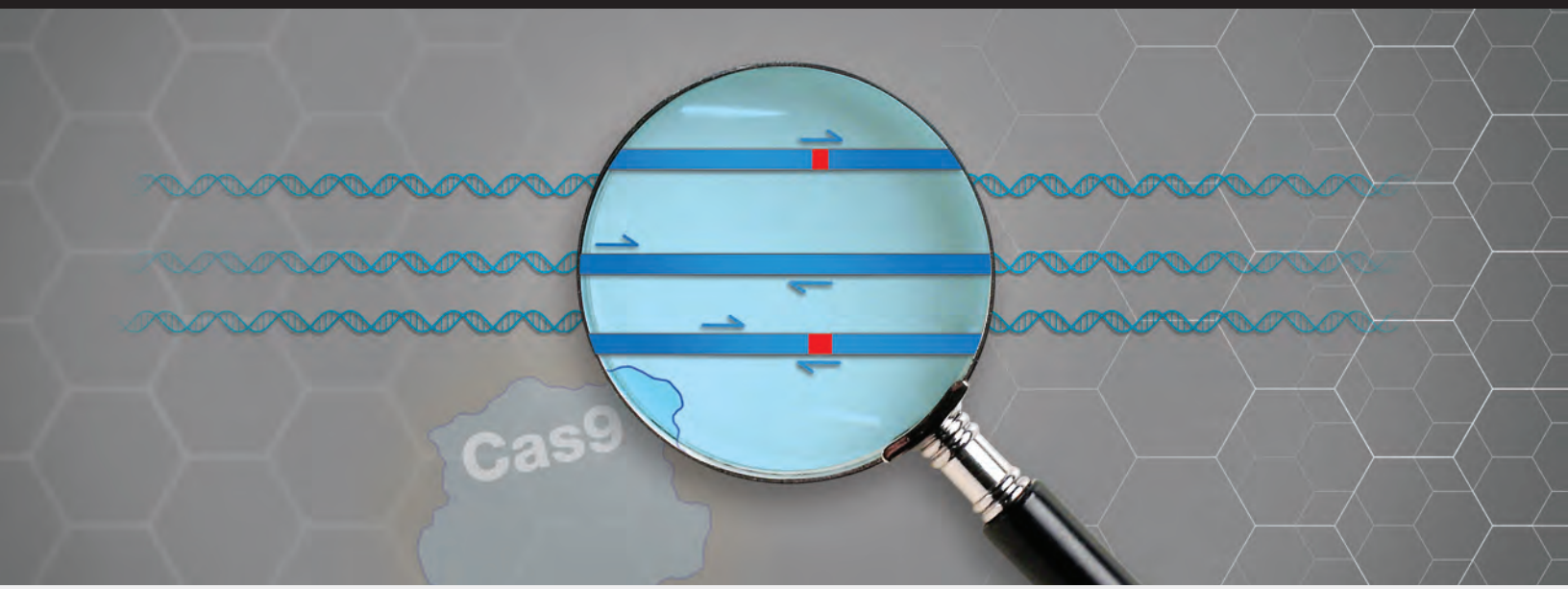
BIO-RAD Explorer



Out of the Blue Genotyping Extension

Catalog #12012607EDU

Instructor Guide

This product is not for self-administration.

For technical support, call your local Bio-Rad office, or in the U.S. call **1-800-4BIORAD** (1-800-424-6723) option 2.

BIO-RAD

Dear Instructor

In the recent past, as researchers chipped away at the mysteries of the newly discovered “CRISPR” it became apparent that our understanding of immunity in bacteria was incomplete. When the full picture came into focus — that of an adaptive immune response in bacteria — the scientific community was presented with yet another example of the power of the scientific process. Researchers in many labs around the world carefully designed experiments, challenged each other’s data, and made claims using multiple sources of evidence. In the end, the struggle to understand this tiny sliver of nature led to a breakthrough in life science research and diagnostics with an impact that will affect our lives for decades to come.

In this extension activity, students will use the polymerase chain reaction (PCR) to gather molecular evidence of CRISPR gene editing in bacteria, complementing their previous results from the Out of the Blue CRISPR Kit activities. The extension is not only a key step in a true research workflow but also an opportunity for students to engage in a practice of the scientific process: gathering and using evidence from multiple sources. Just as researchers reveal more of the complexities of CRISPR with each new experiment, your students too will participate in the ongoing process of scientific discovery.

The activities included in this kit were developed in partnership with Sherry Annee, former president of the National Association of Biology Teachers and biotechnology teacher at Brebeuf Preparatory Academy in Indianapolis, IN, and Thomas Tubon Jr., Professor and Director of the Stem Cell Program at Madison Area Technical College in Madison, WI.

