

Name \_\_\_\_\_

## **Background:**

In nature, random **mutations** occur when DNA changes. These mutations can be caused by mistakes made during replication, or by **mutagenic agents** such as **UV radiation**, or certain **chemicals**. Organisms that carry these mutations may appear different than others of their species. This may lead to **variation** within a species. Some new traits may help an organism survive in its environment, while some may hurt the organism's chances of survival.

You will be given seeds from a plant called ***Brachypodium distachyon*** ("**Brachy**"), which has been mutagenized with the chemical **Ethyl Methanesulfonate (EMS)** that is known to produce random mutations by changing one base pair at a time. Since Brachy has 270 million base pairs, these mutations may occur anywhere. Large regions of DNA are **non-coding**, and many mutations may be **silent**. Therefore, there is a strong possibility that the mutations will not result in any new trait that an observer can easily detect. However, many new traits have already been discovered using this method.

Your job will be to grow plants from the seeds of one of these mutated plants, compare them against the known normal or "**wild type**" Brachy, and hypothesize whether any new trait you discover will help the organism's chances of survival, hurt it, or have no adaptive value at all.

## **Materials:**

12 EMS treated seeds

Soil Liner

Plant Labels

Marker

Miracle-Grow Potting Soil

Thermometer

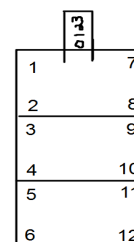
Lux Meter

Humidity Detector

Light Bank

## **I. Planting Seeds:**

1. Fill clean a soil liner with new potting soil.
2. Add water as needed until the soil is thoroughly saturated.
3. Fill out the plant tag with the following information
  - a. Side one: Family Number, date planted
  - b. Side two: Your name
4. Using a pencil, place 4 holes in each section of the soil liner: one in each corner.
5. Using forceps, place one seed in each hole, then gently cover with soil.
6. Place your soil liner, with its 12 seeds and plant tag into a tray.



7. Add water to the bottom of the tray, keeping the water level just above the “ribs” on the bottom of the tray. Check your water levels daily, and add water as needed.
8. Place the tray under the light bank
9. **Record the environmental conditions in the data table.**

## **II. Screen 1:**

1. A few days after germination (plants are about 4-8 cm high) observe the plants.
2. Record how many have germinated and their characteristics as compared to the wild type (WT). Refer to the mutant photo library on the BTI website for specific terms for each trait.
3. **Record the environmental conditions in the data table.**

## **III. Screen 2:**

1. About three weeks after planting (plants are about 20 cm high) observe the plants.
2. Branching of wild type plants has now occurred, and new traits may be evident.
3. Once again, refer to the mutant library for any necessary clarification, and record your observations in your data table. Begin to check plants daily for flowering and record the day they flower.
4. **Record the environmental conditions in the data table.**

## **IV. Screen 3:**

1. About 7 weeks after planting, the wild type should have matured and produced seeds.
2. Check for the appearance of any new traits, as well as the development of the previously identified traits. **Record all information in the data table.**

## **Collection of Seeds:**

1. If you had any phenotypic mutations, take a picture of them alongside the family # tag and a ruler.
2. Collect and count the seeds from those plants.
3. Record the number of seeds, and place them in a new envelope with the family number.

**Questions:**

1. Calculate the percentage of your seed were mutants by dividing the number of mutants by the total number of seeds that germinated then multiply by 100. \_\_\_\_\_%

2. How did your mutation rate compare to others in your class? Why do you think this is so?

3. How can any differences in new traits between families be explained?

4. Observe your mutants(s), or one provided for you by the teacher. How does it differ from the wild type?

5. Do you think this new trait is beneficial? Why or why not?

6. Calculate the average number of days it took for your wild type plants to flower. \_\_\_\_\_ days.

Were there any mutants in the class with a different flowering time?

7. How many seeds did the mutant produce under these laboratory conditions? \_\_\_\_\_ How does this compare to the wild type's average seed production under the same conditions?

