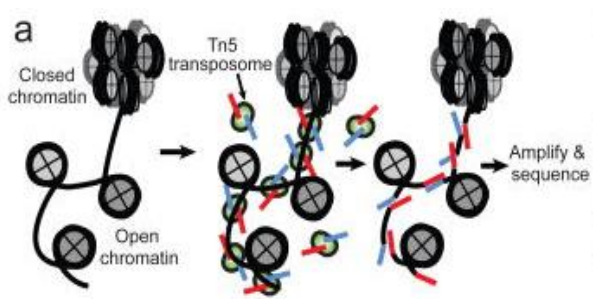
snATAC-seq

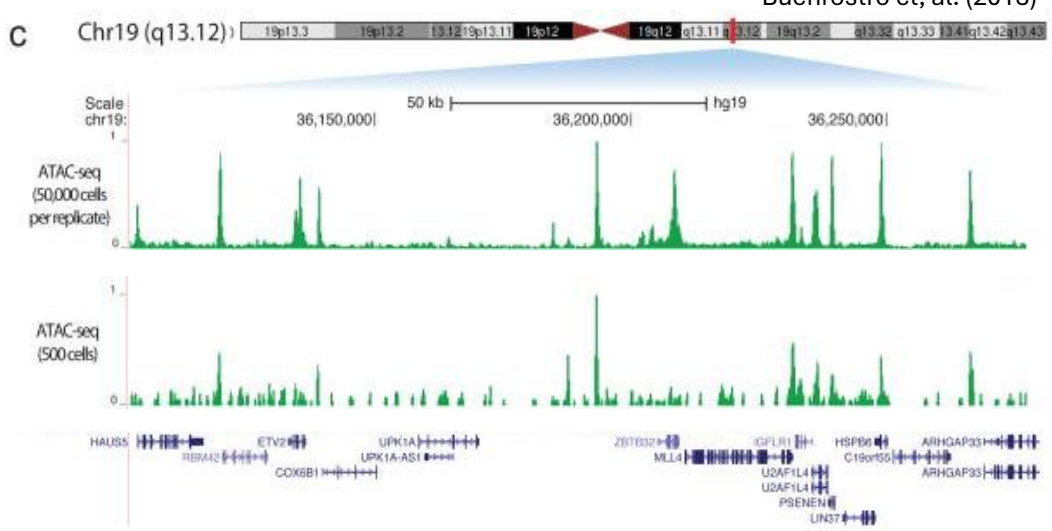
Signac

- 10x single-nucleus Multiome, for ATAC library part still uses a Tn5 transposase.

- Tn5 cuts and tags open chromatin, while 10x barcoded gel beads capture nuclear RNA and transposed DNA fragments from the same nucleus inside the same GEM droplet.



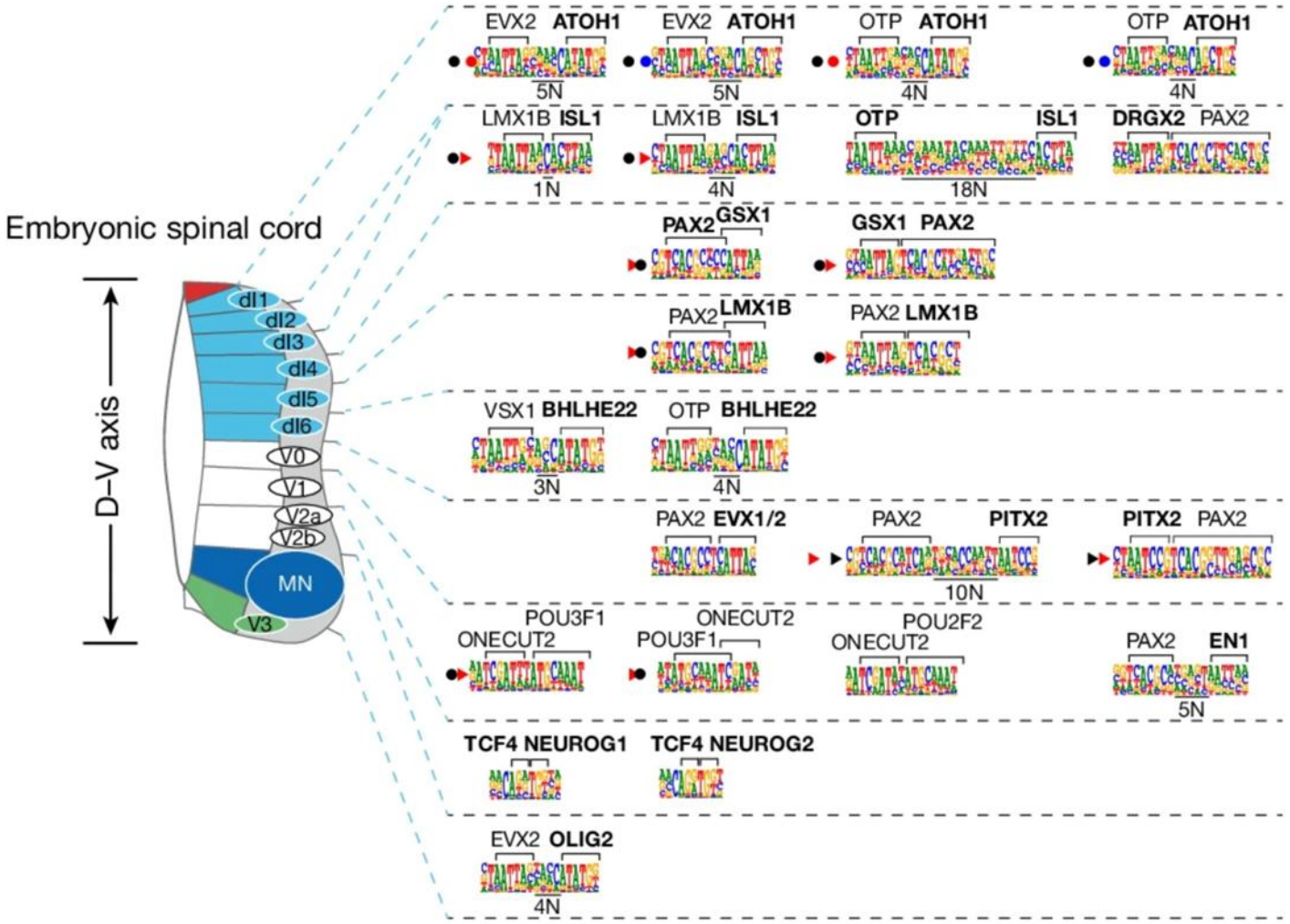
Buenrostro et, al. (2013)



