



Breast Cancer Genetics Reagent  
Pack  
Student Protocol

**Cat# M3054**

Version 031824



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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.

## Part I: Electrophoresis

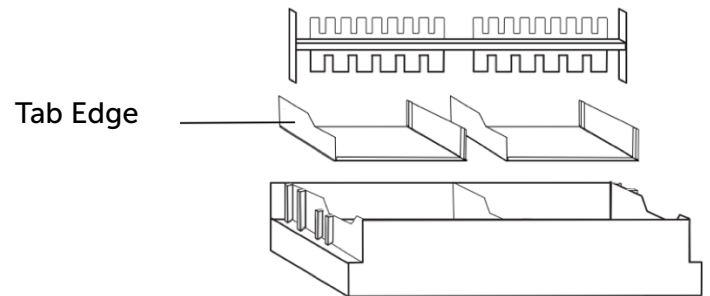
### Materials

- 1 MiniOne® Casting System
- 1 MiniOne® Electrophoresis System
- 1 agarose GreenGel™ cup (1.5 %)
- 6 DNA sample aliquots
- 135 mL of running buffer
- 1 micropipette (2-20µL)
- 10 pipette tips

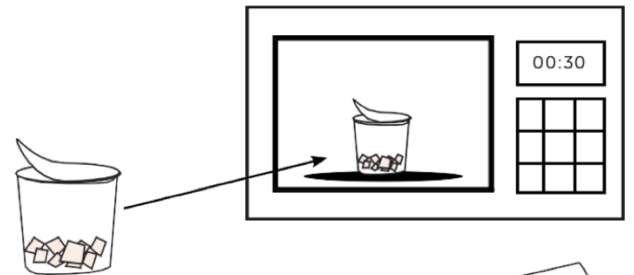
Lane #	Sample Name	Volume
1	MiniOne® marker (100, 300, 500, 1K, 2K bp) (M1M)	10 µL
2	Diana	10 µL
3	Jennifer	10 µL
4	Laura	10 µL
5	Judy	10 µL
6	Negative Control (N)	10 µL

### 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.

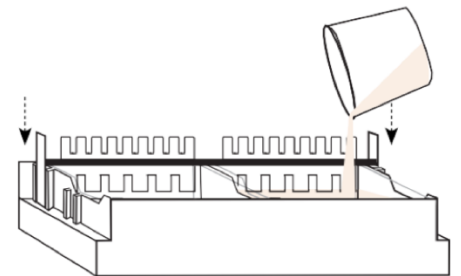


2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.



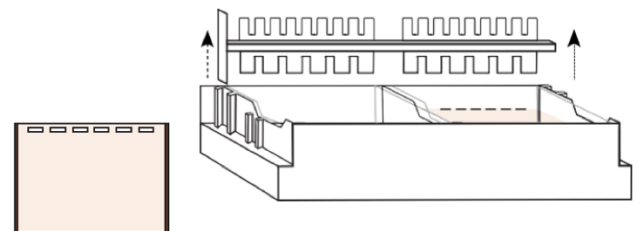
**Safety requirement: Adult supervision required if students are handling gel cups!**

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.




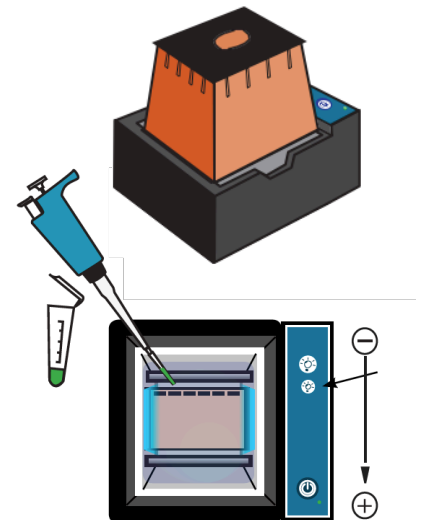
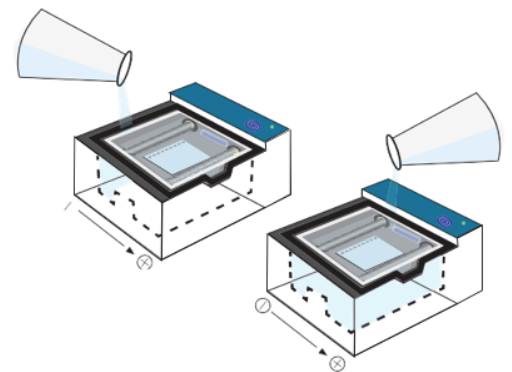
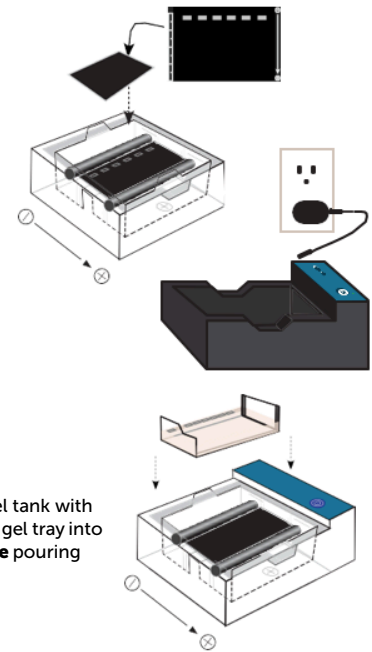
**DO NOT disturb the gel until time is up.**