We strive to continually improve our curriculum and products, and your input is extremely important to us. We welcome your stories, comments, and suggestions.

Share your students’ success on social media @bioradeducation.

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Table of Contents

Kit Storage

When you receive the Out of the Blue Genotyping Extension:

1. Record storage location and batch numbers from the product labels.
2. Store the **Out of the Blue Genotyping Extension Reagent Refill Pack** in the freezer (–20°C).

3. Store the **InstaGene Matrix** and **UView 6x Loading Dye and Stain** (if purchased) at 4°C.

4. Store the kit box and plastic materials at room temperature.

5. Visit **bio-rad.com/outoftheblue** to download the instructor and student guides.

Technical Support is available at support@bio-rad.com or 1-800-4BIORAD, option 2.

Safety Guidelines

Wearing protective eyewear and gloves is strongly recommended. Students should wash their hands with soap before and after this exercise. If any solution gets into a student's eyes, flush with water for 15 minutes. Lab coats or other protective clothing should be worn to avoid staining clothes.

The LB agar media, which students will handle as part of the genotyping extension activity, contains both kanamycin and spectinomycin, antibiotics that may cause allergic reactions or irritation. Those with an allergy to these or similar antibiotics should consult with their physician before handling kit materials and reagents. Following the laboratory activities, place all bacteria plates and any materials that contacted bacteria in a 10% bleach solution for at least 20 min to decontaminate. Follow local regulations for further disposal recommendations.

The bacterial strain *E. coli* HB101-pBRKan used in this activity is nonpathogenic and has been genetically modified to require an enriched medium for growth. However, standard microbiological practices should be used.

Use special caution when working with hot molten agar. Heat resistant gloves and other standard personal protective equipment including goggles and a laboratory coat are recommended.

Kit Components

Each kit contains materials for 8 student workstations.

Item	Quantity
InstaGene Matrix	20 ml
2x PCR Master Mix	1,200 µl
Out of the Blue Primer Mix, 50x	20 µl
Out of the Blue PCR Positive Control DNA	150 µl
PCR MW Ruler	200 µl
Orange G Loading Dye, 5x	1 ml
1.5 ml conical tube, O-ring screw cap, sterile	50
Microtube with cap, 1.5 ml	60
PCR tube, 0.2 ml	100
Instructor Answer Guide	1
Small Fast Blast DNA Electrophoresis Pack	
Fast Blast DNA Stain, 500x	100 ml
Certified Molecular Biology Agarose	25 g
TAE Electrophoresis Buffer, 50x	100 ml
Small UView DNA Electrophoresis Pack	
UView 6x Loading Dye and Stain	1 ml
Certified Molecular Biology Agarose	25 g
TAE Electrophoresis Buffer, 50x	100 ml

Required Materials (not included in this kit)	Quantity
Thermal cycler with at least 56 wells	1
100–1,000 µl adjustable-volume micropipet and tips	8
20–200 µl adjustable-volume micropipet and tips	1
2–20 µl adjustable-volume micropipet and tips	8
Horizontal gel electrophoresis chamber with gel casting tray and comb	4–8
Power supply	1–8
Dry bath or water bath with floating tube racks set to 56°C	1–2
Dry bath or water bath with floating tube racks set to 95°C	1–2
Microcentrifuge, ≥2,000 x g	4–8
UV transilluminator (if using UView 6x Loading Dye and Stain)	1
Gel staining tray (if using Fast Blast DNA Stain)	4–8
Tube racks	8
Microwave oven	1
Distilled water	750 ml
Graduated cylinders, 1 L, 200 ml, and 50 ml, for preparing buffers	1 each
Beaker or plastic carboy, >2.5 L, for preparing buffers	1
Beaker, 500 ml, for preparing Fast Blast DNA stain (if using)	1
Erlenmeyer flask, ≥500 ml, for preparing agarose	1
Permanent marking pen	8

Recommended Materials (not included in this kit)	Quantity
Vortexer	4–8
PCR tube adapter for centrifuge	1–8
PCR tube rack	8

