Practical Example: Analysis of Open Chromatin Data

Ivan Gesteira Costa & Zhijian Li
Institute for Computational Genomics

Institute for Computational Genomics
RWTH AACHEN UNIVERSITY

Contact Information

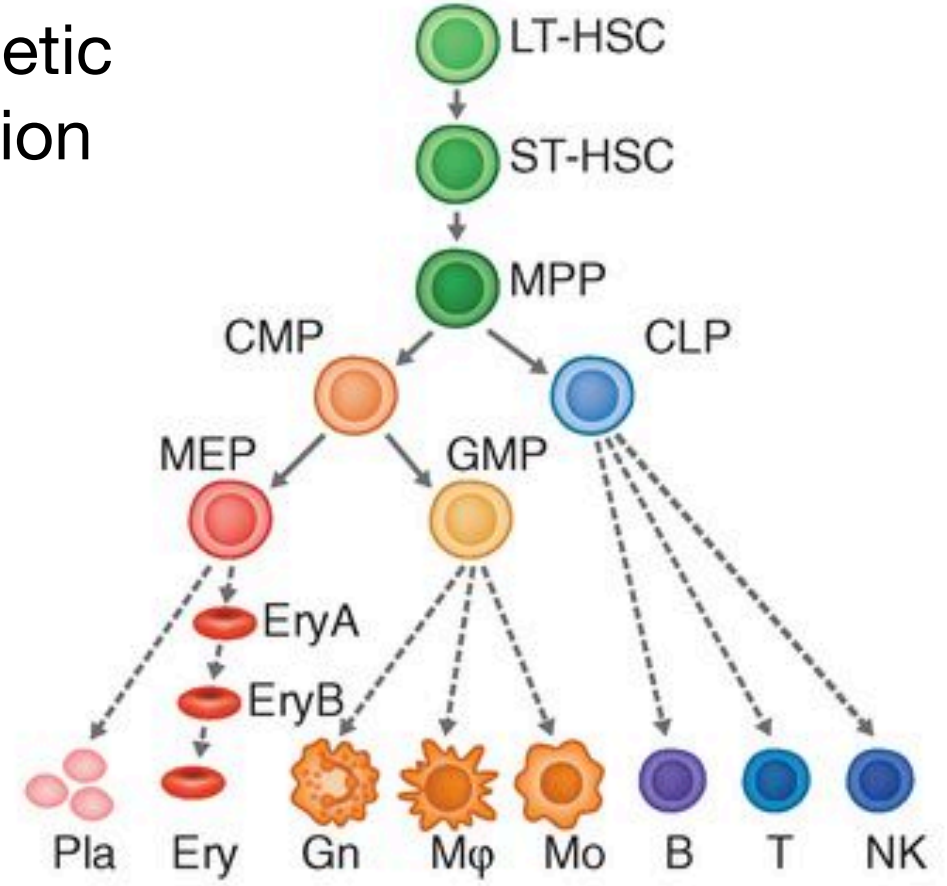
Zhijian Li

zhijian.li@rwth-aachen.de

MTZ, Room 3.02

Chromatin dynamics during blood formation

hematopoietic differentiation



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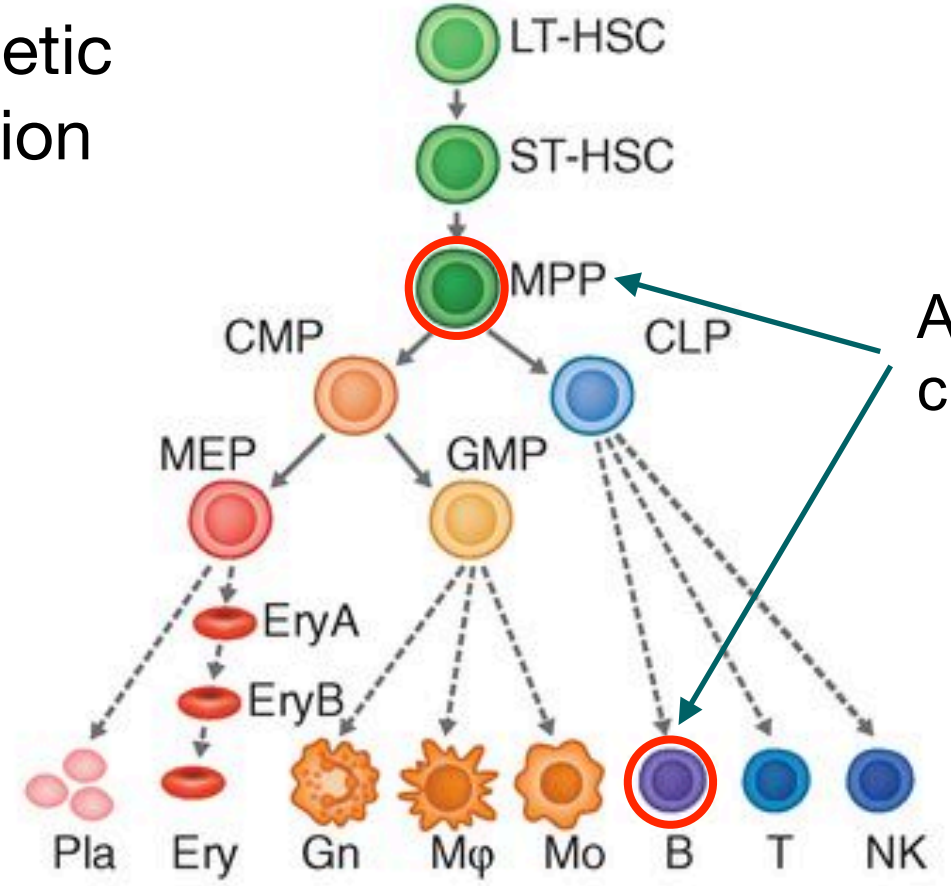
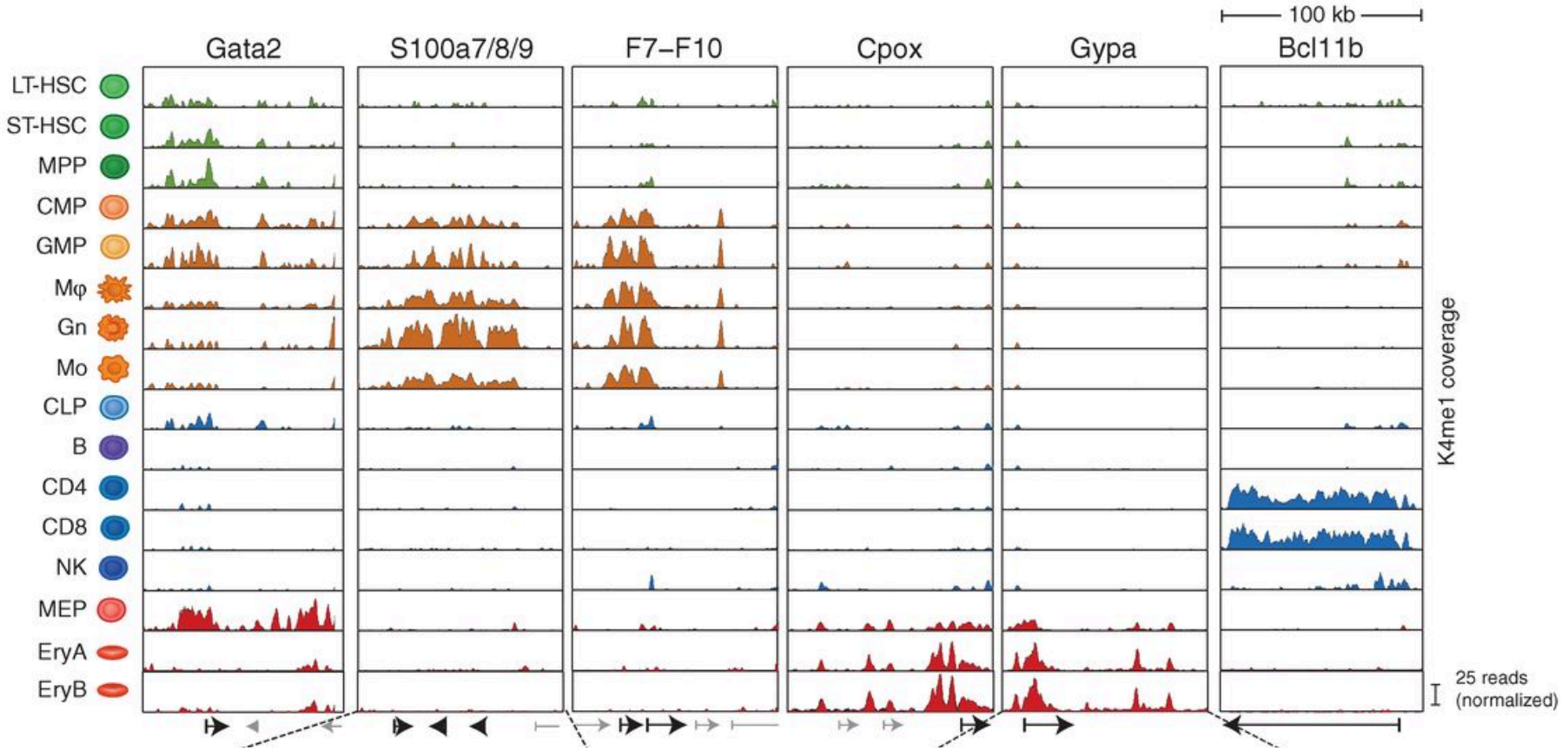


