Other chromatin capture/targeted sequencing

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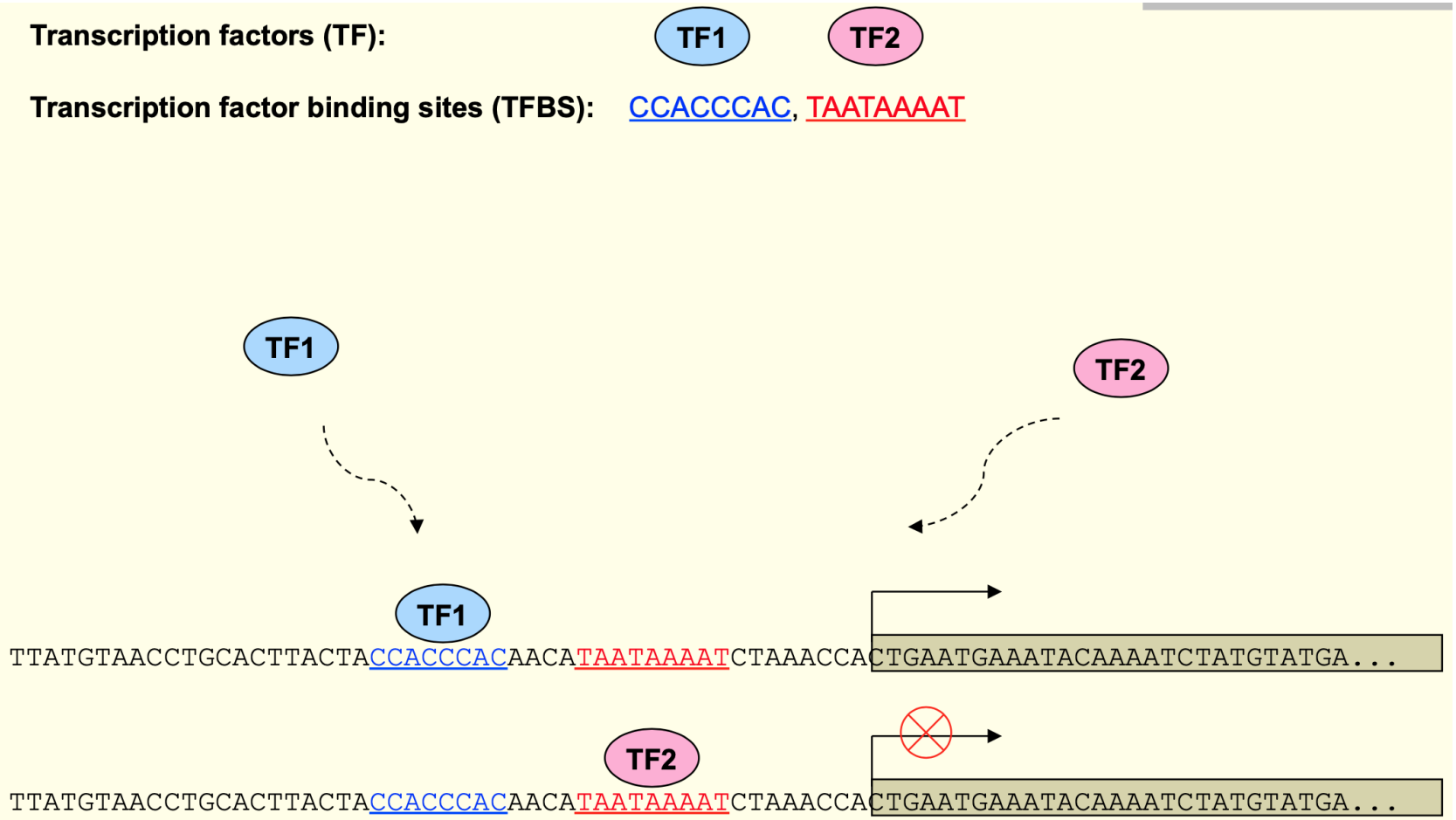
