Biotechnology Basics™ by Ellyn Daugherty

Gel Box Science

(Cat. # BBED-4J)

Developed in partnership with

Gel Box Science

Teacher's Guide

The following laboratory activity was inspired by two lab activities. One, "Using Gel Electrophoresis to Study DNA Molecules" from *Biotechnology: Science for the New Millennium Laboratory Manual* by Ellyn Daugherty and the other one, "The Chemistry and Physics of a Gel Box" originally developed by the Gene Connection Partnership of San Mateo County in 1989. For more information about *Biotechnology: Laboratory Manual* curriculum, please visit www.emcp.com/biotechnology. This kit is produced under license from Paradigm Publishing, Inc., a division of New Mountain Learning.

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About Ellyn Daugherty: Ellyn Daugherty is a veteran biotechnology educator and recipient of the Biotechnology Institute's National Biotechnology Teacher-Leader Award. She is the founder of the San Mateo Biotechnology Career Pathway (SMBCP). Started in 1993, SMBCP has instructed more than 10,000 high school and adult students. Annually, 30-40 SMBCP students complete internships with mentors at local biotechnology facilities.

About G-Biosciences: In addition to the Biotechnology by Ellyn Daugherty laboratory kit line and recognizing the significance and challenges of life sciences education, G-Biosciences has initiated the BioScience Excellence™ program. The program features hands-on teaching kits based on inquiry and curiosity that explore the fundamentals of life sciences and relate the techniques to the real world around us. The BioScience Excellence™ teaching tools will capture the imagination of young minds and deepen their understanding of various principles and techniques in biotechnology and improve their understanding of various social and ethical issues.

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MATERIALS INCLUDED

This kit has enough materials and reagents for 4 set-ups of gel box testing partly as a teacher-led demonstration and partly as student-loaded gel electrophoresis.

- 4 vials of 1M Sodium Chloride (NaCl), 1mL
- 4 tubes of 50X TAE buffer concentrate, 8 mL
- 4 vials of phenol red, 100 µL
- 12 large disposable transfer pipets
- 1 bottle of 0.8% agarose in 1X TAE
- 4 vials of DNA Loading Buffer [6X] for practice loading, 350 µL
- 4 vials of Bromophenol blue in clear loading dye, 20 µL
- 4 vials of Xylene cyanol in clear loading dye, 20 µL
- 4 vials of Crystal violet in clear loading dye, 20 µL
- 4 vials of Methyl red in clear loading dye, 20 µL
- 4 vials of Lambda/HindIII sizing markers in DNA loading dye, 20 µL
- 4 vials of *E. coli* genomic DNA in DNA loading dye, 20 µL
- 4 vials of Plasmid DNA in DNA loading dye, 20 µL
- 4 vials of Lambda DNA, uncut, in DNA loading dye, 20 µL
- 12 small disposable transfer pipets
- 1 vial LabSafe™ Nucleic Acid Stain (50µl)
- Large Weigh boats, 4

ADDITIONAL EQUIPMENT & MATERIALS REQUIRED

- Ziploc® bags, 4, quart-size
- Permanent lab markers, 4
- Paper towels
- Deionized or distilled water, one-gallon jugs or 4L
- Beakers, 500 mL
- Horizontal Gel Boxes with either 8-well, 9-well, or 10-well combs, 4
- Power Supplies, 4
- A UV Transilluminator or Blue LED light box
- "TRASH" beaker or container, 4

OPTIONAL MATERIALS:

- P1000, P200, P10 micropipette and tips
  These can be purchased separately

Cat. #	Fisher Sci Cat #	
BT1501	501059650	Pipette, Variable, P10
BT1502	501059651	Pipette, Variable, P100
BT1503	501059652	Pipette, Variable, P1000
BT1504	501059653	Pipette, Variable, P2
BT1505	501059654	Pipette, Variable, P20
BT1506	501059655	Pipette, Variable, P200

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SPECIAL HANDLING INSTRUCTIONS

- All components except the DNA samples can be stored at room temperature until ready to use.
- DNA samples should be stored in a freezer.
- Use paper towels to wipe spills when using the electrophoresis equipment.

