334 MBIO
Biochemical Instrumentation Techniques

- Lab 7 -

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2025

DNA Extraction and Analysis

Introduction

- **DNA isolation** is a process of purification of DNA from samples using a combination of physical and chemical methods.
- The methods used for this isolation are **depends on the sample:**
  - Source
  - Age
  - Size

Introduction

- These methods aim to **separate DNA present in the nucleus** of the cell from other cellular components.
- Isolation of DNA is needed for **genetic analysis**, which is used for **scientific, medical, or forensic** purposes.
- Presence of proteins, lipids, polysaccharides and some other organic and nonorganic compounds in the DNA preparation can **interfere** with DNA analysis methods.

DNA Sources

- Sources for DNA isolation are **very diverse.**
- Basically, it can be isolated from any living or dead organisms.
- Common sources for DNA include whole blood, hair, sperm, bones, nails, tissues, blood stains, saliva, cheek swabs, epithelial cells, urine, bacterial/animal/plant tissues.
- Stored samples can come from archived tissue samples, frozen blood or tissue, exhumed bones or tissues, and ancient human/animal/plants samples.

DNA Isolation Procedure

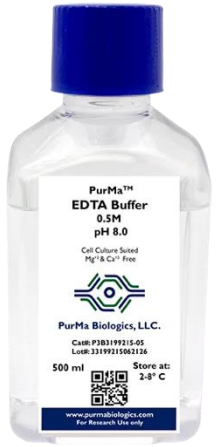
- Isolation of DNA basically consists of three major steps:

1. **Preparation of a cell extract**
2. **Purification of DNA from cell extract**
3. **Measurement of purity of DNA concentration**

1. Preparation of Cell Extract

- To extract DNA from a tissue/cells of interest, the **cells must be separated**, and the **cell membranes must be disrupted**.
- The "**Extraction buffers**" helps in carrying out these processes.

Common extraction buffers used for cell extraction



Ethylene Diamine Tetra Acetate (EDTA)

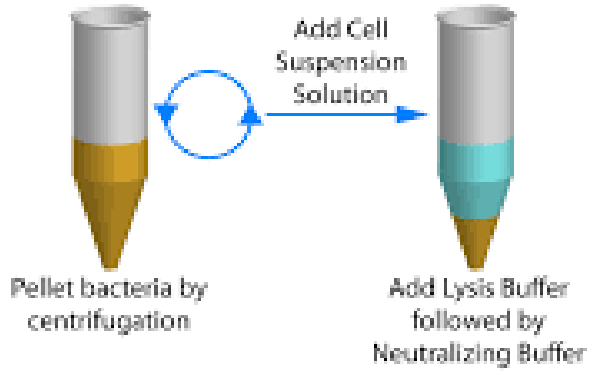
Removes **Mg^{2+} ions** that are **essential for preserving the overall structure** of the cell membrane.

Sodium Dodecyl Sulfate (SDS)

Aids in disrupting **the cell membranes** by **removing the lipids** of the cell membranes.

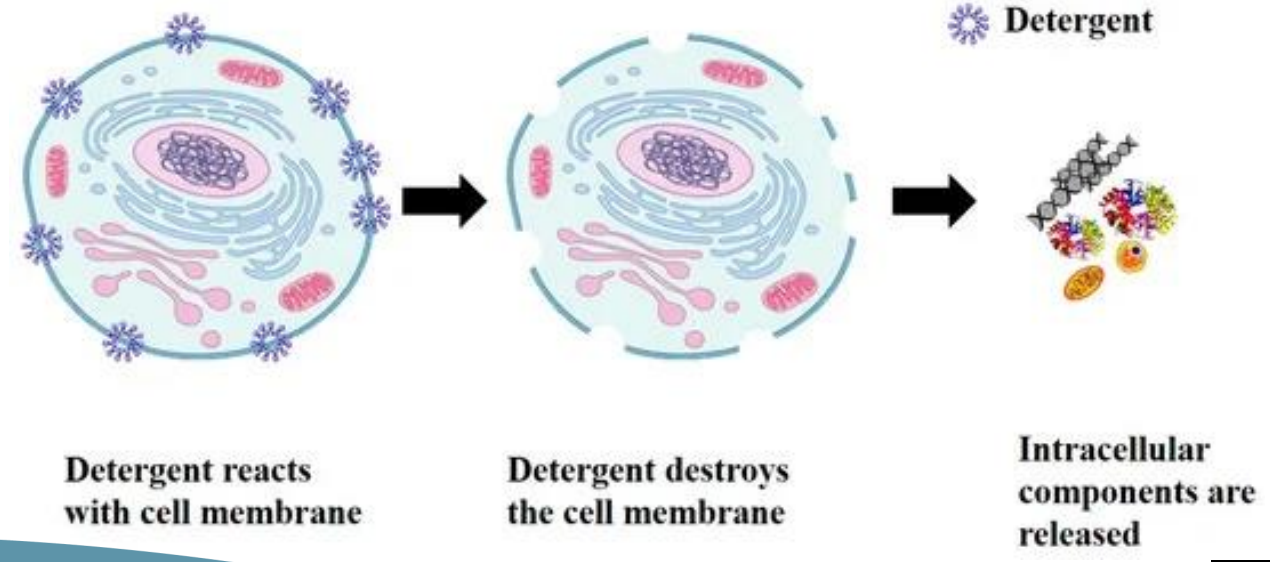
1. Preparation of Cell Extract

- Having lysed the cells, the final step in the preparation of a cell extract is **removal of insoluble cell debris.**
- Cell debris and partially digested organelles etc. **can be pelleted by centrifugation** leaving the cell extract as a reasonably **clear supernatant**.



2. Purification of DNA from Cell Extract

- In addition to DNA, **the cell extract will contain significant quantities of detergents, proteins, salts and reagents used during cell lysis step and RNA**.
- A variety of procedures can be used to remove these contaminants, leaving the DNA in a pure form.

