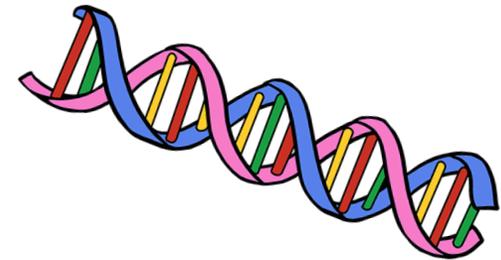
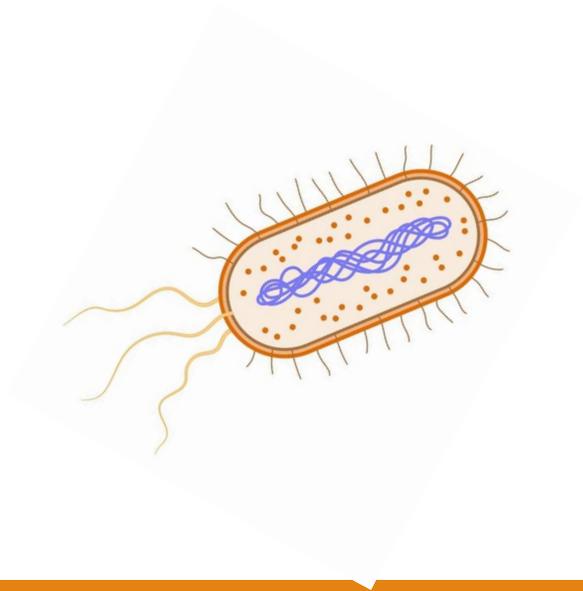


MBIO 240
LABORATORY SKILLS

DNA Isolation and Extraction, Agarose Gel Preparation, and Electrophoresis



Aim and Objectives

1. Isolation and extraction of **genomic DNA** from biological samples using a standard solution-based (chemical) methodology, leading to a visible and transferable DNA precipitate
2. Preparation of agarose gel for visualization of DNA
3. Separation of DNA based on molecular weight with gel electrophoresis

The processes involve 3 steps;

- 1. DNA isolation and extraction from the bacterial cells**
- 2. Preparation of agarose gel**
- 3. Separation of DNA with gel electrophoresis**

Procedure

1. **Cellular lysis:** Add 1 ml of an overnight culture to a Eppendorf tube. then Centrifuge at 10,000 RPM for 1 min. Remove the supernatant. Add 100 µl of Lysis Solution then incubate the samples at 0°C for 10 minutes.
2. **Protein precipitation** Add 100 µl of Protein precipitation solution (1 min) then Centrifuge at 10,000 RPM for 1 minutes.
3. **DNA Precipitation:** Transfer the supernatant containing the DNA to new Eppendorf tube containing 500 µl of isopropanol then Centrifuge at 10,000 RPM (1 minutes). Remove supernatant. Add 500 µl of 70% ethanol then centrifuge at 10,000 RPM (1 minutes). Carefully remove all the ethanol. Watch not lose the DNA pellet, Invert the tube and allow to dry on absorbent paper for 10 minutes.
4. **Add 50 µl RNase and incubate for 30 minutes at 37 °C.**
5. **Repeat step (3).**
6. **Hydration of DNA:** Add 500 µl of DNA hydration Solution. Resuspend by micropipette the white pellet. Incubation at 10°C which aid in the dissolution of the DNA.
7. Store at -20°C.

