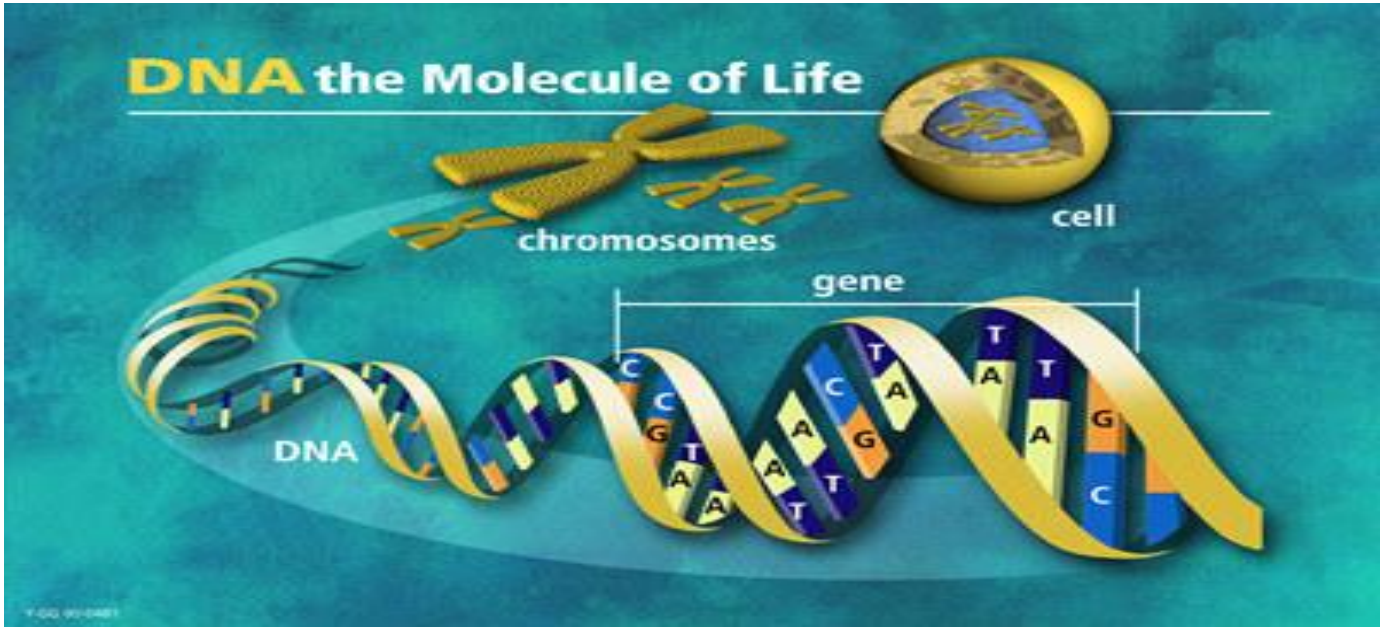
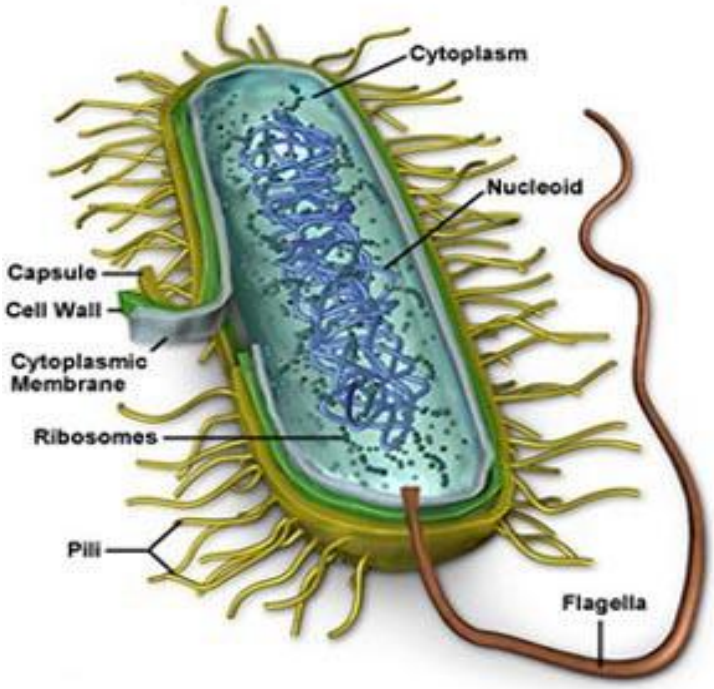
