Analysis of Open Chromatin Data

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Objectives

- Understand open chromatin from biological point of view
- Analyse bulk open chromatin data
- Visualise the result using IGV
- Extend the analysis to single cell open chromatin data

Open Chromatin

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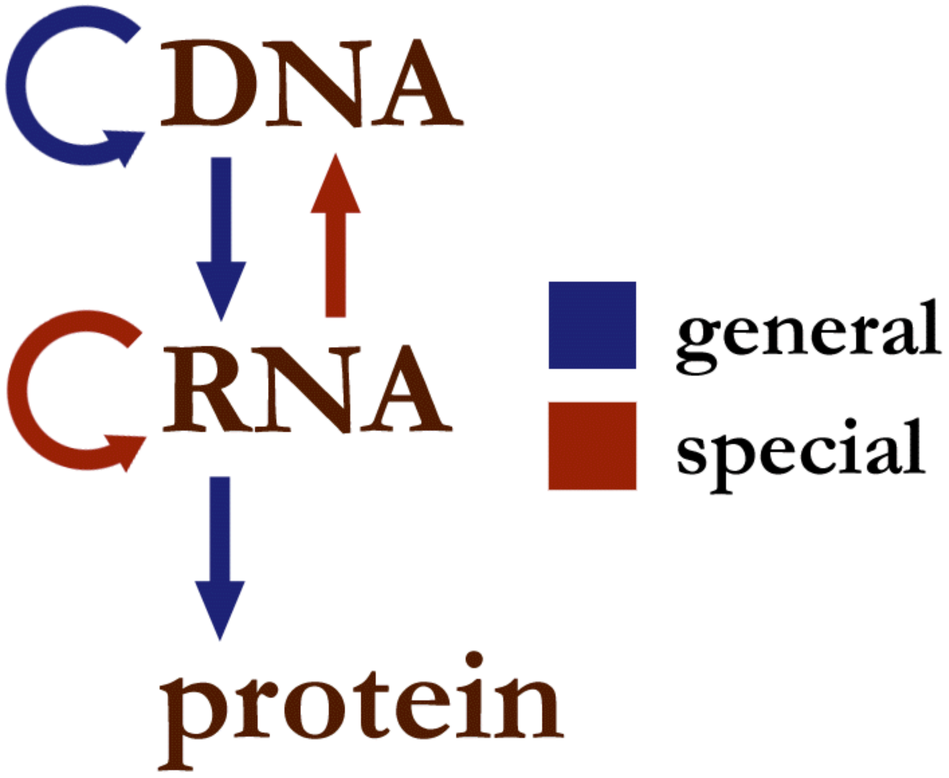