Out of the Blue Genotyping Extension

Small Fast Blast DNA Electrophoresis Pack

Small UView DNA Electrophoresis Pack

Ordering Information

Catalog #	Description
Kits and Refill Packs	
12012607EDU	**Out of the Blue Genotyping Extension**
12012708EDU	**Out of the Blue Genotyping Extension Refill Pack**
17006070EDU	**Out of the Blue Genotyping Extension plus Small Fast Blast Electrophoresis Pack**
17006284EDU	**Out of the Blue Genotyping Extension plus Small UView Electrophoresis Pack**
12012608EDU	**Out of the Blue CRISPR Kit**
12012620EDU	**Out of the Blue CRISPR Kit Refill Pack**
17006081EDU	**Out of the Blue CRISPR and Genotyping Extension Kits**
17006286EDU	**Out of the Blue CRISPR and Genotyping Extension kits plus Small Fast Blast DNA Electrophoresis Pack**
17006285EDU	**Out of the Blue CRISPR and Genotyping Extension kits plus Small UView DNA Electrophoresis Pack**
Consumables	
7326030EDU	**InstaGene Matrix**, 20 ml
1660450EDU	**Small Fast Blast Electrophoresis Reagent Pack**, includes 25 g agarose powder, 100 ml 500x Fast Blast DNA Stain, 100 ml 50x TAE electrophoresis buffer
1660462EDU	**Small UView Electrophoresis Reagent Pack**, includes 25 g agarose powder, 1 ml UView 6x Loading Dye and Stain, 100 ml 50x TAE electrophoresis buffer
1613015EDU	**1% TAE Mini ReadyAgarose Precast Gel**, 7.1 x 10 cm, 8-well
Equipment and Laboratory Supplies	
1660506EDU	**Professional Adjustable-Volume Micropipet**, 2–20 µl
1660507EDU	**Professional Adjustable-Volume Micropipet**, 20–200 µl
1660508EDU	**Professional Adjustable-Volume Micropipet**, 100–1,000 µl
1861096EDU	**T100 Thermal Cycler**
1664000EDU	**Mini-Sub™ Cell GT Cell**
1645050EDU	**PowerPac Basic Power Supply**
1660562EDU	**Digital Dry Bath**, 120 V
1660504EDU	**Temperature-Controlled Water Bath**, 120 V
12011919EDU	**Mini Centrifuge**, 100–240 V
1660531EDU	**UView Mini Transilluminator**
1660610EDU	**BR-2000 Vortexer**, 120 V
1660477EDU	**Gel Staining Trays**, pack of 4
1660481EDU	**Green Racks**, set of 5
TRC0501EDU	**96-Place PCR Tube Racks and Covers**, set of 5, multicolor
2240110EDU	**1.5 ml Conical Tubes**, O-ring screw caps, sterile, 500
TWI0201EDU	**0.2 ml PCR Tubes with Domed Caps**, clear, 1,000

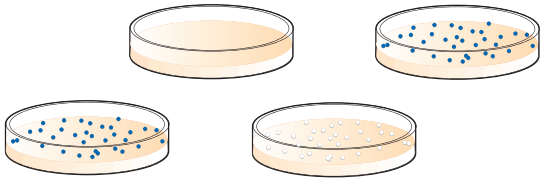
Visit **explorer.bio-rad.com** for a full list of 220–240 V equipment.

Kit Activity Overview

Part 1

Explaining Results from *lacZ* CRISPR Gene Editing Laboratory

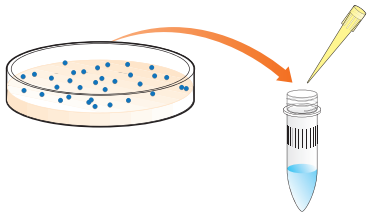
Students review their results from the *lacZ* CRISPR bacterial gene editing laboratory activity and provide two explanations of the results. Then, they brainstorm what additional information could help them to rule out one of the two.



Part 2

Bacterial Colony DNA Extraction and PCR

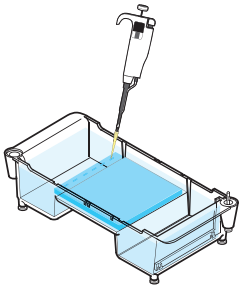
Students extract genomic DNA from bacterial colonies and prepare multiplex PCR samples to collect molecular evidence of whether or not the *lacZ* gene was modified in the chosen bacterial colonies.



Part 3

Gel Electrophoresis and Visualization

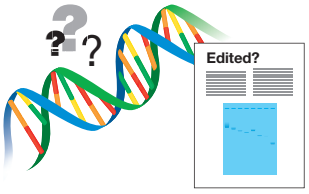
Students analyze their PCR samples via agarose gel electrophoresis and visualize using either Fast Blast DNA Stain or fluorescent DNA staining.



Part 4

Data Analysis and Argumentation

Students analyze the electrophoresis results to determine the genotype of the source bacteria and compile their evidence into claims about gene editing.



Activity Timelines

Start the Out of the Blue Genotyping Extension activity within two weeks of completing Activity 2: *lacZ* CRISPR Bacterial Gene Editing Laboratory from the Out of the Blue CRISPR Kit but before completing the capstone activity. The genotyping extension activity is designed to take two to three 90-minute or longer class periods as shown in Table 1. An alternative timeline for ~50-minute periods is outlined in Table 2.

