Effects of Spaceflight on Plants Using GLDS-38

Curricular Unit
Teacher Materials

OVERVIEW

Plants play a central role in human survival on earth. Conversely, they will play a critical in life support systems for space exploration. To understand the effects of space flight on Plants NASA's plant science research focuses on understanding how plants sense and respond to gravity, the role of gravity and microgravity in plant development and reproduction, the effects of gravity and microgravity on plant metabolism and transport, the synergistic effects of microgravity, radiation, and electromagnetism, and the role plants will play in recycling life support systems for space exploration.

In this unit students will describe the basic structure and function of plants, understand how plants grow, acquire energy and reproduce, learn about plant genes, genomes and genetics, and analyze plant omics data to explore the effects of spaceflight on plants.

Video: Science at NASA Astronauts Learn Gardening in Space (Astronauts Learn Gardening in Space | Video - YouTube)

CONTENT OBJECTIVES

- Describe the structure and function of a plant cell
- Identify major plant organs and describe the structures and functions of stems, leaves, and roots
- List requirements for plant growth and consider the challenges of growing plants in space
- Identify characteristics of a model organism for plant research
- Interpret data visualizations
- Use evidence to evaluate the impact of spaceflight on plants

PACING AND SCHEDULING

This set of five activities can take between 3 – 5 days in a traditional 55-minute long class period, depending on depth of knowledge expected, prior student knowledge associated with positioning of the unit in existent curriculum, and time allotments for intended student groupwork within the activities. Additional suggestions are included in the Teacher Materials (Answer Keys) of each activity.

This unit could be taught after an overview of eukaryotic organisms or as an introduction to plant sciences. It can also be incorporated proximally to or within a unit on gene expression and/or cell signaling.

TEACHING METHODS

Background Information

The first lesson focuses on understanding the basic structure and function of flowering plants or **angiosperms**. These are the dominant form of plants on earth today. A flowering plant has an above ground component, the **shoot,** and a below ground component, the **root**. The shoot includes more obvious structures such as the **stem** and **leaves** but is also where you will find the **shoot apex**, **leaf primordium,** and **axillary buds**. Below the soil line you will find the root system which includes the **root apical meristem**, **primary root, lateral roots, and root hairs.**

Teachers may choose to include this background information, if relevant to their curriculum, but this information is not directly informative to the lessons in this unit: Angiosperms are divided into **monocots** and **dicots** based on differences in some of these main structural features. The most well-known of these are the number of **cotyledons** or seed leaves. Monocots have one cotyledon and dicots have two. Some other key features to look at include the vein patterns on leaves, the number of petals or flowering parts, the type of root system, and organization of **vascular tissue** in the stem.

Vascular tissue along with the **epidermis** and **ground** tissue make up the plants tissue systems. The epidermis of the leaf is usually covered in a **waxy cuticle** to prevent water loss. Pores called **stomata** are found in the epidermis of the leaf. The size of these pores are controlled by **guard cells.** The stomata allow CO_2 to enter the leaf and O_2 to escape. They also allow water vapor to escape the plant so that water may be drawn up through the roots.

In the root epidermis, some of the cells will develop into root hairs. These are thin-walled cells that will facilitate the absorption of water and nutrients from the surrounding environment. These cells also interact with soil bacteria and play a role in the **symbiotic relationship** between roots and these bacteria. Root hairs develop near the root apical meristem and are lost as the root ages just like the cells of our skin.

Vascular tissue is arranged in bundles of **xylem** and **phloem** that travel through the plant. Xylem is responsible for transporting water up through the plant from the roots. The phloem transports carbohydrates from the leaves throughout the plant.

Plants share many of the same cellular structures present in other **eukaryotic** cells. Their cells are surrounded by a **plasma membrane**, their genetic material is in the form of **DNA,** and they synthesize their proteins using **ribosomes.** Although plants share many structures with animals, they differ in important ways. Plant cells are enclosed by a **cell wall,** made of cellulose. The cell walls are traversed by tunnels called **plasmodesmata.** They contain **chloroplast** which are the site of **photosynthesis** and a prominent **central vacuole.**

This lesson introduces the basic structure and function of plants and begins to unfold the idea that plants are impacted by conditions in space. The lessons that follow will provide a more detailed look at the biology of plants including plant development, physiology, metabolism, and plant genes and the potential impacts of spaceflight on those processes.

Discussion and Direction of Instruction

Further instructions and information are provided in the supplementary file that accompanies this curricular unit, especially for items 4 & 5 in this list.

1. Engage students in a discussion about the major plant organs. Emphasize that plants only have a few major organs, roots, stems, buds, flowers, and leaves. Teachers may choose to focus on the roots, stems, and leaves, but should note that flowers develop from buds which are distinct organs from leaves. Teachers could show buds in the "axils" of leaves (where the petiole meets the stem) and explain that those buds can develop into flowers or into lateral stems that can bear many flowers and/or leaves. Gene expression within a bud determines how it develops (if it stays dormant or develops into a flower or lateral stem etc). Have students complete **Activity 1: Labeling the parts of a flowering plant.**

2. Engage students in a discussion about model organisms. Encourage students to think about why we use model organisms and what would make an organism an ideal candidate for plant research. Have students complete **Activity 2: Model organisms.**

3. Introduce students to factors of space flight. Break students out in groups of two to three students. Give each group a large piece of paper and have them research and think about how and why plants would be impacted in space. Have students report and discuss what they found with the class.

4. Once students have a general understanding of the impacts of spaceflight on plants, introduce the Metadata and have students follow along using **Activity 3: Meta Data Analysis.** Once the students understand the protocols and factors, have them look at **Activity 4: Arabidopsis GLDS-38 MA-plot for Spaceflight: FLT vs GC.** Guide students in understanding the significance of the MA-plot and how this relates to their previous discussion on the effects of spaceflight on plants.

5. Now that students have the general understanding that gene expression is altered in space, let's look at a volcano plot in order to quickly identify specific genes that have significantly changed as a result of spaceflight. Introduce students to volcano plots. Have students complete **Activity 5: Arabidopsis GLDS-38 Volcano Plot.** Guide students in understanding the volcano plot.

CURRICULAR CONTENT

For this curricular unit, all of the associated worksheets (teacher versions) are embedded into the next pages of this document but each of the materials are also available in its own student worksheet + teacher answer reference file.

Supplemental Teacher Instructions are provided after the Activity sheets in order to help teachers prepare for **Activity 4** and **Activity 5** of this unit.

Red text indicates additional notes to/annotations for teachers or target responses expected of students.

Activity 1: Labeling the Parts of a Flowering Plant

Instructions: Label the parts of the model plant and write a function for each part.

Teacher's Note: If a live sample of this plant is available, have students observe each of the structures on the live plant. Wild mustard plants can often be found as a common weed in parking lots or fields.

Flower; Reproduction

Stem; Support, transport, storage

Leaves; Site of photosynthesis, transpiration, gas exchange

Root; Anchor the plant, absorption, transport,storage, vegetative growth

Brassica Image Credit: Yang, C., Zhang, C., Lu, Y., Jin, J., & Wang, X. (2011). The mechanisms of brassinosteroids' action: from signal transduction to plant development. *Molecular plant, 4 4*, 588-600
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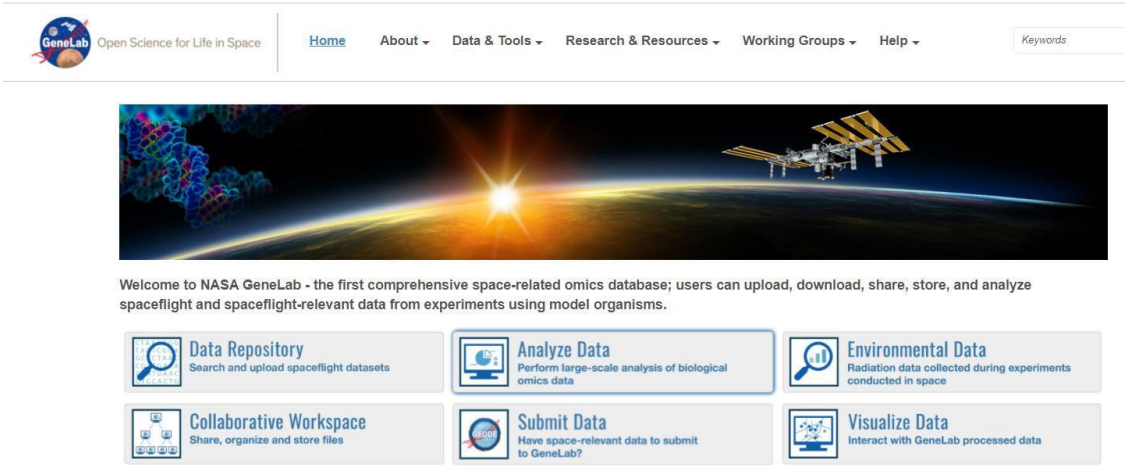
Activity 2: Model Organisms