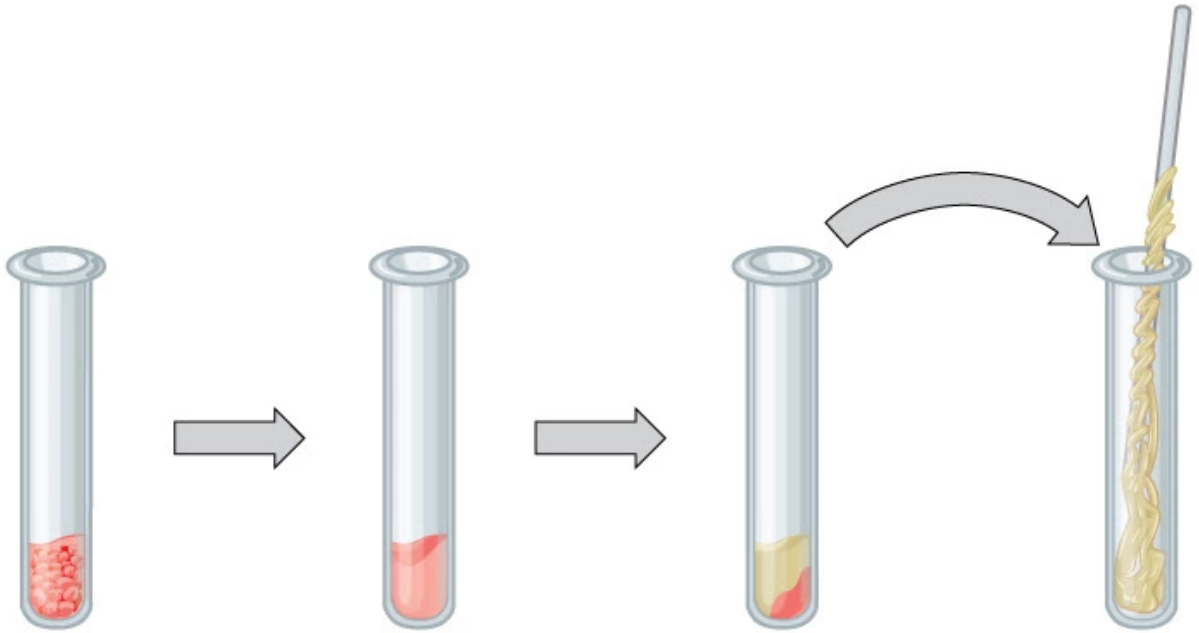


DNA Purification Procedures

1. Ethanol precipitation	2. Phenol/Chloroform extraction	3. Minicolumn purification
- Usually by **ice-cold** ethanol or isopropanol - Since DNA is **insoluble** in alcohols, it **aggregate** together giving a **pellet** upon centrifugation. - Precipitation of DNA improved by increasing of ionic strength, usually by adding **sodium acetate.**	- The phenol /chloroform **denature proteins** in the sample. - After sample centrifugation, denatured proteins stay in the organic phase while aqueous phase containing the DNA is mixed with chloroform that **removes phenol residues from solution**.	- This protocol relies on the fact that may **bind or adsorbed to the solid phase (e.g., silica),** depending on **the pH and the salt concentration** of the buffer.

- **DNA can be stored at 4°C for extended periods, but for long term storage, - 20°C is usually utilised.**

Ethanol Precipitation



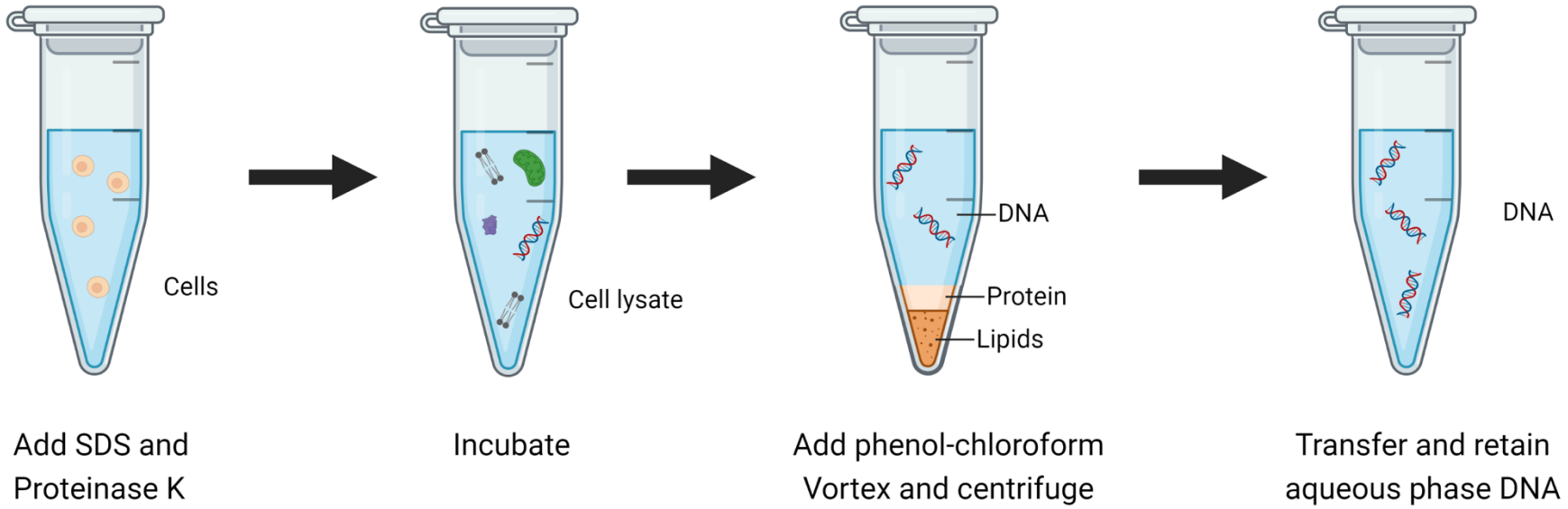
Cells are lysed using a detergent that disrupts the plasma membrane.

Cell contents are treated with protease to destroy protein, and RNAase to destroy RNA.

Cell debris is pelleted in a centrifuge. The supernatant (liquid) containing the DNA is transferred to a clean tube.

The DNA is precipitated with ethanol. It forms viscous strands that can be spooled on a glass rod.

