LSL course

Analyzing Genome-wide Chromatin Accessibility

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Outline

- ATAC-seq
- Data processing pipeline
  - Preprocessing
  - Peak calling
- Post-alignment analysis
  - Accessible regions profiling

Methods for measuring chromatin accessibility

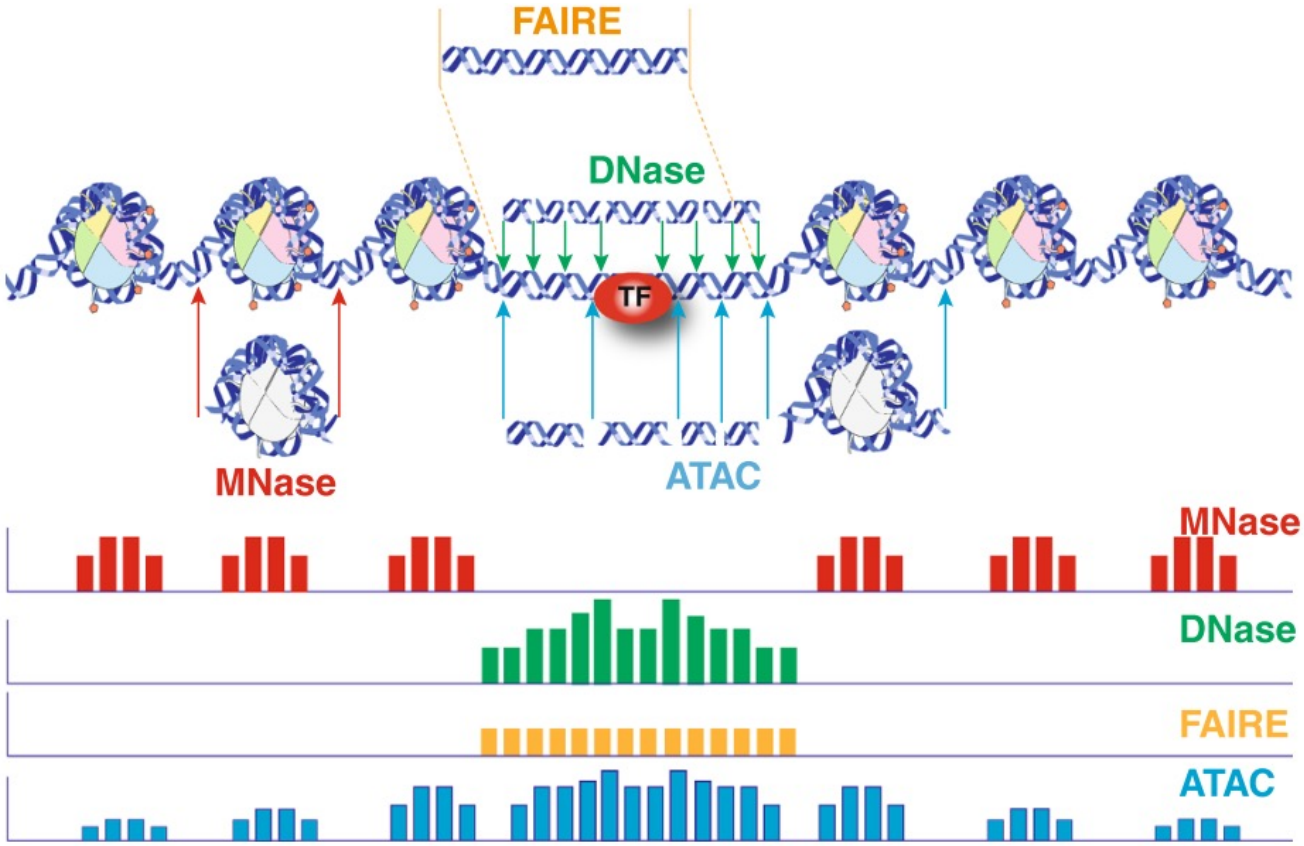
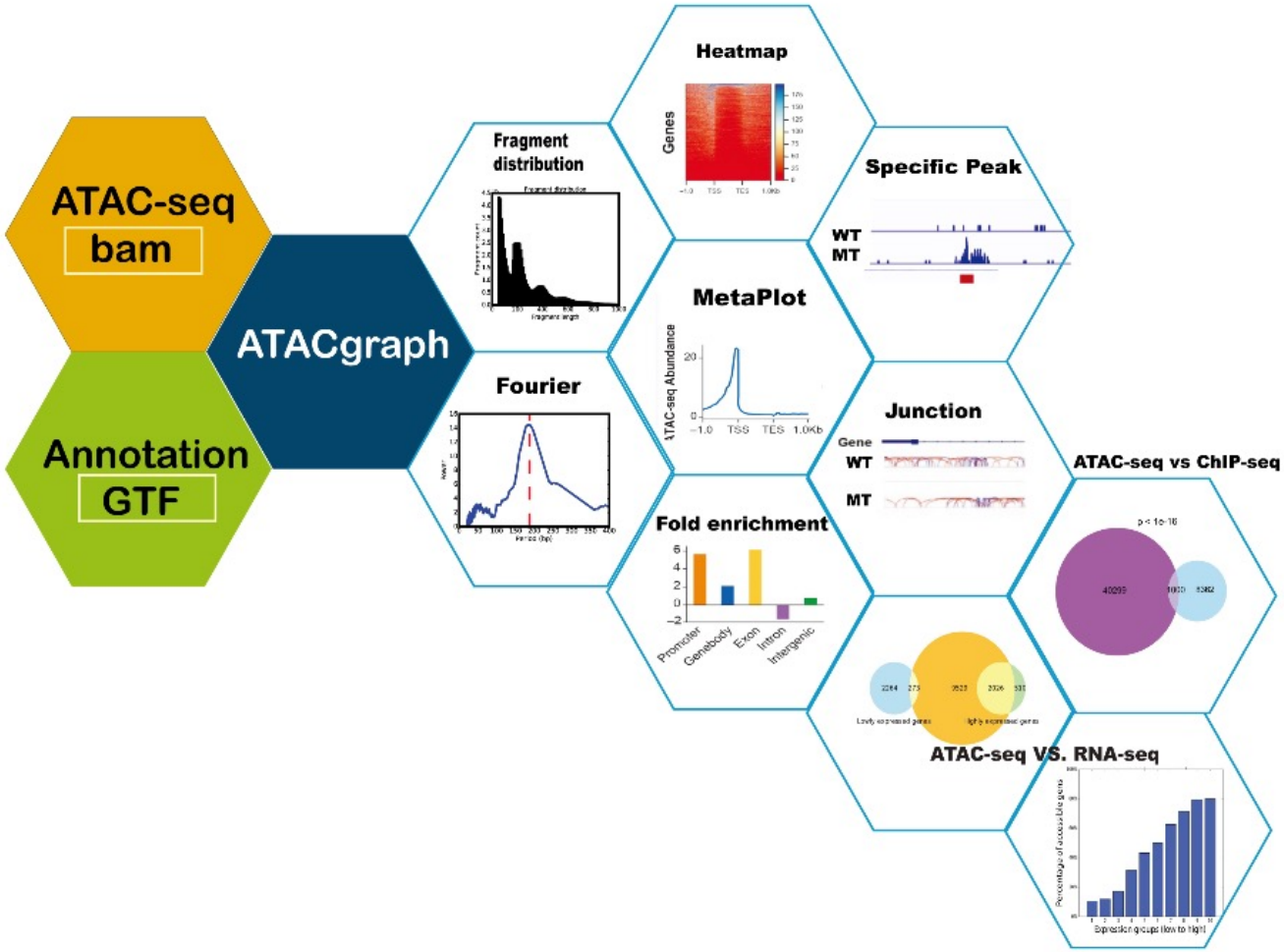