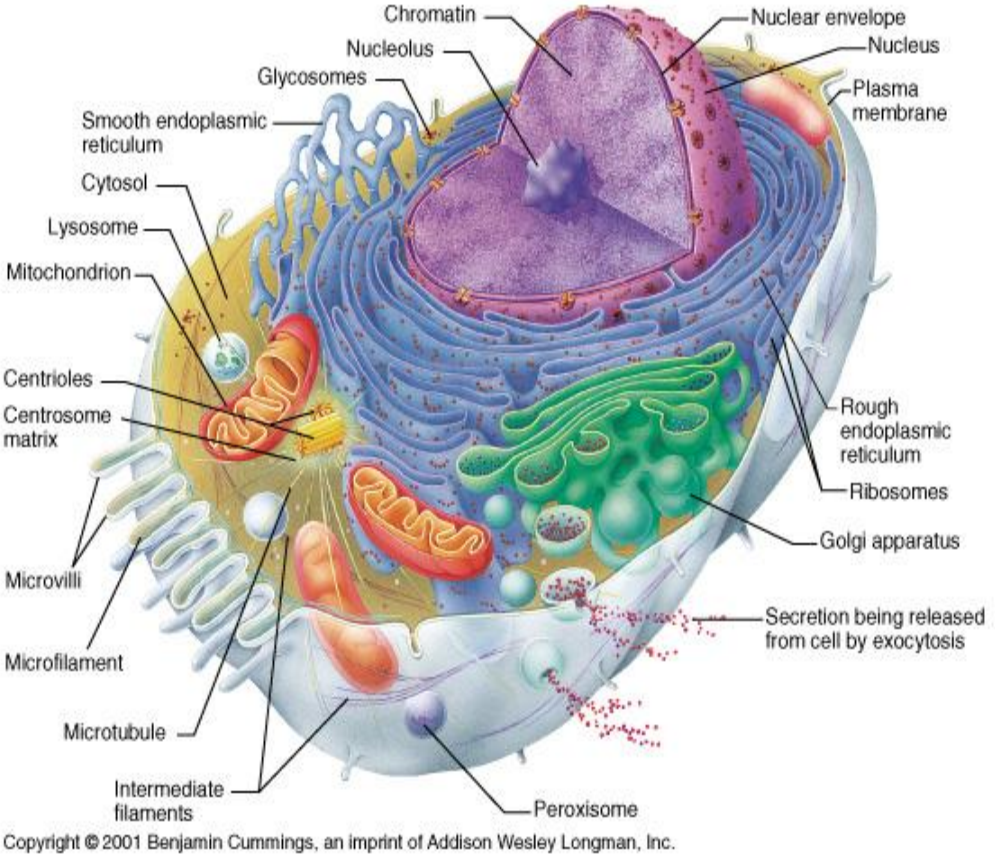
Extraction of DNA



