Dye Electrophoresis Lab

Mendel's Peas

miniPCR bio Learning Lab™
Dye Electrophoresis Lab:
Mendel's Peas

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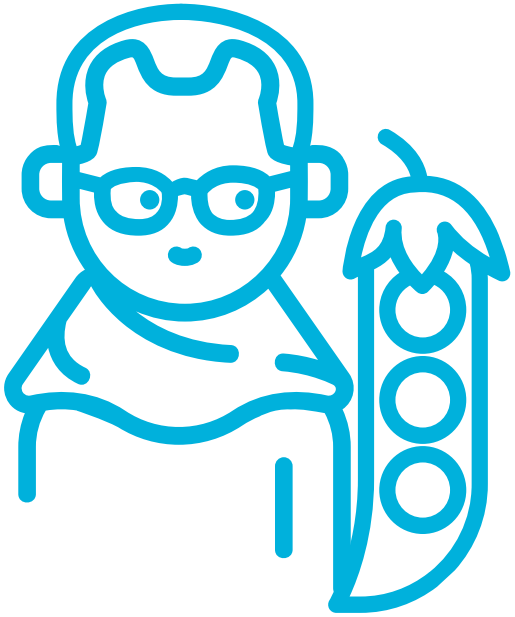
Student's Guide

Background information

A pioneering geneticist: Gregor Mendel

1

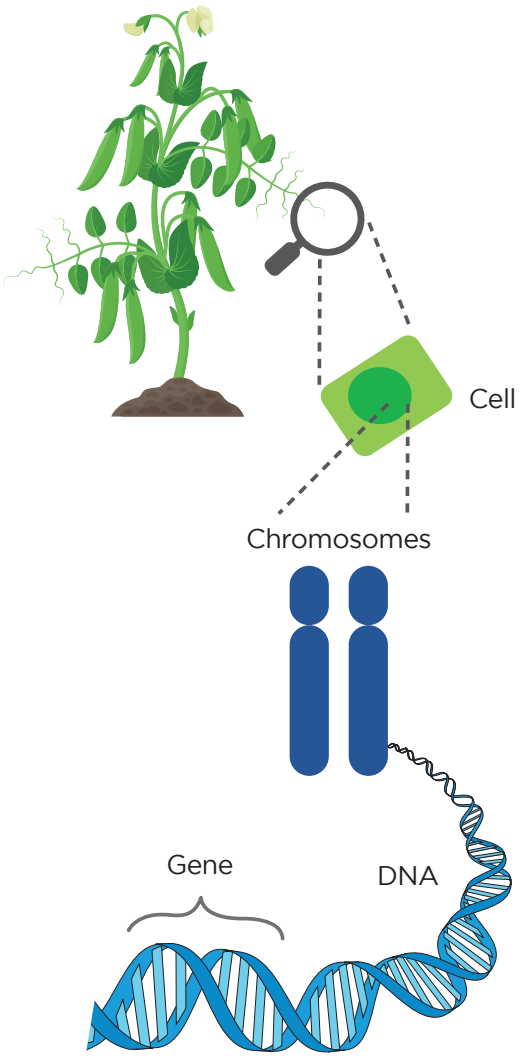
- Many traits are **inherited**, meaning they are passed from parent to offspring.
- In the 1800s, Gregor Mendel figured out basic patterns of inheritance by breeding pea plants and observing whether the offspring looked like either parent plant.
- Mendel studied inherited traits that had two forms in pea plants. For example, peas can either be round or wrinkled.



2

- Through his experiments, Mendel came to believe that pea plants receive something from each parent that defines the plant's appearance.
- He did not know what this material was, but he symbolized it using capital and lowercase letters like A and a.
- Your goal in today's lab is to connect Mendel's A and a with our modern understanding of inheritance.

DNA and genes



1

- Today, we know that all organisms inherit traits from their parents through DNA.
- **DNA** contains instructions for the cell and is found in structures called **chromosomes**.
- The cells of many organisms, including humans and pea plants, have two copies of each chromosome, one inherited from each parent.

2

- A **gene** is a section of DNA that contains a specific instruction for the cell.
- The instructions in an organism's genes determine its inherited traits.
- For example, pea plants have a gene that contains instructions that determine the shape of the pea.

