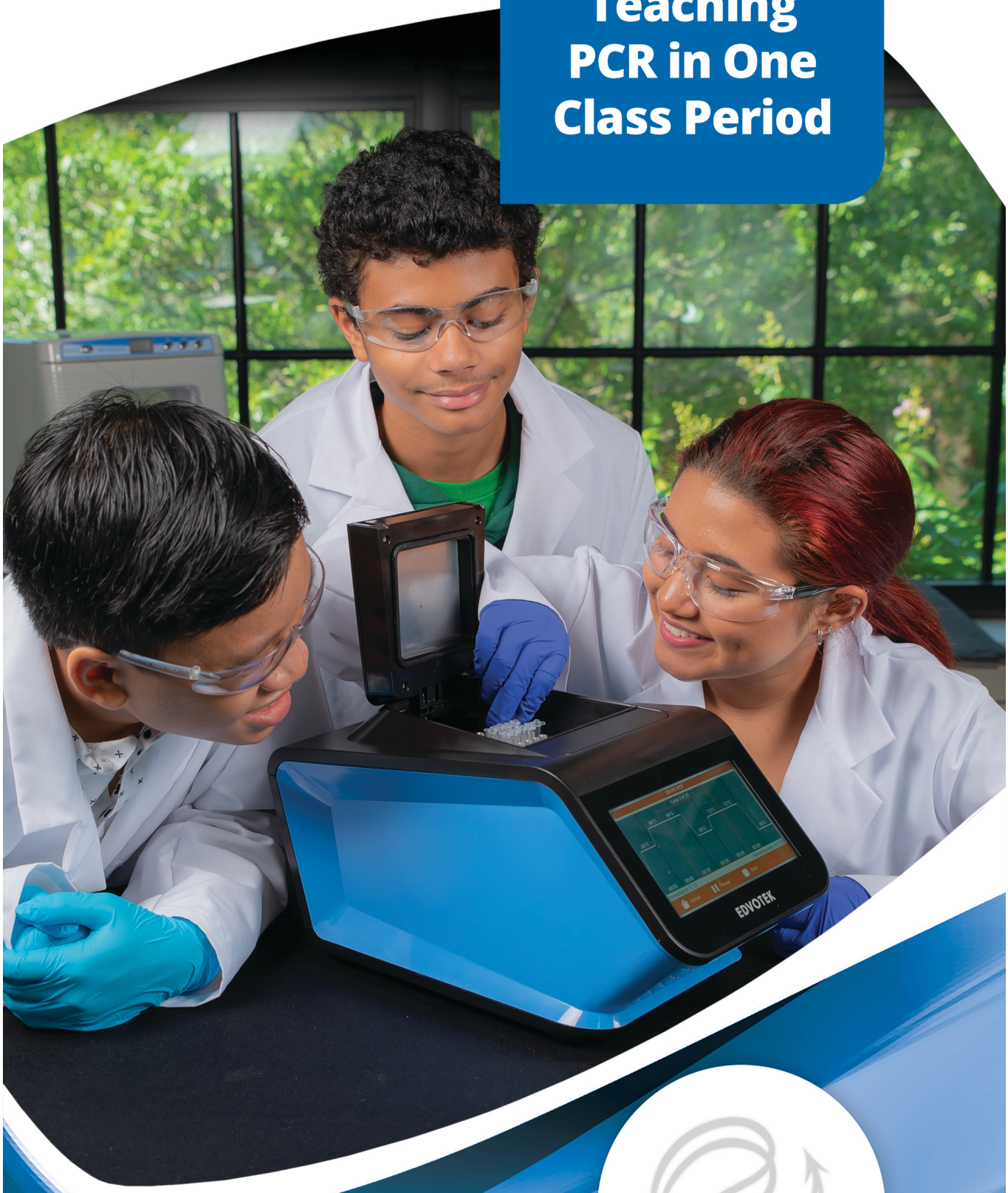
WORKSHOP

Teaching PCR in One Class Period



EDVOTEK®

Designed for the Classroom

SINCE 1987

Introduction

In this experiment, students will gain an understanding of the traditional three-step Polymerase Chain Reaction (PCR). Using PCR and Agarose Gel Electrophoresis, they will analyze a small section of Lambda DNA in a time-saving two-step process.

