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INTRUMENTASI BIOTEKNOLOGI

Program Studi Bioteknologi

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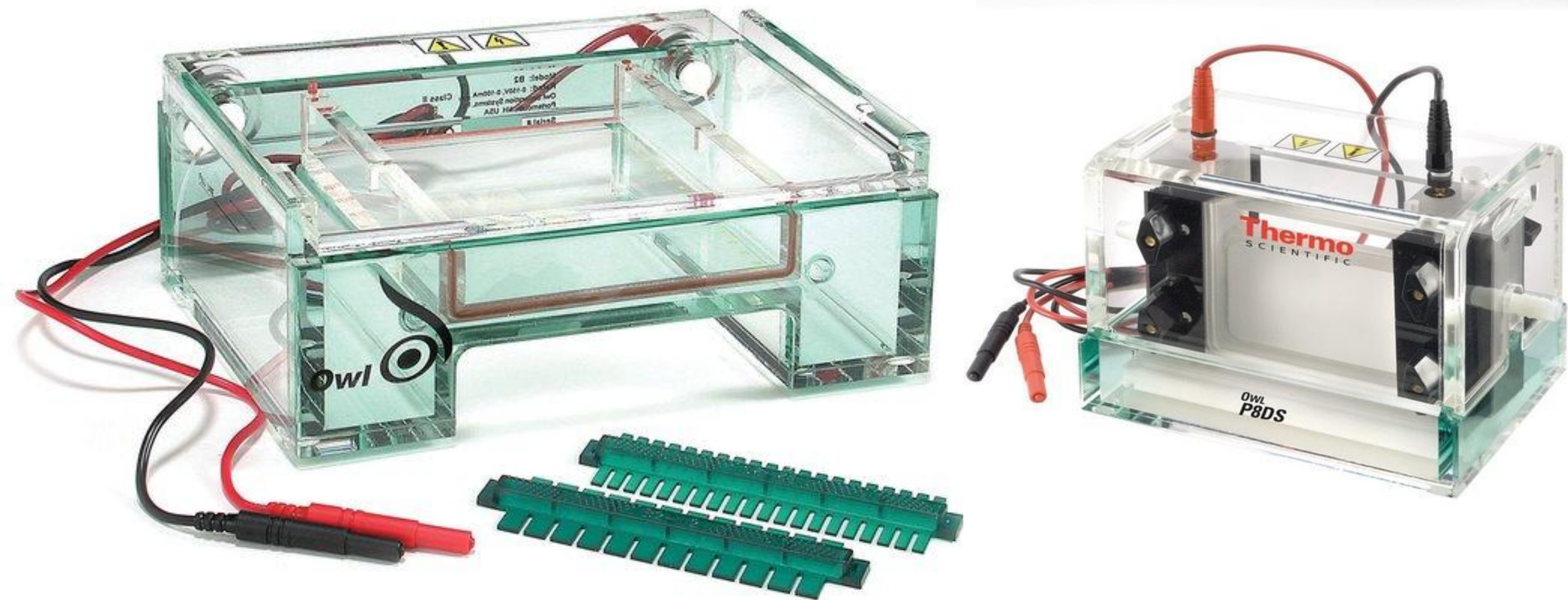
Universitas Esa Unggul

Electrophoresis and Gel Documentation System

Tujuan Perkuliahan

- Mahasiswa dapat mengidentifikasi dan mengetahui prinsip bekerjanya peralatan Elektroforesis DNA dan Protein
- Mengetahui prinsip bekerjanya alat-alat Gel Documentation system (DNA dan Protein)



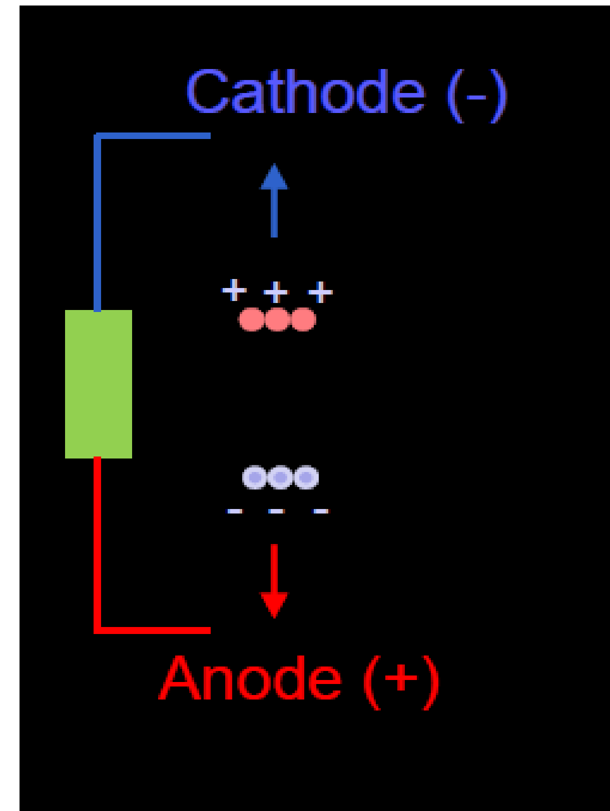
Electrophoresis Chamber

Electrophoresis

- Electrophoresis is the migration of charged molecules, particles or ion in a medium under the influence of an electric field
- Gel electrophoresis : A method of separating bio-molecules (DNA/RNA/Protein) in a gelatin-like material using an electrical field
- Electrophoresis used to determine the presence and size of PCR products
- General steps of electrophoresis:
  - Preparation
  - Separation
  - Detection

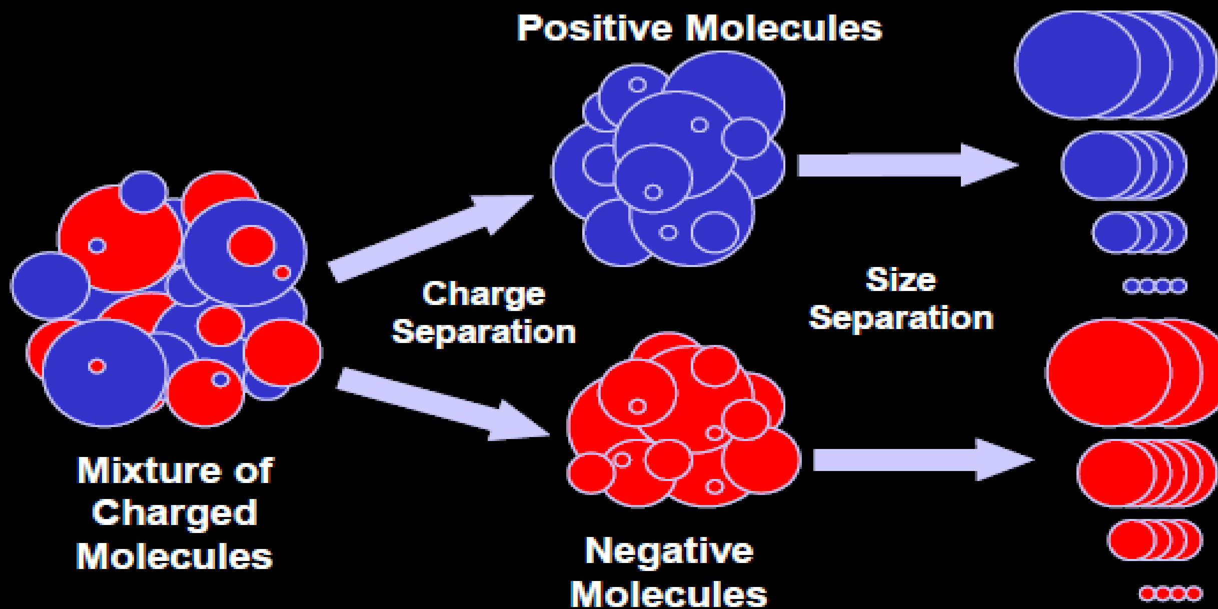
Electrophoresis : basic principles of Separation

- Many molecules have naturally occurring negative and positive charges on them.
- When introduced to an electrical current, negatively charged molecules are attracted to the positive electrode and positively charged molecules are attracted to the negative electrode.



Electrophoresis : basic principles of Separation

Charged molecules are separated based on their electrical charge and size.



Electrophoresis : basic principles of Separation

Factors influenced electrophoresis mobility/ migration:

- Sample : charge, size
- Electric field : current, voltage, temperature
- Supporting media : adsoprtion, electro-osmosis, molecular sieving
- Buffer : composition, concentration, pH

- Separation Media

Agarosa--→DNA/RNA
poliakrilamide→ DNA,protein
pati/*starch*→isoenzim

What is needed?

- Agarose - a polysaccharide made from seaweed. Agarose is dissolved (dilarutkan) in buffer and heated, then cools to a gelatinous solid with a network of crosslinked molecules
- Some gels are made with acrylamide if sharper bands are required

What is needed?

- Buffer - in this case TBE 1 x, TAE 1 x
- The buffer provides ions in solution to ensure electrical conductivity.
- Not only is the agarose dissolved in buffer, but the gel slab is submerged (direndam) (submarine gel) in buffer after hardening (mengeras)

Buffer Composition

Buffer	Properties	Descriptions
TAE (Tris-acetate EDTA)	• Low buffering capacity • Periodic replacement of the buffer may be necessary for long electrophoresis (>6h)	• Used when DNA is to be recovered • Better resolution for high molecular weight DNA than TBE • Migration rate of linear dsDNA fragments is ~10% faster than TBE • Slightly cheaper than TBE
TBE (Tris-borate EDTA)	• High buffering capacity • Borate forms complex with agarose and nucleic acid • Borate is a strong inhibitor for many enzymes	• Not suitable for DNA recovery • Not suitable for downstream application using enzymatic reaction • Suitable for electrophoresis of small (< 1 kb) DNA when DNA recovery is not required • Increased resolution of small (< 1 kb) DNA • Decreased DNA mobility

What is needed?

- Also needed are a power supply and a gel chamber
- Gel chambers come in a variety of models, from commercial through home-made, and a variety of sizes

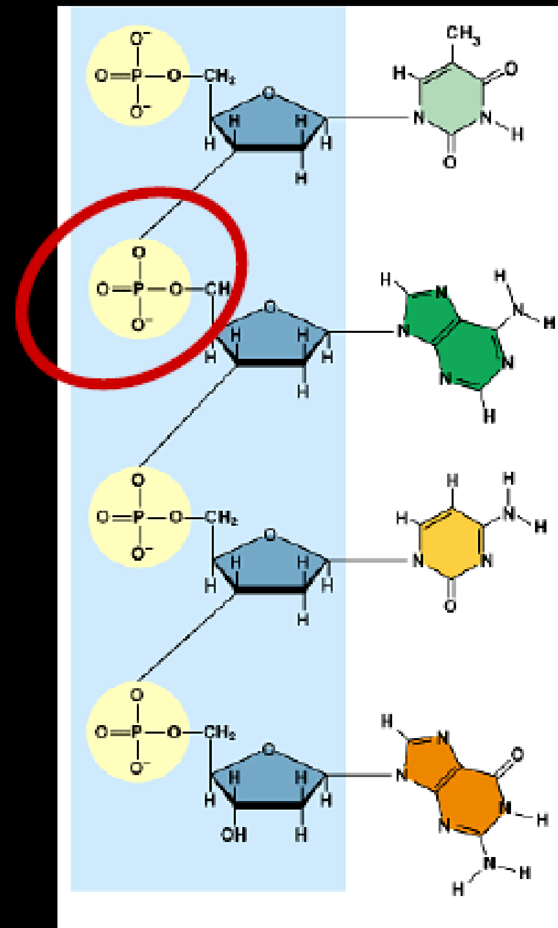
How does it work?

- DNA is an organic acid, and is negatively charged *(remember, DNA for Negative)*
- When the DNA is exposed to an electrical field, the particles migrate toward the positive electrode
- Smaller pieces of DNA can travel further in a given time than larger pieces

DNA negatively charged

Nature of samples:

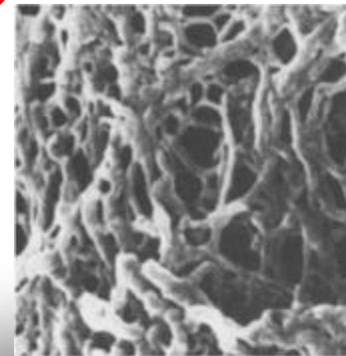
- **Nucleic acids** (DNA/ RNA), unlike proteins, are not amphoteric.
- DNA is negatively charged due to the phosphates in its backbone
- They remain negative at any pH used for electrophoresis
- DNA moves toward the positive side in an electrical field



- When placed in an electrical field, DNA will migrate toward the positive pole (Anode).
- An agarose gel is used to slow the movement of DNA and separate by size.

