Lecture One

12 February 2020

BTG 314: Molecular Genetics Practical (2 Units: PH 90)

Course Lecturer

Prof. Onasanya Amos

Course Lecture Time Table

Thursday
12-2pm

Continuous Assessment

S/N	MARKS
Class Attendance	10
Class Test	10
Practical	20
Examination	60
Total	100

Course Attendance

S/N	NAME OF STUDENT	MATRIC NO	DATE	SIGNATURE

Course Outline

BTG 314: Molecular Genetics Practical (2 Units: PH 90)

Experiments designed to achieve the practical components of BTG 301 and enrich students' grasp of the theoretical foundation of the course.

BTG 301: **Molecular Genetics (3 Units: LH 30)**
Principles of genetics at the molecular level. Chemical nature of hereditary material. The genetic code, regulatory mechanisms, the molecular basis of mutation. DNA replication and recombination.

Gel electrophoresis Techniques

Electrophoresis

The movement of charged particules (ions) in an electric field resulting in their migration towards the oppositely charged electrode is known as Electrophoresis

Electrophoresis

Molecules with a net positive charge (cations) move towards the negative cathode while those with net negative charge (anions) migrate towards positive anode

Electrophoresis

It is a widely used analytical technique for the separation of biological molecules such as

1. Plasma proteins
2. Lipoproteins
3. Immunoglobulins
4. DNA
5. RNA

Electrophoresis

The rate of migration of ions in an electric field depends on several factors such as

1. Shape
2. Size
3. Net charge
4. Salvation of the ions
5. Viscosity of the solution
6. Magnitude of the current used

Electrophoresis

Different types of electrophoresis

1. Zone electrophoresis
    1.Paper electrophoresis
    2.Gel electrophoresis
        1. Agarose gel electrophoresis
        2. Polyacrylamide gel electrophoresis (PAGE)
        3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
2. Isoelectric focusing
3. Immunoelectrophoresis

Agarose gel electrophoresis

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

For most applications, only a single-component agarose is needed and no polymerization catalysts are required

Agarose gel electrophoresis is a widely used procedure in various areas of biotechnology

It is also used in

1. Research
2. Biomedical
3. Forensic laboratories

Agarose gel electrophoresis

Of the various types of electrophoresis, agarose gel electrophoresis is one of the most common and widely used methods

It is a powerful separation method frequently used to

1. Analyze DNA fragments generated by restriction enzymes
2. Determining the size of DNA molecules in the range of 500 to 30,000 base pairs.
3. Separate other charged biomolecules such as dyes, RNA and proteins

Agarose gel electrophoresis technique practical steps

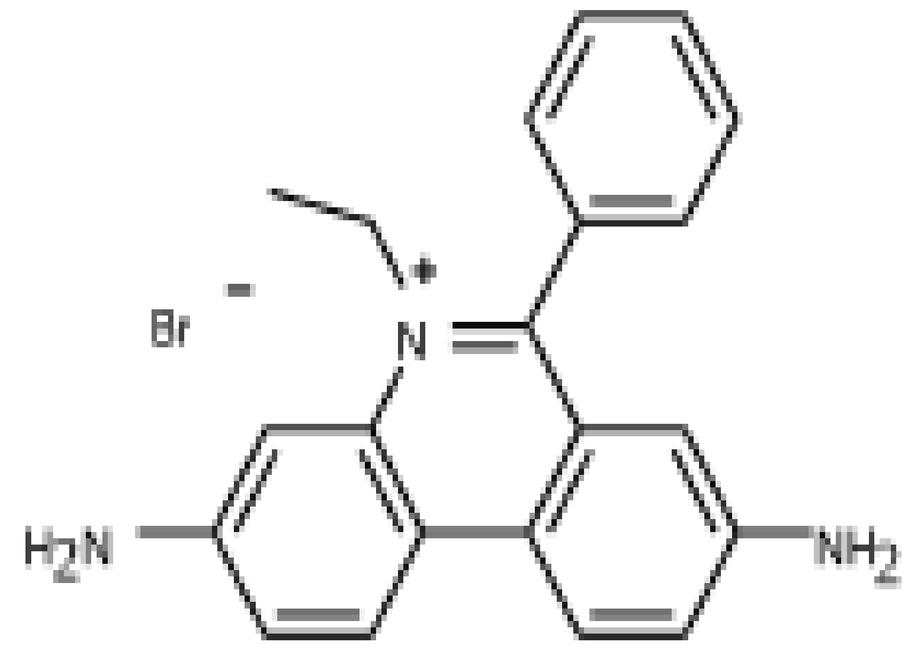
1. Sample type
   - DNA
   - RNA
   - PCR products
   - Restriction digested DNA