Table 1. Suggested timeline for 90-minute or longer class periods meeting once or twice a week. The schedule includes the activities from both the CRISPR Kit and Genotyping Extension.

	Class Period 1	Class Period 2	Class Period 3
In-class work	Begin Part 1. Explaining Results from *lacZ* CRISPR Gene Editing Laboratory (requires students' results from *lacZ* CRISPR Bacterial Gene Editing Laboratory) Begin Part 2. Bacterial Colony DNA Extraction and PCR Pre-Laboratory Questions	Part 2. Bacterial Colony DNA Extraction and PCR (requires students' bacterial plates from *lacZ* CRISPR Bacterial Gene Editing Laboratory) Begin Part 3. Gel Electrophoresis and Visualization Pre-Laboratory Questions	Part 3. Gel Electrophoresis and Visualization Begin Part 4. Data Analysis and Argumentation
Outside-of-class work	Finish Part 2. Pre-Laboratory Questions	Finish PCR and store samples refrigerated Continue Part 3. Pre-Laboratory Questions	Retrieve overnight stained agarose gels for analysis Finish Part 4.

Table 2. Alternative timeline for daily ~50-minute class periods.

	Class Period 1	Class Period 2	Class Period 3	Class Period 4	Class Period 5
In-class work	Begin Part 1. Explaining Results from *lacZ* CRISPR Gene Editing Laboratory (requires students' results from *lacZ* CRISPR Bacterial Gene Editing Laboratory)	Begin Part 2. Bacterial Colony DNA Extraction and PCR (requires students' bacterial plates from *lacZ* CRISPR Bacterial Gene Editing Laboratory) Stop after students extract DNA	Finish Part 2. PCR	Part 3. Gel Electrophoresis and Visualization	Retrieve overnight stained agarose gels for analysis Part 4. Data Analysis and Argumentation
Outside-of-class work	(Optional) finish Part 1. Part 2. Bacterial Colony DNA Extraction and PCR Pre-Laboratory Questions		Run PCR and store samples refrigerated Begin Part 3. Gel Electrophoresis and Visualization Pre-Laboratory Questions	(Optional) begin Part 4. Data Analysis and Argumentation	Finish Part 4.

Curriculum Fit

Required prior knowledge and skills

- Basic DNA structure and function, including nucleotide base-pairing rules
- Basic function of Cas9 and single guide RNA (sgRNA) in targeted DNA cleavage
- Basic function and process of homology directed DNA repair, including the role of donor template DNA
- Central dogma (DNA > RNA > Protein > Trait)
- *lacZ* gene expression and how the presence of β-galactosidase can be detected using blue-white screening
- Key steps and purpose of the polymerase chain reaction (PCR)
- How to use a micropipet
- How to perform agarose gel electrophoresis and interpret gel results

Concepts, topics, and skills

- **Genetics** — students will determine whether bacteria have wild type or edited *lacZ* genes using PCR amplification. The genotyping results will provide evidence for students to confirm that CRISPR-mediated gene editing has occurred in certain bacterial samples

- **Genetic engineering** — students will transform bacteria and perform CRISPR gene editing, which are two key techniques within genetic engineering. Manipulating or engineering genomes to elicit a particular phenotype is the underlying goal of genetic engineering

- **Multiplex PCR** — students will use multiple primer sets in a single PCR sample, which is a convenient way to detect multiple DNA targets at once

- **Central dogma** — students will disrupt the function of the *lacZ* gene, which codes for β-galactosidase (β-gal), an enzyme that hydrolyzes lactose, a milk sugar. Students will edit the *lacZ* gene and observe a visible change in phenotype

- **Argumentation in science** — students will construct arguments about gene editing that include claims backed by evidence they collect in the Out of the Blue CRISPR and Genotyping Extension Kits

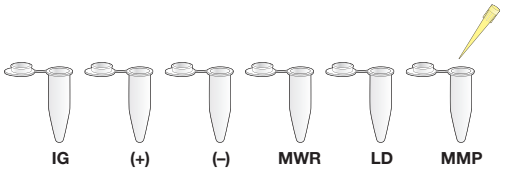
Preparation Instructions

Prepare and dispense solutions

Prepare reagents, except the PCR master mix and primers, up to four weeks before the activity and store refrigerated at 4°C. The PCR master mix plus primers solution should be prepared *no more than 30 min before the students prepare their PCR samples.*

1. Prepare and dispense solutions into labeled tubes as specified in the table below.

   To help prevent cross-contamination, label tubes and dispense one solution at a time. Be sure to use a new pipet tip for each solution.



