



Gene Expression in the Spleen

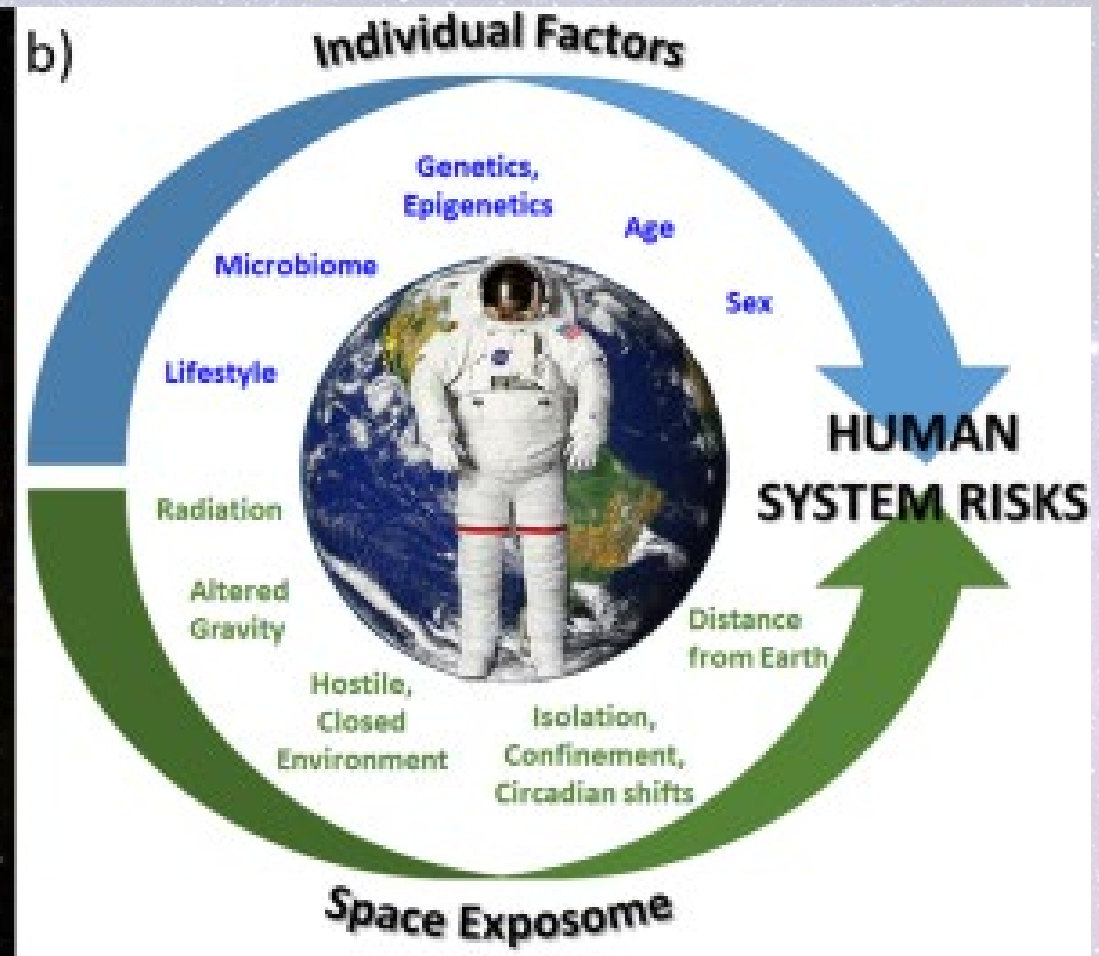
Supplementary slides for the Curricular Unit:
Exploring Gene Expression with Bioinformatics

Spaceflight Factors



How do these
factor affect
humans in
space?





Spaceflight Factors (Image Credit: NASA)

As space biologists, we work on determining the mechanisms behind **phenotypic changes** we see during spaceflight. This is often due to changes in **gene expression** NOT mutation.

Mouse-stronauts?

