Network Analysis of Rodent Transcriptomes in Spaceflight

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Abstract

Network analysis methods leverage prior knowledge of cellular systems and the statistical and conceptual relationships between analyte measurements to determine gene connectivity. Correlation and conditional metrics are used to infer a network topology and provide a systems-level context for cellular responses. Integration across multiple experimental conditions and omics domains can reveal the regulatory mechanisms that underlie gene expression. GeneLab has assembled rich multi-omic (transcriptomics, proteomics, epigenomics, and epitranscriptomics) datasets for multiple murine tissues from the Rodent Research 1 (RR-1) experiment. RR-1 assesses the impact of 37 days of spaceflight on gene expression across a variety of tissue types, such as adrenal glands, quadriceps, gastrocnemius, tibalius anterior, extensor digitorum longus, soleus, eye, and kidney. Network analysis is particularly useful for RR-1 -omics datasets because it reinforces subtle relationships that may be overlooked in isolated analyses and subdues confounding factors. Our objective is to use network analysis to determine potential target nodes for therapeutic intervention and identify similarities with existing disease models. Multiple network algorithms are used for a higher confidence consensus.

Background

Rodent Research-1

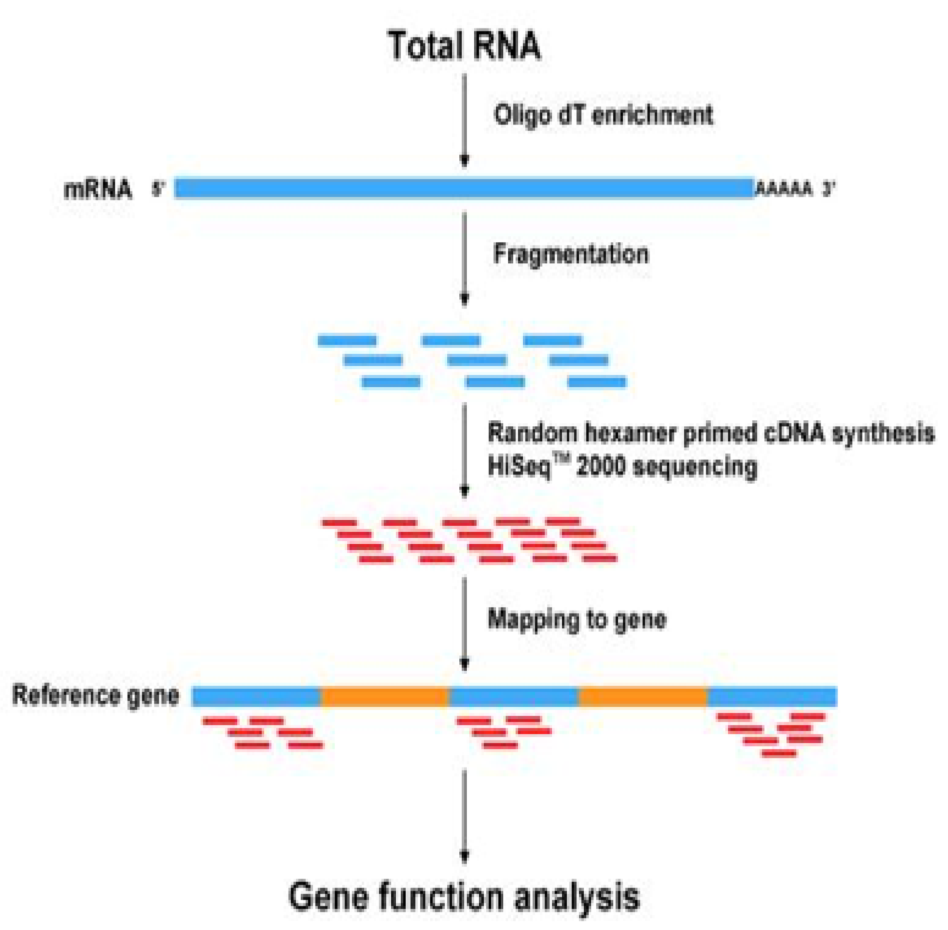
- 37 days on the International Space Station
- 7 tissue types:
  - Adrenal gland
  - Kidney
  - Type I & II muscles
    - Soleus
    - Extensor digitorum longus
    - Gastrocnemius
    - Tibialis anterior

Objective: Visualize & analyze the effects of microgravity on rodent gene expression

- Which genes are affected by microgravity across all tissues?
- How significant & how large are the effects?