Any differences at chromatin level?

Chromatin dynamics during blood formation



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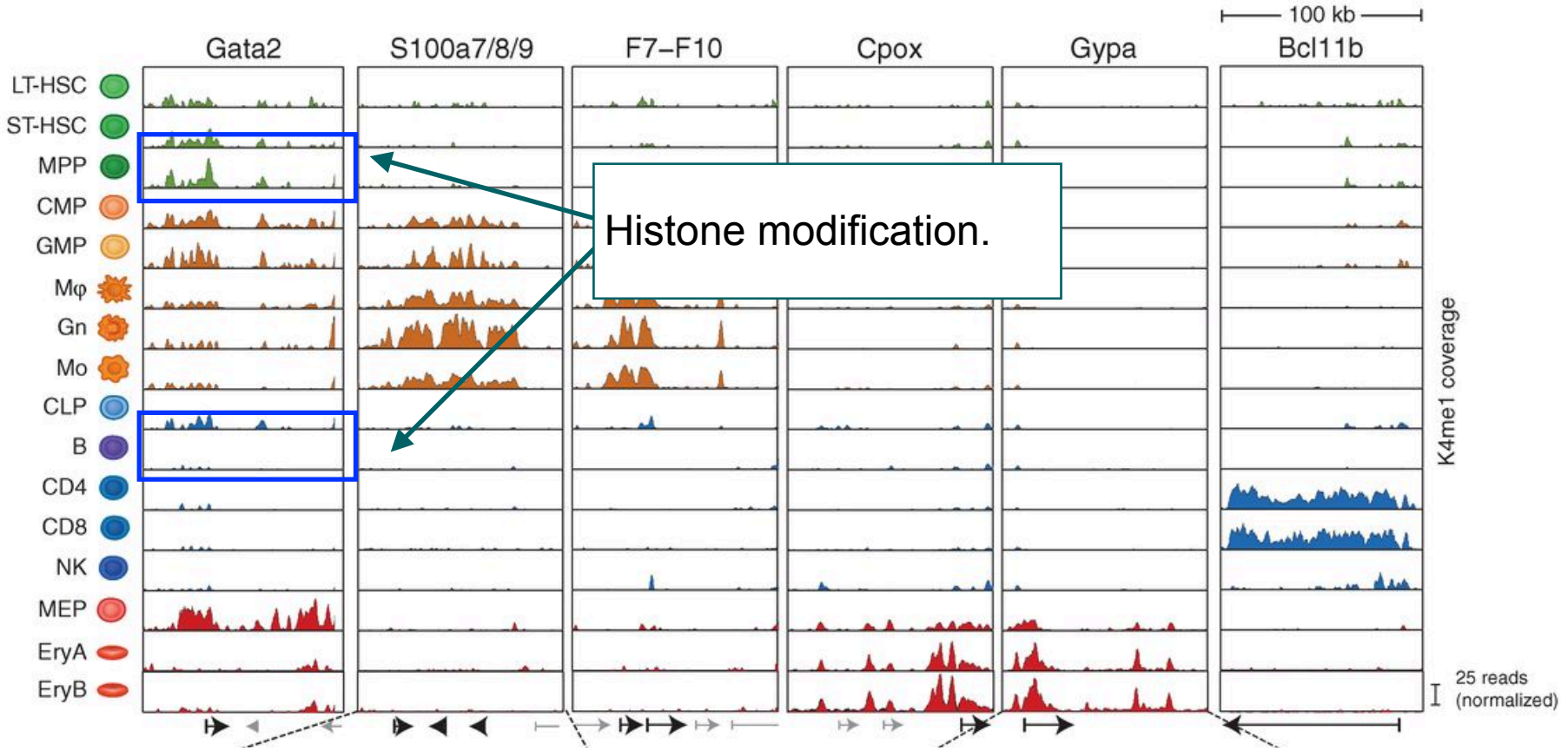


Analysis pipeline

- Download sequencing data
  - SRA toolkit, FASTA, FASTQ and SRA file
- Sequence alignment
  - Bowtie2, samtools, SAM and BAM file
- Peak calling
  - MACS
- Footprinting and motif analysis
  - RGT, bed file

1. Download Data

SRA tools

- A collection of tools and libraries for using data in NCBI Sequence Read Archives

- More information:
  - https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=toolkit_doc&f=std

