What's in my Lunch?

Testing Food for GMOs

Danielle R. Snowflack, Ph.D.
Edvotek ®
Washington, D.C.

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Genetic Modifications through the Ages

- Mutations (variations in the DNA sequence) can change the way an organism interacts with its environment.
- Some mutations grant an organism an advantage that promotes survival.
- Humans have long taken advantage of genetic variation using traditional plant and animal husbandry techniques.

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What is a Genetically Modified Organism (GMO)?

- While it used to take years of selective breeding to produce the genomic changes necessary to give rise to such desirable traits, the advent of biotechnology has accelerated this pace.

- **Genetic engineering** allows scientists to directly manipulate a DNA sequence in order to generate desirable traits.

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GM Ingredients are Present in Many Different Foods

In order to determine whether students are consuming GM foods, the FDA commissioned a study to analyze common snacks.

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Identification of GMOs Using Polymerase Chain Reaction

Step 1: DNA is extracted from food samples

Step 2: Extracted DNA is amplified using PCR

Step 3: PCR is analyzed using electrophoresis

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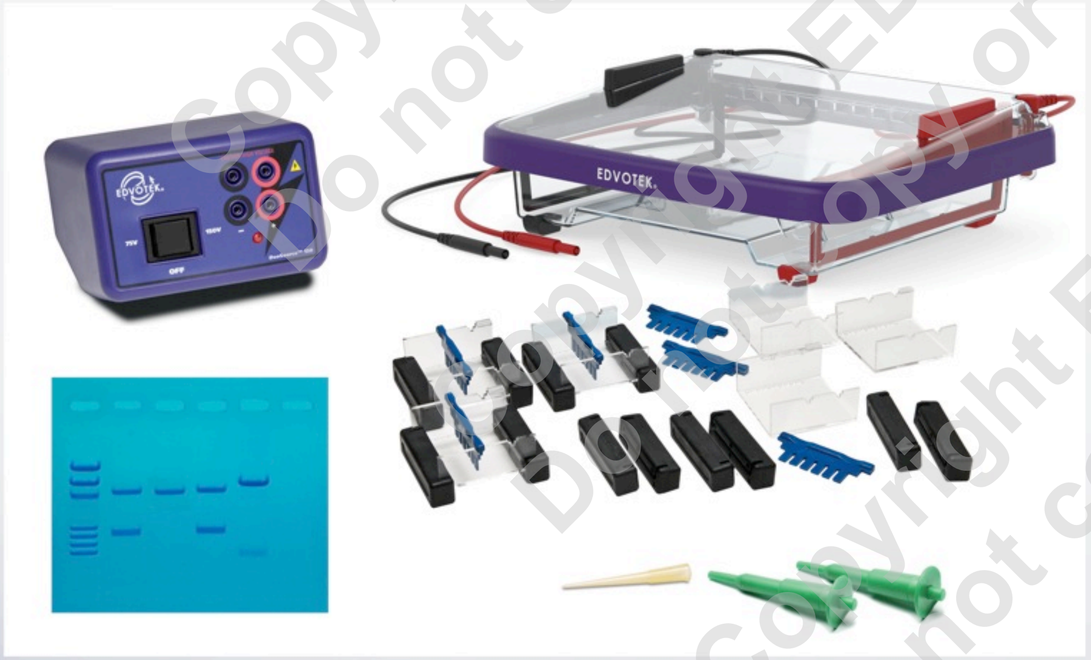
What Do I Need to Perform Electrophoresis Experiments?



LabStation™ #5062

- Horizontal electrophoresis apparatus
- D.C. power source
- Micropipet or transfer pipet
- Agarose
- Electrophoresis Buffer
- Samples – dye, DNA, RNA
  - PCR products
- A way to visualize samples

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Let's run our gels!

Load 30 microliters of sample per well.

Lane	Sample
1	DNA Molecular Marker
2	GMO Positive Control
3	GMO Negative Control
4	Kansas High School
5	Florida High School
6	California High School

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Electrophoresis Chambers for Classrooms of all Sizes

Cat. # 502
Model M12
Two gels

Cat. # 515
Model M36
Six gels

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Power Supplies Provide Current for Electrophoresis

Cat. #509
DuoSource™ 150
(75/150 V)

Cat. #5010
TetraSource™ 300
(30-300V)

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How Do Scientists Create GM Organisms?

- Recombinant DNA technology allows us to combine DNA from different sources.
  - Identify a gene of interest and amplify it using PCR
  - Move it into a transformation vector
  - Integrate the transgene into the target genome.
  - Gene is present in subsequent generations.

GloFish – the first GM pet!

