



Sci-On[®] Biology

S-48

EDVO-Kit #

What is PCR and How Does It Work?

Storage:

Store this experiment at room temperature

EXPERIMENT OBJECTIVES:

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.

All 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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Experiment Components

ELECTROPHORESIS SAMPLES

- Ready-to-Load™ Dye samples
 - A Standard dyes with assigned base pair equivalents
 - B Sample after 10 cycles
 - C Sample after 20 cycles
 - D Sample after 30 cycles
 - E Sample after 40 cycles

Storage:
Store entire experiment
at room temperature.

REAGENTS & SUPPLIES:

- Practice Gel Loading Solution
- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- 1 ml pipet
- 100 ml graduated cylinder (packaging for samples)
- 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
- DNA visualization system (white light)
- Distilled or deionized water

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EDVO-Kit # S-48
What is PCR and How Does It
Work?

Polymerase Chain Reaction (PCR)

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.

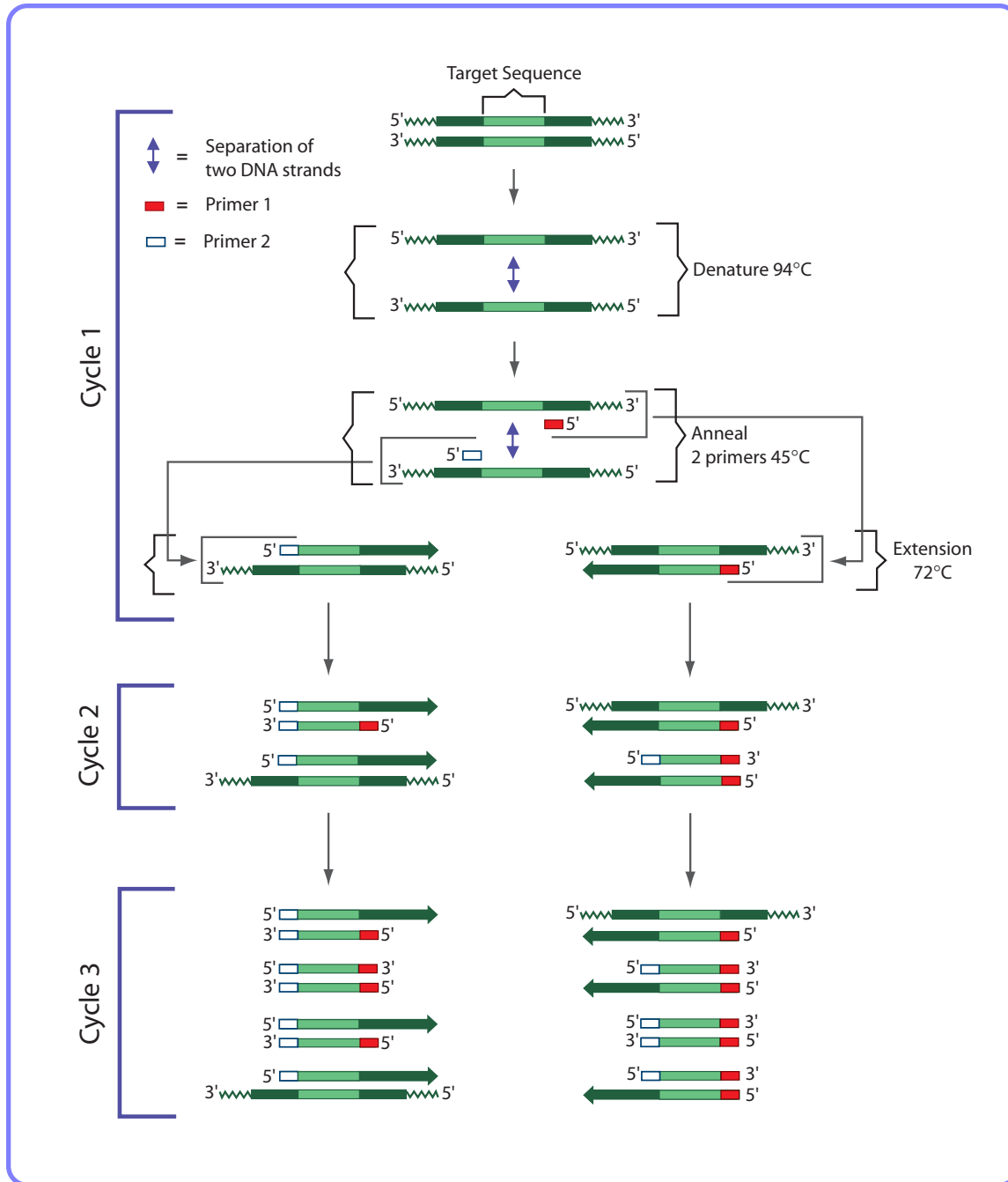
The specificity of DNA synthesis is dictated by the Watson - Crick base pairing rules and is directed by the template DNA. The strand being synthesized is complimentary and antiparallel to the template DNA strand. *De novo* DNA synthesis catalyzed by DNA polymerase cannot occur without a primer having 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 on the next page.



