Integrated ATAC-seq Data Analysis with ATACseqQC

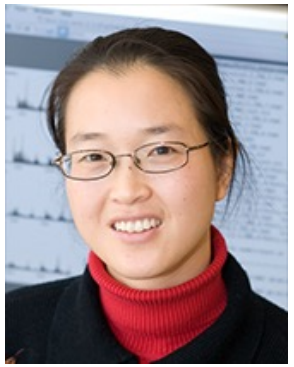
Kai Hu, Haibo Liu, Lihua Julie Zhu

University of Massachusetts Medical School (UMMS)

Acknowledgments

Core developers of **ATACseqQC**

Haibo Liu



Lihua Julie Zhu

Jianhong Ou

Other contributors

Nathan Lawson

Michelle Kelliher

Lucio Castilla

Agenda

Mini-lecture (15~20min)

- Introduction to ATAC-seq technology
- A common ATAC-seq workflow
- Best practices in ATAC-seq assays and data analysis

Demo (15~20min)

- ATACseqQC functions
- Demo plots

Q/A (5~10min)

- Bioconductor support site

How to run the workshop

Step1: install Docker engine: https://docs.docker.com/get-docker/

Step2: download the docker image:

- docker pull hukai916/integrated_atacseq_analysis_workshop2021:latest

Step3: start the container:

- docker run -e PASSWORD=yourpassword -p 8787:8787 hukai916/integrated_atacseq_analysis_workshop2021:latest

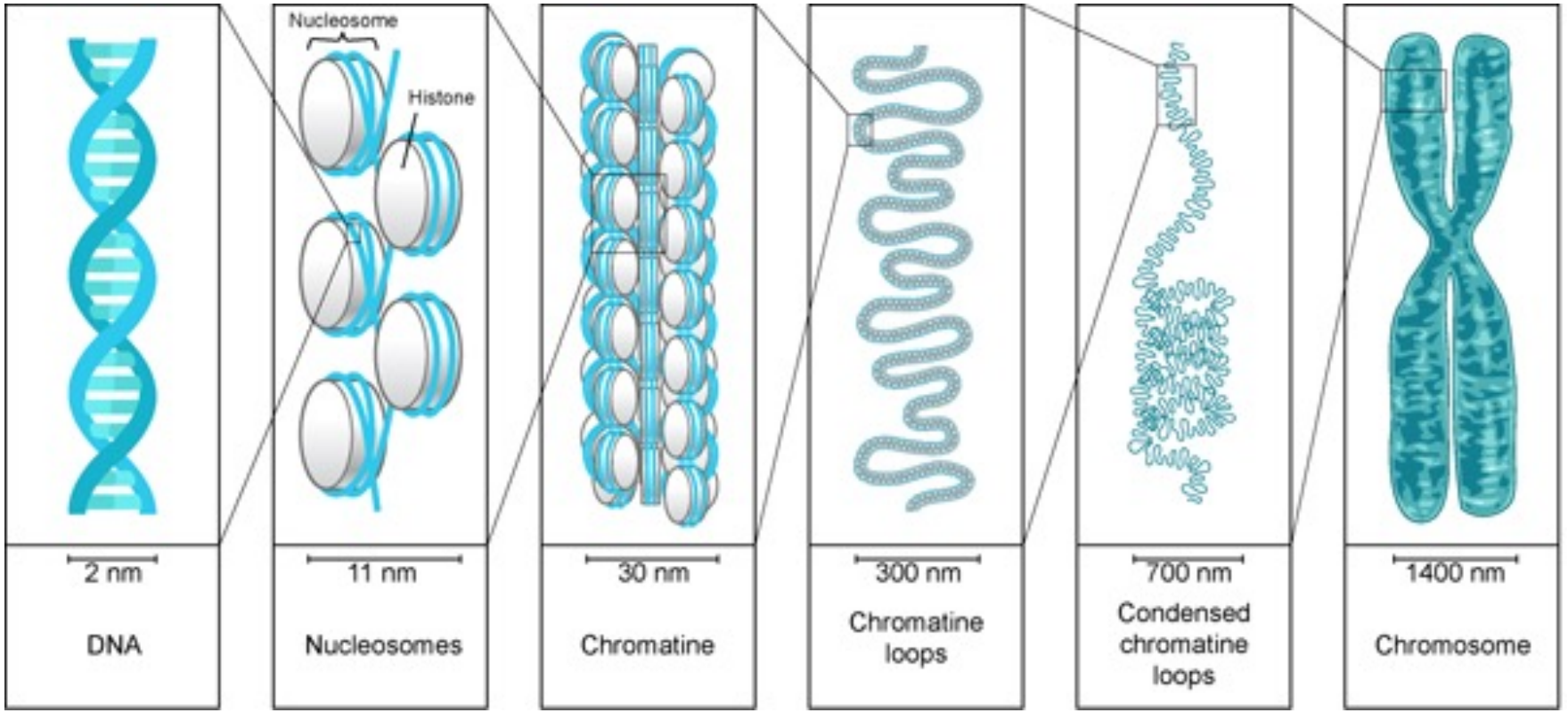
Step4: in your web browser:

- Enter http://localhost:8787 using username rstudio and password yourpassword

More instructions:
https://github.com/hukai916/IntegratedATACseqAnalysisWorkshop2021

- Introduction to ATAC-seq technology
- A common ATAC-seq workflow
- Best practices in ATAC-seq assays and data analysis

