

# Exploring Antibiotic Resistance using Gel Electrophoresis

## Teacher Materials

In this lab students will learn about the rapid spread of antibiotic resistance and will take on the role of doctor to determine which antibiotic they should prescribe to a fictional family. They will use agarose gel electrophoresis to separate PCR products and compare the bands on the gel to a DNA ladder in order to determine if each bacteria sample has the gene(s) for antibiotic resistance .

<b>Learning Goals, Objectives, and Skills .....</b>	<b>2</b>
<b>Instructor Planning Guide .....</b>	<b>3</b>
<b>Instructor Preparation Guide .....</b>	<b>5</b>
<b>Answers to Student Questions .....</b>	<b>8</b>
<b>Standards Alignments .....</b>	<b>11</b>
<b>Calculation Tool for ordering NEB Reagents.....</b>	<b>12</b>

# Exploring Antibiotic Resistance

## Learning Goals

### Student Learning Goals:

- Students will understand what an antibiotic is and what it is used for.
- Students will understand what a plasmid is and its relationship to antibiotic resistance.
- Students will understand the process of agarose gel electrophoresis.
- Students will understand how both PCR and gel electrophoresis are used for DNA profiling.

### Student Learning Objectives:

- Students will perform the technique of agarose gel electrophoresis.
- Students will estimate the size of DNA fragment from agarose gel data.
- Students will analyze the results of the molecule separation by gel electrophoresis.
- Students will identify genes based on PCR product size using gel data.

### Scientific Inquiry Skills:

- Students will pose questions and form hypotheses.
- Students will design and conduct scientific investigations.
- Students will use experimental data to make conclusions about the initial question and to support or refute the stated hypothesis.
- Students will follow laboratory safety rules and regulations.

### Laboratory Technical Skills:

- Students will demonstrate proper use of micropipettes.
- Students will consider safety considerations when working with an electric current.
- Students will demonstrate proper use of gel electrophoresis equipment.
- Students will prepare and pour agarose gels.

# Exploring Antibiotic Resistance

## Instructor Planning Guide

### Experimental Timing:

From start to finish this lab takes a single 45-50 minute class period. Additional time may be required if you choose to have students prepare their own gels.

### ① Notes:

- Time required for electrophoresis is approximately 20-25 minutes, but may vary depending on the type of electrophoresis equipment and voltage.
- If this is the first agarose gel students have experienced, you should expect the students to need extra time to load the gel.

### Specialized Equipment:

- p20, p200\*, p1000\* micropipettes (\*Teacher prep only)
- gel electrophoresis units with power supplies (as written, each student group will run 4 samples plus a ladder)
- UV or blue light source
- centrifuge (optional)

### Ordering Information:

The NoLimits™ DNA fragments used in this lab can be ordered through Fisher Scientific using the product numbers provided in the materials section of this guide. Each tube of NoLimits™ DNA contains 10 µg of DNA in 20 µL (at a concentration 500 ng/µL). If you follow the set-up procedures described here, a single set of NoLimits™ DNA fragments (400, 600, 800) will provide enough DNA for 50 groups to perform this lab, assuming that each group is provided with bacterial DNA samples from three patient plus the prepared DNA ladder.

\*The reagents from New England BioLabs can be ordered (at no cost) by going to their website (<https://www.neb.com>). A calculation tool for ordering NEB Reagents for this lab can be found on the final page of this document.

### Procedure Tips:

1. Before starting the experiment, ask students to check their materials list to make sure they have everything.
2. Demonstrate how to pipet very small volumes of liquid and load a gel. If your students have not had the opportunity to run many gels, you may want to have them practice loading gels before you begin this experiment. Refer to “A Guide to Agarose Gel Electrophoresis” on the website (<https://www.massbioed.org/educators/curriculum>), for instructions on how to make practice gels.

3. Remind students to use a fresh pipette tip with each sample and to record the location of each of the samples as they load them into the gel.

### **Teaching Tips:**

1. The protocol for preparing the electrophoresis gels is not included in this version of the lab. You can download “A Guide to Agarose Gel Electrophoresis” document from the website (<https://www.massbioed.org/educators/curriculum>), adapt it to your equipment and insert it in the lab.
2. This lab provides an opportunity to discuss PCR without actually doing a PCR reaction. Consider using the BioTeach video “The Ups and Downs of PCR” to reinforce the concepts behind PCR.
3. Although we have chosen to use antibiotic resistance as the scenario for this lab, you could easily use this protocol to create your own crime scene analysis, paternity test, or other heredity study.

