Analyzing a Crime Scene with DNA Supplemental Reagent Pack

Teacher's Prep and Student Protocol

Cat# M3053

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The digital version of the Student Worksheet can be found at
www.theminione.com/minione-teacher-and-student-guides/

In this hands-on MiniLab kit, students use the DNA evidence of a crime scene to determine whose blood has been left behind at the crime scene by evaluating the DNA fingerprints using the MiniOne® Electrophoresis System.

This MiniLab contains enough supplies for **10** groups to perform experiments.

Experimental Timing

From start to finish this lab will take 1 class period or 50 minute block.

Activity	Time Required
Pour gels	15 minutes
Load gels	10 minutes
Run and document gels	25 minutes

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.

MiniLab Components

Supplies included	Quantity
5 Ready-To-Load DNA samples Crime Scene Blood Sample - Table Crime Scene Blood Sample - Floor Eric Dom Anna	130 µL each
MiniOne® Universal DNA Marker (200, 400, 600, 800, 1000, 2000, 3000, 6000, and 10000 bp)	130 µL
GreenGel™ Cups, 1% agarose with GelGreen DNA stain, TBE buffer	10 cups
MiniOne® TBE buffer concentrate (enough to make 2L of 1X running buffer)	100 mL
0.65 mL microcentrifuge tubes	70 tubes
Universal fit micropipette tips	70 tips

Additional material Required (not included)

MiniOne Systems	Quantity Needed
MiniOne® Electrophoresis Systems - Carriage base, Running tank, Photo hood, 42 V Power supply	1 each per group
MiniOne® Casting System - Casting stand, Lid, Gel tray, Gel comb	1 each per group
MiniOne® Micropipette 2-20 µL adjustable volume	1 per group
Other Equipment	**Quantity Needed**
(dH_2O) for diluting running buffer	1900 mL
Benchtop microcentrifuge	1-3 per class
Microwave oven	1 per class
Fine tip permanent markers	1 each per group
Waste container for tubes and tips	1 per group
Lab coats/aprons, gloves, eye protection	1 per student
Microcentrifuge tube racks (optional)	1 per group

Pre-Lab Preparation

This MiniLab provides enough materials to run 10 workstations. The following suggested preparation steps assume that you are preparing to run all 10 workstations.

Prepare Running Buffer

1. The Reagent Pack provides 100 mL of concentrated Tris-Borate EDTA (TBE) Concentrate. Dilute 1 volume of the TBE Concentrate with 19 volumes of DI water to get TBE running buffer. Estimate that each experiment will need 135 mL of TBE running buffer.

   Sample calculation: (enough for 10 runs) Final volume: 2000 mL TBE Concentrate needed: 2000 mL / 20 =100 mL DI water needed: 2000 mL - 100 mL = 1900 mL

2. Following the calculation, add 100 mL of TBE Concentrate to 1900 mL of DI water to get 2L of TBE running buffer.

Optional: Aliquot 135 mL of the TBE running buffer for each group of students before class starts, using conical flasks or beakers.

Prepare Sample Aliquots

The Reagent Pack provides a total of 130 µL for each sample. With the provided microcentrifuge tubes, label and aliquot samples as follows:

Tube Label	Sample Name	Aliquot volume per tube
A	MiniOne® Universal DNA Marker	12 µL
B	Blood - Floor	12 µL
C	Blood - Table	12 µL
D	Eric	12 µL
E	Dom	12 µL
F	Anna	12 µL

Set up common workstations

- Benchtop microcentrifuge (optional for spinning tubes down if needed)
- Microwave
- Gloves, eye protection, lab coats

Set up student group workstations:

- 2–20 µL micropipette and 2-20 µL adjustable volume micropipette
- Universal fit micropipette tips, at least 6 tips per student group
- Aliquoted DNA samples (5 samples, plus DNA marker)
- MiniOne® Electrophoresis, and MiniOne Gel Casting System
- Waste container for tips and tubes
- Microcentrifuge tube rack (optional)
- Fine point permanent marker
- Gloves, eye protection, lab coats

MiniOne Visual Instructions for Electrophoresis

Materials

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

Lane #	Tube Label	Tube Contents	Load Volume
1	A	MiniOne Universal Marker	10 µL
2	B	Blood from crime scene: Table	10 µL
3	C	Blood from crime scene: Floor	10 µL
4	D	Eric	10 µL
5	E	Dom	10 µL
6	F	Anna	10 µL