- Sensitive open chromatin detection is maintained even when using 5,000 or 500 human nuclei.

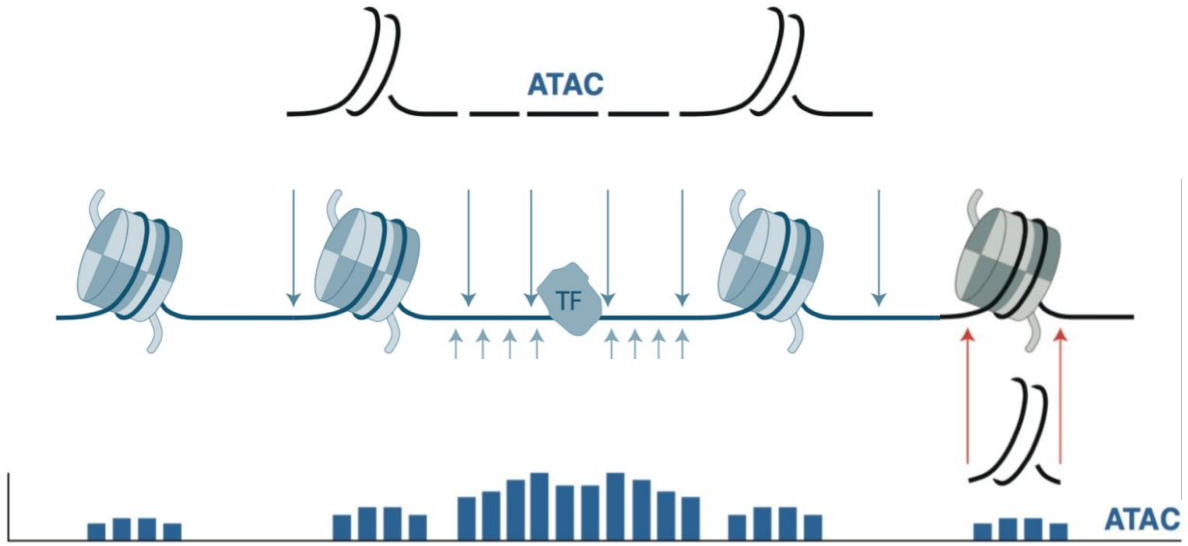
- Low coverage per peak: It means fewer DNA fragments are detected at each genomic region

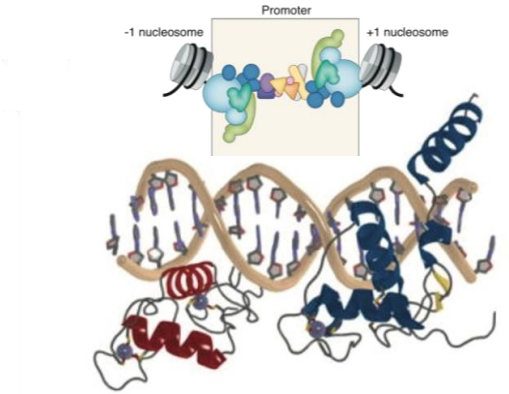
A motif is a short sequence recognized by a Regulator

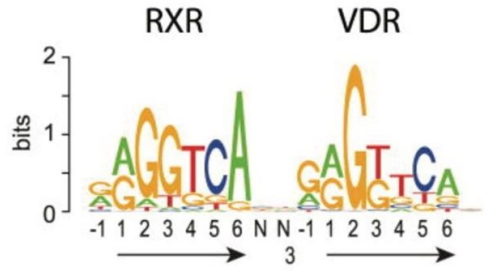


Motifs can be found in regulatory regions (promoters, enhancers, introns, or other)