### **Safety Considerations:**

- Gloves, lab coats and eye protection should be used whenever possible, as a part of good laboratory practice.
- Practice sterile techniques whenever possible to avoid contamination of reagents.
- Exercise caution when heating and/or melting reagents during gel preparation.
- Exercise caution when working with electrical equipment.
- UV protective shields and/or glasses must be used if visualizing gels with a UV transilluminator.
- Always wash hands thoroughly after handling biological materials or reagents.
- Obtain the Material Safety Data Sheets (MSDS) available from the suppliers and follow all safety precautions and disposal directions as described in the MSDS.
- Check with your school’s lab safety coordinator about proper disposal of all reagents and gels containing DNA stains.

# Exploring Antibiotic Resistance

## Instructor Preparation Guide

**Materials:** This guide assumes 30 students, working in groups of two, for a total of 15 groups.

<b>Materials for Teacher Advanced Preparation:</b>	
1 tube	NoLimits™ DNA Fragment 400 bp (Fisher#FERSM1631) Each tube contains 10 µg of DNA in 20 µL at a concentration 500 ng/µL
1 tube	NoLimits™ DNA Fragment 600 bp (Fisher#FERSM1461) Each tube contains 10 µg of DNA in 20 µL at a concentration 500 ng/µL
1 tube	NoLimits™ DNA Fragment 800 bp (Fisher#FERSM1481) Each tube contains 10 µg of DNA in 20 µL at a concentration 500 ng/µL
1 mL	6X loading Dye (NEB#B7024S)
3 mL	1X sterile TE (Tris-EDTA) or sterile distilled water
1	p20 micropipette and pipette tips
1	p200 micropipette and pipette tips
1	p1000 micropipette and pipette tips
80	microcentrifuge tubes (0.5 mL or 1.5 mL)
10	microcentrifuge tubes (1.5 mL or 2.0 mL)
5	microcentrifuge tube racks
1	ultrafine point permanent marker
1	centrifuge (optional)
all	reagents and equipment to prepare gels for gel electrophoresis *see Teaching Tips

<b>Materials for each Student Workstation:</b>	<b>Materials for the Common Workstation:</b>	
3 tubes	bacterial DNA from each patient (Pat 1, Pat 2, Pat 3)	1X electrophoresis buffer
1 tube	Control DNA	UV or blue light source
1 tube	DNA ladder	centrifuge (optional)
1	agarose gel (1.8%) with DNA stain	
1	p20 micropipette and tips	
1	microcentrifuge tube rack	
1	waste container	
1	gel electrophoresis unit with power supply	

## Easy Substitutions:

- Sterile distilled water can be used instead of TE to prepare the DNA samples.
- If you do not have a centrifuge, have students gently tap the microcentrifuge tubes on the lab bench to collect all the reagents at the bottom of the tube.

## Set-up Calendar:

### 2 weeks before lab:

- Check supplies. Order any needed materials.
- If making any substitutions to the supply list, edit the student protocol accordingly.
- Mix “Patient” bacterial DNA samples using NoLimits™ DNA and the information in Table 1. Alternatively, you can create your own “Patients”

**Table 1. Patient bacteria samples**

Patient	NoLimits™ DNA	Antibiotic Resistance Gene(s)
Patient 1 (labeled Pat 1)	800	Erythromycin
Patient 2 (labeled Pat 2)	400	Amoxicillin
Patient 3 (labeled Pat 3)	400 + 800	Amoxicillin + Erythromycin

1. Dilute 6X loading dye to 2X – You will need 300  $\mu\text{L}$  of 2X loading for each sample and ladder that you are preparing. As written, this lab directs you to prepare 3 patients + 1 control + DNA Ladder.

Prepare 1500  $\mu\text{L}$  of 2X loading dye: Mix 500  $\mu\text{L}$  of 6X loading dye with 1000  $\mu\text{L}$  distilled  $\text{H}_2\text{O}$ .

2. You will need to mix and dilute stock DNA to create the patient bacteria DNA samples.

To make Patient 1 bacteria DNA sample you will mix:

6  $\mu\text{L}$  of 800 bp DNA + 294  $\mu\text{L}$  1X TE (or sterile distilled water).

