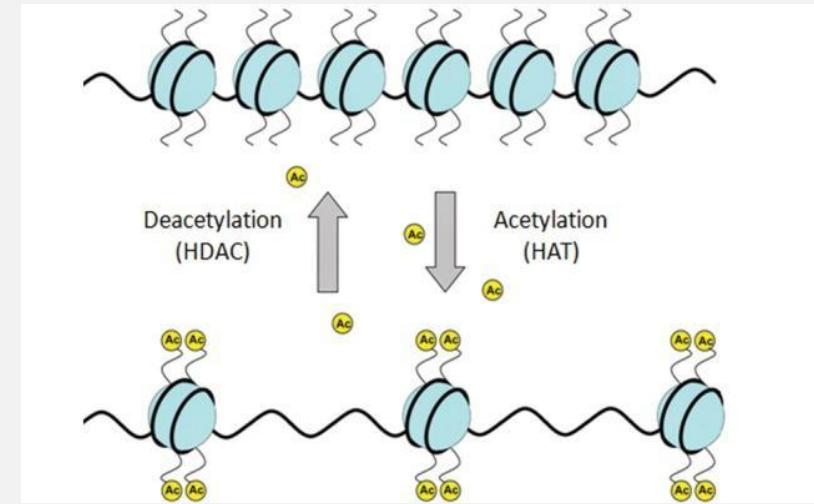


Tissue-Specific Analysis of H3K27ac ChIP-seq Data in Dogs

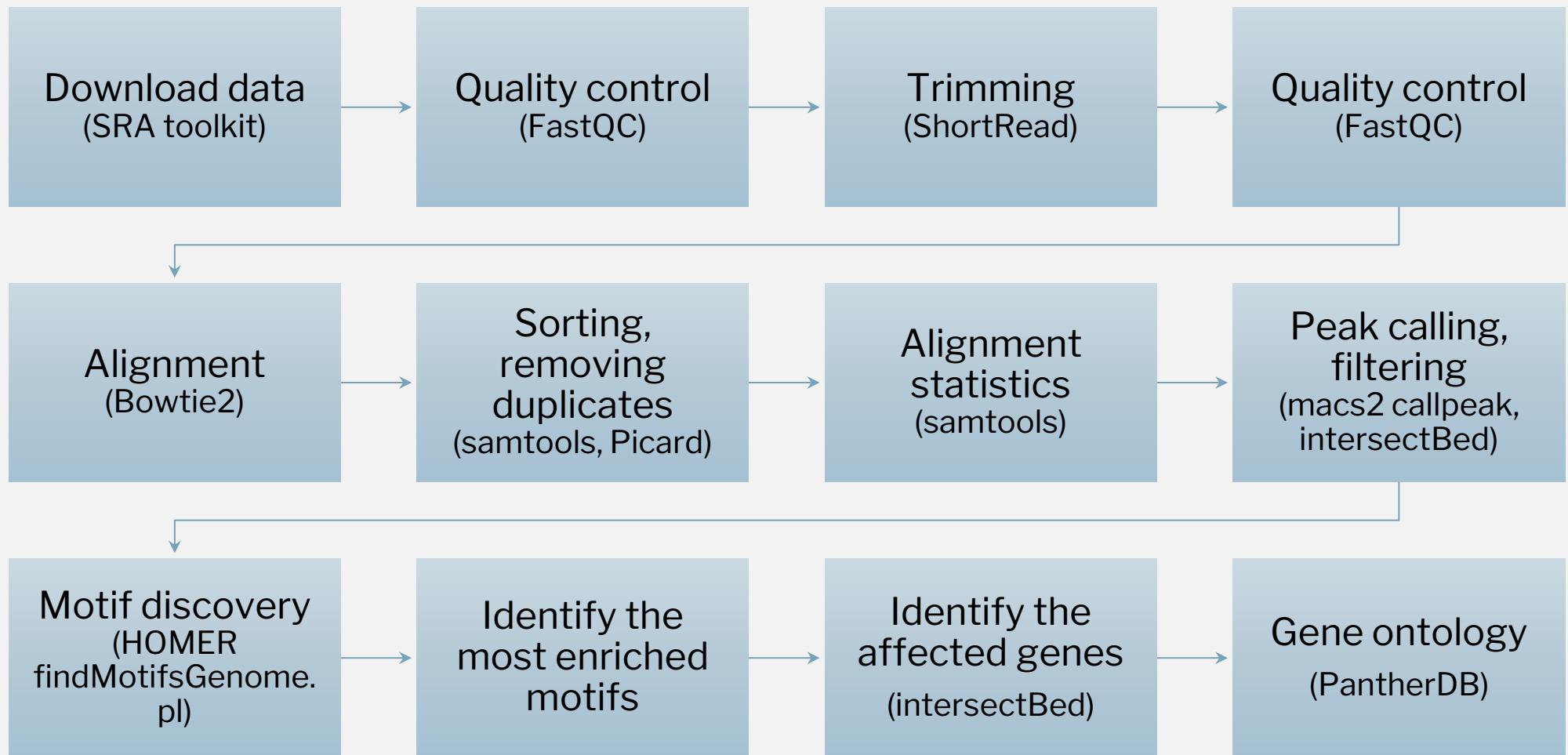
Dataset

- Dataset: ChIP-seq data derived from adult **beagles** (Son et al., 2023)
 - 4 tissues: cerebrum, kidney, liver, pancreas – 2 samples/tissue
 - One control for each tissue
- **Immunoprecipitation** with antibody
 - Identify protein binding sites (e.g. TFs) or histone modifications
 - **H3K27ac modification** → acetylation of lysine 27 on the tail of histone H3 → indicate **active promoters** and **enhancers**