- Mice are often flown on the ISS because they are excellent **model organisms** for humans
- 1949: First mice were sent to space to study radiation on biological processes
- The current series of rodent studies is called Rodent Research (RR) and RR10 was the most recent mission



Rodent habitat for the ISS- up to 5 mice per side (Image Credit: NASA)

The Experiment

- Go to genelab.nasa.gov > Click on Data Repository > Search GLDS-288
- Explore the details of the experiment to answer questions 1-4 under Background

GLDS-95
Version 1

DESCRIPTION

PROTOCOLS

SAMPLES

ASSAYS

PUBLICATIONS

STUDY FILES

VISUALIZATION

GLDS-95: A Molecular Genetic Basis Explaining Altered Bacterial Behavior in Space Version 1

Select a Version: 1

DOI: 10.26030/ne00-p770
Source Accession Number(s) GSE82341
Total Data Volume: 36 GB

Submitted Date: 07-JUN-2016
Release Date: 15-NOV-2016

DESCRIPTION

Study Description

Bacterial behavior has been observed to change during spaceflight. Higher final cell counts, enhanced biofilm formation, increased virulence, and reduced susceptibility to antibiotics have been reported to occur for cells cultured in space. Most of these phenomena are theorized as being an indirect effect of an altered extracellular environment, where the carbon source uptake is inhibited and excreted acidic byproducts buildup around the cell due to the lack of gravity-driven transport forces. However, to date neither spaceflight results, ground-based studies, physical measurement techniques nor computational approaches have provided sufficient evidence needed to confirm this model. Gene expression data from the Antibiotic Effectiveness in Space (AES-1) experiment, however, have now allowed us to look into the biomolecular processes behind these observations and showed a systematic activation of glucose starvation and acid resistance genes. These results corroborate the reduced mass transport model proposed to govern bacterial responses to spaceflight. Furthermore, the gene expression data suggests that metabolism was stimulated in space, which could play a role in causing the observed increase in bacterial cell concentrations in microgravity. Similarly, the decrease in extracellular pH may also be involved with the reported increase in virulence in space.

Gene Expression in Space

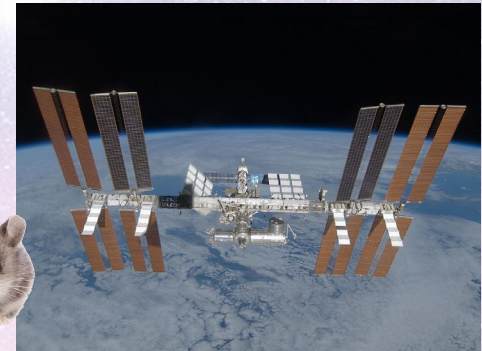
The Goal: Use the GeneLab platform to help us analyze data to determine possible biological pathways contributing to **differential gene expression** in the spleens of mice.

The Experiment:

Mice on Earth



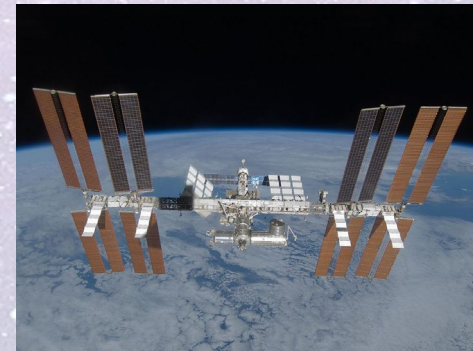
1. Mice flown for 35 days
2. Mice were euthanized
3. Spleens were collected and froze
4. RNAseq done after landing for data collection



