EDVOTEK WORKSHOP:

Biotechnology Basics:
DNA Isolation, PCR, & Electrophoresis

EDVOTEK®

www.edvotek.com

Introduction

Feeling overwhelmed by all the topics, procedures, and equipment used in the diverse biotechnology field? If so, this workshop is for you! Join us for some hands-on experimentation - you will learn about three biotechnology techniques commonly used in research labs: DNA isolation, PCR, and gel electrophoresis.

Receive a 4GB flash drive and be entered for a T-shirt drawing!

Background Information

WHAT IS BIOTECHNOLOGY?

Simply put, biotechnology is the manipulation of living things to create a product that is useful for humans. Biotechnology has its roots in traditional practices that have been used since the ancient times. For example, fermentation has been used to preserve food for over 6000 years. Scientists later discovered that microorganisms are responsible for fermentation. Fermented products like yogurt, sauerkraut and wine continue to be consumed on a regular basis. Furthermore, selective breeding techniques have been used to promote desirable qualities in livestock and food crops like corn. Early farmers may have crossed a corn plant with larger kernels with another plant that produced more flavorful corn (Figure 1A). Plants in the next generation would produce corn with large, delicious kernels (Figure 1B).

Figure 1A - Traditional varieties of corn.

Figure 1B - Cultivated corn

Biotechnology continues to evolve as new techniques are developed. Advances in genetic engineering allow scientists to directly manipulate DNA sequences, dramatically reducing the time necessary to create useful organisms. For example, bacteria can be engineered to produce large amounts of important proteins, essentially converting them into living factories. Insulin, which is used to control diabetes, was the first medication for human use that was produced by genetic engineering.

DNA ISOLATION

The basic unit of all living organisms, from bacteria to humans, is the cell. Contained within cells is DNA, which acts as a blueprint for all processes within an organism. A strand of DNA is composed of building blocks known as nucleotides. Each nucleotide comprises three basic parts: a phosphate group, a deoxyribose sugar, and a nitrogen-containing base (adenine, cytosine, guanine, or thymine — abbreviated as A, C, G, or T). The sugar of one nucleotide forms a covalent bond with the phosphate group of its neighbor, making DNA a stable scaffold for genetic information. The order of these nucleotides gives rise to genes, each with a unique sequence.

Background Information

Two strands of DNA come together to form a double helix, which resembles a ladder that spirals around a central axis. The rungs of the ladder are formed by hydrogen bonds between bases (shown as dashed lines in Figure 2). Base-pairing interactions are highly specific – A will only pair with T, and G with C – but are weaker than covalent bonds, which allows paired DNA strands to be "unzipped" during DNA replication.

Before performing many biotechnology experiments, cellular membranes must be broken down, or "lysed", to release the DNA. Then, ice-cold alcohol (ethanol or isopropanol) is added to cellular lysate. The alcohol disrupts the electrostatic interactions between the water molecules and the sugar-phosphate backbone, forcing the DNA out of solution. The addition of salt enhances DNA precipitation because the positively charged sodium ions further disrupt the interactions between the DNA molecules and water. If the cellular lysate is overlaid with alcohol, the alcohol "floats" on top of the lysate due to its lower density. This causes the DNA to precipitate at the interface of the two liquids as sticky white fibers that are collected using a stirrer or glass rod.

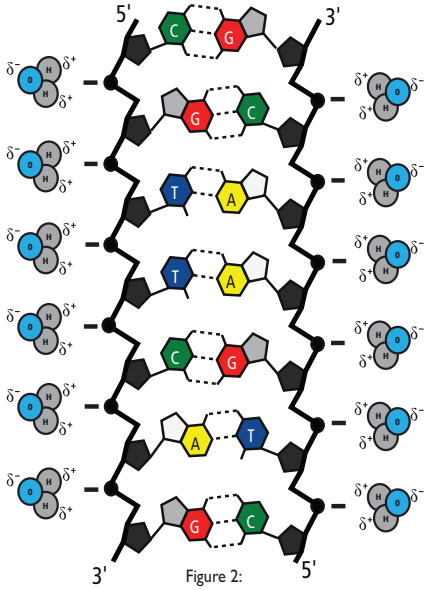


Figure 2:
The Structure of DNA

THE POLYMERASE CHAIN REACTION (PCR)

