

# **GEL ELECTROPHORESIS**

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# Gel electrophoresis

It is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge.

## **It is used in**

1. clinical chemistry to separate proteins by charge and/or size.
2. Biochemistry and Molecular biology to separate DNA and RNA fragments by length, or to separate proteins by charge.

# Gel electrophoresis

## **This phenomenon is called Sieving**

1. Nucleic acid and Proteins' molecules are separated by applying an electric field to move the negatively charged molecules through **a matrix** of agarose or other substances.

*Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel.*

**Electrophoresis** is a technique used for sorting of macromolecules molecules based on size and charge.

- The gel is placed in an electrophoresis chamber, which is then connected to a power source.
- The electric field consists of a negative charge at one end which pushes the molecules through the gel and a positive charge at the other end that pulls the molecules through the gel.

- Positively charged (cations) will migrate towards the (cathode) which is negatively charged.
- Negatively charged (anions) they will migrate towards the positively charged anode.
- The larger molecules move more slowly through the gel while the smaller molecules move faster.
- The different sized molecules form distinct bands on the gel.
- If several samples have been loaded into adjacent wells in the gel, they will run parallel in individual lanes.

# Gel Types

**There are two types of gel most typically used are agarose and polyacrylamide gels.**

Each type of gel is well-suited to different types and sizes of analyte.

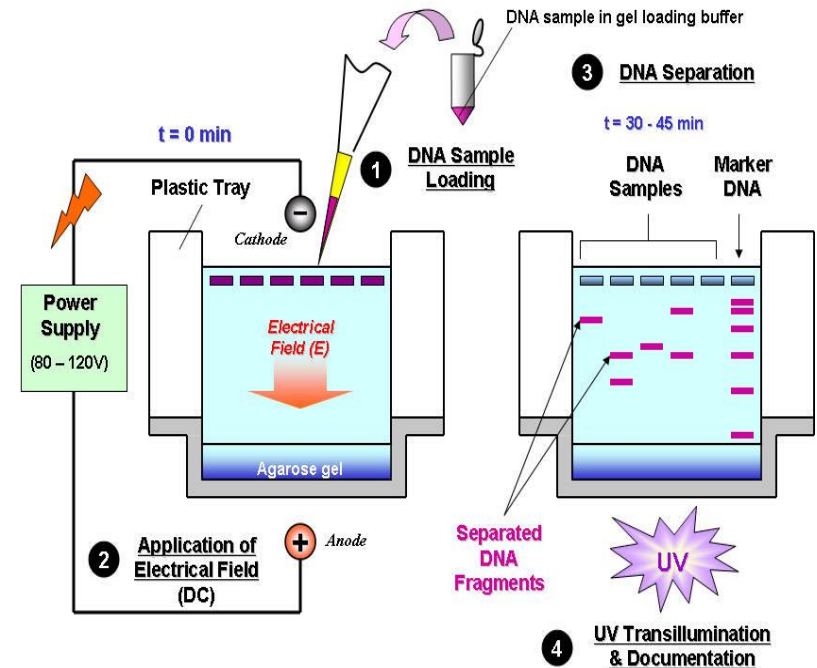
- 1. Agarose** is a polysaccharide extracted from seaweed.
- It is typically used at concentrations of 0.5 to 2%.

*The higher the agarose concentration the "stiffer" the gel.*

*Higher percentages requiring longer run times*

# Agarose

- Agarose gels have greater range of separation, and are therefore used for DNA fragments of usually 50-20,000 bp in size.
- Agarose gels are typically run horizontally in a submarine mode.