Basic structure of the cell





Prokaryotic cell

Eukaryotic cell

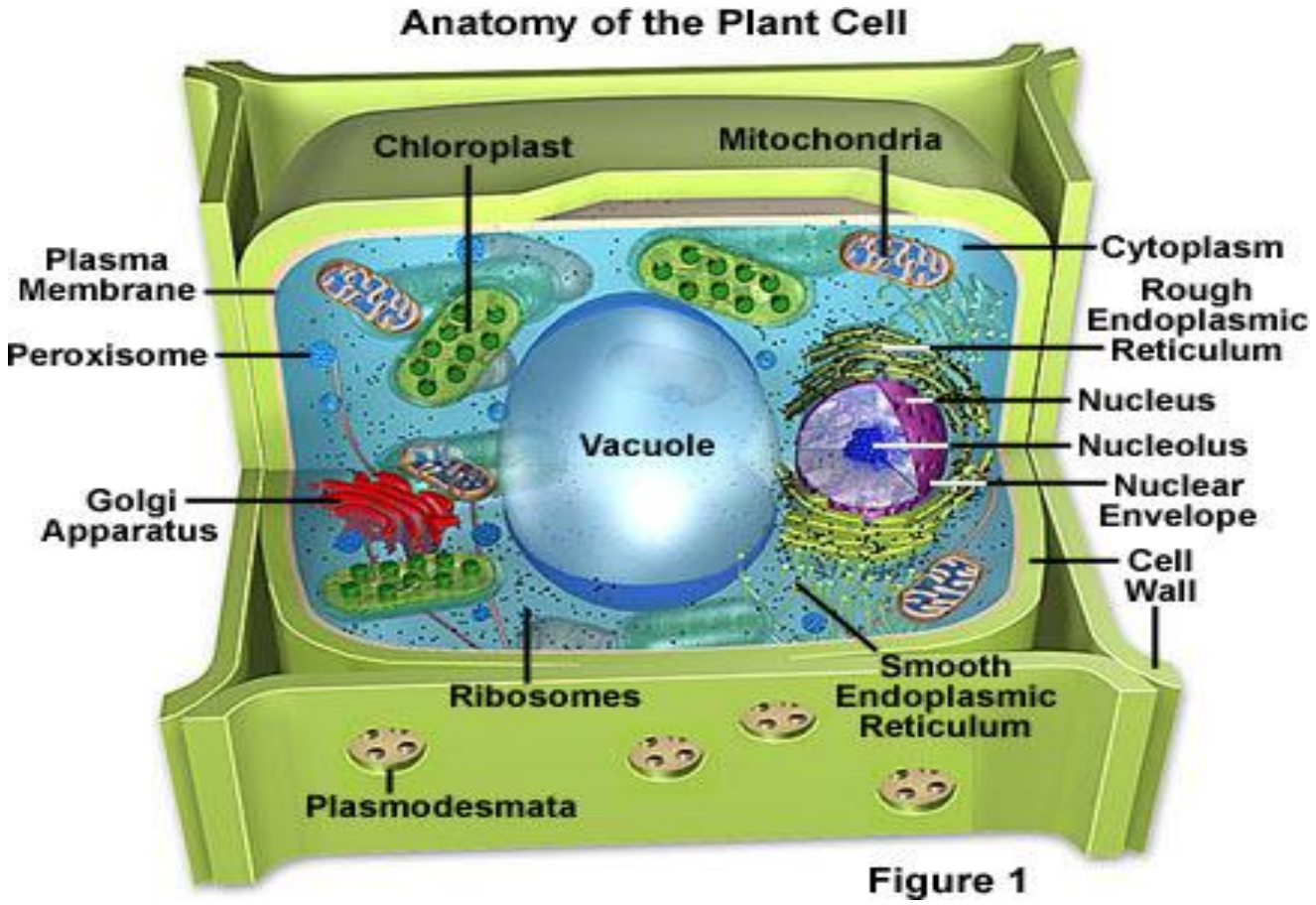


Figure 1

Exceptions, RBCs, …..etc

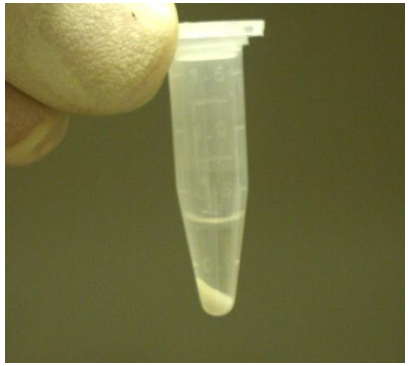
PCR from bacteria

Freshly cultured bacterial colonies

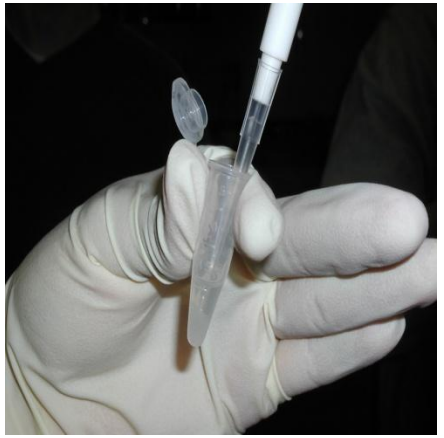
1 colony in 100µl water, 95°C for 5 minutes.