GENERAL SAFETY PRECAUTIONS

- The reagents and components supplied in the Biotechnology Basics™ by Ellyn Daugherty kits are considered non-toxic and are safe to handle (unless otherwise noted), however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
- The teacher should 1) be familiar with safety practices and regulations in his/her school (district and state) and 2) know what needs to be treated as hazardous waste and how to properly dispose of non-hazardous chemicals or biological material.
- Students should know where all emergency equipment (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located and be versed in general lab safety.
- Remind students to read all instructions including Safety Data Sheets (SDSs) before starting the lab activities. A link for SDSs for chemicals in this kit is posted at www.gbiosciences.com
- At the end of the lab, all laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. All used culture vials, strips, tips, or may be discarded in regular trash.

TEACHER'S PRE EXPERIMENT SET UP

1. Set up a gel box (with either an 8-well, 9-well, or 10-well comb) and a power supply at 4 stations surrounding a spot where the teacher can stand and instruct. In Part I, four teams of students will surround each gel box and follow the teacher's directions.
2. Prepare the agarose for gel pouring gel as follows:
    - Loosen the caps on the bottle of prepared agarose, so they vent steam and pressure when heating. The agarose gets very hot, very quickly and can cause severe burns. Wear protective goggles when preparing agarose solution. Use heat-protective gloves or "hot hand" mitts when handling hot agarose solutions. Do not use latex gloves.
    - One at a time, heat each solution in a microwave on 50% power for about 3-4 minutes. Keeping the container away from your face, check to see that all the agarose has dissolved completely and that no crystals or lumps of agarose are left unmelted. Heat for 0.5-1 minute intervals, if necessary, until agarose has dissolved.
    - Allow the agarose solution to cool to about 60°C. 60°C is still pretty hot but it is cool enough to hold for pouring. While it cools, orient the gel electrophoresis trays into position for casting (pouring) the gel(s). Some gel trays use tape on the ends, some use gates or buffer dams, and some are wedged into the gel box for pouring. Please refer to the manufacturer's instructions for how to set up and use your gel box.
    - Once the agarose has cooled to the point it can be held comfortably in one's hand (around 60°C), add 50µl of the LabSafe™ Nucleic Acid Stain to the warm agarose. Swirl gently to mix.
3. Pour a 7-mm thick gel in each gel tray **making sure that the gel comb is placed in the center slot**. Depending on the comb, that should result in a row of 8-10 wells across the center of the gel.

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- Quickly, pour the agarose into one or more gel casting trays, depending on your electrophoresis equipment, to about 7mm thick. Using an appropriate size gel-well comb (8-, 9- or 10-well comb), place the comb in the center slots of the hot agarose tray to create wells that will hold at least 25 µl. Make sure that the comb is level so that all the wells will be of the same dimensions.
- Let the gel(s) solidify, 30-40 minutes depending on the size of each gel. This is longer than "usual" to make sure the gels are firm. Since the gels will be used during Part III (maybe on the 2nd day) of the lab activity after Part II, place the gel, still in its tray with the comb still in the gel, into a weigh boat, and add enough 1X TAE buffer to keep it damp until ready to use. Slide the weigh boat with the gel into a quart-sized Ziploc® bag. Seal it and keep in a cool, dark place for up to 24 hours or in a refrigerator for several days. Do not allow the gels to freeze.
- Before students do Procedure Part III, gently lower a tray with the pre-cast gel with the comb in it into the gel box filled with 1X TAE buffer. Hold the ends of the gel and tray as it lowers into the buffer so that it settles straight down and doesn't break.

3. At each of the four gel box set-ups, put out:

- 1 vial of 1*M* NaCl, 1mL
- 1 tube of 50X TAE buffer concentrate, 8 mL
- 1 vial of phenol red, 100 µL
- 3 large disposable transfer pipets (or P-200 and P-1000 Micropipettes)
- 1 vial of DNA Loading Buffer for practice, 350 µL
- 1 vial of Bromophenol blue in clear loading dye, 20 µL
- 1 vial of Xylene cyanol in clear loading dye, 20 µL
- 1 vial of Crystal violet in clear loading dye, 20 µL
- 1 vial of Methyl red in clear loading dye, 20 µL
- 1 vial of Lambda/HindIII sizing markers in DNA loading dye, 20 µL
- 1 vial of *E. coli* genomic DNA in DNA loading dye, 20 µL
- 1 vial of Plasmid DNA in DNA loading dye, 20 µL
- 1 vial of Lambda DNA, uncut, in DNA loading dye, 20 µL
- 3 small transfer pipets (or P20 Micropipette)
- Large Weigh boat
- Permanent lab marker
- Paper towels
- Beakers, 500 mL
- Ziploc® bag, quart-size
- "TRASH" beaker or container,
- Paper towels
- Beakers, 500 mL
- Horizontal Gel Box with either an 8-well, 9-well, or 10-well combs
- Power Supplies