FASTA File

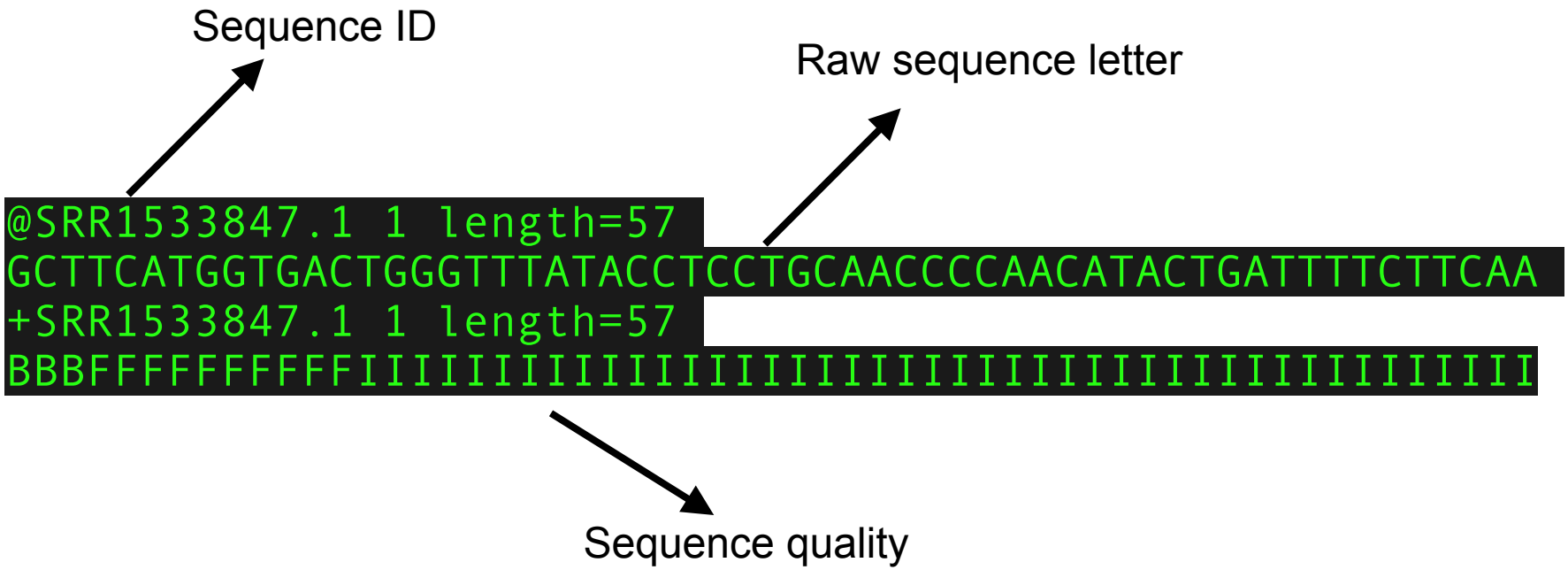
- Store DNA sequences in a text-based file
- Mainly used to store large genomic sequences
- Header (lines that start with ‘>’) + DNA sequence
- DNA alphabeta: A, C, G, T, N

```
>SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
```

FASTQ File

- Also text-based file
- Mainly used to store short DNA sequences
- Normally use four lines per sequence
  - Line 1 begins with '@' and is followed by a sequence ID
  - Line 2 is the raw sequence letter
  - Line 3 begins with a '+' character and is followed by the same sequence ID
  - Line 4 encodes the sequencing quality values

FASTQ File: example



SRA File

- A compressed version of FASTQ file

Download data

- Download and unpack our reference sequence
  - wget http://hgdownload.soe.ucsc.edu/goldenPath/mm10/chromosomes/chr19.fa.gz
  - gunzip chr19.fa.gz
- Download sequencing reads
  - prefetch SRR1533863 SRR1533847
- Use SRA toolkit to convert SRA to FASTQ
  - fastq-dump ~/ncbi/public/sra/SRR1533847.sra
  - fastq-dump ~/ncbi/public/sra/SRR1533863.sra

Practice for data download

5 minutes

2. Short DNA Sequence Alignment

Sequence Alignment

- Input data
  - A large reference sequence (chr19.fa)
  - Millions of short DNA reads (SRR1533863.fastq, SRR1533847.fastq)
- Sequence alignment:
  - Find most probable position for each read in the genome (allow insertion and deletion)

Bowtie2

- Align reads to the genome:
  - Extract ‘seed’ substrings from the read
  - Align the substrings to the reference
  - Calculate the position information
  - Extend the seeds to full alignments using dynamic programming
- More information:
  - Paper: https://www.nature.com/articles/nmeth.1923
  - Website: http://bowtie-bio.sourceforge.net/bowtie2

Samtools

- Provides various utilities for manipulating alignments in SAM format
- More information:
  - Paper: https://www.ncbi.nlm.nih.gov/pubmed/19505943
  - Website: http://www.htslib.org/doc/samtools.html

Perform alignment

- Build genome's index:
  - bowtie2-build chr19.fa chr19
- Align reads to the genome:
  - bowtie2 -x ./chr19 - U MPP.fastq -S MPP.sam
  - bowtie2 -x ./chr19 - U B.fastq -S B.sam

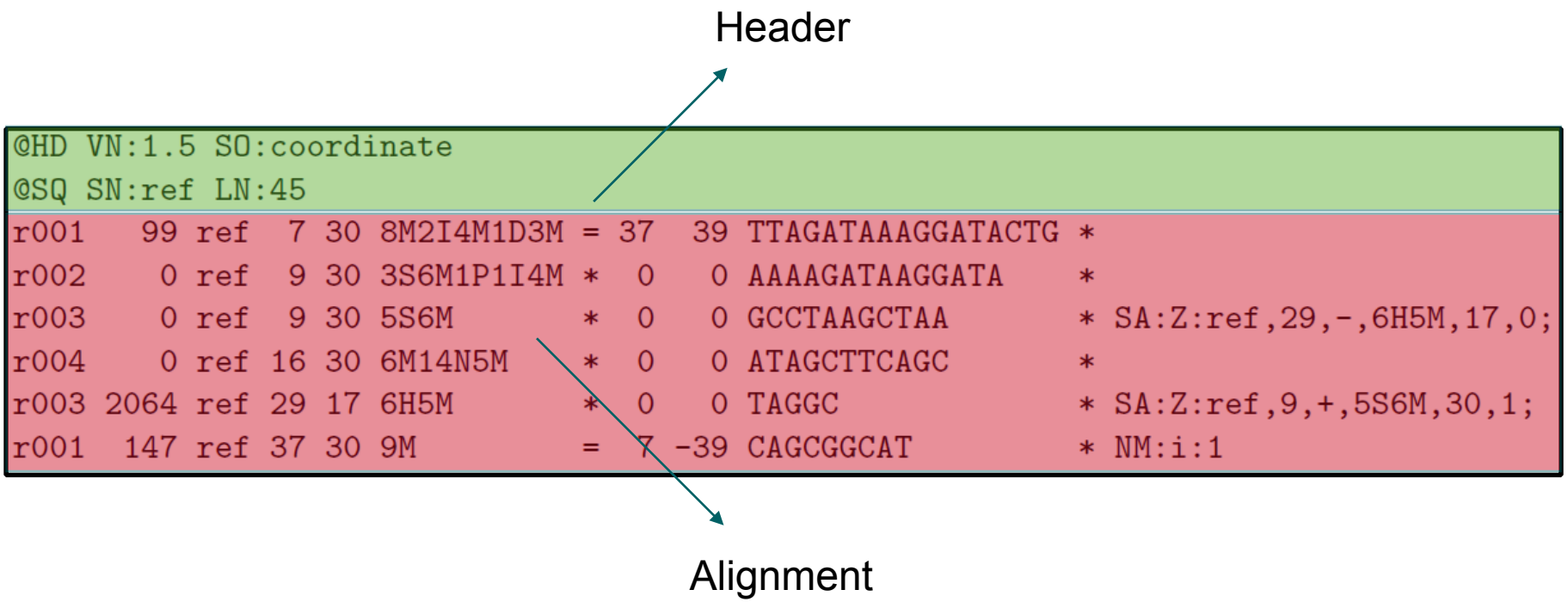
Perform alignment

- Build genome's index:
  - bowtie2-build chr19.fa chr19
- Align reads to the genome:
  - bowtie2 -x ./chr19 - U MPP.fastq -S MPP.sam
  - bowtie2 -x ./chr19 - U B.fastq -S B.sam

Use -p to speed up your alignment!

