EDVOTEK® • The Biotechnology Education Company®

NOTE:
This experiment is designed to work with EDVOTEK® Kits 222, 223, or 303. Please refer to page 20 for specifics.

Edvo-Kit # 323

Edvo-Kit #323

GFP Transformation Extension: Colony PCR

Experiment Objective:

In this experiment, students will use colony PCR to analyze bacteria transformed with pFluoroGreen.

See page 3 for storage instructions.

Version 323.240214

PROTOCOL HAS BEEN UPDATED!
Please review before beginning experiment!

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Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

	Component	Storage	Check (√)
A	PCR EdvoBeads™ PLUS*	-20°C Freezer	❑
B	Colony Primer mix concentrate	-20°C Freezer	❑
C	Control DNA concentrate	-20°C Freezer	❑
D	Ampicillin	-20°C Freezer	❑
E	IPTG	-20°C Freezer	❑
F	EdvoQuick™ DNA Ladder	-20°C Freezer	❑
G	TE Buffer	-20°C Freezer	❑
•	BactoBeads™ *E.coli* pt-GFP (*pre*-transformed with GFP)	4°C Refrigerator	❑

**Each PCR EdvoBead™ PLUS contains: dNTP Mixture, Taq DNA Polymerase Buffer, Taq DNA Polymerase, $MgCl_2$, and Reaction Buffer).*

This experiment is designed for 10 groups.

Sample volumes are very small. It is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.

Reagents & Supplies

Store components below at Room Temperature.

	Component	Check (√)
•	ReadyPour™ Luria Broth Agar (ReadyPour™ Agar)	❑
•	Petri Dishes	❑
•	Sterile Loops	❑
•	UltraSpec-Agarose™	❑
•	Electrophoresis Buffer (50x)	❑
•	10x Gel Loading Solution	❑
•	SYBR® Safe DNA Stain	❑
•	FlashBlue™ Liquid Stain	❑
•	0.5 mL microcentrifuge tubes	❑
•	0.2 mL thin-walled PCR tubes	❑

PCR EdvoBeads™ are now conveniently stored at -20°C with the other perishable components.

NOTE:
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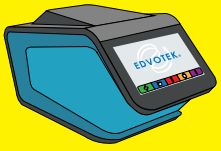


NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

Requirements

- Thermal cycler (EDVOTEK® Cat. #540 or #541-542 highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- UV transilluminator or blue light visualization for SYBR® Safe stained gels (EDVOTEK® Cat #557 recommended)
- UV safety goggles (for visualizing SYBR® Safe stained gels)
- White light visualization system (for visualizing FlashBlue™ stained gels) (EDVOTEK® Cat. #552 recommended)
- Automatic micropipettes (5-50 µL, 20-200 µL 100-1000 µL) with tips
- Microwave or hot plate
- Pipet pump
- Hot gloves
- Disposable laboratory gloves
- Sterile water
- Ice buckets and ice
- Bleach solution or laboratory disinfectant

Background Information

Biotechnology is the modification of living organisms to create a product that is useful for industrial or other purposes. Processes central to biotechnology include traditional practices such as fermentation and selective breeding, as well as more modern developments such as genetic engineering. Scientists utilize genetic engineering and recombinant DNA techniques to effectively re-program cells to create living factories, capable of producing large amounts of important proteins, antibiotics, or metabolites. This technology can be used to manufacture products as diverse as biofuels, biopharmaceuticals and fine chemicals. The demand for these products has driven development of novel technologies for microbial growth, large-scale production of biomolecules and the subsequent purification of those molecules.

In order to create a recombinant DNA molecule, the sequence of interest is isolated and inserted into a plasmid vector using molecular cloning techniques (Figure 1). The modified plasmid is transformed into *E.coli*, plated on selective media, and allowed to grow overnight. Because the plasmid contains a selectable marker, only cells transformed with this plasmid should grow into colonies. However, just because the plasmid is present does not mean the sequence of interest has been incorporated properly. For example, the DNA could have inserted in the reverse orientation, or the plasmid could have ligated to itself – there is no way to tell whether the cloning was successful from the transformation alone. As such, scientists must analyze the plasmid to ensure that the correct DNA molecule was assembled before performing complex experiments.

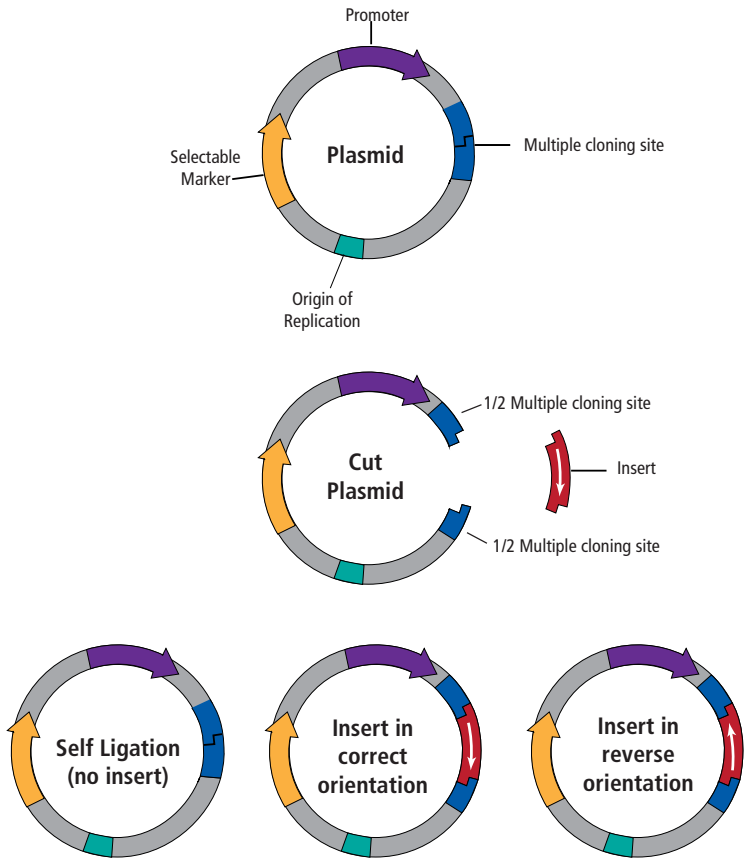


Figure 1: Creating a recombinant DNA molecule.