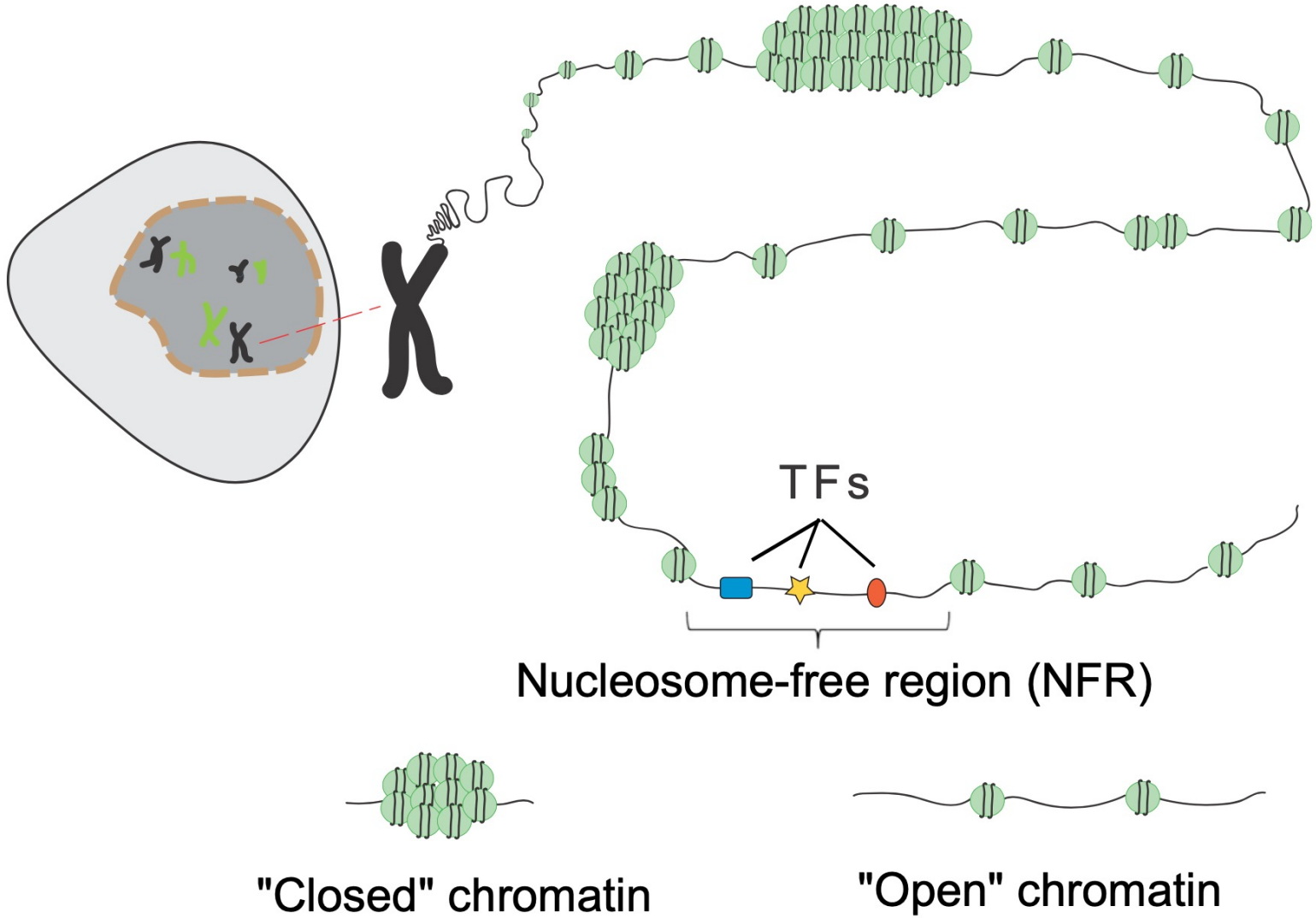
DNA packaging in eukaryotes



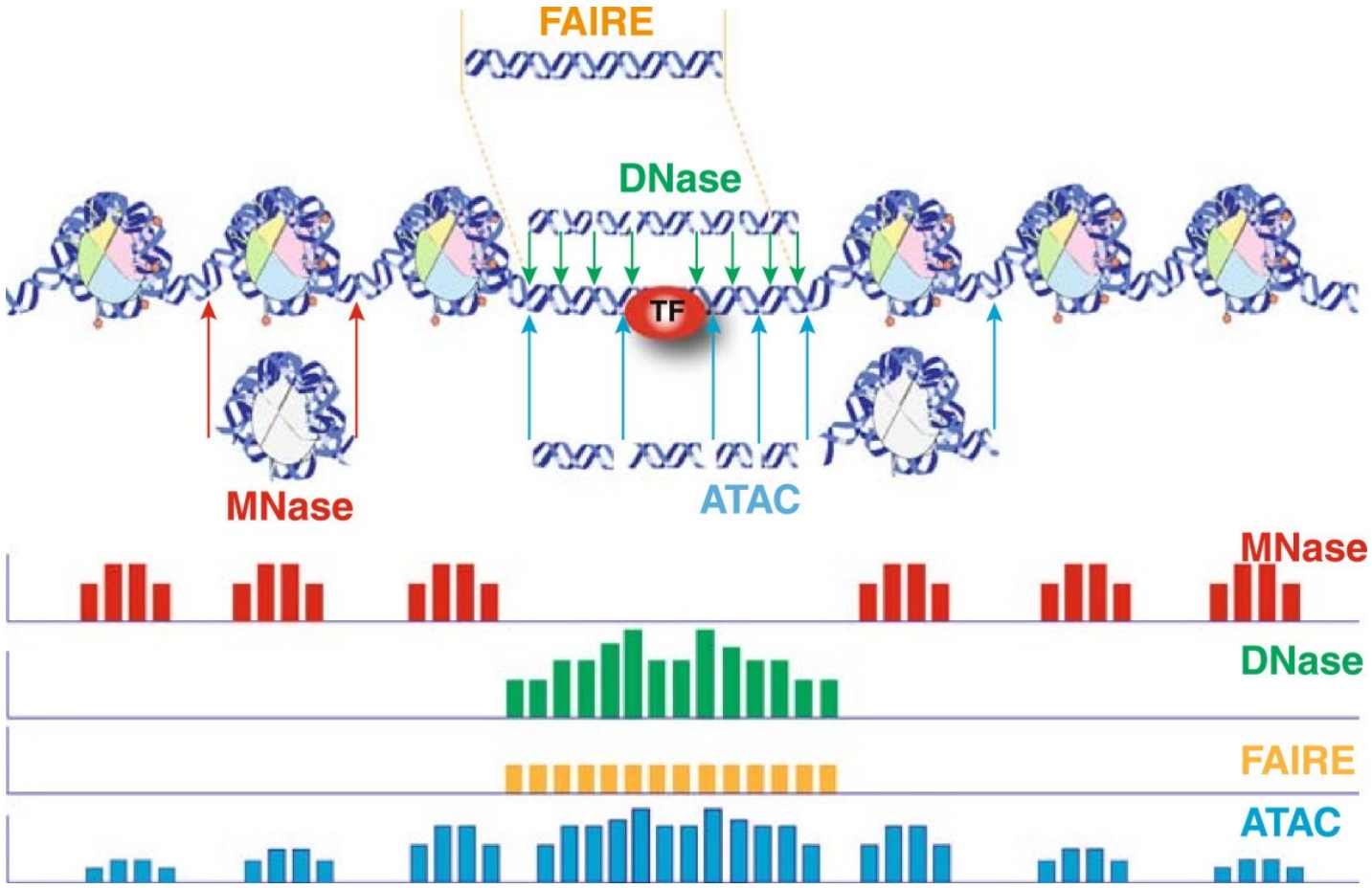
Condensed approximately 10,000 times.

https://www.shmoop.com/study-guides/biology/dna/dna-packaging

“Open” vs “closed” chromatin



Methods for profiling chromatin accessibility landscape



MNase: endo-exonuclease

DNAase: endonuclease, preferentially cut nucleosome free region.

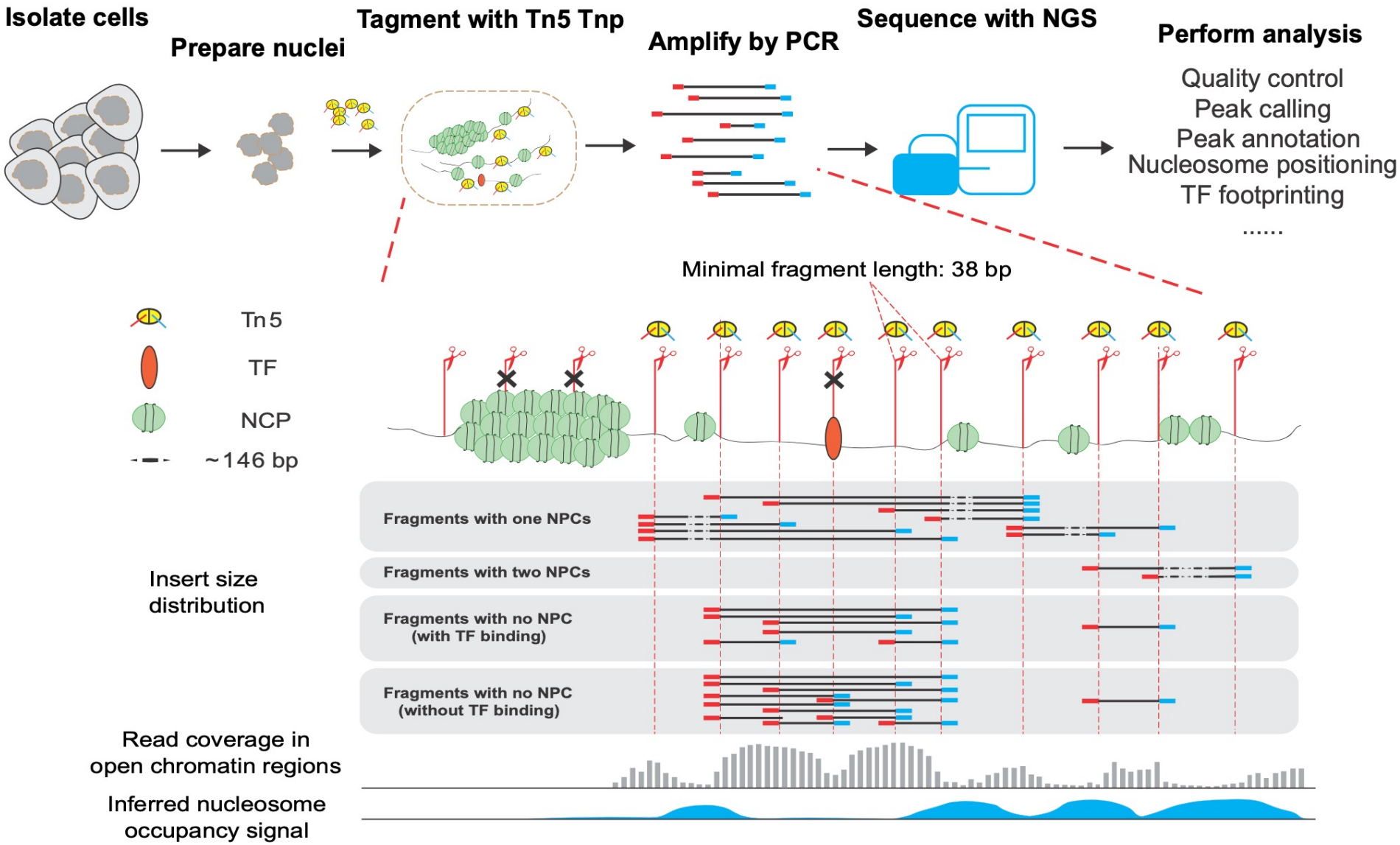
FAIRE: regulatory DNA fragments will be preferentially released to extraction solution after sonication.