SAM file

- Store DNA sequences in a text-based file
- Mainly used to store aligned sequences
- Consists of a header and an alignment section



SAM Header

- Begins with character '@' followed with some tags
  - @HD - Header line
  - @SQ - Reference genome information.
  - @RG - Group information
  - @PG - Program (software) information.
  - @CO – Commentary line.

SAM Alignment

- Includes mandatory and optional fields

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

Mandatory

Optional

- More information:
  - https://samtools.github.io/hts-specs/SAMv1.pdf

BAM file

- Binary Alignment/Map format - compressed version of SAM
- Efficient random access
- BAI index files

Manipulating alignments

- Convert SAM to BAM
  - samtools view -bS MPP.sam > MPP.bam
  - samtools view -bS B.sam > B.bam
- Sort BAM
  - samtools sort MPP.bam MPP.sorted
  - samtools sort B.bam B.sorted
- Remove low map quality reads
  - samtools view -bq 30 MPP.sorted.bam > MPP.final.bam
  - samtools view -bq 30 B.sorted.bam > B.final.bam

Manipulating alignments

- You can download the results here:
  - http://134.130.18.8/open_data/bioinfolab_2018/Practices/MPP.final.bam
  - http://134.130.18.8/open_data/bioinfolab_2018/Practices/B.final.bam
- Create index files
  - samtools index MPP.final.bam
  - samtools index B.final.bam

IGV visualization

IGV visualization

Practice for short DNA sequence alignment

10 minutes

3. Peak Calling

Peak Calling

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

Aligned Reads

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
http://www.regulatory-genomics.org/rgt/tutorial/implementing-your-own-peak-caller/

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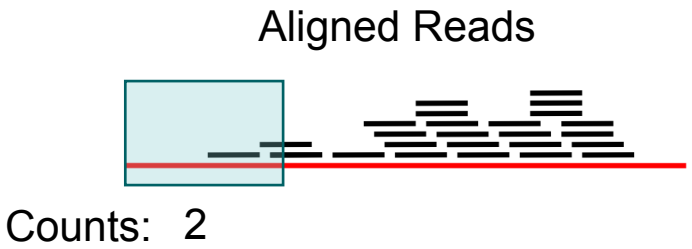

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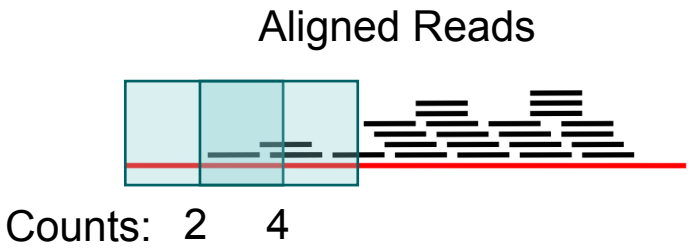

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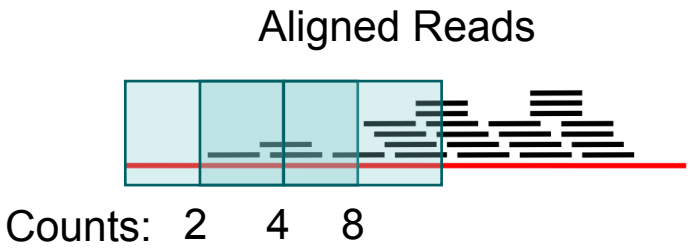

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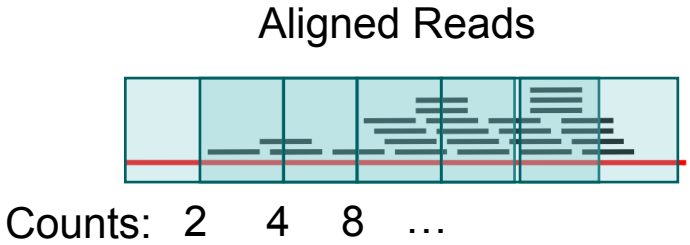

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Aligned Reads

Counts: 2 4 8 …

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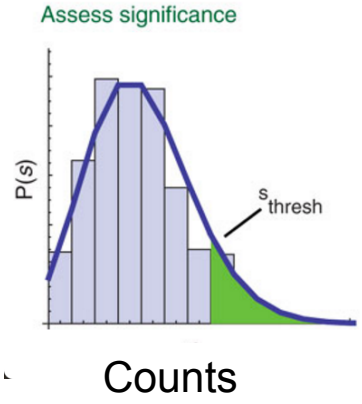

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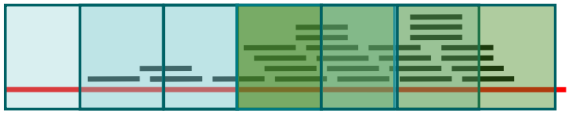

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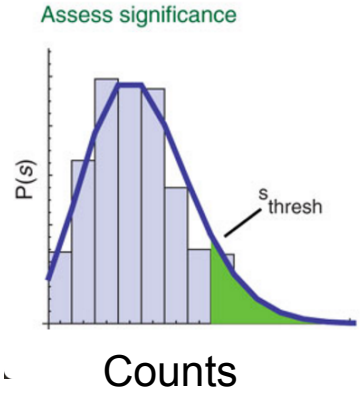

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Peak Calling

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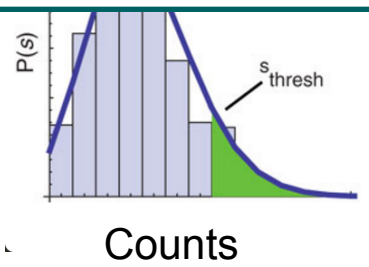
Aligned Reads

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, …

if the number of reads in higher than expected by change



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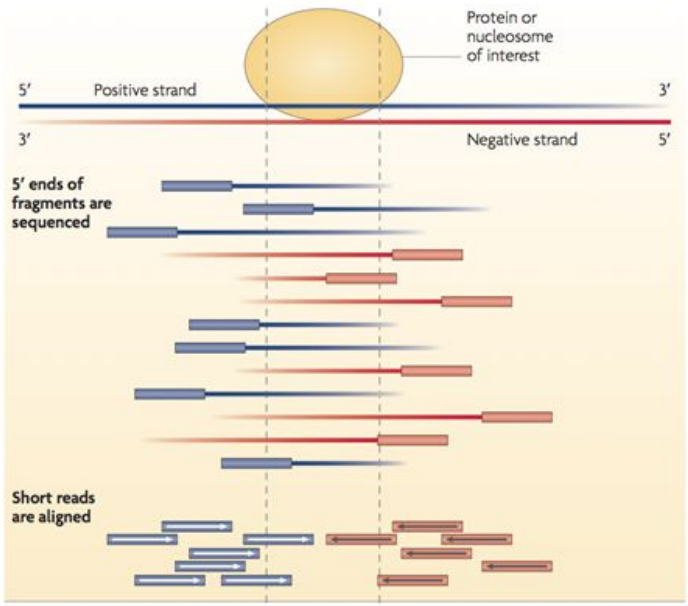
MACS Peak Caller