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At a common use area, set up:

- A UV Transilluminator or Blue LED light box
- Deionized or distilled water, 4L

OPTIONAL MATERIALS:

- Micropipettes, P20 and micropipette tips

TIME REQUIRED

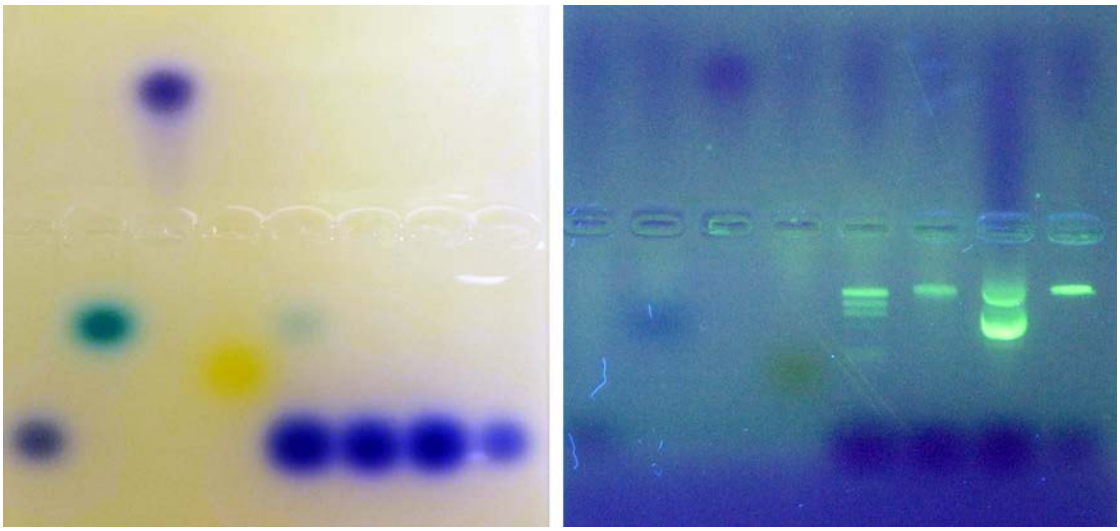
- 1-hour for teacher preparation of gels and distribution of reagents
- 1-hour lab period for study of voltage and current in the gel box.
- 1-hour lab period for loading and running samples on the gel

NEXT GENERATION SCIENCE STANDARDS ADDRESSED

- HS-LS1: From Molecules to Organisms: Structures and Processes
- LS1.A: Structure and Function

For more information about Next Generation Science Standards, visit: http://www.nextgenscience.org/

EXPECTED RESULTS



How samples may appear on a gel:

1. Lane 1 contains Bromophenol blue, moves to positive electrode so it is negatively-charged. It is a molecule used in loading dyes and it is smaller than xylene cyanol, so migrates (moves) further in the gel.
2. Lane 2 contains Xylene cyanol, moves to positive electrode so it is negatively-charged. It is a molecule used in loading dyes and it is larger than bromophenol blue, so migrates (moves) slower in the gel.
3. Lane 3 contains Crystal violet, moves to the negative electrode so it is positively-charged.
4. Lane 4 contains Methyl Red, moves to the positive electrode so it is negatively-charged.
5. Lane 5 contains a sample of lambda virus DNA digested with HindIII restriction enzyme that results in pieces of DNA ranging in size. These molecules are linear pieces of DNA with a variety of sizes from 500 base pairs to about 23,000 base pairs. The largest group of DNA molecules are in the lane in the band nearest to the well. The

bands farther from the well contain DNA molecules of smaller size. From these known pieces of lambda DNA, other DNA samples of unknown size can be estimated. Lambda/HindIII sizing standards are some of the most common sizing standards used in biotechnology.

6. Lane 6 contains a sample of *E. coli* genomic DNA (the single "chromosomal" DNA of *E. coli* bacteria). Even the smallest bacteria DNA molecule is about 3,000,000 bp in size. So, it doesn't enter the gel easily or go very far.
7. Lane 7 contains plasmid DNA. Plasmids are found in some bacteria and are small rings of DNA, usually only 2000-8000 base pairs in size. Plasmids are important in genetic engineering. Remember, to be able to actually see the band of a gel, there must be thousands or millions of (plasmid) molecules in the band.
8. Lane 8 contain Lambda DNA that has not been treated with the HindIII restriction enzyme so it is uncut. It is the size of the original viral DNA.