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Future Directions for GM Technology

- "Golden rice", named for its yellow color, produces β-carotene, a precursor of vitamin A.
  - Vitamin A deficiency is a widespread problem in developing countries, so nutrient-supplemented crops represents major progress in combating malnutrition.
- GMOs for the synthesis/delivery of pharmaceuticals.
  - In "pharming", GM plants make proteins with medicinal value like insulin or growth hormone.

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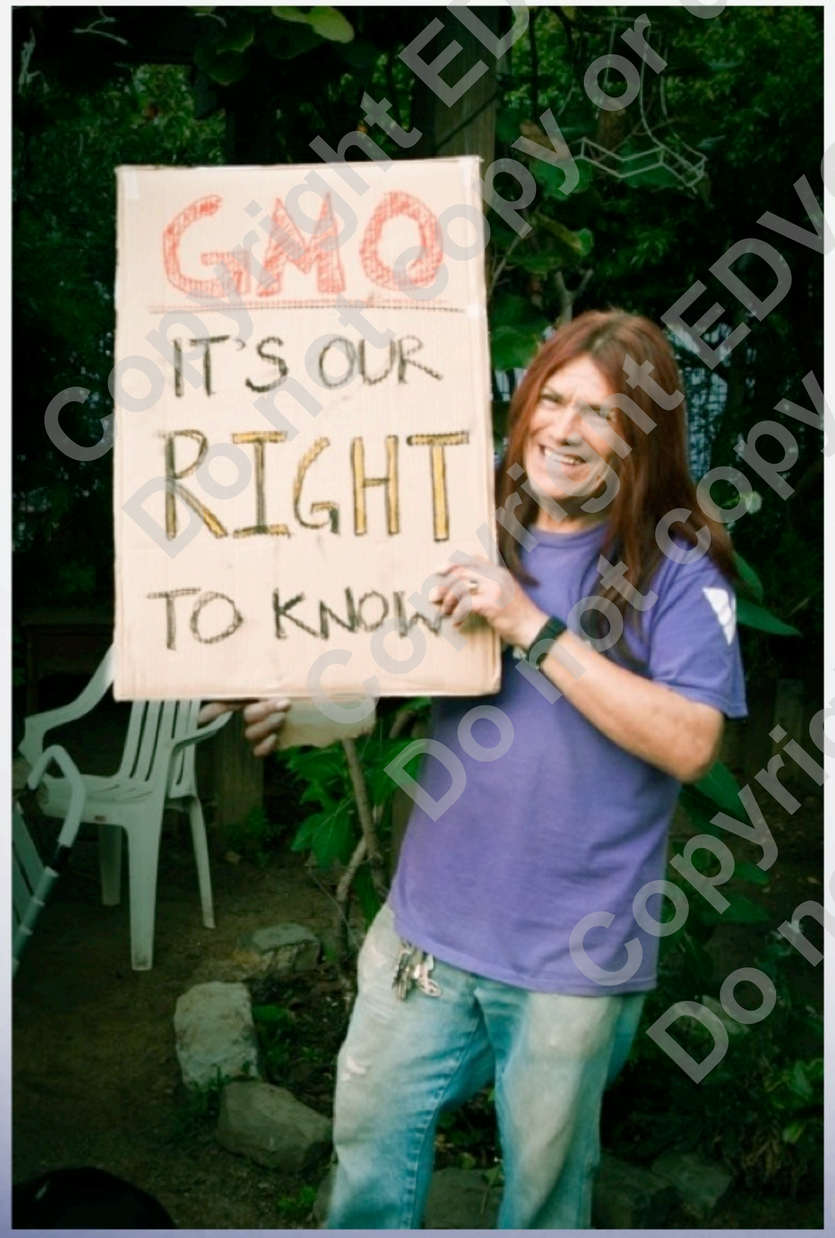
The Pros and Cons of GM Technology



- **Benefits**
  - Improved quality and quantity of crops
  - Decreased cost to farmers
  - Less environmental impact
  - Increased nutritional values
- **Concerns**
  - Food safety
  - Large corporations controlling small farmers
  - Horizontal gene transfer
  - Unanticipated risks to health and environment

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Extraction of DNA from Food Samples

- Chip samples are homogenized in a solution that releases the DNA from the cell by breaking down cellular and nuclear membranes in a process called **lysis**.
  - a buffer stabilizes the sample's pH (Tris-HCl)
  - a chelating agent removes ions (inactivates DNAses)
  - a detergent dissolves lipids (SDS)
  - an enzyme breaks down proteins (Proteinase K).
- Next, ice-cold alcohol (ethanol or isopropanol) is used to precipitate DNA from cellular lysate as sticky white fibers.
- Salt helps the DNA molecules precipitate.
- Centrifugation moves the DNA to the bottom of the tube, creating a pellet.