Tube Type and Quantity for 8 Lab Workstations	Tube Label	Tube Contents and Preparation Instructions
For DNA Extraction and PCR Activity		
8 microtubes with caps, 1.5 ml	**IG**	1.3 ml InstaGene Matrix **Preparation:** Before dispensing, resuspend the resin by shaking, stirring manually, or stirring on a stir plate using the magnetic stir bar included in the bottle.
8 microtubes with caps, 1.5 ml	**+**	15 µl Out of the Blue PCR Positive Control DNA
8 microtubes with caps, 1.5 ml	**–**	15 µl distilled water
8 microtubes with caps, 1.5 ml	**MMP**	80 µl Master Mix and Primer solution **Preparation:** Just before the PCR activity, add 735 µl 2x PCR Master Mix and 15 µl Out of the Blue Primer Mix to a separate 1.5 ml microtube, pipet up and down to mix, and then dispense.
For Electrophoresis Activity		
8 microtubes with caps, 1.5 ml	**LD**	40 µl loading dye
8 microtubes with caps, 1.5 ml	**MWR**	15 µl molecular weight ruler with loading dye **Preparation:** Before dispensing, add 50 µl loading dye directly to the PCR molecular weight ruler tube and pipet up and down to mix.

Prepare agarose gels, TAE buffer, and stain *(Fast Gel Protocol only)**

2. Prepare eight 1% agarose gels with 8 wells each. It is essential to use 1x TAE (not 0.25x TAE) to make these gels. See Appendix for gel casting instructions.

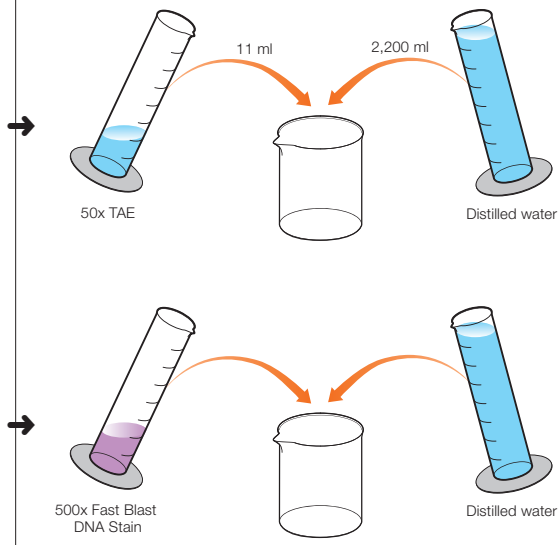
3. Prepare 2.2 L of 0.25x TAE electrophoresis buffer by adding 11 ml of 50x TAE to 2,200 ml of distilled water to use with the Fast DNA Gel Protocol.*

   Note: the correct volume of distilled water would be 2,189 ml, but the difference does not affect results.

4. If using, prepare 100x Fast Blast DNA Stain for staining of agarose gels. See Appendix for instructions.

   Note: Fast Blast DNA Stain is not required if using UView 6x Loading Dye and Stain for visualization.

* This activity is designed to take advantage of the Bio-Rad Fast Gel Protocol using 0.25x TAE running buffer and higher voltages. See Appendix for details and alternate electrophoresis options with preparation instructions.



Instructor Background

Multiple methods of detecting gene editing

Following a gene editing protocol, it is essential to verify not only that an edit was made in the correct location but also that the edit was successful as intended. An example of the consequences of incomplete gene edit verification can be found in the resources section (see Regalado 2019). Researchers can use a number of different techniques to determine the genotype of the organism they are studying or to verify a gene edit. The choice of technique depends on the numbers of genes, the types of allelic differences being investigated, as well as the cost and time of analysis. Some allelic variations can, for example, result in changes in restriction fragment patterns (restriction fragment length polymorphisms, RFLPs), while others may be detected only through sequencing. In the case of the *lacZ* gene edit (insertion) from the Out of the Blue CRISPR Kit Activity 2. *lacZ* Gene Editing Laboratory, the modification can be detected by PCR, which is a common step in a molecular biology workflow. In addition to providing students with the true research experience, collecting this type of molecular evidence highlights a crucial tenet of scientific practice — the gathering and using of evidence from multiple sources to strengthen a claim and rule out alternative explanations. See Resources for related articles and tools.