ATAC: hypersensitive transposase that cuts accessible regions.

https://epigeneticsandchromatin.biomedcentral.com/articles/10.1186/1756-8935-7-33

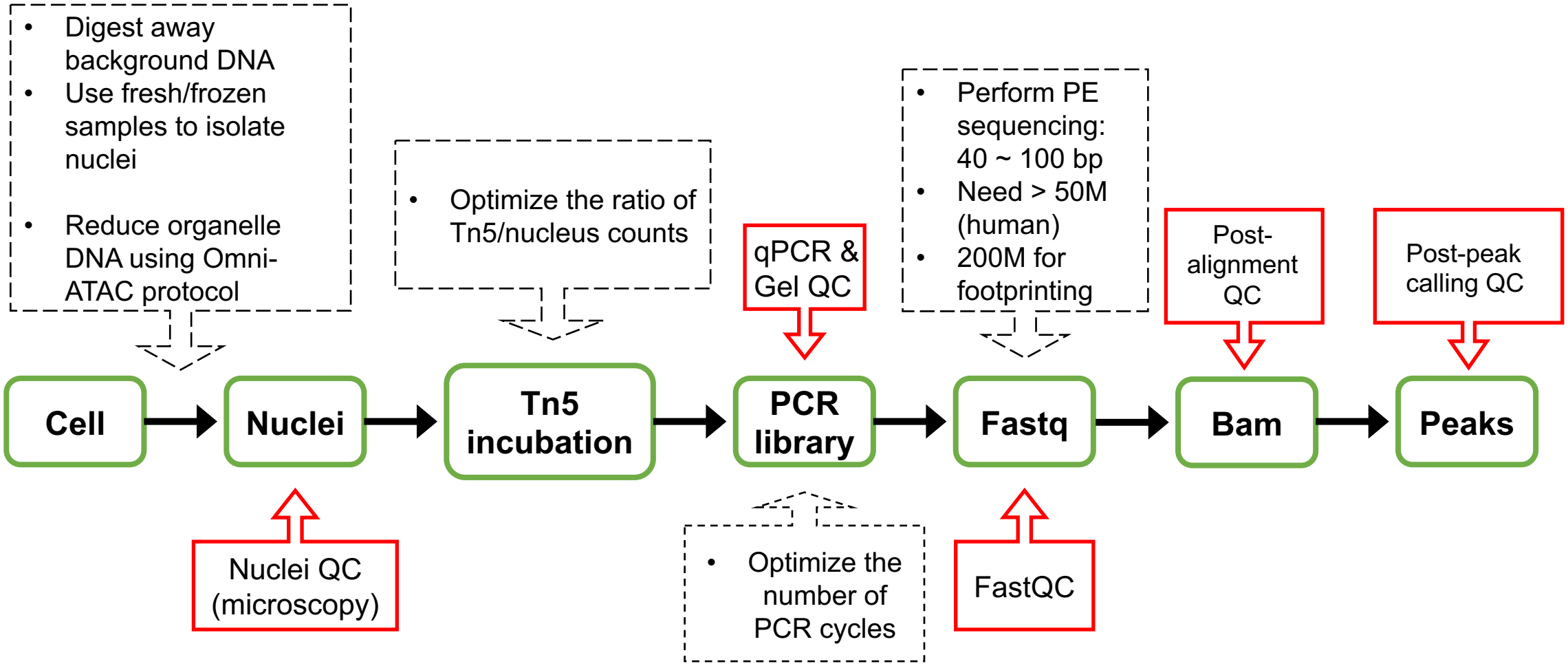
- Introduction to ATAC-seq technology
- A common ATAC-seq workflow
- Best practices in ATAC-seq assays and data analysis

A typical ATAC-seq workflow

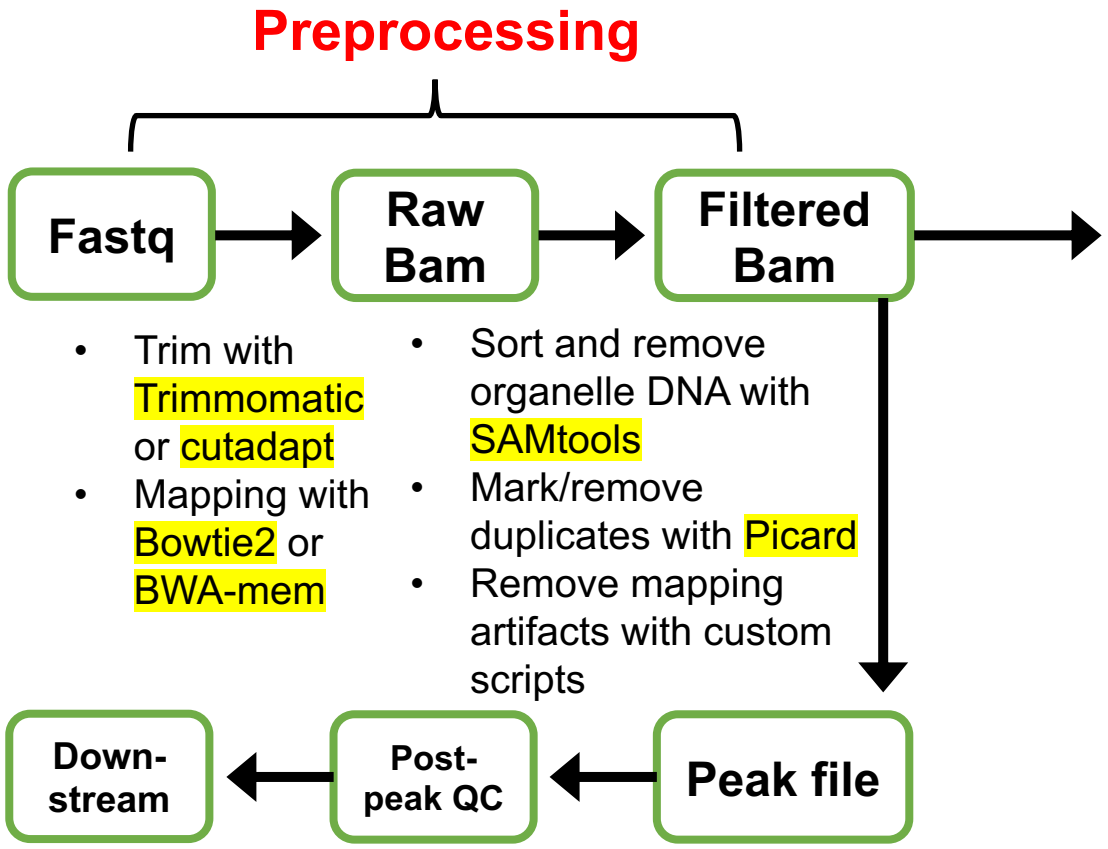


- Introduction to ATAC-seq technology
- A common ATAC-seq workflow
- Best practices in ATAC-seq assays and data analysis

Important steps, best practices, and important QCs



Popular software tools in each step



ATACseqQC workflow

i. Assessing mapping status: **bamQC()** (Alternatives: samtools, picard)
ii. Assessing sequencing depth and library complexity: **saturationPlot()** & **estimateLibComplexity()**
iii. Assessing insert size distribution: **fragSizeDist()**
iv. Assessing similarities of replicates: **plotCorrelation()**
v. Shifting aligned reads: **shiftGAlignmentsList()**
vi. Splitting BAM files: **splitGAlignmentsByCuts()**
vii. Plotting aggregate signals around TSSs: **featureAlignedHeatmap()**, **featureAlignedDistribution()**
viii. Streamlining IGV snapshots: **IGVSnapshot()**
ix. Assessing DNA-binding factor footprints: **factorFootprints()**

- ATACseqQC functions
- Demo plots

Demo I: assessing mapping status

bamQC():

