

Abstract

Transcription factors recognize specific patterns on DNA and are one of the key components in controlling gene expression. FoxA1 is a pioneer transcription factor that facilitates chromatin binding, function of lineage, and is a regulator of liver specific transcripts. ***The ability to make chromatin accessible during cell development is important for determining the cell's fate.*** When FoxA1 is introduced into mouse embryonic stem cells, it successfully binds to DNA; however, does not show significant liver gene activation.

Proteins that
turn our
genes off &
on!

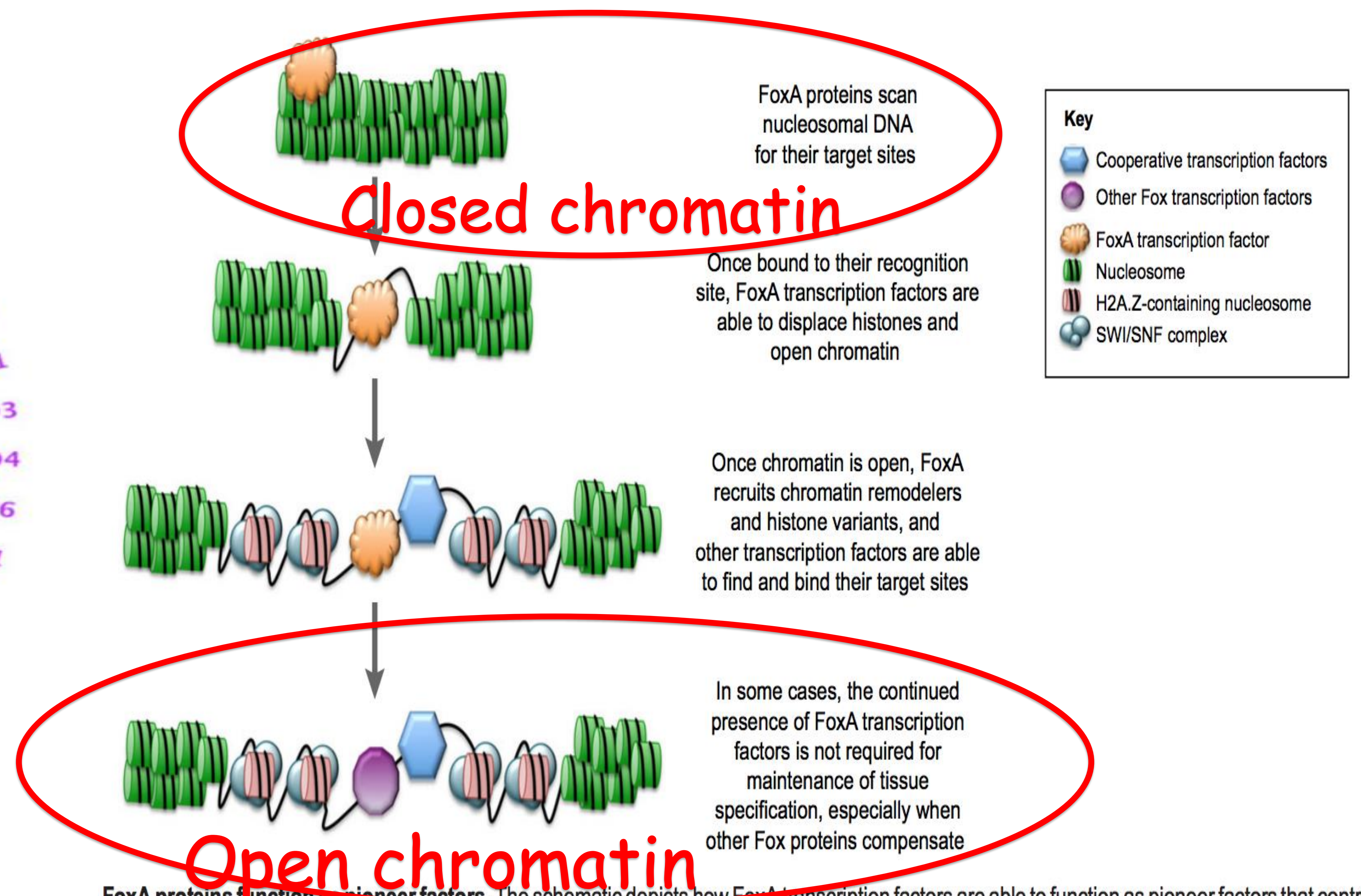
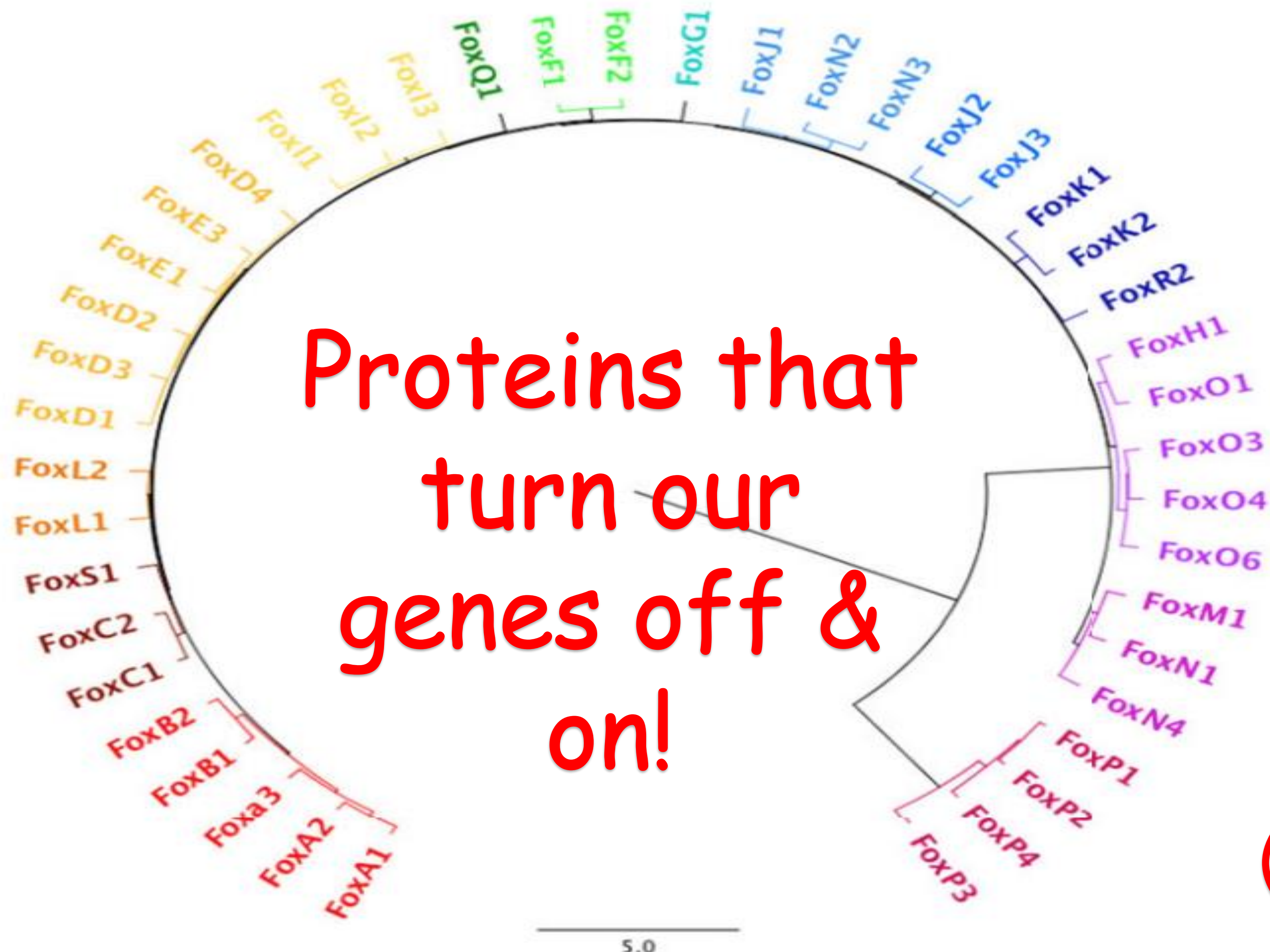
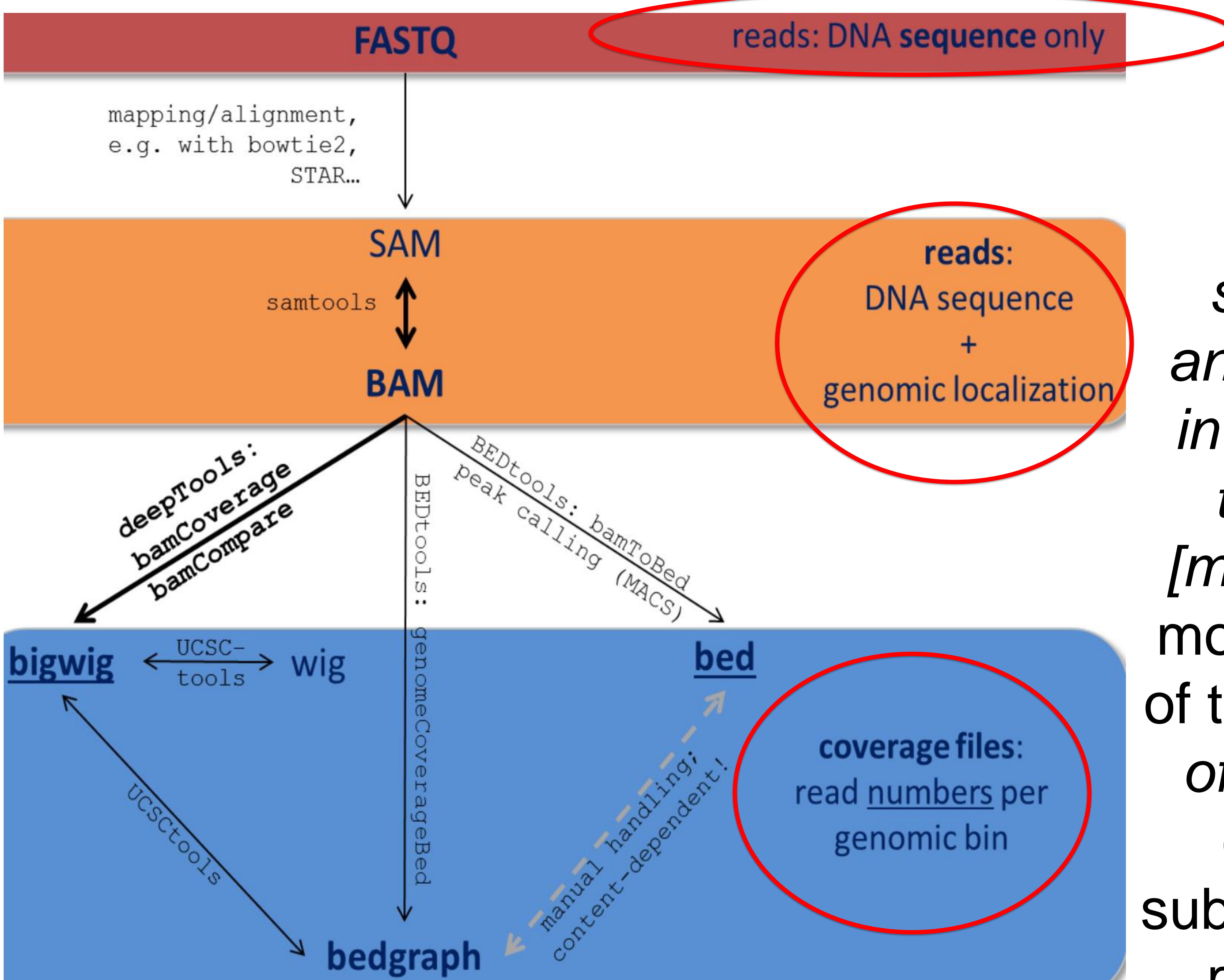
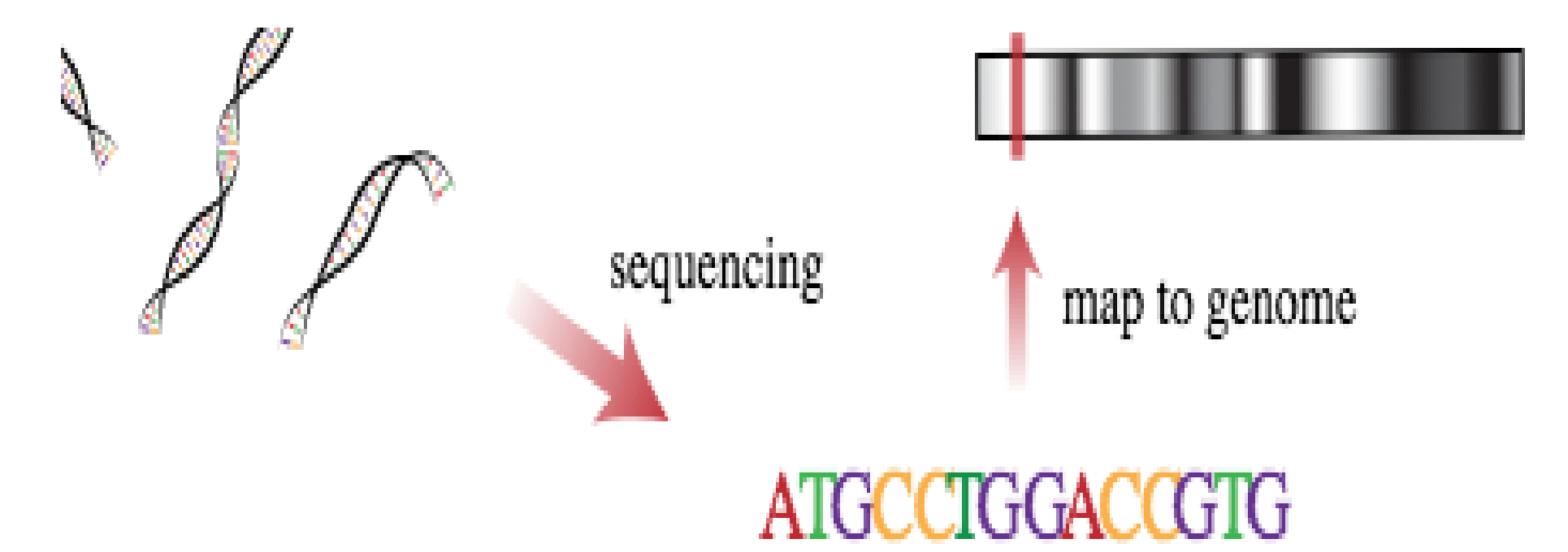