Transcription factors are recruited (directly) to DNA motifs

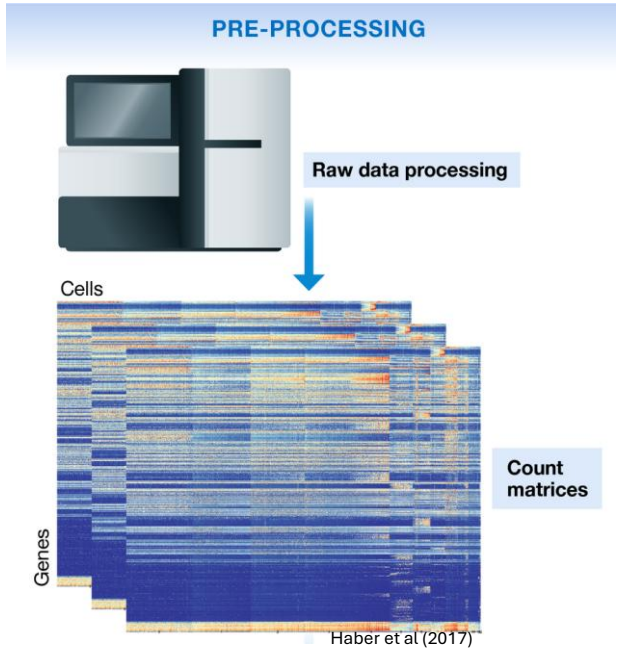






Pairs of transcription factors can bind DNA together and form a regulatory code. Example: RXR y VDR (half-sites).

In a motif logo, numbers like -1, 1, 2, 3, etc. are positions inside the transcription factor binding site.



10x Multiome **H5** file

Gene Expression matrix

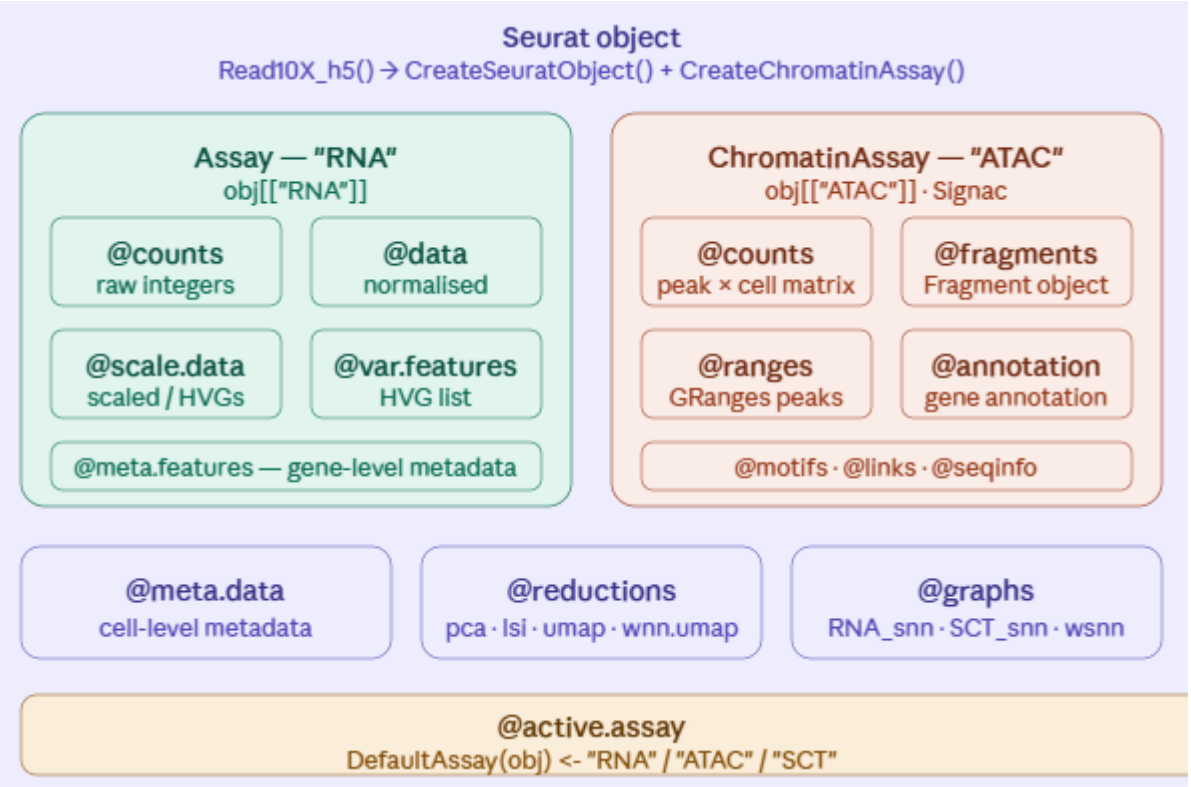
	AAAC-1	AAAG-1	AACT-1
OTX2	5	0	3
MITF	2	1	0
RPE65	0	4	6

