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EDUCATION COMPANY®

# LESSON PLAN:

## Modeling DNA Amplification by Polymerase Chain Reaction (PCR)

Grade level: 9-12, College  
Subject area: Biotechnology

# General Overview:

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## LEARNING OBJECTIVES:

The objective of this experiment is for students to gain hands-on experience of the principles and practice of Polymerase Chain Reaction (PCR). At the completion of this activity, students should understand the process by which PCR amplifies DNA.

## ESSENTIAL KEY QUESTIONS:

- How does PCR amplify DNA?
- What reagents are necessary to perform PCR?
- What kinds of questions can PCR answer?

## CONTENT STANDARDS:

### NGSS Alignment:

HS-PS1: Matter and Its Interactions

PS1.A: Structure and Properties of Matter

HS-PS2: Motion and Stability: Forces and Interactions

PS2.B: Types of Interactions

HS-LS1: From Molecules to Organisms: Structures and Processes

HS-LS3: Heredity: Inheritance and Variation of Traits

LS3.A: Inheritance of Traits

Science and Engineering Practices:

Developing and Using Models

Analyzing and Interpreting Data

Crosscutting Concepts:

Cause and Effect

Structure and Function

### Common Core State Standards:

ELA/Literacy:

RST.11-12.1

RST.11-12.9

WHST.9-12.9

Mathematics:

MP.2

## Lesson Introduction:

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. Recognizing that an initial step in DNA replication in a cell's nucleus is the binding of RNA primers, Mullis discovered that he could replicate DNA in vitro using short, synthetic DNA primers and DNA polymerase I. Furthermore, because researchers can specify a primer's sequence to target a specific gene, this method allowed for the rapid amplification of a selected DNA sequence in the laboratory. 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.

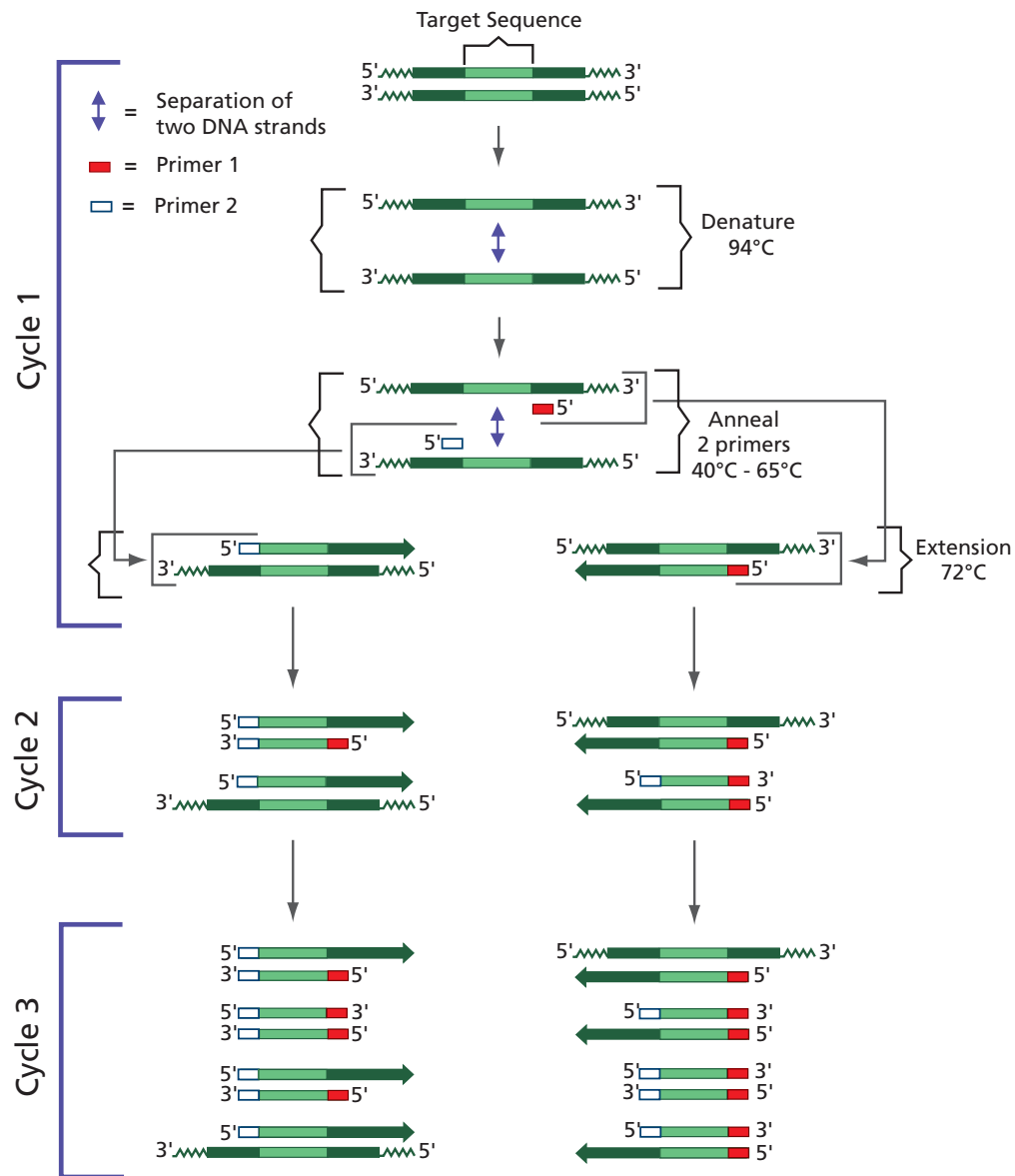
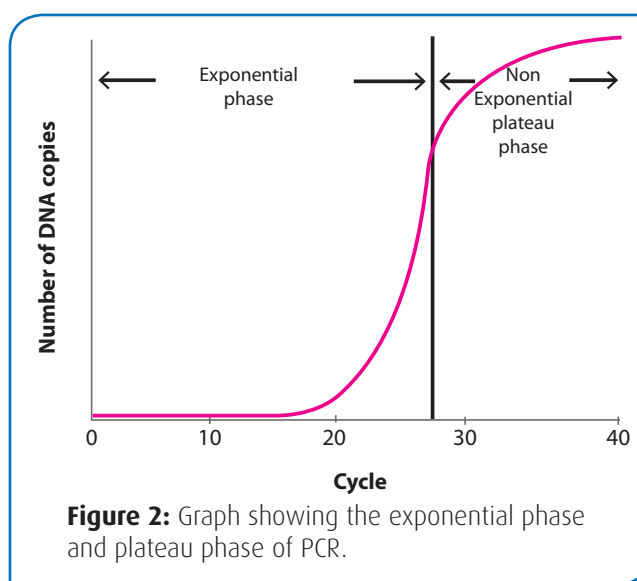