1-3 µl for PCR directly

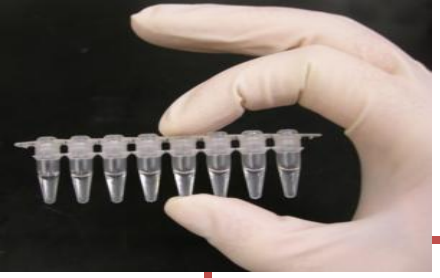
centrifugation



1- 5 µl for PCR



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

What are the Most Commonly used DNA Extraction Procedures ?

- Organic (Phenol-Chloroform) Extraction
- Non-Organic (Proteinase K and SDS)
- Commercial kits (column technology)

The method utilized may be sample dependant or researcher preferences

Most DNA extraction protocols consist of three parts

1. A technique to lyse the cells gently and solubilize the DNA
2. Enzymatic or chemical methods to remove contaminating proteins, lipids, RNA, or macromolecules
3. Washing

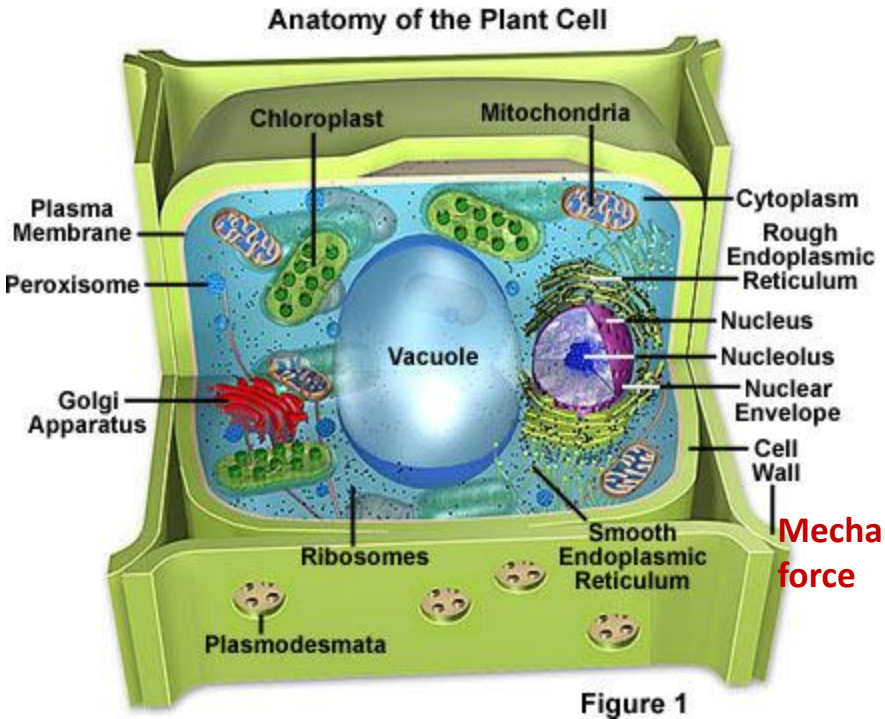