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he discovered that he could replicate DNA in vitro using short, synthetic DNA primers and DNA polymerase I. Furthermore, because the primers were designed to match a specific DNA sequence, this technique only replicated the targeted gene. For the development of this simple and elegant method to copy specific pieces of DNA, known as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

To perform PCR, purified DNA is mixed with primers, a thermostable 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 (known as "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 cycle (denaturation, annealing, extension) doubles the amount of target DNA. Today, a specialized machine, called a "thermal cycler" or "PCR machine", is used to rapidly heat and cool the samples. As a result, a PCR cycle can be completed in less than 5 minutes; 20-40 cycles produce sufficient DNA for analysis.

Because of its ease of use and its ability to rapidly amplify DNA, PCR has become indispensible in medical and life sciences labs. For example, research laboratories can amplify a specific region of DNA for cloning applications. Medical tests 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.

Background Information

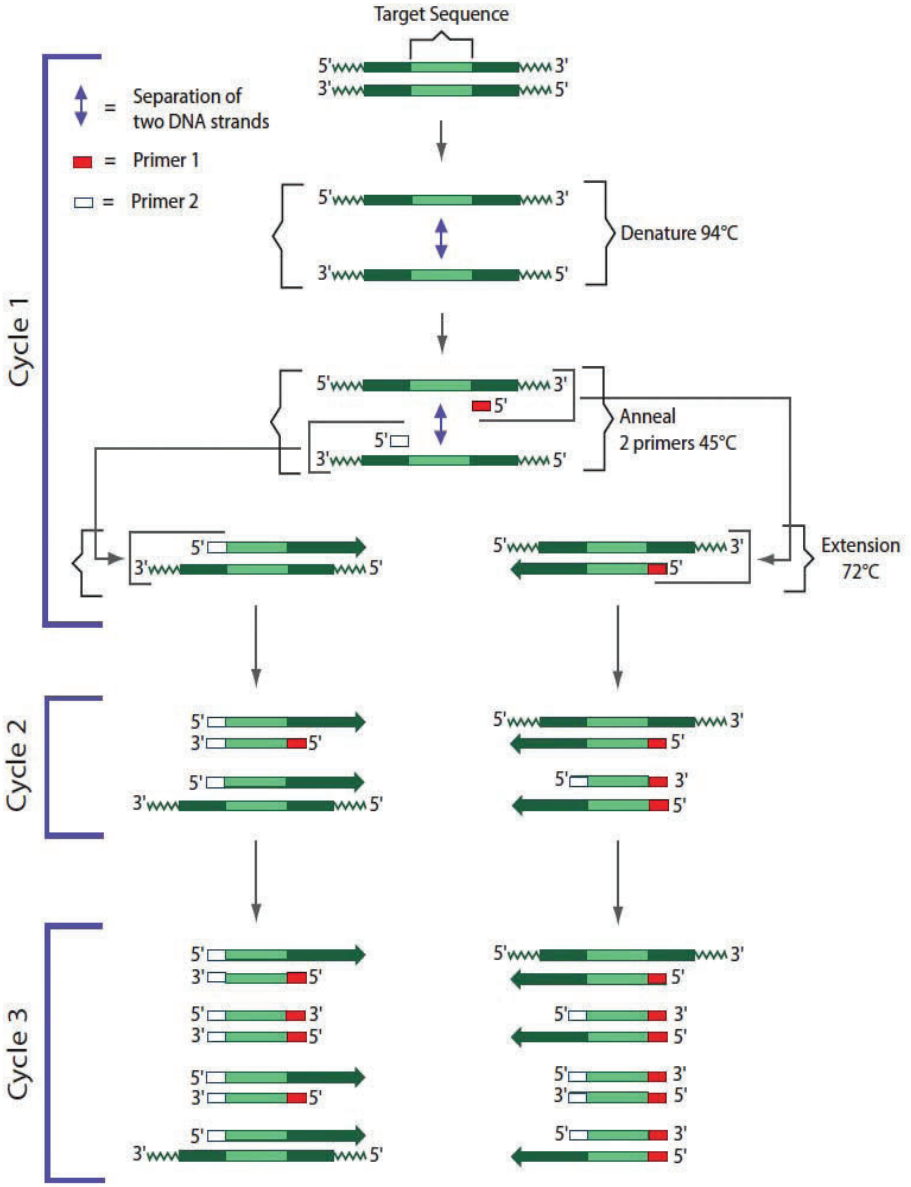


Figure 3: The Polymerase Chain Reaction

Background Information

PRINCIPLES OF GEL ELECTROPHORESIS

Agarose gel electrophoresis is a technique that separates a mixture of DNA molecules based upon charge, size and shape. First, the agarose gel is placed in a horizontal electrophoresis chamber and covered with an aqueous, pH-balanced buffer. After the DNA samples are added into depressions (or "wells") within the gel, an electrical current is passed through the gel. The current drives the negatively charged DNA fragments through the gel towards the positive electrode.