Agarose gel electrophoresis technique practical steps

2. Staining dye

For staining DNA or RNA in agarose gel thus making the separated in the agarose gel visible under UV fluorescence or Blue light is also convenient for visualization

1. Ethidium bromide
   (UV fluorescence)



2. SYBR Green I (Blue light)
3. SYBR Safe (Blue light)

Agarose gel electrophoresis technique practical steps

3. Loading buffer

Negatively charged loading buffers are commonly added to DNA or RNA prior to loading to the gel

Loading buffers are particularly useful because

1. they are visible in natural light
2. co-sediment with DNA or RNA

Examples of loading buffer include

1. Xylene cyanol
2. Bromophenol blue
3. Cresol Red
4. Orange G

Agarose gel electrophoresis technique practical steps

4. Standard DNA marker

Standard DNA marker is a powerful tool for estimating the molecular weight of linear, double ***stranded DNA*** fragments

Usually contains a set of known DNA fragments with different sizes in base pairs (bp) or kilo bases (kb)

An example is, 1 kb DNA ladder/DNA marker

4. Standard DNA marker

1 kb DNA ladder/DNA marker

Base pairs	Nanogram in each loading			
bps	**10ul**	**5ul**	**2ul**	**1ul**
15k	40	20	8	4
10k	20	10	4	2
7k	50	25	10	5
5k	80	40	16	8
3k	100	50	20	10
2k	200	100	40	20
1.5k	150	75	30	15
1k	100	50	20	10
800	80	40	16	8
700	70	35	14	7
500	50	25	10	5
400	40	20	8	4
300	30	15	6	3
200	20	10	4	2
100	10	5	2	1
50	20	10	4	2

5. Electrophoresis buffer

Several electrophoresis buffers can be used for fractionating DNA or RNA such as

1. Trisacetate-EDTA (TAE) pH 8
2. Tris-borate-EDTA (TBE) pH 8

Agarose gel electrophoresis technique practical steps

6. Preparing the agarose gel

Agarose gel volume (cm^3) = L x B x T

Where L = tray length, cm

B = tray width, cm

T = gel thickness, cm

Selecting the right gel tray,
L=10 cm, B=8 cm, T=0.5 cm

Agarose gel volume= 10 x 8 x 0.5 = 40 cm^3 or ml

Consider also the agarose gel concentration (%) needed to be prepared

Agarose gel concentration (%)

Concentration of agarose (%)	**DNA size range (bp)**
0.2	5000-40000
0.4	5000-30000
0.6	3000-10000
0.8	**1000-7000**
1	500-5000
1.5	300-3000
2	200-1500
3	100-1000

Consider also the agarose gel concentration (%) needed to be prepared

If the concentrations of agarose gel to be prepared is 0.8%

Then the amount of agarose (g) to weigh

$$= \frac{\text{Agarose gel volume}}{100} \times 0.8$$

$$= 40 \times 0.8/100$$

$$= 0.32 \text{ g}$$

Thus 0.32 g agarose powder will be mixed with 40 ml electrophoresis buffer (TAE or TBE), then heat in a microwave oven until completely dissolved.