There are three main ways by which a recombinant plasmid can be analyzed: restriction digestion, DNA sequencing, and polymerase chain reaction. The restriction digestion approach uses enzymes called restriction endonucleases to cut the DNA at specific target sequences. Different digestion patterns are generated depending on whether the insert is present or absent. The second analytical technique, Sanger DNA sequencing, can determine the exact nucleotide sequence of the plasmid. The third approach, the polymerase chain reaction (PCR), uses synthetic oligonucleotide primers to target the inserted gene for amplification. Depending on the primers chosen, PCR can be used to determine the size and the orientation of the insert.

Both DNA sequencing and restriction digestion require that the plasmid be purified before analysis. First, a single colony from the transformation plate is used to inoculate liquid media. The bacteria are then allowed to grow and multiply in the media overnight, producing millions of copies of the plasmid. Finally, the plasmid is purified from the bacteria and analyzed. This process can be very time consuming, especially because each plasmid to be tested must be purified individually. In contrast, colony PCR analysis can be completed in a few short hours on the day after the transformation because it eliminates the need for plasmid

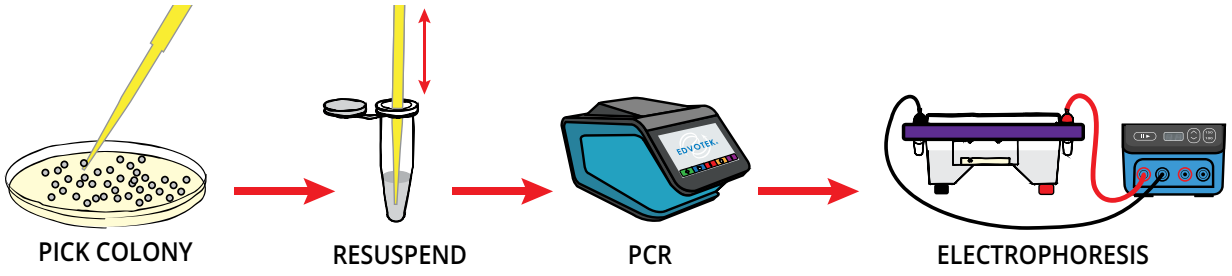


Figure 2: Flowchart of colony PCR.

purification. PCR is very sensitive and can detect a recombinant insert using only the DNA within the cells on the transformation plate as a template.

As a result, colony PCR represents a simple, fast and reliable method to determine whether gene cloning was successful (summarized in Figure 2). To perform colony PCR, a sterile pipet tip is used to collect a small amount of cells from the transformation plate. These cells are resuspended in a buffered solution that contains nucleotides, a thermostable DNA polymerase (*Taq*) and sequence specific primers. This mixture is heated to 94°C to lyse the cells, releasing the plasmid into the solution. Additionally, at this high temperature, double-stranded DNA is denatured (unzipping it into single strands). Next, the sample is cooled to 45-60°C, allowing the primers to base pair with their target DNA sequences (a step known as annealing). Lastly, the temperature is raised again, to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer and synthesize a new strand of DNA. Each cycle (denaturation, annealing, extension) doubles the amount of target DNA (Figure 3). A specialized machine—called a "thermal cycler" or "PCR machine"—is used to rapidly heat and cool the samples. Using this machine, a single PCR cycle can be completed in less than five minutes; 20-40 cycles produce a sufficient amount of DNA for analysis.

Following PCR, the samples can be analyzed using agarose gel electrophoresis, which separates DNA fragments according to size. The sample is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current draws the DNA through the gel toward the positive electrode.

At first glance, an agarose gel appears to be a solid at room temperature. At the molecular level, the gel contains small channels through which DNA can pass. Small DNA fragments move through these holes without restriction, but larger DNA fragments have a difficult time navigating this matrix and so proceed more slowly. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized using a stain that sticks to DNA. If PCR produces a band of the correct size, it is likely that the cloning was successful. If further confirmation is desired, the plasmid can be analyzed separately by sequencing or restriction digestion.

In this experiment, students will utilize colony PCR to analyze bacteria containing pFluoroGreen, a bacterial plasmid containing the GFP gene from the jellyfish *A. victoria*. After transforming the recombinant plasmid into *E.coli*, a single colony will be used as the DNA template for PCR. The resulting PCR sample will then be analyzed using agarose gel electrophoresis. If the bacteria have been transformed successfully, a 475 bp segment representing the GFP gene will be produced. A bacterial housekeeping gene is simultaneously amplified as a positive control; this PCR product is 190 bp. The presence of both of these bands is indicative of a successful colony PCR experiment.

