
Nucleic acids
Extraction
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DNA

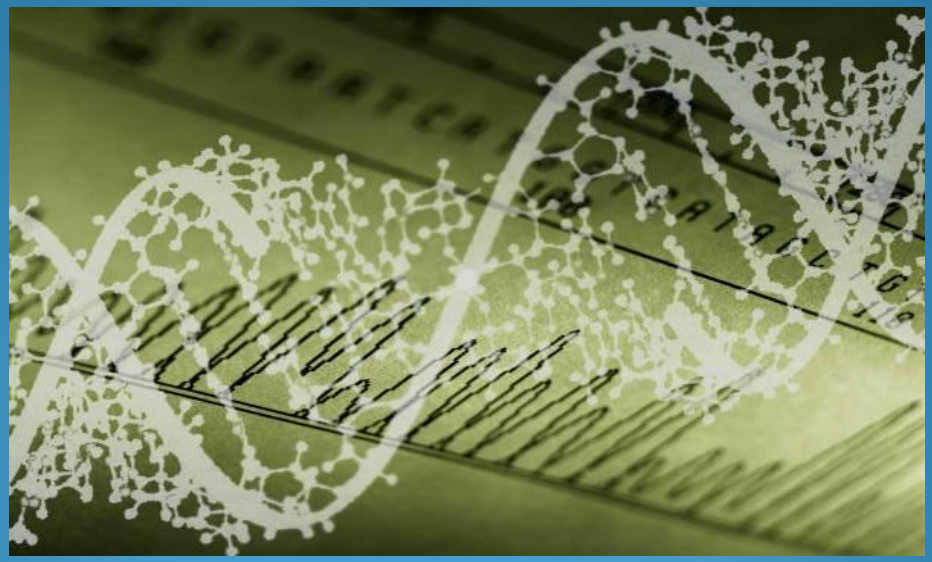
- DNA determines the characteristics of all living organisms.
- DNA is composed of a *four*-letter nucleotide/molecule alphabet referred to as A, T, C, and G.
- The order of the alphabet determines the characteristics of the living organism, much like the order of letters in our alphabet determines the words.
- Each cell in the human body contains >3 BILLION letters.

DNA

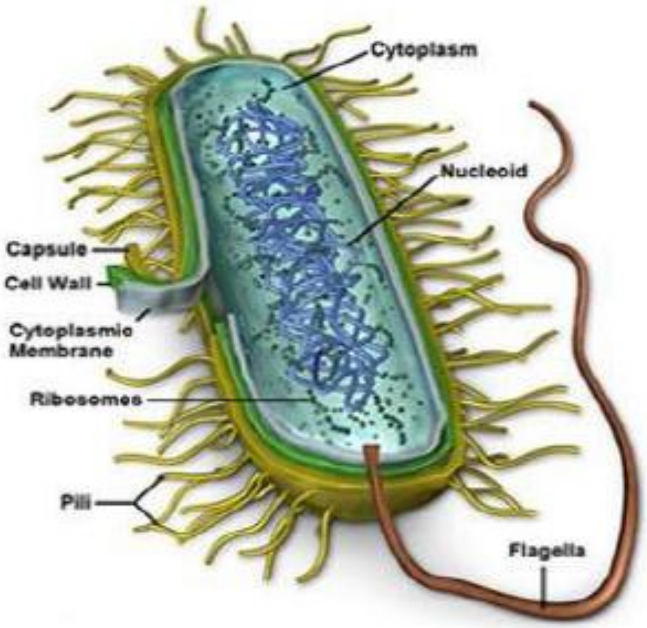
The ***only*** difference between living organisms is the amount and order of the DNA alphabet.