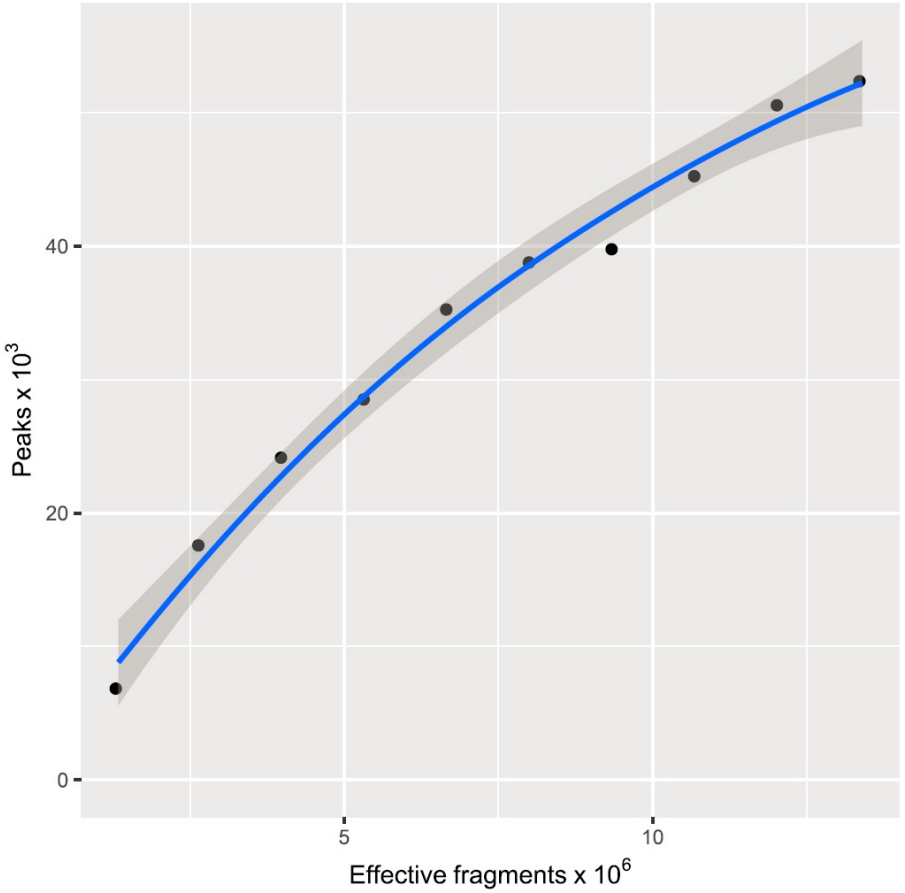
- Input: sorted BAM files with duplicates marked
- Output: mapping rate, duplicate rate, mapping quality, *etc.*

Alternative tools:

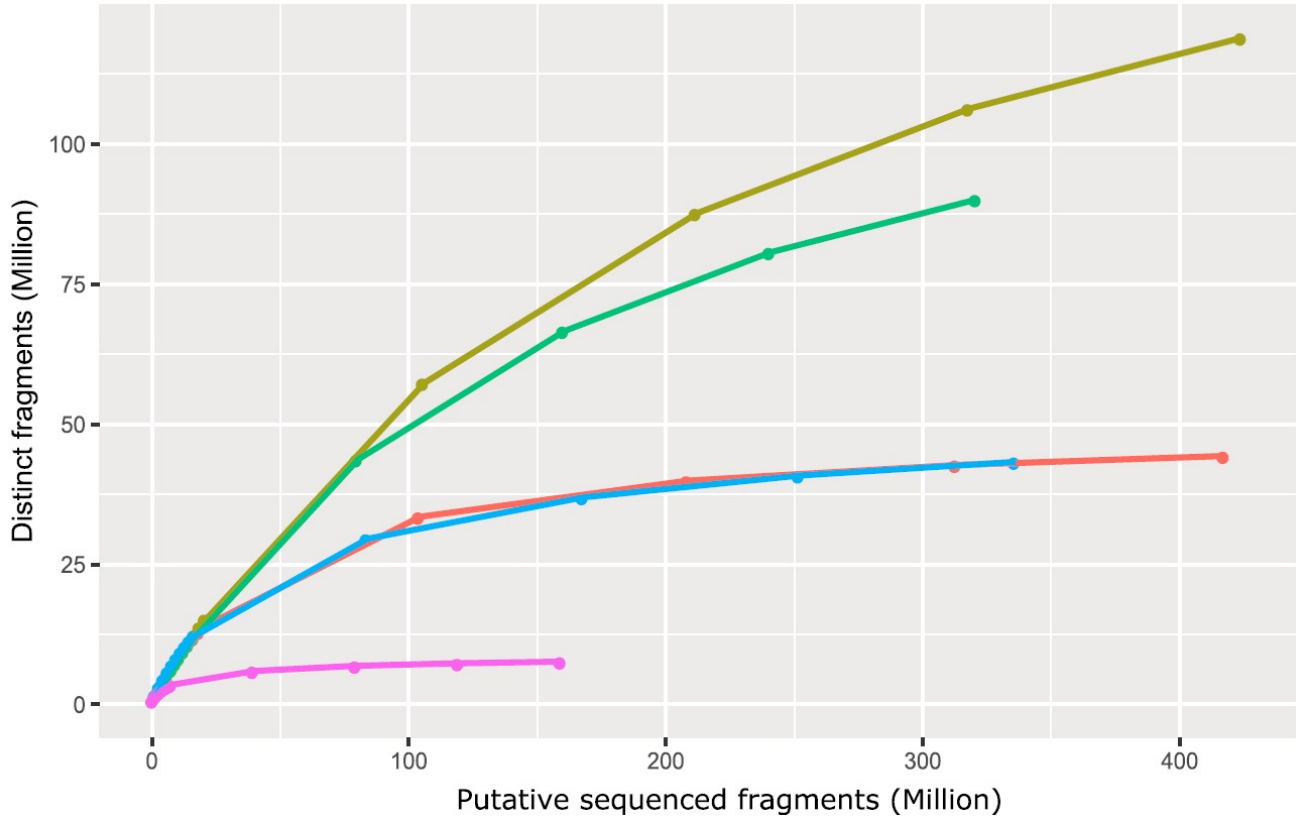
- SAMtools
- picard tools

Demo II: assessing sequencing depth and library complexity

saturationPlot()



estimateLibComplexity()

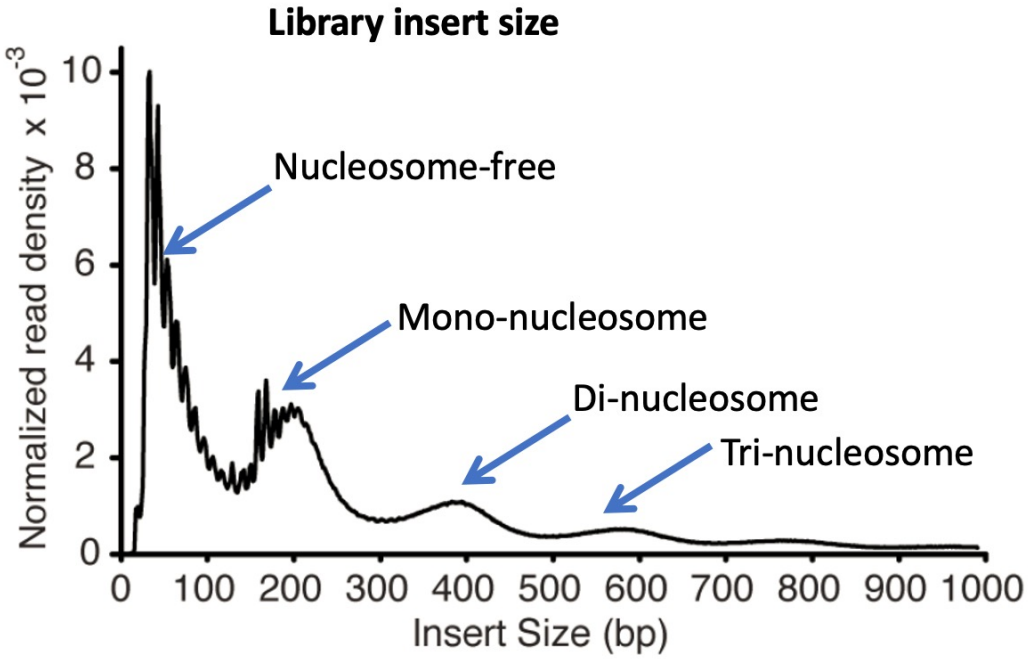


Ou, J., Liu, H., Yu, J. *et al.* ATACseqQC: a Bioconductor package for post-alignment quality assessment of ATAC-seq data. *BMC Genomics* **19,** 169 (2018).

