Dye Electrophoresis Lab

Molecular Rainbow

miniPCR bio Learning Lab™
Dye Electrophoresis Lab: Molecular Rainbow
Instructor's and Student's Guide

Version: 1.0
Release: April 2022

Contents

At a glance

Bring a molecular rainbow to your classroom!

This lab offers students an engaging introduction to the theory and practice of a fundamental biotechnology technique: gel electrophoresis. Students will run dye samples to demonstrate how gel electrophoresis can be used to characterize molecules and separate molecular mixtures.

TECHNIQUES

Micropipetting
Gel electrophoresis

TOPICS

Electric charge
Electric forces
Molecular separation

LEVEL

Middle school
General high school

WHAT YOU NEED

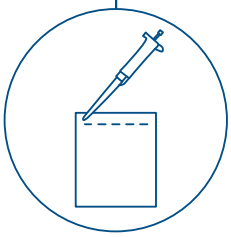
Micropipette
Gel electrophoresis system

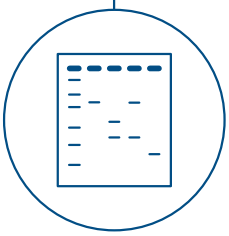
Reagents sufficient for eight lab groups.

Planning your time

SINGLE CLASS PERIOD: 45 MINUTES* if gels are prepared in advance

Gel electrophoresis → **Interpret results**





* Allow an additional 25 minutes if students will be preparing the gels in class.

Additional supports

Help your students build proficiency in pipetting and gel electrophoresis with additional instructional videos, worksheets, and activities available at: minipcr.com/tutorials/

For answers to the lab study questions, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Materials needed

Supplied in kit (KT-1400-01)

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
Rainbow dyes (red, orange, yellow, green, blue, and purple)	**180 µl each**	**20 µl each**	Room temp.	
Agarose tabs™	**10 tabs**	**1 tab** per gel	Room temp.	
TBE electrophoresis buffer	**Supplied as powder** Sufficient to prepare 600 ml of 1X working solution	**60 ml 1X solution** per gel (if using a Bandit™ or blueGel™ electrophoresis system)	Room temp.	
Plastic microtubes to distribute samples	**50**	**6**	Room temp.	

Materials needed (cont.)

Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed per lab group	Teacher's checklist
Horizontal gel electrophoresis apparatus e.g. BandIt™ STEM electrophoresis kit or blueGel™ electrophoresis system	1	
Micropipettes • 2-20 µl adjustable volume or 10 µl fixed volume	1	
Disposable micropipette tips	At least 6	
Distilled water for making agarose gels and diluting TBE buffer	600 ml total	
Flask or beaker to dissolve agarose		
Microwave or hot plate to dissolve agarose		
Other supplies: • Disposable laboratory gloves • Protective eyewear • Permanent marker • Cup to dispose of tips		

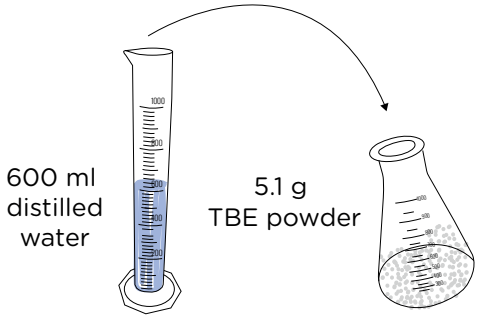
Lab setup

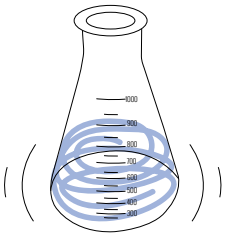
The following activities should be carried out by the instructor ahead of class. Reagents are sufficient to be used with 8 student groups. Reagents are stable at room temperature.

! Gloves and protective eyewear should be worn for the entirety of this lab.

A. Prepare 1X TBE buffer

- The lab kit comes with a 5.1 g pouch of TBE powder. Mix TBE powder and 600 ml distilled water
  - Obtain a heat resistant container such as a glass Erlenmeyer flask or beaker that is at least 1 L in volume.
  - Empty entire container of TBE powder (5.1 g) into the flask or beaker.
  - Add 600 ml distilled water.
  - Optional: Pre-warming the water will make the TBE powder dissolve faster.
- Mix solution for 10-15 minutes by stirring or shaking
  - Stir or intermittently shake solution for 10-15 minutes.
  - Warm as necessary to help dissolve powder. it is normal for a small amount of powder to remain undissolved after 15 minutes. Small amounts of undissolved powder will not affect performance.
- Store 1X TBE at room temperature in a closed container
  - 1X TBE can be stored in an airtight container at room temperature for at least three months.
  - Discard unused 1X TBE buffer if it becomes cloudy or if gel runs appear smeared.



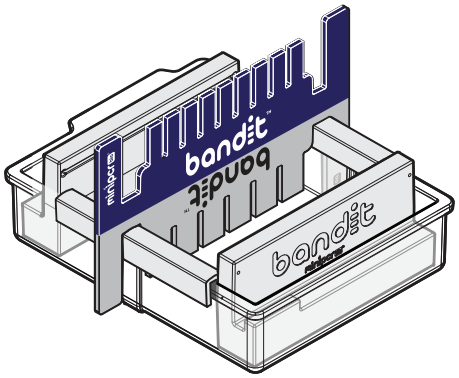


10-15 minutes

B. Optional: prepare gels in advance of class

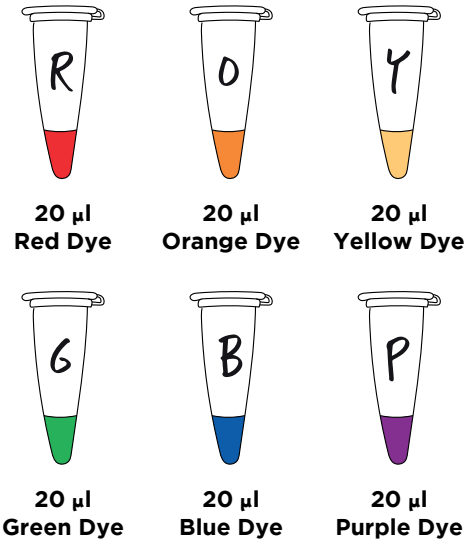
- Students can prepare gels in class, or to save time, the instructor can prepare the gels in advance.
- Pre-poured gels can be stored for up to five days at room temperature if in an airtight container.
- For detailed instructions on preparing agarose gels, see page 29.
- Make sure to place the comb in the center of the gel.

Comb in center!



