QUICK GUIDE

Biotech Basics

INNOVATIONS IN YOUR CLASSROOM

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.

Table of Contents

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

TIMSTAR

Part of WF Education Group

EDVOTEK®

What is the Polymerase Chain Reaction (PCR)?

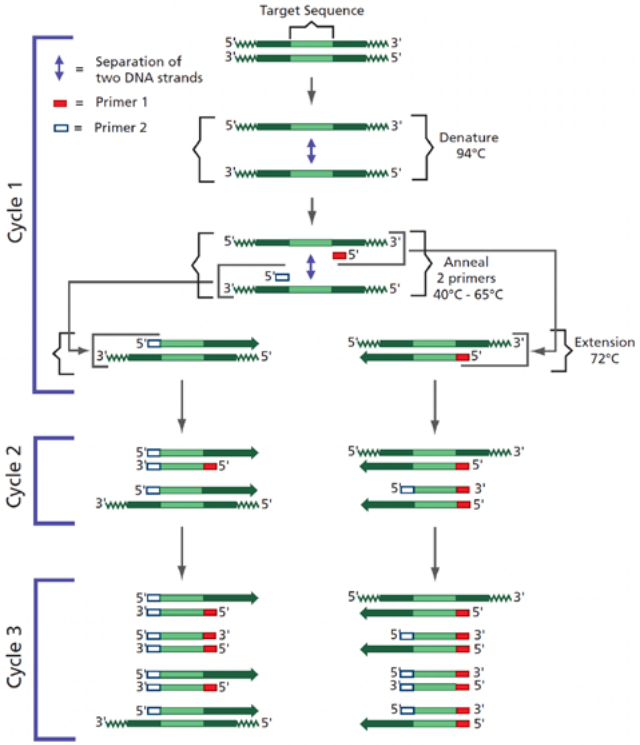
PCR is a technique that allows researchers to quickly create many copies of a specific region of DNA *in vitro*.

WHAT DO I NEED TO PERFORM PCR?

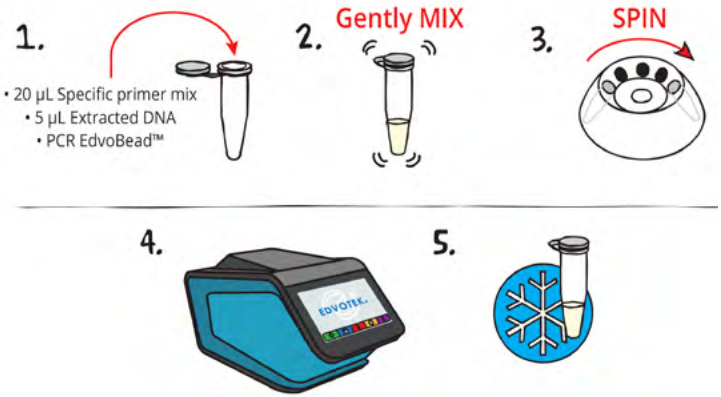
- **Template** – the purified, double-stranded piece of DNA we want to copy
- **Primers** – short synthetic DNA molecules that target a specific DNA sequence for amplification
- ***Taq* DNA Polymerase** – thermostable enzyme used to copy DNA
- **Free nucleotides** – the building blocks of DNA
- **Thermal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

HOW DOES PCR WORK?

To perform PCR, the template is mixed with primers, *Taq* polymerase and nucleotides. The mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target sequence in less than five minutes. In order to produce enough DNA for analysis, twenty to forty cycles may be required.



POLYMERASE CHAIN REACTION (PCR)



1. **ADD** 20 µL specific primer mix, 5 µL extracted DNA and the PCR EdvoBead™ to a labeled 0.2 mL tube.
2. **MIX** the PCR sample. Make sure the PCR EdvoBead™ is completely dissolved.
3. **CENTRIFUGE** to collect the sample at the bottom of the tube.
4. **AMPLIFY** DNA using PCR. ***(NOTE: Actual PCR cycling conditions will vary. Reference your experiment's instructions for specific times and temperatures.)***
   PCR cycling conditions:
   Initial denaturation 94°C for 3-5 min.
   94°C for 30-60 sec.
   45-65°C for 30-60 sec. } 20-40 cycles
   72°C for 30-60 sec.
   Final Extension 72°C for 5-10 min.
5. **PLACE** tubes on ice. **ANALYZE** samples using agarose gel electrophoresis.