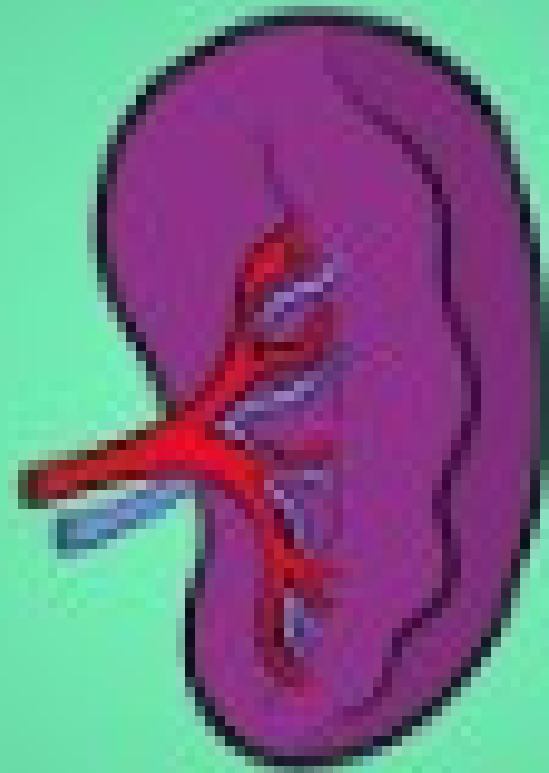
Mice on ISS in **microgravity**



Mice on ISS in **artificial gravity**

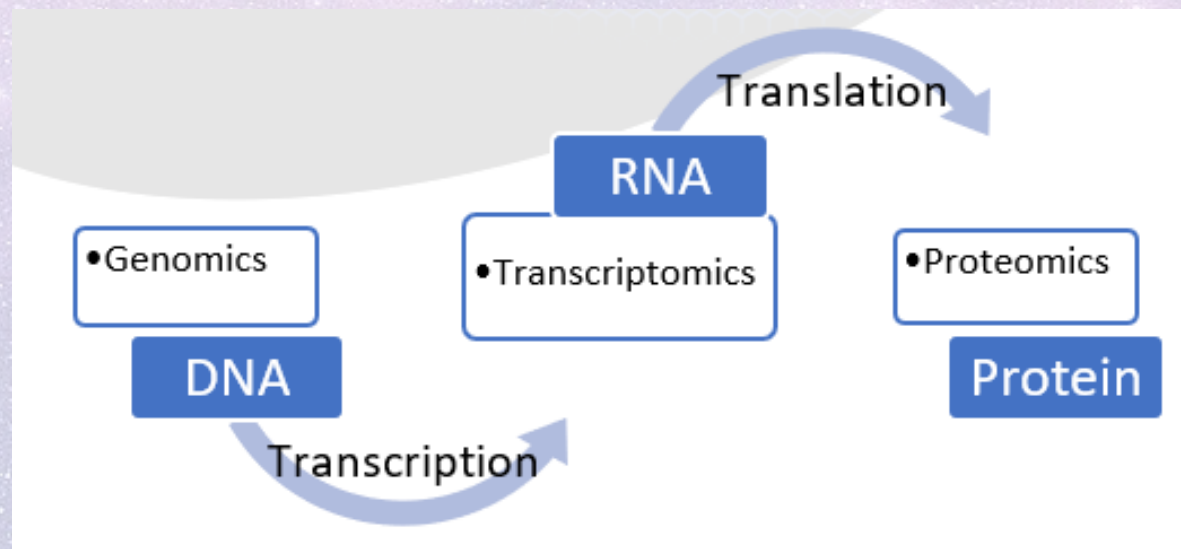


What on Earth is a Spleen?



Transcriptomics

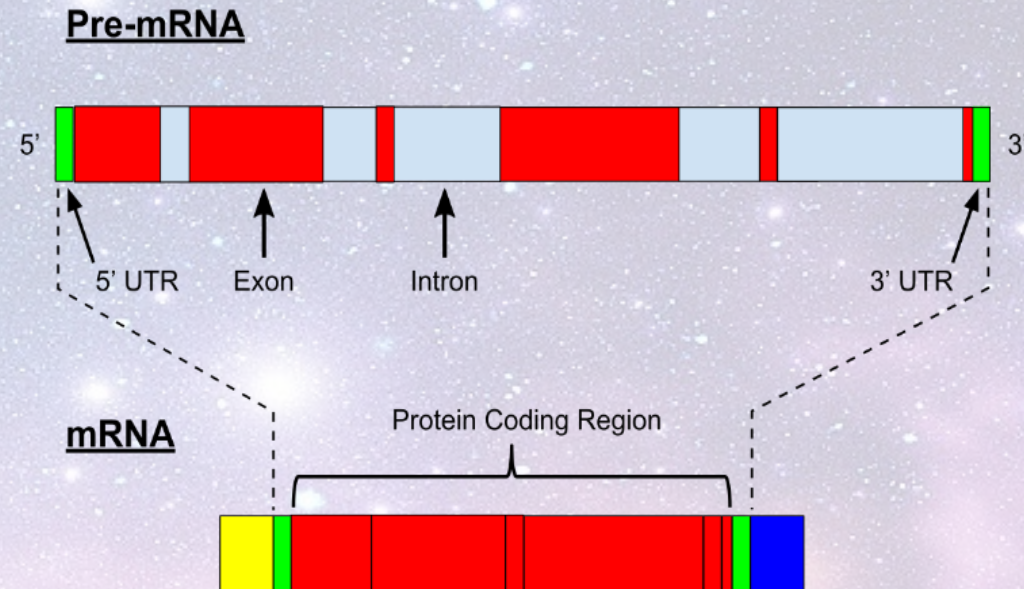
- In space, many of the differences we see are due to changes in gene expression, NOT mutations
 - Genes are being turned off or on in different ways than on Earth
- This is why we want to look at transcript data instead of genetic data



