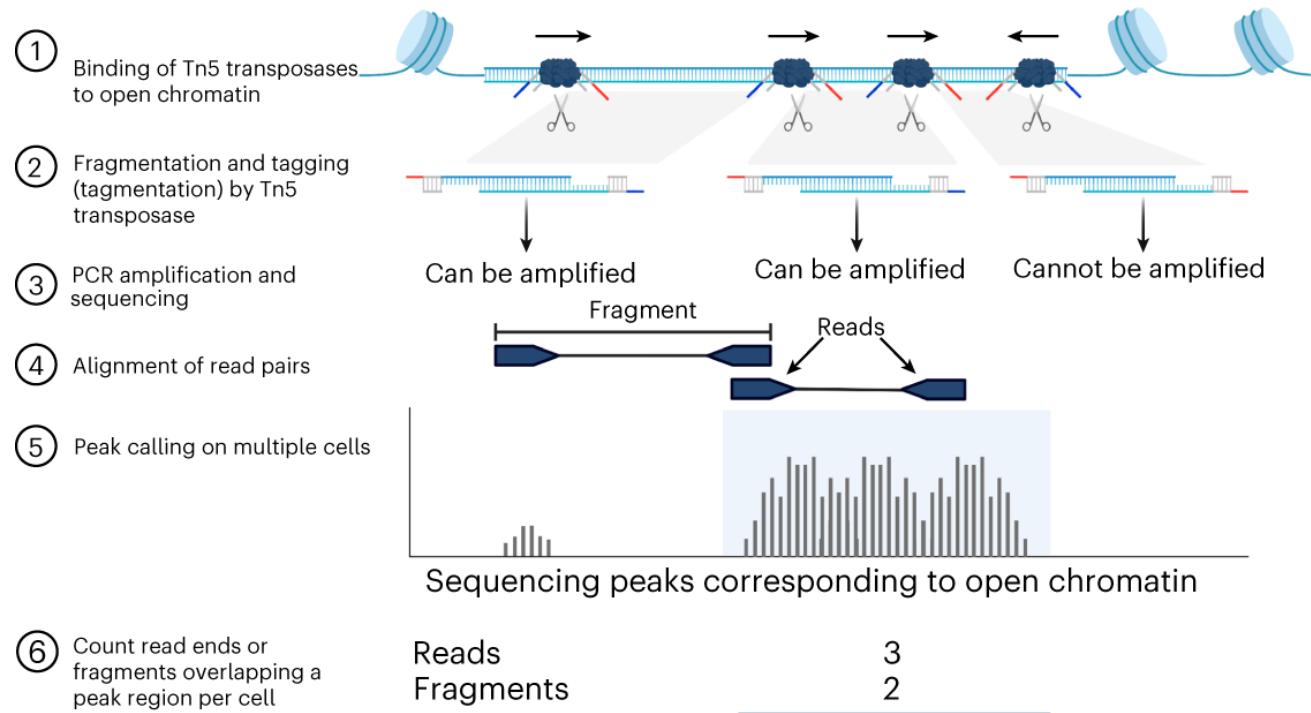
- Though the bead softgel follows super Poisson distribution in 10X genomics, there can still be barcode multiplets
 - About 5% barcode multiplets in 10X scATAC-seq (80% single-bead droplets)
- Barcode multiplets in scRNA-seq can be challenging to detect
- Detect barcode multiplets in scATAC-seq
 - Based on molecular diversity of Tn5 insertion sites across genome



$$J(A, B) = \frac{|A \cap B|}{|A \cup B|} = \frac{|A \cap B|}{|A| + |B| - |A \cap B|}$$

scATAC-seq count matrix (Martens et. al. Nature Methods 2024)

- Peak * cell count matrix
 - Count number of reads or number of fragments that overlap with peak region
 - Should count number of fragments to avoid bias
 - For a specific region, number of fragments follow Poisson distribution across cells
- Binarize the peak * cell matrix:
 - Entry = 1 if there are any fragments detected overlap with the peak region
 - Binarization is shown to hide quantitative information and not helpful

