Introduction to ATAC-seq

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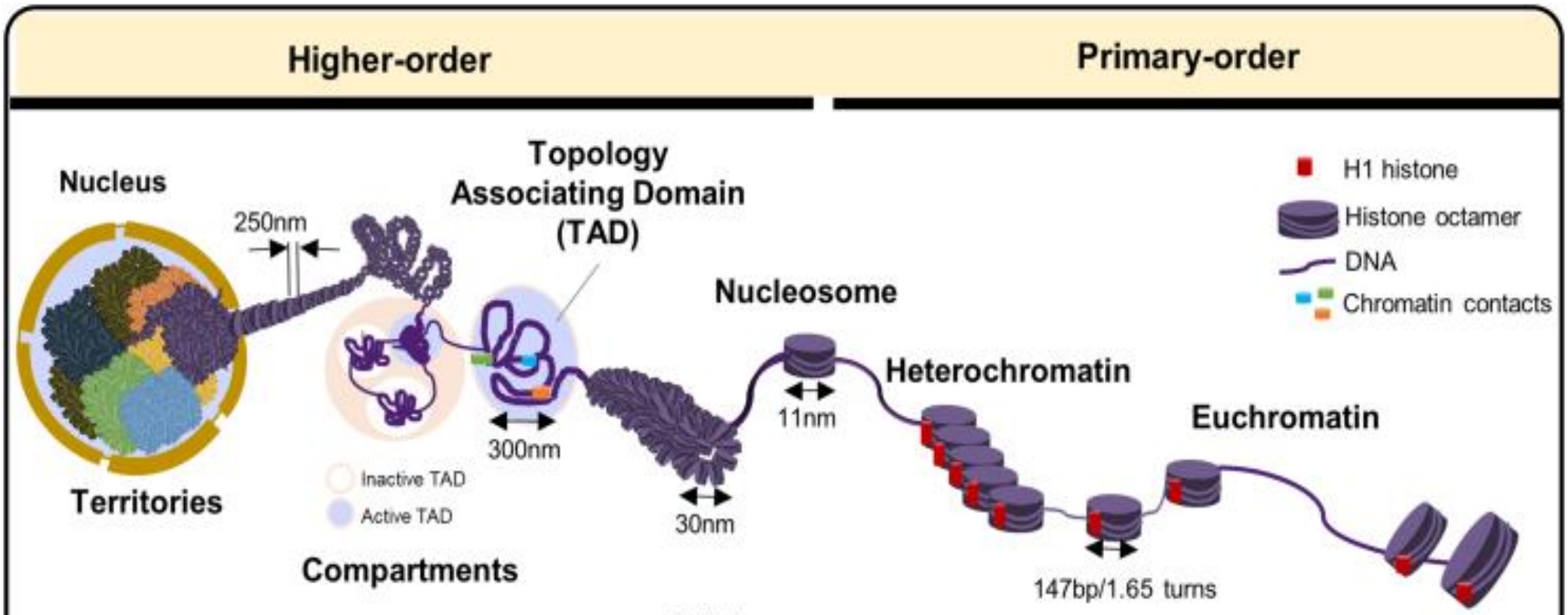