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Extraction of DNA from Food Samples

THE LYSIS HAS ALREADY BEEN PERFORMED

1. **Add an equal volume of 100% isopropanol to the supernatant to precipitate DNA.**
2. **Incubate sample on ice for five minutes.**
3. **Spin the tube at maximum speed for five minutes**
4. **Remove and discard the supernatant from the pellet. (Do not disturb the pellet!)**
5. **Allow the pellet to dry completely (~10 minutes).**
6. **Dissolve the DNA pellet in 300 µl of TE buffer.**

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DNA Amplification using PCR

- DNA template
- Primers
  - Short piece of DNA that defines the area to amplify
- Thermostable DNA polymerase (*Taq*)
- Free nucleotides (dNTPs)
- Buffer

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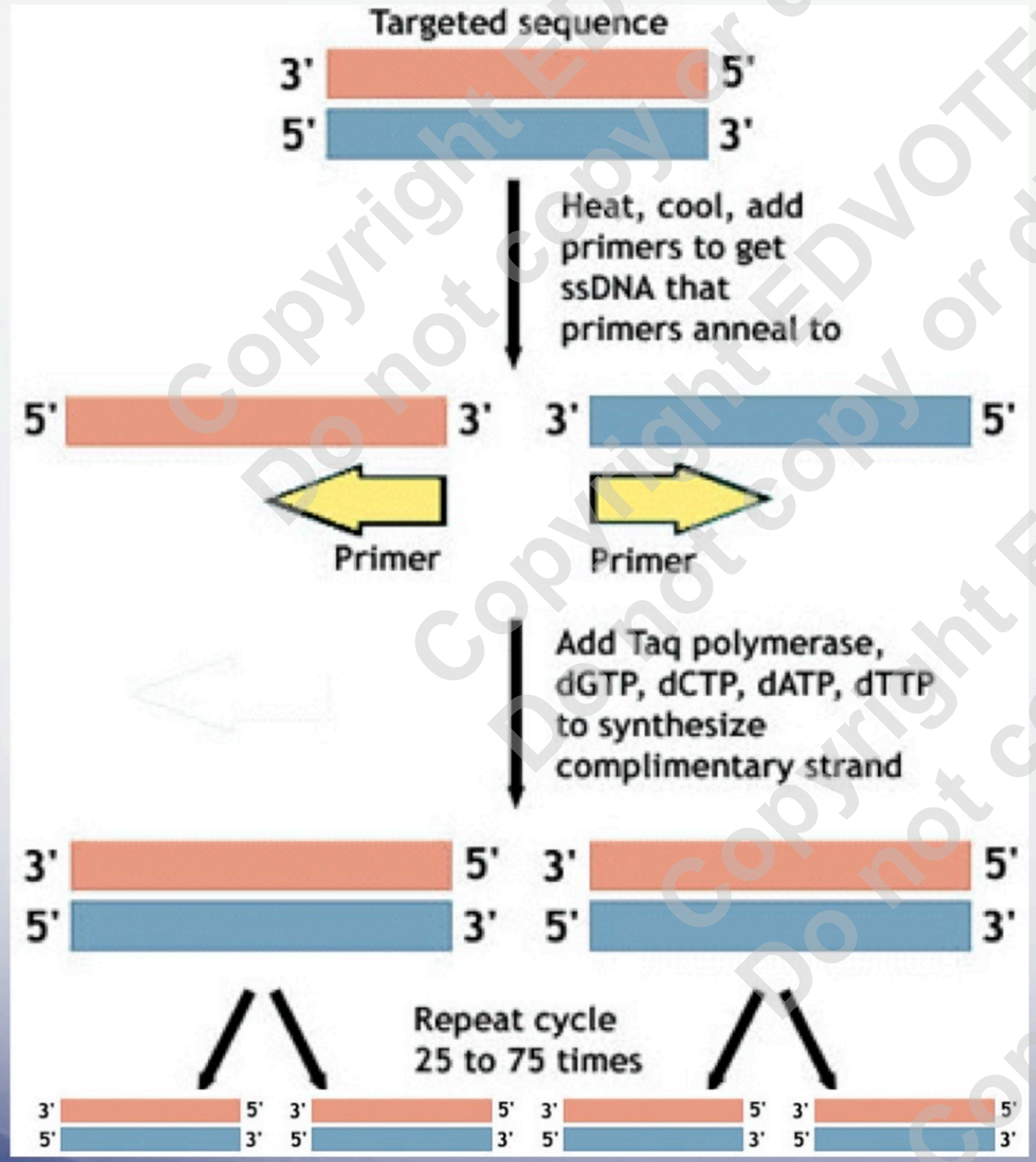
PCR Amplifies Specific DNA Sequences



- **Denaturation (94°C)**
  Double-stranded DNA is "unzipped" into single strands.
- **Annealing (40-65°C)**
  Primers base pair with the target DNA sequence.
- **Extension (72°C)**
  Taq polymerase extends the primer and synthesizes a new strand of DNA.

