Genomic Insights into ATF3 Regulation of cell proliferation of HepG2 cells

Rameesha Syed, Chandrima Modak, Sahiti Somalraju

Introduction

Background: ATF3

- AMP-dependent transcription factor (ATF-3) protein is a stress-induced transcription factor: vital roles in modulating metabolism, immunity, and oncogenesis.
- ATF3 acts as a hub of the cellular adaptive-response network.
- Member ATF/cAMP response element-binding (CREB) family.

Project Objectives

- Earlier studies have shown that overexpression of ATF3 protein decreases cell proliferation, cell migration and cell growth in HepG2 cells (liver tumor cells).
- Our study aims to use ChIP-seq data to identify the genomic regions that AFT3 protein binds to in the DNA to facilitate HepG2 cells suppression.

The Dataset

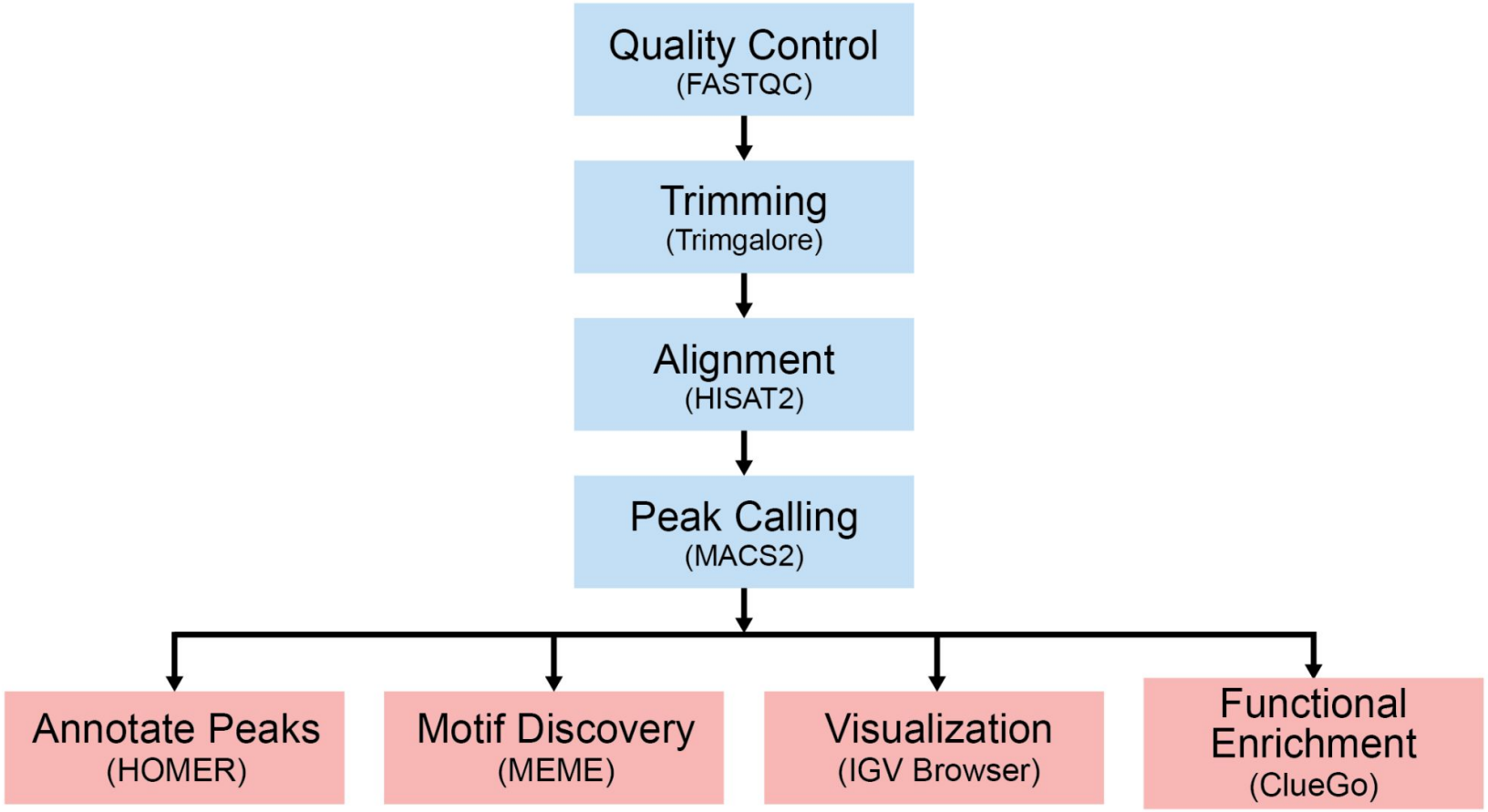
- Data for treatment and control groups were extracted from ENCODE databases with IDs:

  Treatment: ENCFF522PUA, ENCFF094LXX

  Control: ENCFF522PUA, ENCFF094LXX

- HepG2 Cell line was used for our samples.
- Each read size was 50 bp long and was sequenced using Illumina HiSeq 2000

Pipeline


Quality Control
(FASTQC)
Trimming
(Trimgalore)
Alignment
(HISAT2)
Peak Calling
(MACS2)
Annotate Peaks
(HOMER)
Motif Discovery
(MEME)
Visualization
(IGV Browser)
Functional Enrichment
(ClueGo)

Results

Quality Control and Trimming

- FastQC and Trim Galore were used for reads quality analysis.
- Processing of the reads based on phred33 score of each nucleotide.
- Any overrepresented sequence were remove for downstream analysis.

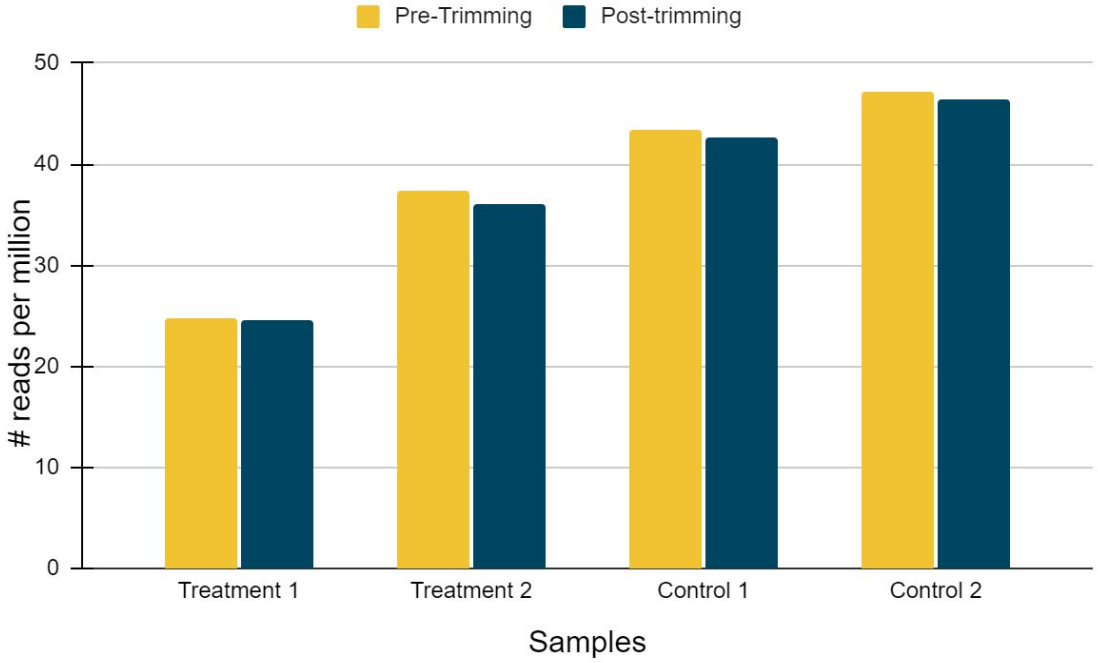


Fig: Read counts report pre-QC and post-QC for both the group. Blue are the treatment group and Oranges are control