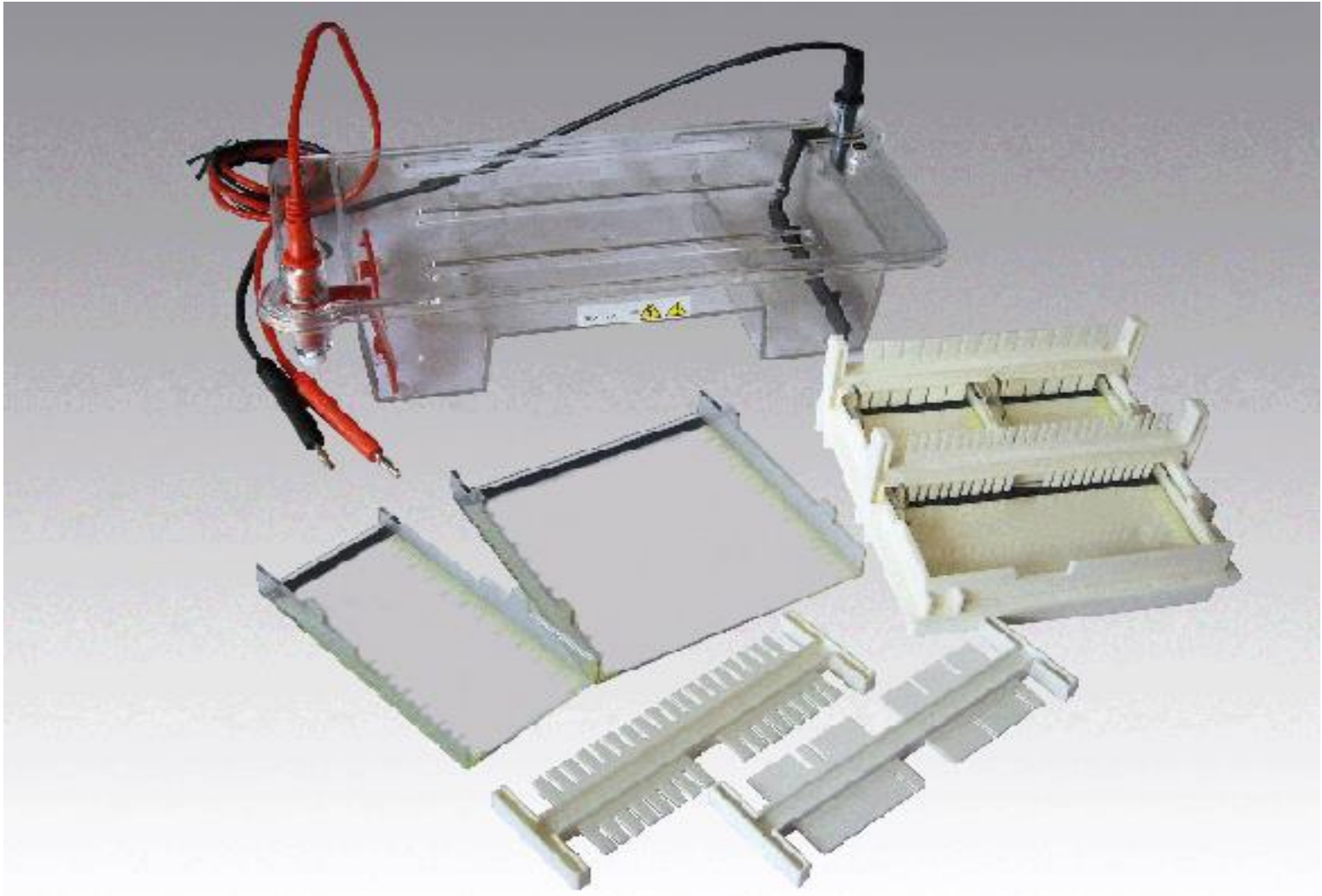


## **Preparation of agarose:**

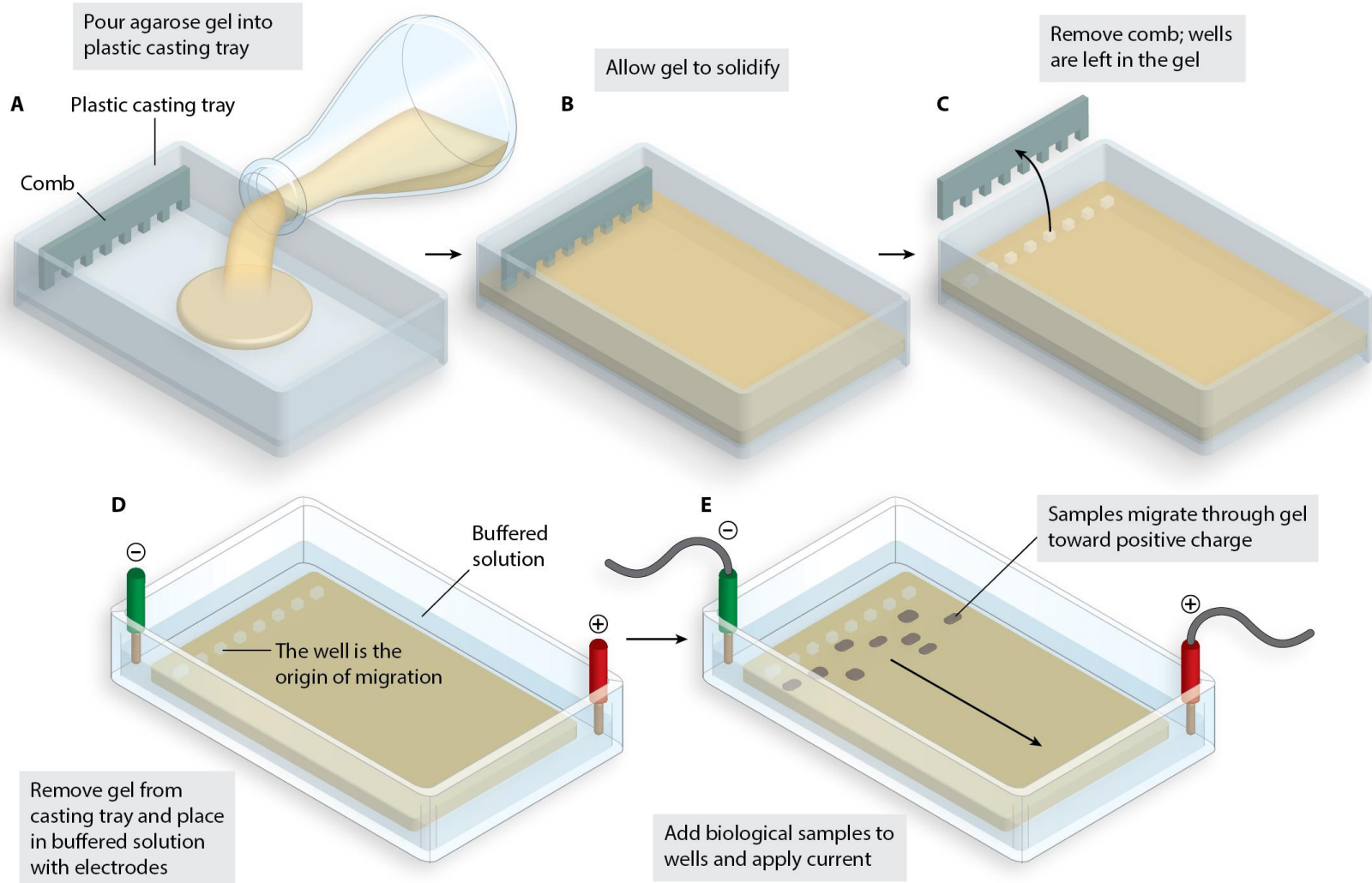
- Agarose sets thermally.
- Agarose gels are extremely easy to prepare you simply mix agarose powder with buffer solution, melt it by heating, and pour the gel.
- It is also non-toxic except after adding of ethidium bromide.



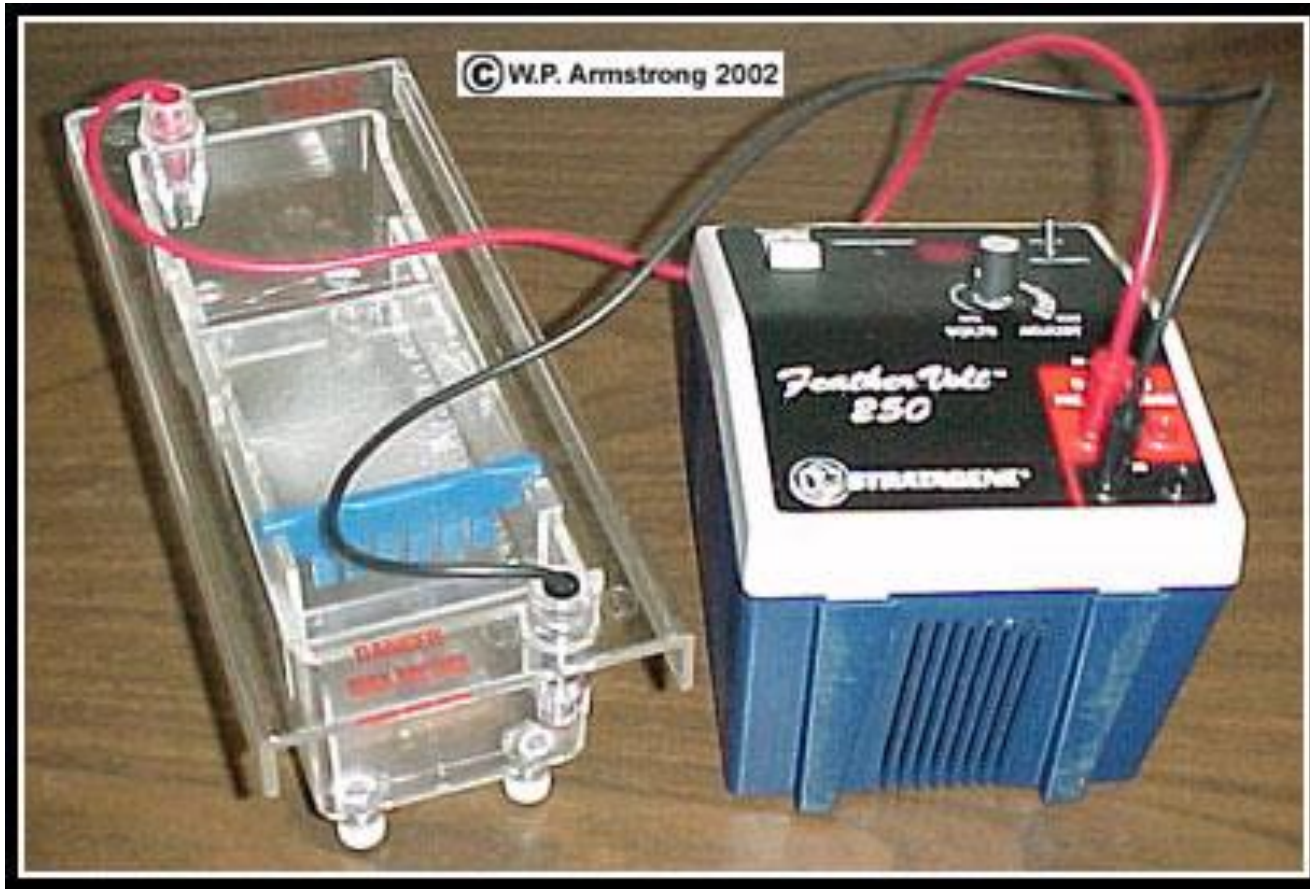
# Preparation of agarose:



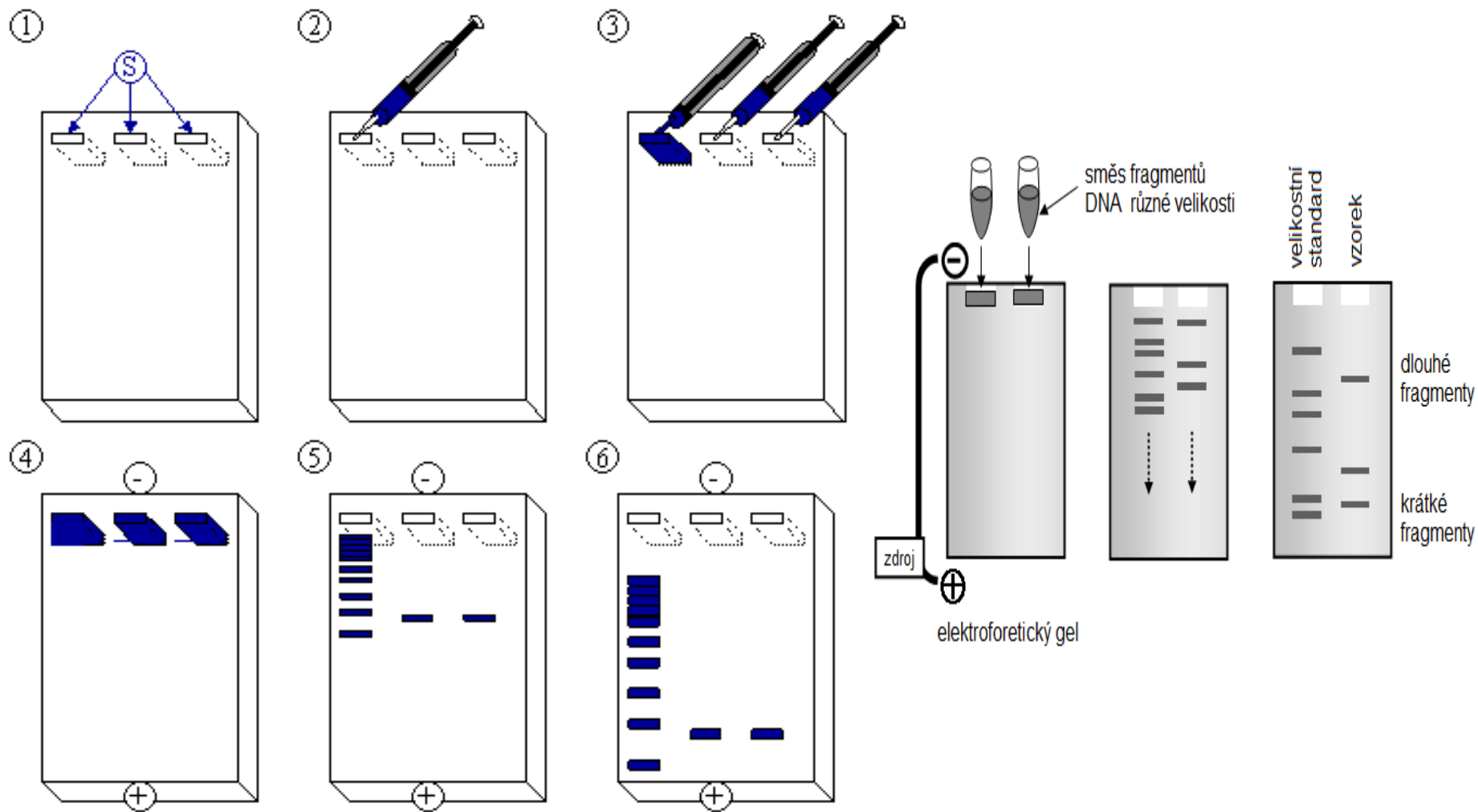
# Preparation of agarose:



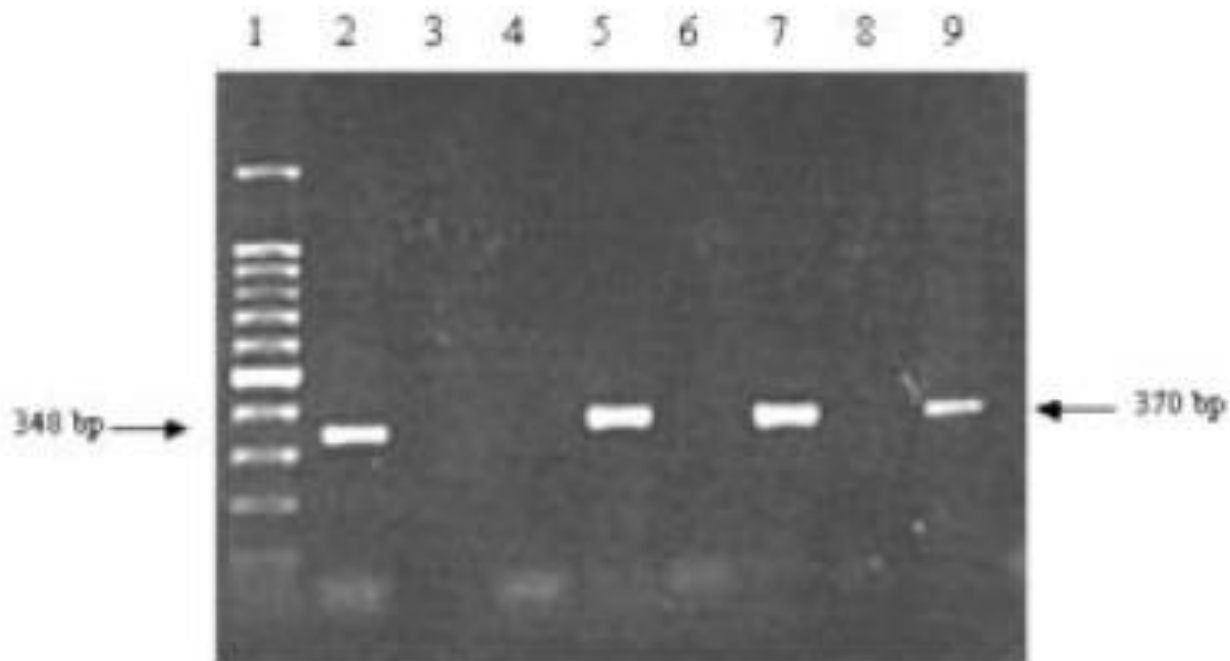
# Preparation of agarose:



# Agarose Electrophoresis



# Agarose





Hey You!

