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Edvo-Kit #

S-48

Edvo-Kit #S-48

What is PCR and How Does it Work?

Experiment Objective:

The objective of this experiment is for students to gain hands-on experience in the principles and practice of Polymerase Chain Reaction (PCR). Students will understand the relationship between the number of cycles of PCR and the quantity of DNA amplified.

See page 3 for storage instructions.

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Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store QuickStrip™ samples in the refrigerator immediately upon receipt.
All other components can be stored at room temperature.

Components (in QuickStrip™ format)		Check (√)
A	Standard Dye Marker	❑
B	Sample after 10 cycles	❑
C	Sample after 20 cycles	❑
D	Sample after 30 cycles	❑
E	Sample after 40 cycles	❑

Experiment #S-48 is designed for 10 gels.

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

REAGENTS & SUPPLIES

- Practice Gel Loading Solution ❑
- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50X) ❑
- 1 ml pipet ❑
- Microtipped Transfer Pipets ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Visualization system (white light box)
- Distilled or deionized water

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Background Information

POLMERASE CHAIN REACTION

Polymerase Chain Reaction (PCR) has had an extraordinary impact on various aspects of biotechnology. PCR has revolutionized research and diagnostics based molecular biology. PCR is a simple, accurate and highly reproducible procedure. The technology introduced an important advantage to molecular biology. It provides the ability to start with a small amount of DNA and to be able to amplify it so that there will be a sufficient amount of DNA to perform experiments. It is analogous to a radio or stereo amplifier where radiowave signals which are normally not heard are amplified so we can hear music.

Since the first application of PCR to detect sickle cell anemia, a large number of diagnostic tests have been developed and are becoming routine tests. PCR is also used in genome projects for DNA mapping and sequencing and is being applied to forensics, paternity determinations, as well as the determination of evolutionary relationships. In all these cases the DNA samples that are extracted are limited and PCR amplifies segments of DNA that become the subject for further analysis and study.

In a PCR reaction, the first step is the preparation of the DNA sample that is extracted from tissues or various biological sources. In PCR experiments, the DNA or gene to be amplified is referred to as the target and the synthetic oligonucleotides used are referred to as primers. A set of two primers (a forward and reverse primer) usually ranging between 20 and 45 nucleotides are chemically synthesized to correspond to the two ends of the gene to be amplified. Each primer binds to one of the two DNA strands and is the initiation point of the amplification. The primer concentrations are always in excess of the target gene to make possible subsequent priming. The exact nucleotide primer sequences for a specific amplification reaction are determined to yield the best conditions (hybridization) for template-primer formation.

The specificity of DNA synthesis is dictated by the Watson - Crick base pairing rules and is directed by the template DNA. The strand being synthesized is complimentary and antiparallel to the template DNA strand. *De novo* DNA synthesis catalyzed by DNA polymerase cannot occur without a primer having a free 3′ terminal hydroxyl group, which is required for the addition of the next nucleotide. The primer is antiparallel and is base paired to the template strand. An overview of the PCR reaction is shown in Figure 1.

A typical PCR reaction mixture contains DNA, *Taq* DNA polymerase, and the four deoxynucleotide triphosphates in the appropriate buffer. The total incubation reaction is usually 10-20 µl or smaller in volume. The incubation mixture is then exposed to a three step temperature cycle which is repeated. The first temperature is 94°C to melt the hydrogen bonds between the two strands of DNA. The temperature is then dropped to between 42° and 60°C to hybridize the two primers on the two DNA target strands. The temperature is then increased to 72°C, which is the optimum temperature for *Taq* DNA polymerase. At this temperature, the DNA polymerase synthesizes the opposite strand of DNA using the original strands as templates. These temperature cycles are repeated 20 to 50 times. This process is made efficient by placing the reaction tubes in specifically designed thermal cyclers which are programmed to alternate temperatures rapidly and accurately. The amplified product is then detected by separating the reaction mixture by gel electrophoresis and analysis.