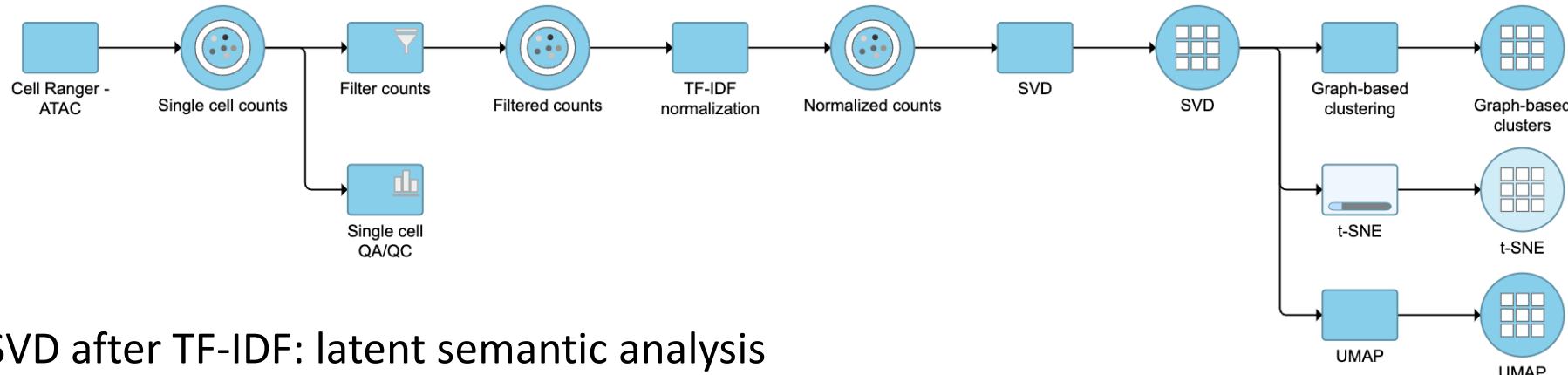


scATAC-seq normalization

- Normalize by total number of fragments per cell
- TF-IDF matrix transformation (Cusanovich et. al., Cell, 2018)
 - Normalize by gene and cell at the same time
 - Term Frequency (TF) - Inverse Document Frequency (IDF)
 - TF: total fragment normalization per cell $TF = C_{ij}/F_j$
 - F_j : total number of fragments in cell j
 - IDF: $\log(1 + N/N_i)$ N_i total counts per peak across all cells
 - Can also directly use N/N_i as IDF (Stuart et. al., Nature Methods 2021)