Multiplex PCR

Multiplex PCR is the simultaneous amplification of multiple amplicons in a single reaction well using a unique primer pair for each. Instead of running multiple individual reactions, all the reactions can be performed in a single sample. After a quick DNA extraction from each colony, students will use multiplex PCR to detect the presence/absence of the CRISPR-modified insertion: one primer pair will generate a ~1,100 bp amplicon in the presence of wild-type *lacZ* gene while a different primer pair will generate a ~650 bp amplicon in the presence of the edited *lacZ* (see the Student Guide for primer target details). In many multiplex experiments, the DNA target sequences do not overlap. In this activity, however, the primers are intentionally designed to overlap at the edit site so that either the “edited” primer set or the “unedited” primer set will generate an amplicon allowing for genotype determination. As an internal control, a third primer pair generates a ~350 bp amplicon only if chromosomal DNA was successfully extracted and amplified.

Though multiplex PCR offers convenience and can help save valuable samples, a multiplex PCR experiment requires careful design so that all targets can be successfully amplified using the same cycling parameters (in a single sample). Some considerations include:

- **Amplicon size** — if they are to be analyzed by gel electrophoresis, each amplicon must be of a different size for adequate separation, but they cannot be too different because shorter amplicons are preferentially amplified
- **Primer pairs** — all must have a similar melting temperature (to allow them to all work well using the same cycling parameters), should not form dimers with one another, and must be specific for their targets

The extra effort, however, is rewarded with the ability to analyze more than one target in a single sample and increased accuracy of comparative analysis and quantitation in research, forensics, and diagnostics.

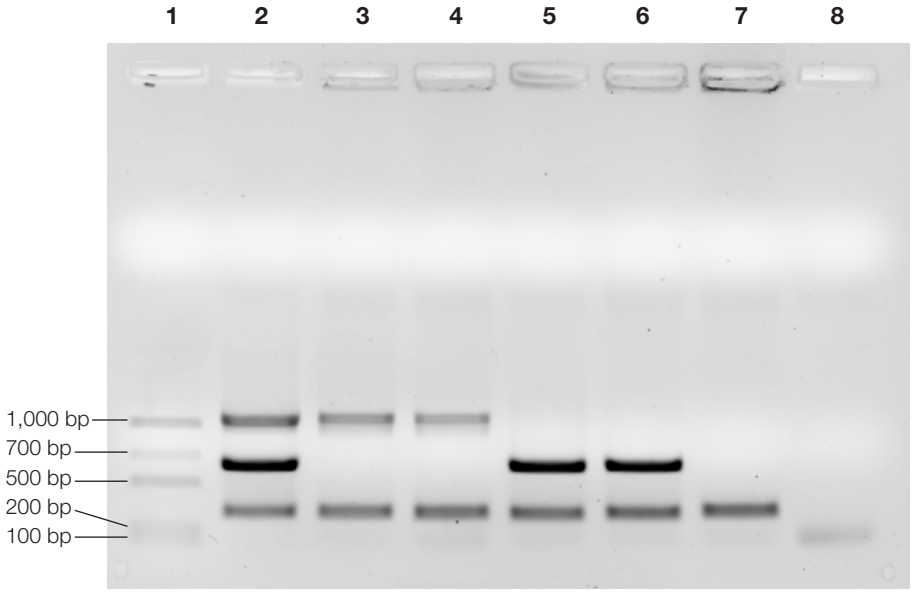
Bacterial Colony DNA Extraction and PCR

Goal: Students collect DNA evidence and combine it with their previous evidence to support their claims of CRISPR-mediated gene editing.