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Polymerase Chain Reaction (PCR)



Background Information

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EDVO-Kit # S-48
What is PCR and How Does It Work?

Polymerase Chain Reaction (PCR)

Background Information

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 μ l 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 several hundred 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.



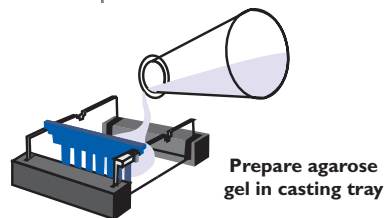
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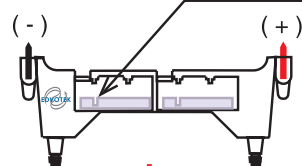
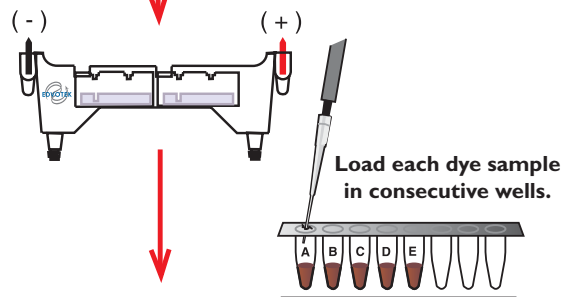
Experiment Overview

BEFORE YOU START THE EXPERIMENT

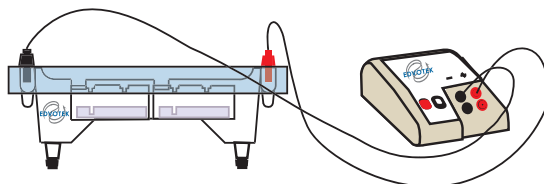
1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.



Remove end blocks, comb and submerge gel under buffer in electrophoresis chamber



Attach safety cover, connect leads to power source and conduct electrophoresis



EXPERIMENT CONTENT 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.

WORKING HYPOTHESIS

If the major product of one round of DNA is amplified exponentially after each cycle, then increasing the number of cycles from one to thirty will result in exponentially produced quantities of DNA.

EDVO-Kit # S-48
What is PCR and How Does It Work?

Activity One - Agarose Gel Preparation and Practice Gel Loading

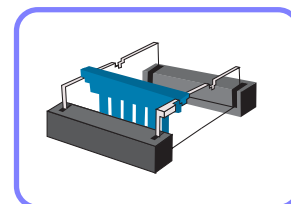
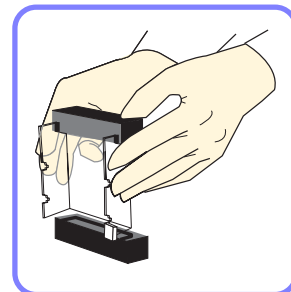


LABORATORY SAFETY

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.

PREPARING THE GEL BED

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
 - A. Using Rubber dams:
 - Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
 - B. Taping with labeling or masking tape:
 - With 3/4 inch wide tape, extend the tape over the sides and bottom edge of the bed.
 - Fold the extended edges of the tape back onto the sides and bottom. Press contact points firmly to form a good seal.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.