Figure 1 Schematic diagram of current chromatin accessibility assays performed with typical experimental conditions. Representative DNA fragments generated by each assay are shown, with end locations within chromatin defined by colored arrows. Bar diagrams represent data signal obtained from each assay across the entire region. The footprint created by a transcription factor (TF) is shown for ATAC-seq and DNase-seq experiments.

(Tsompana and Buck ., 2014)

Chromatin accessibility analysis workflow

All work is done in Linux environment



Data processing – peak calling

1. Remove mitochondria DNA
2. Peak calling

Download and environment setup for ATAC-seq

1. Download ATAC-graph

   git clone https://github.com/RitataLU/ATACgraph.git
   cd ATACgraph

2. Create python2.7 environment for ATAC-graph

   sh ./ATACgraph/base.txt

(https://github.com/beritlin/ATACgraph)

Example data Human (人類)

- Example:
  cd ATACgraph/demo

01_for_data_processing

human genome annotation:
demo_gene.gtf
human gene and promoter bed files:
demo_gene_body_bed6.bed

02_for_data_visualisation

Raw reads bam file:
data.bam
Raw reads bam index file:
data.bam.bai
BigWig file:
demo_rmM_peakcall_coverage.bw
Peak location BED file:
demo_rmM_peakcall_peaks.narrowPeak
A genes list of overlapping with peaks locations :
demo_rmM_peakcall_peak_gene_list.txt

03_for_downstream_analysis

Peak location BED file:
demo_rmM_peakcall_peaks.narrowPeak

Remove mitochondria DNA

20-80% sequences in ATAC-seq are from mitochondria genomes

- Remove mitochondria chromosome

  ./script/ATACgraph 00_rmChr demo/demo.bam demo/demo_rmM.bam chrM

  Input.bam **Output.bam** **chromosomes**

```
Remove chrM 69628 reads
Remove total 69628 out of 71843 (0.969)
```

- Transform GTF file to BED files

  ./script/ATACgraph 02_gtftoBed demo/demo_gene.gtf demo/demo -p 2000

  Reference genome.gtf **Output.bed** **Promoter regein**

Peak calling

- Peak calling

./script/ATACgraph 03_callPeak demo/demo_rmM.bam demo/demo_rmM_peakcall demo_gene_body_bed6.bed

input.bam **Output name**

Gene body.bed

•Peak location BED file
demo_rmM_peakcall_peaks.narrowPeak
•Peak intensity bigWigfile
demo_rmM_peakcall_coverage.bw
•A genes list of overlapping with peaks locations
demo_rmM_peakcall_peak_gene_list.txt

Post-alignment analysis

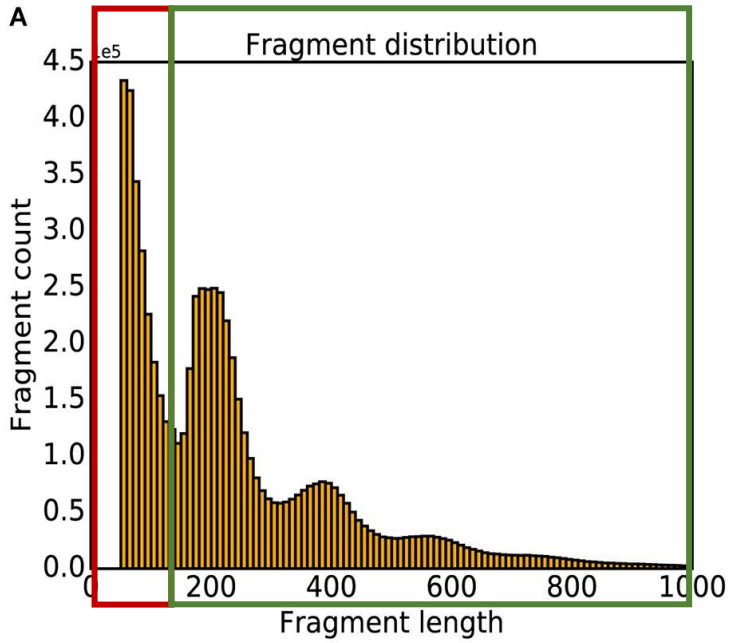
Fragment length distribution and Fast Fourier Transform (FFT)