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Background Information

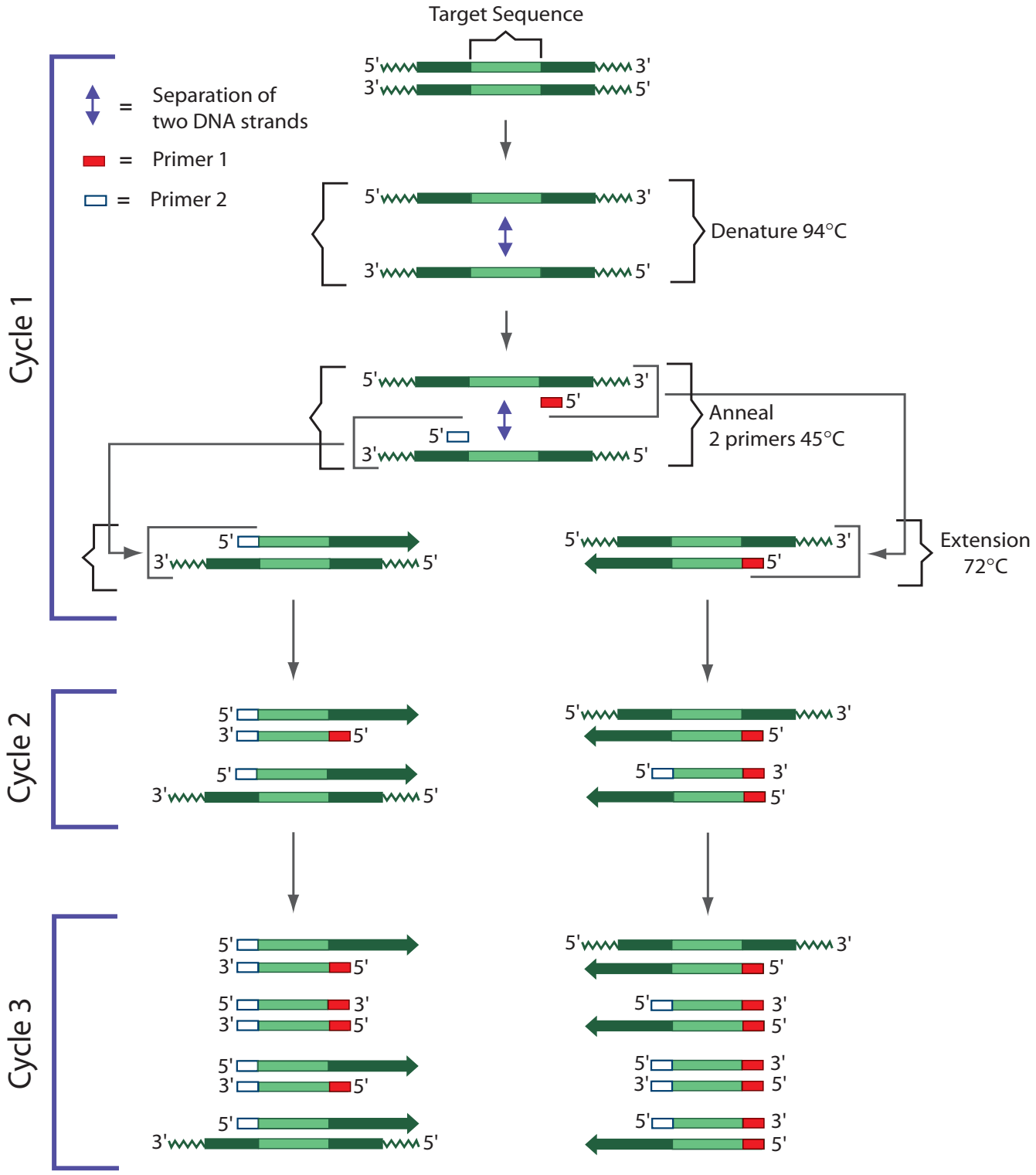

Figure 1:
The Polymerase Chain Reaction

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Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is for students to gain hands-on experience in the principles and practice of Polymerase Chain Reaction (PCR). Students will understand the relationship between the number of cycles of PCR and the quantity of DNA amplified.

LABORATORY SAFETY:

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that 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.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

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.