DNA Isolation and Extraction Flow Chart

Collect Sample (e.g., Bacterial Cells)

↓ (Step 1: LYSIS)

Add Lysis Buffer (Detergent + Protease) → Incubate

(Detergent breaks membranes; Protease digests proteins)



Centrifugation (High Speed)

(Forms a solid pellet of cell debris and a liquid supernatant with DNA)



(Step 2: SEPARATION)

Carefully Transfer Supernatant to a New Tube



(Step 3: PRECIPITATION)

Slowly Add Ice-Cold 95% Ethanol (2-3 volumes)

(DNA precipitates out of solution at the interface as a white mass)



Spool/Pellet DNA

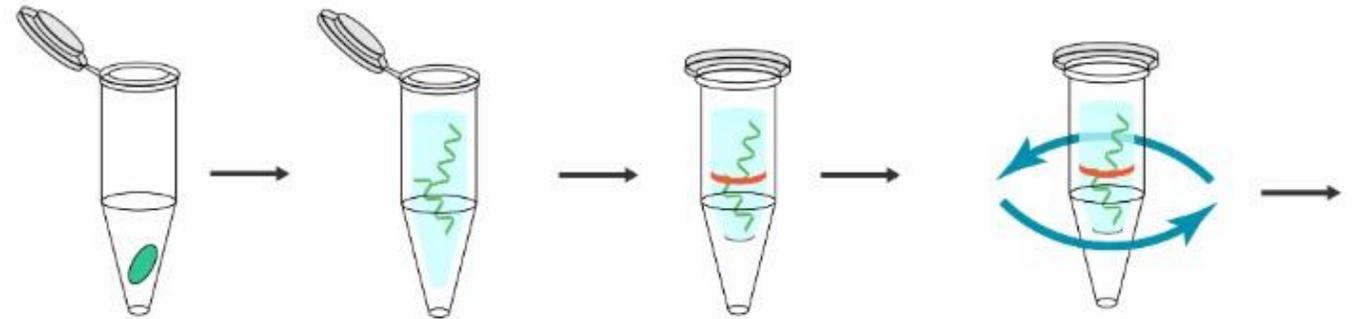


Wash with 70% Ethanol (removes residual salt)