Fig. 1. Phylogenetic tree of mouse Fox family members. The entire FoxA proteins function as pioneer factors. The schematic depicts how FoxA transcription factors are able to function as pioneer factors that control gene expression via their interaction with chromatin.

Background

The use of sequence specific transcription factors [TFs] such as FoxA1, has fate-changing implications. They can scan and target genomic DNA initiating cooperative events that open up chromatin, allowing for the regulation of gene expression in different cell types. The differentiation of *FoxA1* in embryonic stem cells can be observed through several computational techniques.



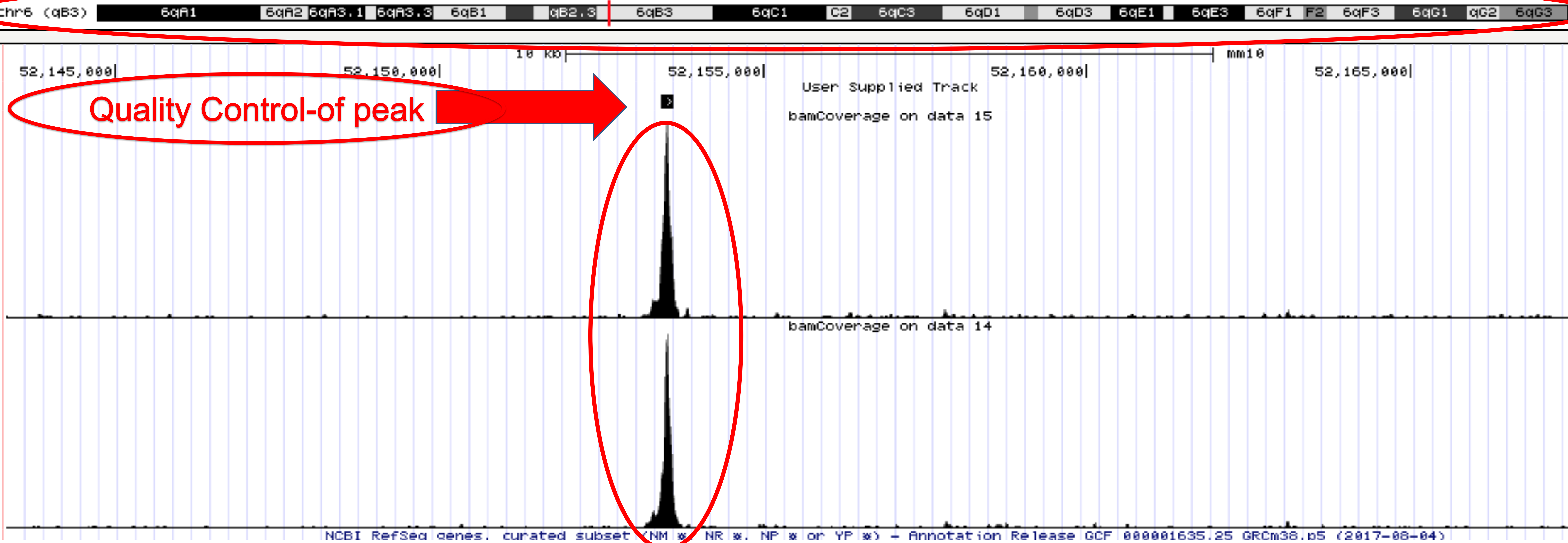
Chromatin-immunoprecipitation sequencing [ChIP-seq] analyzed the DNA-Protein interactions and identified the binding preference [motif] of FoxA1. Once the motif is located, the validity of the data and visualization of phenotypic expression can be checked using subsequent data processing protocols & deep tools.

