21–1208

Outbreak! Fingerprinting
Virus DNA

Teacher’s Manual
Overview

Students become virus hunters as they try to identify the viral agent for a potentially deadly, fictitious disease outbreak. Students perform electrophoresis using harmless predigested DNA samples that simulate samples from the fictitious viruses. In this lab exercise, students simulate the use of DNA fingerprinting to identify a particular strain of virus. They load precut DNA into agarose gels and perform electrophoresis to separate DNA fragments in three different samples—two representing two known virus strains, and one representing the unknown strain to be identified. They compare the banding patterns to identify the unknown virus strain.

Materials

The materials in this kit are sufficient for six complete setups of the experiment. The materials are supplied for use with the exercise in this kit only. Carolina Biological Supply Company disclaims all responsibility for any other use of these materials.

Materials included in the kit:

- 6 Vials Alabama Virus DNA*
- 6 Vials Pennsylvania Virus DNA*
- 6 Vials Missouri Virus DNA*
- 24 Plastic Transfer Pipets
- 150 mL 20x TBE Buffer Concentrate (for 3 L buffer)
- 4g Agarose (for 400 mL solution)
- 250 mL Carolina BLU™ Final Stain**
- 7 mL Carolina BLU™ Buffer/Agarose Stain**
- 6 Staining Trays
- 2 Pairs Disposable Gloves
- Teacher’s Manual
- Student Worksheet

*Note: Kit contains no human virus DNA—the DNA samples are from a harmless bacterial virus.

This kit may be stored at room temperature. During prolonged storage at room temperature, however, water may evaporate from the DNA solution. Although this does not damage the DNA, it decreases the solution volume.
and increases its viscosity. We therefore recommend that the DNA samples be refrigerated if they are to be stored for more than six weeks. Should you find that your samples have become viscous, simply add 10–15 µl water immediately before loading.

**The kit contains the nontoxic DNA stain Carolina BLU™. For the most efficient and sensitive staining with this dye, low concentrations of it are added to both the agarose gel and the electrophoresis buffer. During electrophoresis, Carolina BLU™ intercalates into the DNA, allowing the DNA bands to be seen during or at the end of electrophoresis. This allows students to immediately view their results, without the lengthy staining and destaining steps required for methylene blue. Following electrophoresis, the DNA bands can be stained more intensely by soaking the gel in a dilute solution of Carolina BLU™ (large bottle) for 15 minutes. Background stain in the gel can be removed by several washes in deionized water over a period of 30–40 minutes. This additional staining and destaining intensifies the staining of all the fragments, making the smallest fragments more visible than is usually possible with other blue stains.

## Scheduling

DNA electrophoresis requires several different activities. Plan your time as follows:

<table>
<thead>
<tr>
<th>Day</th>
<th>Time Needed</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several days before lab</td>
<td>30 min</td>
<td><strong>Pre-lab:</strong> Mix TBE Buffer</td>
</tr>
<tr>
<td>Lab Day 1</td>
<td>30 min</td>
<td><strong>Pre-lab:</strong> Prepare agarose solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Set up work stations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pool small volumes of reagents</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td><strong>Lab:</strong> Practice pipetting, gel loading (optional)</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>Cast agarose gel</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>Load gel</td>
</tr>
<tr>
<td></td>
<td>40 min</td>
<td><strong>Post-lab:</strong> Electrophorese</td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>Stain gels</td>
</tr>
<tr>
<td></td>
<td>30+ min</td>
<td>Destain gels</td>
</tr>
<tr>
<td>Lab Day 2</td>
<td>40 min</td>
<td>Results and discussion</td>
</tr>
</tbody>
</table>
Pre-Lab Preparation

Mix TBE Buffer
Because tris-borate-EDTA (TBE) buffer solution is stable, it can be made ahead of time and stored in a carboy or other container until ready to use. Add entire bottle (150 mL) of TBE buffer concentrate to 2850 mL of distilled or deionized water. Stir until all the concentrate is completely mixed.

Prepare Agarose Solution
Before class on Lab Day 1, prepare 1.0 % agarose solution. Add 4 g (entire bottle) of agarose to 400 mL TBE electrophoresis buffer in a clean 1-L flask or beaker. Cover with aluminum foil and heat in a boiling water bath (double boiler) for 10 to 20 min. Solution will become clear as agarose dissolves. Swirl and observe bottom to insure that no undissolved agarose remains. Alternatively, heat solution at high setting of microwave oven for 5 to 10 min, without aluminum foil. Cool solution to approximately 65° C before use. Cover with aluminum foil and keep warm in 60° C water bath until ready to use.