Ethidium bromide is usually added to the gel at concentration of 0.5 µg/ml for nucleic acid visualization

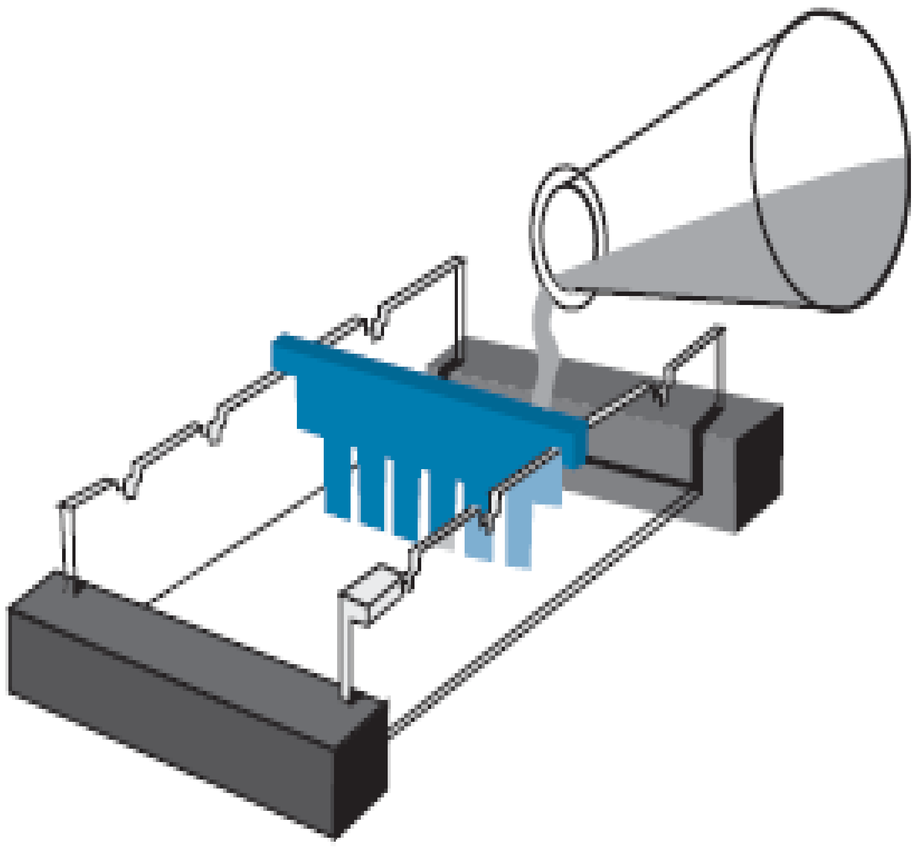
Agarose gel electrophoresis technique practical steps

7. Casting the agarose gel inside Electrophoretic tray

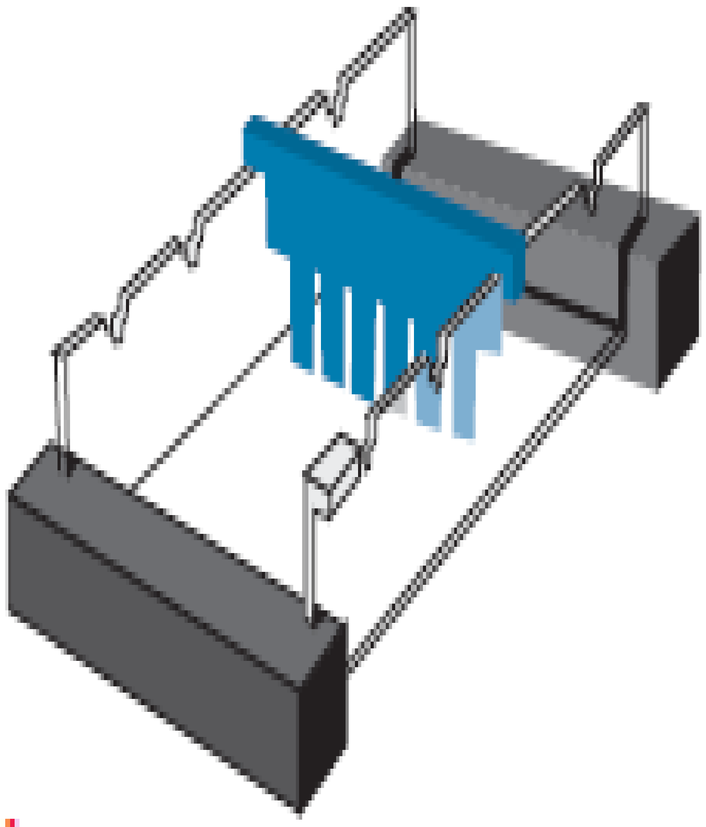
The mixture is cooled to 60^0C and poured into the casting tray to solidify