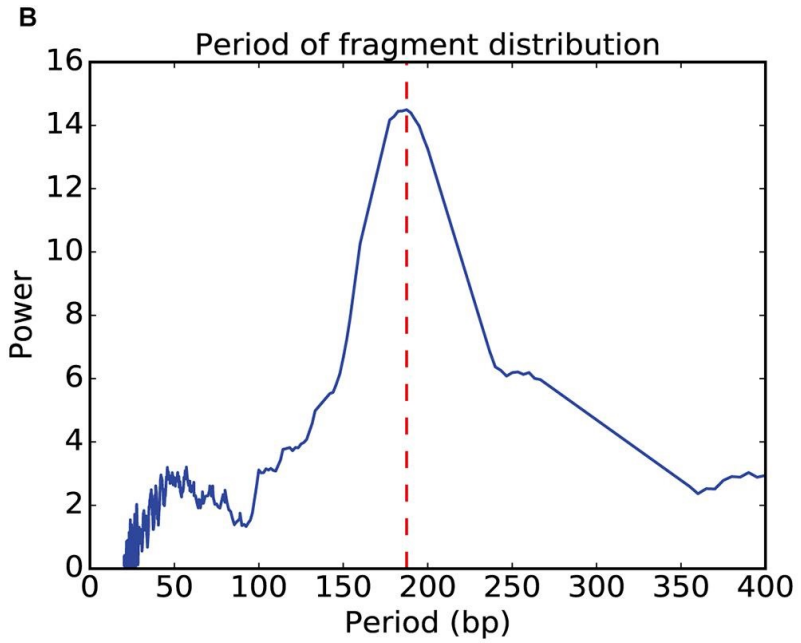
- Find fragment

./script/ATACgraph 01_calFragDist demo/demo_rmM.bam demo/demo_rmM_fragment demo/demo_rmM_FFT

input.bam **Output (fragment)** **Output (FFT)**

NFR (nucleosome free region)





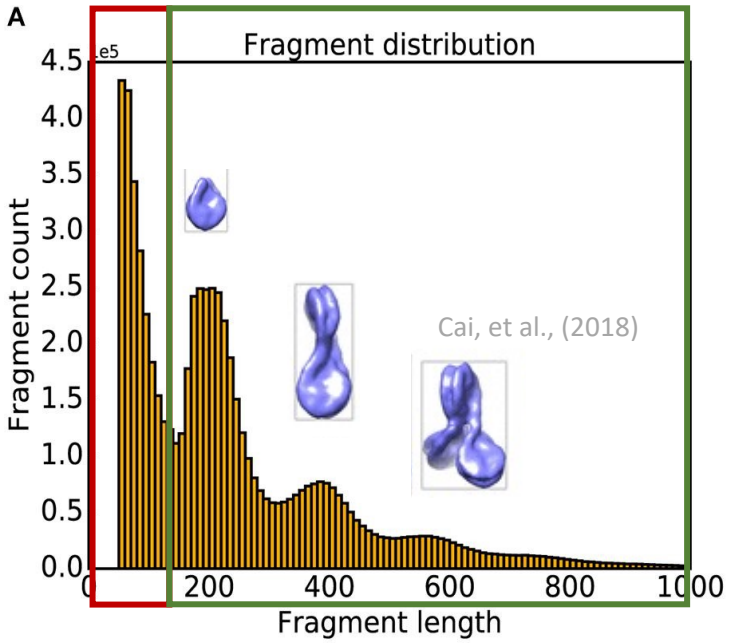
Fragment length distribution and Fast Fourier Transform (FFT)

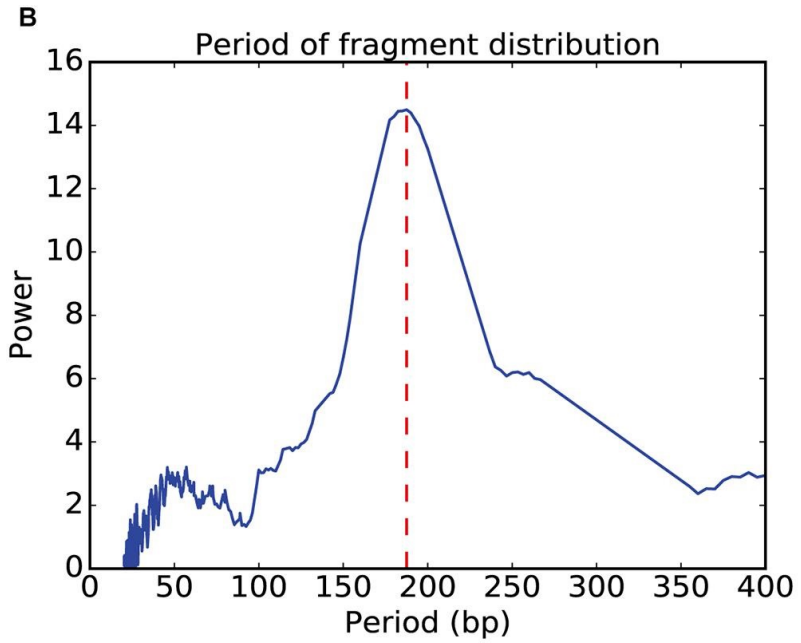
- Find fragment

./script/ATACgraph 01_calFragDist demo/demo_rmM.bam demo/demo_rmM_fragment demo/demo_rmM_FFT