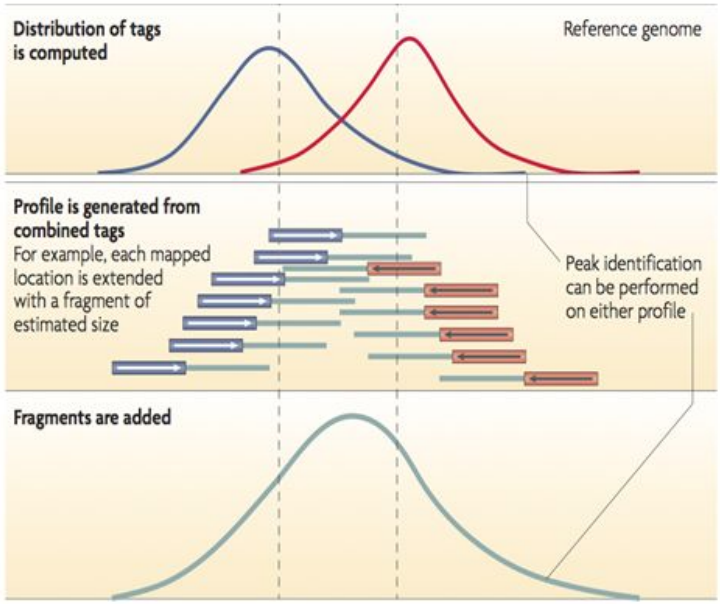
- Model-based Analysis of ChIP-seq
- Two important steps
  - models the shift size of ChIP-seq reads and uses it to improve the spatial resolution of inferred TF binding sites
  - estimates a dynamic background reads distribution to effectively capture local biases in the genome, allowing for more robust identifications

MACS Peak Caller

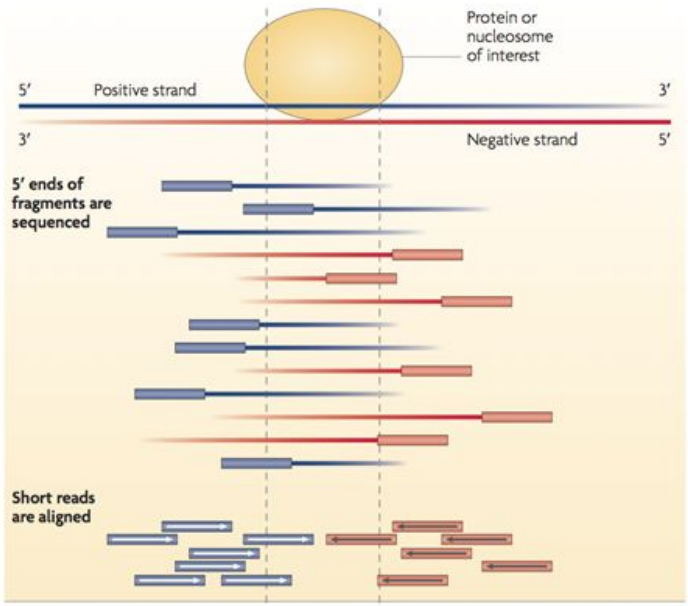


ChIP-seq peaks

- Only 5’ end of fragments are sequenced
- Tags from both + and - strand aligned to reference genome



MACS Peak Caller



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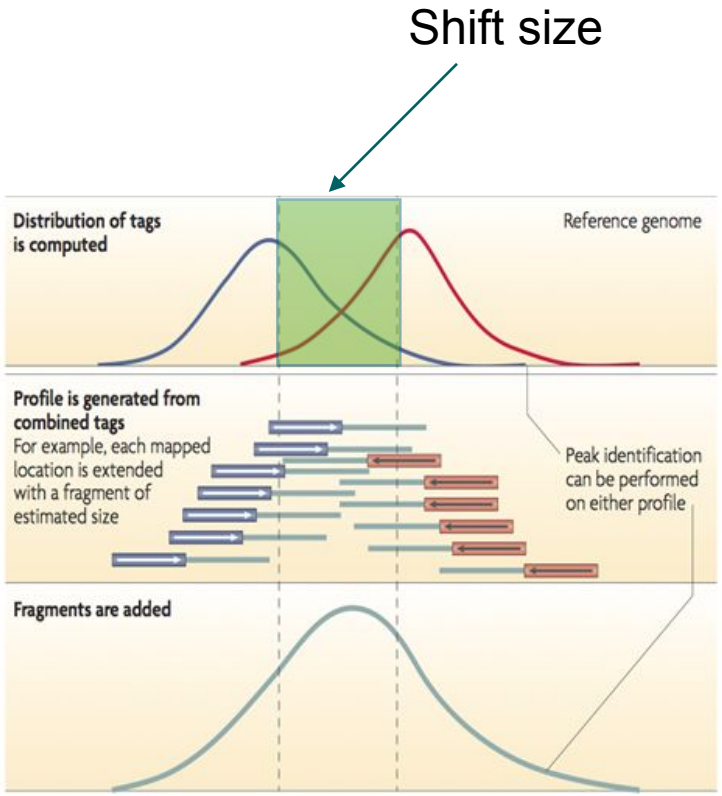
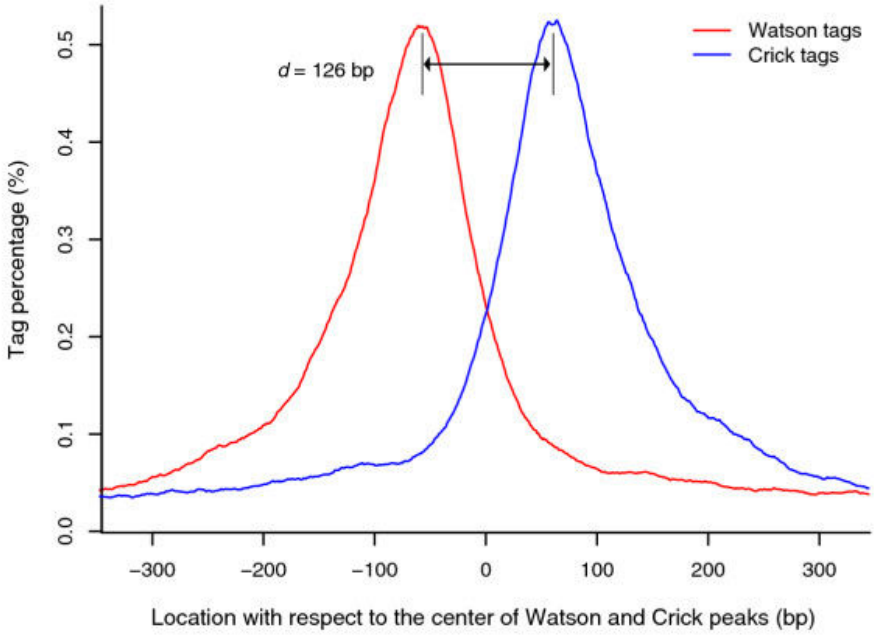
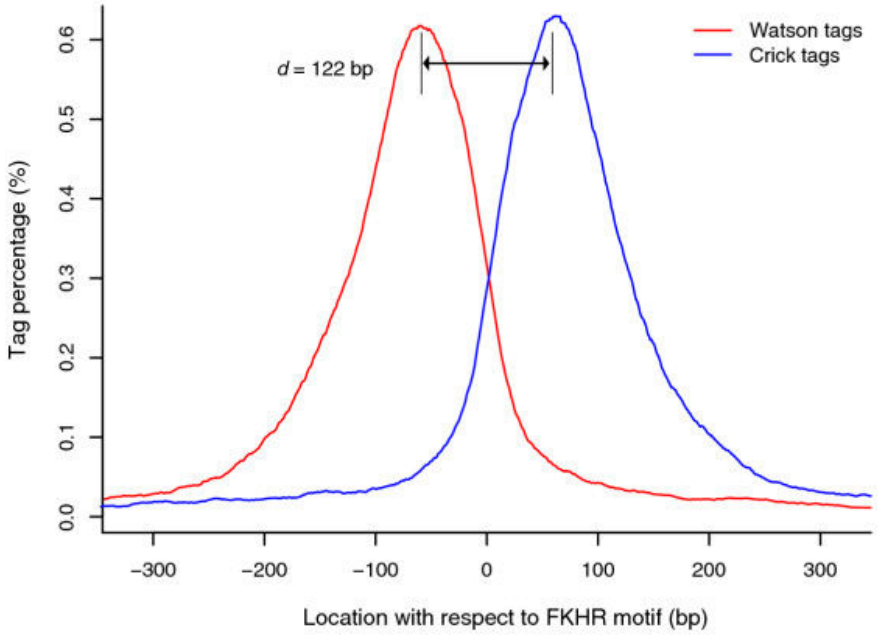