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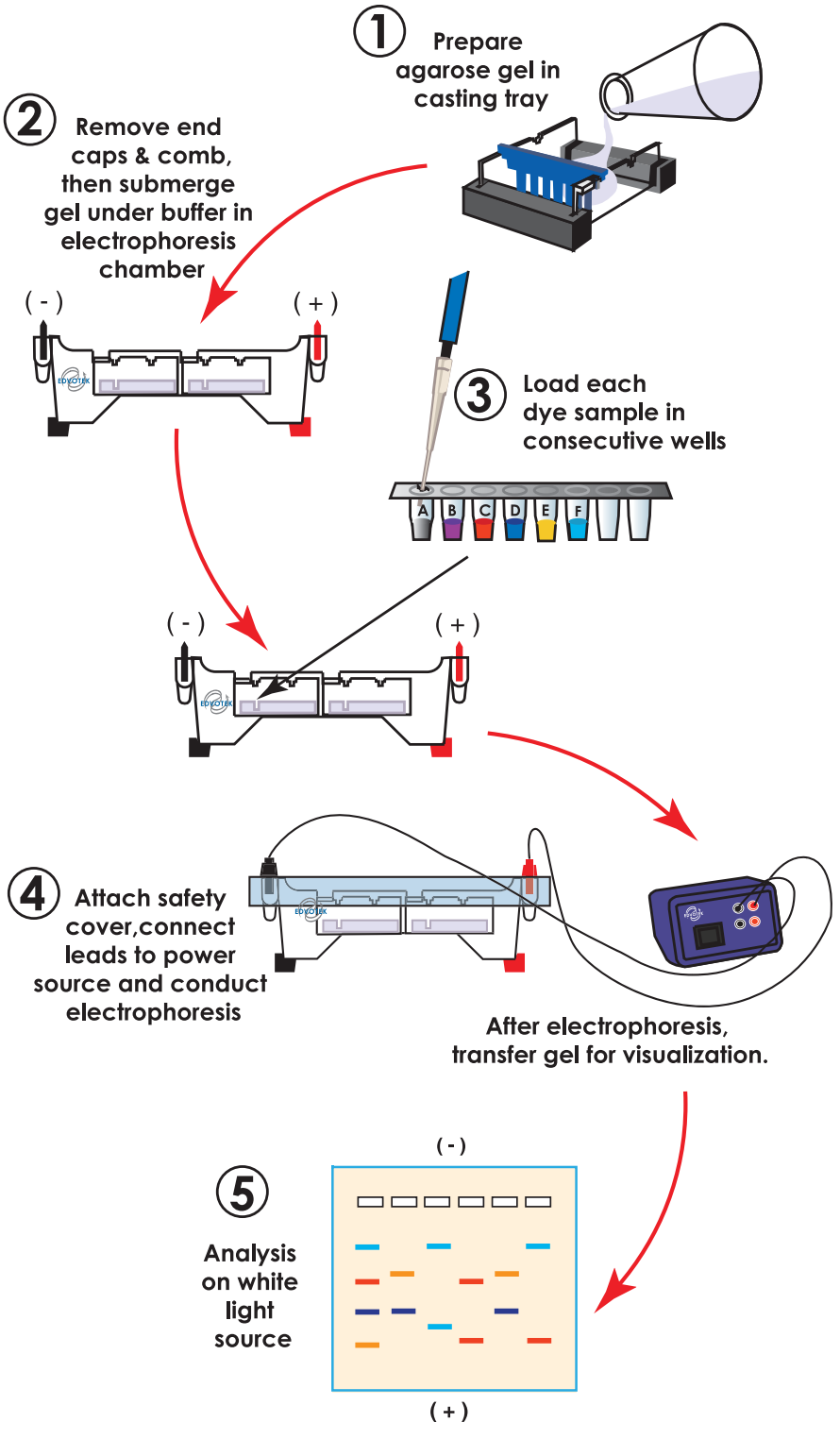
Experiment Overview



Gel pattern will vary depending upon the experiment.