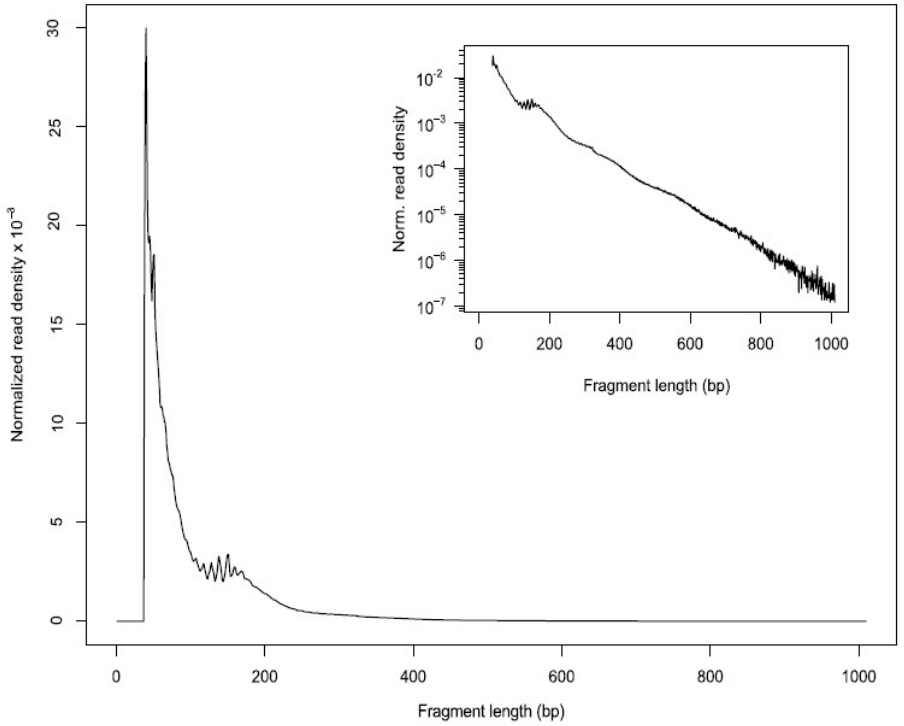
Demo III: assessing insert size distribution

fragSizeDist()

High quality

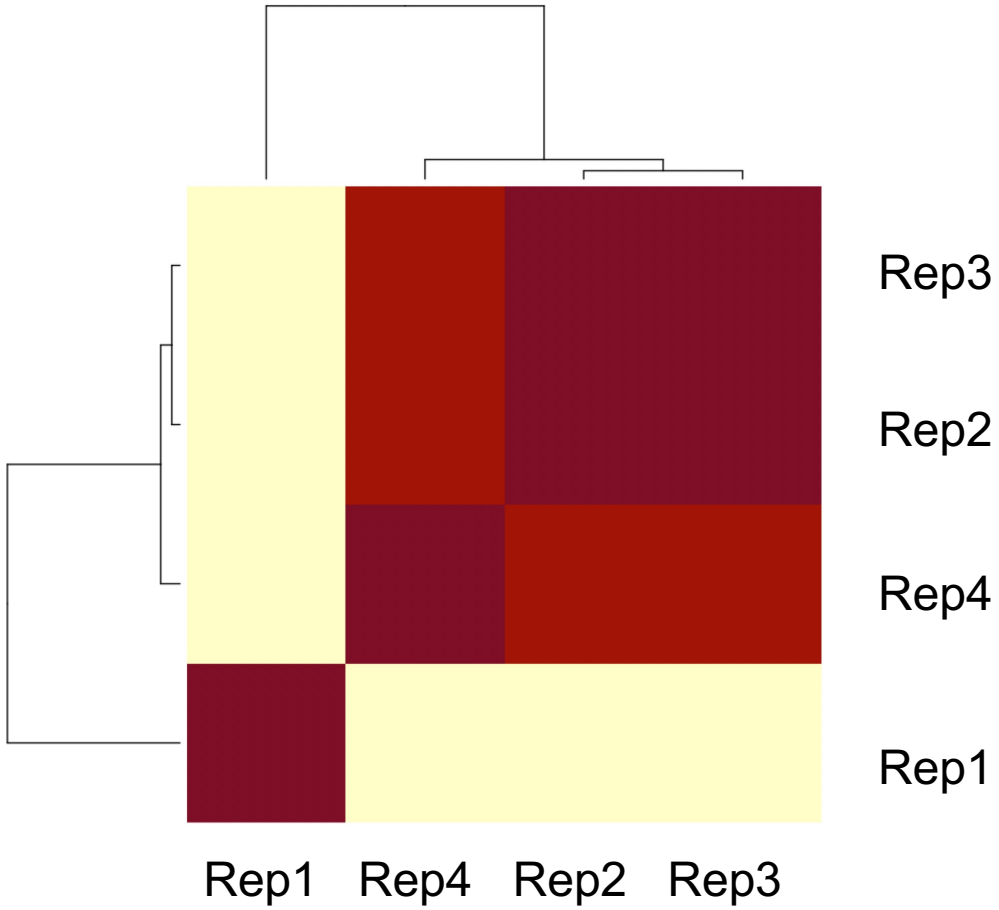


Poor quality



Ou, J., Liu, H., Yu, J. *et al.* ATACseqQC: a Bioconductor package for post-alignment quality assessment of ATAC-seq data. *BMC Genomics* **19,** 169 (2018).

Demo IV: assessing similarities of replicates:

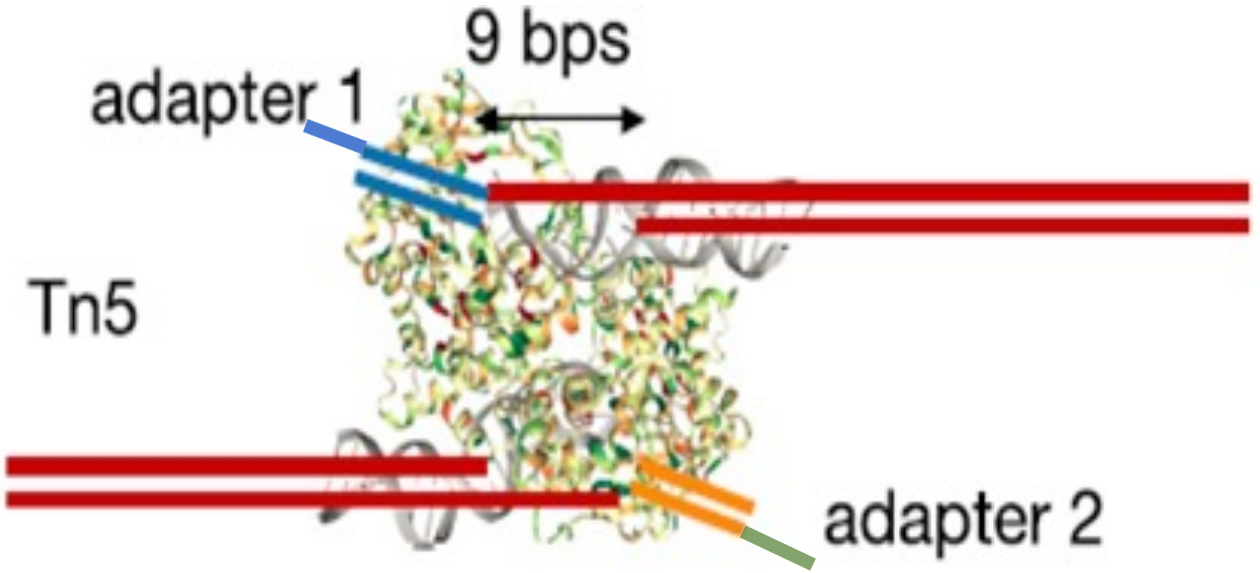


plotCorrelation()

- Can plot PCA or heatmap.
- The correlation is calculated by the counts in the promoter regions.

Rep1 is quite different from other replicates.