How to Cast a Gel

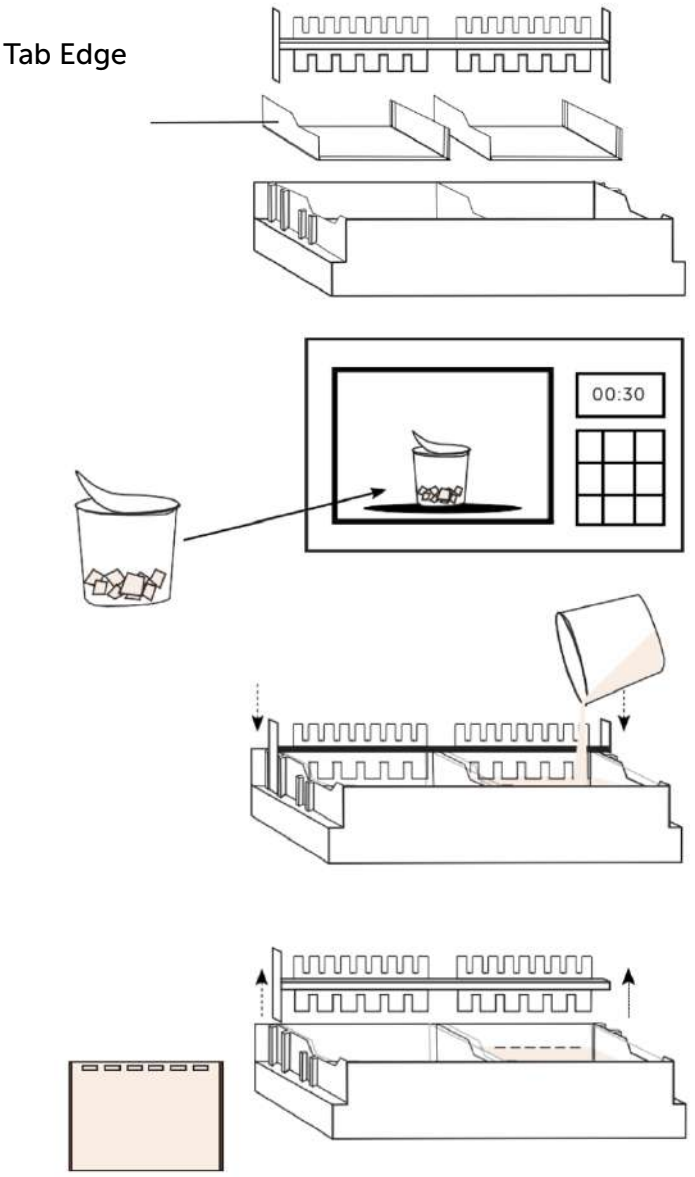
1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

2. **Partially** peel the film off of 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.

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.

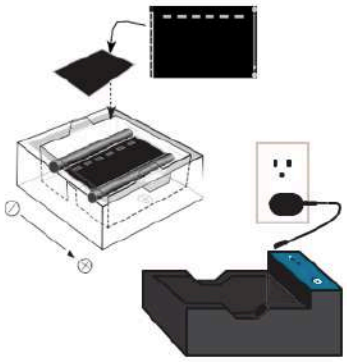
   DO NOT disturb the gel until time is up.

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

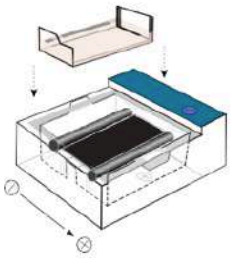
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

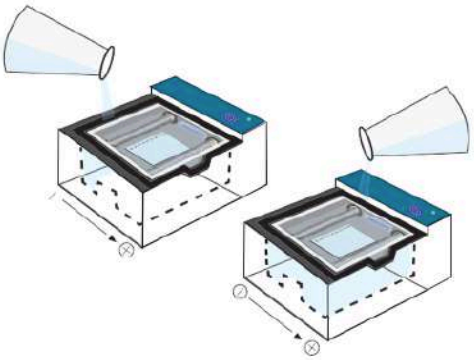
3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

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.



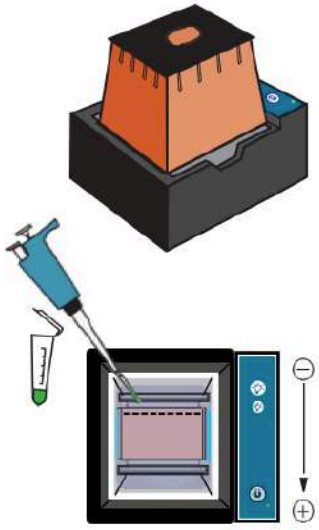
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.