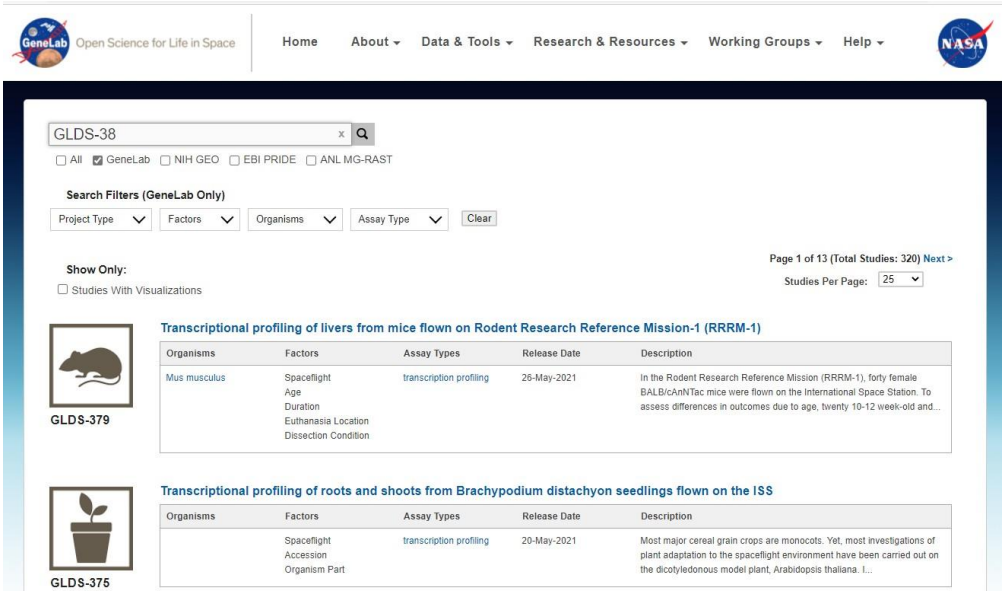
	What is one area of research in which this organism is used?	Why is it a good model organism for this area of study?	What else makes it a good model organism?
Fruit fly ***(Drosophila melanogaster)***	Learning and memory	Human genes involved in learning and memory are similar to those in thefly.	• short life cycle • inexpensive and easyto raise • studied extensively, soa lot is known about it • genome sequenced
Wall cress ***(Arabidopsis thaliana)***	Plant development	Small number of genes compared to many plants, and thegenes can be easily manipulated.	• short generations • easy to pollinate • lots of seeds • can be grown year-round in a greenhouse • genome sequenced • no specialized growth reqs. • Primarily self-fertilizing
Nematode(EXAMPLE: ***Caenorhabditis elegans*—called *C. elegans***)	Animal development	Its nearly transparent body lets researchers see and image stages of development.	• multicellular, but not an extensive number of different cells • easy to raise • short life cycle • genome sequenced
Rodents (EXAMPLE: **mouse,** ***Mus musculus***)	Human disease	The mouse is a mammal, so it sharesmore genes with us than nonmammalian model organisms.	Relative to other mammals that are studied, such as dogs,the mouse • has short generations • is easy to raise • genome sequenced

Activity 3: GLDS-38 Meta Data Analysis

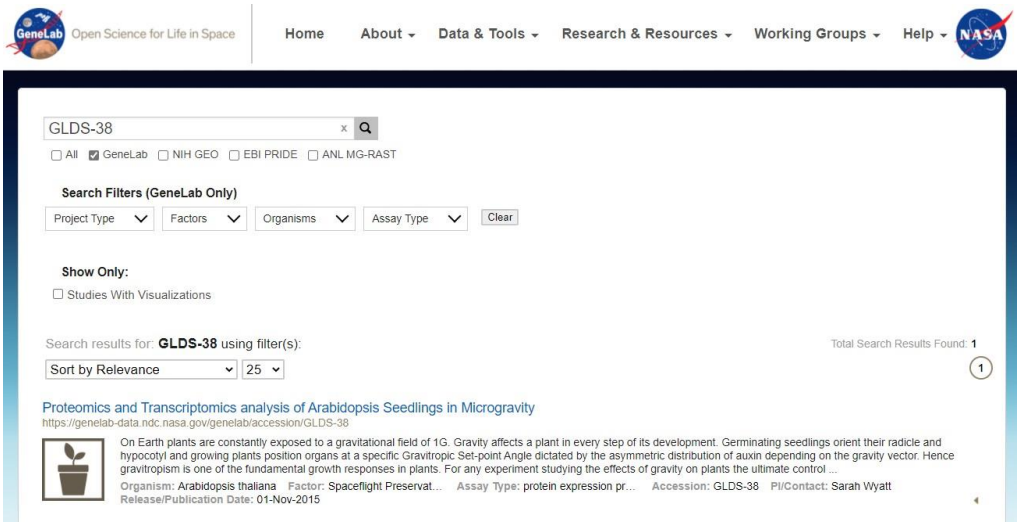
Instructions: Navigate a web browser to [NASA GeneLab: Open Science for Life in Space]



Once on the site, click on **Data Repository** and search for **GLDS-38**.



This will bring you to the study that we will be analyzing in this curricular unit**,** Proteomics andTranscriptomics analysis of Arabidopsis Seedlings in Microgravity.



Click on the hyperlink that corresponds to the study "Proteomics and Transcriptomicsanalysis of Arabidopsis Seedlings in Microgravity".

1. According to the **Study Description**, what is one of the fundamental growth responses in plants? What is the ultimate control for any experiment studyingthe effects of gravity in space?

   Gravitropism

   Microgravity in space

2. What is the model organism for this study?

   Arabidopsis thaliana

3. In the **Samples** section under **Factor Value**, what two groups are being compared in the study? What is being compared?

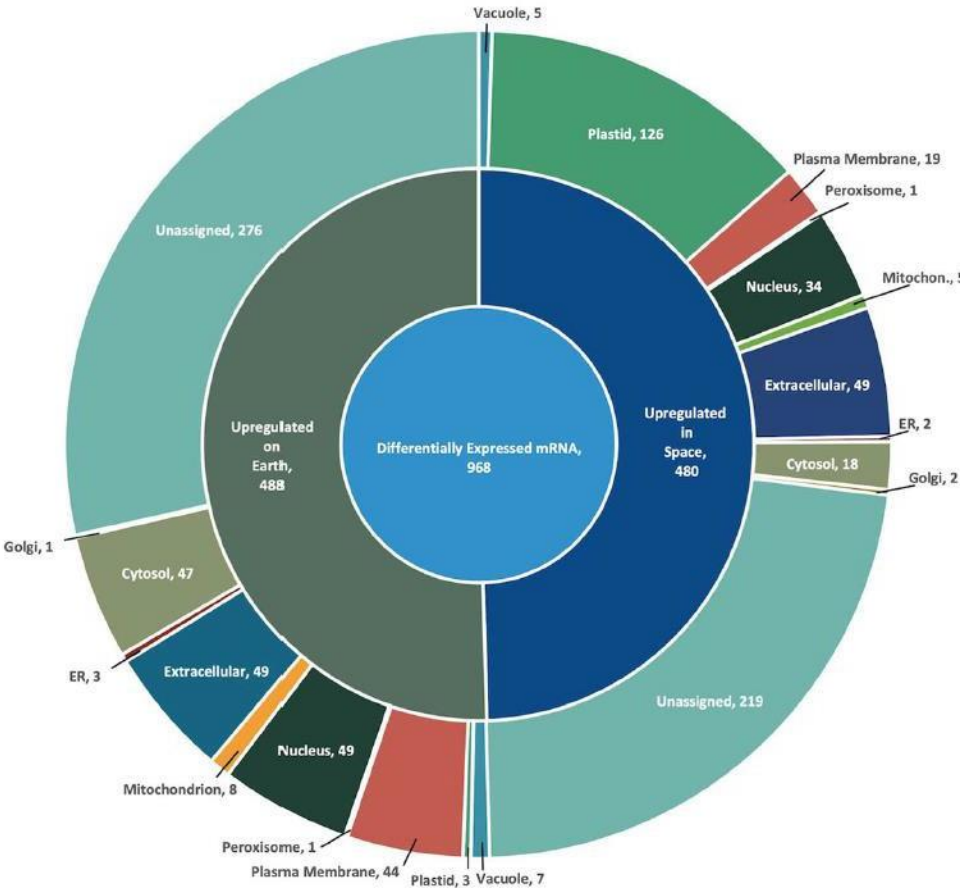
   Possible Answers: Spaceflight and ground control (conditions) OR RNAlater or liquid nitrogen (preservation method)

