Assay for Transposase-Accessible Chromatin – sequencing (ATAC-seq)

Epigenomics Data Analysis Workshop

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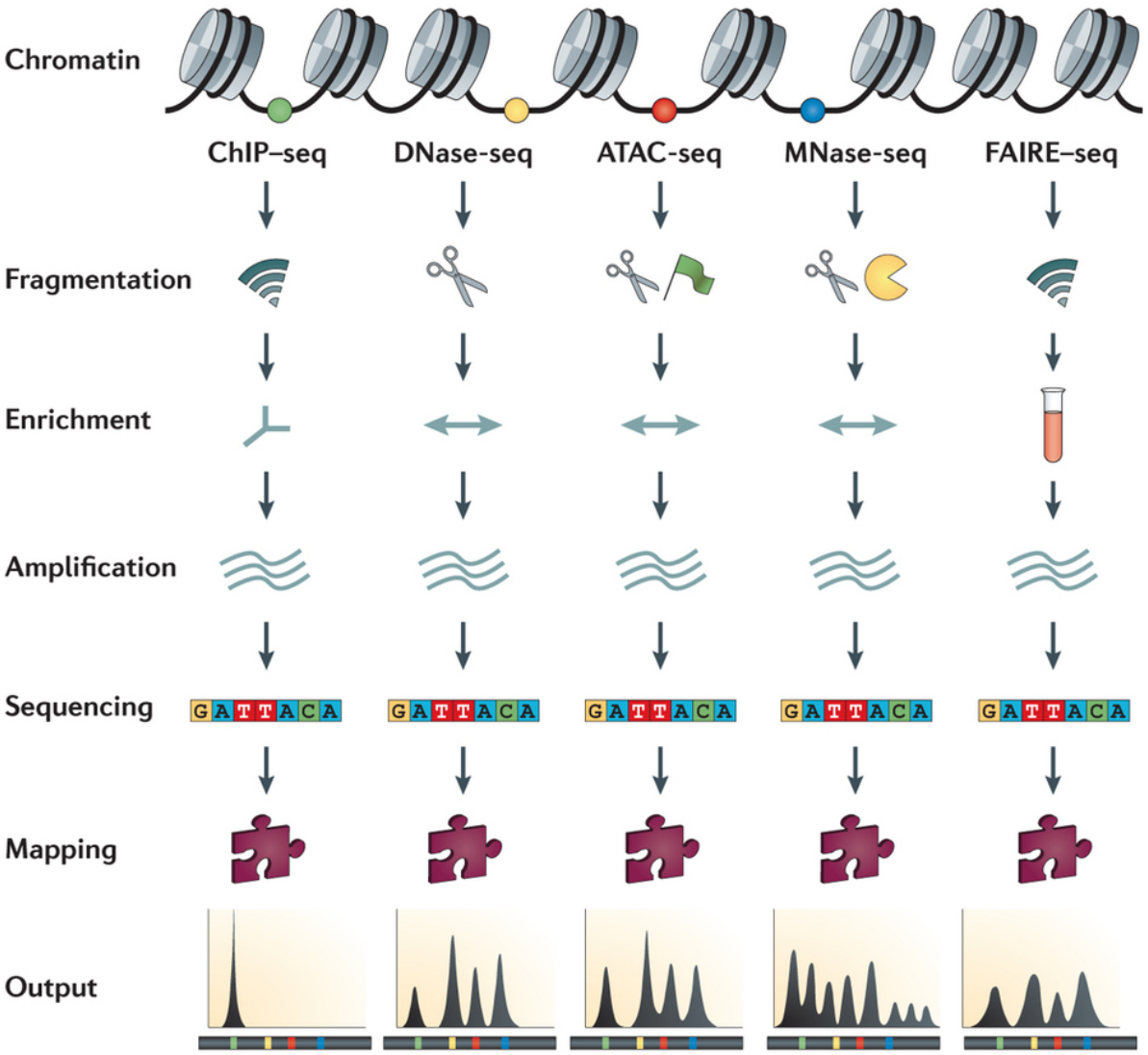
SciLifeLab