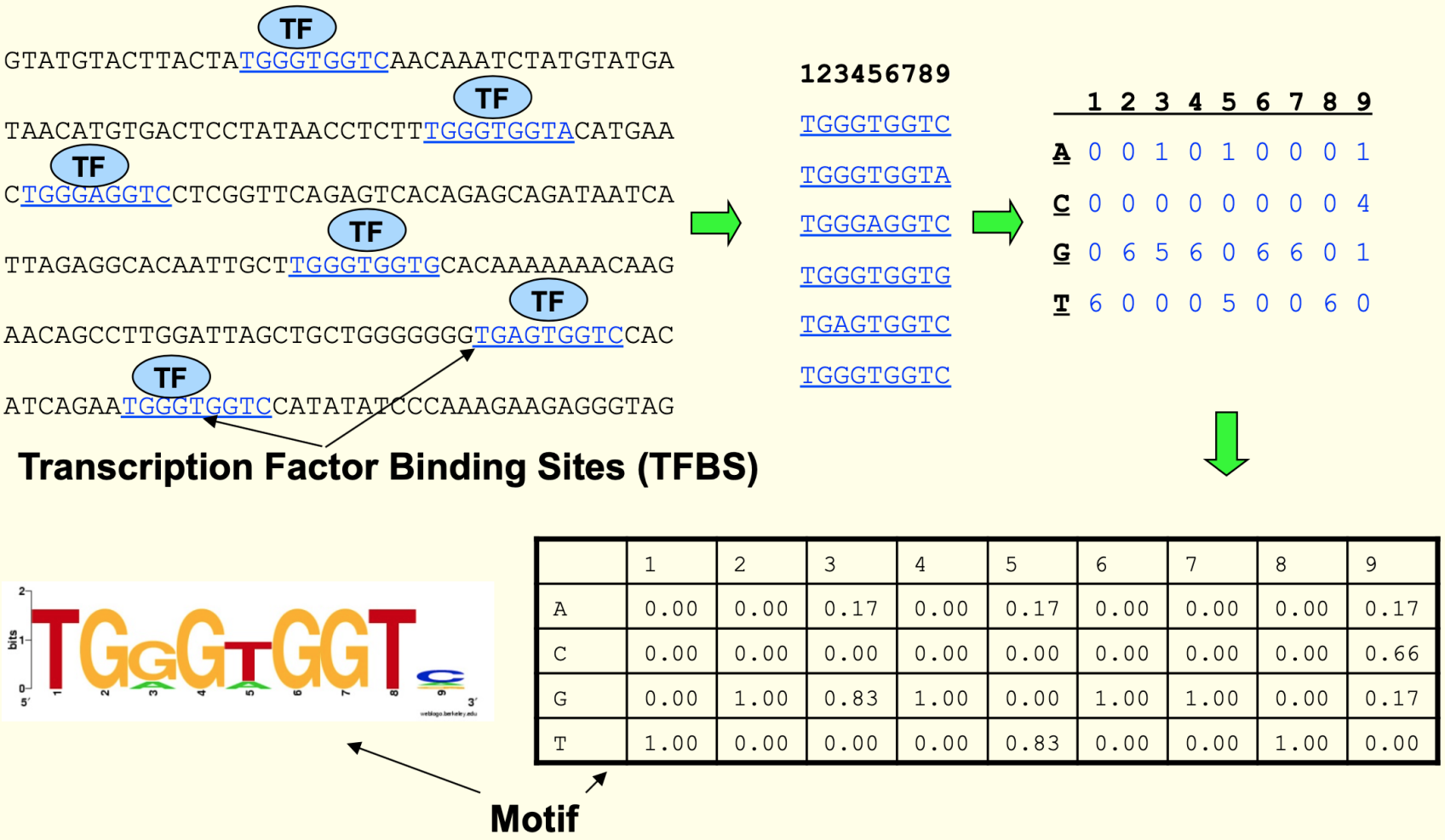
Transcription factor binding regulates gene expression