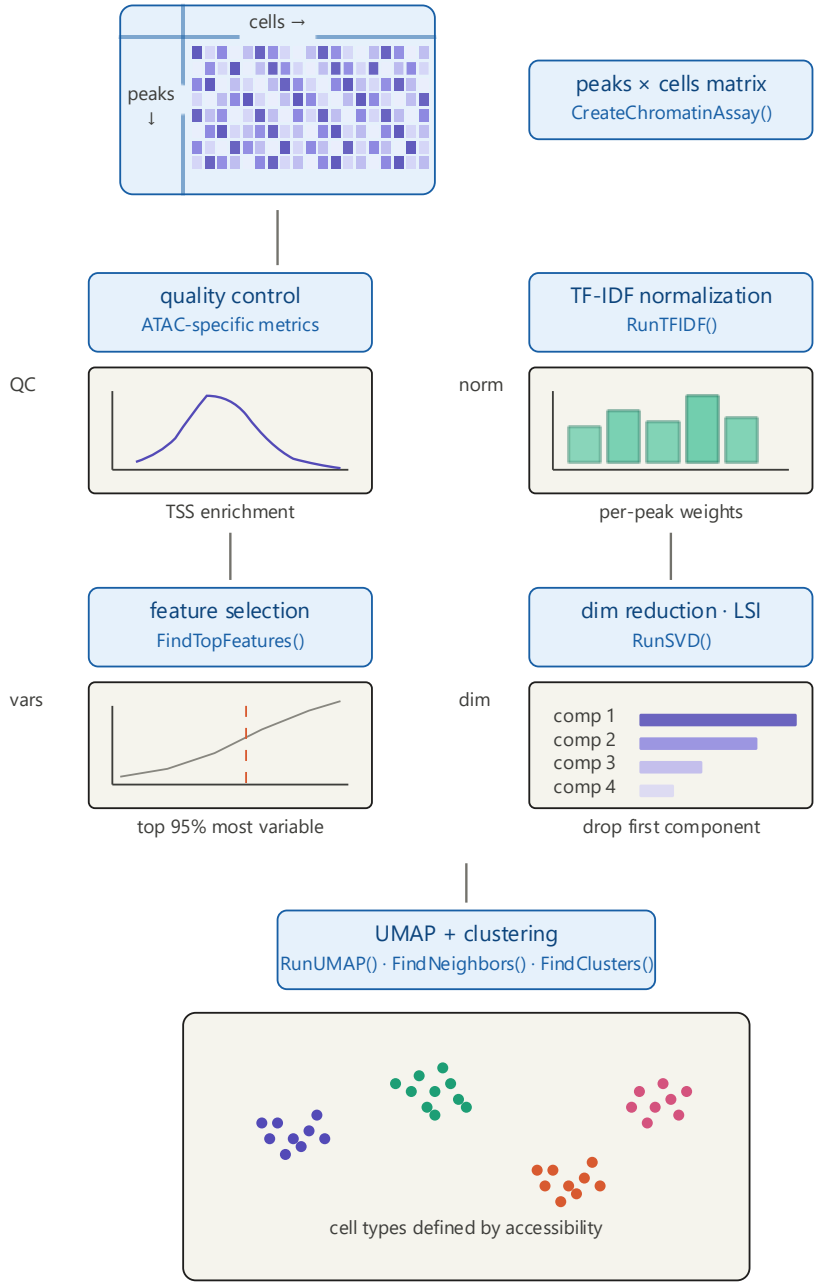
Peaks matrix

	AAAC-1	AAAG-1	AACT-1
chr1-1000-1500	2	0	1
chr1-9000-9500	0	3	0
chr2-20000-20500	1	1	4