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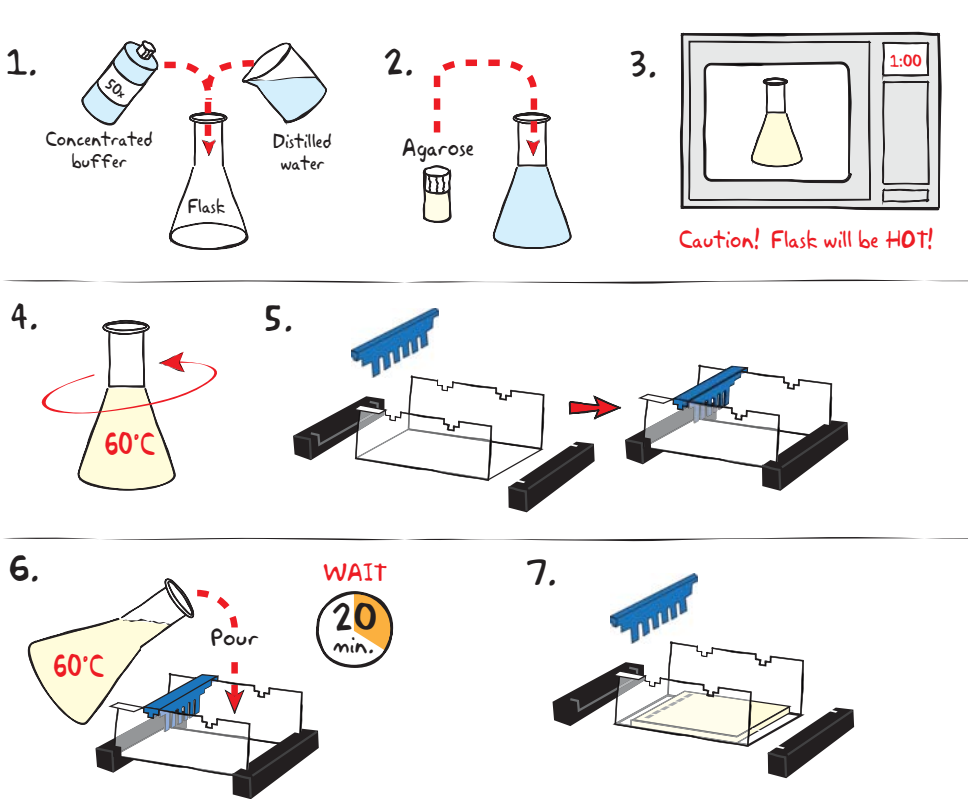
Agarose Gel Electrophoresis



IMPORTANT:

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

Wear gloves and safety goggles

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.
6. **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.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

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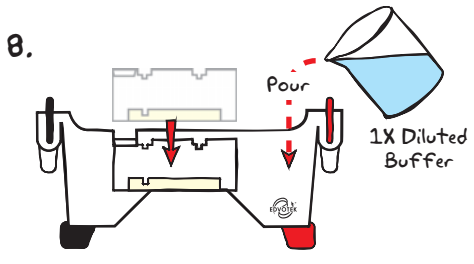
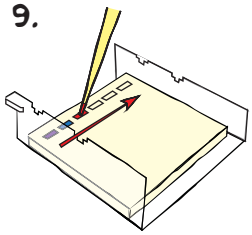
Agarose Gel Electrophoresis

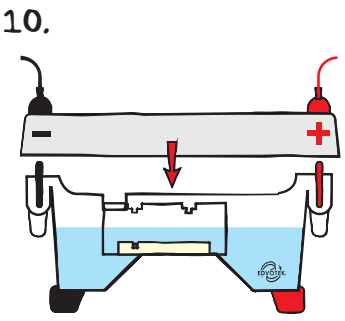


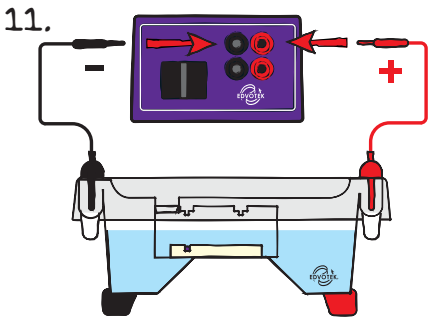


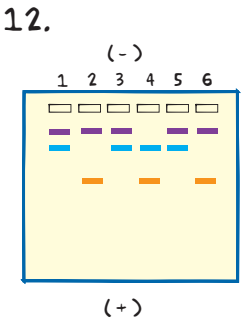
Reminders:

If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

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







8. **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.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38 µL) into the well in consecutive order. The identity of each sample is provided in Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Standard Dye Marker
2	Tube B	Sample after 10 cycles
3	Tube C	Sample after 20 cucles
4	Tube D	Sample after 30 cycles
5	Tube E	Sample after 40 cycles

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 (0.8% Agarose Gel)

	Electrophoresis Model: M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

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Study Questions

1. What is PCR and why is it important?
2. What are the three temperatures steps in a PCR cycle and what does each step accomplish?
3. What is the function of the four free nucleotides (dATP, dCTP, dGTP, dTTP)?
4. Why are two different primers required for PCR?