Eslaminejad et al, 2013

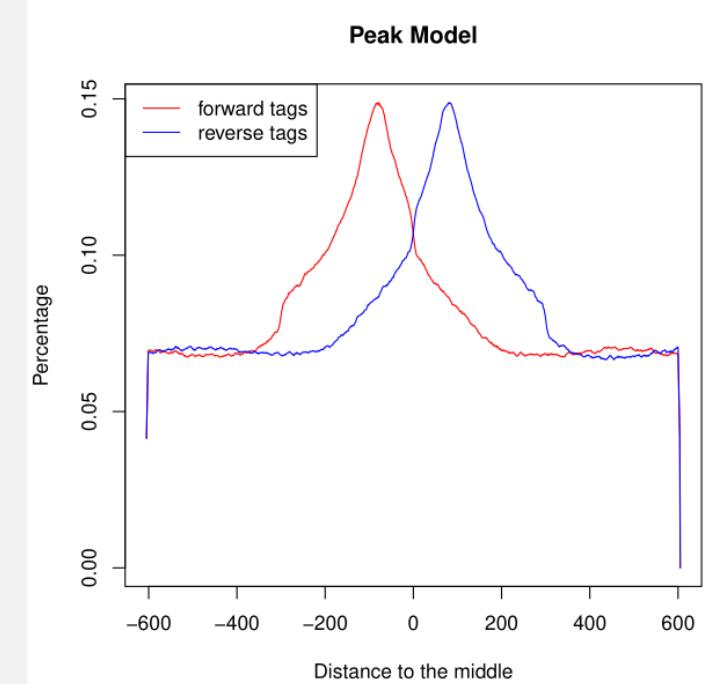
Pipeline



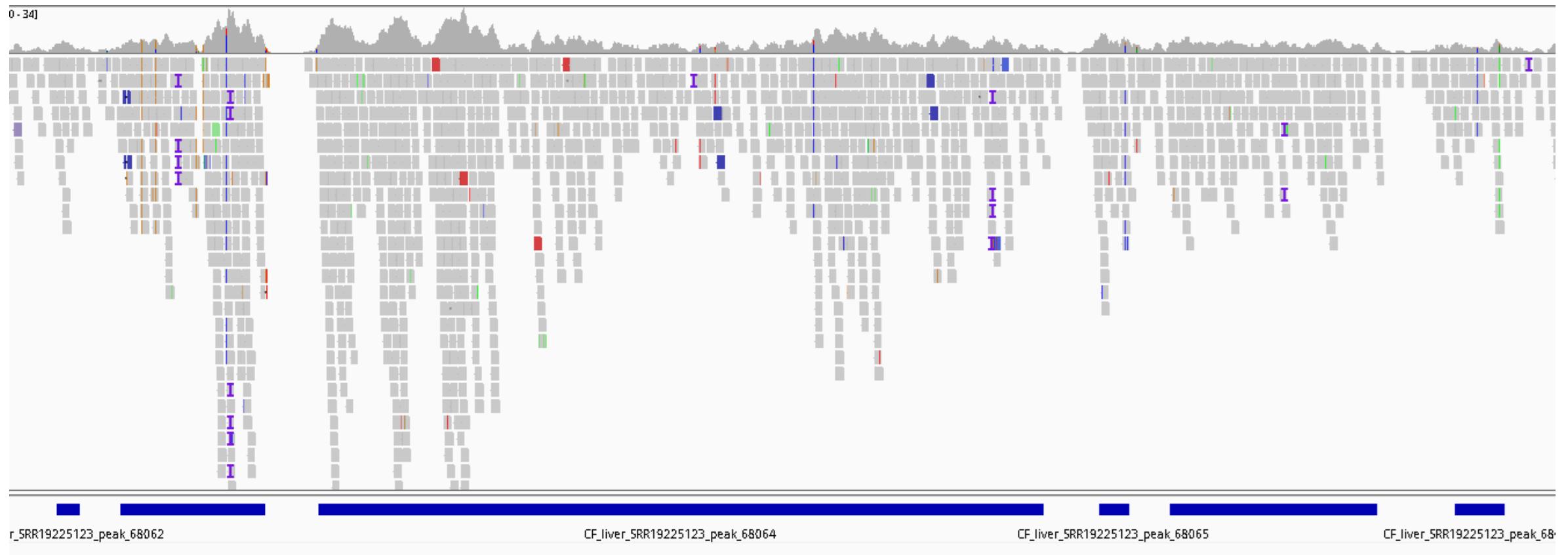
Peak Calling

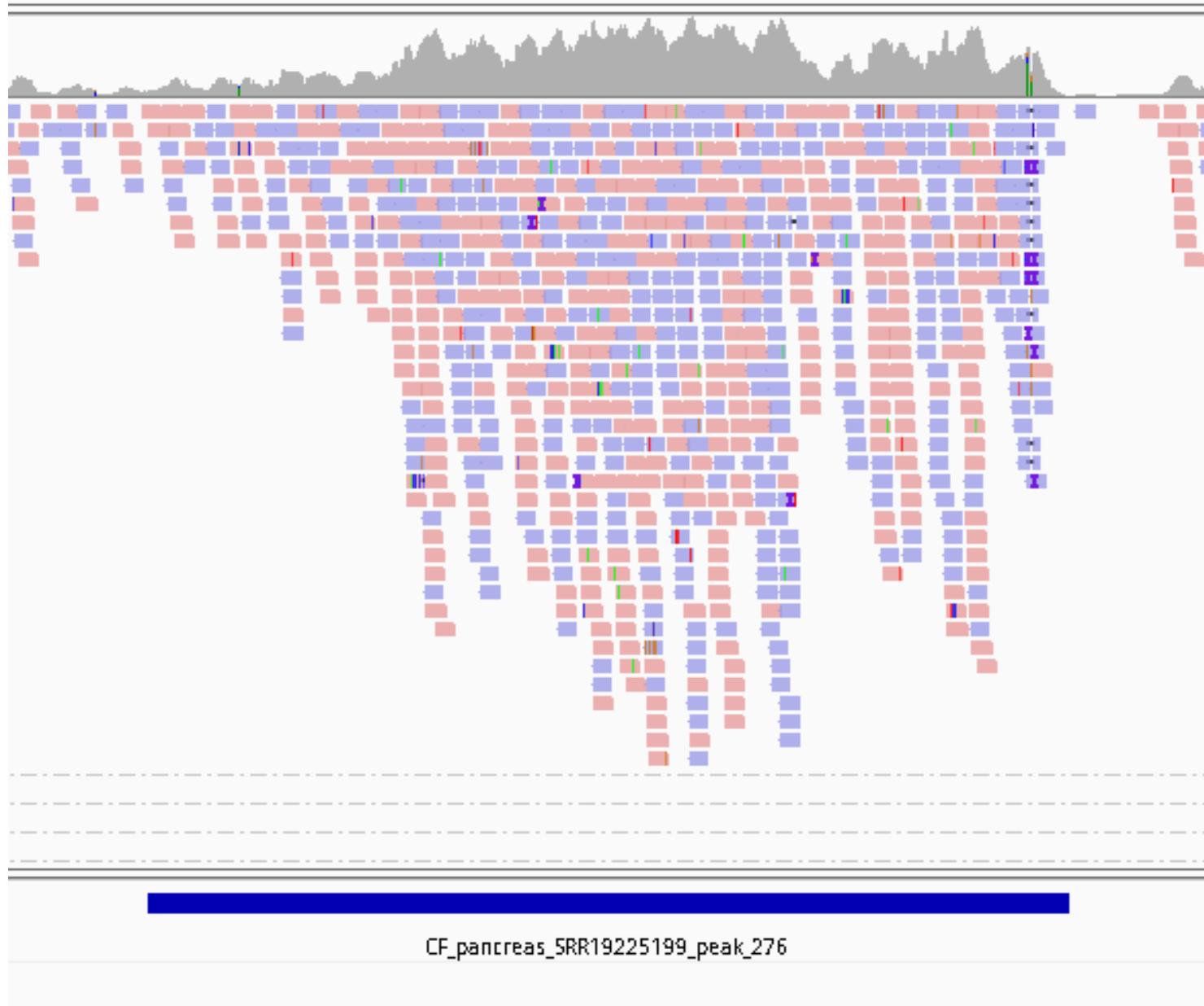
- Identify areas in the genome that have been enriched with aligned reads
- Two types of enrichment:
 - a) Narrow peaks → e.g. TFs binding sites
 - b) **Broad peaks** → e.g histone modifications such as H3K27ac, H3K4me3, H3K36me3 etc.
 - Affect wide regions, on several nucleosomes

Yan et al., 2020



Check the detected peaks with IGV





Detected Peaks

Within tissues

Statistical power is too weak with only two samples per tissue →
presence/absence approach using bedtools intersect

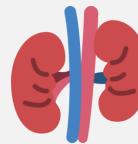
Tissues	Within tissue	Tissue specific	Shared
Cerebrum	10.835	936	
Kidney	25.006	4.809	
Liver	60.473	35.835	
Pancreas	29.668	8.084	6.722

TFs of Most Enriched Known Motifs

Fold enrichment (FE) ≥ 2

Shared:

- ETS
- YY1
- Ronin
- GFY-Staf
- GFY
- GFX
- SPL3
- T1ISRE
- DEL1
- ZML1
- DUX4



Cerebrum:

- Dorsal
- ZNF382
- At5g22890
- OCT
- AT4G27900
- AT5G59990
- REST/NRSF
- DREF
- Pax7

Kidney:

- OCT
- DEL1
- AT4G27900
- LIN-15B

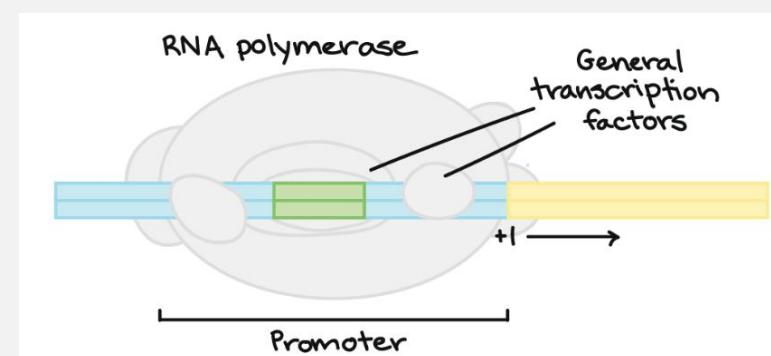
Pancreas:

- AT4G27900
- bZIP42

Liver:

- ZIM
- DUX
- ZFP3

www.khanacademy.org



Most Enriched New Motifs

- Not known to which TF they belong → Compare results with database (if there is one containing dog TFs)
- Novel motifs have much high FE

Liver: 38

Cerebrum: 41

Kidney: 23

Pancreas: 37

Shared: 14

Liver:

A sequence logo for a liver motif. The sequence is GATATTGAGTA. Each nucleotide is represented by a colored triangle pointing to its position: G (green), A (orange), T (red), A (green), T (orange), G (green), A (orange), G (green), T (red), A (orange), T (red), A (green). Below the sequence, small letters indicate the frequency of each base at each position: G, A, G, C, T, A, T, G, A, T, T, A.

Kidney:

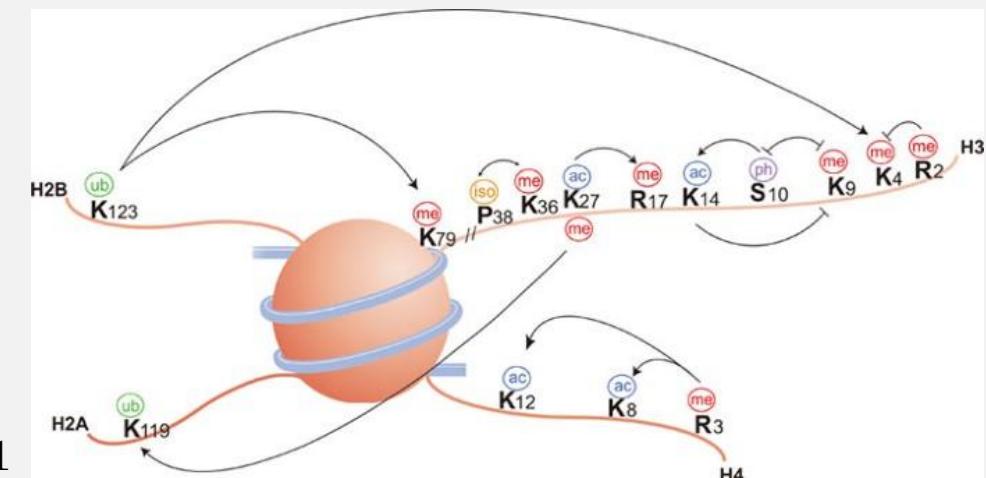
A sequence logo for a kidney motif. The sequence is ACCCTTT. Each nucleotide is represented by a colored triangle pointing to its position: A (green), C (orange), C (blue), C (blue), T (red), T (red), T (red). Below the sequence, small letters indicate the frequency of each base at each position: T, C, A, C, G, T, A, G, C, A.

Pancreas:

A sequence logo for a pancreas motif. The sequence is TTGGACTTGGTC. Each nucleotide is represented by a colored triangle pointing to its position: T (red), T (red), G (green), G (green), A (orange), C (blue), T (red), G (green), G (green), T (red), C (blue). Below the sequence, small letters indicate the frequency of each base at each position: A, C, A, C, T, A, G, C, A, T, C, G.