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Background Information *Excerpts from EDVO-Kit 372*

THE POLYMERASE CHAIN REACTION

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Mullis recognized that he could replicate DNA *in vitro* using short, synthetic DNA oligonucleotides (known as primers) and DNA polymerase I in a process similar to DNA replication in a cell's nucleus. Because researchers can customize the primers to target a specific gene, this method allows for the rapid amplification of a selected DNA sequence. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

Before performing PCR, template DNA is extracted from a biological sample. Two primers are designed to correspond to the 5' and 3' ends of the target sequence. The template DNA and primers are mixed with buffer, the four "free" deoxynucleotides (dATP, dCTP, dGTP, and dTTP), and a thermostable DNA polymerase (*Taq*). Next, the PCR mixture is subjected to sequential heating/cooling cycles at three different temperatures to amplify DNA.

- In the first step, known as "denaturation", the mixture is heated to 94° C to disrupt the hydrogen bonds between the complementarity strands. This causes the target DNA to unzip into single strands (or melt). It is important to use a thermostable DNA polymerase for PCR because this enzyme remains stable at high temperatures.
- In the second step, known as "annealing", the reaction mixture is cooled to 45° C - 65° C. This allows the primers to base pair with the target DNA sequence.
- In the third step, known as "extension", the temperature is raised to 72° C. This temperature is optimal for *Taq* polymerase to add nucleotides to the 3' end of the primer, synthesizing a new strand of DNA.

Together, these three steps - denaturation, annealing, and extension – make up one PCR "cycle" (Figure 1). To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to heat and cool the samples rapidly.

Each PCR cycle doubles the amount of the target DNA in less than five minutes. This makes PCR a very sensitive technique, as only a few copies of the template DNA are required to produce a large amount of signal. Mathematically, PCR is described as an exponential relationship – if we begin with a starting copy number of m, then after n cycles, we will have $m \times 2^n$ copies of our DNA target. For example, if we start with one copy of our target, we will have two copies after the first PCR cycle, four after the second PCR cycle, eight after the third PCR cycle,

