GEL ELECTROPHORESIS

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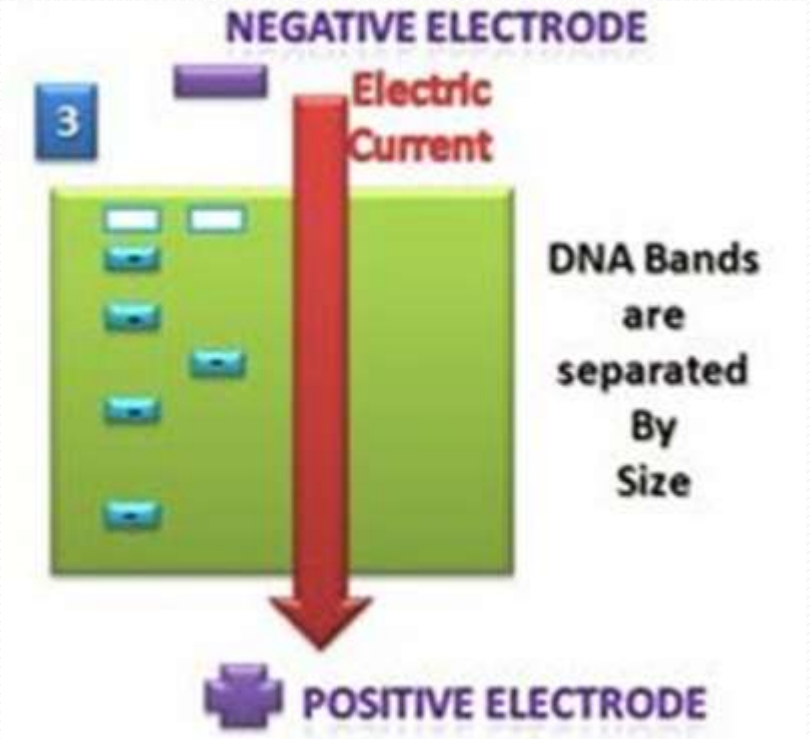
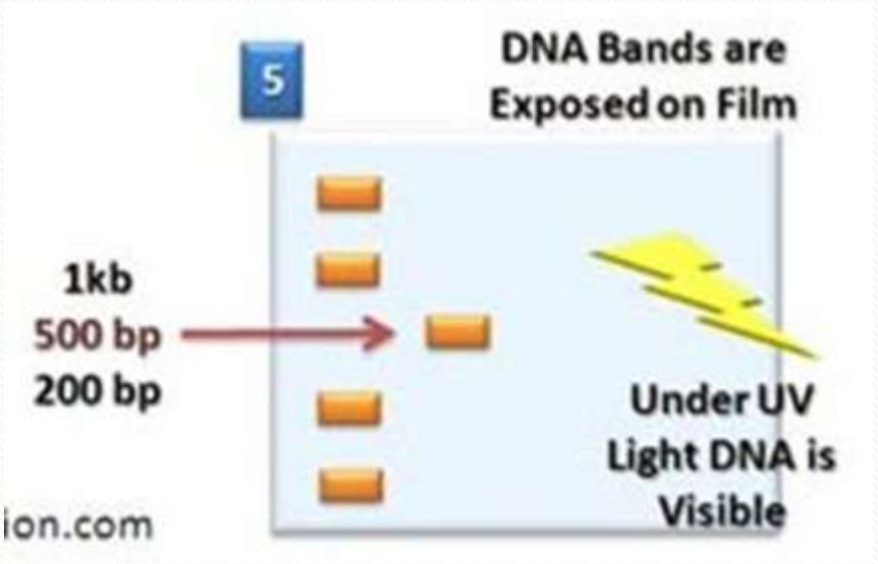
Outlines

- Electrophoresis
- Gel Electrophoresis
- Agarose Gel Electrophoresis
- Polyacrylamide Gel Electrophoresis
- Pulsed Field Gel Electrophoresis
- Procedure steps and factors affecting separation in gel electrophoresis
- Applications of Gel Electrophoresis

Electrophoresis

- Electrophoresis is a laboratory technique used to separate charged molecules like DNA, RNA, or protein molecules on a matrix in an applied electric field dependent on the phenomenon of electrostatic attraction.



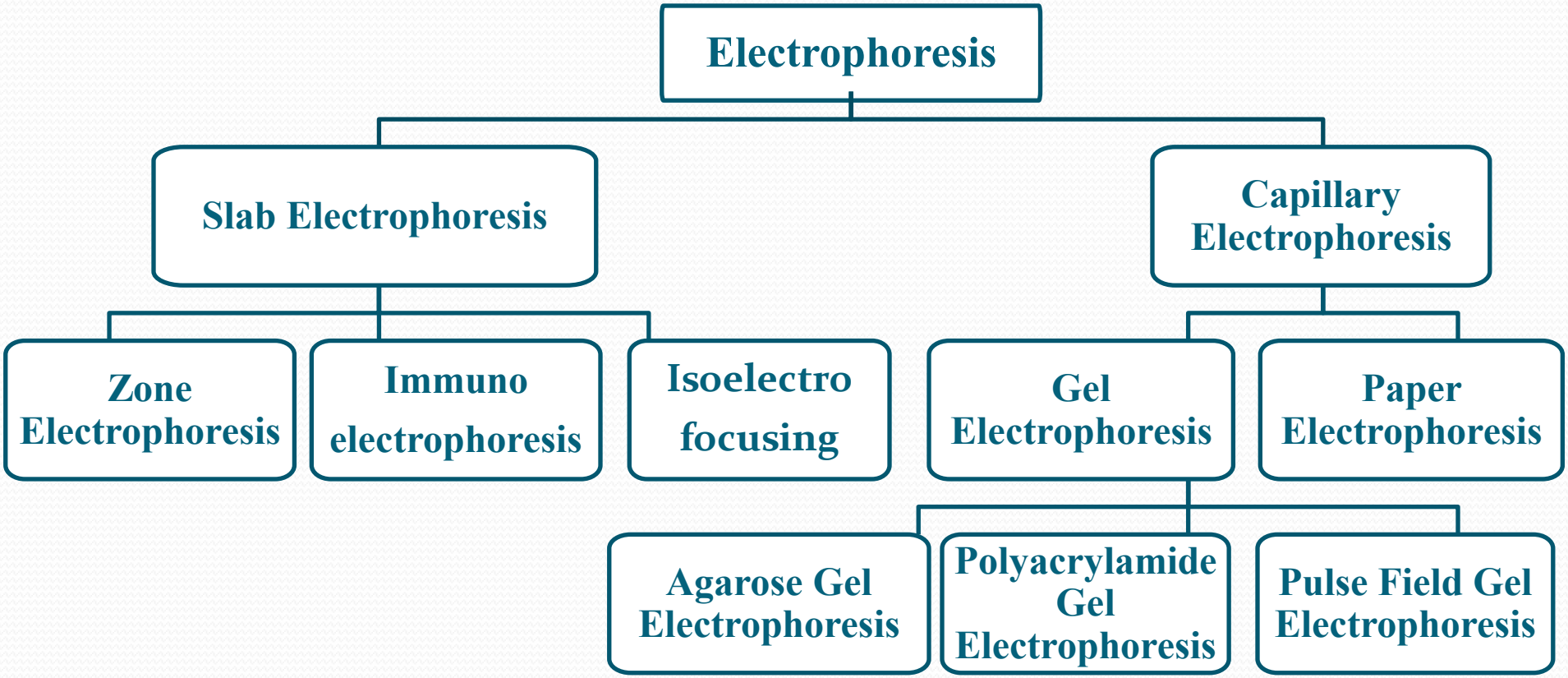


Principle of Electrophoresis

In separation the biomolecules through the matrix is based on:

- **Charge:** When charged molecules are placed in an electric field, they migrate toward either the positive (anode) if they are negatively charged or negative (cathode) pole if they are positively charged.
- **Size:** The smaller molecules move more swiftly than the larger-sized ones, as they can travel through the pores more easily than the later.

Types of Electrophoresis



Gel Electrophoresis

- The gels are an inert matrix and have crosslink structure, so they form pores, and the size of the pores relative to that of the molecule determines whether the molecule will enter the pore and be retarded or will bypass it. Thus, Pores in the gel work like a sieve, allowing smaller molecules to move faster than larger molecules.
- Samples are loaded into wells (indentations) at one end of a gel, and an electric current is applied to pull them through the gel.
- A simple, rapid, and sensitive analytical technique for the separation of charged particles.
- The resolution of a sample is better in a gel than in any other type of medium.

Factors affecting separation in gel electrophoresis

- **The sample**
  - The charge/mass ratio of the sample dictates its electrophoretic mobility.
    - ✓ **Charge**: The Higher the charge, the greater the electrophoretic mobility.
    - ✓ **Size**: Size is inversely proportional to electrophoretic mobility, thus the larger molecules are separated nearer the well and the smaller molecules farther away.
    - ✓ **Shape**: Globular substances move faster than the fibrous ones.

