Bioinformatics Lab

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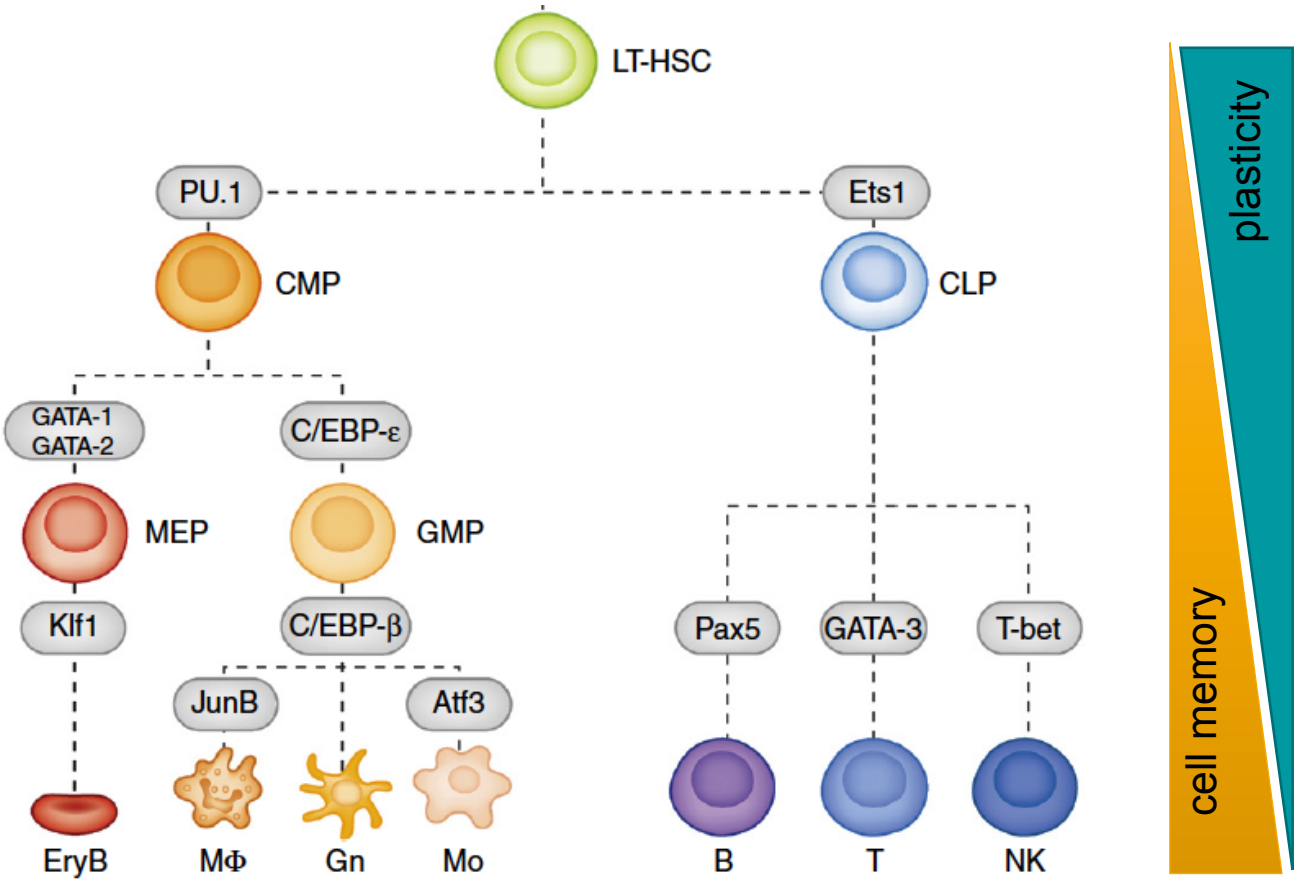
Resume

- Review basic biological/computational aspects
  1. basics of molecular biology
  2. basics of sequencing
  3. basics bioinformatics problems
     - short sequences read alignment
     - gene expression quantification
     - single cell approaches
     - **computational epigenetic (today)**

