Discovering Epigenetics: It's a *bal!*

Purpose and Overview

Arabidopsis thaliana, commonly called 'mouse-eared cress' is in the mustard family, is small in size, and grows rapidly in laboratory and classroom settings. It has a small genome and was the first plant to have a fully sequenced genome. It is a **model organism** used by plant scientists around the world to study a variety of biological processes, including growth, development, flowering time, circadian rhythms, environmental stress response, disease resistance, and **epigenetics.**

Epigenetics is the study of inheritable changes in gene function that cannot be explained by changes in the gene sequence. One of the most common mechanisms to encode epigenetic information is the addition of chemical groups directly to the DNA, thereby marking the region without changing the underlying nucleotide sequence. Methylated DNA is DNA with one or methyl groups attached to a cytosine nucleotide. This is known as "**methylated DNA**" and is an important **epigenetic marker**.

Adding a small methyl group, consisting of one carbon atom and three hydrogen atoms (CH_3) to cytosine is the most common form of DNA modification in plants and in humans. Cytosine **methylation** helps ensure the stability of the genetic information, and loss of this DNA modification can lead to the formation of heritable **mutations**.

Not all mutations cause visible changes in plants, still scientists aim to better understand the frequency and longevity of the effects of epigenetic changes in plants; when they occur and how they show up in future generations.

Scientists at the Boyce Thompson Institute for Plant Research are working with middle and high school students to identify the transmission of new mutations that occur in *A. thaliana* **lines** that have dramatically reduced levels of cytosine methylation.

These lessons can be taught before, during or after students have studies topics of Genetics, Heredity, and Evolution, and can be used to engage students in questions about mechanisms of phenotypic diversity and the interactions between genes and environment, and how these interactions shape genetic diversity and fitness. In earlier grades these lessons can also be taught as a stand-alone scientific inquiry unit or to illustrate plant growth, development and life cycles.

Grade Level

This lab is designed for students in grades 9-12 in introductory to advanced biology courses and can be modified for students in grades 7-16.

Learning Objectives

- Students will observe plant growth and development from germination to seed set
- Students will compare developmental and phenotypic **variation** across plants lines with three genetic backgrounds: experimental plants (unknown mutations), *bal* plants (known mutations) and wild type plants will be grown as controls to determine if any observable **mutations** have occurred in the experimental line
- Students will characterize mutant growth and **phenotypes**
- Students will calculate the frequency of mutant appearance in the population
- Students will research and write an explanation for the mechanisms that cause diverse mutations
- Students share their results with BTI scientists including lab reports, and photos of any evidence of mutations
- Students will collect and return seeds from plants exhibiting mutations to BTI

Learning Standards

Next Generation Science Standards (NGSS)

HS-LS-3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

HS-LS-3-2. Make and defend a claim based on evidence that inheritable genetic **variations** may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

NGSS Cross Cutting Concepts

Patterns
Cause and Effect: Mechanisms and Explanations
Stability and Change

National Common Core Standards, Dimension 3: Science:

1. Asking questions (for science) and defining problems (for engineering)
2. Developing and using models
3. Planning and carrying out investigations
4. Analyzing and interpreting data
5. Using mathematics and computational thinking
6. Constructing explanations (for science) and designing solutions (for engineering)
7. Engaging in argument from evidence
8. Obtaining, evaluating, and communicating information

New York State Living Environment Core Curriculum

Standard 1: Key Idea 1 and 3:

1.1a: Scientific explanations are built by combining evidence that can be observed with what people already know about the world.

1.3a: Scientific explanations are accepted when they are consistent with experimental and observational evidence and when they lead to accurate predictions.

1.3b: All scientific explanations are tentative and subject to change or improvement. Each new bit of evidence can create more questions than it answers. This leads to increasingly better understanding of how things work in the living world.

3.1a: Interpretation of data leads to development of additional hypotheses, the formulation of generalizations, or explanations of natural phenomena.

Standard 4: Key Idea 2:

2a: For thousands of years, new varieties of cultivated plants and domestic animals have resulted from selective breeding for particular traits.

2b: In recent years new varieties of farm plants and animals have been engineered by manipulating their genetic instructions to produce new characteristics.