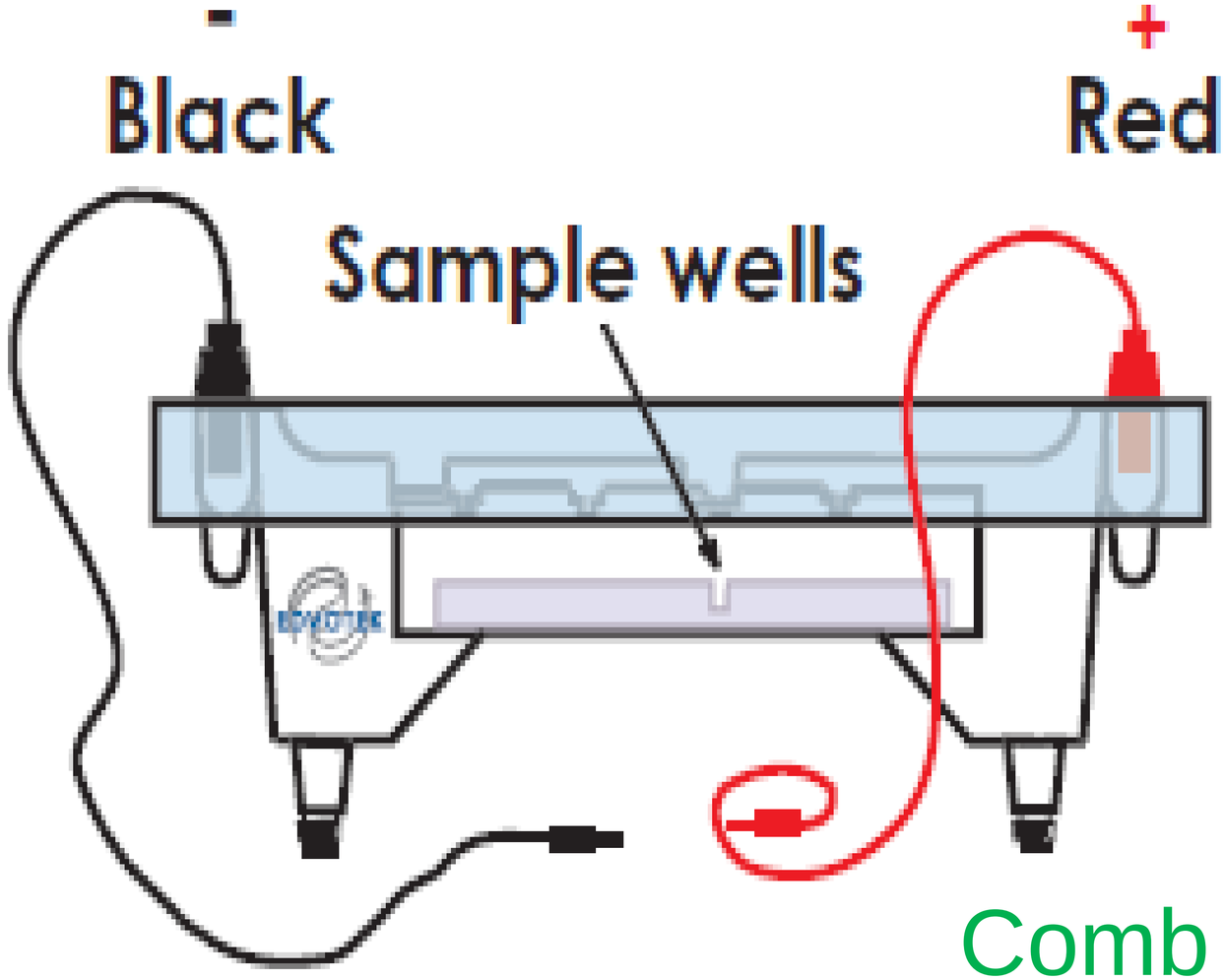
Immediately after the gel solidification, the tray is put inside electrophoresis tank already contained the electrophoresis buffer, after which the comb is removed