Although the gel appears to be solid, it actually contains microscopic channels through which the molecules can pass. These pores act as a molecular sieve that affects the rate at which DNA travels through the gel. Small DNA fragments move through the holes in the gel 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.

While electrophoresis is a powerful separation technique, it is not without its technical limitations. Most significantly, if two different fragments share a similar size, they will migrate together through the gel and may appear as a single band. In addition, if digestion results in a broad distribution of DNA sizes, the fragments may stain as a smear. Lastly, DNA with a streamlined secondary structure (such as supercoiled DNA) can pass through the gel more quickly than similarly-sized linear DNA, which prevents an accurate comparison of size.

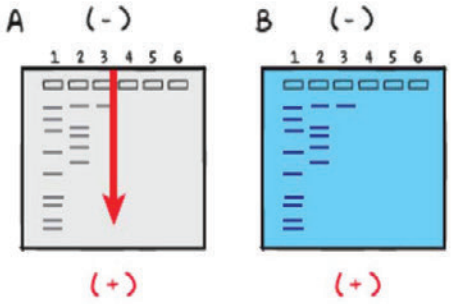
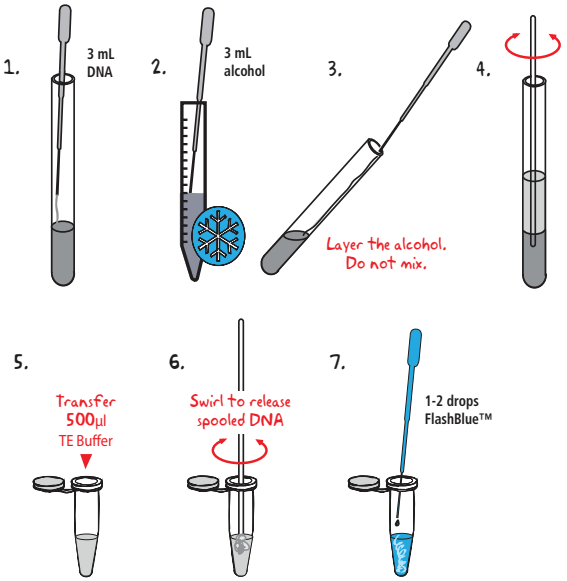


Figure 4: Overview of agarose gel electrophoresis

Module I: DNA Extraction

1. Using a calibrated transfer pipet, **TRANSFER** 3 mL of ready-to-spool DNA to a disposable plastic beaker.
2. **MEASURE** 3 mL of ice-cold alcohol using a calibrated transfer pipet.
3. **PLACE** the tip of the pipet on the inside wall of the beaker containing the DNA solution. **LAYER** the alcohol on top of the DNA solution by gently streaming the alcohol down the side of the tube. **DO NOT mix the two solutions.**
4. **PLACE** the end of the spooling rod into the beaker, just below the interface between the alcohol and DNA solutions. Quickly **TWIRL** the rod in a circular motion to spool out the DNA.
5. **TRANSFER** 500µl of TE Buffer to the Genes in a Tube™ necklace.
6. Gently **SWIRL** the rod to release the spooled DNA into the the Genes in a Tube™ necklace.
7. **ADD** 1-2 drops of FlashBlue™ solution to the tube containing the DNA. Your necklace is ready to wear!