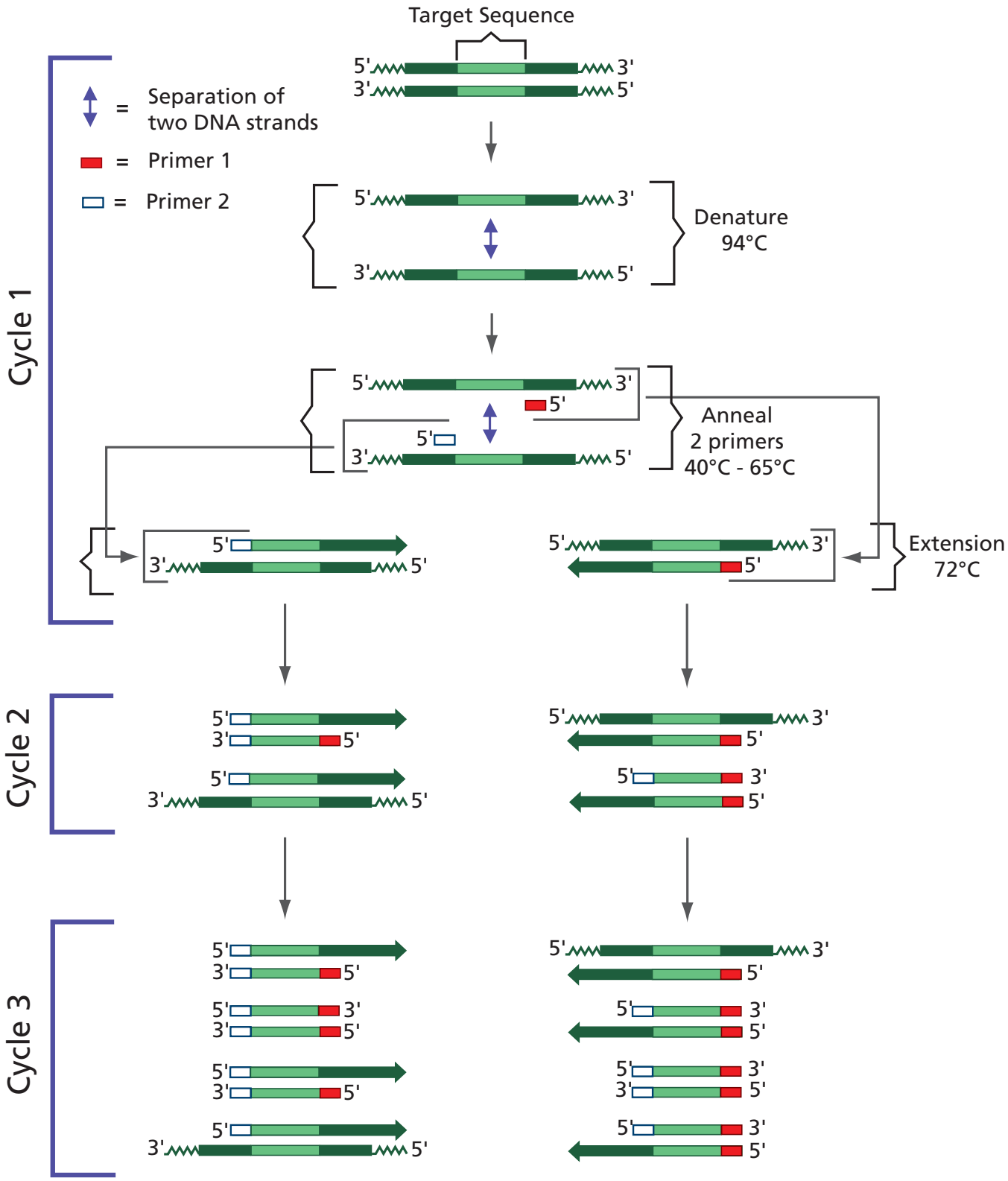


Figure 1: The Polymerase Chain Reaction (PCR) - Three Cycles

and so on. In numbers, cycle 1 equals $1x2^1$, cycle 2 equals $1x2^2$, cycle 3 equals $1x2^3$. After n cycles, we will have $1x2^n$ copies of our DNA target. In order to produce enough DNA for analysis, twenty to forty cycles may be required. After many cycles (regardless of the quantity of DNA present in the starting material) the amount of DNA produced reaches a maximum amount of product known as the plateau. This is due to depletion of reaction components like primers and nucleotides and the loss of *Taq* polymerase activity.

Because of its ease of use and its ability to amplify DNA rapidly, PCR has become indispensable in medical and life sciences labs, replacing the time-intensive Southern blot as the method of choice. For example, today's research laboratories can quickly create copies of a specific region of DNA for cloning applications. Medical diagnostics use PCR to identify genetic mutations and infectious agents. In addition, because amplification by PCR requires a small amount of starting material, it is ideal for forensic analysis of biological samples or determination of paternity.

REINVENTING PCR

While PCR is relatively fast and easy compared to techniques like the Southern blot, it still takes several hours to complete the experiment. In response, researchers have devised several strategies to reduce the time necessary to amplify a specific sequence. One timesaving strategy involves designing the primers so that the annealing temperature and the extension temperature are very close. This allows researchers to combine the annealing and extension steps of the traditional PCR cycle. Another timesaving strategy involves reducing the time spent at each temperature. By modifying the PCR program, researchers could reduce the length of each cycle from 90-150 seconds to 60 seconds or less (Table 1). These changes reduce the time required for this experiment by over 50%.

Table 1: Comparison of Traditional and Quick PCR

	Traditional PCR	Quick PCR
Denaturation (95° C)	45s	15s
Annealing (40° C - 60° C)	45s	0s
Extension (72° C)	45s	15s
TOTAL TIME (30 cycles)	**~70 minutes**	**~30 minutes**

In this exploration, we will use quick PCR to analyze genomic DNA isolated from a virus that infects *E.coli*, known as bacteriophage lambda. Historically, lambda is an important virus for molecular biology. Early studies of the lambda genome contributed to our understanding of DNA replication, transcription, and translation. The 48,500 base pair genome contains information necessary for the virus's entry into the cell, production of new virions, and lysis of the host cell (Figure 2). The primers used in this experiment have been designed to amplify a 500 base pair region of a viral capsid protein. They are engineered to have an annealing temperature of 71° C, which is close to the optimum temperature for *Taq*'s DNA polymerase activity. This allows us to combine the annealing and extension steps of PCR. As a result, the entire amplification can be performed in about thirty minutes, allowing your students to perform PCR in a single lab period.