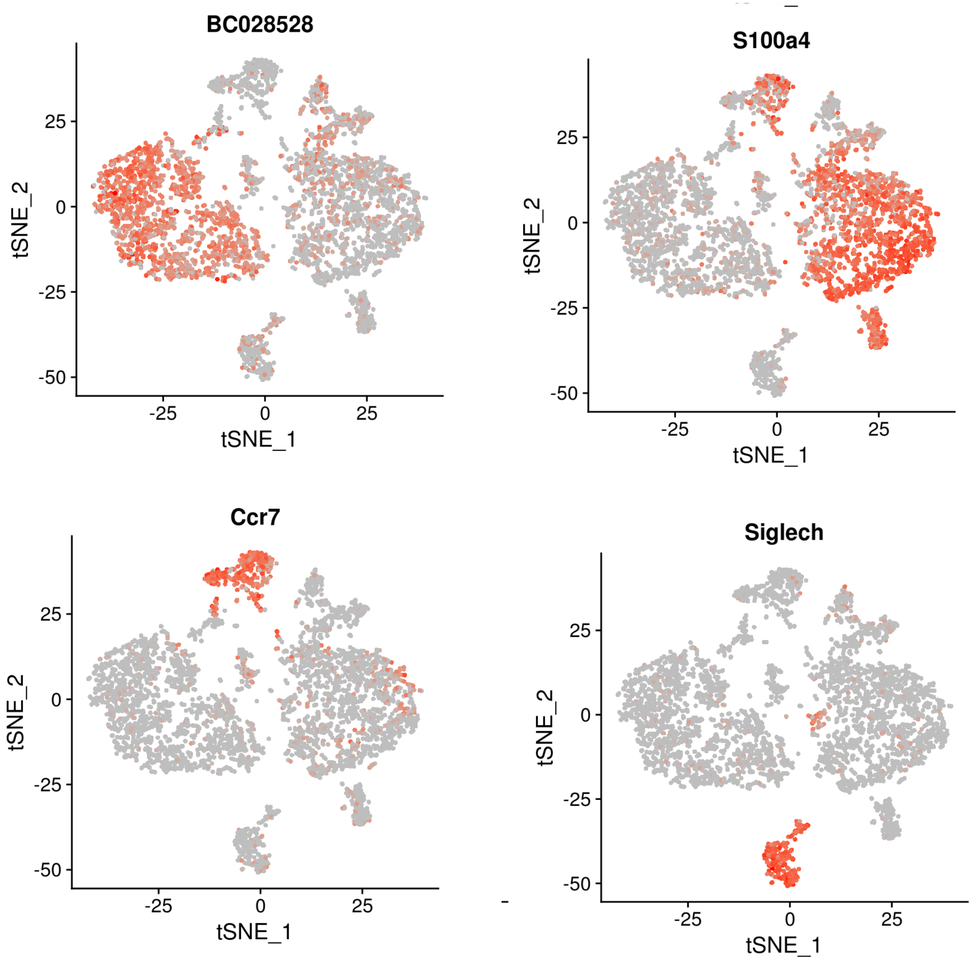
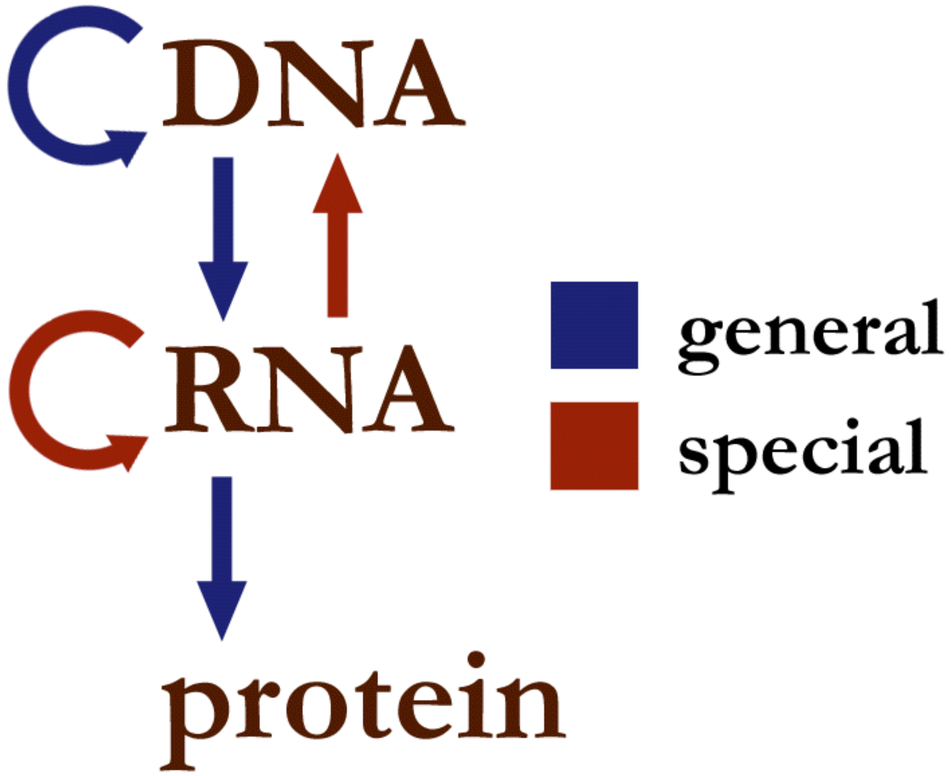
Central dogma of molecular biology





https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

One genome vs. many cell types



Why do the cells have different gene expression, given that they have the exactly same genome?