Figure 1: DNA Amplification by the Polymerase Chain Reaction

In order to amplify DNA, purified double-stranded DNA is mixed with the short DNA primers, a thermo-stable DNA polymerase (*Taq*) and nucleotides. The mixture is heated to 94°C to “denature” (i.e., unzip into single strands by breaking hydrogen bonds) the DNA duplex. Next, the sample is cooled to 45° C - 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 to synthesize a new strand of DNA. Each cycle (denaturation, annealing, extension) doubles the amount of target DNA (Figure 1). A specialized machine, called a “thermal cycler” or “PCR machine”, is used to rapidly heat and cool the samples.

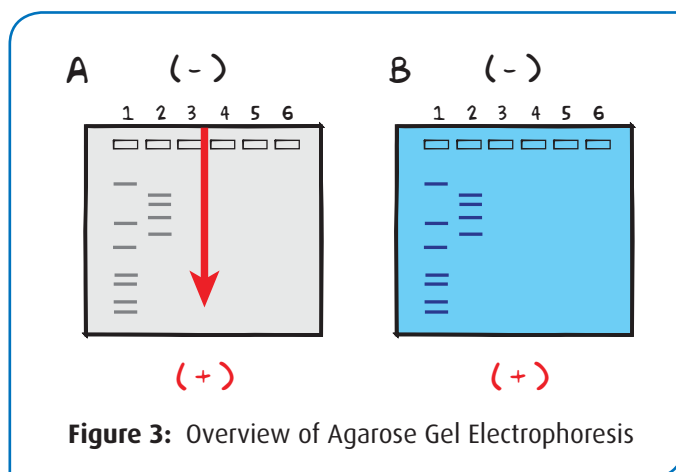
Each cycle of PCR doubles the amount of the DNA in the sample (Figure 2). Mathematically, this doubling can be expressed as an exponential relationship – if we begin with a starting copy number of  $m$ , then after  $n$  cycles, we will have  $m \times 2^n$  copies of our DNA target. For example, if 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, and so on. In general, 20-40 cycles produce sufficient DNA for analysis. In theory, this process could continue indefinitely. In practice, after many cycles the amount of DNA produced reaches a maximum known as the plateau (Figure 2). This leveling off of the curve is due to the 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 rapidly amplify DNA, 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 very little starting material, it is ideal for forensic analysis of biological samples or determination of paternity.