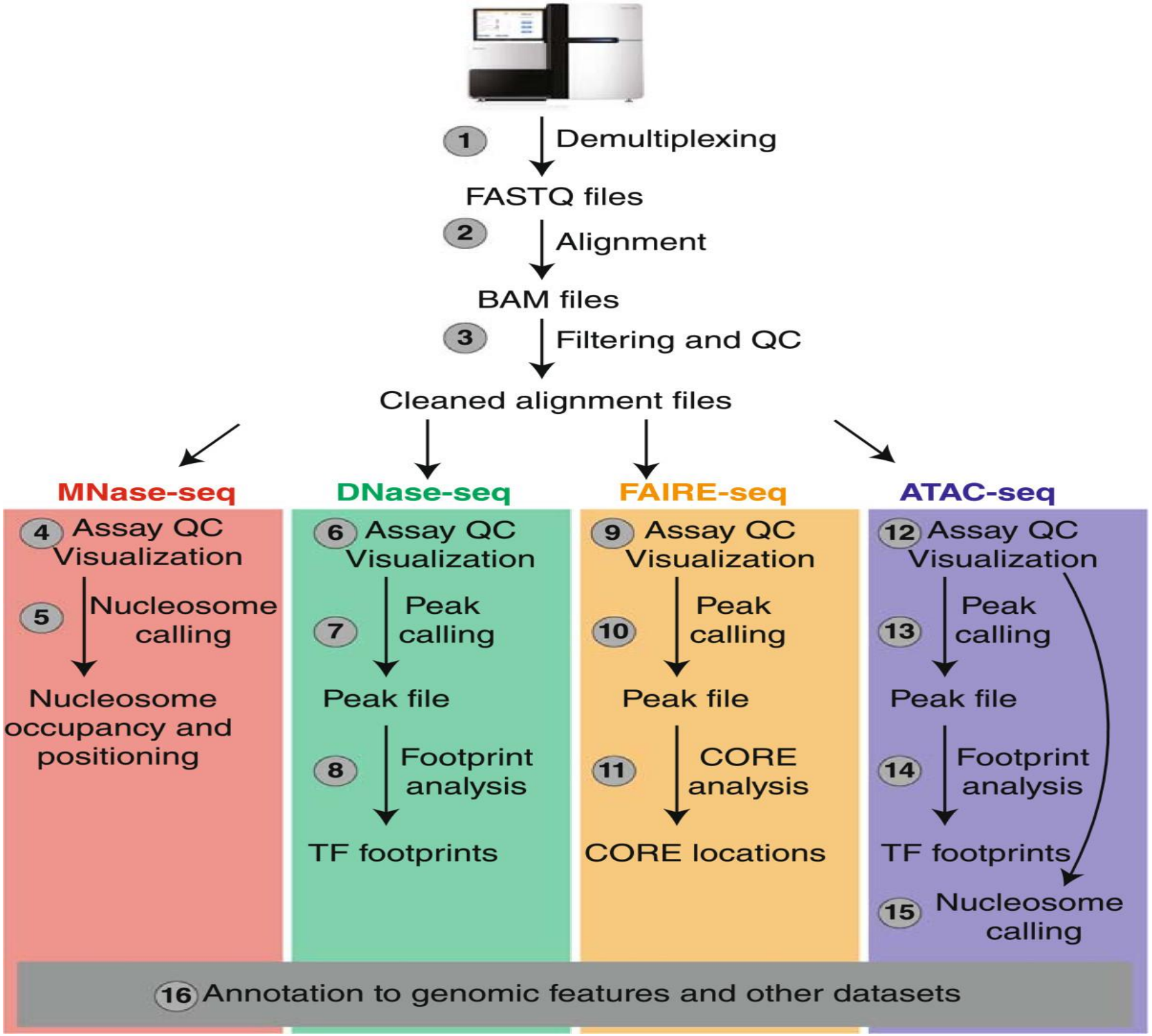
Techniques

Validation

4C	5C	Hi-C	ChIP, Imaging etc.	MNase-seq	DNase-seq	FAIRE-seq	ATAC-seq
1-vs-Many	*Many-vs-Many*	*All-vs-All*		*Inferred closed regions*	*Open chromatin*	*Open chromatin*	*Open/Closed chromatin*