https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

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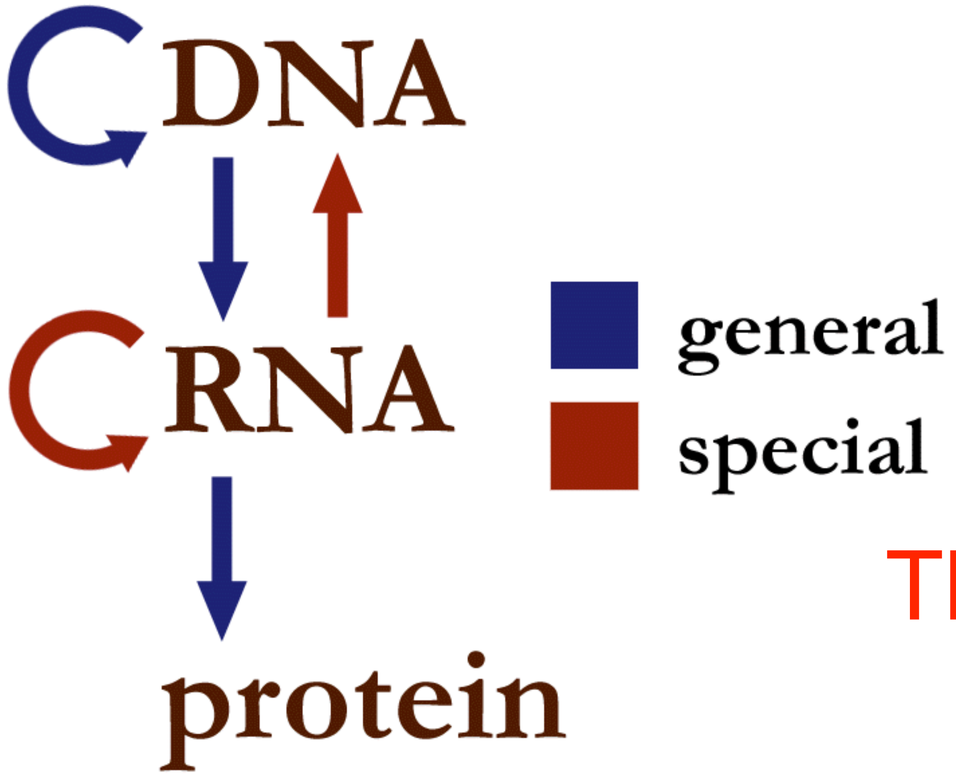
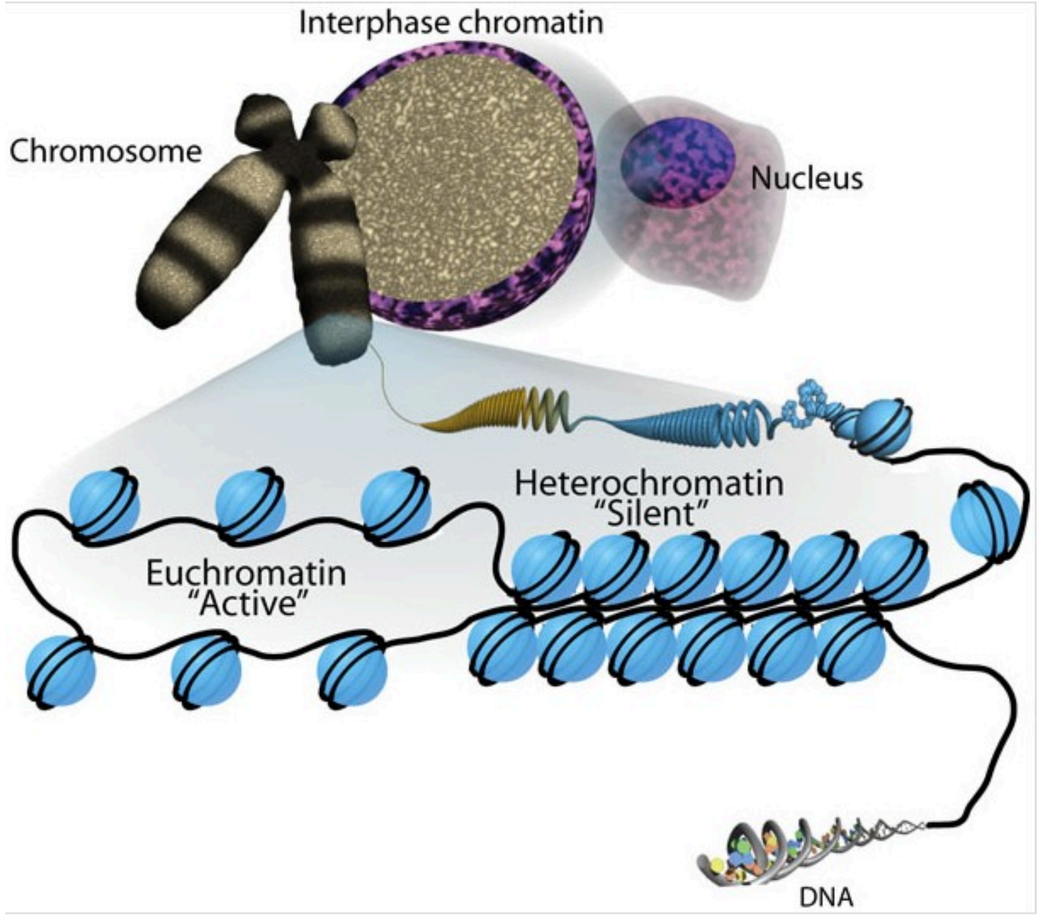