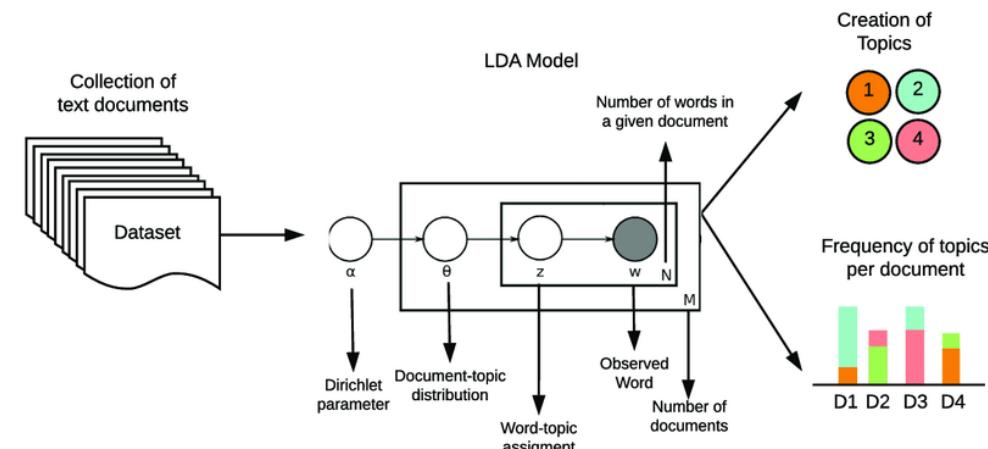
$$TF-IDF = TF * IDF$$

- TF-IDF
 - Can take log transformation if needed



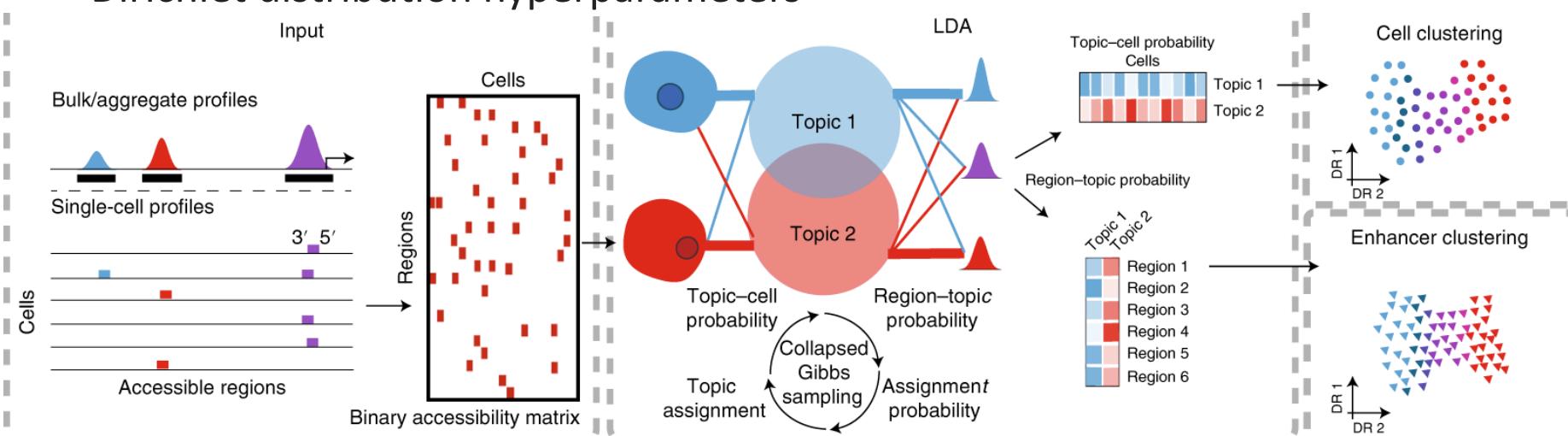
cisTopic (Gonzalez-Blas et. al., Nature Methods, 2019)

- Core steps:
 - Binarize the count matrix
 - Topic modeling using Latent Dirichlet Allocation (LDA, Blei et. al., JMLR 2003)
 - Generative process
 - Choose $N \sim \text{Poisson}(\xi)$.
 - Choose $\theta \sim \text{Dir}(\alpha)$.
 - For each of the N words w_n :
 - Choose a topic $z_n \sim \text{Multinomial}(\theta)$.
 - Choose a word w_n from $p(w_n | z_n, \beta)$, a multinomial probability conditioned on the topic z_n .
 - Each position in a document independently choose a topic
 - Each topic has a topic-specific Multinomial distribution of words
 - Solve the model:
Gibbs sampling / variational Bayes /
Expectation-propagation