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Activity One - Agarose Gel Preparation and Practice Gel Loading

CASTING AGAROSE GELS

- Use a 250 ml flask to prepare the gel solution. Add the following components to the flask as specified for your experiment (refer to Table A).
 - Buffer concentrate
 - Distilled water
 - Agarose powder

Table A Individual 0.8% UltraSpec-Agarose™ Gel
Electrophoresis of Dyes

Size of EDVOTEK Casting Tray (cm)	Amt of Agarose (g)	+ Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
7 × 7	0.24	0.6	29.4	30
7 × 15	0.48	1.2	58.8	60

- Swirl the mixture to disperse clumps of agarose powder.
- With a marking pen, indicate the level of the solution volume on the outside of the flask.
- Heat the mixture to dissolve the agarose powder. The final solution should appear clear (like water) without any undissolved particles.
 - Microwave method:
 - Cover the flask with plastic wrap to minimize evaporation.
 - Heat the mixture on High for 1 minute.
 - Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.
 - Hot plate method:
 - Cover the flask with aluminum foil to prevent excess evaporation.
 - Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

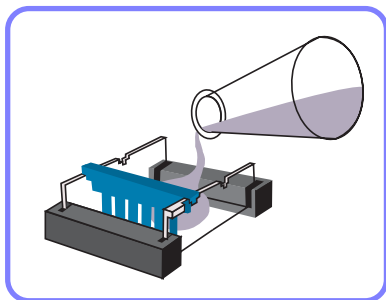
At high altitudes, it is recommended to use a microwave oven to reach boiling temperatures.

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 What is PCR and How Does It
 Work?

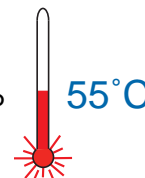
Activity One - Agarose Gel Preparation and Practice Gel Loading

Experiment Procedures

7. Cool the agarose solution to 55°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 5.



Cool the
 agarose to



**DO NOT POUR BOILING
 HOT AGAROSE INTO THE
 GEL BED.**

Hot agarose solution may
 irreversibly warp the bed.

After the gel is cooled to 55°C:

**If you are using rubber dams, go to step 9.
 If you are using tape, continue with step 8.**

8. Seal the interface of the gel bed and tape to prevent the agarose solution from leaking.
- Use a transfer pipet to deposit a small amount of cooled agarose to both inside ends of the bed.
 - Wait approximately 1 minute for the agarose to solidify.
9. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.



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Activity One - Agarose Gel Preparation and Practice Gel Loading

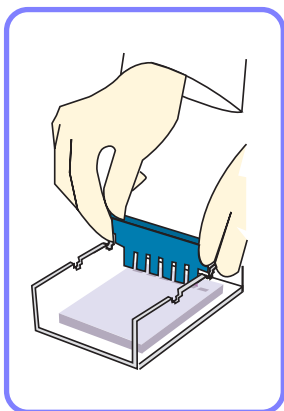
PREPARING THE GEL FOR ELECTROPHORESIS

- After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed.

Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.

- Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.
- Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
- Fill the electrophoresis apparatus chamber with the required volume of diluted buffer for the specific unit you are using (see guidelines in Table B).

For DNA analysis, the same EDVOTEK 50x Electrophoresis Buffer is used for preparing both the agarose gel buffer and the chamber buffer. The formula for diluting EDVOTEK (50x) concentrated buffer is 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water.



The electrophoresis (chamber) buffer recommended is Tris-acetate-EDTA (20 mM tris, 6 mM sodium acetate, 1 mM disodium ethylenediamine tetraacetic acid) pH 7.8. Prepare the buffer as required for your electrophoresis apparatus.

Table B Dilution of Electrophoresis (Chamber) Buffer

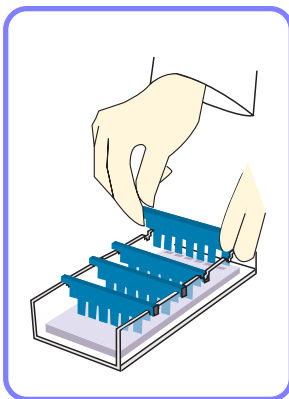
EDVOTEK Model #	Concentrated Buffer (50x) (ml)	+ Distilled Water (ml)	= Total Volume (ml)
M6+	6	294	300
M12	8	392	400
M36 (blue)	10	490	500
M36 (clear)	20	980	1000

- Make sure the gel is completely covered with buffer.
- Proceed to loading the samples and conducting electrophoresis.