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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.

What to do:	When:	Time Required:
Prepare QuickStrips™	Up to one day before performing the experiment.	40 min.
Prepare diluted TAE buffer		
Prepare molten agarose and pour gel		

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Pre-Lab Preparations:

SEPARATION OF PCR PRODUCTS BY AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. 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.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

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 stored under buffer in the refrigerator for up to 2 weeks.

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.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results.

If students are unfamiliar with using micropipets, we recommend performing the optional activity found in Appendix C, Practice Gel Loading, prior to conducting the experiment.

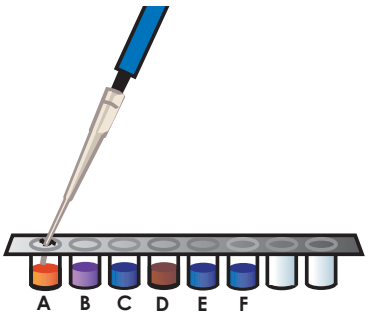
Each Student Group should receive:
- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

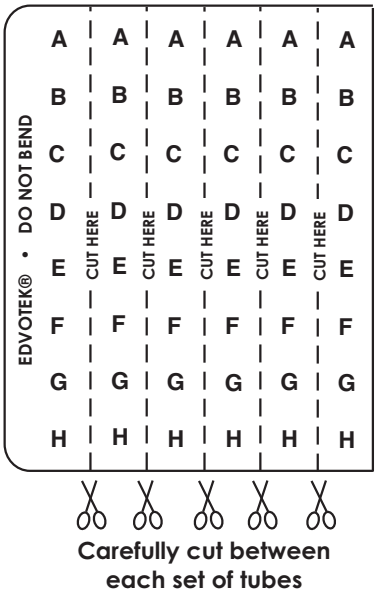
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted dyes.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.





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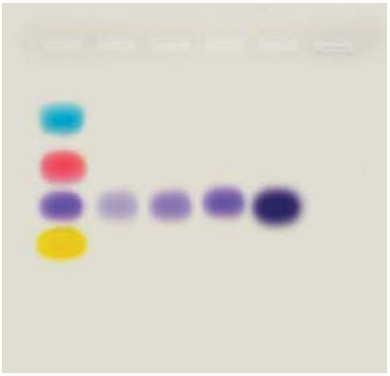
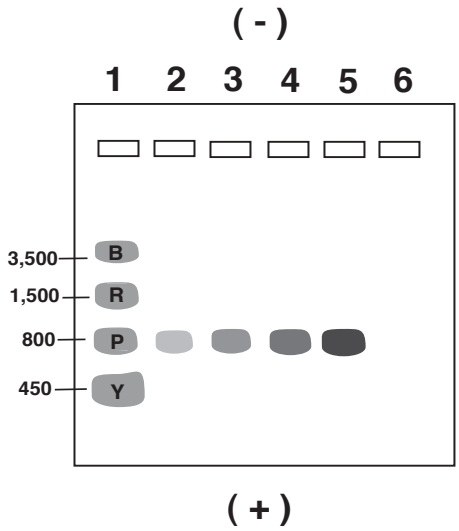
Experiment Results and Analysis





S-48 Gel Idealized schematic: The relative positions of dye molecules are shown but are not depicted to scale.

Lane	Tube	
1	A	Standard Dye Marker B=Blue R=Red P=Purple Y=Yellow
2	B	Sample after 10 cycles
3	C	Sample after 20 cycles
4	D	Sample after 30 cycles
5	E	Sample after 40 cycles

In this PCR simulation, the amount of dye increases as it proceeds through the PCR reaction cycles, as shown in Lanes 2 through 5 in the above figures.