What Does DNA Look Like?

Cat. #S-10 $39

This fun and easy lab activity shows your students what real chromosomal DNA looks like and allows them to explore the procedures involved in DNA extraction. Just overlay with 95% ethanol or isopropyl alcohol and spool the DNA on the glass rod!

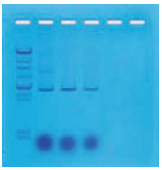
Genes in a Tube™

Cat. #119 $99

Teach your students how to extract and spool their own DNA in this exciting and easy activity. Students can transfer their DNA to a tube that can be used as a pendant on a necklace!

Mini-Prep Isolation of Plasmid DNA

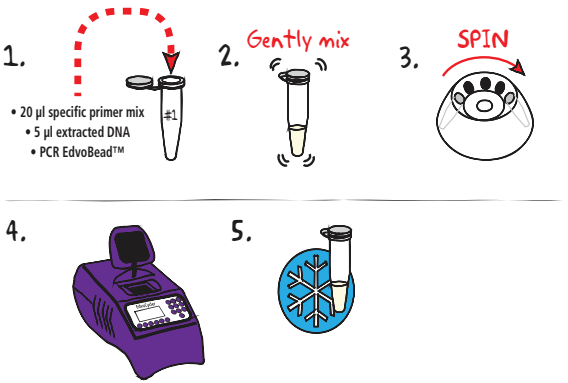
Cat. #202 $95



Small-scale rapid isolation of plasmid DNA is a routine procedure used for screening and analysis of recombinant DNAs in cloning and subcloning experiments. In this experiment, students isolate plasmid DNA without the use of toxic chemicals such as phenol or chloroform.

Module II: PCR

Excerpts from Kit 330



1. **ADD** 20 µL specific primer mix, 5 µl extracted DNA (or control DNA) and one PCR EdvoBead™ to a labeled 0.2 ml or 0.5 ml PCR tube (depending on the Thermal Cycler).
2. **MIX** the PCR sample. Make sure the PCR Edvo-Bead™ is completely dissolved.
3. **CENTRIFUGE** to collect the sample at the bottom of the tube.
4. **AMPLIFY** DNA using PCR:

   PCR cycling conditions:
   Initial denaturation 94°C for 5 minutes
   94°C for 30 seconds
   61°C for 30 seconds } 32 cycles
   72°C for 45 seconds
   Final Extension 72°C for 4 minutes

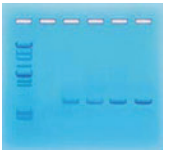
5. **ADD** 5 µL 10x gel loading solution to each tube. **PROCEED** to Module III: Electrophoresis of PCR product.

OPTIONAL STOPPING POINT

The PCR samples may be stored at -20°C for electrophoresis at a later time.

PCR Amplification of DNA

Cat. #330 $145



In this easy PCR experiment, students will make billions of copies of a small amount of DNA in just 90 minutes! They will just need to mix template DNA & primers with PCR beads that contain all of the other components required to carry out a PCR reaction. Students will see the increasing amounts of DNA for themselves, taking samples every few cycles and analyzing them on a DNA gel.

EdvoCycler™ & MegaCycler™

The Edvoycler™ and MegaCycler™ are stand alone classroom PCR machines that are easy to use! Both come pre-programmed with all EDVOTEK PCR protocols. These programs may be modified or deleted, plus there is extra memory slots for more!