Air Dry Pellet and Resuspend in TE Buffer/Water

Pure DNA sample



Harvesting
Bacterial cells

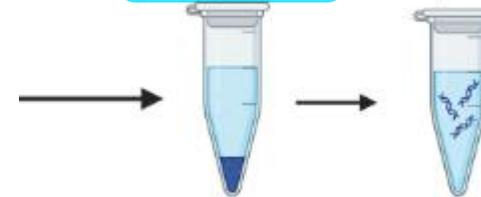
Cell Lysis

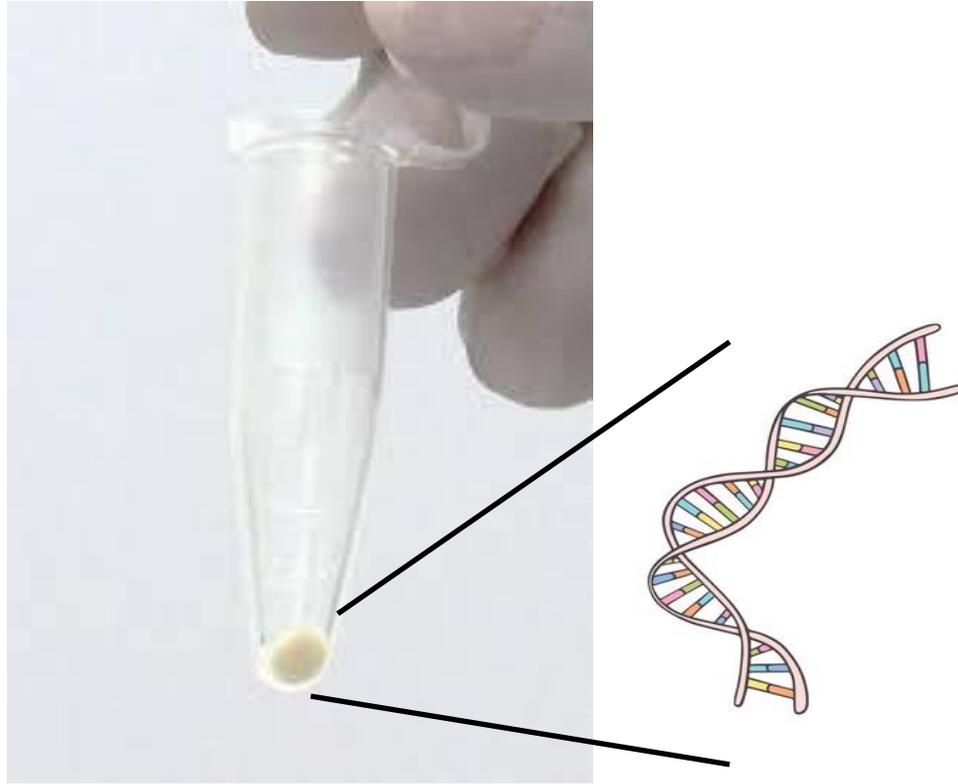