Why do the cells have different gene expression, given that they have the exactly same genome?

Their chromatin states are different!

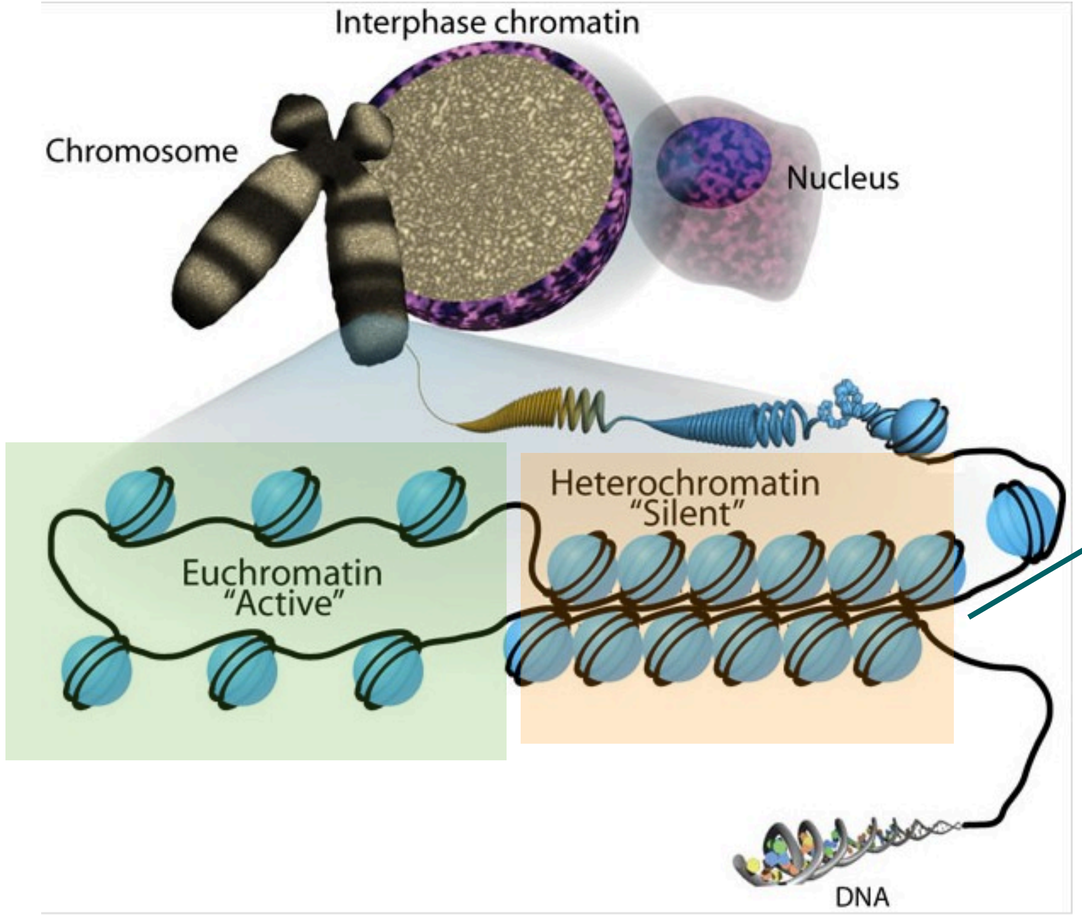
https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