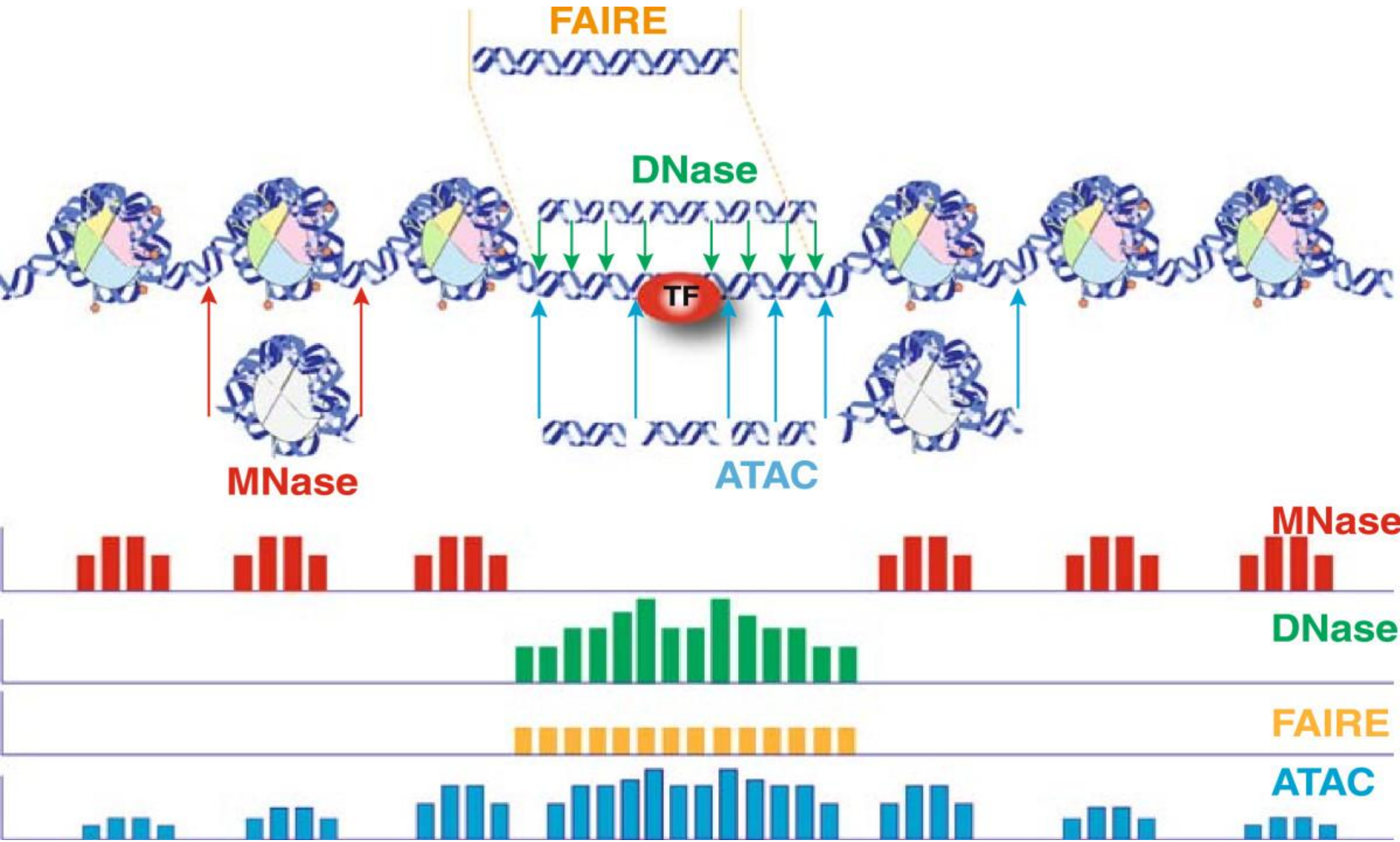
Procedure

Experimental	Computational	Experimental	Computational
1. Crosslinking	1. Alignment	1. Crosslinking*	1. Size-selection
2. Fragmentation	2. Filtering	2. Fragmentation	2. Alignment
3. Ligation	3. Binning	3. Size-selection	3. Peak calling
4. Detection	4. Normalization	4. Sequencing	4. Normalization
	5. Identifying interactions		5. Visualization
	6. Visualization		