Factors affecting separation in gel electrophoresis

The other conditions used during electrophoresis also affect the electrophoretic mobility of separated biomolecules, so they must to be adjusted to separate molecules in a specific size range.

- ✓ **The electric field:** An increase in the potential gradient increases the rate of migration.

Size	Voltage	Buffer	
		Recovery	Analytical
≤1 kb	5 V/cm	TAE	TBE
1 kb to 12 kb	4 - 10 V/cm	TAE	TAE/TBE
>12 kb	1 - 2 V/cm	TAE	TAE

Factors affecting separation in gel electrophoresis

The medium: The inert medium can exert molecular sieving, influencing the rate of particle migration, such as agar, polyacrylamide, which have cross-linked structures giving rise to pores within the gel beads.

1X TAE Buffer			1X TBE Buffer	
XC	BPB	% Agarose	XC	BPB
24,800	2,900	0.30	19,400	2,850
11,000	1,650	0.50	12,000	1,350
10,200	1,000	0.75	9,200	720
6,100	500	1.00	4,100	400
3,560	370	1.25	2,500	260
2,800	300	1.50	1,800	200
1,800	200	1.75	1,100	110
1,300	150	2.00	850	70

Factors affecting separation in gel electrophoresis

The buffer: the buffer can affect the electrophoretic mobility by:

Ionic strength: increase in ionic strength of buffer means a larger share of current is carried by buffer and smaller proportion by sample, while decrease in ionic strength is vice-versa.

Most routinely used buffers are:

TAE- (Tris-acetate-EDTA), it has lower buffering capacity and higher ionic strength, thus it is generally used to separate larger nucleic acid fragments

TBE- (Tris-borate-EDTA), it has high buffering capacity with lower ionic strength, thus it is generally used for the separation of low molecular weight compounds

Factors affecting separation in gel electrophoresis

1X TAE Buffer			1X TBE Buffer	
XC	BPB	% Agarose	XC	BPB
24,800	2,900	0.30	19,400	2,850
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2,800	300	1.50	1,800	200
1,800	200	1.75	1,100	110
1,300	150	2.00	850	70

Equipment of Gel Electrophoresis

✓ Instrument

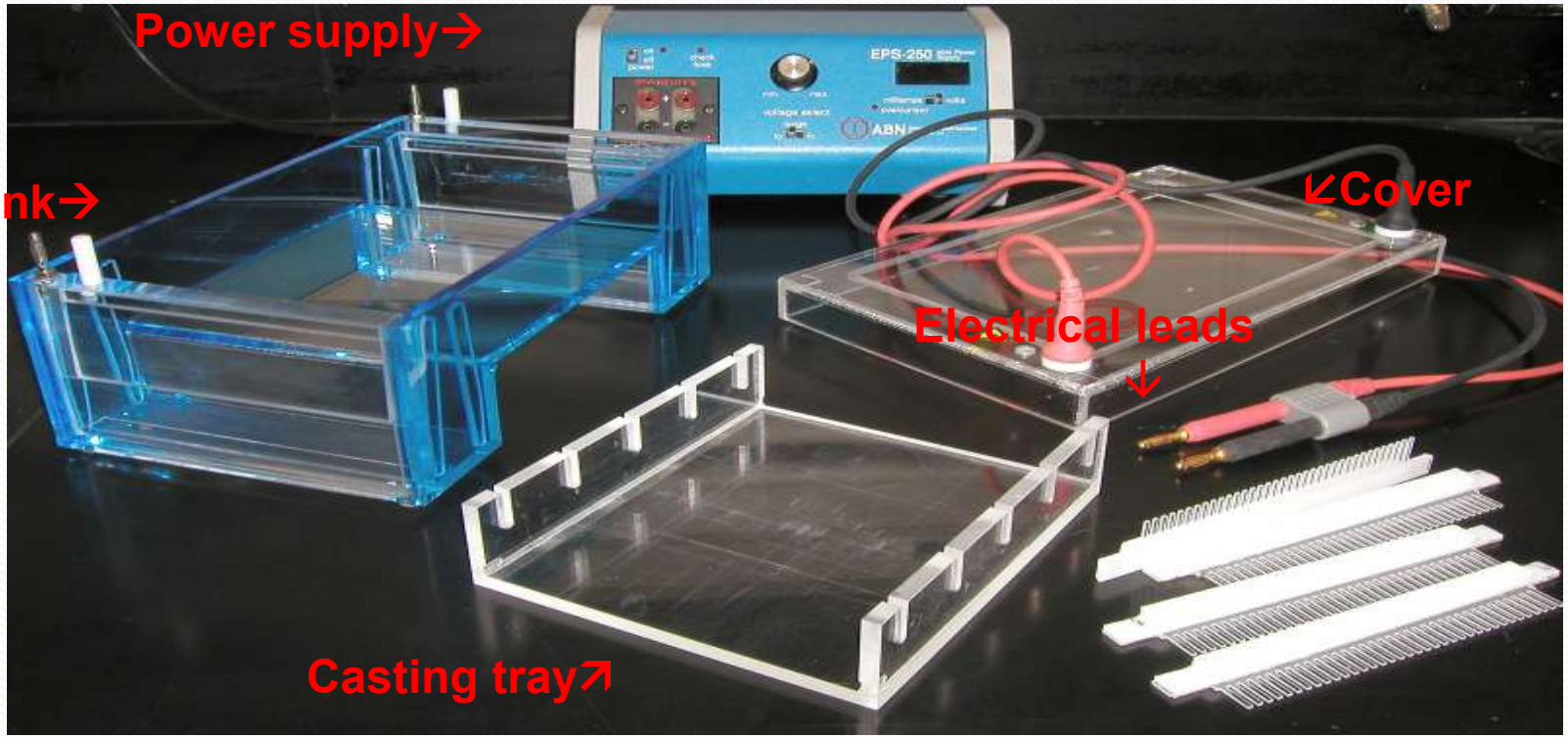
- Electrophoresis apparatus
- Power supply
- Transilluminator (An ultraviolet light box)
- Blot techniques apparatus for quantification the samples

✓ Reagents

- Electrophoresis Buffer
- Supporting media (Gels)
- Stains for detection

Electrophoresis apparatus

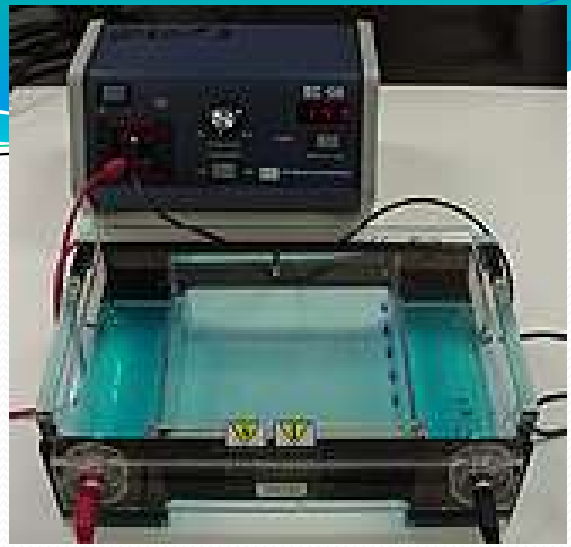
- The casting tray is made up of glass or plastic.
- The comb contains varying number of teeth in order to help in formation of well.



Set up the Electrophoresis apparatus

- Electrophoresis chamber with buffer solution
- Casting tray
- Electrodes

Power supply



- The electrodes are connected to their respective terminals of the electrophoresis chamber and the power supplier controls the rate of current flow and voltages depend on
- The size of the gel tank
- The sizes of sample bands that you want to separate.

So we use 10 Volts per centimetre between the gel tank electrodes (not the gel length) for particle sizes between (1Kb to 12Kb).

		Buffer	
Size	Voltage	Recovery	Analytical
≤1 kb	5 V/cm	TAE	TBE
1 kb to 12 kb	4 - 10 V/cm	TAE	TAE/TBE
>12 kb	1 - 2 V/cm	TAE	TAE

- Blot techniques apparatus for quantification the samples

  - Southern blotting apparatus (for DNA)
  - Northern blotting apparatus (for RNA)
  - Western blotting apparatus (for protein)