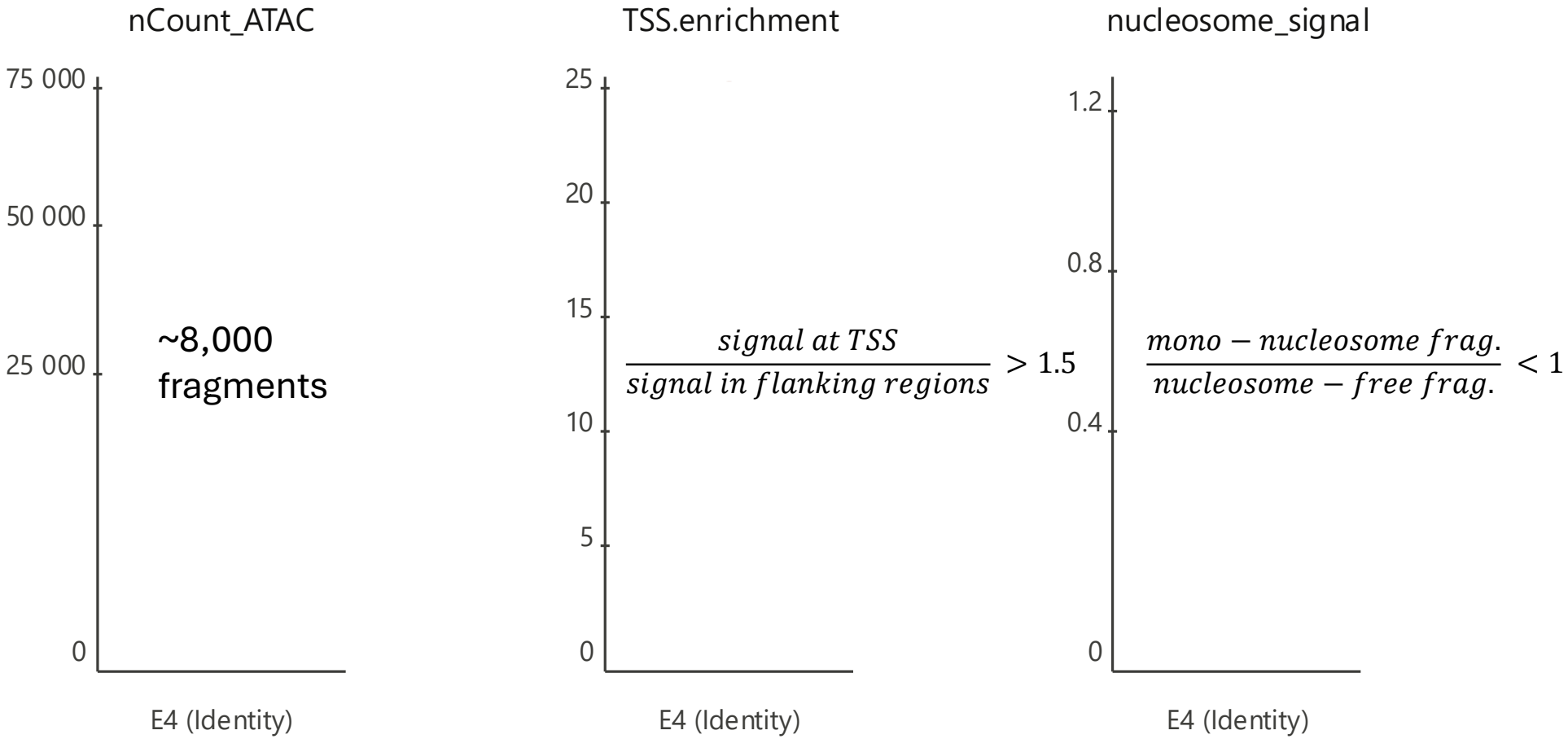
- Feature-barcode **matrix** with two feature types: Gene Expression and Peaks
- Count matrices have the dimension:
  number of **feature** (number of transcripts, etc) x **barcodes**.
- En Seurat or Signac, containers are organized by keys.