UNIVERSITET STOCKHOLMS

Functional genomics techniques to probe chromatin states



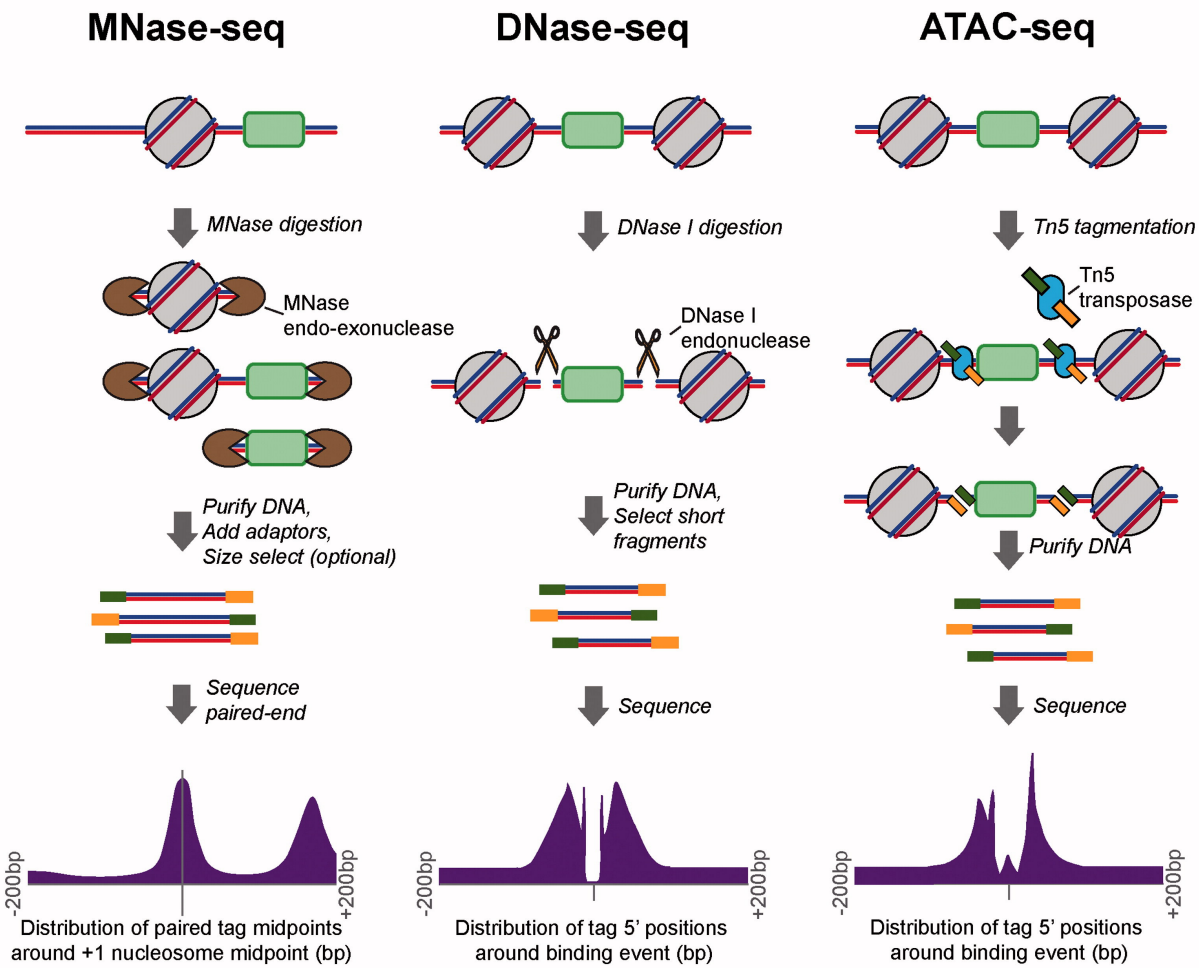
Accessibility – targeting nucleosome-depleted DNA:

DNase-seq
ATAC-seq
FAIRE-seq (Formaldehyde-Assisted Isolation of Regulatory Elements)

Nucleosome positioning:
MNase-seq

Clifford et al, Nature Rev Genet, 2014

Functional genomics techniques to identify open chromatin regions



Pugh 2015

Assay for Transposase-Accessible Chromatin (ATAC)-seq

- The method published recently in bulk (Buenrostro et al., 2015) and single cell (Buenrostro et al., 2015)

Current Protocols in Molecular Biology / Volume 109, Issue 1

UNIT

ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide

Jason D. Buenrostro, Beijing Wu, Howard Y. Chang, William J. Greenleaf

First published: 05 January 2015
https://doi.org/10.1002/0471142727.mb2129s109
Citations: 696