C. Dispense reagents

- For each lab group, dispense the following reagents into six microtubes:
  - Red Dye 20 µl
  - Orange Dye 20 µl
  - Yellow Dye 20 µl
  - Green Dye 20 µl
  - Blue Dye 20 µl
  - Purple Dye 20 µl



20 µl Red Dye **20 µl Orange Dye** **20 µl Yellow Dye**

20 µl Green Dye **20 µl Blue Dye** **20 µl Purple Dye**

D. Distribute supplies and reagents to lab groups

Check	At the start of this experiment, every lab group should have:	Amount
	Red, orange, yellow, green, blue, and purple dyes	20 µl each
	2-20 µl micropipette or 10 µl fixed volume micropipette	1
	Micropipette tips	At least 6
	Six wells in an electrophoresis gel	

Student's Guide Contents

Background information

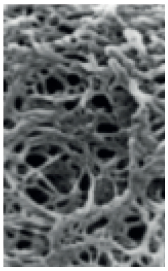
Introduction to gel electrophoresis

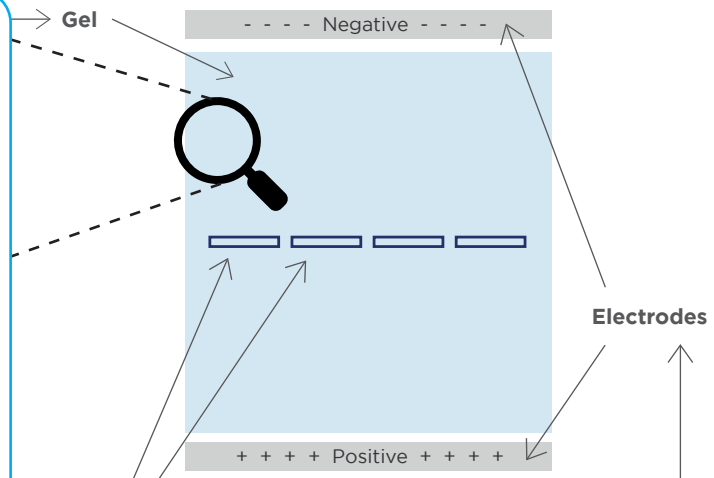
One of the most common methods for separating and observing biological molecules in the lab is called **gel electrophoresis**. The word electrophoresis means carried by electricity. During gel electrophoresis, an electric field causes molecules with an electric charge to move through a gel.

To perform gel electrophoresis you need (1) a gel, usually made of a substance called agarose, and (2) a way to conduct electricity through the gel.

1

Agarose gels feel like firm Jell-O. At the microscopic level, the inside of a gel looks like a web or a sponge. Small molecules can move through the holes without much trouble, but larger molecules get slowed down. This allows us to separate molecules of different sizes.





2

When we make a gel, we make it with small pockets called **wells**. These wells are arranged in a line across the gel. The wells allow us to add our samples into the gel.

Wells

3

Metal bars or wires are placed on either side of the gel. These act as positive and negative **electrodes**. If there is a conductive material between the electrodes, then electrical current can flow between them. During gel electrophoresis, charged molecules will move through the gel towards the oppositely charged electrode.

The gel is covered in a liquid called **electrophoresis buffer**, which helps conduct electricity between the two electrodes and through the gel. When the power is turned on, any charged molecules will move through the gel towards the electrode of the opposite charge.

Review

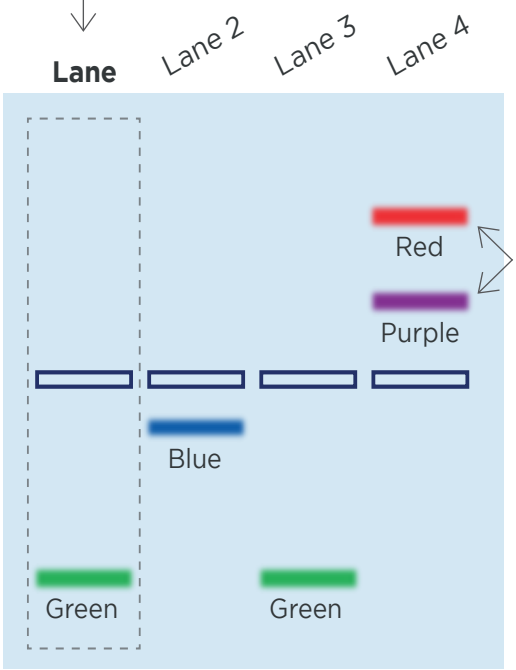
1. What causes molecules to move through the gel during gel electrophoresis?

2. What role does the gel play in gel electrophoresis?

When scientists discuss molecules separating in a gel, they talk about **bands** moving in **lanes**. Let's take a closer look at what these two terms mean.

1

Charged molecules that are put in a well will move towards the electrode that has an opposite charge. As they move through the gel, they travel in a straight line. Like runners on a track, when we talk about the molecules moving through a gel, we talk about them moving in **lanes**.



2

There are usually billions of molecules in each well of the gel. As the molecules move towards an electrode with the opposite charge, all of the molecules that have the same charge and size will move through the gel in the same direction and at the same speed. This group of molecules moving together will stay in about the same shape as the well they started in. When you look at the gel, the group of molecules will look like a small horizontal line on the gel. We call these lines **bands**.

In Lane 4, there are two bands: a red band made of billions of red molecules and a purple band made of billions of purple molecules. Both of these bands started in the well in Lane 4, but as the molecules moved through the gel the two bands separated because the red and purple molecules are different sizes and traveled through the gel at different speeds.

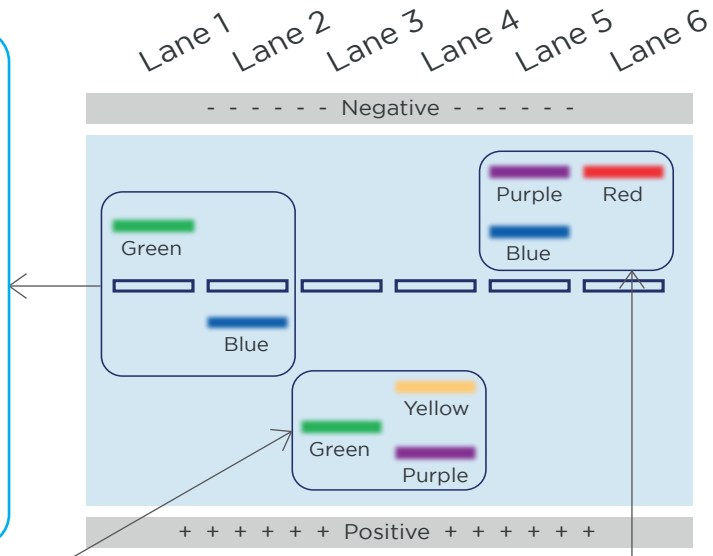
Interpreting gel results

You can get a lot of information from gel electrophoresis results! There are three pieces of information that we will focus on today: charge, composition, and size.

1

What is the charge of the molecules that were in the sample?

- Charged molecules will be attracted to the *opposite* charge.
- The attractive force will cause positive molecules to move towards the negative electrode. The green band in Lane 1 is made of positively charged molecules.
- Negative molecules will move towards the positive electrode. The blue band in Lane 2 is made of negatively charged molecules.



2

What is the composition of the molecules that were in the sample? Put another way, how many types of molecules were in the sample?

- If there is only one band in a lane it usually means that there is just one type of molecule in that sample. Lane 3 has only one green band, meaning only one type of molecule was placed in the well.
- If there is more than one band in the lane, it means there is more than one type of molecule in that sample. Lane 4 has two bands, a yellow band and a purple band, meaning a mixture of two molecules was placed in the well.

3

How big were the molecules in the sample compared to each other?

- Smaller molecules can move through the gel easily and will travel farther from the wells than larger molecules with the same charge. In Lane 5, the purple band moved farther from the well than the blue band. If we assume the purple and blue molecules have the same net charge, we can infer that the purple molecules are smaller than the blue molecules.
- Molecules that are the same size and charge will travel the same distance. The purple band in Lane 5 and the red band in Lane 6 traveled the same distance from the well. If we assume the purple and red molecules have the same net charge, we can infer that the purple and red molecules are about the same size.