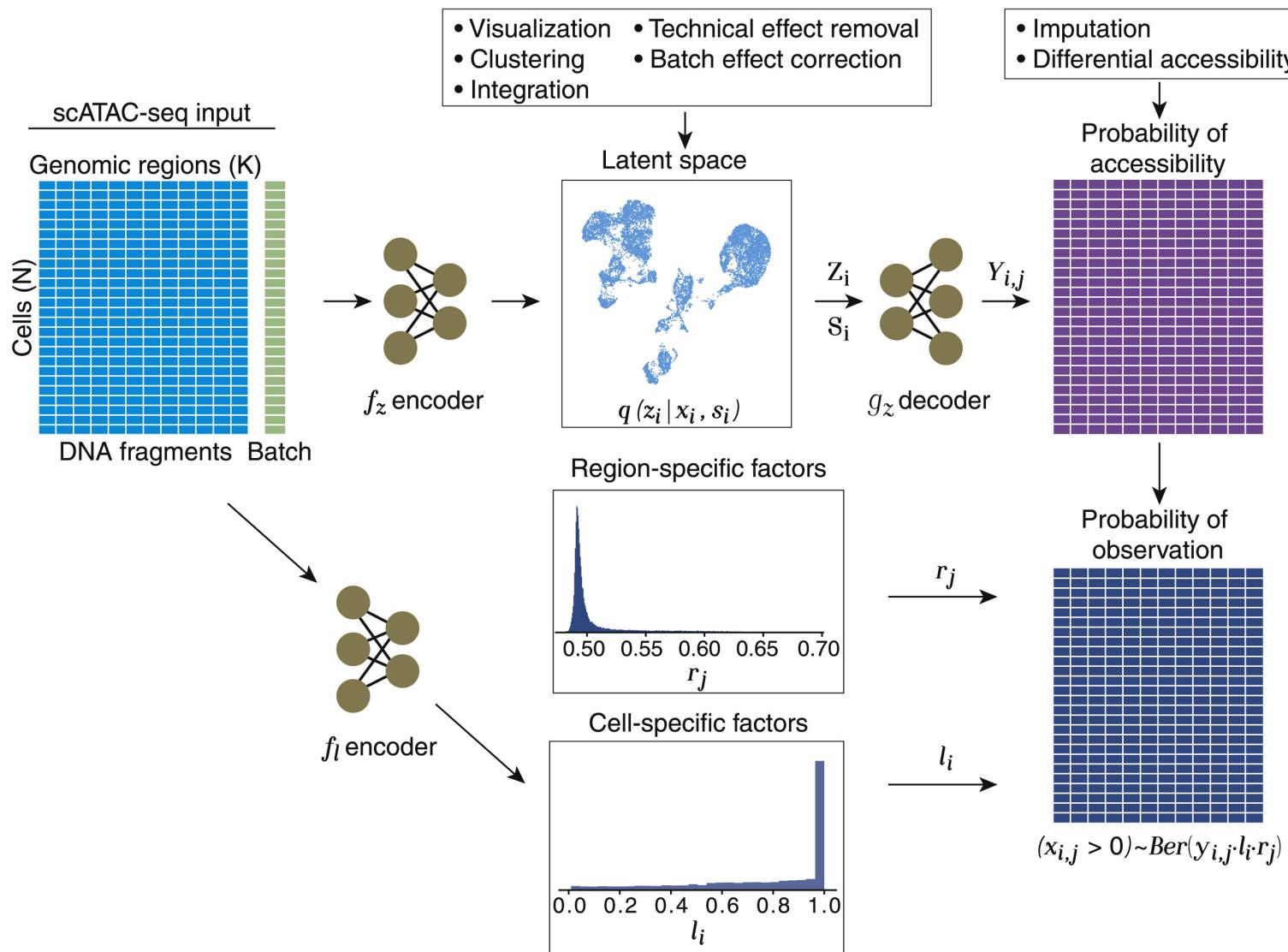
cisTopic (Gonzalez-Blas et. al., Nature Methods, 2019)

- Core steps:
 - Binarize the count matrix
 - Topic modeling using Latent Dirichlet Allocation (LDA, Blei et. al., JMLR 2003)
 - Treat each cell as a document and each region (peak) as a word
 - Use Gibbs sampler to iteratively optimize two probability distributions:
 - Region-topic distribution: the probability of a region belonging to a topic
 - Topic-cell distribution: the contribution of a topic within a cell
 - Determine the hyperparameters
 - Number of topics K : fit a model with different K , find the smallest K that stabilize the log-likelihood
 - Dirichlet distribution hyperparameters



peakVI (Gonzalez-Blas et. al., Nature Methods, 2019)