Published: 17 June 2015

Single-cell chromatin accessibility reveals principles of regulatory variation

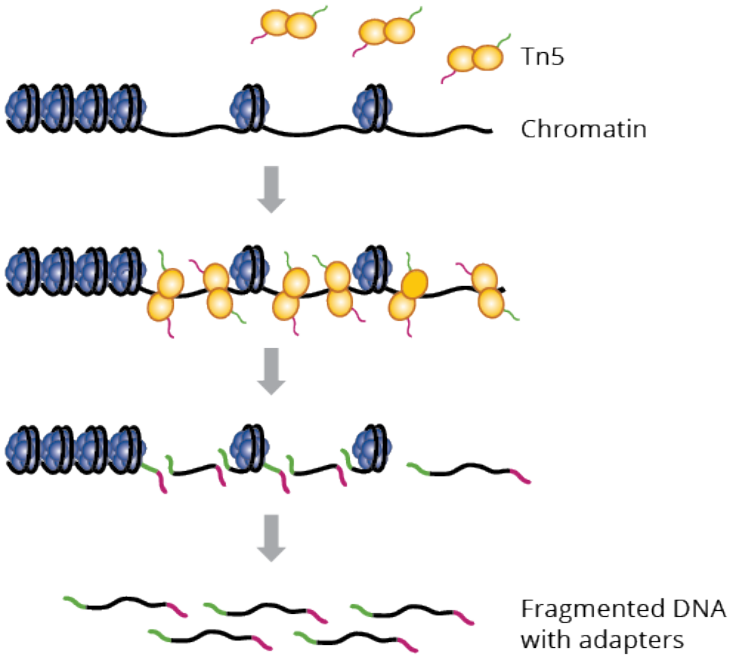
Jason D. Buenrostro, Beijing Wu, Ulrike M. Litzenburger, Dave Ruff, Michael L. Gonzales, Michael P. Snyder, Howard Y. Chang & William J. Greenleaf

Nature **523**, 486–490(2015) | Cite this article

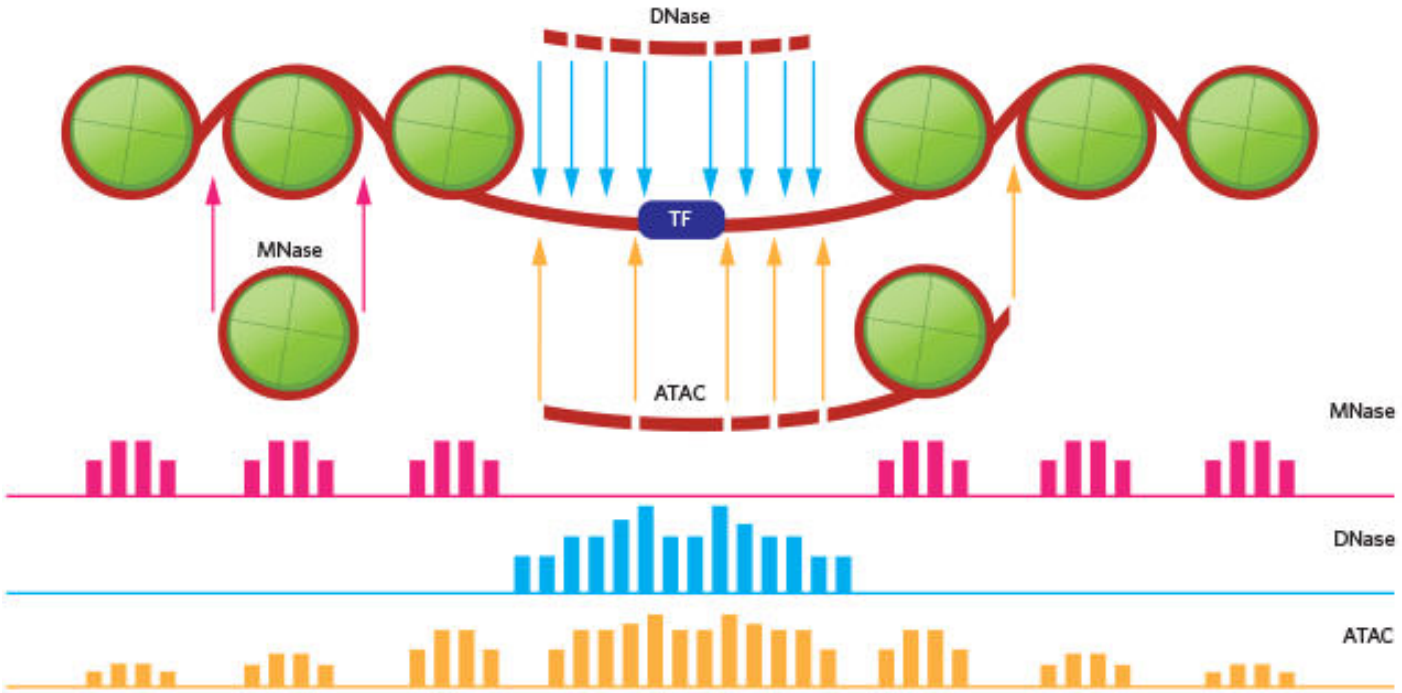
21k Accesses | **600** Citations | **100** Altmetric | Metrics