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DNA Amplification using PCR

1. To prepare the PCR reaction mix, add the following to the pellet:

Food DNA Template:	5 µl
Primer Mix:	20 µl

2. Gently mix the reaction tube. Make sure the PCR reaction pellet™ is completely dissolved.

3. Place the tubes in the Edvocycler.

4. After PCR, add five µl of 10X Gel Loading Dye to each tube.

5. Proceed to electrophoresis of amplified DNA.

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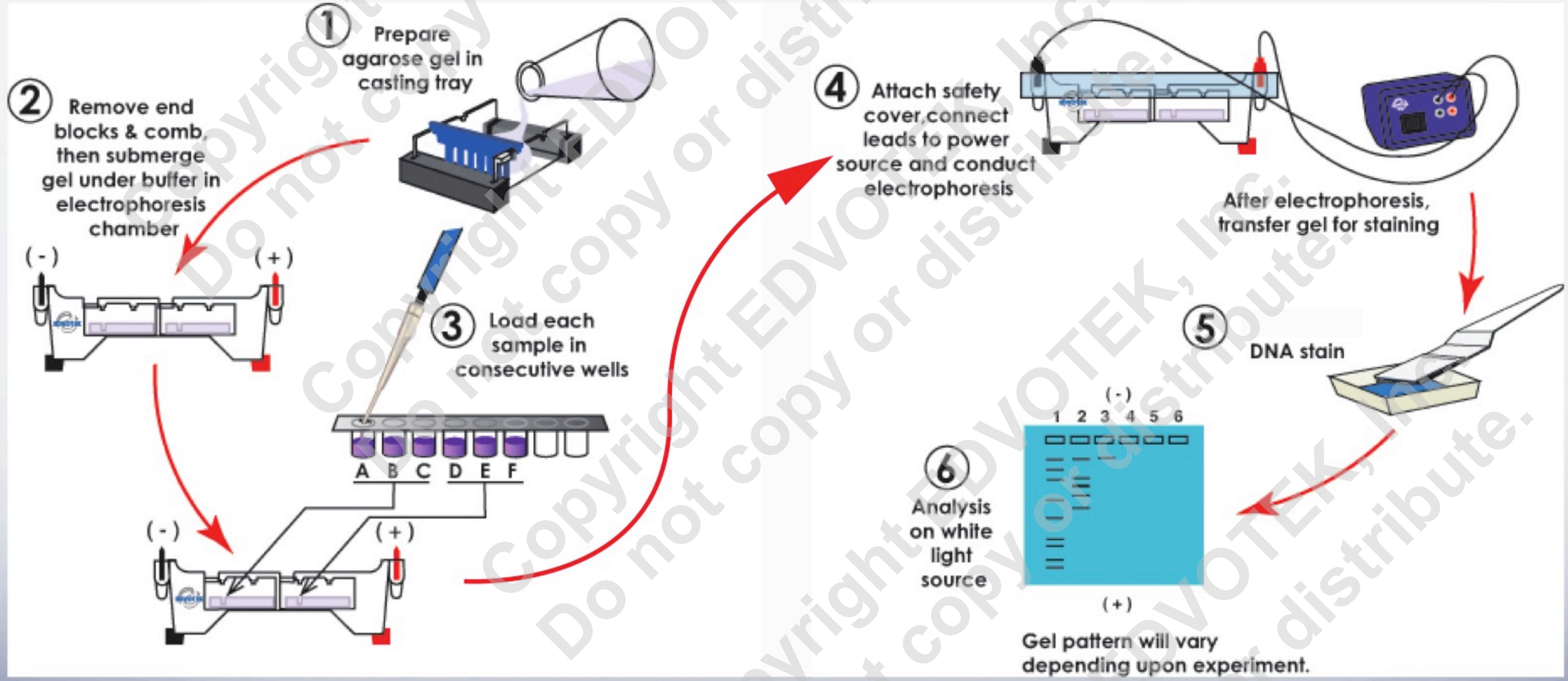
Electrophoresis at a Glance