Protein digestion
and DNA extraction

Washing and
DNA elution

DNA
precipitation

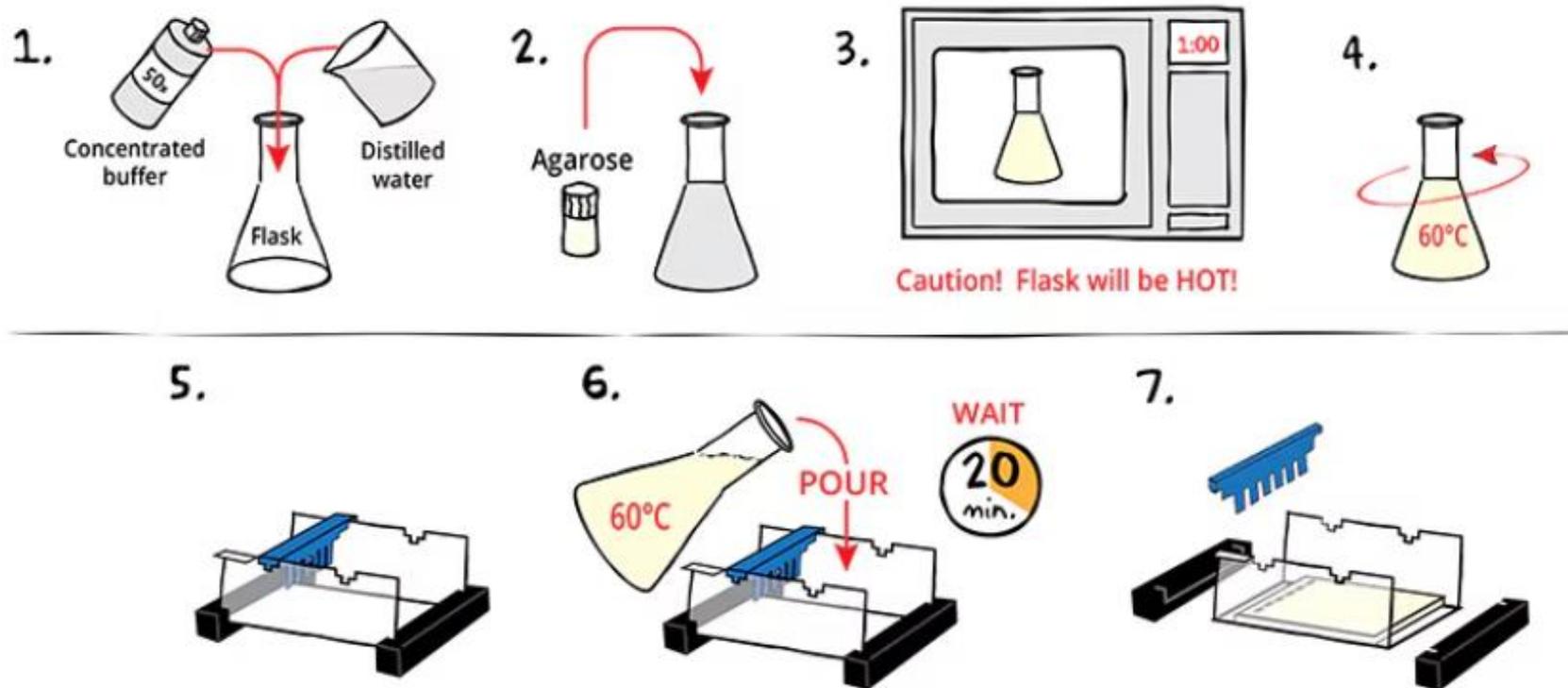
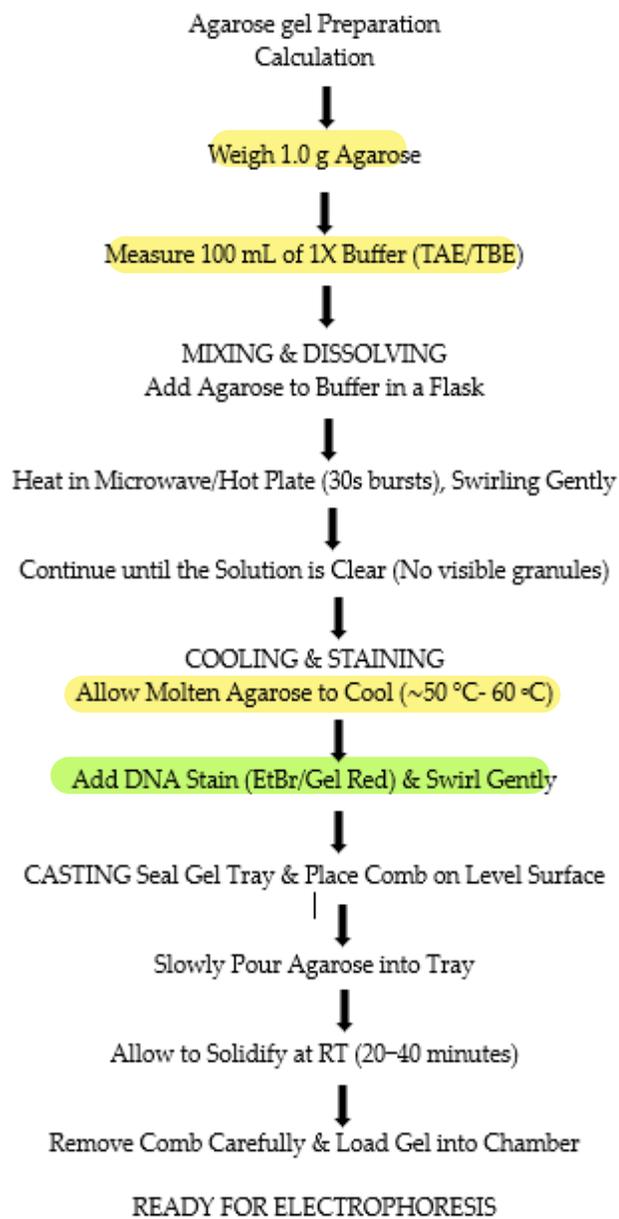
DNA
resuspension