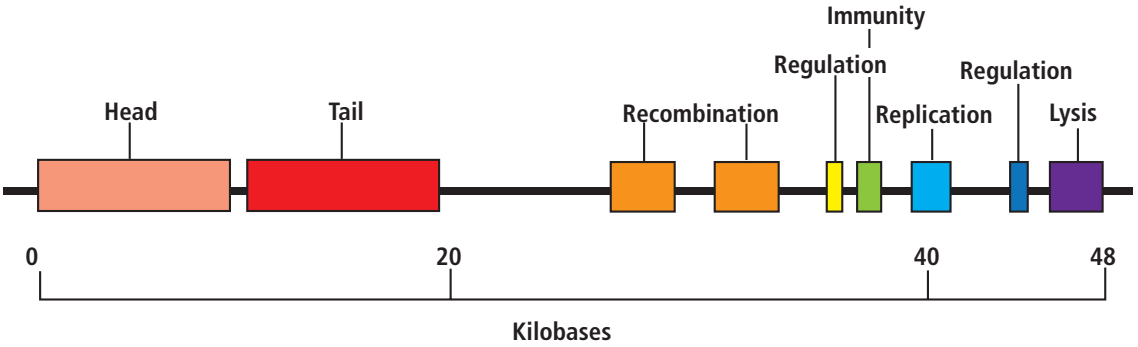


Figure 2: Lambda Phage Map

Experiment Overview

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
   - Turn off power and then unplug the equipment when not in use.
   - Electrical current from the power source is automatically disrupted when the electrophoresis cover is removed from the apparatus on all EDVOTEK models. However, exercise caution when working with electrophoresis equipment. After electrophoresis is completed, turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
5. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.
6. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Module I - 25 min.
Amplification of Lambda DNA

↓

Module II - 30-45 min.
Separation of PCR Products by Electrophoresis

↓

Module III - 5-30 min.
Staining with FlashBlue™ Stain (OPTIONAL)

NOTE: Experimental times are approximate.

GEL SPECIFICATIONS

This experiment requires a gel with the following specifications:

Recommended Gel Size:	7 x 7 cm
Number of Samples Wells:	6
Placement of the Well-former Template:	First set of notches
Gel Concentration Required:	1.0%

Online Resources

Here at EDVOTEK®, we've created Quick Guide manuals, FREE for you to download off our website. We have also filmed several Instructional Videos that show step-by-step procedures. We hope you take advantage of these resources and enjoy teaching and learning with EDVOTEK®!

Quick Guide www.edvotek.com/Quick-Guides

Tech Video www.youtube.com/EdvotekInc

Overview of 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: Amplification of Lambda DNA	Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)	One day to 30 min. before performing the experiment.	30 min.
	Program Thermal Cycler	One hour before performing the experiment.	15 min.
Module II: Separation of PCR Product by Electrophoresis	Prepare diluted electrophoresis buffer and SYBR® Safe Stain.	Up to one day before performing the experiment.	45 min.
	Prepare molten agarose and pour gel		
Module III: Staining with FlashBlue™ (OPTIONAL)	Prepare staining components	The class period or overnight after the class period.	10 min.

Related Products

Cat. # 540

EdvoCycler™ Jr.

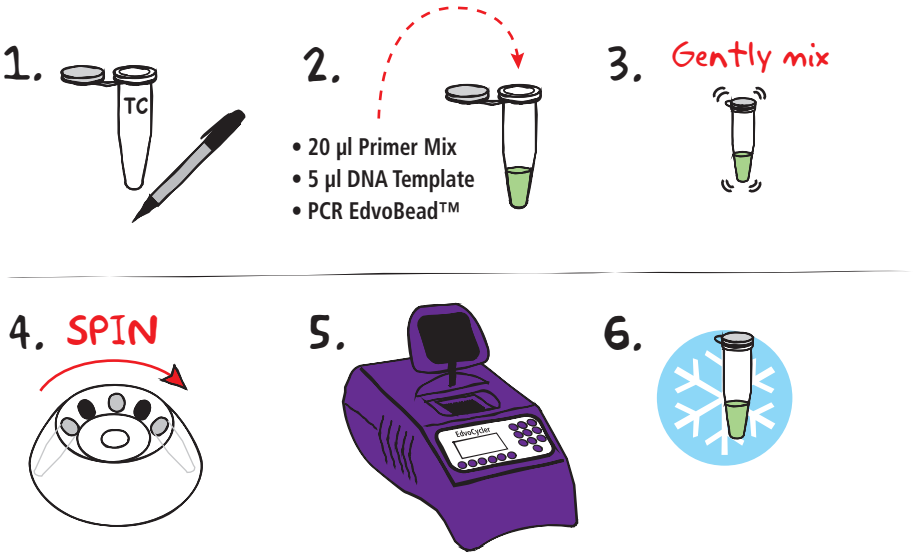
The EdvoCycler™ Jr. is the newest member of our PCR family and is based on the advanced EdvoCycler™ 2 platform. The sleek form factor has been miniaturized with 16 wells to run individual PCR experiments. A vivid and intuitive touchscreen and onboard computer simplify operation by not requiring a secondary device. And it's backed by an industry-leading 3-year warranty!