Computational Epigenomics

Cell Differentiation

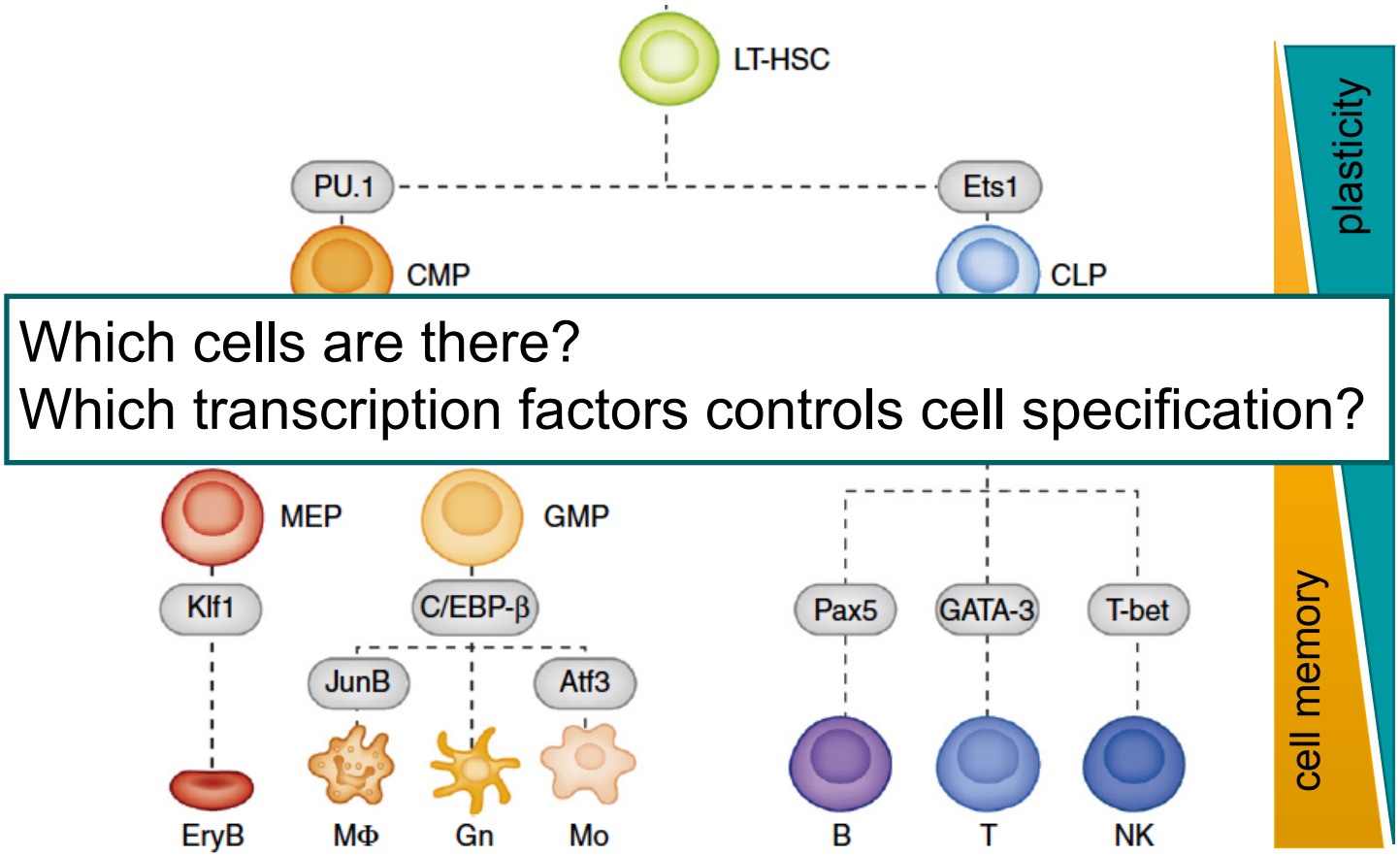
Hematopoiesis



Source: Amit (2016), *Nature Immunoloy.*

Cell Differentiation

Hematopoiesis



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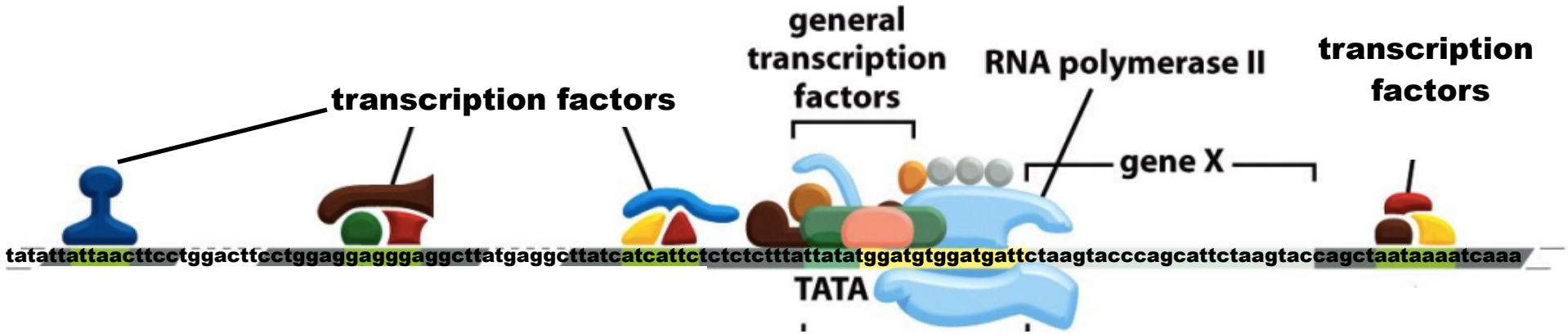
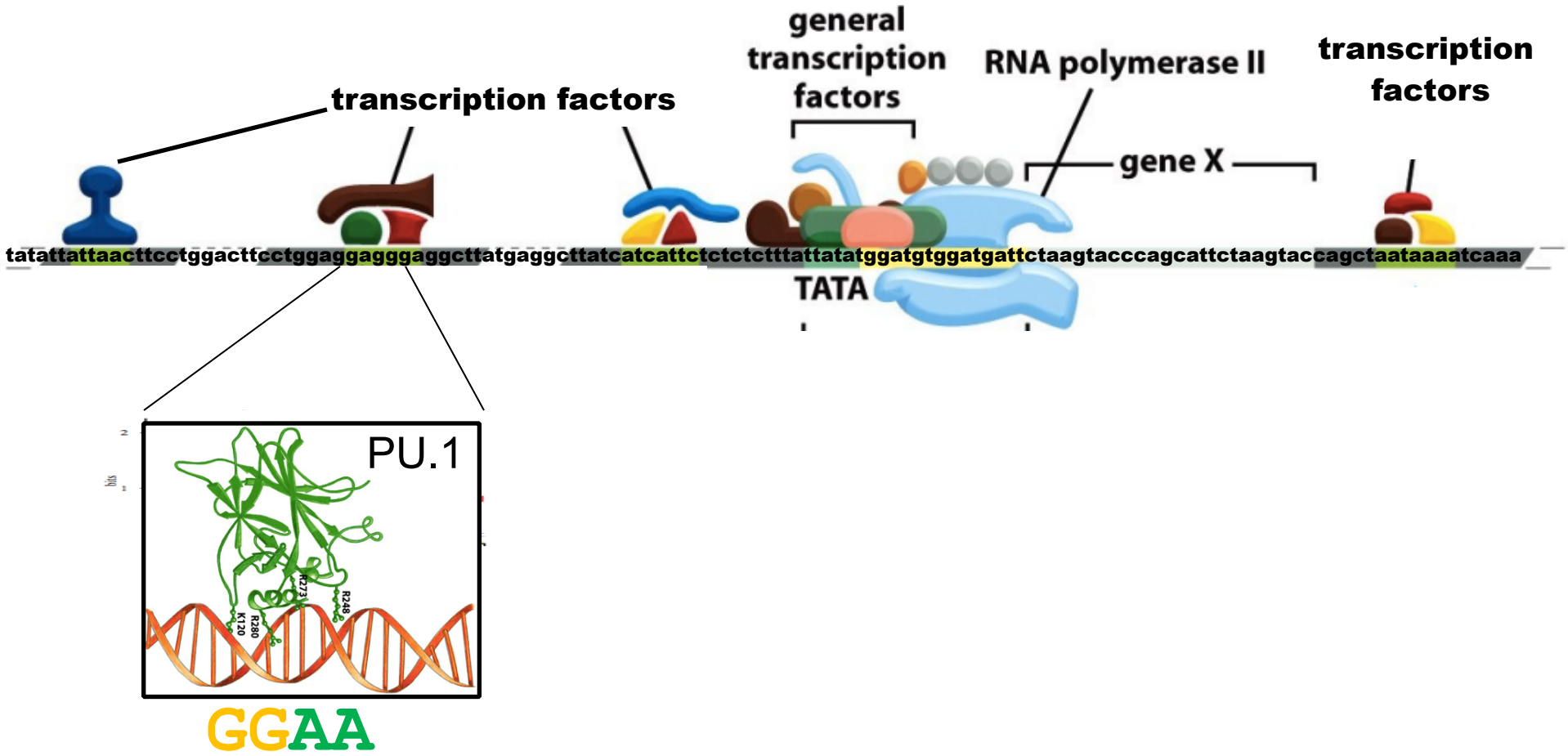
Regulatory Control – Transcription Factor Binding



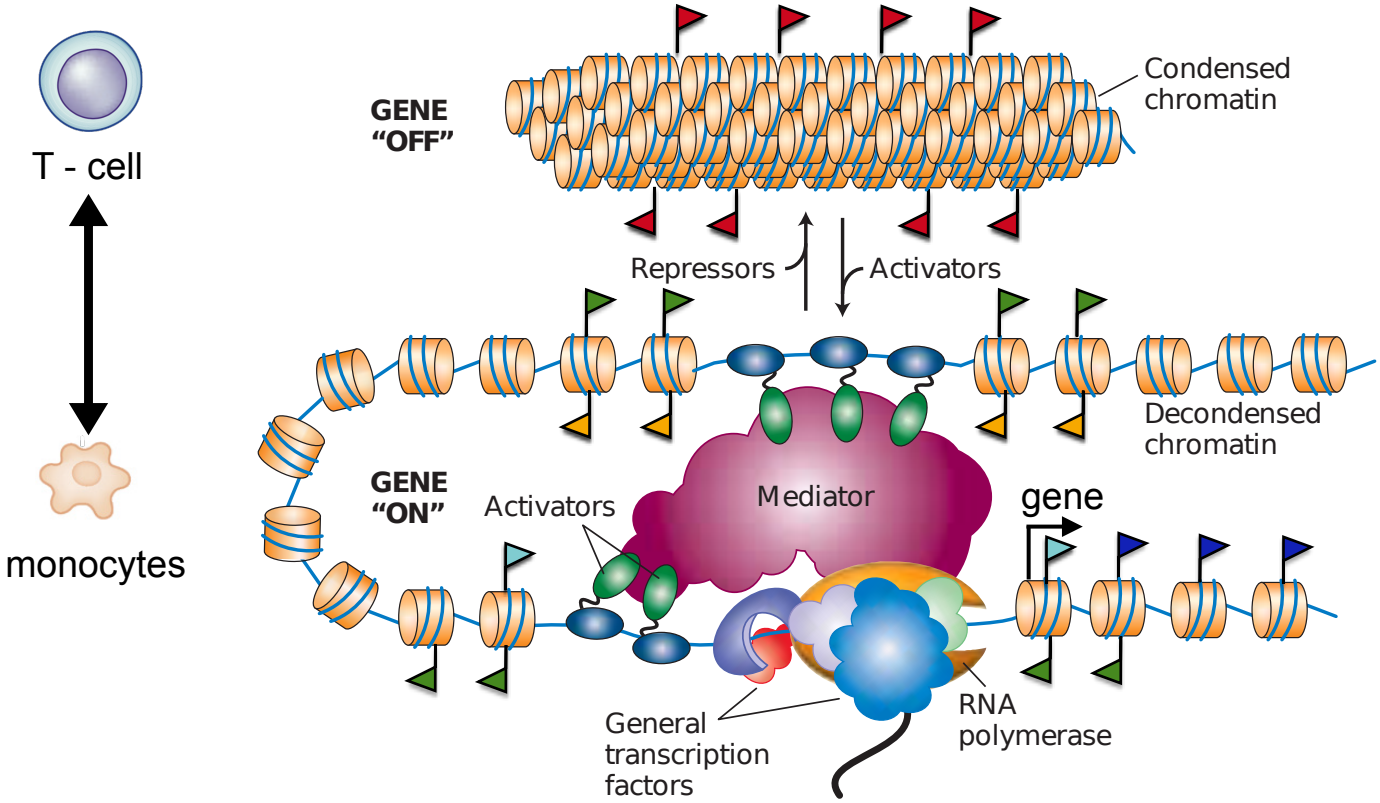
Source: Alberts, B. et al. (2008) Garland Science, 5th ed.