http://www.biostat.jhsph.edu/~khansen/teaching/2014/140.668/GeneRegulation.pdf

Transcription factors recognize specific motifs



	1	2	3	4	5	6	7	8	9
A	0.00	0.00	0.17	0.00	0.17	0.00	0.00	0.00	0.17
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.66
G	0.00	1.00	0.83	1.00	0.00	1.00	1.00	0.00	0.17
T	1.00	0.00	0.00	0.00	0.83	0.00	0.00	1.00	0.00

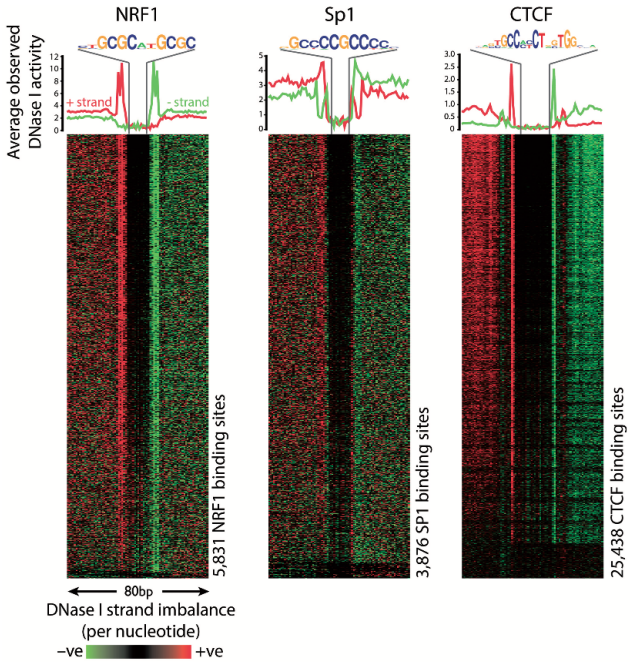
http://www.biostat.jhsph.edu/~khansen/teaching/2014/140.668/GeneRegulation.pdf