Add Carolina BLU™ Stain to Agarose and Buffer
The concentration of stain added to the agarose/buffer is dependent on the voltage used for electrophoresis. If electrophoresing at voltages less than 50 volts, a slightly lower concentration is used than if running at voltages greater than 50 volts. The stain may be added to the entire volume of agarose and distributed, or the agarose may be distributed to each lab station and the stain added by the students at the rates listed below.

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Agarose Volume</th>
<th>Stain Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 Volts</td>
<td>30 mL</td>
<td>40 µL (1 drop)</td>
</tr>
<tr>
<td></td>
<td>60 mL</td>
<td>80 µL (2 drops)</td>
</tr>
<tr>
<td></td>
<td>400 mL</td>
<td>533 µL (13 drops)</td>
</tr>
<tr>
<td>&gt; 50 Volts</td>
<td>50 mL</td>
<td>80 µL (2 drops)</td>
</tr>
<tr>
<td></td>
<td>400 mL</td>
<td>640 µL (16 drops)</td>
</tr>
</tbody>
</table>
After addition of the stain to the agarose, swirl to mix and immediately pour the gel. Gels may be prepared one day ahead of the lab day, if necessary. Gels stored longer tend to fade and lose their ability to stain bands during electrophoresis. Store covered with a small amount of buffer (leaving masking tape in place), or store covered in the gel box. Don’t try using more stain than recommended in your gel. This leads to precipitation of the DNA in the wells and can create artifactual aggregated DNA bands in the agarose gel.

Use the chart below for addition of the stain to 1× TBE electrophoresis buffer:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Buffer Volume</th>
<th>Stain Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 Volts</td>
<td>500 mL</td>
<td>500 µL (12 drop)</td>
</tr>
<tr>
<td></td>
<td>2.6 L</td>
<td>2.6 mL (65 drops)</td>
</tr>
<tr>
<td>&gt; 50 Volts</td>
<td>500 mL</td>
<td>960 µL (24 drops)</td>
</tr>
<tr>
<td></td>
<td>2.6 L</td>
<td>5 mL (125 drops)</td>
</tr>
</tbody>
</table>

The dropper bottle provided delivers 40 µL/drop. If a calibrated pipet is available, the dropper tip can be removed for quicker addition of larger volumes of stain. The volume of buffer and agarose required for some gel box options are listed below:

<table>
<thead>
<tr>
<th>Type Gel Box</th>
<th>Volume Buffer Required</th>
<th>Volume Agarose Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini Gel System Box</td>
<td>200 mL</td>
<td>30 mL/ casting tray</td>
</tr>
<tr>
<td>Carolina Gel Box, 1 tray</td>
<td>250 mL</td>
<td>50 mL</td>
</tr>
<tr>
<td>Carolina Gel Box, 2 trays</td>
<td>450 mL</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

While *Carolina BLU™* is not toxic, we recommend that the students wear gloves to prevent staining the skin. TBE buffer containing *Carolina BLU™* stain may be reused if the reuse occurs within a day or so. If left longer, the dye loses its ability to stain DNA during electrophoresis. If reusing the buffer for several days is important, we recommend using *Carolina BLU™* in the gel and as a final stain only.
Pool Small Volumes of Reagents
Because of the small volumes supplied, restriction enzymes and lambda DNA often become spread in a film around storage tube wall or cap during shipping. Therefore, it is recommended that these reagents be pooled at the bottom of their storage tubes prior to setting up workstations, using one of three methods:

1. Spin tubes briefly in a microfuge.
2. Spin tubes briefly in a preparatory centrifuge, using adaptor collars for 1.5-mL tubes. Alternately, spin tubes within 15-mL tube, and remove carefully.
3. Tap tubes sharply on bench top.

Set Up Student Stations
1. Prepare six student stations, each with the following materials:
   - Vial Alabama virus DNA
   - Vial Pennsylvania virus DNA
   - Vial Missouri virus DNA
   - 3 plastic transfer pipets
   - Permanent marker
   - Test tube rack
   - Beaker for waste
   - Power supply
   - Gel electrophoresis chamber
   - Staining tray
   - Gel-casting tray
   - Well-forming comb
   - Masking tape
   - Student worksheets

2. Groups must share the following materials: agarose solution; TBE electrophoresis buffer; and Carolina BLU™ stain.

3. Hold agarose solution at 60° C in a water bath.

Background For Students
Discuss as much of this background information with your students as you deem appropriate.