Glossary

Gel electrophoresis: *A method used to separate molecules by size and charge.*
During gel electrophoresis, an electric force is used to move molecules through a gel in a direction dictated by their net electrical charge. The gel acts as a size filter; smaller molecules move more easily through the gel and travel further than larger molecules. This allows scientists to separate molecules of different sizes.

Agarose gel: *A type of gel commonly used for gel electrophoresis.*
Agarose is a sugar 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 scientists to separate molecules of different sizes.

Well: *A pocket in a gel where samples are placed at the start of a gel electrophoresis experiment.*
When making a gel, a mold called a comb is used to create pockets called wells. Samples can be placed into these wells. Then, when an electric force is applied, the molecules in the sample will move through the gel and travel towards the oppositely charged electrode.

Electrode: *A conductor that is used to establish a current through an object or material.*
During a gel electrophoresis experiment, an electrical current is used to move charged molecules through the gel. Metal wires or bars are placed on either end of the gel and connected to a power source. These wires serve as negative and positive electrodes that drive the flow of electricity through the gel.

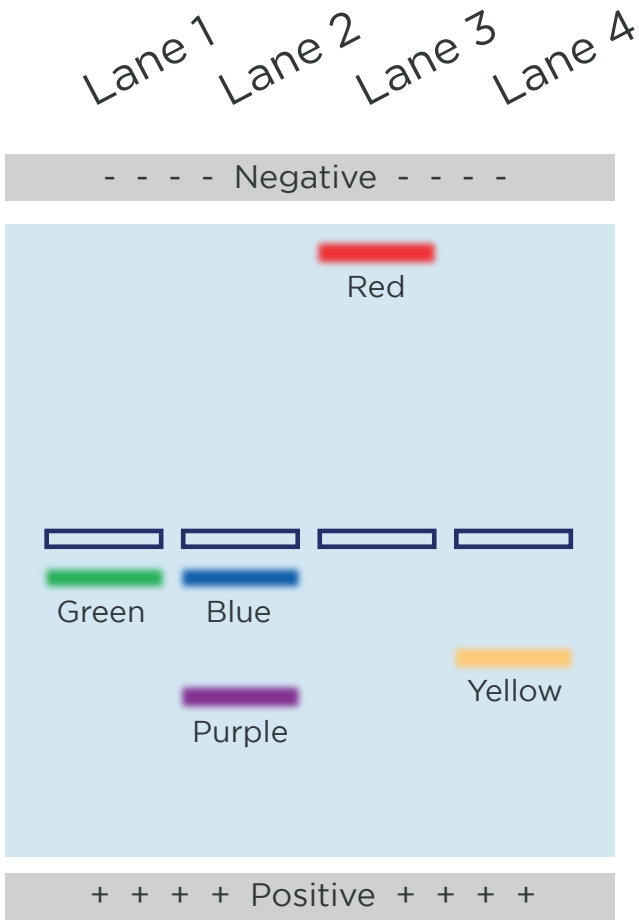
Electrophoresis buffer: *A solution used to conduct electricity between the electrodes during gel electrophoresis.*
During a gel electrophoresis experiment, charged molecules that are put in a well will move towards the electrode that has an opposite charge. The gel is covered in a salty liquid that conducts electricity between the electrodes and through the gel.

Lane: *The straight line through which molecules travel through a gel.*
During a gel electrophoresis experiment, charged molecules that are put in a well will move towards the electrode that has an opposite charge. As they move through the gel, molecules travel in a straight line that is the same width as the well. This area is referred to as a lane.

Band: *A visible group of molecules that traveled together through a gel.*
During a gel electrophoresis experiment, the molecules in a sample move through the gel towards an electrode with the opposite charge. All of the molecules that have the same charge and size will move through the gel in the same direction and at the same speed. The molecules will stay in about the same shape as the well they started in. When you look at the gel, the group of molecules will look like a small horizontal line, or band, on the gel.

Review

Refer to the gel on the right to answer the following questions:



Charge:

1. Which band(s) are made up of negatively charged molecules? Select as many answers as are correct.
   a. Green band
   b. Blue band
   c. Purple band
   d. Red band
   e. Yellow band

   Explain how you can tell:

2. Which band(s) are made up of positively charged molecules? Select as many answers as are correct.
   a. Green band
   b. Blue band
   c. Purple band
   d. Red band
   e. Yellow band

   Explain how you can tell:

Composition of samples:

3. Which lane(s) contained a single type of dye? Select as many answers as are correct.
    a. Lane 1
    b. Lane 2
    c. Lane 3
    d. Lane 4

    Explain how you can tell:

4. Which lane(s) were mixtures and contained more than one type of dye? Select as many answers as are correct.
    a. Lane 1
    b. Lane 2
    c. Lane 3
    d. Lane 4

    Explain how you can tell:

Size:

5. Let's focus on the green, blue, purple and yellow bands on the bottom half of the gel. If you assume all the molecules have the same net charge, which band(s) in the gel are made up of the smallest molecules? Select as many answers as are correct.
    a. Green band
    b. Blue band
    c. Purple band
    d. Yellow band

    Explain how you can tell:

6. If you assume all the molecules have the same net charge, which band(s) in the gel are made up of the largest molecules? Select as many answers as are correct.
    a. Green band
    b. Blue band
    c. Purple band
    d. Yellow band

Explain how you can tell:

Today's lab

Today, you will use gel electrophoresis to analyze dyes.

The way each dye will move through the gel will depend on the characteristics of the molecules that make that dye.

- The direction that dye molecules will move through the gel depends on the charge of the molecules. Negatively charged molecules will move towards the positive electrode, while positively charged molecules will move towards the negative electrode.
- How fast molecules will move through the gel will depend on the size of the molecules—smaller molecules will typically move faster.

The dyes:

Your task is to use gel electrophoresis to study the dye samples. You will determine:

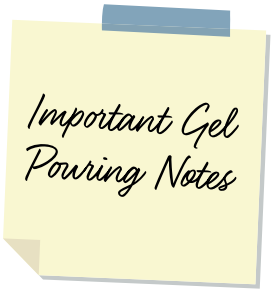
- **Charge:** Which dye molecules are positively charged and which ones are negatively charged?
- **Composition:** Which dye samples contain only one type of dye and which ones are mixtures of more than one dye?
- **Size:** Which dye molecules appear to be large and which ones seem small compared to each other?

Time to analyze the molecular rainbow!

Laboratory guide

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

Pouring gels (before or during class period)



- Gels can be prepared up to five days ahead of time and stored at room temperature if placed in an airtight container.
- These instructions are designed for use with the pre-weighed Agarose Tabs™ provided in the lab kit.
- One Agarose Tab™ will yield one gel for use in either a Bandit™ or blueGel™ electrophoresis system by miniPCR bio™.
- If using a different electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions. You can use gels of any percentage between 1-2%.

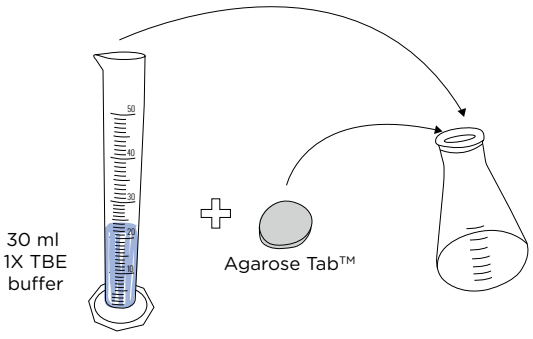
If using a Bandit™ STEM Electrophoresis Kit, detailed instructions for pouring and running gels can be found on page 29

A. Prepare 1X TBE buffer (to be completed by teacher in advance)

- Prepare at least 60 ml of buffer for every Bandit™ or blueGel™ electrophoresis system you plan to use.
- 30 ml of the buffer will be used to make your gel and 30 ml will be used as running buffer.
- Refer to page 6 for detailed instructions on preparing 1X TBE buffer.