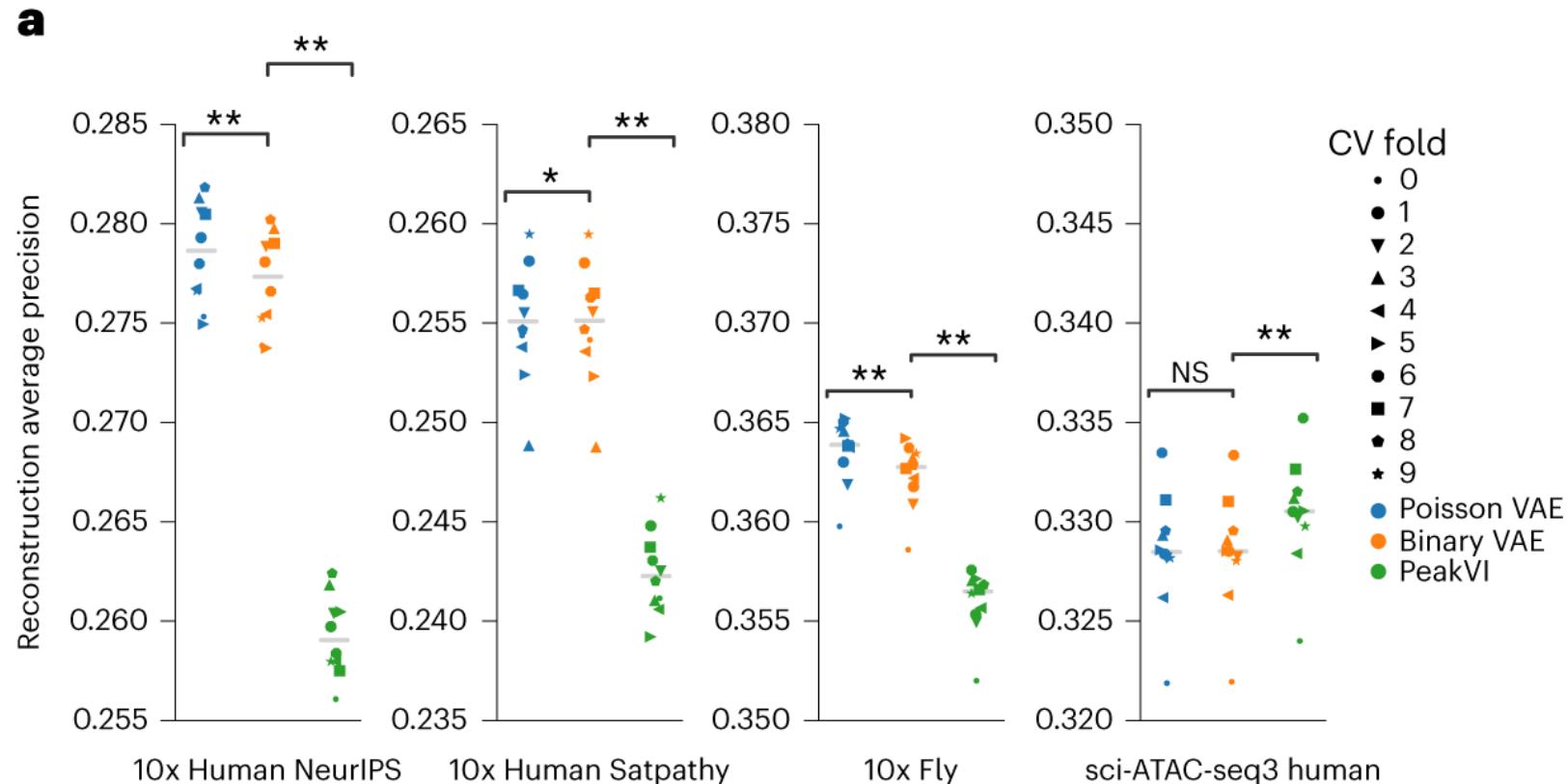
A



- Adaptation of scVI to correct batch effects, denoising, and perform dimension reduction
- Main change: distributional assumption on count data

peakVI (Gonzalez-Blas et. al., Nature Methods, 2019)