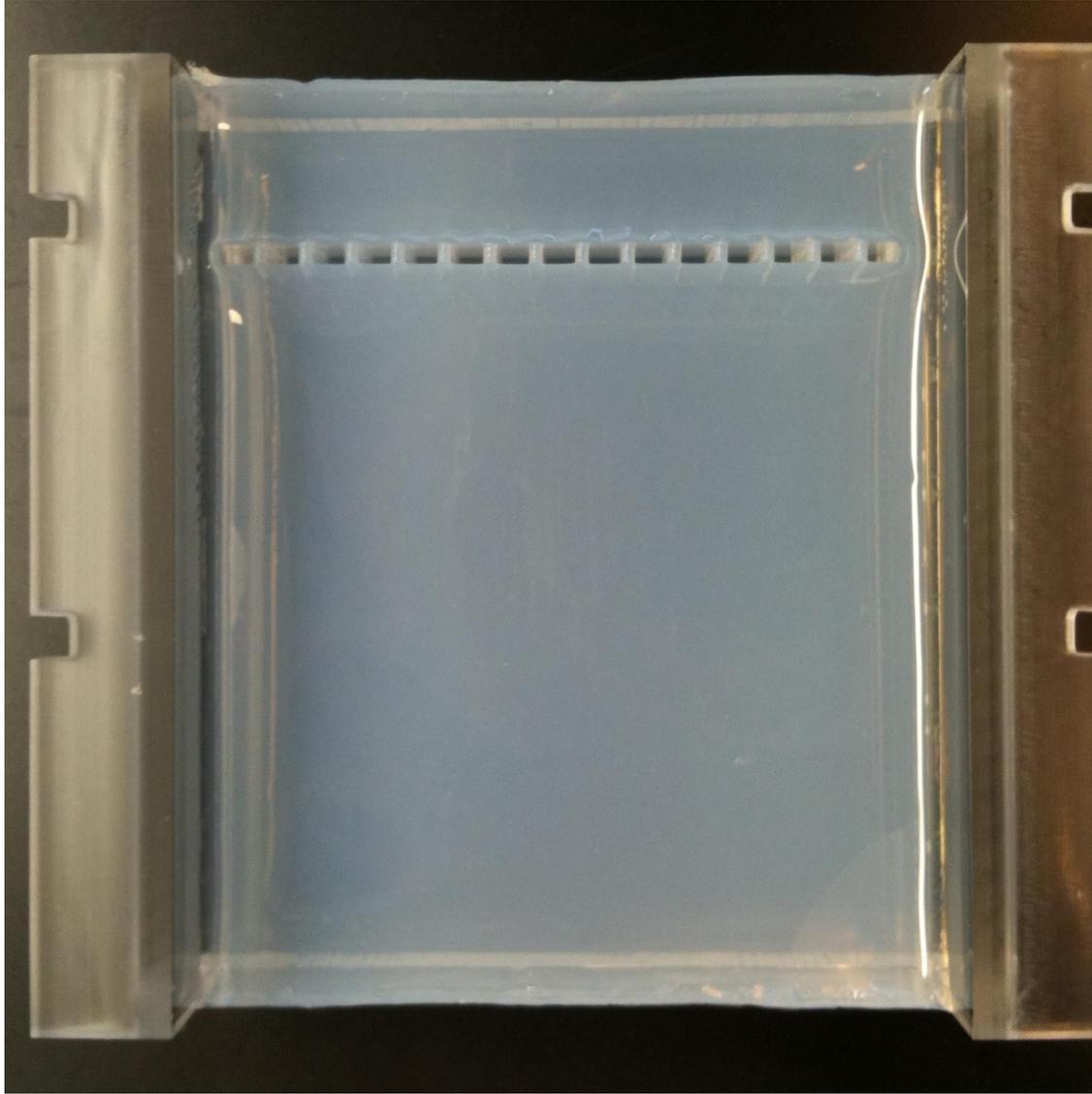




Pure DNA

Preparation of Agarose gel - Flow Chart





**Agarose gel with well and
coasting plate**

Preparation of Gel Electrophoresis Flow Chart

Gel Electrophoresis process
Agarose gel Preparation (Casting)

Place Solidified Gel in Chamber (Wells near - Electrode)