Cat. # 541-542

EdvoCycler™ 2

The successor to the bestselling EdvoCycler™ has been redesigned with improved PCR technology to be the best value among classroom thermal cycler. The EdvoCycler™ 2 doubles the capacity of the original at 48 wells and features an intuitive touchscreen. Proudly made in the USA and backed by an industry-leading 3 year warranty!

Module I: Amplification of Lambda DNA



REMINDER:

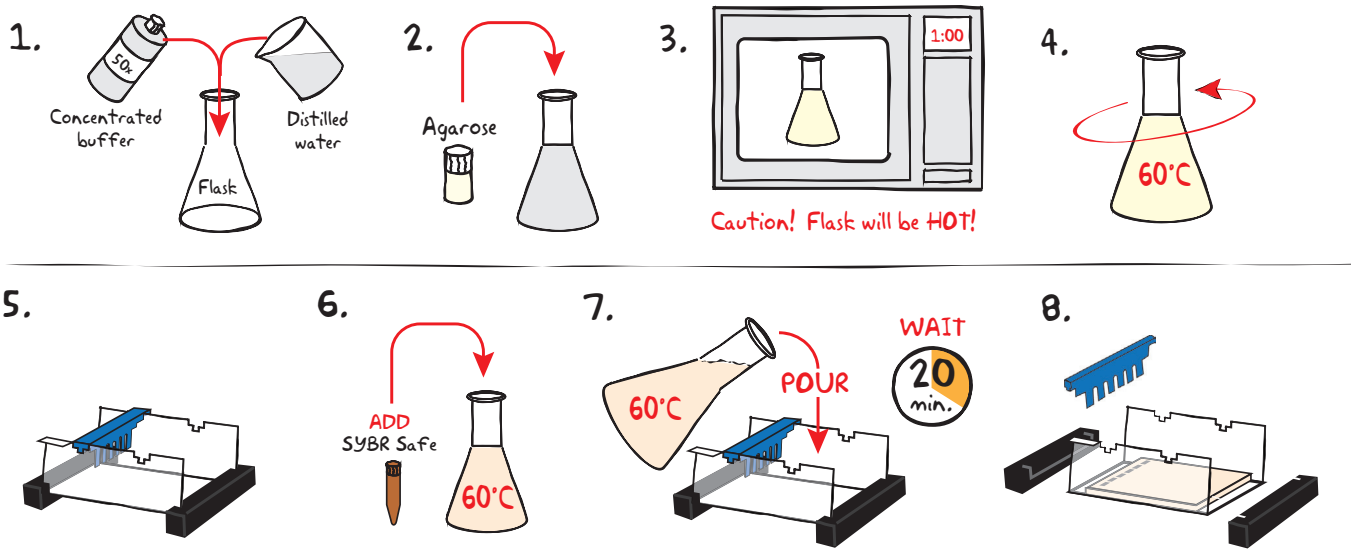
At least one negative control should be performed per class. To prepare the control sample, add 20 µl Primer Mix and 5 µl Lambda DNA template to a labeled PCR tube. **NO PCR EdvoBead™ IS ADDED.**

If your thermal cycler does not have a heated lid, it is necessary to overlay the PCR reaction with wax or mineral oil to prevent evaporation. See **www.edvotek.com** for more information.