- (Martens et. al. Nature Methods 2024) finds that using the Poisson model with counts without binarization and use observed region-specific and cell-specific factors instead of estimated factors can improve performance of peakVI

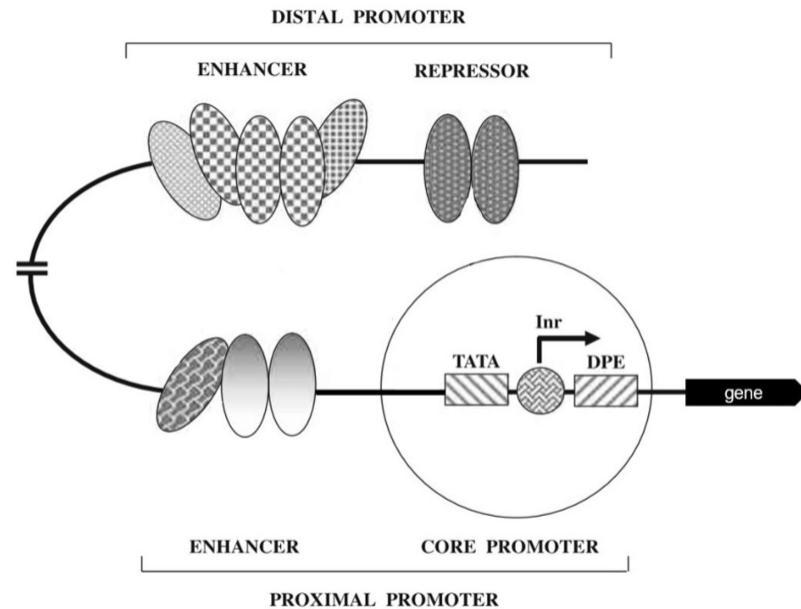


Gene activity score

- Transfer the peak * cell matrix to gene * cell matrix
 - Aggregate peaks around promoter region of a gene
- Cicero (Pliner et. al. Molecular Cell, 2018)
 - Overall measure linked to each gene k using peaks that belong to proximal or distal sites of gene TSS

$$R_{ki} = \sum_{p \in P} \sum_{j \in D_p} A_{ji} \frac{u_{pj}}{\sum_{k \in D_p} u_{pk}} + A_{pi}$$

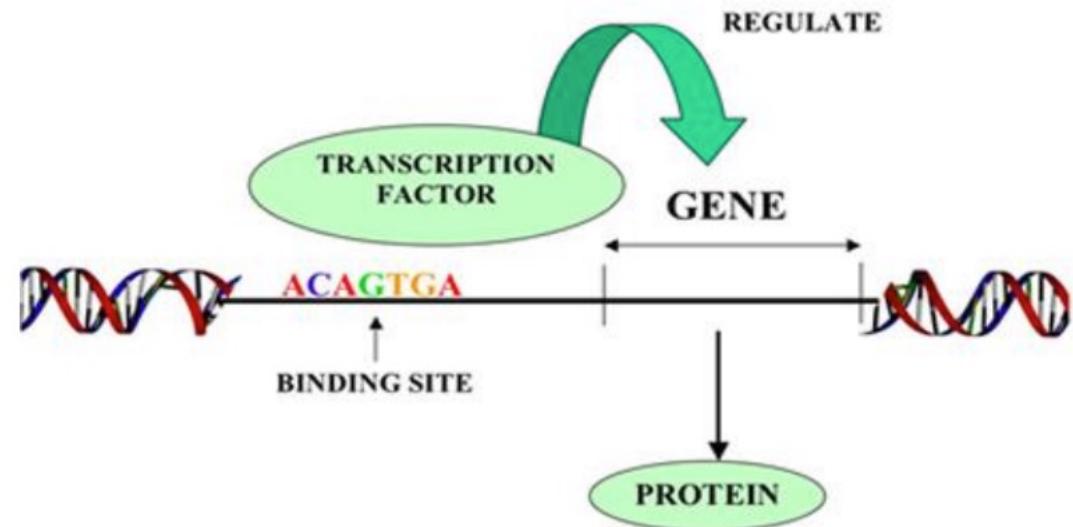
- u_{pk} peak-peak co-accessibility score
- Signac (Stuart et. al. Nature Methods, 2018)
 - Count number of fragments overlapping the gene body and a 2-kb upstream region for each gene in each cell
- Apply scRNA-seq methods on gene activity score matrix