Alignment

- HISAT2 was used to align the reads to the reference genome before peak calling of all the samples.
- GRCh38 was used as a reference genome for alignment of the reads using seed-and-extend algorithm

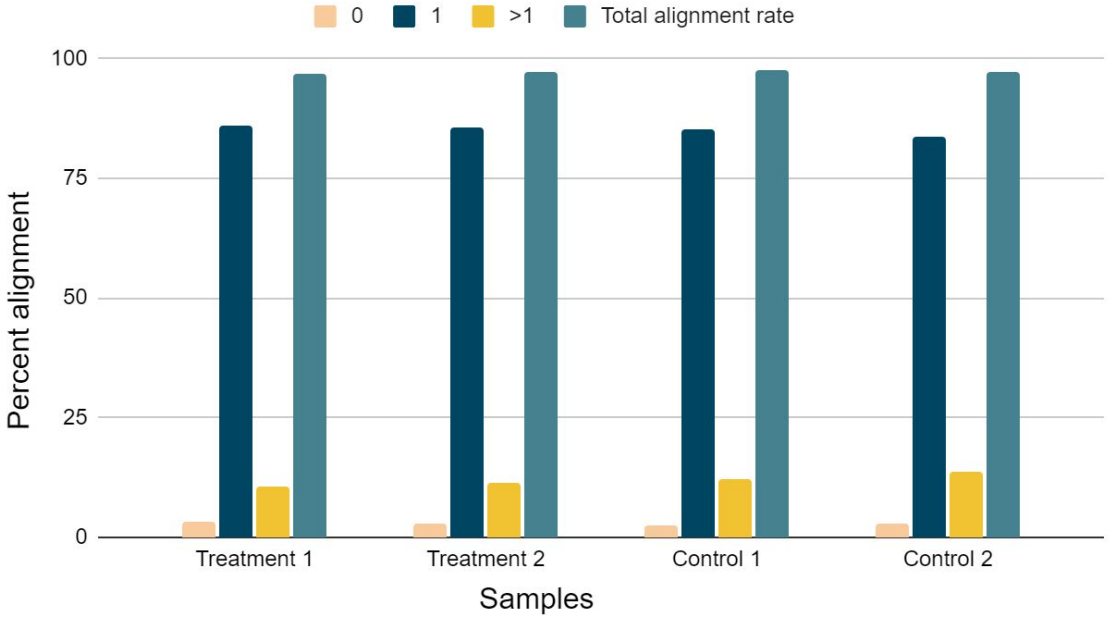
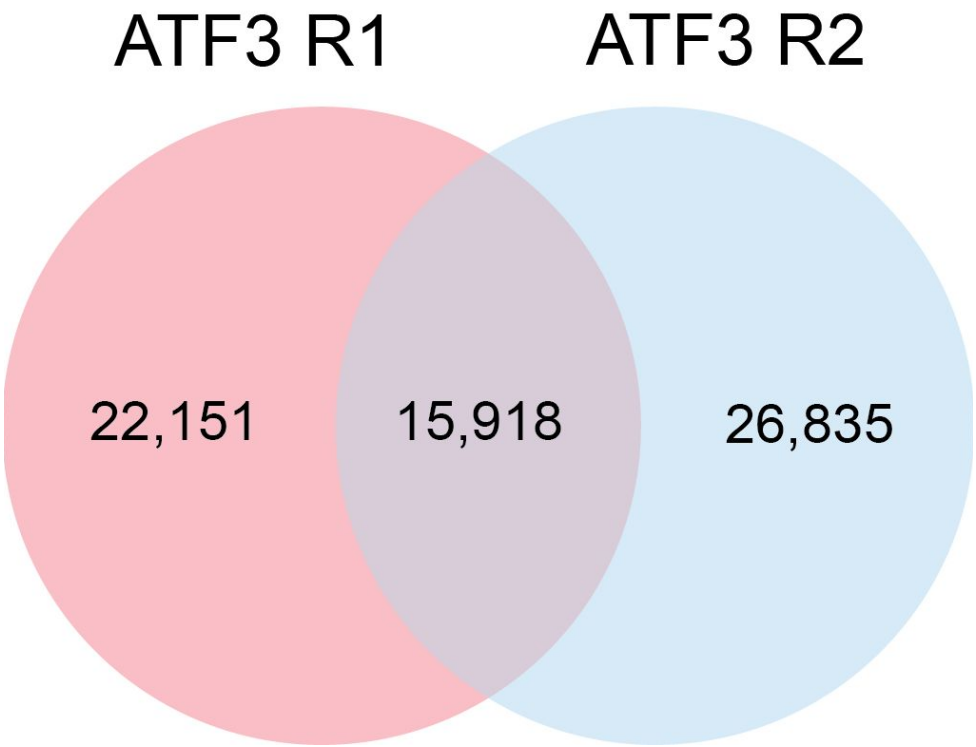