6. Measure 135 mL of TBE 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.

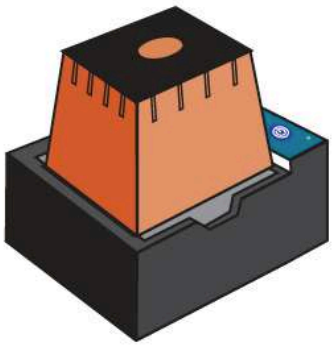
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

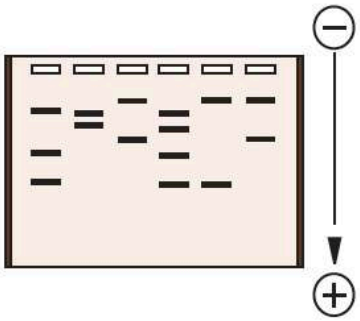
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage.**
   - **There is no or too little buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage.**
   - **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**



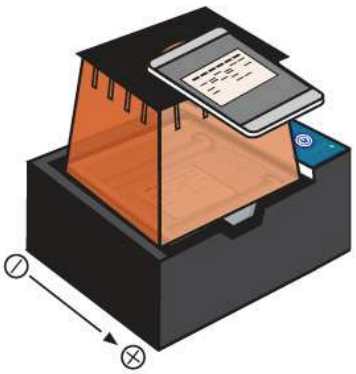
2. Have students periodically check the migration of the bands (~every five minutes).



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.

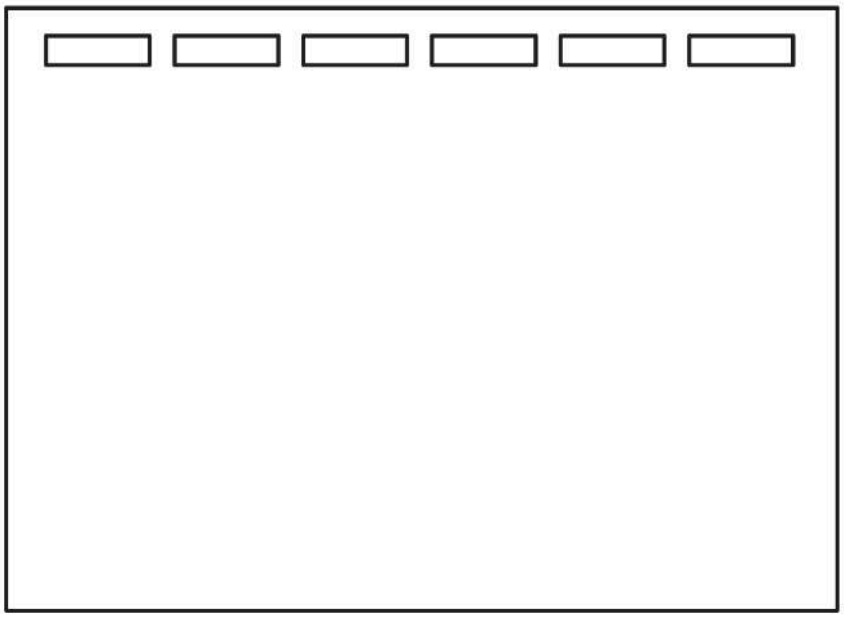
Clean Up

Note: All reagents in this lab can be disposed 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.

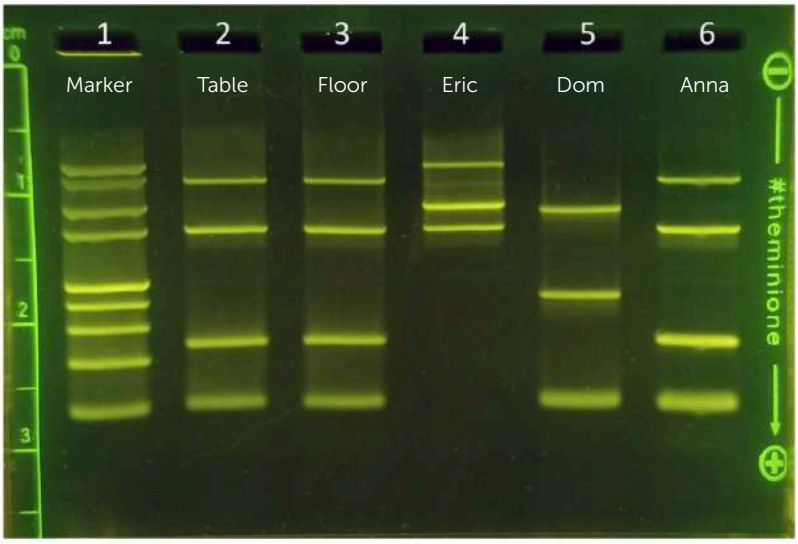
Part II: Results

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



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

Example Results:



Lane #	Tube Label	Tube Contents
1	A	MiniOne Universal marker (200, 400, 600, 800, 1000, 2000, 3000, 6000, and 10000 bp)
2	B	Blood from crime scene: Table
3	C	Blood from crime scene: Floor
4	D	Eric
5	E	Dom
6	F	Anna

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