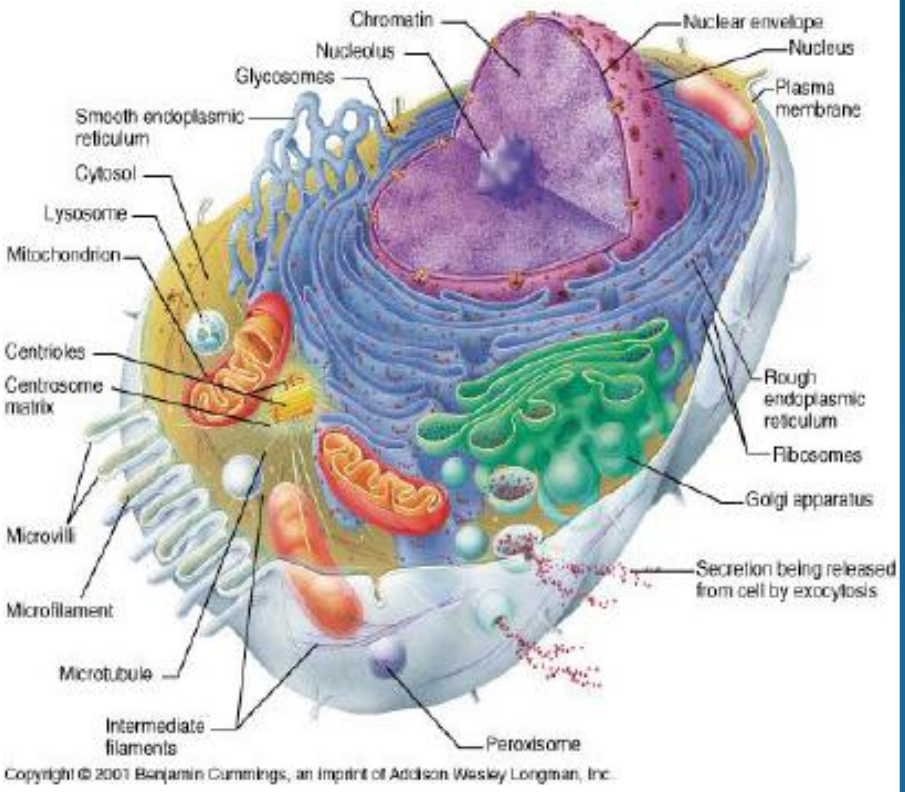
Purpose of DNA Extraction

To obtain DNA in a relatively purified form which can be used for further investigations, i.e. PCR, sequencing, etc



Basic structure of the cell





Prokaryotic cell

Eukaryotic cell

Most DNA extraction protocols consist of

Cells lysis

Precipitation

Washing

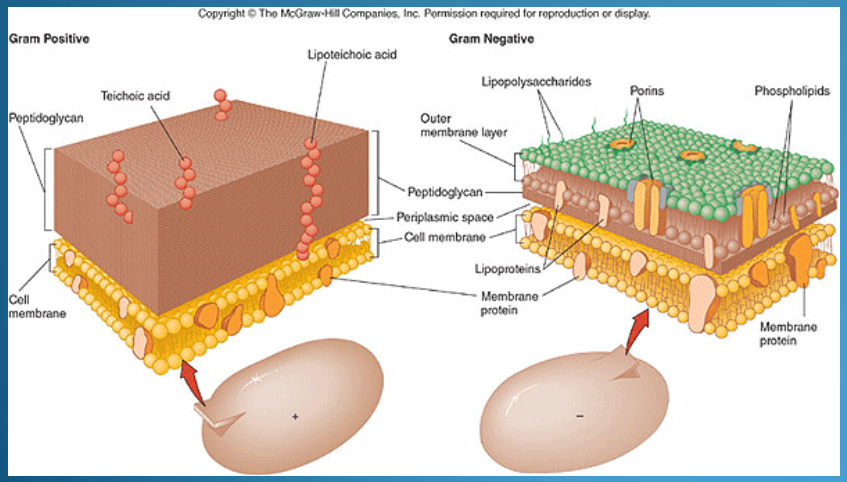
Resuspension

Cells lysis

Bacteria

Lysozymes
(Tears, egg white, milk, mm)