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 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  $\mu\text{L}$  per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

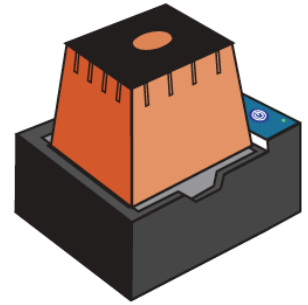



## Run, Visualize and Capture Image

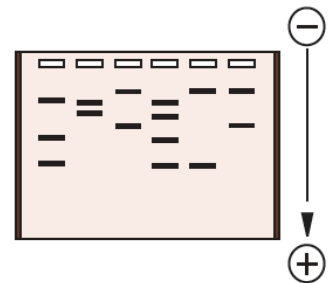
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the  button. The green LED next to the button will turn on.

**The green power LED will not turn on if:**

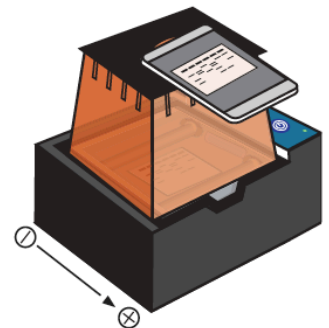
- The tank is not properly placed inside the carriage.
- There is no buffer in the tank.
- The buffer is too diluted.
- The photo hood is not on the carriage.
- There is too little running buffer.
- The power supply is not plugged in. Check by turning on the blue LEDs.
- If the green power LED is blinking, 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). (Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.)
3. Allow the gel to run **25 - 30 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.
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.

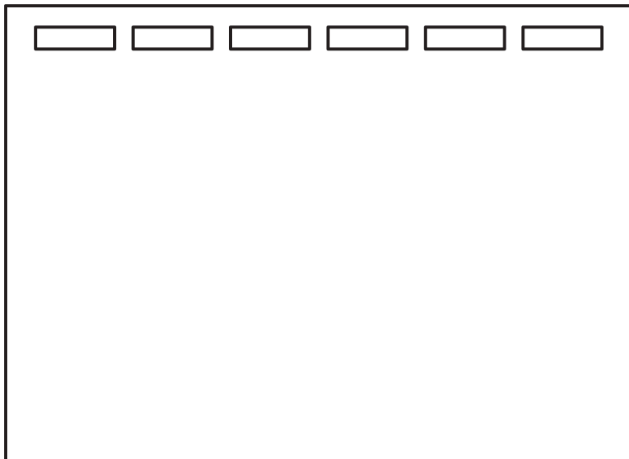
## Clean Up

**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 BUT 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:** \_\_\_\_\_

## Part III: Data Analysis

- Using a ruler, measure the distance traveled from the bottom of the well to the bottom edge of each fragment band (Fragments 1 - 5, this is Distance A in Table 1. Then measure the distance from the bottom of the well to the bottom edge of the gel, this distance B (this is the same for all 5 fragments). Calculate the R<sub>f</sub> value for each fragment by dividing the A and B values. Record your data in Table 1.



DNA Size Markers	Fragment length in base pairs (bp)	A = Distance Migrated (mm)	B = Distance to Reference Point (mm)	R <sub>f</sub> (A/B)
Fragment 1	2000			
Fragment 2	1000			
Fragment 3	500			
Fragment 4	300			
Fragment 5	100			

- Plot a standard curve (fragment size vs R<sub>f</sub>) on Log-Y (semi-log) paper. Plot R<sub>f</sub> values on the X-axis, and fragment size on the Y-axis.
- Next, calculate the R<sub>f</sub> for the patient samples by measuring the A and B values for each fragment, and dividing the two values, as you previously did in Step 1. Record your values in Table 2 below.

DNA Sample	Fragment	A = Distance Migrated (mm)	B = Distance to Reference Point (mm)	R <sub>f</sub> (A/B)
Tube 2 - Diana	Fragment 1			
	Fragment 2			
Tube 3 - Jennifer	Fragment 1			
	Fragment 2			
Tube 4 - Laura	Fragment 1			
	Fragment 2			
Tube 5 - Judy	Fragment 1			
	Fragment 2			

4. Use your standard curve that you drew in Step 2 to determine the fragment lengths of each of your DNA samples. Record in Table 3.

Table 3			
DNA Sample	Fragment	Fragment length calculated from the graph (bp)	Allele Present
Tube 2 - Diana	Fragment 1		
	Fragment 2		
Tube 3 - Jennifer	Fragment 1		
	Fragment 2		
Tube 4 - Laura	Fragment 1		
	Fragment 2		
Tube 5 - Judy	Fragment 1		
	Fragment 2		

5. Look up each fragment length calculated from the graph and find the number of corresponding alleles present from Table 4. For example, if you calculated that the fragment length is 800 bp, the number of alleles present would be 7. Record in Table 3.


Table 4	
Fragment Length in Base Pairs:	Allele:
200	Allele 1
300	Allele 2
400	Allele 3
500	Allele 4
600	Allele 5
700	Allele 6
800	Allele 7
1050	Allele 8



# miniOne®

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Patents issued: US 10,641,731 B2, US 20110253541 A1, US-11879118-B2