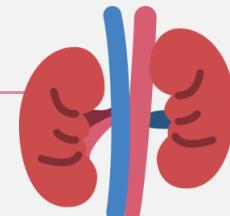
Gene Ontology for Tissue Specific and Shared Peaks

- Demonstrate only **biological processes**
- **Enrichment** → active processes; presumably, genes related to the specific process are expressed
- **Under-represented** → less active processes or processes regulated with other histone modifications (such as H3K4me3) or condition dependent

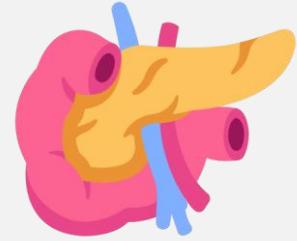


Bannister and Kouzarides, 2011

GO Biological Processes of Shared Genes

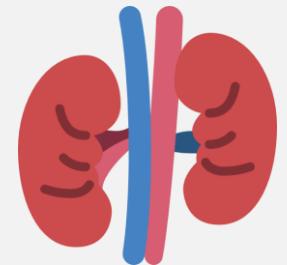
Biological process	Fold enrichment	
regulation of circadian rhythm	3,81	
protein quality control for misfolded or incompletely synthesized proteins	3,81	
alternative mRNA splicing, via spliceosome	2,96	
transcription by RNA polymerase III	2,72	
T cell receptor signaling pathway	0,14	
inflammatory response	0,1	

GO Biological Processes of Pancreas



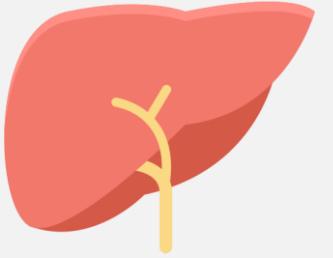
Biological process	Fold enrichment
regulation of transforming growth factor beta receptor signaling pathway	5,89
substrate adhesion-dependent cell spreading	5,35
regulation of cell shape	3,93
establishment of cell polarity	3,66

GO Biological Processes of Kidney



Biological process	Fold enrichment
regulation of filopodium assembly	9,18
sprouting angiogenesis	6,89
positive regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction	6,03
response to oxidative stress	3,76

GO Biological Processes of Liver



Biological process	Fold enrichment
platelet aggregation	3,31
negative regulation of coagulation	3,31
receptor signaling pathway via JAK-STAT	3,01
heme biosynthetic process	2,84
fatty acid beta-oxidation	2,7
lipid storage	2,58
cholesterol homeostasis	2,49

GO Biological Processes of Cerebrum

Biological process	Fold enrichment
postsynapse organization	17,32
microtubule depolymerization	15,4
homophilic cell adhesion via plasma membrane adhesion molecules	10,66
regulation of microtubule polymerization or depolymerization	10,1
regulation of neuron projection development	8,21



Conclusion and Final Thoughts

- Combine with **other histone modification ChIP-seq data** and also **ATAC-seq data**
- More sample
 - Within and between tissues peaks based on statistical methods
- Histone modification ChIP-seq data is primarily used for **define chromatin state**
 - Enhancer and promoter regions are **rich in TF motifs**
 - Species-specific TFs

Thank you for your attention!

References

Son KH, Aldonza MBD, Nam AR, Lee KH, Lee JW, Shin KJ, Kang K, Cho JY. Integrative mapping of the dog epigenome: Reference annotation for comparative intertissue and cross-species studies. *Sci Adv.* 2023 Jul 7;9(27):eade3399. doi: 10.1126/sciadv.ad3399. Epub 2023 Jul 5. PMID: 37406108; PMCID: PMC10321747.

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Eslaminejad MB, Fani N, Shahhoseini M. Epigenetic regulation of osteogenic and chondrogenic differentiation of mesenchymal stem cells in culture. *Cell J.* 2013 Spring;15(1):1-10. Epub 2013 May 5. PMID: 23700555; PMCID: PMC3660019.

Bannister, A., Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res* 21, 381–395 (2011). <https://doi.org/10.1038/cr.2011.22>

Sources of Pictures

- <https://www.khanacademy.org/>
- https://hbctraining.github.io/Intro-to-ChIPseq/lessons/05_peak_calling_macs.html
- <https://www.freepik.com>