Agarose gel pouring



Agarose gel solidified



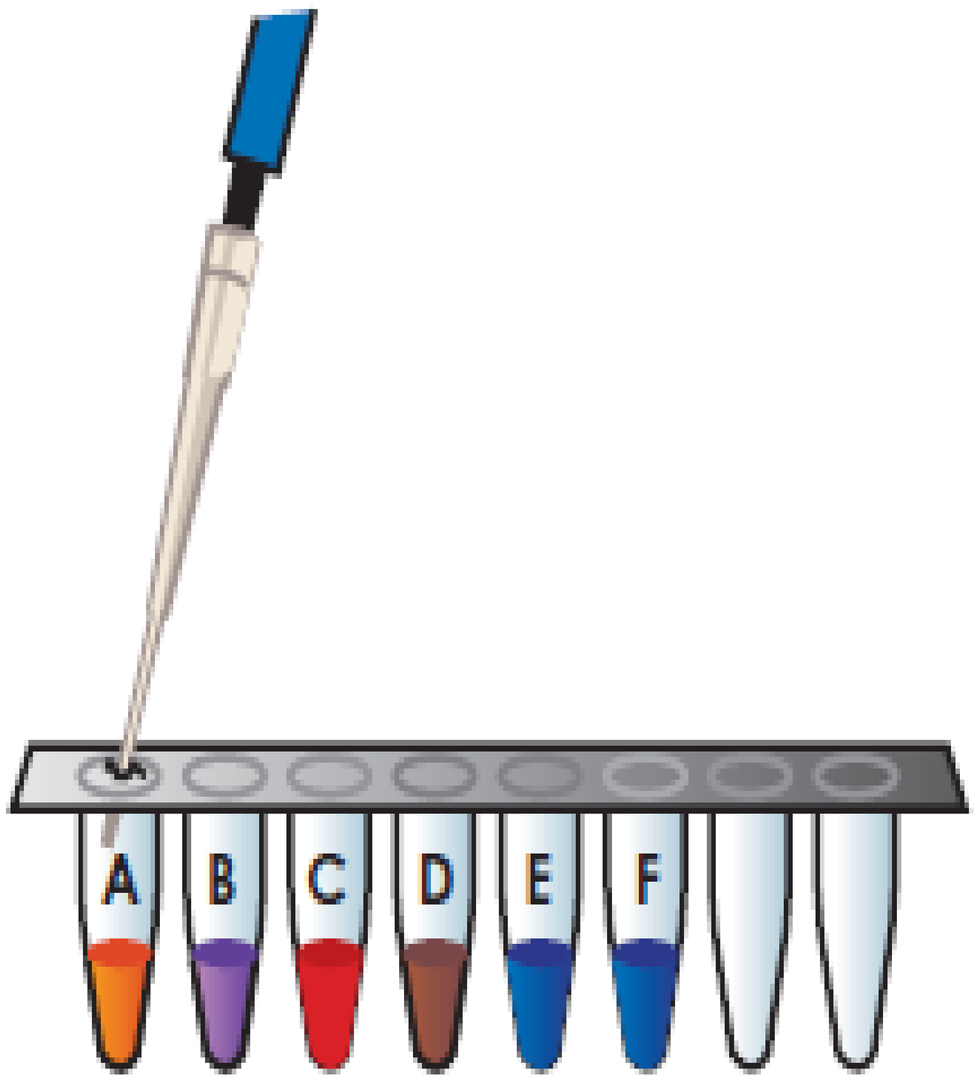


Comb is removed

