ChIP-seq module

BD2K Course 2018

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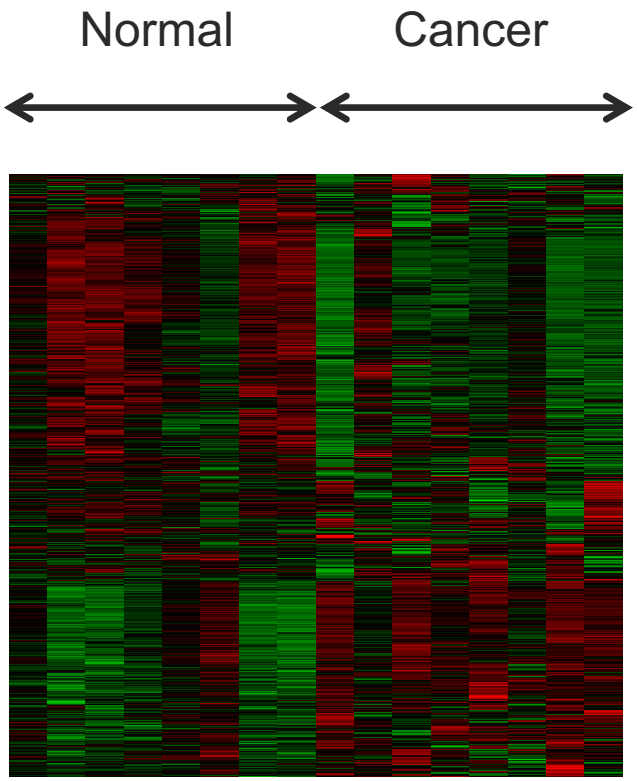
The Jackson Laboratory
Leading the search for tomorrow's cures

Introduction

Learn from RNA-Seq

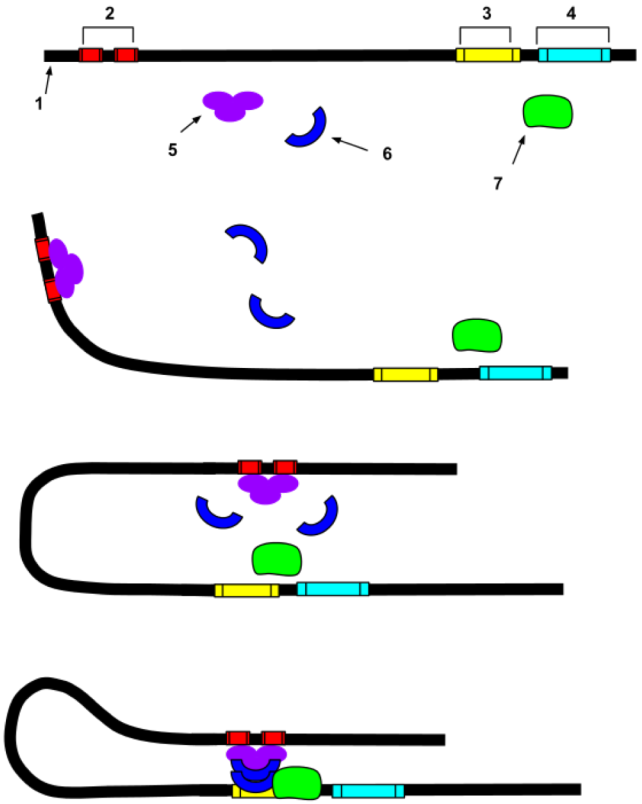


Differential Gene Expression

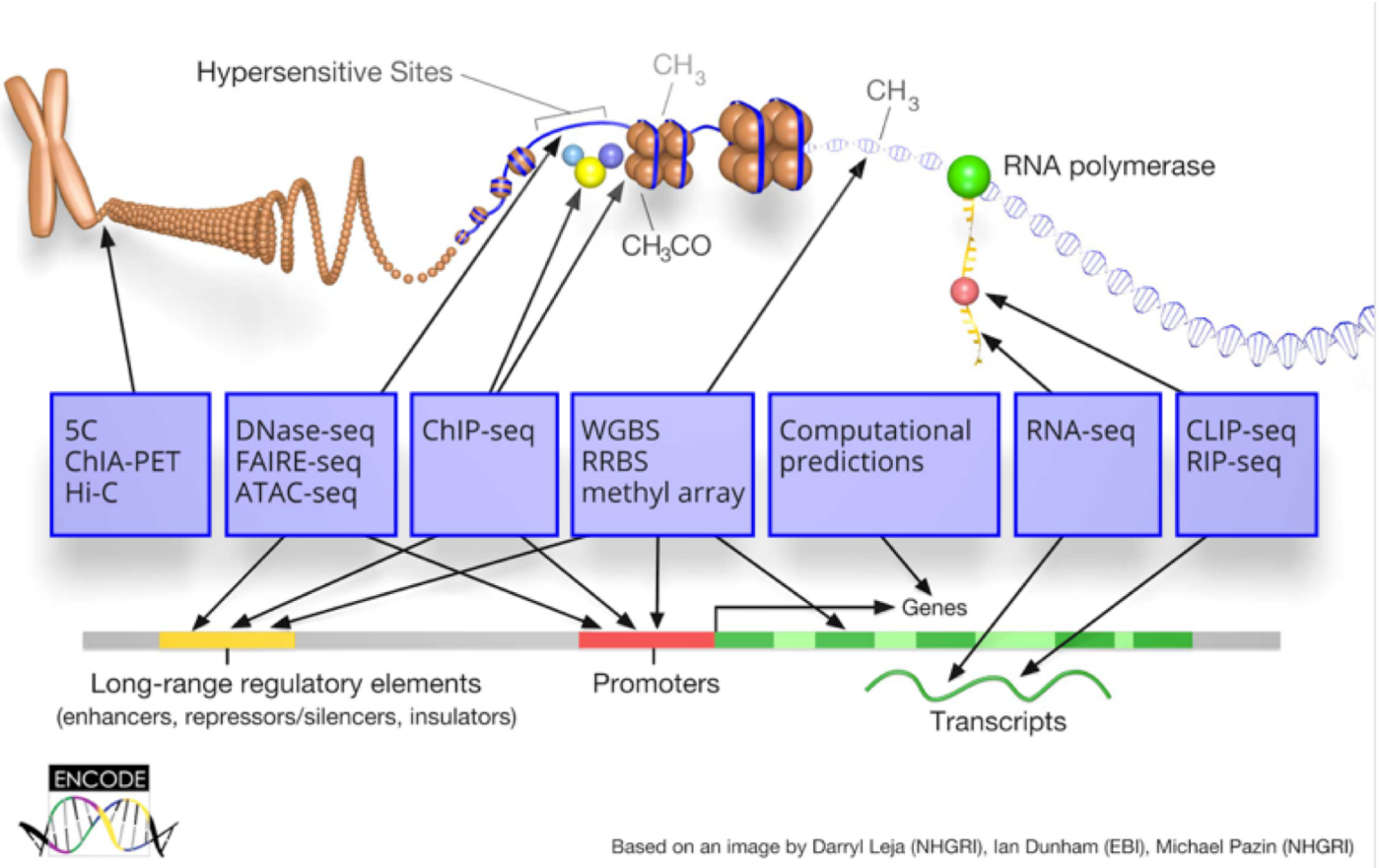
What may cause the differences?