Demo V: shifting aligned reads

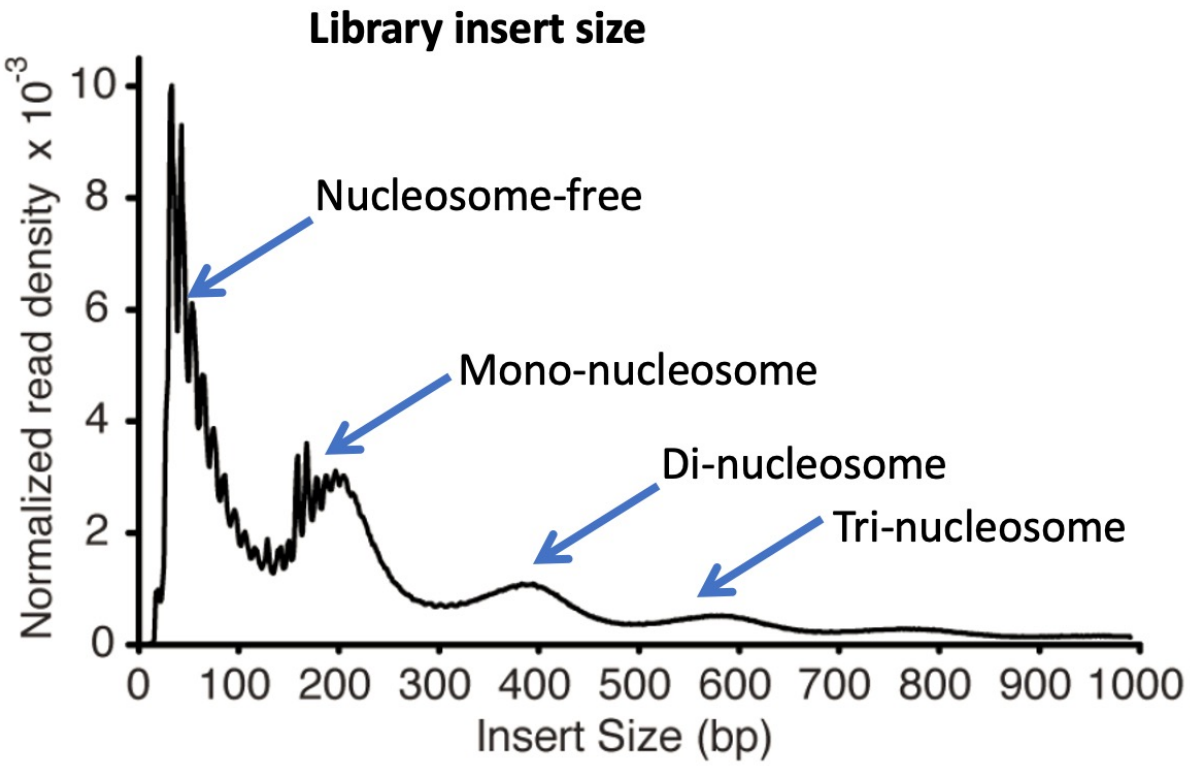


shiftGAlignmentsList()

- Tn5 tagmentation produces fragments with 9bp overhang at the 5’ ends
- To center the cleavage events:
  - shift +4 bp for reads mapped to forward strand
  - shift -5 bp for reads mapped to reverse strand

(Li *et al.* 2019)

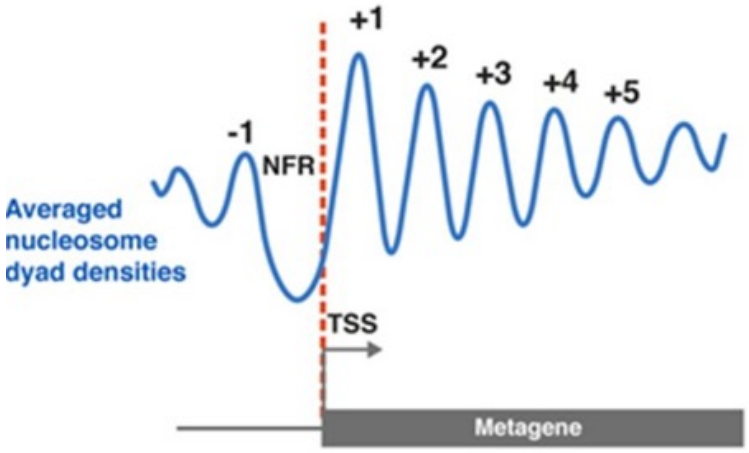
Demo VI: splitting BAM files



splitGAlignmentsByCut()

- You can split the Bam into different bins:
  - Nucleosome-free fragments
  - Inter1
  - Mono-nucleosome fragments
  - Inter2
  - Di-nucleosome fragments
  - *etc.*
- The reads from different bins can be used to visualized different signals:
  - promoter/enhancer/insulators are localized in NFR

Demo VII: plotting aggregate signals around TSSs

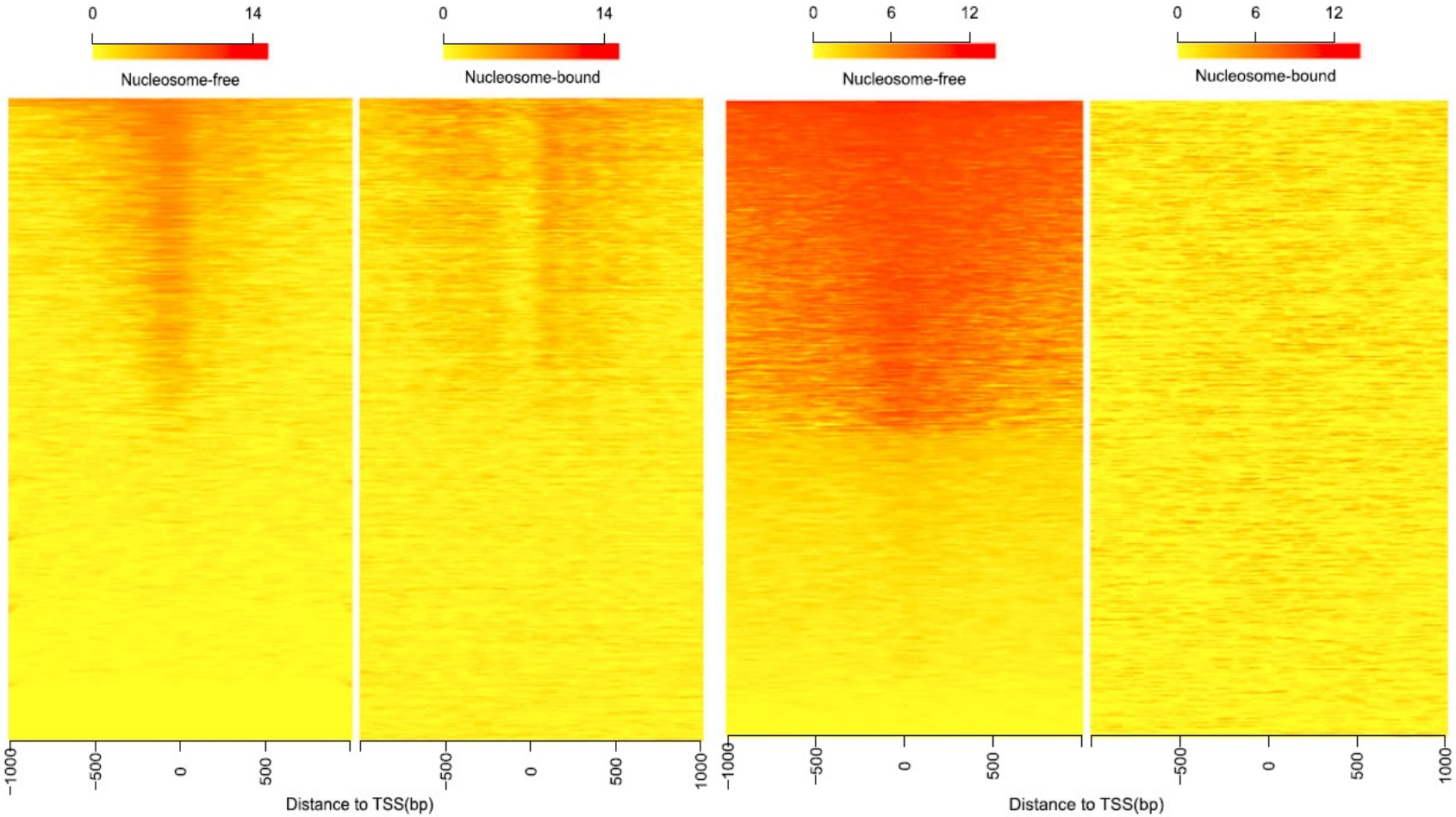


Nucleosome positioning around TSSs

featureAlignedHeatmap()