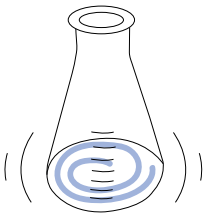
B. Prepare an agarose solution

- Obtain a heat-resistant container such as a glass Erlenmeyer flask or beaker that is at least three times the volume you wish to add.
- Combine 30 ml room temperature 1X TBE buffer and one Agarose Tab™ for each gel you plan to pour.
- Allow the tabs to soak until they fully disintegrate (this could take a few minutes).
- Swirl the flask or beaker to ensure the tabs have fully disintegrated before heating.



C. Heat solution

- Expect to heat for about 60 seconds per 30 ml of liquid in a standard microwave.
- Heat until the solution boils and continue until agarose is fully dissolved. No agarose particles should remain.



Caution: The solution may boil over the top of some containers. The solution will be very hot.

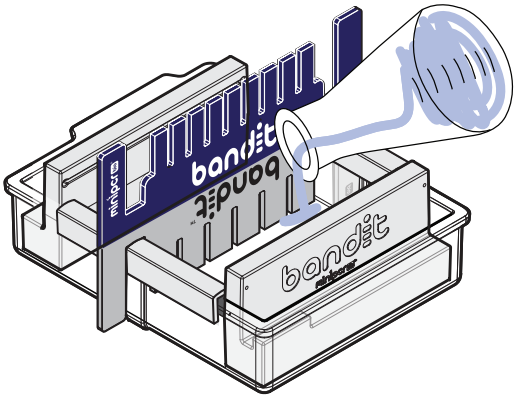
D. Set up your gel casting system with the comb in the center position

- You will need six lanes per gel.
- If using a Bandit™ STEM electrophoresis kit, make sure Electrodams™ are firmly in place before pouring the gel. Refer to page 29 for detailed assembly instructions.
- If using a different electrophoresis system, refer to the manufacturer's instructions for how to set up your particular gel casting system.

E. Pour the agarose solution into the prepared casting platform with a gel tray and comb

- The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).
- Note: Because this lab uses colored dyes as experimental samples, there is no need to add DNA stain.

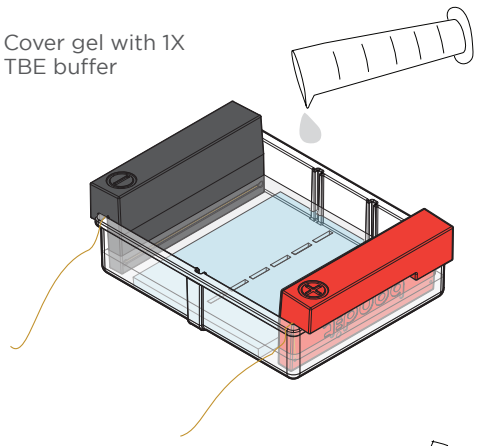
Prepare an agarose gel with comb in center position



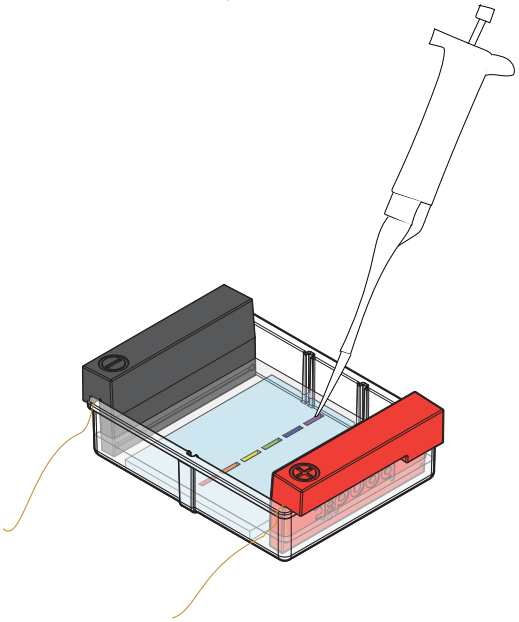
F. Allow gel to solidify completely

- Gel is ready when cool and firm to the touch.
- Gels will typically be ready in about 10 minutes.
- Gels can be stored in an airtight container at room temperature for up to five days before use.
- You can remove the comb and disassemble the gel casting apparatus before storing the gel.

Running the gel



1. **Submerge your gel in enough 1X TBE buffer to just cover the gel and fill the wells**
   - If using a Bandit™ STEM electrophoresis kit, your gel is already in the buffer chamber. Ensure that the Electrodams™ are in the correct orientation and have the electrode wires threaded (refer to page 33 for detailed instructions).
   - If using a Bandit™ or blueGel™ electrophoresis system you will need approximately 30 ml of 1X TBE buffer.



2. **Use a micropipette to load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl **Red Dye**
   - **Lane 2:** 10 µl **Orange Dye**
   - **Lane 3:** 10 µl **Yellow Dye**
   - **Lane 4:** 10 µl **Green Dye**
   - **Lane 5:** 10 µl **Blue Dye**
   - **Lane 6:** 10 µl **Purple Dye**

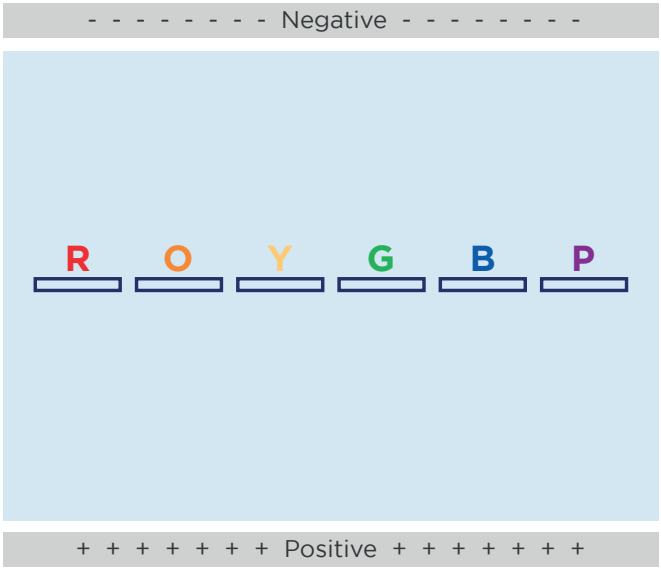
3. **Connect the electrodes and turn on your gel electrophoresis system**
   - If using a Bandit™ STEM electrophoresis kit, refer to page 33 for detailed assembly instructions.

4. **Conduct electrophoresis for 15-25 minutes**
   - Longer electrophoresis times will result in better separation.
   - Placing the gel over a white background will make it easier to see your results.

Post-lab study questions

Interpreting results

1. Use the image on the right to draw the bands that you see on your gel. There are six lanes on the gel: one for each dye sample.