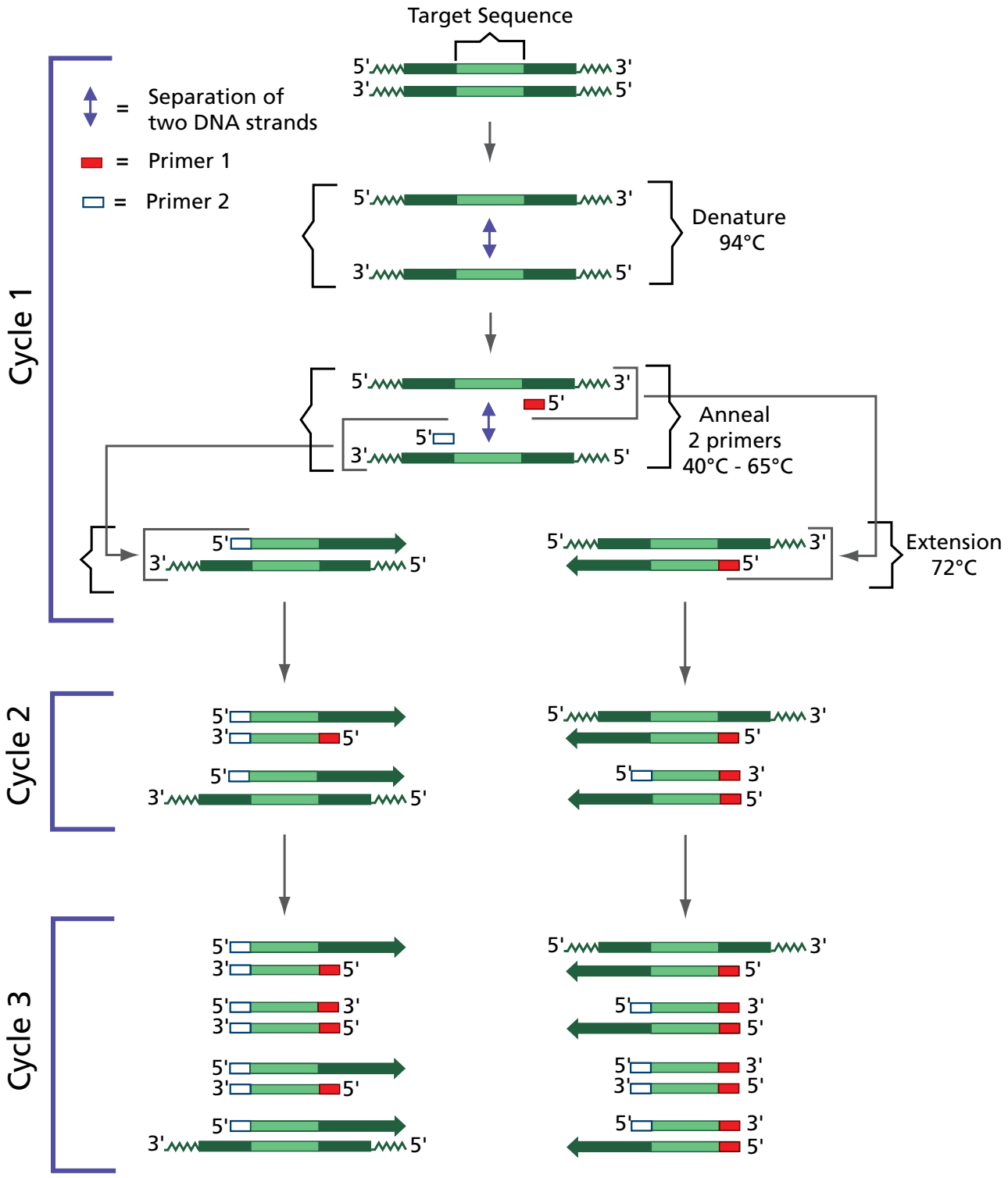


Figure 3: DNA Amplification by the Polymerase Chain Reaction.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will use colony PCR to analyze bacteria transformed with pFluoroGreen.

This experiment is designed to work with Edvotek Kits #222, 223, or 303. Please refer to page 20 for specifics.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations.

After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

PRELAB QUESTION:

- Of your transformation plates, which will have the GFP gene? Which will not? Form a hypothesis and use this experiment to test two of these conditions.

NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

Laboratory Safety

IMPORTANT READ ME!

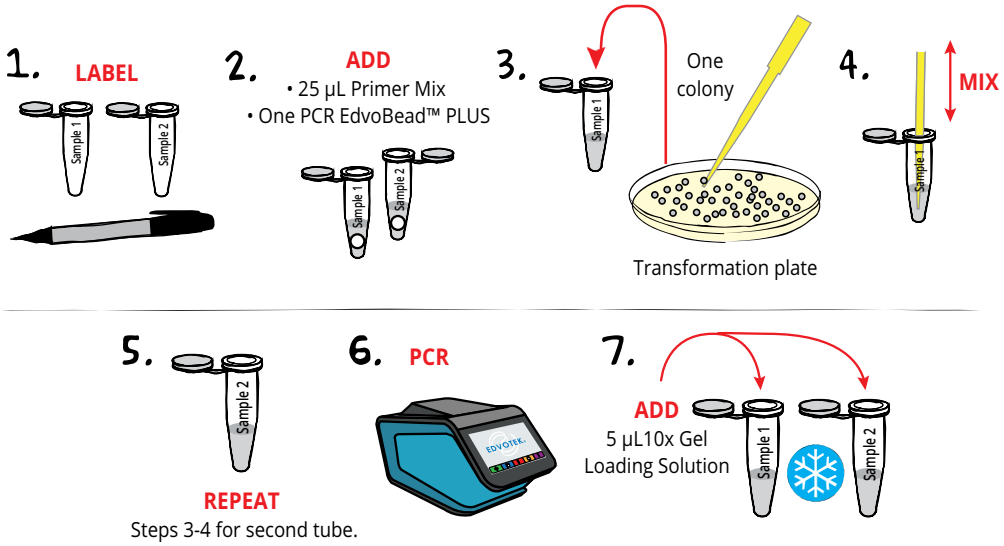
Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicillin, ampicillin, or other related antibiotics should not participate in this experiment.

Wear gloves and safety goggles

1. Wear gloves and goggles while working in the laboratory.
2. Exercise extreme caution when working in the laboratory - you will be heating and melting agar, which could be dangerous if performed incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. The *E.coli* bacteria used in this experiment is not considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
   - Autoclave at 121°C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
5. Always wash hands thoroughly with soap and water after working in the laboratory.

6. If you are unsure of something, ASK YOUR INSTRUCTOR!

Module I: Colony PCR



IMPORTANT:

The transformants from bacterial transformation plates (kits 222, 223 or 303) will be picked up and used as the template to amplify the GFP gene. Each group will choose two colonies from different plates.

Before performing PCR, each group should create an experiment to determine which cells have the GFP DNA and which do not. We recommend picking one colony from a +DNA plate and one colony from a -DNA plate.

1. **LABEL** two 0.2 mL PCR tubes with the sample name and your initials.
2. **ADD** 25 µL primer mix and one PCR EdvoBead™ PLUS to each PCR tube. Spin down if necessary to get all of the liquid to the bottom of the tube.
3. Using a sterile yellow pipet tip, **REMOVE** one small colony from a transformation plate. **ADD** the cells to the PCR reaction by placing the pipet tip into the liquid and gently shaking.
4. **MIX** the sample by gently pipetting up and down with the same pipet tip used to pick the colony.
5. **REPEAT** steps 3-4 with a second colony and the second labeled PCR tube.
6. **AMPLIFY** DNA using PCR:

- Initial denaturation 95°C for 5 minutes.
- 95°C for 30 seconds
- 55°C for 30 seconds } 35 cycles
- 72°C for 45 seconds
- Final extension at 72°C for 5 minutes.

7. After PCR, **ADD** 5 µL of 10x Gel Loading Solution to the samples. **PLACE** tubes on ice. **PROCEED** to Module II: Separation of PCR Products by Electrophoresis.

OPTIONAL STOPPING POINT:
The PCR samples may be stored at -20°C for electrophoresis at a later time.

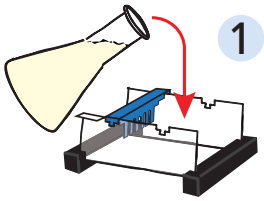
NOTES:

- **At least one control sample should be performed per class. To prepare the control sample, add 25 µL Primer Mix, 5 µL control DNA, and one PCR EdvoBead™ PLUS to a 0.2 mL PCR tube.**
- When picking colonies, use a sterile pipet tip. DO NOT scoop up LB agar since this inhibits the PCR reaction.
- Put one colony in each PCR tube - do not put multiple colonies in the same reaction. If colonies are too small to pick one, then put the plate back in the incubator and allow the cells to grow more. If colonies are too crowded, have students pick a single colony from the prepared control bacteria plate.
- DO NOT spin the tube down after adding the single colony- this might pellet the cells that were added.