With Signac, we can connect chromatin accessibility with gene regulation





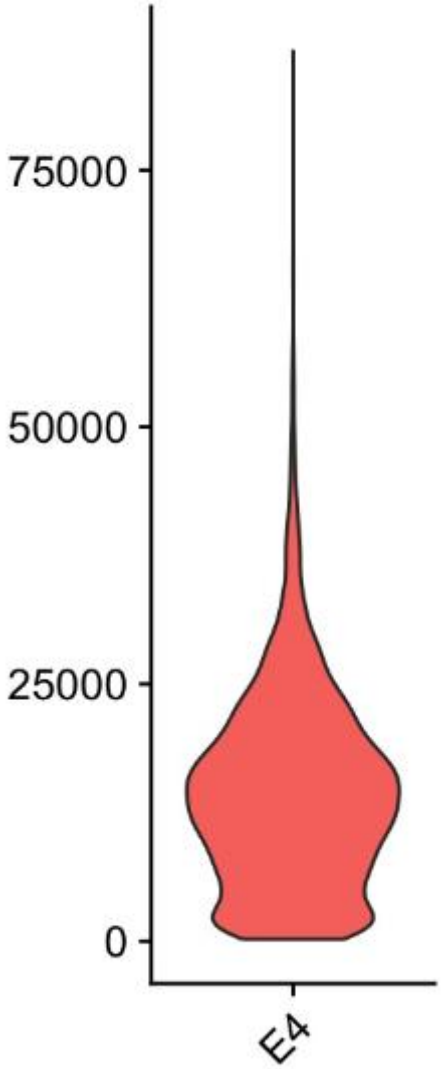
QC violin panel for scATACseq



500/1k < nCount_ATAC < 50000/1k

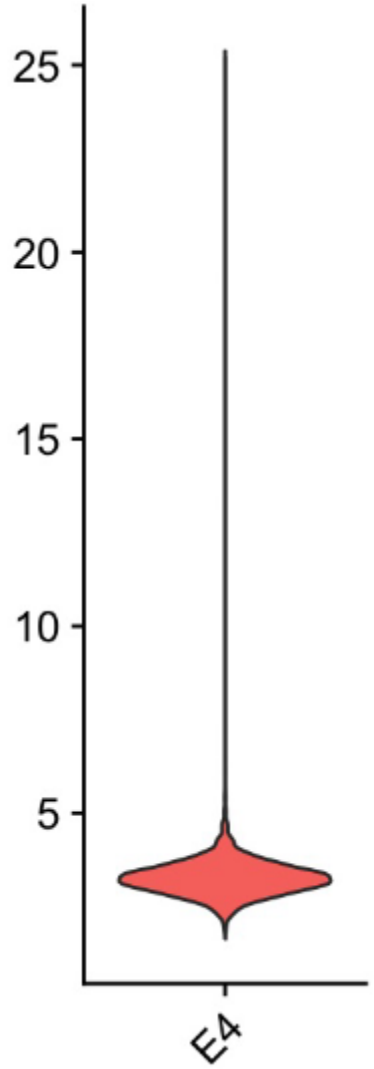
Total fragments means how many ATAC DNA pieces we detect from one nucleus.

