Version: 1.0
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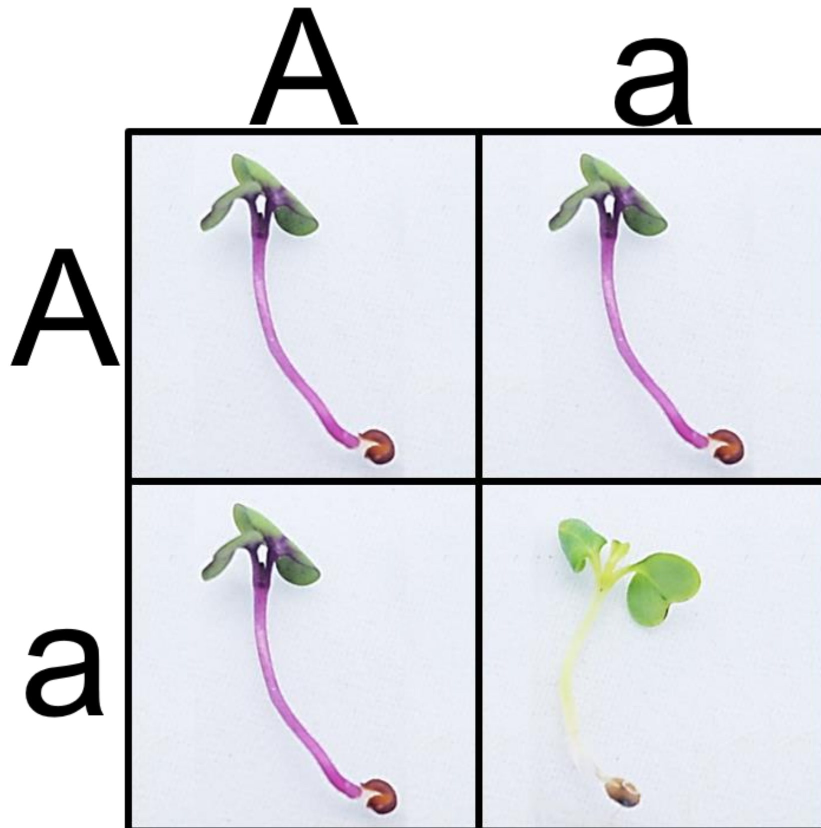
miniPCR™ Plant Genetics Lab: Taking Mendel Molecular



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1. Background and significance

Overview

In this lab you will investigate the genotypic basis of an observable phenotype using Rapid Cycling *Brassica rapa* (RCBr), also known by the trademark name Wisconsin Fast Plants®. Wild-type plants grow with a distinctive purple stem, best observed in the first few days after germination. This purple color is due to the presence of anthocyanin, a common plant pigment. In some RCBr plants, however, a mutation disrupts the anthocyanin production pathway and leads to green stems with no purple color. The gene responsible for the purple vs. green color is named *anthocyaninless* because the mutant form leads to decreased production of the pigment anthocyanin. There are two alleles for the *anthocyaninless* gene, *A*, which results in purple stems, and *a*, which leads to green stems.

A wild-type anthocyanin producing seedling beside an anthocyaninless *aa* homozygote

For this trait, purple color (anthocyanin production) is dominant to green (lack of anthocyanin production). For this reason, both *AA* homozygotes and *Aa* heterozygotes will be purple stemmed, while only *aa* homozygotes will be green stemmed. Using classical crossing techniques, these alleles can be tracked over generations with predictable results. In this lab, you will test the *anthocyaninless* gene from different plants for the presence of the mutation that differentiates the two alleles. In this way, you will be able to directly link an organism's phenotype to its genotype.

Mendelian genetics goes molecular

In 1865 Gregor Mendel described his work studying inheritance in pea plants. His work, at the time, was largely overlooked. Around the turn of the 20th century, however, other scientists studying inheritance rediscovered Mendel's manuscripts and the profundity of his findings was recognized. The laws of Mendelian genetics were first observed in pea plants, but it was quickly appreciated that these laws applied broadly to sexually reproducing eukaryotes in general. Not only did Mendel explain how traits are passed on, but in doing so, he provided a mechanistic basis for many other areas of biology, most notably evolution.

Mendel's careful observations led him to propose two basic rules: the *law of segregation* and the *law of independent assortmen*t. The law of segregation states that for any gene, an individual possesses two copies, or alleles. When an individual makes gametes (sex cells), each

gamete contains only one of the two alleles. In other words, the alleles are segregated into different gametes, and only one of those two alleles will be passed on to each offspring.

The *law of independent assortment* states that the segregation of alleles responsible for one trait will occur independently of the segregation of other alleles. In other words, in pea plants, knowing the flower color allele will tell you nothing about the pea pod color allele.

Mendel's laws and basic crossing techniques helped crack open the field of genetics in many organisms. By crossing organisms and tracking the different phenotypes in the offspring, scientists were able to create genetic maps of chromosomes some 30 years before DNA was confirmed to be the genetic material. In other words, people had made accurate genetic maps before they knew it was DNA that they were mapping.

Today we know that Mendel's laws work because what is being inherited are sequences of DNA on chromosomes. We know that when we see a trait that is inherited in a Mendelian fashion, it is because there is a physical place in the DNA that leads to differences between the two traits. Using molecular techniques, such as PCR and gel electrophoresis, we can peer inside of phenotypes and Mendelian ratios to determine their molecular basis. For example, we know that the offspring of two heterozygotes (the F2 generation of a monohybrid cross) should result in a 3:1 phenotypic ratio and 1:2:1 genotypic ratio. With classical breeding, we can observe the 3:1 phenotypic ratios in offspring, but now with molecular techniques, we can go further, determining the genotypes that led to that 3:1 ratio.

Anthocyanin: It makes plants purple

Anthocyanin is a common plant pigment that is typically purple but can appear red to purple to blue depending on where it is found. It is the reason blueberries and blue corn are blue, and the reason eggplants and cabbage are purple. In Rapid Cycling *Brassica rapa*, anthocyanin can be seen in the stems of plants, and it is best observed in the first few days after germination. When anthocyanin production is disrupted for some reason, the purple color will be absent.

Varieties of eggplant that vary in anthocyanin content.
Image courtesy of J. E. Fee

Classical mutants are named for what happens to a phenotype when a gene is mutated.

Somewhat paradoxically, this leads to genes being named after what happens when they don't function properly. The *anthocyaninless* gene is named for the fact that, if mutated, anthocyanin will no longer be produced. This means that the normal role for the *anthocyaninless* gene is likely in a pathway that produces the anthocyanin pigment. Genes are given such names based on observable outcomes because, historically, mutant phenotypes were recognized long before the actual DNA sequence responsible for those traits could be identified. It is only more recently that scientists may match the cause of a phenotype to an actual molecular cause.

In 2016, scientists identified the genetic location and sequence of *anthocyaninless*, a sequence that codes for the enzyme dihydroflavonol 4-reductase, or DFR[1]. DFR is an enzyme known to function in the anthocyanin production pathway and knocking out the production of the DFR protein would almost certainly lead to the loss of anthocyanin production.

