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Bioinformatics Bite #4:
Intro to Experimental Design

Teacher Version

OVERVIEW

This worksheet is part of a set of short lessons to help students become familiar with the basics of experimental design.

KEY CONCEPTS

- Independent variables are defined by the researcher's choice based on factors of interest when designing an experiment.
- Dependent variables are measured in response to the experiment.
- Control variables are held constant in all groups within an experiment.

OBJECTIVES

- Students will be able to define and identify a control variable.
- Students will be able to differentiate between an independent and a dependent variable.
- Students will be able to locate information related to variables in the GeneLab Data Repository

TEACHING TIPS

- Prior knowledge of the central dogma of molecular biology, particularly understanding that gene expression comes from transcription of RNA is important background information.
- Students may work individually or in groups.
- It is recommended that students complete one section and then have a class discussion on the section.
- It may be beneficial to select a separate experiment for part 3 either for differentiation (this particular set was selected as beneficial for middle ability students, but a more difficult or straightforward data set could benefit your students more) or as a second example to reinforce the concepts before moving on to part 4.
- One challenge on this particular data set is the variation of both spaceflight and gravity. Often, space biology involves having a ground control at the same conditions as the spaceflight to essentially equilibrate criteria of the experiment itself, then features a separate factor that is altered in spaceflight (as is the case in this experiment where simulated gravity and microgravity are compared to each other in spaceflight).
- For Part 4, you may have students work individually or in groups of 2-3 students, depending on the class size and ability level. You may also choose to have students present these findings to the class if time permits as it will offer more opportunity to review and extract the information from different scenarios.

Bioinformatics Bite #4: Intro to experimental design

Part 1: Why is a control important in experimental design?

If you are conducting an experiment, how many factors should you be changing at once?

Ideally only one. (Spaceflight presents challenges to this and in many instances the experiments are structured to look at two different factors - but in a way that you can examine one at a time).

Why is it important to do this?

If you vary more than one factor, you don't know what is causing the change you observe.

Imagine you were interested in whether sunlight was an important factor in plant growth. Think about the design parameters for this experiment. Describe an experiment that could help you test this idea. What would you have to change? What would you have to keep the same? How many different groups would you need to show an effect?

Various answers are acceptable here - but they should reflect on varying the amount of sunlight plants receive while controlling all other variables, such as water, temperature, soil, pH, vessel size, seed depth.

Since you are trying to show that sunlight has an effect on plant growth (perhaps you believe this is a positive effect and is needed to make plants grow, or perhaps you believe this is a negative effect and prevents plants from growing), anything you change between the group you are trying to study and the group you are trying to use to compare the study group to may be the cause of the change you are seeing in your results.

The group that receives no treatment to establish a background condition is called the control group.

The group(s) that involves a treatment or change in factor is/are called the experimental group(s).

What is your control group? Responses will vary with experimental design - focus on consistency within the experiment they design more than on an absolute correct answer.

What is your experimental group? _____

What factor did you vary? _____

What factors did you control? _____

_____

_____

Part 2: What exactly are dependent variables depending on?

Imagine your class has a 10 gallon aquarium and you have 10 minnows in the tank which you have been raising since September. You have fed them the same food all year, but one of your classmates suggested a change in food would help the fish grow larger.

What is a possible hypothesis that your classmate is suggesting? Answers will vary - a typical response is that some ingredient in the new food will cause the fish to grow larger when it consumes it than the old food. Students could also hypothesize more thoroughly that a particular ingredient in the food contributes to more nutritional value, hence leading to increased fish growth.

What do you need to change to be able to gather evidence to support or refute this hypothesis?

The food given to the fish, by identifying or establishing the nutritional content breakdown of the food. Experiments to test this hypothesis could include gradations of types of foods

This is your independent variable. You know that you will change it in order to see a different outcome in the experiment. You also know how you will change it. Since these changes do not depend on you carrying out the experiment, you can consider them to be independent of the experiment.