High quality

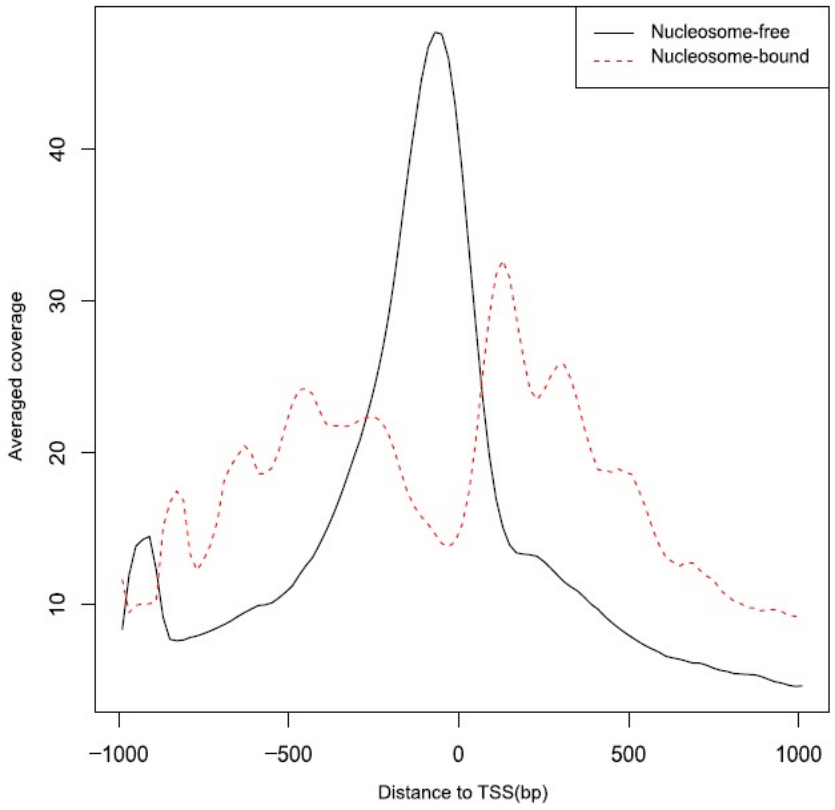
Poor quality



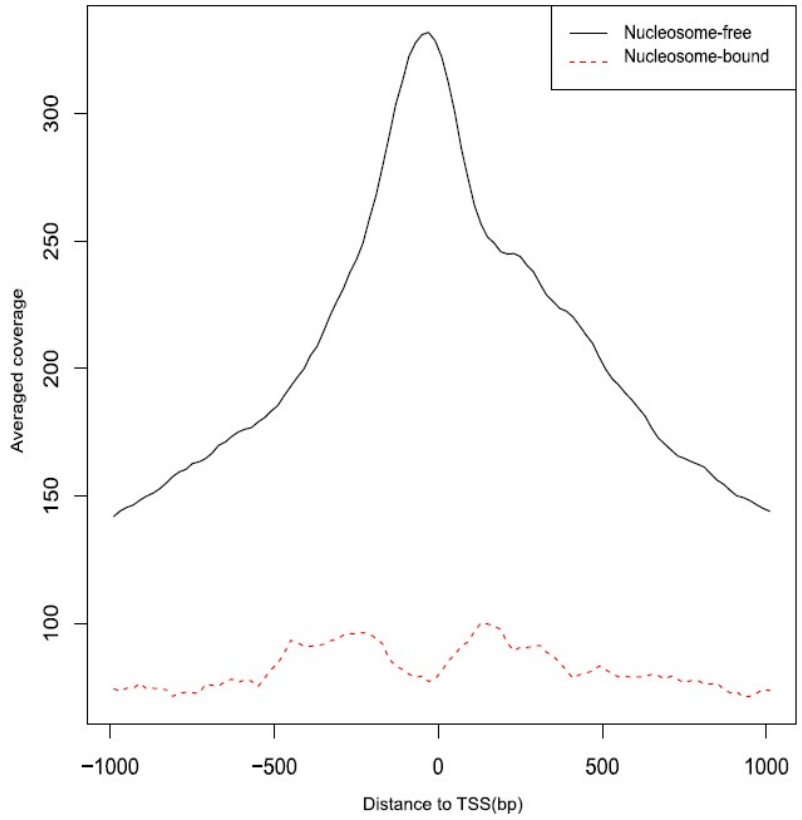
Demo VII: plotting aggregate signals around TSSs

featureAlignedDistribution()

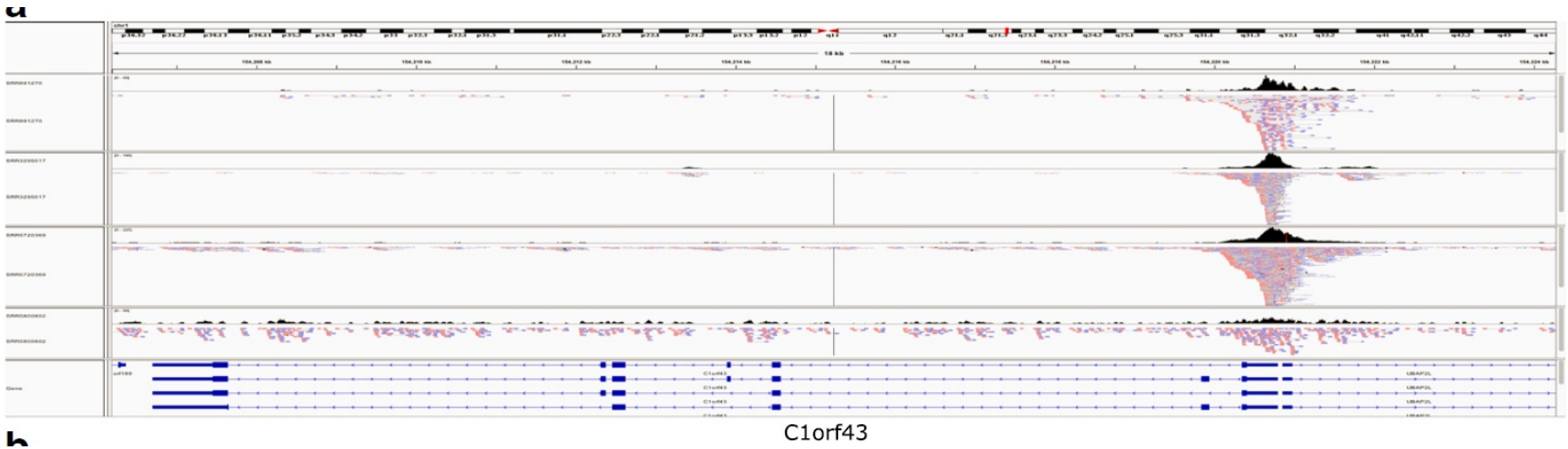
High quality



Poor quality



Demo VIII: streamlining IGV snapshots



IGVSnapshot()

- Need IGV
- For high quality data:
  - read signals are enriched to open regions
- For poor quality data:
  - read signals distribute across the genome