2.1a: Genes are inherited, but their expression can be modified by interactions with the environment.

2.1b: Every organism requires a set of coded instructions for specifying its traits. For offspring to resemble their parents, there must be a reliable way to transfer information from one generation to the next. Heredity is the passage of these instructions from one generation to another.

2.1c: Hereditary information is contained in genes, located in the chromosomes of each cell. An inherited trait of an individual can be determined by one or by many genes, and a single gene can influence more than one trait. A human cell contains many thousands of different genes in its nucleus.

Time Frame

The activity should take a total of minutes 160 minutes of class time, with an overall time frame of 6-8 weeks.

Teacher Laboratory Preparation

- One hour to prepare materials, assemble light rack
- Watering as needed

Class Time over a span of four weeks:

- 60 min: Background information, introduction to *Arabidopsis* and project goals, make seed paper
- 40 min: Planting and labeling seeds
- 20 min: Check for growth at 2 weeks, characterize mutations and photograph
- 40 min: Monitor growth and characterize mutations at 4 weeks; collect data; separate mutants, take photographs

Teacher Wrap Up

- 2-4 more weeks of plant maintenance until seed pods are ready for harvest
- One hour to report data, upload photos and return any identified mutant seeds to BTI

Background Information

Epigenetics research aims to understand how environmental factors influence gene regulation and expression. Briefly stated, epigenetic mechanisms regulate the activity levels of genes. It has long been known that genes are turned on or shut off during the process of differentiation in multicellular organisms, and this is one aspect of epigenetics. The concept of a single, totipotent **zygote** that gives rise to evermore specialized yet genetically identical cells is a major concept in the Living Environment curriculum.

Gene regulation occurs and is influenced by multiple complex factors. The method of gene regulation that this project investigates is gene repression associated with methylation of cytosine. Methyl groups are added to the DNA, effectively preventing the gene from being read for transcription and silencing the gene. Cytosine methylation can also suppress gene mutations, such as gene duplications that occur from DNA replication and recombination errors. In this way, cytosine methylation protects the genome from mutation.

With the development of the Common Core Standards and Next Generation Science Standards, an increasing emphasis is being placed on the authentic science experience. Because the field of epigenetics is relatively new, many aspects of it are still being explored. This provides an excellent opportunity for students to gain experience in the discovery process, while introducing them to concepts that are so new as to not to have entered the arena of their textbooks. As students are being encouraged to form opinions based on prior knowledge and their own experiences, and to defend those opinions based on scientific observation, the field of epigenetics provides a forum full of examples of questions to which there are currently no answers.

The "Let's Have a *bal*!" Project

Dr. Eric Richards, a research scientist at the Boyce Thompson Institute for Plant Research (BTI), studies the function of **DNA methylation** on the model plant *Arabidopsis thaliana*. ***DDM1*** is a gene found in *Arabidopsis* that when active, maintains methylation throughout the genome, ensuring that genes and other genetic elements are properly regulated.

Dr. Richards has identified a number of **mutations** that occur in plants with a faulty *DDM1* gene. One of these changes occurs at the *BAL* gene due to a duplication of the gene. This extra copy of the *BAL* gene results in an abnormally small plant with twisted leaves. This dwarf-like **phenotype** is referred to as "*bal*". The *bal* phenotype is caused by overexpression of a resistance like gene (R-gene) which activates a defense response**.** In this case, the extra copy of the BAL gene causes the plant to respond as if it is under attack by pathogens, even when pathogens are absent. The plant sacrifices normal growth, resulting in abnormally small plants.

The objective of the project is to isolate new, independent mutations that are generated in *ddm1* lines to determine whether they affect the *BAL* gene or a similar region in the genome. In the process, scientists will learn more about how cytosine methylation protects the genome against mutations. These insights may lead to a better understanding of human conditions, such as cancer, which have been linked to changes in cytosine methylation.

Arabidopsis Wild Type

Figure 1: A normal *Arabidopsis* plant prior to flowering. Note the diameter of the **rosette** of leaves at the base, and the flattened characteristics of the leaves.

Arabidopsis bal

Figure 2: A *bal* phenotype at the time of bolting. Note the shorter bolt height, the smaller rosette, and the curled nature of the leaves.