4. Under the **Protocol** section, how was nucleic acid extracted? What platform wasused to sequence it?

   Extracted using RNeasy Plant mini extraction kit

   Sequenced using Illumina HiSeq 2500

5. Look at the figure below.

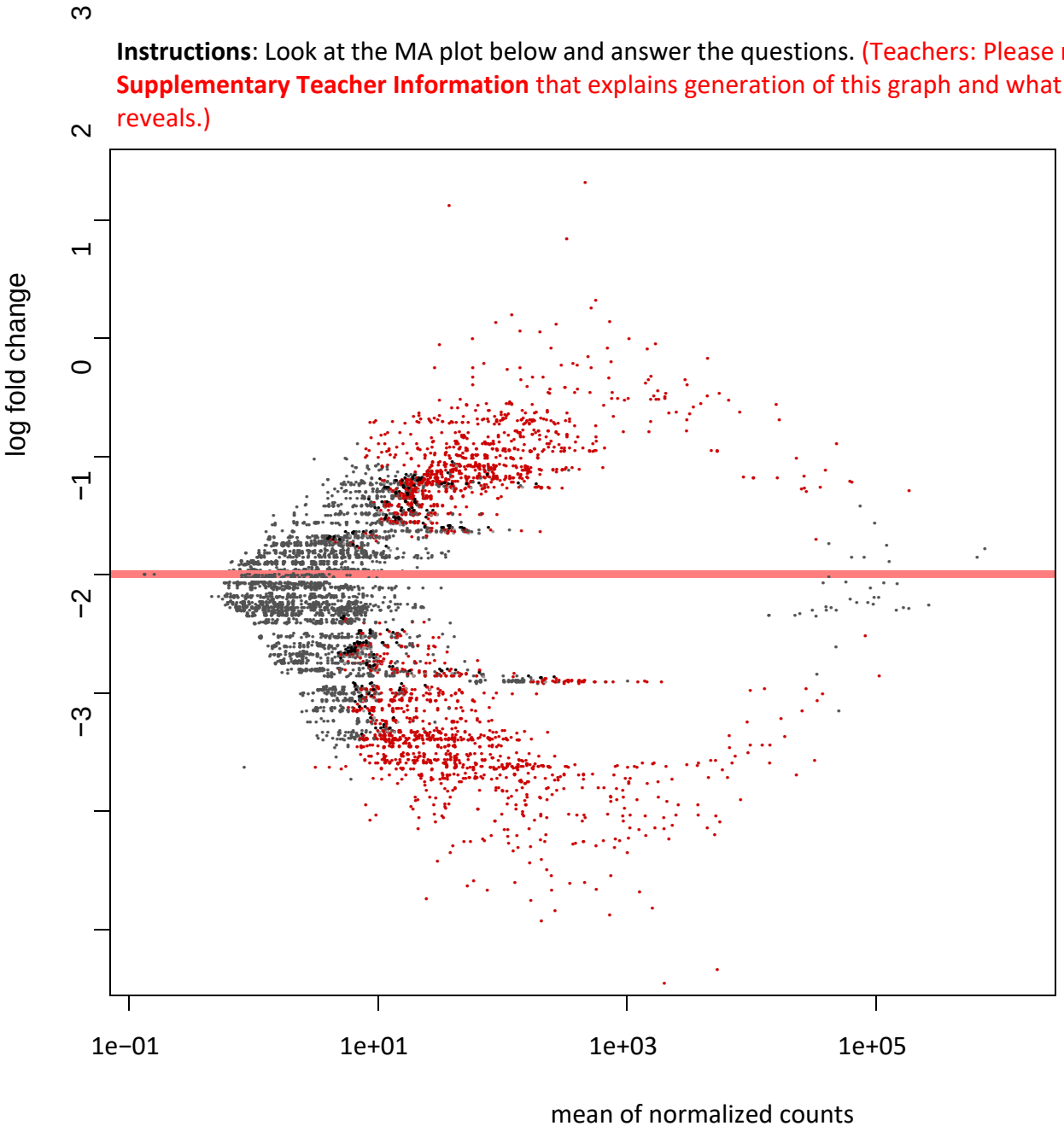


The figure shows an overview of transcripts differentially expressed duringspaceflight. How do the upregulated in space compare to those on earth?

Answers will vary
Example: More plastid genes are upregulated in space than on Earth

Activity 4: Arabidopsis GLDS-38 MA–plot for Spaceflight: FLT vs GC

Instructions: Look at the MA plot below and answer the questions. (Teachers: Please reference the **Supplementary Teacher Information** that explains generation of this graph and what the information in it reveals.)



1. **What do the dots represent?**

   A gene or feature

2. **What do the red dots represent?**

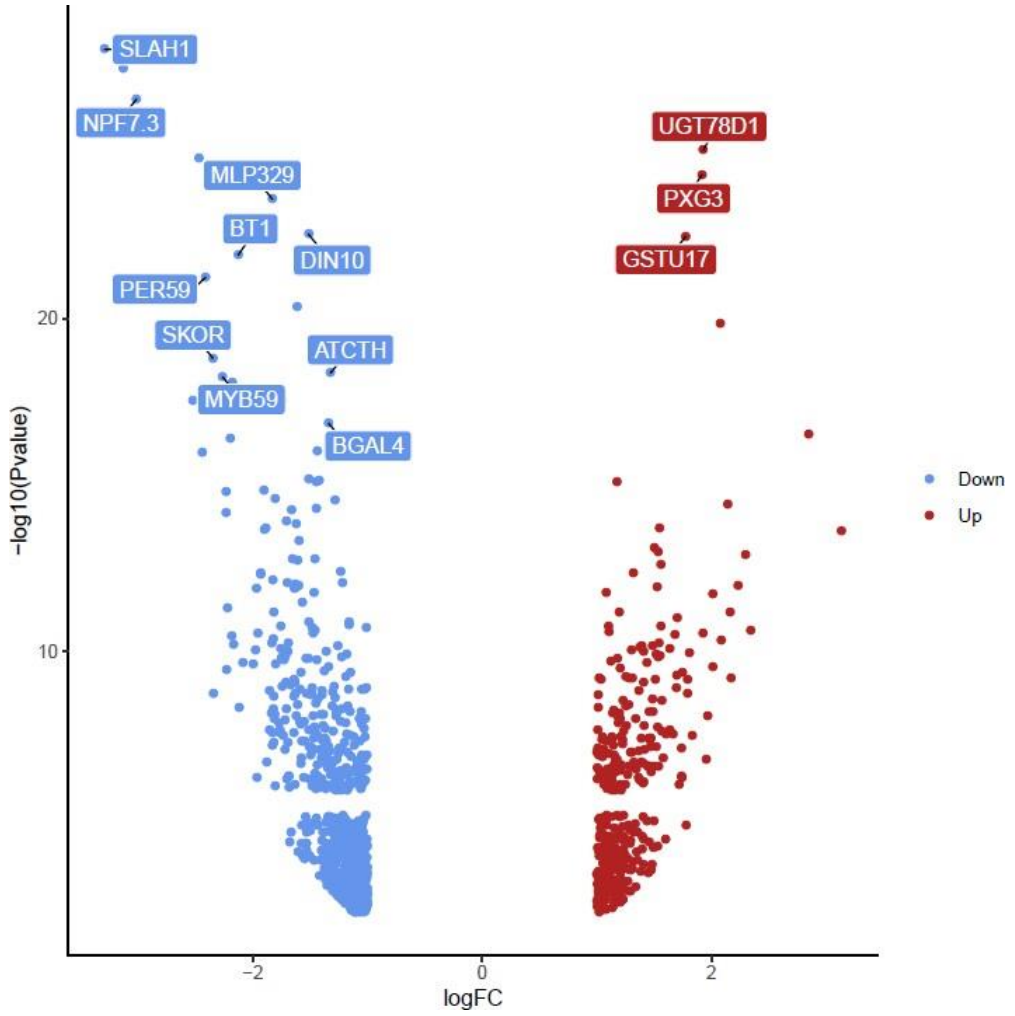
   These are genes that were significantly changed

3. **After discussing the impacts of spaceflight on plants and looking at the MAplot, what can you infer?**

   Answers will vary.
   Example: Gene expression in plants is affected by space flight. Somegenes are impacted more than others.

Activity 5: Arabidopsis GLDS-38 Volcano Plot

Instructions: Look at the volcano plot below and answer the questions. (Teachers: Please reference the **Supplementary Teacher Information** that explains generation of this graph and what the information in it reveals.)



1. **What do the blue dots on the volcano plot represent?**
   Significantly down regulated genes
2. **What do the red dots on the volcano plot represent?**
   Significantly upregulated genes
3. **Which gene was the most downregulated? Upregulated?**
   Down regulated-SLAH1, Upregulated-UGT78D1
4. **Go to TAIR - Home Page (arabidopsis.org) and search for the function of these genes.** Propose reasons that explain why regulation of these genes would change in space. (*You will learn more about genes and their expression in a future lesson.)*
   Answers will vary

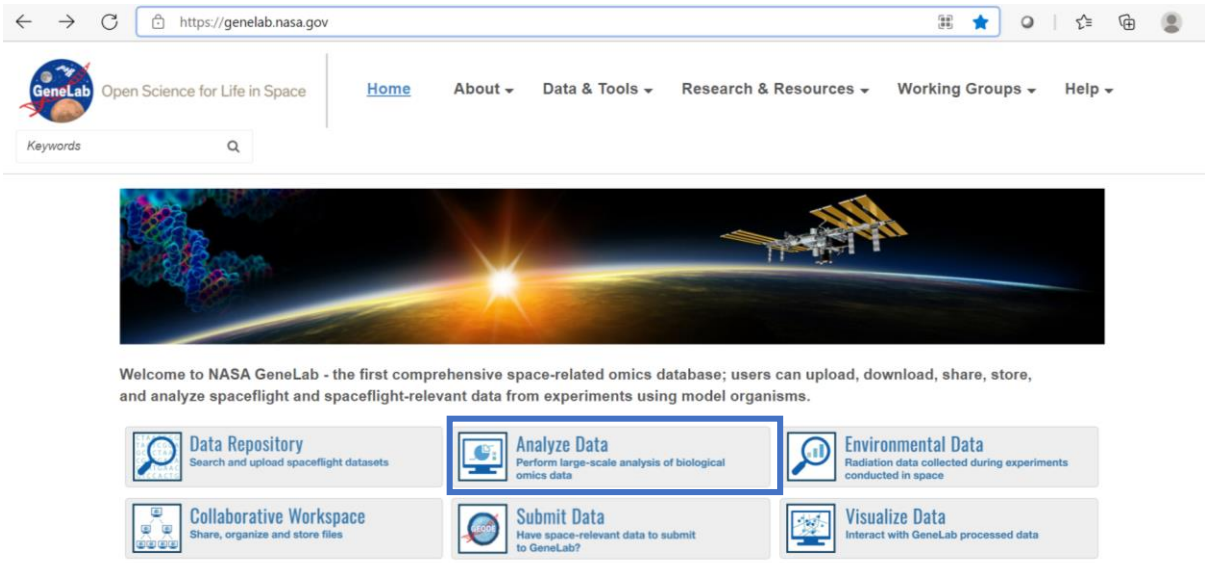
SUPPLEMENTARY TEACHER INSTRUCTIONS

The plots used in Activity 4 and 5 have already been generated and provided for you, but for teachers who are interested in learning how to use the analysis platform and to customized a dataset, here are the instructions used in the plot generation using the GeneLab Analysis Platform.