What is it that you will measure or collect to be able to substantiate or refute your hypothesis?

The growth of the fish as measured by mass, length, etc.

This will be a measure of the dependent variable. You can not predict with certainty how much this factor will change, that depends on you conducting the experiment.

What do you need to be able to keep consistent in the experiment while gathering evidence to be able to say with certainty that the changes you observed came from the factor you changed?

Answers again will vary, but should include many of the following: the number of fish, type of fish, pH of the water, size of the aquarium, presence of rocks/horticulture, temperature of the water, ambient light received in the aquarium.

These are controlled variables. If they are not the same for each experimental group, you are introducing additional variables to the experiment and your results are no longer based on changing just one variable. This makes it impossible to state which variable caused the change you observed.

Part 3: What does this look like in the GeneLab Data Repository?

Navigate to https://genelab.nasa.gov/ . Select the Data & Tools tab and select Data Repository from the drop down menu (Figure 1).

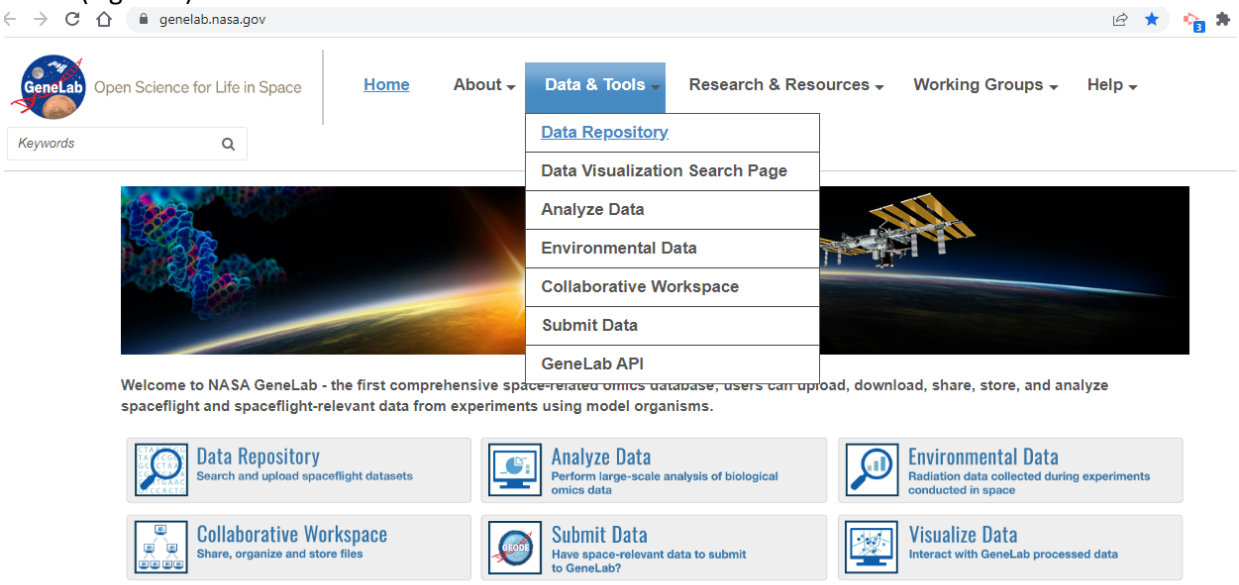

Figure 1. GeneLab website homepage

In the search box at Data Repository, type in “GLDS-213” and select the resulting file. Let’s take a moment to see what information is here (Figure 2) .