Tsompana and Buck, *Epigenetics & Chromatin* 2014

Assay for Transposase Accessible Chromatin

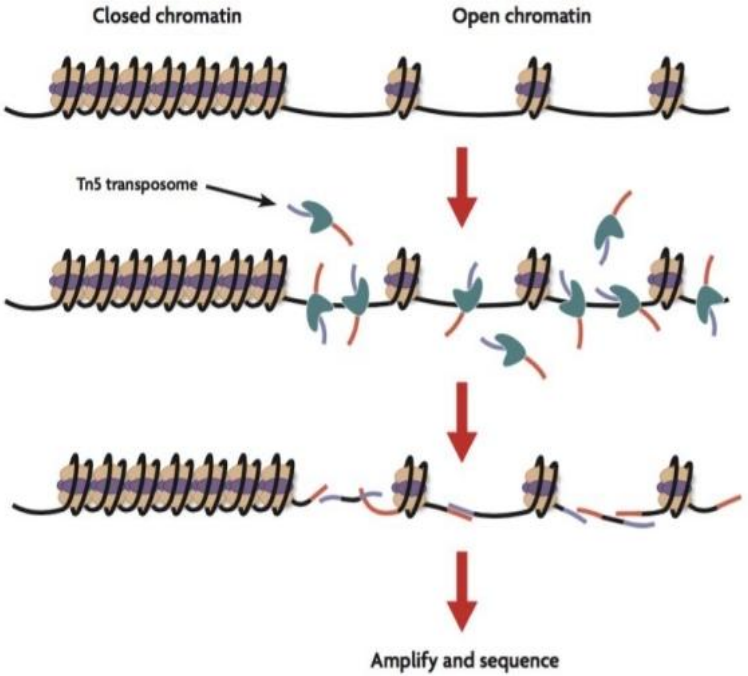


Tsompana and Buck, *Epigenetics & Chromatin* 2014

ATAC-seq

- Measure chromatin accessibility and nucleosome free regions
- Can also detect nucleosome packing, positioning and TF footprints

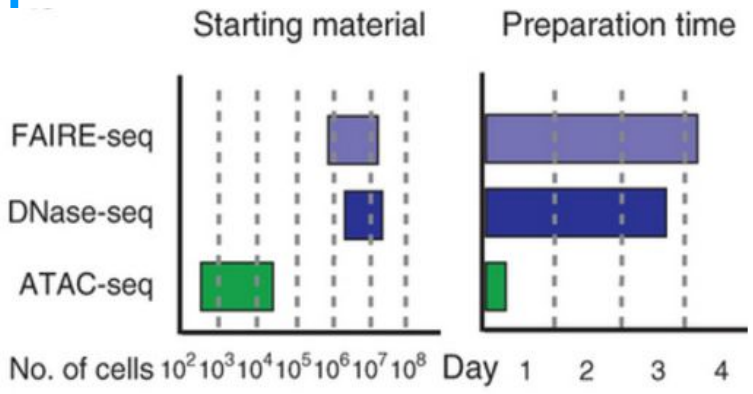
- Does not require sonication and phenol chloroform extractions, antibodies or sensitive enzymatic digestions that can introduce potential bias
- A hyperactive Tn5 transposase is used to fragment DNA and integrate into active regulatory regions
- During ATAC-seq, 500–50,000 unfixed nuclei are tagged *in vitro* with sequencing adapters by Tn5 transposase