Charge:

2. Which lane(s) in the gel contain bands made up of negatively charged molecules? Explain how you can tell.

3. Which lanes(s) in the gel contain bands made up of positively charged molecules? Explain how you can tell.

Composition of samples:

4. Which sample(s) contained a single type of dye molecule? Explain how you can tell.

5. Which sample(s) were mixtures and contained more than one type of dye molecule? Explain how you can tell.

Size:

6. Let's focus on the negatively charged dyes. If you assume all the molecules have the same net charge, which color band(s) in the gel seem to be made up of the smallest molecules? Explain how you can tell.

7. If you assume all the negatively charged molecules have the same net charge, which color band(s) in the gel seem to be made up of the largest molecules? Explain how you can tell.

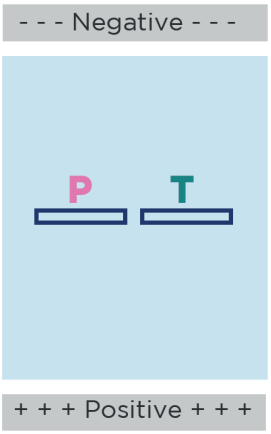
Critical thinking

8. Were the same types of molecules present in more than one sample? Explain how you can tell.

9. In questions 6 and 7 above, you were asked to assume the molecules you were comparing had the same net charge. Imagine you run another gel where one sample contains pink molecules and the other sample contains teal molecules. The pink and teal molecules are the same shape and size, but the pink molecules have a net charge of +3 while the teal molecules have a net charge of +1.

   A. Use the image on the right to draw the expected results from this experiment.

   B. In the gel you ran, all of the molecules had a similar net charge. Why was that important for interpreting the relative size of the molecules? Use the example given here in your answer.



CER Table

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

Question:

Which dye(s) contained negatively charged molecules and which dye(s) contained positively charged molecules?

Claim Make a clear statement that answers the above question.	
Evidence Provide data from the lab that supports your claim *(hint: you may want to consult other lab groups' results, in order to have more data to evaluate)*	
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 highly 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.

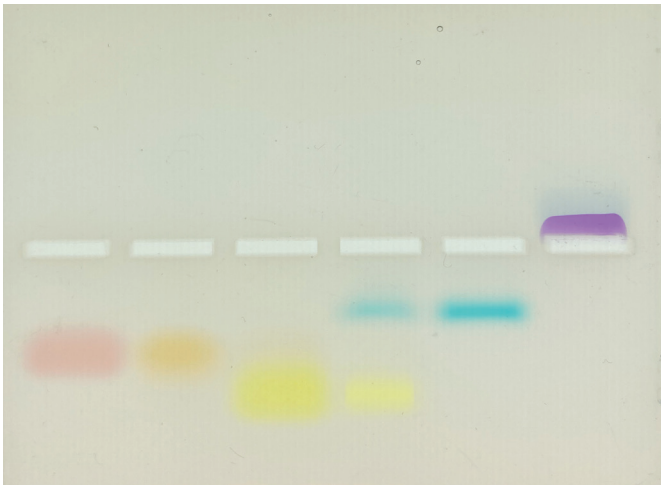
We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

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

Instructor's Guide

Expected results

Gel electrophoresis results are expected to resemble the gel image below.



This image represents results obtained after a 15 minute run using a Bandit™ STEM electrophoresis kit.

- Red Dye: a single band composed of negatively charged red molecules.
- Orange Dye: a single band composed of negatively charged orange molecules.
- Yellow Dye: a single band composed of negatively charged yellow molecules.
- Green Dye: two bands, one composed of negatively charged blue molecules and one composed of negatively charged yellow molecules.
- Blue Dye: a single band composed of negatively charged blue molecules.
- Purple Dye: a single band composed of positively charged purple molecules.

Interpretation

Charge:

- The Red, Orange, Yellow, Green, and Blue Dyes contain negatively charges molecules.
- The Purple Dye contains positively charged molecules.

Composition:

- The Red, Orange, Yellow, Blue, and Purple Dyes contain a single type of molecule.
- The Green Dye is a mixture of two types of molecules: the Blue Dye and the Yellow Dye.

Size: For simplicity, students are asked to compare the relative sizes of the dye molecules only in the dye samples that contain negatively charged molecules. These dyes all have similar net charges, so we can make the following conclusions:

- The blue molecules in the Blue Dye and Green Dye are the largest molecules.
- The yellow molecules in the Yellow Dye and the Green Dye are the smallest molecules.

Notes on lab design

This lab serves as an introduction to gel electrophoresis. We believe our approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals.

- While agarose gel electrophoresis is most often used to separate DNA samples, using charged dyes allows for the samples to be directly visualized in the gel without the need for additional staining.
- During gel electrophoresis, both the shape and the charge-to-mass ratio of a molecule will affect migration. In this lab all of the negatively charged dyes have a similar net charge, allowing students to focus on size as the determining factor in how quickly a molecule migrates through the gel. The positively charged dye has a smaller net charge, so you cannot draw conclusions about the relative size of the positively charged dye compared to the negatively charged dyes.

Additional student supports

At miniPCR bio™, we are committed to preparing students to be successful in the laboratory through high quality curriculum and training. We have created an extensive set of resources to help your students succeed in molecular biology techniques, all of which are available for free download at the miniPCR bio™ tutorials page of our website.
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https://www.minipcr.com/tutorials/
Those activities most relevant to this lab are listed below.

Micropipetting: Video and activity resources to train students in the basic use of a micropipette.

Gel electrophoresis: Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

Learning goals and skills developed

Student Learning Goals - students will:

- Explain how gel electrophoresis can be used for molecular separation
- Analyze the components of unknown mixtures using gel electrophoresis
- Identify how charged molecules will behave in an electric field

Scientific Inquiry Skills - students will:

- Identify or pose a testable question
- Follow detailed experimental protocols
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular Biology Skills:

- Micropipetting
- Preparation of agarose gels
- Agarose gel electrophoresis

Standards alignment

Next Generation Science Standards

Students who demonstrate understanding can:

MS-PS2-3 Motion and Stability: Forces and Interactions
Ask questions about data to determine the factors that affect the strength of electric and magnetic forces.

MS-PS2-5 Motion and Stability: Forces and Interactions
Conduct an investigation and evaluate the experimental design to provide evidence that fields exist between objects exerting forces on each other even though the objects are not in contact.

Science and Engineering Practice	Disciplinary Core Ideas	Crosscutting Concepts
• Asking Questions and Defining Problems • Planning and Carrying Out Investigations • Analyzing and Interpreting Data • Constructing Explanations and Designing Solutions • Engaging in Argument from Evidence • Obtaining, Evaluating, and Communicating Information	**PS2B:** Types of interactions	• Patterns • Interdependence of Science, Engineering, and Technology • Influence of Engineering, Technology, and Science on Society and the Natural World