	Teaching tips and notes
Part 1. Results from *lacZ* CRISPR Gene Editing Laboratory	• There are many alternative explanations for the results of the previous bacterial transformation laboratory activity. For example, colony color may be influenced by the introduction of a DNA binding protein, protein-protein interactions that reduce the activity of β-galactosidase, or even a cancellation effect between the elements that were introduced to the bacteria. Many alternate explanations are highly unlikely given our understanding of bacterial cell biology. However, they can be ruled out with additional evidence • Asking students to think of and share example experimental evidence that would rule out one of two alternative explanations is an opportunity for students to practice articulating how evidence supports a claim • Option: if students need more scaffolding, provide them with an alternative explanation to use when answering the questions. For example: the Cas9-sgRNA complex suppresses expression of important genes, including *lacZ,* which leads to cell death; the HDR machinery with donor template DNA interferes with the function of the Cas9-sgRNA complex • Option: ask students to design an experiment that would provide the information needed to rule out one of their alternative explanations
Part 2. Bacterial Colony DNA Extraction and PCR	• The DNA extraction protocol includes necessary heating and centrifugation steps. In order to complete these steps within a 50 min class period, you may need multiple dry/water baths and centrifuges. Be sure to plan ahead for the time it will take to accommodate all student samples • If any student groups found a bacterial colony on plate B, we recommend replacing sample D3 with the colony from B. Bacterial growth on plate B is unexpected and is an opportunity for authentic inquiry in your classroom • Your students may predict the generation of a ~1,750 bp amplicon produced by the reverse wild type and forward edited primers. This is a real possibility. Elongation of the 1,750 bp amplicon takes longer, however, and is suppressed by the design of the PCR program • The supernatant from InstaGene Matrix is technically a better negative control than water. However, decanting supernatant renders the remaining InstaGene Matrix unusable. Water is used instead to prevent waste • The positive control DNA template includes all three PCR targets and the reaction will produce ~1,100, ~650, and ~350 bp amplicons • Formative assessment opportunity: review student PCR predictions to check for comprehension. Look out for misconceptions about primer sets being added to samples separately or that both the 1,100 and 650 bp amplicons would be produced in a single sample. It is possible for both amplicons to be produced from a colony with a mixed population, but if a colony grows from a single transformed cell as intended, then only one should be observed
Part 3. Gel Electrophoresis and Visualization	• The agarose gel electrophoresis laboratory activity is designed to take advantage of the Bio-Rad 10-minute Fast Gel protocol. A power supply that can deliver 300 V, such as the Bio-Rad PowerPac Basic Power Supply, is required for this protocol. See Appendix for more information

Bacterial Colony DNA Extraction and PCR (continued)

	Teaching tips and notes
Part 4. Data Analysis and Argumentation	• Example student gel:



Lane	Sample	*lacZ* gene status
1	Molecular weight ruler	N/A
2	Positive control DNA (+)	N/A
3	PCR Sample (S)	Wild type
4	PCR Sample (C)	Wild type
5	PCR Sample (D1)	Edited
6	PCR Sample (D2)	Edited
7	PCR Sample (B/D3)	???
8	Negative control (–)	N/A

- Lane 7 represents a rare result observed when bacteria repair the double-strand break caused by Cas9 without using the donor DNA template and may be observed in colonies on plate B or D. Although the *lacZ* gene is edited in these cases, the edited *lacZ* primer set binding sequence, which is introduced by the donor template DNA, is missing. The exact cause of these rare events is uncertain. For most students, lane 7 will look identical to lanes 5 and 6
- Ensure that students include evidence from both the multiplex PCR and bacterial transformation experiments when making their claims
- Assessment opportunity: show students the example student gel image and have them propose explanations for the result in lane 7 AND further experiments that could help determine the cause. For example, sequencing could provide detailed information about the status of the *lacZ* gene

Appendix

Fast Gel Protocol and Electrophoresis Preparation Instructions

See Resources for how-to videos on casting, loading, and running agarose gels.

Fast Gel Protocol

There are multiple ways to hasten visualization of DNA bands on an agarose gel using modified conditions and alternate reagents. Options, as well as the required materials and protocols, are provided below.

1. Cast 1% agarose gels with 1x TAE buffer.
2. Prepare 0.25x TAE electrophoresis buffer.
3. Load samples, run gel using conditions in Table 1, and visualize DNA using one of the stain options below.

Table 1. Electrophoresis options.

Electrophoresis Buffer and Voltage	Electrophoresis Time
0.25x TAE at 300 V*	10 min
0.25x TAE at 200 V	20 min
1x TAE at 100 V	30 min

* Requires power supply capable of voltages of 300 V, such as the PowerPac Basic Power Supply (catalog #1645050EDU).

UView 6x Loading Dye and Stain: Prepare DNA samples for electrophoresis using 6x UView Loading Dye and Stain. After gel electrophoresis, visualize instantly with a UV transilluminator or a handheld UV lamp in the dark.

Fast Blast DNA Stain: Prepare DNA samples before electrophoresis with 5x Orange G Loading Dye. After electrophoresis, stain DNA with Fast Blast DNA Stain and visualize the next day.

Preparing Agarose Gels

Cast either eight 7 x 7 cm gels with one 8-well comb for eight workstations or four 7 x 10 cm gels with two 8-well combs each to be shared between two workstations. Table 2 provides measurements for a variety of options.

Table 2. Volumes and quantities of reagents for agarose gels.

Number of Gels	1	4	8	16
1% TAE Agarose Gel (7 x 7 cm) — serves one workstation				
Purified water, ml	39	156	312	624
50x TAE, ml	0.8	3.2	6.4	12.8
Agarose, g	0.4	1.6	3.2	6.4
Total volume of molten agarose, ml	40	160	320	640
1% TAE Agarose Gel (7 x 10 cm) — serves two workstations				
Purified water, ml	49	196	392	784
50x TAE, ml	1.0	4.0	8.0	16.0
Agarose, g	0.5	2.0	4.0	8.0
Total volume of molten agarose, ml	50	200	400	800

Prepare molten agarose

1. Add the appropriate amount of agarose powder and then the liquids to a suitable container; fill to less than 50% of the container volume. Swirl to mix.