3

- Each gene can come in different versions. We call different versions of the same gene **alleles**.
- Alleles of the same gene carry different instructions.
- For example, there are two alleles for the gene that determines pea shape. One allele has instructions that lead to round peas, and the other allele has instructions that lead to wrinkled peas.

4

- Even though Mendel performed his experiments before we knew that DNA underlies inheritance, his work showed how alleles pass from parents to offspring.
- Today, we know that different alleles of the same gene carry different instructions because they have differences in their DNA.

Background: Stop and think

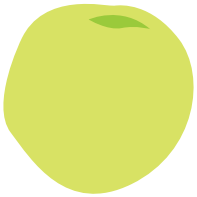
Circle the words that complete the sentence:

Q1. (Alleles/DNA molecules) are different versions of the same (gene/chromosome).

Mendelian inheritance

1

- Today, we know that Mendel was tracking alleles in his pea breeding experiments.
- Mendel coined the terms dominant and recessive to explain how some traits can skip a generation. Now we use the same language to describe the relationship between alleles of the same gene.
- A single copy of a **dominant** allele of a gene will produce the dominant trait.
- A **recessive** trait will only be present if both alleles are recessive.



Round peas
Dominant trait
AA or Aa

Wrinkled peas
Recessive trait
aa

2

- Mendel showed that having round peas is dominant and having wrinkled peas is recessive. This means that peas can only be wrinkled with two copies of the recessive allele.
- Scientists today represent alleles the same way Mendel did: an uppercase letter symbolizes the dominant allele, and a lowercase letter symbolizes the recessive allele.

Background: Stop and think

Circle the word(s) that complete each sentence:

Q2. Mendel showed that having (round/wrinkled) peas is dominant. He used capital and lowercase letters to track the inheritance of the pea shape trait. Mendel showed that a pea that was Aa would have (round/wrinkled) peas.

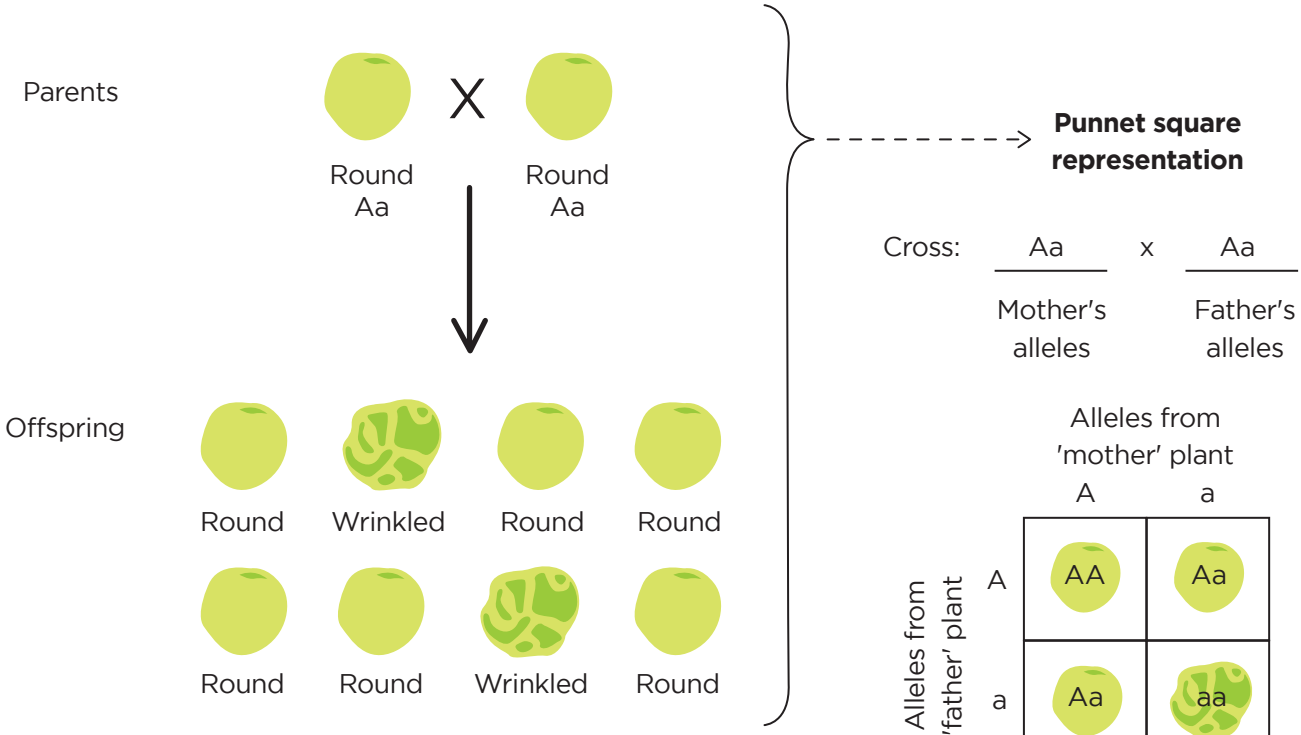
Genetics is complicated!