Regulatory Control – Transcription Factor Binding



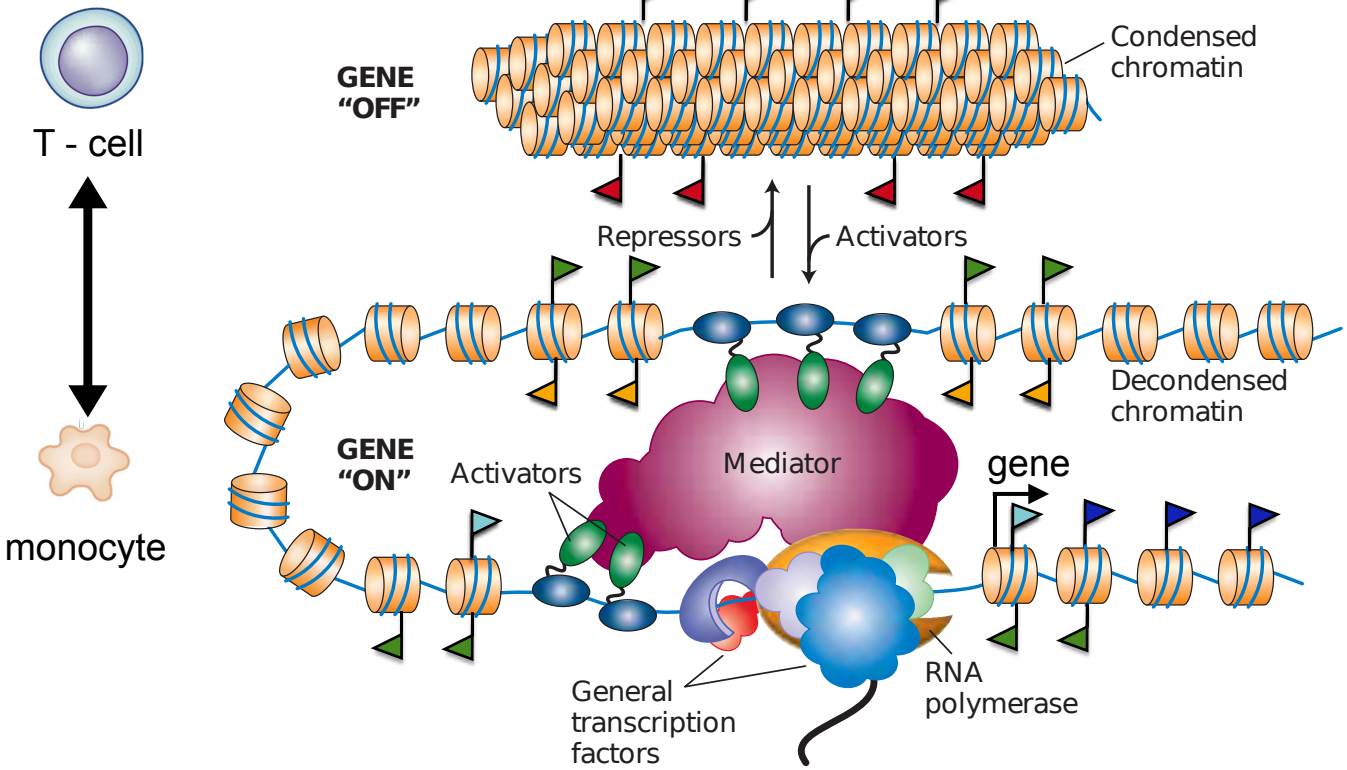
Source: Alberts, B. et al. (2008) Garland Science, 5th ed.

Chromatin, Regulation and Cellular Memory



Adapted from Lodish, B. et al. (2004) 5th ed.