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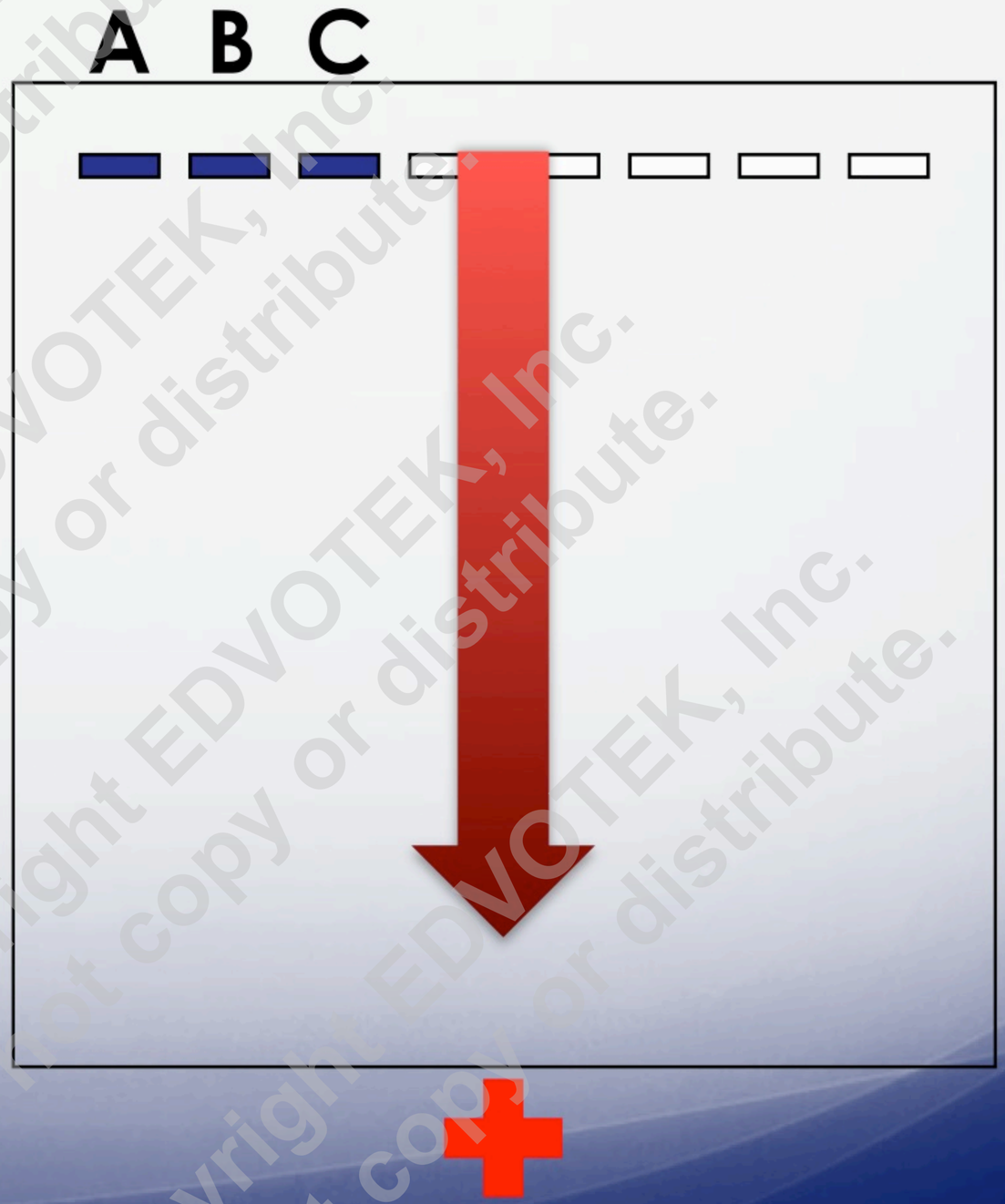
Electrophoresis Separates DNA Fragments By Size

- The sugar-phosphate backbone of DNA has a strong negative charge.
- When an electrical current is passed through the gel, the current drives the DNA fragments through the gel towards the positive electrode.