Other "captured/targeted" sequencing technologies

- Enrich and then sequence selected genomic regions.
  - **DNase-seq**, **MNase-seq**, **FAIRE-seq**, **ATAC-seq**: detect open chromatin sites.
  - **CUT&RUN**, **CUT&TAG**: improved ChIP-seq profiling.
  - **MeDIP-seq**: measure methylated DNA.
  - **GRO-seq**: map the position, amount and orientation of transcriptionally engaged RNA polymerases.
  - **Ribo-seq**: detect ribosome occupancy on mRNA. This is captured RNA-seq.

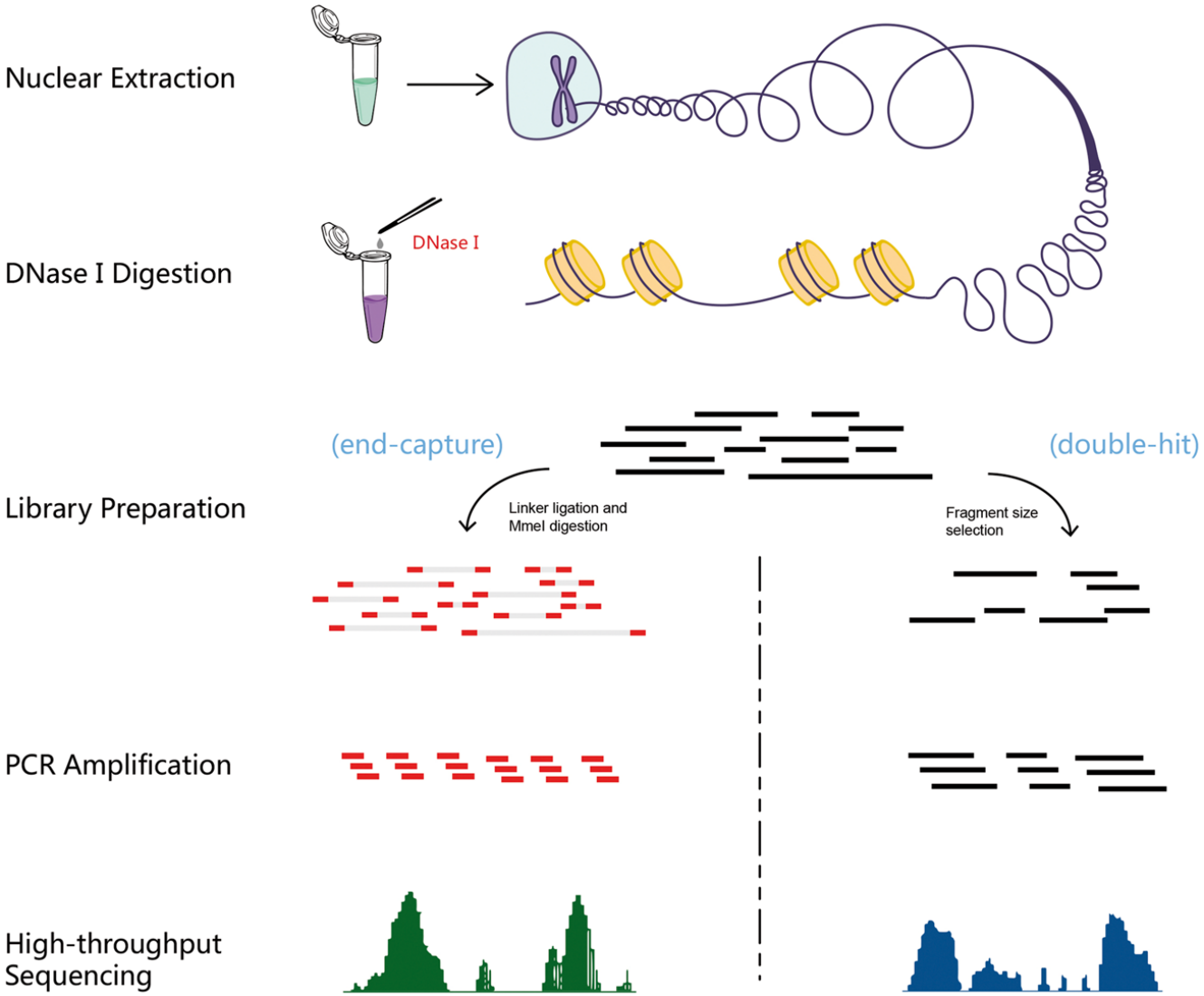
DNAse-seq

- A widely used approach in gene regulation studies uses DNase I as a tool to identify DNase I Hypersensitive Sites (DHSs) within chromatin
- DHSs represent open chromatin regions that are normally only accessible at sites of active regulatory elements such as transcriptional enhancers



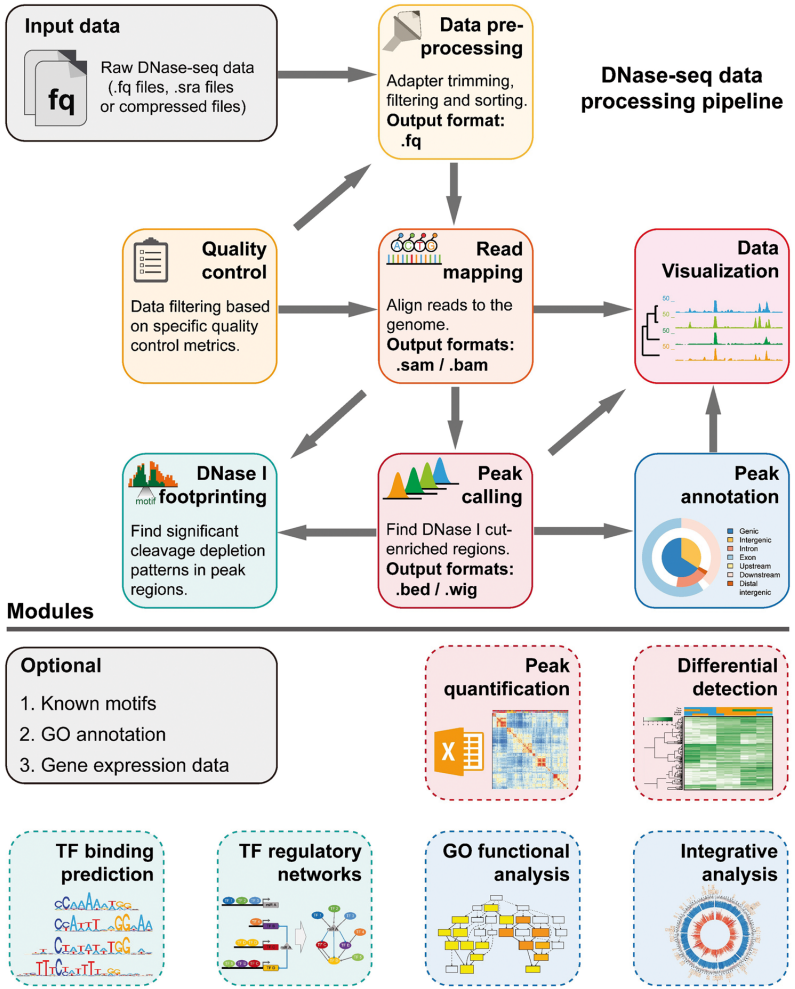
Cockerill,P.N. (2011) Structure and function of active chromatin and DNase I hypersensitive sites. FEBS J., 278, 2182–2210.

Overview of DNase-seq experimental protocols



Yongjing Liu et al. A practical guide for DNase-seq data analysis: from data management to common applications, September