Read through the information on RNA Sequencing and answer 1-3

RNA Splicing

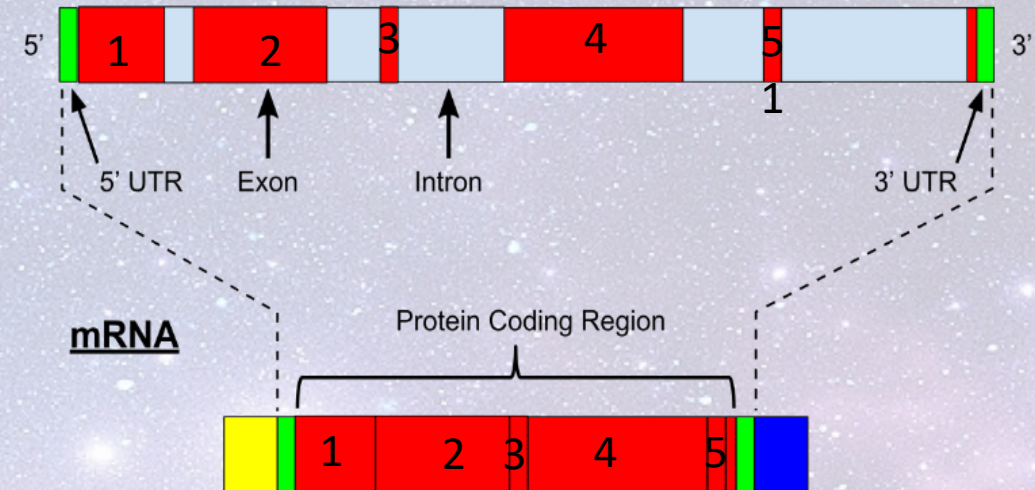
- One gene DOES NOT equal one protein
- **Exons** are **expressed**
- **Introns** are **interrupting**
- We need to use alignment algorithms that are splice-aware to ensure that we are getting correct data



Answer questions 1-2 under RNASTar

RNA STAR

- Splicing means that parts of an mRNA transcript correspond to different locations in the genome



If we searched the genome for the entire mRNA transcript, would we find a match?

- RNA STAR breaks the transcript data into pieces and then finds where they match to the genome and then stitches them back together to determine which gene they came from



Getting to the Galaxy Platform

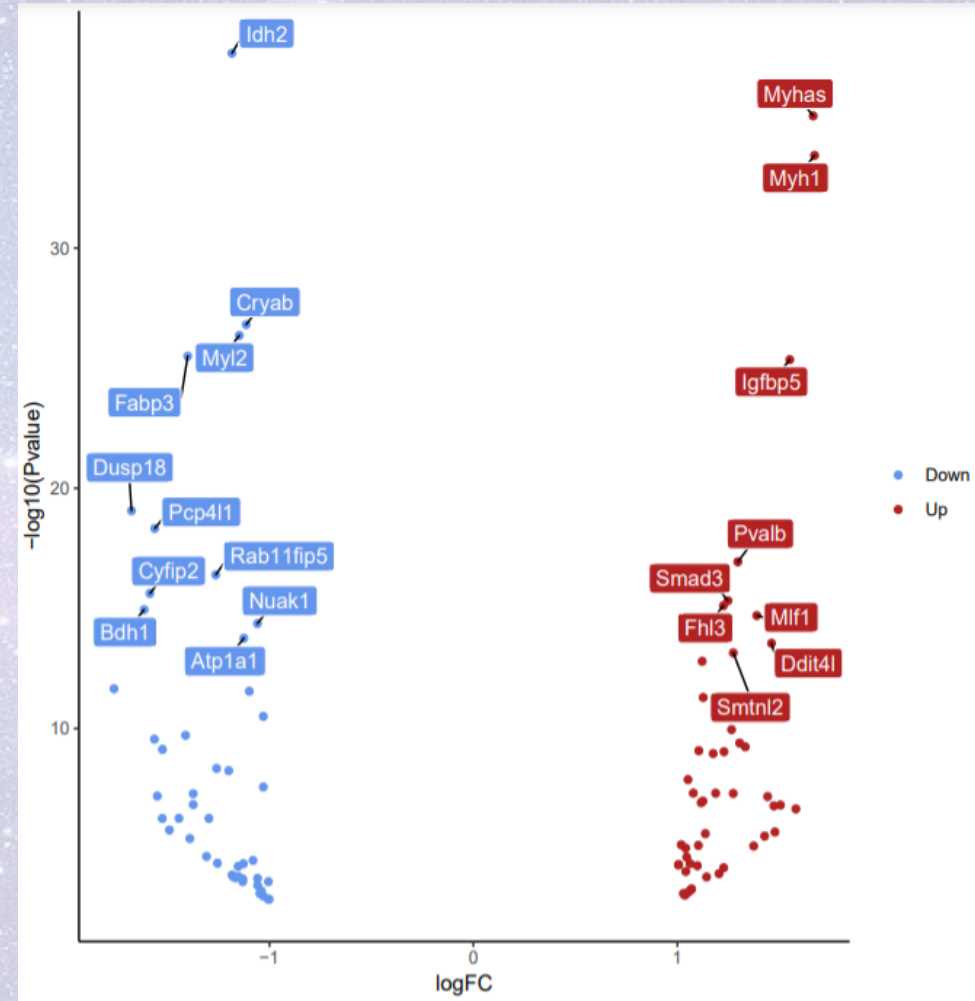
- Go to genelab.nasa.gov
- Click on Analyze Data
- Use the Sign-in with Google option- use your Gmail account to log in
- Use the link in the handout to access the shared history called