Related Products

PCR Tubes
Thin-walled 0.2 mL PCR microtest tubes, 100 pack.
Code: BT100562

PCR EdvoBeads™
Bottle of 25 beads.
Code: BT140596

EdvoCycler™ Junior
Holds 16 x 0.2 mL PCR Samples
Code: BT200806

View all these products and MORE on our website!

What is Electrophoresis?

Electrophoresis is a technique that separates DNA, RNA or proteins according to their size.

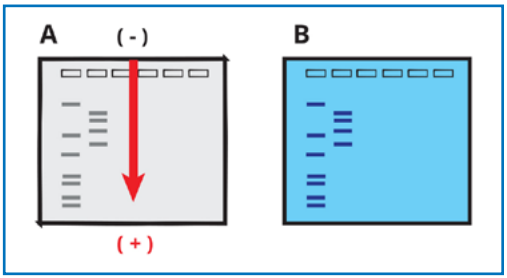
WHAT DO I NEED TO SEPARATE A MIXTURE OF DNA MOLECULES?

In addition to your DNA sample, you will need:

- GEL LOADING SOLUTION – includes glycerol to help DNA samples enter into the wells and a visible dye to monitor migration through the gel.
- AGAROSE – a polysaccharide used as the separation matrix.
- ELECTROPHORESIS BUFFER – contains ions necessary to conduct an electrical current, maintains pH of experiment.
- HORIZONTAL ELECTROPHORESIS APPARATUS – holds the buffer and the gel, has positive and negative electrodes.
- POWER SUPPLY – generates the current necessary to move DNA through gel.
- MICROPIPETTE – used to transfer samples into wells.
- A special STAIN that allows us to visualize DNA.

HOW DOES ELECTROPHORESIS SEPARATE DNA FRAGMENTS?

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 A).



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 B).

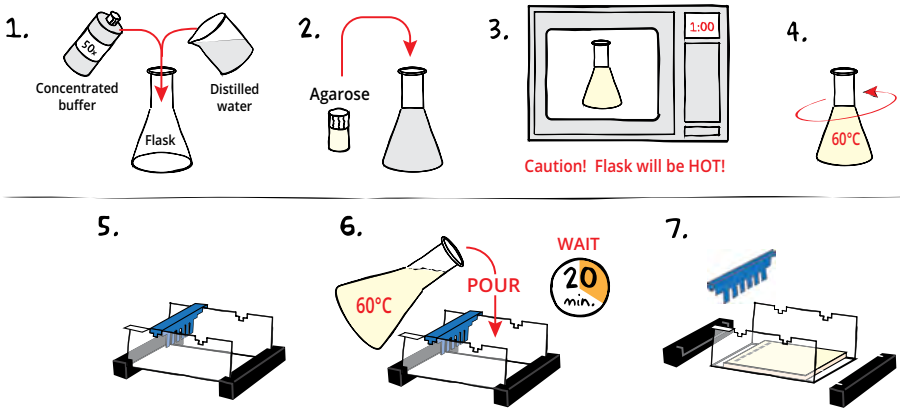
Related Products

M12 Complete Electrophoresis Package
Code: BT180800

M36 HexaGel Electrophoresis Apparatus
Code: BT97820

View all these products and MORE on our website!

ELECTROPHORESIS: CASTING THE AGAROSE GEL



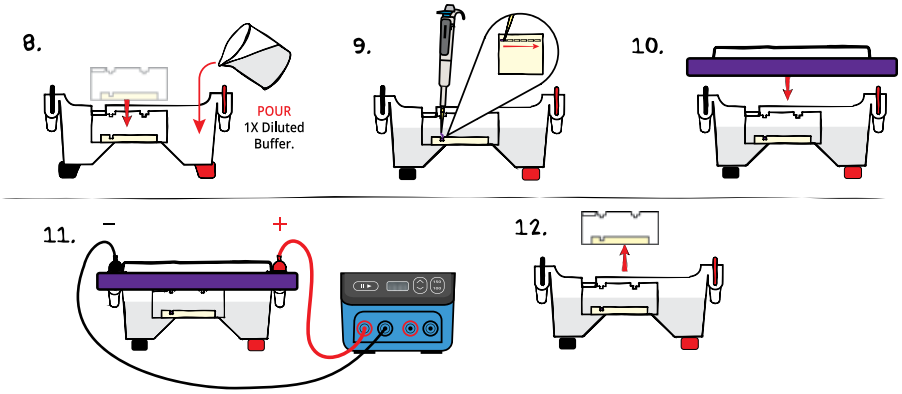

Wear gloves and safety goggles

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to 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™ Gels**