Transcriptional factor activity matrix

- Motifs: DNA binding sites (has a specific structure)
- ChromVAR (Schep et. al., Nature Methods 2017)
- Motif enrichment of each cell
 - Motif matching matrix W : motif by peak matrix
 - for a list of motifs, calculate the frequency of each motif within any peak regions

$$Y = \frac{M \times X^T - M \times E^T}{M \times E^T} \quad E = \frac{\sum_{i=1} x_{i,j}}{\sum_{j=1} \sum_{i=1} x_{i,j}} \times \sum_{j=1} x_{i,j}$$



- Can adjust for other peaks that contain similar motifs (background peaks) to adjust for local bias – reduce the motif enrichment if
- Transcriptional factor activity of each cell
 - For each TF, select a representative subset of motifs

Related papers

- Satpathy, A. T., Granja, J. M., Yost, K. E., Qi, Y., Meschi, F., McDermott, G. P., ... & Chang, H. Y. (2019). Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nature biotechnology*, 37(8), 925-936.
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- Fang, R., Preissl, S., Li, Y., Hou, X., Lucero, J., Wang, X., ... & Ren, B. (2021). Comprehensive analysis of single cell ATAC-seq data with SnapATAC. *Nature communications*, 12(1), 1337.
- Feng, J., Liu, T., Qin, B., Zhang, Y., & Liu, X. S. (2012). Identifying ChIP-seq enrichment using MACS. *Nature protocols*, 7(9), 1728-1740.
- Thibodeau, A., Eroglu, A., McGinnis, C. S., Lawlor, N., Nehar-Belaid, D., Kursawe, R., ... & Ucar, D. (2021). AMULET: a novel read count-based method for effective multiplet detection from single nucleus ATAC-seq data. *Genome biology*, 22, 1-19.
- Lareau, C. A., Ma, S., Duarte, F. M., & Buenrostro, J. D. (2020). Inference and effects of barcode multiplets in droplet-based single-cell assays. *Nature Communications*, 11(1), 866.
- Cusanovich, D. A., Hill, A. J., Aghamirzaie, D., Daza, R. M., Pliner, H. A., Berletch, J. B., ... & Shendure, J. (2018). A single-cell atlas of in vivo mammalian chromatin accessibility. *Cell*, 174(5), 1309-1324.
- Bravo González-Blas, C., Minnoye, L., Papasokrati, D., Aibar, S., Hulselmans, G., Christiaens, V., ... & Aerts, S. (2019). cisTopic: cis-regulatory topic modeling on single-cell ATAC-seq data. *Nature methods*, 16(5), 397-400.
- Blei, D. M., Ng, A. Y., & Jordan, M. I. (2003). Latent dirichlet allocation. *Journal of machine Learning research*, 3(Jan), 993-1022.
- Ashuach, T., Reidenbach, D. A., Gayoso, A., & Yosef, N. (2022). PeakVI: A deep generative model for single-cell chromatin accessibility analysis. *Cell reports methods*, 2(3).
- Pliner, H. A., Packer, J. S., McFaline-Figueroa, J. L., Cusanovich, D. A., Daza, R. M., Aghamirzaie, D., ... & Trapnell, C. (2018). Cicero predicts cis-regulatory DNA interactions from single-cell chromatin accessibility data. *Molecular cell*, 71(5), 858-871.
- Stuart, T., Srivastava, A., Madad, S., Lareau, C. A., & Satija, R. (2021). Single-cell chromatin state analysis with Signac. *Nature methods*, 18(11), 1333-1341.
- Schep, A. N., Wu, B., Buenrostro, J. D., & Greenleaf, W. J. (2017). chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data. *Nature methods*, 14(10), 975-978.