- In this lab, we are studying simple **Mendelian inheritance** where a single gene with a dominant allele and a recessive allele determines pea shape.
- Examining a simple trait like pea shape serves as a good entryway to inheritance, but it is important to keep in mind that most heritable traits are influenced by many genes and/or environmental factors. For example, human height is influenced by many genes, but it is also highly dependent on environmental factors such as diet.

Punnett squares

1

- Alleles are often shown in the context of a **Punnett square**, a diagram that shows the possible combinations of alleles in offspring that could be born to a pair of parents.
- Punnett squares are a useful tool to see how traits are passed from parents to offspring.



2

- Like Mendel, Punnett squares use letters to represent alleles of a gene.
- In this lab, you will connect Mendel's A and a with our modern understanding of DNA and alleles!

Background: Stop and think

Q3. Mendel studied seven different traits in pea plants. He also showed that having purple flowers is dominant and having white flowers is recessive. Use this information to answer the following questions.

- Use the letter B to represent dominant purple flower allele.
- Use the letter b to represent the recessive white flower allele.

Cross: **Bb** (Mother's alleles) x **bb** (Father's alleles)

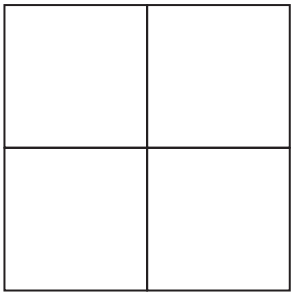
A. You have a mother plant that is Bb. This plant will have (purple/white) flowers.

B. You have a father plant that is bb. This plant will have (purple/white) flowers.

C. Use the Punnett square to the right to predict the result of mating these two plants.

Alleles from 'mother' plant

Alleles from 'father' plant



D. What are the expected allele ratios for the offspring plants?

__ BB : __ Bb : __ bb

E. What ratios do you expect for the offspring peas' appearance?

__ Purple : __ White

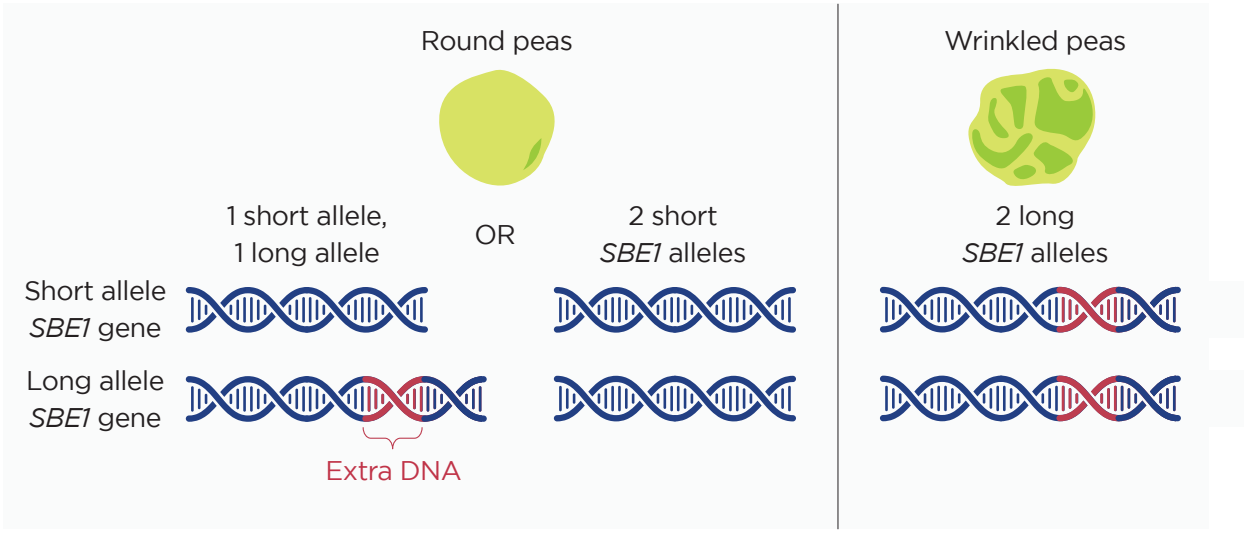
The search for Mendel's genes!