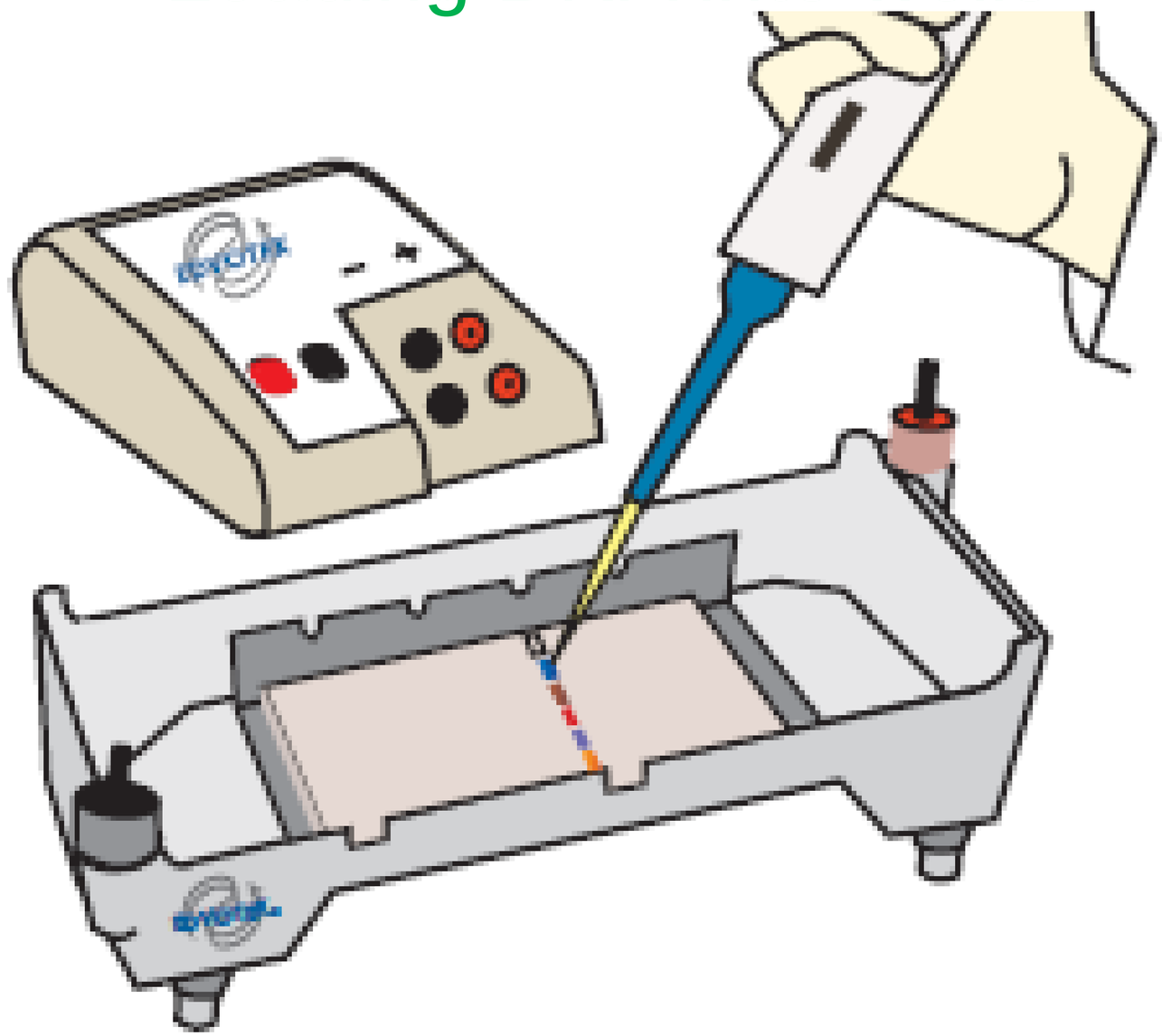
Agarose gel electrophoresis technique practical steps