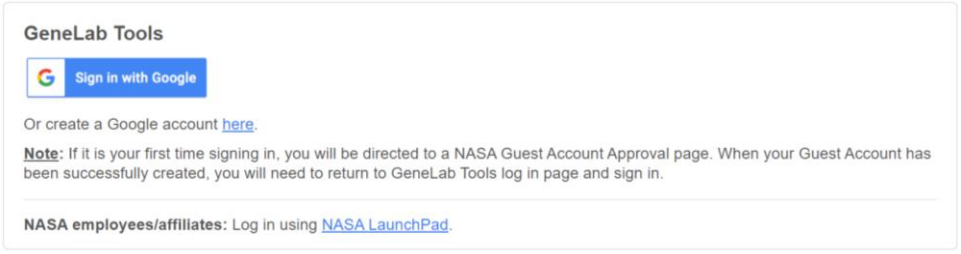
Accessing the Data Base

For this analysis, we will use a version of the Galaxy software that is hosted by the GeneLab project called the GeneLab Analysis Platform. Please not that for this curriculum unit you will be using a curated data set and these instructions will bypass those provided in the original GL4HS Bioinformatics Manual. To begin, navigate a browser to the Galaxy website at https://galaxy.genelab.nasa.gov/. Please note that the GeneLab Analysis Platform provides tools for transcriptomics analysis.

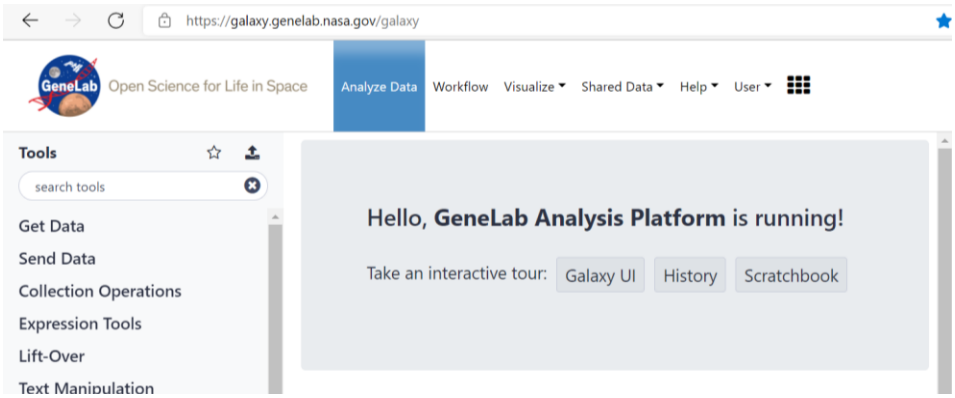
The tools and workflows are part of the standardized pipelines used by GeneLab and the Analysis Working Groups. To access the Analysis Platform, navigate to the GeneLab homepage NASA GeneLab: Open Science for Life in Space and click on the 'Analyze Data' button or navigate through the top menu 'Data & Tools' and select 'Analyze Data'.



To sign in, click on the 'Sign in with Google' button. Sign in using a Google account.

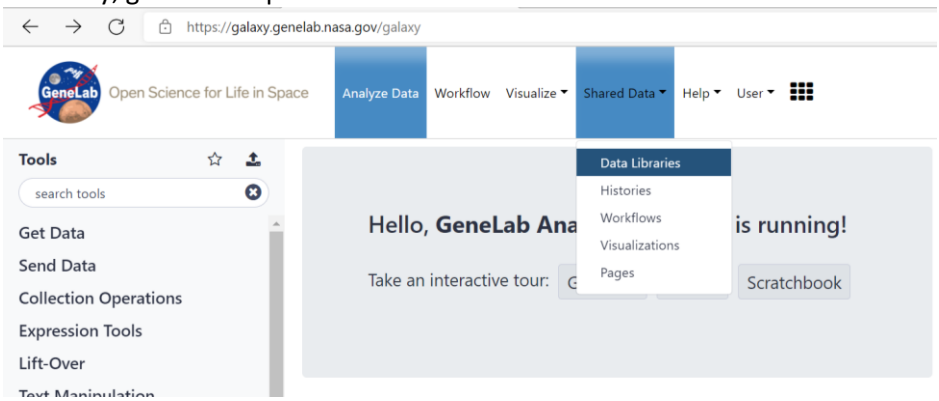


Register your Google Account with NASA and enter your Citizenship information. Click Yes, Register. Navigate back to https://galaxy.genelab.nasa.gov and sign in. You have now successfully created an account and logged into GeneLab Analysis Platform (Galaxy). Please note for security reasons you will have to re-login periodically as you use the platform. From now on, you will just need to navigate to https://galaxy.genelab.nasa.gov and log in.

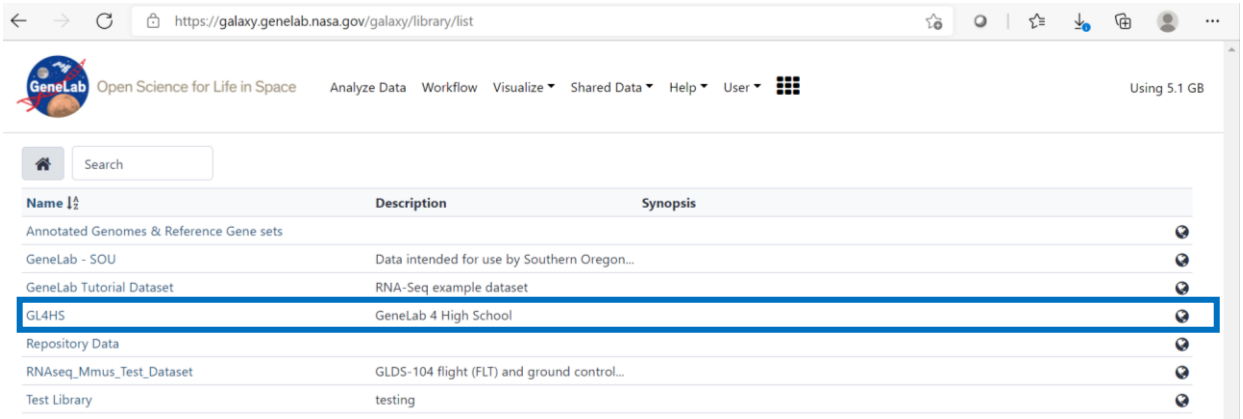


Creating Your History (Data Export)

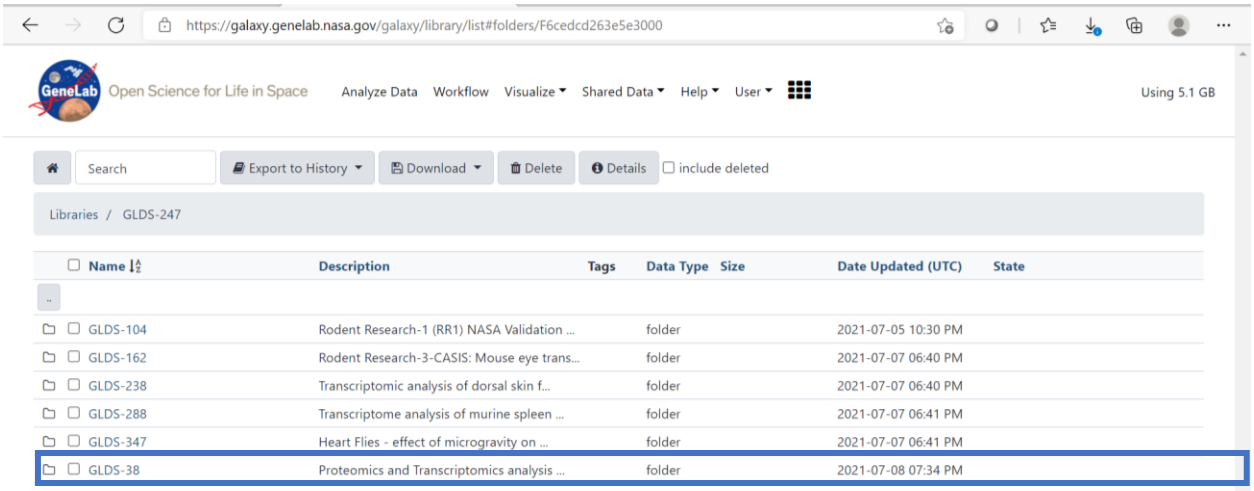
In Galaxy, go to the top menu and select Shared Data → Data Libraries.