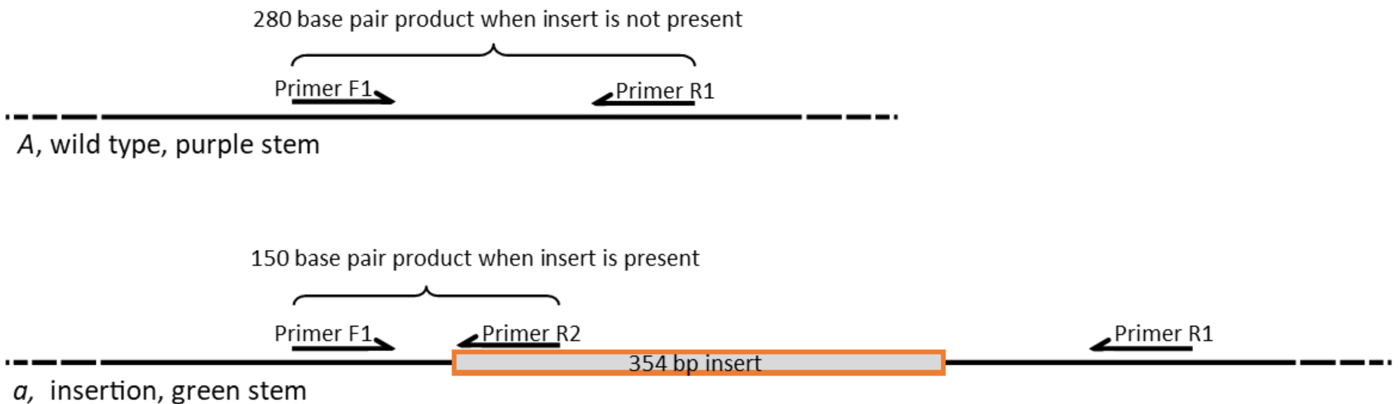
After sequencing the DFR gene from both purple and nonpurple RCBr, the scientists identified the presence of a 354 base pair sequence that was present in the green allele (*a*), but absent from the purple (*A*) allele. By analyzing the DNA sequence, they hypothesized that this 354 base pair difference is the result of an insertion from a transposable element in the coding sequence of the DFR gene. This insertion introduces a premature stop codon to the DFR coding sequence, making the resulting protein truncated and non-functional.

Classical Mendelian traits refer to individual traits being caused by a single gene with dominant or recessive alleles. In *anthocyaninless*, this is because one allele has been made completely non-functional, but anthocyanin production can be maintained with only one functional allele. While most observable variation is caused by alleles that are inherited in a Mendelian fashion, it is somewhat rare for natural variation to be due to one allele producing a non-functional form of the protein. Most phenotypic variation is based on the inheritance of many genes with multiple alleles and complex interactions between them. For example, DFR is one of many genes involved in the production of anthocyanin, and changes to any of them have the potential to modify an anthocyanin related phenotype. The connection between DFR and *anthocyaninless* is an excellent model for demonstrating how alleles are inherited and linking that inheritance to actual sequence changes on the chromosome. But it should be remembered that most traits have more complex bases than seen in this example.

[1] Wendell, D.L. Vaziri, A. Shergill, G. (2016) The Gene Encoding Dihydroflavonol 4-Reductase Is a Candidate for the *Anthocyaninless* Locus of Rapid Cycling *Brassica rapa* (Fast Plants Type) *PLoS One,* 11(8)

Today's lab

In this lab, you will use PCR to amplify (make copies of) the specific region of the DFR coding sequence that is responsible for producing the purple (dominant) and green (recessive) *anthocyaninless* alleles. The reaction will use three primers, one forward primer and two different reverse primers, to identify whether the insertion that disrupts the DFR gene is present. The first forward primer in this design (F1) will bind to both alleles, regardless of whether the *anthocyaninless* insertion is present. A reverse primer (R1) will bind 280 base pairs downstream from the F1 primer in the wild type (*A*) allele. Together, these primers will make a 280-base pair PCR product in plants that produce the anthocyanin pigment. A second reverse primer (R2) will bind only when the insertion is present. In the green allele (*a*), together with the F1 primer, the R2 primer will produce a 150-base pair fragment. R2 will not bind in the purple allele (*A*) because the insertion is not present, and so no 150 base pair product will form. To be clear, both R1 and R2 primers will bind to the *a* allele, but because of differences in the relative efficiency of PCR due to size and structural constraints of the DNA, only the 150 base pair amplicon will be produced.



Segment of DFR gene showing primers designed to identify specific alleles responsible for purple or green *Anthocyaninless* phenotypes. In the *A* (wild type) DFR allele, no insertion is present and primer F1 and R1 will produce an approximate 280 base pair product. In the *a* (insertion) DFR allele, primer F1 and R2 will produce an approximately 150 base pair product. Primer R1 will bind to both sequences, but will only produce a product in the *A*, wild type DFR sequence.

2. Laboratory guide

A: Phenotype seedlings (if using F_2 seeds)

1. **Identify how many of the germinated plants are purple and how many are green.**
    - Record the total number of plants that show the purple and green phenotypes in your class

	# of Seedlings
Purple	
Green	
Total	

B: DNA Extraction

Note: Usable DNA can be extracted from many different plant tissues using the following basic procedure. We recommend using **leaf tissue**, either from the cotyledon in seedlings or mature leaf tissue if present. If using seedlings, wait at least 5 days after starting germination before extracting DNA for best PCR results.

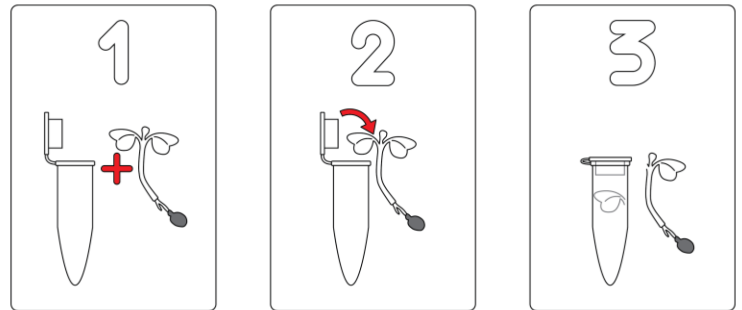
1. **Choose up to four plants that your group will genotype.** Your teacher will instruct you on which plants to choose. If testing adherence to predicted Mendelian ratios, it is important to choose plants randomly.
    - Record the phenotype of the plant sample you are testing (purple or green.)
    - Record any other information that you know about the plant sample as instructed by your teacher. For example, is it a pure breeding plant, an F2 plant, etc.

Sample #	Phenotype	Additional information
1		
2		
3		
4 (or blank control)		

2. **Label four 200 µL thin-walled PCR tubes** per lab group on the side, not cap, of the tube
   - Label tubes 1-4 to correspond with your plant samples.
   - Also label each tube with the group's name on the side wall.

3. **Add 50 µL of DPX Buffer** to each tube

4. **Use numbered PCR tubes to collect portion of leaf**
   - To avoid contamination, try not to touch the part of the plant you will use for your DNA sample.
   - Hold the plant so that the leaf is over the opening of your tube.
   - Close the cap of the tube, using the cap as a punch to cut a sample of the plant.
   - With the tube closed, discard any plant tissue that is not in the tube.
   - The plant sample remaining in the tube will be used to isolate DNA.
   - If using mature plants, close the tube on a mature leaf using the top as a punch.



5. **Use a pipette tip to macerate plant tissues for DNA extraction**
   - Carefully open the tube to avoid losing plant sample.
   - Use a pipette tip to crush and grind the plant tissue in the DPX buffer solution in order to break cell walls.
   - Tissue should be clearly broken up leaving the solution greenish and/or cloudy.
   - Use a new tip for each tube to avoid contamination.