ChIP-seq detection of FoxA1 & Genomic visualization

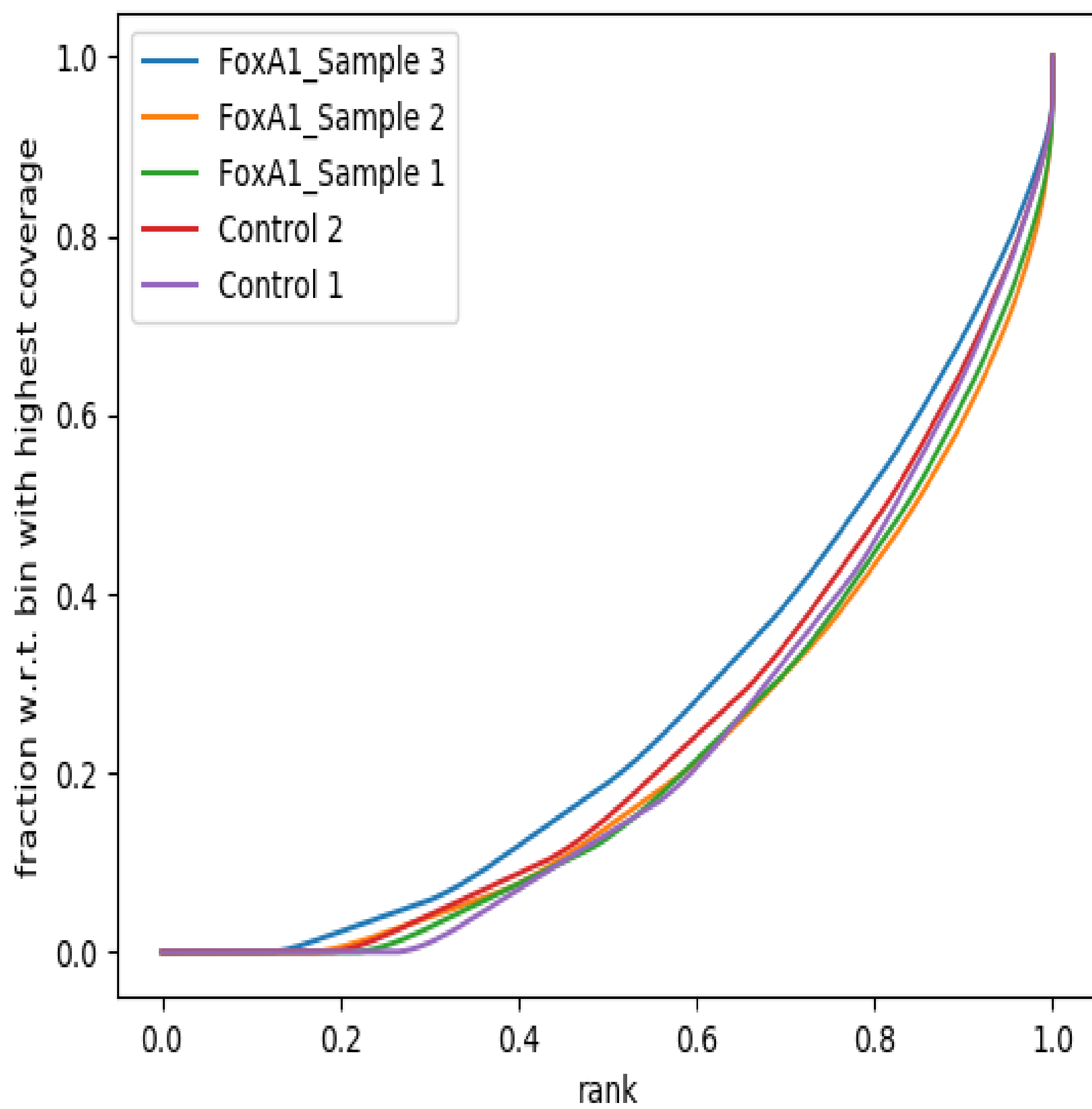
UCSC Genome Browser on Mouse Dec. 2011 (GRCm38/mm10) Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x 100x

chr6:52,143,314-52,170,593 27,280 bp. enter position, gene symbol or search terms go

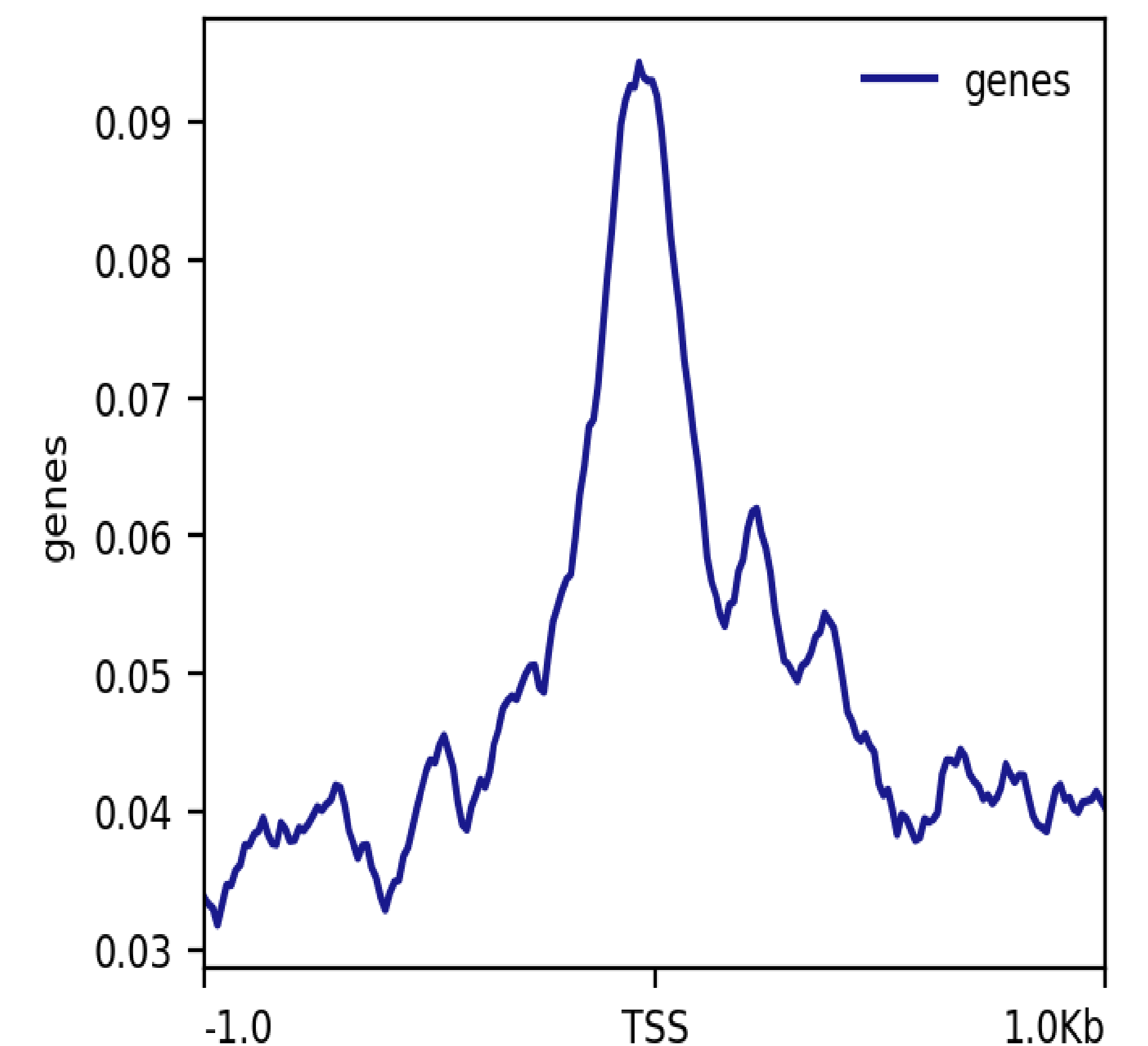


Quality Control-of peak



Quality Control-measurement of signal strength

Control 1 Normalized _ob_ bamCompare on data 1 ar



Data & Analysis

Next generation sequencing [NGS] produces large amounts of data sets available for a variety of deep analysis.

MEME-ChIP data:

MOTIFS

The significant motifs (E-value ≤ 0.05) found

Expand All Clusters



Visualization of FoxA1 in Embryonic stem cells and confirmation of binding preference [motif]

CIS-BP database:

**Foxa1 (*Mus musculus*)
Forkhead**

TF Information

Gene ID	CIS-BP ID
ENSMUSG00000035451	T186352_2.00

Directly determined binding motifs

Forward	Reverse
 TRTTKACWYW	 WRWGTMAAYA

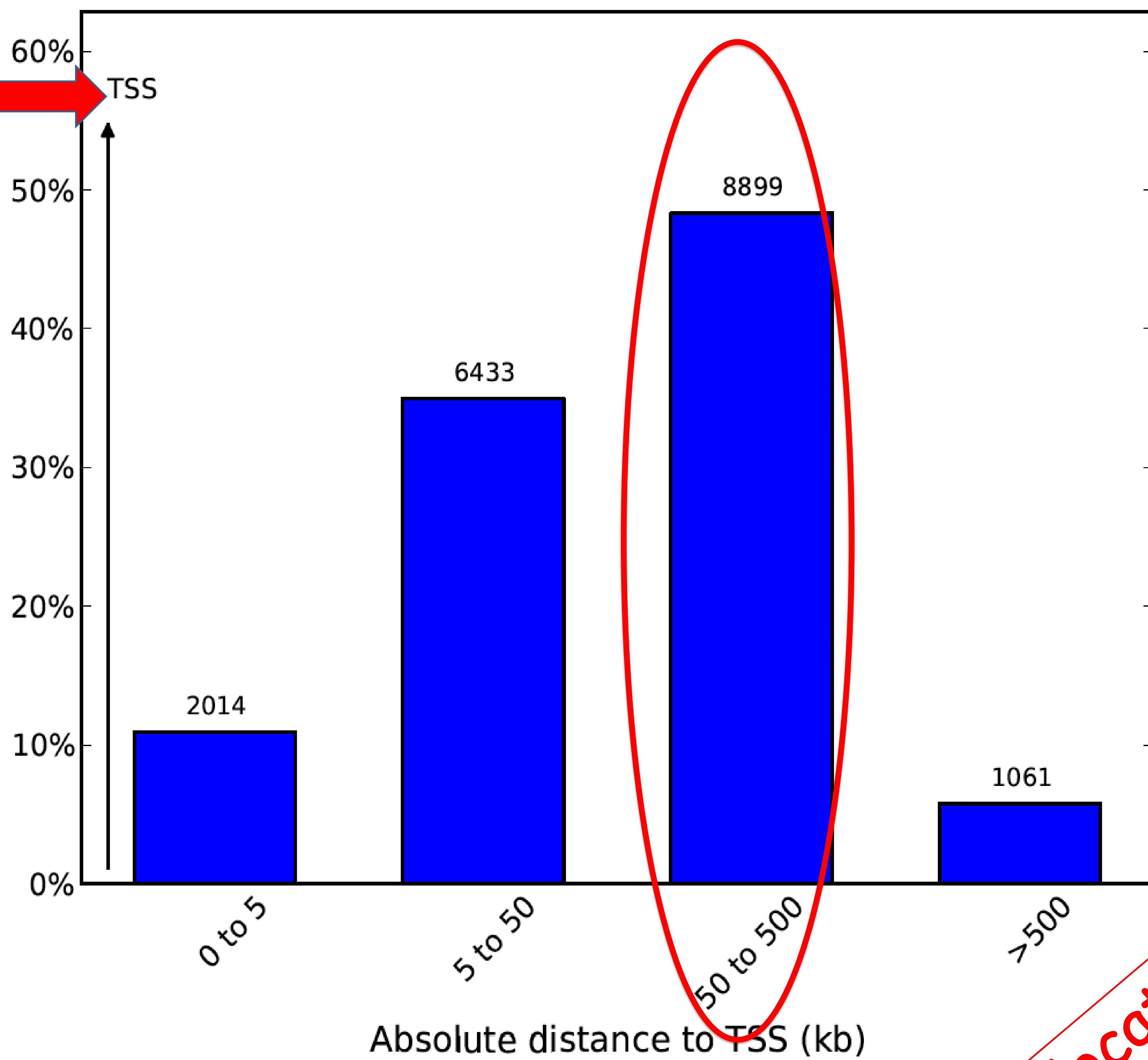
My results

Known TF binding site motifs

Data & Analysis

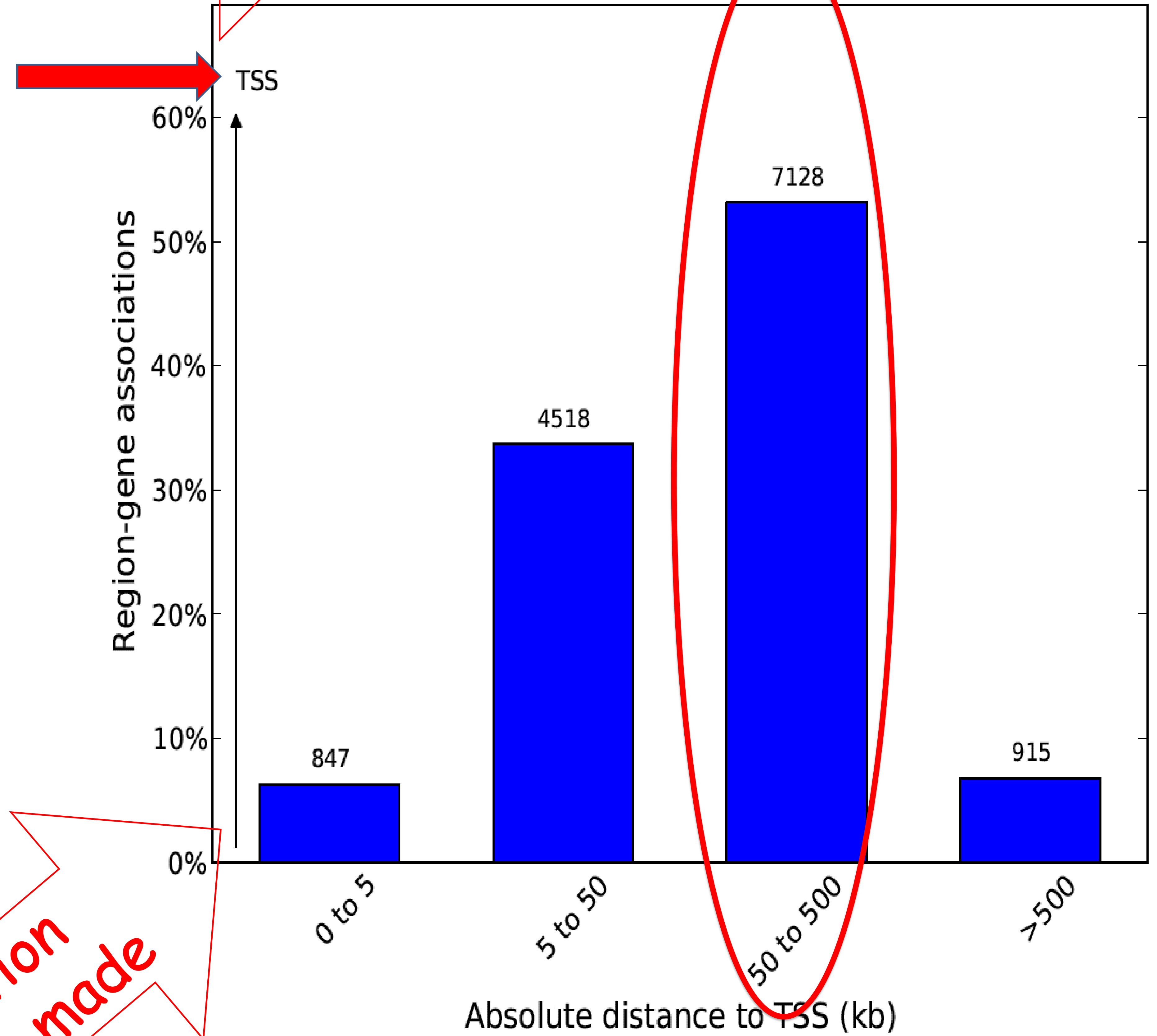
Identification of *cis*-regulatory regions using GREAT

Fox A1-liver cells:



My results

Fox A1- Mouse embryonic stem cells:



TSS= location proteins are made

Promoter region [needed to turn genes on /off] = highest column

Data & Analysis

The phenotypes [physical appearance] of genes expressed using GREAT

Fox A1-liver cells:

abnormal hepatobiliary system physiology
abnormal liver physiology
abnormal triglyceride level
increased triglyceride level
abnormal circulating triglyceride level
abnormal liver lobule morphology
abnormal liver parenchyma morphology
abnormal hepatocyte physiology
enlarged liver
increased liver triglyceride level
decreased erythrocyte cell number
hepatic steatosis
abnormal liver triglyceride level
increased circulating alanine transaminase level
increased insulin sensitivity
decreased circulating triglyceride level
abnormal circulating VLDL cholesterol level
abnormal glycogen homeostasis
abnormal hepatocyte morphology
abnormal circulating non-HDL cholesterol level

Fox A1-Mouse embryonic stem cells:

My results

abnormal notochord morphology
abnormal fibroblast migration
abnormal tail development
abnormal liver development
abnormal neural fold morphology
failure of somite differentiation
decreased cell migration
abnormal ectoderm development
abnormal fetal cardiomyocyte proliferation
decreased trophoblast giant cell number
decreased fibroblast cell migration
abnormal urethra morphology
rectal prolapse
abnormal neural fold formation
midline facial cleft
increased fibroblast apoptosis
facial cleft
abnormal embryonic cloaca morphology
absent trophoblast giant cells
disorganized extraembryonic tissue

Conclusion:

The pioneer transcription factor FoxA1 increases the rate of transcription by opening up chromatin making DNA accessible for transcription. Depending upon the regulatory environment of the cell type, phenotypic expression varies.

In the liver cells, the genes expressed by FoxA1 are all related to liver development and metabolism. However, in the stem cells, the majority of the genes expressed are unrelated to liver function indicating in this cell type, the cellular environment must contain other factors effecting gene regulation.

The data from ChIP-seq shows ***FoxA1 successfully binds to mouse embryonic stem cell DNA with a strong signal strength and correct motif.***

Further genomic analysis reveals there are very ***few liver related genes expressed*** suggesting that it may not activate them in this cell type.

Additional research is needed on the surrounding genes to identify the cause of the lack of protein expression or if other members of the Fox family have the same results.

Acknowledgements

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Dr. Kathleen Hill,
Dr. Matthew Johnson,
Tiffany Lewis,
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and Kyle Simon,

Thank you for your continuous support and
guidance throughout this outstanding research
experience.