input.bam **Output (fragment)** **Output (FFT)**

NFR (nucleosome free region)





mono-nucleosomes, di-nucleosomes, and tri-nucleosomes

Visualision of peaks

- Peaks analyses

./script/ATACgraph 03_genePlot demo/demo_rmM_peakcall_peaks.narrowPeak demo/demo_rmM_peakcall_coverage.bw demo/demo

input.narrowpeak

Input.bw

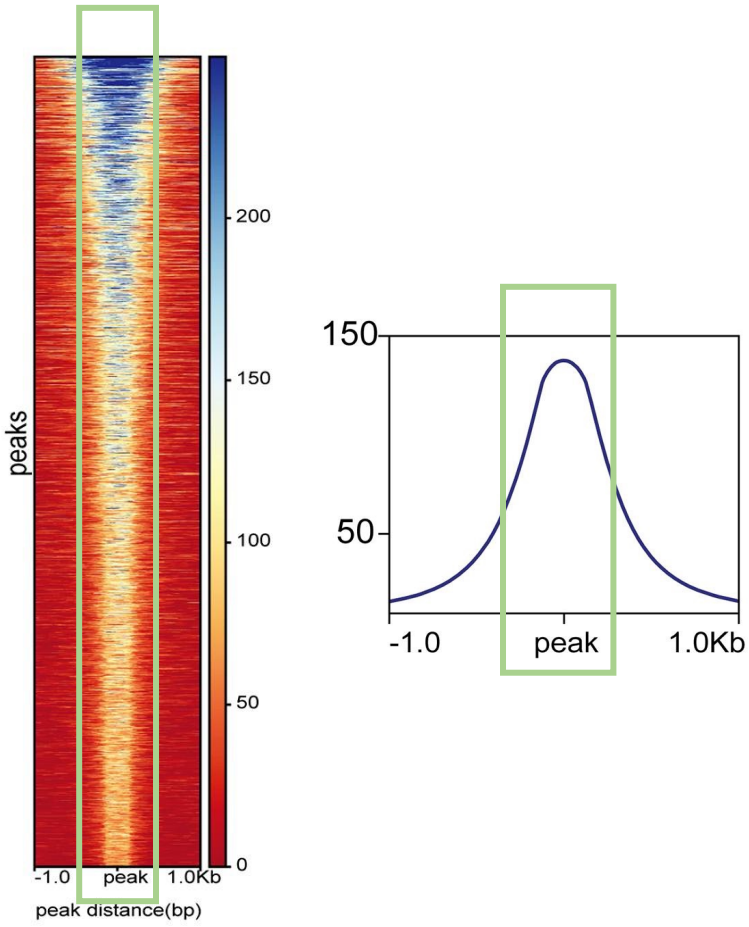
Output name

3 Figures

- The enrichment status of accessible region in genome
  Fold_Enrichment.pdf
- The accessibility – or read abundance – around genes
  gene_body_heatmap.pdf
- The accessibility – or read abundance – around peaks
  Peak_heatmap.pdf

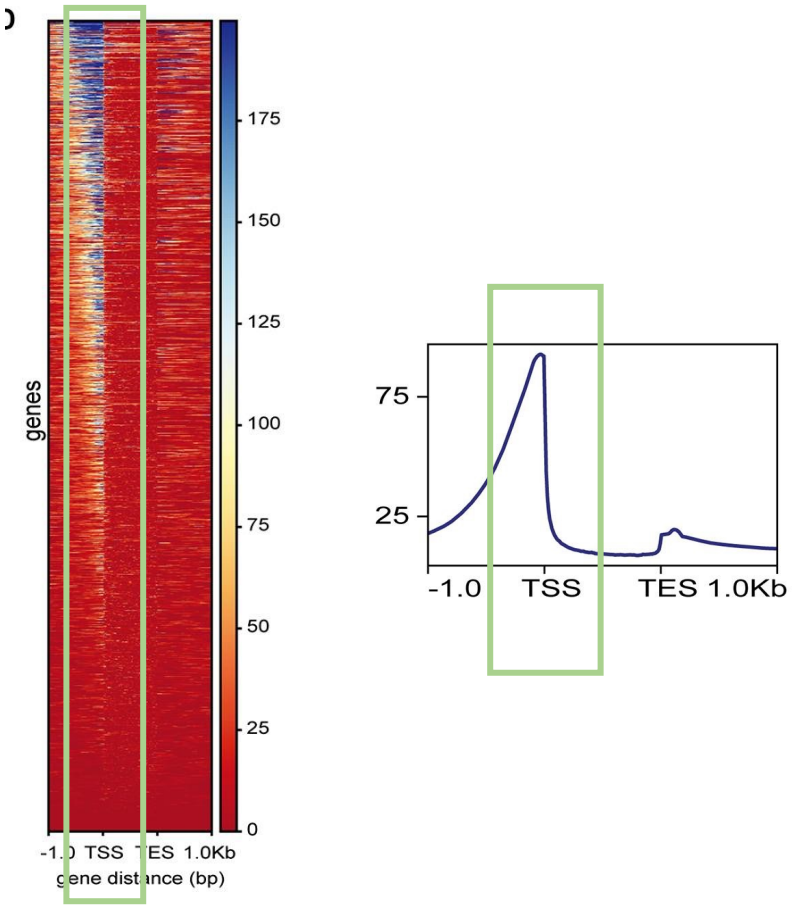
ATAC-seq abundance near the peak regions