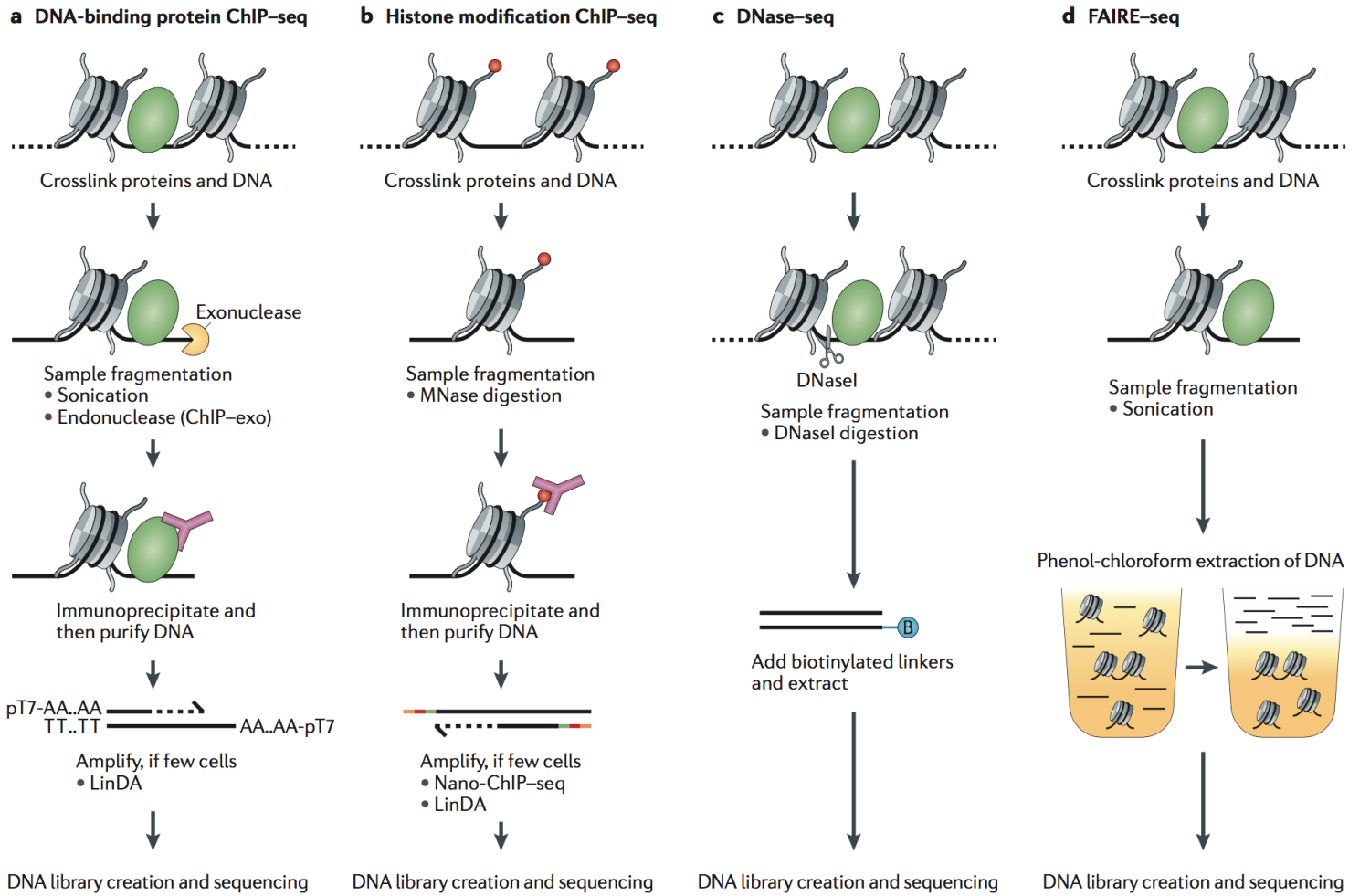
General analysis pipeline for sequence-tag experiments



Other technologies to assess open chromatin

- **FAIRE-seq** - Formaldehyde-Assisted Isolation of Regulatory Elements followed by sequencing.
- **MNase-seq** - Micrococcal Nuclease-assisted assessment of open chromatin.
- **ATAC-seq** - Assay for transposase- accessible chromatin using sequencing. Tn5 transposase is used to transpose sequencing adapters into the genomic DNA