Chromatin & Histone Code



Histone Code

Transcription

H3K79me2, H3k36me3

Active Regions

H3K27ac, H3K9ac

Active Promoters

H3K4me3

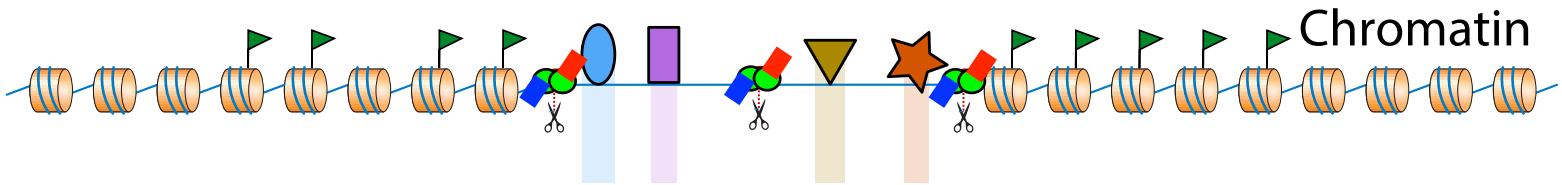
Active Enhancers

H3K4me1

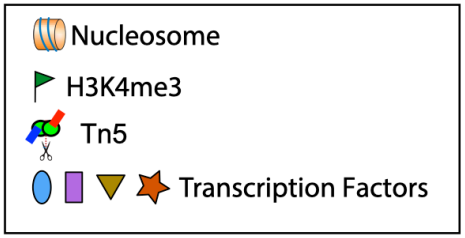
Repressed regions

H3K27me3, H3K9me3

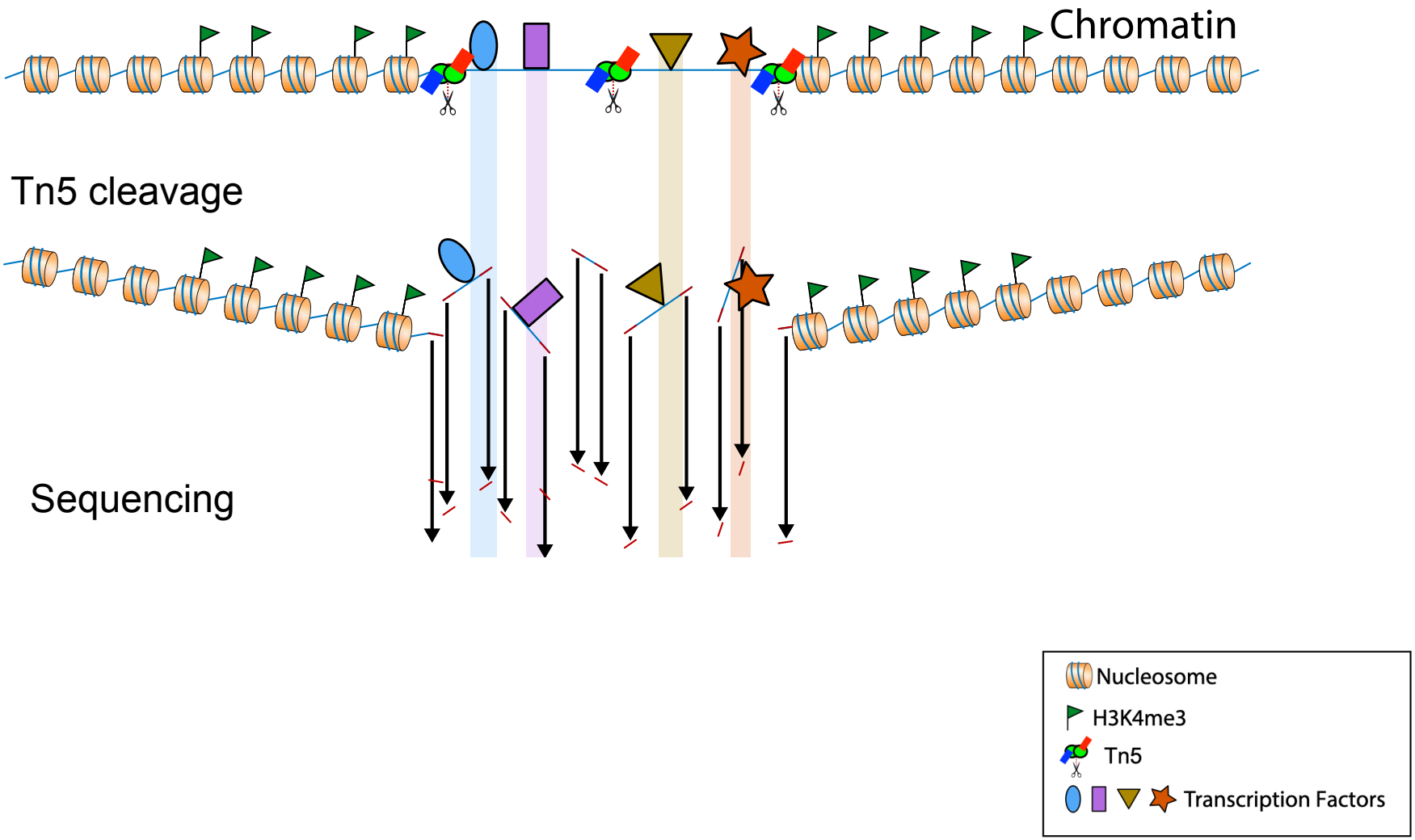
Open Chromatin with ATAC-seq



Tn5 cleavage



Open Chromatin with ATAC-seq



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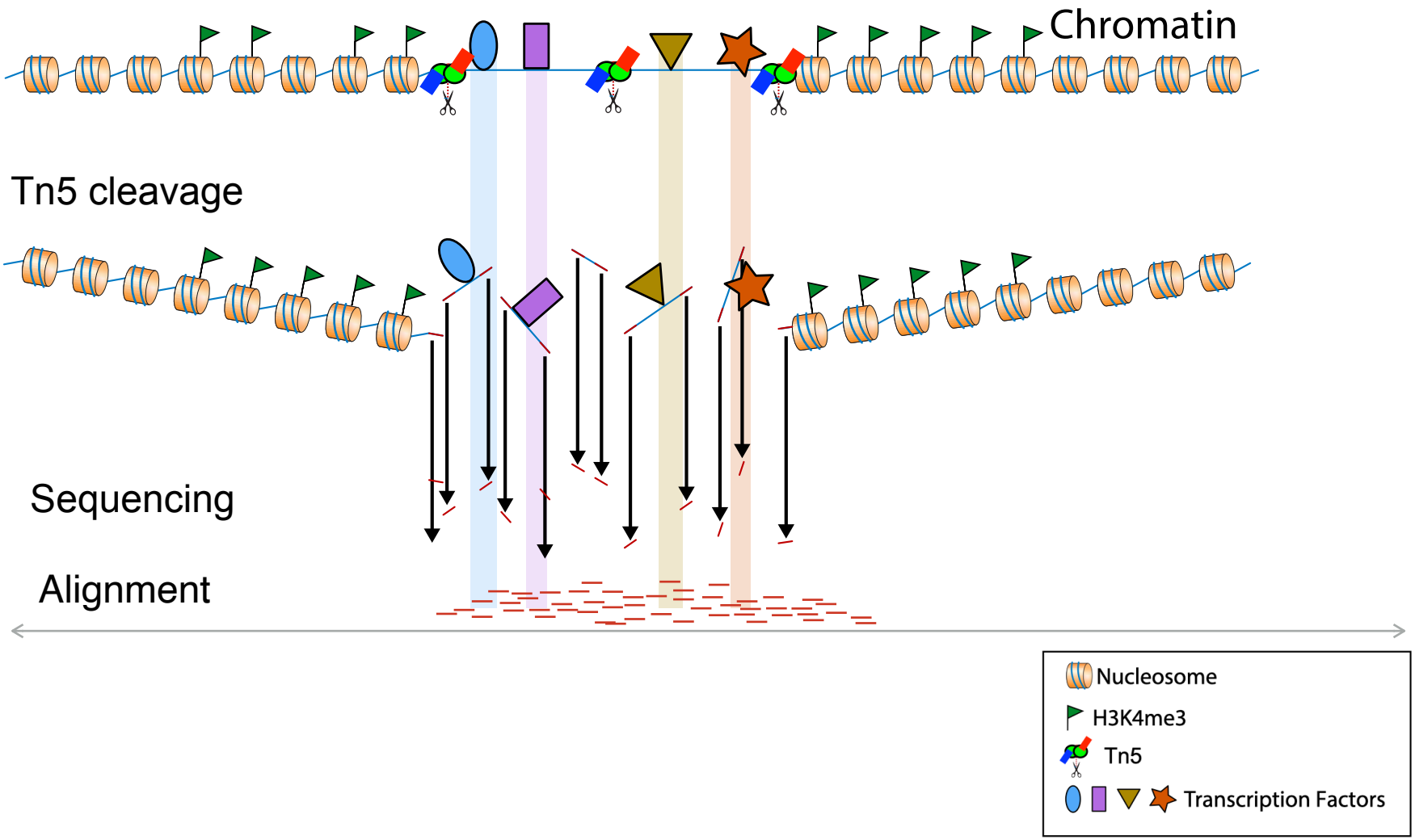