The fast growing nature of *Arabidopsis*, the high volume of plants that must be screened, and the very obvious appearance of the *bal* mutation make the investigation of the *bal* mutation a rare opportunity to involve students in the research process. Not only will students experience an authentic scientific experiment, they will have the opportunity to collaborate with a plant scientists and educators.

The Boyce Thompson Institute will supply high school classrooms with seeds derived from a line of *Arabidopsis* plants that have a mutation in the *DDM1* gene. Students will grow the plants, and report back to BTI the number of mutant plants, including those with the *bal* phenotype, that they observe. This information and the seeds from mutant plants will then be returned to Dr. Richards for further studies.

Key Scientific Vocabulary

Bolting: The production of a flowering stem

Chord: A straight line joining two points on a circle

Dehiscence: The spontaneous opening at maturity of a plant structure, releasing its contents (usually seeds)

DNA Methylation: The addition of a methyl group to a DNA molecule, which alters the expression of the gene

Epigenetic: The study of changes in gene expression or phenotype caused by mechanisms other than changes in the DNA sequence

Epigenetic Trait: A stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence

Genotype: The two specific alleles a diploid individual has for a trait

Genetic Marker: A variation in easily identify piece of genetic material, usually DNA, at a known location in the genome

Model Organism: An organism chosen by scientists for study, usually based on characteristics such as short generation time, high reproductive rates, easily observed characteristics, and close relative relationship to other organisms.

Mutation: A change in the DNA sequence.

Phenotype: The physical appearance of an individual

Plant or Seed Lines: A variety of species that is relatively uniform genetically because of continued inbreeding and artificial selection. Certain characters appear in successive generations as a result of inbreeding or self-fertilization, creating varieties within a single species that share similar, but unique genetic backgrounds.

Plant Architecture: Structures of the plant, leaves, stems roots, and the patterns they take during growth and development

Rosette: A circular arrangement of leaves

Silique: Any dry fruit that separates at maturity into two or four segments called valves, leaving a persistent partition that bears the seeds. A typical silique is an elongated capsule and is characteristic of plants in the mustard family

Stratification: A brief cold-treatment (few hours - days) of water soaked seeds to break seed dormancy, results in an even germination

Variation: Diversity within a species or population

Wild Type: Considered the typical phenotype for the species, considered "normal" when compared to new, possibly mutant traits

Zygote: a eukaryotic cell formed by a fertilization event between two gametes containing all genetic information necessary to form a new individual

Lab Materials

BTI Provided Materials

Light Rack:

- This will be picked up from BTI or purchased from Carolina Biological and sent to your school address if requested.

Environmental Monitoring Meters:

- Light meter for measuring light intensity in lux
- Temperature and humidity meter- measures min and max of both as well as current readings

Arabidopsis Seeds (seed packets)

- Columbia wild type (a control)
- known *bal* mutant (a control)
- 1-2 different experimental *ddm1* lines

Planting Materials:

- 2 x 2 potting cells (32 cells per large flat)
- 4 large watering flats or 8 small flats
- 4 humidity domes (Applied until seeds germinate, then remove)
- Plant labels
- 3 ml Pipettes

You will need to provide:

- A sunny classroom location, where temperatures are fairly constant throughout the experiment
- Three full Spectrum Florescent Light Bulbs for light rack (standard shop light size)
    - Example: Florescent light bulbs: Ex: Sylvania Octron XPS 32 W 4100K and F032/841/XPS/ECO3
- Soil, Seedling Starter Mix (fine particles size to promote germination)
- Refrigeration (seed stratification overnight)
- Markers
- Scissors
- 100 ml beakers
- Rulers (10 cm)
- Camera (cell phone cameras fine too) (at weeks 2 and 4 following germination)
- Watering Can (.5, 1, and 2 liter markings recommended but not required)
- Toilet paper Roll – 1 ply or 2 ply that has been separated

Safety

- Any students with potential allergies to specific plants should notify the teacher and should avoid contact with the plants
- Any injuries while working with the laboratory materials should be reported to the teacher immediately
- Students should not manipulate the light set-up while experiment is in progress

Lesson Outline

Lab Preparation

You will receive seeds from one to two experimental *ddm1* lines labeled with a plant identification number. These are the experimental groups. You will also be sent Columbia (Col) Wild Type seeds (to be grown as a control and to compare typical growth and development) and known *bal* mutant seeds for phenotypic comparison to the experimental seeds.