Add 300  $\mu\text{L}$  of the 2X loading dye you prepared in step 1. You will have 600  $\mu\text{L}$  of DNA.

To make Patient 2 bacteria DNA sample you will mix:

6  $\mu\text{L}$  of 400 bp DNA + 294  $\mu\text{L}$  1X TE (or sterile distilled water).

Add 300  $\mu\text{L}$  of the 2X loading dye you prepared in step 1. You will have 600  $\mu\text{L}$  of DNA.

To make Patient 3 bacteria DNA sample you will mix:

6  $\mu\text{L}$  of 400 bp DNA + 6  $\mu\text{L}$  of 800 bp DNA + 288  $\mu\text{L}$  1X TE (or sterile distilled water).

Add 300  $\mu\text{L}$  of the 2X loading dye you prepared in step 1. You will have 600  $\mu\text{L}$  of DNA.

To make Control bacteria DNA sample with no antibiotic resistance you will mix:

300  $\mu\text{L}$  1X TE (or sterile distilled water) + 300  $\mu\text{L}$  of the 2X loading dye you prepared in step 1.

You will have 600  $\mu\text{L}$  1X loading dye.

**① Note: The final volume of each Patient bacteria DNA sample will be 400  $\mu\text{L}$  at a concentration of 5ng/ $\mu\text{L}$  of DNA or enough DNA for 33 groups.**

- Mix and dilute the stock DNA to create a DNA ladder consisting of 3 bands (400bp, 600 bp, 800 bp).

To make the DNA Ladder you will mix:

6  $\mu\text{L}$  of 400 bp DNA + 6  $\mu\text{L}$  of 600 bp DNA + 6  $\mu\text{L}$  of 800 bp DNA + 282  $\mu\text{L}$  1X TE (or sterile distilled water).

Add 300  $\mu\text{L}$  of the 2X loading dye you prepared in step 1. You will have 600  $\mu\text{L}$  of DNA Ladder.

**Ⓢ Note: You should have 2  $\mu\text{L}$  remaining in the 800 bp stock NoLimits™ DNA tube, 14  $\mu\text{L}$  remaining in the 600 bp stock NoLimits™ DNA tube, and 2  $\mu\text{L}$  remaining in the 400 bp stock NoLimits™ DNA tube. Keep this DNA frozen. It will be stable in the freezer indefinitely.**

- Aliquot out DNA samples, ladder DNA.
  - Aliquot 12  $\mu\text{L}$  of each Patient bacteria DNA into microfuge tubes labeled Pat 1, Pat 2, Pat 3. As the lab is written, each student group will need one tube each of the 3 Patient bacteria DNA samples, but you can adapt as needed.
  - Aliquot 12  $\mu\text{L}$  of each Control bacteria “DNA” into microfuge tubes labeled Control. Prepare one Control tube per lab group.
  - Aliquot 12  $\mu\text{L}$  of the DNA ladder you prepared into microfuge tubes labeled Ladder. Prepare one tube of ladder per lab group.

**⚠ Caution: Store all DNA samples and ladder in the freezer until the morning of the lab.**

*Note, the band for the patients were suggested because the size difference between the 400 bp and 600 or 800 bp fragments will allow a quick and clear separation.*

*The suggested patients are optional. You can create your own by using the NoLimits™ DNA sizes recommended or by purchasing different sized NoLimits™ DNA fragments.*

### 1 day before lab:

- Set up student lab stations with all durable materials according to the materials listed above.
- Prepare TAE or buffer of your choice.
- Prepare 1.8% agarose gels with DNA Stain. Each group will load 3 samples, 1 control and 1 ladder (5 lanes total).

**Ⓢ Tip: Gels can be prepared ahead of time. If you pour the gels several days before the lab, they should be stored in a plastic container/bag with a damp paper towel to keep them from drying out. Gels should be stored in a cool location.**

**⚠ Caution: DNA stains such as GelGreen are light sensitive. Gels should be stored in the dark.**

### Morning of lab:

- Set out DNA samples and ladder at each student lab station.
- Set up Common Workstation according to the materials list.