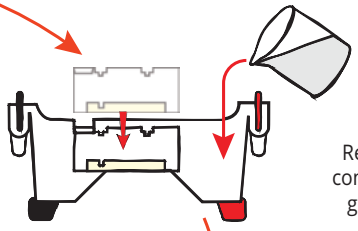
OVERVIEW

MODULE II: Separation & Analysis of PCR Products by Electrophoresis

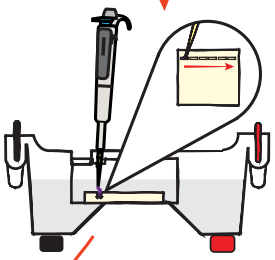
Time required: See Table C



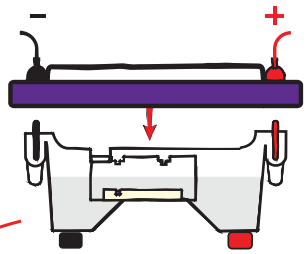
1 Prepare agarose gel in casting tray.



2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.



3 Load each sample in consecutive wells

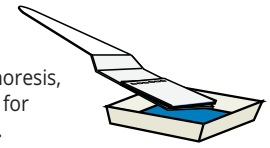


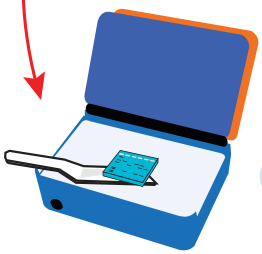
4 Attach safety cover & connect leads to power source to conduct electrophoresis.

(OPTIONAL) MODULE III: Staining Using FlashBlue™

Time required: 30 min.

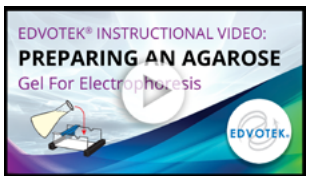
5 After electrophoresis, transfer gel for staining.

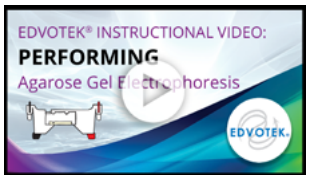




6 Analysis on white light source.

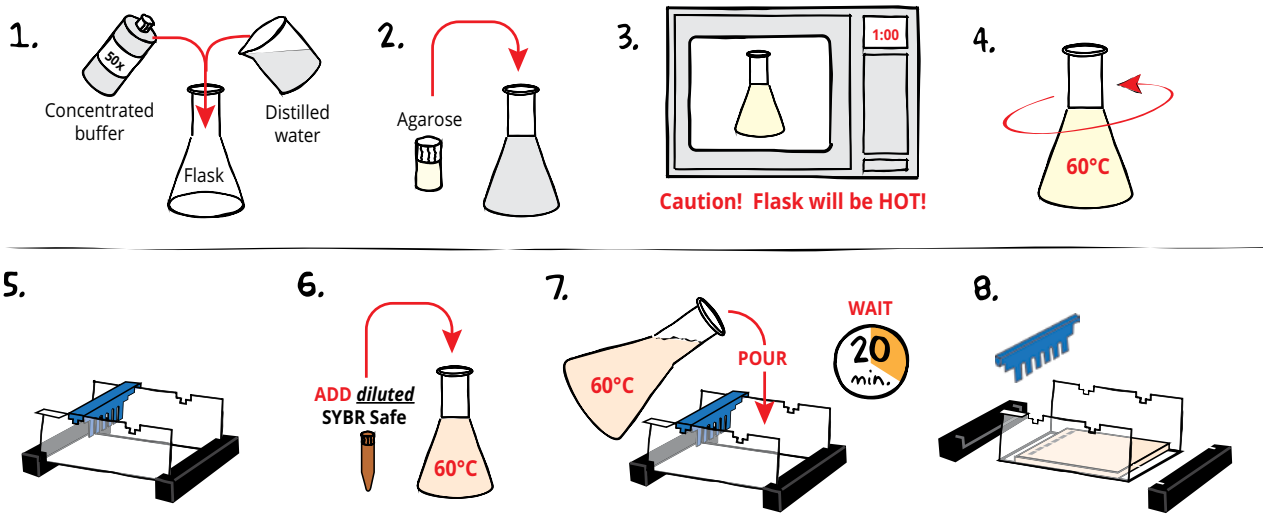
Related EDVOTEK® Instructional Videos





www.youtube.com/edvotekinc

Module II: Separation & Analysis of PCR Products by Electrophoresis



CASTING THE AGAROSE GEL

Wear gloves and safety goggles

Reminder: Two groups will share one 1.5% agarose gel with at least five sample wells for electrophoresis. We strongly recommend 10 x 7 cm or 14 x 7 cm gels for this experiment.