Breaking peptidoglycan cell wall in Bacteria



Alexander Fleming
Nobel Prize 1945

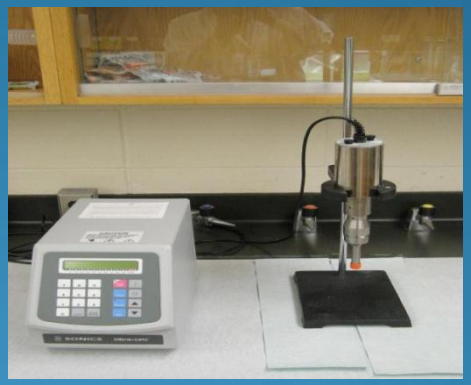
DNA Extraction

Cells lysis

Plant, Fungi

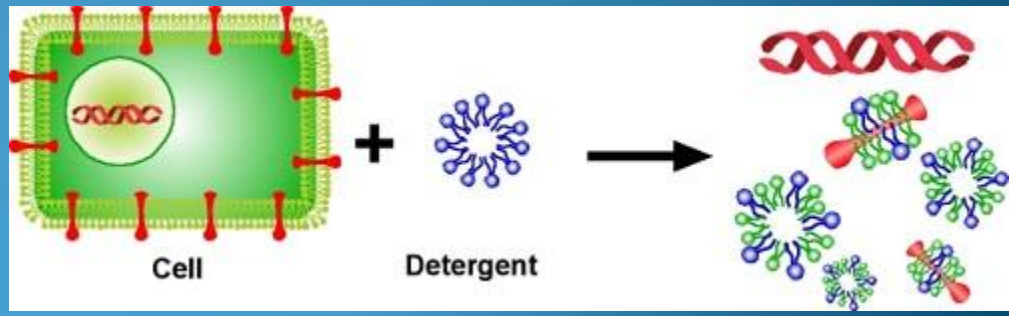
Mechanical Force

Liquid nitrogen and grinding , Sonication, grinding



Animal cell

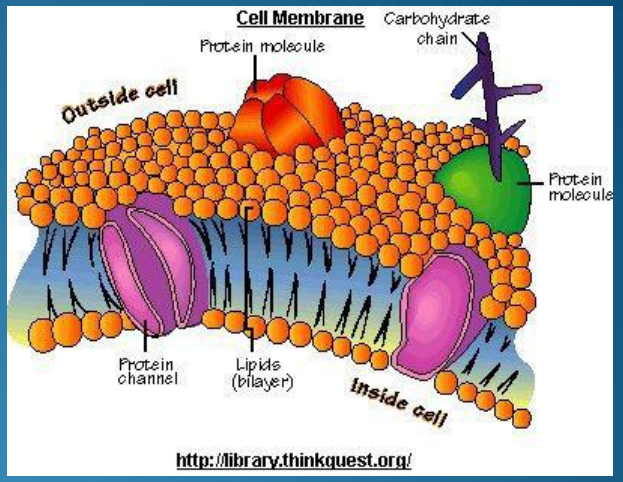
Mild Detergent



Cells lysis

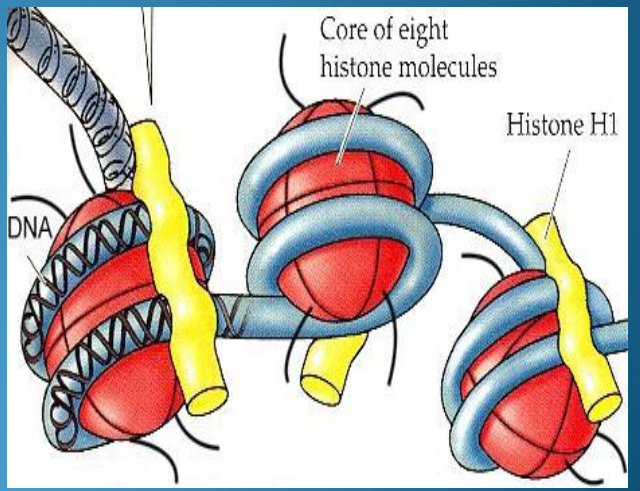
SDS(sodium dodecyl sulfate)