6. **Tightly cap the 200 µL tubes** containing DPX Buffer and macerated plant sample
   - Ensure that plant fragments are well mixed into the DPX Buffer.
   - Avoid touching the inside of PCR tube caps to avoid contamination.

7. **Incubate the macerated plant sample in DPX Buffer for 10 minutes at 95°C**
   - Use a miniPCR™ machine in Heat Block mode, or use a 95°C heat block or water bath.

8. **After 10 minutes remove tubes from heat**
   - This solution is your DNA extract.
   - The DNA extract can be stored frozen for at least two weeks.

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PCR set up

1. **Label 4 clean 200 µL thin-walled PCR tubes** per group on the side wall
   A. Label the tubes 1-4 to correspond with your DNA extract.
   B. Also label each tube with the group's name on the side wall.

2. **Add PCR reagents** to each 200 µL PCR tube

	Tube 1	Tube 2	Tube 3	Tube 4
Plant Lab Primers	18 µL	18 µL	18 µL	18 µL
EZ PCR Master Mix	5 µL	5 µL	5 µL	5 µL

Use a micropipette to add each of the reagents.
Remember to change tips at each step!

3. **Add DNA samples to each tube,** using a clean tip for each sample

CRITICAL STEP

Add 2 µL of DNA extract avoiding large plant particles, as these will clog your pipette tip. If clogging occurs, pipette up and down to unclog.

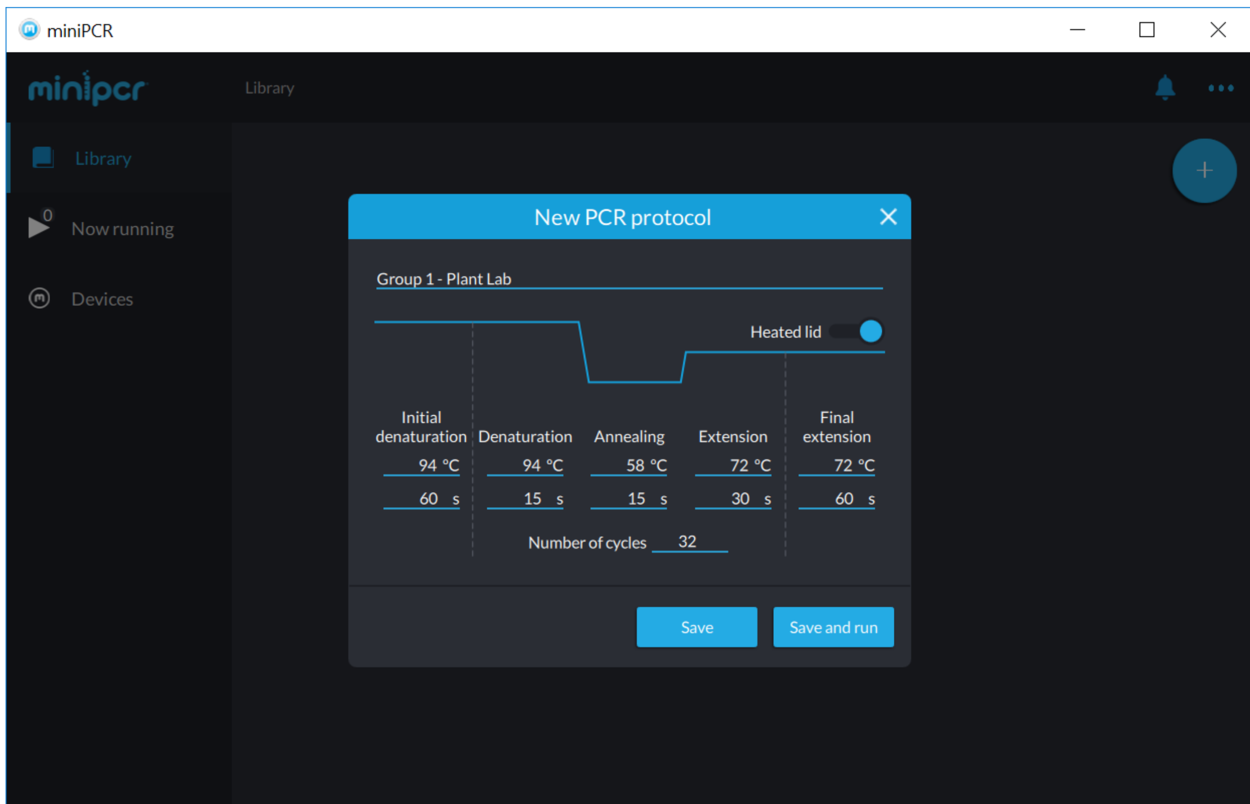
	Tube 1	Tube 2	Tube 3	Tube 4
Template DNA	**2**	**2**	**2**	**2**
FINAL VOLUME	***25 µL***	***25 µL***	***25 µL***	***25 µL***

4. **Cap the tubes**
   - Make sure all the liquid volume collects at the bottom of the tube.
   - If necessary, spin the tubes briefly using a microcentrifuge.

5. **Place the tubes inside the PCR machine**
   - Press firmly on the tube caps to ensure a tight fit.
   - Close the PCR machine lid and gently tighten the lid.

PCR programming and monitoring (illustrated using miniPCR™ software)

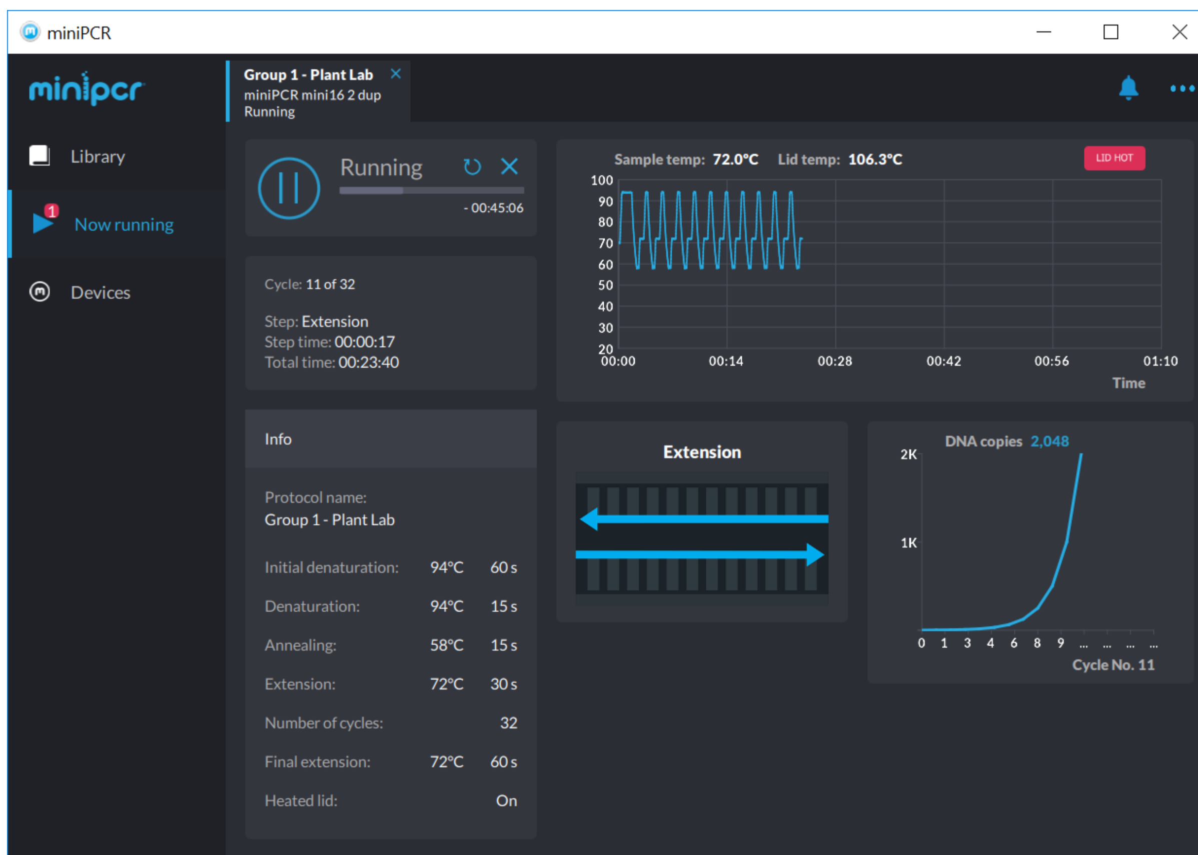
1. Open the miniPCR software app and remain on the **"Library"** tab.
2. Click the ⊕ button on the top right corner.
3. Select the **PCR** from the top drop-down menu.
4. Enter a name for the Protocol; for example, **"Group 1 – Plant Lab".**
5. Enter the PCR protocol parameters:
    - **Initial denaturation** 94°C, 60 sec
    - **Denaturation** 94°C, 15 sec
    - **Annealing** 58°C, 15 sec
    - **Extension** 72°C, 30 sec
    - **Number of cycles** 32
    - **Final extension** 72°C, 60 sec
    - **Heated lid** ON
6. Click **"Save"** to store the protocol or **"Save and Run"** to start the protocol.