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Reflections/Thinking Like a Biotechnician:

1. The sizes of the lambda/HindIII standard fragments (Lane #5), in base pairs, follows.

   23130 9416 6557 4361 2322 2027 564 125

   (these are hard to see)

   Estimations of the unknown DNA fragments (in lanes 6-8) can be made by comparing their location on the gel to the location of these known lambda DNA fragments. Make an approximation of the size of the plasmid in DNA molecules in Lane #7.

   Answer: The plasmid rings in Lane #7 are approximately 4,000 base pairs in size.

2. List 2 or 3 reasons why samples that are loaded onto a gel might not electrophorese through a gel or be visible on a gel after electrophoresis.

   Answers: There are several including:

   - Buffer is not prepared correctly
   - Samples are not loaded correctly
   - There is no or little current running through the gel
   - Gel ends are blocked by plastic or tape
   - Power supply is not set correctly

3. DNA gel electrophoresis in research and testing is conducted in a similar way to what you have just completed. However, in all DNA gel electrophoresis, the wells are created on the negative end of the gel slab (not in the center). Explain why?

   Answer: DNA molecules are negatively-charged (due to the phosphate groups in the sugar-phosphate backbone strands). DNA always moves from the negative to the positive side of a gel. Placing the wells at the negative end gives more room for similarly sized DNA pieces to separate.

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OBJECTIVES

How is an electric field created in a gel box? How do molecules behave during an electrophoresis? What information can be learned about DNA molecules during a gel electrophoresis?

BACKGROUND

The molecules studied in biotechnology are colorless and submicroscopic. Scientists use gel electrophoresis technology to separate large biomolecules on a gel slab. Using electrophoresis, researchers can easily separate and visualize charged molecules, such as DNA fragments, RNA, and proteins which is important in several applications including genetic testing, criminal investigations, and genetic engineering.

But how does an electrophoresis work and what information can a researcher learn during a gel electrophoresis?

A gel electrophoresis is conducted in a gel box unit that is connected to a power supply that supplies electricity. If the gel box is filled with a solution that will conduct electricity, an electric field is set up producing a negatively-charged and positively-charged region at opposite sides of the gel box (Figure 1).

To separate molecules in the electric field, a gel material is prepared and molded into a slab.