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What is PCR and How Does It Work?

Activity One - Agarose Gel Preparation and Practice Gel Loading

Experiment Procedures



PRACTICE GEL LOADING

Accurate sample delivery technique ensures the best possible gel results. Pipeting 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 practice gel loading is performed in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. A small amount of practice gel loading solution (filling up to 12 wells) will not interfere with the experiment, so it is not necessary to prepare fresh buffer.

If you are using transfer pipets, gently squeeze the pipet stem, instead of the bulb to help control the delivery of small sample volumes.



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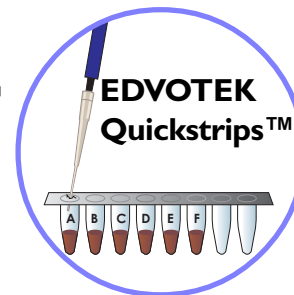
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Activity Two - Conducting Agarose Gel Electrophoresis

ELECTROPHORESIS SAMPLES

Samples in this electrophoresis experiment are packaged in pre-aliquoted Quickstrip™ connected tubes.

To remove samples from the Quickstrip™ tubes, simply pierce the foil top with the micropipet tip and withdraw the sample.



Quickstrips
patent pending

LOADING THE SAMPLES

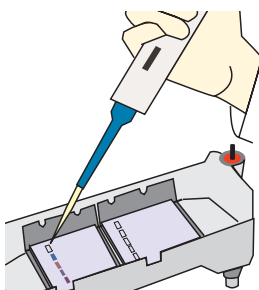
1. Check the Sample Volumes

Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the bottom of the tubes before starting to load the gel.

- If your samples are in Quickstrip™ connected tubes, tap the foil top of the strip so samples fall to the bottom of the tubes.
- If your samples are in individual 1.5 ml or 0.5 ml microtest tubes, briefly centrifuge the sample tubes, or tap each tube on the tabletop to get all the sample to the bottom of the tube.

2. Load Samples

Load each of the dye samples in tubes A - E into the wells in consecutive order. The amount of sample that should be loaded is 35-38 μ l.



Lane	Label	Sample
1	A	Standard dyes with assigned base pair equivalents
2	B	Sample after 10 cycles
3	C	Sample after 20 cycles
4	D	Sample after 30 cycles
5	E	Sample after 40 cycles

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What is PCR and How Does It
Work?

Activity Two - Conducting Agarose Gel Electrophoresis

Experiment Procedures

RUNNING THE GEL

- After the samples are loaded, carefully snap the cover down onto the electrode terminals.

Make sure that the negative and positive color-coded indicators on the cover and apparatus chamber are properly oriented.

- Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).
- Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.
- Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

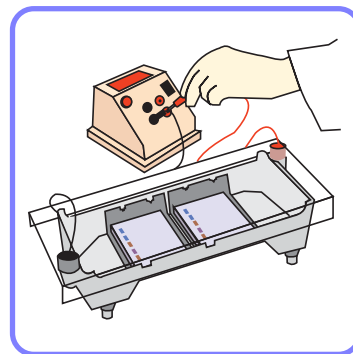


Table C Time and Voltage
Electrophoresis of Dyes

Volts	Recommended Time
125	20 min
70	40 min
50	60 min

- After approximately 10 minutes, you will begin to see separation of the colored dyes.
- After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
- Document the gel results.

A variety of documentation methods can be used, including drawing a picture of the gel, taking a photograph, or scanning an image of the gel on a flatbed scanner.

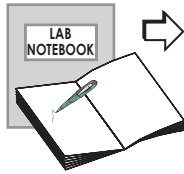
Staining is not required for Experiment # S-48, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small they will diffuse out of the gel. Thus, the gel cannot be saved.



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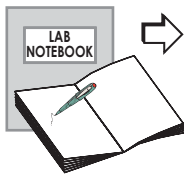
Critical Thinking and Hypothesis Development



Record the following in your Laboratory Notebook or as instructed by your teacher.