Common Core ELA/Literacy Standards

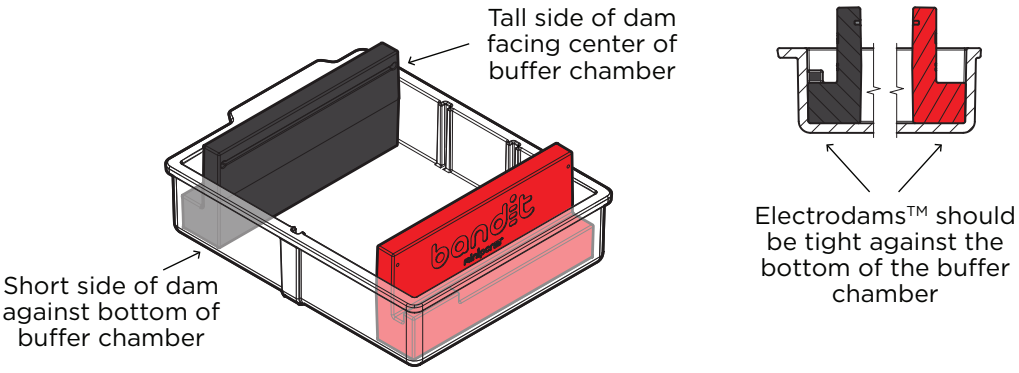
RST.6-8.1	Cite specific textual evidence to support analysis of science and technical texts.
RST.6-8.3	Follow precisely a multistep procedure when carrying out experiments, taking measurements, or performing technical tasks.
RST.6-8.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 6-8 texts and topics.
RST.6-8.5	Analyze the structure an author uses to organize a text, including how the major sections contribute to the whole and to an understanding of the topic.
RST.6-8.7	Integrate quantitative or technical information expressed in words in a text with a version of that information expressed visually (e.g., in a flowchart, diagram, model, graph, or table).
WHST.6-8.1	Write arguments focused on discipline-specific content.
WHST.6-8.1.A	Introduce claim(s) about a topic or issue, acknowledge and distinguish the claim(s) from alternate or opposing claims, and organize the reasons and evidence logically.
WHST.6-8.1.B	Support claim(s) with logical reasoning and relevant, accurate data and evidence that demonstrate an understanding of the topic or text, using credible sources.

For simplicity, this activity has been aligned to high school NGSS and grades 6-8 Common Core standards. This activity also aligns with equivalent grade 9-10 standards.

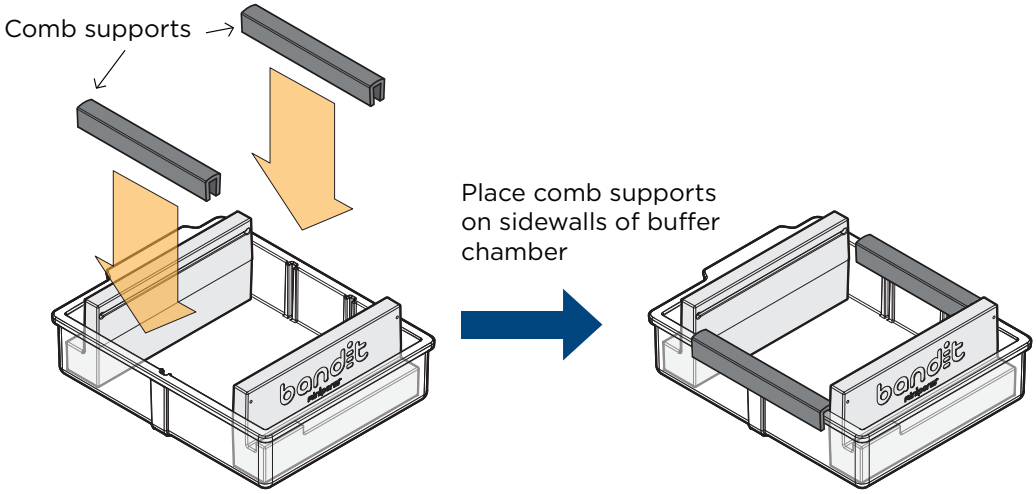
Detailed instructions for running the Molecular Rainbow lab using the Bandit™ STEM electrophoresis kit

A. Set up the Bandit™ buffer chamber and Electrodams™ for gel casting

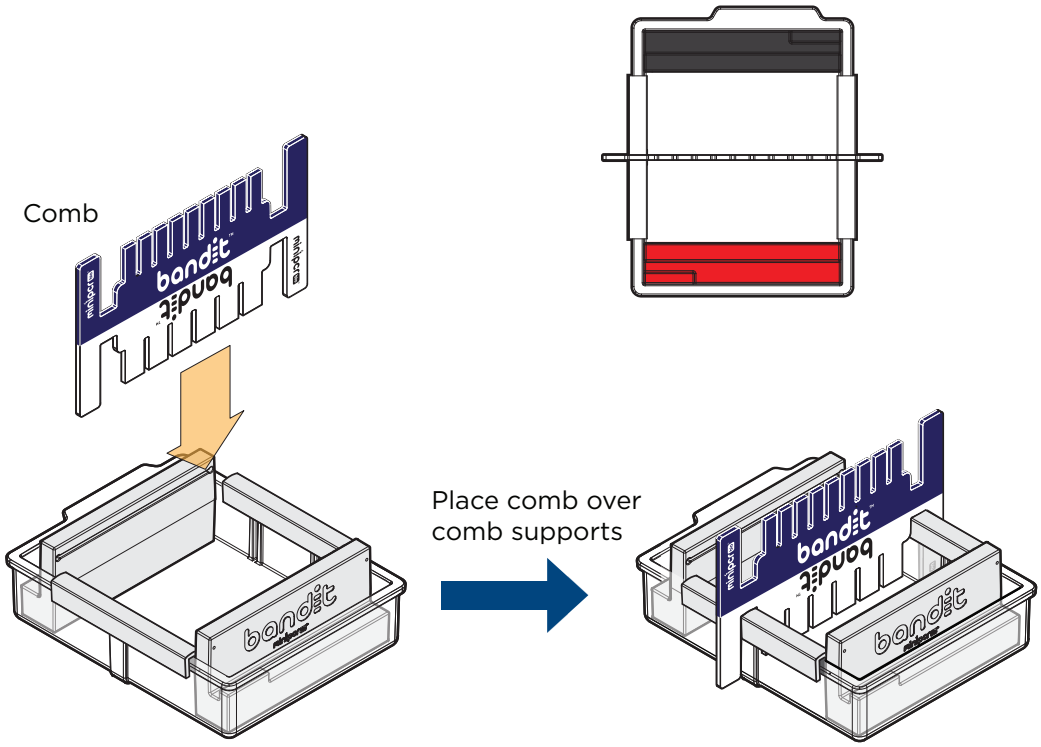
1. **Place the Eletrodams™ at the ends of the buffer chamber**
   - The Electrodams™ must be inserted in the correct orientation.
   - The short side of the Electrodams™ should sit flat against the bottom of the buffer chamber and the tall side should face the center of the buffer chamber.
   - Be sure that the Electrodams™ sit tightly against the bottom of the buffer chamber.



2. **Place the comb supports over the sidewalls of the buffer chamber**



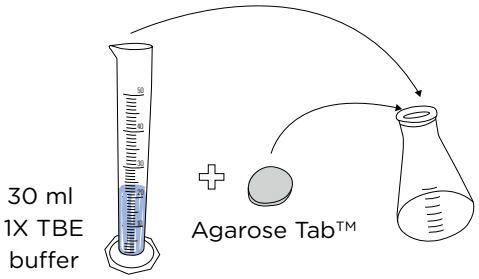
3. **Place the comb in the buffer chamber, resting over the comb supports**
    - Place the comb in the center of the buffer chamber.
    - The comb should be straight (parallel with the back of the buffer chamber).
    - The white side of the comb with six teeth should be facing down.



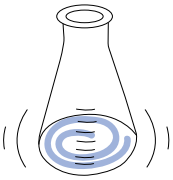
B. Prepare the gel

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - Prepare at least 60 ml of buffer for every Bandit™ or blueGel™ electrophoresis system you plan to use.
   - 30 ml of the buffer will be used to make your gel and 30 ml will be used as running buffer.
   - Refer to page 6 for detailed instructions on preparing 1X TBE buffer.