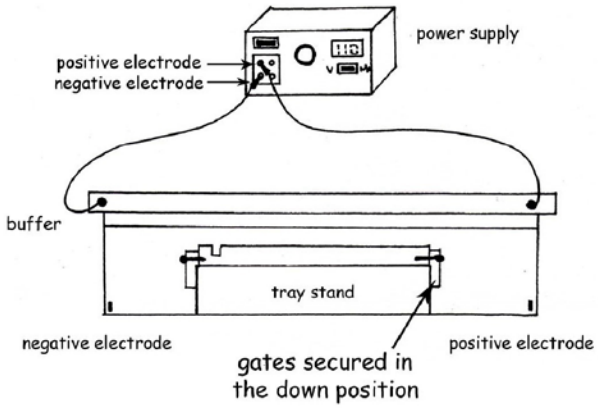

Figure 1

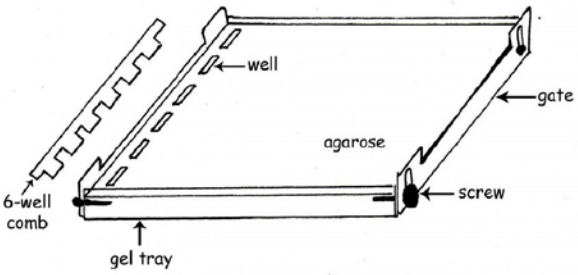

Figure 2

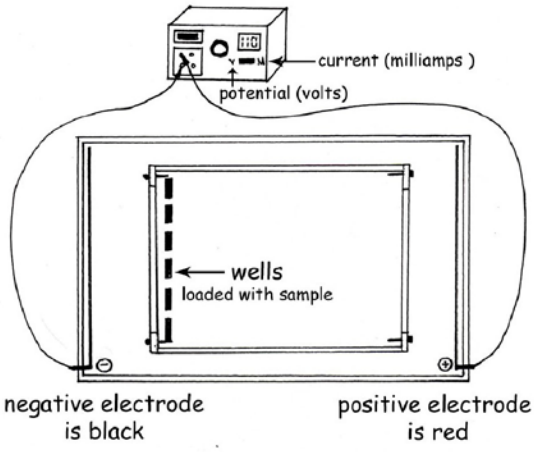

Figure 3

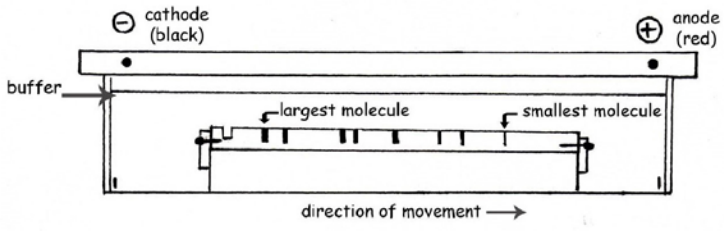

Figure 4

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Depending on the gel material, molecules can be added to wells created in the gel slab. The molecules can be moved in the electric field through the gel and separated based on their size, shape, and charge (Figure 2).

A sample of charged molecules is loaded into the sample wells of the gel slab. When the power is turned on, and the electric field is established, the charged molecules move into the gel from the wells. If the molecules have a net negative charge, they move toward the positive end of the gel. If the molecules have a net positive charge, they move toward the negative end of the gel. The gel material acts as a molecular strainer, separating longer molecules from shorter ones, fatter ones from thinner ones, and positively-charged molecules from negatively-charged molecules (Figure 3 & 4).

MATERIALS FOR EACH GROUP

Each group should have the following components from the kit:

At each of the four gel box set-ups, put out:

- Horizontal Gel Box with either an 8-well, 9-well, or 10-well combs
- Power Supply, 300V
- 1 vial of 1M NaCl, 1mL
- 1 tube of 50X TAE buffer concentrate, 8 mL
- 1 vial of phenol red, 100 µL
- 3 large disposable transfer pipets (or P-200 and P-1000 Micropipettes)
- 1 vial of DNA Loading Buffer for practice, 350 µL
- 1 vial of Bromophenol blue in clear loading dye, 20 µL
- 1 vial of Xylene cyanol in clear loading dye, 20 µL
- 1 vial of Crystal violet in clear loading dye, 20 µL
- 1 vial of Methyl red in clear loading dye, 20 µL
- 1 vial of Lambda/HindIII sizing markers in DNA loading dye, 20 µL
- 1 vial of *E. coli* genomic DNA in DNA loading dye, 20 µL
- 1 vial of Plasmid DNA in DNA loading dye, 20 µL
- 1 vial of Lambda DNA, uncut, in DNA loading dye, 20 µL
- 3 small disposable transfer pipets (or P20 Micropipet)
- Permanent lab marker
- Paper towels
- Beakers, 500 mL
- "TRASH" beaker or container
- Paper towels
- Deionized or distilled water

At a common use area:

- UV Transillumator or Blue LED Light Box
- Deionized or distilled water, 4L

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PROCEDURE

Parts I and II are completed during teacher-lead observation. Record your observations as instructed.

Part I: The Parts of a Gel Box Unit

Check off each step below to show that you have completed your observation of the parts of the gel box and power supply.

1. At the gel electrophoresis station, identify the equipment and materials found on the Materials List. If any item is missing, inform your instructor/supervisor. _____
   NOTE: *The electrophoresis gel box is a delicate instrument. Don't pick it up or use it before instructed to below.*

2. Examine the gel box and power supply. Make sure that the power supply is not plugged into the outlets and that the power supply is turned off. _____

3. Locate the two electrodes, on the top (cover) of the gel box unit that are used to connect the gel box to the power supply. For the gel box to conduct electricity it must have an established electric field between the positive end (red wire) and a negative end (black wire). _____

4. Make sure the electrodes are securely attached to the gel box cover. Never pull on the electrodes as this could irreversibly damage the unit. Open the gel box cover by sliding the cover off using the plastic sides of the cover. _____

5. Before using the metal ends of electrodes will be plugged into the similarly colored electrode receptacle in the power supply. *Do **not** do that now.*

6. Look at the inside of the gel box. Notice that two very thin wire filaments are found running from the colored electrodes. ***Never** touch these for any reason*. If they are broken the gel box will not work.
   The thin wire filaments are continuations of the electrodes, meaning that the positive electrode continues into one side of the box and the negative electrode continues into the opposite side of the box. _____

7. In the center of the gel box unit is a platform to place a gel slab for electrophoresis. If there is a gel tray and/or a gel-well comb, remove these and set these aside. _____