- Polymerized agarose is porous, allowing for the movement of DNA

Scanning Electron Micrograph of Agarose Gel (1×1 µm) →



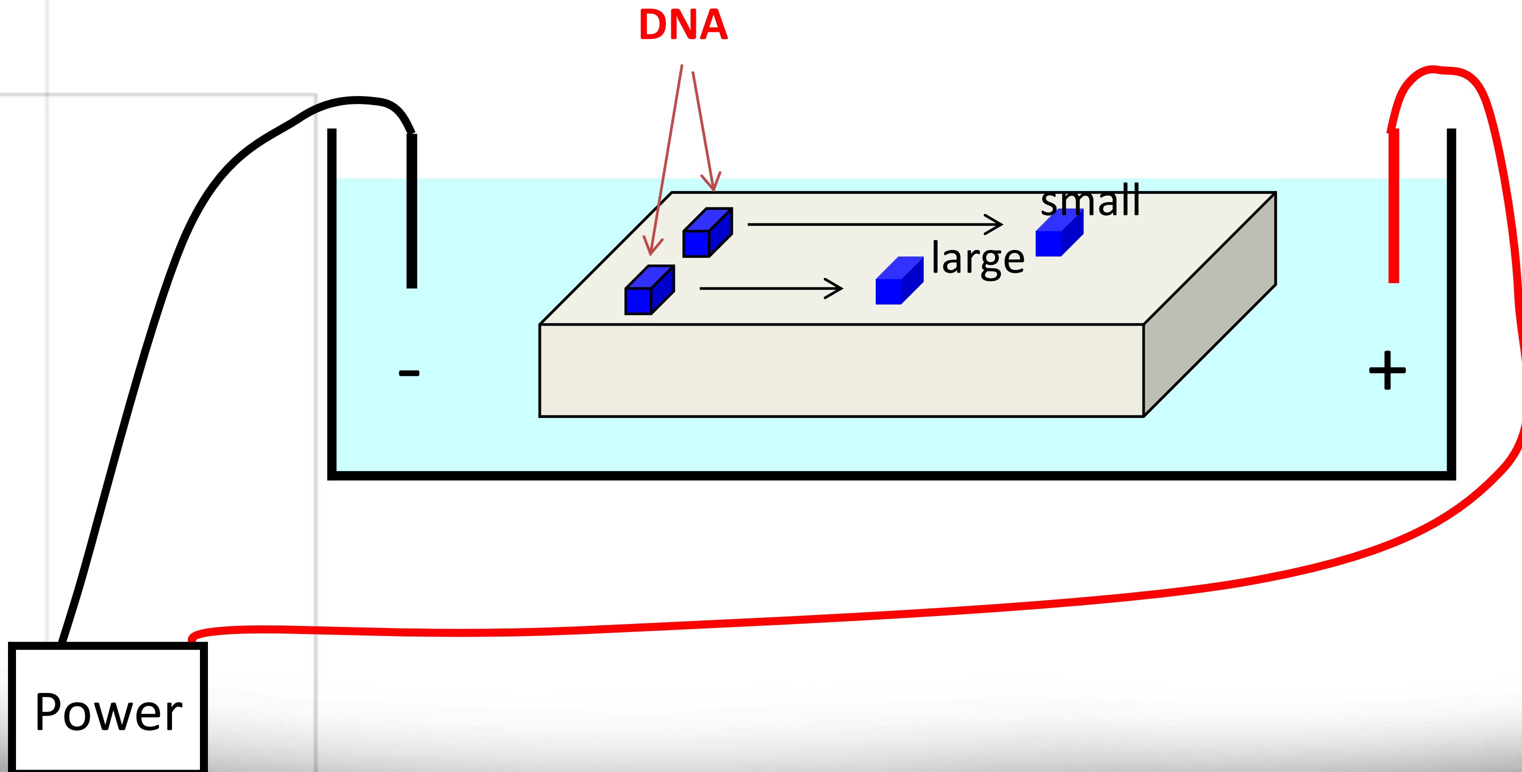
How fast will the DNA migrate?

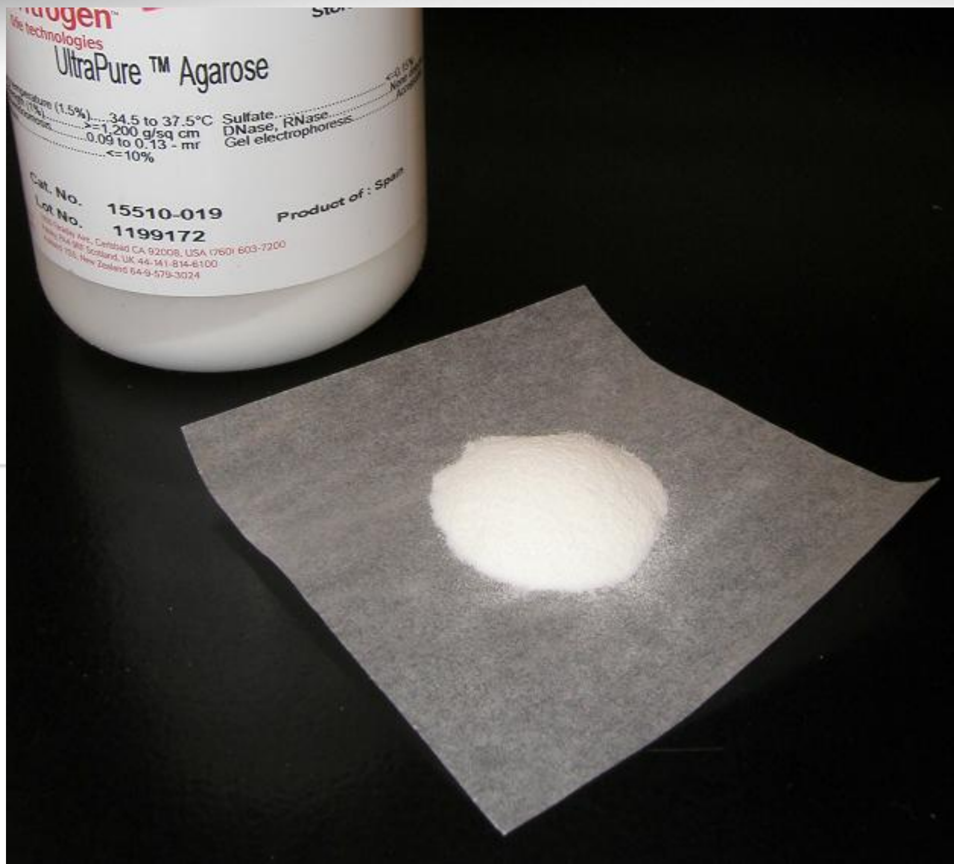
strength of the electrical field, buffer, density of agarose gel…

Size of the DNA!

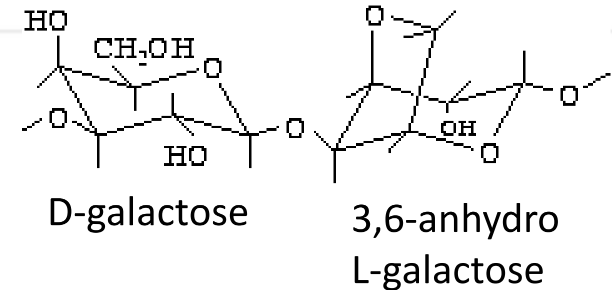
*Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to size





Agarose



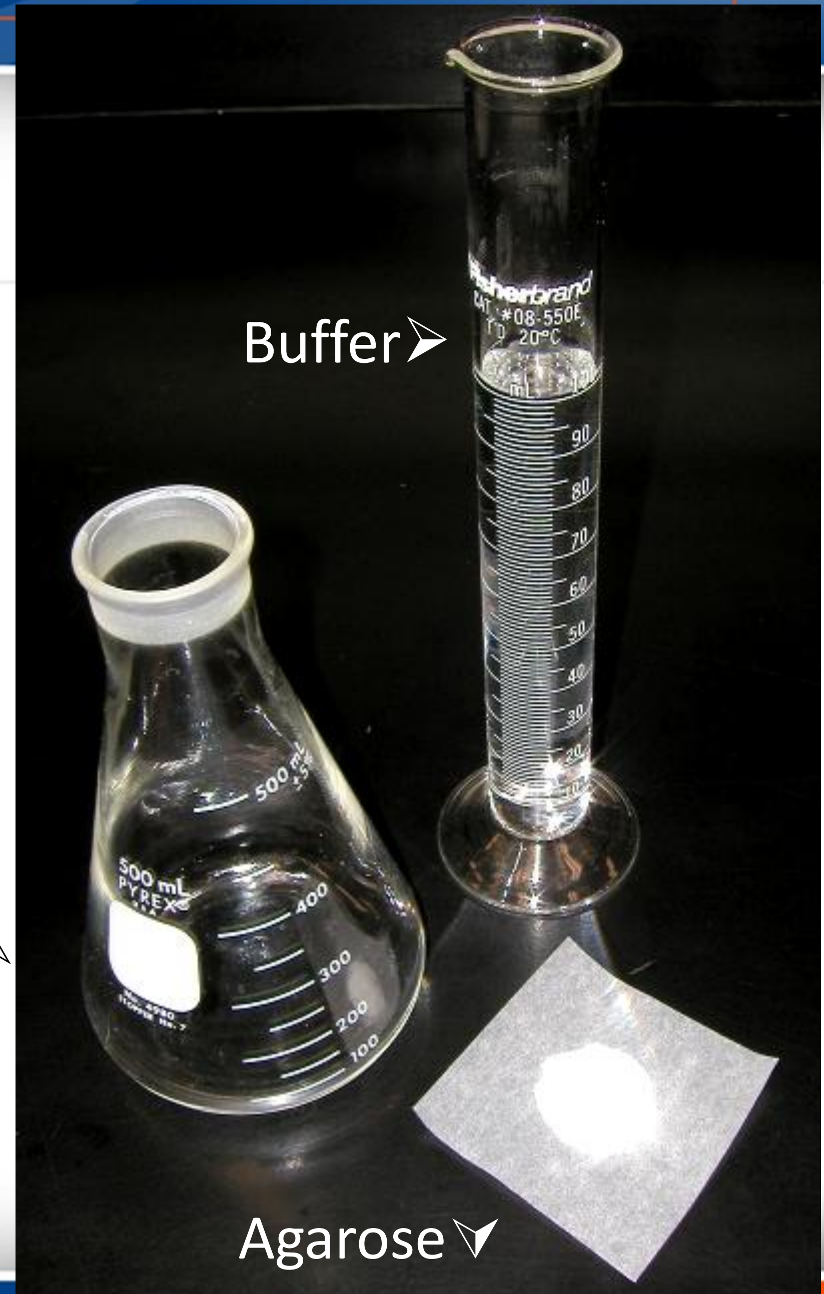
•Sweetened agarose gels have been eaten in the Far East since the 17th century.

•Agarose was first used in biology when Robert Koch* used it as a culture medium for Tuberculosis bacteria in 1882

***Lina Hesse**, technician and illustrator for a colleague of Koch was the first to suggest agar for use in culturing bacteria

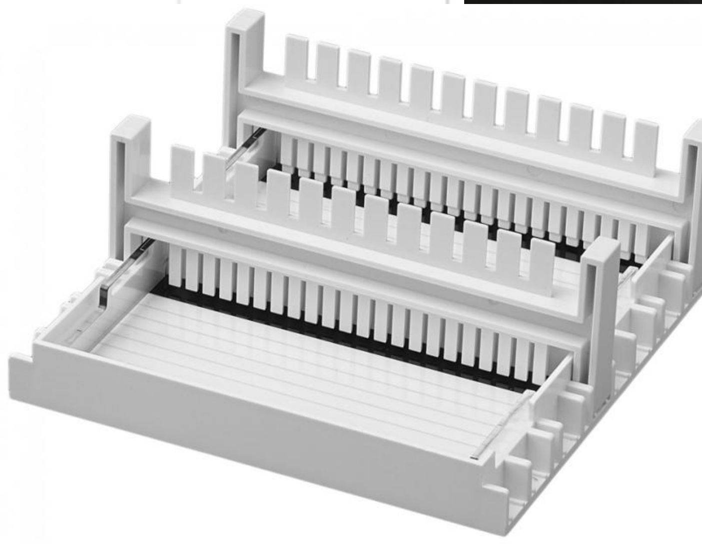
Agarose is a linear polymer extracted from seaweed.