Figure 1

Mechanical force

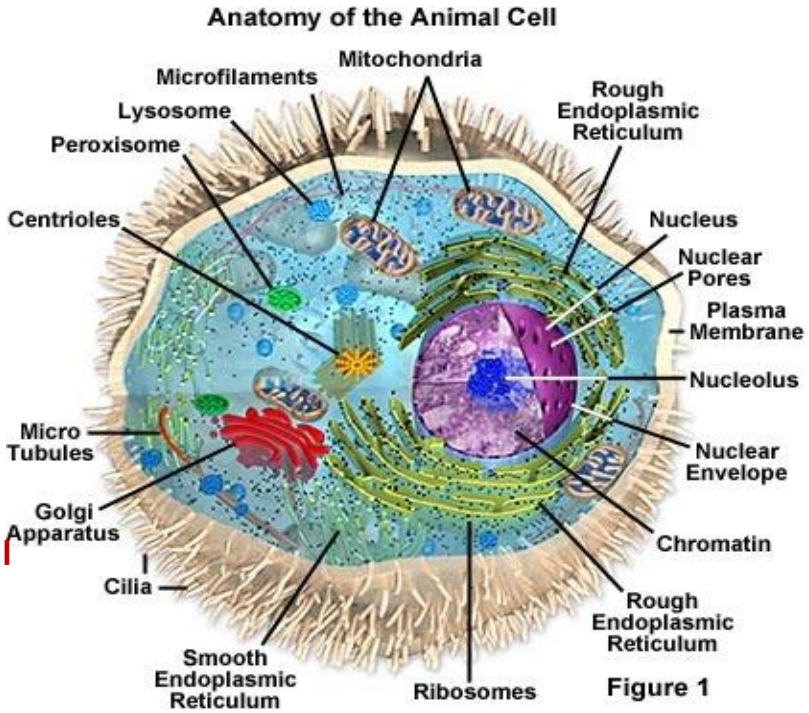


Figure 1

Cell lysis
or Cell disruption

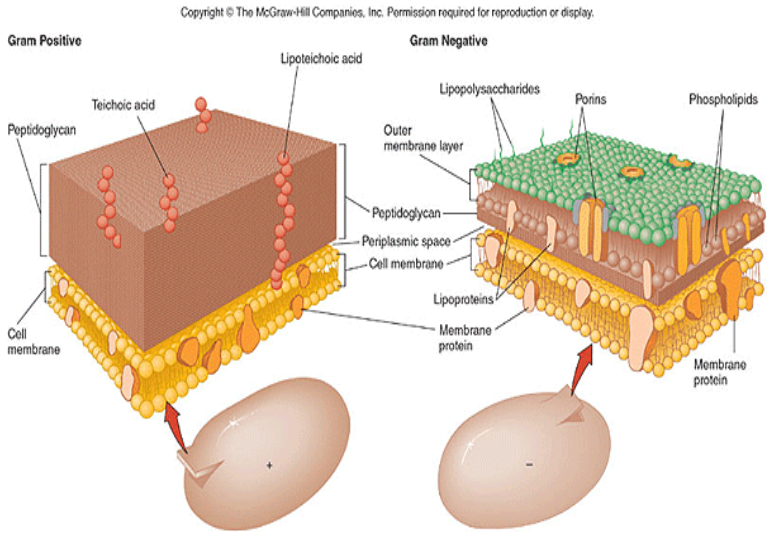
Mechanical Force
(Plant, Fungi and Bacteria)

Liquid nitrogen and grinding , Sonication, grinding

Cell lysis
or Cell disruption

Lysozymes Freshly prepared
(Tears, egg white, milk, mm)

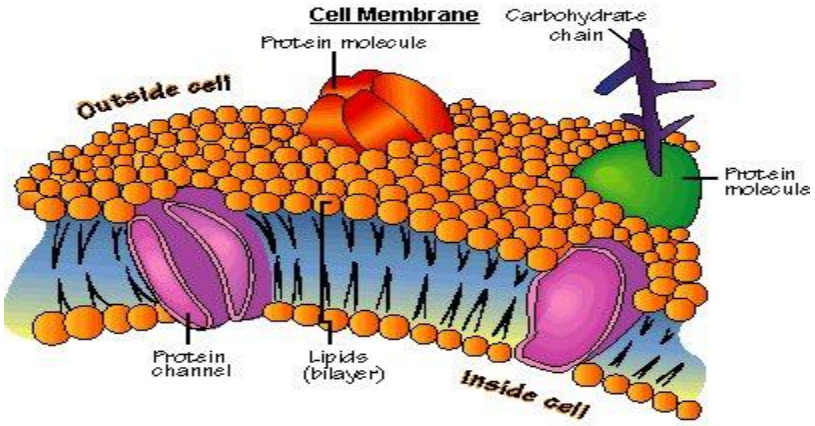
Breaking peptidoglycan
cell wall in Bacteria



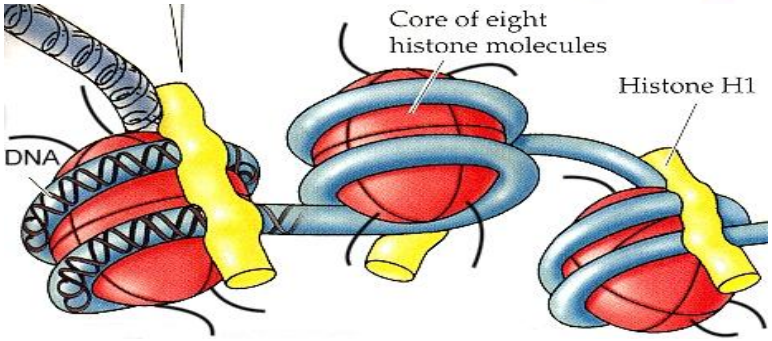
Alexander Fleming
Nobel Prize 1945

Cell Lyses

SDS (sodium dodecyl sulfate) SLS
•Remove lipids
•Denature proteins
(Shampoo, Body wash detergent , car wash)
Swine Flu vs Hepatitis A virus