Supporting media (Gel) Preparation

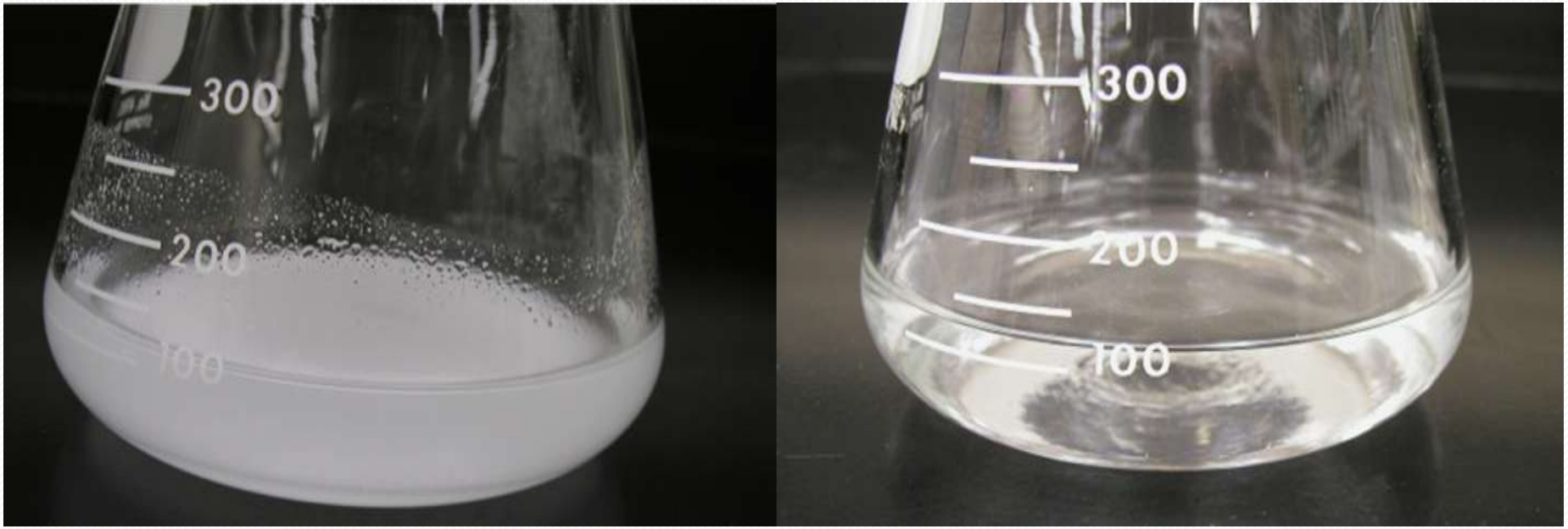
1. Agar/agarose
2. Polyacrylamide

- Agarose and polyacrylamide gels are cross-linked structures; choosing one of them depends on the type and size of molecules to be separated
- It is important that the support media is electrically neutral as the presence of a charge group may cause migration retardation

Agarose gel solution preparation

Agarose Solution 0.3-2%

- 0.3-2g agarose boiled in 100 ml of TBE buffer 0.5x



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.

Polyacrylamide gel solution preparation

Polyacrylamide gel Solution 3.5-20%

- 30-1% acrylamide to bisacrylamide and 3% ammonium persulphate in H2O and completed to 100 ml of TBE buffer 1x

	Polyacrylamide gel percentage				
Reagents	3.5%	5.0%	8.0%	12.0%	20.0%
30% acrylamide/bis acrylamid	11.6	16.6	26.6	40.0	66.6
H_2O	76.3	71.3	61.3	47.9	21.3
3% ammonium persulphate	2.1	2.1	2.1	2.1	2.1
1x TBE	10.0	10.0	10.0	10.0	10.0
Total	100.0	100.0	100.0	100.0	100.0

Electrophoresis running buffer

TBE 5x (stock)1L

- 850 ml of dH2O,
- 20 ml of 0.5M EDTA (pH 8), 46.5 EDTA in 200ml of water, 5 g of NaOH pellets to adjust pH to 8.0, as EDTA won't dissolve until pH is 8.0
- 54g Tris base
- 27.5 g Boric Acid
- Pour into a graduated cylinder and fill up to 1 L with dH2O, autoclave

Working solution for agarose gel preparation is 0.5x

Whereas for polyacrylamide preparation gel is 1x

Loading buffer and dye solution preparation

Loading buffer: Nucleic acid is before loading on to a gel is first mixed with the gel loading buffer, which usually consists of:-

Salts Tris-HCL : It creates an environment with favorable ionic strength and pH for the sample

Metal chelator EDTA: It prevents nucleases to degrade the nucleic acid

Loading dyes: It provides color for tracking and easy monitoring of sample. Such as, **xylene cyanol, bromophenol blue** that contain :

0.25 % Bromophenol blue.

40 % W\V Sucrose in water.

Stains for detection

Stains for detection DNA

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

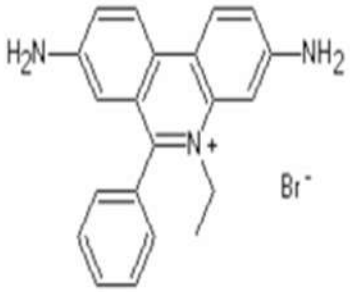
Ethidium bromide can be added to the gel and/or running buffer before the gel is run or the gel can be stained after it has run with **(0.5 µg\ml)** for about **20 min** and **stock solution** is **10mg\ml** that stored at **RT**.

SYBR Green I is more expensive, but 25 times more sensitive and possibly safer than ethidium bromide.

SYBR Safe is a variant of SYBR Green, and shows low level of mutagenicity and toxicity.

Cresol red and **Orange G** are less frequently used markers.

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



Safer alternatives to ethidium bromid staining

- Safer alternatives to Ethidium Bromid

- Methylene Blue
- BioRAD - Bio-Safe DNA Stain
- Ward's - QUIKView DNA Stain
- Carolina BLU Stain
- …others

advantages

Inexpensive

Less toxic

No UV light required

No hazardous waste disposal

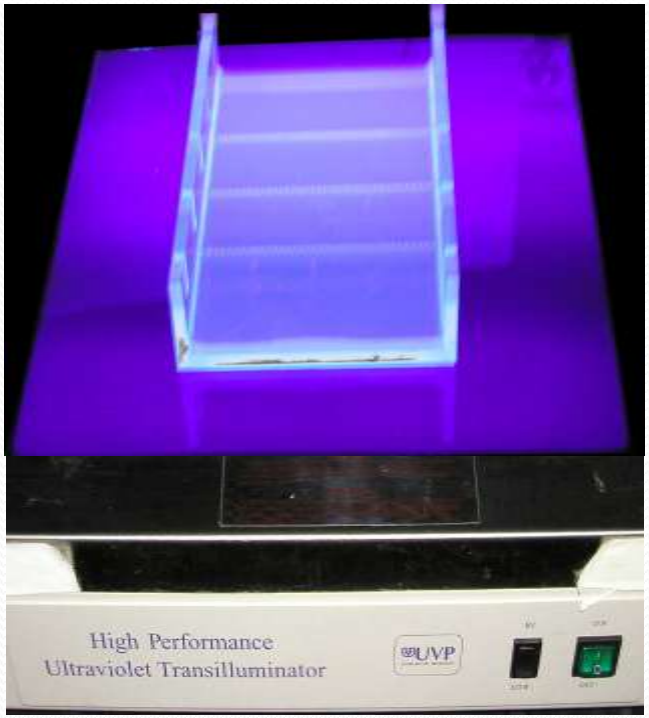
disadvantages

Less sensitive

More DNA needed on gel

Longer staining/destaining time

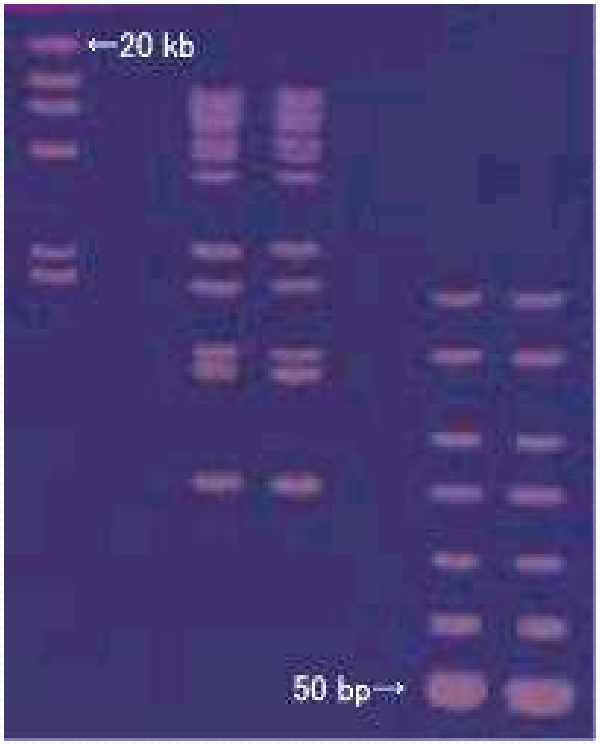
Visualize DNA stained with Ethidium Bromide on Ultraviolet light source



Transilluminator:

(Ultraviolet light box)

used to visualize bands in gels.