An agarose gel is prepared by combining agarose powder and a buffer solution.

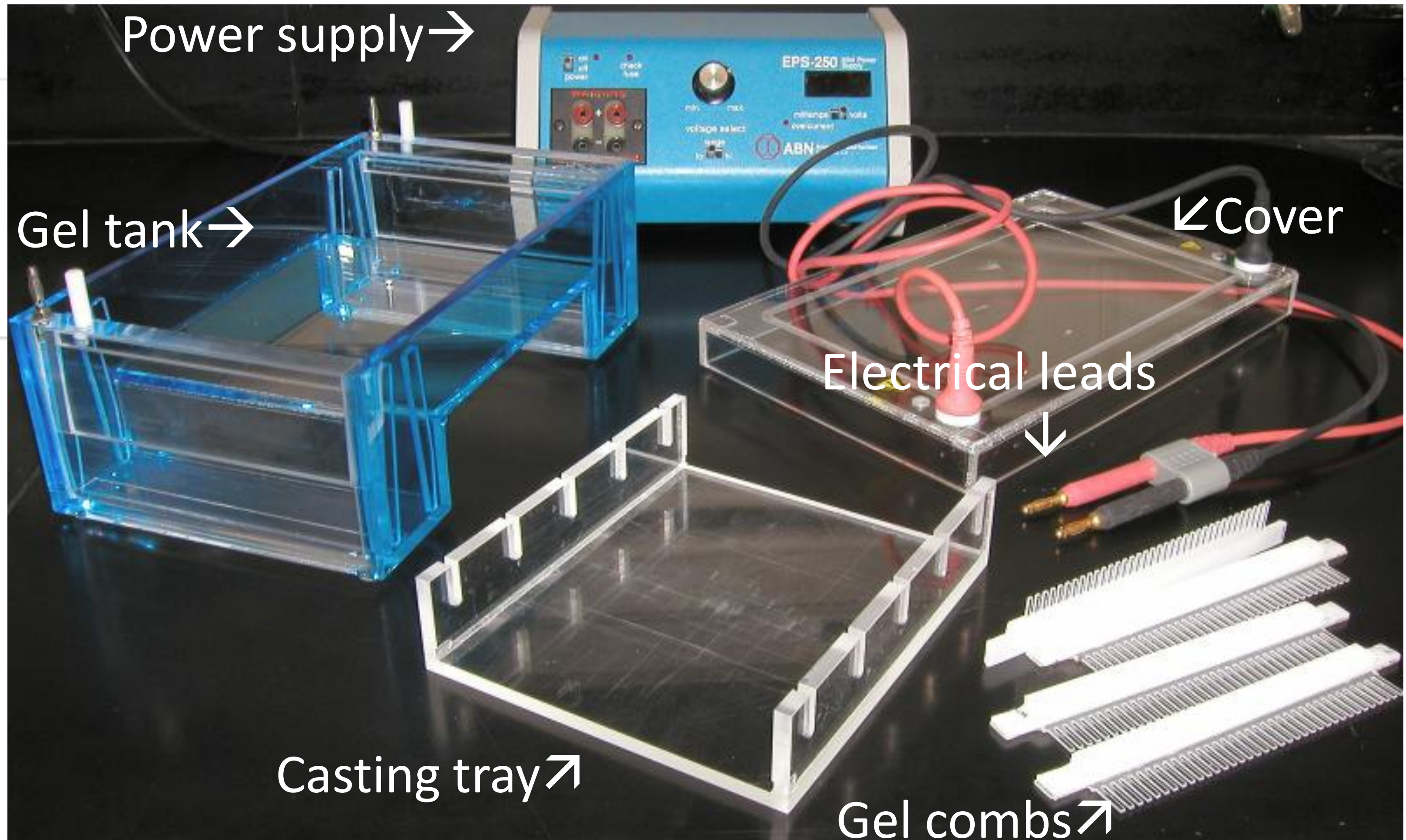


Flask for boiling

Gel casting tray & combs

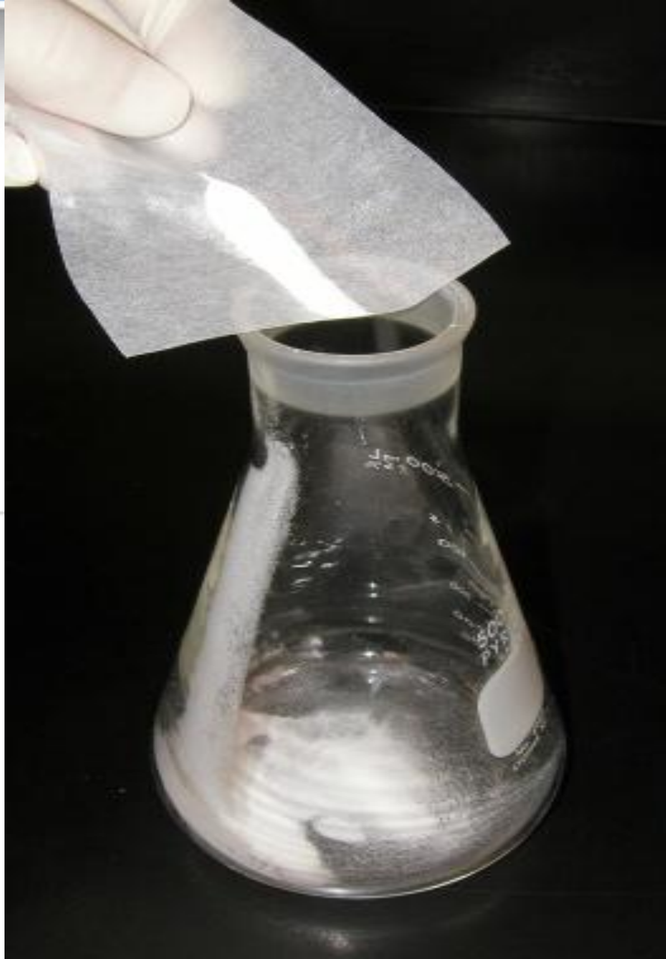


Electrophoresis Equipment



Preparing the Casting Tray

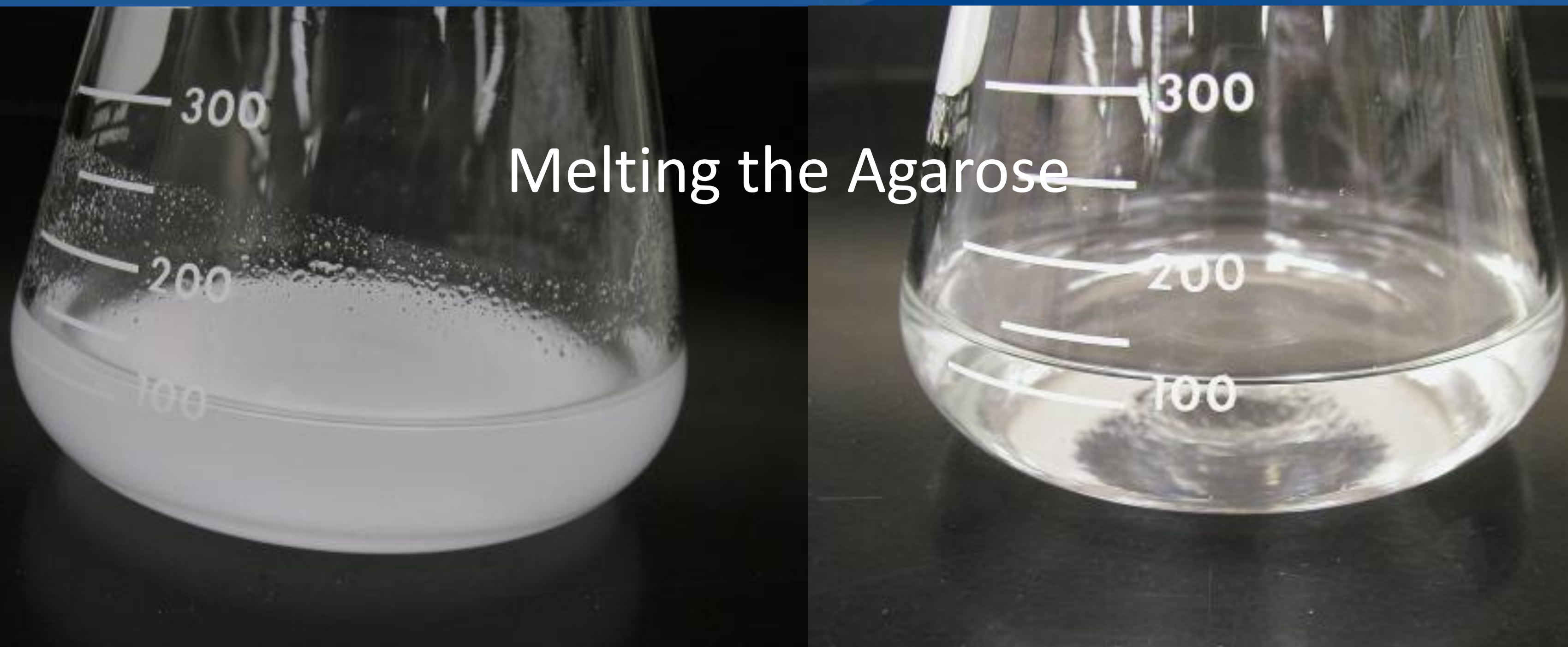
Seal the edges of the casting tray and put in the combs. Place the casting tray on a level surface. None of the gel combs should be touching the surface of the casting tray.



Agarose

Buffer Solution

Combine the agarose powder and buffer solution. Use a flask that is several times larger than the volume of buffer.