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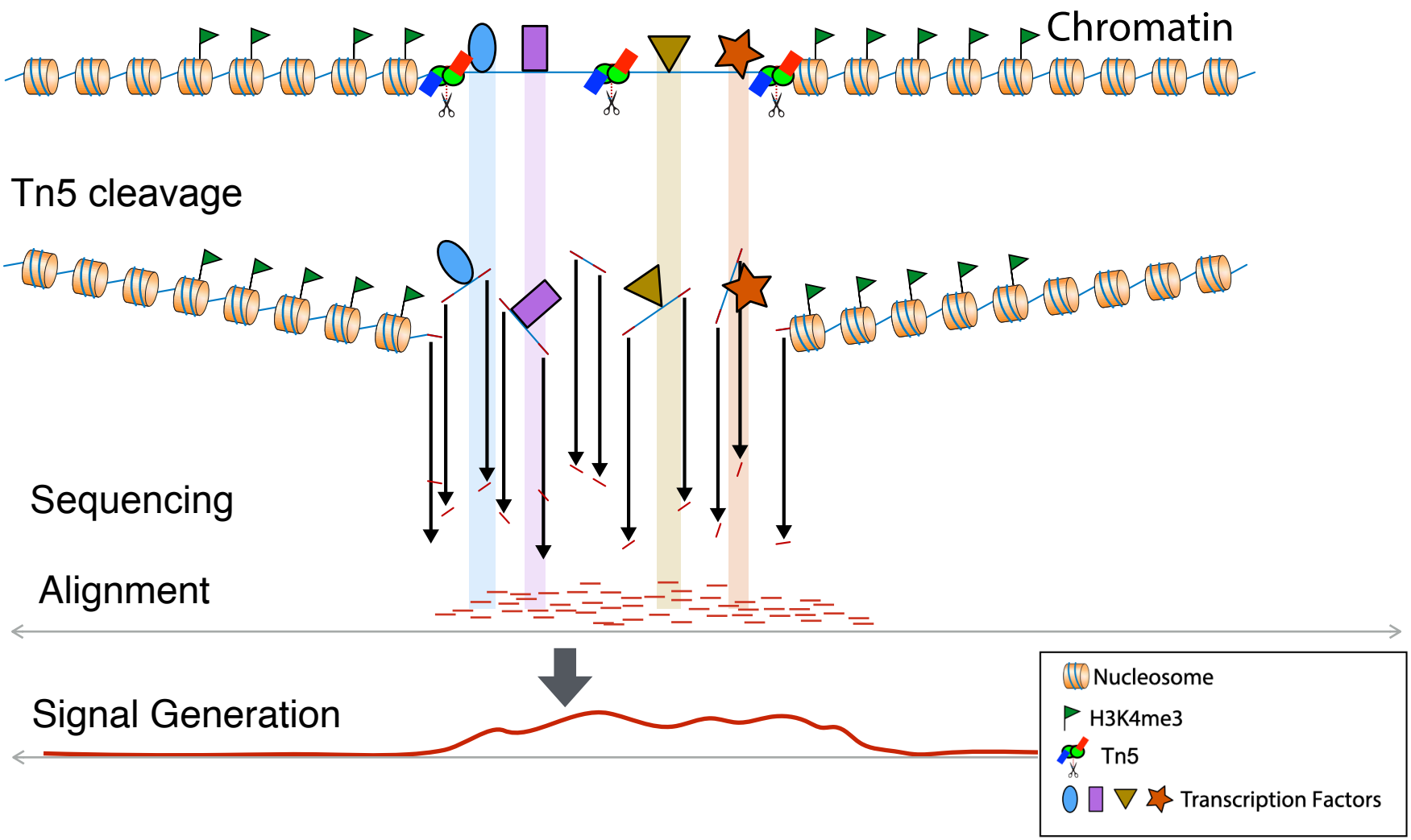

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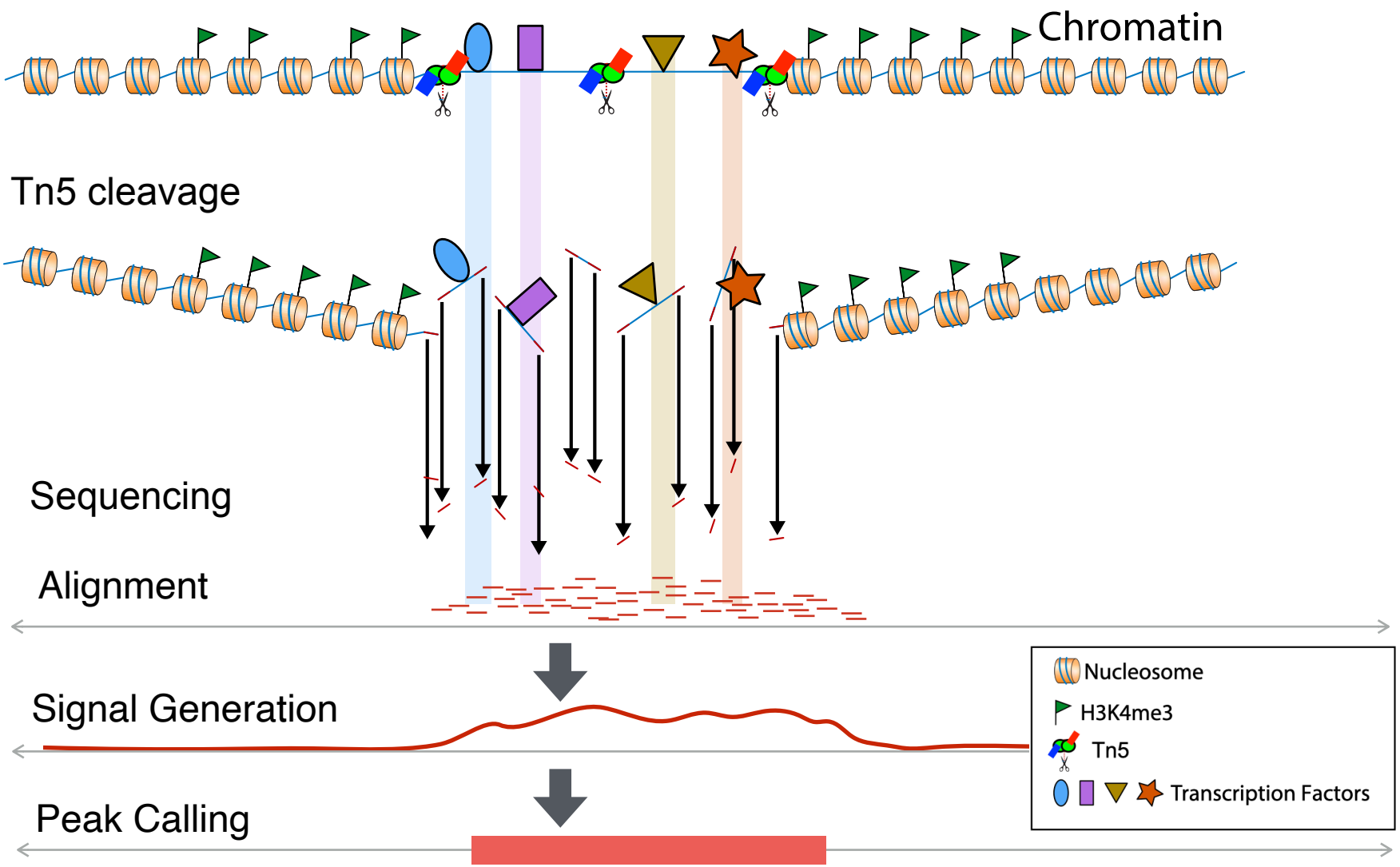
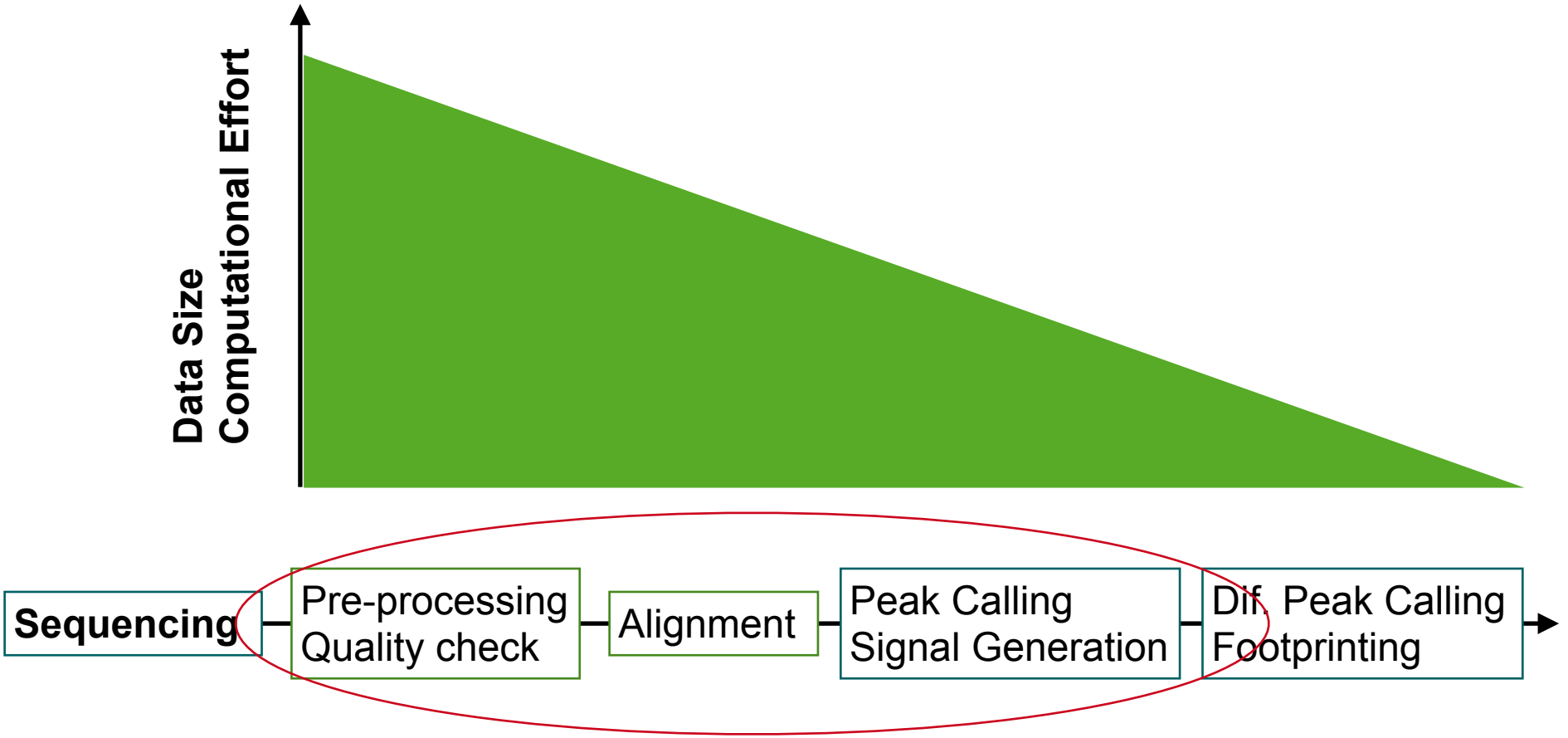