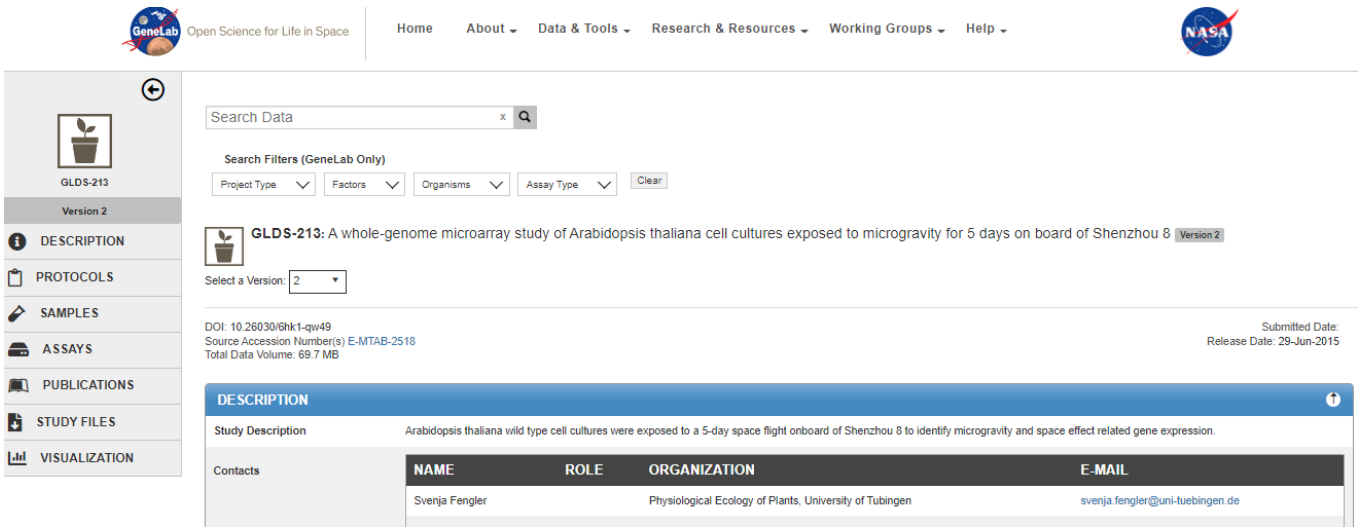

FIgure 2. GLDS-213 Study page

It is beneficial to review the study in advance. This activity can be done with any of the GLDS data sets, so you may alternatively choose to use an alternate study that is in line with your personal course objectives.

- *Description*: Details the plan of the study conducted and often contains abstract information. This is particularly useful in trying to understand the premise of the research conducted.
- *Protocols*: Explains the particular methods used for the study.
- *Samples*: Lists the samples involved in the study and information about each sample.
- *Assays*: Details specific tests that are run on the samples.
- *Publications*: Has direct links to published, open source studies from the research.
- *Study files*: Typically contain data files with more information about the data and findings.
- *Visualization*: Will show a range of different plots that you may learn about in other Biotechnology Bites (not prepared for each study).

To better understand what this research is about, two sections are particularly beneficial and we will look at them first: *Description* and *Publications*. For this study, the description is brief, so looking into the publication is necessary, but this is not always the case. Select the description and read the information on the study. Then navigate to the *Publications* tab and open the publication to read the abstract.

Summarize what the researchers are looking into: The effect of spaceflight AND microgravity on gene expression in cell culture of *Arabidopsis thaliana*.

What is the factor(s) that they are investigating and how do you know? Spaceflight AND microgravity. The description states "to identify microgravity and space effect" and the abstract discusses ground control against simulated 1g in spaceflight as well as microgravity during spaceflight.
Students may also be directed to examine the samples tab, as the list of samples includes the factors of study and the conditions for each factor.

What is the independent variable? Gravity is varied in spaceflight.

What is the dependent variable? Gene expression and transcription activity

What are the controlled variables? Launch age, growth time, habitat, genotype and cell type are all controlled variables. Study conditions are controlled with the ground control samples - these are compared to the 1g spaceflight samples to ensure that the container is not causing changes. This enables comparison in spaceflight of microgravity vs gravity conditions.