RNA Sequencing

- RNA Seq was performed using Illumina paired-end HISEQ by collaborators at University of California-Davis



Differential Expression Analysis

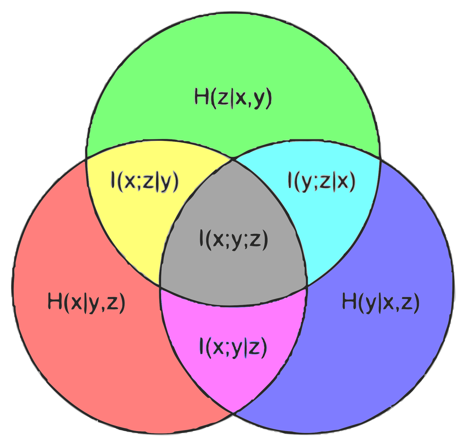
1. **For each gene,** compare RNA counts between space & ground control mice (log2 fold change)
2. **For each gene,** calculate the significance of the change (t-test, p-value)
3. **For each tissue,** Identify genes with large effect sizes & high significance (volcano plot)

Network Analysis Workflow

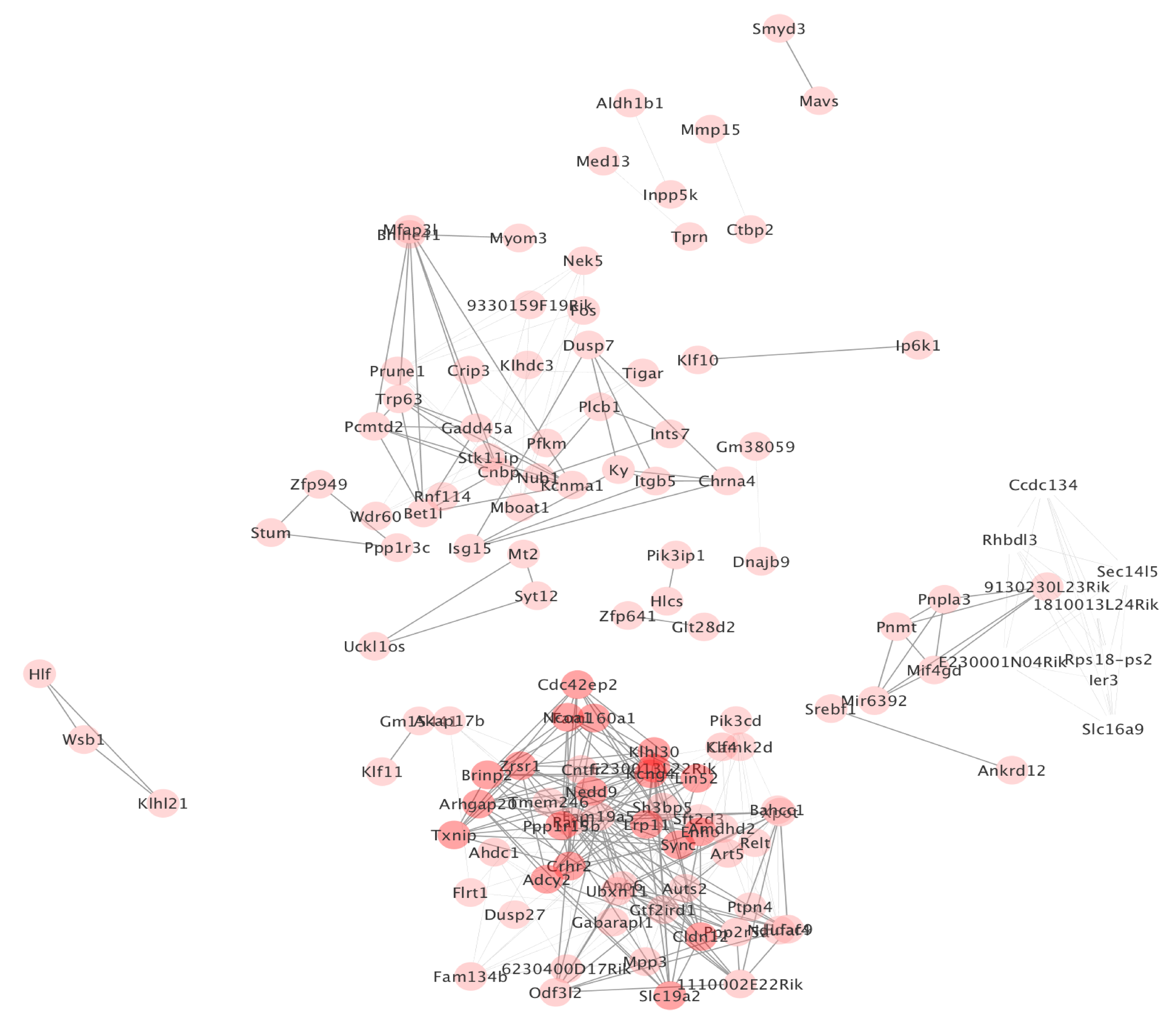
4. For each gene pair, compute the relatedness of expression in space vs. ground across all tissues (Mutual Information of $\log_2$ fold changes)
5. Create a network of the genes with the most interrelated expression & collapse the weakest links (ARACNE network inference algorithm)
6. Mine databases for relevant pathways and known gene & protein interactions to understand underlying biological processes (Functional Enrichment)