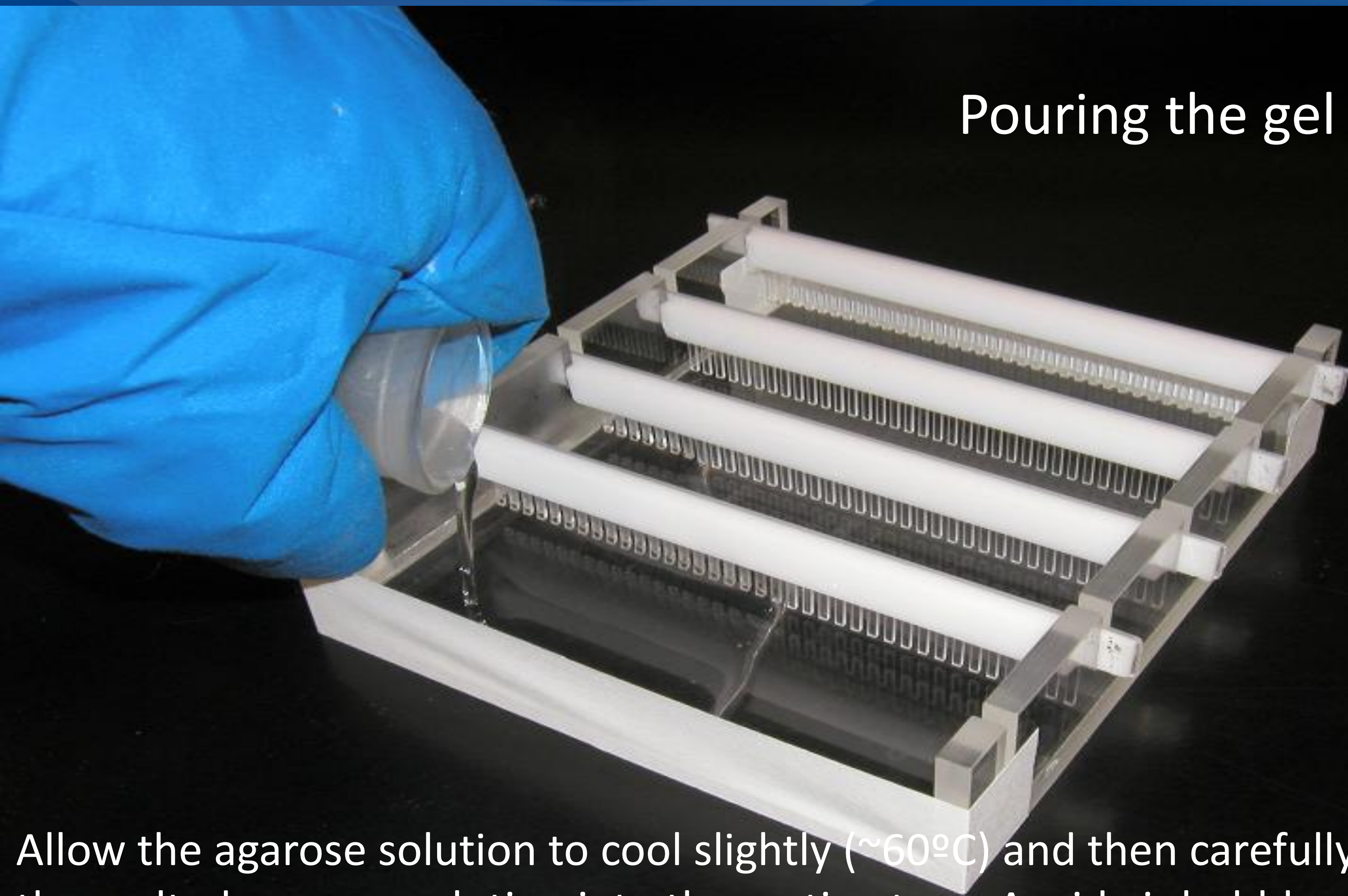


Melting the Agarose

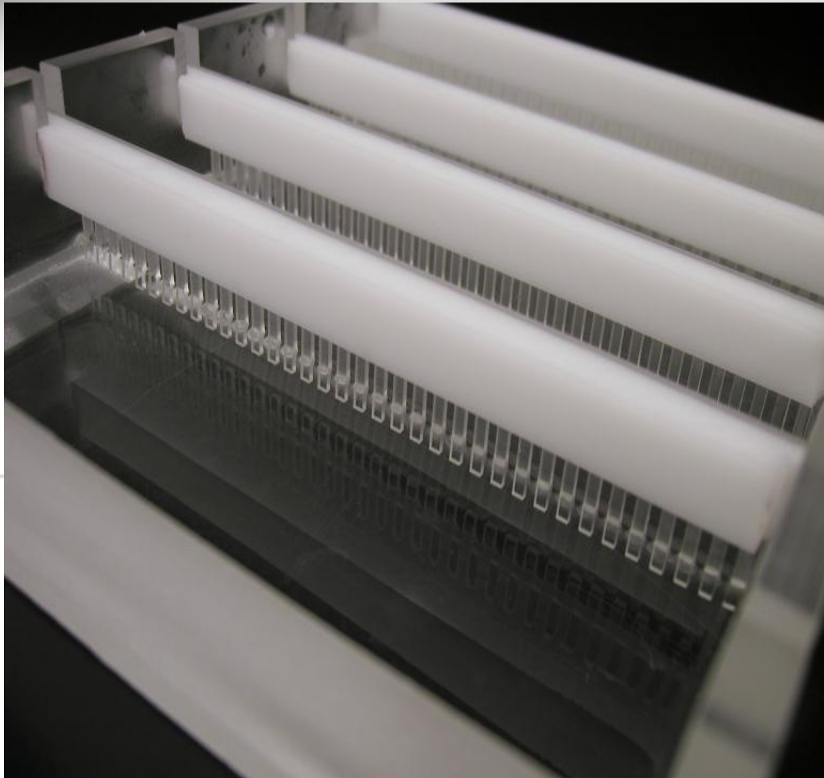
Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

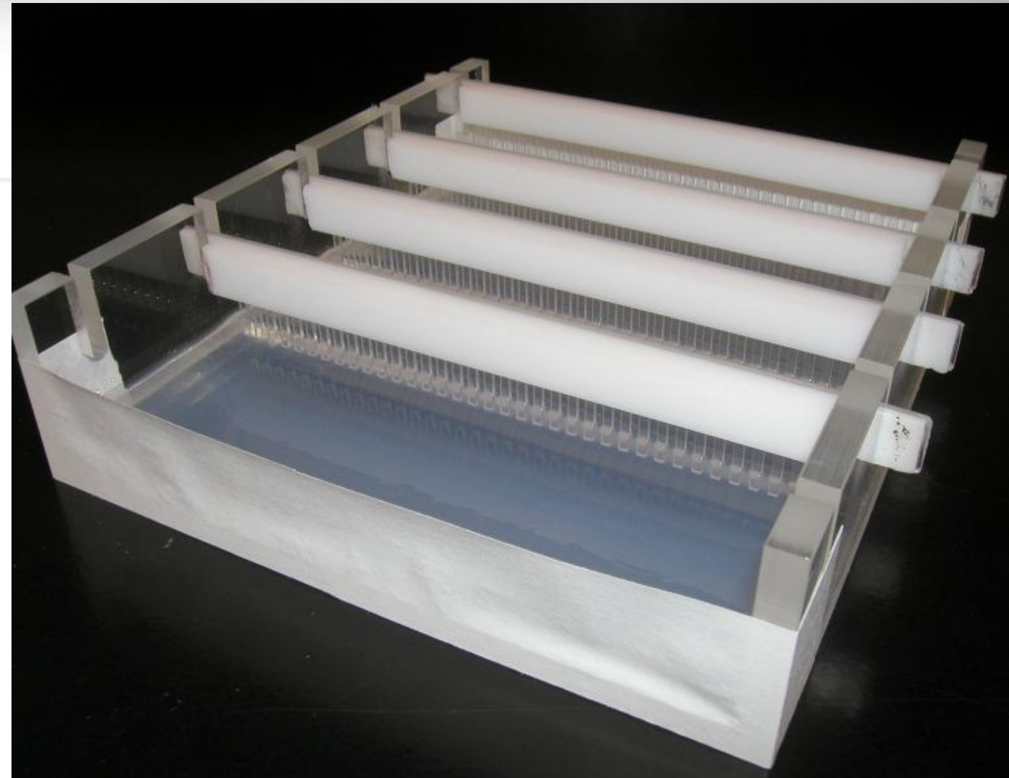
Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.
***Be careful when boiling - the agarose solution may become superheated and may boil violently if it has been heated too long in a microwave oven.

Pouring the gel



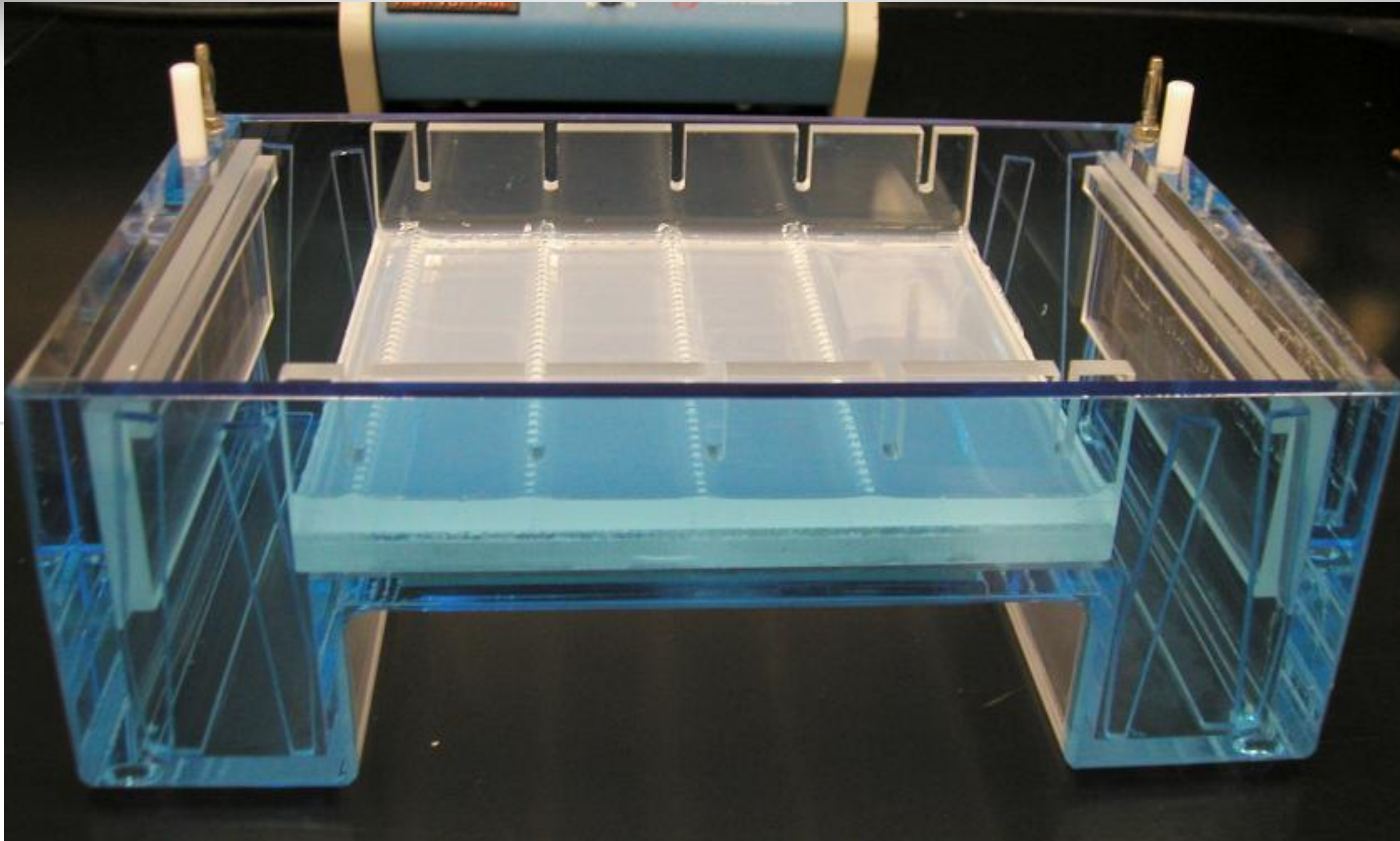
Allow the agarose solution to cool slightly (~60ºC) and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.