Phenol/Chloroform Extraction

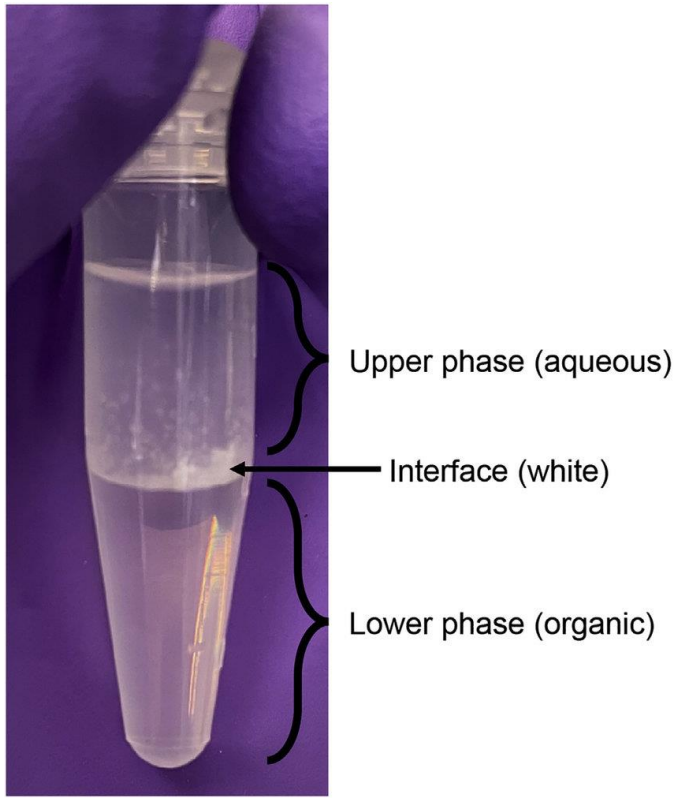


Add SDS and Proteinase K

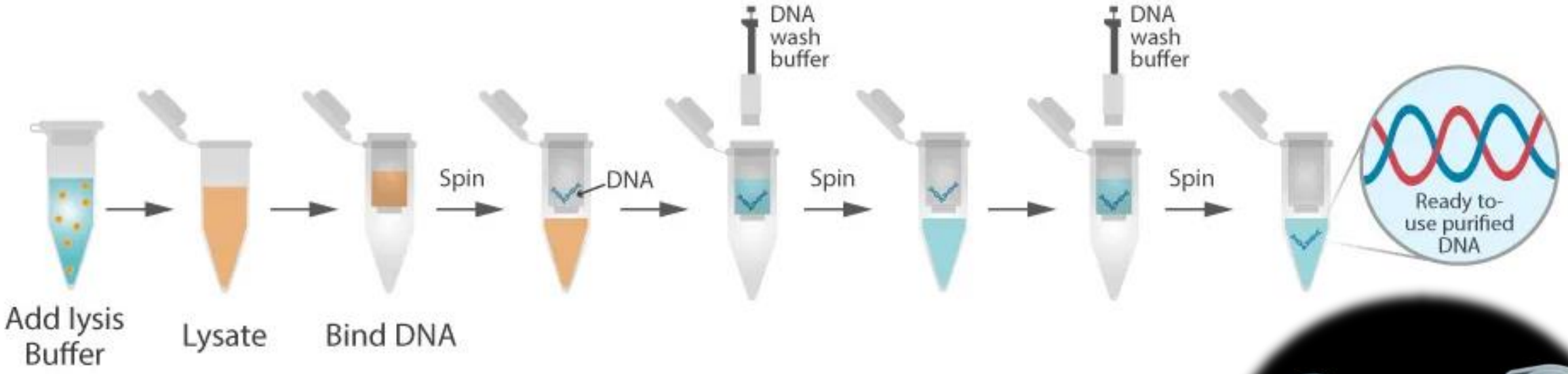
Incubate

Add phenol-chloroform Vortex and centrifuge

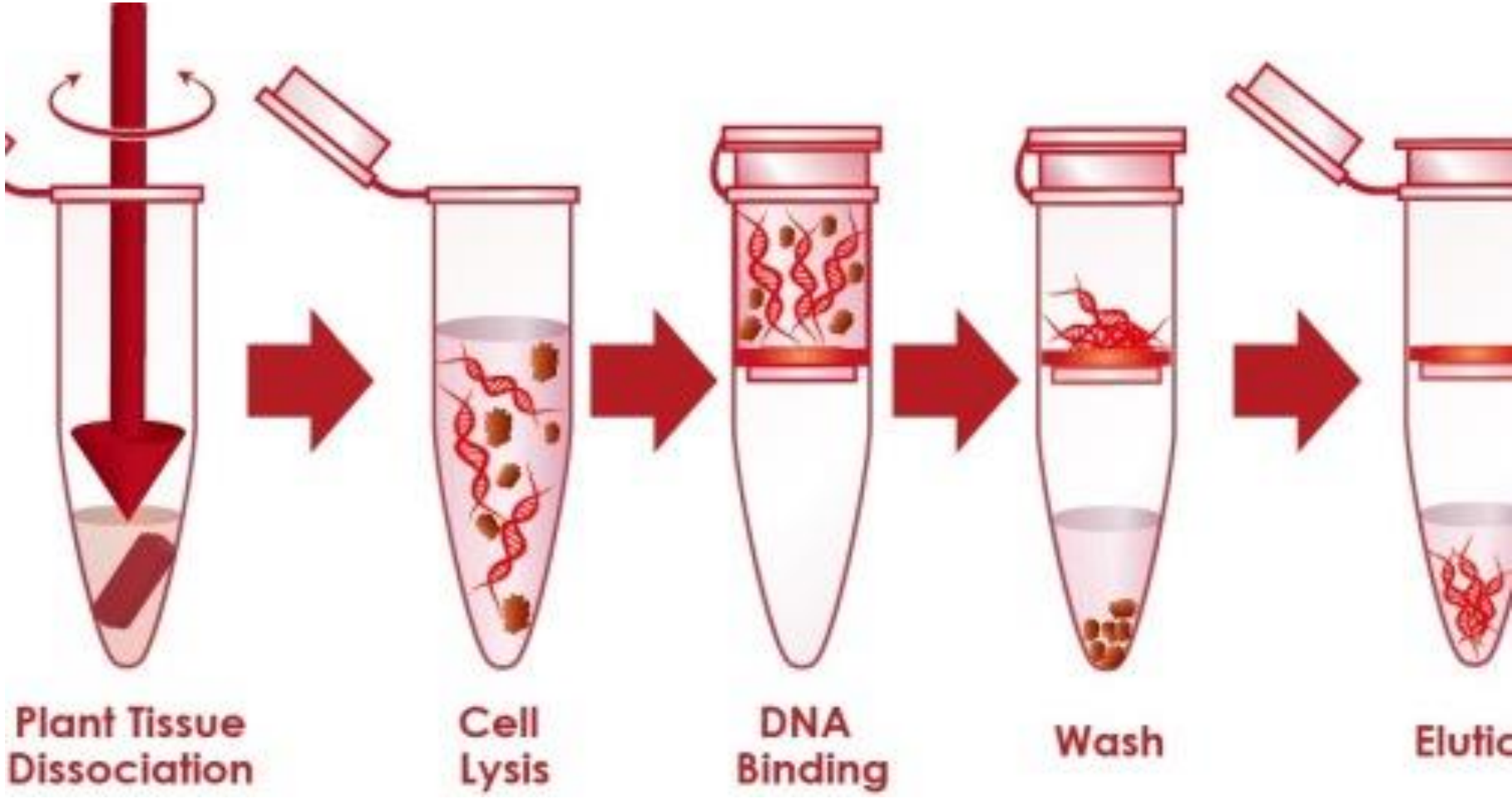
Transfer and retain aqueous phase DNA



Minicolumn purification



Minicolumn purification

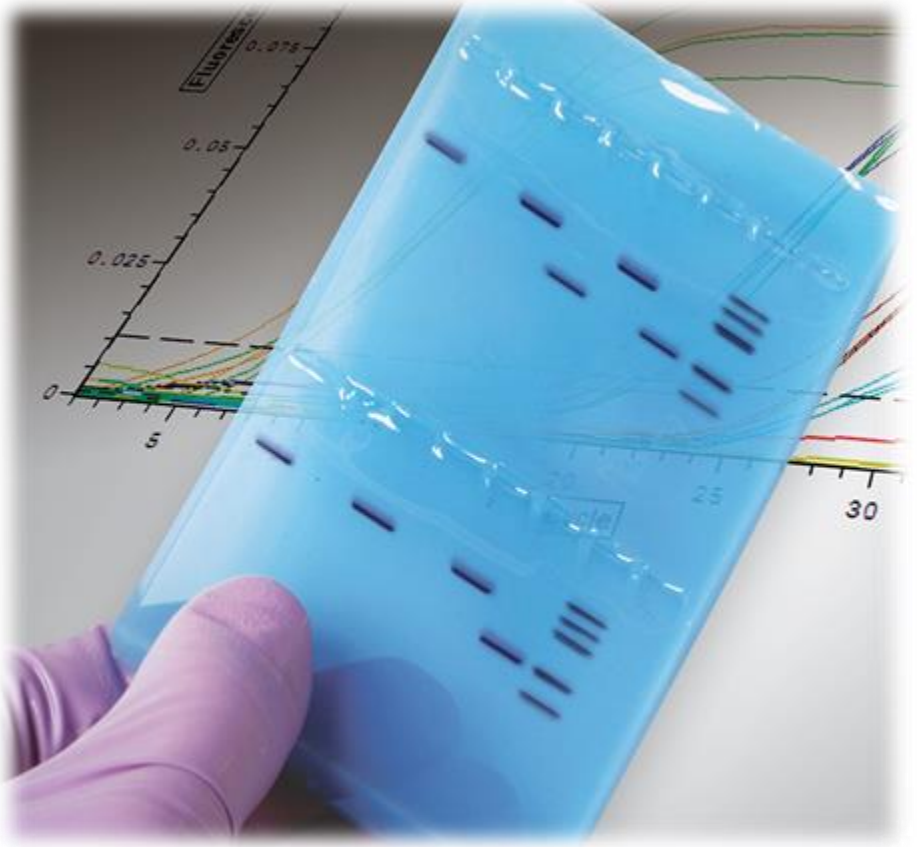


3. Measurement of Purity of DNA Concentration

- **DNA concentration** can be accurately measured by UV absorbance spectrophotometer **(Nanodrop).**
- DNA absorbance is measured **at 260 nm**. A pure DNA sample typically has **an A260/A280 ratio of ~1:8.**
- Ratio **less than 1:8** indicate that the preparation **is contaminated** either with proteins or phenol.
- The unit for DNA concentration is **ng/µL.**