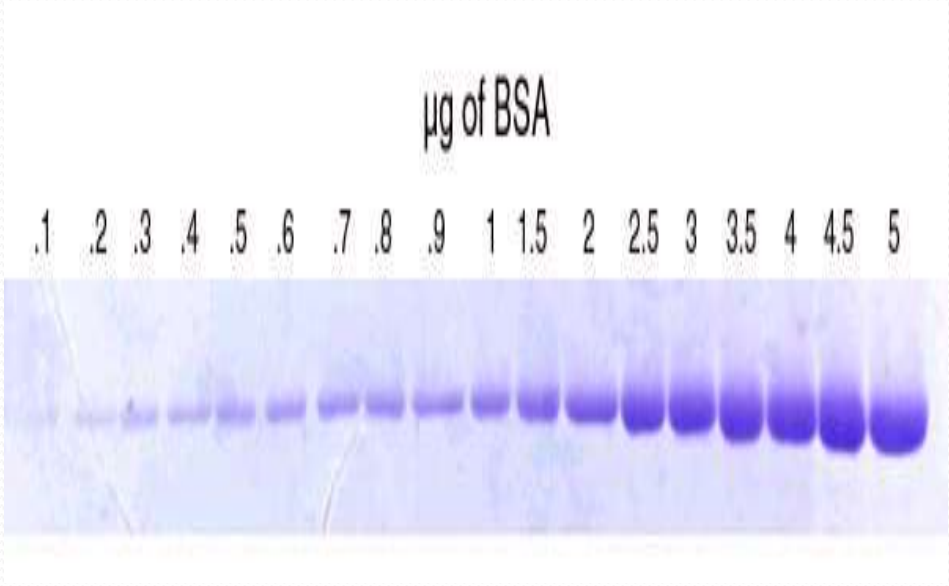


Ethidium bromide

Stains for detection proteins

Stains for detection proteins

Proteins visualized using Silver stain or Coomassie Brilliant Blue dye.



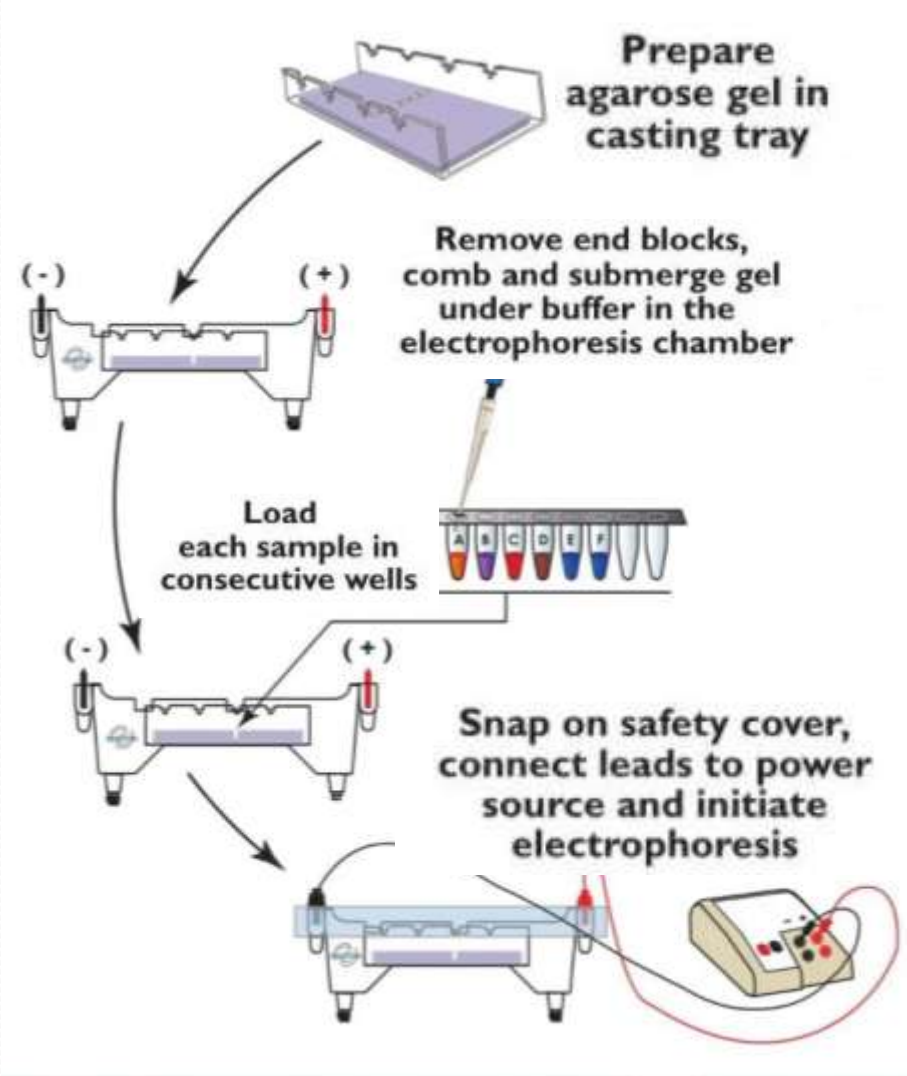
Characterization and procedures of gel Electrophoresis types

❑ Agarose Gel Electrophoresis

- Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool.
- They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation.
- Potential difference is applied across the electrodes in a horizontal electrophoretic tank containing agarose gel and biomolecules (such as nucleic acid or proteins) is loaded, then molecules migrated to their respective electrodes.

Steps Involved in AGE

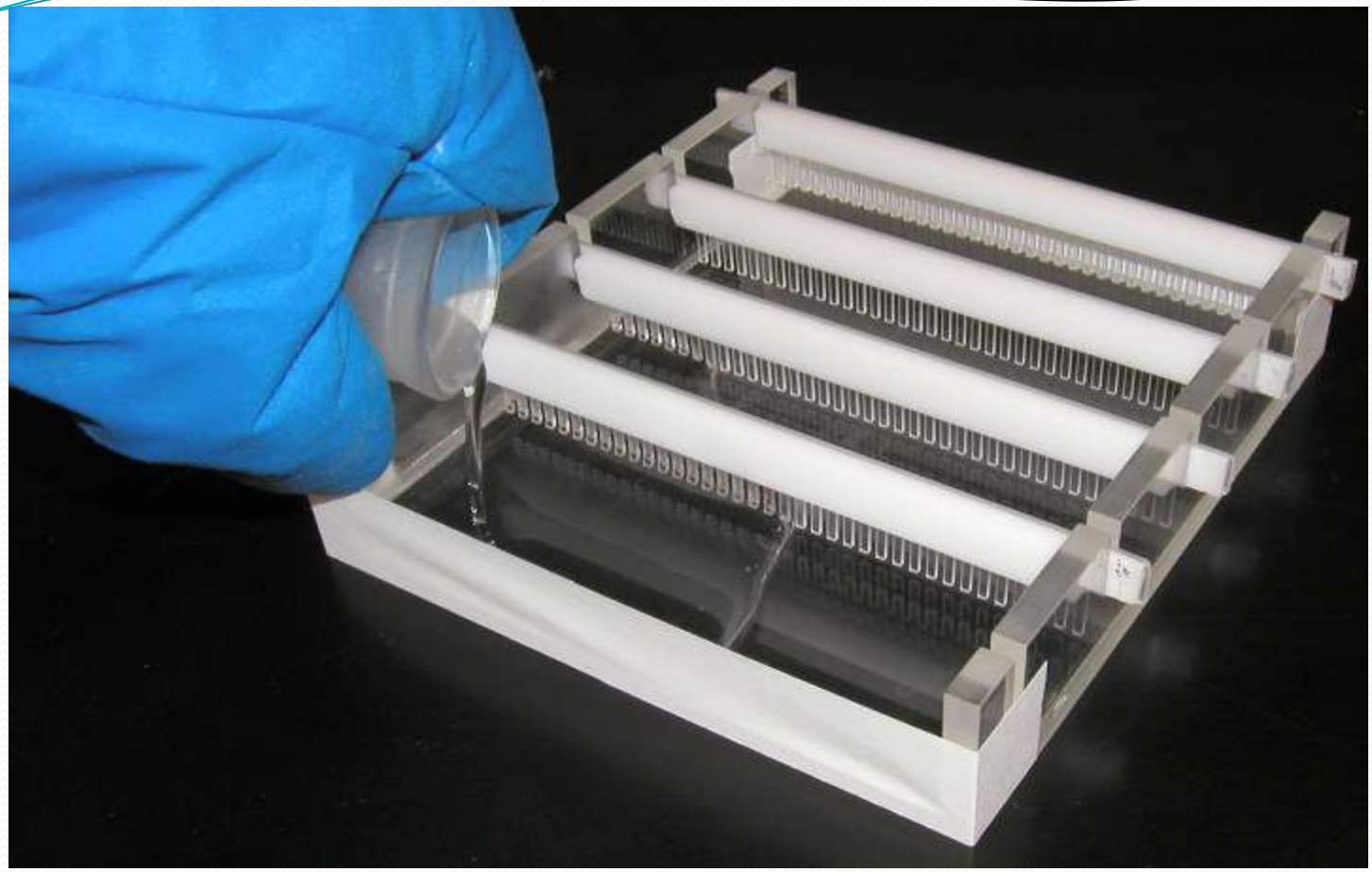
- Prepare sample and buffer
- Prepare an agarose gel solution
- Gel casting
- Setting up the electrophoresis chamber
- Gel loading and sample loading
- Process of electrophoresis
- Observe the DNA by exposing it to UV light