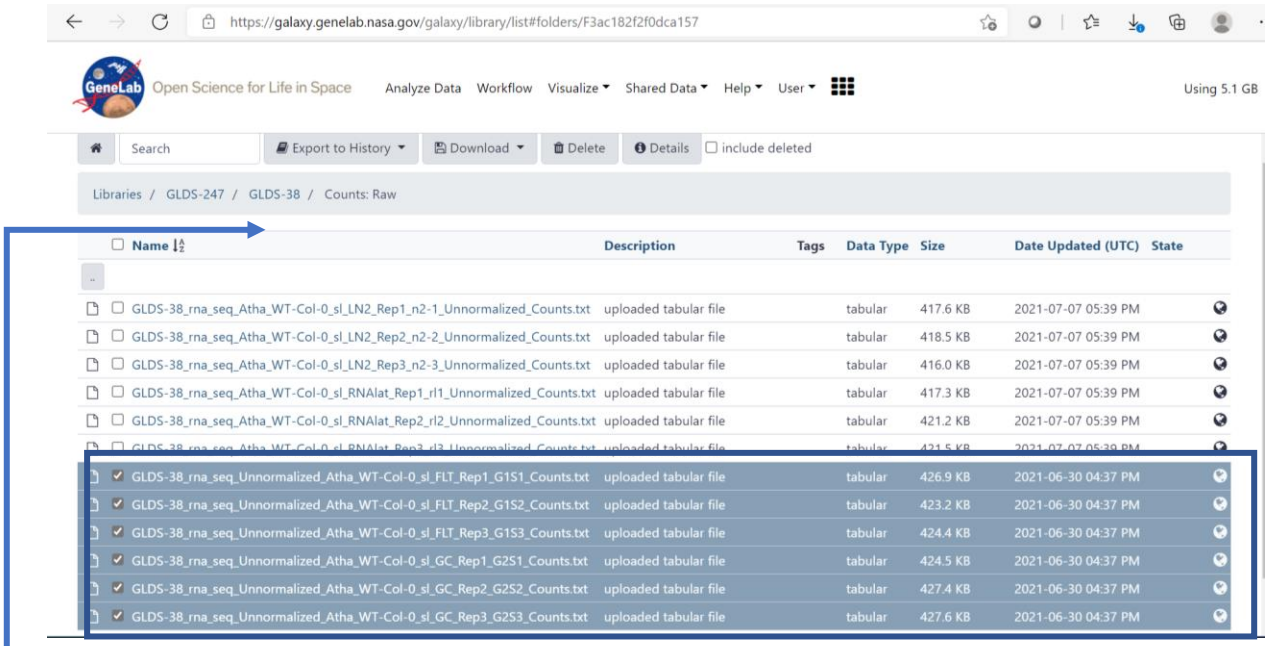
Once in the data libraries click on GL4HS.



Once in the GL4HS folder, click on the GLDS-38 folder.

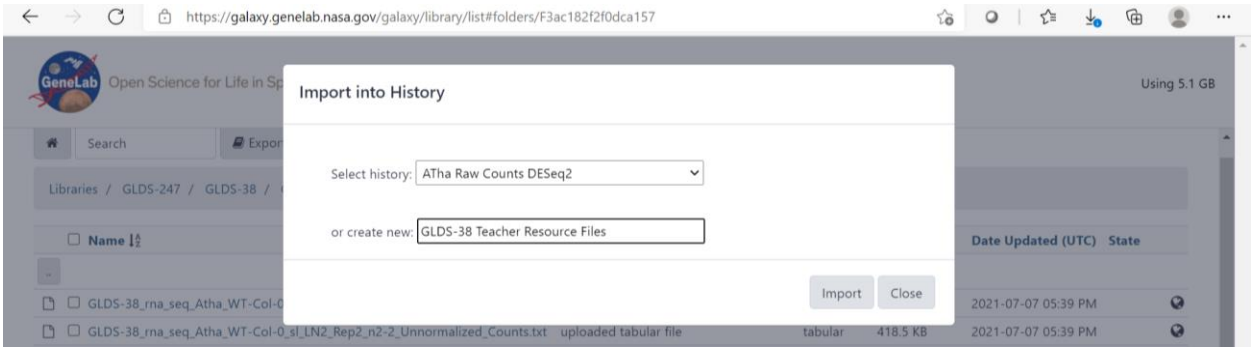


This folder will contain the raw data files and additional files required for further analysis. Click on the raw data files and select the ground control (GC) and spaceflight (FLT) raw data files.

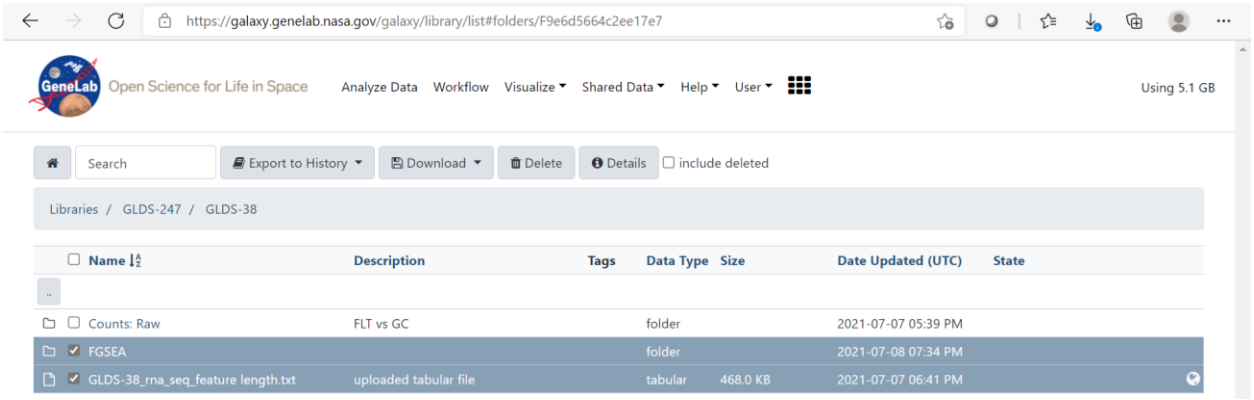


Once the 6 raw data files have been selected, click on Export to History.

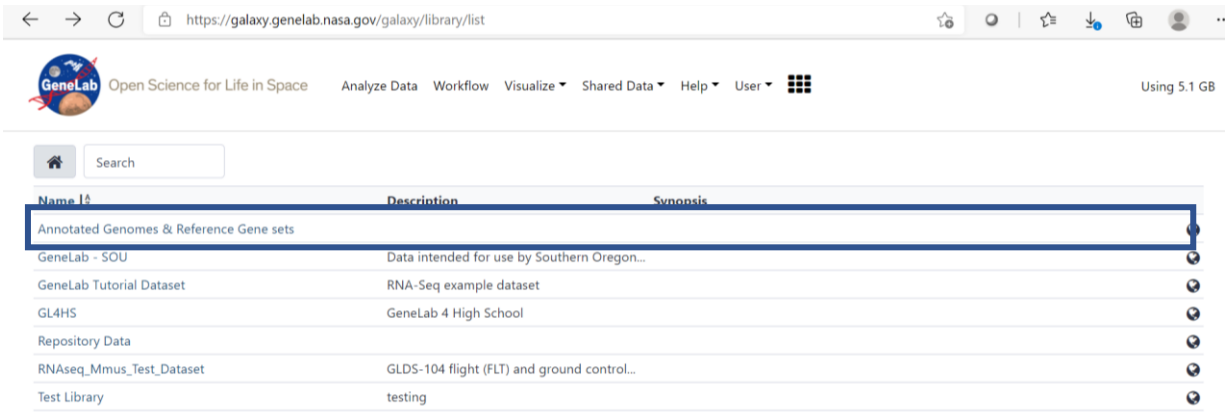
Import to histories as a new history. I have named the files GLDS-38 Teacher Resource Files but you may name according to classes or preferences.



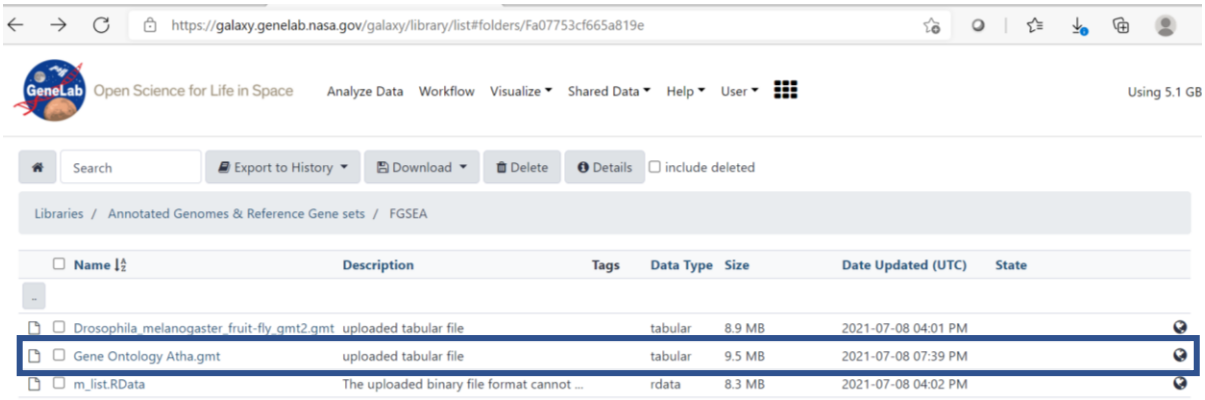
Since this is a curated data set you will want to go back and import a few additional files for use in later analysis. Follow the previous steps to access the GLDS-38 folder and export the FGSEA and the Feature Length Files into your current histories.