8. Locate the following on the power supply:

   a. Master Power Switch (often on the back of the power supply) _____
   b. Black Power Cord (to attach power supply to room electricity) _____
   c. On/OFF/RUN or similar switch (to start the unit) _____
   d. Voltage meter [potential energy (ability to produce electricity if conditions are right) measured in "volts" or "V"] _____
   e. Current Meter [kinetic energy (electricity flowing) measured in "milliamps" or "mA"]
   f. Timer (if present) _____

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Part II: The Science of a Gel Box

Remind students that electricity can be conducted through water. So, when using the electrophoresis equipment, to reduce the chance of electric shock, students must wipe up all spills before turning on a power supply.

1. Set up the gel box approximately 6 inches for the edge of a lab table. This will make for easy access. Plug in the power supply to the room outlet. Make sure the power supply is turned off. Make sure the power supply is close enough to the gel box (about 6 inches) so that the electrodes will reach the connections. _____

2. Gently remove the gel box cover as instructed by your teacher and place it to the side. If the gel tray and gel-well comb are in the box, place them aside as well.

3. Replace the cover on the empty gel box and hook it up to the power supply by plugging in the positive and negative electrodes (to the similar color plug holes on the power supply). Turn on the power and set the voltage to 100V. Record the current flowing through the empty box. _____mA. Does this amount of current seem reasonable? ***Turn off the power supply***.

4. Open the cover on the empty gel box and add 300 mL of deionized water to the inside of the gel box. Close the cover and hook it up to the power supply. Wipe up any liquid that spills with the paper towels provided.

5. Turn on the power and set the voltage to 100V. Record the current flowing through the deionized water-filled gel box. _____mA. Does this amount of current seem reasonable? ***Turn off the power supply***.

6. Open the cover on the gel box and add 1 mL of NaCl solution to the 300 mL of deionized water inside the gel box. Gently tilt the gel box back and forth to even distribute the NaCl. Wipe up any liquid that spills with the paper towels provided.

7. Close the cover and hook it up to the power supply. Turn on the power and set the voltage to 100V. Record the current flowing through the salt water-filled gel box. _____mA. Does this amount of current seem reasonable? ***Turn off the power supply***.

8. When was current (mA) found flowing through the gel box? When was no current flowing through the box? Can you explain why? *Hint: NaCl ionizes to Na+ and Cl- ions in water.*

   _____
   _____
   _____
   _____

9. The current shows that electricity is flowing through the gel box. To verify that there is a positive and negative end to the electric field in the gel box, add the 100 µl volume of phenol red to the salt water solution in the gel box. Gently tilt the gel box back and forth to even distribute the phenol red. Wipe up any liquid that spills with the paper towels provided.
   Phenol Red is an acid/base indicator and turns golden color when there are excess H+ ions in solution (acidic conditions). Phenol red is a dark pink color when there is a deficit of H+ and an excess on OH- ions in solution (alkaline or basic conditions).

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10. Close the cover and hook it up to the power supply. Turn on the power and set the voltage to 100V. Record the current flowing through the phenol red/salt water-filled gel box. _____mA. After 5-10 minutes of current running look closely at the color of the solution inside at each end of the gel box?
Is there any evidence that the charge is different at the opposite ends? Explain.

_____

_____

_____

_____

Turn off the power supply and pour the salt water out of the gel box at the lab sink.

11. Ions must be present to conduct electricity. For biotechnology gel electrophoresis, special salt solutions made up of other ions are used. A common electrophoresis solution is prepared as 1X TAE Buffer. In TAE, the salt that conducts electricity is TRIS ($C_4H_{11}NO_3$). There are 2 other compounds in TAE Buffer. A quick search on the Internet will give a recipe for TAE Buffer.
To prepare 1X TAE Electrophoresis Running Buffer from the 50X TAE concentrate provided, label a 500 mL beaker "1X TAE" and add 8 mL of the 50X TAE buffer (provided) and 392 mL of deionized water. Stir until thoroughly mixed.

12. Open the cover on the gel box and add 350 mL of 1X TAE Electrophoresis Running Buffer to the inside of the gel box. Wipe up any liquid that spills with the paper towels provided.

13. Close the cover and hook it up to the power supply. Turn on the power and set the voltage to 100V. Record the current flowing through the buffer-filled gel box. _____mA. What can you say about the difference between the NaCl's and the buffer's ability to conduct electricity? Look at the electrode ends of the gel box. If you see bubbling, current is flowing. ***Turn off the power supply***.