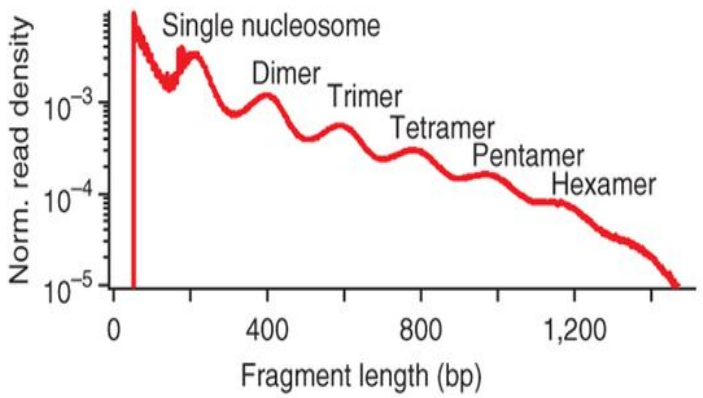


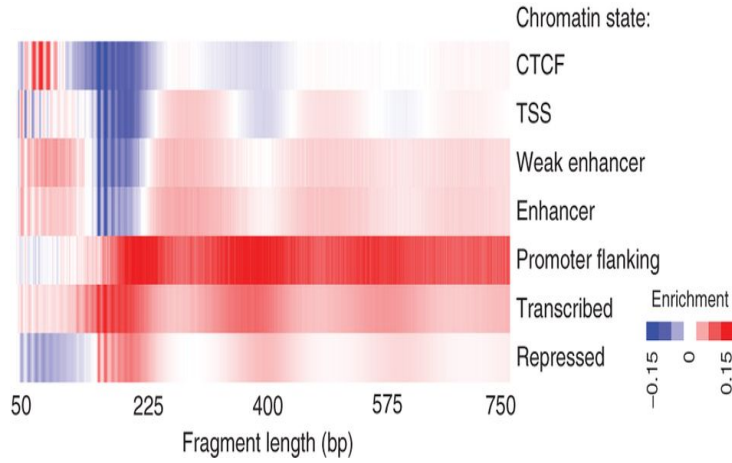
JD Buenrostro et al, Nature Methods, 2013.

ATAC-seq

- Two-step protocol
  - Insertion of Tn5 transposase with adaptors
  - PCR amplification
- Needs ~500-50,000 cells
- Paired-end reads produce information about nucleosome positioning
- Insert size distribution of fragments has a periodicity of ~200 bp, suggesting that fragments are protected by multiples of nucleosomes
- Different fragmentation patterns can be associated with different functional states (eg. TSSs are more accessible than promoter flanking or transcribed regions)