1

- We have known the inheritance pattern for pea seed shape since Mendel's experiment: round is dominant and wrinkled is recessive.
- But even with modern tools, linking inherited traits to specific genes can be tricky. In fact, scientists are still trying to figure out exactly which genes Mendel was studying!

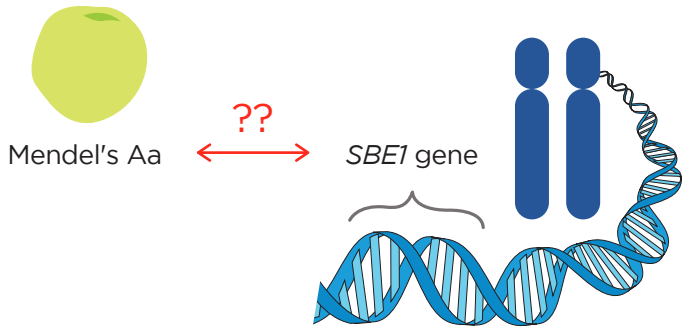
2

- You are a plant geneticist, and your lab discovered two alleles of a gene called ***SBE1*** that controls pea shape.
- The *SBE1* alleles are the same, except one has extra DNA near the end of the gene. We will refer to them as the short allele and the long allele.
- Your team has shown that the short *SBE1* allele is dominant and leads to round seeds.



3

- You suspect that the short and long alleles of the *SBE1* gene might be the A and a alleles that Mendel was tracking...
- But without access to the actual peas Mendel was using in the 1800s, there is no way to know for sure.



Background: Stop and think

Q4. Indicate which *SBE1* allele each description corresponds to:

A. Two copies of this allele yields wrinkled peas
B. Dominant *SBE1* allele
C. This allele has extra DNA compared to the other allele
D. Recessive *SBE1* allele

I. Short *SBE1* allele
II. Long *SBE1* allele

Today's lab

Historians have just discovered one of Mendel's lost notebooks. Included in Mendel's notes are six dried peas. You can now test your hypothesis that the short and long alleles of the *SBE1* gene correspond to the A and a alleles that Mendel was tracking!

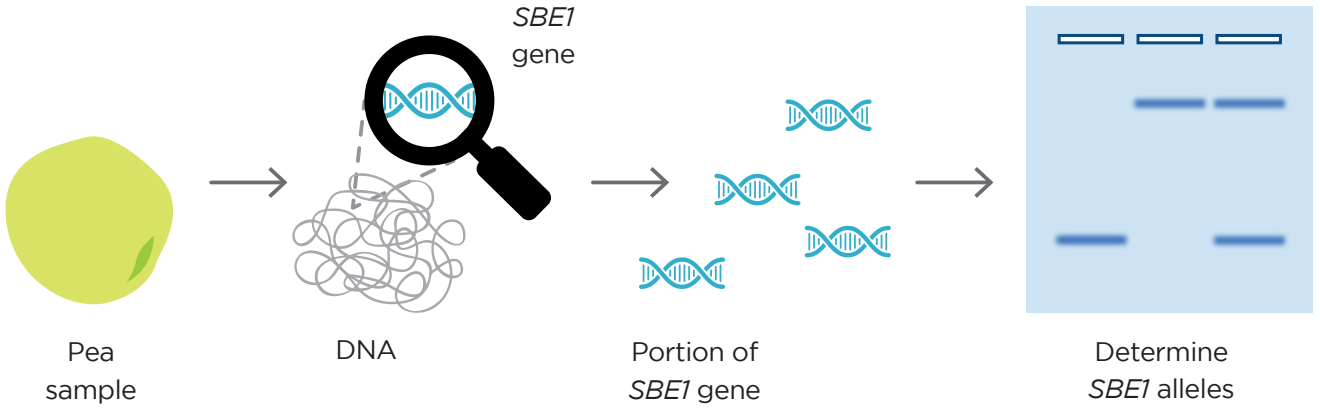
To accomplish this you will:

- **Determine which *SBE1* alleles are present in the peas from Mendel's notebook**
- **Compare each pea's *SBE1* alleles with Mendel's notes**

The ink in Mendel's notebook was smeared and you can't make out his notes for some of the peas

DNA testing

To study the peas' *SBE1* alleles, the samples will be tested as follows:



Step 1: DNA collection → **Step 2: PCR** → **Step 3: Gel electrophoresis**

Already completed

You'll do this step

1

- First, DNA is taken out of the pea cells. Only a tiny piece of pea is needed.
- Someone else in your research lab already performed this step.

2

- Next, a process called **PCR** is used to copy just the *SBE1* gene from the pea's DNA.
- Someone else already performed this step too.

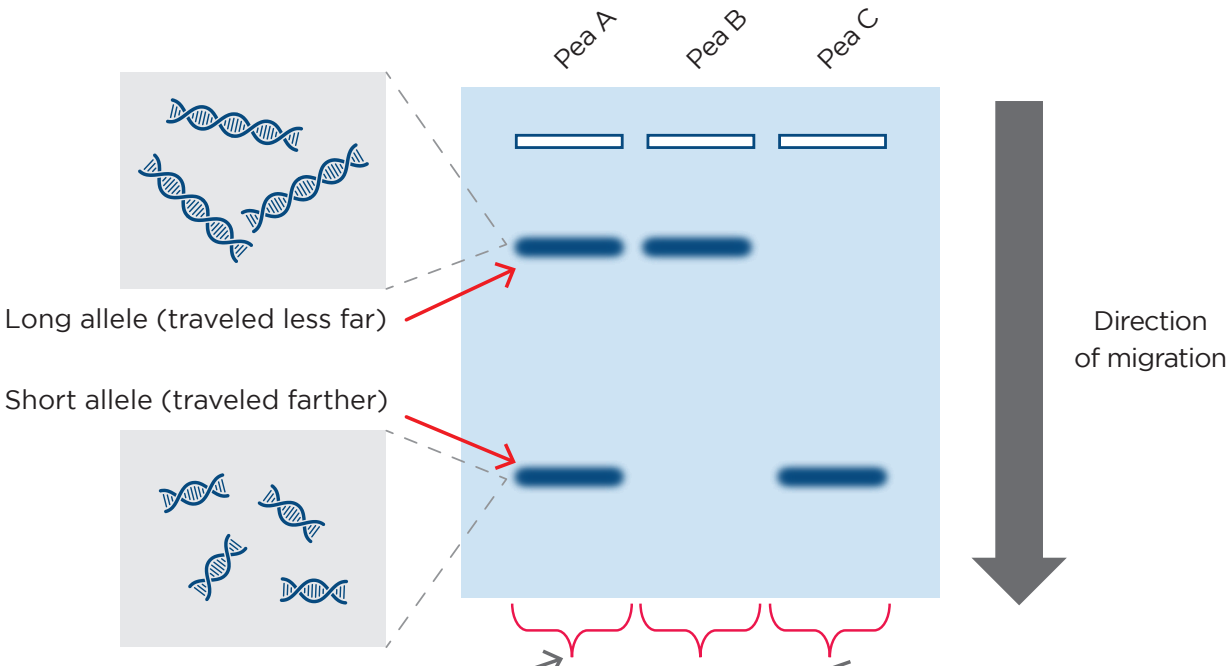
3

- Your assignment is to perform the last step of the DNA testing: gel electrophoresis.
- This will allow you to determine which *SBE1* alleles each pea has.