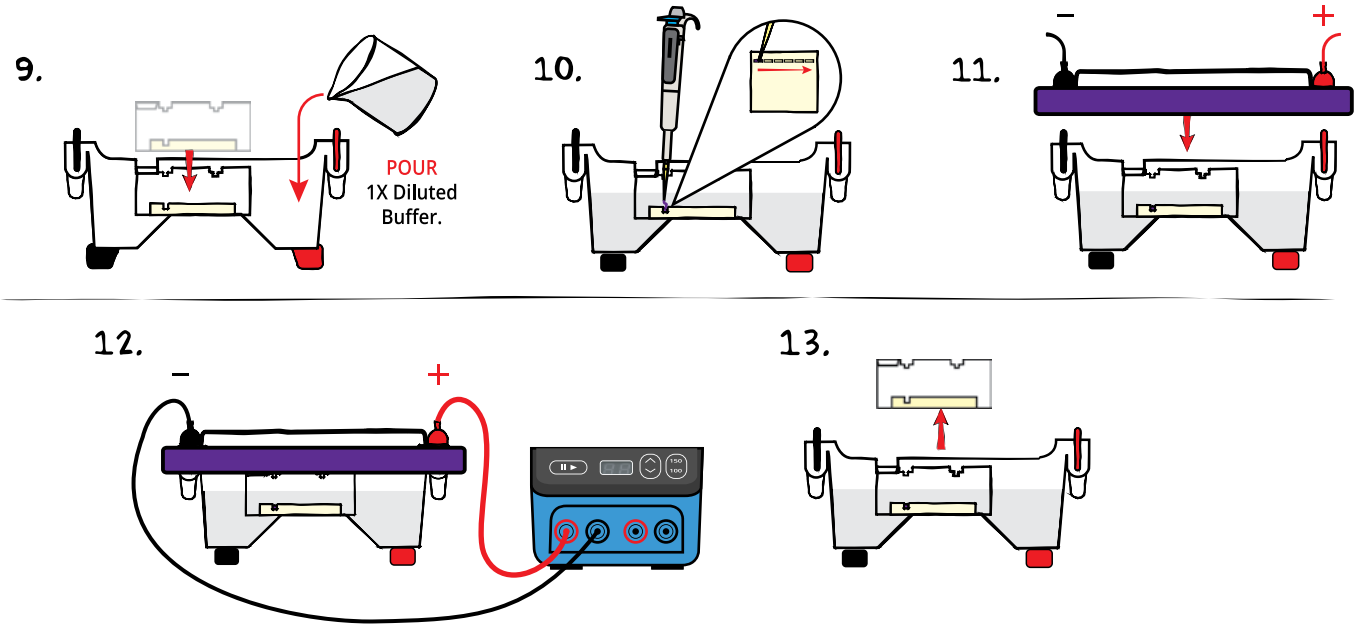
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60°C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch. ***NOTE: This experiment requires 6 wells.***
6. Before casting the gel, **ADD *diluted* SYBR® Safe** to the molten agarose and swirl to mix (see Table A).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — **Individual 1.5% UltraSpec-Agarose™ Gel with SYBR® Safe Stain**

Size of Gel Casting Tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.45 g	30 mL	30 µL
10 x 7 cm*	0.9 mL	44.1 mL	0.68 g	45 mL	45 µL
14 x 7 cm	1.2 mL	58.8 mL	0.9 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

Module II: Separation and Analysis of PCR Products by Electrophoresis, continued



RUNNING THE GEL

9. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.

10. Using Table 1 as a guide, **LOAD** the entire sample (30 µL) into the well. **RECORD** the position of each sample in the table.

11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.

12. **CONNECT** leads to the power source and **PERFORM** electrophoresis. (See Table C for time and voltage guidelines.)

13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

TABLE 1: Gel Loading

Lane	Recommended	Sample Name
1	EdvoQuick™ DNA Ladder	
2	Control DNA	
3	Group 1, Sample 1	
4	Group 1, Sample 2	
5	Group 2, Sample 1	
6	Group 2, Sample 2	

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

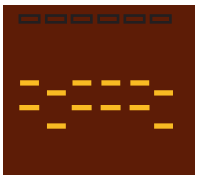
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

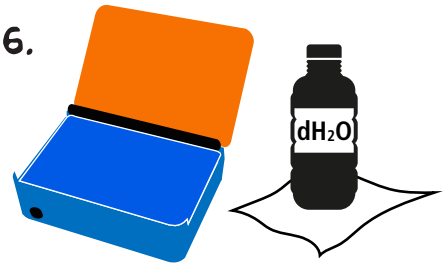
Table C — **Time and Voltage Guidelines (1.5% - Agarose Gel)**

	Electrophoresis Model: EDGE	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	15/30	20/40
125	N/A	30/50
100	30/40	40/60

Module II: Separation and Analysis of PCR Products by Electrophoresis, continued

14. 

15. 

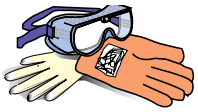
16. 

VISUALIZING THE SYBR® GEL

14. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.

15. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **PHOTOGRAPH** results.

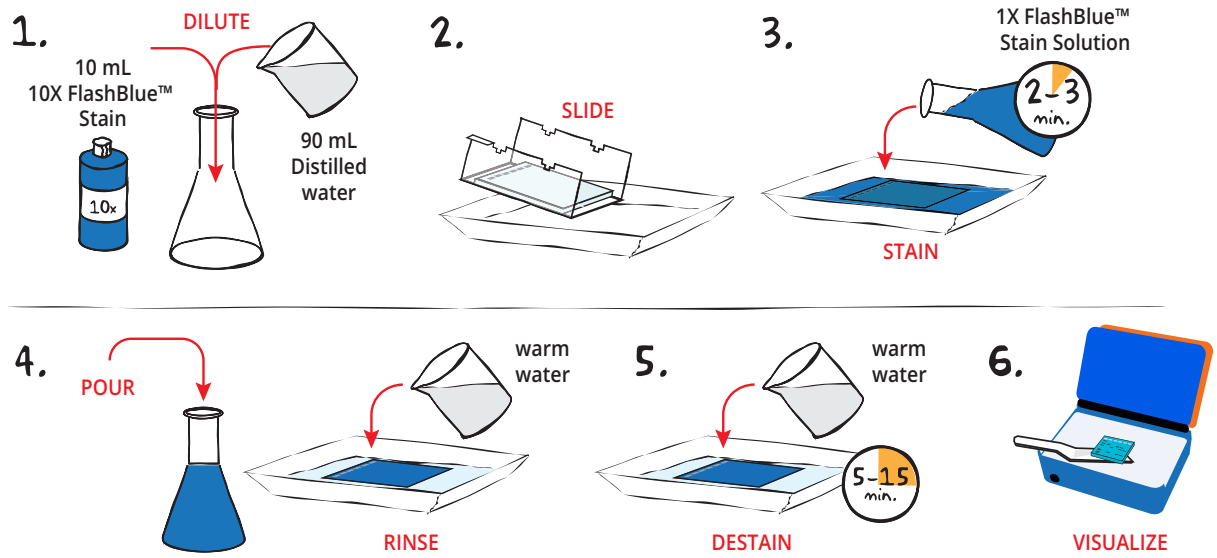
16. **TURN** the unit off. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.



Be sure to wear UV goggles if using a UV transilluminator.

Module III: Staining Agarose Gels with FlashBlue™ (OPTIONAL)

FlashBlue™ Stain is a simple and effective visible DNA stain that can be used as an alternative, or in addition to, UV-reactive DNA stains like SYBR® Safe. ***IF staining with both SYBR® Safe and FlashBlue™, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45°C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45°C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.


Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Study Questions

1. Why is it important to analyze recombinant DNA molecules after transforming them into bacteria?

2. What is colony PCR? What are some advantages to using this technique?

3. List and describe the three steps of PCR.

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
Module I: Colony PCR	Perform Transformation Kit 222 (GFP only), 223, or 303.*	The transformation can be performed up to one week before experiment. The plates should be stored at 4° C until necessary.	60 min.
	Prepare LB-agar plates	2-7 days before the experiment	30 min.
	Prepare source plates with Bacto-Beads™ containing E.coli transformed with GFP	At least one day before the experiment	20 min. to streak plates; 16-20 hours to incubate plates
	Program thermal cycler	Any time before performing the experiment.	15 min.
	Prepare & aliquot various reagents (primer, DNA template, ladder, etc.)	One day to 30 minutes before performing the experiment.	30 min.
Module II: Separation & Analysis of PCR Products by Electrophoresis	Prepare diluted TAE buffer and diluted SYBR® Safe Stain	Up to one day before performing the experiment.	10 min.
	Prepare molten agarose and pour gel	One day to 30 minutes before performing the experiment.	30 min.
Module III: Staining Agarose Gels Using FlashBlue™ (OPTIONAL)	Prepare staining components	Up to 10 min. before the class period.	10 min.

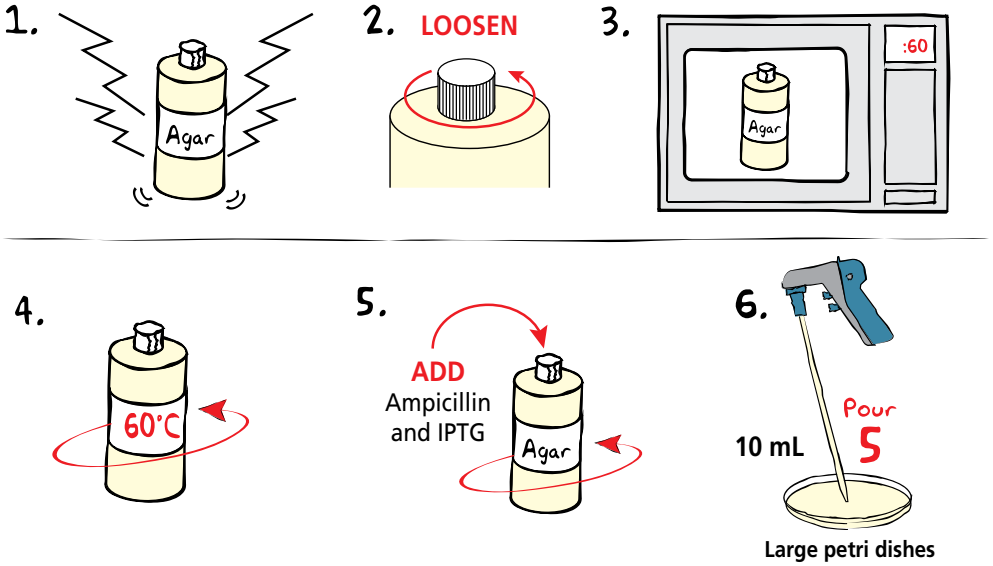
**** NOTE: The Transformation kits are not included with this experiment. See page 20 for specifics.***

IMPORTANT NOTE: Before running the experiment, confirm that your PCR program matches the settings below.

- Initial denaturation 95°C for 5 minutes.
- 95°C for 30 seconds
- 55°C for 30 seconds } 35 cycles
- 72°C for 45 seconds
- Final extension at 72°C for 5 minutes.

Pre-Lab Preparations: Module I - Colony PCR

PREPARATION OF LB-AGAR PLATES FOR CONTROL BACTERIA



1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.

2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. CAUTION: Failure to loosen the cap prior to heating may cause the bottle to break or explode.

3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and MIX by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).

4. **COOL** the ReadyPour™ Agar to 60°C with careful swirling to promote even dissipation of heat.

5. **ADD** the entire amount of IPTG (E) and ampicillin (D) to the cooled ReadyPour Agar. **RECAP** the bottle and **SWIRL** to mix the reagents. ONLY ADD REAGENTS TO COOLED AGAR. Reagents like ampicillin and IPTG degrade at high temperature.

6. **POUR** 10 mL of the cooled ReadyPour™ Agar into each of the five large petri dishes using a 10 mL pipet and pipet pump.

Wear Hot Gloves and Goggles during all steps involving heating.

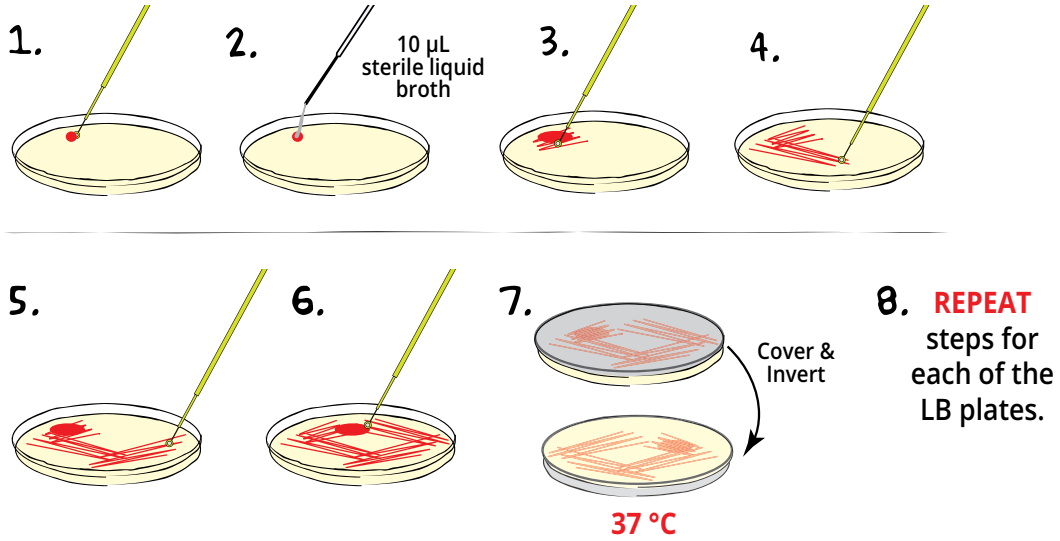
NOTE FOR STEP 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

Pre-Lab Preparations: Module I - Colony PCR

PREPARATION OF CONTROL BACTERIA PLATES

The BactoBeads™ containing *E.coli* transformed with GFP should be streaked 16-20 hours before the experiment is performed. **If necessary, the plates can be streaked up to one week in advance and stored in the refrigerator.** If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.

NOTE: These plates are only to be used if the students do not get any transformed colonies after performing kits #222, 223 or 303.