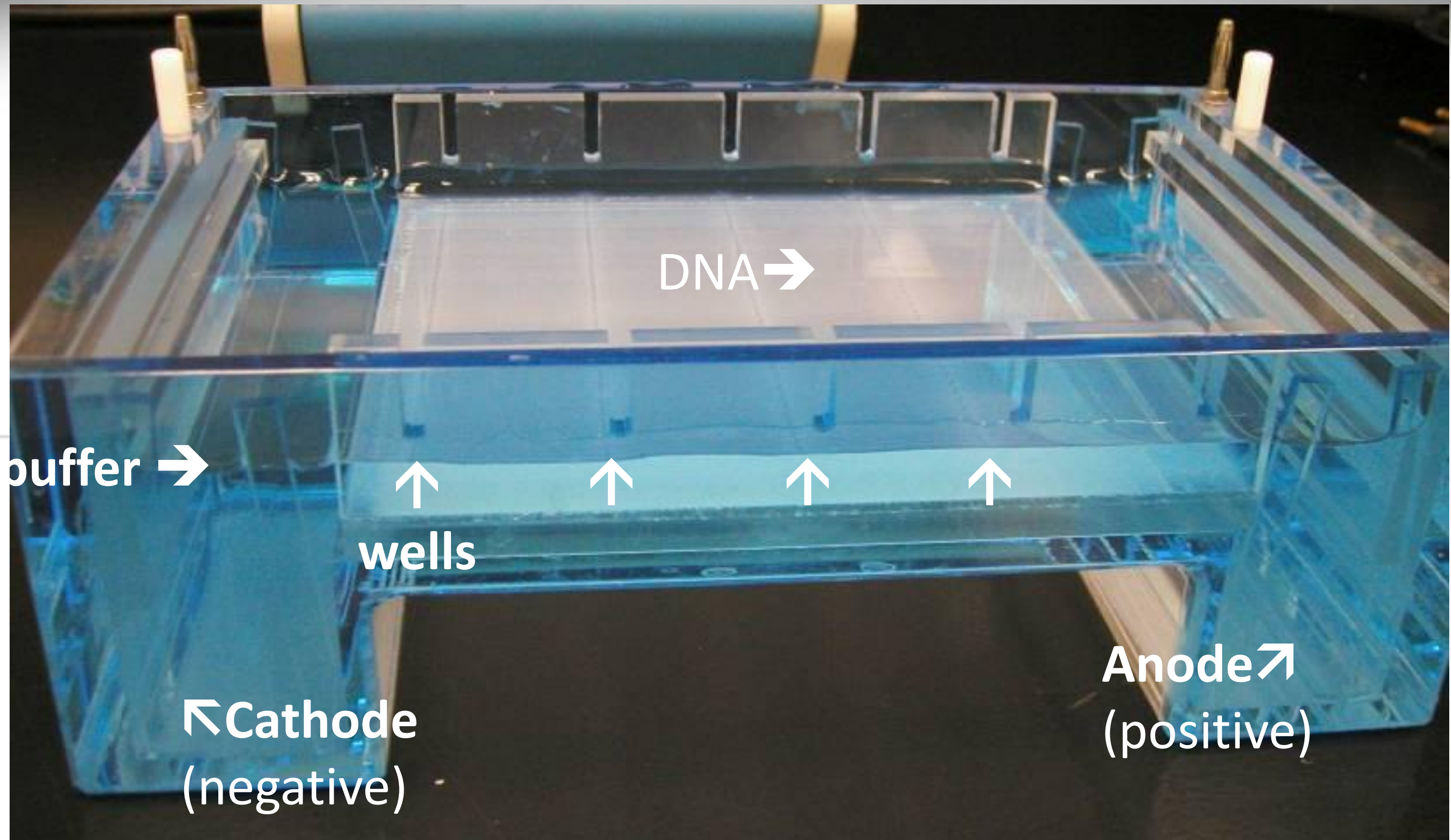


Each of the gel combs should be submerged in the melted agarose solution.

When cooled, the agarose polymerizes, forming a flexible gel. It should appear lighter in color when completely cooled (30-45 minutes). Carefully remove the combs and tape.



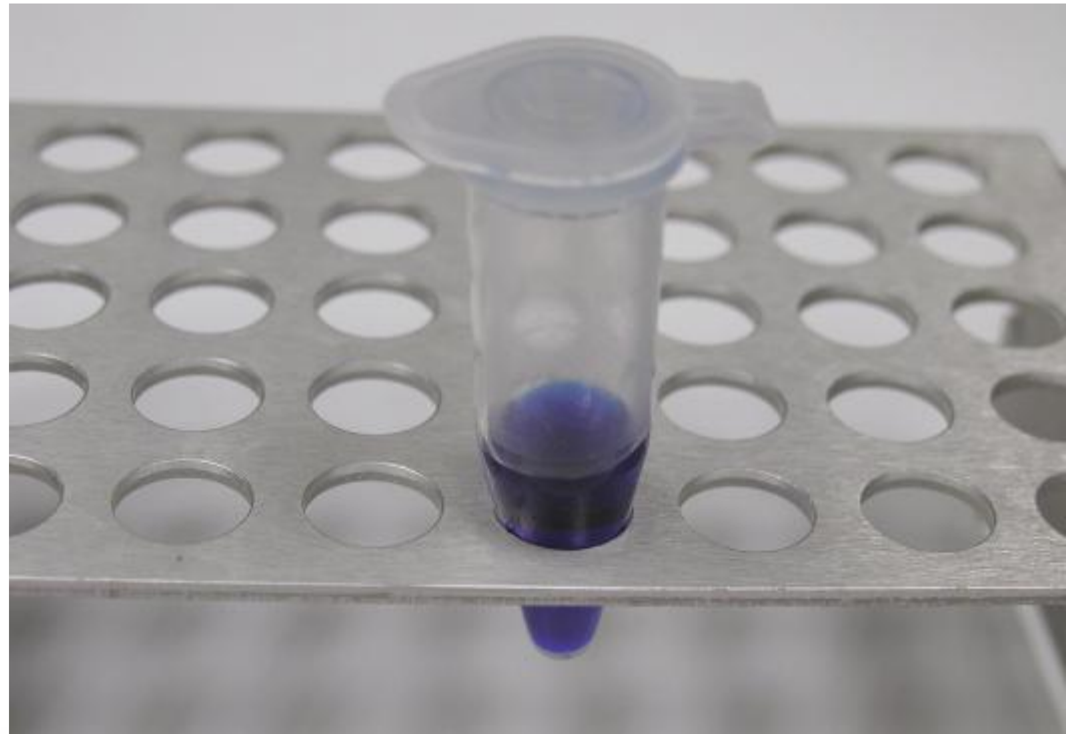
Place the gel in the electrophoresis chamber.



Add enough electrophoresis buffer to cover the gel to a depth of at least 1 mm. Make sure each well is filled with buffer.

Sample Preparation

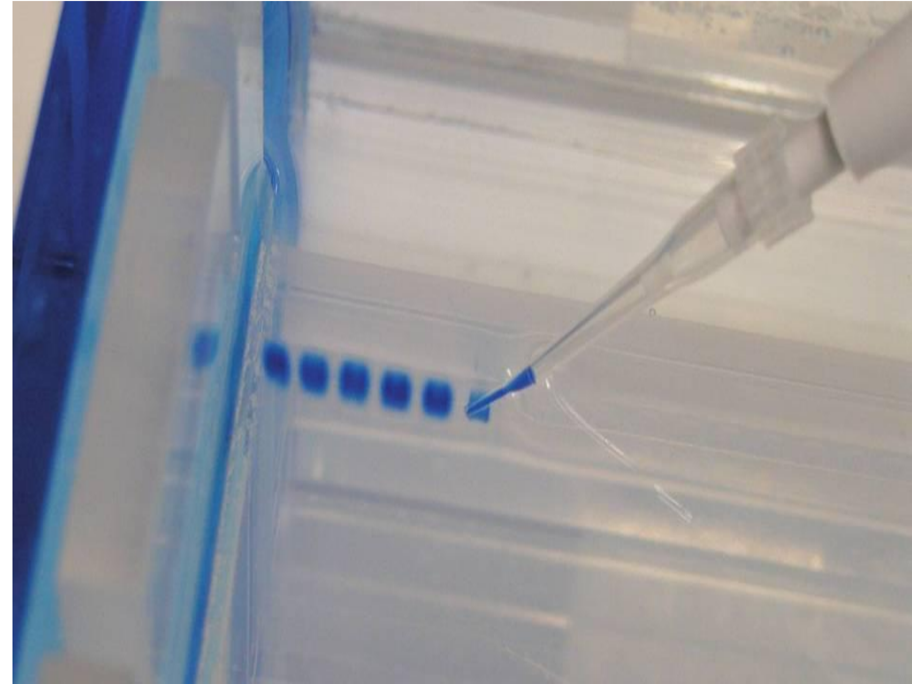
Mix the samples of DNA with the 6X sample loading buffer (w/ tracking dye). This allows the samples to be seen when loading onto the gel, and increases the density of the samples, causing them to sink into the gel wells.



6X Loading Buffer: →

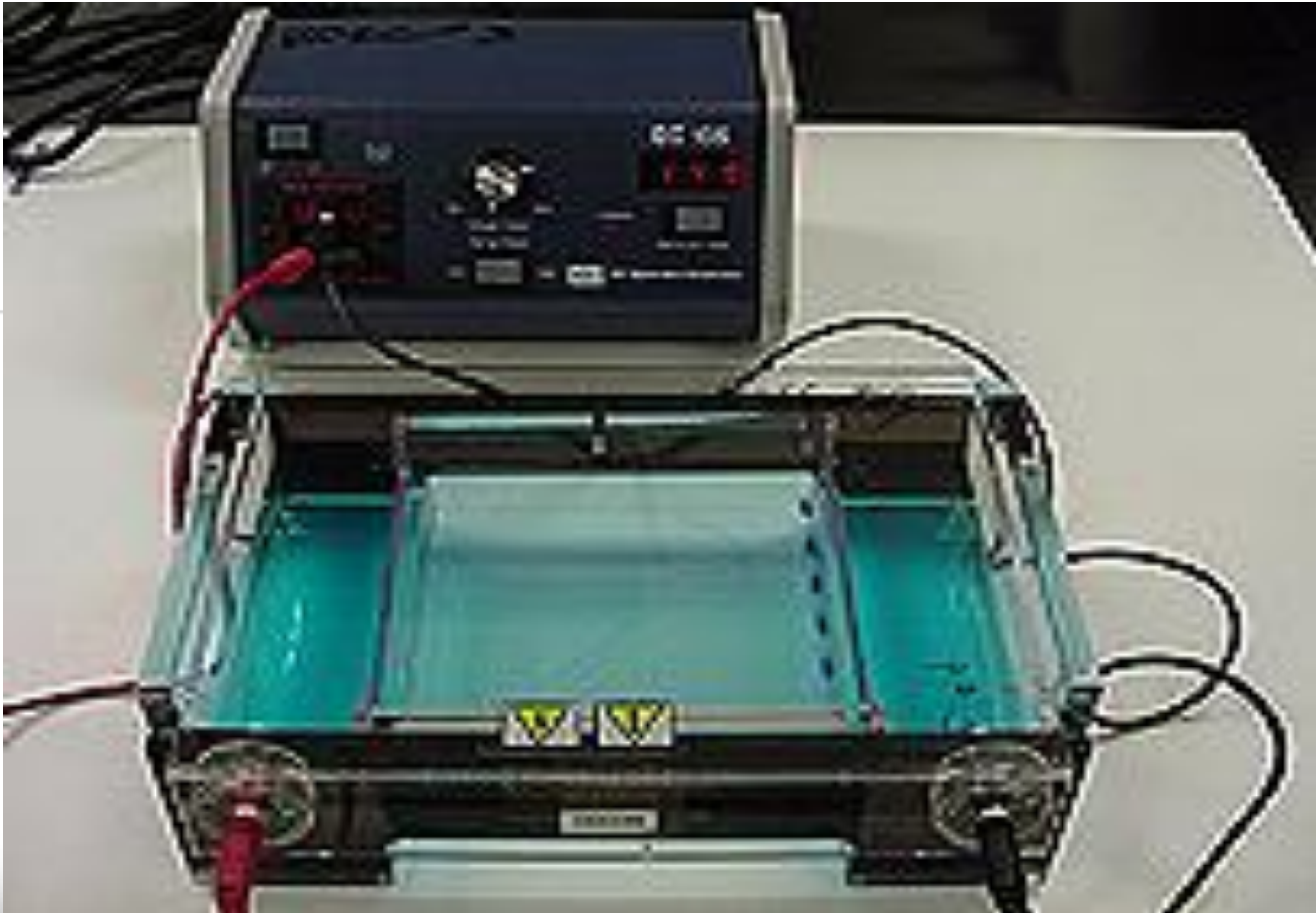
- Bromophenol Blue (for color)
- Glycerol (for weight)

The DNA samples are mixed with a dense loading dye so they sink into their wells and can be seen



Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well. Be careful not to puncture the gel with the pipette tip.