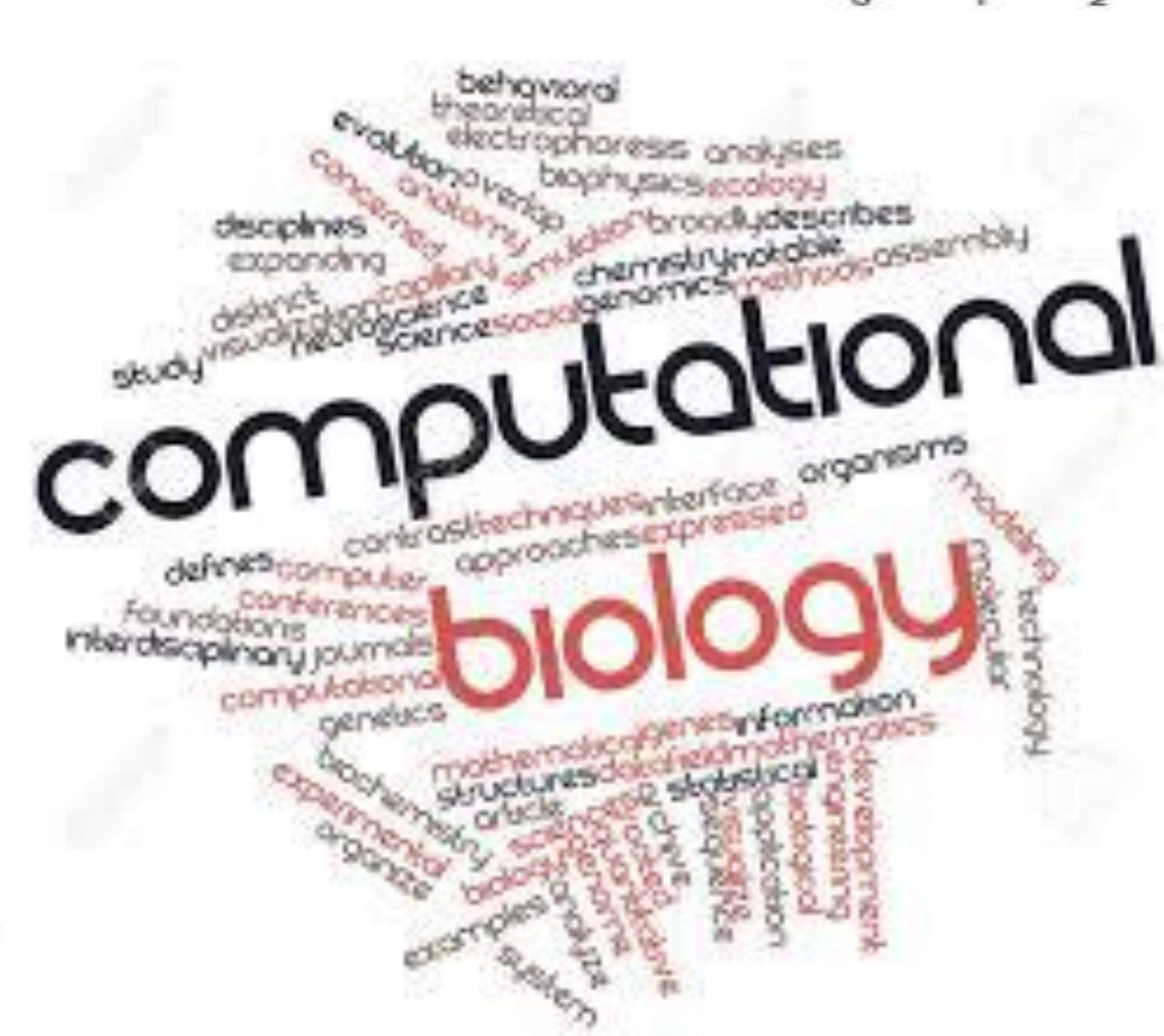
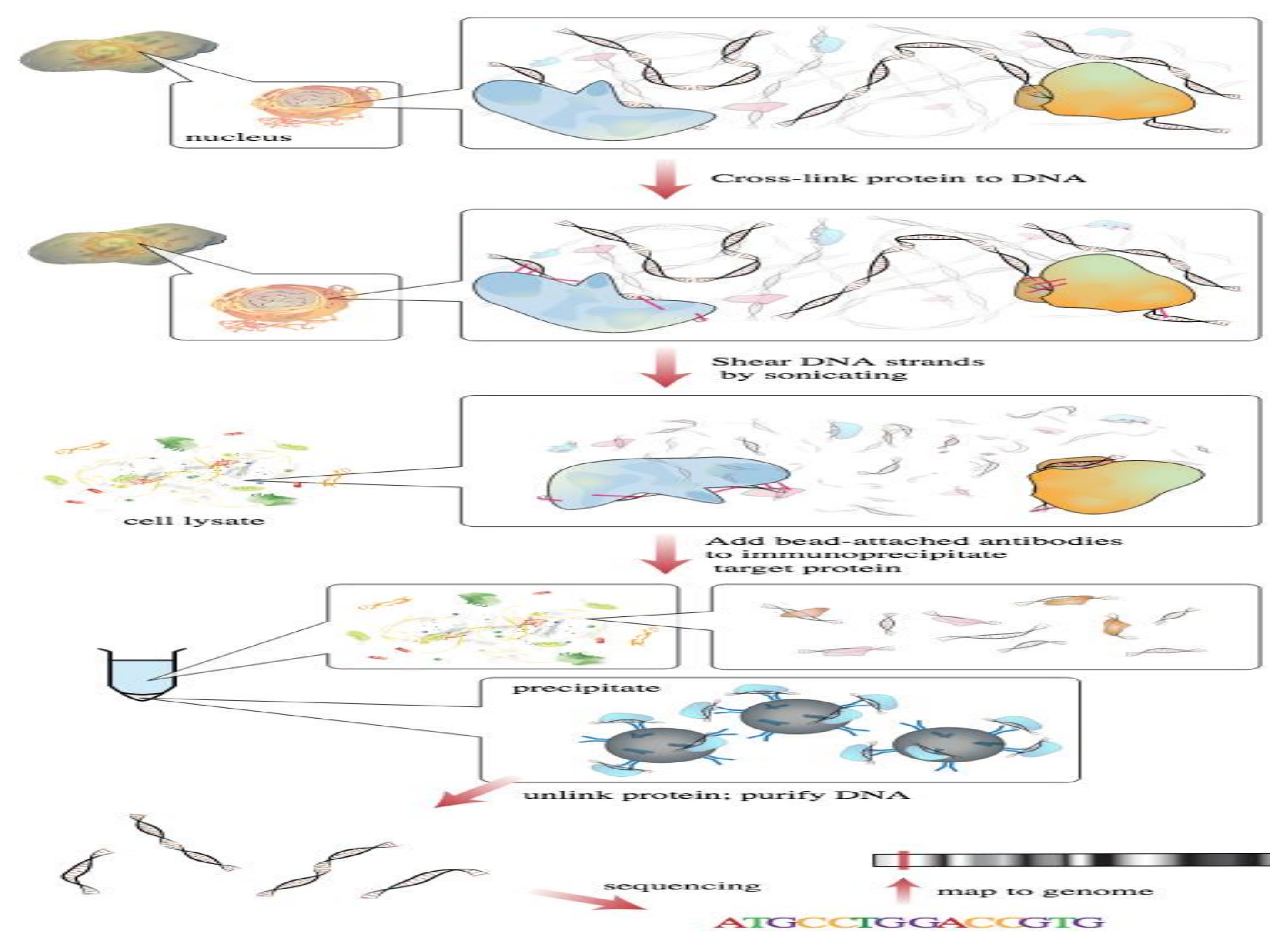
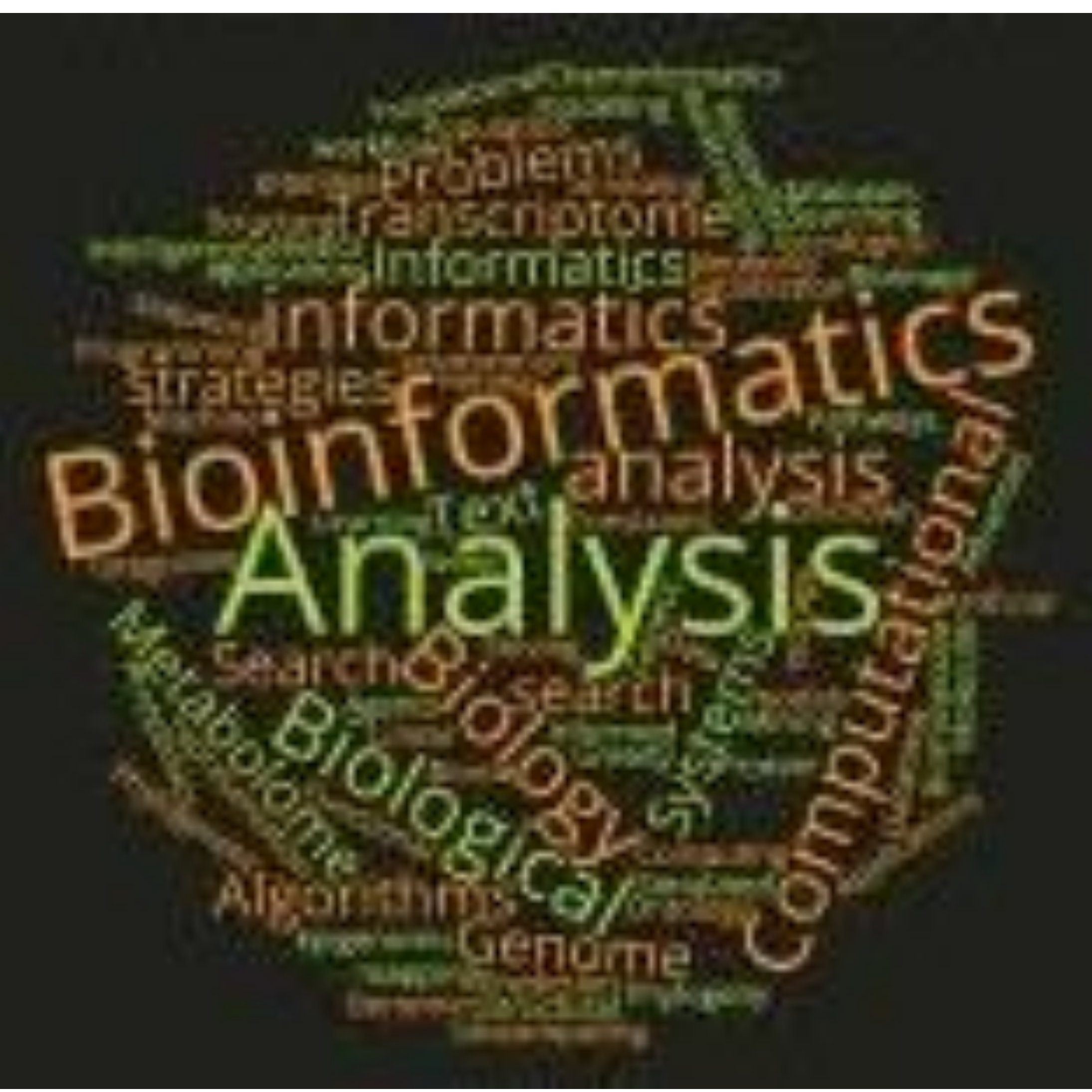
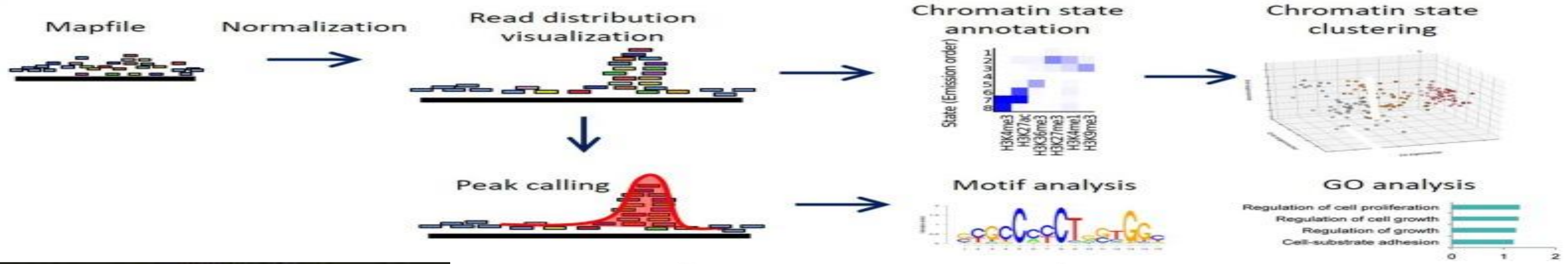