Submerge Gel in 1X Running Buffer

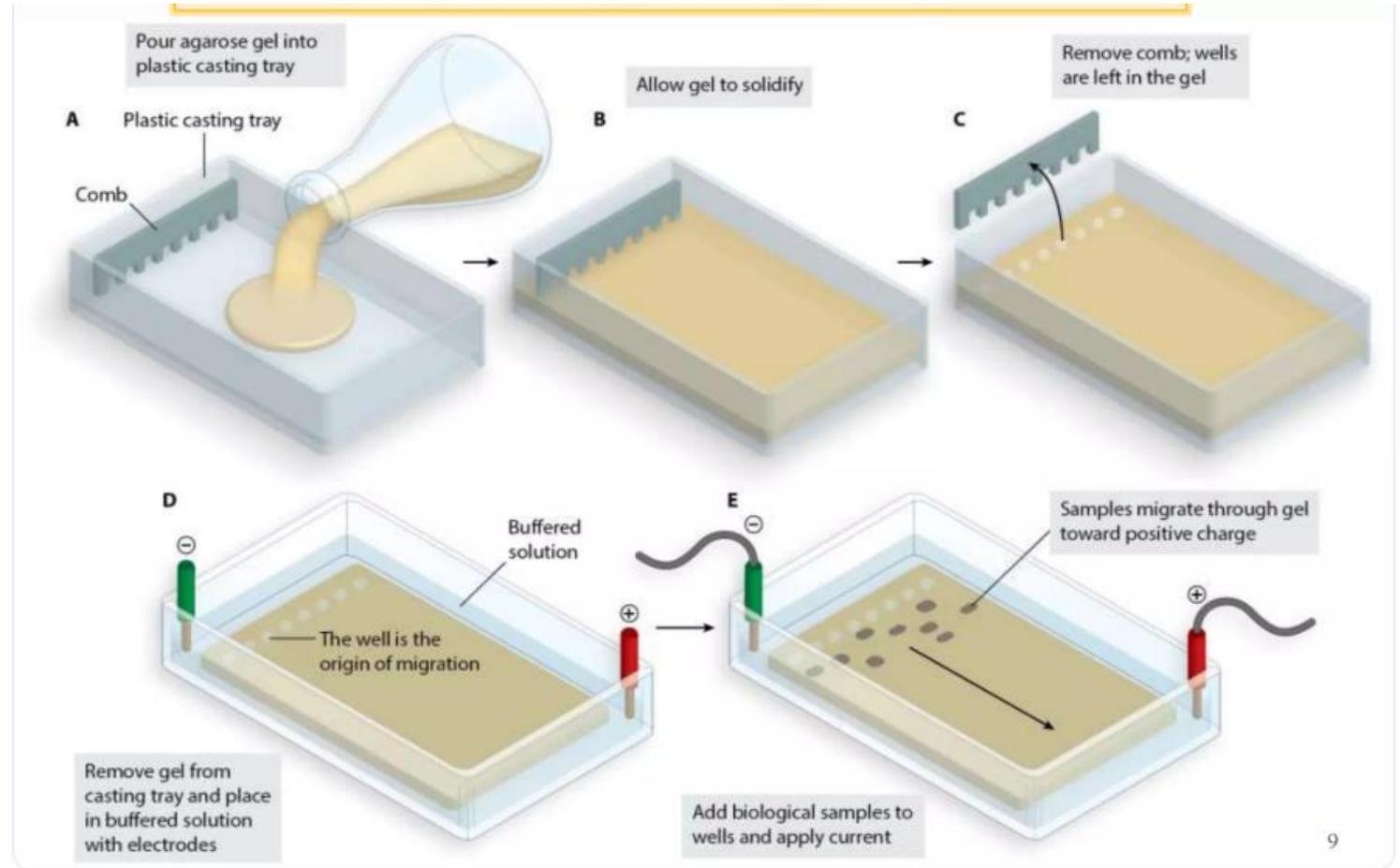
Load DNA Samples (Mixed with Loading Dye) into Wells
Load the DNA Ladder into a separate well

APPLY ELECTRICAL CURRENT (Run to Red)