What can go wrong or be different?

1. DNA
2. Enhancer
3. Promoter
4. Gene
5. Transcription Activator Protein
6. Mediator Protein
7. RNA Polymerase

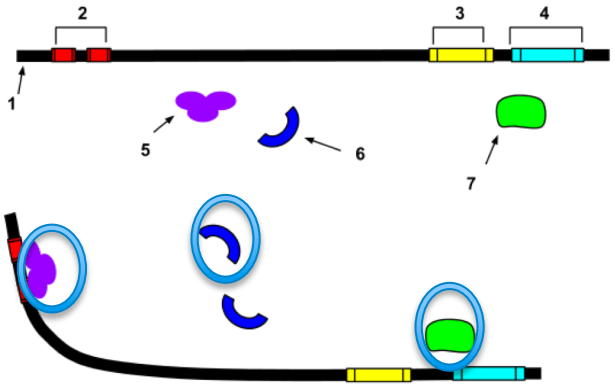


- Structure variation (mutation,deletion, translocation etc. in DNA sequences)
- Chemical modifications on DNA (methylation, acetylation, etc.)
- Chemical modifications on histone


Hypersensitive Sites
CH3
CH3
CH3CO
RNA polymerase
5C
ChIA-PET
Hi-C
DNase-seq
FAIRE-seq
ATAC-seq
ChIP-seq
WGBS
RRBS
methyl array
Computational
predictions
RNA-seq
CLIP-seq
RIP-seq
Genes
Long-range regulatory elements
(enhancers, repressors/silencers, insulators)
Promoters
Transcripts
ENCODE
Based on an image by Darryl Leja (NHGRI), Ian Dunham (EBI), Michael Pazin (NHGRI)

ChIP-seq

Chromatin **I**mmuno**P**recipitation followed by **Seq**uencing



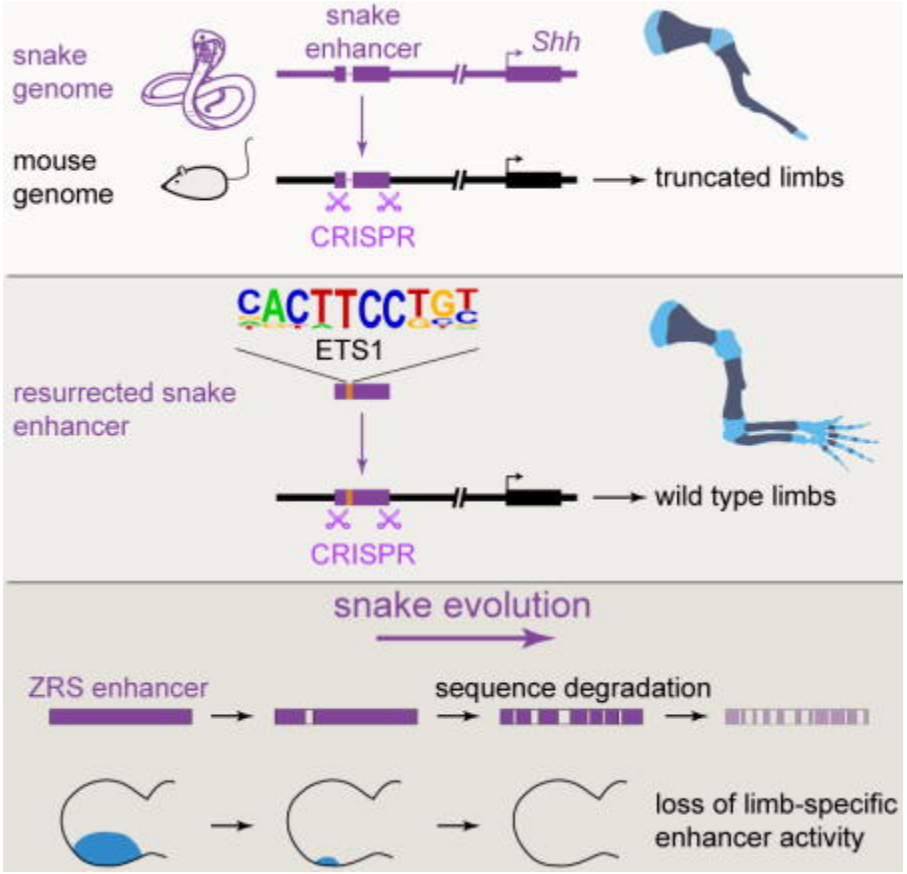
- Work on proteins that interact with DNA
- Get the DNA sequences that are bound by the protein of interest
- Explore how genes are regulated

Applications:

To detect interesting regions in genome at base-pair resolution for

- DNA-binding proteins, e.g. transcription factor, RNAPII, etc
- Histone modifications  → status of enhancers

