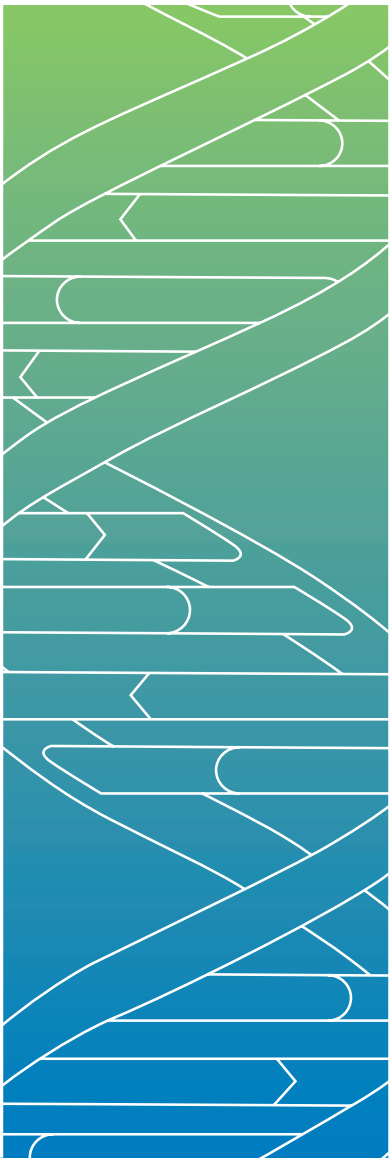
AMGEN® Biotech Experience

Scientific Discovery for the Classroom

FOUNDATIONS OF BIOTECH EXTENSION



GEL ELECTROPHORESIS TROUBLESHOOTING

Student Guide

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INTRODUCTION

In the ABE *Foundations of Biotech* labs, you used gel electrophoresis to verify bacterial transformation (to ensure that the plasmid contained the red fluorescent protein (*rfp*) gene from a sea anemone). In that laboratory, you performed gel electrophoresis of your samples of transformed plasmids and compared it to a 1 kb DNA ladder from New England Biolabs (NEB). You may have used GelGreen to stain the DNA and make bands visible under blue LED light. Some students have noticed a problem with their gels; however, for them, the larger DNA bands exhibited inconsistent migration through the gel; that is, **the fragments of the plasmids did not end up where they should have, according to the 1 kb ladder.**

In this extension, you'll explore why this inconsistent migration happens and what can be done to correct it.

GOALS

By the end of this extension, you will be able to do the following:

- Design and carry out an experiment to investigate why inconsistent migration happens in gel electrophoresis
- Develop and test strategies to minimize the issues in DNA migration

WHAT DO YOU ALREADY KNOW?

Discuss the following questions with your lab partner, and write your ideas in your notebook. Be prepared to discuss your responses with the class. Don't worry if you don't know all the answers; discussing these questions will help you think about what you already know about gene cloning and gel electrophoresis, and what questions you may have.

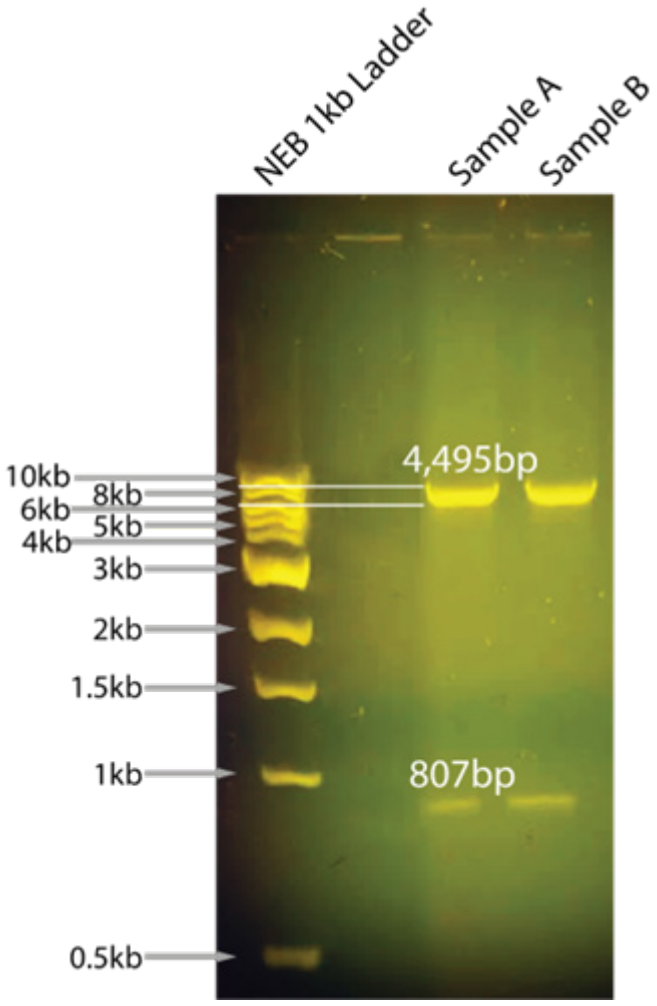
1. Why is it important for the DNA ladder in the *Foundations of Biotech* labs to accurately reflect the sizes of the DNA fragments?
2. What do you think might happen if a researcher didn't know that the placement of fragments compared to the ladder was inaccurate?