After PCR is performed, the samples will contain millions of DNA fragments. To analyze this mixture, scientists use a technique called agarose gel electrophoresis, which separates DNA fragments according to size. The mixture of DNA molecules 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 drives the DNA through the gel towards the positive electrode (Figure 3A).

At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. 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 (Figure 3B).



## Pre-Lab Assessment

Before performing the exercise, be sure that students are comfortable with the following topics:

1. Structure of DNA
  - a. DNA is built of four different nucleotides
  - b. The nucleotides are linked by phosphodiester bonds
  - c. DNA strands hydrogen bond to one another in an anti parallel orientation
2. Function of DNA in an organism
  - a. DNA contains genetic information as genes
  - b. Each gene contains the instructions to build a protein
3. Fundamentals of PCR
  - a. Three steps per PCR cycle
    - i. Denaturation – unzip DNA into single strands by breaking hydrogen bonds
    - ii. Annealing - primers to base pair with their target DNA sequences
    - iii. Extension - *Taq* polymerase will extend the primer to synthesize a new strand of DNA
  - b. Exponential amplification of DNA -- each PCR cycle doubles the amount of DNA in the sample.
4. Analysis by Electrophoresis
  - a. Agarose gel electrophoresis separates DNA fragments by size
  - b. The percent agarose used in the gel will affect the migration of DNA through the gel
    - i. High percent gel separates small fragments better
    - ii. Low percent gel separates large fragments better

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## Activity #1: Modeling PCR

*Students should work in groups of 2-4.*

### REQUIRED MATERIALS:

- Pop-beads: Each group will need at least 32 beads per color.
- Stopwatch or timer
- Digital Camera or Cell Phone (optional)

### PROCEDURE:

1. Each group will receive a set of colored beads. The beads have been assigned a base designation by color as detailed below.
  - a. Red = adenine (A)
  - b. Blue = thymine (T)
  - c. White = guanine (G)
  - d. Yellow = cytosine (C)
2. Assemble the beads into two strands to form the DNA template. The “nub” end of the bead represents the 5’ phosphate.
  - a. Forward Strand: 5’-ATAGGCACTGCCTAGT-3’
  - b. Reverse Strand: 3’-TATCCGTGACGGATCA -5’
3. Assemble three sets of DNA primers:
  - a. Forward Primer: 3’-TATCCG-5’
  - b. Reverse Primer: 5’-CCTAGT-3’
4. Line up the two DNA strands.
5. Perform PCR: (The instructor announce each step at 30-45 second intervals)
  - a. Denaturation at 94°C: the two DNA strands separate.
  - b. Annealing at 45-60°C: the primers anneal to the DNA strands
  - c. Extension at 72°C: *Taq* polymerase extends the complementary strand of DNA using the primer as a starting point.
  - d. Repeat a-c two more times.

### ASSESSMENT:

After three cycles of PCR are completed, go around the room to check each student group’s final products. Students should have four DNA duplexes (eight strands of DNA). Alternatively, students can take pictures of their final products for submission.

## Activity #1: Discussion Questions

1. *Taq* polymerase is estimated to make one mistake per 9000 bases copied. Do you see any “mistakes” (i.e. incorrect bases) in your PCR products? What is your error rate?

Answers will vary with each student group.

2. Imagine you are starting with one copy of the DNA template. After performing 10 cycles of PCR, how many copies of DNA are present in the sample? After 20 cycles? 30?

After 10 cycles –  $1 \times 2^{10} = 1024$  copies

After 20 cycles –  $1 \times 2^{20} = 1,048,576$  copies

After 30 cycles –  $1 \times 2^{30} = 1,073,741,824$  copies

### Required Materials

Cat. 1500

#### Colored DNA Beads

A set of colored beads that can be designated to represent the Watson-Crick DNA bases (A, T, G, C). The beads can be used in a variety of ways to demonstrate concepts related to the structure and biology of DNA. Includes detailed outline of various sample demonstrations.

Includes 150 beads of each color.