   Note: If using an Erlenmyer flask, invert a small 25 ml beaker over the opening to minimize evaporation. If using a bottle, loosen the cap so that air and steam can escape.

2. Place the agarose solution into the microwave. Microwave for 3 min. Continue to heat in 30 sec increments until the solution boils and all agarose has dissolved.

   Caution: Always wear heat-protective gloves, goggles, and a lab coat while preparing agarose gels. Hot molten agarose can cause severe burns.

3. Let the agarose cool to about 60°C before pouring the gels.

Cast agarose gels

There are a variety of ways to cast agarose gels. This section outlines the tape method. Consult the instruction manual for your horizontal electrophoresis system for alternate methods.

1. Firmly seal the ends of a gel tray with standard laboratory tape (not regular sticky tape).

2. Place the comb into the appropriate slot in the gel tray. If pouring a double-well gel, place a comb at one end of the tray and another in the middle.

3. Once the molten agarose has cooled at least to 60°C, pour enough agarose to cover the gel comb teeth or to a depth of 0.5–0.75 cm.

4. Allow the gel to solidify at room temperature for 10–20 min.

5. Carefully remove the comb(s) and the tape.

6. Store gels in a sealable plastic bag at room temperature for up to 1 day or in the refrigerator for up to 1 week.

Preparing TAE Buffer

Conventionally, 1x TAE buffer is used both for gel casting and as running buffer. The electrophoresis time can be greatly reduced by instead running the gels with 0.25x TAE buffer at 200 or 300 V. When using this faster protocol, gels should still be cast using 1x TAE buffer. See Table 3.

1. Combine distilled water with the volume of 50x TAE buffer indicated in Table 10 and mix well.

   Note: If you are using 0.25x TAE buffer sequentially between classes, ensure the buffer is at or below room temperature before using. If the buffer starts out warm, it may become hot enough to melt the agarose gel during a high voltage run.

Table 3. Volumes and quantities of reagents for electrophoresis buffer.

Number of Electrophoresis Chambers	1	4	8	16
0.25% TAE Buffer				
Purified water, ml	274	1,094	2,189	4,378
50x TAE, ml	1.4	5.6	11	22
Total, volume of 0.25% TAE buffer, ml	275	1,100	2,200	4,400

Visualizing DNA

UView 6x Loading Dye and Stain

No additional preparation is needed when using UView 6x Loading Dye and Stain.

1. Replace Orange G Loading Dye with UView 6x Loading Dye and Stain when preparing DNA samples.

2. Directly after electrophoresis, carefully place gels on a UV transilluminator, lower UV shield, and turn on UV light to visualize.

Fast Blast DNA Stain

1. Prepare 100x Fast Blast DNA Stain according to volumes in Table 4.

2. Add ~50 ml of 100x stain per gel in a gel staining tray and gently rock for 2–3 min.

3. After 2 min pour off stain and retain for future use.* Staining longer will greatly increase background.

4. Rinse gel with tap water for 30 sec to 1 min or until all surface stain is removed. Completely cover the gel with a large volume of tap water and gently rock overnight to destain. DNA will be visible as dark blue bands against a lighter blue background after a few hours with contrast gradually increasing overnight.

   Note: Using 100x Fast Blast DNA Stain prevents the small DNA fragments from diffusing in the gel during an overnight stain. Because it is lower concentration, 1x Fast Blast DNA Stain will not prevent DNA fragment diffusion and so should not be used for overnight staining.

   * 100x Fast Blast DNA Stain can be reused at least six times.

Table 4. 100x Fast Blast DNA Stain Preparation.

Number of 7 x 10 cm gels to stain	1	4	8	16
500x Fast Blast DNA Stain, ml	10	40	100	200
Distilled water, ml	40	160	400	800
Total volume of 100x Fast Blast DNA Stain, ml	50	200	500	1,000

Resources

How to Load and Run Agarose Gel Electrophoresis: https://www.youtube.com/watch?v=uAttNVEEEwY. Accessed February 6, 2020.

How to Cast an Agarose Gel: https://www.youtube.com/watch?v=qbUMRDrNnu8. Accessed March 5, 2020.

Polymerase Chain Reaction interactive animation: https://www.bio-rad.com/webroot/web/movies/lse/global/english/what-is-polymerase-chain-reaction/tutorial.html. Accessed February 6, 2020.

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