Is everything  
alright?

# Polyacrylamide (PAGE)

2. **Polyacrylamide** is a cross-linked polymer of acrylamide. The length of the polymer chains is dictated by the concentration of acrylamide used, which is typically between 3.5 and 20%.

- It is used for separating **proteins** ranging in size from 5 to 2,000 kDa due to the uniform pore size provided by the polyacrylamide gel.

*In contrast to agarose, polyacrylamide gels are used extensively for separating and characterizing mixtures of proteins.*

# Polyacrylamide (PAGE)

- Traditional DNA sequencing techniques used polyacrylamide gels to separate DNA fragments differing by a **single base-pair in length** so the sequence could be read.

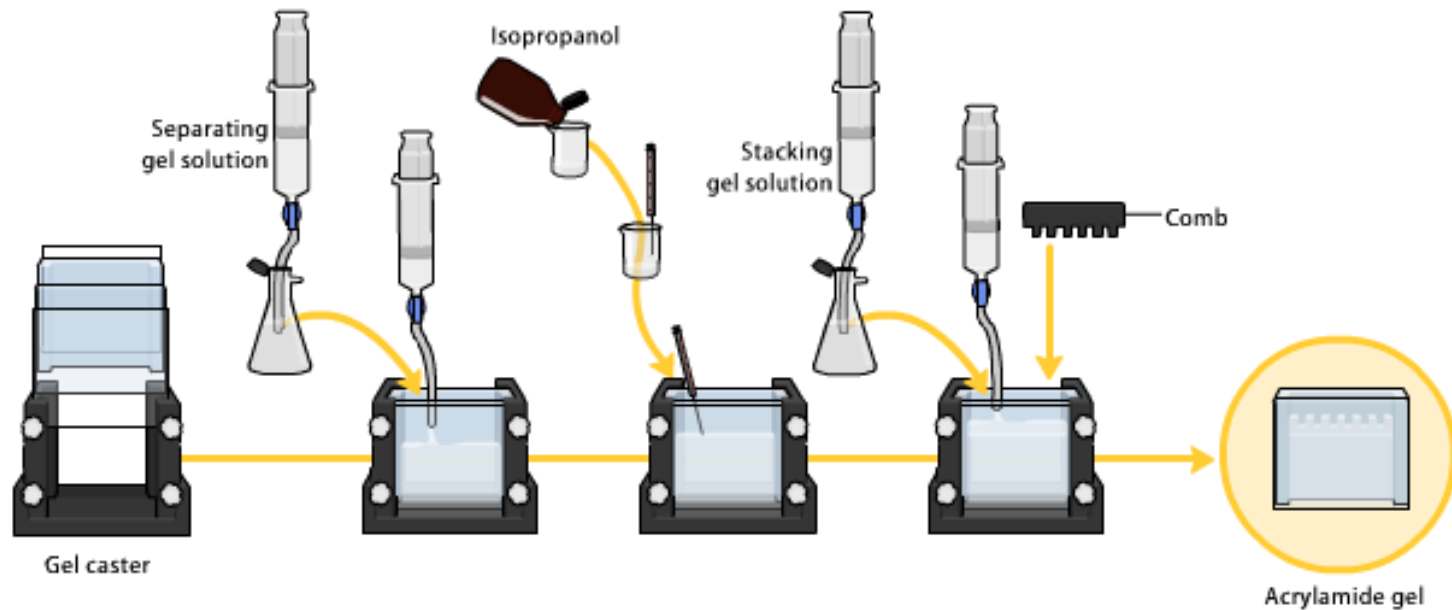
*However, under appropriate conditions, fragments of DNA differing in length by a single base pair are easily resolved*

- In the case of DNA, polyacrylamide is used for separating fragments of **less** than about 500 bp.



# Preparation of Polyacrylamide

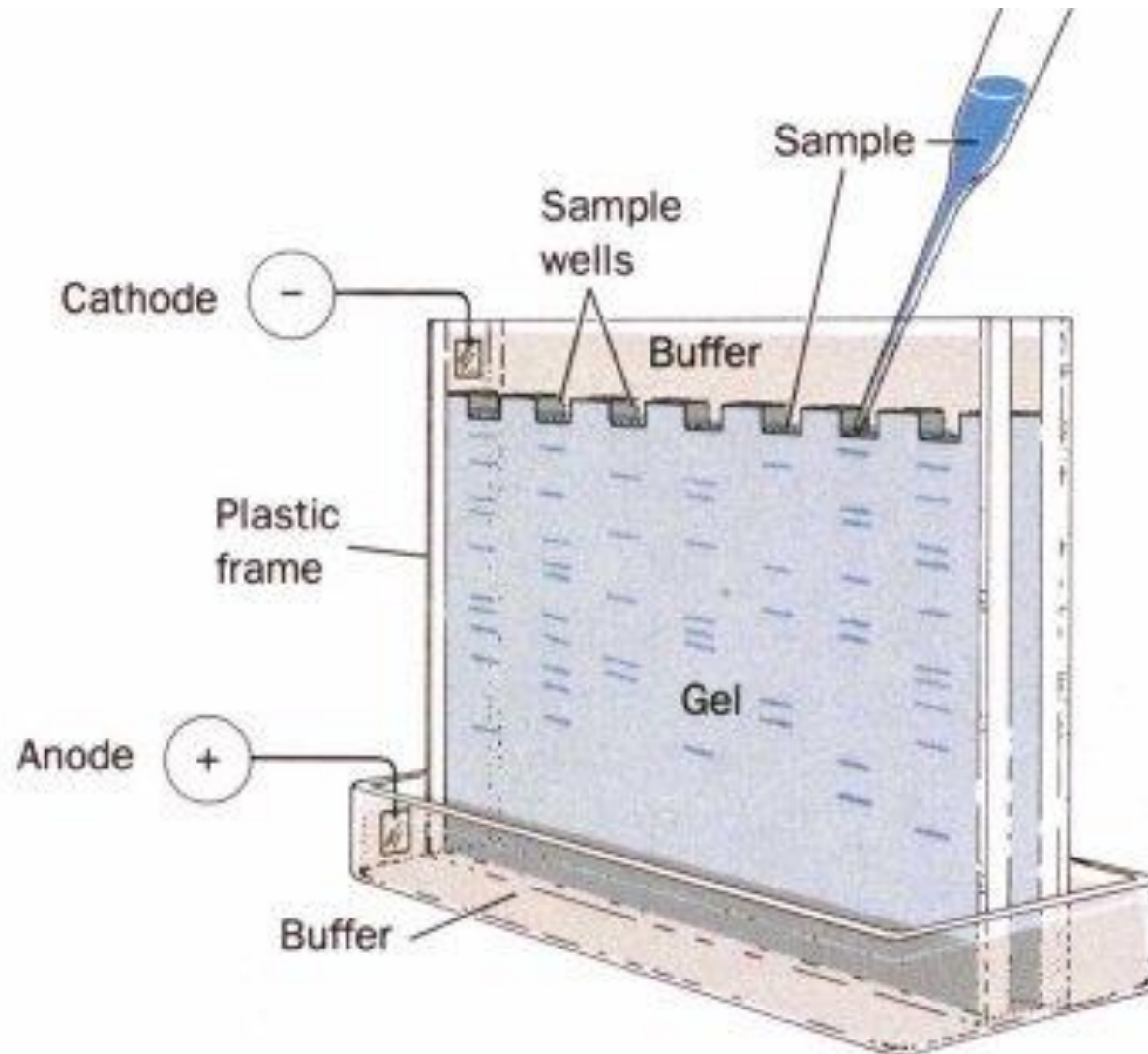
- Polyacrylamide gels are significantly more annoying to prepare than agarose gels.
- polyacrylamide forms in a chemical polymerization reaction.
- Oxygen inhibits the polymerization process, they must be poured between glass plates (or cylinders).