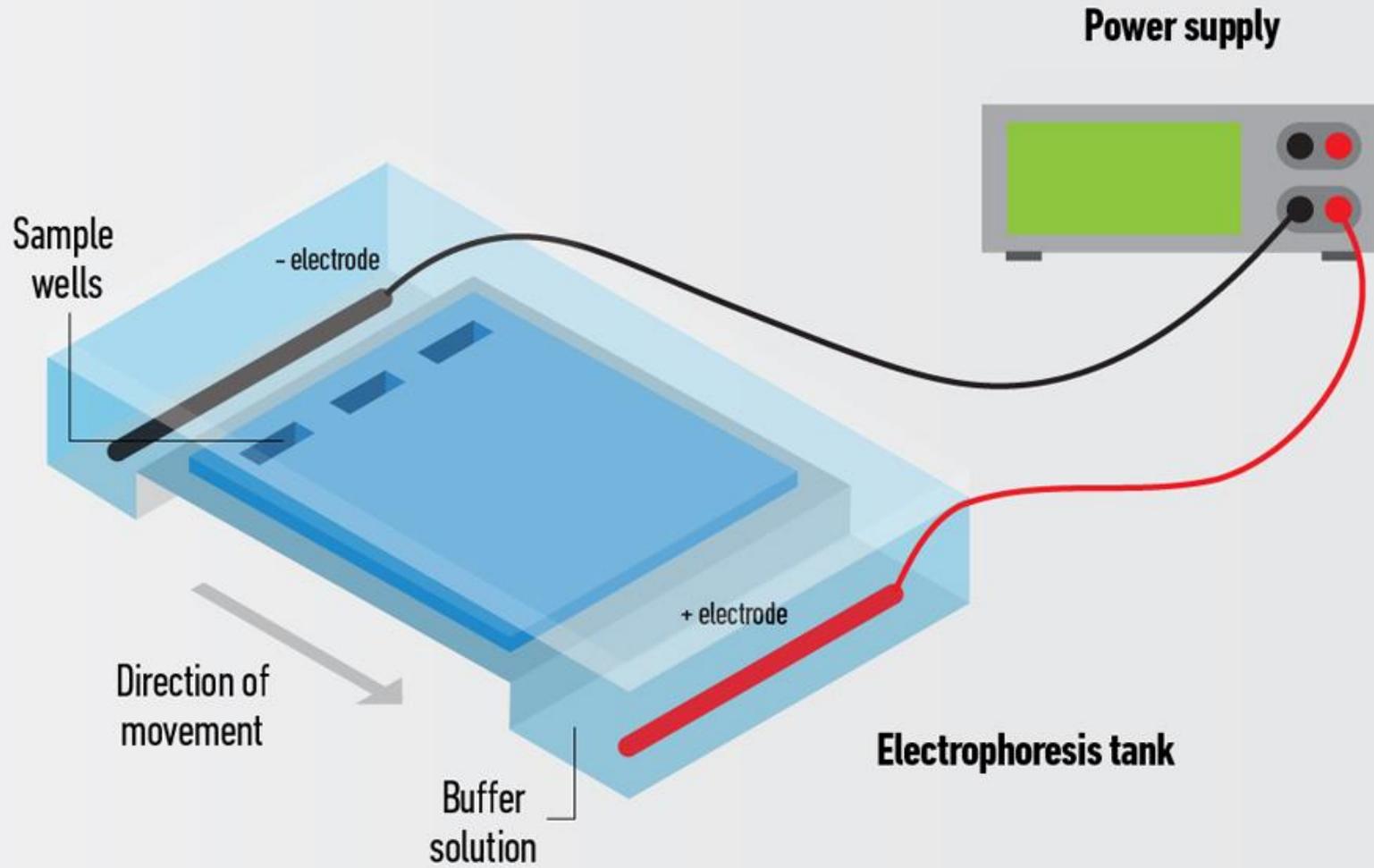
DNA Migrates: Small Fragments → Fast; Large Fragments → Slow

Stop running when the Tracking Dye Reaches the Desired Distance

VISUALIZATION Image Gel on a UV Transilluminator
SEPARATED DNA FRAGMENTS (BANDS)



Agarose Gel and Electrophoresis Unit with power bank



Agarose gel with DNA bands