1. What is the variable in this experiment?
2. What is the control in this experiment?
3. What could one change in the experiment if this experiment was repeated?
4. Write a hypothesis that would reflect a change.

Study Questions




Record the answers to the following Study Questions in your Laboratory Notebook or as instructed by your teacher.

1. Why is DNA amplification important?
2. What is the difference between the PCR reaction and replication in cells?
3. What is the function of the four dXTPs (dATP, dCTP, dGTP, dTTP)?
4. Why are two different primers required for PCR?

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) Agarose			
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.			
Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850			
Emergency Telephone Number (301) 251-5990			
Telephone Number for information (301) 251-5990			
Date Prepared 07/01/03			
Signature of Preparer (optional)			
Section II - Hazardous Ingredients/Identify Information Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.			
CAS #9012-36-6			
Section III - Physical/Chemical Characteristics			
Boiling Point For 1% solution	194° F	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Insoluble - cold			
Appearance and Odor White powder, no odor			
Section IV - Physical/Chemical Characteristics N.D. = No data			
Flash Point (Method Used)	No data	Flammable Limits	LEL N.D. UEL N.D.
Extinguishing Media Water spray, dry chemical, carbon dioxide, halon or standard foam			
Special Fire Fighting Procedures Possible fire hazard when exposed to heat or flame			
Unusual Fire and Explosion Hazards None			

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility No data available			
Hazardous Decomposition or Byproducts			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes			
Health Hazards (Acute and Chronic) Inhalation: No data available Ingestion: Large amounts may cause diarrhea			
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure No data available			
Medical Conditions Generally Aggravated by Exposure No data available			
Emergency First Aid Procedures Treat symptomatically and supportively			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Sweep up and place in suitable container for disposal			
Waste Disposal Method Normal solid waste disposal			
Precautions to be Taken in Handling and Storing None			
Other Precautions None			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) Chemical cartridge respirator with full facepiece.			
Ventilation	Local Exhaust	Special	
	Mechanical (General)	Gen. dilution ventilation	Other
Protective Gloves	Yes	Eye Protection	Splash proof goggles
Other Protective Clothing or Equipment Impervious clothing to prevent skin contact			
Work/Hygienic Practices None			

 Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.			
IDENTITY (As Used on Label and List) 50x Electrophoresis Buffer			
Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.			
Section I Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850			
Emergency Telephone Number (301) 251-5990			
Telephone Number for information (301) 251-5990			
Date Prepared 07/01/03			
Signature of Preparer (optional)			
Section II - Hazardous Ingredients/Identify Information Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)			
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Appreciable, (greater than 10%)			
Appearance and Odor Clear, liquid, slight vinegar odor			
Section IV - Physical/Chemical Characteristics N.D. = No data			
Flash Point (Method Used)	No data	Flammable Limits	LEL N.D. UEL N.D.
Extinguishing Media Use extinguishing media appropriate for surrounding fire.			
Special Fire Fighting Procedures Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.			
Unusual Fire and Explosion Hazards None identified			

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility Strong oxidizing agents			
Hazardous Decomposition or Byproducts Carbon monoxide, Carbon dioxide			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion?			
Health Hazards (Acute and Chronic) None			
Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?			
Signs and Symptoms of Exposure Irritation to upper respiratory tract, skin, eyes			
Medical Conditions Generally Aggravated by Exposure None			
Emergency First Aid Procedures Ingestion: If conscious, give large amounts of water Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorbent material and dispose of the absorbent material.			
Waste Disposal Method Dispose in accordance with all applicable federal, state, and local environmental regulations.			
Precautions to be Taken in Handling and Storing Avoid eye and skin contact.			
Other Precautions None			
Section VIII - Control Measures			
Respiratory Protection (Specify Type)			
Ventilation	Local Exhaust	Yes	Special None
	Mechanical (General)	Yes	Other None
Protective Gloves	Yes	Eye Protection	Safety goggles
Other Protective Clothing or Equipment None			
Work/Hygienic Practices None			