1. **LABEL** a PCR tube with the sample and your initials
2. **ADD** 20 µl Primer mix (orange), 5 µl Lambda DNA Template (blue) and one PCR EdvoBead™ to a labeled PCR tube.
3. **MIX** the sample by gently flicking the tube. The solution should be pale green in color, and the PCR EdvoBead™ should be completely dissolved. **NOTE: If the solution is not pale green, the PCR sample has not been correctly assembled.**
4. **CENTRIFUGE** the sample for a few seconds to collect the sample at the bottom of the tube.
5. **AMPLIFY** DNA using PCR.
6. After PCR, **ADD** 5 µl of 10x Gel Loading Solution to the sample. **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.

Module II: Separation of PCR Products by Agarose Gel Electrophoresis



PREPARING THE AGAROSE GEL WITH SYBR® SAFE STAIN

1. **DILUTE** the concentrated (50X) electrophoresis buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** the agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** the 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** the agarose to 60° C by carefully swirling the flask to promote even dissipation of heat.
5. While the agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the comb in the appropriate notch.
6. Before casting the gel, **ADD** the diluted SYBR® Safe stain to the cooled 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** the end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Wear gloves and safety goggles

IMPORTANT:

7 x 7 cm gels are recommended. Place the comb in the first set of notches.

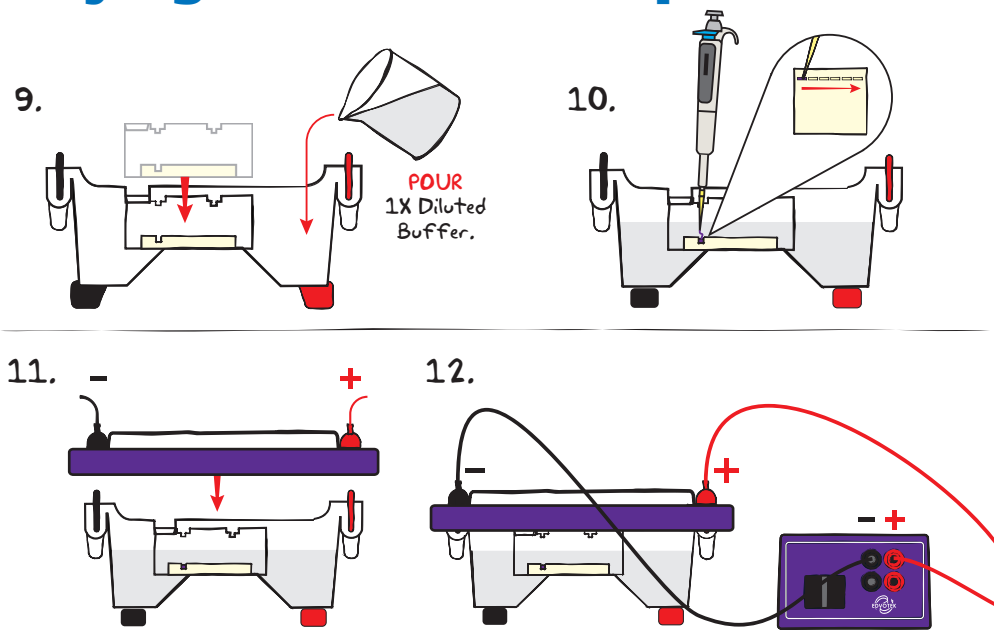
If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

Table A

Individual 1.0% UltraSpec-Agarose™ Gel with SYBR® Safe Stain

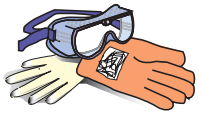
Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Add SYBR® (Step 6)
7 x 7 cm	0.5 mL	24.5 mL	0.25g	25 mL	25 µL
7 x 14 cm	1.0 mL	49.0 mL	0.50 g	50 mL	50 µL

Module II: Separation of Digested Products by Agarose Gel Electrophoresis



REMINDER:

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



Wear gloves and safety goggles

RUNNING THE GEL

9. **PLACE** the gel (on the tray) into the electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** the entire volume (25 µL) into the well in the order indicated by Table 1, right.
11. **CHECK** that the gel is properly oriented, then **PLACE** the safety cover onto the chamber. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** the 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.

OPTIONAL STOPPING POINT:
Gels can be stored for several days. Place gel in a watertight plastic bag with 2 mL of electrophoresis buffer and store in the refrigerator.