This kit focuses on virus DNA fingerprints because the genomes of viruses are small enough to give discernible patterns using only restriction enzyme digestion and normal agarose gel electrophoresis. Genomes of other organisms, even bacteria, are too large to give recognizable fingerprints following digestion with most restriction enzymes. For example, the genome of the bacterium Escherichia coli is approximately 4 million base pairs in length. The restriction enzyme EcoRI cleaves E. coli DNA into approximately 800 fragments. In a normal agarose gel, these fragments overlap and form a smear rather than a distinct pattern.
See the Further Resources section on pg. 10 for a variety of appropriate background materials.

**Fine Points Of Lab Procedure**

**Storing Cast Agarose Gels**
If needed, students may cast gels a day before use. Keep gels covered with TBE buffer to prevent their drying out.

**Electrophoresis**
The migration of DNA through the agarose gel is dependent upon voltage—the higher the voltage the faster the rate of migration. Best separation is achieved when the bromophenol blue band (the faster-moving band of the loading dye) nears the end of the gel. Stop electrophoresis before the bromophenol blue runs off the end of the gel. During electrophoresis, *Carolina BLU™* intercalates into the DNA, allowing for its immediate visualization. Refer to chart below for approximate running times at various voltages for the CBS gel box with a gel of 84 × 96 mm. Times for other apparatus will vary according to size.

![Graph showing voltage vs. time for electrophoresis](image)
Viewing and Photographing Gels

Transillumination, in which light passes up through a gel, gives superior viewing of gels stained with Carolina BLU™. A fluorescent light box for viewing slides and negatives provides ideal illumination for Carolina BLU™-stained gels. An overhead projector may also be used. Cover the surface of light box or projector with plastic wrap to keep liquid off the apparatus. A Polaroid® “gun” camera, equipped with close-up diopter lens, can be used to photograph gels. A plastic hood extending from the front of the camera forms a mini darkroom and provides correct lens-to-subject distance. A close-focusing 35-mm camera can also be used to photograph transilluminated gels.

Lab Procedure

Read the student lab instructions (reprinted for you in this manual) and be aware of the steps students will need your help with (e.g., setting voltage for electrophoresis, staining gels).

Procedure A: Cast Agarose Gel

1. Seal ends of gel-casting tray with tape and insert well-forming comb. Place gel-casting tray out of the way on lab bench, so that agarose poured in next step can set undisturbed.

2. Carefully pour enough agarose solution into casting tray to fill to a depth of about 5 mm. Gel should cover only about \( \frac{3}{4} \) the height of comb teeth. Use a pipet tip or toothpick to move large bubbles or solid debris to sides or end of tray while gel is still liquid.

3. Gel will become cloudy as it solidifies (about 10 min). Do not move or jar casting tray while agarose is solidifying.

4. When agarose has set, unseal ends of casting tray. Place tray on platform of gel box, so that comb is at negative (black) end.

5. Fill box with tris-borate-EDTA (TBE) buffer to level that just covers entire surface of gel.

6. Gently remove comb, taking care not to rip wells.

7. Make certain that sample wells left by comb are completely submerged. If “dimples” are noticed around wells, slowly add buffer until they disappear.

8. The gel is now ready to load with DNA.
**Note:** This is an optional stopping point. Students should cover the electrophoresis chamber to prevent gel from drying out.

**Procedure B: Load Gel**

Use micropipet to load contents of each reaction tube into a separate well in gel. Use a fresh tip for each reaction tube.

1. Draw sample into pipet tip or other gel-loading device.

2. Steady pipet over well using two hands.

Be careful to expel any air in micropipet tip end before loading gel. (If air bubble forms “cap” over well, DNA/loading dye will flow into buffer around edges of well.)

Dip pipet tip through surface of buffer, position it over the well, and slowly expel the mixture. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. Be careful not to punch tip of pipet through the bottom of the gel.

**Procedure C: Electrophorese**

1. Close top of electrophoresis chamber, and connect electrical leads to an approved power supply, anode to anode (red-red) and cathode to cathode (black-black). Make sure both electrodes are connected to same channel of power supply.