Bioinformatics Pipeline / ATAC-seq



Adapted from Rasmussen:
http://www.cbs.dtu.dk/courses/27626/programme.php

Peak Calling

Problem definition: Find genomic regions (of arbitrary size) with more aligned reads than expected by chance.

Example of a simple peak caller :

1. use a fix window to scan through the genome and obtain a distribution of counts per bin
2. define a statistical test to evaluate if the number of reads in higher than expected by change

See for an example of a code for a peak caller
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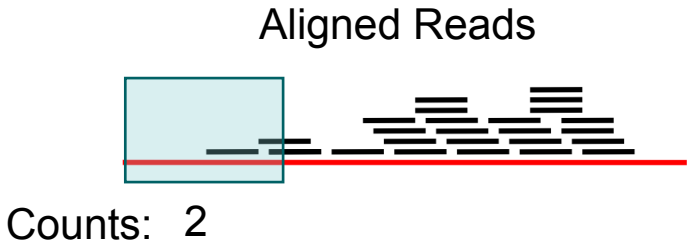

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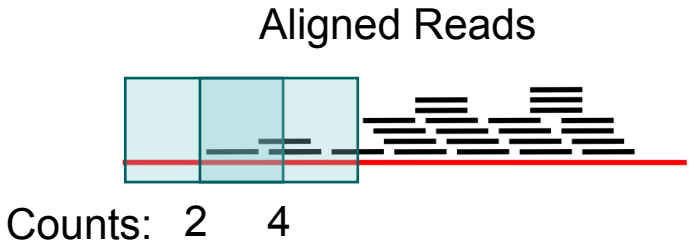

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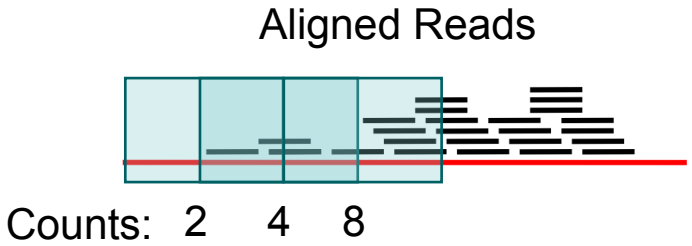

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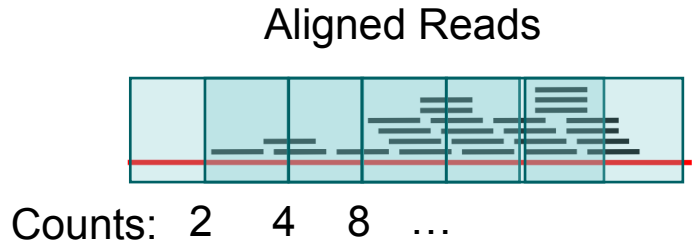

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Aligned Reads

Counts: 2 4 8 …

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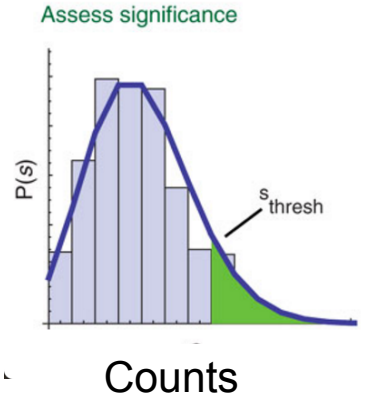

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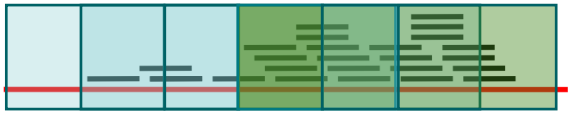

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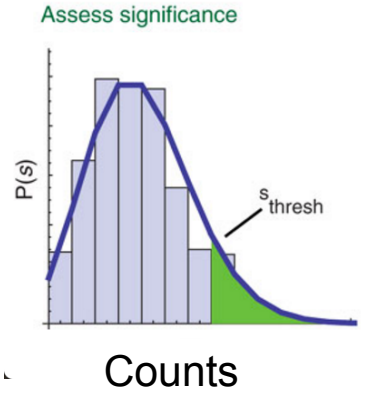

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Peak Calling

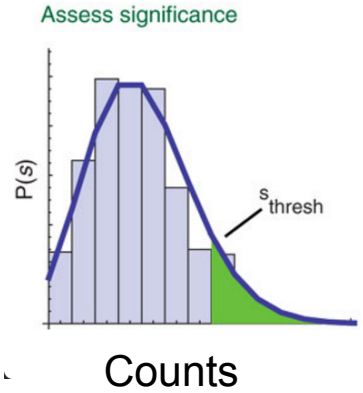
Problem definition: Find genomic regions (of arbitrary size) with more aligned reads than expected by chance.

Problems:

- which window size to use?
- proper quantification of read counts require several further steps: CG bias correction, duplicated reads, mappability, **fragment size**, …