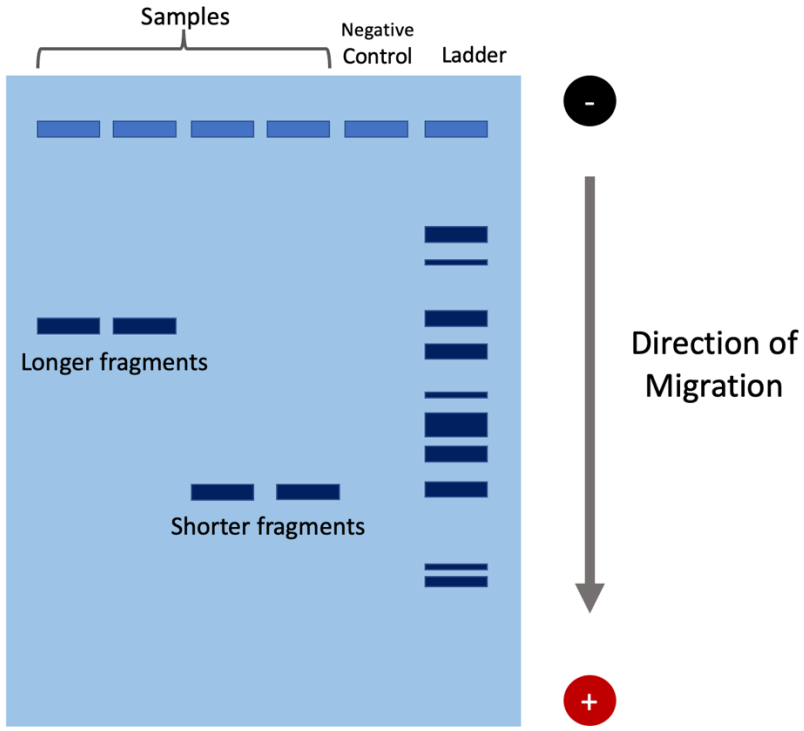
DNA Analysis by Gel Electrophoresis

- A technique used to **separate DNA, RNA, or proteins** according to their **size and charge**.
- It is essential for analysing DNA fragments following PCR restriction enzyme digestion or after the release of genomic DNA from cells.
- The principle involves an **electric field causing negatively charged DNA molecules to move towards a positive electrode.**



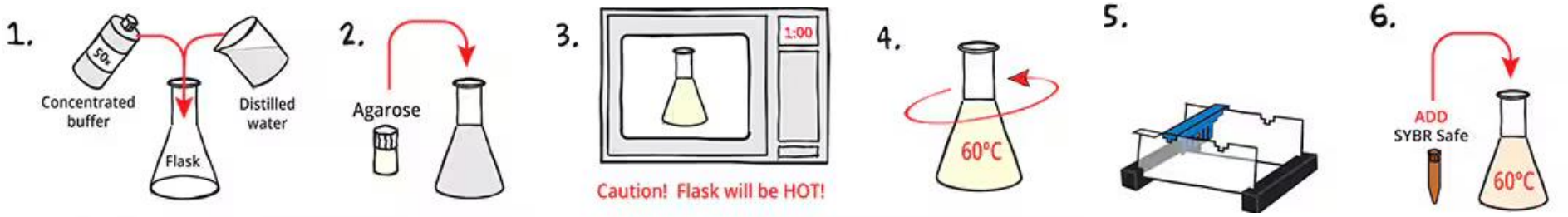
DNA Analysis by Gel Electrophoresis

- **Smaller DNA** fragments move through the gel **faster than larger ones**, enabling separation by **size**.
- Applications include DNA fingerprinting, genetic analysis, and verifying molecular cloning experiment success.