1. **REMOVE** a single BactoBead™ from the vial using a sterile inoculating loop. Using aseptic technique, **TRANSFER** the bead to the edge of a large petri plate (LB source plate) and replace lid. **CAP** the vial immediately after using to limit exposure to moisture in the air.
2. Instantly **DISSOLVE** the bead by adding 10 µL of sterile liquid broth or sterile water.
3. **STREAK** the loop back and forth through the dissolved BactoBead™ to make a primary streak at the top of the plate. Try not to gouge the loop into the medium.
4. **STREAK** the loop through primary streak to a clean part of the agar several times to create a secondary streak.
5. **ROTATE** the plate. **STREAK** the loop through the secondary streak to a clean part of the agar several times.
6. **ROTATE** the plate once more. **STREAK** the loop through the third streak to a clean part of the agar. This should produce isolated colonies.
7. **COVER** the plate and **INCUBATE INVERTED** at 37°C for 16 to 20 hours. If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.
8. **REPEAT** the above steps for each of the LB source plates.

Pre-Lab Preparations: Module I - Colony PCR

Student transformation plates from kit #222, 223, or 303 are required for this experiment. Students should work in the same groups that they did for the transformation.

Each group will be able to perform two colony PCR experiments. Allow students to choose plates they would like to analyze. We recommend having students pick one colony from the +DNA/+Amp or +DNA/+Amp/+IPTG plates. These plates will have cells containing the GFP gene. The students should pick a second colony from the -DNA plate, as this plate will contain untransformed bacteria.

If colonies are too small to pick using a pipet tip, incubate the plates at 37°C for an additional 4-8 hours. If plates are overcrowded, or if transformation was unsuccessful, have students pick a single colony from the prepared control bacteria plate.

Preparation of Colony PCR Primer Mix

1. Thaw the Primer Mix Concentrate (B) on ice.
2. Add 1 mL of TE buffer (G) to the tube of Primer Mix Concentrate. Cap tube and mix.
3. Aliquot 75 µL of the diluted Colony PCR primer mix into 10 labeled snap-top microcentrifuge tubes.
4. Distribute one tube of diluted Colony PCR primer mix to each student group.

FOR MODULE I
Each Group should receive:
- Transformation plates from previous experiment
- 1 0.5 mL snap-top tube containing GFP Primer Mix
- 2 PCR EdvoBeads™ PLUS
- 2 0.2 mL snap-top PCR tubes

Preparation of the Control DNA

1. Thaw the tube of Control DNA Concentrate (C) on ice.
2. Add 20 µL of TE buffer (G) to the tube containing the Control DNA Concentrate. Pipet up and down to mix.
3. Dispense 6 µL of the diluted Control DNA for each control reaction. At least one control reaction should be performed per class to confirm that the PCR was successful.

IMPORTANT:
The PCR EdvoBeads™ absorb moisture if left open, even for a short period of time. Only open the vial when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C (desiccated) until needed.

PCR EdvoBeads™

NOTE: The beads are vacuum-sealed and may move around once the stopper is removed. Only open the vial when you are ready to use the beads and quickly recap the vial.

Dispense the PCR EdvoBeads™ just prior to the students performing their PCR reactions. Alternatively, student groups can dispense PCR EdvoBeads™ during Module I. Each group will require 2 beads and 2 tubes.

1. Open the vial of PCR EdvoBeads™ (carefully remove metal crimp) and gently/slowly remove the gray rubber stopper.
2. With gloved hand, forceps or sterile loop, remove one single bead and transfer to a PCR tube. Do this for the number of reactions you wish to perform. Quickly cap each of the tubes and distribute to the student groups.

PCR Amplification

The Thermal cycler should be programmed as outlined on page 17 and in Module I in the Student's Guide.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 min.) is recommended to check that the thermal cycler is properly programmed.

Pre-Lab Preparations: Module II

AGAROSE GEL ELECTROPHORESIS

This experiment requires a 1.5% agarose gels. Each gel can be shared by 2 student groups. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Prepare SYBR® Safe Stain:

1. Following the instructions in Appendix B, prepare 1x Electrophoresis Buffer by combining 20 µL of 50X Concentrated Buffer with 980 µL of distilled water.

2. Add 300 µL of the 1X buffer from step 1 to the tube of SYBR® Safe and mix by tapping the tube several times. The diluted SYBR® Safe Stain is now ready to be used during agarose gel preparation.

Individual Gel Preparation:
Each student group can be responsible for casting their own individual gel prior to conducing the experiment. See Part 1 in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water, agarose powder, and *diluted* SYBR® Safe Stain.

FOR MODULE II
Each Group should receive:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- Diluted SYBR® Safe Stain
- EdvoQuick™ DNA Ladder (30 µL)

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B.

Preparing Gels in Advance:
Gels may be prepared ahead and stored for later use. Solidified gels can be store in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels.

Do not freeze gels at -20°C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials:
Each 1.5% gel should be loaded with the EdvoQuick™ DNA ladder. Aliquot 30 µL of the EdvoQuick™ DNA ladder (F) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.

Visualizing SYBR® Safe-Stained Gels:
SYBR® Safe is a DNA stain that fluoresces when bound to double-stranded DNA, allowing us to visualize our samples. This DNA stain is compatible with both UV and blue-light transilluminators. For best results, we recommend the TruBlu™ 2 Blue Light Transilluminator (**Cat. #557**).

Pre-Lab Preparations: Module III

STAINING WITH FLASHBLUE™ (OPTIONAL)

MODULE III (OPTIONAL)
Each Group should receive:
- 10 mL 10X concentrated FlashBlue™
- Small plastic tray or weight boat
- Distilled or deionized water

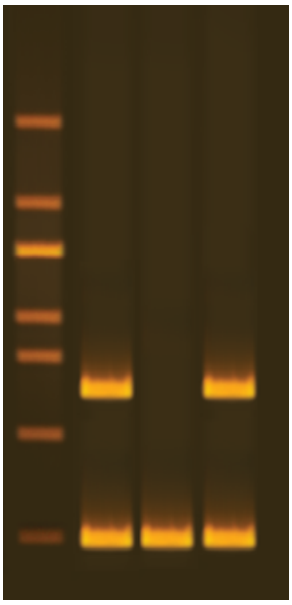
FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to "equilibrate" in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (**Cat. #552**) or the white light feature of the TruBlu™ 2 (**Cat. #557**) is recommended for visualizing gels stained with FlashBlue™.