unts: 2 4 8 …

if the number of reads in higher than expected by change



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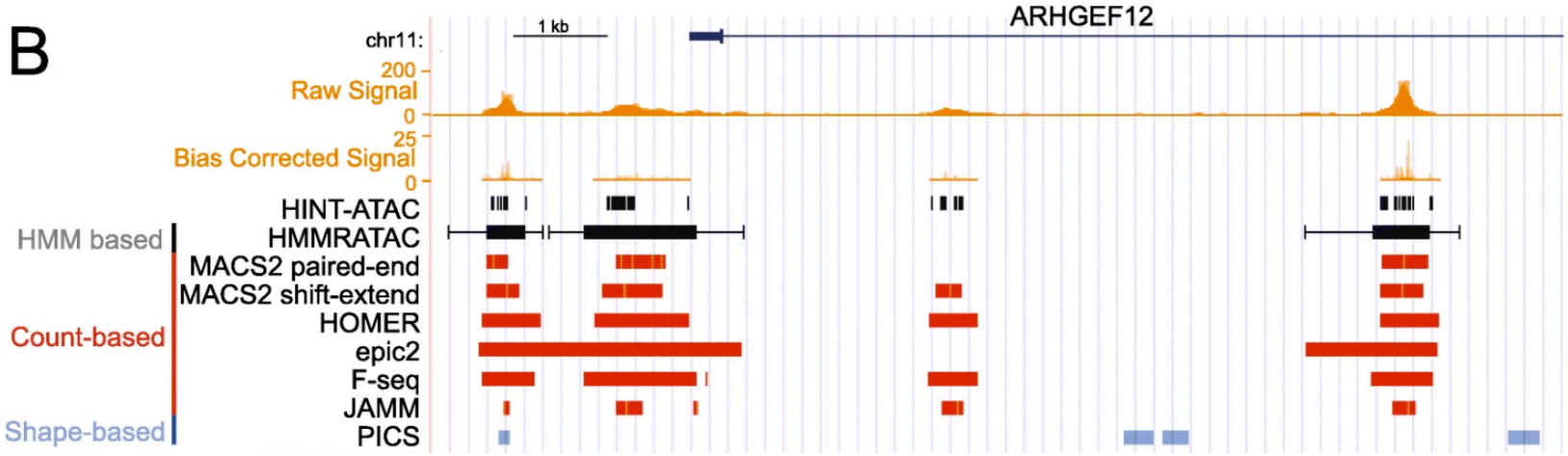
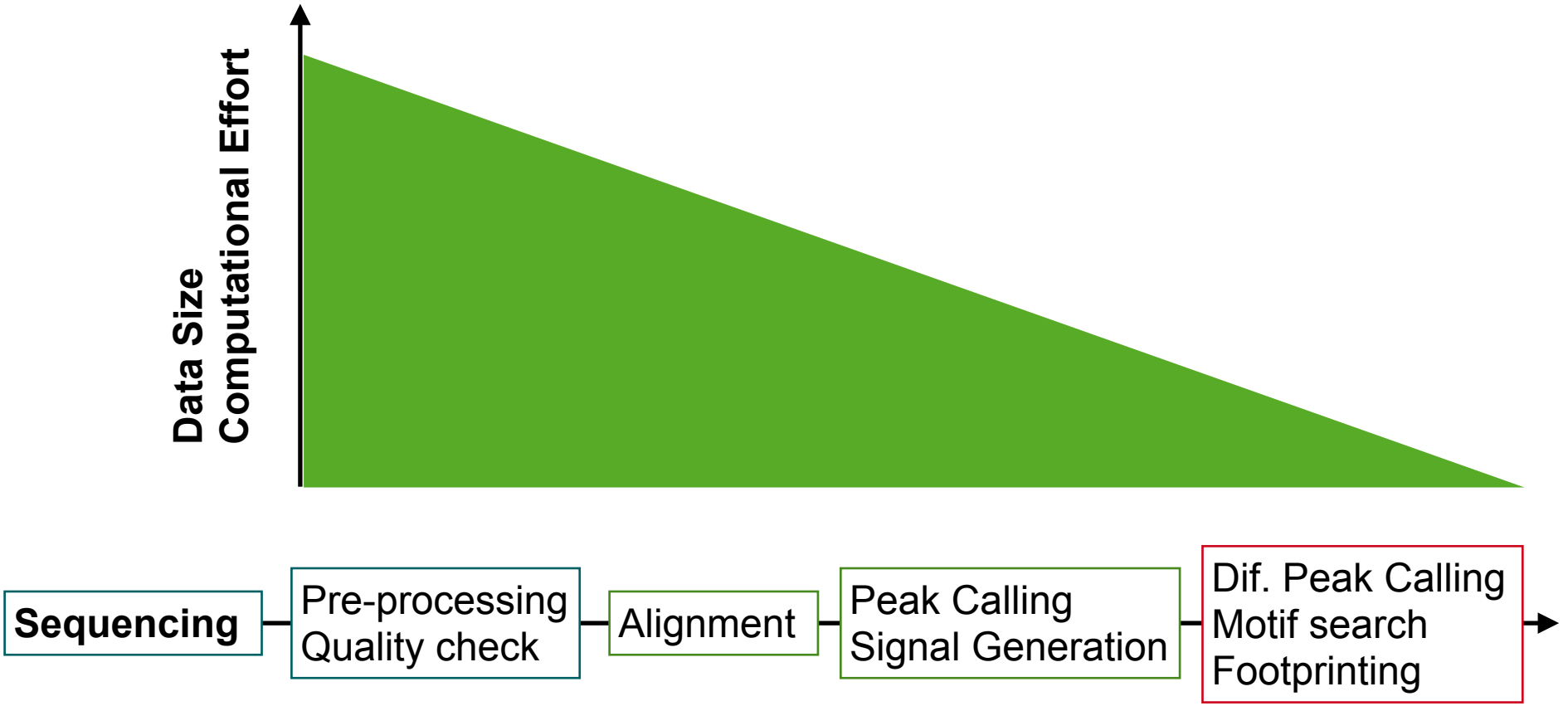
Peak calling in ATAC-seq



- MACS2
  - most frequently used
- HMMRATAC
  - ATAC-seq specific peak caller
  - ignores reads from large fragments / linker cleavage sites

Source: Yan, Genome Biology, 2020.

Bioinformatics Pipeline / ATAC-seq



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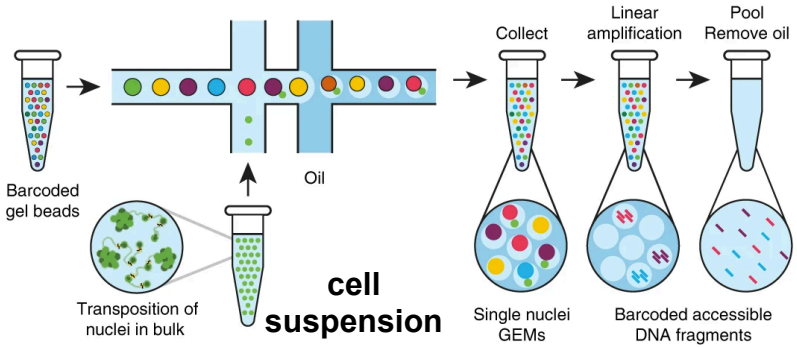
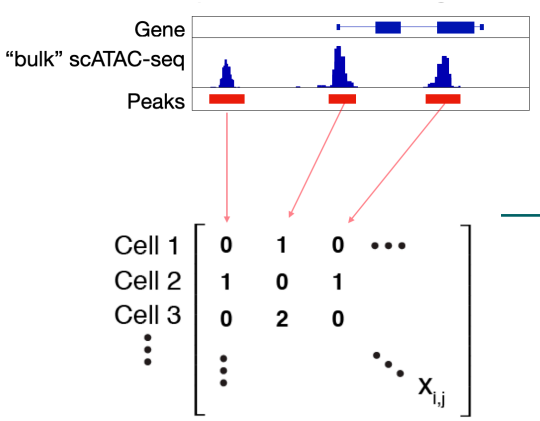
Open chromatin with scATAC-seq

Droplet based scATAC-seq

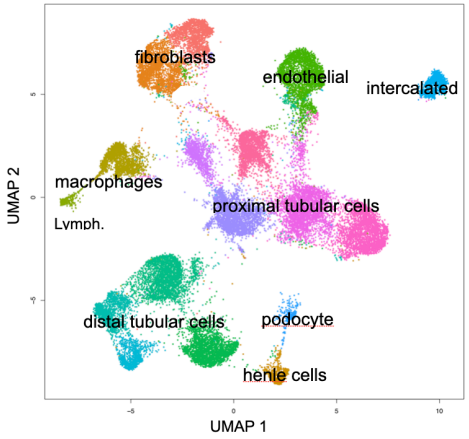


Adapted from Satpathy,
Nature biotechnology, 2019

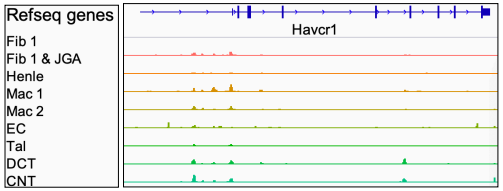
Open Chromatin Matrix

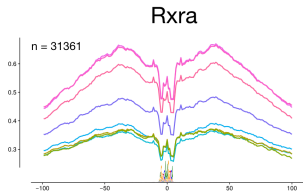


UMAP / Clustering



cluster pseudo bulk ATAC-seq





Footprinting & TF Activity

Li, …, **Kramann, Costa**, Biorvx, https://doi.org/10.1101/865931.