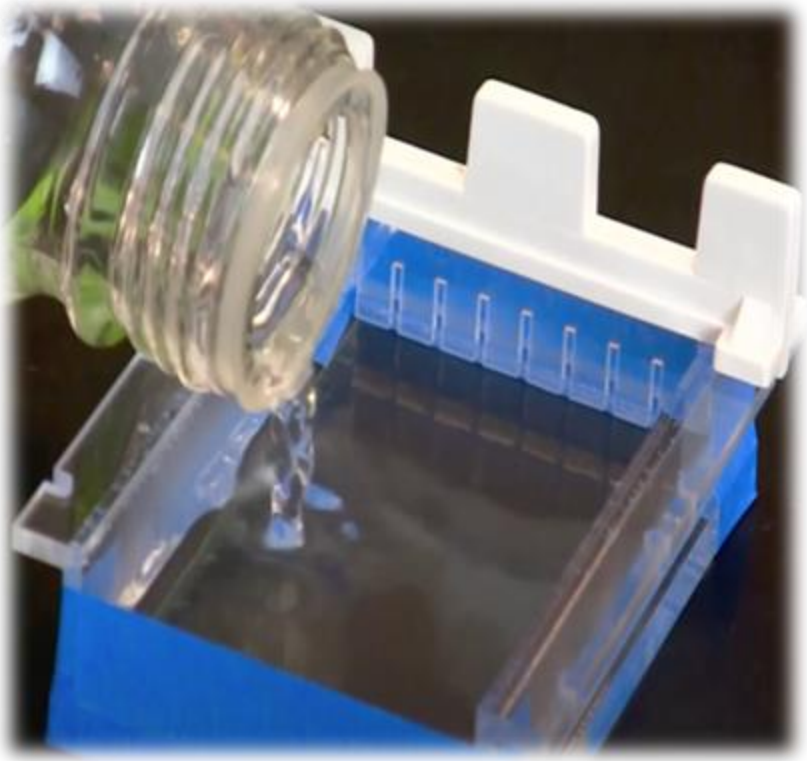
Making the gel

- Add 0.5g of agarose to 50ml TAE buffer (tris-acetate-EDTA) to make a 1% agarose solution.
- Dissolve the agarose by heating the solution in a microwave.
- Allow the solution to cool down to around 60°C.
- Add the gel stain for **DNA visualisation** (e.g., ethidium bromide, SafeView).