GLDS-288 GeneExpressionActivity

- Click the + in the upper right corner to import the history
- Name your new history **GLDS-288 Analysis**

Volcano Plots

- Each point represents one gene.
- The x-axis is showing the **fold change** which is how many times more or less the gene was expressed during spaceflight.
- The y-axis is showing statistical significance, or how confident we are that these genes changed because of the environment (space).
- Red points are genes that are **upregulated**.
- Blue points are genes that are **downregulated**.
- The labels are the 20 most significantly changed gene IDs.



Analyzing Volcano Plots- GeneCards


- GeneCards is a database for many different vertebrate genes
- Search the gene ID labeled in the volcano plot to determine what your gene does and major biological pathways it's involved in

GeneCards®
THE HUMAN GENE DATABASE

Free for academic non-profit institutions. Other users need a [Commercial license](#)

WEIZMANN INSTITUTE OF SCIENCE

LifeMap SCIENCES


Keywords ▾ Search Term  Advanced

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IL6 Gene (Protein Coding) ★

Interleukin 6 Protein Name

GCID: GC07P022725 ?

Genes Participants 

GIFts: 50 ?

[Jump to section](#) [Aliases](#) [Disorders](#) [Domains](#) [Drugs](#) [Expression](#) [Function](#) [Genomics](#) [Localization](#) [Orthologs](#)

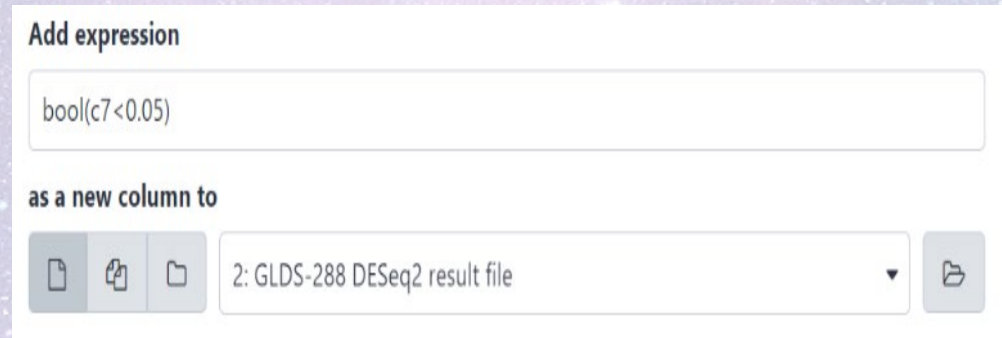
[Paralogs](#) [Pathways](#) [Products](#) [Proteins](#) [Publications](#) [Sources](#) [Summaries](#) [Transcripts](#) [Variants](#)

Gene Ontology Plots

- Gene Ontology (GO) is a database with gene functions
- Pathway analysis and categories of genes instead of analyzing genes separately
- Reduces complexity of analysis!
 - Looking at biological pathways is easier to make hypothesis about why the changes we see are occurring
- To run goseq, we need to create 2 files from the DESeq2 results file
 - Add a T/F column
 - Cut out c1, c8

Running goseq

Step 1: Adding a column with T/F data for genes that are statistically significant



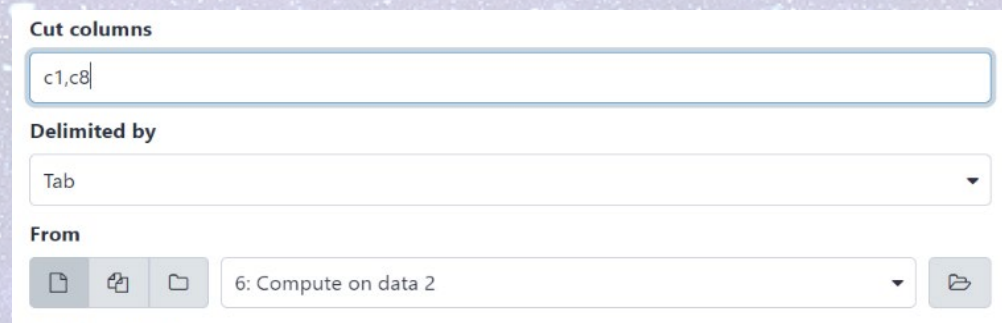
Add expression

`bool(c7<0.05)`

as a new column to

2: GLDS-288 DESeq2 result file

Step 2: Cutting out all the columns of the file **except** the genes and T/F we added. **RENAME THIS FILE ONCE IT TURNS GREEN**