Mutual Information & ARACNE

- Measures interrelatedness of gene expression by assessing the dependence of every gene's expression on each other
- Positive and symmetric
- ARACNE is a network inference tool that removes the weakest edge between every triplet of genes to avoid capturing indirect relationships

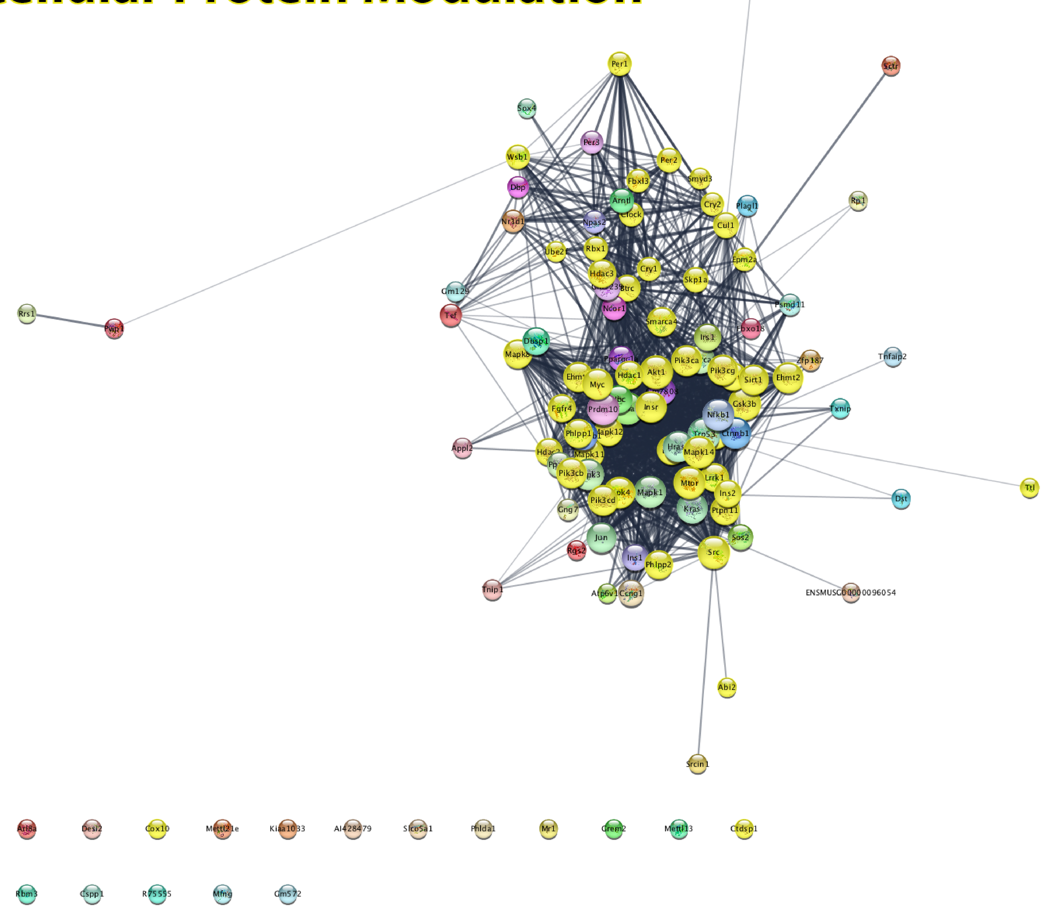


Differential Gene Network



STRING Functional Enrichment

Cellular Protein Modulation



Anatomical Structure Development