MACS Peak Caller

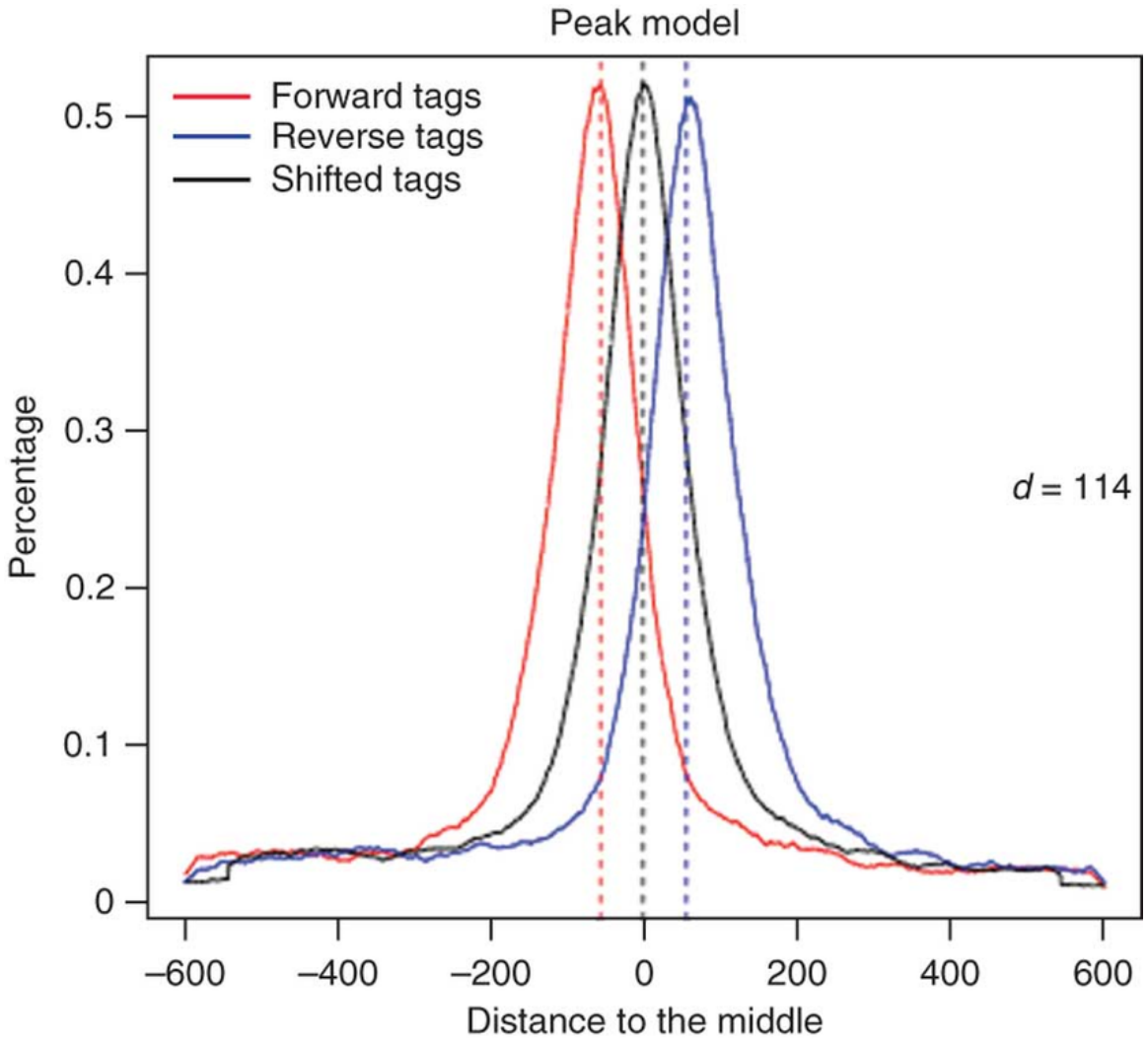
Estimated shift size



shift size based on motif



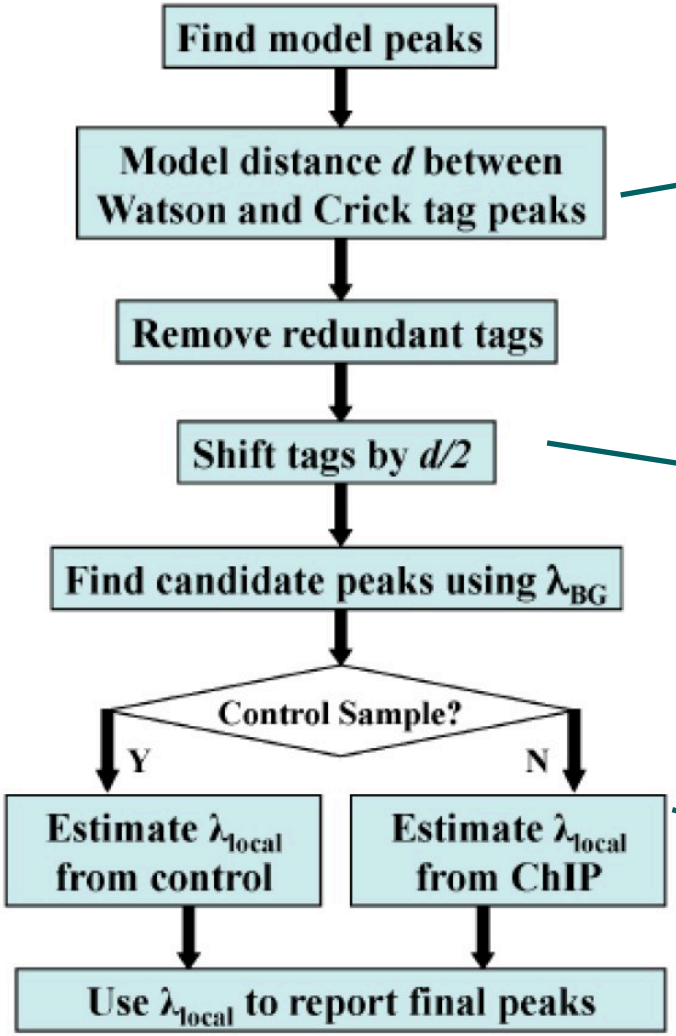
MACS Peak Caller

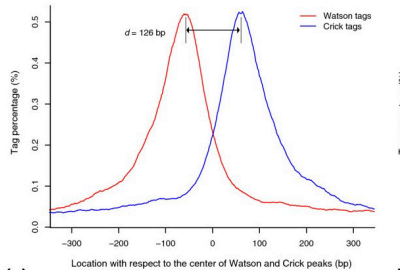


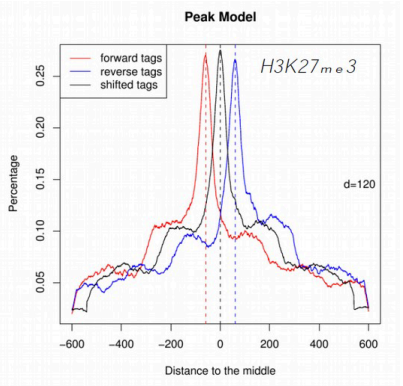
MACS Peak Caller

- Model the reads using a Poisson distribution
- Advantage: only one parameter (λ) which models mean and variance
- Peaks are defined given a p-value on the Poisson model

MACS Peak Caller







$$\lambda_{local} = \max(\lambda_{BG}, [\lambda_{1k},] \lambda_{5k}, \lambda_{10k})$$

Perform peak calling with MACS

```
mkdir PeaksMPP
macs2 callpeak -t MPP.final.bam -n MPP --outdir PeaksMPP -g mm
```

Treatment file

Experiment name

Output directory

Genome (necessary to calculate length)

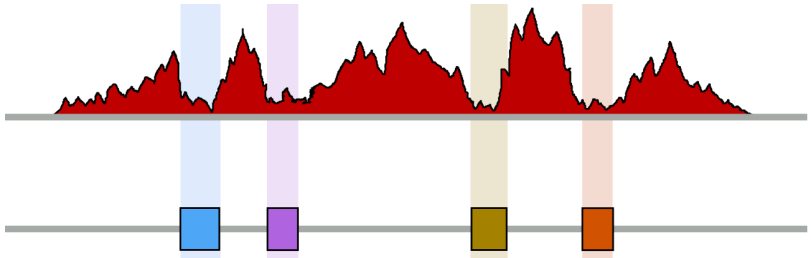
```
mkdir PeaksB
macs2 callpeak -t B.final.bam -n B --outdir PeaksB -g mm
```

4. Footprint & Motif Analysis

Institute for
Computational Genomics
RWTH AACHEN
UNIVERSITY

Digital Footprinting