2. **Prepare an agarose solution**
   - Obtain a heat-resistant container such as a glass Erlenmeyer flask or beaker that is at least three times the volume you wish to add.
   - Combine 30 ml room temperature 1X TBE buffer and one Agarose Tab™ for each gel you plan to pour.
   - Allow the tabs to soak until they fully disintegrate (this could take a few minutes).
   - Swirl the flask or beaker to ensure the tabs have fully disintegrated before heating.

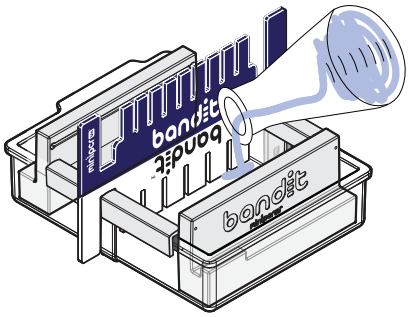


3. **Heat solution**
   - Expect to heat for about 60 seconds per 30 ml of liquid in a standard microwave.
   - Heat until the solution boils and continue until agarose is dissolved and the solution becomes fully transparent.



Caution: The solution may boil over the top of some containers. The solution will be very hot.

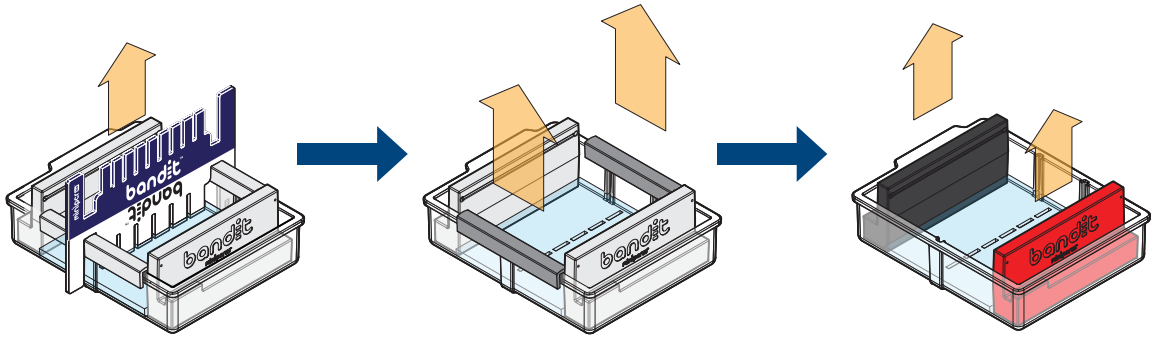
4. **Pour the agarose solution into the prepared Bandit™**
   - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).
   - Note: Because this lab uses colored dyes as experimental samples, there is no need to add DNA stain.



5. **Allow gel to solidify completely**
   - Gels will typically be ready in about 10 minutes.
   - Gel is ready when cool and firm to the touch.

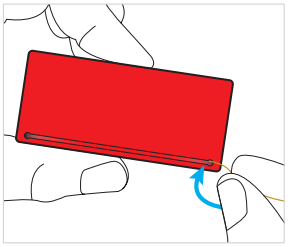
C. Set up Bandit™ for gel run

1. **Remove the comb, comb supports, and Electrodams™ by pulling firmly upwards**



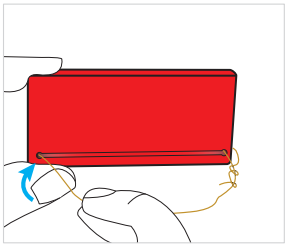
Remove comb | Remove comb supports | Remove Electrodams™

2. **Prepare the electrodes**
   - Note: The electrode wire can remain attached to the Electrodams™ for several uses. If the electrode wires are already in place, proceed to step 3.
   - If the Electrodams™ do not already have wire attached, cut two approximately 15 cm (6 inch) pieces of electrode wire from the included spool.

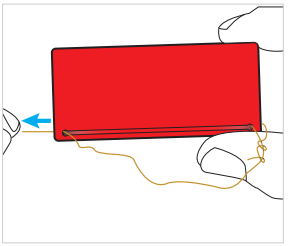


- Starting from the tall side of the Electrodam™ with a channel present, thread the wire through one of the small holes.
- You only need about 2 cm of wire to come through on the other side.

- Flip the Electrodam™ over.
- Twist the short free end of the wire that you just threaded through the hole around the longer piece of the wire to prevent it from slipping back through the hole.

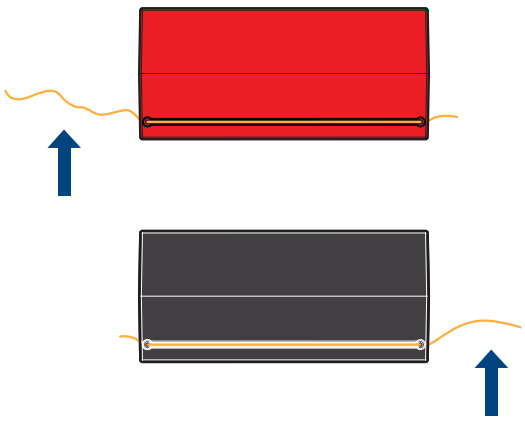


- Flip the Electrodam™ back over so you are looking at the side with the channel again.
- Thread the loose end of the wire through the small hole on the other side of the Electrodam™.



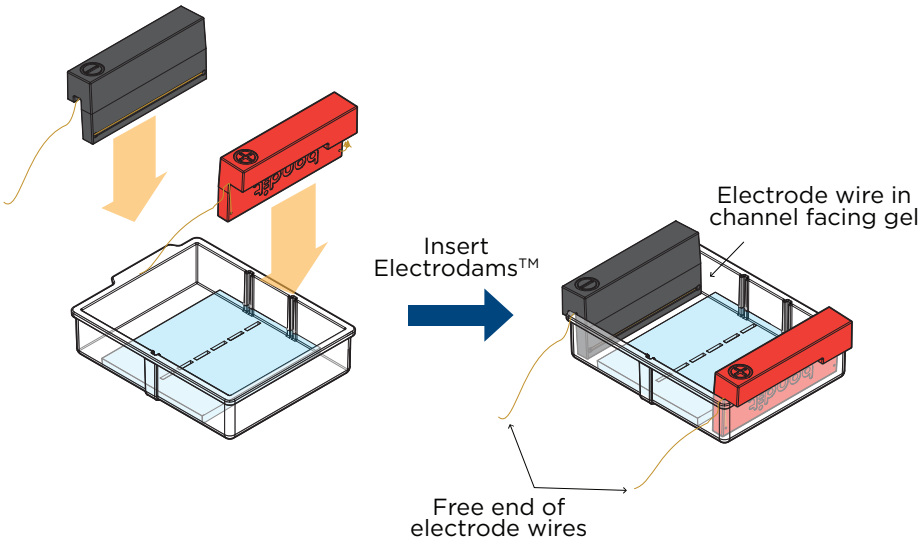
- Slowly pull the long free end of the wire until is it taut and the electrode wire sits flush in the channel.

- Repeat for the other Electrodam™, but thread the electrode wire from the opposite side so that the long free ends of the electrode wires extend in opposite directions.