**For preparation of 100 ml on TBE buffer**

- 1. Acrylamide**
- 2. Bisacrylamide A**
- 3. Ammonium persulfate**
- 4. TEMED**
- 5. Glycerol**

# Polyacrylamide



# Polyacrylamide

- *Acrylamide is a potent neurotoxin and should be handled with care!*

Wear disposable gloves when handling solutions of acrylamide, and a mask when weighing out powder.

Polyacrylamide is considered to be non-toxic, but polyacrylamide gels should also be handled with gloves due to the possible presence of free acrylamide.

# Starch

- Partially hydrolysed potato starch makes for another non-toxic medium for protein electrophoresis.
- The gels are slightly more opaque than acrylamide or agarose.
- Non-denatured proteins can be separated according to charge and size.
- They are visualized using **Napthal Black** or **Amido Black** staining.
- Typical starch gel concentrations are 5% to 10%.

# Buffers

- Buffers in gel electrophoresis are used to provide ions that carry a current and to maintain the pH at a relatively constant value.
- There are a number of buffers used for electrophoresis.

The most common being, for nucleic acids

1. Tris/Acetate/EDTA (TAE).
2. Tris/Borate/EDTA(TBE).
3. Lithium borate (LB).

# Buffers

- **TAE is better than TBE**, it has the lowest buffering capacity but provides the best resolution for larger DNA.

This means a lower voltage and more time, but a better product.

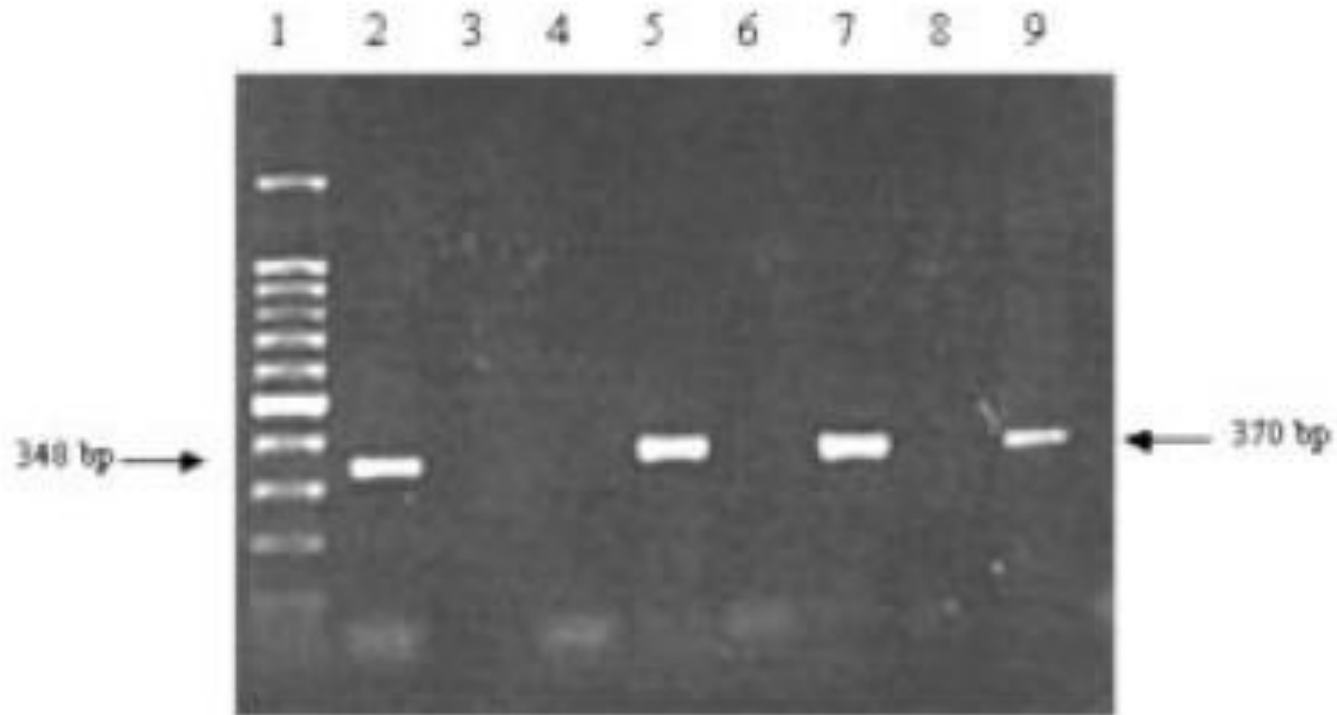
- **Lithium borate (LB)** is relatively new but it has low conductivity, so much higher voltage could be used, which means a shorter analysis time for routine electrophoresis.