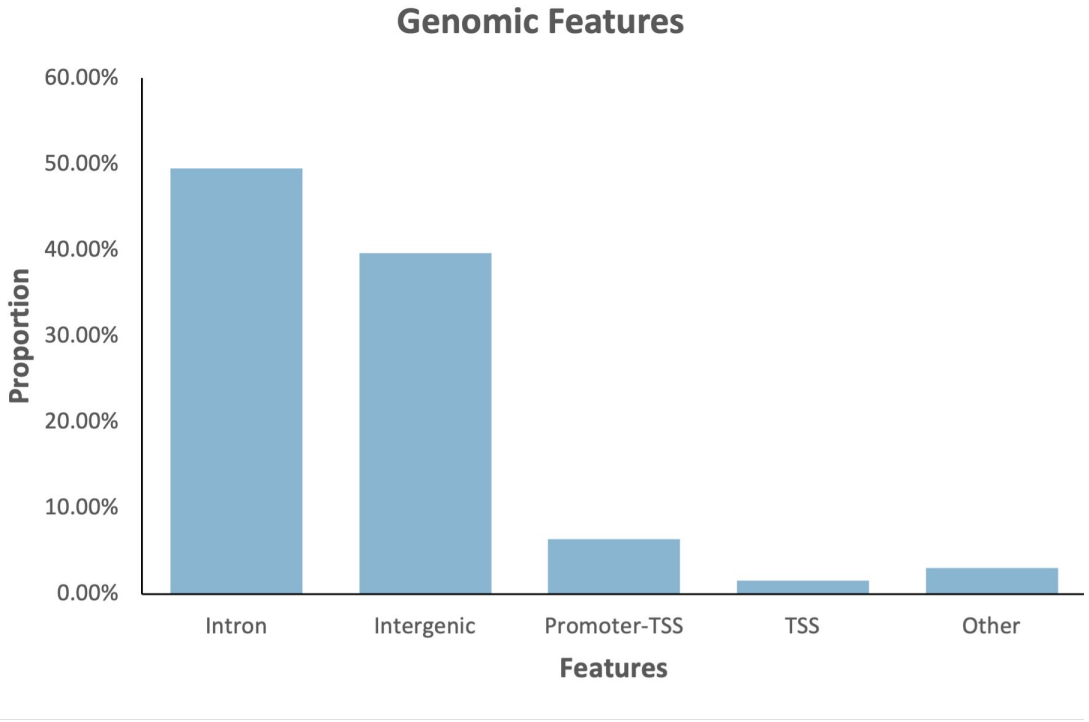
Fig: Percentage alignment for Treatment and Control group reads with the reference genome

Peak calling

- Peak calling: MACS2 callpeak
- Consensus peaks: BEDtools intersect



Annotate Peaks: Genomic Features



Other:

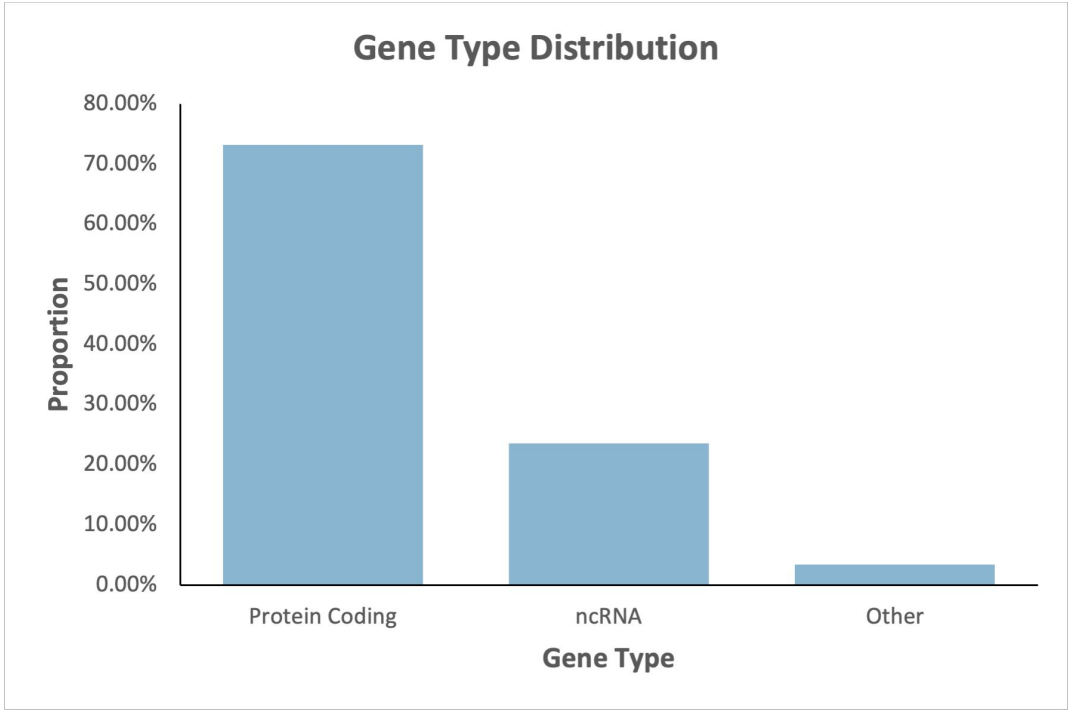
3’ UTR

Exon

Noncoding

5’ UTR

Annotate Peaks: Gene Types



Other:

Pseudo

snoRNA

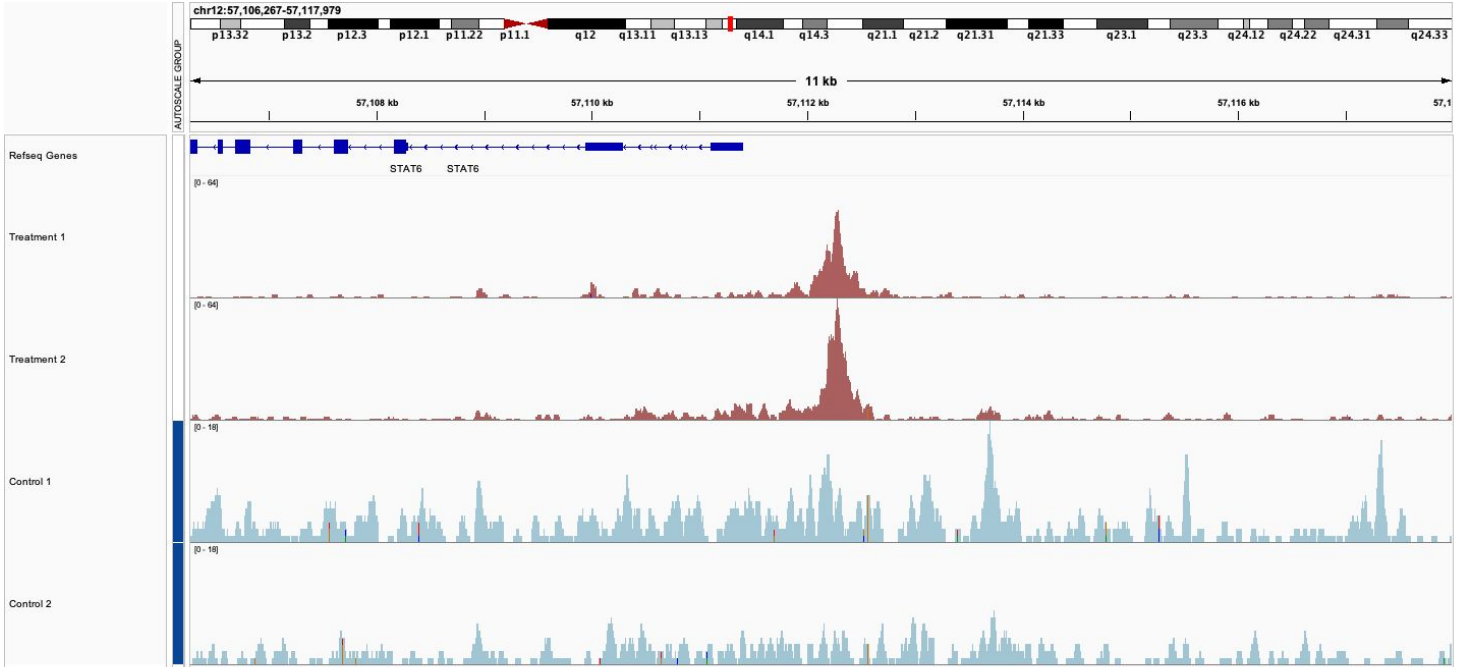
snRNA

scRNA

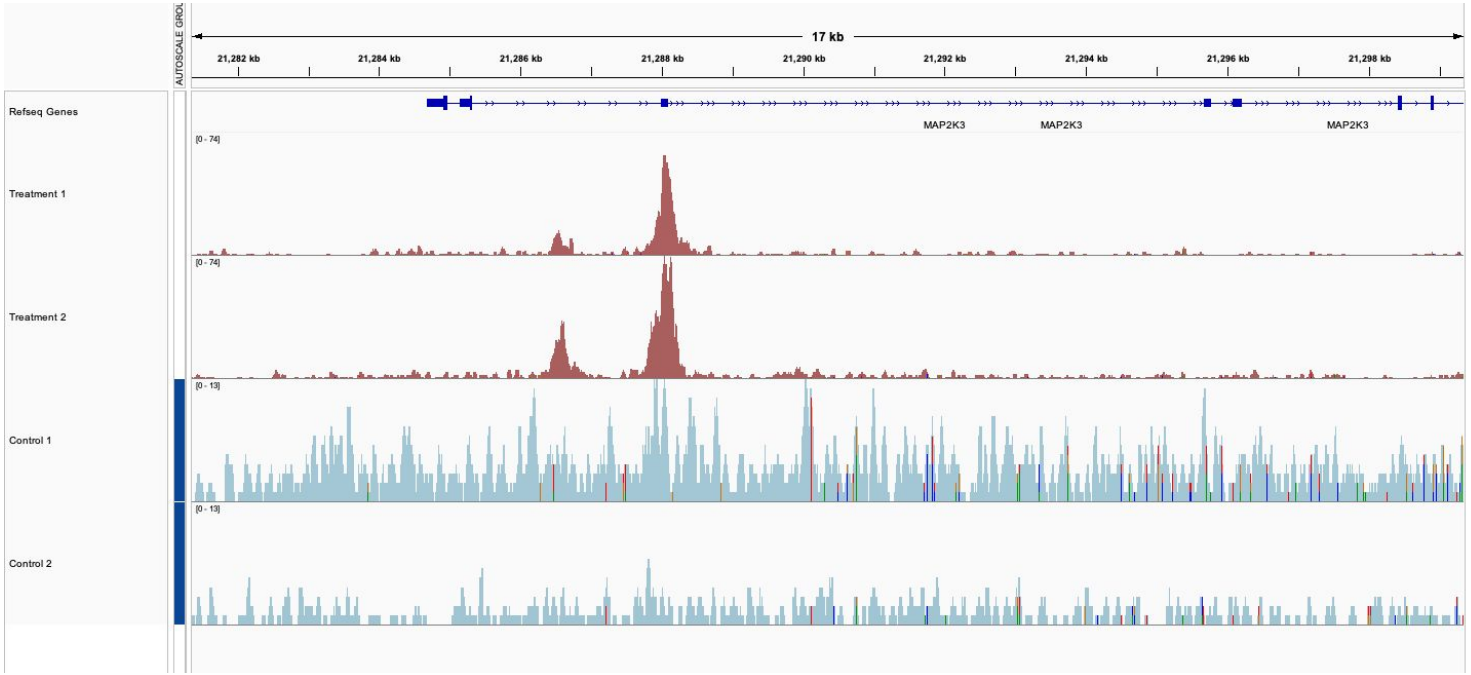
rRNA

Visualizing Peaks: STAT6

Is highly expressed in HCC

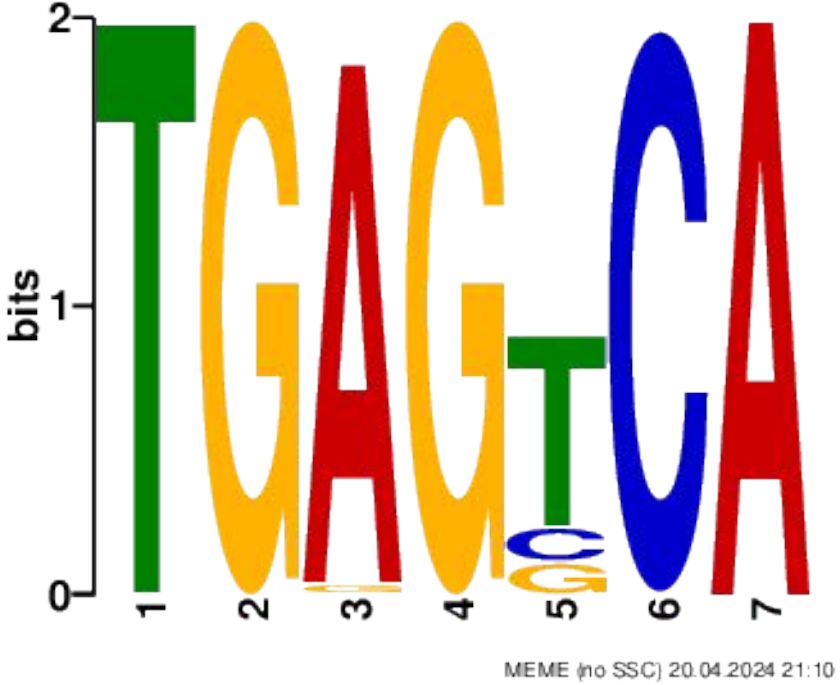


Visualizing Peaks: MAP2K3

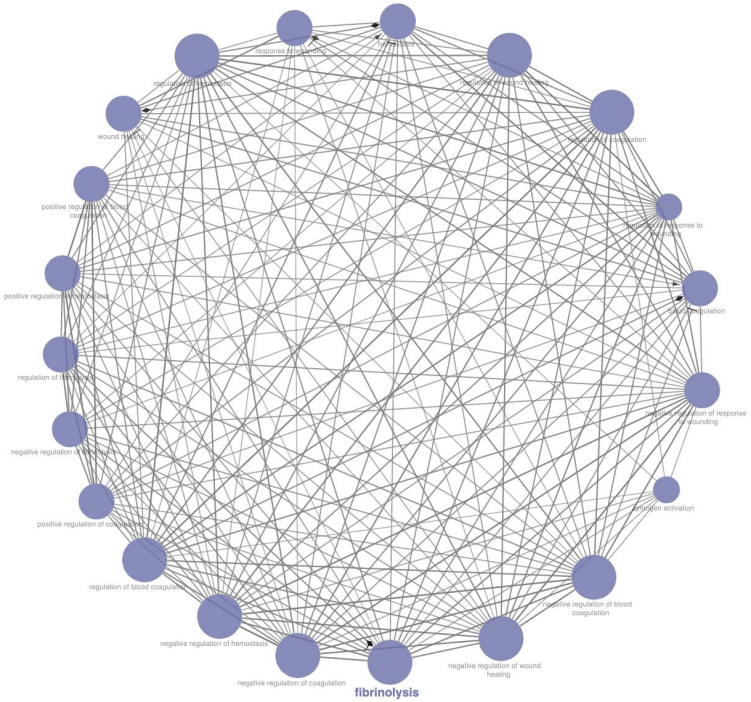


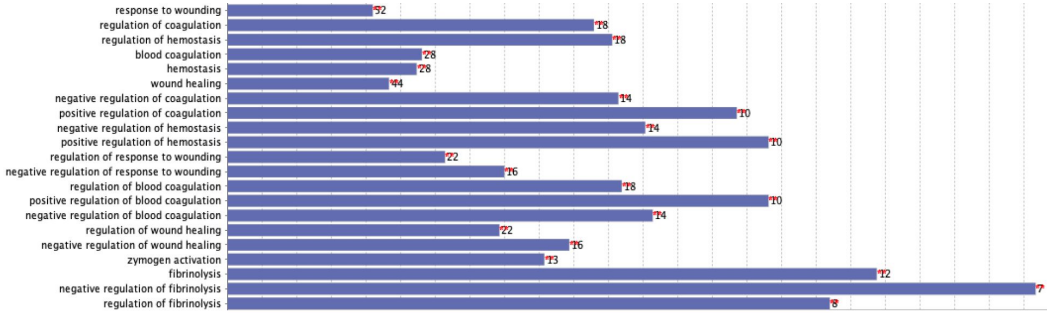
Motif Discovery

- Site Count: 5043