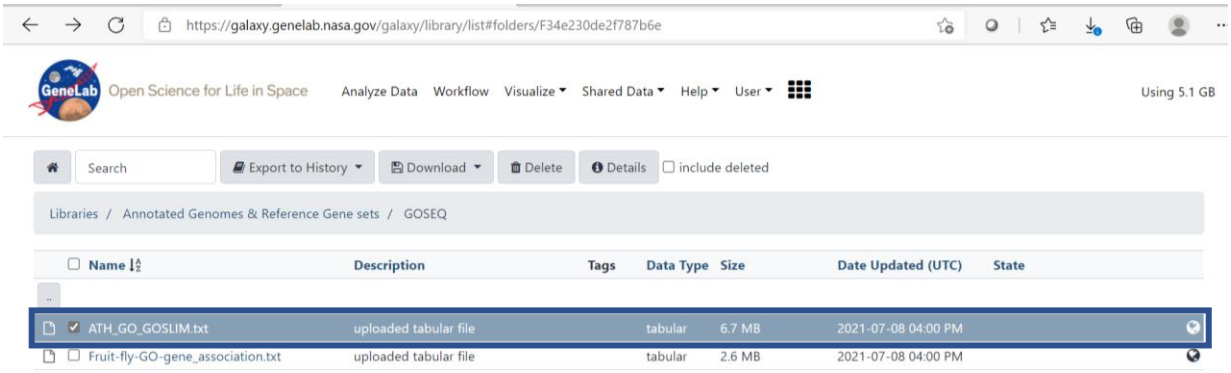
You will now go back to the GL4HS folder and export three additional files for use in later analysis. In the GL4HS folder click on the Annotated Genomes & Reference Gene Sets folder.



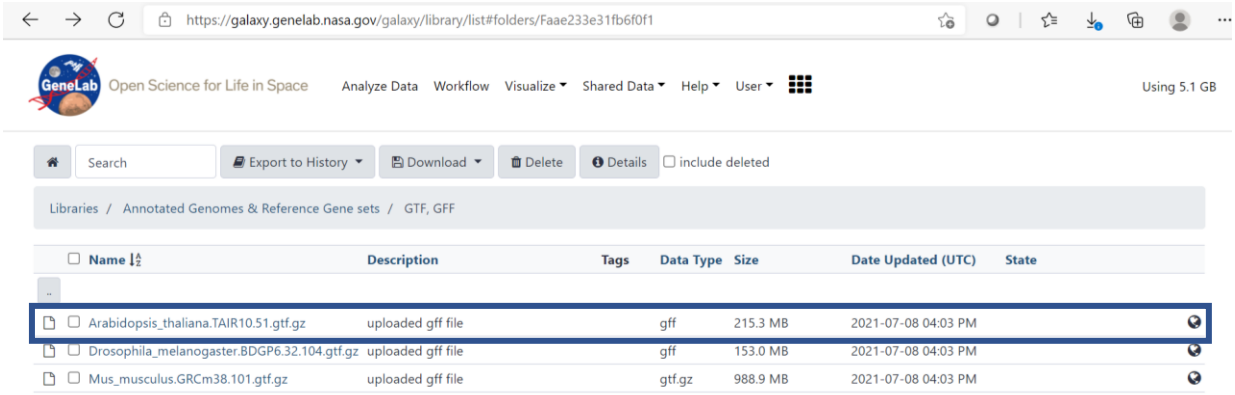
Click on the FGSEA folder and export the Gene Ontology Atha.gmt file. Import this file into your current history.



You will now go back to the GL4HS folder. In the GL4HS folder click on the Annotated Genomes & Reference Gene Sets folder. Click on the GOSEQ folder and export the ATH_GO_GOSLIM.txt. Import this file into your current history.



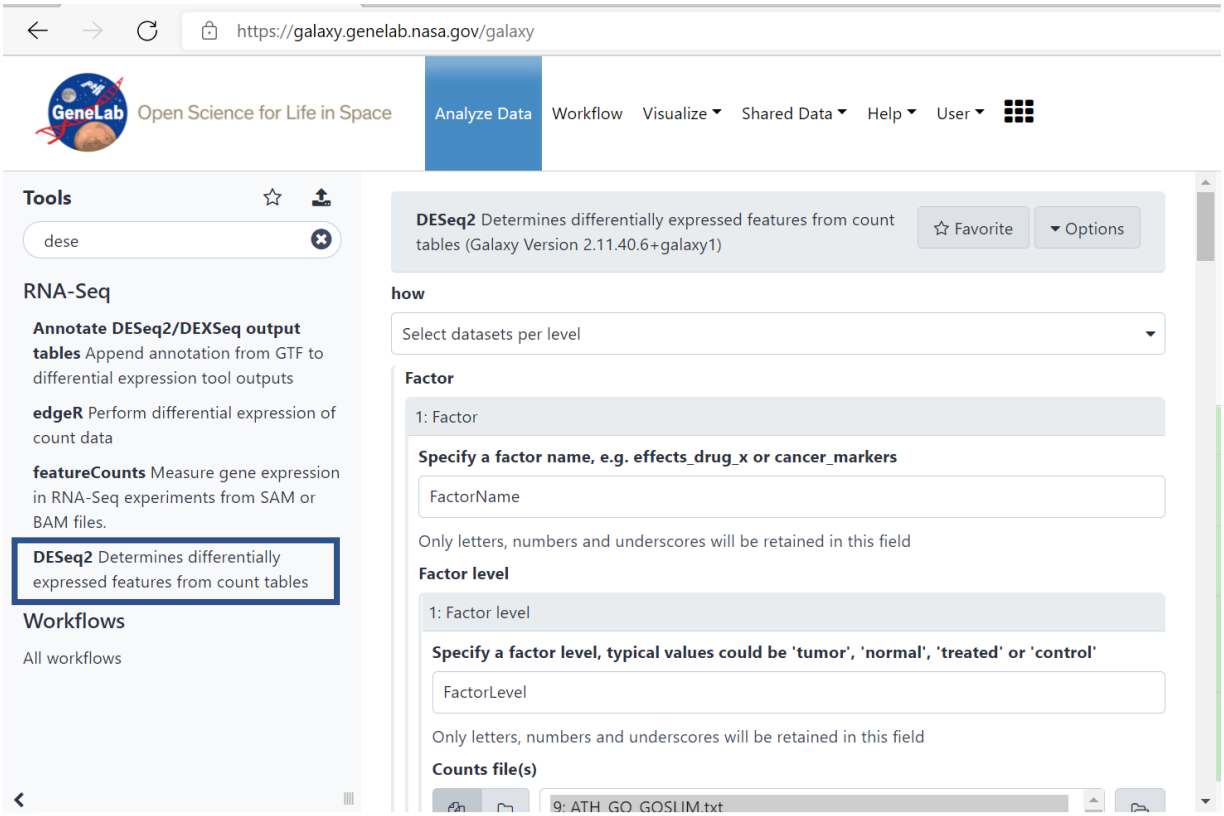
You will now go back to the GL4HS folder. In the GL4HS folder click on the Annotated Genomes & Reference Gene Sets folder. Click on the GTF, GFF folder and export the Arabidopsis_thaliana.TAIR10.51.gtf.gz. Import this file into your current history.



Note that because this is a curated data set, you will begin your analysis with DESeq2.

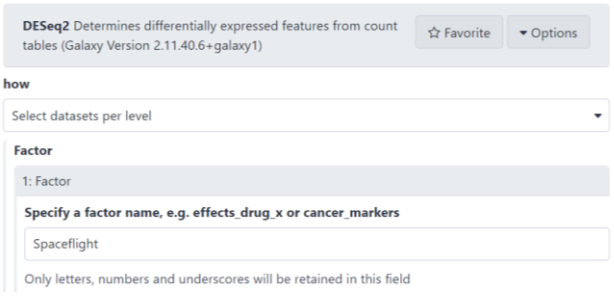
Instructions on How to Generate the DESeq2 Data Plots

From the tools panel, search for **DESeq2** or select it from the **RNAseq_tools** folder.

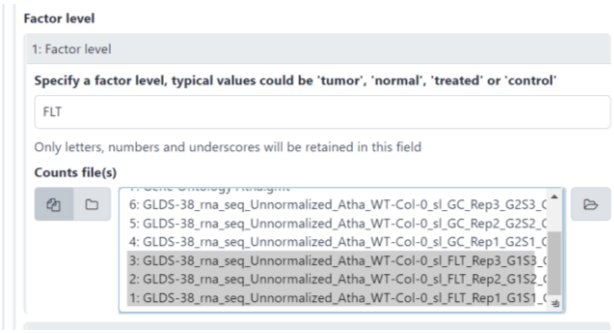


Once the tool parameters have populated the center panel, we can select options and run the tool.