7. If prompted, choose the serial number of the miniPCR you are using from the list.
    - Serial number can be found on the white sticker below the power switch.

8. Make sure that the **power switch in the back** of miniPCR is in the **ON** position.

9. To monitor the PCR reaction in real time, choose the "**Now running**" tab on the left ("Monitor" on a smartphone or tablet.) If more than one miniPCR is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window.



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.

Once the PCR run is completed (approximately 70-80 min), the screen will display: **"Status: Completed". All LEDs on the miniPCR machine will light up.**

You can now open the miniPCR lid and remove your PCR tubes.
Be careful opening the miniPCR, lid and heat block may still be hot

PCR products can be stored for up to 1 week in the fridge or 1 year in a freezer.

Gel electrophoresis – Pouring agarose gels (Preparatory activity)

1. Prepare a clean and dry agarose gel casting tray
   - Seal off the ends of the tray as needed for your apparatus.
   - Place a well-forming comb at the top of the gel (5 lanes or more).

2. For each lab group, prepare a 2% agarose gel using electrophoresis buffer
   - For example, add 0.4 g of agarose to 20 ml of TBE buffer (for blueGel™).
   - Mix reagents in glass flask or beaker and swirl to mix.
   - If using blueGel Tabs, use one 0.4 g tab per 20 ml of TBE buffer. Allow the blueGel Tab to disintegrate completely before heating.
   - Adjust volumes and weights according to the size of your gel tray.
   - Mix reagents in glass flask or beaker and swirl to mix.

3. Heat the mixture using a microwave or hot plate.
   - Heat until agarose powder is dissolved and the solution becomes clear.
   - Use caution, as the mix tends to bubble over the top and is very hot.

4. Let the agarose solution cool for about 2-3 min at room temperature.
   - Swirl the flask intermittently.

5. Add gel staining dye (e.g. GelGreen™).
   - Follow dye manufacturer instructions.
   - e.g., 2 µL of GelGreen 10,000 X per 20 mL agarose gel.

6. Pour the agarose solution into the gel-casting tray with comb.

7. Allow gel to completely solidify (until firm to the touch) and remove the comb.
   - Typically, ~10 minutes for blueGel™ gels.

Gel electrophoresis – Running the gel

1. Place the gel into the electrophoresis chamber and cover it with run buffer.
    - Add just enough buffer to fill reservoirs on both ends of the gel and to just barely cover the gel.

2. Make sure the gel is completely submerged in electrophoresis buffer.
    - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged.)
    - Fill reservoirs at both ends of the electrophoresis chamber and add just enough buffer to cover the gel and wells.

3. Load DNA samples onto the gel in the following sequence
    - **Lane 1:** 7 µL DNA ladder.
    - **Lane 2**: 15µL PCR product from tube 1.
    - **Lane 3**: 15µL PCR product from tube 2.
    - **Lane 4**: 15µL PCR product from tube 3.
    - **Lane 5**: 15µL PCR product from tube 4.

Note: there is **no need to add gel loading dye to your samples**.
The *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* come premixed with loading dye, and ready to load on your gel.

4. Place the cover on the gel electrophoresis box.

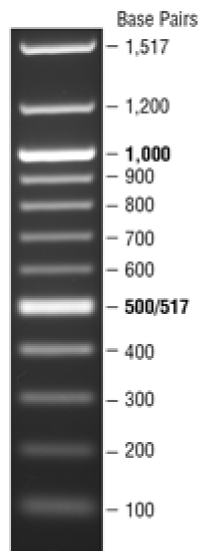
5. Conduct electrophoresis for ~25 minutes, or until the colored dye has progressed to at least half the length of the gel.
    - Check that small bubbles are forming near the terminals in the box.
    - Longer electrophoresis times will result in better size resolution.
    - Lower voltages will result in longer electrophoresis times.

Size determination and interpretation

1. Turn on the blueGel™ blue light illuminator.
    - *Or place the gel on a transilluminator if not using blueGel™.*

2. Verify the presence of PCR product.

3. Ensure there is sufficient DNA band resolution in the 100-300 bp range of the 100 bp DNA ladder.
    - Run the gel longer if needed to increase resolution.
    - DNA ladder should look approximately as shown.



100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.
Source: New England Biolabs

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100 bp DNA ladder).
    - Capture an image with a smartphone camera or other gel documentation system.

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3. Study questions

Questions before experimental set-up

1. What are the two alleles that we are using to investigate Mendel's laws in this experiment? Please describe them phenotypically.

2. In this lab we are tracking the inheritance of two alleles of the same gene through different generations. Is this a better investigation of the law of independent assortment or the law of segregation? Explain.

3. In organisms used for genetics, quite often genes end up with two names. Names like *anthocyaninless* are reflect the mutant phenotype, while names like DFR refer to the protein the gene produces. Why not just have one name?

4. If phenotypes are caused by genotypes, can you explain why the genotypic and phenotypic ratios for Mendelian traits are different? How is it that a 3:1 phenotypic ratio can be caused by a 1:2:1 genotypic ratio?

5. This lab demonstrates how the *anthocyaninless* gene affects stem color in *Brassica rapa.* Think of another trait you are familiar with, for example human height. In what ways do you think *anthocyaninless* is a good model for studying the inheritance of phenotypes? In what ways may it not be the best model?

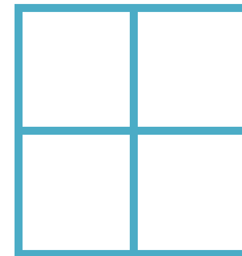
Questions using Punnett squares.

