Updated: July 2019

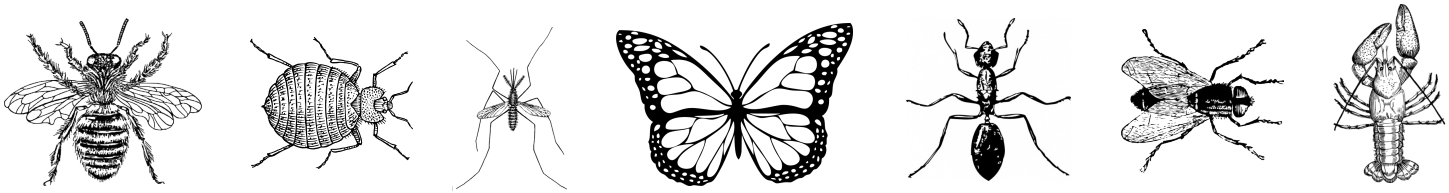
The Wolbachia Project

Discover the Microbes Within!

Lab 4: Gel Electrophoresis

MiniOne

Project Guide



The *Wolbachia* Project

1. Arthropod Identification
2. DNA Extraction
3. PCR
4. Gel Electrophoresis
5. Bioinformatics

The *Wolbachia* Project: Discover the Microbes Within! was developed by a collaboration of scientists, educators, and outreach specialists. It is directed by the Bordenstein Lab at Vanderbilt University.

https://www.vanderbilt.edu/wolbachiaproject

Preparation

Goal
The methods used during this lab will determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product.

Learning Objectives
In this activity you will learn how DNA samples separate based upon different sizes and learn how to visualize DNA samples. We will be using agarose gel electrophoresis to determine the presence and size of two different gene fragments (mitochondrial Cytochrome Oxidase I, and *Wolbachia* 16S rRNA) amplified by our PCR.

MiniOne Gel Electrophoresis System
This protocol uses the MiniOne electrophoresis system (https://theminione.com/) and GreenGel cups (MiniOne #M3102TBE). The unit contains a built-in transilluminator (blue light) and imaging doc. Students are able to visualize the DNA as it migrates through the gel by turning on the blue light. Be careful to not overuse the light feature as the stain is photosensitive and will bleach out with prolonged exposure to light.

If you wish to make your own gels, you will need a DNA stain that is compatible with blue light, such as GelGreen or SYBR Safe. The MiniOne casting trays hold about 11 ml of molten agarose and the electrophoresis system will need about 135 ml of running buffer.

Students will need a cell phone or other mobile device to take pictures.

Pre-Lab Preparation
If using the GreenGel cups, little to no pre-lab preparation is required. We recommend that you prepare a working solution of the TBE running buffer prior to class.

Prepare electrophoresis running buffer by adding 1 part TBE concentrate to 19 parts deionized or distilled water.

Note: Distilled water from the grocery store is suitable. Do not use tap water. Review the documents below for more information about using the MiniOne system.

Visit the MiniOne Resource Center for:
MiniOne Benchtop Guide
MiniOne User Manual
MiniOne Instructional Videos

https://theminione.com/minione-resource-center/

Overview

Introduction

Electrophoresis is a method of separating substances based on the rate of movement while under the influence of an electric field. Agarose is a polysaccharide purified from seaweed. An agarose gel is created by suspending dry agarose in a buffer solution, boiling until the solution becomes clear, and then pouring it into a casting tray and allowing it to cool. The result is a flexible gelatin-like slab.

During electrophoresis, the gel is submersed in a chamber containing a buffer solution and a positive and negative electrode. The DNA to be analyzed is pulled through the pores of the gel by the electrical current. Under an electrical field, DNA will move to the positive electrode (+) and away from the negative electrode (-). Several factors influence how fast the DNA moves, including; the strength of the electrical field, the concentration of agarose, and most importantly, the size of the DNA molecules. Smaller DNA molecules move through the agarose faster than larger molecules. DNA itself is not visible within an agarose gel. The DNA will be visualized by the use of a dye that binds to DNA.