- ATAC-seq enriched at the center of the predicted peak locations



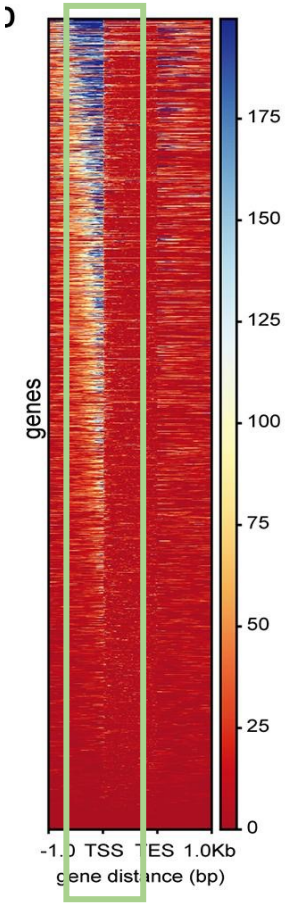
ATAC-seq abundance of the gene body and flanking regions

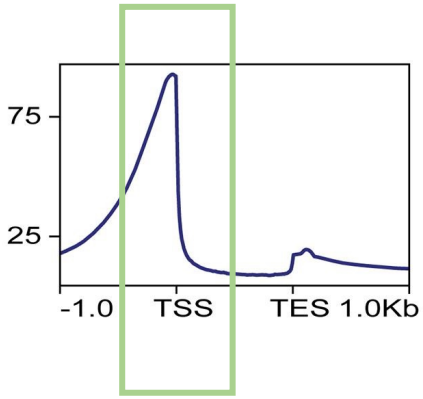
- The accessible regions are located before the transcription start sites (TSSs) in two-thirds of the genes

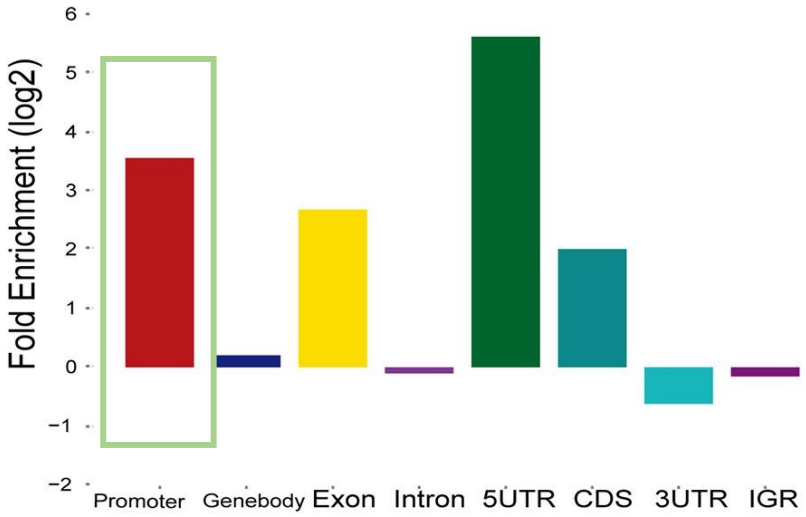


ATAC-seq abundance of the gene body and flanking regions

- the ATAC-seq abundance is clearly enriched at promoters close to TSSs, depleted in the gene body, and slightly enriched after the transcription end sites (TESs)







Thank you for listening!