Cat. 763

#### 4 Channel Timer



[youtube.com/edvotekinc](https://youtube.com/edvotekinc)

Watch us on



**Video: Preparing  
Agarose Gels**



**Video: Staining with  
InstaStain® Blue**



**Video: Staining with  
InstaStain®  
Ethidium Bromide**



**Video: Performing  
Agarose Gel  
Electrophoresis**



**Video: Staining  
with FlashBlue™**



**Video: Staining  
with SYBR® Safe**



## Activity #2: Performing PCR

Students should work in groups of 2-4.

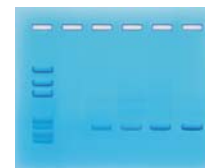
### REQUIRED MATERIALS:

- EDVOTEK® Kit #330 – PCR Amplification of DNA
- 5-50 µl adjustable micropipets
- Disposable micropipet tips
- Thermal Cycler
- Electrophoresis Apparatus
- Power Supply
- UV Transilluminator
- Microwave or Hot Plate

Cat. 300

### PCR Amplification of DNA

In this easy, authentic PCR experiment, students will make billions of copies of a small amount of DNA in just 90 minutes! Students will see the increasing amounts of DNA for themselves, taking samples every few cycles and analyzing them on a DNA gel. For 10 Lab Groups.



### PROCEDURE:

Before performing the experiment, be sure to emphasize good laboratory practices (proper personal protective equipment, exercise caution when using equipment, hand washing, etc.) Perform experiment as outlined in the lab packet (Available online at <http://www.edvotek.com/site/pdf/330.pdf>.) Figure 4 provides approximate times for each module, so plan the lab activities accordingly.

#### Before starting the experiment, students should:

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

#### During the experiment, students should:

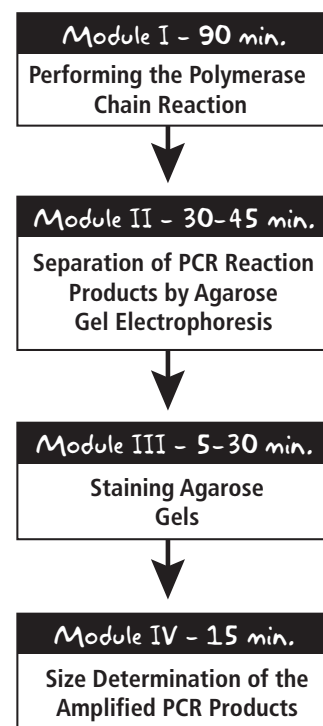
- Record any observations in a lab notebook.

#### After the experiment, students should ask the following questions:

- Interpret the results – does the data support or contradict the hypothesis?
- If the experiment was repeated, what should change? Revise the hypothesis to reflect this change.

Be sure to have your students reflect upon these points in their lab notebooks. Students should also submit a formal lab report upon completion of the exercise.

Figure 4: Lesson Timeline



NOTE: Experimental times are approximate.



## Activity #2: Discussion Questions

### 1. What are the components of a PCR sample? What does each component contribute?

- Template – the purified, double-stranded piece of DNA we want to copy
- Primers – short synthetic DNA molecules that target a specific DNA sequence for amplification
- *Taq* DNA Polymerase – thermostable enzyme used to copy DNA
- Free nucleotides – the building blocks of DNA
- Thermal Cycler (a.k.a. PCR machine) – a specialized machine that rapidly heats and cools the samples.

### 2. *Taq* is a thermostable DNA polymerase. Why is that important?

Amplification of DNA by PCR requires heating the sample 94° C to denature the hydrogen bonds between the DNA strands. Unlike non-thermostable DNA polymerases, *Taq* DNA polymerase is stable at these high temperatures. Therefore, it will retain its enzymatic activity through the multiple PCR cycles.

### 3. What are some applications of PCR in the real world? Where would you use PCR?

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 very little starting material, it is ideal for forensic analysis of biological samples or determination of paternity.

Encourage students to think creatively on applications to which they would apply PCR.

### Required Materials



**Cat. 504**  
**M12 Dual Electrophoresis Apparatus**

Run up to two groups of student samples at the same time!



**Cat. 509**  
**DuoSource™ 150 75/150 V Power Supply**

Runs gels quickly for 2 units in only 20-30 minutes (at 150 V)!



**Cat. 541**  
**EdvoCycler™**

This stand alone PCR machine arrives ready-to-use and pre-programmed with all EDVOTEK PCR protocols!



**Cat. 590**  
**EDVOTEK® 5-50 µl Variable Micropipet**

**Cat. 636**  
**1-200 µl Yellow Micropipet Tips**

2 racks of 96 each



**Cat. 558**  
**Midrange UV Transilluminator**

Visualize DNA stained with Ethidium Bromide or SYBR® Safe. Includes a 7 x 14 cm UV filter.