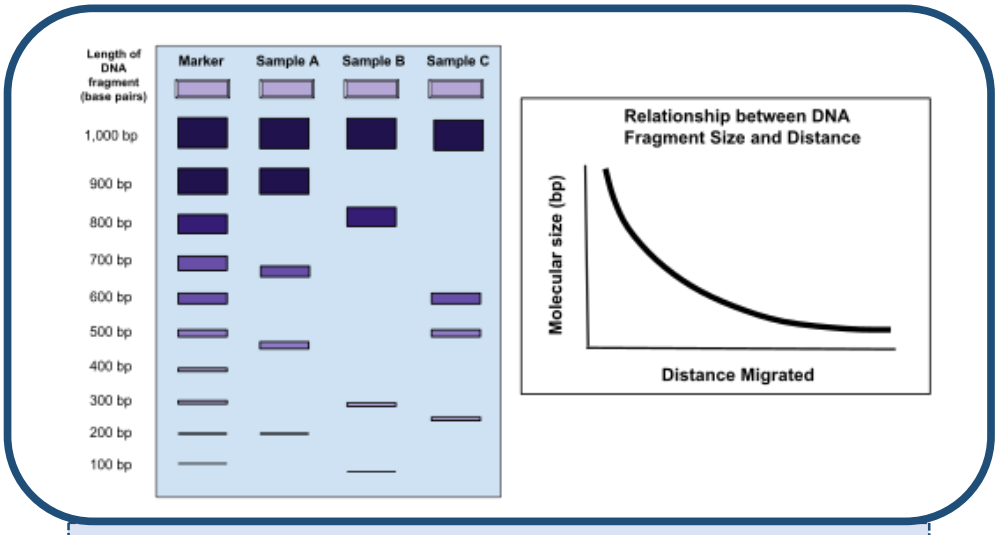


Figure 4.1. (A) A typical gel electrophoresis run in regards to the size of DNA fragments and the distance migrated through the agarose gel. In Lane 1, a DNA ladder is used as both an experimental control and as a reference for the length of the DNA (in base pairs). Individual DNA samples are loaded into Lanes 2-4. Smaller DNA fragments move farther throughout the agarose gel than larger fragments of DNA. These distances can be used to identify or match specific DNA sequences. (B) There is a nonlinear relationship between the size of the DNA fragments and the distance migrated. It is a negative curve because as DNA fragments get larger, they migrate less distance through the gel. Figure available via CC BY-SA 4.0 by Mckenzielower.

Gel Electrophoresis Protocol

Materials

- ❑ MiniOne - electrophoresis system
- ❑ MiniOne casting stand with gel trays & comb
- ❑ Microwave for class
- ❑ GreenGel cup, 1% TBE
- ❑ TBE running buffer
- ❑ Deionized or distilled water
- ❑ 250 ml graduated cylinder
- ❑ Your PCR products
- ❑ DNA ladder
- ❑ PCR tube rack
- ❑ Gloves
- ❑ Sharpie
- ❑ P20 pipette
- ❑ 1 box of P20 pipet tips
- ❑ Waste cup for tips
- ❑ Mobile device with camera

Class Preparation

1. Prepare a working solution of electrophoresis running buffer by adding 1 part TBE concentrate to 19 parts deionized or distilled water. This may have already been prepared by your teacher. *(See Note 4.1)*

Note 4.1: *Download the Benchtop Guide from https://theminione.com for details about preparing the buffer.*

Prepare the Gel

2. Place the MiniOne casting stand on a level surface and insert two gel trays.
3. Select the 9-tooth comb (in order to accommodate the DNA ladder and 6 PCR products) and insert into the top ridges of the casting tray.
4. Partially peel the film of a GreenGel cup to vent and place in the microwave for 20 seconds.
5. Allow the cup to cool for 15 seconds. *(See Note 4.2)*
6. Carefully pour the hot gel into one gel tray and let it sit for at least 10 minutes. Do not move the tray.
7. While you wait for the gel to solidify, measure out 135 ml of the diluted TBE running buffer.
8. Once the gel is solidified, carefully remove the comb. Lift the gel tray from the tray and wipe off excess agarose from the bottom of the tray. Do not remove the gel from the tray.