Material Safety Data Sheet
 May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List) **Practice Gel Loading Solution** Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I
 Manufacturer's Name: **EDVOTEK, Inc.**
 Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**
 Emergency Telephone Number: **(301) 251-5990**
 Telephone Number for information: **(301) 251-5990**
 Date Prepared: **07/01/03**
 Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information
 Hazardous Components [Specific Chemical Identity, Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)
 This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water	Soluble		
Appearance and Odor	Blue liquid, no odor		

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	No data	Flammable Limits	LEL No data	UEL No data
Extinguishing Media	Dry chemical, carbon dioxide, water spray or foam			
Special Fire Fighting Procedures	Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.			
Unusual Fire and Explosion Hazards	Unknown			

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None
Incompatibility	None		
Hazardous Decomposition or Byproducts	Sulfur oxides, and bromides		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Yes	Skin?	Yes	Ingestion?	Yes
Health Hazards (Acute and Chronic)	Acute eye contact: May cause irritation. No data available for other routes.					
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?			
	No data available		None			
Signs and Symptoms of Exposure	May cause skin or eye irritation					
Medical Conditions Generally Aggravated by Exposure	None reported					
Emergency First Aid Procedures	Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.					

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop spill area. Rinse with water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves	Yes	Eye Protection	Splash proof goggles
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Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact



Material Safety Data Sheet
 May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List) **Bromophenol Blue** Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I
 Manufacturer's Name: **EDVOTEK, Inc.**
 Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive, Rockville, MD 20850**
 Emergency Telephone Number: **(301) 251-5990**
 Telephone Number for information: **(301) 251-5990**
 Date Prepared: **07/01/03**
 Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information
 Hazardous Components [Specific Chemical Identity, Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)
 This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 62625-28-9

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water	Soluble		
Appearance and Odor	Blue color, liquid, no odor		

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	No data	Flammable Limits	LEL No data	UEL No data
Extinguishing Media	N/A			
Special Fire Fighting Procedures	N/A			
Unusual Fire and Explosion Hazards	None			

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	Unknown
Incompatibility	None		
Hazardous Decomposition or Byproducts	Sulfur oxides and bromides		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	No	Skin?	Yes	Ingestion?	Yes
Health Hazards (Acute and Chronic)	Acute eye contact: may cause irritation					
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?			
	None	No data	No data	No		
Signs and Symptoms of Exposure	May cause skin or eye irritation					
Medical Conditions Generally Aggravated by Exposure	None reported					
Emergency First Aid Procedures	Rinse contacted areas with copious amounts of water					

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear eye and skin protection and mop/wipe spill area. Rinse with water.

Waste Disposal Method: Can be disposed in the trash or down the sink

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact

Other Precautions: None

Section VIII - Control Measures


Respiratory Protection (Specify Type): NIOSH/MSHA - approved respirator

Ventilation	Local Exhaust	No	Special	None
	Mechanical (General)	No	Other	None


Protective Gloves	Yes	Eye Protection	Splash proof goggles
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Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Avoid eye and skin contact

 <p align="center">Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.</p>			
IDENTITY (As Used on Label and List) Orange G		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 1936-15-8			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Soluble			
Appearance and Odor yellow-orange color, liquid, no odor			
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	No data	Flammable Limits	LEL No data UEL No data
Extinguishing Media N/A			
Special Fire Fighting Procedures N/A			
Unusual Fire and Explosion Hazards None			

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	Unknown
Incompatibility None			
Hazardous Decomposition or Byproducts Sulfur oxides and bromides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? No Skin? Yes Ingestion? Yes			
Health Hazards (Acute and Chronic) Acute eye contact: may cause irritation			
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No
Signs and Symptoms of Exposure May cause skin or eye irritation			
Medical Conditions Generally Aggravated by Exposure None reported			
Emergency First Aid Procedures Rinse contacted areas with copious amounts of water			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled			
Wear eye and skin protection and mop/wipe spill area. Rinse with water.			
Waste Disposal Method Can be disposed in the trash or down the sink			
Precautions to be Taken in Handling and Storing Avoid eye and skin contact			
Other Precautions None			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA - approved respirator			
Ventilation	Local Exhaust	No	Special None
	Mechanical (General)	No	Other None
Protective Gloves	Yes	Eye Protection	Splash prof goggles
Other Protective Clothing or Equipment None required			
Work/Hygienic Practices Avoid eye and skin contact			