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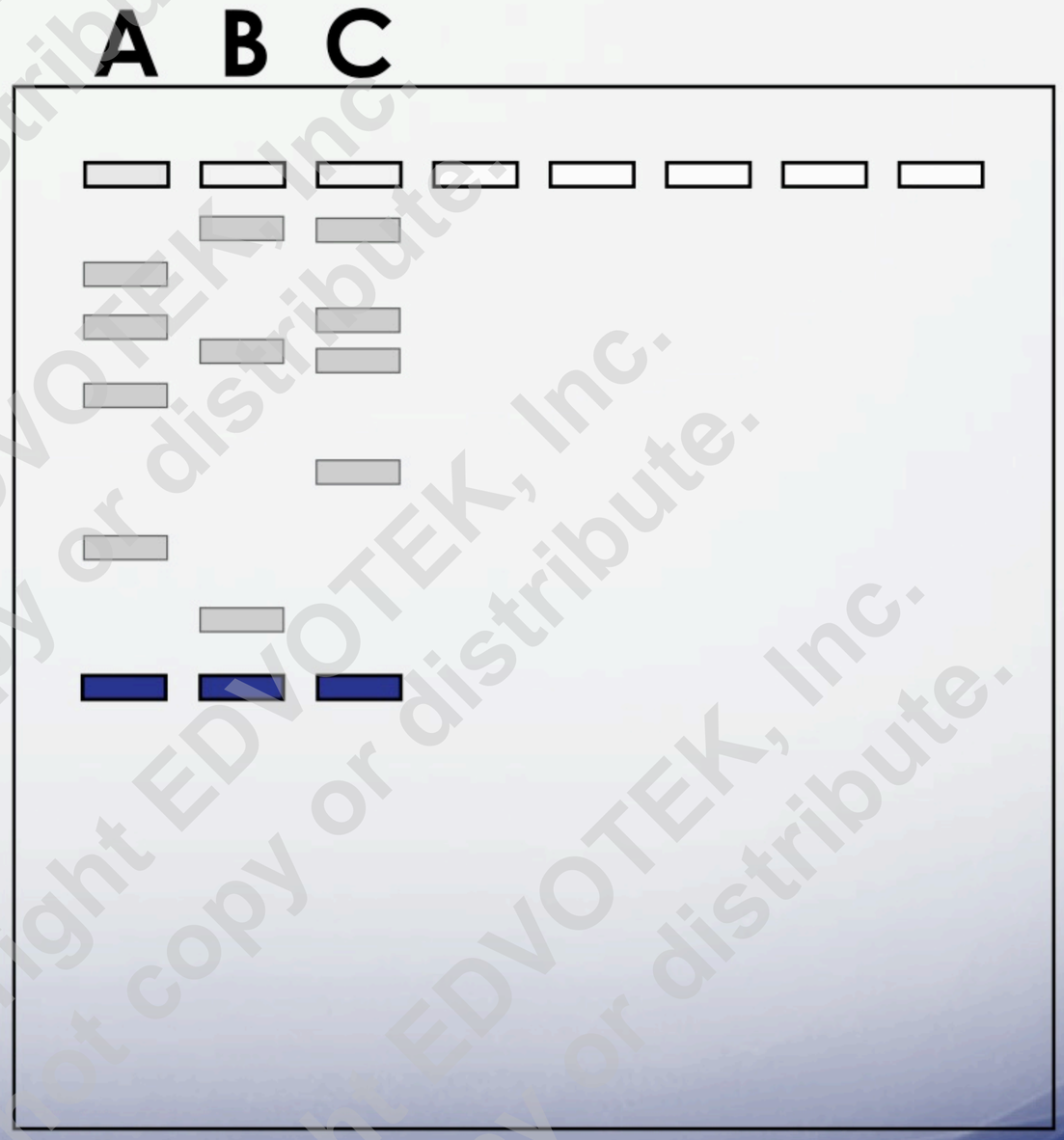
Electrophoresis Separates DNA Fragments By Size

- 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.