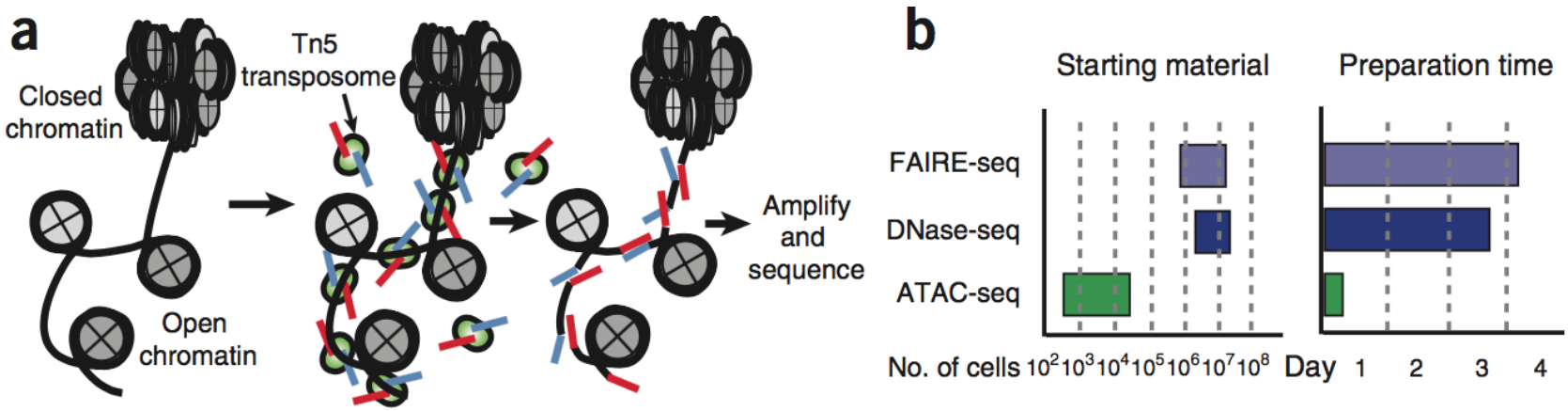
Comparison of experimental protocols



https://zhonglab.gitbook.io/3dgenome/chap2-experiment-tools-for-exploring-genome-interaction/2.2-primary-order

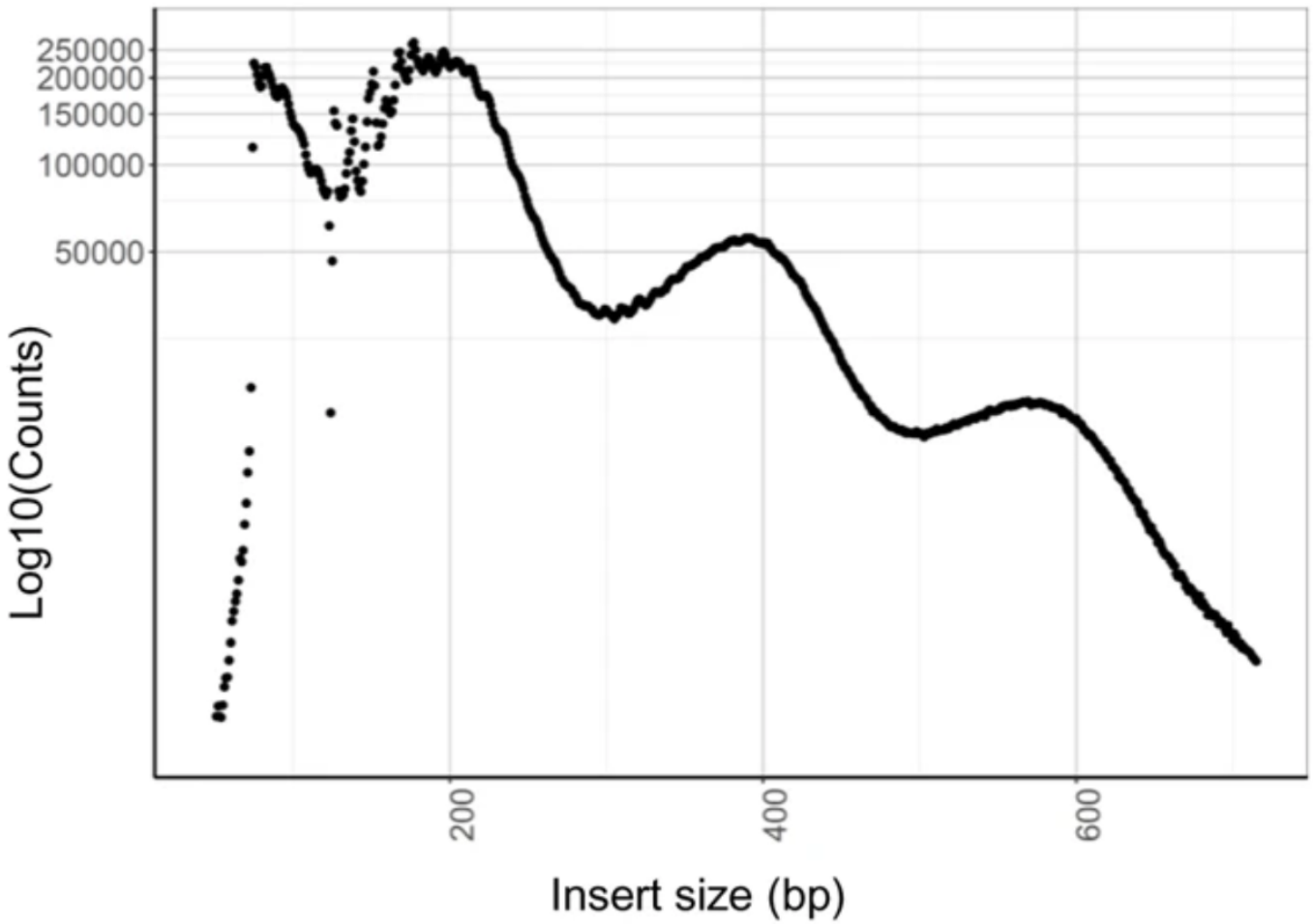
ATAC-seq: finding open chromatin regions