Pre-Laboratory Activities (20 minutes)

Assigned either in class or as homework, a Pre-Lab activity will introduce students to ideas such as effect of the environment of gene expression, topics in human Epigenetics, and the benefits of using **model organisms**. Epigenetics through current event articles or online videos, examples of which can be found through the resources provided at the end of this manual.

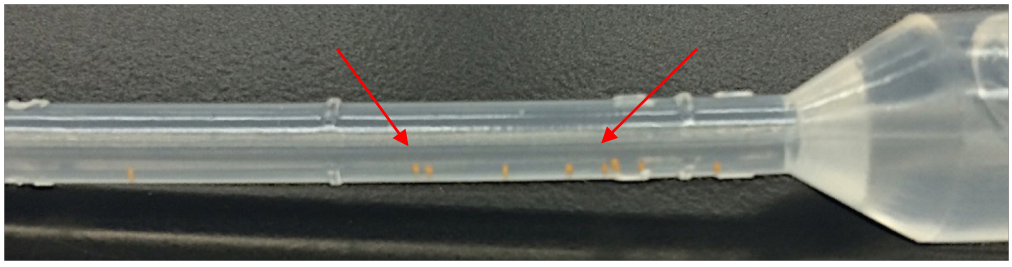
Introduction: Motivating the Students (20 minutes)

- Use the Power Point presentation provided, or create your own
- Expose students to some of the concepts of authentic scientific research and the need for people to participate in citizen science projects
- Online Nova Video: Epigenetics (see Resources Guide) (7 min)

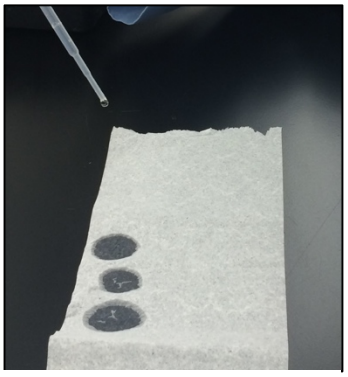
Lab Day 1: Making Seed Paper (20 min)

Arabidopsis thaliana seeds are very, very small, almost dust-like, and require patience, as well as a plan for careful handling throughout the project to be successful.

1. Fill your beaker about halfway with water. Open seed packet and **very gently** tap on the sides until you see some seeds go into the beaker.
2. Take a square of toilet paper and lay it down flat.
3. Place the transfer pipet in the beaker and squeeze until it fills with water. You should also get some seeds. Look in the pipet to make sure there are seeds inside (shown below).



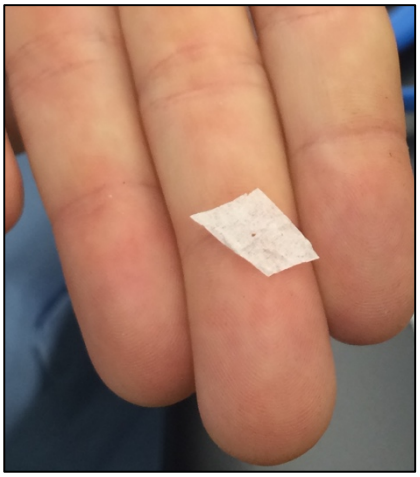
4. One drop at a time, squeeze the seed-water onto the toilet paper, making rows of drops. Not every drop will have a seed in it, and some drops will have more than one seed. Allow the paper to dry on the lab bench (20-40 min. depending on classroom conditions).



Step 4. Adding seed-water to the paper one drop at a time

Lab Day 2: Planting the Seeds (40 min)

1. Fill all planting cells to the top with soil
2. Place the cells in the planting trays
3. Add about 2 liters of water to the bottom of the trays and allow the water to soak up through the soil from below (at least 30 minutes)
4. While soil is soaking, Label the planting tags with the Plant ID numbers of the seeds to be planted. Students' names and planting dates should be written on the back of the planting labels
5. A piece of masking tape with the plant ID number should be put on the outside of the 2x2 cell pack, just in case planting labels are lost or moved
6. Take your dry seed paper created in the previous lab. Use scissors to cut out small squares containing a single seed (shown on right).
7. Take one seed square from your seed paper and press it gently into the soil. Do not cover the seeds with soil; doing so will prevent germination.



