Outbreak!
Fingerprinting Virus DNA Kit

TEACHER'S MANUAL AND
STUDENT GUIDE

CAROLINA®
World-Class Support for Science & Math

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TEACHER'S MANUAL

STUDENT GUIDE*

*Photocopy the Student Guide as needed for use in your classroom.

Storage Note: The DNA in this kit has been stabilized for short-term storage at room temperature. However, if the DNA is to be kept for more than 6 weeks it should be frozen or refrigerated. All other components in this kit may be stored at room temperature.

Outbreak! Fingerprinting Virus DNA Kit

Overview

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

Objectives

Students will

- perform electrophoresis, a basic technique for separating DNA fragments by size.
- learn about the use of restriction enzymes in DNA fingerprinting.
- understand some of the thought processes involved in analyzing an infectious disease outbreak.

Content Standards

This kit is appropriate for high school students and addresses the following National Science Education Standards:

Grades 9–12

Science as Inquiry

- Abilities necessary to do scientific inquiry

Science and Technology

- Understandings about science and technology

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Time Requirements

Plan your time as follows.

Day	Time Required	Activity	Description
Several Days Before the Lab	30 min	Pre-lab:	Mix TAE buffer Make copies of the Student Guide
Lab Day 1	60–90 min	Pre-lab:	Prepare agarose solution Set up workstations Pool small volumes of DNA
	15 min	Lab:	Practice pipetting and gel loading (optional)
	20 min		Cast agarose gels
	15 min		Load gels
	40+ min		Electrophorese
	20 min	Post-lab:	Stain gels
	40 min to overnight		Destain gels
Lab Day 2	40 min		Results and discussion

Materials

The materials in the 8-station kit are sufficient for eight complete setups of the experiment. The materials in the 4-station kit are sufficient for four complete setups of the experiment. Refer to the table below. The materials are supplied for use with the exercise in this kit only. Carolina Biological Supply Company disclaims all responsibility for any other uses of these materials. The kits include:

Kit Component	4-Station Kit (211206)	8-Station Kit (211207)
vials of Alabama Virus DNA* (20 µL)	4	8
vials of Missouri Virus DNA* (20 µL)	4	8
vials of Pennsylvania Virus DNA* (20 µL)	4	8
TAE buffer 50× concentrate	50 mL	100 mL
agarose	2.4 g	5 g
disposable plastic needle-nose transfer pipets	16	32
*Carolina*BLU™ Gel and Buffer Stain (7 mL)	1	1
*Carolina*BLU™ Final Stain (250 mL)	1	1
staining trays	4	8
pairs of disposable gloves	4	8
Teacher's Manual and reproducible Student Guide	1	1

***Note:** This kit contains no human virus DNA. The DNA in the samples is from bacteriophage lambda, a harmless bacterial virus. The DNA samples contain loading dye.

The quantities of agarose and 50× TAE buffer provided are sufficient for electrophoresis chambers requiring 50-mL gels and 250–300 mL of buffer in the chamber.

Storage Note: The DNA in this kit has been stabilized for short-term storage at room temperature. However, if the DNA is to be kept for more than 6 weeks, it should be frozen or refrigerated. For storage longer than 6 months the DNA should be frozen. All other components in this kit may be stored at room temperature.

Needed, but not supplied:

PPE for instructor and students, as appropriate

gel electrophoresis chambers (for 4 gels or 8 gels depending upon which kit is used)

power supplies (to accommodate the required number of chambers; can be shared by stations)

masking tape (if needed to seal gel casting trays)

racks for holding samples (optional; 4 or 8 depending upon how many stations you have)

1-L graduated cylinder for diluting and measuring TAE buffer

distilled or deionized water

container for holding 3 L (4-station kit) or 5 L (8-station kit) 1× TAE buffer

0.5-L (4-station) or 1-L (8-station) flask for melting agarose

boiling water bath, hot plate, or microwave oven for melting agarose

60°C water bath for keeping agarose liquid until poured (optional, but convenient)

aluminum foil

permanent markers (4 or 8)

microcentrifuge or preparatory centrifuge for pooling DNA (optional)

funnel (optional; for pouring stain back into bottle after use)

white-light box, slide viewer, or overhead projector for viewing *Carolina*BLU-stained gels

resealable plastic bags, plastic wrap, or containers with lids (optional; if storing gels overnight)

instant or digital camera for recording results

Safety

Individuals should use this kit only in accordance with prudent laboratory safety precautions and under the supervision of a person familiar with such precautions. Use of this kit by unsupervised or improperly supervised individuals could result in serious injury.

Ensure that students understand and adhere to safe laboratory practices when performing any activity in the classroom or lab. Demonstrate the protocol for correctly using the instruments and materials necessary to complete the activities, and emphasize the importance of proper usage. Use personal protective equipment such as safety glasses or goggles, gloves, and aprons when appropriate. Model

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proper laboratory safety practices for your students and require them to adhere to all laboratory safety rules.

The gels run at a significantly high voltage. Under no circumstance should students place their hands in the buffer when there is current running through it. Either use gel boxes that do not run unless the lids are securely in place, or instruct your students not to remove the lids unless the power supply has been turned off and the gel boxes disconnected.

Background

The Student Guide includes both basic and more in-depth background information relevant to the lab. The background information preceding the laboratory procedure assumes that students already are familiar with PCR and restriction enzyme digestion. Two additional handouts, one on Polymerase Chain Reaction and the other on DNA fingerprinting of viruses, have been included at the end of the Student Guide. You may find these useful if your students are not already familiar with these subjects.

See the Resources section for a variety of other sources of appropriate background material.

Preparation

Review the Student Guide and make one copy for each student in your class. Then conduct the following preparations for the lab.

Mix TAE Buffer

Because tris-acetate-EDTA (TAE) buffer solution is stable, it can be made ahead of time and stored in a carboy or other container until you are ready to use it.

If you are using the 4-station kit, add the entire 50-mL bottle of 50× TAE to 2.45 L of distilled or deionized water. Mix well.

If you are using the 8-station kit, add the entire 100-mL bottle of 50× TAE to 4.9 L of distilled or deionized water. Mix well.

If you are going to use *Carolina*BLU Gel and Buffer stain in the gel and buffer, do not add *Carolina*BLU stain to the mixed buffer at this time.

Prepare Agarose Gels

Prepare 0.8% agarose before class on Lab Day 1. Depending upon the apparatus, you will need approximately 50 mL of agarose per gel. Maintain the agarose solution in its liquid state by placing it in a 55–65°C water bath. Cover the top of the agarose container with aluminum foil to minimize evaporation.

4-station kit: To make 0.8% agarose for the 4-station kit, add both of the 1.2-g vials of agarose (2.4 g total) to 300 mL of 1× TAE. Melt the agarose using one of the methods described in "Methods for Melting Agarose."

8-station kit: To make 0.8% agarose for the 8-station kit, add the 5 g of agarose provided to 625 mL of 1× TAE. Melt the agarose using one the methods described in "Methods for Melting Agarose."