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	0.24 g	30 mL
10 x 7 cm	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

ELECTROPHORESIS: RUNNING THE GEL



8. **PLACE** the gel (still 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.
9. **LOAD** the samples into the wells in the order indicated by your instructor.
10. **PLACE** safety cover on the unit. **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). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

PROCEED to staining and visualizing agarose gels using FlashBlue™ Stain.

Table B

1x Electrophoresis Buffer (Chamber Buffer)			
Timstar Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
BT180800 (M12)	400 mL	8 mL	392 mL
BT97820 (M36)	1000 mL	20 mL	980 mL

Table C

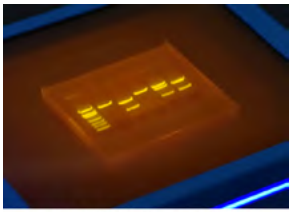
Time and Voltage (0.8% Agarose Gel)	
Model	BT180800 (M12) or BT97820 (M36)
Volts	Min/Max (minutes)
150	20/35
125	30/45
100	40/60

Visualizing DNA

Agarose gel electrophoresis is used to separate DNA fragments in complex mixtures according to their size. However, because DNA is clear and colorless, these bands cannot be seen with the naked eye. We offer different methods for visualizing the DNA separated by electrophoresis.

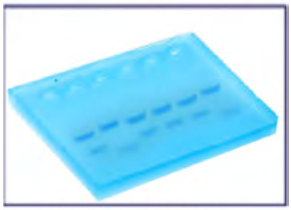
SYBR® SAFE DNA STAIN:

Research laboratories commonly use fluorescent DNA stains because they are extremely sensitive, making it easy to quantify small amounts of DNA. In order to visualize the DNA fragments, an ultraviolet (UV) light source (such as a transilluminator) is used to excite the fluorescent molecules.



SYBR® Safe DNA Stain

SYBR Safe® is a fluorescent DNA stain that binds specifically to the DNA double helix. When excited with UV or blue light, any SYBR Safe® that is bound to DNA fluoresces with a bright green color. Fluorescent DNA stains like SYBR Safe® are perfect for technically challenging experiments like PCR because they are extremely sensitive, making it easy to quantify small amounts of DNA. In contrast with other fluorescent stains, SYBR Safe® has been engineered to be non-mutagenic, making it much safer to use in the classroom.



FlashBlue™ DNA Stain

FLASHBLUE™ DNA STAIN:

Although they are less sensitive than fluorescent stains, dye-based DNA stains are an excellent alternative for the teaching classroom, as they are non-toxic and require no special equipment for visualization. The molecules of the DNA stain possess a positive charge, which allows them to bind to the negatively charged backbone of DNA. The DNA fragments are easily visualized because the bound dye molecules stain them with an intense blue color.

WHICH DNA STAIN SHOULD I USE?

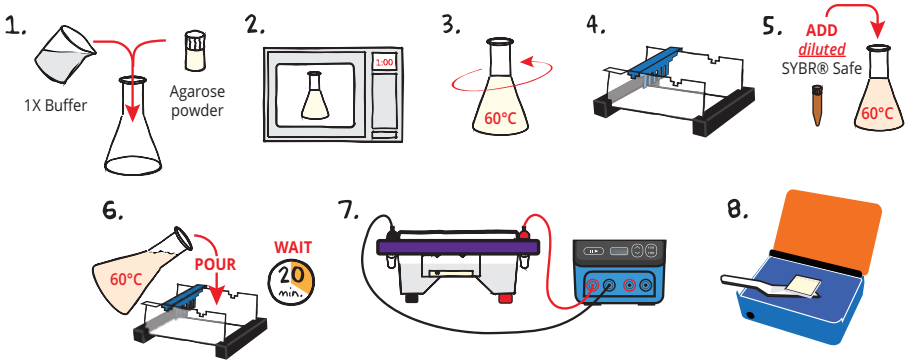
STAIN	ADVANTAGES	DISADVANTAGES
SYBR® Safe	Very sensitive Non-mutagenic	Requires UV transilluminator More expensive
FlashBlue™	Simple and fast Reusable, inexpensive	Less sensitive Disposal of liquid

SYBR® SAFE METHOD I: IN-GEL STAINING PROTOCOL (PREFERRED METHOD)

This fast, easy staining protocol incorporates SYBR® Safe into the molten agarose before the gel is poured into the casting tray. This means that the DNA is staining while the electrophoresis experiment is running! Results can be visualized immediately post electrophoresis.