Cut columns

`c1,c8`

Delimited by

Tab

From

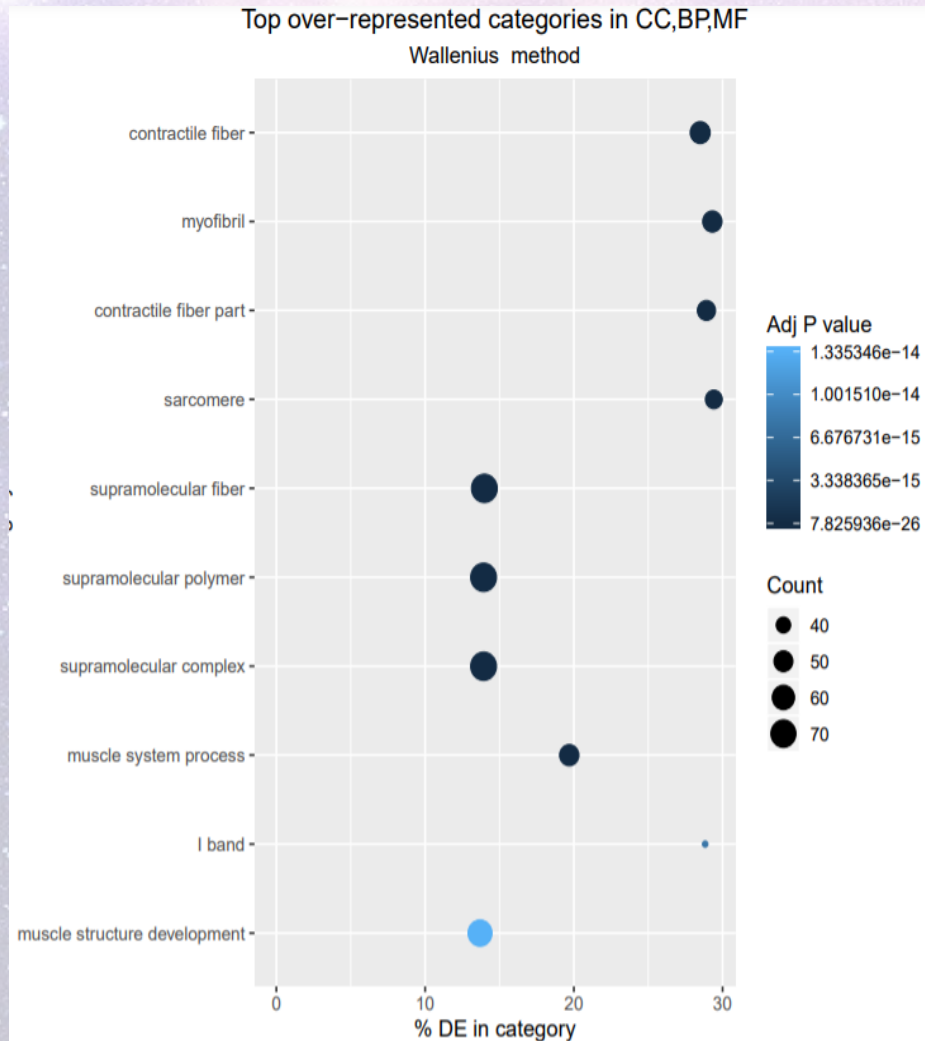
6: Compute on data 2

Running goseq

Step 3: Running the new file with 2 columns through the goseq algorithm

- Search goseq in the toolbar menu
- Choose the **Genes and T/F file** for the first file
- ***Gene Lengths:*** Select the **GLDS-288 Gene lengths file**
- ***Select a Genome:*** Mouse
- Find ***Output Options***
 - ***Output Top GO Terms Plot?:*** Yes
 - ***Extract the DE genes?*** Yes
- Execute

GO Terms Plots



- Each dot represents ALL of the genes in one GO pathway
- Each pathway is labeled on the left
- The color of the dot is the P value (statistical significance)
 - The darker the color, the MORE significant the changes are
- The x-axis how much change in gene expression there is
- The size of the points represents how many genes were altered in that pathway

GeneOntology.org

- Copy the pathway you are interested in into the search bar
- On the next page, click Ontology > find the entry that matches what you searched and open that

The screenshot shows the Gene Ontology website homepage. The header includes the Gene Ontology logo and navigation links: About, Ontology, and Annotations. The main content area has three large buttons: 'Ontology' (circled in red), 'Genes and gene products', and 'Annotations'. The 'Ontology' button is highlighted with a red circle. Below the buttons, there is a search bar with the text 'sarcomere' entered. At the bottom, there are radio buttons for 'Any', 'Ontology' (selected), and 'Gene Product'.