Interpreting gel electrophoresis results

1

- You will use gel electrophoresis to study the *SBE1* gene.
- **Gel electrophoresis** separates pieces of DNA by size. Smaller pieces of DNA travel farther through an **agarose gel**.
- Because the alleles of the *SBE1* gene differ in length, you can use gel electrophoresis to identify which *SBE1* alleles each of Mendel's peas has.
- Let's walk through gel electrophoresis results for three hypothetical peas to practice interpreting *SBE1* gel results.



2

- Pea A has two bands. This tells us that the *SBE1* DNA was two sizes.
- Because Pea A has both bands, we know it has one copy of the long *SBE1* allele and one copy of the short *SBE1* allele.

3

- Pea B only has one band. This tells us that all of the *SBE1* DNA was the same size.
- Because the DNA traveled less far, we know Pea B has two copies of the long *SBE1* allele.

4

- Pea C only has one band. This tells us that all of the *SBE1* DNA was the same size.
- Because the DNA traveled farther, we know Pea C has two copies of the short *SBE1* allele.

Background: Stop and think

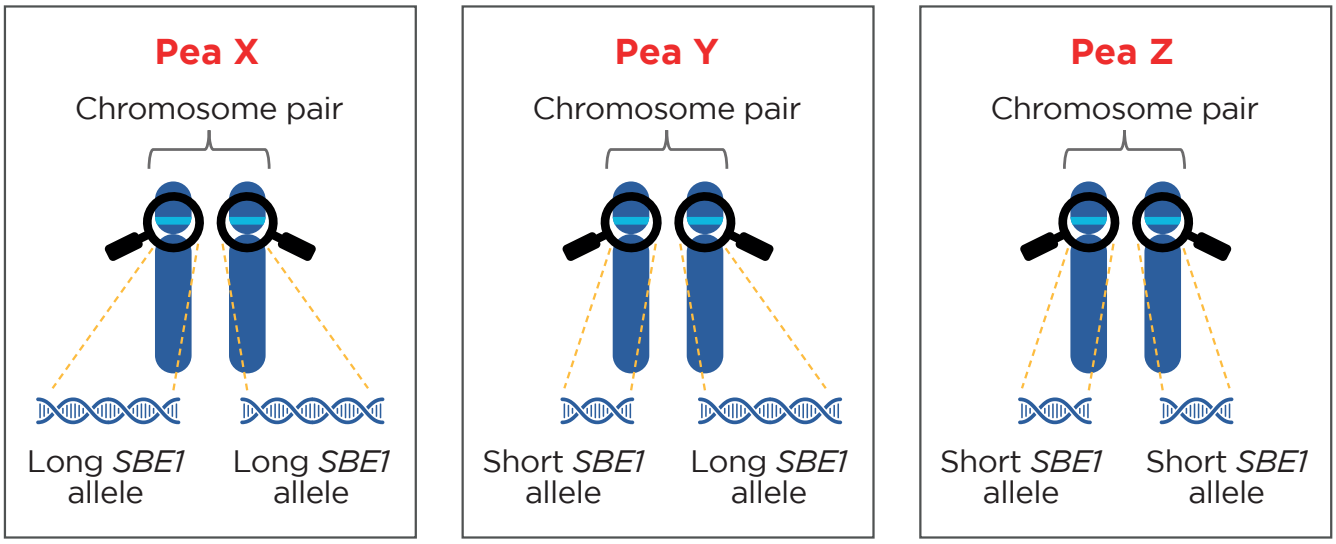
Q5. Why is gel electrophoresis a good tool for studying the different alleles of the *SBE1* gene in peas?

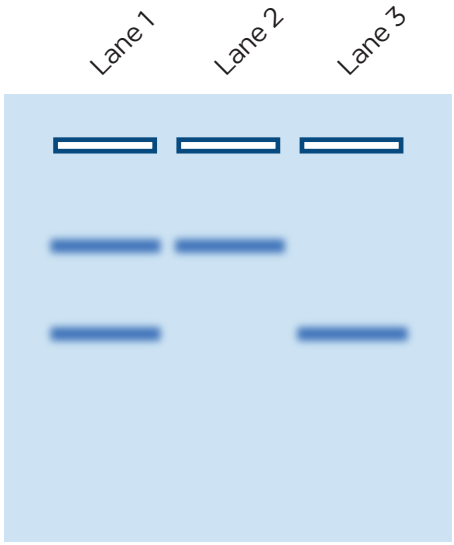
A. Because it allows you to make copies of the *SBE1* gene to study
B. Because peas have two copies of each chromosome
C. Because it allows you to extract DNA from cells
D. Because the *SBE1* alleles are different lengths

Q6. Match the potential allele combinations shown below for Peas X, Y, and Z with the corresponding gel results shown in lanes 1, 2, or 3.