- It probes access to chromatin by using Tn5 transposase to insert sequencing adapters into DNA which allows simultaneous fragmentation of chromatin and integration of those adapters into open chromatin regions

- Significantly fewer cells needed (~ 50,000 cells for ATAC-Seq compared to millions of cells for the other methods (DNase-Seq or FAIRE-Seq)
- Two step process, one day of work



Functional genomics techniques to probe chromatin states



http://www.the-scientist.com/?articles.view/articleNo/44772/title/Reveling-in-the-Revealed

Analysis workflow

Sample_Read1

Sample_Read2

QC

cutadapt

Read trimming, filtering

Trimmed_Read1

Trimmed_Read2

Read mapping

bowtie2

QC

Alignments

Filtering alignments

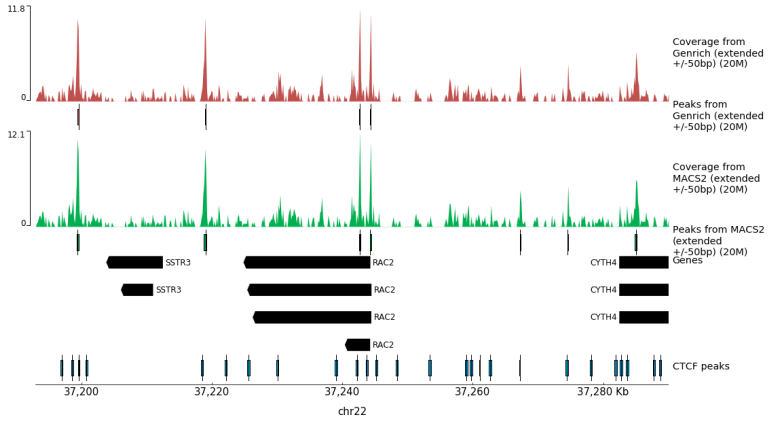
- MAPQ
- Duplicates
- Blacklists
- Mt reads

QC

samtools

Filtered alignments

Peaks: Genrich MACS2



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Replicate congruency

IDR, overlaps

Differential accessibility

DiffBind
...

Signal visualisation

deepTools
IGV ...

Annotation to genes

Bedtools
ChIPpeakAnno
ChIPSeeker

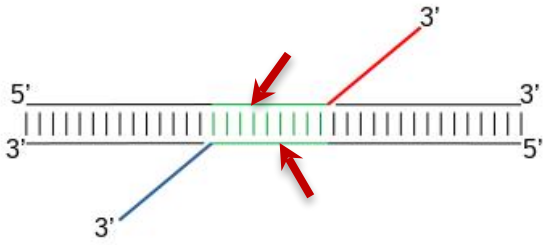
Motifs in peaks

Motif enrichment tools

Special considerations for ATAC-seq data analysis

- Paired end (PE) sequencing is recommended
- QC: fragment length distribution – mononucleosome peak should be evident
- QC: fraction of Mt reads – it can be high (up to 40%) – calculate sequencing depth accordingly
- For current data quality standards, refer to ENCODE; currently 25 million non-duplicate, non-mitochondrial aligned read pairs (i.e. 50M PE reads); non-redundant fraction >0.9; fraction of reads in called peak regions (FRiP) >0.3; TSS enrichment observed

- Peak calling
  - Genrich – peak caller dedicated to ATAC-seq data (has an ATAC-seq mode); PE data only
  - MACS2 – can be used BUT some adjustments are required to center fragments on the Tn5 insertion sites



Special considerations for ATAC-seq differential accessibility analysis: effect of normalisation

Methodology | Open Access | Published: 22 April 2020

ATAC-seq normalization method can significantly affect differential accessibility analysis and interpretation

Jake J. Reske, Mike R. Wilson & Ronald L. Chandler

Epigenetics & Chromatin **13**, Article number: 22 (2020) | Cite this article

doi:https://doi.org/10.1186/s13072-020-00342-y

Normalization benchmark of ATAC-seq datasets shows the importance of accounting for GC-content effects