Procedure of agarose gel electrophoresis

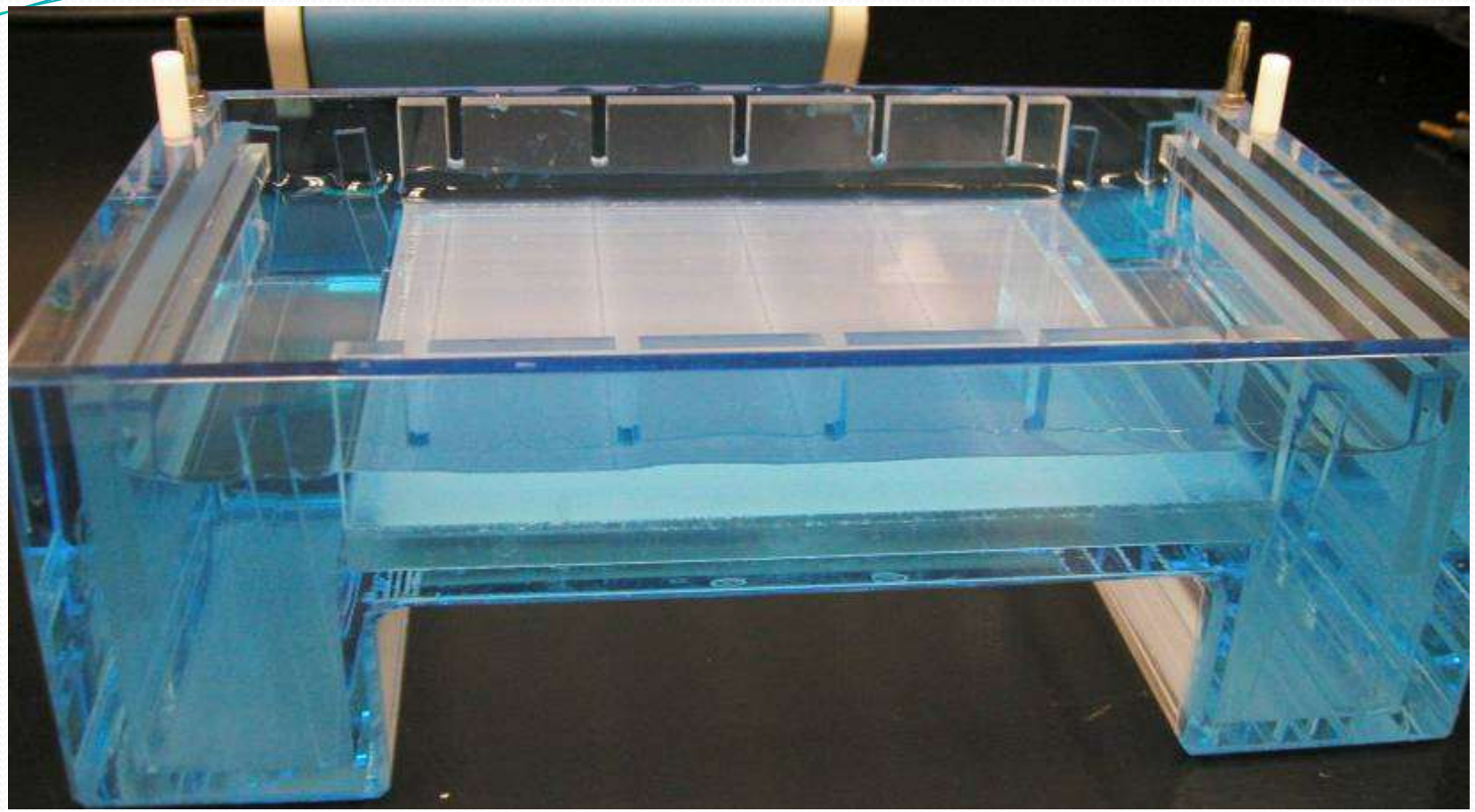
1. Pour the 100 ml of melted agarose into the gel casting tray.
2. Apply combs.
3. Let the agarose solidify for at least 15 minutes. Place the casting tray with the gel on it in the gel box.
4. Add running buffer until the gel is completely submerged, and the gel is now ready for use.
5. Use a micropipette and a clean tip to transfer the samples to wells.
6. Place the lid on the gel box and fit the power cords over the two electrodes.
7. Normally, you would want to stop the gel when the bromophenol blue (dark blue) dye line is near the end edge of the gel. Turn off the power supply and disconnect the leads
8. Carefully remove the gel tray and gel from it.
9. After gel running UV light or UV illuminator used to visualize the bands.

Pouring the gel



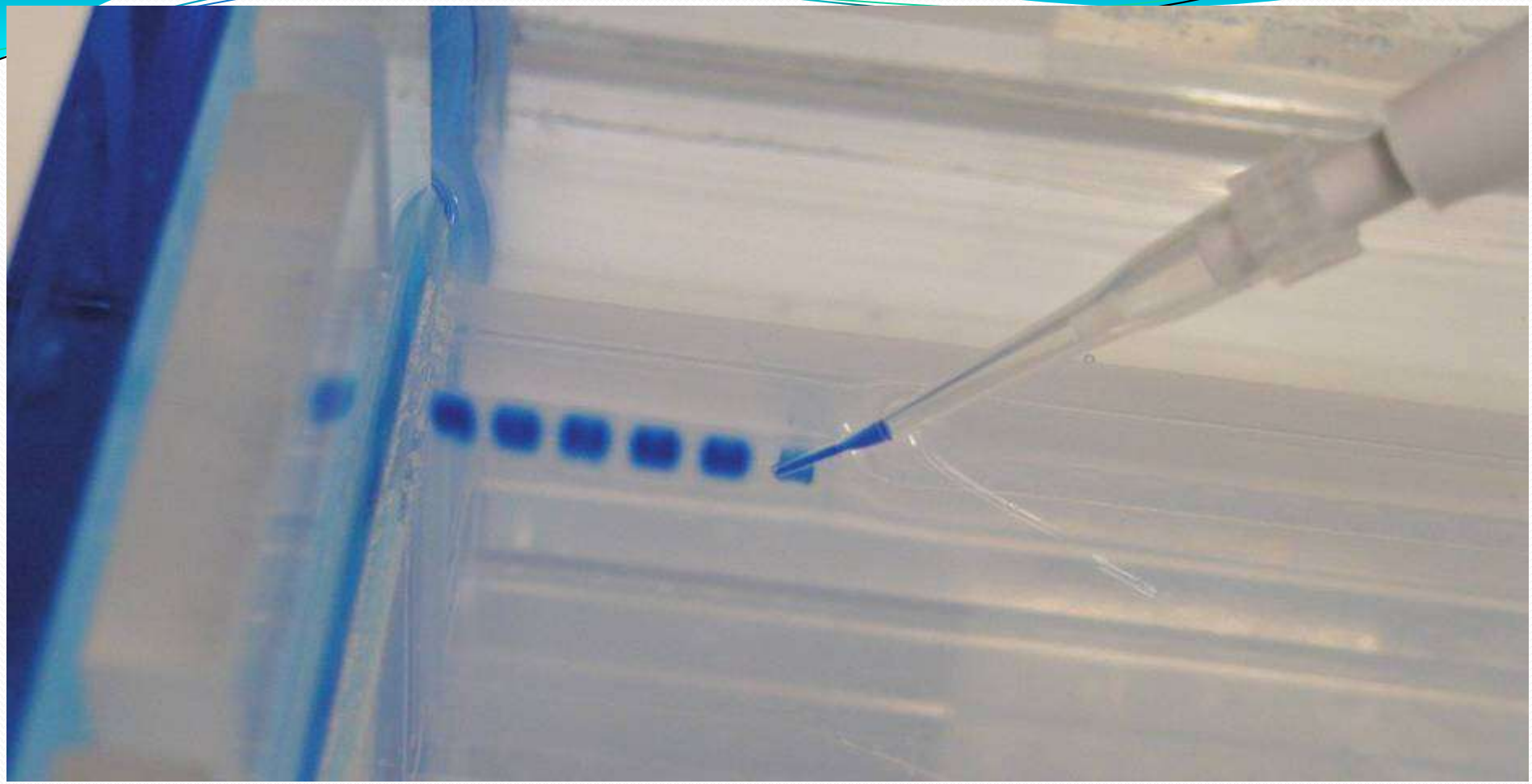
Allow the agarose solution to cool slightly (~60°C) and then carefully pour the melted agarose solution into the casting tray. Avoid the bubbles.