Table 2

Lane	Recommended	Sample Name
1	EdvoQuick™ DNA ladder	
2	Control DNA*	
3	Student Group #1	
4	Student Group #2	
5	Student Group #3	
6	Student Group #4	

** Optional, or additional student group sample.*

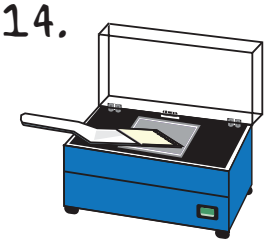
Table B — 1x Electrophoresis Buffer (Chamber Buffer)

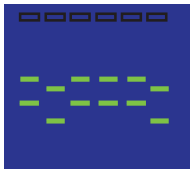
EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+ & M12 (new)	300 ml	6 ml	294 ml
M12 (classic)	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

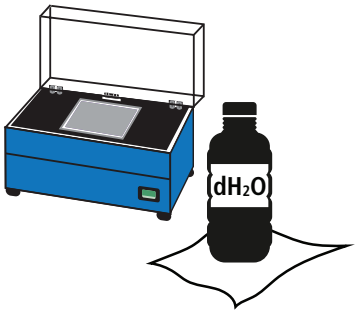
Table C — Time and Voltage Guidelines (1.0% Agarose Gels)

Volts	Recommended Times: Minimum	Maximum
150	15 min.	20 min.
125	20 min.	35 min.
70	35 min.	60 min.
50	60 min.	90 min.

Module II: Separation of Digested Products by Agarose Gel Electrophoresis

14. 

15. 

16. 

VISUALIZING THE SYBR® GEL

14. **SLIDE** the gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.
15. **PHOTOGRAPH** the results.
16. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

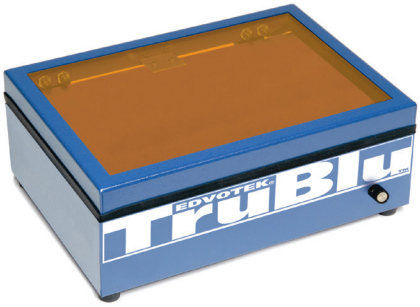
Be sure to wear UV goggles if using a UV transilluminator.

Related Products

Cat. # 557

TruBlue™ Blue Light Transilluminator

The all-new TruBlu™ Blue Light Transilluminator is ideal for viewing DNA gels stained with SYBR® Safe, thus eliminating the need for UV light or ethidium bromide. It's optimized to fit Edvotek® Gels as well as any other agarose gel. The high intensity control and orange lid ensure superior visualization. Developed in concert with the inventor of the technology under license from Clare Chemical Research, Inc.



Cat. # 608

SYBR® Safe DNA Stain

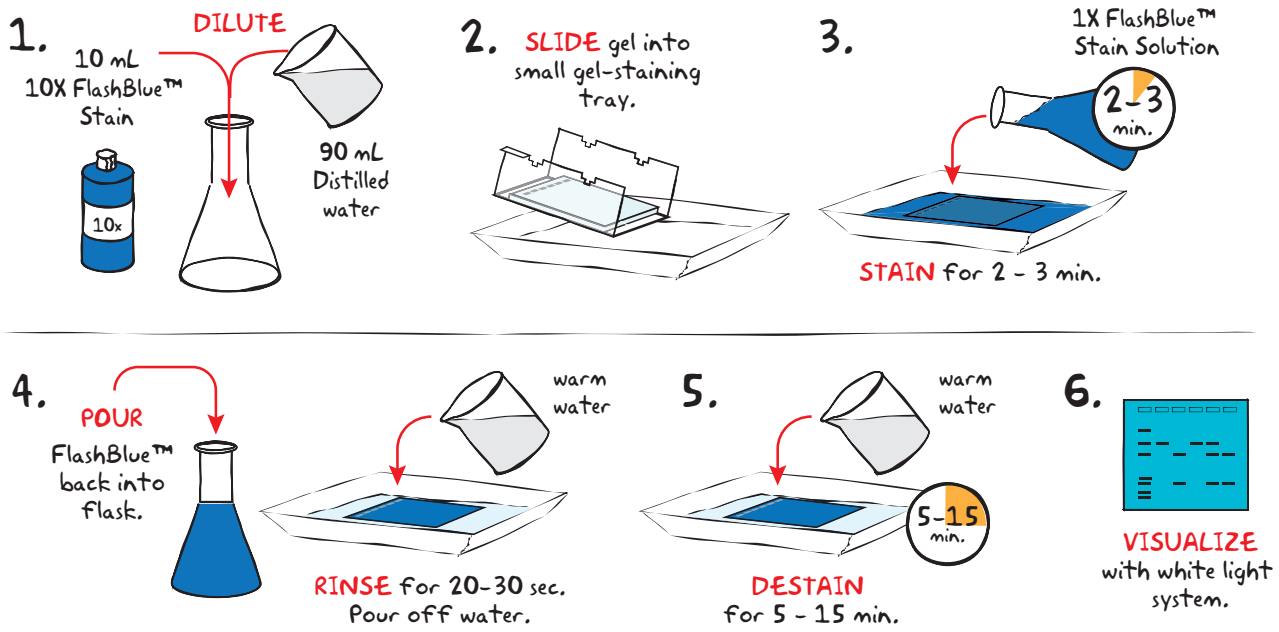
Non-mutagenic and SAFE for the Biotechnology Classroom!
More sensitive than ethidium bromide. Excellent gel results!