Open chromatin vs. closed chromatin



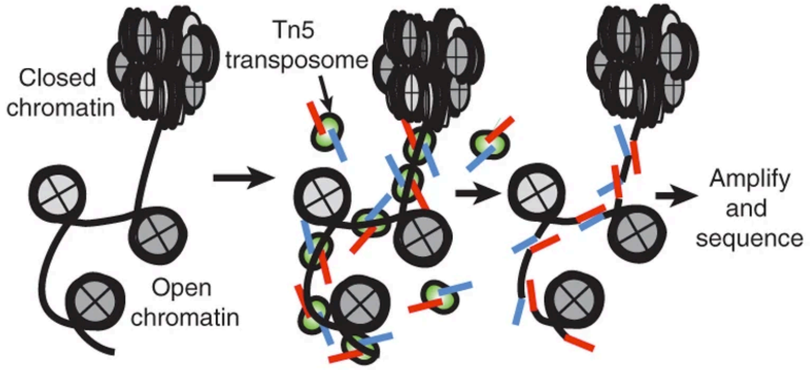
Open chromatin vs. closed chromatin

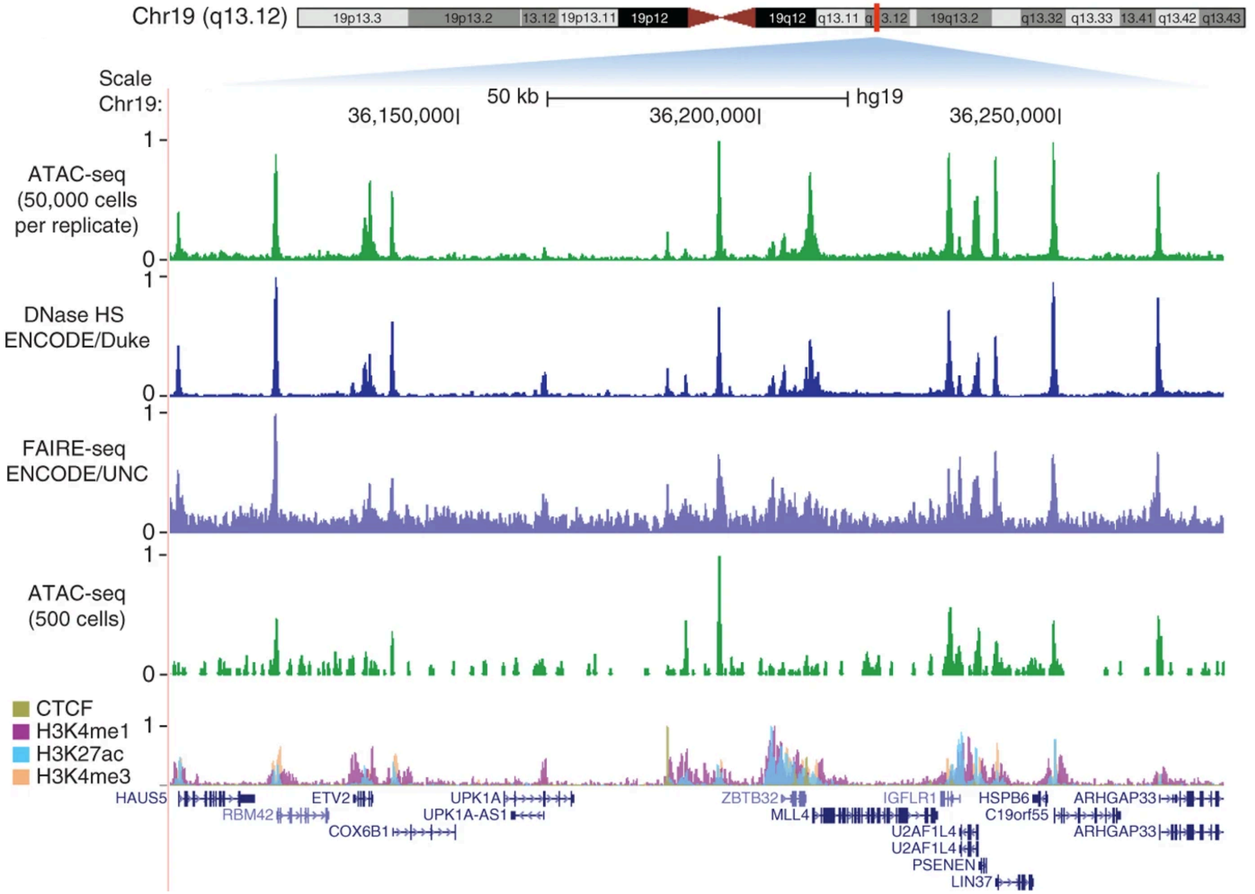
Open chromatin



Close chromatin

ATAC-seq probes open chromatin state