8. What is the purpose of the wild type in this activity?

9. Do you think this plant will be more, less, or equally successful as the wild type? Explain why.

10. What would you expect to happen to the frequency of this mutation in the Brachy population over time?

11. What, if any climate conditions would this mutation survive exceptionally well?

**Family #** \_\_\_\_\_

**Planting Date:** \_\_\_\_\_ °C \_\_\_\_\_ %Humidity min\_\_\_\_/max\_\_\_\_  
**Lux** \_\_\_\_\_

**Screen One:** \_\_\_\_\_ °C \_\_\_\_\_ %Humidity min\_\_\_\_/max\_\_\_\_  
**Lux** \_\_\_\_\_

**Screen Two:** \_\_\_\_\_ °C \_\_\_\_\_ %Humidity min\_\_\_\_/max\_\_\_\_  
**Lux** \_\_\_\_\_

**Screen Three:** \_\_\_\_\_ °C \_\_\_\_\_ %Humidity min\_\_\_\_/max\_\_\_\_  
**Lux** \_\_\_\_\_

<b>Seed</b>	<b>Screen One</b>	<b>Screen Two</b>	<b>Screen Three</b>	<b>Flowering Date</b>	<b># of Seeds Harvested</b>
<b>1</b>					
<b>2</b>					
<b>3</b>					
<b>4</b>					
<b>5</b>					
<b>6</b>					
<b>7</b>					
<b>8</b>					
<b>9</b>					
<b>10</b>					
<b>11</b>					
<b>12</b>					

### **Glossary of Standard Brachypodium Phenotype Terms**

<b>Phenotype</b>	<b>Phenotype Sub Category</b>	<b>Mutant Descriptions</b>
<b>Color</b>	Green	Dark Pale
	Yellow	Dark Pale
	Variegated	Horizontal Vertical Other
	Virescent	Slow Greening
	White	Albino
	Other	Brown Red
<b>Disease</b>	Necrosis	Tissue Color Lesion Type # of Leaves infected
	Other	Other
<b>Flowering Time</b>	Development	Days to Flower Early Late
<b>Architecture</b>	Branching	Less More
	Flower	Abnormal Shape Less Spikelets More Spikelets Large Small # of Florets Sterility
	Leaf	Drooping Upright Hairy Smooth Long Short Narrow Wide Serrated Spiral
	Size	Dwarf (wide leaf) Tiny (narrow leaf) Small

		Big
	Stem	Long Short Wide Narrow Spiral Hairy Smooth
	Roots	Abnormal
<b>Other</b>		

**Compiling Class Data:**

Did you have any mutants in your family?

If you did, your data is important to the scientists at the Boyce Thompson Institute. Take a picture of your mutant. Make sure the tag with the family number is clearly visible, as well as a ruler (metric) to illustrate the size of the plant. You will upload your data and pictures of your mutants on the BrachyBio! Datacentral Webpage.

**Optional Extensions:**

**Reporting:**

Scientists really do want your input! By enlisting the help of students like you in cataloguing all the new traits that arise randomly through EMS treatment, scientists can get far more work done than by working alone. If any mutant trait appeared within your family of seeds, prepare a brief Power Point presentation reporting your findings. The presentation should include:

Slide 1: Name, School, Brachy Family Number

Slide 2: A description of the trait, the percentage of the plants effected, Number of seeds produced per mutant plant

Slide 3: A summary of the abiotic conditions in the classroom during the course of the experiment. This is important because sometimes differences in temperature, light or humidity can affect the way genes are expressed.

Slide 4: Photos of the mutated plant, photo of a wild type plant, and a ruler for comparison

Slide 5: A discussion of whether you feel this new trait has any adaptive value. Include your reasoning, as well as whether you believe the trait would be beneficial to a plant in nature or if it might be a trait that scientists may want to use in selective breeding.

### **Heredity & Evolution:**

Mutations have contributed to variety of life on Earth! New traits may start with a mutation. Often mutations are harmful, and the organisms that display them do not compete well. Sometimes a new trait helps the organism that inherits it to survive, and in turn have more offspring with that trait. Humans often purposely breed organisms with desired traits (artificial selection). You have just completed a project that helped document new mutations and the characteristics they produce.

- Will the new genes generated by the EMS treatment of the Brachy seeds be passed on to future generations?
  - Can you select for the trait to produce an entirely NEW variety of Brachypodia?
  - Will the seeds of your mutant grow better under different conditions?
- Based on your knowledge and the procedures from this activity, develop a research project that will answer one of these questions, or one of your own.