DNA Detectives Investigations

Student Protocol

Cat# M3052

Version 123019

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Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Experimental Protocol
Part I - Set Up and Run Your Restriction Digests

1. Check your workstation to make sure you have all of the materials listed above.
2. Label six PCR tubes with your group name and the sample # according to Table 1.
3. Set up your restriction digests by adding the reagents to the six tubes according to Table 1. (Use PCR tubes if the incubation is going to be in a thermal cycler or use microcentrifuge tubes if the incubation is going to be in a water bath.)

Table 1: Experimental Setup

Reagent	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
DNA Sample Source	Missing Person#1	Missing Person#1	Missing Person#1	Missing Person#2	Missing Person#2	Missing Person#2
DNA Sample Volume	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL
Enzyme DilutionBuffer	5 µL	-	-	5 µL	-	-
Diluted EcoRV	-	5 µL	-	-	5 µL	-
Diluted HindIII	-	-	5 µL	-	-	5 µL
Total Volume	**15 µL**	**15 µL**	**15 µL**	**15 µL**	**15 µL**	**15 µL**

4. Gently flick the tubes with your finger to mix the reagents. Centrifuge for 15 seconds at 8,000 RPM to bring the reagents to the bottom of the tubes.
5. Place your tubes in the MiniOne® PCR System and close the lid. Incubate the restriction digest at 37°C for 1800 seconds using constant temperature mode on your MiniOne® PCR Mobile App. Enter 4°C for the final incubation temperature. If you do not have a PCR system, a water bath set to 37°C can be used, incubating for 30 minutes.
6. While you are waiting for your digest, prepare the MiniOne® Agarose Gel (Part II).
7. When the incubation is complete, add 4 µL of Sample loading dye (5X) to each of your experimental tubes. Use a new micropipette tip when adding to each sample. Gently flick each tube with your finger to mix the dye with the samples.
8. Centrifuge for 15 seconds at 8,000 RPM to bring the reagents to the bottom of the tubes.
9. Give your samples to your teacher for storage until the next lesson.

Part II: Electrophoresis

Materials

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

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.

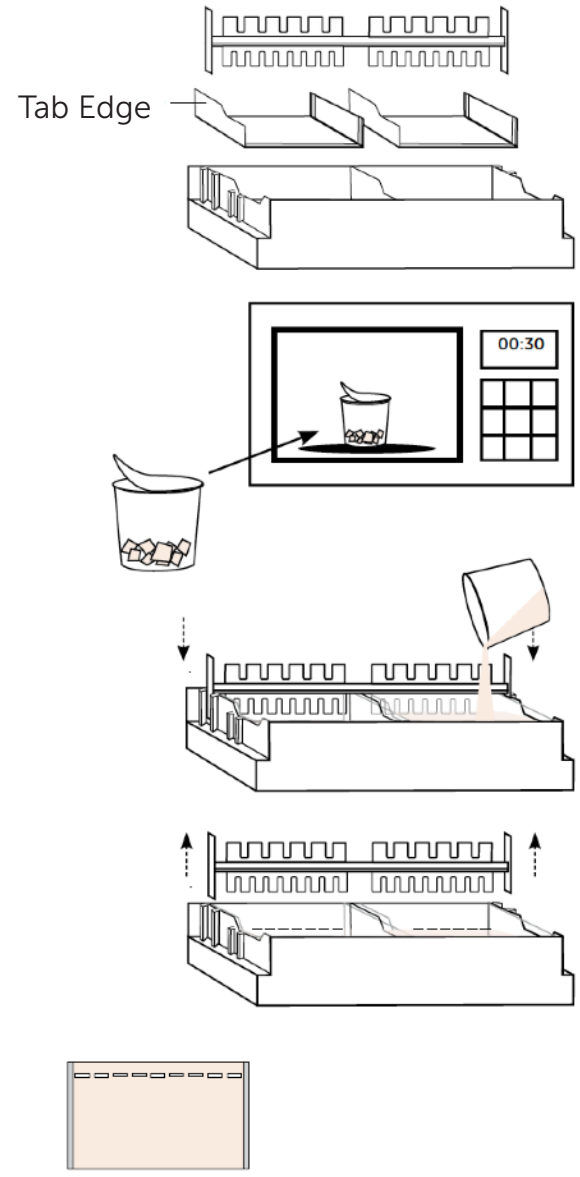
Safety requirement: Adult supervision required if students are handling gel cups!

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.

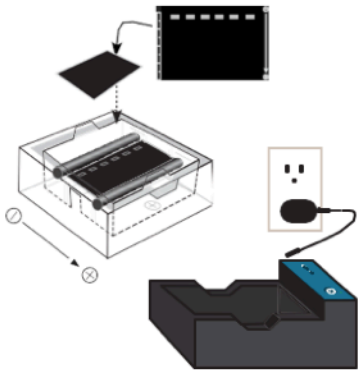
4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.