Buenrostro, Jason D., et al. *Nat. Method* 2013

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Analysis of ATAC-seq Data

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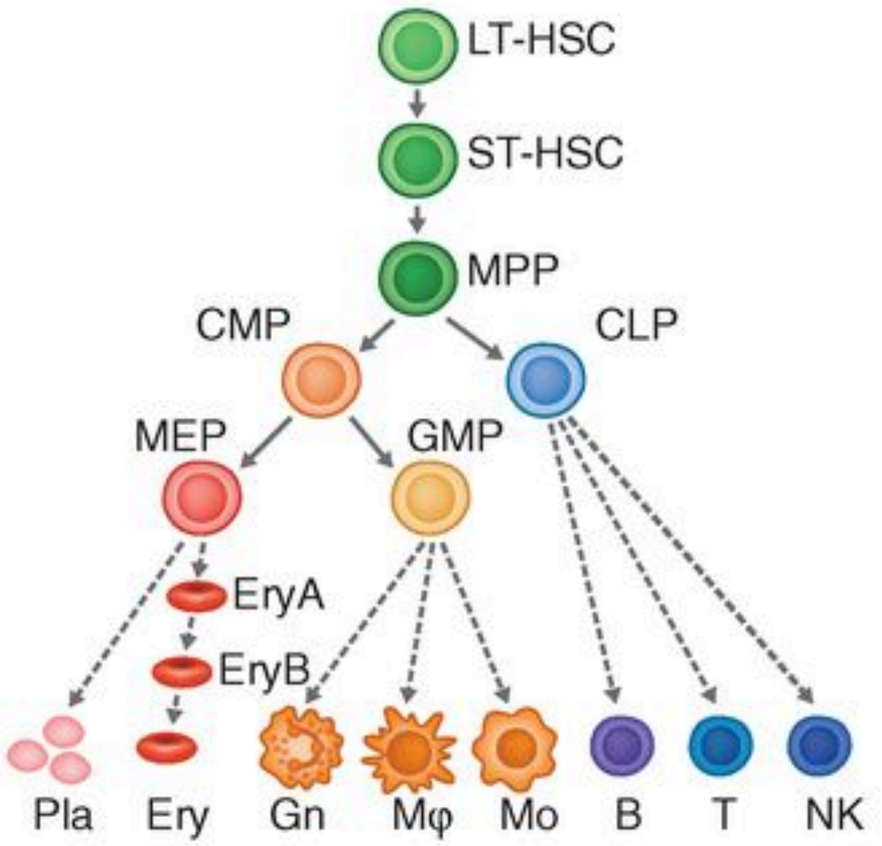
Chromatin dynamic during blood formation