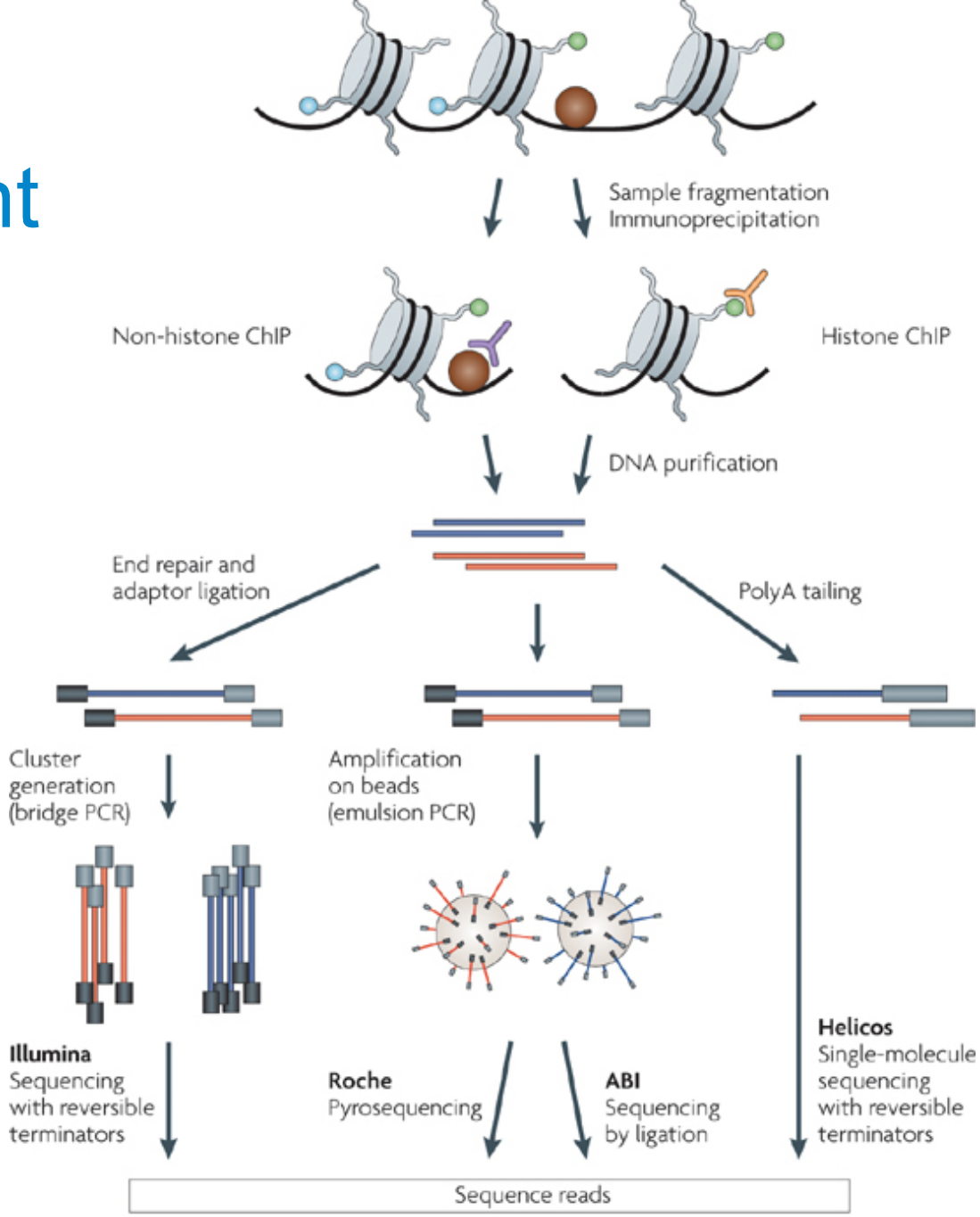
Example on enhancer



- ChIP-Seq can help find enhancers
- Other techniques, like eQTL, Hi-C or ChIA-PET, are required to identify the interactions between particular enhancers and genes.

Kvon et. al, cell 2016

ChIP-seq Experiment



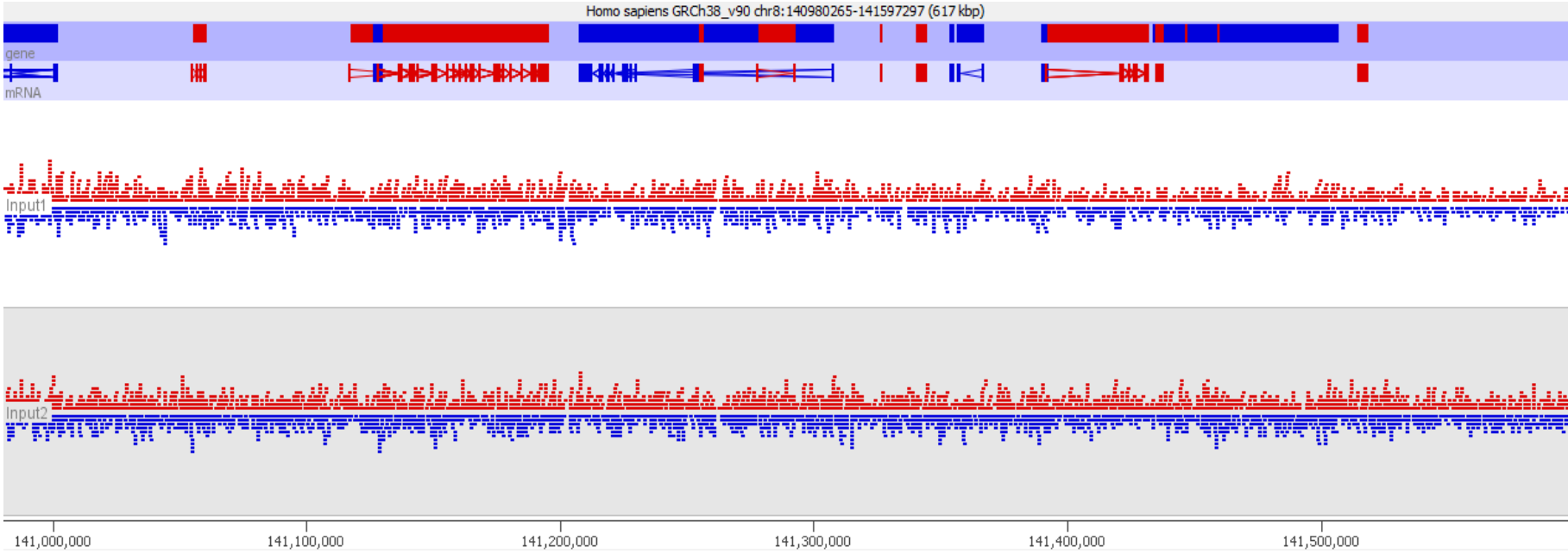
Nature Reviews | Genetics

Experimental Considerations

- Antibodies: High sensitivity and specificity
- Cell number: More cells, better signal-to-noise (typically 10^6 to 10^7 cells)
- Controls: To remove background noise
  - IgG (Reads are only informative if the ChIP hasn't worked.)
  - Input Chromatin (sonicated / Mnase etc)
    - Genomic library - everywhere equally
    - Technical issues can cause variation
- Replicates:

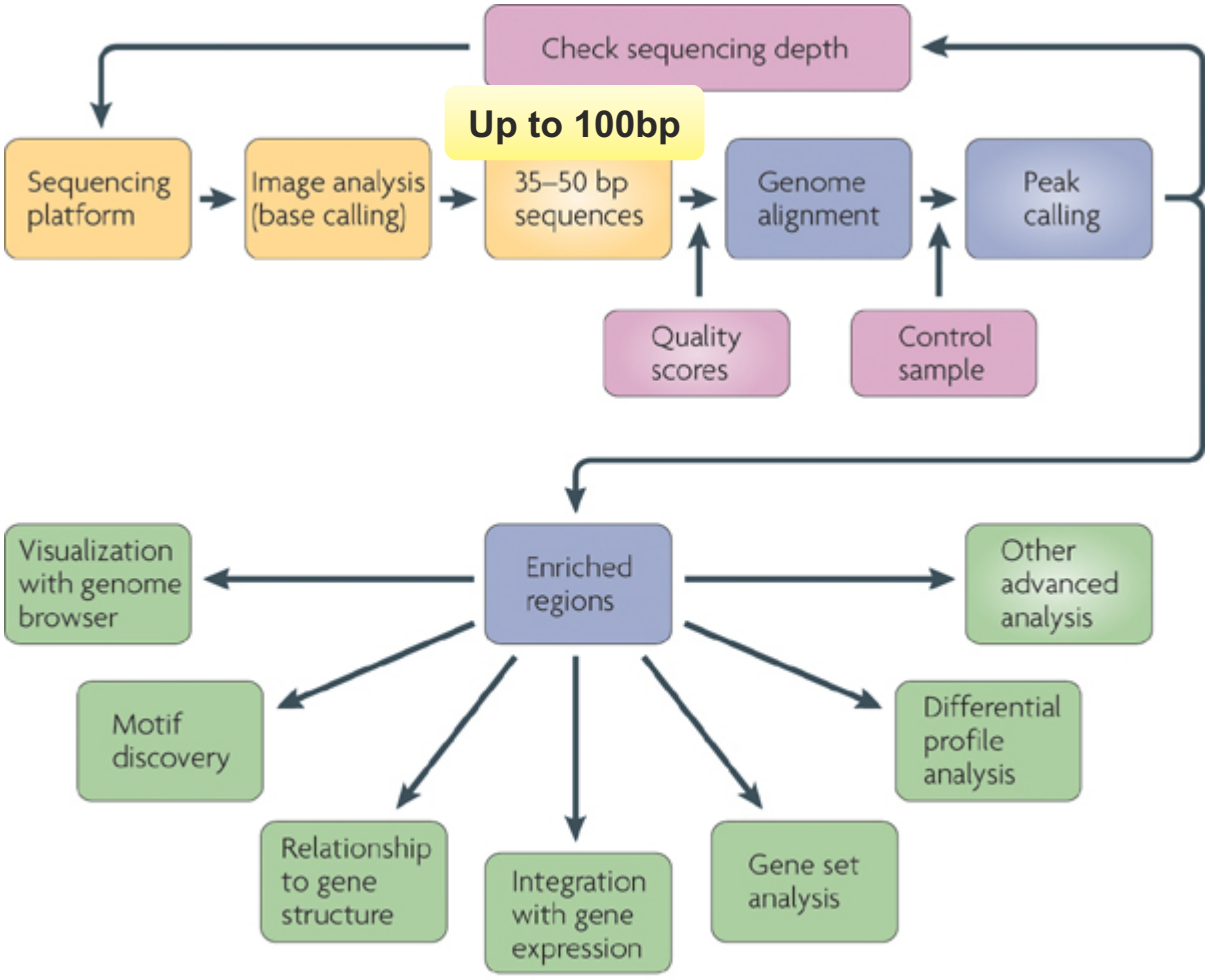
B. Kidder et al. Nature Immunology 12, 918-922 (2011)

Examine controls

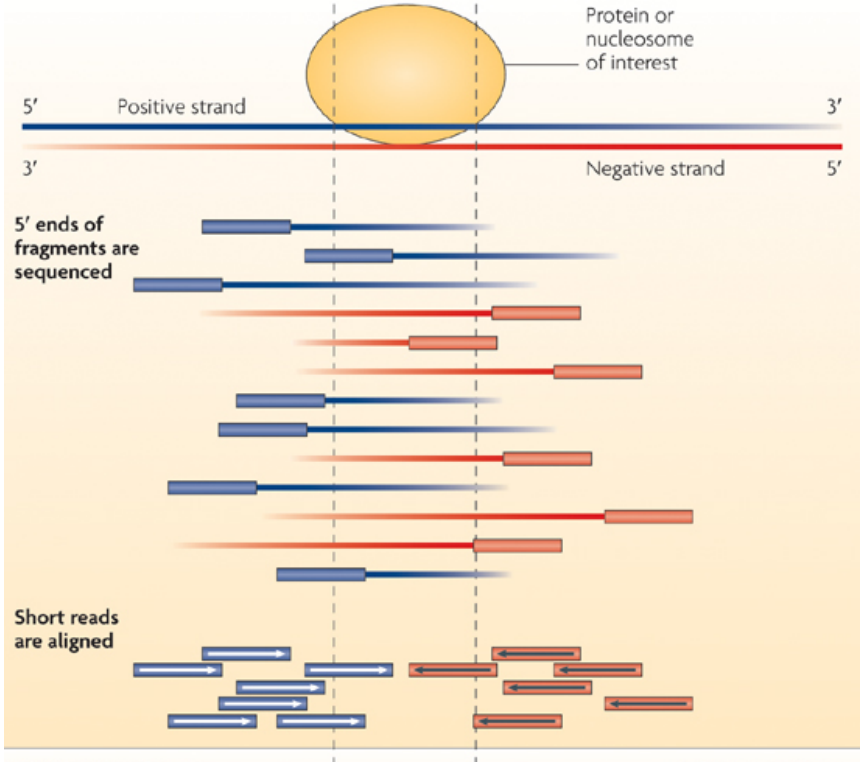


- Does the coverage look even
- If there are multiple inputs to do they look similar