Thank you for your interest in my presentation!

(A) Sample preparation and sequencing



(B) Computational analysis



References

Eeckhoute, Jerome, Mathieu Lupien, Clifford A. Meyer, Michael P. Verzi, Ramesh A. Shivdasani, X. Shirley Liu, and Myles Brown. “Cell-type selective chromatin remodeling defines the active subset of FOXA1-bound enhancers”. *Genome Research*. Retrieved 7.25.2020.

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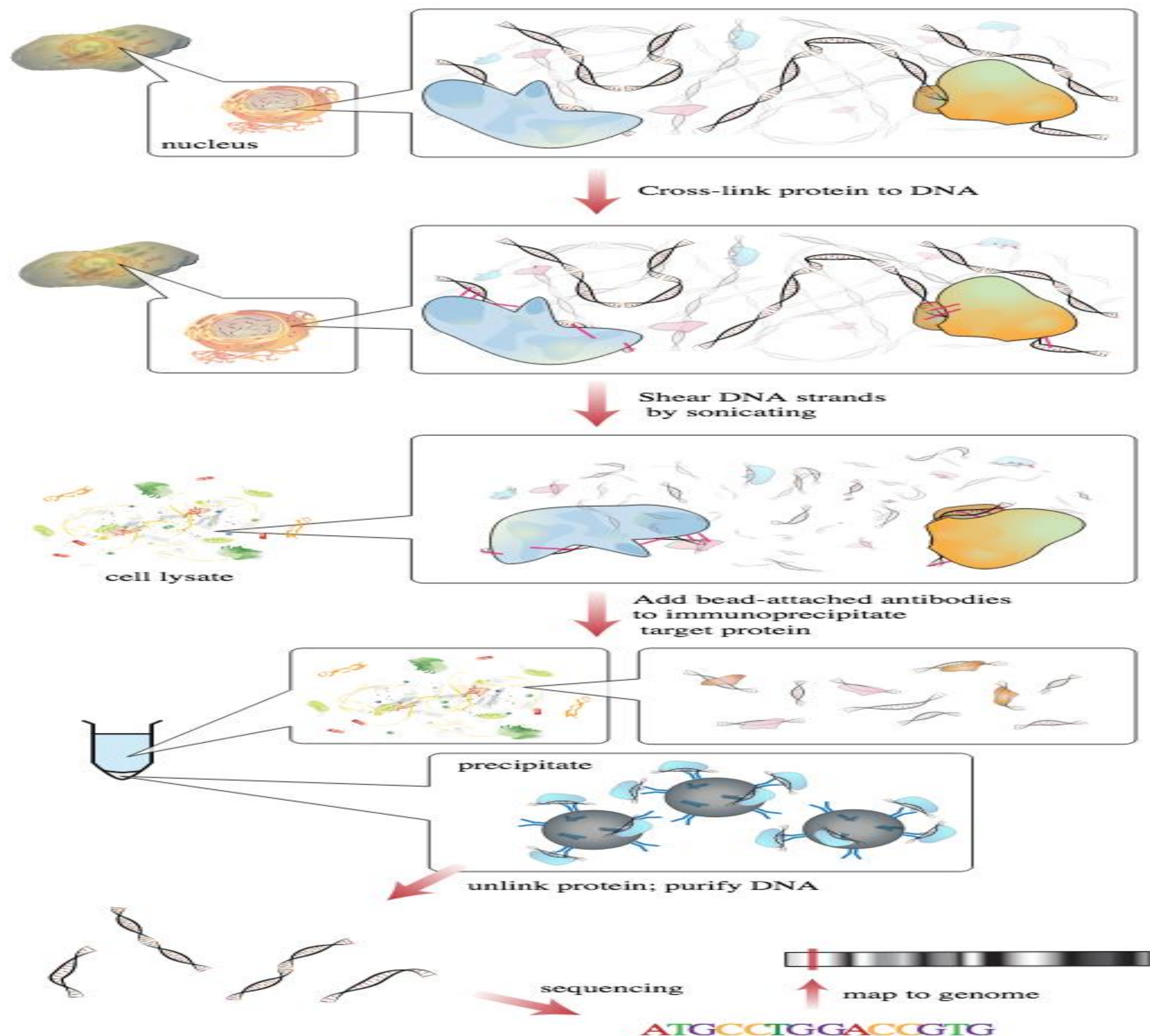
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Where did my data come from?