Hematopoietic differentiation



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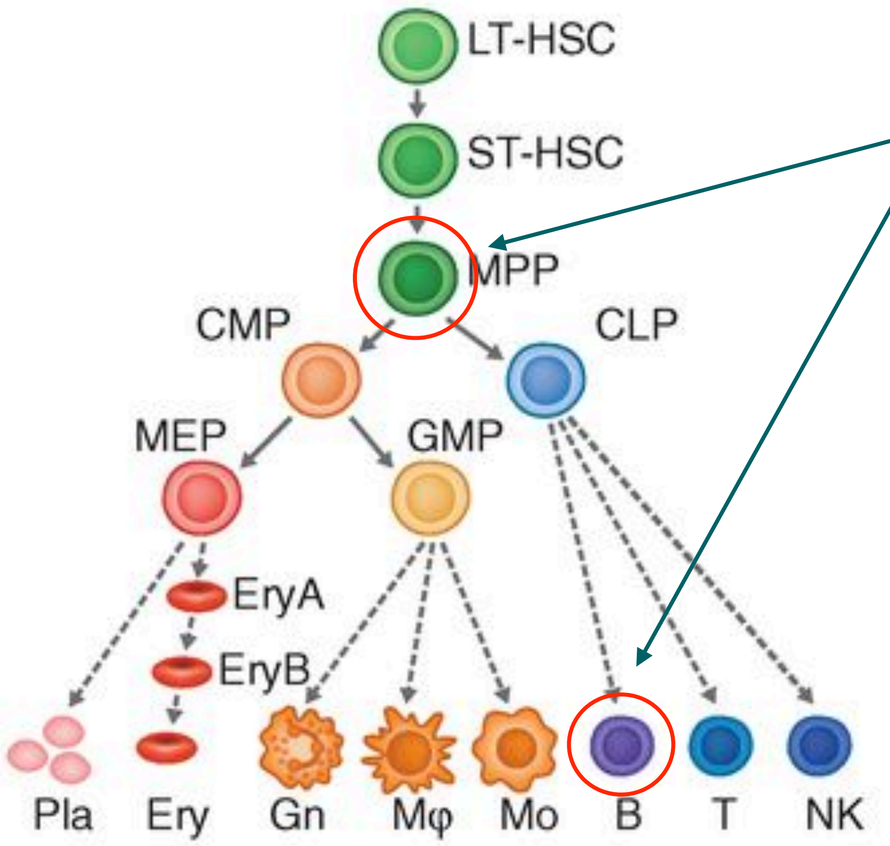


Any differences at chromatin level?

Analysis pipeline

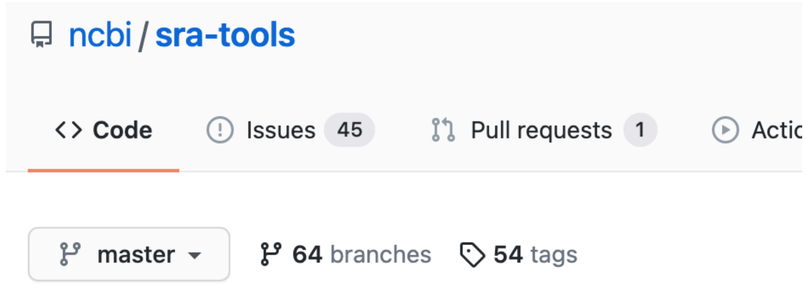
- Download data (SRA toolkit)
- Sequence alignment (Bowtie2)
- Peak calling (MACS2)
- Motif matching (RGT)

https://github.com/CostaLab/SOSE2022/blob/main/Practical_ATAC.md

1. Download data

SRA toolkit

The SRA Toolkit is a collection of tools and libraries for using data in the NCBI Sequence Read Archives.



Two common sub commands:

- *prefetch*
- *fastq-dump*

Download data

10 minutes

2. Short DNA Sequence Alignment

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Sequence alignment

Input data

- A large reference genome (chr19.fa)
- Millions of short DNA reads (MPP.fastq, B.fastq)

Sequence alignment

- Find most probable position for each read in the genome (allow insertion and deletion)

Output data

- Aligned file (MPP.sam, B.sam)

Bowtie 2

Align reads to reference genome

- Extract ‘seed’ substrings from the reads
- Align the substrings to the reference
- Calculate the position information
- Extend the seeds to full alignment using dynamic programming

More information

- Paper: https://www.nature.com/articles/nmeth.1923
- Website: http://bowtie-bio.sourceforge.net/bowtie2/index.shtml

Alignment

10 minutes

3. Peak Calling

Peak calling

Problem definition: Find genomic regions with more aligned reads than expected by chance.