- Remove lipids
- Denature proteins



Proteinase K (65 °C)

- Digest protein
- Inactivate DNAse
- Remove Histones
- Very active with SDS



Cells lysis

Phenol/Chloroform

- Separate DNA and RNA from other components
- Denature Proteins

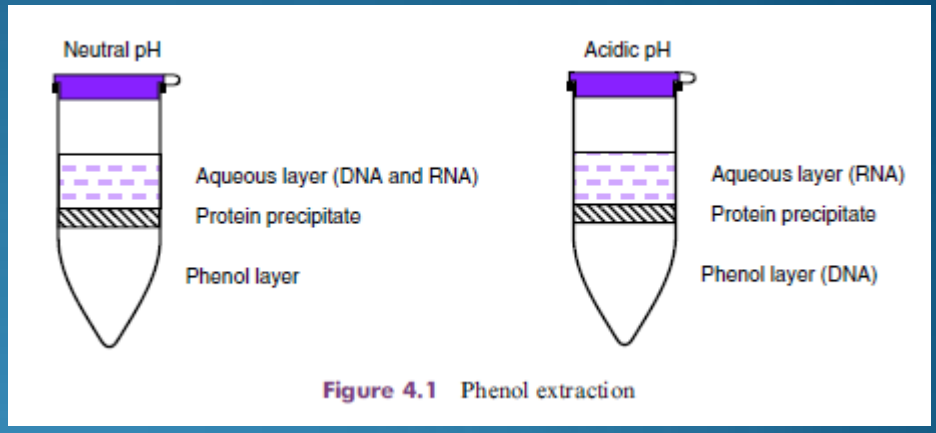
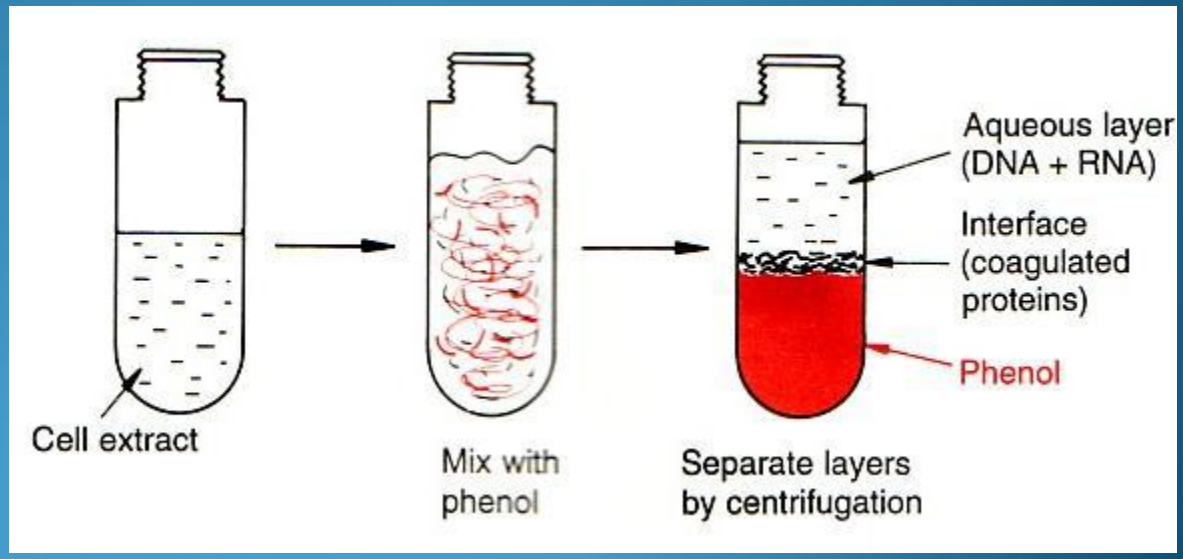