Predict the genotypic and phenotypic ratios for the possible offspring from each of the following crosses:

1. A homozygous purple plant crossed to a green plant.

Phenotypic ratio of offspring: Purple _____

Green _____



Genotypic ratio of offspring: *AA* _____

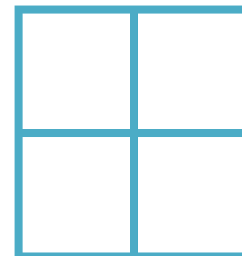
Aa _____

Aa _____

2. A heterozygous purple plant crossed to a green plant.

Phenotypic ratio of offspring: Purple _____

Green _____



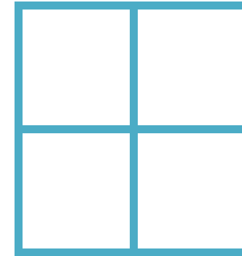
Genotypic ratio of offspring: *AA* _____

Aa _____

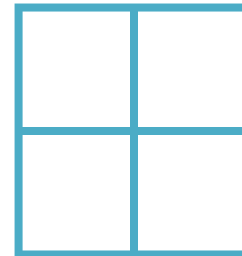
Aa _____

3. Two heterozygous plants.
   Phenotypic ratio of offspring: Purple _____
   Green _____

   Genotypic ratio of offspring: *AA* _____
   Aa _____
   Aa _____



4. If you had a plant that developed with a purple stem, what type of plant could you cross it to tell if it were homozygous or heterozygous? Explain using a Punnett square.



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Predict your possible results for this experiment.

Sample #	Phenotype	Possible genotype(s)
1		
2		
3		
4		

On the image of a gel to the right, draw in your expected results. In some cases, you cannot predict whether a particular band will be present or absent on your gel. If it is uncertain if a certain band will be present, draw it as a dotted line.

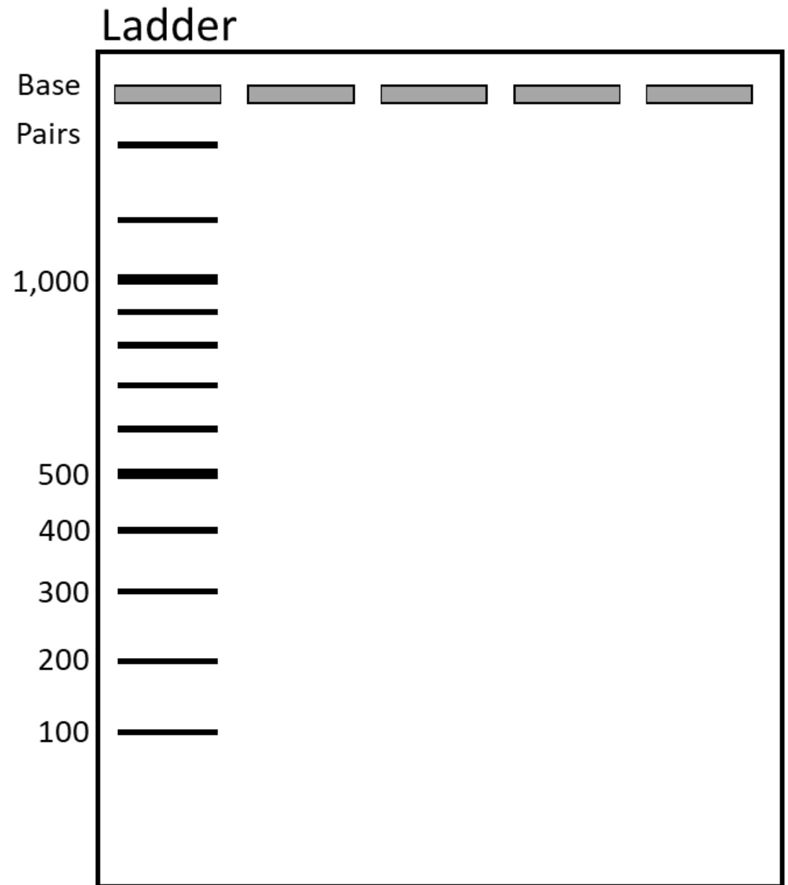
For each lane, explain why you predict the results that you drew.

Lane 1:

Lane 2:

Lane 3:

Lane 4:



Questions after gel electrophoresis and visualization

1. What are the genotypes of each plant you tested? Explain whether your results match what you predicted.
   a. Plant 1 genotype:
      Does this result match your prediction?

   b. Plant 2 genotype:
      Does this result match your prediction?

   c. Plant 3 genotype:
      Does this result match your prediction?

   d. Plant 4 genotype:
      Does this result match your prediction?

2. Look at the first two lanes of your gel. If you were able to breed those two plants, what would the expected genotypic and phenotypic ratios of the offspring be?

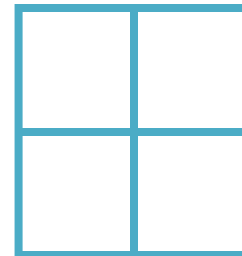
Phenotypic ratio of offspring: Purple _____

Green _____

Genotypic ratio of offspring: *AA* _____

Aa _____

Aa _____



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Class data:

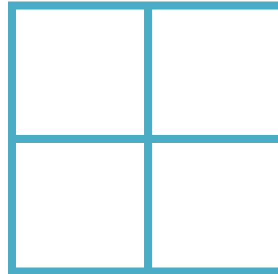
Predicted:

F_2 plants are the result of an *Aa* x *Aa* cross.

Phenotypic ratio of offspring:

Purple ______

Green ______



Genotypic ratio of offspring:

AA ______

Aa ______

Aa ______

Record the total number of plants in your class that displayed each phenotype and genotype.

**Note you may test the phenotypes of more plants than you are able to genotype.*

	Total plants
Purple	
Green	

	Total plants
AA	
Aa	
aa	

How closely do your predicted phenotype and genotype ratios match your class data?

AP biology link

Testing adherence to predicted phenotypic and genotypic ratios - for classes growing F2 seeds. Use the chi-squared statistical test to determine if class data matches Mendelian expectations.

Compile class data for phenotypes.

	Observed	Expected
Purple		
Green		

$$\sum \frac{(o-e)^2}{e}$$

State the null hypothesis:

Degrees of freedom: ________ χ^2 value: ________

χ^2 critical value: ________ (from table)

Do you reject or fail to reject the null hypothesis?

Explain what your statistical results mean in common language.

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Compile class data for genotypes.

	Observed	Expected
AA		
Aa		
aa		

$$\sum \frac{(o-e)^2}{e}$$

State the null hypothesis:

Degrees of freedom: ________ χ^2 value: ________

χ^2 critical value: ________ (from table)

Do you reject or fail to reject the null hypothesis?

Explain what your statistical results mean in common language.