Electrophoresis buffer



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

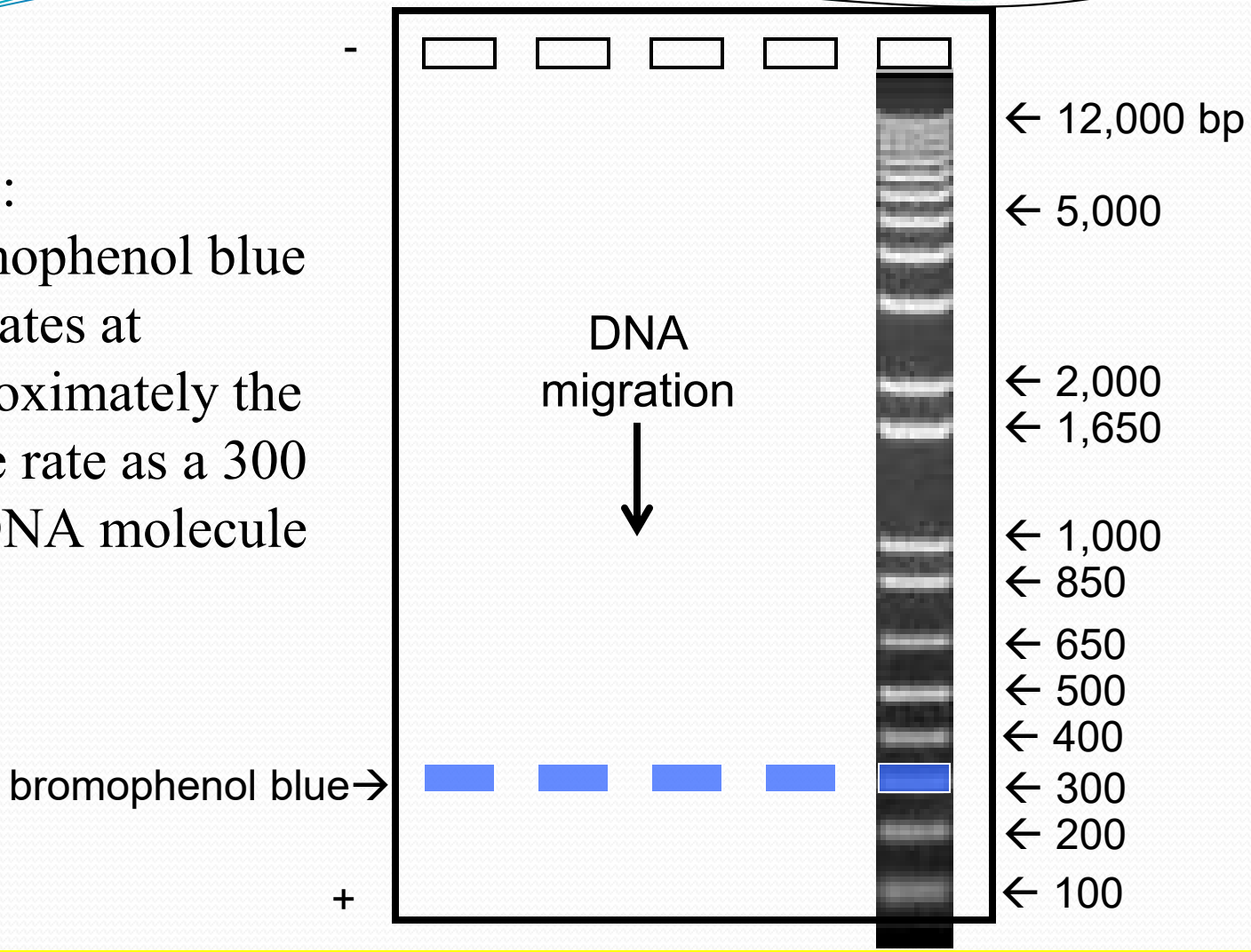
Loading the samples



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

DNA Ladder Standard

Note:
bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule



Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

Polyacrylamide Gel Electrophoresis (PAGE)

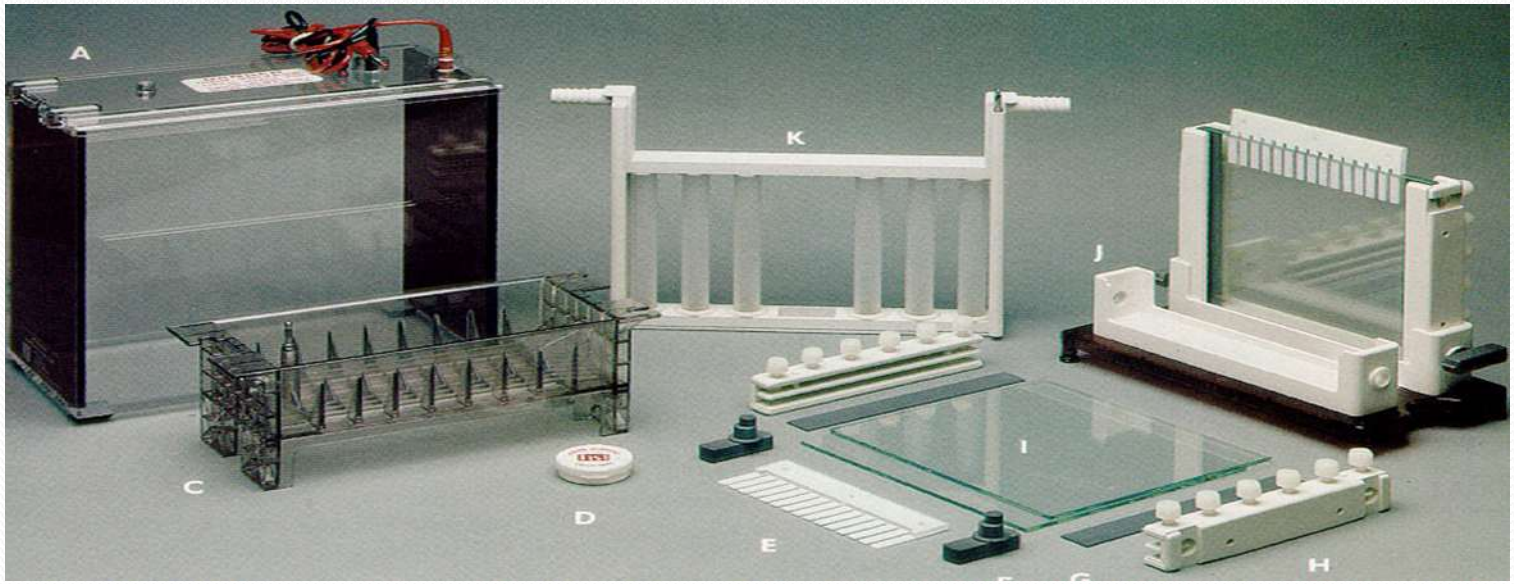
- A technique widely used in biochemistry, genetics, molecular biology and biotechnology to separate small molecular weights of proteins or nucleic acids, according to their electrophoretic mobility.
- The most commonly used form of polyacrylamide gel electrophoresis is the Sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS- PAGE) used mostly for the separation of proteins.
- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N, N'-methylenebisacrylamide.

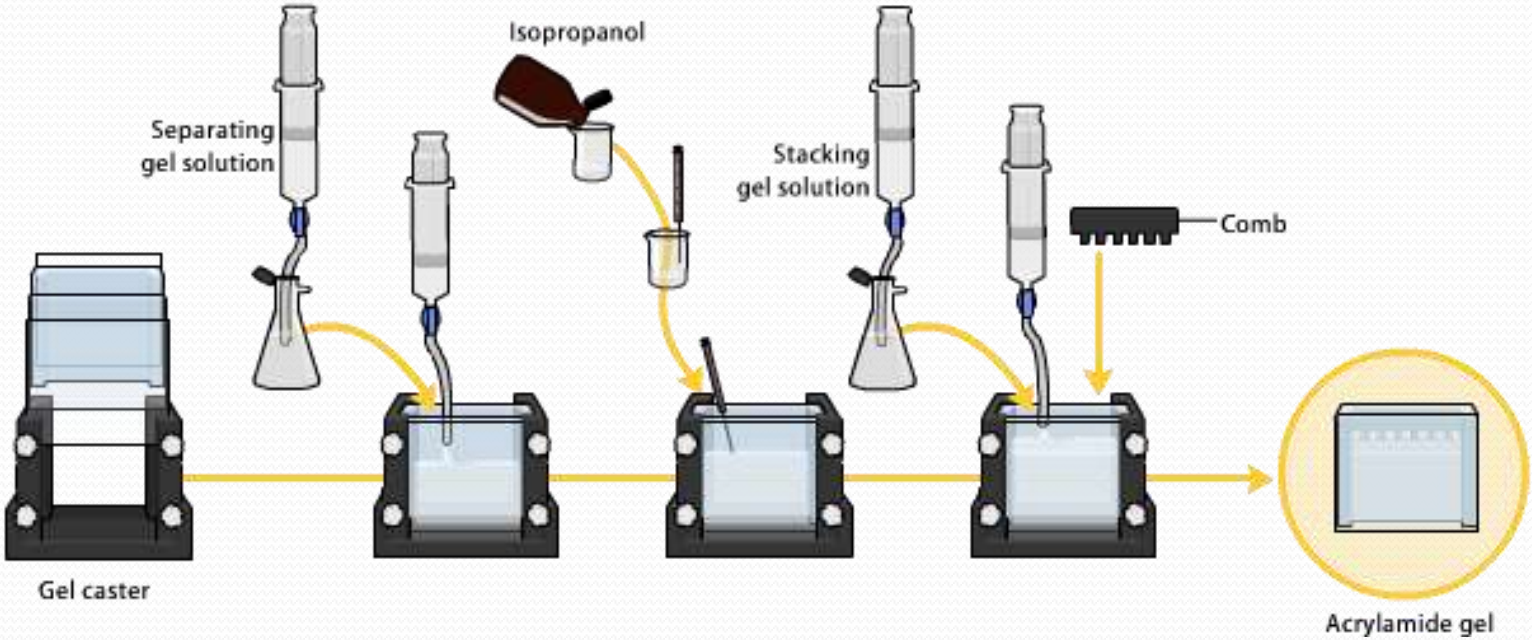
Steps Involved in Polyacrylamide Gel Electrophoresis

Preparation of polyacrylamide gel

- The gels typically consist of acrylamide, bisacrylamide, produces cross-linked polymerized structure.
- The ratio of bisacrylamide to acrylamide can be varied for special purposes, but is generally about 1 part in 30 (1:30). The acrylamide concentration of the gel can also be varied, generally in the range from 3.5% to 25%.
- Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages of acrylamide are needed to resolve smaller proteins.
- Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells.
- After the gel is polymerized 30-60 minutes at room temperature, the comb can be removed and the gel is ready for electrophoresis.