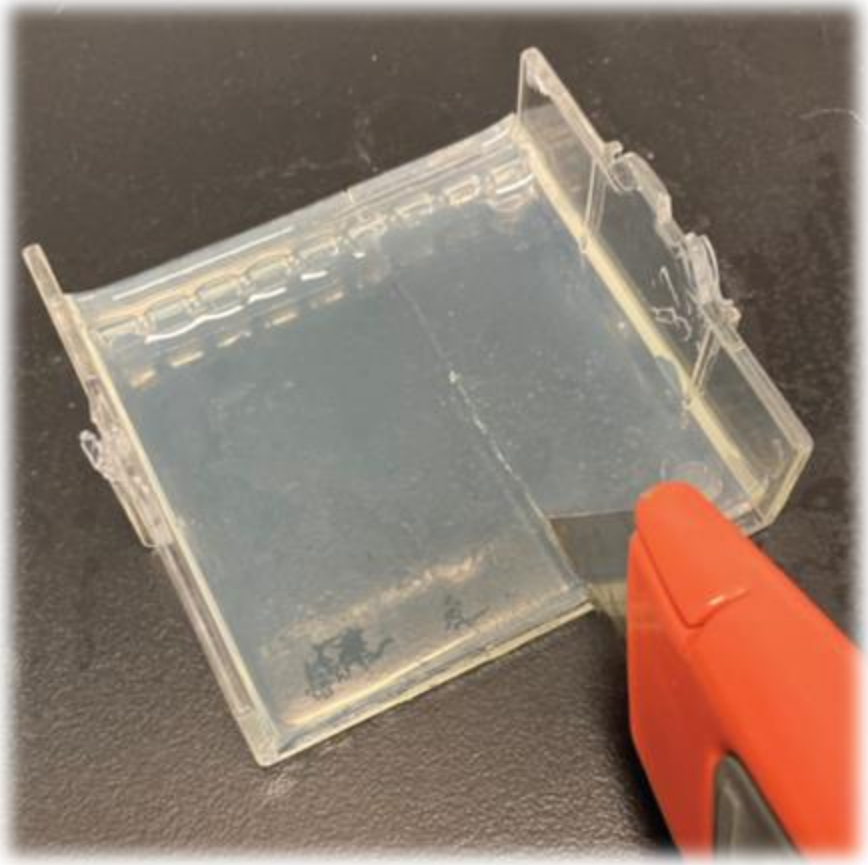


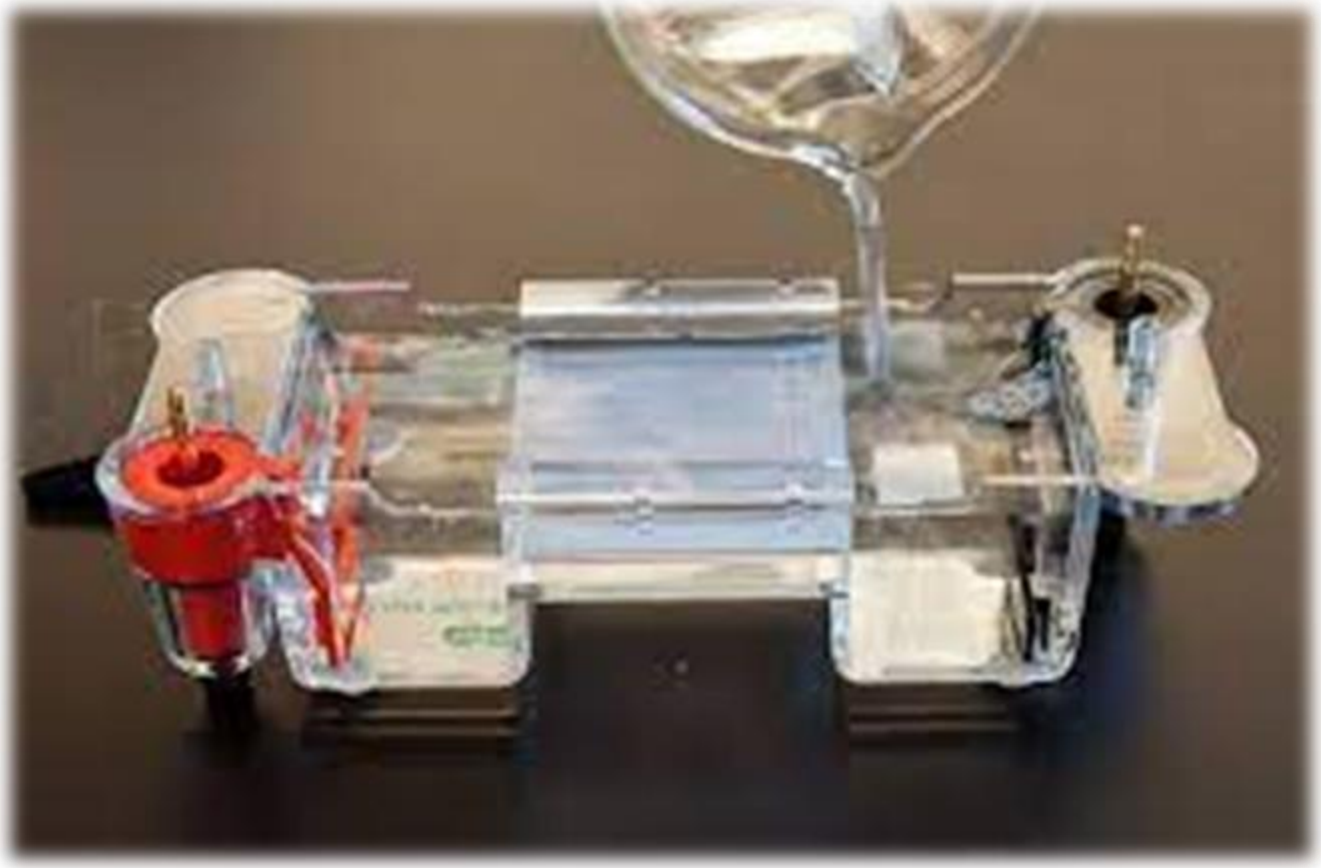
Casting the Gel

- Pour the agarose solution into the gel tray and place **the comb in position**.
- Pump and air bubbles immediately.
- Let the gel harden at room temperature for 20 -30 minutes.
- **Remove the comb** and place the gel tray in the electrophoresis chamber.
- Fill the chamber with TAE buffer till it covers the gel by 2 –3 mm.



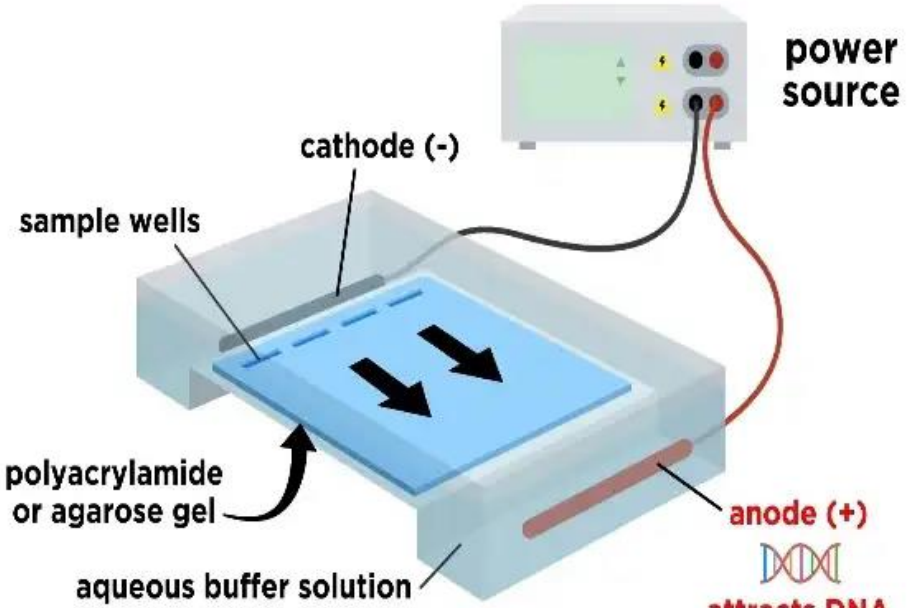
Casting the Gel





Loading the sample and running the gel

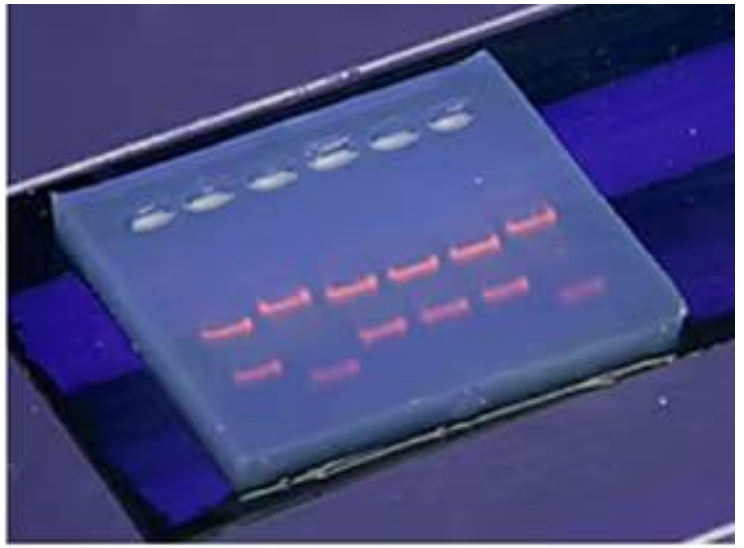
- Mix each DNA sample with loading dye.
- Use a micropipette to load DNA samples and ladder into wells.
- Close the electrophoresis chamber lid and connect the power supply.
- **Ensure that the DNA samples migrate towards the anode.**
- Set voltage to 100 V, run the gel horizontally for 30–45 minutes.