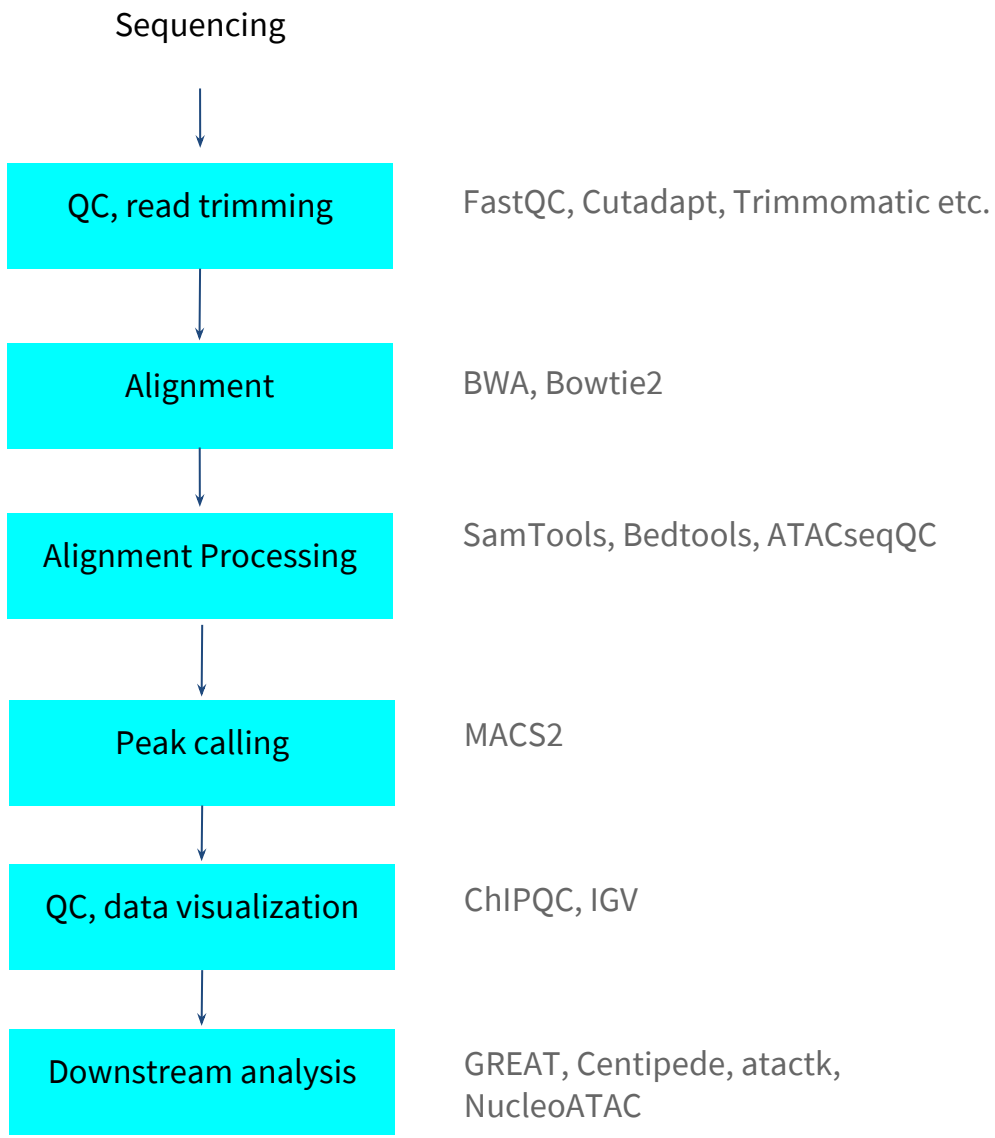






[1] JD Buenrostro et al, *Nature Methods*, 2013.

Workflow of ATAC-seq data processing



Working with ATAC-seq

- Remove mitochondrial reads
  - A large fraction of ATAC-seq reads map to the mitochondrial genome (up to 40-60%)
- Remove blacklisted regions before peak calling
  - hg19 or hg38 blacklisted regions can be obtained from ENCODE

- The signal (open chromatin where the transposase was inserted) is a mixture of feature types:
  - Short fragments - signal from nucleosome free regions (NFRs) and open regions around DNA bound transcription factors. TSS rich.
  - Longer fragments - open regions from around nucleosomes. Includes +1 and -1 nucleosome positions

Differences from ChIP-seq data processing

- Peak calling: Use fragment size for smoothing when calling peaks with MACS2
- Alternatively, to identify open regions following MACS parameters are used:

  MACS2 callpeak -t bamfile --nomodel --shift -100 --extsize 200 --format BAM -g hg38

  For nucleosome occupancy shift and extension can centre the signal on nucleosomes (147 bp DNA is wrapped in a nucleosome)

  MACS2 callpeak -t bamfile --nomodel --shift 37 --extsize 73 --format BAM -g hg38

  Optionally, for paired end data

  MACS2 callpeak -t bamfile --format BAMPE -g hg38

  - --nomodel: don't build shifting model
  - --shift: when this value is negative, ends will be moved toward 3'->5' direction
  - --extsize: extend reads in 5'->3' direction to fix-sized fragments

Normalization and Differential Accessibility of ATAC-seq

- Normalisation across samples might be needed
  - Efficiency of the ATAC-seq protocol in assaying open regions is affected by how many transposons get into the nuclei
  - One normalisation solution is to use signal from 'essential or housekeeping genes'. see: *Sarah K. Denny et al, Cell, 2016.*

- THOR is an HMM-based approach to detect and analyze differential peaks in two sets of ChIP-seq data from distinct biological conditions with replicates
  - Normalizes bam files (given a bed file of housekeeping genes)
  - Can compare two ATAC-seq datasets to perform differential openness analysis

Cutting site and TF Footprinting analysis

- ATAC-seq produces shorter fragments around smaller protected regions such as TF bound regions.
- Cut sites pileup signal is a good guide to TF binding sites
- Shifting reads +4/-5 nt depending on strand, should adjust for expected shift from Tn5 insertion.
- TF footprinting of these regions enables the detection of motifs for bound TFs
  - Centipede
  - msCentipede
  - PIQ
  - Mocap