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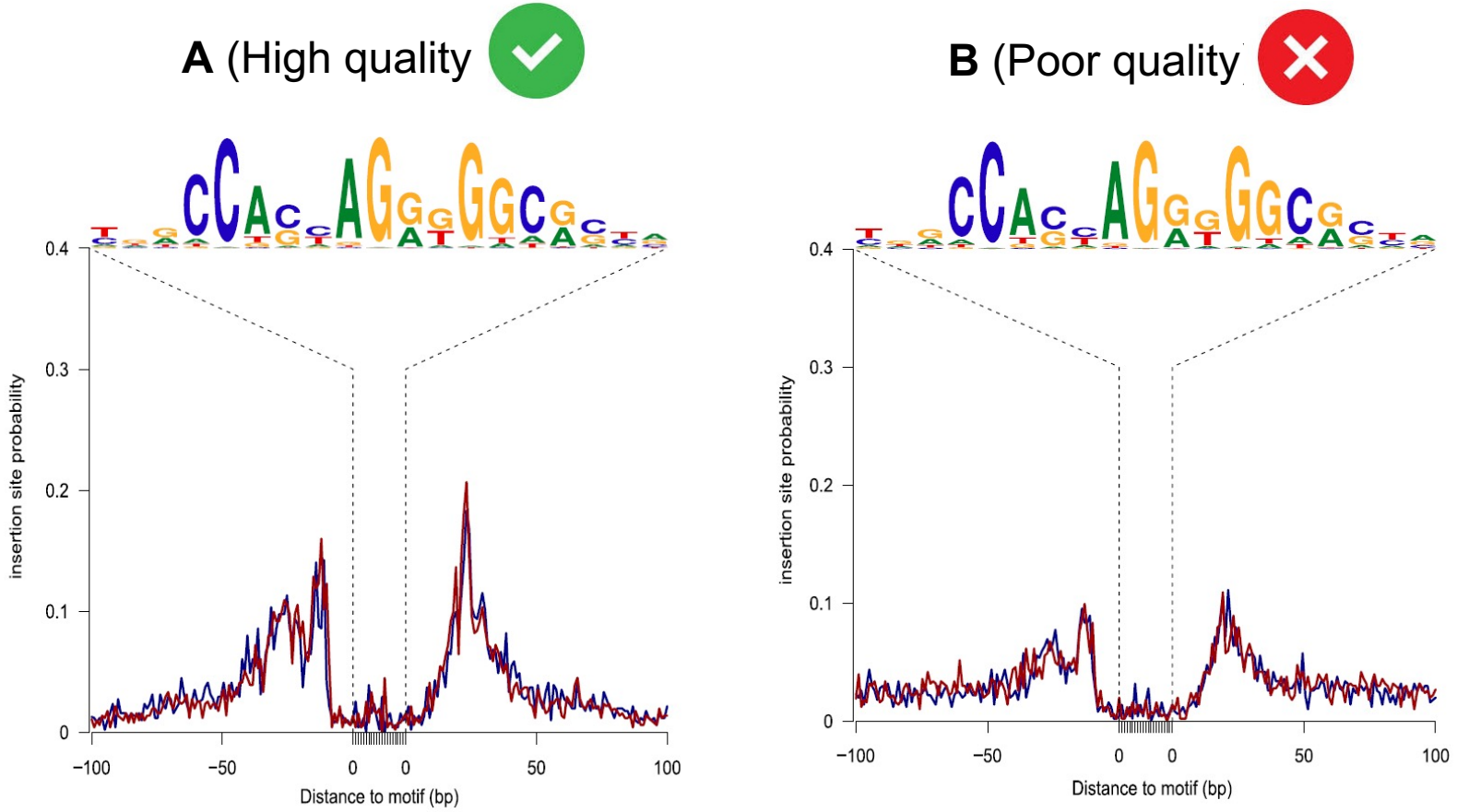
Demo IX: assessing DNA-binding factor footprints

factorFootprints()



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- ATACseqQC functions
- Demo plots

Datasets for demo with ATACseqQC

Dataset1: good quality
SRR891269 and SRR891270 are ATAC-seq data for two biological replicates of 50K cells from EBV-transformed lymphoblastoid cell line GM12878 (Buenrostro *et al.* 2013).

Dataset2: bad quality
SRR5800801 and SRR5800802 are ATAC-seq data for two replicates of 75k cells from a breast cancer cell line T47 (Valles and Izquierd-Bouldstridge, unpublished).

Preprocessing already performed for you:

- FastqQC on raw fastq files
- Mapped to hg38
- Bam file filtered according to the steps aforementioned

- Download link to the preprocessed bam files and preprocessing scripts: Google Drive
- A small subset of the bam files are included in the workshop package for quick demo

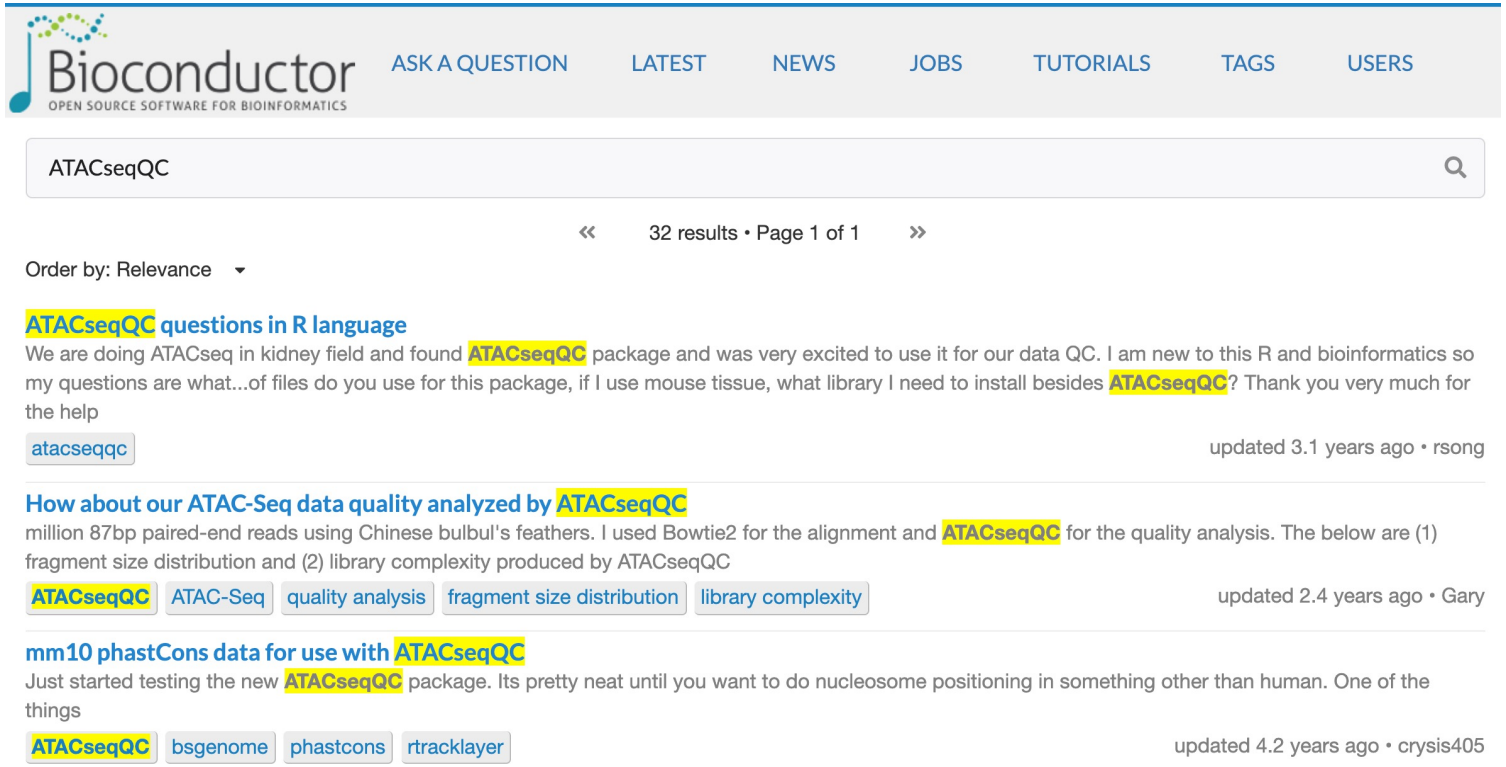
Demo with Rstudio

- demo.R
- demo.Rmd
- demo.html

Q & A

View and submit your questions to **Bioconductor support site**:
https://support.bioconductor.org/post/search/?query=ATACseqQC

Developers actively monitor questions posted there.



References

1. ATAC-seq technology: Buenrostro, J., Giresi, P., Zaba, L. *et al.* Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods* **10,** 1213–1218 (2013). https://doi.org/10.1038/nmeth.2688
2. ATACseqQC package: Ou, J., Liu, H., Yu, J. *et al.* ATACseqQC: a Bioconductor package for post-alignment quality assessment of ATAC-seq data. *BMC Genomics* **19,** 169 (2018). https://doi.org/10.1186/s12864-018-4559-3
3. ChIPpeakAnno package: Zhu, L.J., Gazin, C., Lawson, N.D. *et al.* ChIPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. *BMC Bioinformatics* **11,** 237 (2010). https://doi.org/10.1186/1471-2105-11-237
4. ATACseqQC workshop: https://github.com/haibol2016/ATACseqQCWorkshop

Read more:

1. ChIPpeakAnno workshop: https://github.com/hukai916/IntegratedChIPseqWorkshop