# An Introduction to Biotechnology

Biotechnology represents the use of cellular, molecular, and biochemical technologies to improve society and the environment. Basic biotechnology techniques have been used for centuries for the production and preservation of food, selective breeding of livestock, and to improve human health. Modern laboratories that discover many of these biotechnological innovations use simple but powerful techniques to visualize and manipulate DNA and proteins.

Edvotek® offers multiple biotech products to outfit your classroom. From gel electrophoresis and PCR, to bacterial transformation and exciting forensics experiments, you can now bring a wide variety of inquiry-based activities into your classroom!

## Electrophoresis

Electrophoresis is a technique that allows scientists to separate DNA, RNA, or proteins according to their size. The molecules are pulled by an electrical field through a defined gel, forcing them to move through microscopic pores. This process separates the molecules based on size, with smaller fragments moving more easily through the gel. Because of this, electrophoresis experiments can be used to detect the presence and size of DNA from a variety of sources. We offer a broad range of exciting electrophoresis experiments for the classroom, including both dye and DNA-based gel electrophoresis.

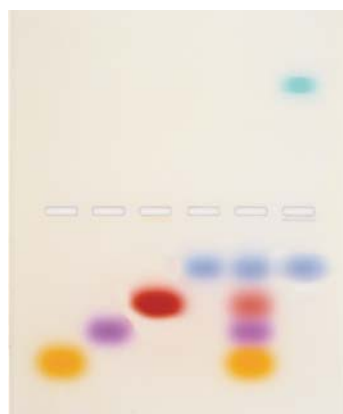
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#### Classroom DNA Electrophoresis LabStation™

Supports up to 24 students

##### Includes:

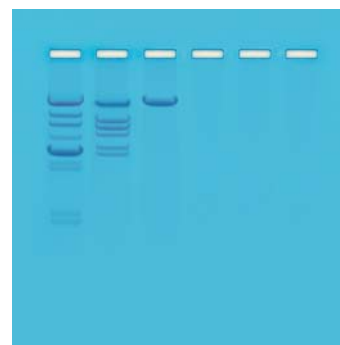
- 1 Cat. #515 M36 HexaGel™ Electrophoresis Apparatus
- 1 Cat. #509 DuoSource™ 150 (75/150 V for 1 or 2 units)
- 2 Cat. #588 Fixed Volume MiniPipet (40 µl)
- 1 Cat. #636 Yellow Micropipet Tips (1 - 200 µl / 2 Racks of 96)
- 1 Cat. #130 DNA Fingerprinting Classroom Experiment



### Cat. 101

#### Principles & Practice of Agarose Gel Electrophoresis

In this safe, colorful and fun experiment, students learn the basics of agarose gel electrophoresis. Groups of students will cast gels, load samples, and observe the separation of visible bands. This experiment arrives Ready-to-Load and does not require DNA or toxic chemicals.



### Cat. 112

#### Restriction Enzyme Analysis of DNA

Restriction enzyme analysis of DNA Introduce your students to the concept of DNA digestion by restriction enzymes, followed by electrophoresis to examine the results. This Ready-to-load experiment examines digestion of lambda DNA at specific nucleotide sequences. You can also Save money with one of our convenient Classroom DNA Electrophoresis LabStations, designed to provide everything you need to perform many of our electrophoresis experiments!

## Staining Agarose Gels

**InstaStain®  
Ethidium  
Bromide**



**SYBR®  
Safe  
DNA Stain**



**InstaStain®  
Blue and  
FlashBlue™**



After electrophoresis, DNA fragments will have separated according to their size. However, DNA bands are colorless, and it is necessary to first stain the DNA before it can be seen. EDVOTEK® offers several methods for visualizing DNA within agarose gels.

### Fluorescent DNA Stains:

The most frequently used method for visualizing DNA is through the use of fluorescent DNA stains. These stains are extremely sensitive and rapid, making them perfect for use in the classroom. Fluorescent stains must be visualized using an ultraviolet (UV) light source. We offer two options for fluorescent stains: InstaStain® Ethidium Bromide and SYBR® Safe DNA Stain.

### Visible Dye-based DNA Stains:

Dye-based DNA stains are an excellent alternative to fluorescent stains, requiring no special equipment or waste-disposal. Although they are less sensitive to DNA concentrations, our visible stains provide excellent results and dye DNA with an intense blue color. EDVOTEK® offers two visible dye-based DNA stains: InstaStain® Blue and FlashBlue™ stain.