WHAT IS THE PROBLEM?

In the *Foundations of Biotech* labs, the *rfp* gene from the sea anemone is added to a plasmid that has been cut using restriction enzymes. The plasmid is taken up by bacterial cells in a process called bacterial transformation. The bacterial cells then make their own proteins along with the red fluorescent protein encoded by the inserted gene. As part of the lab, gel electrophoresis is used to ensure

that the bacterial transformation has taken place. If it has, the gel will show two bands: one between 4 kb and 5 kb in size and the other just under 1 kb in size.

In the gel below, Lane 1 contains the 1 kb ladder and Lanes 2 and 3 contain samples that should show fragments that measure 4,495 bp and 807 bp, but the 4,495 bp band doesn't seem to be in the right place. This gel was run under normal conditions: 0.8% agarose gel with 1:10,000 GelGreen in the agarose.



So, what's the problem? This lab is designed to verify that the digested plasmid contains the *rfp* gene. If proper ligation and digestion have taken place, you should see bands at approximately 4,500 bp (between the 4 kb and 5 kb bands on the ladder) and just below the 1 kb band on the ladder. But in the gel above, the 4,495 bp band seems to be pretty close to the 8,000 bp band on the ladder. We know that the digestion was successful because the fragments of DNA were later sequenced, and the sequences and sizes of the fragments were correct. This makes it difficult for teachers and students to assess the success of their restriction digest and their gel verification. If the fragment is not migrating with

the corresponding DNA ladder fragment, then you may draw the conclusion that the correct size of DNA was not separated during digestion; therefore, the plasmid does not contain the *rfp* gene.

WHAT IS HAPPENING AND WHAT CAN BE DONE ABOUT IT?

It turns out that the inconsistent DNA migration for DNA bands above 400 bp is a known issue with GelGreen. In 2019, researchers published a paper that compared how a variety of stains and staining methods affect DNA migration through agarose gels. They found that different DNA stains vary in how they affect DNA migration through agarose gels. You will read this paper and answer questions about how the authors conducted their research.

Remember, a DNA ladder (or DNA marker) is a solution that contains a series of DNA fragments of particular lengths. Researchers use DNA ladders as "molecular rulers" that allow them to visualize the approximate number of base pairs (or size) of a DNA band in a gel. Different DNA ladders act as different rulers and are selected based on the expected sizes of the DNA fragments in an experimental procedure. For the *Foundations of Biotech* labs, a 1 kb (1,000 bp) ladder is used, which contains known fragment sizes from 500 bp to 10 kb (10,000 bp). The laboratory traditionally uses the 1 kb ladder because the procedure is designed to measure fragments that are between 337 bp and 4,535 bp (337 bp, 807 bp, 4,705 bp, 4,495 bp, and 4,535 bp).

YOUR TASK

As an ABE student-researcher, your objective is to design and execute an experiment, or a series of experiments, to determine what factors might be contributing to the DNA ladder migrating incorrectly and how to reduce the effect. You task is as follows:

- Research your experiment and fill out **Student Data Sheet 1: Conducting Background Research**.
- Fill out **Student Data Sheet 2: Identifying Your Research Project**.
- Develop your experimental protocol by filling out **Student Data Sheet 3: Designing Your Experiment**.
- Collect your equipment and prepare your workspace.
- Carry out your experimental protocol, keep track of your data, and write up the results. of your experiments using **Student Data Sheet 4: Carry Out Your Experiment**.
- Present your results to the rest of the class.