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

Appendices

A EDVOTEK® Troubleshooting Guide
B Bulk Preparation of Agarose Gels
C Practice Gel Loading

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Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands not visible on the gel	The electrophoresis buffer was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The dyes ran off of the gel because the polarity of the leads was reversed.	Ensure that leads are attached in the correct orientation.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
Very light colored band seen after electrophoresis	Pipetting error.	Make sure students pipet 35 µl of dye sample per well.
Poor separation of bands	Gel was not prepared properly.	Make sure to prepare a 0.8% gel.
Dye bands disappear when the gels are kept at 4° C.	The dye molecules are small and will diffuse out of the gel.	The results must be analyzed upon the completion of electrophoresis

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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

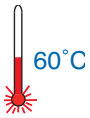
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

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.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 3.0 grams 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. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
7. 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.

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

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Appendix C
Practice Gel Loading

Accurate sample delivery technique ensures the best possible gel results. Pipetting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel.

If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

1. Cast a gel with the maximum number of wells possible.

2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge it under buffer or water.

Note:
The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

3. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.

   - For electrophoresis of dyes, load the sample well with 35-38 microliters of sample.

   - If using transfer pipets for sample delivery, load each sample well until it is full.

4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.

5. Replace the practice gel with a fresh gel for the actual experiment. **Note: If practicing gel loading in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. It will not interfere with the experiment, so it is not necessary to prepare fresh buffer.**

Note:
If you do not wish to pour extra agarose gels, Edvotek® DuraGels™ (Cat. S-43) can be used as a substitute. Edvotek® DuraGels™ are reusable polymer gel models that allows students to gain experience with gel loading before performing agarose gel electrophoresis. The use of DuraGels™ eliminates the preparation time, expense, and waste of pouring actual agarose practice gels.

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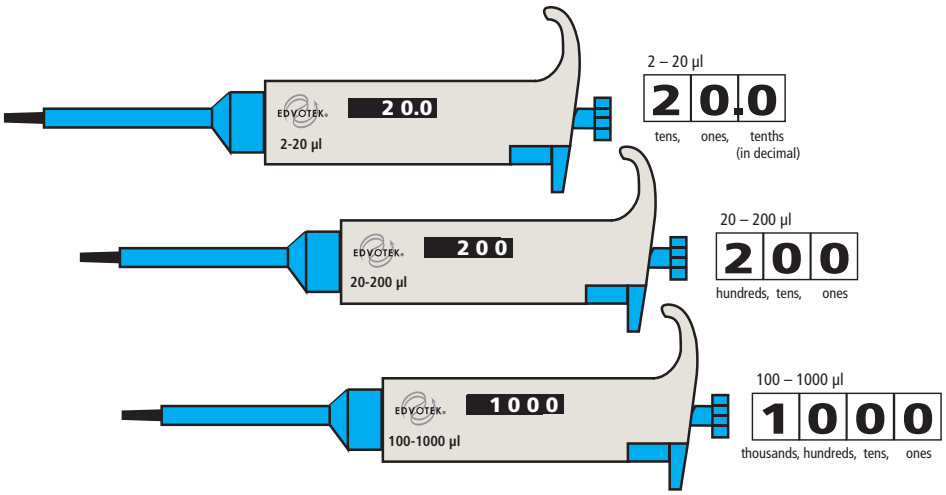
Appendix C
Practice Gel Loading



Wear gloves and safety goggles

SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

1. **CHOOSE** the correct micropipet for the volume you are measuring. Make sure that the volume to be measured **DOES NOT EXCEED** the upper or lower volume setting of the micropipet.

2. **DETERMINE** the units measured by the micropipet by looking at the volume setting. The setting will appear in the window on the side of the micropipet. Note that the different micropipets use different scales for their measurements. Some micropipets are accurate to a tenth of a microliter, while others are accurate to one microliter.

3. **SET** the volume by twisting the top of the plunger. In general, twisting the plunger clockwise reduces the volume, and twisting the plunger counter clockwise increases the volume.