Overview of ChIP-seq analysis



Sequence Alignment

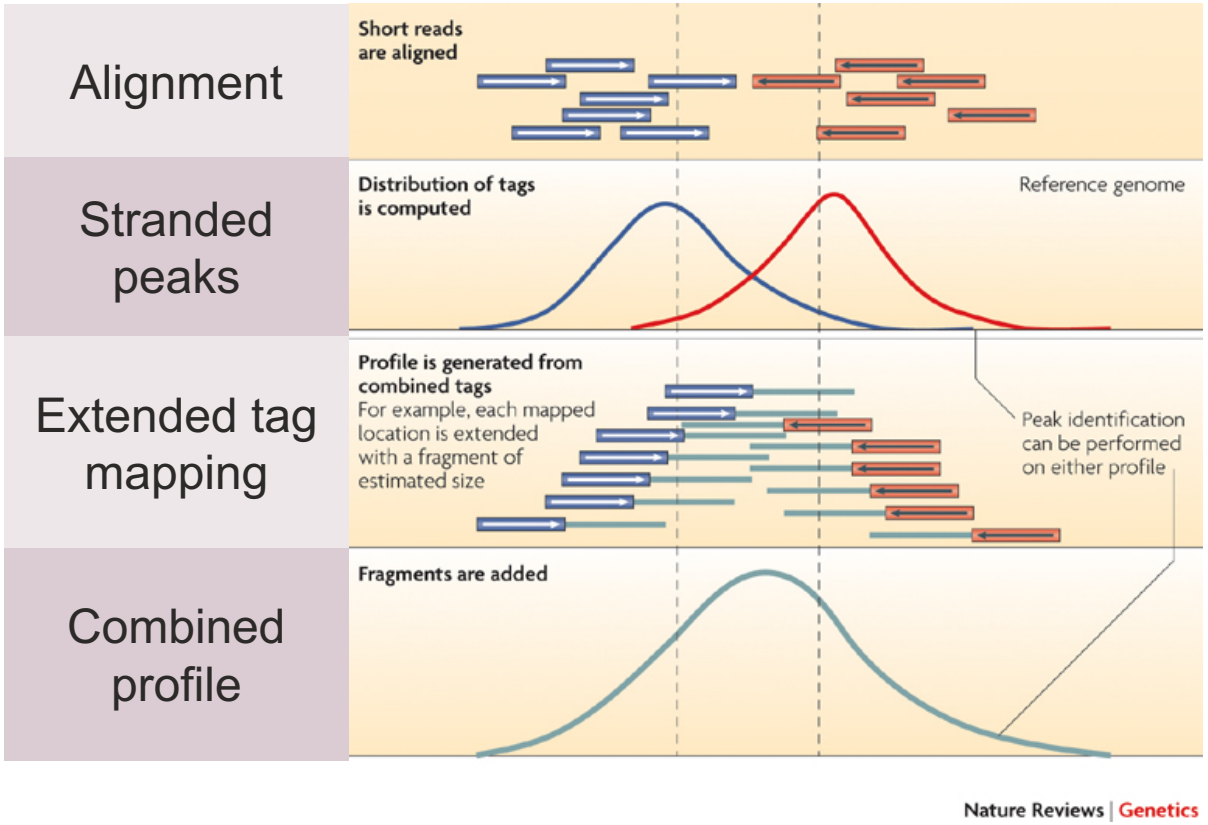


Sequence Alignment

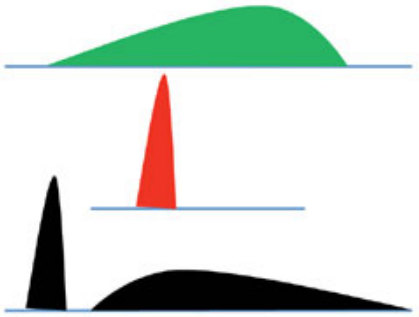
- Bowtie (35 – 50 bp)
- Bowtie2 (50 – 100 bp, no upper limit actually)
- BWA (different algorithms for shorter and longer sequences than 70bp)
- Others: MAQ, ELAND, …

Peak calling

Identify enriched regions above controls



Peak calling
Software



Peak calling (choose the right tool)

Type of peak	Example	Representative tools
Broad	H3K27me3	CCAT, SICER
Sharp	CTCF	MACS
Sharp & broad	Pol II	ZINBA

B. Kidder et al. Nature Immunology 12, 918-922 (2011)

Peak calling

Software used in Encode pipeline

- **SPP**

  A ChIP-seq peak calling algorithm, implemented as an R package, that accounts for the offset in forward-strand and reverse-strand reads to improve resolution, compares enrichment in signal to background or control experiments, and can also estimate whether the available number of reads is sufficient to achieve saturation, meaning that additional reads would not allow identification of additional peaks. SPP will be used in the ENCODE 3 uniform peak calling pipeline.

  Kharchenko PV, Tolstorukov MY, Park PJ. Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nat Biotechnol*. 2008 Dec;26(12):1351-9. PMID: 19029915; PMC: PMC2597701

- **GEM**

  GEM is a Java software package for analyzing genome wide ChIP-seq/ChIP-exo data. GEM can decompose single observed peaks into multiple binding events, determine binding event location at high spatial resolution, and discover explanatory DNA sequence motifs with an integrated model of ChIP reads and proximal DNA sequences. GEM is able to process single-end or paired-end data and can be run in single-condition mode or multi-condition mode. GEM will be used in the ENCODE 3 uniform peak calling pipeline.

  Guo Y, Mahony S, Gifford DK. High resolution genome wide binding event finding and motif discovery reveals transcription factor spatial binding constraints. *PLoS Comput Biol*. 2012;8(8):e1002638. PMID: 22912568; PMC: PMC3415389