# Bands

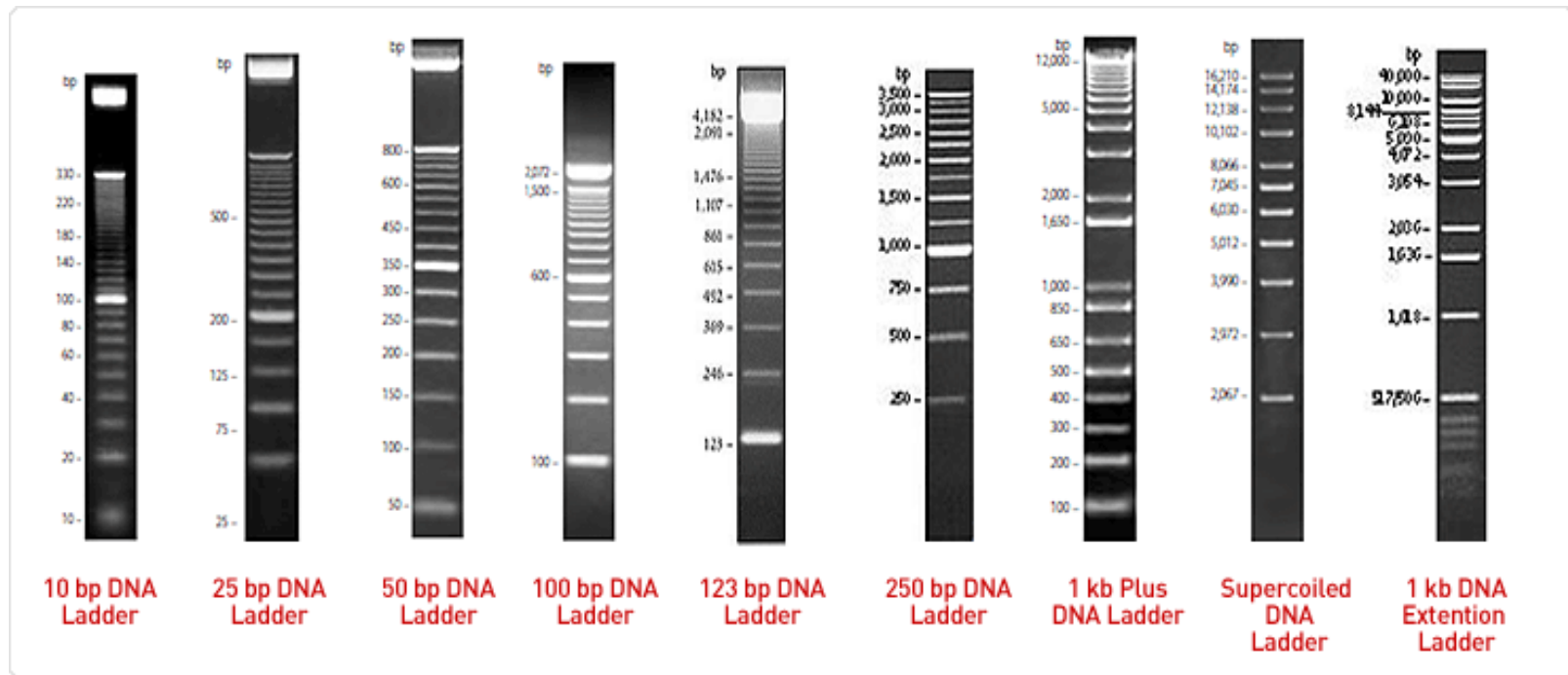
- **Bands** in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size.
  - *This size usually measured by Markers.*
- **Molecular weight size Marker/Ladder** it is a substance that contains a mixture of molecules of known sizes. It runs on one lane parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size.



# Marker and samples



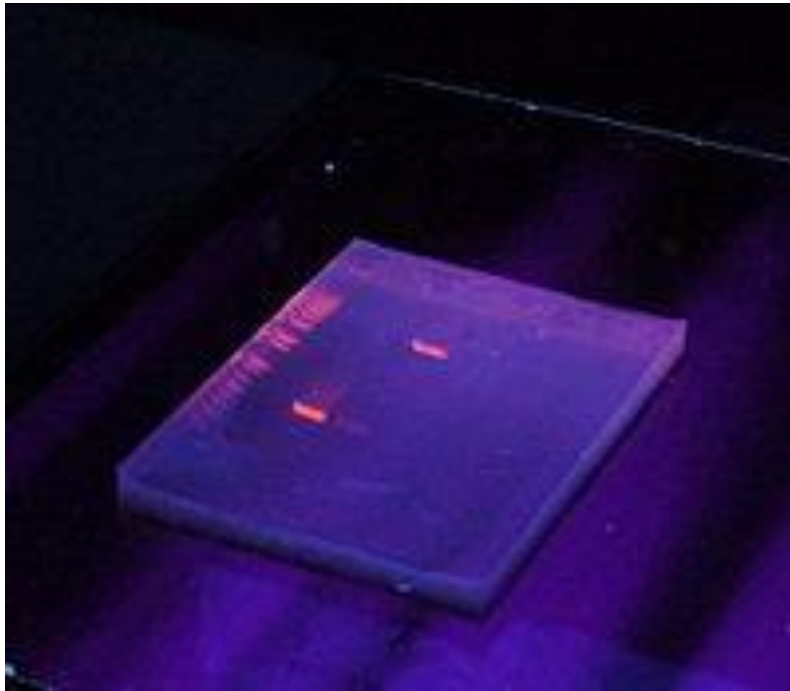
# Molecular-weight size Ladder



# Visualization

- After the electrophoresis is complete, the molecules in the gel can be stained to make them visible.
- DNA may be visualized using **Ethidium bromide** which, when intercalated into DNA → fluoresce under ultraviolet light.
- Protein may be visualised using **Silver stain** or **Coomassie Brilliant Blue dye**.
- If the molecules to be separated contain radioactivity, for example in a DNA sequencing gel, an autoradiogram can be recorded of the gel.

# Visualization



Visualization by using **Ethidium bromide**

## **Ethidium bromide:**

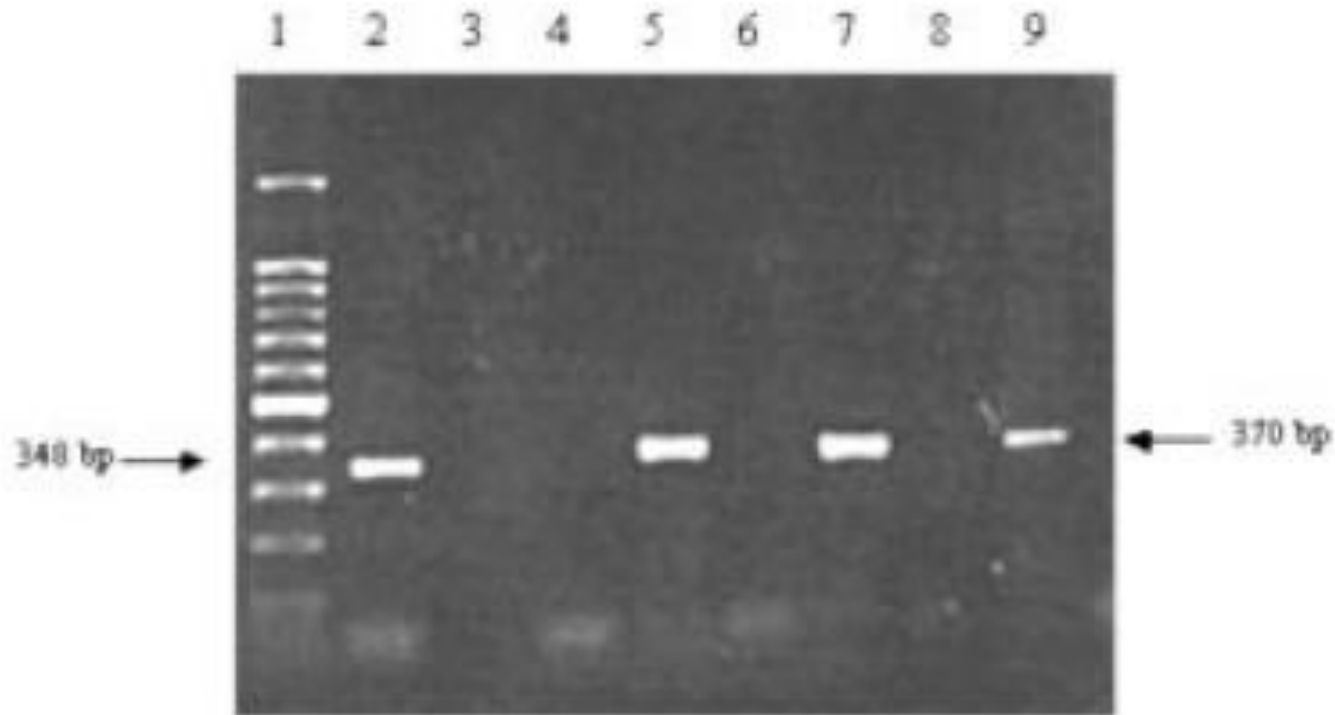
It is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) mixed with the gel during its preparation.