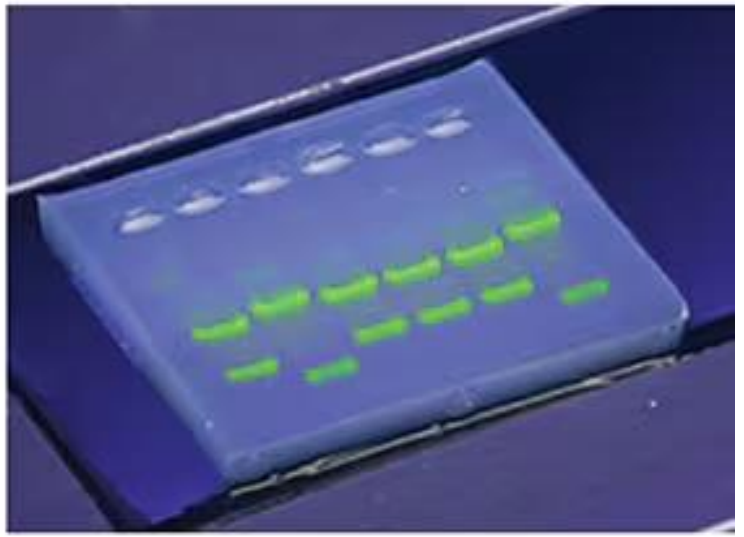
DNA Visualisation

- Visualize the DNA bands under a UV transilluminator.
- Compare the sample DNA bands to the DNA ladder to determine fragment sizes.
- Record any observations and take photographs for documentation.

InstaStain®
Ethidium
Bromide

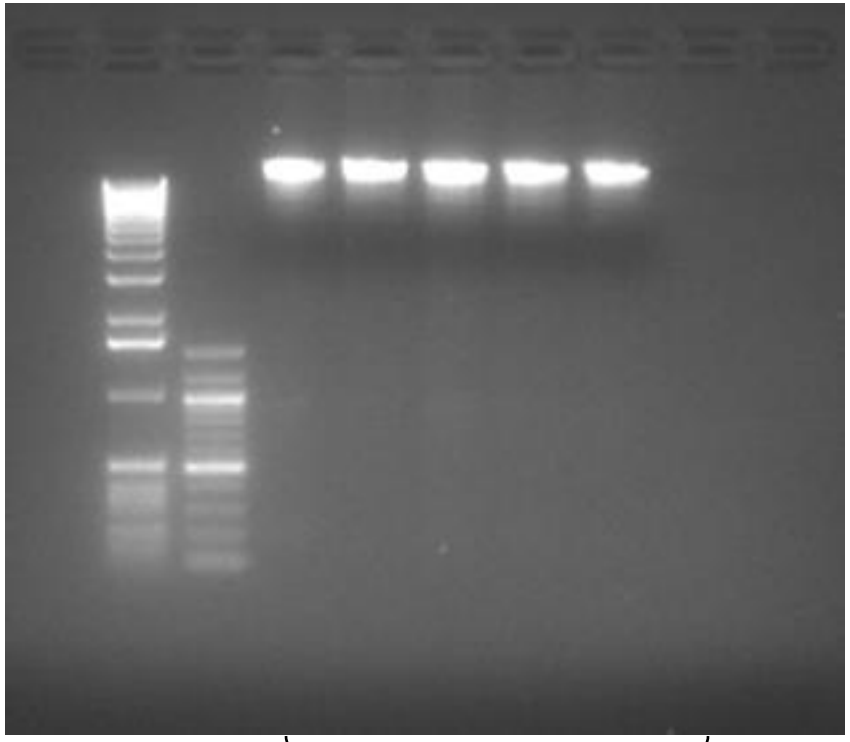


SYBR®
Safe
DNA Stain



Analysing DNA Samples

- Below is an agarose gel that has 5 genomic DNA samples from various plants.
- the DNA runs at a **very high molecular weight and as a clear, thick band.**
- This DNA was extracted in a research lab under optimal conditions.



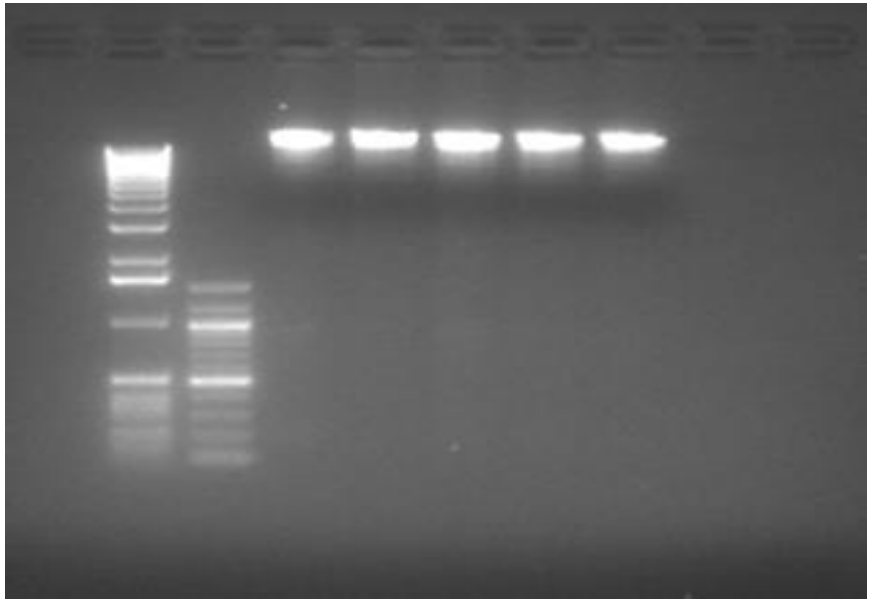
1 kbp and 100 bp ladders

Genomic DNA of 5 species of cereals

Analysing DNA Samples

- If properly done, **genomic extraction should result in bright bands** in the very high base pair range of a gel electrophoresis.