EdvoCycler™
Holds 25 x 0.2 ml sample tubes.
Cat. #541 $1,799

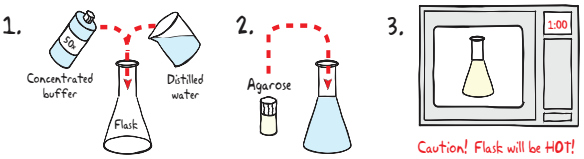
MegaCycler™
Holds 49 x 0.2 ml sample tubes.
Cat. #542 $2,499

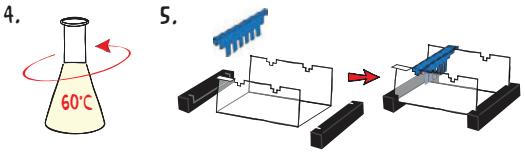
NIH Research supported in part by NIH SBIR NCRR Grant #R44RR18670

Module III: Analyzing PCR Products using Agarose Gel Electrophoresis

Excerpts from Kit 103

Wear gloves and safety goggles





NOTES:

7 x 14 cm gels are recommended. Each gel can be shared by 4 students. Place well-former template (comb) in the first set of notches.

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

Table A — Individual 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

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.

Module III: Analyzing PCR Products using Agarose Gel Electrophoresis

Excerpts from Kit 103

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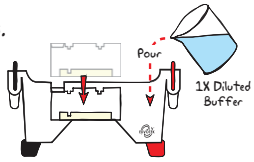
8.



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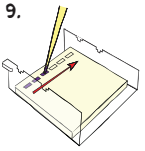

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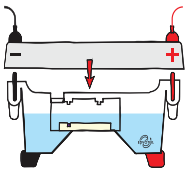

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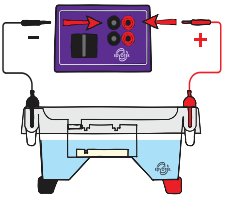


Reminder:

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

Wear gloves and safety goggles

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. **LOAD** the entire sample (30 µL) into the well.
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 proceed to **STAINING** the agarose gel.

Table 1

Lane	Recommended
1	Standard DNA Marker
2	0 Cycles
3	10 Cycles
4	30 Cycles
5	50 Cycles
6	70 Cycles

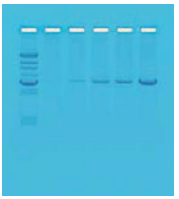


Table B 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	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 & M36
Volts	Min. / Max.	Min. / Max.
150	10 / 15 min.	20 / 30 min.
125	15 / 20 min.	35 / 45 min.
70	35 / 45 min.	60 / 90 min.
50	50 / 80 min.	95 / 130 min.

Module IV: Staining Agarose Gels

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. Edvotek® offers several different methods for visualizing the DNA separated by electrophoresis.

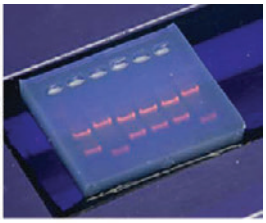
Fluorescent DNA Stains:

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. We offer two fluorescent DNA stains: InstaStain® Ethidium Bromide and SYBR® Safe DNA Stain.

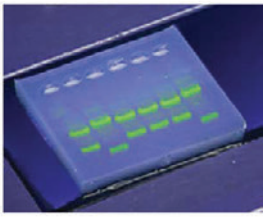
Visible Dye-based DNA Stains:

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. We offer two visible dye-based DNA Stains: InstaStain® Blue and Flash Blue Stain.

InstaStain® Ethidium Bromide



SYBR® Safe DNA Stain



InstaStain® Blue and FlashBlue™

Which DNA Stain Should I Use?

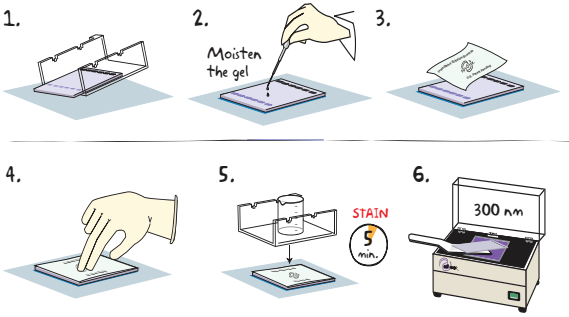
Stain	Advantages	Disadvantages
InstaStain® EtBr	Very sensitive Very fast	Requires UV transilluminator Potentially mutagenic
SYBR® Safe	Very sensitive Non-mutagenic	Requires UV transilluminator More expensive
InstaStain® Blue	Easy to use Generates minimal waste	Less sensitive More time
FlashBlue™	Simple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

Midrange UV Transilluminator

Cat. #558 $499

EDVOTEK®'s Midrange UV Transilluminator is designed to visualize DNA stained with either ethidium bromide or SYBR® Safe. The UV filter measures 7 x 14 cm which is optimized for viewing gels cast from EDVOTEK® electrophoresis chambers. Safety features include a UV blocking cover and an automatic power-cut off when the cover is opened.