_____

_____

_____

_____

14. With the gel box filled with 350 mL of 1X TAE electrophoresis buffer, determine how current is affected by changes in voltage. Wipe up any liquid that spills with the paper towels provided. Make sure to turn off the power supply after you have collected data.
Measure the current (in mA) at every 20 volts from 0-120 volts and record these on a data table similar to the one below.

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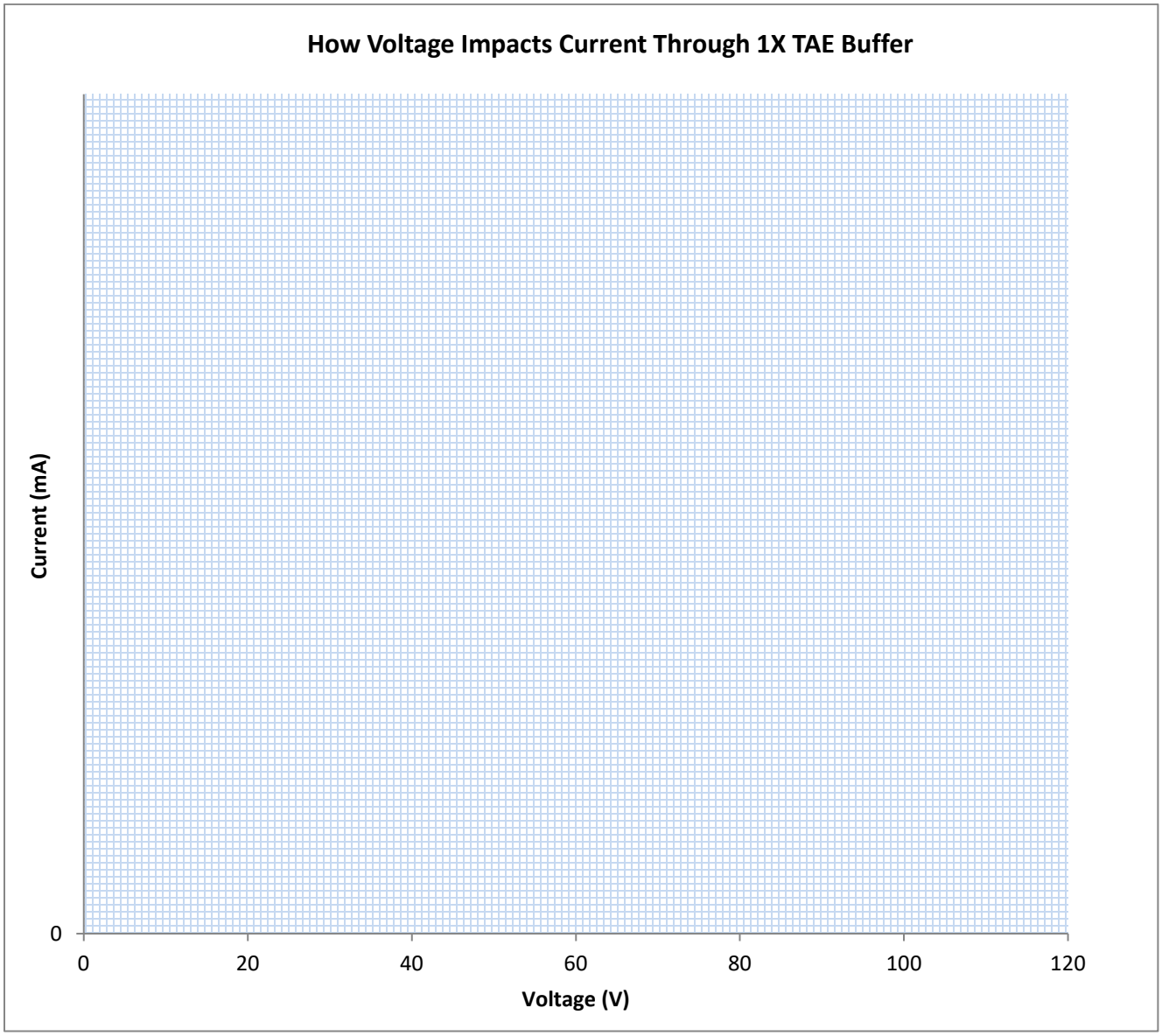
How Voltage Impacts Current Through 1X TAE Buffer

Voltage (V)	Current (mA)	Comments
0		
20		
40		
60		
80		
100		
120		

Create a line graph below to show how voltage impacts current through 1X TAE Buffer.