Total number of fragments



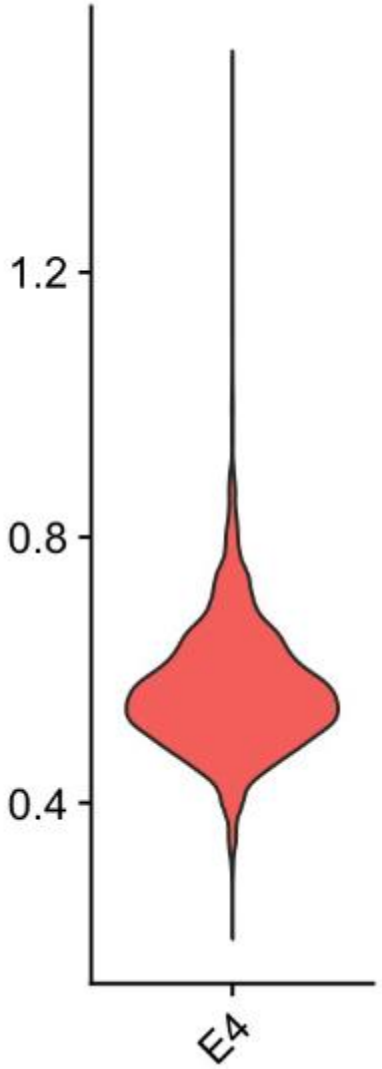
nCount_ATAC

Ratio of fragments at TSS vs flanking regions

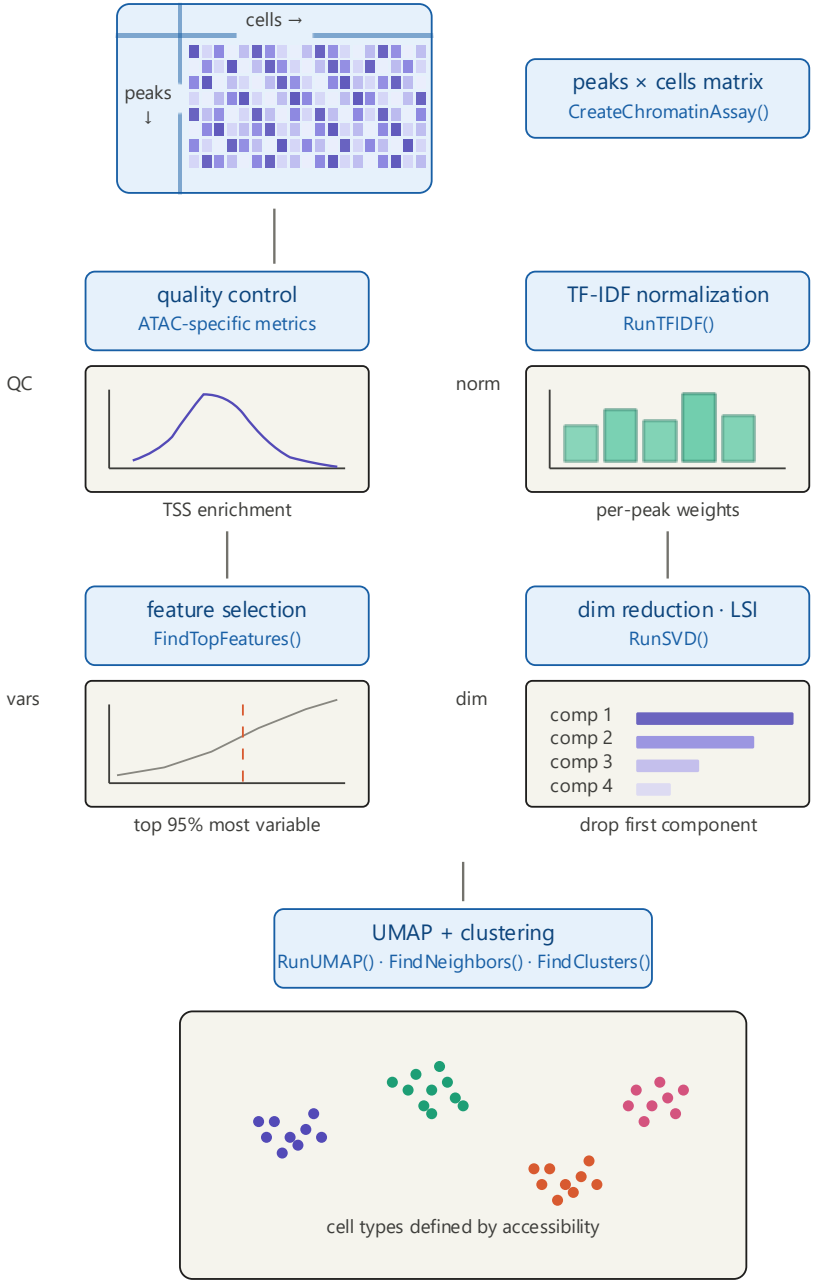


TSS.enrichment

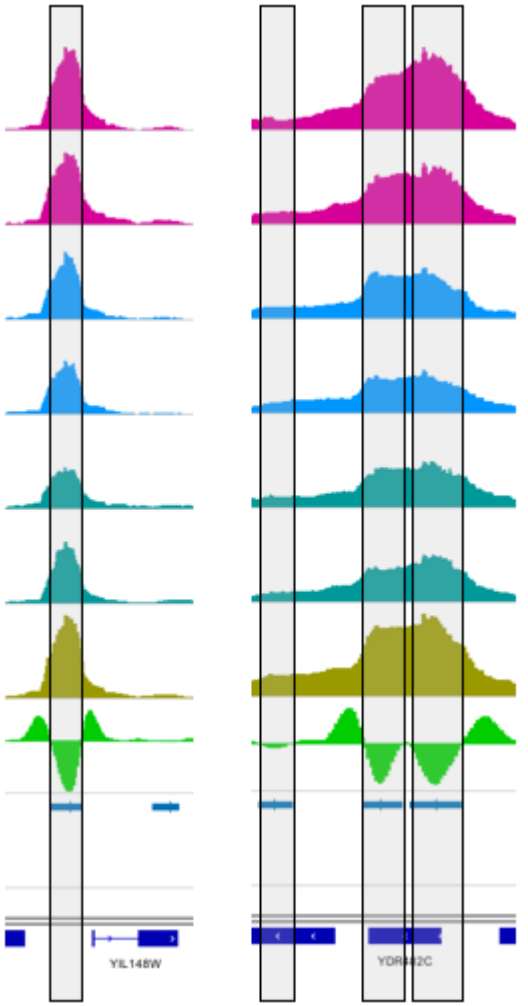
Ratio of mono-nucleosome vs nucleosome-free fragments