- 31.68%
- Validated through Zhao et al., where they noticed the top 12 motifs contained the AP-1 sequence TGAGTCA.



Functional Enrichment





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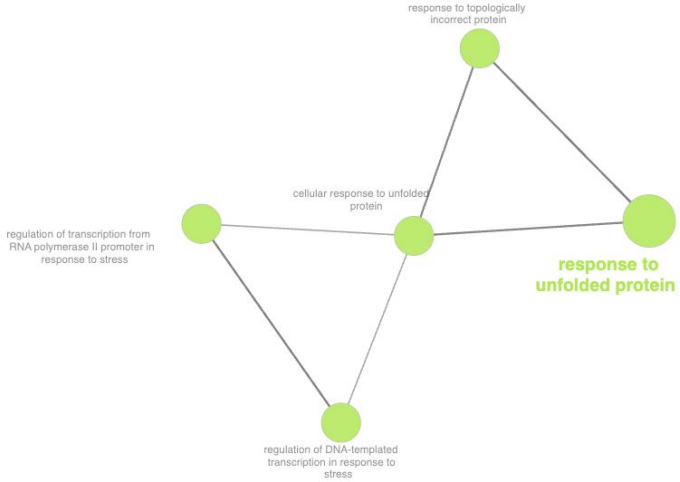

Conclusion

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- RT-qPCR to validate the expression changes of genes upon ATF3 modulation
- ChIP-qPCR to validate the direct binding between ATF3 and the genes of interest

Key Takeaways:

- Explored ATF3’s role in modulating liver cancer-related pathways through genome interactions.
- Uncovered novel gene interactions, offering fresh research avenues.
    - Offers potential for further investigation into ATF3's impact on gene regulation.