Lane #	Sample Name	Volume
1	Missing Person 1, uncut	10 µL
2	Missing Person 1, cut with EcoRV	10 µL
3	Missing Person 1, cut with HindIII	10 µL
4	Missing Person 2, uncut	10 µL
5	Missing Person 2, cut with EcoRV	10 µL
6	Missing Person 2, cut with HindIII	10 µL
7	Skeleton DNA sample cut with EcoRV	10 µL
8	Skeleton DNA sample cut with HindIII	10 µL
9	MiniOne DNA Marker	10 µL



How to Load a Gel

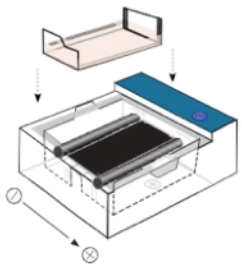
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



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.

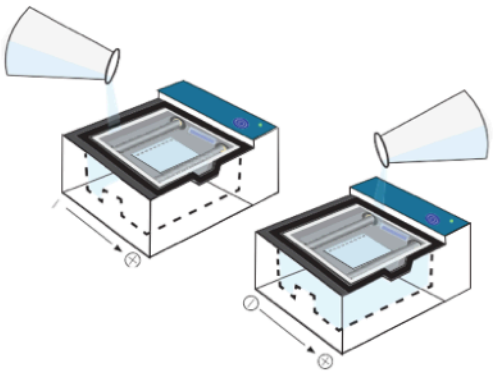
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



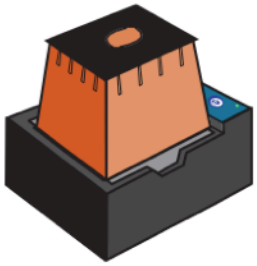
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

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.**

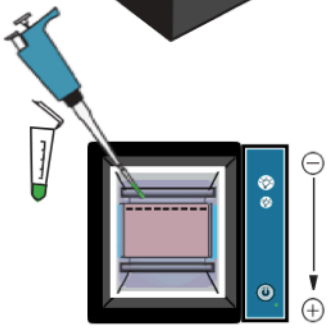


7. Place photo hood on the carriage.



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.



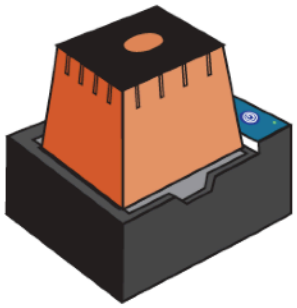
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

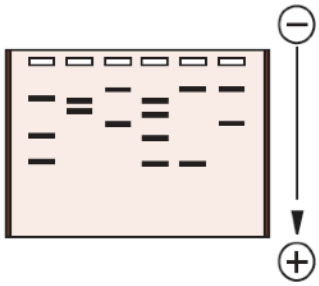
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   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**



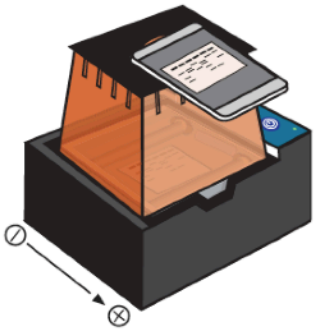
2. Have students periodically check the migration of the bands (~every five minutes).



3. Allow the gel to run **25 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.

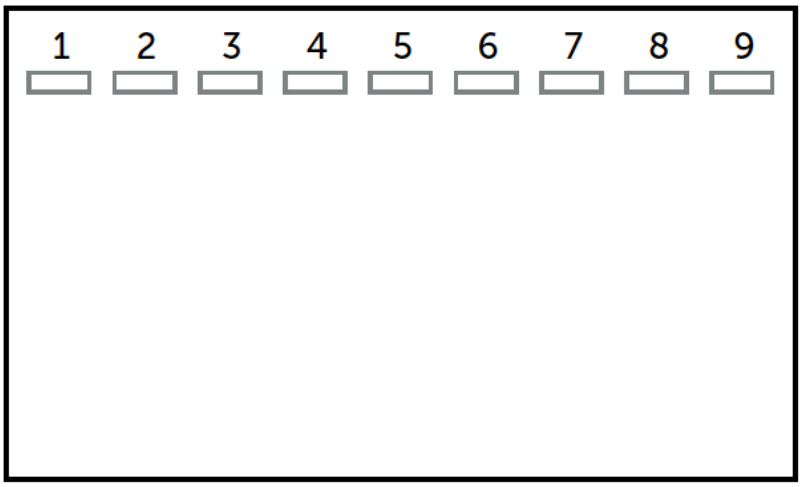
Clean Up

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 III: Results

What does your gel look like? Record images of the gel in the gel below



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____
Lane 7: _____
Lane 8: _____
Lane 9: _____

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