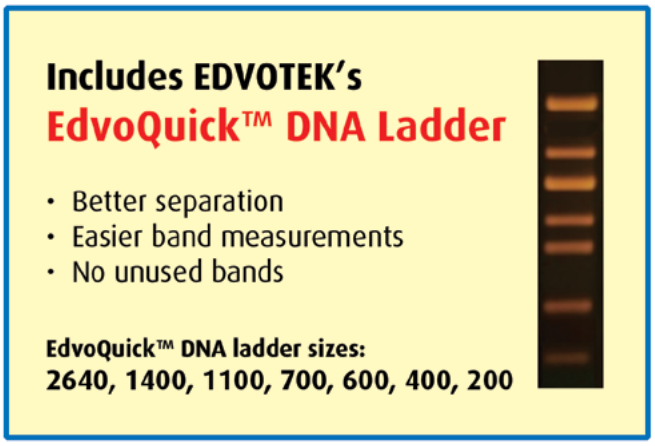
- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

Experiment Results and Analysis





Student results will vary depending upon the plates chosen for the colony PCR. The photo represents the results when bacteria from a -DNA plate and bacteria from a +DNA plate are analyzed.

LANE	SAMPLE	MOLECULAR WEIGHT	RESULT
1	EdvoQuick™ DNA Ladder	---	---
2	Control DNA	190, 475 bp	Contains both pFluoroGreen DNA and *E. coli* DNA
3	Bacteria from -DNA plate (-DNA or -DNA/+Amp)	190 bp	Contains *E. coli* DNA, but does not contain pFluoroGreen DNA
4	Bacteria from +DNA plate (+DNA/+Amp or +DNA/+Amp/+IPTG)	190, 475 bp	Contains both pFluoroGreen DNA and *E. coli* DNA

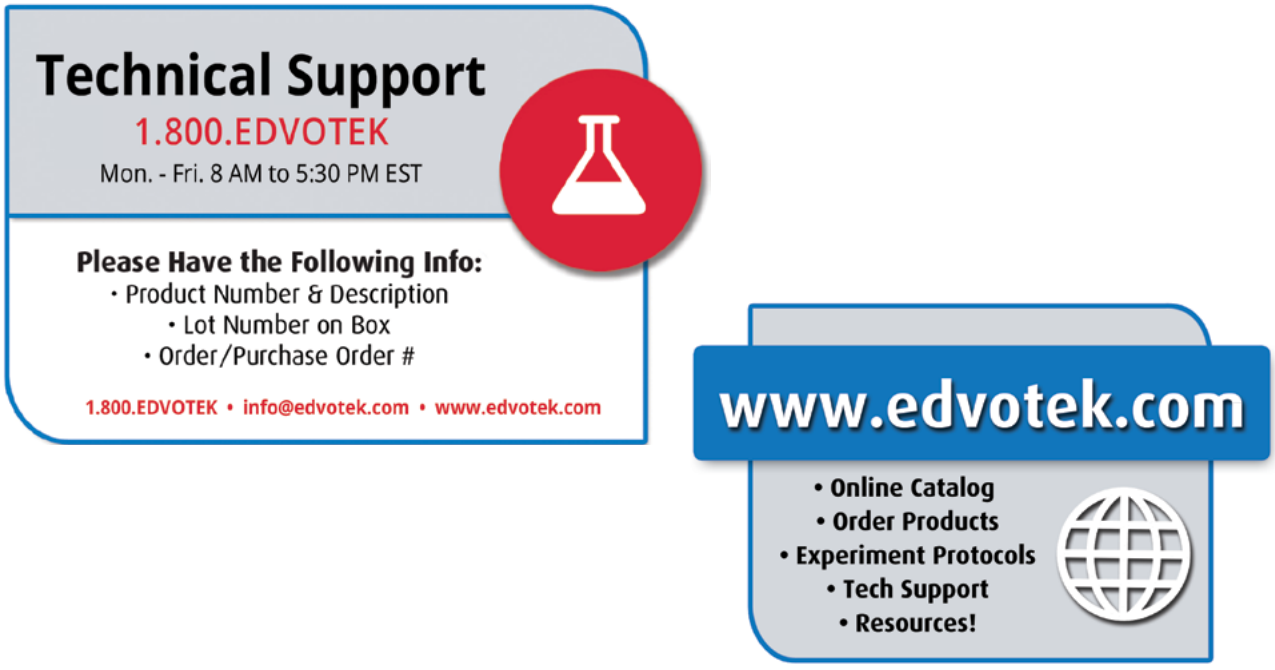
Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Agarose Gels

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets



Appendix A
EDVOTEK® Troubleshooting Guides

PCR AND ELECTROPHORESIS

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR	Sample has evaporated	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 25 µL primer mix into the 0.2 mL tube.
The ladder, control DNA, and bacterial PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
		The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
There is only a small amount of SYBR® Safe in my tube.	SYBR® Safe is a concentrate that is diluted before use.	Centrifuge or tap the tube to move the contents to the bottom of the tube. Dilute the SYBR® Safe before use as outlined on page 20.
After staining, the ladder and control PCR products are visible on the gel but some bacterial samples are not present.	Bacterial DNA sample was not concentrated enough.	Colony was not transferred into the tube.
	Wrong volumes of DNA and primer added to PCR reaction.	Practice using pipettes
Some bacterial samples have more/less amplification than others.	Concentration of bacteria varies by sample.	There is an inherent variability in Colony PCR.
Low molecular weight band in PCR samples	Primer dimer	Low concentration of bacterial DNA in PCR reaction. Colony may have been too small.
DNA bands were not resolved.	Tracking dye should migrate at least 6 cm from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™

Appendix B
Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2940 mL	3000 mL (3 L)

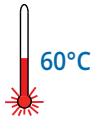
Batch Agarose Gels (1.5%)

For quantity (batch) preparation of 1.5% agarose gels, see Table E.

Table E — Batch Preparation of 1.5% UltraSpec-Agarose™

Amt of Agarose	+	50x Conc. Buffer	+	Distilled Water	=	Diluted Buffer (1x)
4.5 g		6.0 mL		294 mL		300 mL

1. Use a 1 L flask or bottle to prepare the diluted gel buffer.
2. Pour the appropriate amount of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Add the entire tube of ***diluted*** **SYBR® Safe** stain (from page 21) to the cooled agarose and mix well.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 30 mL for a 7 x 7 cm tray, 45 mL for a 10 x 7 cm tray, and 60 mL for a 14 x 7 cm tray. ***NOTE: For this experiment, 10 x 7 cm or 14 x 7 cm gels are recommended.***
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

NOTE:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.