nucleosome_signal



Identify accessible/differential peaks



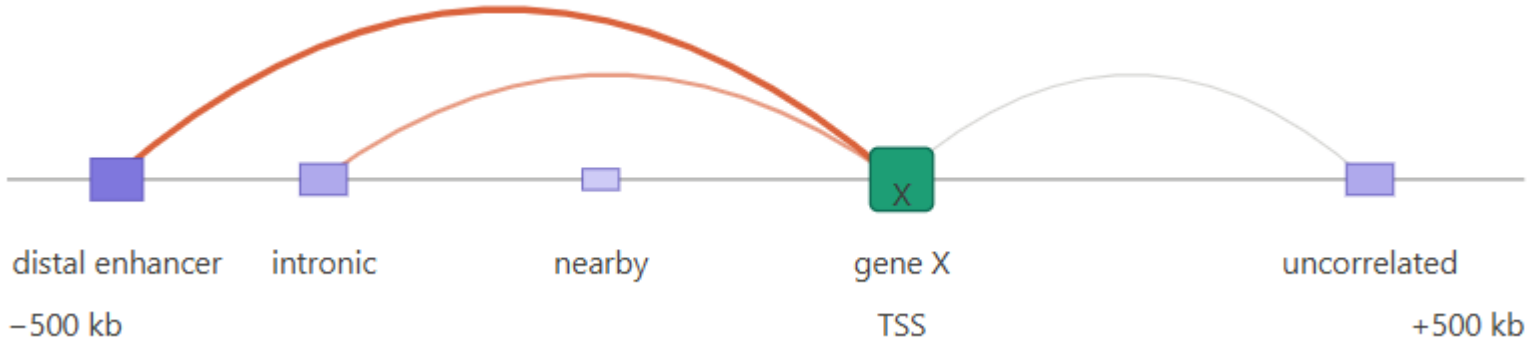
Sample 1

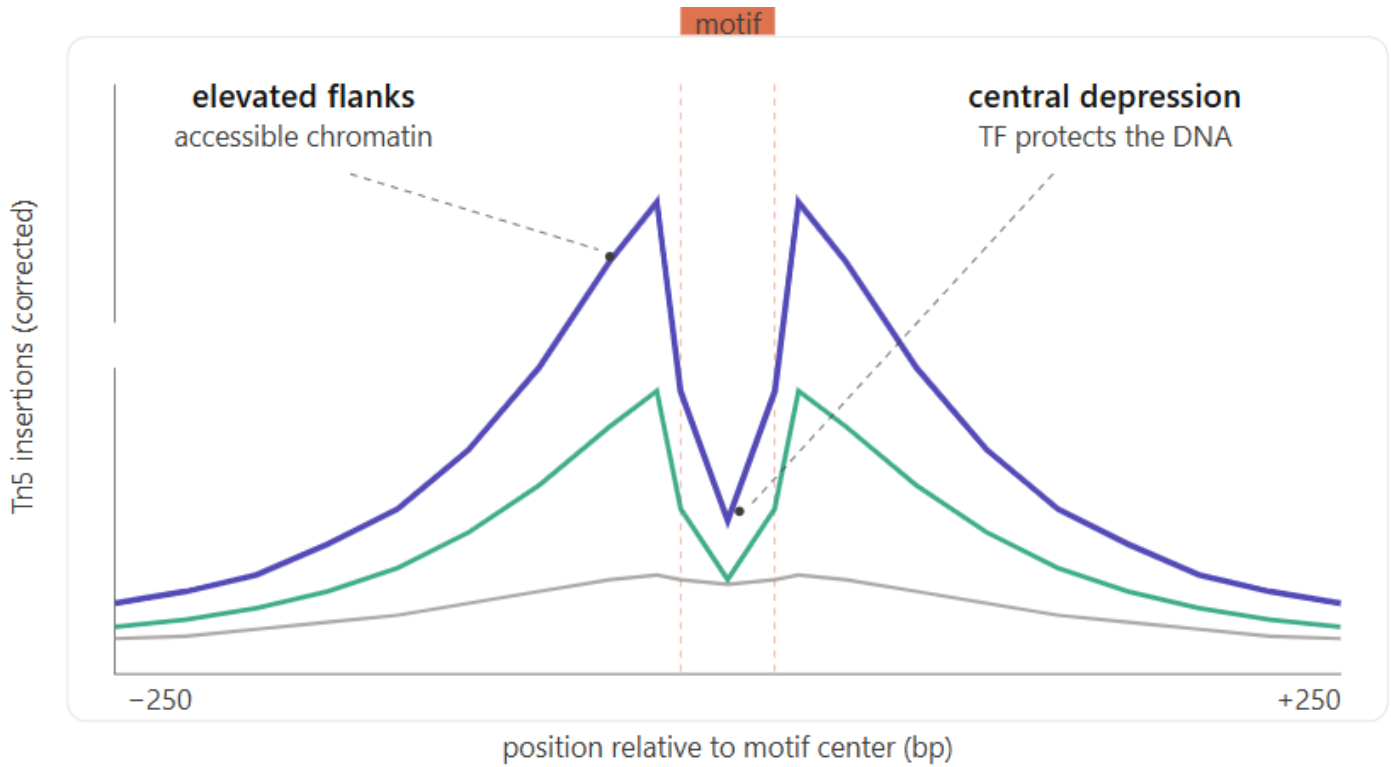
.
.
.

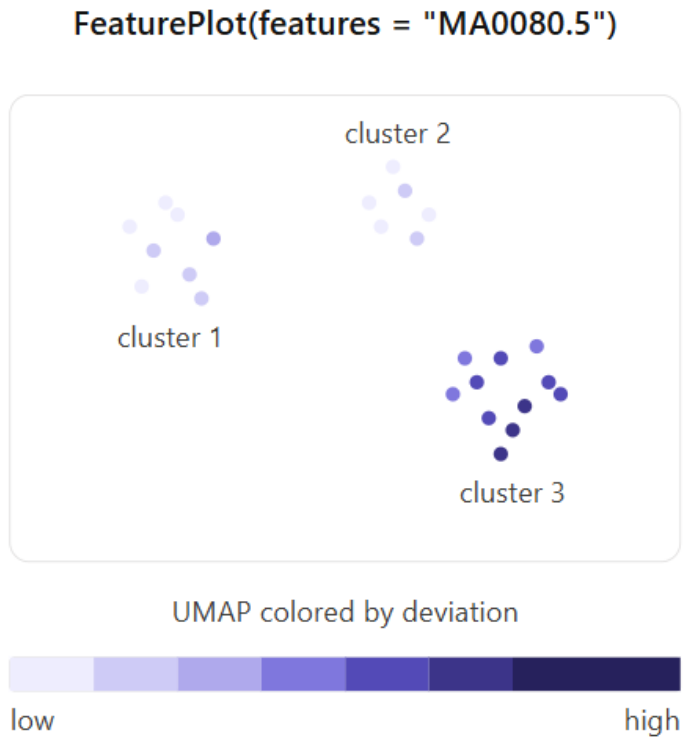
Sample N

Peaks

Gene annot.


X
distal enhancer
intronic
nearby
gene X
uncorrelated
−500 kb
TSS
+500 kb


motif
elevated flanks
accessible chromatin
central depression
TF protects the DNA
Tn5 insertions (corrected)
−250
+250
position relative to motif center (bp)


FeaturePlot(features = "MA0080.5")
cluster 2
cluster 1
cluster 3
UMAP colored by deviation
low
high