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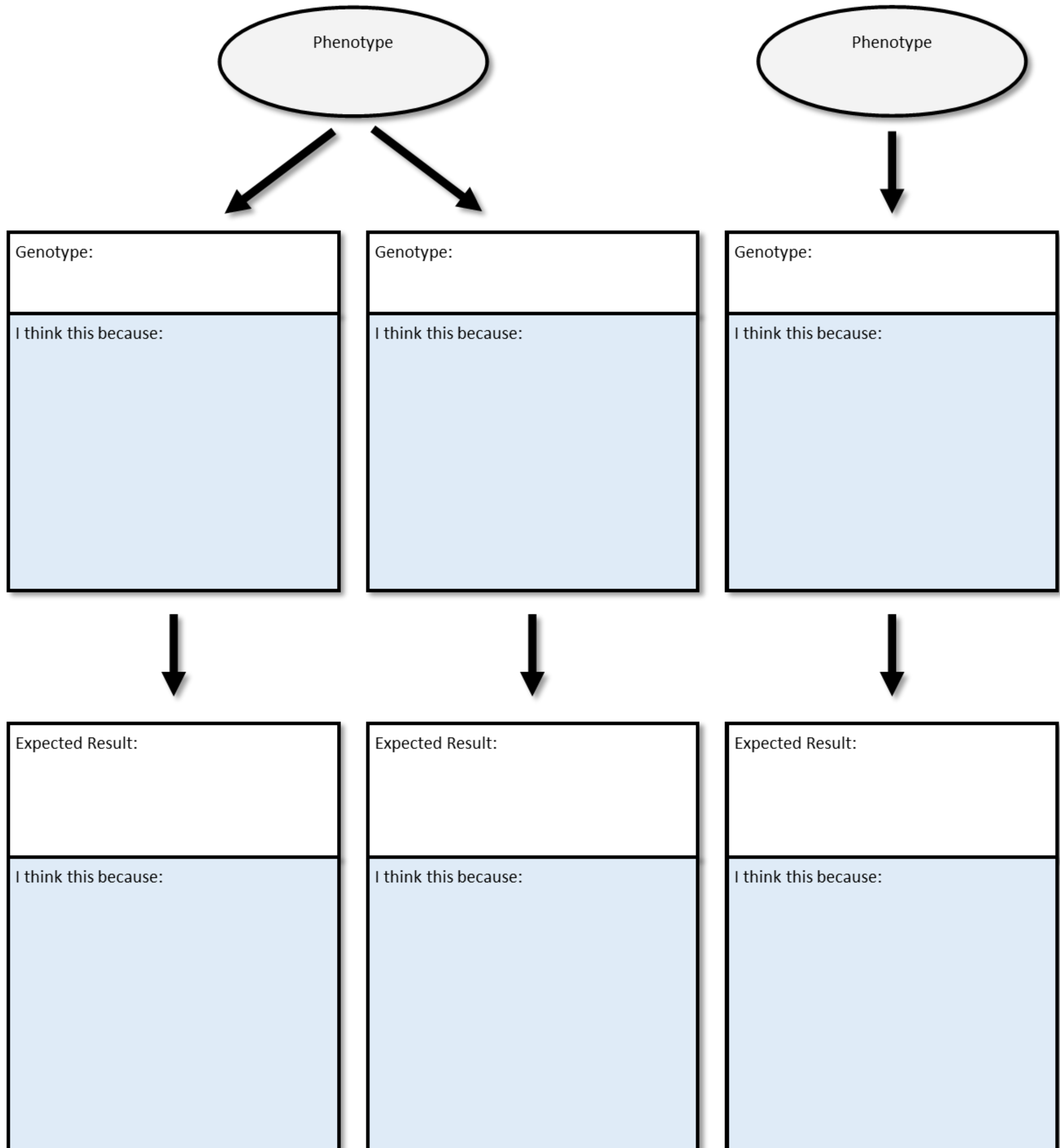
Plant Genetics Lab Concept Map

In the appropriate box, fill in the two possible phenotypes, each possible genotype, and what you expect to observe on a gel when testing that genotype. In the larger boxes, explain how your thinking that led you to answer the way that you did.



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Extension: Exploring the *anthocyaninless* mutation

In Rapid Cycling *Brassica rapa*, lack of anthocyanin production is due to the recessive form of the gene *anthocyaninless*. The trait occurs because the *anthocyaninless* gene codes for the enzyme dihydroflavonol 4-reductase, or DFR, an enzyme central to anthocyanin production. In the *a* allele, the version of the protein produced by the cell is non-functional.

DFR is a 385 amino acid protein and the DNA sequence that codes for DFR is broken up into six exons. The difference between the *A* and *a anthocyaninless* alleles lies in exon 4 – in the *a* allele, exon four is 354 base pairs longer than in the *A* allele. Within this extra 354 base pairs is a stop codon, which ends translation prematurely, making the protein non-functional.

These extra 354 base pairs bear the hallmarks of a transposable element insertion event. Transposable elements, also known as transposons, are sequences of DNA that seem to exist for no other function than to make more of themselves in the genome, and they are sometimes thought of as “genetic parasites”. There are several variations on how transposable elements function, but the basic premise is this: a transposable element is a sequence of DNA present in a genome that, every now and then, will engineer a copy of itself, or actually cut itself out of the genome, and that copy or excised DNA will then be inserted somewhere else in the genome. The ability of these DNA sequences to move from one place to another in the genome has led them to be nicknamed “jumping genes”. This movement of DNA is very rare in any one individual; it is unlikely that the transposable elements in you have moved in your lifetime. But over timespans of many generations, their movement is fairly regular. Over time, their movement and copying causes them to spread, taking up more and more of the genome. It is estimated that around 45% of the human genome is made up of transposable elements or transposable element like repeats—most of which are now inactive.

When transposable elements insert into a segment of DNA they leave a telltale sign, a direct repeat on each end of the insertion. On one end of the insertion will be a segment of DNA that was present before the insertion, and on the other end will be the exact same sequence repeated again. In the *anthocyaninless a* allele, there are identical 10 bp sequences separated by 344 base pairs. These 10 base pairs are present in the *A* allele, but only once, and the 344 base pairs between them is not present at all—exactly what is expected if a transposable element inserted itself in the gene.

Because transposable elements make copies and spread in genomes over time, you would expect to find many copies of this suspected transposable element throughout the *Brassica rapa* genome, showing the history of its transpositions. But when scientists went looking, there

was no such common 344 base pair sequence. In *anthocyaninless*, however, the middle 340 of the 344 base pairs shows a pattern that is unusual; it is a perfect inverted repeat. In other words, the sequence of the first 170 base pairs is exactly the same as the second 170 base pairs, only oriented in the opposite direction. When scientists looked for a 170 base pair sequence that matched one half of the inverted repeat, they found what they were looking for - it was present over 100 times in the *Brassica rapa* genome. The hypothesis is that this 170 base pair repeat represents the transposable element. In the particular instance of *anthocyaninless*, a rare event occurred that caused it to be basically inserted twice back to back in opposite orientations. How or why this occurred is unknown.

Sequences like this are called palindromes. A palindrome is a word or sentence that reads the same in both directions, such as, "Madam, I'm Adam." In genetics, a palindromic sequence reads the same when each DNA strand is read in the 5' to 3' direction. Palindromic sequences are important for different biological processes. Restriction enzymes usually recognize palindromic sequences. Longer palindromic sequences can be the basis for secondary structures in nucleic acids as you will explore in the following questions.

When a transposable element inserts itself in the genome it can have different effects. If it lands in a non-coding region, the newly inserted element may have no effect on the overall phenotype of the organism. If it inserts itself into a coding region, as happened with *anthocyaninless*, it has the potential to disrupt that gene's function. Landing in regulatory regions of DNA may have more subtle effects. And in some cases, if the inserted element transposes again, function can return. Transposons may themselves undergo mutation, making them incapable of transposing again, leaving the sequences in the genome to be passed down like any other DNA. And while often considered junk or parasitic, some transposable element sequences have clearly been conserved over hundreds of millions of years of evolution, suggesting that in some scenarios, they may have a functional role. In corn, transposable elements and similar sequences are thought to make up about 90% of the genome, in humans about 45%. If one thing is certain, they aren't going away.