- ATAC-seq is an ensemble measure of open chromatin that uses the prokaryotic Tn5 transposase to tag regulatory regions by inserting sequencing adapters into accessible regions of the genome



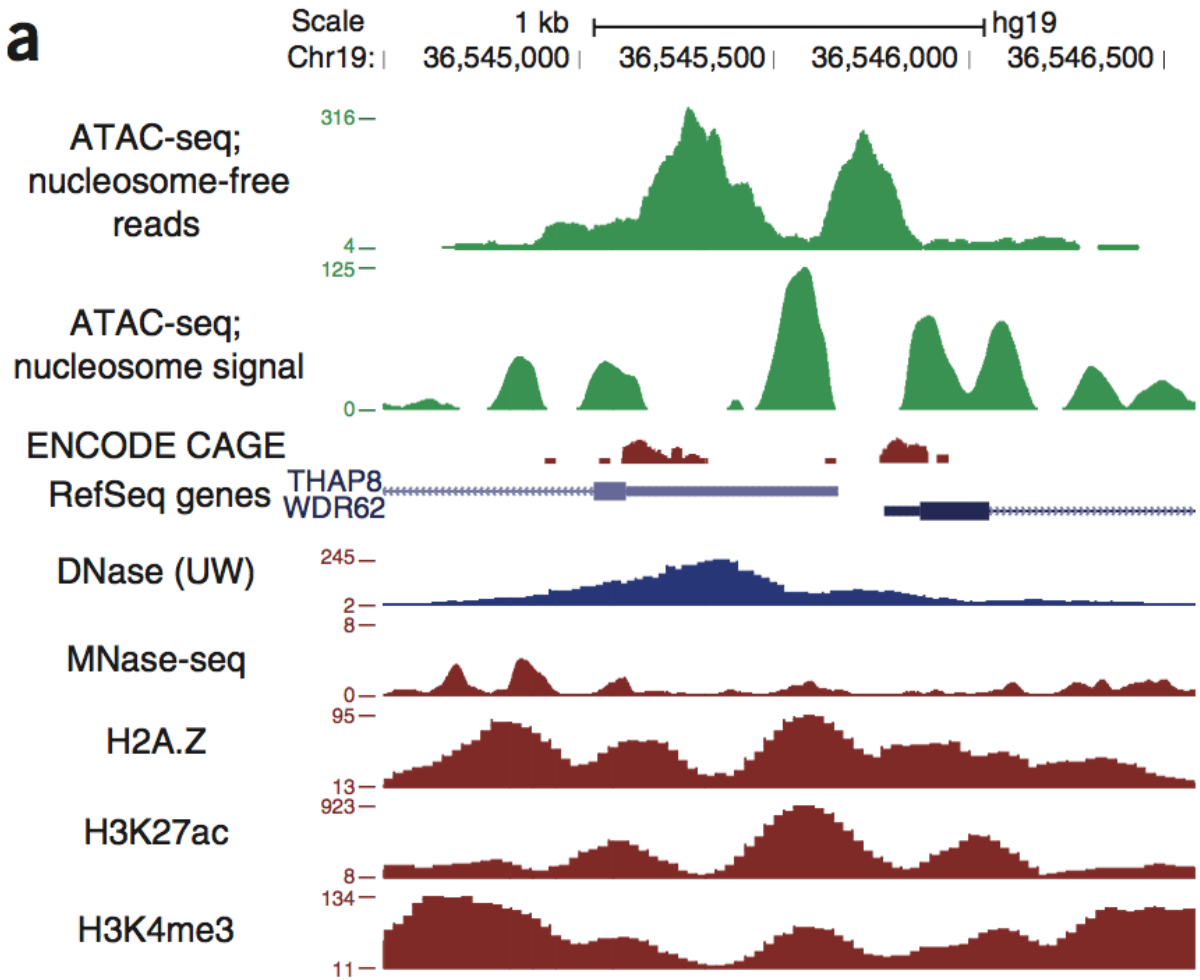
Jason D Buenrostro et al., "Transposition of Native Chromatin for Fast and Sensitive Epigenomic Profiling of Open Chromatin, DNA-Binding Proteins and Nucleosome Position," Nature Methods 10, no. 12 (December 2013): 1213–18, https://doi.org/10.1038/nmeth.2688.