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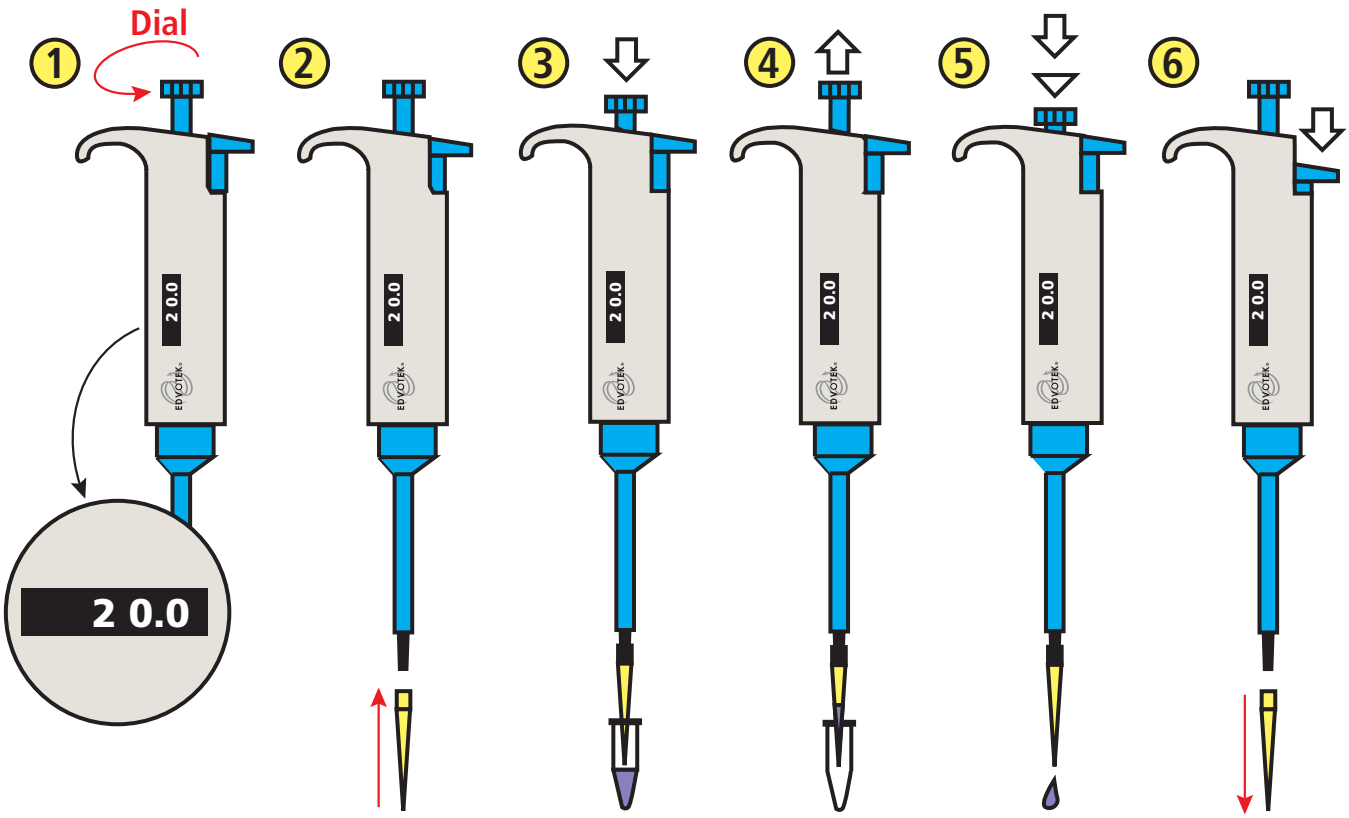
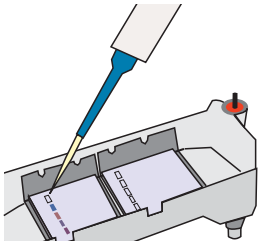
Appendix C
Practice Gel Loading



MEASURING LIQUIDS WITH A MICROPIPET

Wear gloves and safety goggles

1. **SET** the micropipet to the appropriate volume by adjusting the dial.
2. **PLACE** a clean tip on the micropipet.
3. **PRESS** the plunger down to the first stop. **HOLD** the plunger down while placing the tip beneath the surface of the liquid.
4. Slowly **RELEASE** the plunger to draw sample into the pipette tip. Position the pipet tip over the well. Be careful not to puncture or damage the well with the pipet tip.



5. **DELIVER** the sample by slowly pressing the plunger to the first stop. Depress the plunger to the second stop to expel any remaining sample. **DO NOT RELEASE** the plunger until the tip is out of the buffer.
6. **DISCARD** the tip by pressing the ejector button. Use a new clean tip for the next sample.

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