- **PeakSeq**

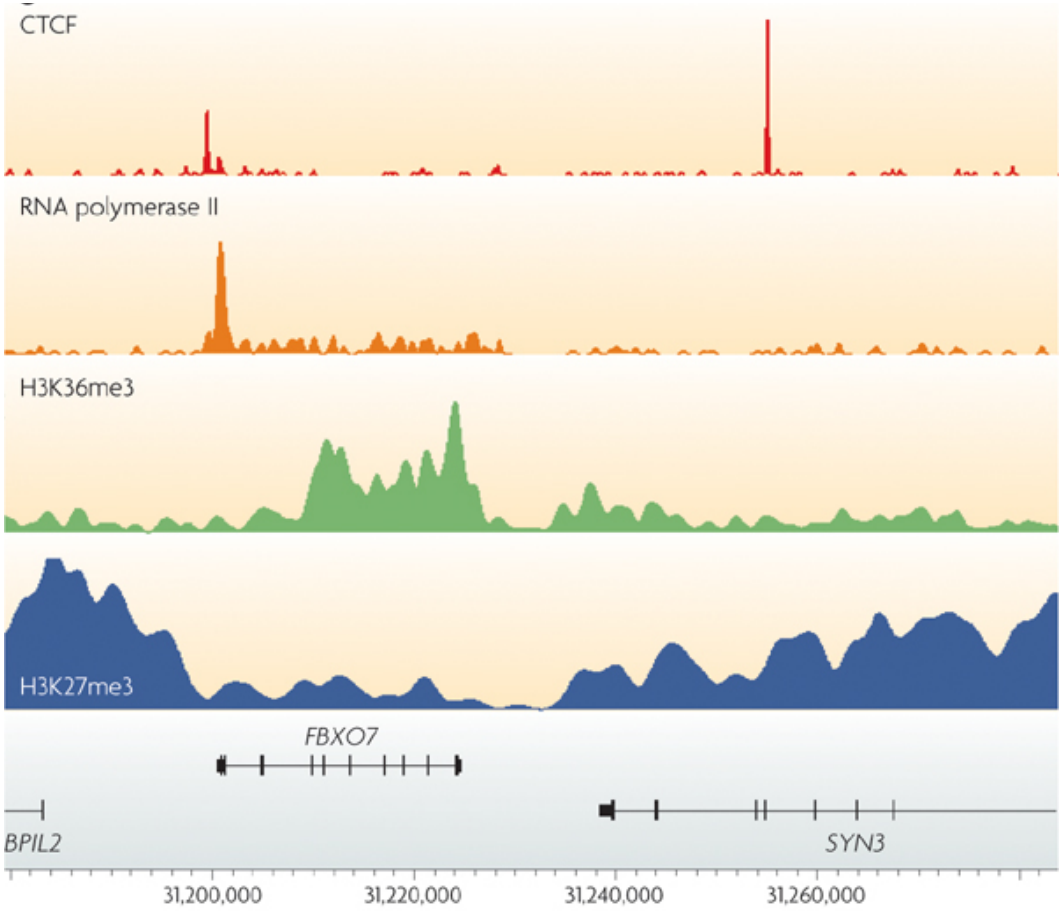
  Identifies enriched regions in ChIP-seq type experiments and explicitly compares signal experiments to control experiments. PeakSeq will be used in the ENCODE 3 uniform peak calling pipeline.

  Rozowsky J, Euskirchen G, Auerbach RK, Zhang ZD, Gibson T, Bjornson R, Carriero N, Snyder M, Gerstein MB. PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls. *Nat Biotechnol*. 2009 Jan;27(1):66-75. PMID: 19122651; PMC: PMC2924752

- **MACS**

  A widely-used, fast, robust ChIP-seq peak-finding algorithm that accounts for the offset in forward-strand and reverse-strand reads to improve resolution and uses a dynamic Poisson distribution to effectively capture local biases in the genome. MACS 1.4 was used in

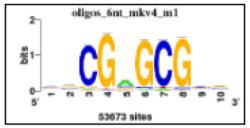
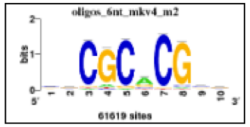
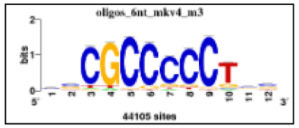
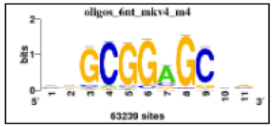
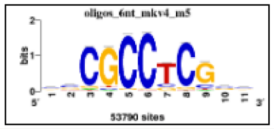
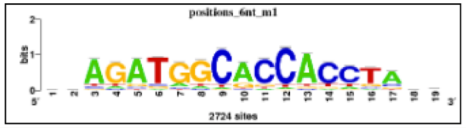
ChIP-seq profile



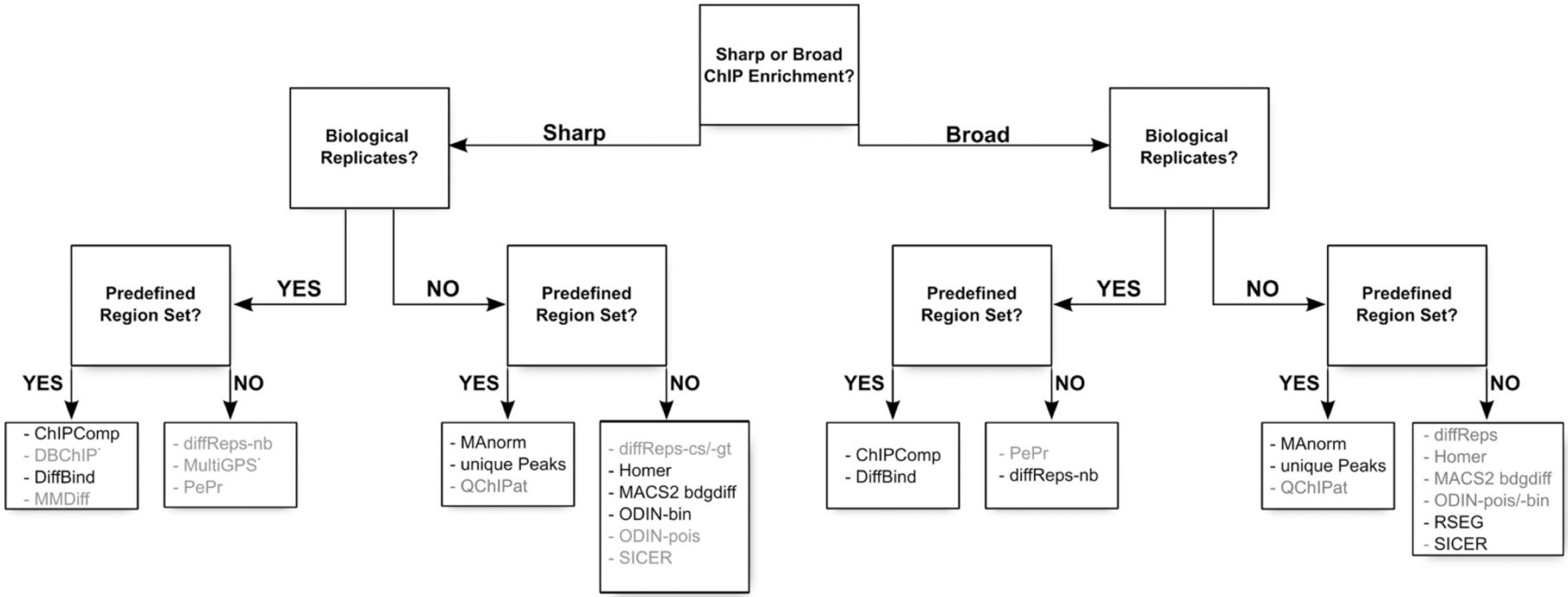
After peak calling

- Motif analysis (MEME, HOMER, …)
- Integrative analysis with expression data
- Differential binding
- Peak annotation
- …