# Exploring Antibiotic Resistance

## Answers to Student Questions

### Background Reading

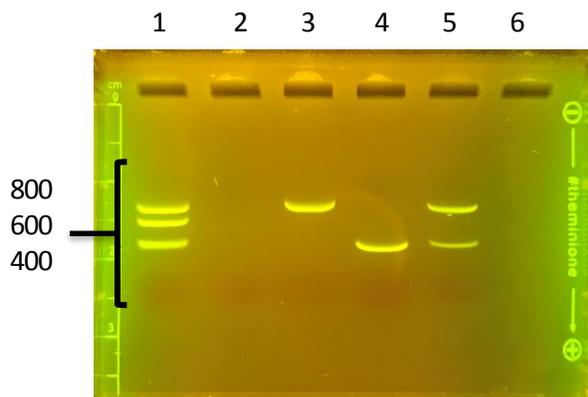
1. Antibiotics are medicines that fight bacterial infections. They either kill bacteria or keep them from reproducing. They are not effective against viruses or other microbes.
2. A bacterial cell is said to be resistant to antibiotics if it carries a gene that allows it to survive in the presence of antibiotics.
3. The spread of antibiotic resistance is making it harder for doctors to treat infections that were once easily treated by antibiotics.
4. Antibiotic resistance can spread so quickly because bacteria can pass genes on to their offspring (through reproduction) but also through the transfer of plasmids.
5. Everyone should get vaccinations, wash their hands carefully, prepare food safely and stay away from others when they are sick. Antibiotics should only be used if a doctor prescribes them and they should be used as directed by a doctor.

### Introduction

1. Smaller molecules will move faster through the gel than larger molecules because they can get through the small holes in the gel easier than bigger molecules.
2. DNA moves towards the positive electrode because it is negatively charged. (Opposites attract!) It is important to make sure that the gels are made properly, and the gel chamber is set up so that the wells are closest to the negative electrode.
3. The control bacteria's DNA in this experiment is DNA from a strain of bacteria that is known to not have antibiotic resistance genes. We are using this strain of bacteria as a negative control. We expect to see no bands (no antibiotic resistance genes) on the gel. A negative control is included to "control for" possible contamination during the preparation of the PCR samples and gel electrophoresis. If students see bands in the control lane then they cannot trust that the patient results are valid.
4. A DNA ladder is a set of standard fragments or molecules of DNA that are used to estimate the size of the unknown fragments/molecules run on a gel.
5. The  $amx^R$  gene will move furthest on the gel because it is the smallest piece of DNA (400 bp).
6. The  $ery^R$  gene will move the shortest distance on the gel because it is the largest piece of DNA (800 bp)

## Observations and Conclusions

1. Sample gel.



### Lanes

- 1 – DNA Ladder
- 2 – Control (no band)
- 3 – Patient 1 = 800 bp = ery<sup>R</sup>
- 4 – Patient 2 = 400 bp = amx<sup>R</sup>
- 5 – Patient 3 = 400 bp + 800 bp = amx<sup>R</sup> + ery<sup>R</sup>
- 6 – Empty

2. A band on the gel represents a molecule or piece of DNA.
3. Lane 2 – the control – has no band which means the DNA from the bacterial control does not have any of the antibiotic resistance genes that we are studying. This result is as we expected.
4. Lanes 3-5 or the Patients 1,2,3 all have one or more band which mean the bacteria collected from these patients all have one or more gene for antibiotic resistance.
5. Based on what your gel, circle the antibiotic resistant gene(s) that each bacteria sample has:  
Results are shaded.

Lane	Bacteria from this patient have what genes?			
Control	amx <sup>R</sup>	ery <sup>R</sup>	kan <sup>R</sup>	none of these
Pat 1	amx <sup>R</sup>	ery <sup>R</sup>	kan <sup>R</sup>	none of these
Pat 2	amx <sup>R</sup>	ery <sup>R</sup>	kan <sup>R</sup>	none of these
Pat 3	amx <sup>R</sup>	ery <sup>R</sup>	kan <sup>R</sup>	none of these

6. Patient 1 – could be prescribed amoxicillin or kanamycin  
Patient 2 – could be prescribed erythromycin or kanamycin  
Patient 3 – could be prescribed only kanamycin

Student will hopefully pick up on the fact that Patient 2 should have gotten better when he/she was given amoxicillin the first time and prompt a discussion about why it might not have worked. During the discussion you can guide students toward the idea that it's important to take antibiotics as directed by the doctor and to complete the entire course of the antibiotics even if you start to feel better.