Preparation of polyacrylamide gel

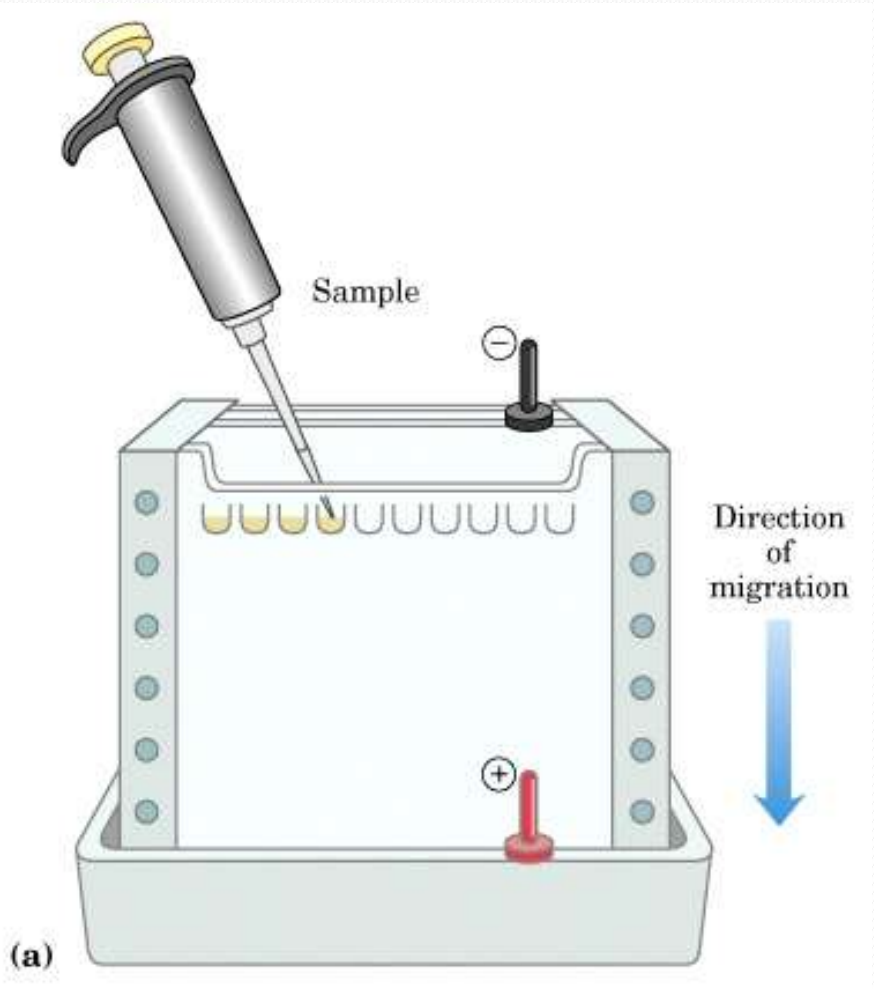




Procedure of polyacrylamide gel electrophoresis

- Use a Pasteur pipette to mix the samples with the appropriate amount of gel loading buffer. Load the mixture into the wells.
- Connect the electrodes to a power pack, turn on the power, and begin the electrophoresis run.
- Run the gel until the marker dyes have migrated the target distance. Turn off the electric power, disconnect the leads, and discard the electrophoresis buffer from the reservoirs.
- Detach the glass plates. Lay the glass plates on the bench. Use a spacer or plastic wedge to lift a corner of the upper glass plate. Check that the gel remains attached to the lower (white) plate. Pull the upper plate smoothly away. Remove the spacers.
- Stain gels with SYBR gold or dry gels are exposed to film or Phospho-Imager screen.

Polyacrylamide electrophoresis



Differences bw Agarose and Polyacrylamide Electrophoresis

Agarose Gel

- Run in horizontal configuration.
- Great range of separation for nucleic acids (200 bp -50 kb) and for proteins more than 200 KDa.
- Agarose is natural product extracted from seaweed
- Non-toxic
- Staining before or after pouring the gel
- Simple to prepare and handle.
- Low resolution separations for larger DNA molecules (Restriction Fragment Length Polymorphism Analysis)

Polyacrylamide Gel

- Run in vertical configuration.
- Low range separation for nucleic acids (5-500 bp) and for proteins ranging from molecular weight 5-200 KDa.
- Made of chemicals only
- Neurotoxin
- Staining after pouring the gel
- Difficult to prepare and handle
- High resolution separations for smaller DNA molecules ((STR) Short Tandem Repeat analysis and DNA sequence analysis)

Applications

Gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA , RNA or proteins in a matrix of gel.

Analysis of PCR products.

Used in molecular genetic diagnosis or genetic fingerprinting.

Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis, or proteins before Western blotting.

Widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g., in restriction mapping of cloned DNA.

Estimation of protein size and its molecular weight.

Determination of protein subunits or aggregation structures.

❑Pulsed-field Gel Electrophoresis (PFGE)

- Pulsed Field Gel Electrophoresis (PFGE) is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction so used by scientists to produce a DNA fingerprint for a bacterial isolate.
- As DNA larger than 50kb migrates through a gel together in a size-independent manner, the standard gel electrophoresis technique was unable to separate very large molecules of DNA effectively which led to the practice of pulsed field gel electrophoresis.
- In 1982, Schwartz introduced the concept that DNA molecules larger than 50 kb can be separated by using two alternating electric fields

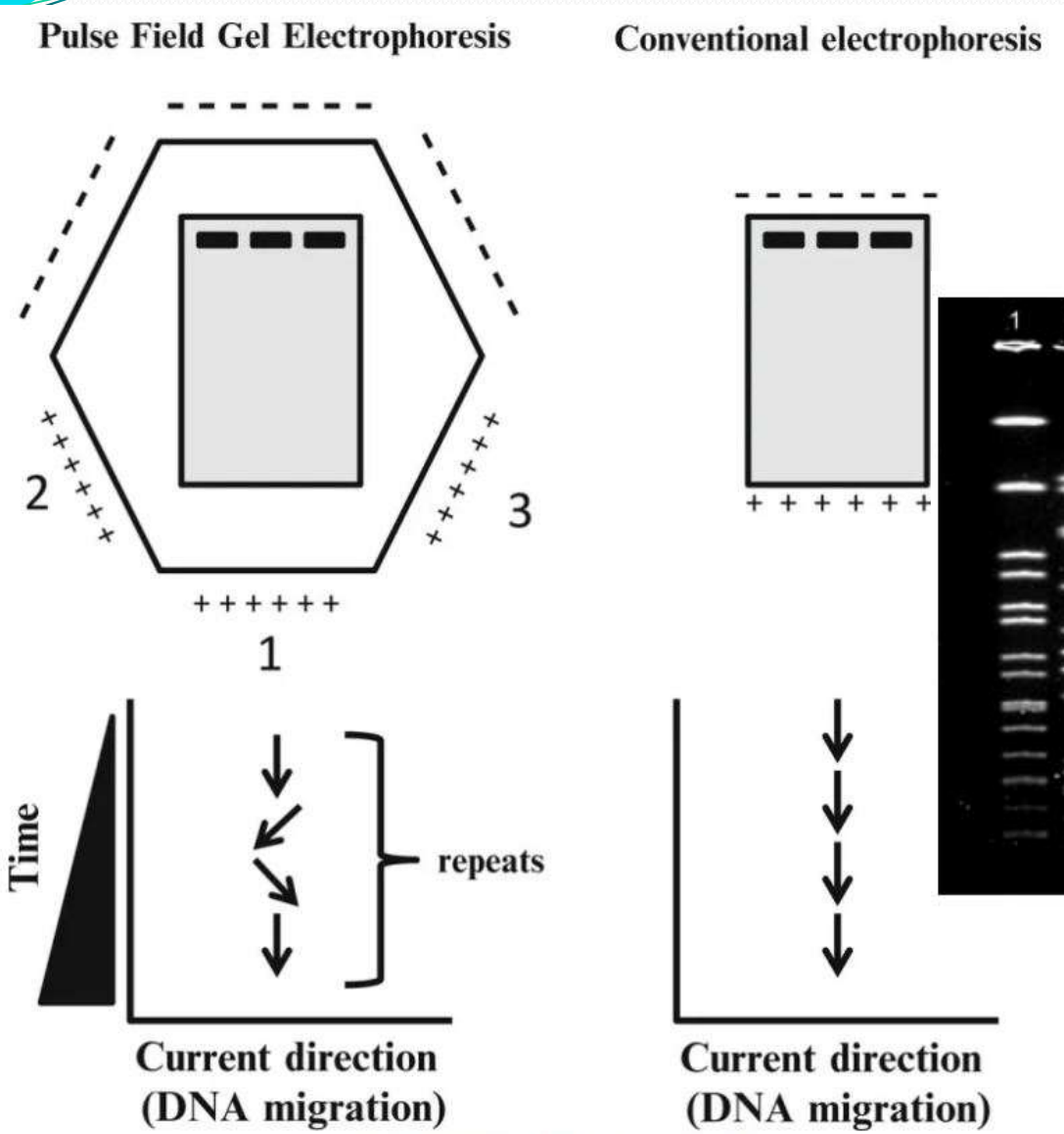
Principle of Pulsed Field Gel Electrophoresis (PFGE)