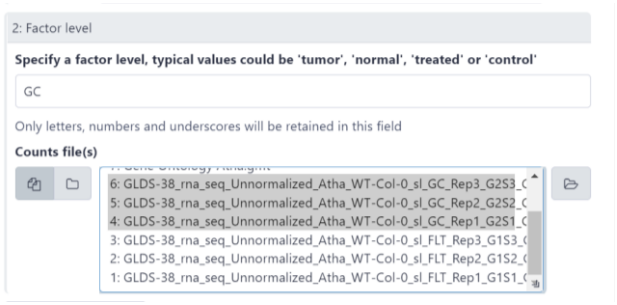
For how, **Select datasets per level**
For factor, enter **Spaceflight** under name.



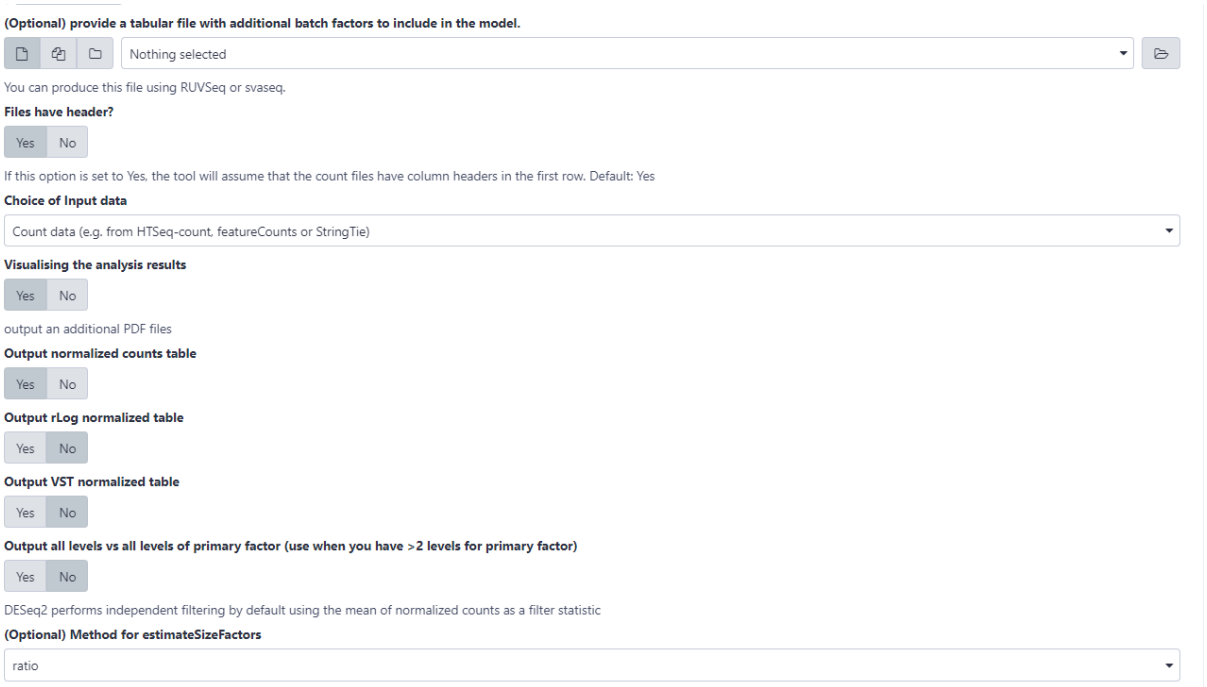
For first Factor level, **enter FLT** (no spaces)
Select the Count files for all your **FLT** samples (3 files)



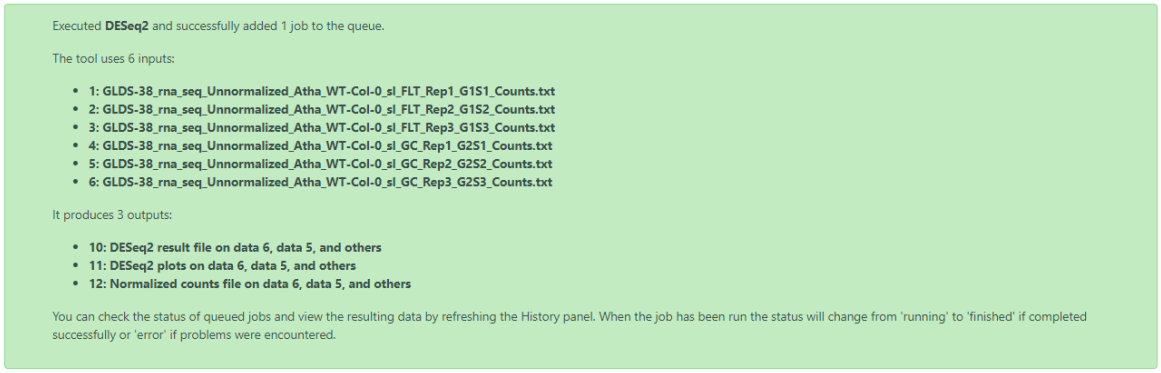
For second Factor level, **enter GC** (no spaces)
Select the Count files for all you **GC** samples (3 files)



(Optional) provide a tabular file with additional batch factors to include in model remains at default – **Nothing selected**.
Select **Yes** for Files have header
Select **Yes** for Visualizing the analysis results
Select **Yes** for Normalized table counts
Select **No** rLog normalized table, VST normalized table, all levels vs all levels of primary factors
Select **ratio** for Method of estimate size factors



Select **parametric** for Fit type
All other parameters to be left in the default setting
Click **Execute**

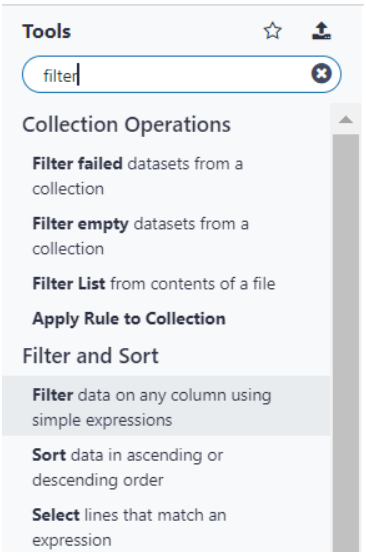


Remember that DESeq2 generates several output files including:
- A table with normalized counts
- A summary table with statistics and DGE results per gene
- A graphical summary of the results, which includes the MA Plot that you will use in - **Activity 4.**

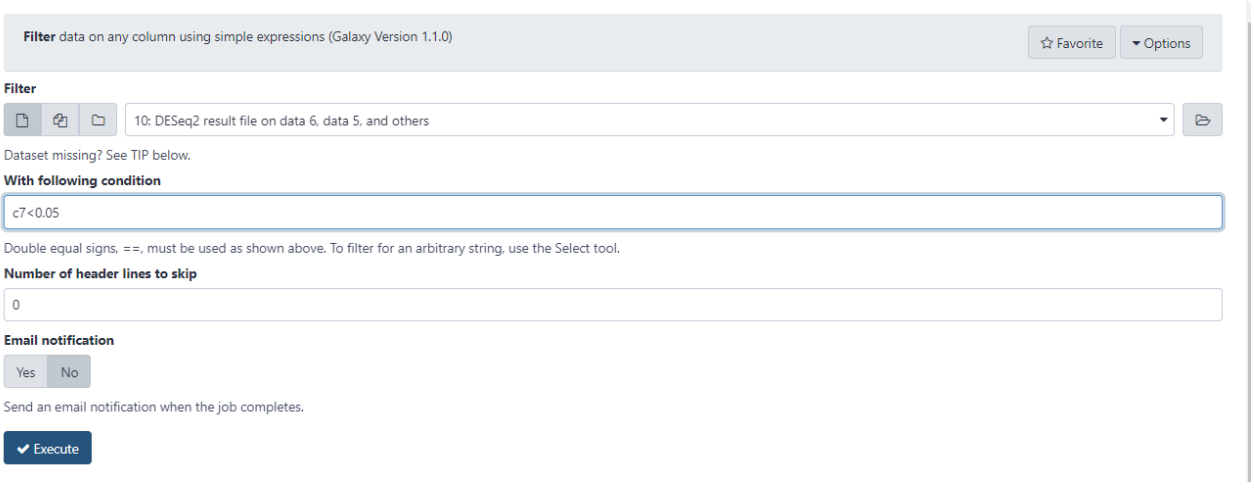
Filter and Annotate Differentially Expressed Genes

Remember that this is a curated data set and steps may differ from those provided in the GL4HS Bioinformatics Manual.

In the **Tools** panel on the right-hand side of the screen, select **Filter and Sort**. Then select **Filter** data on any column using simple expressions.