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The structure of the *anthocyaninless* mutation: Questions

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TACGCGAAGGAAAAAGGAATGAGAATTCGGCCAAAAAAAACCTCAACTTAAGTTGAGGTTTTTTTTGGCCGAATTCCCAAAAAGGAATAGATTTC
ATGCGCTTCCTTTTTCCTTACTCTTAAGCCGGTTTTTTTTGGAGTTGAATTCAACTCCAAAAAAAACCGGCTTAAGGGTTTTTCCTTATCTAAAG
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The sequence above is taken from the *anthocyaninless a* (non-purple) allele and shows both strands of DNA from the insertion sequence, flanking direct repeats, and several base pairs on either side of the direct repeats. In this example, a significant portion of the inserted sequence has been removed to make the exercise more manageable, but the basic structure the same.

1. On the sequence above, identify the direct flanking repeats. There should be two sequences, separated by some distance, that are identical and oriented in the same direction. Draw a box around the two sequences.

2. Between the direct repeats is a single a long inverted repeat. Draw a vertical line marking the center of the inverted repeat

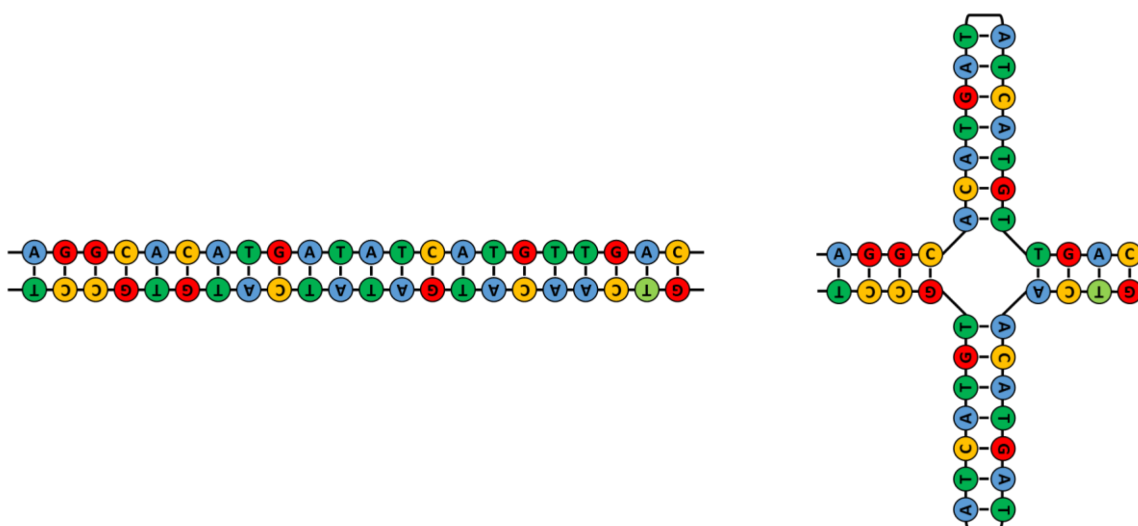
3. From the line you drew, compare the bases on the top strand going to the right with the bottom strand going to the left. What do you notice?

4. The sequence to the right is the top strand of the DNA sequence, ending at the middle of the inverted repeat. Continue copying the top line of DNA but going down the page instead of up. Align the base pairs you write with the letters already on the page.

5. When you are done copying look at what bases are next to each other. Draw a box around the portion of the sequence that is perfectly complementary (each letter is matched with its complementary base).

TACGCGAAGGAAAAAGGAATGAGAATTCGGCCAAAAAAAACCTCAACTT

The structure you drew the box around is called a “hairpin” or a “stem and loop”. Structures like this one can form whenever single stranded DNA or RNA molecules that have inverted repeat sections are allowed to anneal. Portions outside the box you drew typically will not anneal because there is not enough complementarity. Below is another example of how DNA containing an inverted repeat can form a hairpin. The DNA sequences for both structures are the same.



6. Both of the above two structures obey base pairing rules. Which would you expect to find in the cell? Explain why you think this.

7. Do you think a hairpin structure like this is more likely to form in DNA or RNA in the cell? Explain your reasoning.

8. Think of the steps of a PCR reaction. For a DNA sequence like this, why may the hairpin structure be much more likely to likely to form during a PCR reaction?

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Extension: Focus on Women in Science

Barbara McClintock – the discoverer of transposable elements

When Barbara McClintock was born in 1902, her parents actually named her Eleanor. As a young girl, however, they felt that Eleanor was too gentle a name for their daughter's independent and outgoing personality, so they began calling her Barbara. When Barbara decided to enroll as a student at Cornell in 1919, her mother objected, believing it would make it too difficult for her to find a husband.

A husband was clearly not Barbara's chief concern. McClintock excelled at Cornell and, impressed by her performance in his Plant Genetics class, C.B. Hutchinson called McClintock to invite her to join the Cornell Plant Breeding program as a graduate student. In 1925, she was awarded her Master's degree; two years later, she received her Ph.D.

McClintock studied cytogenetics, the relationship of inheritance and chromosome structure, in maize (corn). As a young researcher, McClintock was the first to describe the structure of maize chromosomes and was able to show that traits and chromosomes were inherited in the same patterns, linking chromosomes definitively to genetic inheritance.

In 1930, McClintock became the first person to describe how during meiosis homologous chromosomes form cross-like structures—the structural basis for genetic recombination. The following year, she demonstrated that this crossing over between chromosomes was responsible for genetic recombination between linked traits. Also, in 1931, she described the location of three genes on the maize ninth chromosome, the first genetic map of maize. All of this was accomplished before the structure of DNA was known and even before it was definitively established that DNA was the genetic material.

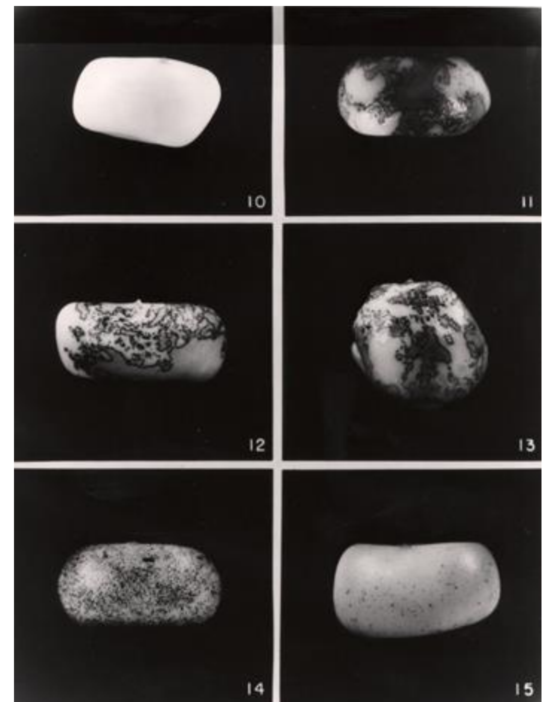
Over the next ten years, McClintock worked at several institutions, first in Germany, where she left due to the rise of Nazism, and then Cornell and University of Missouri. At both Cornell and Missouri, despite making a name for herself in her field and contributing important discoveries to the study chromosomal structure in maize, she found considerable obstacles to becoming a full professor. She believed this was largely due to the fact that she was a woman.

