



Sexing by DNA is For The Birds!

Supplemental Reagent Pack
Student Protocol

Cat# M3056TAE

Version 032426



Part I: Electrophoresis

Materials

- 1 Minione® Casting System
- 1 MiniOne® Electrophoresis System
- 1 agarose GreenGel™ cup (2%)
- 6 DNA sample aliquots
- 135 mL of running buffer
- 1 micropipette (2-20µL)
- 6 pipette tips

Lane #	Sample Name	Volume
1	Sample 1	10 µL
2	Sample 2	10 µL
3	Sample 3	10 µL
4	Sample 4	10 µL
5	Sample 5	10 µL
6	Sample 6	10 µL

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

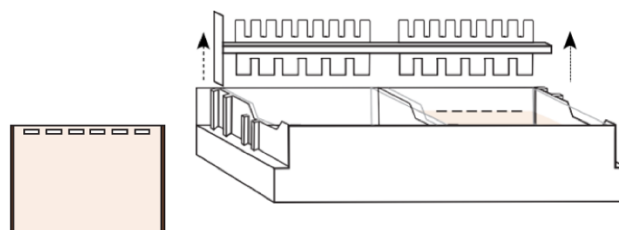
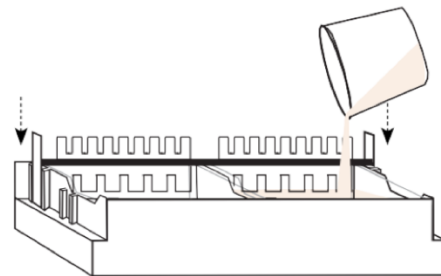
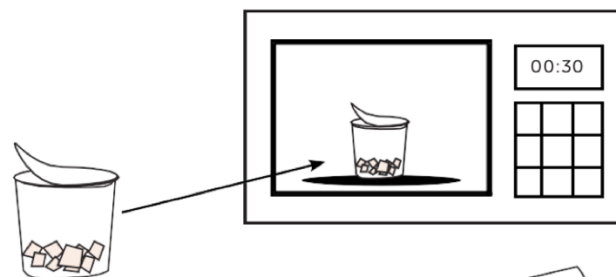
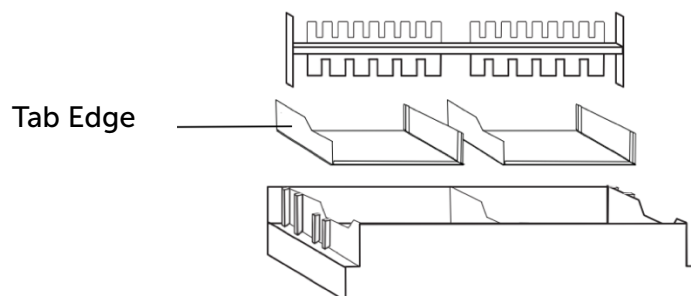
2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.

Safety requirement: Adult supervision required if students are handling gel cups!


3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.

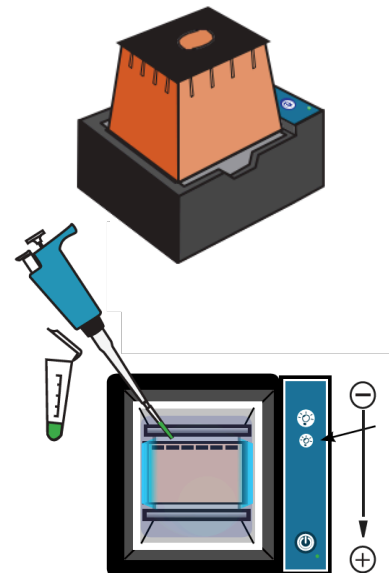
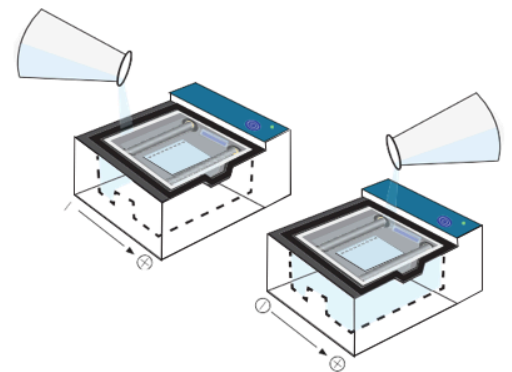
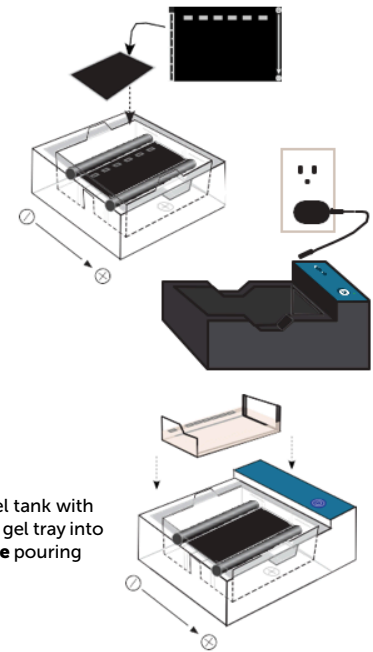
DO NOT disturb the gel until time is up.