A. Pea X
B. Pea Y
C. Pea Z

1. Lane 1
2. Lane 2
3. Lane 3





Glossary

Inherited: A trait is inherited when it is passed from parents to offspring through DNA .

DNA: DNA contains the instructions for the cell and is passed down from parent to offspring.

Chromosome: The structures that store DNA in the cell. Organisms like humans and pea plants have pairs of chromosomes, with one copy of each chromosome inherited from each biological parent.

Gene: A region of DNA that contains a single set of instructions. Different genes correspond to different traits.

Allele: One of two or more alternative versions of the same gene. Different alleles of the same gene have differences in the DNA.

> **Dominant:** Some alleles have a relationship called dominant/recessive. A single copy of a dominant allele of a gene will produce the corresponding dominant trait.
>
> **Recessive:** Some alleles have a relationship called dominant/recessive. A recessive trait will only be present if both alleles of a gene are recessive.

Mendelian inheritance: The inheritance pattern described by Mendel in his study of pea plants. Mendel tracked traits that are determined by a single gene with two alleles. The alleles have a fully dominant/recessive relationship.

Punnett square: A diagram that shows allele combinations in offspring that could be born to a set of parents.

***SBE1* gene:** A gene that controls whether peas are round or wrinkled. Scientists suspect this was the gene that Mendel was tracking in his experiments.

Polymerase chain reaction (PCR): A method used to make many copies of a DNA segment you are interested in studying. For more detailed information on PCR, refer to https://www.minipcr.com/polymerase-chain-reaction/.

Gel electrophoresis: A method that separates pieces of DNA by length. For more detailed information on electrophoresis, refer to https://www.minipcr.com/gel-electrophoresis/.

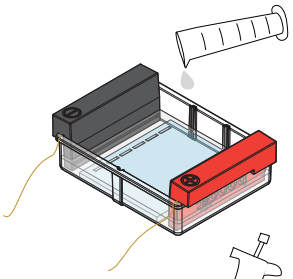
Agarose gel: A type of gel commonly used for gel electrophoresis. Agarose is a sugar that comes from seaweed. At the microscopic level, the inside of an agarose gel looks like a web or a sponge. Small molecules can move through the holes with relative ease, but larger molecules get slowed down. This allows you to separate molecules of different sizes.

Laboratory guide

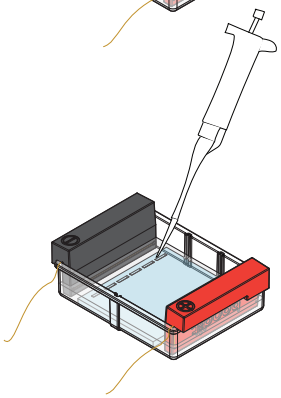
Protective gloves and eyewear should be worn for the entirety of this experiment.

See detailed assembly and gel pouring instructions for the Bandit™ STEM Electrophoresis Kit https://www.minipcr.com/bandit-assembly/

1. **Submerge your gel in enough TBE buffer to just cover the gel and fill the wells.**
   - If using a Bandit™ or blueGel™ electrophoresis system you will need approximately 30 ml of TBE buffer.



2. **Use a micropipette to load samples onto the gel from the corresponding tubes in your Load Ready™ Strip.**
   - Lane 1: 10 µl Pea 1 DNA
   - Lane 2: 10 µl Pea 2 DNA
   - Lane 3: 10 µl Pea 3 DNA
   - Lane 4: 10 µl Pea 4 DNA
   - Lane 5: 10 µl Pea 5 DNA
   - Lane 6: 10 µl Pea 6 DNA