Problem definition: Find genomic regions (of small size) with depletion in DNase-seq signals



BED file: Storing genomic regions

- Text-based tab-delimited file to store genomic signals.
- Fields:
  - chrom: The name of chromosome
  - chromStart: The starting position of the coordinate (start = 0)
  - chromEnd: The ending position of the coordinate
  - name: Label of the coordinate.
  - score: A score between 0 and 1000.
  - Strand: either '+' or '-'
- Example:

chr1	714057	714099	chr1:714057-714099	424	+
chr1	714102	714120	chr1:714102-714120	463	-
chr1	714121	714135	chr1:714121-714135	473	+
chr1	714137	714148	chr1:714137-714148	429	-
chr1	714220	714228	chr1:714220-714228	419	+

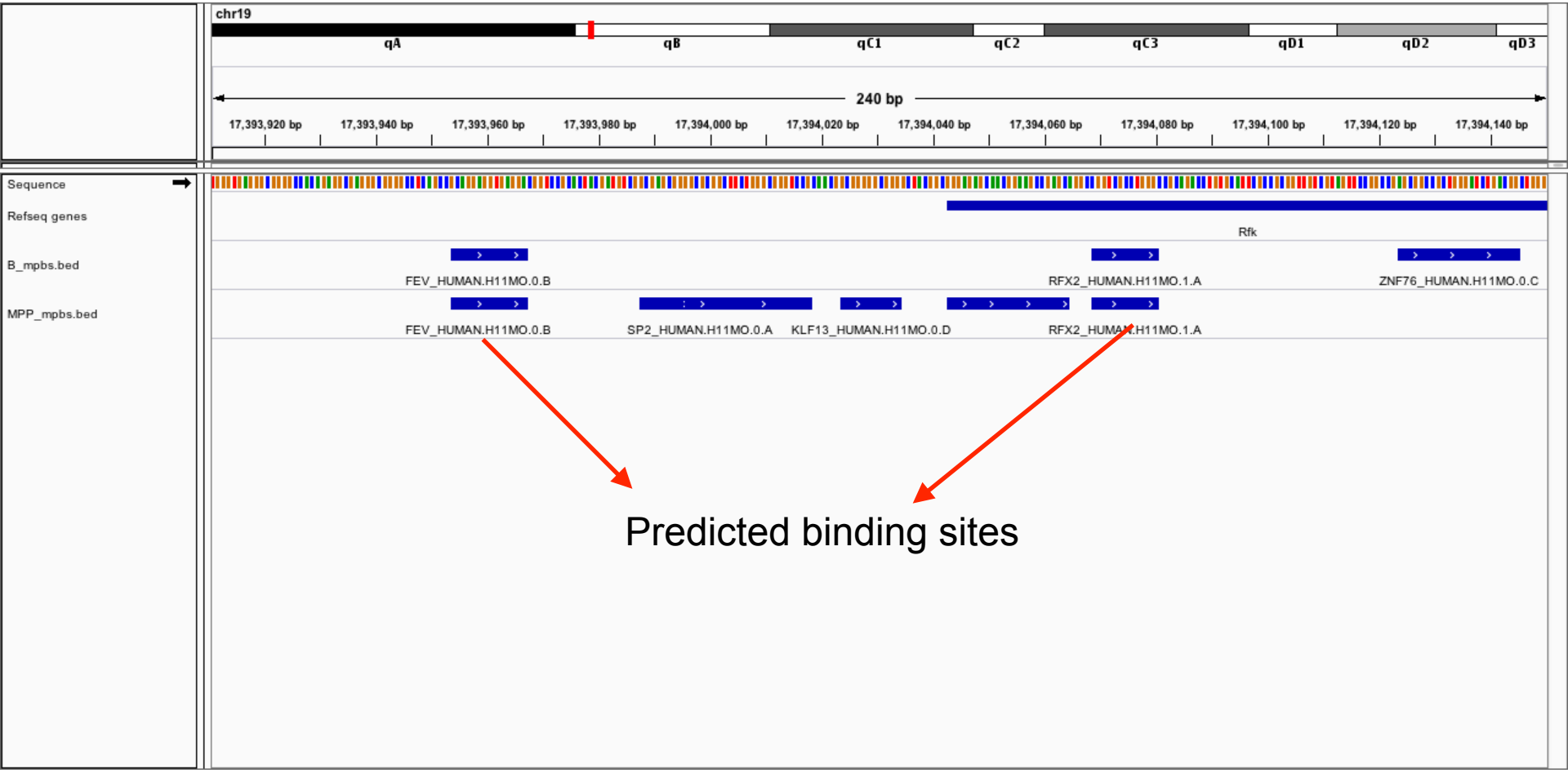
Detect Footprints with HINT

- Detect footprints using HINT:
  - rgt-hint footprinting --atac-seq --organism mm10 --output-prefix=MPP MPP.final.bam ./PeaksMPP/ MPP_peaks.narrowPeak
  - rgt-hint footprinting --atac-seq --organism mm10 --output-prefix=B B.final.bam ./PeaksB/ B_peaks.narrowPeak