 <p align="center">Material Safety Data Sheet May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.</p>			
IDENTITY (As Used on Label and List) Phenol Red		Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.	
Section I		Emergency Telephone Number (301) 251-5990	
Manufacturer's Name EDVOTEK, Inc. Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850		Telephone Number for information (301) 251-5990 Date Prepared 07/01/03 Signature of Preparer (optional)	
Section II - Hazardous Ingredients/Identify Information			
Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional) This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 7114-03-6			
Section III - Physical/Chemical Characteristics			
Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water Soluble			
Appearance and Odor Red color, liquid, no odor			
Section IV - Physical/Chemical Characteristics			
Flash Point (Method Used)	No data	Flammable Limits	LEL No data UEL No data
Extinguishing Media N/A			
Special Fire Fighting Procedures N/A			
Unusual Fire and Explosion Hazards None			

Section V - Reactivity Data			
Stability	Unstable		Conditions to Avoid
	Stable	X	Unknown
Incompatibility None			
Hazardous Decomposition or Byproducts Sulfur oxides and bromides			
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None
Section VI - Health Hazard Data			
Route(s) of Entry: Inhalation? No Skin? Yes Ingestion? Yes			
Health Hazards (Acute and Chronic) Acute eye contact: may cause irritation			
Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No
Signs and Symptoms of Exposure May cause skin or eye irritation			
Medical Conditions Generally Aggravated by Exposure None reported			
Emergency First Aid Procedures Rinse contacted areas with copious amounts of water			
Section VII - Precautions for Safe Handling and Use			
Steps to be Taken in case Material is Released for Spilled			
Wear eye and skin protection and mop/wipe spill area. Rinse with water.			
Waste Disposal Method Can be disposed in the trash or down the sink			
Precautions to be Taken in Handling and Storing Avoid eye and skin contact			
Other Precautions None			
Section VIII - Control Measures			
Respiratory Protection (Specify Type) NIOSH/MSHA - approved respirator			
Ventilation	Local Exhaust	No	Special None
	Mechanical (General)	No	Other None
Protective Gloves	Yes	Eye Protection	Splash prof goggles
Other Protective Clothing or Equipment None required			
Work/Hygienic Practices Avoid eye and skin contact			

**Material Safety Data Sheet**

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List)
Xylene Cyanol

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name EDVOTEK, Inc.	Emergency Telephone Number (301) 251-5990
Address (Number, Street, City, State, Zip Code) 14676 Rothgeb Drive Rockville, MD 20850	Telephone Number for information (301) 251-5990
	Date Prepared 07/01/03
	Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)] OSHA PEL ACGIH TLV Other Limits Recommended % (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard. CAS # 2650-17-1

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H ₂ O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data
Solubility in Water	Soluble		
Appearance and Odor	_____ color, liquid, no odor		

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	No data	Flammable Limits	LEL No data	UEL No data
Extinguishing Media	N/A			
Special Fire Fighting Procedures	N/A			
Unusual Fire and Explosion Hazards	None			

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	Unknown
Incompatibility	None		
Hazardous Decomposition or Byproducts	Sulfur oxides and bromides		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation? No	Skin? Yes	Ingestion? Yes
Health Hazards (Acute and Chronic)	Acute eye contact: may cause irritation		
Carcinogenicity:	NTP? No data	IARC Monographs? No data	OSHA Regulation? No
Signs and Symptoms of Exposure	May cause skin or eye irritation		
Medical Conditions Generally Aggravated by Exposure	None reported		
Emergency First Aid Procedures	Rinse contacted areas with copious amounts of water		

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled	Wear eye and skin protection and mop/wipe spill area. Rinse with water.
Waste Disposal Method	Can be disposed in the trash or down the sink
Precautions to be Taken in Handling and Storing	Avoid eye and skin contact
Other Precautions	None

Section VIII - Control Measures

Respiratory Protection (Specify Type)	NIOSH/MSHA - approved respirator		
Ventilation	Local Exhaust	No	Special None
	Mechanical (General)	No	Other None
Protective Gloves	Yes	Eye Protection	Splash prof goggles
Other Protective Clothing or Equipment	None required		
Work/Hygienic Practices	Avoid eye and skin contact		