- While in general small fragments can find their way through the gel matrix more easily than large DNA fragments, a threshold length exists above 50 kb where all large fragments will run at the same rate, and appear in a gel as a single large diffuse band.
- However, with periodic changing of field direction, the various lengths of DNA react to this change at differing rates.
- That is, larger pieces of DNA will be slower to realign their charge when field direction is changed, while smaller pieces will be quicker.
- Over the course of time, with the consistent changing of electric field directions, each band will begin to separate more and more even at very large lengths.

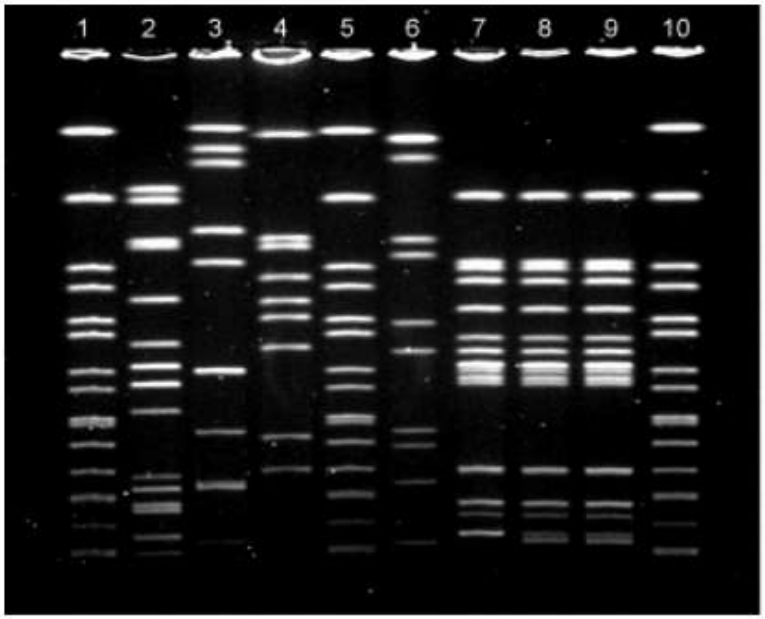
Procedure of Pulsed Field Gel Electrophoresis

- The procedure for 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
  - two that run at an angle of 60 degrees on either side.
- The pulse times are equal for each direction, resulting in a net forward migration of the DNA.

❑Pulsed-field Gel Electrophoresis (PFGE)



PFGE vs Conventional electrophoresis (Image source: Ref-2)



DNA Fingerprint profile generated by PFGE

Steps Involved in Pulsed-field gel electrophoresis

Lysis:

- First, the bacterial suspension is loaded into an agarose solution to protect the chromosomal DNA from mechanical damage by immobilizing it in agarose blocks.
- Then the bacterial cells are lysed to release the DNA by the proteinase K enzyme

Digestion of DNA:

- The bacterial DNA is treated with restriction enzymes to yield less number of larger size DNA fragments .

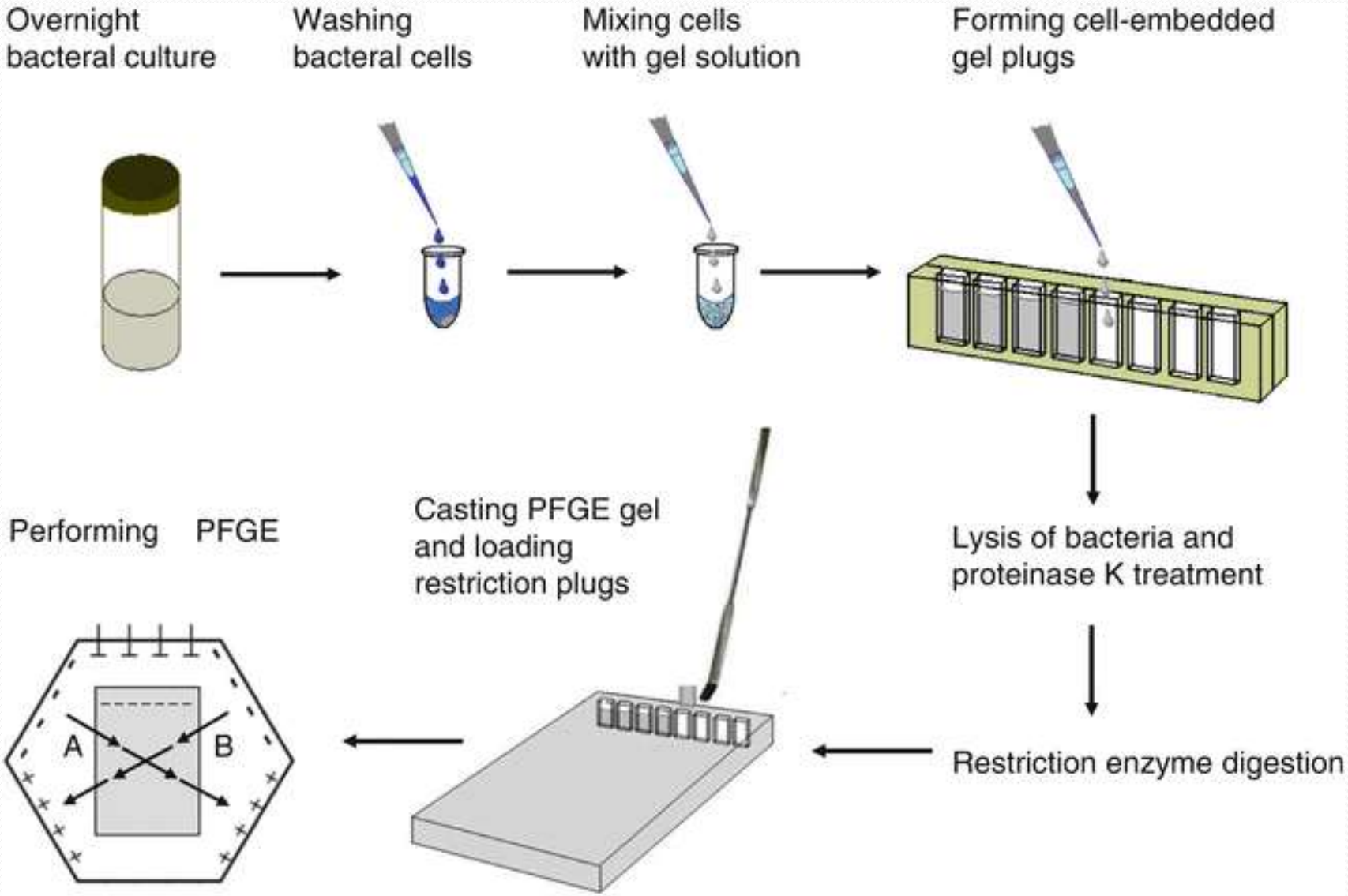
Electrophoresis:

- The larger pieces of DNA are subjected to pulse field gel electrophoresis by applying electric current and altering its direction at regular intervals.

Analysis:

- The fragments of different organisms' DNA generated by PFGE are compared to standards manually or by computer software like BioNumerics.

Steps Involved in Pulsed-field gel electrophoresis



Applications of Pulsed Field Gel Electrophoresis (PFGE)

- Since, PFGE separates DNAs from 50 kb to upto10 Mb pairs

, characterization of such large fragments has allowed construction the physical map for the chromosomes, genotyping and genetic fingerprinting of several bacterial species

- Successfully applied to the subtyping of many pathogenic bacteria and has high concordance with epidemiologically relatedness so it is used to link environmental or food isolates with clinical infections.
- DNA restriction patterns generated by PFGE are stable and can be used for reproducible purpose

References:

- Instrumental methods of chemical analysis. By Dr. B.K.Sharma, Page no. 661-670.
- Instrumental analysis by William Kemp.
- http://www.intechopen.com/books/gel-electrophoresisprinciples-and-basics
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THANK YOU