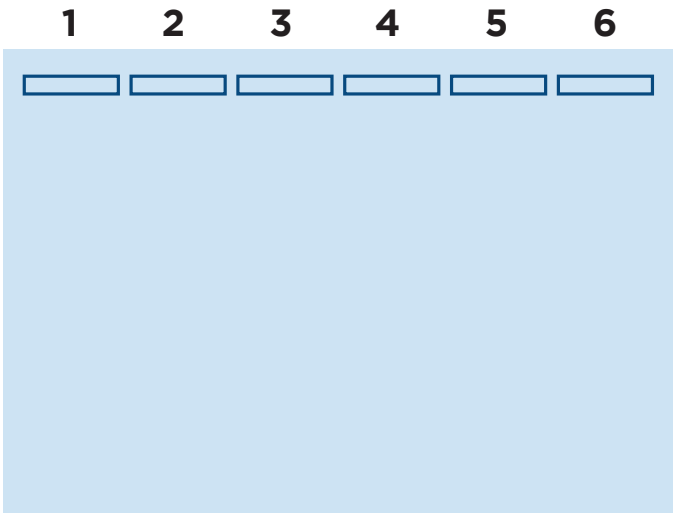
3. **Connect the electrodes and turn on your gel electrophoresis system.**

4. **Run the gel for 15-20 minutes or until there is sufficient separation between the bands.**
   - Times are based on Bandit™ and blueGel™ electrophoresis systems. If using other gel electrophoresis systems, separation time may vary.
   - Longer electrophoresis times will result in better separation.
   - Placing the gel over a white background will make it easier to see your results.

Pre-lab study questions

Critical thinking

Pea	Appearance	Mendel's notes
1	Round	Aa
2	Round	AA
3	Wrinkled	aa
4	Round	??
5	Round	??
6	Wrinkled	??



1. You know the alleles for Peas 1-3 from Mendel's notes. On the gel above, draw what your gel would look like for Peas 1-3 if your experimental results support your hypothesis that the short and long *SBE1* alleles were the A and a alleles Mendel was tracking. Be sure to label the short and long *SBE1* alleles.

2. You can't read Mendel's notes on the alleles for Peas 4-6, but you can make some predictions about which alleles they have based on what you know about the inheritance of pea shape. Abbreviate the alleles as A and a.

   A. Round peas: Peas 4 and 5

   Possible alleles:
   Explain your reasoning:

   B. Wrinkled peas: Pea 6

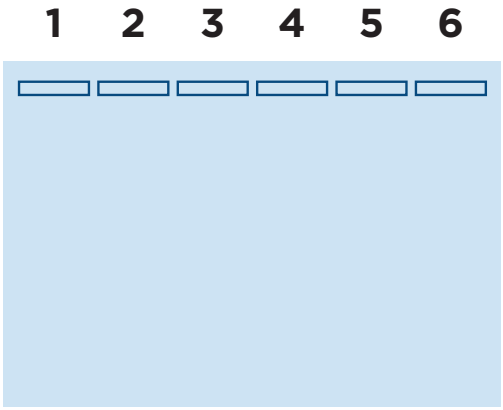
   Possible alleles:
   Explain your reasoning:

3. Use your answers from question 2 to draw what your gel would look like for Peas 4-6 if your experimental results support your hypothesis. Use a dashed line to draw alleles that you cannot predict with certainty based on the pea's appearance.

Post-lab study questions

Interpreting results

1. Use the image of a gel on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.



2. Label each band as either the short allele or the long allele of the *SBE1* gene.

3. Record your results in the table below. The first row has been filled out for you as an example.

Pea	Appearance	Mendel's notes	*SBE1* gel results
1	Round	Aa	Long allele, short allele
2	Round	AA	
3	Wrinkled	aa	
4	Round	??	
5	Round	??	
6	Wrinkled	??	

Critical thinking

Compare the results in the "Mendel's notes" column and the "*SBE1* gel results" column to answer the following questions.

4. Do your experimental results support your hypothesis that the short and long *SBE1* alleles correspond to the A and a alleles Mendel was tracking? Explain your reasoning.

5. Assuming your hypothesis is correct, what would Mendel's notes for Peas 4, 5, and 6 have read before the ink was smeared? Explain your reasoning.

CER table

Fill in the table based on your results from the lab. Use the rubric on the next page to help your answers.

Question:

Do your experimental results confirm your hypothesis that the short and long SBE1 alleles correspond to the A and a alleles that Mendel was tracking?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is hightly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100