ATAC-seq: revealing nucleosome positioning



https://www.nature.com/articles/s41598-019-44076-8

ATAC-seq: finding open chromatin regions



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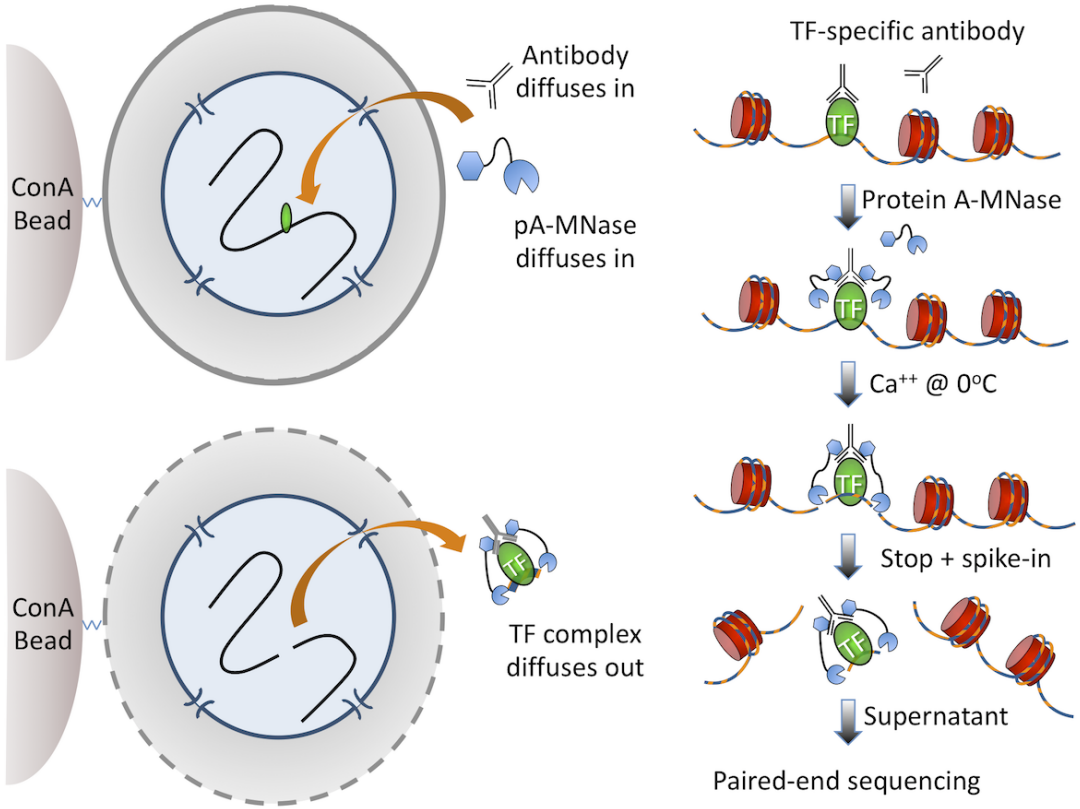
CUT&RUN

- Cleavage Under Targets and Release Using Nuclease
- Antibody-targeted controlled cleavage by micrococcal nuclease



https://data.4dnucleome.org/experiment-types/cut-n-run/

CUT&TAG

- Cleavage Under Targets and Tagmentation
- Tn5 transposase conjugated with adapters inserts them directly into cut sequences

CUT&Tag vs. CUT&RUN vs. ChIP-Seq

	CUT&Tag	CUT&RUN	ChIP-Seq
Performed Under Native Conditions?	Yes	Yes	No
Chromatin Fragmentation Method	Tn5-based tagmentation	MNase digestion	Sonication
Cell Number Requirements	5,000-500,000 cells	500,000 cells	1-10 million cells
Sequencing Depth Required *	2 million reads	8 million reads	20-50 million reads
Integrated Library Preparation?	Yes, uses tagmentation	No, separate library prep required	No, separate library prep required
Compatible Targets	Primarily histone modifications, some transcription factors and co-factors	Wide range of histone modifications, transcription factors, and co-factors	Wide range of histone modifications, transcription factors, and co-factors
Workflow Length	1-2 days	1-2 days	2-3 days

* Kaya-Okur et al. *Nature Communications* (2019) 10:1930

https://www.activemotif.com/blog-cut-tag

More

- ChIP-seq analysis notes from Ming Tang, https://github.com/crazyhottommy/ChIP-seq-analysis
- Notes on ChIP-seq and other-seq-related tools, https://github.com/mdozmorov/ChIP-seq_notes