8. Loading of samples into agarose gel wells

Add loading buffer to DNA



Loading DNA into wells



9. Loading of standard DNA marker into agarose gel wells

Load standard DNA marker inside first and last wells

Agarose gel electrophoresis technique practical steps

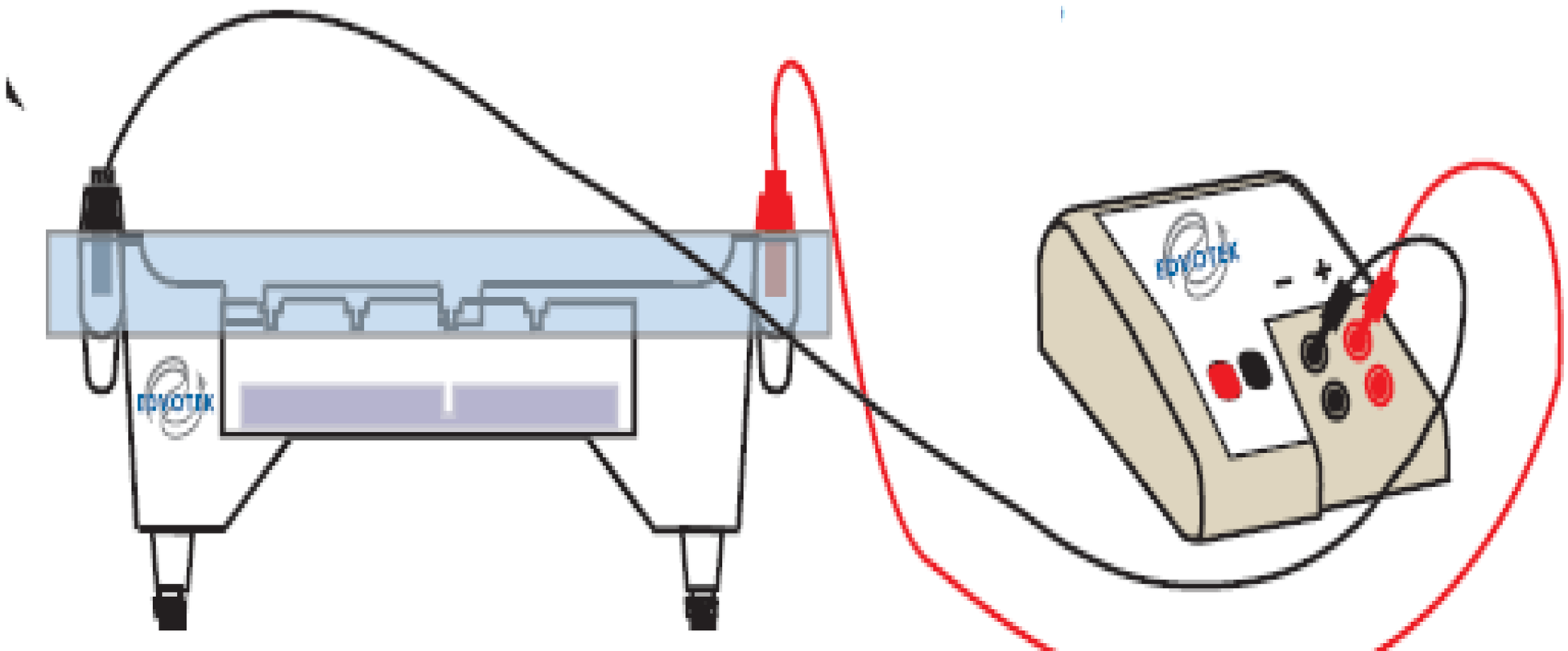
10. Start applying electric current

Migration of fragments in an agarose gel depends on the difference in electric current

Different optimal voltages are required for different fragment sizes

The voltage to apply depends on the electrophoresis tank size

1. Smaller tank with 12 comb wells (80 volts)
2. medium tanks with 20-25 comb wells (100 volts)
3. bigger tanks with comb wells above 30 (120 volts).