Electrical current applied to the chamber

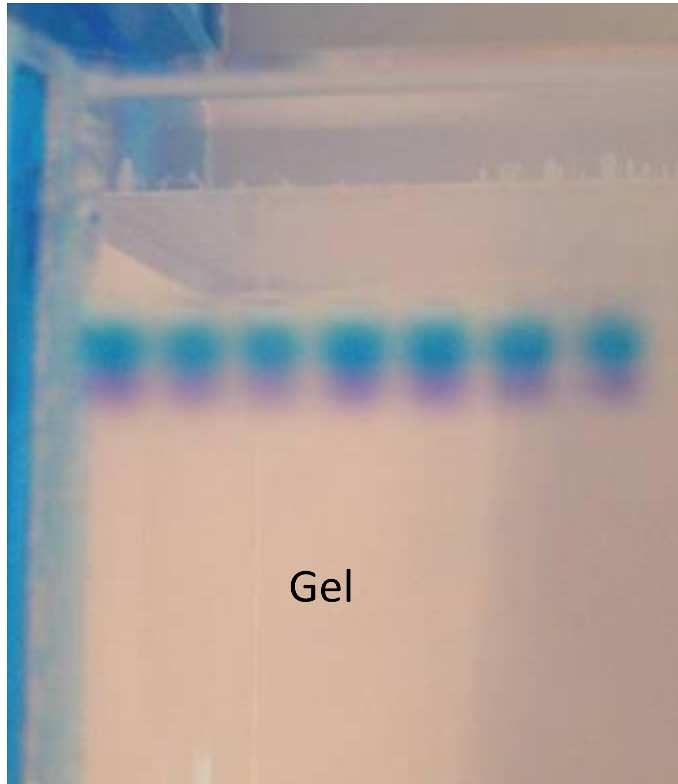


Prinsip Kerja Elekroforesis

Cathode
(-)

DNA
(-)
↓

Anode
(+)

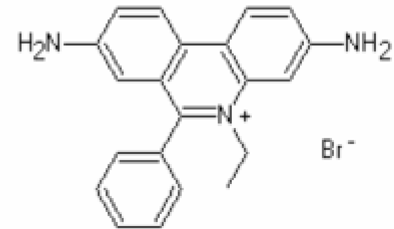


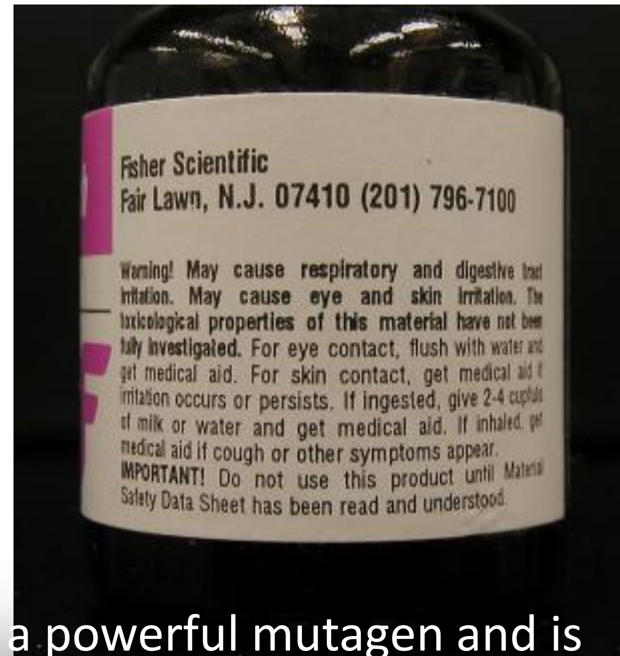
← wells
← Bromophenol Blue

Staining the Gel

- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.

- Ethidium bromide can be added to the **gel** and/or **running buffer** before the gel is run or the gel can be stained after it has run.





***CAUTION! Ethidium bromide is a powerful mutagen and is moderately toxic. **Gloves should be worn at all times**.

Safer alternatives to Ethidium Bromide

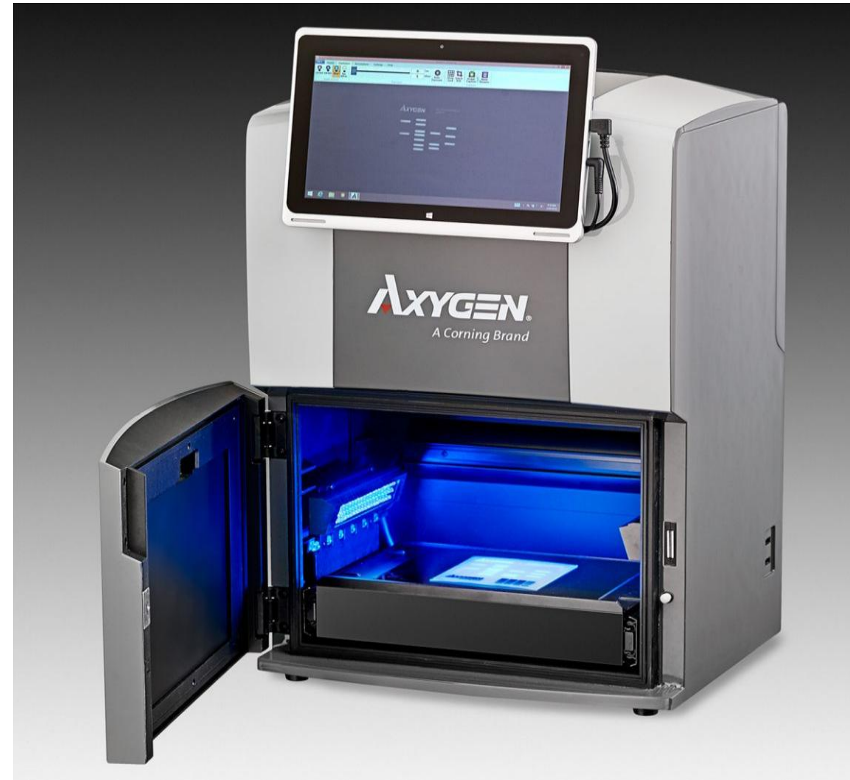
- Methylene Blue
- BioRAD - Bio-Safe DNA Stain
- Ward's - QUIKView DNA Stain
- Carolina BLUe Stain
- SYBR safe stain

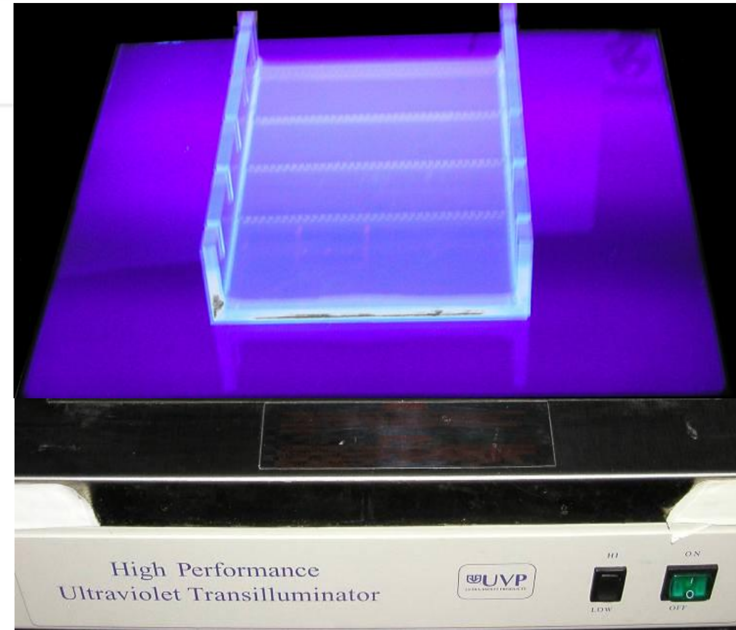
...others

advantages
Inexpensive
Less toxic
No hazardous waste disposal

disadvantages
Less sensitive
More DNA needed on gel
Longer staining/destaining time

Gel documentation system
UV Transilluminator dan Photo polaroid





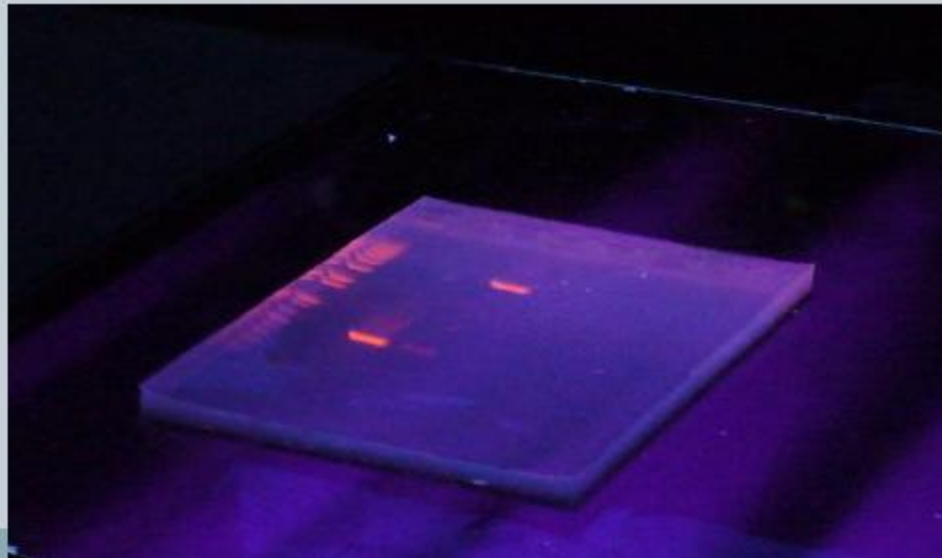
Staining the Gel Allow the gel to stain for 25-30 minutes.