It is commonly abbreviated as "**EtBr**". When exposed to ultraviolet light. It will fluoresce with an orange colour

**N.B.** It is carcinogenic agent Ethidium bromide intercalated between two adenine-thymine base pairs.

The intercalation may cause mutation of DNA.

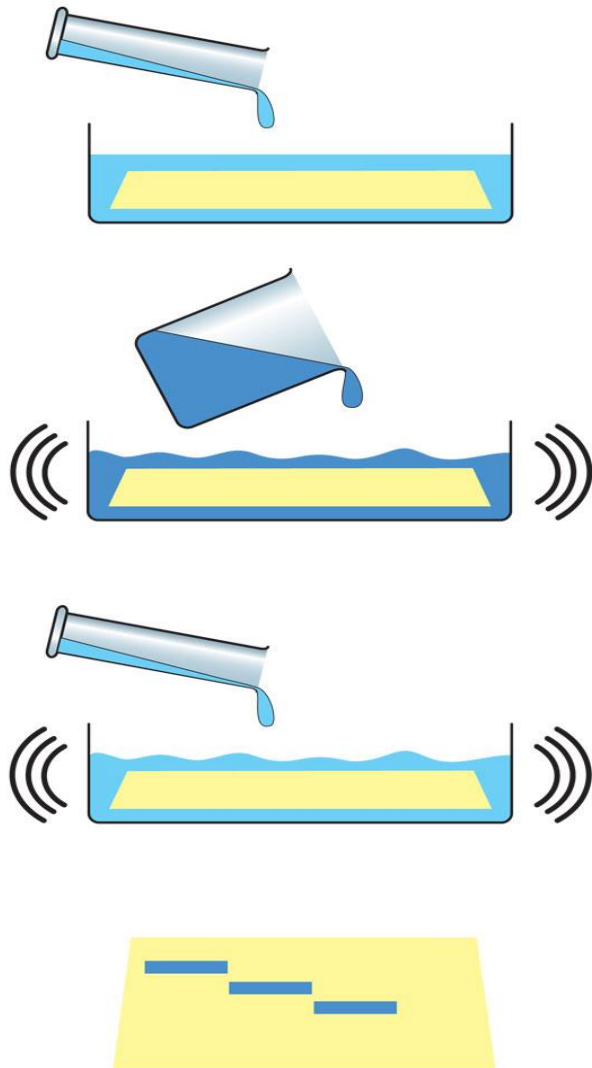
# Visualization



**Photographs can be taken of gels, often using Gel Doc system.**

# Visualization

## Colloidal Coomassie Blue Staining



1

### Wash

Wash Acrylamide or Agarose Gel with purified deionised water. Then dispose of water.

2

### Stain

Add Colloidal Coomassie Blue Safe Stain and agitate Gel until discreet bands appear (20min - 2 hours). Then remove CCB stain.

3

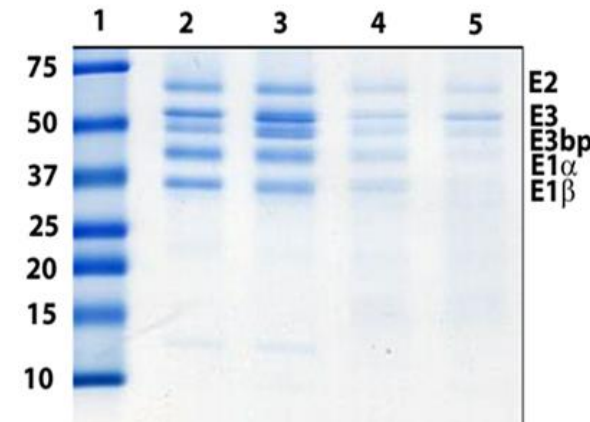
### Destain

Rinse the Gel with purified water and wash several times to remove background staining of Gel. Agitate or swirl to aid destaining of Gel until bands are distinct.