Note 4.2: *Do not microwave more than 5 cups at a time.*

Carefully handle the cup to prevent bubbles from forming in the agarose solution.

Prepare the Electrophoresis System

9. Insert the plastic viewing platform into the middle of the tank and place the solidified gel (along with gel tray) on top of the platform. The wells should be aligned with the (-) end of the tank. *(Note 4.3)*
10. Pour the diluted TBE running buffer into one side of the tank and allow it to flow to the other side. Ensure that air bubbles are not trapped under the tray.
11. Plug the power supply into the wall. Carefully place the tank into the carriage such that it is level and the - /+ ends are properly aligned. The electrodes should be touching the rivets.

Note 4.3: The tank should be freestanding and not inserted in the carriage at this point.

(Continued)

Gel Electrophoresis Protocol

Lane #	PCR Product
1	DNA Ladder
2	
3	
4	Uninfected (−) *Drosophila* Control
5	Infected (+) *Drosophila* Control
6	*Wolbachia* DNA
7	Water

Load the Gel

12. Press the smaller light button to turn on the low intensity blue light. This will help to visualize wells while loading samples.
13. Starting with the ladder, load 5 ul per well. Remember to change tips between each sample. When finished loading, turn off the light. *(See Note 4.4)*

Note 4.4: *5 ul works well for the 9-tooth comb. If using the 6-tooth comb, load 10 ul per well.*

Run the Gel

14. Place the orange photo hood on the carriage.
15. Turn on the electrophoresis system by pressing the power button. A green light should appear. If the green power LED does not appear, consult the MiniOne User Manual: https://theminione.com/minione-resource-center/
16. Allow the gel to run for about 20 minutes. Use the low intensity blue light to occasionally view the bands as they migrate down the gel. Do not leave the light on for an extended period as the DNA stain (GelGreen) is photosensitive and weakens with exposure to light. *(See Note 4.5)*
17. Once the run is complete, turn off the system by pressing the power button.

Note 4.5: *You may notice fuzzy bands at the bottom of the gel (even in the negative water control). These are primer dimers. Your PCR products will appear bright and sharp. They will initially run as a single band and then gradually separate into two – one for arthropod and one for* Wolbachia *DNA.*

Obtain an Image of the Gel

18. Use a soft cloth to wipe off condensation from the orange hood.
19. Place the hood back on the system and press the larger light button to turn on the high intensity blue light.
20. Place your cell phone, or other camera-ready mobile device, directly over the viewing hole to take a picture. There is not need to zoom because it is already at the optimal focal length.
21. Document your results on the next page.

Clean your Work Station

22. Discard used tips and wipe down the bench with 70% ethanol.
23. Refer to page 16 of the MiniOne Electrophoresis System Instruction Manual to clean, dry, and store the system.

https://theminione.com/minione-resource-center/

Results

Lane	DNA Source	Arthropod CO1 Band?	*Wolbachia* 16S Band?
1	DNA Ladder	N/A	N/A
2			
3			
4			
5			
6			
7			

Document your Results

24. Use the table above to record presence (+) or absence (-) of bands.

Interpret your Results

25. This experiment included 5 controls. In the table below, list the following for each lab activity:
    - (+) for positive control
    - (-) for negative control
    - N/A for not applicable

Control	DNA Extraction	PCR	Gel Electrophoresis
DNA Ladder			
Infected *Drosophila*			
Uninfected *Drosophila*			
(+) DNA			
Water			

26. Do electrophoresis results match the expected controls? _____
27. Based on this evaluation, complete the table below.

Label	Arthropod ID	*Wolbachia*-infected? (Yes, No, Unknown)