Ethidium Bromide requires an ultraviolet light source to visualize with GelDoc

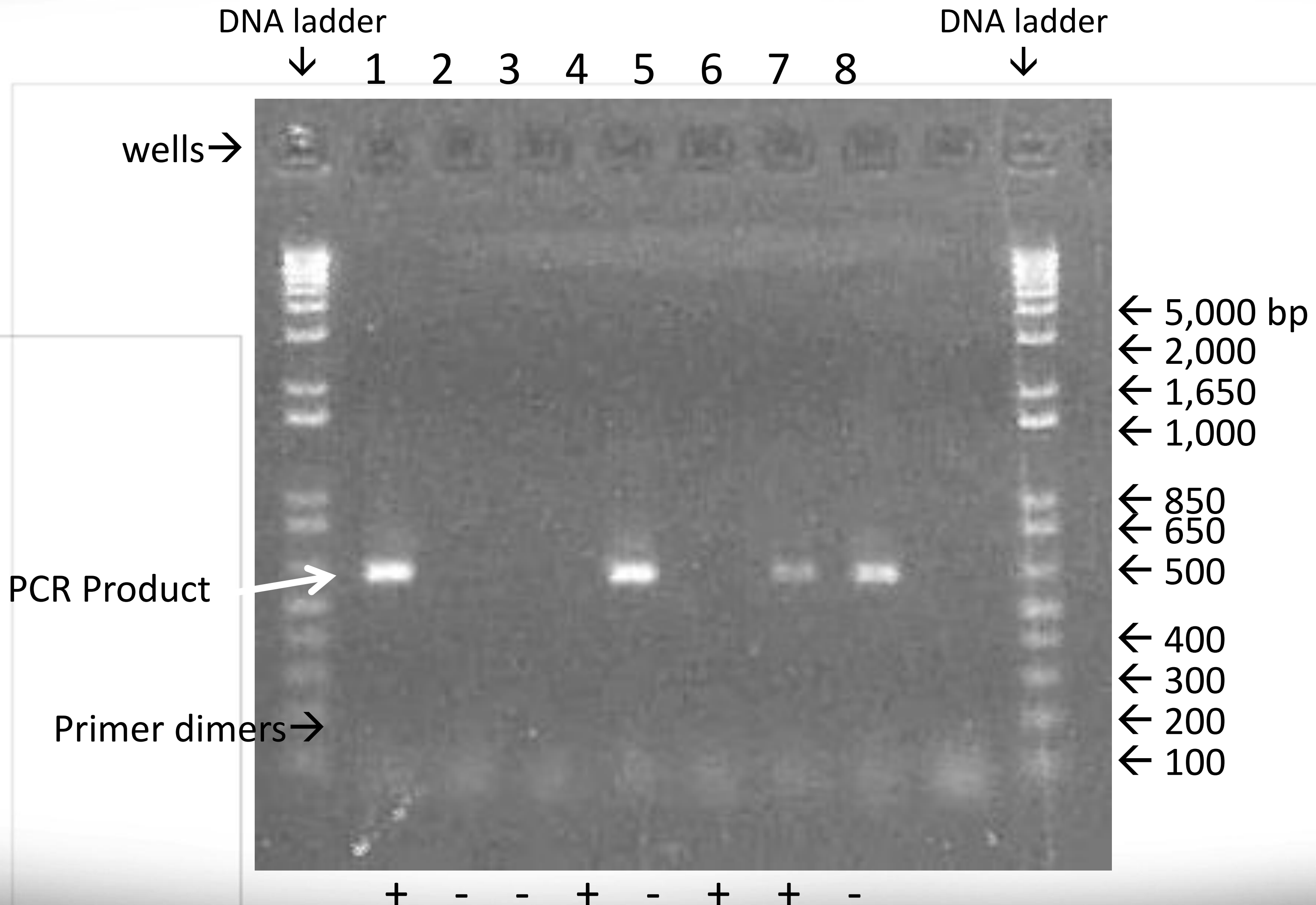
Visualisasi DNA

Sebelum

Sesudah

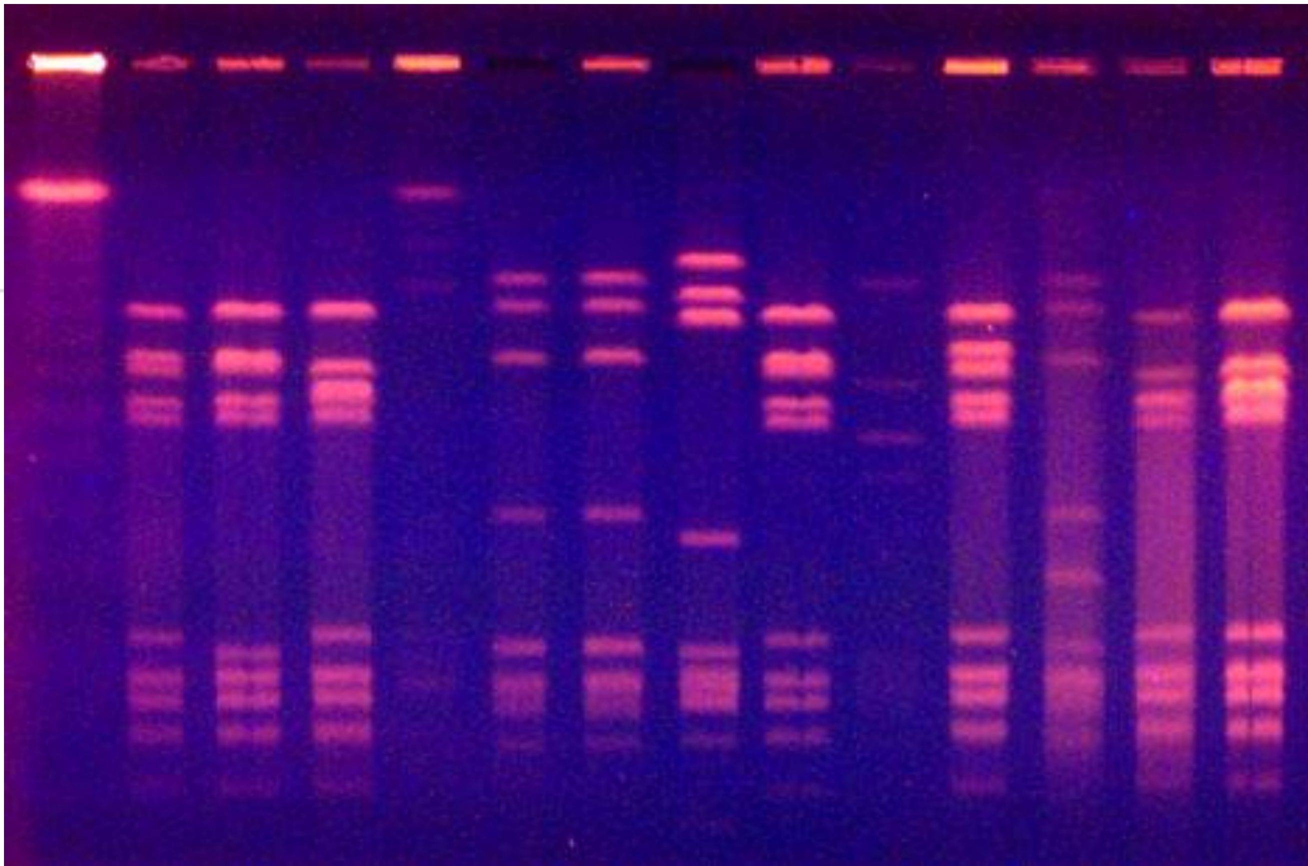


Visualizing the DNA (ethidium bromide)



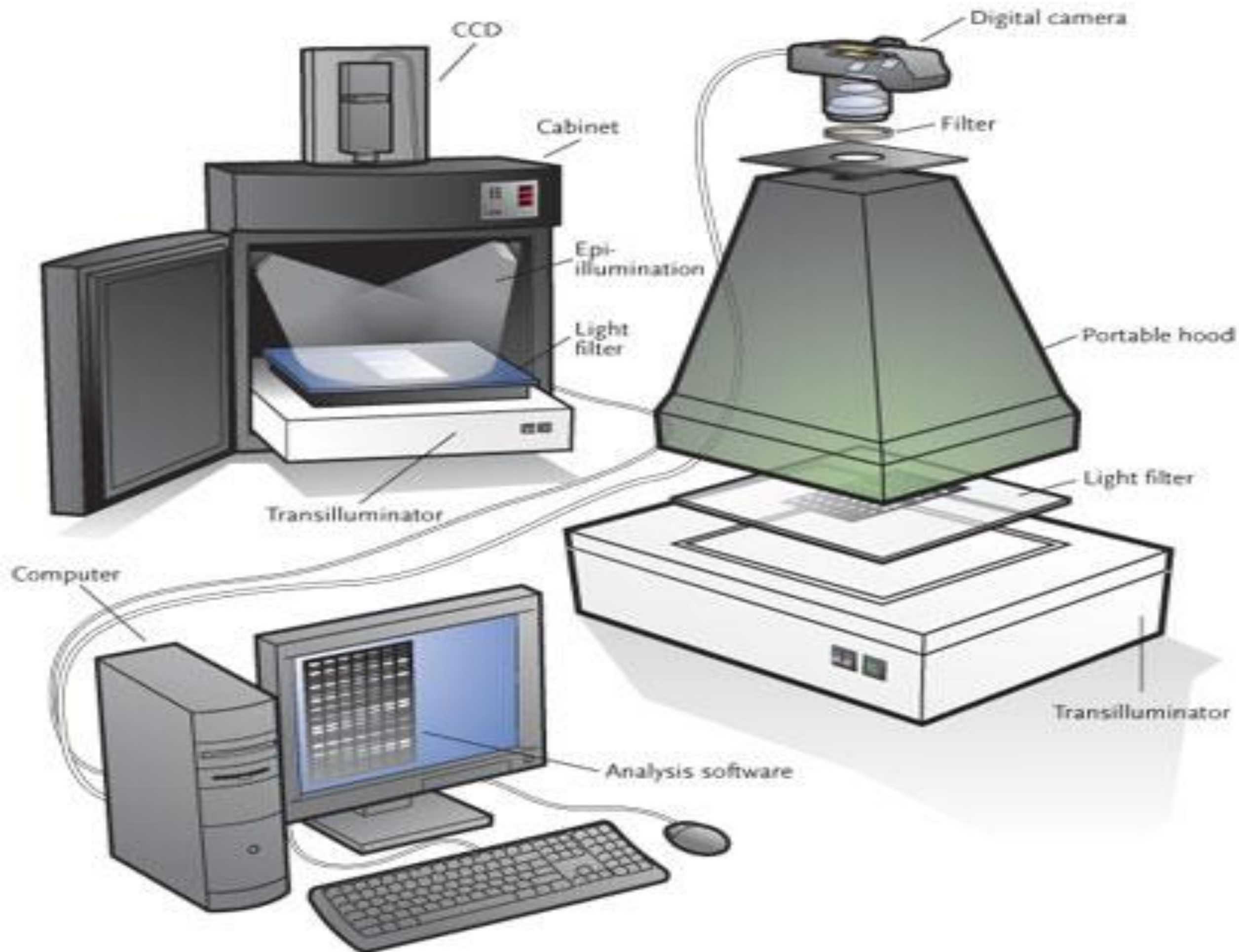
Samples # 1, 4, 6 & 7 were positive for Wolbachia DNA

An ethidium-stained gel photographed under UV light



Each band that you see is a collection of millions of DNA molecules, all of the same length!!

Gel Documentation system



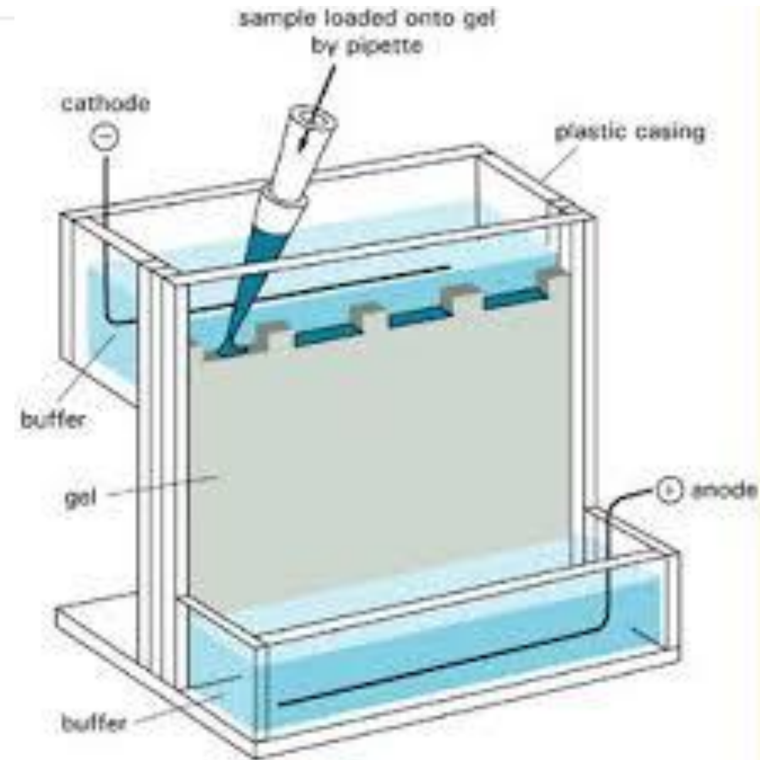
Gel Documentation System Protein

Staining Proteins in Gels

- Coomassie Brilliant Blue
  - The CBB staining can detect about 1 μg of protein in a normal band.
- Silver Staining
  - The silver stain system are about 100 times more sensitive, detecting about 10 ng of the protein.

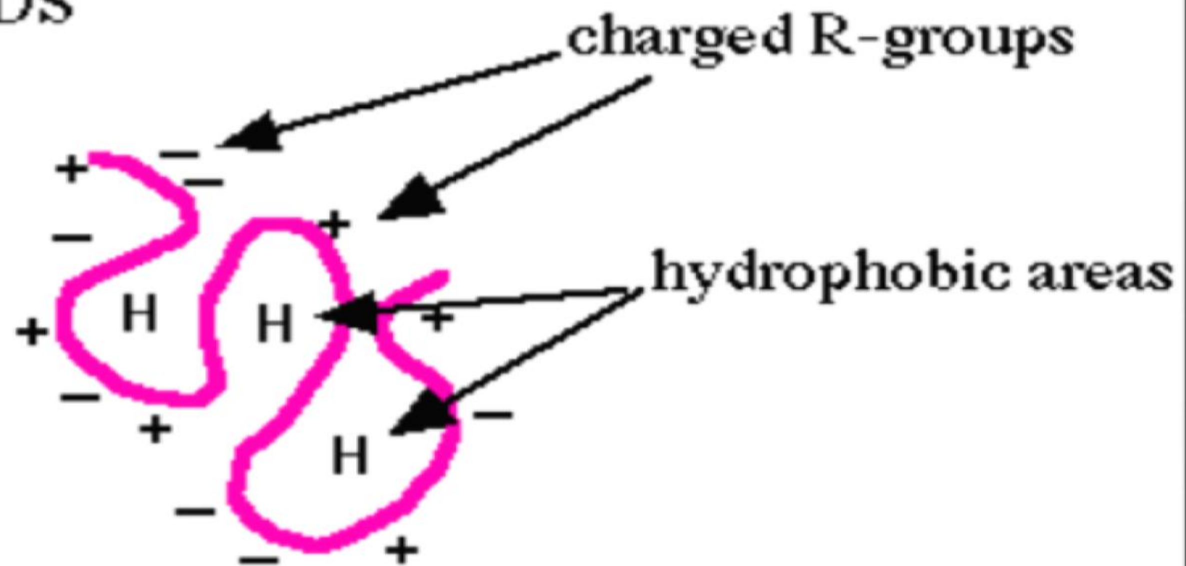
SDS PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Principle of SDS PAGE – Vertikal electroforesis