Part 4: Examining the Data Repository independently

Select a dataset of your own from the Data Repository. You may browse by model organism, search for a particular feature you may be interested in or use any other criteria to select a dataset.

Dataset number : _____

Dataset title: Answers here will be different for each dataset - see tips below

You will now follow the same process you used in part 3 with your class to review your individual dataset. Feel free to look beyond the description and publication sections. The sample list is often helpful in discerning what the researchers are looking for.

Summarize what the researchers are looking into: Correct responses should include the premise of the research and some restatement of their hypothesis

Note: Sometimes spaceflight will test spaceflight vs ground control as one factor, then test a separate factor in spaceflight. When this is the case, you will have more than one factor as an independent variable - but you will only look at one at a time. For instance, in part 3, the researchers compared ground control to 1g spaceflight (gravity controlled, spaceflight varied) then compared 1g spaceflight to microgravity spaceflight (spaceflight controlled, gravity varied).

What is the factor(s) that they are investigating and how do you know? Correct responses should identify the factor or factors being examined and should cite evidence of where the information was obtained from - ideally being more than one section of the dataset.

What is the independent variable? (If multiple factors are examined, explain how)

Correct responses should identify the variable for each factor being examined and should cite evidence of where the information was obtained from - ideally being more than one section of the dataset.

What is the dependent variable? (If multiple factors are examined, explain how)

Correct responses should identify the variable for each factor being examined and should cite evidence of where the information was obtained from - ideally being more than one section of the dataset.

What are the controlled variables? (If multiple factors are examined, explain how)

Correct responses should identify the variables for each factor being examined and should cite evidence of where the information was obtained from - ideally being more than one section of the dataset.

Switch papers with another student and go through their part 4 answers. If after reviewing their data set, you agree with them, you are ready to review this material with the teacher.

Part 5: Learning Aims and Evaluation

Please rate where you personally are at, with regards to the learning aims, at the end of the lesson and why.

Rating Scale

1- I do not understand it at all yet.
2-I understand parts of it, but I need my teacher and/or classmates' support to answer questions.
3-I understand it and can complete an assignment by myself.
4-I understand it so well I can teach others and apply my knowledge to new situations.

Learning Aim #1: Students will be able to define and identify a control variable.

My Evaluation of Learning Aim #1 and Explanation:

Learning Aim #2: Students will be able to differentiate between an independent and a dependent variable.

My Evaluation of Learning Aim #2 and Explanation:

Learning Aim #3: Students will be able to locate information related to variables in the GeneLab Data Repository.

My Evaluation of Learning Aim #3 and Explanation:

NGSS Standards

MS-ETS1-1 Engineering Design

Define the criteria and constraints of a design problem with sufficient precision to ensure a solution, taking into account relevant scientific principles and potential impacts on people and the natural environment that may limit possible solutions.

MS-LS1-3 From Molecules to Organisms: Structures and Processes

Use argument supported by evidence for how the body is a system of interacting subsystems composed of groups of cells.

MS-LS1-5 From Molecules to Organisms: Structures and Processes

Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.

REFERENCES

GLDS-213 Dataset: Fengler, S. "A whole-genome microarray study of Arabidopsis thaliana cell cultures exposed to microgravity for 5 days on board of Shenzhou 8", GeneLab, Version 2, http://doi.org/10.26030/6hk1-qw49

GLDS-213 Publication: Horie K, Sasanuma H, Kudo T, Fujita SI, Miyauchi M, Miyao T, Seki T, Akiyama N, Takakura Y, Shimbo M, Jeon H, Shirakawa M, Shiba D, Yoshida N, Muratani M, Takahashi S, Akiyama T. Down-regulation of GATA1-dependent erythrocyte-related genes in the spleens of mice exposed to
space travel. Sci Rep. 2019 May 21;9(1):7654. doi: 10.1038/s41598-019-44067-9. PMID: 31114014; PMCID: PMC6529412.

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

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