SYBR® Safe

Concentrate-for 750 ml

Module III: Staining with FlashBlue™ Stain (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 Flash Blue, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



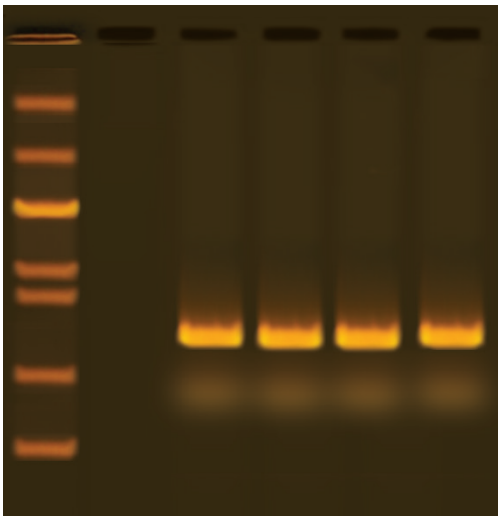
1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ 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 499 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.

Experiment Results and Analysis



This PCR experiment will amplify a 500 base pair region of a viral capsid protein coded for by the lambda genome. The control reaction sample will not produce a PCR product because it is missing the PCR EdvoBead™.

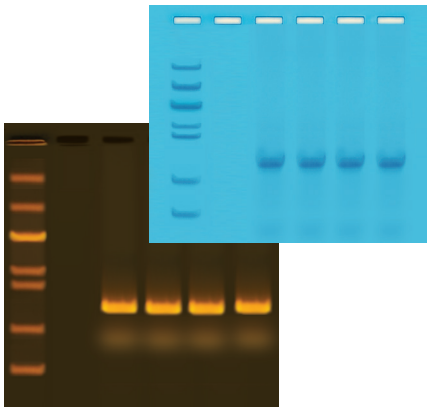
Lane 1 EdvoQuick™ DNA Ladder
Lane 2 Optional control reaction sample (no PCR EdvoBead™)
Lane 3 Student Group #1 PCR Reaction (20 cycles)
Lane 4 Student Group #2 PCR Reaction (20 cycles)
Lane 5 Student Group #3 PCR Reaction (20 cycles)
Lane 6 Student Group #4 PCR Reaction (20 cycles)

Related Products

Cat. #372

Quick PCR

For 10 Groups. In this experiment, students will gain an understanding of the traditional three-step Polymerase Chain Reaction (PCR). Using PCR and Agarose Gel Electrophoresis, they will analyze a small section of Lambda DNA in a time-saving two-step process.



Cat. # 5067

Classroom PCR LabStation™

Supports up to 25 Students

Includes:
1 Cat. #541-542 EdvoCycler™ 2 (48 x 0.2 ml)
6 Cat. #502/504 M12 Complete™ Package (7 x 14 cm Tray & 7 x 7 cm Trays (2)
3 Cat. #5010-Q QuadraSource™ Power Supply (10-300V, for 1 to 4 units)
6 Cat. #590 Variable MicroPipette (5 - 50 µl)
1 Cat. #557 TruBlu™ LED Transilluminator
1 Cat. #539 1.8 L Water Bath