SYBR® Safe is provided as a 10,000X concentrate. Be sure to dilute the SYBR® Safe before adding to the molten agarose (as specified in your experimental protocol).

Agarose gels may be prepared in advance and stored for later use. Place the gels in a plastic container and cover with 1X Electrophoresis Buffer containing SYBR® Safe at a 1:10,000 dilution. Store in the dark at 4°C for up to a week.



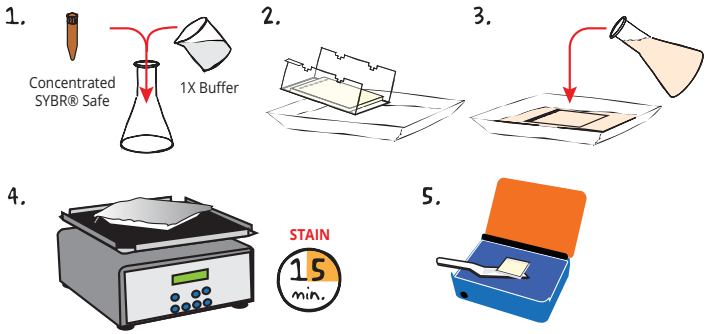
1. **MIX** 1X buffer and agarose powder as specified in your experimental protocol.
2. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for one minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely melted (the solution should be clear like water).
3. **COOL** the molten agarose to 60°C with careful swirling to promote even dissipation of heat.
4. **PREPARE** gel-casting tray while the gel is cooling.
5. Before casting the gel, **ADD** diluted SYBR® Safe to the molten agarose and swirl to mix well. The agarose solution may appear pale orange in color.
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. **LOAD** samples and **PERFORM** electrophoresis as specified in your experimental protocol. ***(Note: For long gels (>10 cm), we recommend adding SYBR® Safe to the Electrophoresis Buffer at a 1:10,000 dilution to avoid dye-front migration issues. Gels under 10 cm in length should not be affected.)***
8. After electrophoresis is complete, **REMOVE** gel and casting tray from the electrophoresis chamber. Carefully **SLIDE** gel off of the casting tray onto the viewing surface of the transilluminator and turn the unit on. DNA should appear as bright green bands on a dark background.

DISPOSAL OF SYBR® SAFE:

SYBR® Safe DNA Stain is not classified as hazardous waste, thus can be safely disposed of down the drain or in the regular trash, providing convenience and reducing cost in waste disposal.

SYBR® SAFE METHOD II: POST-ELECTROPHORESIS STAINING PROTOCOL

Run agarose gel(s) as usual according to your standard protocol. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads, and remove the cover.



1. **DILUTE** SYBR® Safe 1:10,000 by adding 7.5 µL of the concentrated stain to 75 mL of 1x electrophoresis buffer in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **POUR** the 1x SYBR® Safe stain solution over the gel. **COVER** the gel completely with solution.
4. **COVER** the tray with foil to protect the gel from light. **STAIN** the gel for 10-15 minutes. ***(Note: For best results, use an orbital shaker to gently agitate the gel while staining.)***
5. **REMOVE** the gel from the staining solution. **SLIDE** gel off of the casting tray onto the viewing surface of the transilluminator and turn the unit on. DNA should appear as bright green bands on a dark background.