In 1941, McClintock took a full-time research position at the prestigious Cold Spring Harbor Laboratory. It was here that she began the work she is most closely associated with today. McClintock was interested in why some corn kernels show mosaicism, that is, they are multicolored. Not only do these different kernels produce different amounts of anthocyanin, but in some types of corn, different levels

of anthocyanin are produced in different parts of each individual kernel. Furthermore, this mosaicism wasn't inherited in predictable Mendelian ratios.

In 1948, she made one of her biggest discoveries: her system was composed of two genes, one controlling the other. The first gene, named *Dissociator (Ds)*, seemed to be responsible for the color of the kernel, and the second, named *Activator (Ac)*, seemed to turn the *Ds* gene on and off. But when McClintock tried to map the genes, she found it impossible. They literally moved from one place on the chromosome to another. Barbara McClintock had discovered transposable elements.

McClintock spent the next few years meticulously documenting the *Ac* and *Ds* system and another similar system called *Suppressor-mutator (Spm).* In 1953, she published her work in the journal Genetics. McClintock's work was not widely accepted. The system she was working with was complex and dealing with more than one concept that was new to the field of genetics. Recognizing the negative reaction to her work and fearing that her ideas were too radical and would push her out of the science mainstream, she largely stopped publishing or lecturing on the topic.



Different levels of mosaicism in maize kernels due to the interaction of *Ac* and *Ds*. From *Cold Spring Harbor Symposia on Quantitative Biology, 1951*

It took about twenty years for science to catch up to McClintock's discoveries of the late 1940s and early 1950s. Starting in the late 1960s and early 1970s, using more advanced technology, scientists began to discover transposable elements in other organisms. And in the early 1970s scientists showed conclusively that *Ac* and *Ds*, the genes McClintock had characterized two decades prior, were what is now known as class II transposable elements.

McClintock was widely recognized as a prominent scientist in her day, well before her work on transposable element; in 1944, she was the first woman to become president of the Genetics Society of America, and the same year, she joined the National Academy of Sciences, only the third woman ever to be elected. It was only after she retired, however, and her work was reexamined in light of more recent discoveries, that her contributions could be fully appreciated. In 1983, she was awarded the Nobel Prize in Physiology and Medicine for her work on transposable elements. She was the first woman to win the prize unshared, and today Barbara McClintock is widely regarded as one of the great biology minds of the 20th century.

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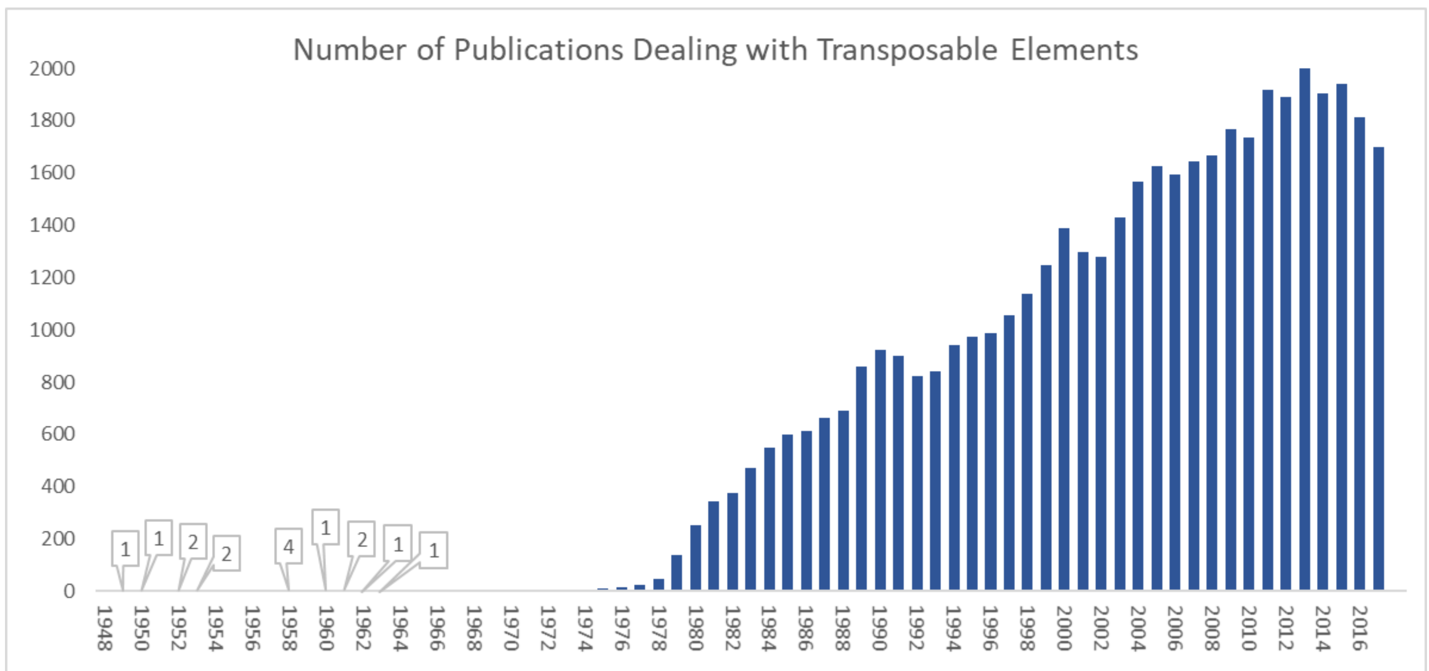
From impacts on human health, to their effect on evolution, to breeding Wisconsin Fast Plants in the classroom, transposable elements remain an active area of research in biology today. In fact, in every year since 1997, well over 1,000 journal articles have been published discussing the topic of transposable elements. The discovery that the *anthocyaninless* mutation was due to a transposon insertion in the DFR gene[2] was one of 1,813 scientific papers that discussed transposable elements published in 2016. Not bad for an idea once considered too radical for mainstream science.



Number of publications discussing transposons, transposase, or transposable elements by year according to a PubMed search. Note, some earlier publications have been later reclassified as having to do with transposable elements.

[2] Wendell, D.L. Vaziri, A. Shergill, G. (2016) The Gene Encoding Dihydroflavonol 4-Reductase Is a Candidate for the *Anthocyaninless* Locus of Rapid Cycling *Brassica rapa* (Fast Plants Type) *PLoS One,* 11(8)

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Focus on Women in Science: Questions

1. Barbara McClintock is most widely recognized for her contributions to the understanding of transposable elements, but she didn't work on transposable elements until fairly late in her career. What evidence from the article shows that she was highly regarded as a scientist even before working on transposable elements?

2. When Barbara McClintock was awarded the Nobel Prize, she was compared to Gregor Mendel. What are some ways in which McClintock's career and discoveries could be compared to Mendel's?

3. In what way is the maize studied by McClintock similar to the *Brassica rapa* studied in this lab?

4. The *anthocyaninless* mutation that causes the non-purple phenotype in *Brassica rapa* is also believed to be caused by a transposable element. In Barbara McClintock's systems, the different phenotypes were caused by the activation and movement of transposable elements. The movement of these elements changed the phenotype, between the generations she was following, which is why phenotypes were not inherited in predictable Mendelian ratios. How is that different than the *Brassica rapa* used in our lab?