Sample	DNA Size (bp)
E. Coli	4,640,000
Yeast	12,100,000
Fruit Fly	140,000,00
Human	3,000,000,000
Pea	4,800,000,000
Wheat	17,000,000,000

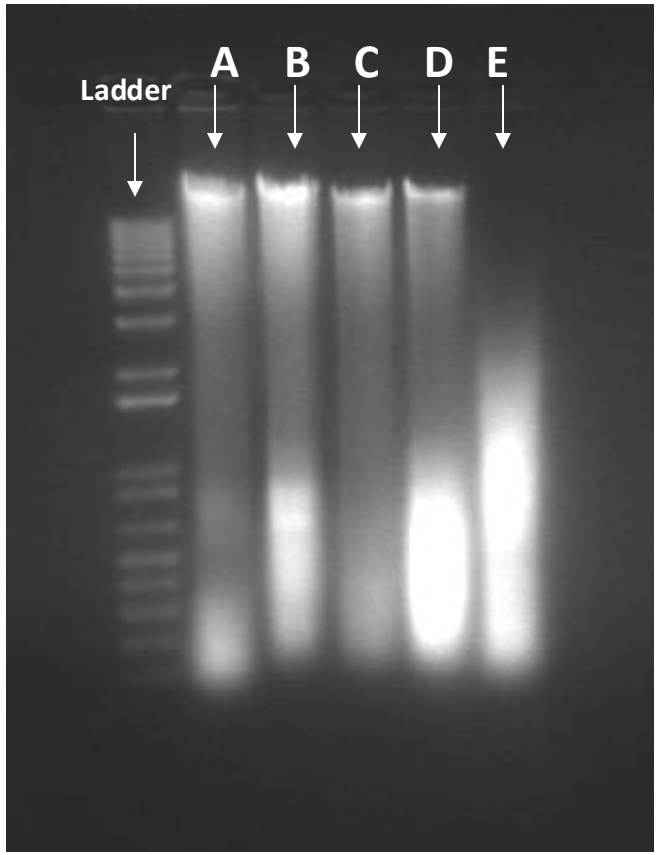


1 kbp and 100 bp ladders

Genomic DNA of 5 species of cereals

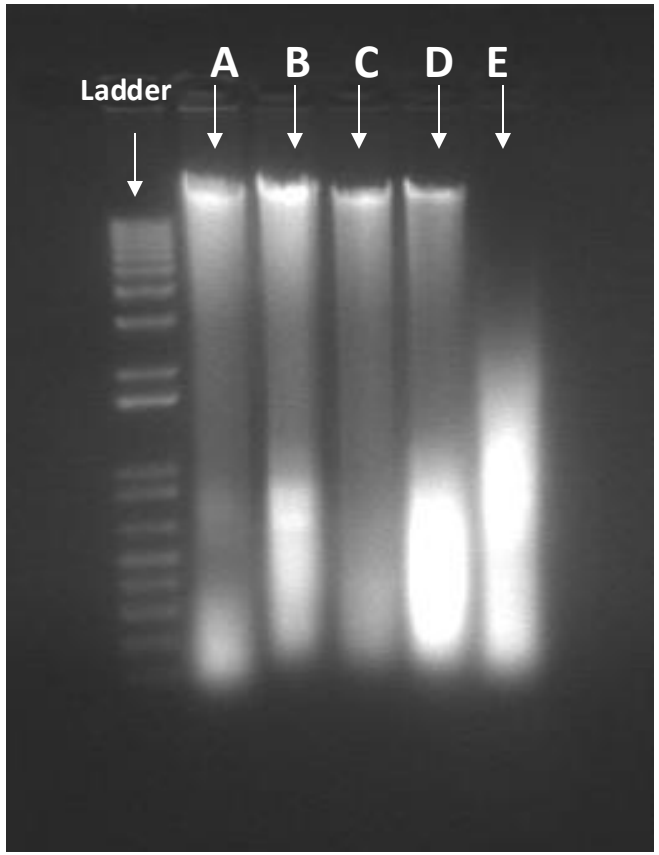
Expected Results

- **This can happen.** Even though this genomic DNA preparation is not perfect, it is suitable for use as a PCR template.
  - Lane A: Barley
  - Lane B: Corn
  - Lane C: Oat
  - Lane D: Rice
  - Lane E: Wheat



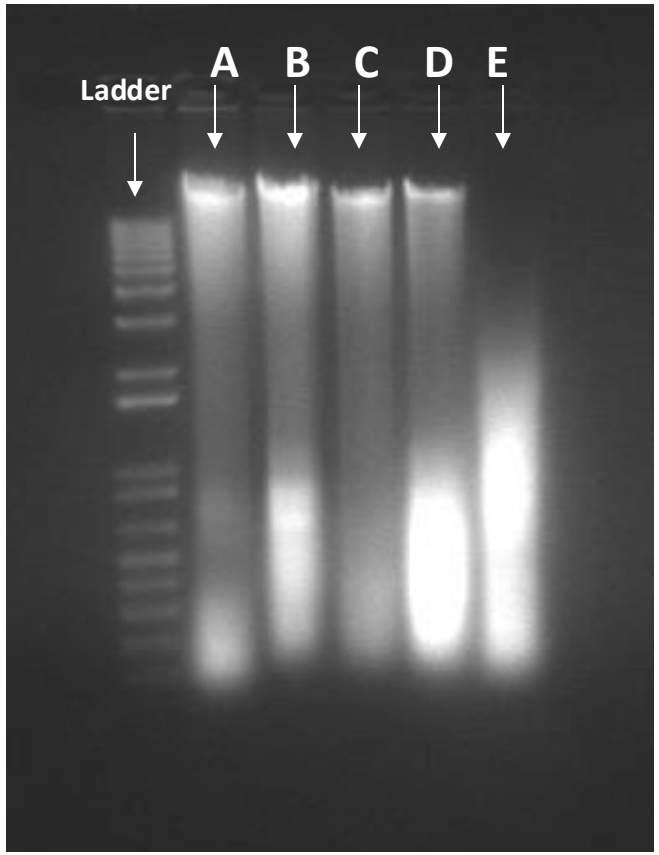
Expected Results

- The DNA **has sheared** (particularly for **Lane E**) – broken up into numerous fragments and is not a clean single band at the top – these are the mid-ranged sized fragments (1000-10,000bp size range).
- The bright bands at the 100 - 1000 bp range are **RNA**, which also gets extracted using this protocol.



Expected Results

- Analysis of the samples:
  - Barley (A) ⟶ This sample is fine
  - Corn (B) ⟶ This sample is fine
  - Oat (C) ⟶ This sample is fine
  - Rice (D) ⟶ This sample is fine
  - Wheat (E) ⟶ This sample has severe degradation, can work for PCR but should re-extract.



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