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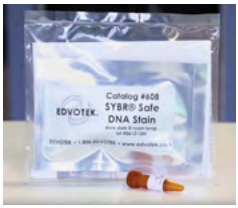
Related Products

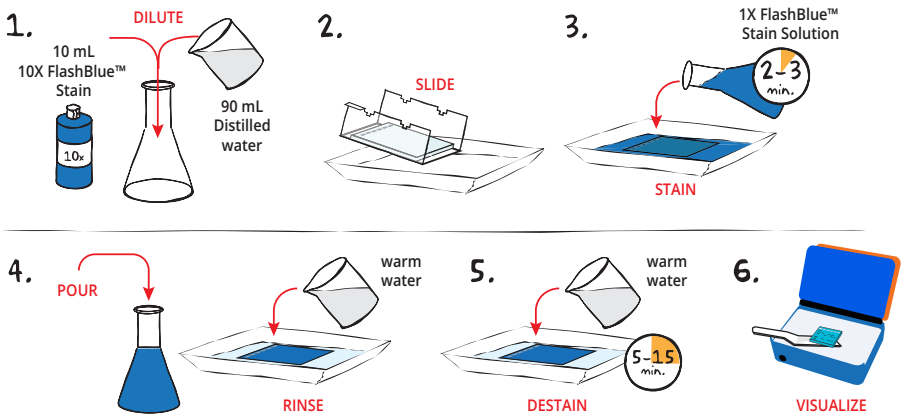


SYBR® Safe DNA Stain
10,000 x concentrate for 750 mL
Code: BT150612

TruBlu™ 2 Blue/White Transilluminator
Accommodates up to eight 7 x 7 cm gels and combines the functions of two units into one!
Code: BT180900

View all these products and MORE on our website!

STAINING AGAROSE GELS USING FLASHBLUE™



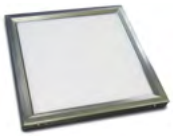
1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™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

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

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

Related Products

Electrophoresis Reagent Package with Flashblue™
Reagents to perform up to 40 electrophoresis experiments.
Code: BT150614



White LED Transilluminator
Spacious 250 x 250 mm viewing area
Code: BT97815

View all these products and MORE on our website!

Troubleshooting Guide

PROBLEM:	CAUSE:	ANSWER:
There is very little liquid left in tube after PCR	Sample has evaporated	Make sure the heated lid reaches the appropriate temperature.
		If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax (see Appendix B for details)
		Make sure students close the lid of the PCR tube properly.
	Pipetting error	Make sure students pipet 20 µL primer mix and 5 µL Lambda DNA template into the PCR tube.
There's not enough sample in my QuickStrip.	The QuickStrip has dried out.	Add 40 uL water, gently pipet up and down to mix before loading.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
		Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of "clumps" and glassy granules before pouring gels.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
After staining, the ladder is visible but no PCR products are present.	PCR amplification was unsuccessful.	Repeat PCR with fresh PCR EdvoBeads™ and primers.
		Ensure that the thermal cycler has been properly programmed. See Module II for guidelines.
After staining, the ladder and control PCR products are visible on gel, but some student samples are not present.	Wrong volumes of DNA and primer added to PCR reaction	Practice using pipettes
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™.

Related Products

Agarose Powder
20 grams
Code: BT100518

100 grams
Code: BT100520

Electrophoresis Buffers
50x TAE, 100 mL
Code: BT100530

50x TAE, 500 mL
Code: BT140585

10x TBE, for 5 L
Code: BT110100

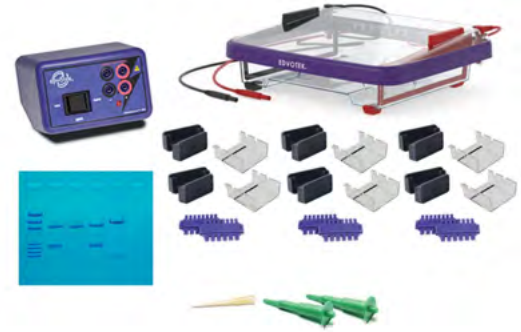
Gel Loading Solution
10x Yields 6 mL final volume of DNA sample.
Code: BT140582

FlashBlue DNA Stain
10x Concentrate for 1.2 L
Code: BT150616

Classroom PCR Labstation™
Supports up to 25 students
Code: BT150886

Fixed Volume Minipipettes

5 µL	***Code: BT97835***
10 µL	***Code: BT97837***
20 µL	***Code: BT97839***
40 µL	***Code: BT97843***
50 µL	***Code: BT97845***
100 µL	***Code: BT97847***



Classroom DNA Electrophoresis LabStation™
An economical way to introduce DNA electrophoresis to the classroom.
Code: BT100510

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