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Part III: How Molecules Behave during an Electrophoresis

The instructor has prepared and poured an agarose gel with wells for loading samples of molecules.

Prior to starting Part III, the gel will be placed in the gel box containing the 1X TAE Buffer.

1. The agarose gel slab should be sitting on a gel tray so that the ends of the gel are facing the electrode ends of the box. There should be enough 1X TAE buffer in the gel box to cover the gel by about 10 mm. Too much buffer slows down the electrophoresis.

2. It is important to remove the gel-well comb while the gel is under buffer to lessen the chance of ripping the well walls. Gently rock the comb lightly back and forth while pulling the comb straight up. This should reveal the sample wells that will be loaded with different samples of molecules. The wells are numbered, starting at 1, from bottom to top, when the negative electrode is on your left side and the positive electrode is on your right side.

3. The instructor will demonstrate how to use a transfer pipet or a micropipette to load a sample into a gel well. Practice loading 20 µL (or so) of practice loading dye into any one the wells. Be careful to never put a loading tip into a well. Instead release the sample to be loaded just above the well and the sample will sink down into the well.

4. Remove the practice samples by rinsing the wells using the buffer in the gel box.

5. Each student should load a sample into the corresponding well as directed in the table below. If using a micropipette change micropipette tips for each sample loading. If using a transfer pipet, rinse the pipet in water before loading each sample. Explain why?

   _____
   _____
   _____
   _____

6. Once the first sample has been loaded, DO NOT MOVE THE GEL BOX. Explain why?

   _____
   _____
   _____
   _____

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Well #	Sample to be loaded	Behavior in electric field (Moves to + or - electrode)	Net Electric Charge of Molecule (+ or -)	Relative size of molecules versus others in samples?
1	Bromophenol blue, 20 µL			
2	Xylene cyanol, 20 µL			
3	Crystal violet, 20 µL			
4	Methyl red, 20 µL			
5	Lambda/HindIII sizing markers in loading dye, 20 µL			
6	*E. coli* genomic DNA in DNA loading dye, 20 µL			
7	Plasmid DNA in DNA loading dye, 20 µL			
8	Lambda (virus) DNA, uncut, in DNA loading dye, 20 µL			

7. Once all samples are loaded, gently replace the gel box cover over the gel box and hook it to the power supply. Wipe up any spills.

8. Turn on the power supply and turn the voltage to 115 volts. Check to see if current is running through the gel box. Record the current. _____mA. There should be at least 30 mA running through the gel box. If the current is less than it was before a gel was included, explain why.

_____
_____
_____
_____

9. Run the electrophoresis for 20-30 minutes or until you can see that the molecules in samples have moved towards one electrode or another. ***Turn off the power supply*** and remove the cover of the gel box. Holding the tray at each end, so the gel doesn't slide off, gently slide the gel into a weigh boat. DNA is colorless so you are only seeing loading dye in samples 5-8. Observe the gel bands and in the table record the behavior, charge and relative size of the molecules in samples 1-4.

10. Gently transfer the gel from weighboat to a UV transilluminator or Blue LED Light Box. The gel has a UV sensitive DNA stain in it. By turning on the UV transilluminator, you should be able to see the DNA in samples 5-8. Observe the gel bands and in the table record the behavior, charge and relative size of the molecules in samples 5-8. Wear UV Safety Specs when working around the UV Light Box.

11. The illuminated or unilluminated gel can be photographed for a permanent record.

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REFLECTIONS/THINKING LIKE A BIOTECHNICIAN:

1. The sizes of the lambda/HindIII standard fragments (Lane #5), in base pairs, follows.

23130 9416 6557 4361 2322 2027 564 125

(these are hard to see)

Estimations of the unknown DNA fragments (in lanes 6-8) can be made by comparing their location on the gel to the location of these known lambda DNA fragments. Make an approximation of the size of the plasmid in DNA molecules in Lane #7.

_____
_____
_____
_____

2. List 2 or 3 reasons why samples that are loaded onto gel might not electrophoresis through a gel or be visible on a gel after electrophoresis.

_____
_____
_____
_____

3. DNA gel electrophoresis in research and testing is conducted in a similar way to what you have just completed. However, in all DNA gel electrophoresis, the wells are created on the negative end of the gel slab (not in the center). Explain why.

_____
_____
_____
_____

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