Motif analysis

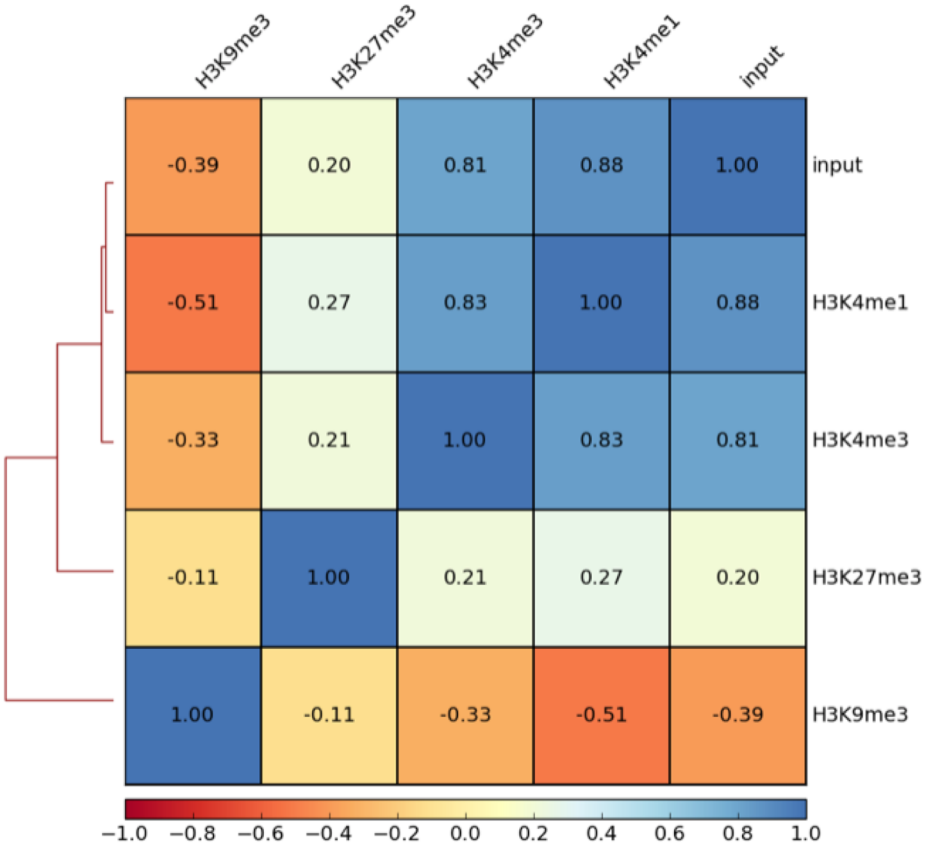
Motif	Logo	3 Top hits in databases
oligos_6nt_mkv4_m1		versus Homer: homer_326.ZNF519, versus jaspar_core_nonredundant_vertebra no match
oligos_6nt_mkv4_m2		versus Homer: homer_123.RTACGTGC, homer_124.HIF2a, homer_38.NCCACGTG, versus jaspar_core_nonredundant_vertebra ARNT::HIF1A, Ahr::Arnt, Hes1,
oligos_6nt_mkv4_m3		versus Homer: homer_25.BORIS, homer_266.Sp1, homer_145.KLF14, versus jaspar_core_nonredundant_vertebra EGR1, SP2, KLF16,
oligos_6nt_mkv4_m4		versus Homer: homer_266.Sp1, versus jaspar_core_nonredundant_vertebra SP1,
oligos_6nt_mkv4_m5		versus Homer: homer_266.Sp1, versus jaspar_core_nonredundant_vertebra no match
positions_6nt_m1		versus Homer: no match versus jaspar_core_nonredundant_vertebra no match