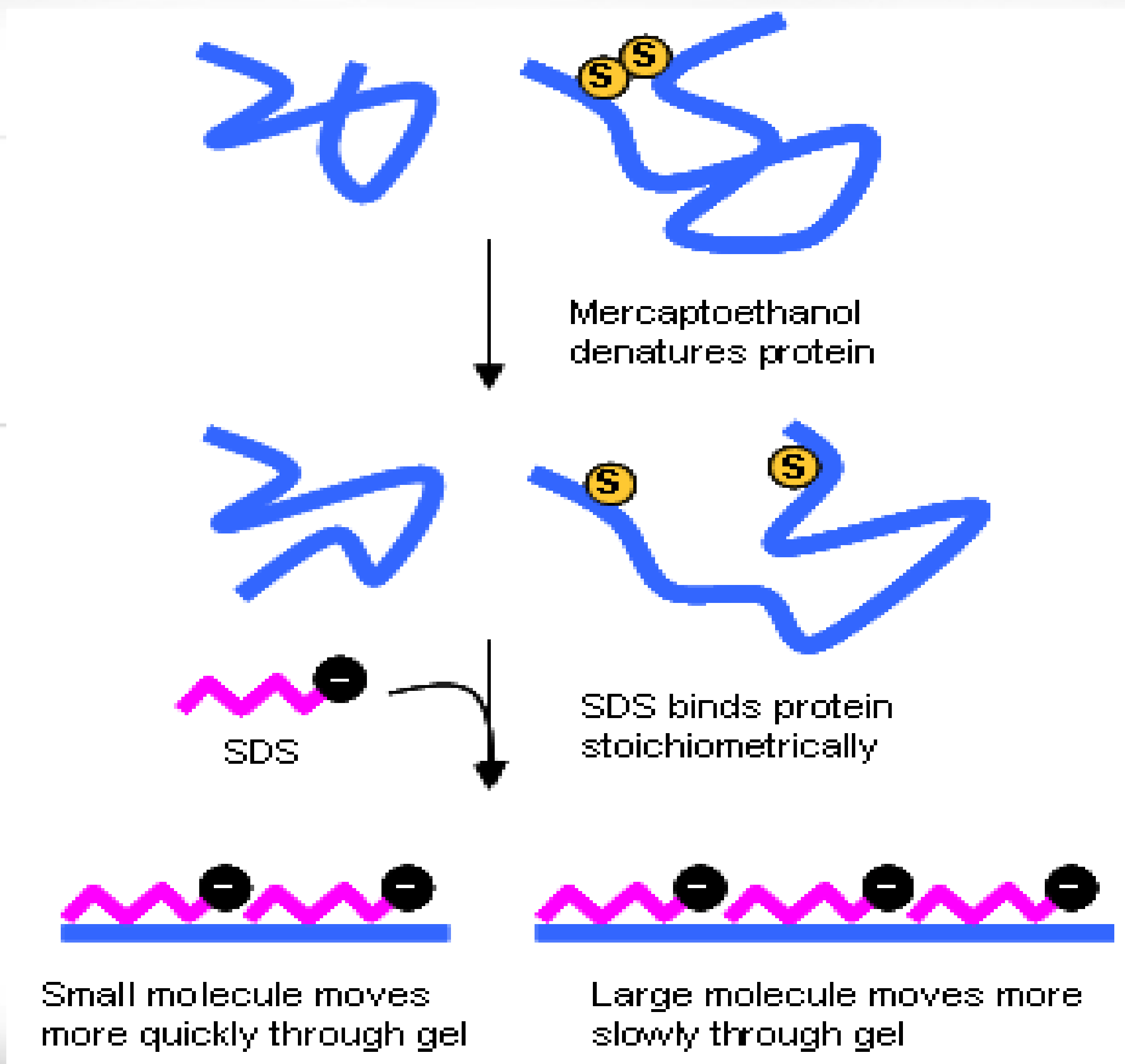


-Sodium Dodecyl Sulfate [SDS]: is a detergent which denature proteins by binding to the hydrophobic regions, all non-covalent bonds will disrupted and the proteins acquire a negative net charge

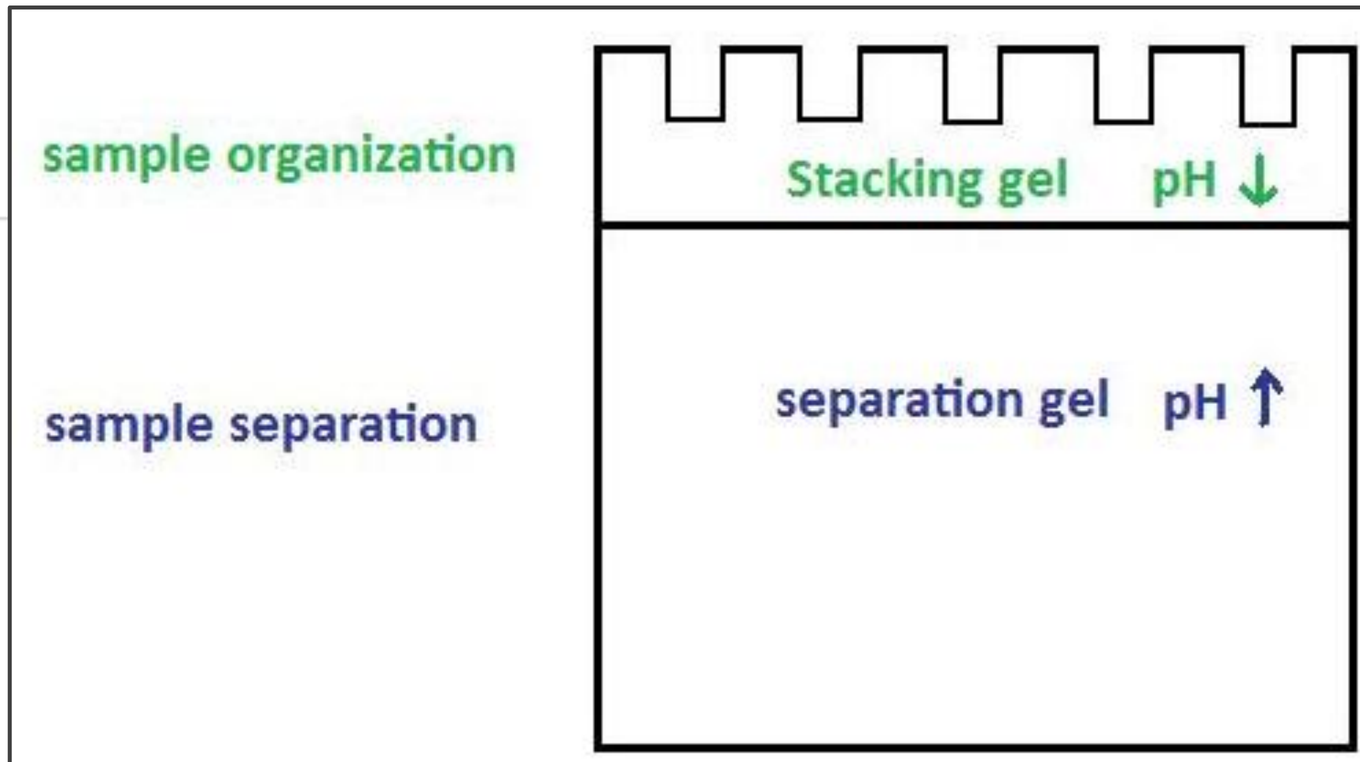
BEFORE SDS



AFTER SDS


S
S
Mercaptoethanol
denatures protein
S
S
SDS
SDS binds protein
stoichiometrically
Small molecule moves
more quickly through gel
Large molecule moves more
slowly through gel

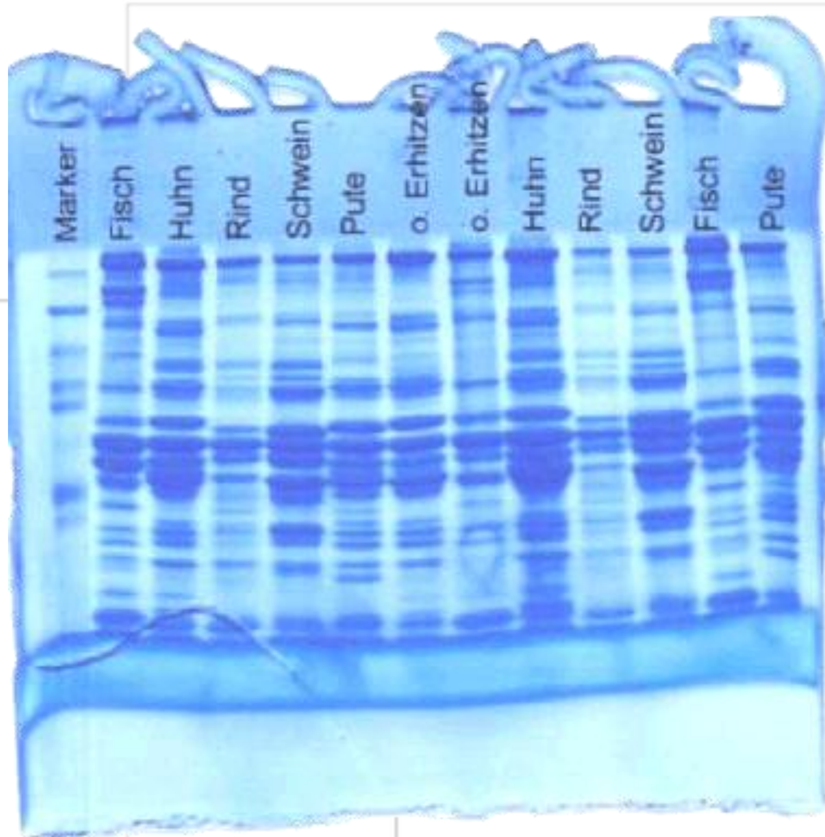
Steps in SDS-PAGE

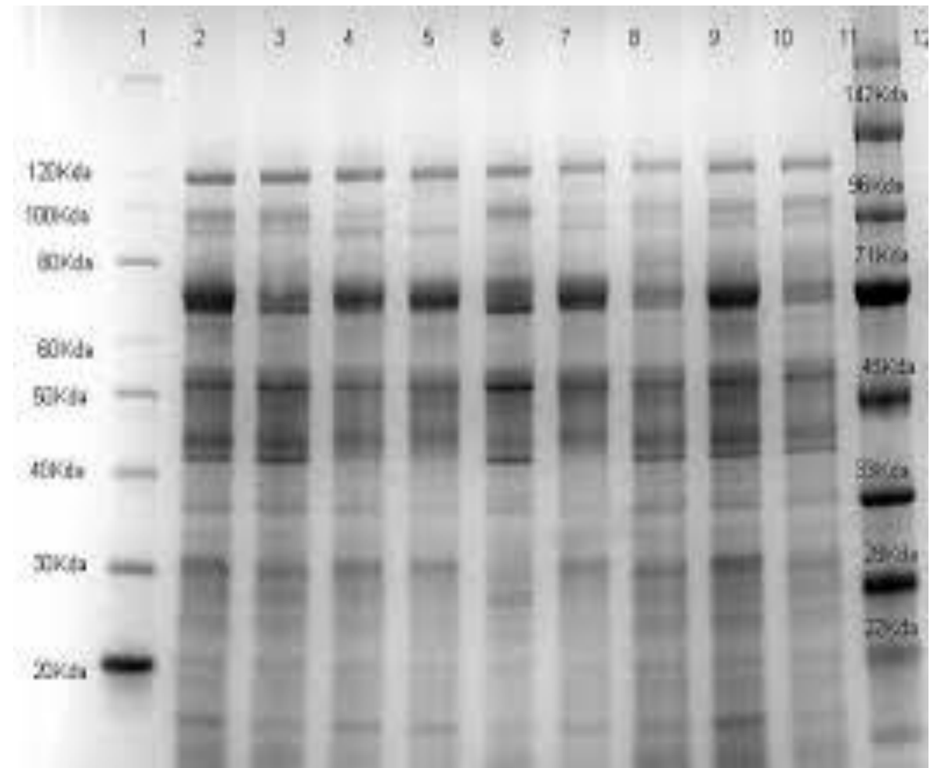


Steps in SDS-PAGE

- Extract Protein
- Solubilize and Denature Protein
- Separate Proteins on a gel
- Stain proteins (visualization)
- Analyze and interpret results

Gel Documentation System Protein





Thank You!!!