Agarose gel electrophoresis technique practical steps

11. Stop applying electric current

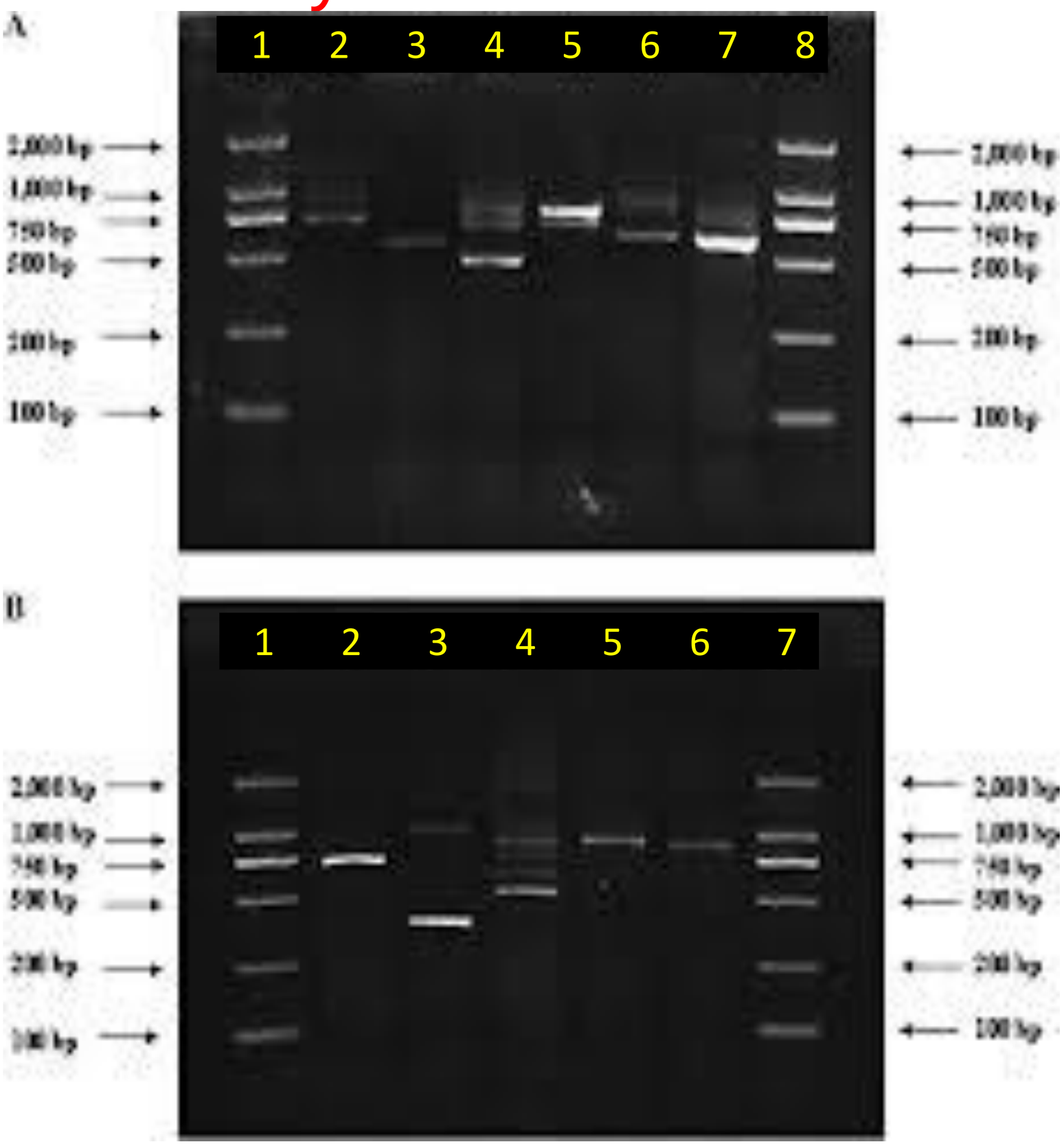
When the DNA samples or dyes have migrated for a sufficient distance through the gel, turn off the electric current and remove the leads and lid from the gel tank

12. Agarose gel staining using staining dye

Stain the gel by immersing it in electrophoresis buffer or H_2O containing ethidium bromide (0.5 µg/ml) for 20-45 minutes at room temperature or by soaking in a 1:10,000-fold dilution of SYBR Green stock solution in electrophoresis buffer

Agarose gel electrophoresis technique practical steps

13. Agarose gel place under UV light to examine separated samples
14. Analysis and documentation of results



Agarose gel electrophoresis technique practical steps

Advantages and disadvantages of agarose gel electrophoresis

S/N	Advantages	Disadvantages
1	Nontoxic gel medium	High cost of agarose
2	Gels are quick and easy to cast	Fuzzy bands
3	Good for separating large DNA molecules	Poor separation of low molecular weight samples
4	Can recover samples by melting the gel, digesting with enzyme agarose or treating with chaotropic salts	

Application of agarose gel electrophoresis

1. To estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.
2. As a routine tool in molecular genetics diagnosis or genetic fingerprinting via analyses of PCR products
3. Separation of restricted genomic DNA prior to Southern blot
4. Separation of RNA prior to Northern blot
5. To resolve circular DNA with different supercoiling topology
6. To resolve fragments that differ due to DNA synthesis
7. As an excellent medium for fragment size analyses
8. Agarose gels allow purification of DNA fragments