InstaStain® Ethidium Bromide

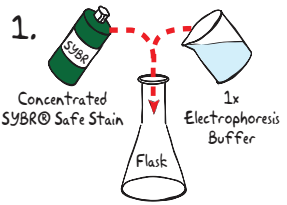


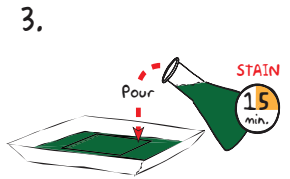
1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface. **DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.**
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **REMOVE** and **DISCARD** the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). **PLACE** the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. Each InstaStain® Ethidium Bromide card will stain 49 cm^2 of gel (7 x 7 cm).
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. **STAIN** the gel for 3-5 min. for an 0.8% gel or 8-10 min. for a gel 1.0% or greater.
6. **REMOVE** the InstaStain® Ethidium Bromide card(s). **VISUALIZE** the gel using a long wavelength ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

SYBR® Safe Stain

1. **DILUTE** SYBR® Safe 1: 10,000 by adding 7.5 microliters 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. **COVER** the gel with the 1x SYBR® Safe stain solution. **COVER** the tray with foil to protect the gel from light. **STAIN** the gel for 10-15 minutes. For best results, use an orbital shaker to gently agitate the gel while staining.
4. **REMOVE** the gel from the staining solution. **VISUALIZE** results using a U.V. transilluminator. DNA bands will appear bright green.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!





InstaStain® Blue

1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing at least 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.

   Note: Appropriate staining trays include large weigh boats and small, plastic food containers.

2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing the gel. Each InstaStain® Blue card will stain 49 cm^2 of gel (7 x 7 cm). **REMOVE** the InstaStain® card(s) after 30 seconds.

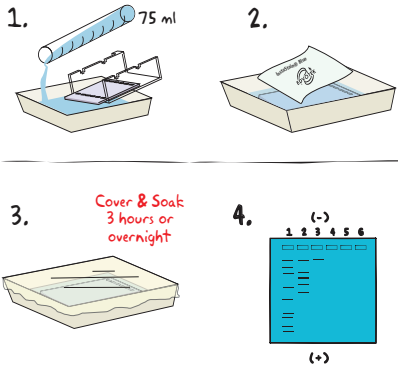
3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.

4. Carefully **REMOVE** the gel from the staining tray and **DOCUMENT** results.

InstaStain® Blue

For 40 gels, 7 x 7 cm
Cat. # 2003 $49

For 100 gels, 7 x 7 cm
Cat. # 2004 $89



FlashBlue™ Stain

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 mL of water in a flask and **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.

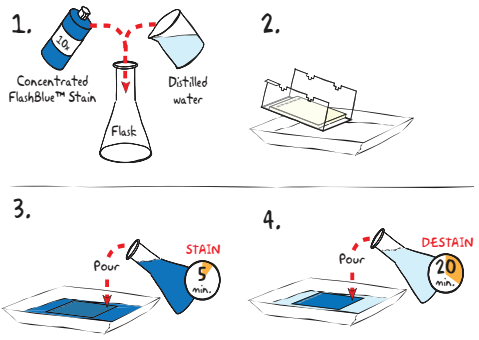
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**

4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.

5. **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.

FlashBlue™ DNA Staining

10x Concentrate for 1.2 L
Cat. # 609 $35



Alternate Protocol:

1. **DILUTE** one mL of concentrated FlashBlue™ stain with 149 mL dH_2O.
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.