Proteinase K (65 °C)
•Digest protein
•Inactivate DNAse
•Remove Histones
•Very active with SDS



EDTA
Eliminate divalent cations (Mg, Ca)
Destabilize cell wall and inhibit DNAse

Cell Lyses

Phenol/Chloroform

- Separate DNA and RNA from other components
- Denature Proteins

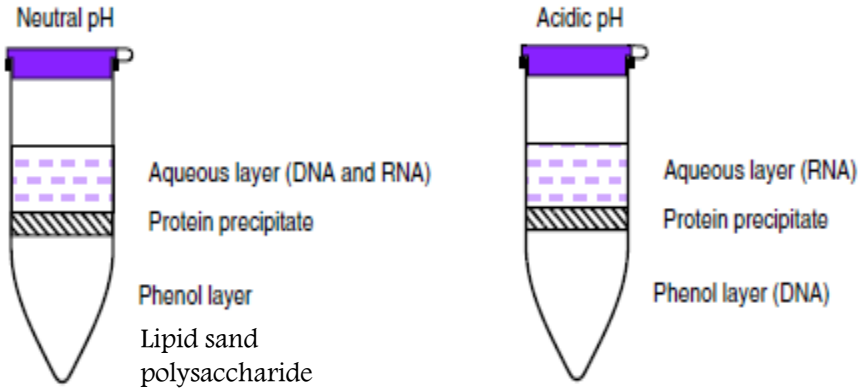
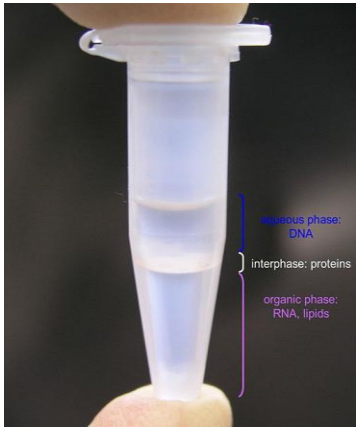


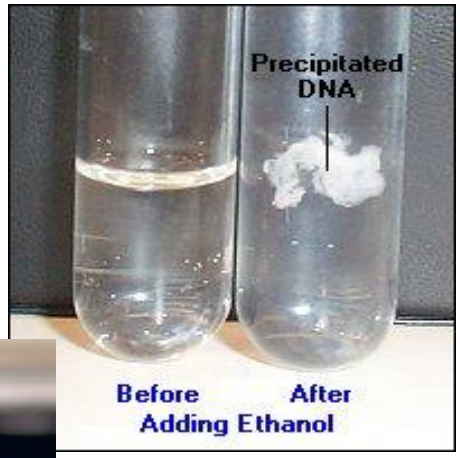
Figure 4.1 Phenol extraction

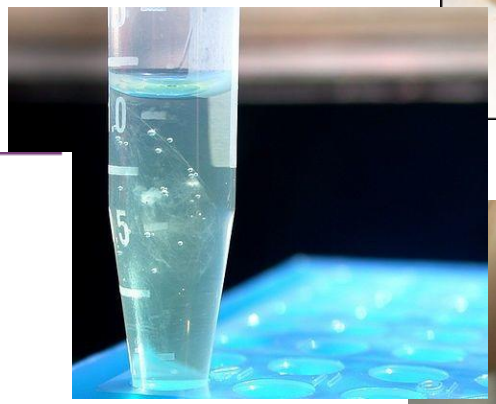


DNA precipitation and washing

Precipitation: 2-3 volumes cold ethanol 95%
with high salt concentration
10% 3 M Na-Ac or NaCl