2. Turn power supply on and set voltage. Use 130 volts for approximately 45-minute runs. Use 10–15 volts for overnight runs (refer to chart on pg. 7). Shortly after current is applied, loading dye should be seen moving through gel toward positive pole of electrophoresis apparatus.

3. The loading dye will eventually resolve into two bands of color. The faster-moving, purple band is the dye bromophenol blue; the slower-moving aqua band is xylene cyanol. Bromophenol blue migrates through gel at same rate as a DNA fragment approximately 300 base pairs long. Xylene cyanol migrates at a rate equivalent to approximately 2000 base pairs.

4. Allow the DNA to electrophorese until the bromophenol blue band is about 2 cm from the end of the gel. You may wish to monitor the electrophoresis after students have left class and turn off the power supplies yourself. You could then stain and destain gels for the students, or simply leave the gels in the covered electrophoresis chambers for students to stain.
5. Turn off power supply, disconnect leads from the inputs, and remove top of electrophoresis chamber.

6. Carefully remove casting tray and slide gel into staining tray. Students should be directed to bring their gels to the instructor for staining. Cover each gel with Final Stain solution and let it sit about 15 minutes. The gel should become darker blue. Destain the gels in several rinses of distilled water to remove the blue background. Allowing the gels to sit in the staining trays covered with distilled water overnight also gives good results.

**Note:** Do not use chlorinated tap water to destain gels; chlorine bleaches the dye.

**Results And Discussion**

Students should analyze the DNA fingerprints in their gels and answer the following questions (found on their worksheets).

1. Compare the fingerprints of the Pennsylvania, Alabama, and Missouri virus isolates. What can you conclude about the virus infecting the Missouri patients?

   *The virus infecting the Missouri patients has the same DNA fingerprint as the Pennsylvania strain. This is the less dangerous virus strain.*

2. Why would the restriction fragment patterns from the two viruses be different?

   *Students should be able to explain that the two viruses are different because their genes, or DNA molecules, are different. Some of the differences in DNA produce changes in restriction enzyme recognition sites, leading to different DNA fingerprints.*

**Further Resources**

The activity in this kit can be used as a starting point for teaching a number of different topics that most students find very interesting. Public awareness of emerging viral diseases has been heightened by popular books such as *The Hot Zone* by R. Preston and *The Coming Plague* by L. Garrett (Carolina item 45-6495), as well as by media coverage of the Ebola outbreaks in Zaire and the hantavirus outbreak in the American Southwest. The resources below might help you extend your study.


This article is especially good for background on hemorrhagic fever.
Good general background on viruses.

To understand what a viral DNA fingerprint is and how it can be used to identify a viral strain, students need to have a basic understanding of DNA structure and function and to know what restriction enzymes do and how electrophoresis works. This book includes background information and models for teaching these topics.


Topics for discussion and further research
• The nature of viruses and viral infection
• How outbreaks of infectious disease are monitored in the United States
• Viral and bacterial epidemics in general
• The history of epidemics in the United States:
  –What epidemics have occurred in this country in the past (such as typhoid fever, yellow fever)
  –How they have been controlled (through means such as mosquito control, water purification, etc.)
  –How likely they are to reoccur
• The importance of basic public health measures such as water purification and sewage systems for preventing epidemics
• Differences between treatments for viral and bacterial diseases
• The threat, if any, posed by the emergence of antibiotic-resistant bacterial strains
• What careers are available in microbiology and epidemiology? (For more information contact the American Society for Microbiology, 1325 Massachusetts Ave. NW, Washington, DC 20005-4171, phone 202-737-3600. ASM has an education outreach program.)
It is the year 2015. You are a molecular biologist working for the Centers for Disease Control and Prevention (CDC). Your job is to help track epidemics and to monitor emerging diseases.

Five years ago, a cluster of cases of hemorrhagic fever occurred in an isolated town in northeastern Alabama. The disease killed approximately 30% of those who caught it. The most alarming aspect of the new disease was that it was highly contagious from human to human, thus posing the threat of an epidemic. Medical authorities, including your office, believe the only reason the outbreak did not erupt into a major epidemic was that the Alabama town was so small and isolated. A CDC team traced the disease to a virus carried by the numerous local squirrels. An extensive trapping campaign was carried out in an attempt to eliminate the virus by eliminating the squirrels infected with it. No further cases have been reported in Alabama.