8. Cover trays with humidity domes and place in the refrigerator overnight. This will help to synchronize seed germination.
    a. If you do not have enough space to refrigerate all of the trays, you may alternatively put the **dry** seed paper in the refrigerator overnight. If this is done, it is essential to plant the seeds immediately upon removing the paper from the refrigerator. (Check with your school cafeteria about using walk-in cooler space)
9. Remove the tray from the refrigerator and place under a 24-hour light source. Leave the humidity dome in place until seeds have germinated. It is recommended to elevate the trays, or lower the light bulb rack just above the surface of the plants in order to provide adequate light for growth. Remember to raise the lights up as the plants grow.
10. Record the date, temperature in °C, % humidity, and light level in Lux.

How Many Seeds of each Line should we Plant?

The kit materials you have been given and the protocol as it is written allows you to plant 32 individual plants per tray, x 4 large trays for a total of 128 individual plants across all seed lines. If you have more than one light rack, or a temperature controlled greenhouse, you could certainly plant more, as you will receive 100 or more seeds for each line. A small student project could examine fewer that the recommended 32 plants per tray.

Make sure you plant ample seeds from all 3 lines: the experimental line, the Columbia (Col) Wild Type line, and the known *bal* mutant line to account for seeds that do not germinate and plant death, so that at weeks 2-4 enough individual plants (replicates) remaining from each line to observe and compare phenotypic differences and growth over time.

Lab Day 3: Germination (20 minutes over 2-3 days)

1. Depending on temperature of your room, germination will occur around Day 3-5. Check the trays daily for any sign of germination. If more than one seedling emerges in a cell, carefully remove them so that just the most vigorous plant is allowed to grow per cell. Choose the best looking seedling to remain. This is called "thinning" your plants.

2. Once seeds germinate, the humidity dome should be slowly removed over the next 2-3 days so as not to stress the seedling and maintain adequate moisture in the pots. On day one after germination, open the domes to the side and keep them loosely propped up (not locked in place) over plants, so water can evaporate and air circulates gently. On day 2 and 3 after germination, remove domes completely during the day to allow air and light to circulate around plants. In the evenings partially cover with the domes. Remove domes completely three days after germination begins.

3. **Maintaining proper soil moisture is CRITICAL**

    - Germinating seeds require consistent soil moisture; however after germination and during seedling establishment overwatering can be problematic and lead to fungi, algae, gnats and other pests that interfere with plant growth
    - At **ALL** times, soil should remain soft to the touch
    - If the top of the soil starts to dry, add 3 ml water with transfer pipet directly around plant.
    - Water plants from the bottom of the tray with 1 L of water only if the soil seems excessively dry, or there is the potential for soil completely drying out over a weekend.

- Do not leave standing water in the watering tray. Remove all water from the tray within one hour of watering.

4. If time permits, encourage students to make and record observations related to plant growth, development and germination time within and across the different **seed lines**. Record phenotypic data and environmental parameters in lab notebooks. Discuss observations, inferences and data students use to draw conclusions about their data.

Lab Day 4: Characterize Plant Phenotypes 2 weeks after germination (40 minutes)

By now, the seedlings should be established. Students should be able to determine not only which plants are growing, but they may see small differences in a few of them. Have students carefully compare their plants to both the Wild type control and the *bal* mutant control plants to gauge if there are any differences in their plants.

Students could collect the following data:

1. number of viable plants, number planted
2. date, temperature, % humidity, and light levels
3. Take pictures of individual plants and entire groups and label the pictures by line, as shown below:

Teacher Name. Plant ID Number. Date

Example: Smith. 1903. 4/3/14

Lab Day 5: Characterize Plant Phenotypes 4 weeks after germination (40 minutes)

Plants are now mature and **bolting** (the shooting up of flowering stems) of control plants has occurred. Students should pay careful attention to those plants that appeared to be different during the first seed check. They should also be looking for new traits that may not manifest themselves until further along in the plants development.