COLLABORATION IN THE WORLD OF SCIENCE

Although you may be just one scientist working at your lab bench independently, your research is not done in isolation. Collaboration within the science community is what leads to discoveries. In this experiment, another lab team's results could impact and inform your own—it will help you better understand the effects of GelGreen if you share your data with other lab teams and they share with you. If you have access to data about the effects of other variables, it will allow you to draw more informed conclusions about the effects of GelGreen on DNA migration. You may also find that you develop new questions that you can answer with further experimentation.

You will be working in a team—remember it is important to cooperate, listen, and share. Other teams may choose different or similar variables to experiment with. This is good! Repeating experiments increases accuracy of data, and diversified experiments can widen the perspective of lab results. The better the collaboration, the better the lab results!

RESOURCES

Biotium. (2018). *GelGreen® quick start protocol*. https://biotium.com/wp-content/uploads/2013/11/PI-GelRed-GelGreen-Quick-Start-Protocol.pdf

González-Candelas, L. (2015, December 5). *Does anybody know why GelRed modifies the migration of DNA fragments in agarose gel electrophoresis?* ResearchGate. https://www.researchgate.net/post/Does-anybody-know-why-GelRed-modifies-the-migration-of-DNA-fragments-in-agarose-gel-electrophoresis

Hall, A. C. (2019). *A comparison of DNA stains and staining methods for agarose gel electrophoresis.* https://doi.org/10.1101/568253

New England Biolabs. (n.d.) *FAQ: Can I use SYBR® and/or GelRed® dyes with the DNA Ladders from NEB?* https://www.neb.com/en-us/faqs/2019/07/29/can-i-use-sybr-and-or-gelred-dyes-with-the-dna-ladders-from-neb

Yun Lee, P. (2012). *Agarose gel electrophoresis for the separation of DNA fragments*. https://doi.org/10.3791/3923

Student Data Sheet 1

CONDUCTING BACKGROUND RESEARCH

Article Title: *"A Comparison of DNA Stains and Staining Methods for Agarose Gel Electrophoresis"*	
Author: *Who was the author?*	*What institution conducted the research?*
Research Question: *What were the scientists trying to figure out?*	
Methodology: *How did they do their research?*	
Conclusions: *What did they find out?*	
Future Research: *What are two further questions or topics the researchers should now explore?*	
Vocabulary: *What words do I need to define to understand the article?*	
Word	***Definition***

Student Data Sheet 2

IDENTIFYING YOUR RESEARCH PROJECT

Now that you have read the research, brainstorm what you could explore to determine if it might be contributing to the ladder not running properly in the gel and list your ideas below.

Next, develop a list of questions you would like to research and possible methods for exploring those questions.

Question	How might we investigate this question?

What variables can you identify in running a gel using GelGreen?

	Variables
1	
2	
3	
4	
5	
6	
7	

Student Data Sheet 3

DESIGNING YOUR EXPERIMENT

With your team, design your experiment using this graphic organizer.

Research question:

Independent variable (What will you change?):

Dependent variable (What will you measure?):

Control (What will stay the same?):

Hypothesis (What are your expected results/predictions? Explain.):

Examine this general lab protocol and consider how you will develop your protocol.

1. Make the appropriate changes to your independent variable.
2. Load and run a gel with three lanes of DNA ladder.
3. Photograph your gel (e.g., using the MyGels app).
4. Record your results in a shared electronic document or lab notebook.
    a. Record the date.
    b. Describe what you did.
    c. Include the photograph of your gel.
    d. Describe the gel (describe the ladder and general results).
    6. Repeat Steps 1–5 as needed.

Design your experiment: Write step-by-step instructions for how you will carry out your experiment. Remember: These instructions should be clear and easy to follow!

Materials/Equipment Needed	Safety Concerns

Student Data Sheet 4

CARRY OUT YOUR EXPERIMENT

Follow your protocol to run your experiment. Make sure that you keep careful records of your procedures and results. It is important for your results to be replicable, so you should run them two or even three times. If possible, as part of your documentation, take photos of your gels to include in your write-up.

RESULTS

To document your results, write about them or create a presentation. Include the following in your documentation:

1. Your data (in the form of tables, images, graphs)
2. An explanation of your data:
    a. What do the results show?
    b. What are your next steps?