FYI – amoxicillin is the most common antibiotic prescribed for strep throat. Erythromycin is sometimes prescribed for recurring infections of strep throat. Kanamycin is used most often for serious bacterial infections like tuberculosis and would not typically be used for strep throat because of the side effects of the antibiotic.

# Exploring Antibiotic Resistance

## Standards Alignments

### MA Science and Technology/Engineering Standards – High School (2016)

#### Biology

**HS-LS1-1.** Construct a model of transcription and translation to explain the roles of DNA and RNA that code for proteins that regulate and carry out essential functions of life.

**HS-LS4-4.** Research and communicate information about key features of viruses and bacteria to explain their ability to adapt and reproduce in a wide variety of environments.

#### NRC Practices

- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing data
- Mathematical and computational thinking
- Constructing explanations and designing solutions
- Engaging in argument from evidence
- Obtaining, evaluating, and communicating information

### Next Generation Science Standards – High School (2013)

#### Life Sciences

**HS-LS1-1.** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

### Common Core State Standards Connections:

#### ELA/Literacy -

- RST.9-10.7** Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) into words.
- RST.9-10.8** Assess the extent to which the reasoning and evidence in a text support the author's claim or a recommendation for solving a scientific or technical problem.
- RST.11-12.1** Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.
- RST.11-12.7** Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.
- RST.11-12.8** Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

- RST.11-12.9** Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.
- WHST.9-12.1** Write arguments focused on *discipline-specific content*.
- WHST.9-12.2** Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
- WHST.9-12.5** Develop and strengthen writing as needed by planning, revising, editing, rewriting, or trying a new approach, focusing on addressing what is most significant for a specific purpose and audience.
- WHST.9-12.7** Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.
- WHST.9-12.9** Draw evidence from informational texts to support analysis, reflection, and research.
- SL.11-12.5** Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

Mathematics -

- MP.2** Reason abstractly and quantitatively.
- MP.4** Model with mathematics.
- HSF-BF.A.1** Write a function that describes a relationship between two quantities.
- HSF-IF.C.7** Graph functions expressed symbolically and show key features of the graph, by hand in simple cases and using technology for more complicated cases.
- HSN.Q.A.1** Use units as a way to understand problems and to guide the solution of multi-step problems; choose and interpret units consistently in formulas; choose and interpret the scale and the origin in graphs and data displays.
- HSN.Q.A.2** Define appropriate quantities for the purpose of descriptive modeling.
- HSN.Q.A.3** Choose a level of accuracy appropriate to limitations on measurement when reporting quantities.
- HSS-IC.A.1** Understand statistics as a process for making inferences about population parameters based on a random sample from that population.
- HSS-IC.B.6** Evaluate reports based on data.

## Calculation tool for ordering NEB Reagents for: Exploring Antibiotic Resistance using Gel Electrophoresis

Please keep in mind that NEB is a fantastic and generous partner and will provide up to \$1000 of reagents for each school. Please check with your colleagues to coordinate your ordering to ensure that your school plans ahead for ALL of the planned labs requiring NEB reagents, and please, only order as much as you need. The calculation tool below will help you determine how much of each reagent to order. *Importantly, the amount needed per group shown below includes the extra needed in case of mistakes or when aliquots are provided for each group.*

Fill out the chart below to determine how many tubes of each of the ladder you need to order.

### Calculation tool:

<i>Example</i>						
NEB Reagent	NEB Catalog #	Amount of Reagent In NEB Tube	Amount Needed per Group	Total Number of Groups Doing the Lab	Total Amount You Will Need	# Tubes Needed
Reagent X	X0000	40 $\mu$ L	4 $\mu$ L	8	32 $\mu$ L	1
				<i>You fill this in</i>	<i>4 <math>\mu</math>L X (# groups)</i>	<i>32 <math>\mu</math>L &lt; 40 <math>\mu</math>L</i>

NEB Reagent	NEB Catalog #	Amount of Reagent In NEB Tube	Amount Needed per Group	Total Number of Groups Doing the Lab	Total Amount You Will Need	NEB Tubes Needed
Gel Loading Dye, Purple (6X)	B7024S	4 mL	< 10 $\mu$ L	Please note that excess gel loading dye is included when NEB sends enzymes. If you have ordered enzymes for other labs, you may already have plenty of this.		1

Once completed, you can submit your order here: <https://www.neb.com/forms/BioTeach>