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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 is higher than expected by change

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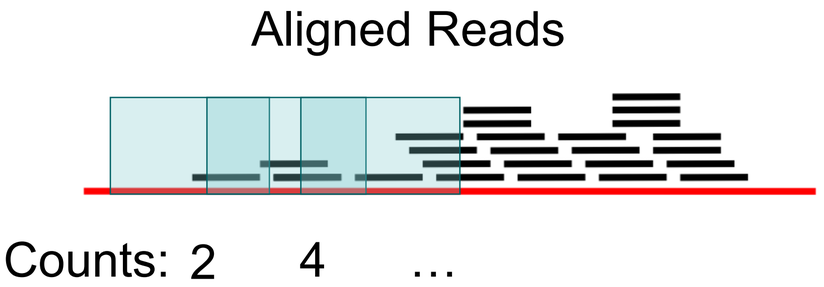

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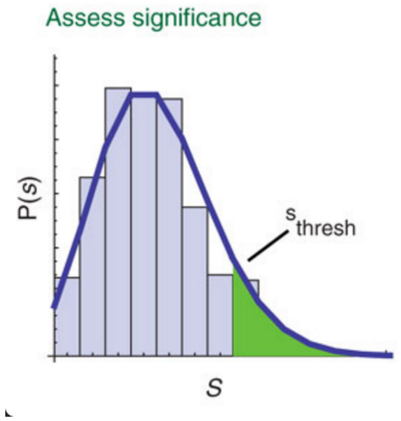
Aligned Reads

Counts: 2 4 …



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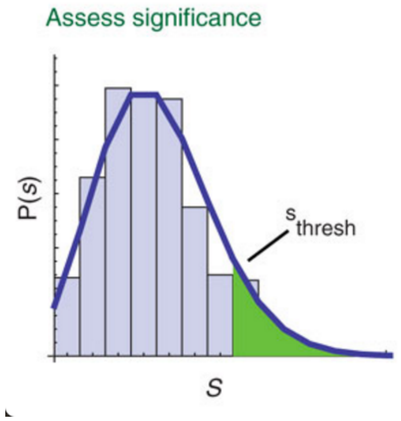

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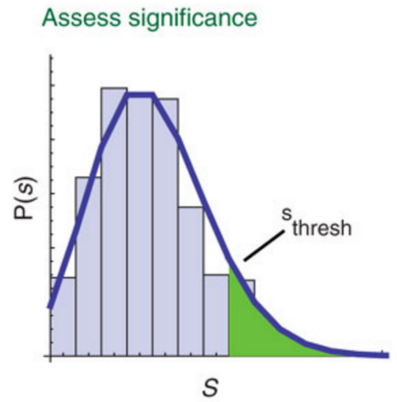
Problems:
- which window size to use?
- distinct proteins have distinct peak sizes
- proper quantification of read counts require several further steps: fragment size estimation, CG bias correction, mappability, …

gned Reads

4 …



Peak calling with MACS2

MACS2

- Models the reads count using a Poisson distribution
  - Only one parameter λ which models mean and variance
  - Estimate a dynamic background reads distribution to capture local biases in the genome, allowing for more robust identification.
- Peaks are defined given a p-value on the Poisson model

More information

- Paper: https://genomebiology.biomedcentral.com/articles/10.1186/gb-2008-9-9-r137

Peak calling

2 minutes

4. Motif Matching

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Motif matching

Problem definition: Matches a set of transcription factor motifs against a set of genomic regions.

Regulatory Genomic Toolbox (RGT)

- is an open-source python library and set of tools for the integrative analysis of high throughput regulatory genomic data
- provides a flexible framework to perform operations related to motif analyses

More information

- Website: https://www.regulatory-genomics.org/

Motif matching

2 minutes

5. Visualizaiton

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Single cell ATAC-seq

R : https://satijalab.org/signac/index.html
Python: https://episcanpy.readthedocs.io/en/latest/