Koen Van den Berge, Hsin-Jung Chou, Hector Roux de Bézieux, Kelly Street, Davide Risso, John Ngai, Sandrine Dudoit

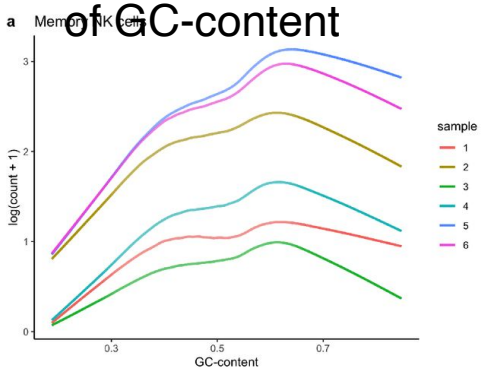
doi: https://doi.org/10.1101/2021.01.26.428252

- GC-content effects are omnipresent in ATAC-seq datasets;
- Since the GC-content effects are sample-specific, they can bias downstream analyses such as clustering and differential accessibility analysis;
- We introduce a GC aware normalization method;
- Our work clearly shows that accounting for GC-content effects in the normalization is crucial for common downstream ATAC-seq data analyses.

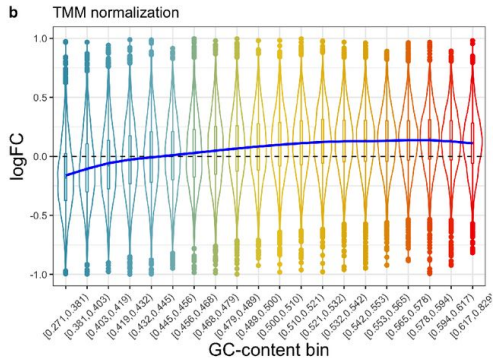
doi: https://doi.org/10.1101/2021.01.26.428252

Special considerations for ATAC-seq differential accessibility analysis: effect of normalisation

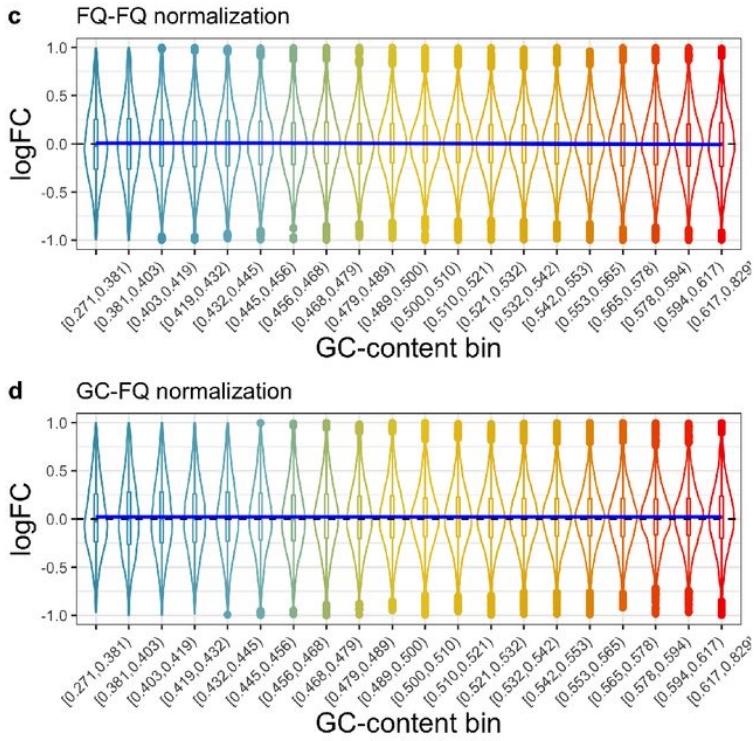
log-count as a function of GC-content



TMM normalisation



GC aware normalisation



Differential accessibility
log-fold change in bins by GC content

A bias for peaks with low and high GC-content
(in a null setting, LFC should be centered around zero)

doi: https://doi.org/10.1101/2021.01.26.428252

Resources

R/Bioconductor workflows

- https://seandavi.github.io/AtacSeqWorkshop/articles/Workflow.html
- https://rockefelleruniversity.github.io/RU_ATAC_Workshop.html
- https://github.com/databio/awesome-atac-analysis

Galaxy workflows

- https://training.galaxyproject.org/training-material/

Biocondutor packages

- ATACseqQC
- esATAC
- ALPS