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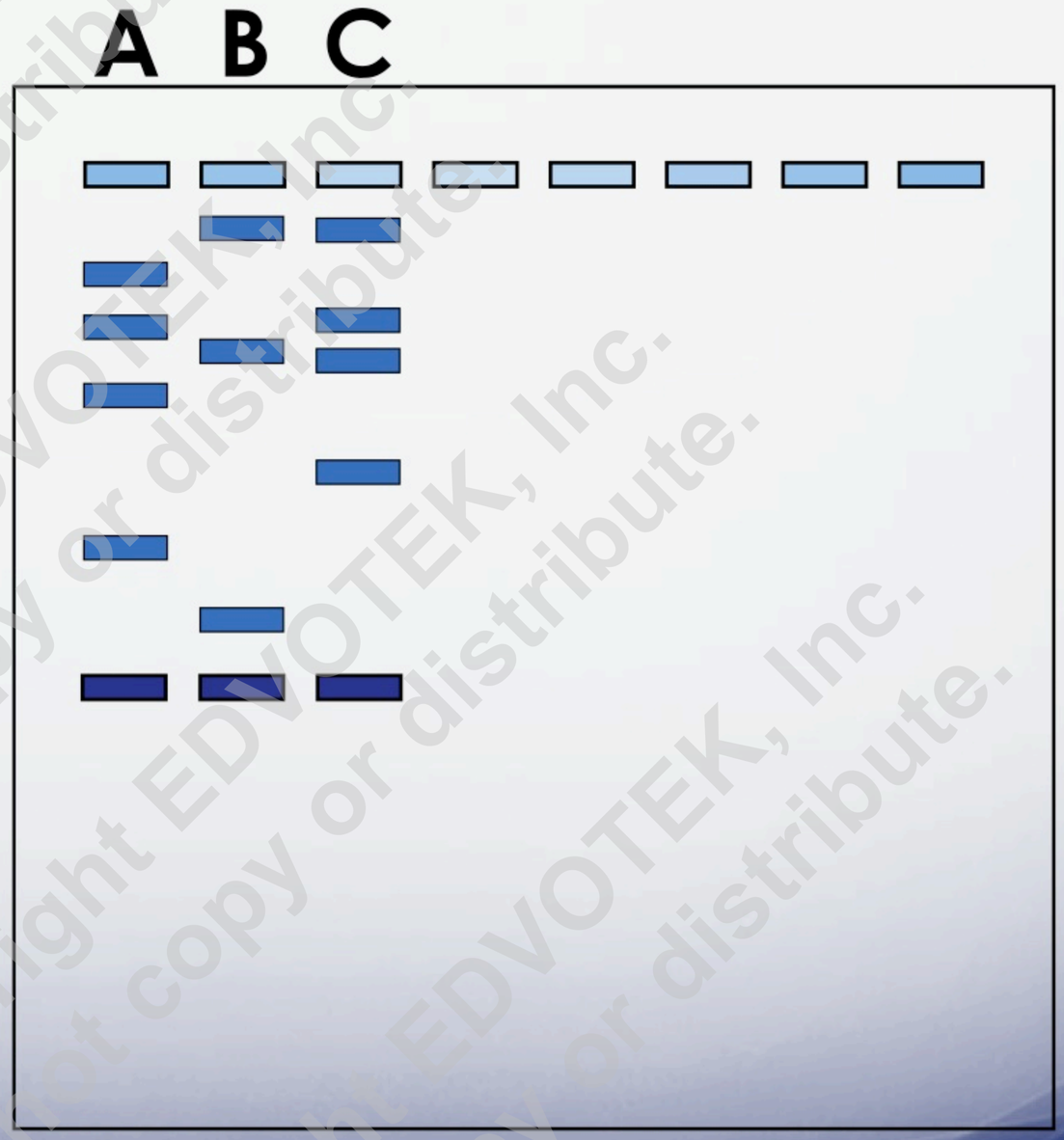
Electrophoresis Separates DNA Fragments By Size

- Because molecules of different sizes travel at different speeds, discrete bands are formed.
- After the current is stopped, the bands can be visualized using a stain that sticks to DNA.
  - InstaStain ® EtBr
  - InstaStain ® Blue
  - FlashBlue™ Stain
  - Sybr® Safe stain



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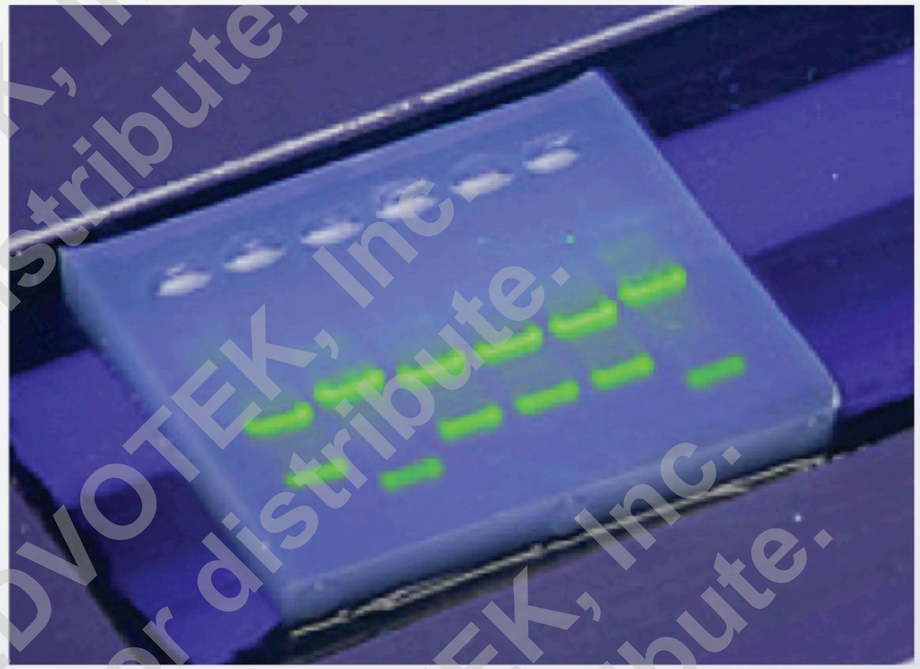
SYBR® Safe DNA Stain

In-gel Staining

- Melt agarose and cool to 65°C.
- Add concentrated Sybr® Safe stain to the molten gel at 1:10,000 dilution (5 µL per 50 mL agarose solution).
- Run DNA samples through gel – no post staining or destaining necessary!