Differential binding

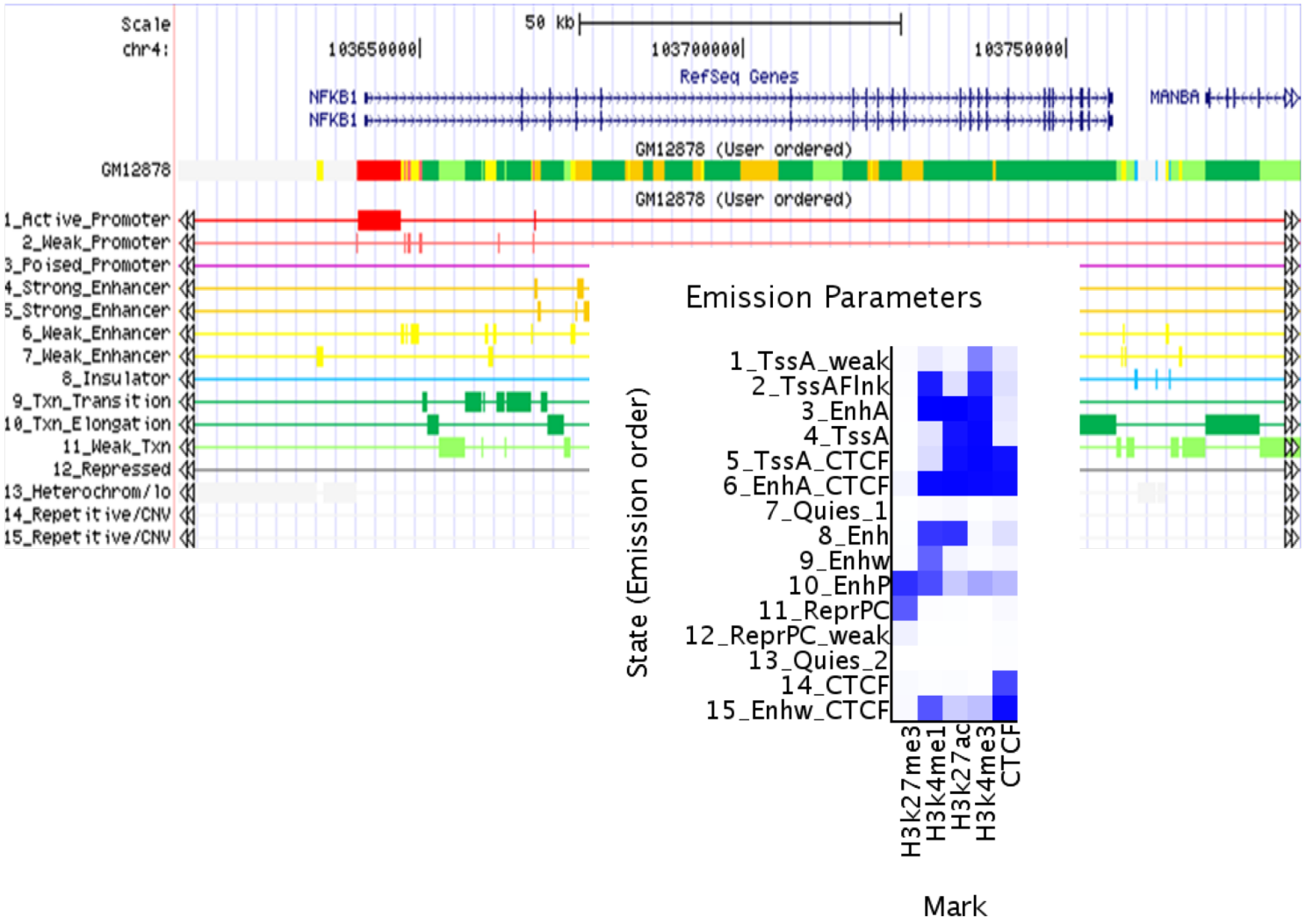


From: A comprehensive comparison of tools for differential ChIP-seq analysis
Brief Bioinform. 2016;17(6):953-966. doi:10.1093/bib/bbv110

Correlation between different binding factors



Chromatin state



ChromHMM: http://compbio.mit.edu/ChromHMM/

ChIP-Seq on-line analysis

http://rsat.ulb.ac.be/dpeaks/

For command line user – Anaconda and bioconda

- Anaconda (package management platform) https://anaconda.org
- Bioconda (package reporsitory for bioinformatics) https://bioconda.github.io
- Deeptools http://deeptools.readthedocs.io/en/develop/

Data from database

- Encode (https://www.encodeproject.org)
- modEncode (http://www.modencode.org/publications/about/index.shtml)
- Roadmap (http://www.roadmapepigenomics.org)
- ArrayExpress (http://www.ebi.ac.uk/arrayexpress/)

- GENCODE (human and mouse genome)

Hands on Work

What we will do?

Objective: Perform a basic ChIP-seq pipeline

- In terminal:
  - Quality check (FASTQC: check the quality of raw sequencing data in fastq format)
  - Mapping (bowtie: align sample sequences to the reference genome)
  - Peak calling (MACS2: locate immuno-enriched regions)
- On MEME online server
  - Motif analysis (MEME-ChIP: identify the binding motifs)

Tools

Software	Version	Usage
fastqc	0.11.3	Quality checking
Bowtie	0.12.8	mapping
Samtools	1.3.1	SAM, BAM file manipulation
MACS2	2.0.10.20120913	Peak calling
bedtools	2.26.0	Bed file manipulation

Note:

- You may install the software through bioconda
- MACS2 requires python2.7.X
- Check software dependencies on their installation page on-line

Datasets

- Assay: ChIP-seq
- Target: CTCF
- Biosample: Homo Sapiens GM12878 (A lymphoblastoid cell line produced from the blood of a female donor with northern and western European ancestry by EBV transformation)
- Sample type: DNA
- Chromosome: Chr1
- Lab: Bradley Bernstein, Broad
- Source: encodeproject.org
- Platform: Illumina Genome Analyzer
- ENCODE accession id:
  - ENCFF000ARP (sample) -> GM12878_CTCF_chr1.fastq
  - ENCFF000ARK (control) -> GM12878_control_chr1.fastq

Getting help

- fastqc --help
- bowtie -h
- samtools or samtools <command>
- macs2 -h

- Check where you are:
  - pwd
- Find out what are in the directory:
  - ls
- Go to ‘ChIPseq’ folder:
  - cd ChIPseq
  - Check what’s in the box: ls
  - Examine files: less filename or cat filename
  - Take a look at two files: readme.txt and workflow.sh
    - readme.txt : describes the data structure in this folder
    - workflow.sh : contains all the commands for this practice

Check the sequencing quality

Go the data folder if you are not there:

cd ChIPseq

Perform fastqc quality check:

fastqc GM12878_control_chr1.fastq

fastqc GM12878_CTCF_chr1.fastq

Check output files

ls

(You will find .zip and .html files for each .fastq. Download .html files and open them in your browser)

Index the genome

To make the alignment more memory efficient, bowtie will index the genome first.
The reference genome is the chromosome 1 of human genome build GRCh38

bowtie-build hg38/GRCh38.chr1.fa hg38/GRCh38.chr1

Sequence alignment

learn bowtie options
bowtie –h

map to reference genome
**bowtie -m 1 -S ./hg38/GRCh38.chr1 GM12878_control_chr1.fastq **
> GM12878_control_chr1.sam

**bowtie -m 1 -S ./hg38/GRCh38.chr1 GM12878_CTCF_chr1.fastq **
> GM12878_CTCF_chr1.sam

Take a look at the first 5 lines of the output
head –n 5 GM12878_CTCF_chr1.sam

Compress SAM

```
# Learn samtools view options
samtools view

# Compress SAM to BAM file to save storage
samtools view -bSo GM12878_control_chr1.bam \
GM12878_control_chr1.sam
samtools view -bSo GM12878_CTCF_chr1.bam \
GM12878_CTCF_chr1.sam
```

Index BAM

Index the BAM files for IGV visualization in two steps

sort the alignment according to chromosome position
**samtools sort GM12878_control_chr1.bam **
GM12878_control_chr1.sorted
**samtools sort GM12878_CTCF_chr1.bam **
GM12878_CTCF_chr1.sorted

index the sorted files
samtools index GM12878_control_chr1.sorted.bam
samtools index GM12878_CTCF_chr1.sorted.bam

Note: make sure the sorted.bam and .bai index files in the same directory for IGV loading

Peak calling

Learn macs2
macs2 –h

Identify peak enrichment
**macs2 callpeak -t GM12878_CTCF_chr1.sorted.bam **
**-c GM12878_control_chr1.sorted.bam **
-f BAM -g 175000000 -n GM12878_CTCF_chr1 -B -q 0.01

- The output files description can be found on MACS2 page : https://github.com/taoliu/MACS
- The R script is one output of macs2.
- The output .bed file can be loaded to IGV directly.

Motif analysis

summit files include the estimation of peak summit at single base level
extend summits 100bp on both directions
**bedtools slop -i GM12878_CTCF_chr1_summits.bed **
**-g hg38/GRCh38.chr1.size -b 100 **
> GM12878_CTCF_chr1_summits_ext.bed

get sequences for the extended summit regions (i.e. fasta)
**bedtools getfasta -fi hg38/GRCh38.chr1.fa **
**-bed GM12878_CTCF_chr1_summits_ext.bed **
-fo GM12878_CTCF_chr1_summits_ext.fa

Motif analysis using MEME

Go to site: http://meme-suite.org/tools/meme-chip