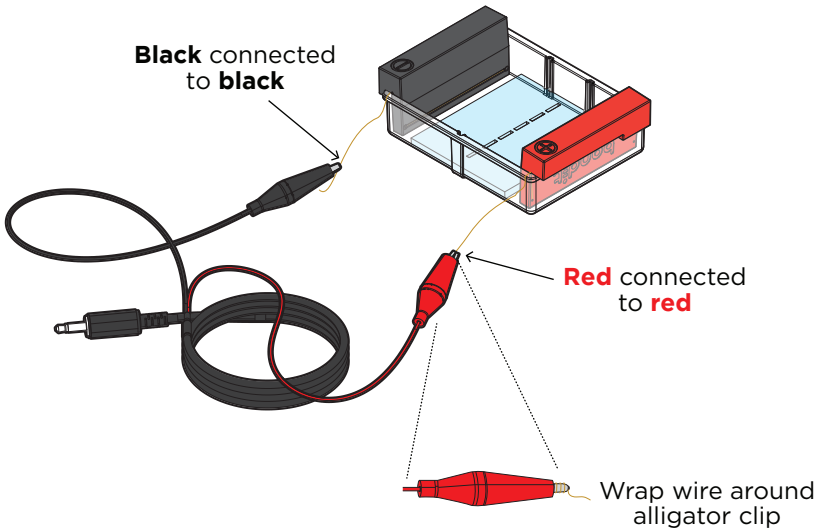
3. Insert the Electrodams™

- The Electrodams™ should be inserted with the electrode channel near the bottom of the buffer chamber, facing the gel.
- Make sure that the long free ends of the electrode are accessible.

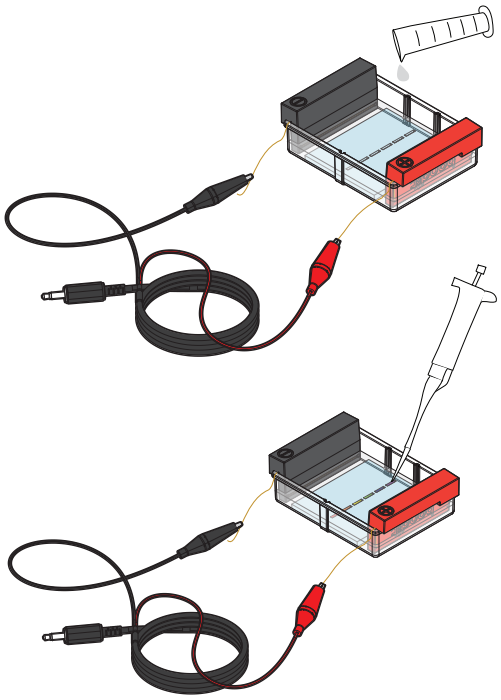


4. Connect the electrodes

- Connect the black alligator clip to the free electrode wire coming out of the black Electrodam™.
- Connect the red alligator clip to the free electrode wire coming out of the red Electrodam™.
- It may help to wrap the electrode wires around the alligator clips to ensure good contact.



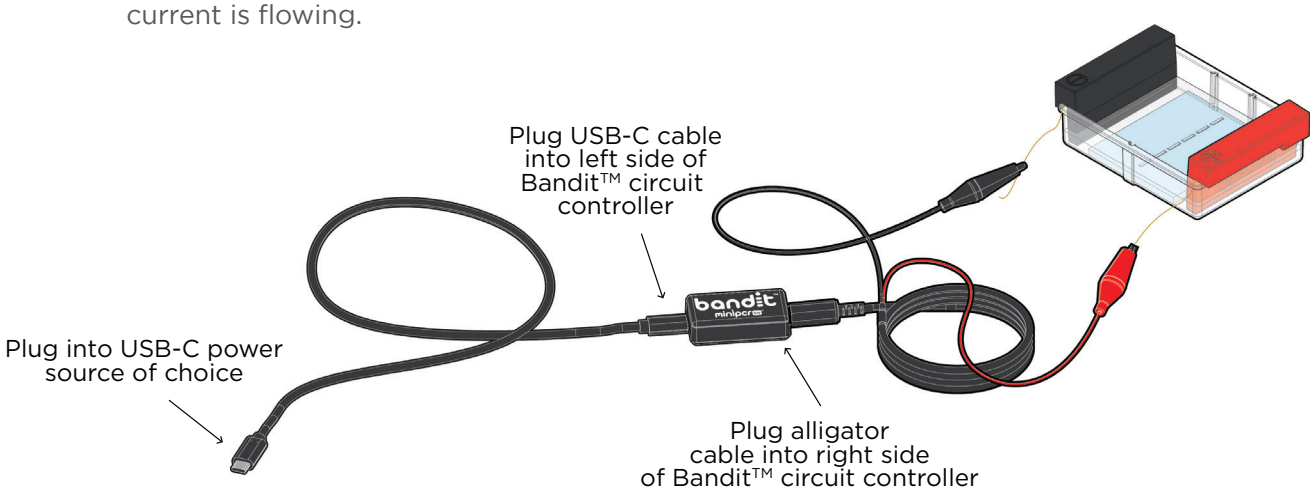
D. Run the gel



1. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells, in addition to the spaces between the Electrodams™ and the gel.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

2. **Use a micropipette to load samples onto the gel in the order specified in the lab guide**
   - **Lane 1:** 10 µl **Red Dye**
   - **Lane 2:** 10 µl **Orange Dye**
   - **Lane 3:** 10 µl **Yellow Dye**
   - **Lane 4:** 10 µl **Green Dye**
   - **Lane 5:** 10 µl **Blue Dye**
   - **Lane 6:** 10 µl **Purple Dye**

3. **Connect the power cords**
   - Plug the wire connected to the alligator clips into the round port on the right side of the Bandit™ circuit controller.
   - Plug the USB-C cord into the USB-C port on the left side of the Bandit™ circuit controller.
   - Plug the other end of the USB-C cord into your power source of choice.
   - Note: USB-C power source is provided by the user.
   - A small light on the right side of the Bandit™ circuit controller will illuminate to indicate that the power is on.
   - Look for bubbles on the electrode wires to verify that all the wires are connected and current is flowing.



4. **Conduct electrophoresis for 15-25 minutes or until adequate separation is achieved**

Ordering information

To order Dye Electrophoresis Lab: Molecular Rainbow kits, you can:


Call (781)-990-8PCR


email us at orders@minipcr.com


visit https://www.minipcr.com

Dye Electrophoresis Lab: Molecular Rainbow (catalog no. KT-1400–01) contains the following reagents and supplies:

- Rainbow dye samples (red, orange, yellow, green, blue, purple)
- Agarose tabs
- TBE electrophoresis buffer
- Plastic microtubes

Materials are sufficient for 8 lab groups
All components can be stored at room temperature
Reagents must be used within 12 months of shipment

About miniPCR bio Learning Labs™

This Learning Lab™ was developed by the miniPCR bio™ team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop miniPCR Learning Labs™ to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The Bandit™ gel electrophoresis kit and accompanying Learning Labs™ were developed to make biotechnology more accessible to classroom settings. Pairing the more affordable Bandit™ gel electrophoresis system with lab activities that use safe and affordable dyes to simulate DNA samples makes authentic molecular biology more accessible. The guiding premise for this lab is that using dyes to simulate DNA samples can recapitulate a real-life biotechnology application and provide the right balance between intellectual engagement, inquiry, and discussion.

Starting on a modest scale working with Massachusetts public schools, miniPCR Learning Labs™ have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.