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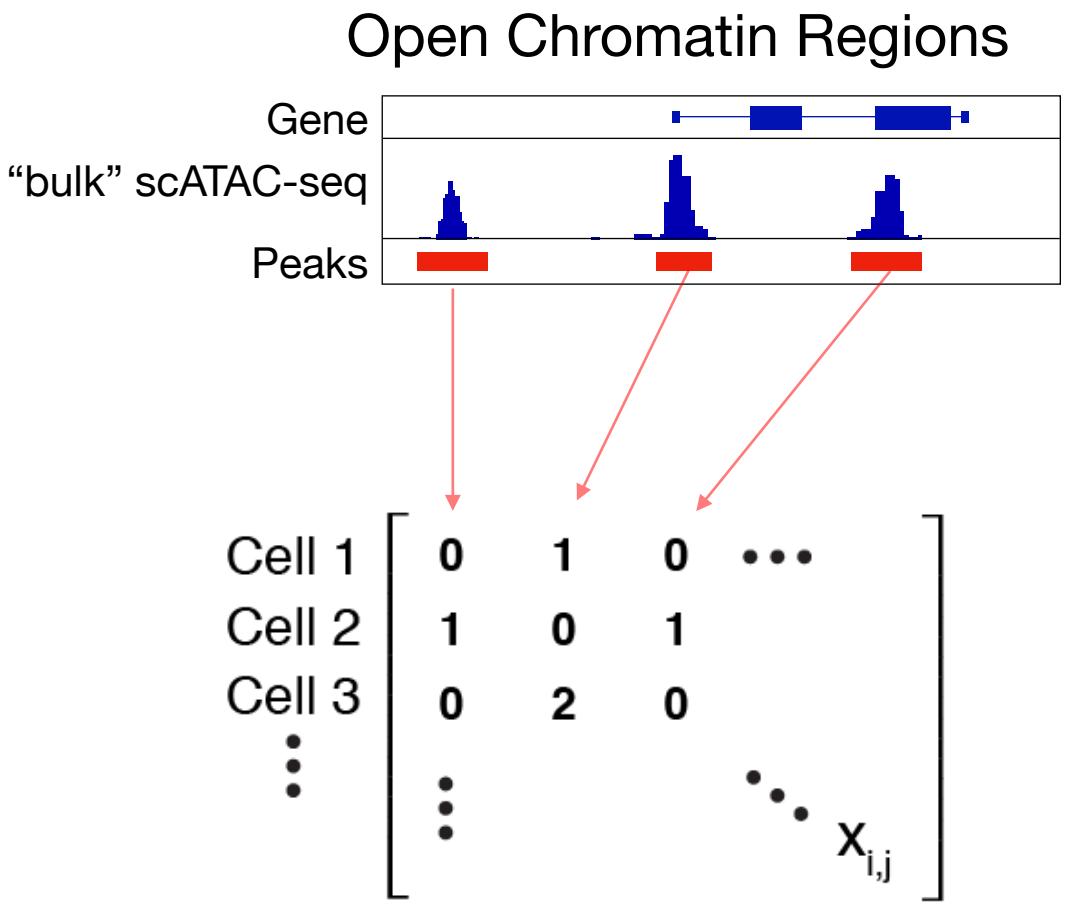
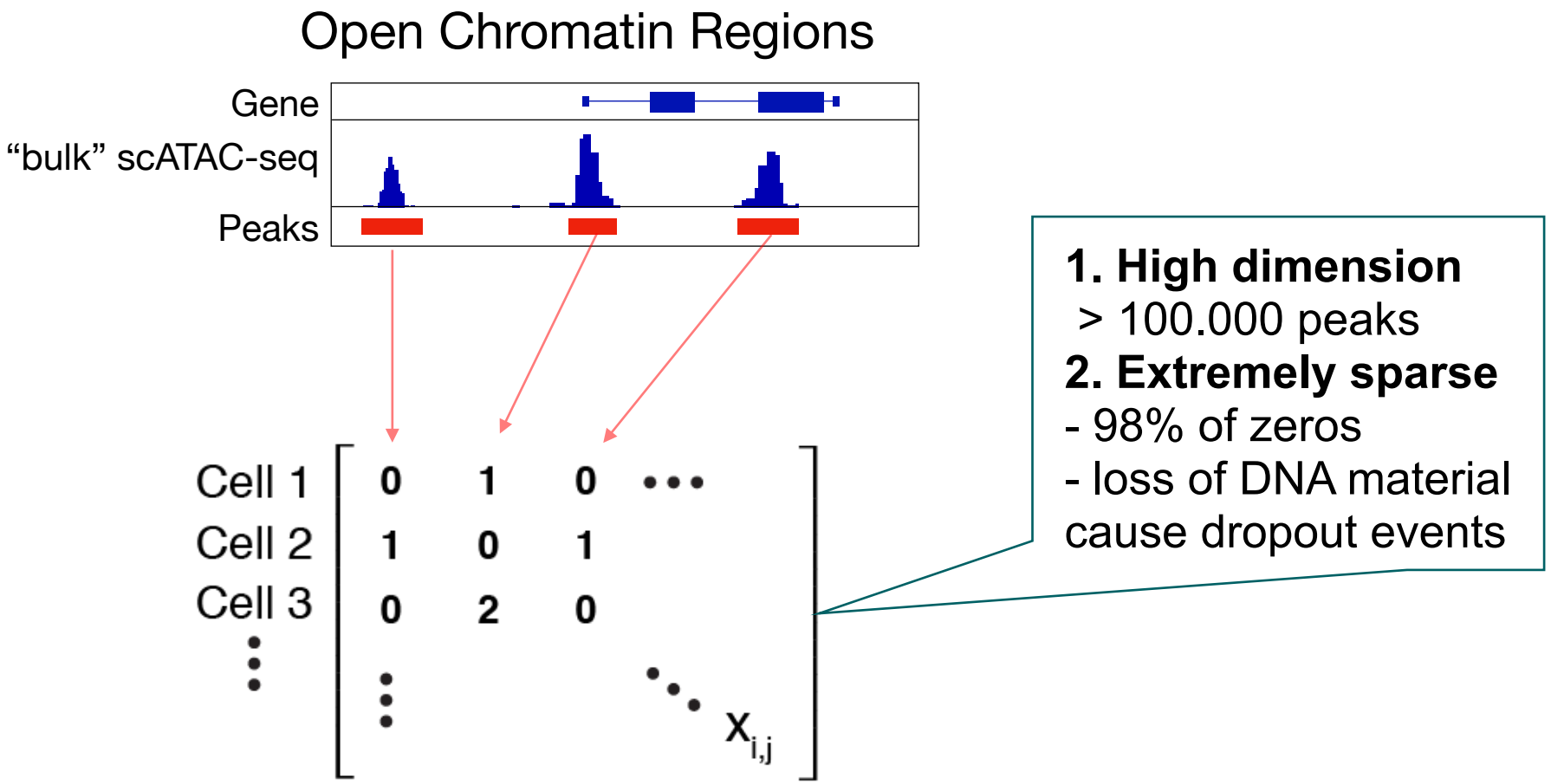
Computational Challenges - Single Cell ATAC



Computational Challenges - Single Cell ATAC



Resume / Single cell clustering

- Finding groups of single cells require complex pipeline:
  - Cell filtering
  - Normalisation
  - Artefact removal
  - Dimension reduction
  - Integration
  - Clustering
  - Cell annotation / visualisation
- Open points:
  - How to deal with sparsity of single cell (scRNA-seq or scATAC-seq) data?

Thank you!