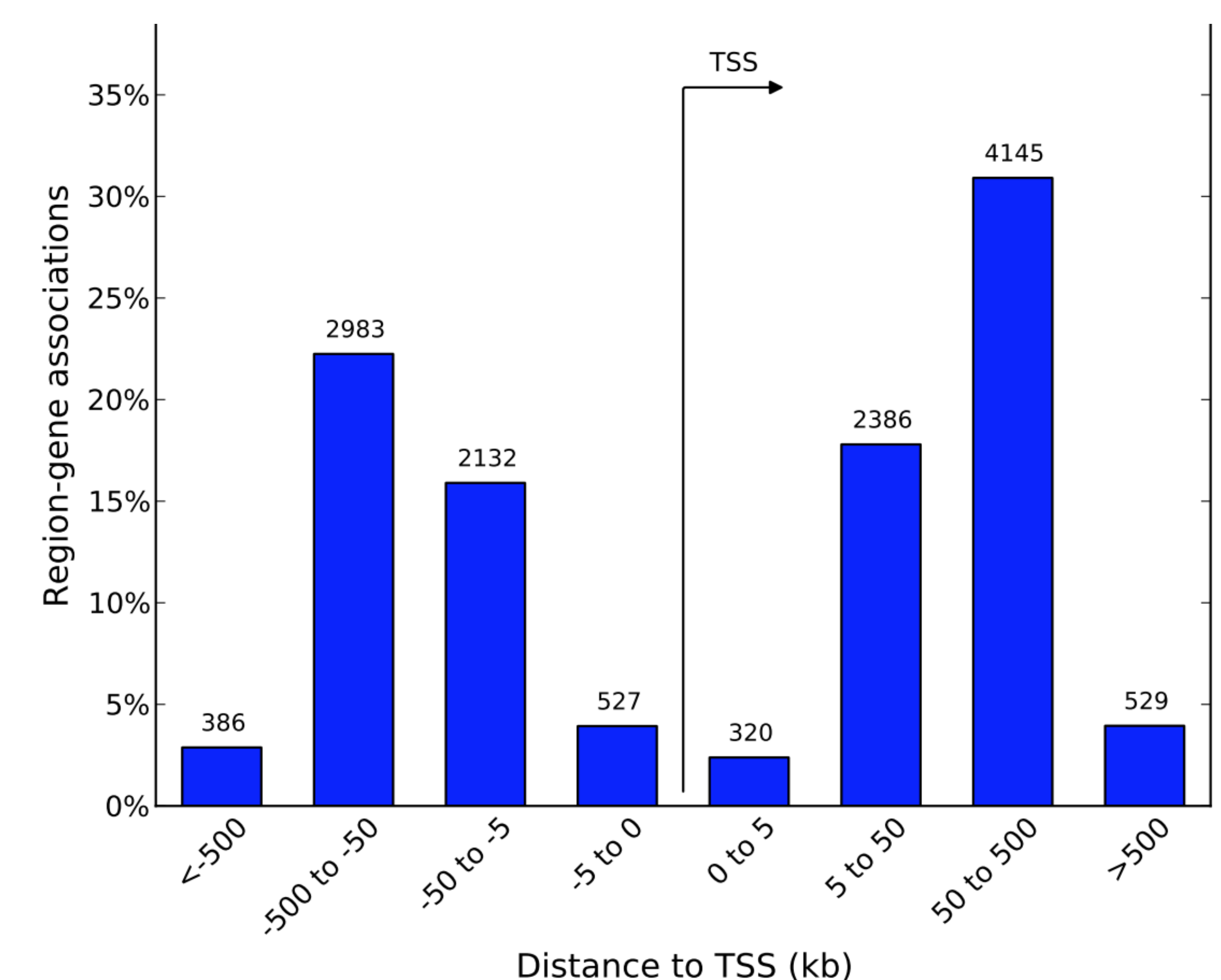
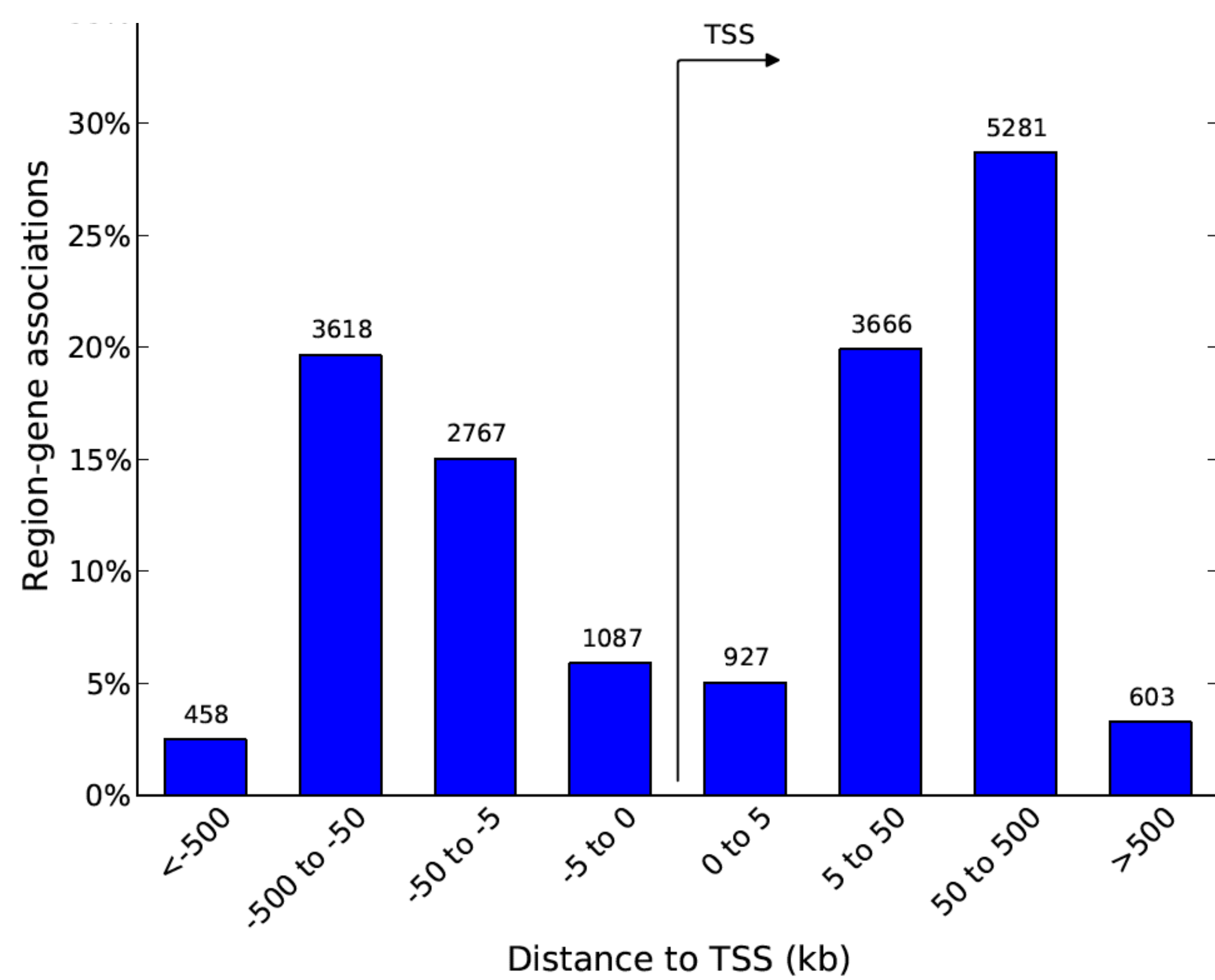
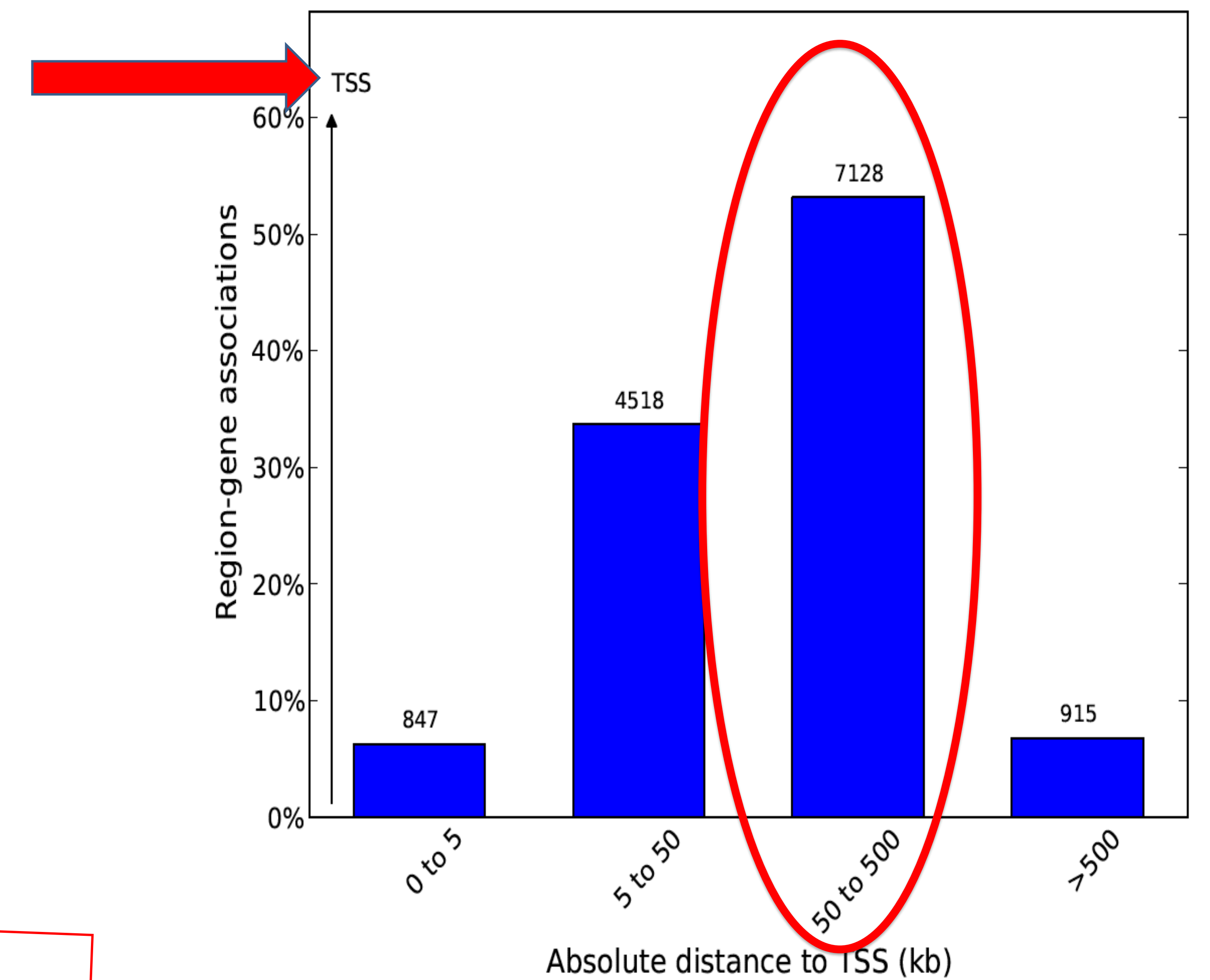
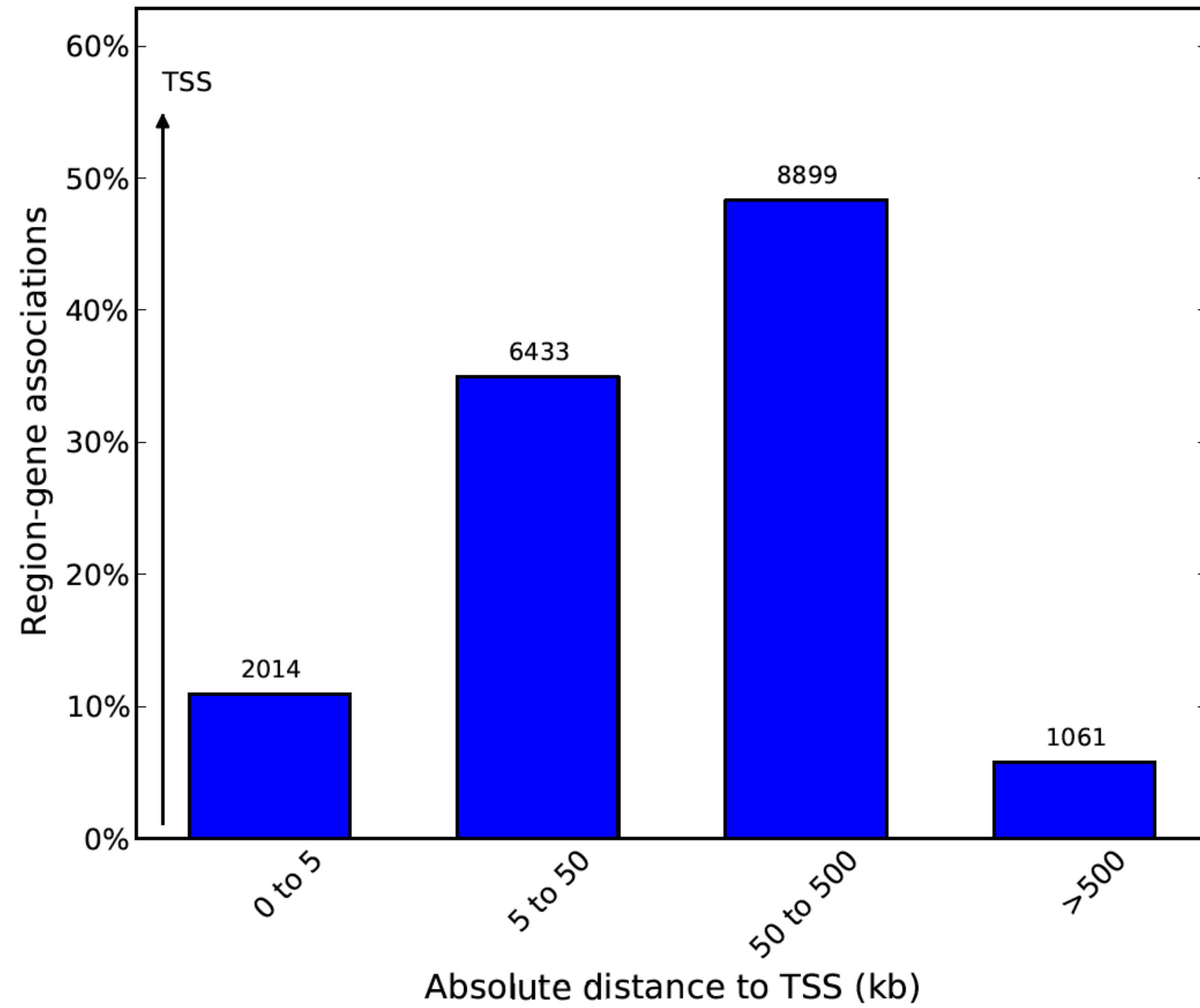
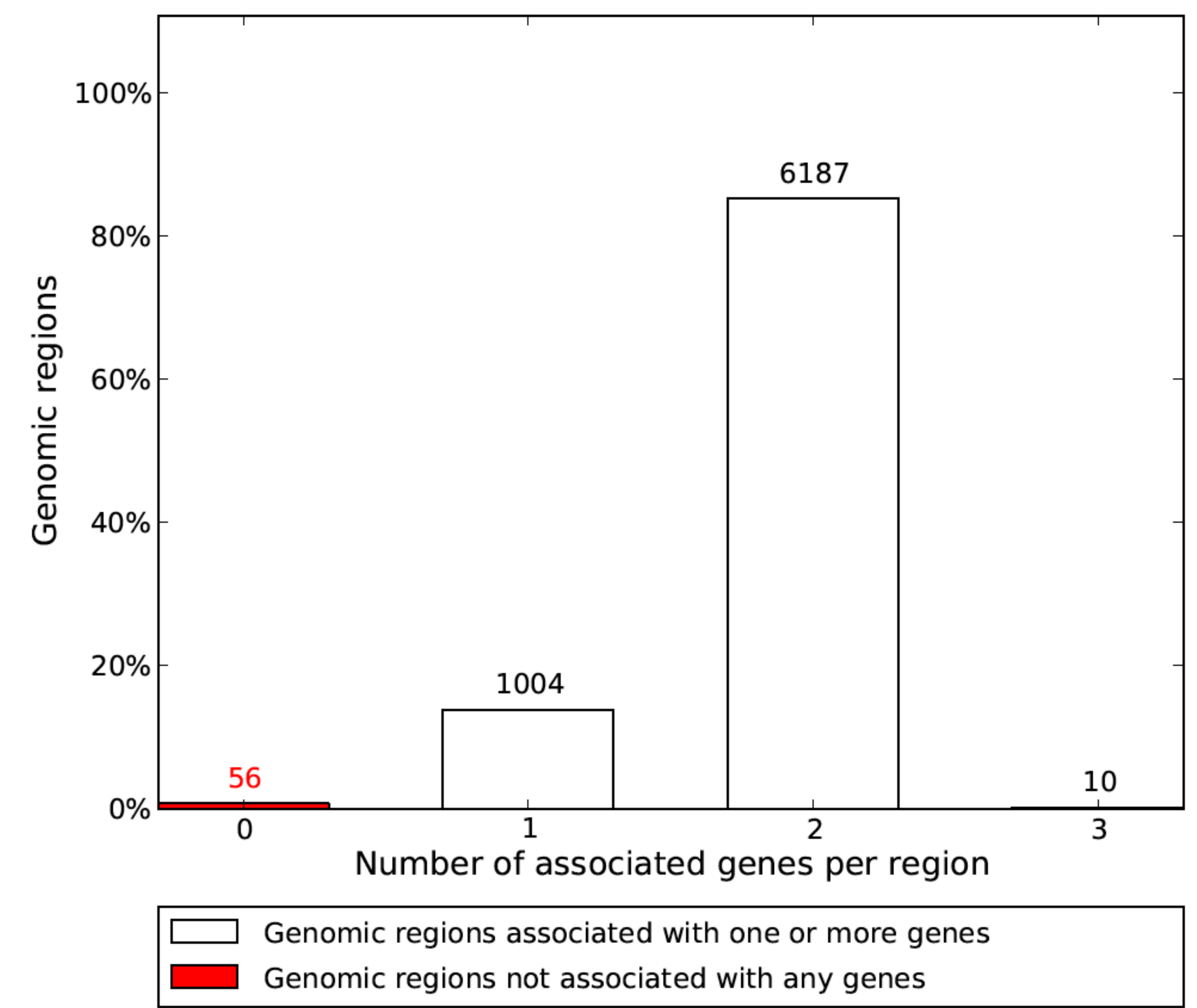
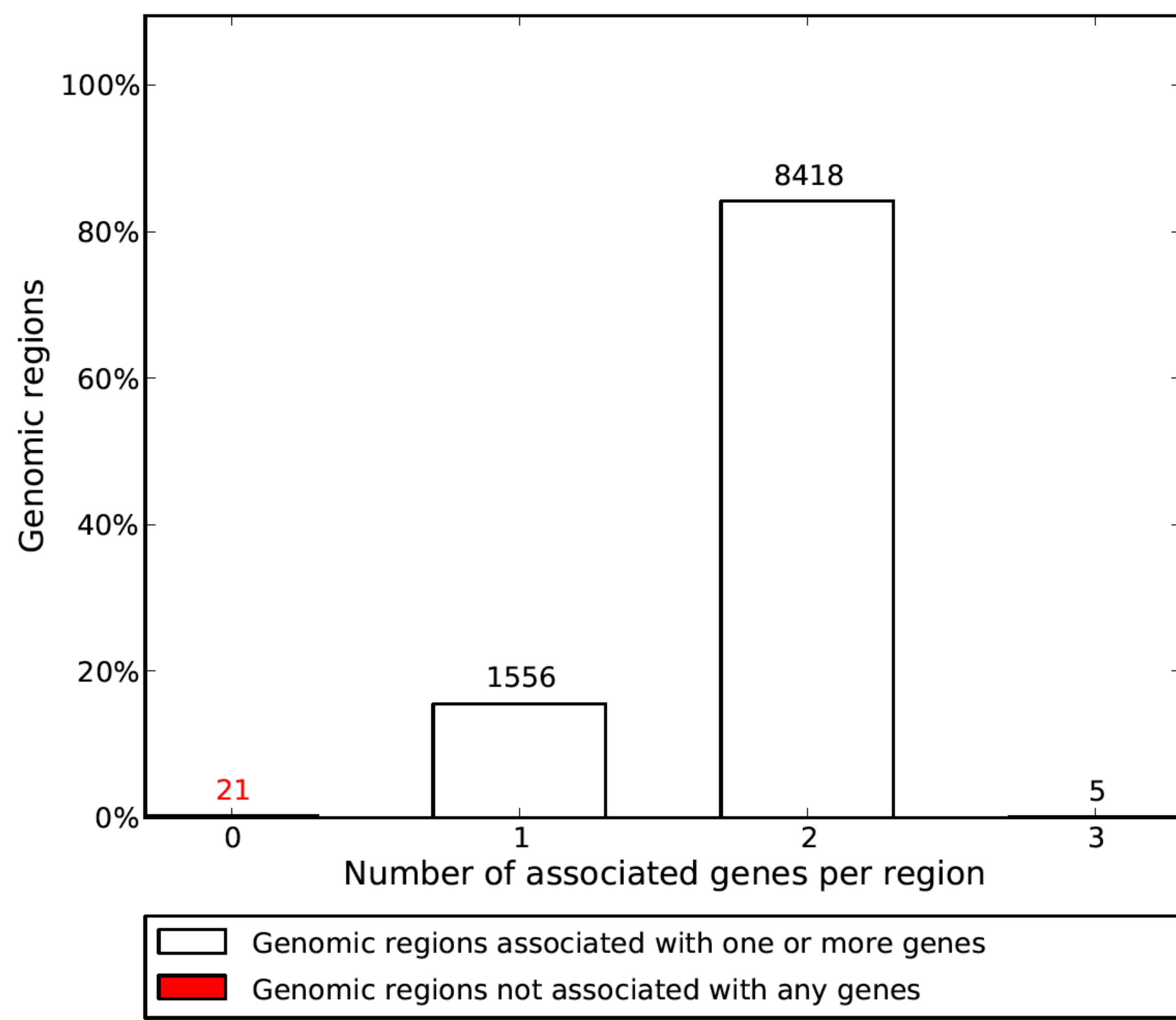
- A lab located in Japan under the supervision of Minoru Ko, grew these cells and processed them with Illumina



<https://www.youtube.com/watch?v=fCd6B5HRaZ8&feature=youtu.be>

Data & Analysis

Identification of *cis*-regulatory regions in Fox A1-liver cells [left] and Embryonic stem cells [right] using GREAT



My results