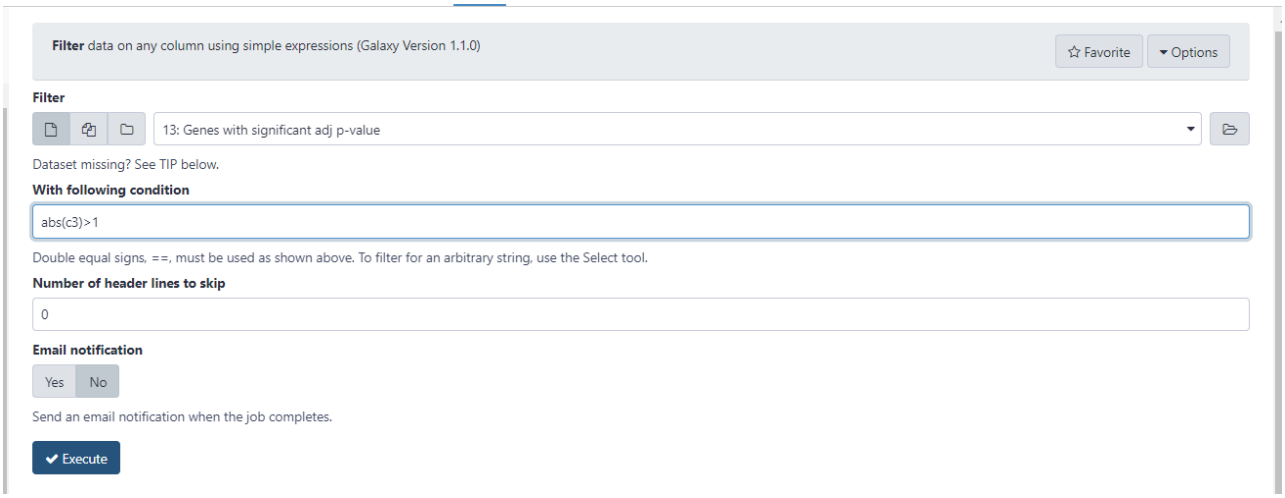


Filter. Select the table from DESeq2 output i.e. **'DESeq2 result file on GLDS-104'.**
With following conditions: Enter c7<0.05
Note column 7 is where the adjusted p-value was found from above
Number of header lines to skip: leave as 0
Click **Execute**



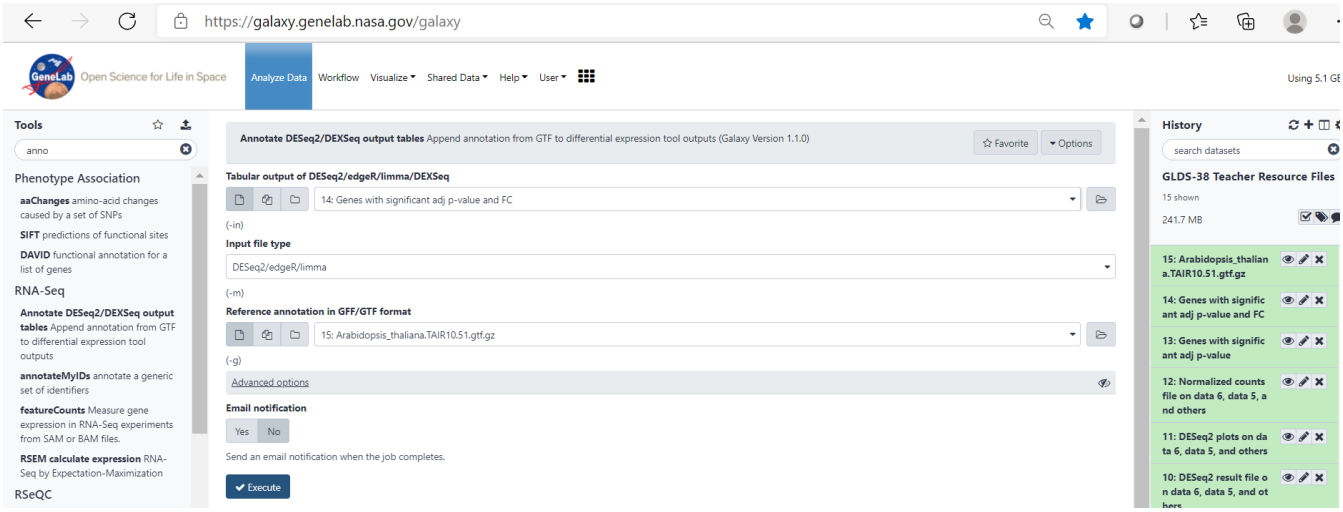
Once your file has successfully completed, rename your output to **Genes with significant adj p-value**

Now we will use Filter again to select only the genes with an absolute fold change (FC) >1
Filter. Select the output *Genes with significant adj p-value* you generated in the previous step.
With following conditions: Enter abs(c3)>1
Number of header lines to skip: leave as 0
Click **Execute**



Once your file has successfully completed, rename your output to **Genes with significant adj p-value and FC**
Search and select **Annotate DESeq2/DEXSeq output tables** from the tools panel under RNASeq.

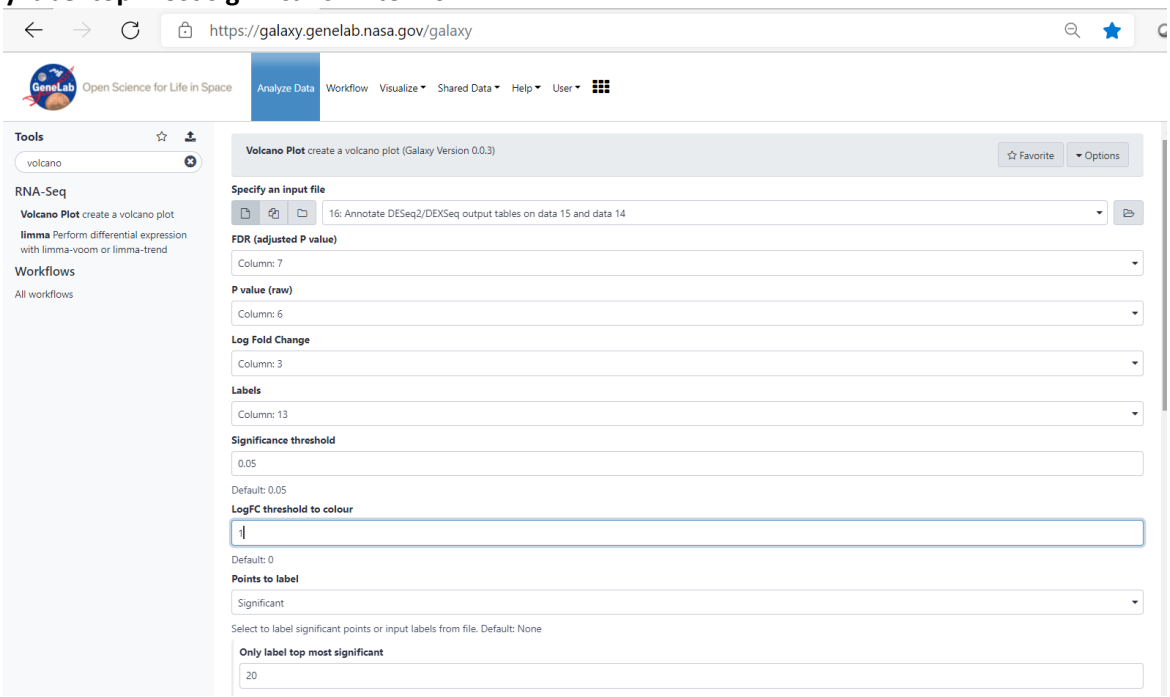
Tabular output of DESeq2/edgeR/limma/DEXSeq – select the file your filtered – *Genes with significant adj p-value and FC*
Input file type – select DESeq2/edgeR/limma
Reference annotation in GFF/GTF format – select the imported GTF file: Arabidopsis_thaliana.TAIR10.51.gtf.gz
All other parameters to be left on default settings.
Click **Execute**



Instructions on How to Generate the Volcano plot

Select **Volcano Plot**
Specify an input file: select file generated after filtering and annotation
FDR (adjusted P value): Column 7
P value (raw): Column 6
Log Fold Change: Column 3
Labels: Column 13

Significance threshold: Enter 0.05
LogFC threshold to color: Enter 1
Points to label: Significant
Only label top most significant: Enter 20



All other parameters to be left on default settings.
Click **Execute**
This will generate the volcano plot that you will use in **Activity 5.**

REFERENCES

GLDS-38 Dataset. Wyatt, SE. (2015). "Proteomics and Transcriptomics analysis of Arabidopsis Seedlings in Microgravity", GeneLab, Version 10, DOI: 10.26030/p03c-xa96.

GL4HS Manual: GeneLab for High School Bioinformatics Manual. Blaber, Elizabeth. 2021.

Video: Science at NASA Astronauts Learn Gardening in Space (Astronauts Learn Gardening in Space | Video - YouTube)

Video: NASA Social: Growing Plants in Space (https://youtu.be/3kspnEQKrcY)

Video: ISS Update: Plants in Space (https://www.youtube.com/watch?v=xLYeZKDv0Ig)

STANDARDS ALIGNMENT

Florida Science Standards: SC.912.N.1.1-7, SC.912.E.5.7-8, SC.912.L.14.3, SC.912.L.14.8, SC.912.L.16.3, SC.912.L.16.5, SC.912.L.16.6, SC.912.L.17.6, SC.912.L.18.5, SC.912.L.18.7, SC.912.L.18.8, SC.912.L.18.9

NGSS AP Biology Science Practices

- Visual Representations 2.C- Explain how biological concepts or processes represented visually relate to larger biological principles, concepts, processes, or theories.
- Representing and Describing Data 4.B- Describe data from a table or graph, including a. Identifying specific data points. b. Describing trends and/or patterns in the data. c. Describing relationships between variables.
- Argumentation 6.C- Provide reasoning to justify a claim by connecting evidence to biological theories.

AP Biology Standards

- IST-2.A.1- Regulatory sequences are stretches of DNA that interact with regulatory proteins to control transcription.
- IST-2.D.1- Gene regulation results in differential gene expression and influences cell products and function.

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