Differential loop analysis of phenotypically and genetically juxtaposed mouse strains using visualization tools

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Introduction

Biological Framework:

- Previous study suggests promoters are key to gene regulation, can be regulated from far away[1]
- Mice strains C57BL/6 (BLK6) and DBA/2J (DBA) have differential gene expression in fear and anxiety genes
- Experiments to isolate these differences, however, are expensive and time-consuming

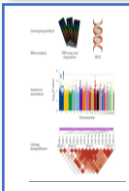
Computational Framework:

- Hi-C data and single-cell data modalities all describe differential expression in slightly different ways[2]
- Data collected using Hi-C bulk and single-cell sequencing

Goals:

- Simultaneous visualization of all modalities
- Classifier for future gene assessment

Background



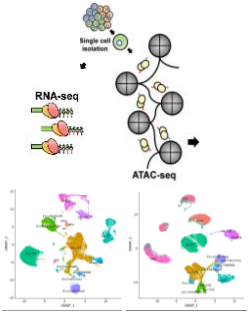
Genome-wide association studies[3]:

- Identified multi-gene (2MB) loci to study further
- Used computational fine mapping to narrow to candidate genes

Knockout Experiment:

- Generate mice with given gene knocked out
- Fear and anxiety phenotype evaluated
- Positive hit → promising genes
- Negative hit → control gene

Methods

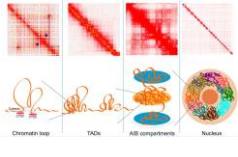


Single-Cell Sequencing[4]:

- **ATAC**: Captures chromatin accessibility
- **RNA**: Captures transcription level
- BLK6 and DBA samples are pooled (n=1, 3 biological replicates, 2 technical replicates) and sequenced together
- De-multiplex/genotyped computationally
- Annotation with Leiden Clustering in low dimensional space
- Aggregated number of mapped reads to pseudobulk (stratified by strain, cell type and normalized by RPKM)

Hi-C[5]:

- Captures long term interactions (loops)
- Collected Bulk Hi-C data for two strains separately
- Controlled for sequencing depth (KR normalized at 5kb resolution)
- Corrected for expected contact frequency
- Visualized by WUSTL Epigenome Browser



Results

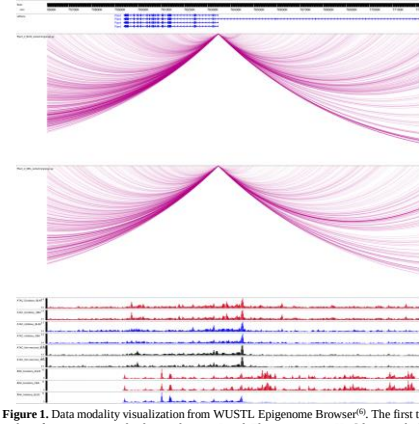


Figure 1. Data modality visualization from WUSTL Epigenome Browser[6]. The first track is the reference genome, displaying the gene Ptprd. The next two are Hi-C loop tracks(5kb). The next six are ATACseq data, and the following six are RNA transcript data.

Findings:

- ATAC, RNA, and Hi-C data demonstrate visually similar peaks
- Visible cell-type differences

Limitations:

- No one modality explains positive hits
- Tool demonstrates limited features for overlapping, multi-colored tracks

Discussion & Conclusions

- Further research is required to draw biologically relevant conclusions
- Accessible regions of a chromosome are distinguishable by all three types of data: RNA transcription, ATACseq, and Hi-C loops
- Promoter regions, are key sections in differential expression
- No distinct long-range connections; higher-resolution loop data may show more

References

(1) Smemo, S., et al. (2014).
(2) Nothjunge, S., et al. (2017).
(3) Tam, V., et al. (2019).
(4) Liu, N. et al. (2020).
(5) Baek, S., & Lee, I. (2020).
(6) Li, D., et al. (2019).