<input type="checkbox"/> Term	Definition
<input type="checkbox"/> positive regulation of sarcomere organization	Any process that increases the rate, frequency or extent of myofibril assembly by organization of mu more...
<input type="checkbox"/> regulation of sarcomere organization	Any process that modulates the rate, frequency or extent of myofibril assembly by organization of mu more...
<input type="checkbox"/> negative regulation of sarcomere organization	Any process that decreases the rate, frequency or extent of myofibril assembly by organization of mu more...
<input type="checkbox"/> sarcomere	The repeating unit of a myofibril in a muscle cell, composed of an array of overlapping thick and thin filaments between two adjacent Z discs.

GeneOntology.org

- You can find the definition of what the category means on this page, but it is not always very helpful
- Scroll down and find Inferred Tree View
 - This shows you what processes your term includes as well as larger pathways your term is part of



A screenshot of the Gene Ontology Inferred Tree View for the term 'sarcomere'. The tree is displayed as a list of terms with blue circular icons containing a 'P' to the left of each term. The terms are: CL:0000187 muscle cell, GO:0099512 supramolecular fiber, GO:0005575 cellular_component, GO:0043292 contractile fiber, CL:0000737 striated muscle cell, GO:0030016 myofibril, and GO:0030017 sarcomere. The 'sarcomere' term is highlighted with a yellow triangle icon to its left. Below 'sarcomere' are four more terms: GO:0031672 A band, GO:0031674 I band, GO:0005863 striated muscle myosin thick filament, and GO:0005865 striated muscle thin filament.

- P CL:0000187 muscle cell
- P GO:0099512 supramolecular fiber
- P GO:0005575 cellular_component
- P GO:0043292 contractile fiber
- P CL:0000737 striated muscle cell
- P GO:0030016 myofibril
- ▼ **GO:0030017 sarcomere**
 - P GO:0031672 A band
 - P GO:0031674 I band
 - P GO:0005863 striated muscle myosin thick filament
 - P GO:0005865 striated muscle thin filament

This is the Inferred Tree View for "sarcomere"

- Sarcomeres are part of Muscle cells and myofibril
- A and I bands are within sarcomeres