4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.




How to Load a Gel

1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**
2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.
5. Turn the low intensity blue LED on by pressing the -button on the carriage.
6. Measure 135 mL of TAE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank**.
7. Place photo hood on the carriage.
8. Press the power button which should now be a solid green light. If **green light is solid**, turn off the unit and proceed to loading gels.
9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.
10. Load 10 μ L per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

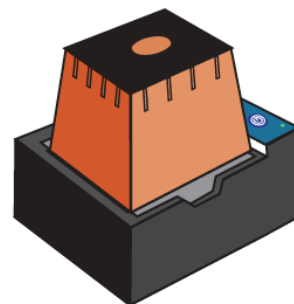



Run, Visualize and Capture Image

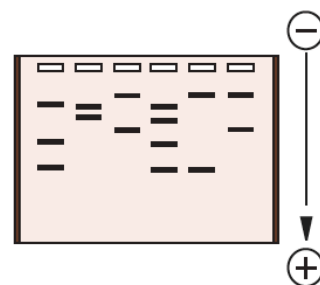
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the  button. The green LED next to the button will turn on.

The green power LED will not turn on if:

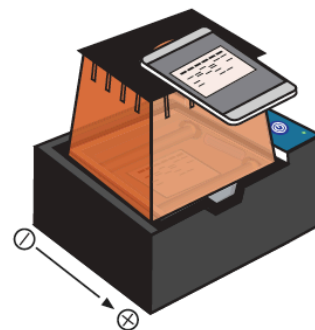
- The tank is not properly placed inside the carriage.
- There is no buffer in the tank.
- The buffer is too diluted.
- The photo hood is not on the carriage.
- There is too little running buffer.
- The power supply is not plugged in. Check by turning on the blue LEDs.
- If the green power LED is blinking, please refer to the troubleshooting steps in the **MiniOne Electrophoresis Instruction Manual**



2. Have students periodically check the migration of the bands (~every five minutes).
3. Allow the gel to run **20 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the  button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.
4. Document your results.



Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)



5. Clean up. Follow teacher's instructions on disposal and clean up.

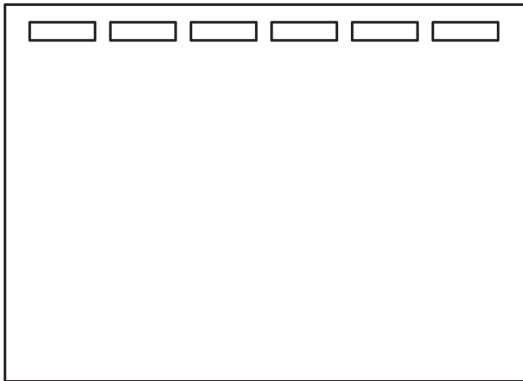
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away AND SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below along with the resulting fragment sizes.



- Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____

Appendix A - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes


A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

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Patents issued: US 10,641,731 B2, US 20110253541 A1, US 11879118-B2, US 12,384,994,
US 11,879,117, US 11,879,118