Have available magnifying glasses and rulers, the Columbia wild type and *bal* mutant plants as a basis for comparison. Encourage students to make as detailed observations as possible.

1. Students should record the date, temperature, % humidity, and light level; as well as the number of viable plants.

2. Students measure the **chord** length of all their plants in cm. Find the longest leaf. Using a piece of string, start from the end of the longest leaf, and measure to the tip of the leaf that gives you the longest line, as shown below.

Measuring the chord length

3. Students should record some detailed observations regarding the appearance of the plant in the **Qualitative Observations** section of their worksheets. For example, compared to the Wild Type, is this plant smaller? Does it have very curly leaves? Is it missing bristles on its leaves? Is it a different color? Does it have a taller flower structure? Does it have fewer leaves? How about compared to the *bal* control plants?

4. If you think any of your plants is very different from the others, it is a **mutant.** Make sure students make a note of this in their observations. If it is determined to be a mutant, set it aside and allowed to grow for several more weeks. Once it has flowered and produced seeds, the seeds will be sent to the scientists at BTI for further study.

Lab Day 6: Wrap up, about 8 weeks after planting (40 minutes)

Seeds from mutant plants should be collected when seedpod or **silique** is brown but not open or showing signs of **dehiscence.** This often occurs between 6-8 weeks after germination depending on growth conditions. When dry, but not open carefully cut off the bolt containing the mature pods and place the entire structure into individual coin envelopes. Seed envelopes should be labeled with the teacher's name, the date collected, and the ddm1 experimental line number from the corresponding planting label. If you assigned an additional unique plant number to each individual plant in your experiment, please indicate that number too. Photos of the mutant plants for which seeds were collected through the experiment are also helpful to share.

Please mail your mutant seed envelops directly to Dr. Richards:

Dr. Eric Richards
Boyce Thompson Institute
533 Tower Road
Ithaca, NY 14853

Compiling Class Data

Send an email to Dr. Richards and Ms. Fleming and attach digital photos (.jpg format) of any mutants as well as the Teacher Data Summary Report to pgrp-outreach@cornell.edu (plant genome research program outreach)

Conclusion & Discussion

Any time after the four-week plant check, discuss the results of the experiment with the class. Discussion topics may include how many mutants were identified; whether or not the mutants were all *bal*, differences in mutation rates among the four different experimental lines, problems that arose, errors that were made, and lessons that were learned. Dr. Richards could be contacted to have a discussion with the class if scheduling permits.

Teacher Data Summary Report

Teacher_____School_____

Date Planted:_____

2 Weeks Post Plant: Date: Temp: Humidity: Lux:

Tray Photos (y/n):

4 Weeks Post Plant: Date: Temp Min: Humidity Min: Lux:

Tray Photos (y/n):____

Temp Max: Humidity Max:

Plant ID Number	Mutant Number	Description	Picture Date	Date Seeds Collected

Interesting Mutant Plant ID Numbers:

_____ _____ _____

_____

Any problems?

Any recommendations?

Optional Extension Activities

The materials provided by this lab provide the opportunity to extend the activity in many different ways.

Graphing Skills:
Students can hone their skills in measuring and graphing any number of data sets:

- -Fluctuations in environmental conditions
- -Size differences between mutants and wild types
- -Plant growth through time

Experimental Design:
The protocol for the lab may be adapted to emphasize major testable concepts in carrying out sound experiments:

- -Identifying the independent and dependent variable
- -Identifying the variables that must be kept constant
- -Quantitative vs. Qualitative analysis
- -Observational skills

Basic Plant Experiments:
By saving the seeds produced from wild type plants students can develop a research plan to test the viability of *Arabidopsis* under specific growing conditions:

- -Exposure to light, humidity, hydroponics
- -Effects of fertilizers
- -Exposure to pollutants (acids, bases, salt)
- -Density of plants per cell

Epigenetic Concepts:
As more advanced students explore the deeper concepts of epigenetics, the background information and results of this lab can be used to enhance understanding of Epigenetics:

- -Provide a clear example of an epigenetic mechanism at work
- -Further investigate *bal* mutations and their mechanisms by examining the primary sources and related articles generated by Dr. Richards' lab