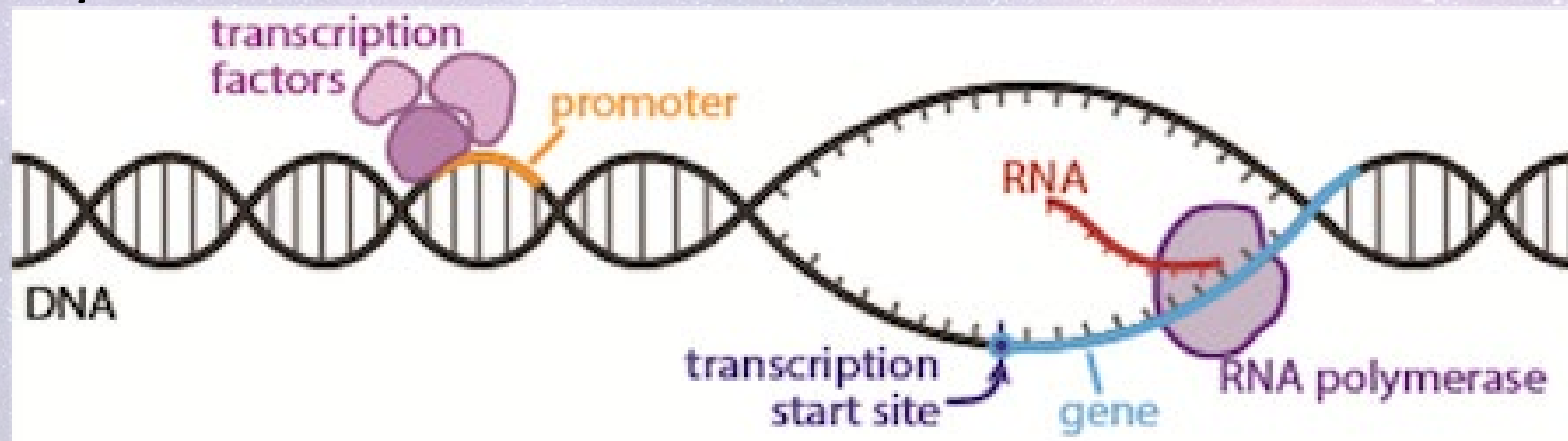
The Findings

Our data further suggest that spaceflight causes a reduction in the expression level of genes related to erythrocytes in the spleen. Spaceflight reportedly caused a reduction of the red cell mass in astronauts³⁹, which was proposed to be due to the suppression of erythropoiesis. In addition, a reduction in the number of erythroid cells in the spleen of rats after 22 day spaceflight was reported²³. Notably, the results of colony formation assays suggest that erythropoiesis is reduced in the bone marrow of flight mice⁴⁰. As extramedullary haematopoiesis occurs in the spleens of mice⁴¹, the mechanisms controlling the extramedullary erythropoiesis may be impaired in mice experiencing spaceflight.

Overall, our data suggest that relatively long-term spaceflight down-regulates the expression of genes related to erythrocytes in the spleen. This down-regulation is likely due to the reduction of transcription factors GATA-1 and Tal1, which control the expression of these genes. Detailed investigation of the possible association between the down-regulation of these gene and the development of anaemia during space flight should be addressed in future studies.

What's Causing These Changes?

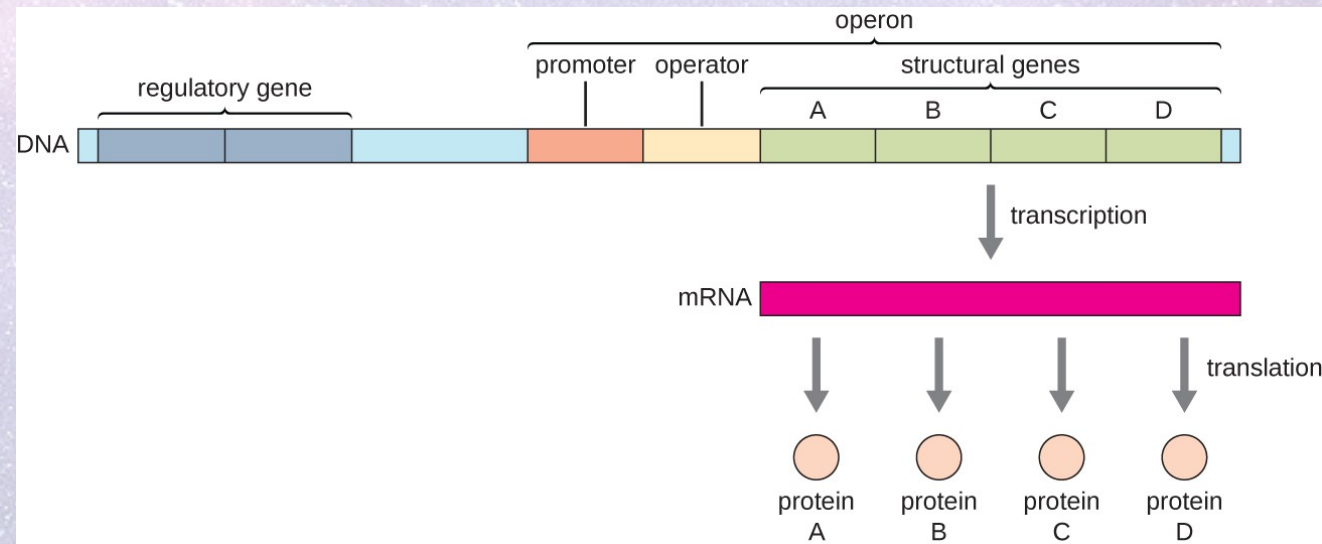
- Proteins involved in the process of transcription → **Transcription Factors**
- May either cause transcription OR stop transcription
 - Genetic on/off switches!
- **Promoters** and **operators** are locations on DNA upstream of a gene where transcription factors may need to bind in order for RNA polymerase to bind



Transcription Factors

- Activators- must be present in order for a gene to be transcribed
- Enhancers- makes transcription happen faster
- Repressors- blocks transcription from occurring

Genes are often arranged into **operons** so that several connected genes can all be made at the same time. Transcription factors are especially important in regulating operons.



Return to the hypothesis you created in the Explore section.

Modify your explanation to include how transcription factors may be changing your pathway.

lac Operon

- Present in *E. coli*
- The operon has 3 genes, all are necessary to metabolize lactose
- CAP is an activator
- *lac* repressor is bound to the operator unless lactose is present
- CAP binds when glucose concentration is low