Figure 4.1 Phenol extraction



DNA Extraction

Precipitation

2-3 volumes cold ethanol 95% with high salt concentration

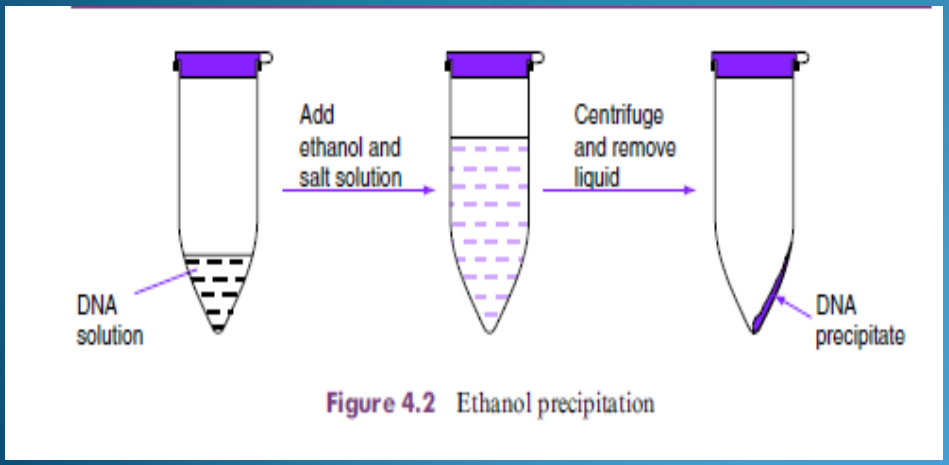
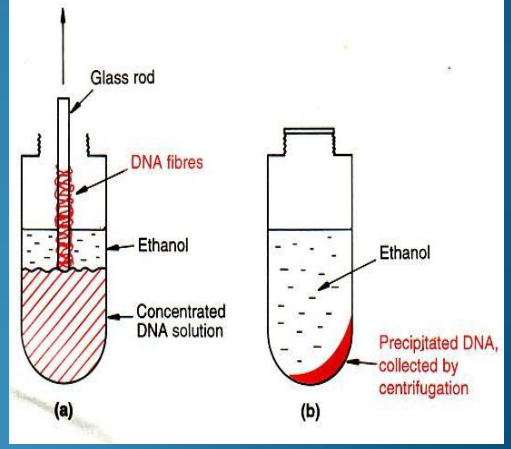


Figure 4.2 Ethanol precipitation



Washing

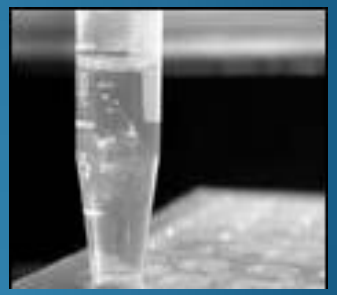
It is “washed” with a 70% ethanol solution to remove salts and other water soluble impurities but not resuspend the DNA.