Post-electrophoresis Staining

- Dilute concentrated stain to 1:20,000 (5 µL per 100 mL distilled water).
- After electrophoresis, place gel in tray. Cover gel with diluted Sybr® Safe stain.
- Stain gel for 10 – 15 minutes.



Kit #109
Transilluminator #558
SybrSafe® Stain #608

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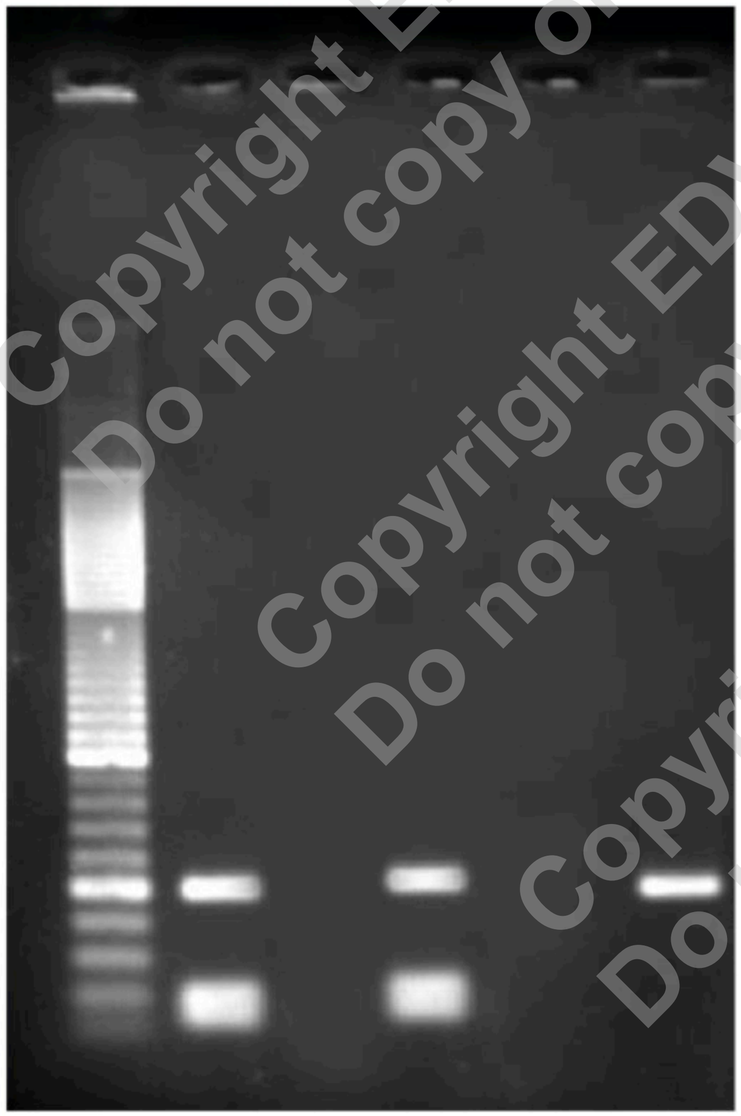
Identification of GM Foods using PCR



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Identification of GM Foods using PCR

- **Genetic engineering allows scientists to directly manipulate a DNA sequence in order to generate desirable traits within an organism.**
- **PCR and agarose gel electrophoresis can be used to identify genetic modifications in an organism.**
- **Like any new technology, GM crops are studied for effects on human health or the environment.**

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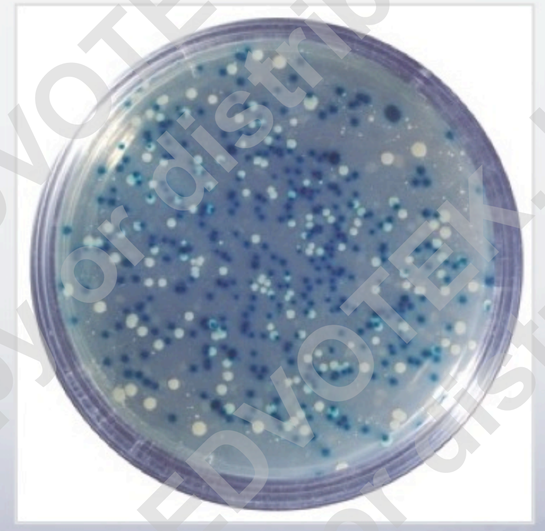
From Peas to PCR:
Genetic Engineering with Edvotek

Bioremediation (#956)

QuickPlant™ Genetics (#336)

Identification of GMOs using PCR (#962)



Gene Cloning and Transformation (#300)

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