Three years ago a suspicious outbreak occurred in Pennsylvania. Several people fell ill with a hemorrhagic fever. The symptoms of the disease were the same as those of the Alabama fever, but no one died. The Pennsylvania fever was apparently less contagious than the Alabama fever since the Pennsylvania patients were exposed to many people who did not come down with the disease. However, the Pennsylvania virus was also traced to the local squirrel population.

You were asked to compare the viruses that caused the two outbreaks. You found that the virus particles looked similar. Both viruses had a DNA genome, but the base sequences of the genomes were different in many places.

Now, three people in Missouri have fallen ill with a hemorrhagic fever. Their symptoms are similar to the symptoms of the Alabama and Pennsylvania fevers. Local medical personnel were alarmed and called the CDC to determine if the dangerous Alabama virus had reappeared. The patients have been placed in quarantine. You are flown to the scene.

You must immediately determine whether the Missouri patients are infected with the highly contagious and deadly Alabama virus, the Pennsylvania virus, or some other agent.

One of the techniques you decide to use is examination of viral DNA by restriction analysis. Wearing protective clothing, you enter the patients’ isolation rooms in the Missouri hospital, carefully draw samples, place them on ice, and rush back to the biological containment laboratory at the CDC for a variety of tests. For the restriction analysis, you isolate virus particles, extract DNA, and subject the samples to restriction digestion and agarose gel electrophoresis. In your gel you include samples of DNA from the Pennsylvania and Alabama viruses for comparison.

**Procedure A: Cast Agarose Gel**

1. Seal ends of gel-casting tray with tape, and insert well-forming comb. Place gel-casting tray out of the way on lab bench, so that agarose poured in next step can set undisturbed.

2. Carefully pour enough agarose solution into casting tray to fill to depth of about 5 mm. Gel should cover only about ¼ the height of comb teeth. Use a pipet tip or toothpick to move large bubbles or solid debris to sides or end of tray, while gel is still liquid.

3. Gel will become cloudy as it solidifies (about 10 min). Do not move or jar casting tray while agarose is solidifying.

4. When agarose has set, unseal ends of casting tray. Place tray on platform of gel box, so that comb is at negative (black) end.

5. Fill box with tris-borate-EDTA (TBE) buffer, to level that just covers entire surface of gel.

6. Gently remove comb, taking care not to rip wells.
7. Make certain that sample wells left by comb are completely submerged. If dimples are noticed around wells, slowly add buffer until they disappear.

8. The gel is now ready to load with DNA.

Note: If this will be your stopping point for the lab period, cover the electrophoresis chamber to prevent gel from drying out.

Procedure B: Load Gel

Use micropipet to load contents of each reaction tube into a separate well in gel. Use a fresh tip for each reaction tube.

1. Draw sample into pipet or gel-loading device.

2. Steady pipet over well using two hands.

3. Be careful to expel any air in micropipet tip end before loading gel. (If air bubble forms a cap over well, DNA/loading dye will flow into buffer around edges of well.)

4. Dip pipet tip through surface of buffer, position it over the well, and slowly expel the mixture. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. Be careful not to punch tip of pipet through the bottom of the gel.

Procedure C: Electrophorese

1. Close top of electrophoresis chamber, and connect electrical leads to an approved power supply, anode to anode (red-red) and cathode to cathode (black-black). Make sure both electrodes are connected to same channel of power supply.

2. Turn power supply on and set voltage as directed by your instructor. Shortly after current is applied, loading dye can be seen moving through gel toward positive pole of electrophoresis apparatus.

3. The loading dye will eventually resolve into two bands of color. The faster-moving, purplish band is the dye bromophenol blue; the slower-moving, aqua band is xylene cyanol. Bromophenol blue migrates through gel at same rate as a DNA fragment approximately 300 base pairs long. Xylene cyanol migrates at a rate equivalent to approximately 2000 base pairs.

4. Allow the DNA to electrophorese until the bromophenol blue band is about 2 cm from the end of the gel. Your instructor may monitor the progress of electrophoresis in your absence; in that case, omit Steps 5 and 6.

5. Turn off power supply, disconnect leads from the inputs, and remove top of electrophoresis chamber.

6. Carefully remove casting tray, and slide gel into staining tray labeled with your group name. Take gel to your instructor for staining.

Questions

1. Compare the fingerprints of the Pennsylvania, Alabama, and Missouri virus isolates. What can you conclude about the virus infecting the Missouri patients?

2. Why would the restriction fragment patterns from two different viruses be different?