4

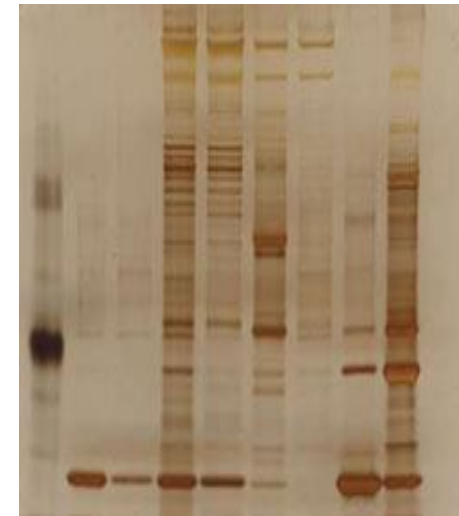
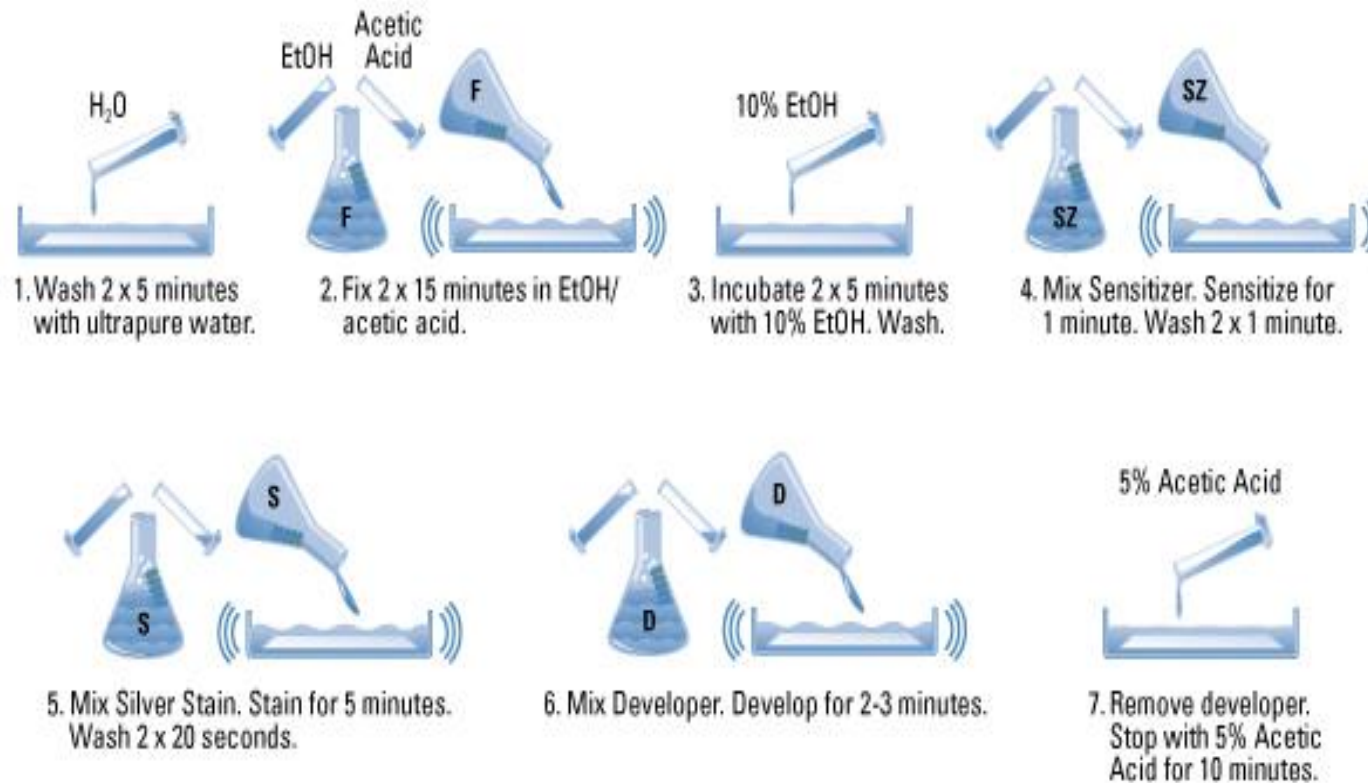
### Observe

Pour off water, dry and observe bands.



# Visualization

## Silver stain



## There are limits to electrophoretic techniques

Since passing current through a gel causes

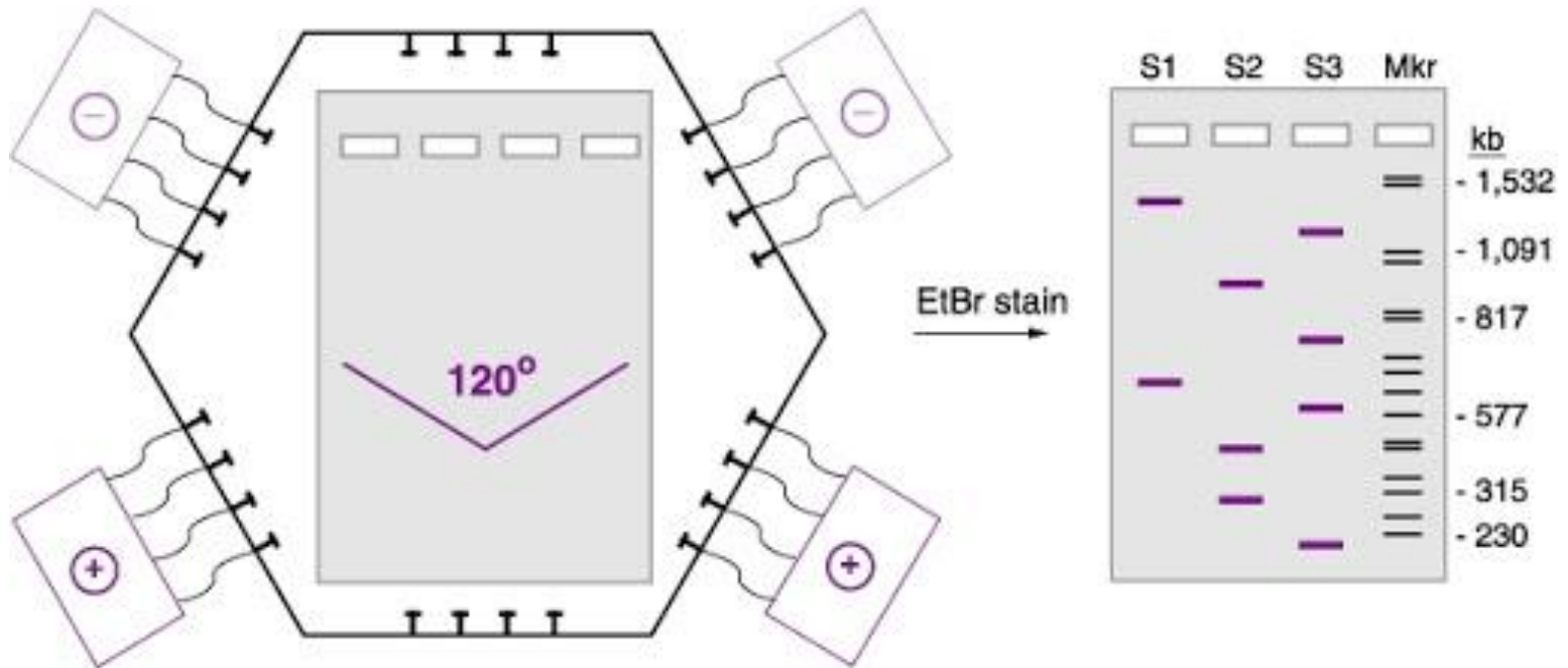
- Heating → Gel may melt during electrophoresis.
- Electrophoresis is performed in buffer solutions (*Electrophoresis buffers TBE*) to reduce pH changes due to the electric field, which is important because the charge of DNA and RNA depends on pH
- Running for too long can exhaust the buffering capacity of the solution so it should be changed from time to time.



# Pulsed-field gel electrophoresis

- This technique is relatively similar to performing a standard gel electrophoresis **except** that instead of constantly running the voltage in one direction, **the voltage is periodically switched among three directions** (one that runs through the central axis of the gel and two that run at an angle of 60 degrees either side).
- The pulse times are equal for each direction resulting in a net forward migration of the DNA.
- This procedure takes longer than normal gel electrophoresis due to the size of the fragments being resolved and the fact that the DNA does not move in a straight line through the gel.

# Pulsed-field gel electrophoresis (PFGE)



Electric field alternates  $120^\circ$  every 90 seconds for 18 to 24 hours at  $14^\circ\text{C}$

Life is like a camera...

**FOCUS** on what's important,  
**CAPTURE** the good times,  
**DEVELOP** from the negatives,  
And if things don't work out.  
Take another shot.



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