Most salts are soluble in 70% ethanol

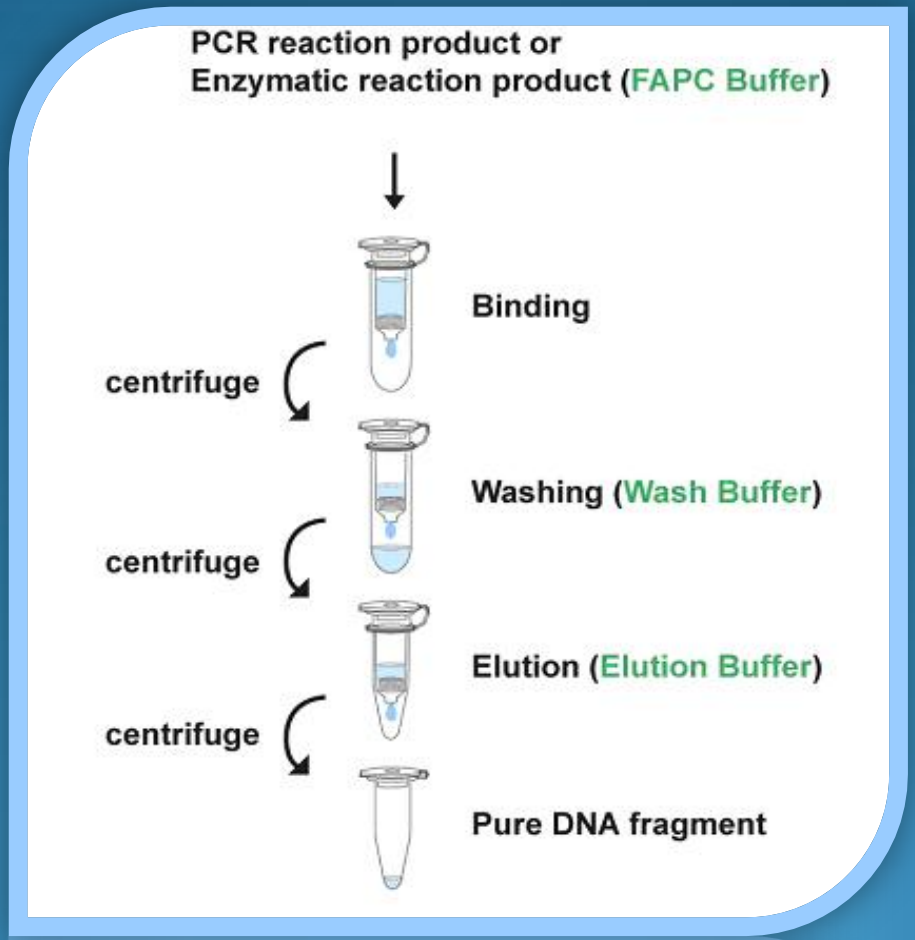
Resuspension

The clean DNA is now resuspended in a buffer or water to ensure stability and long term storage.

The most commonly used buffer for resuspension is called 1xTE or water



Spin columns



PCR from bacteria

Freshly cultured bacterial colonies

1 colony in 100µl water,
95°C for 5 minutes.

1-3 µl for PCR directly

centrifugation

low quality DNA but good enough for routine analyses

Direct PCR from blood, cells, tissues and plants

- **Whole Blood:**

use 1 µl directly in 50 µl reaction
or preheat larger volumes 95 °C 15 min (McCusker *et al., 1992) (Even with anticoagulant)*

- **Cells Resuspend :**

10ul of cell culture in water heat 100 °C in PCR machine for 5 minutes use 1-2 µl for PCR

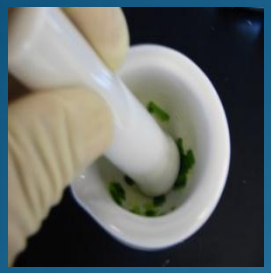
- **Tissues:**

(100 % formamide, heat 95 and 72°C 30 times prior to PCR. Use 2-3 µl for reaction) (Panaccio *et al., 1993)*

- **Plant (seed):**

(Drilling out a sample from the seed, adding NaOH, heating in a microwave oven and neutralizing with Tris-HCl. (Von Post *et al., 2003).Use 1-3 µl for reaction*