Stain	Advantages	Disadvantages
InstaStain® EtBr	Very sensitive Very fast	Requires UV transilluminator Potentially mutagenic
SYBR® Safe	Very sensitive Non-mutagenic	Requires UV transilluminator More expensive
InstaStain® Blue	Easy to use Generates minimal waste	Less sensitive More time
FlashBlue™	Simple and fast Reusable, inexpensive	Less sensitive Disposal of liquid

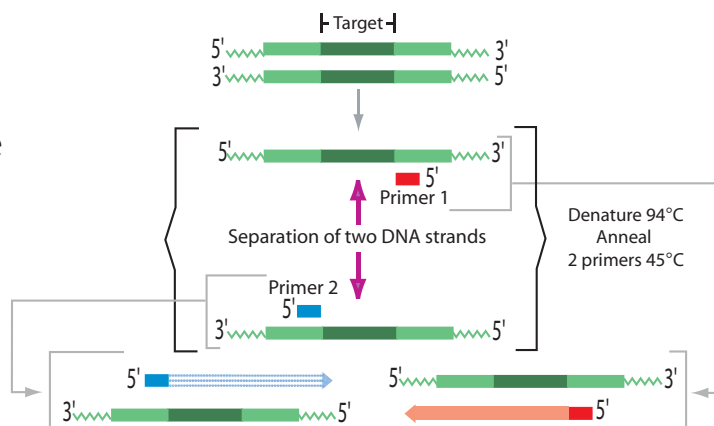


### Cat. 558 Midrange UV Transilluminator

The EDVOTEK® Midrange UV Transilluminator is designed to visualize DNA stained with Ethidium Bromide or SYBR® Safe. The UV filter is 7 x 14 cm and is optimal for visualizing all of our gel sizes. Safety features include a UV-blocking cover and a power cut-off switch when the cover is opened.

# Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a technique that allows researchers to rapidly create many copies of a desired stretch of DNA. PCR is currently used in disease screening, forensic testing, and biological research, and represents a valuable platform for students to explore STEM concepts. Edvotek® has developed a wide variety of PCR experiments and equipment, including the EdvoCycler™ and MegaCycler™ PCR machines.



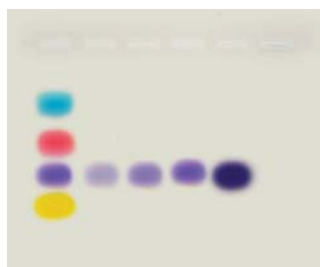
Cat. 541 & 542

## EdvoCycler™ and MegaCycler™

The Edvocycler™ and MegaCycler™ are affordable, stand alone PCR machines. Each machine arrives ready-to-use, and comes pre-programmed with all EDVOTEK PCR protocols, with space for you to make your own!



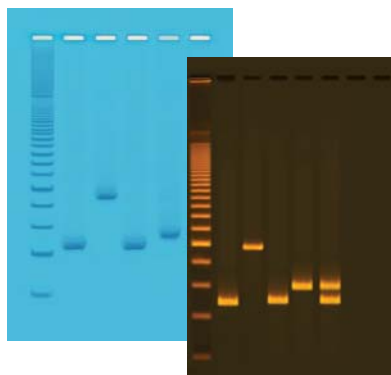
Research supported in part by NIH SBIR NCRR Grant #R44RR18670



Cat. S-48

## What is PCR?

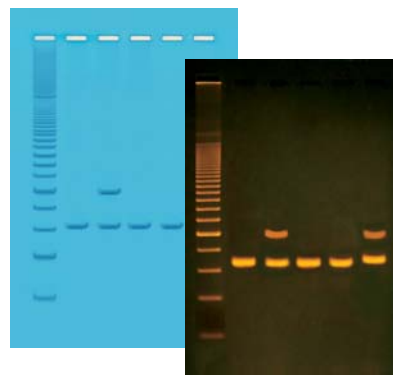
Students will be introduced to PCR with this easy to use kit. This experiment introduces the fundamental principles of PCR without the need for a thermocycler or staining!



Cat. 953

## Multiplex PCR-based Testing of Water Contaminants

PCR is commonly used to test drinking water for contamination. In this classroom-safe experiment, students will test for the presence of three organisms in a water sample using PCR.



Cat. 333

## Alu-Human DNA Typing

Students use with their own DNA to look for a 300 base pair Alu insertion in chromosome 16, allowing them to determine their genotype!



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