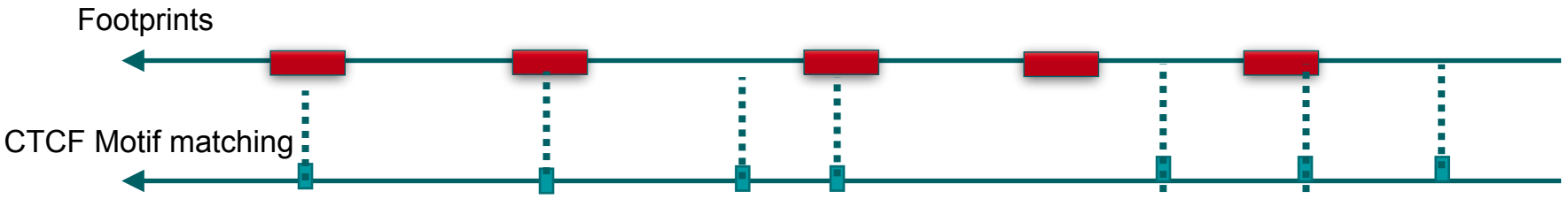
Motif matching

- Motif matching using RGT:
  - rgt-motifanalysis matching --organism mm10 --rand-proportion 10 --input-files B.bed MPP.bed

Visualization

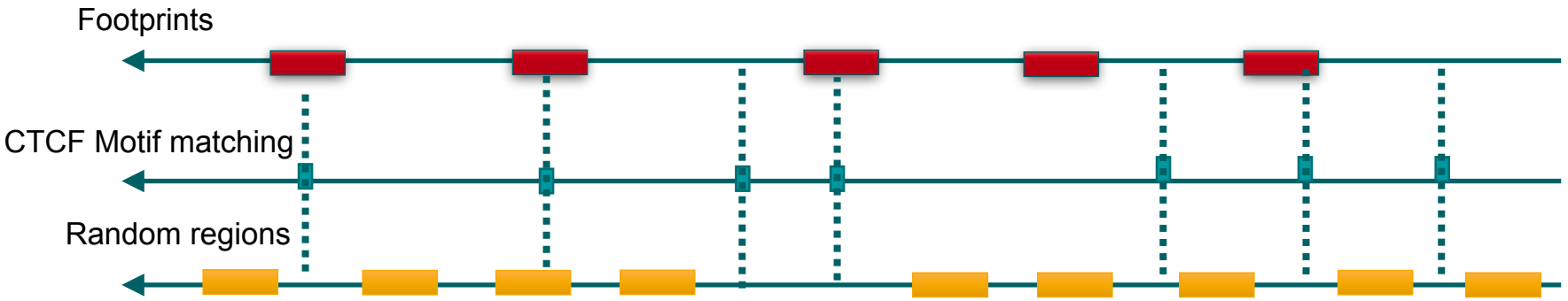


Motif enrichment



Is CTCF more likely to bind in those regions than in background regions?

Motif enrichment



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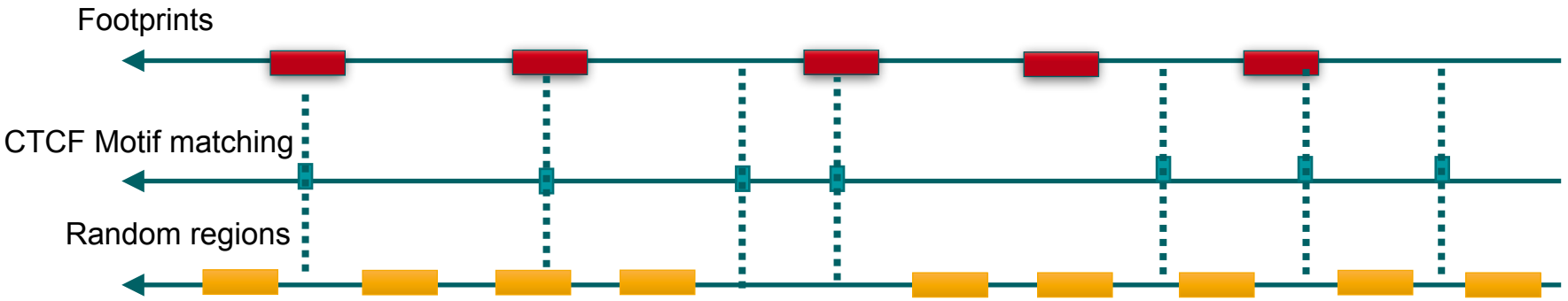
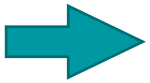
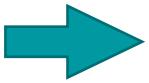


- Performs Fisher's exact test in order to verify if a set of genomic regions are enriched for particular transcription factors.

	With	w/o
Footprints	**a**	**b**
Random	**c**	**d**



	With	w/o
Footprints	**4**	**1**
Random	**1**	**8**



Fisher's exact test

$$p = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} = \frac{(a+b)!\ (c+d)!\ (a+c)!\ (b+d)!}{a!\ \ b!\ \ c!\ \ d!\ \ n!}$$

P = 0.022

Perform motif enrichment

- rgt-motifanalysis enrichment --organism mm10 ./match/random_regions.bed B.bed MPP.bed

Perform motif enrichment

Regulatory Genomics Toolbox - Motif Enrichment Analysis

B MPP

Results for **B** region **Site Test*** using all input regions

* This test considered all input regions against background regions

FACTOR	MOTIF	P-VALUE	CORRECTED P-VALUE	A	B	C	D	FREQUENCY	BACKGROUND FREQUENCY	GO
KLF12_HUMAN.H11MO.0.C		4.8159e-40	3.7131e-37	72	4214	65	45309	1.68%	0.14%	View
SP1_HUMAN.H11MO.1.A		9.7376e-35	3.7538e-32	56	4230	40	45334	1.31%	0.09%	View
MAZ_HUMAN.H11MO.1.A		1.6060e-31	4.1274e-29	85	4201	151	45223	1.98%	0.33%	View
SP2_HUMAN.H11MO.1.B		4.0513e-29	7.6943e-27	70	4216	107	45267	1.63%	0.24%	View
KLF9_HUMAN.H11MO.0.C		4.9898e-29	7.6943e-27	52	4234	48	45326	1.21%	0.11%	View
E2F4_HUMAN.H11MO.0.A		7.0187e-25	9.0190e-23	33	4253	14	45360	0.77%	0.03%	View
KLF1_HUMAN.H11MO.0.A		6.9452e-24	7.6497e-22	56	4230	84	45290	1.31%	0.19%	View

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The data is available on:
http://134.130.18.8/open_data/bioinfolab_2018/Practice.tar.gz

Thank you!