Overview of DNA Extraction



Break down the cell wall & membranes

Centrifuge to separate the solids from the dissolved DNA

Precipitate the DNA using alcohol

Centrifuge to separate the DNA from the dissolved salts and sugars

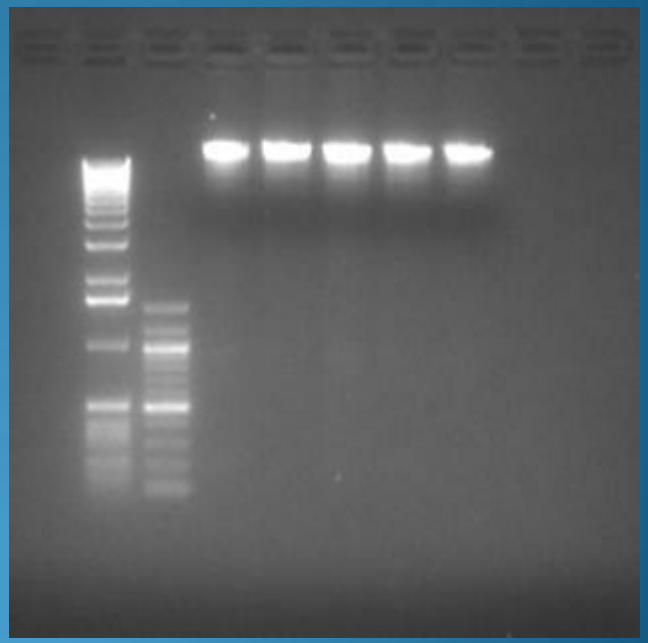
Wash the DNA pellet with Ethanol and dry the pellet

Dissolve DNA

Evaluation of Nucleic Acids

- **Spectrophotometrically**
  - quantity
  - quality

- **Fluorescent dyes**
  - gel electrophoresis



DNA quality and concentration

DNA	A_{260}	1.0 ≈ 50 μg/ml
	A_{260}/A_{280}	1.6 - 1.8
RNA	A_{260}	1.0 ≈ 40 μg/ml
	A_{260}/A_{280}	~2.0

DNA Extraction

Analyzing DNA samples

By using gel electrophoresis

Analysis of samples:

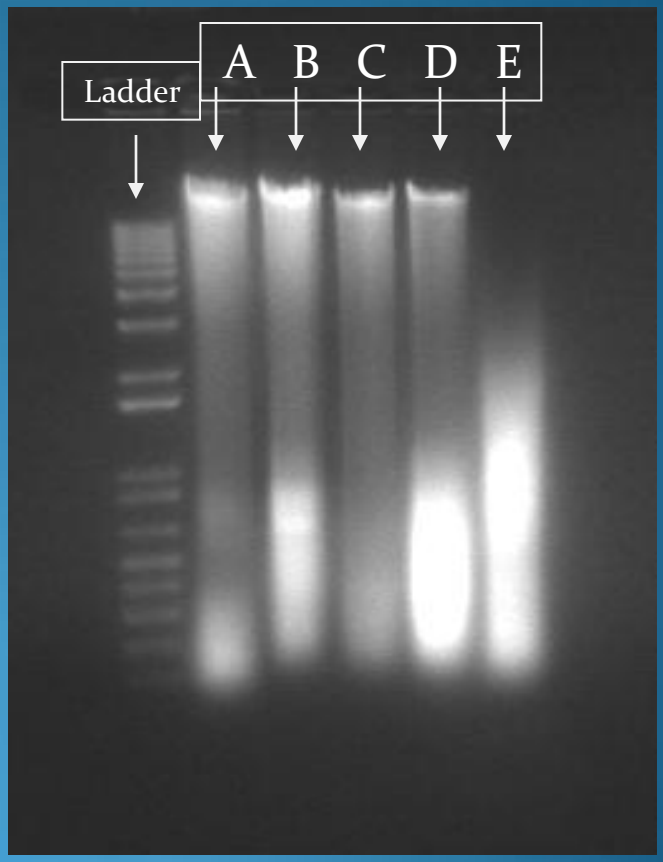
Barley (A): This sample is fine

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




Thank You!