Washing: ethanol 70 %





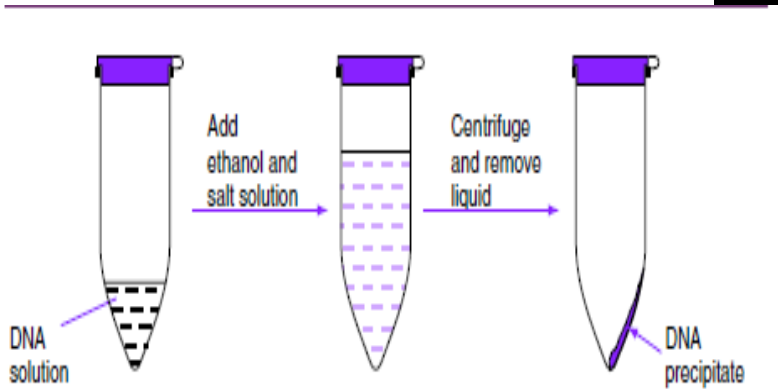
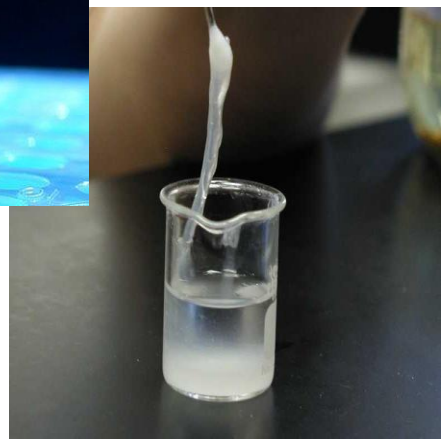


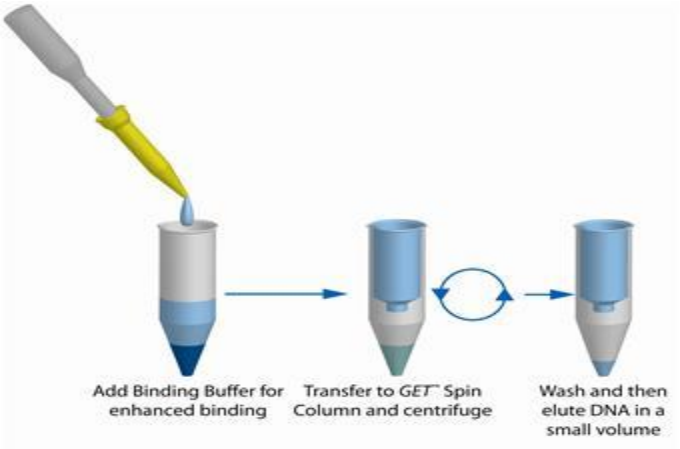
Figure 4.2 Ethanol precipitation



DNA spooling with glass

Spin columns

Silica membrane attach to DNA
in presence of chaotropic salt
(guanidine HCl or urea)



Elution and Storage

- **Buffer (Water and TE)**
- **Best storage - 80 °C (regardless of the buffer)**
- **-20 °C (Water vs. TE)**
- **+ 4°C (Water vs. TE)**
- **Room temperature or shipping**
- **Aliquots**
- **Applications**

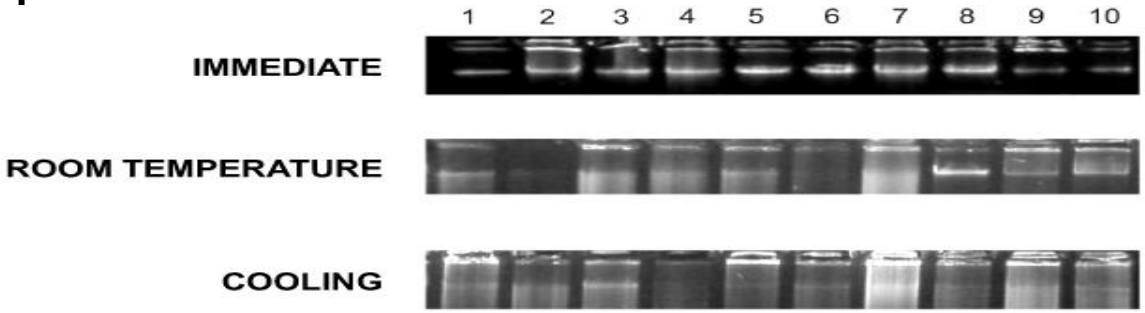
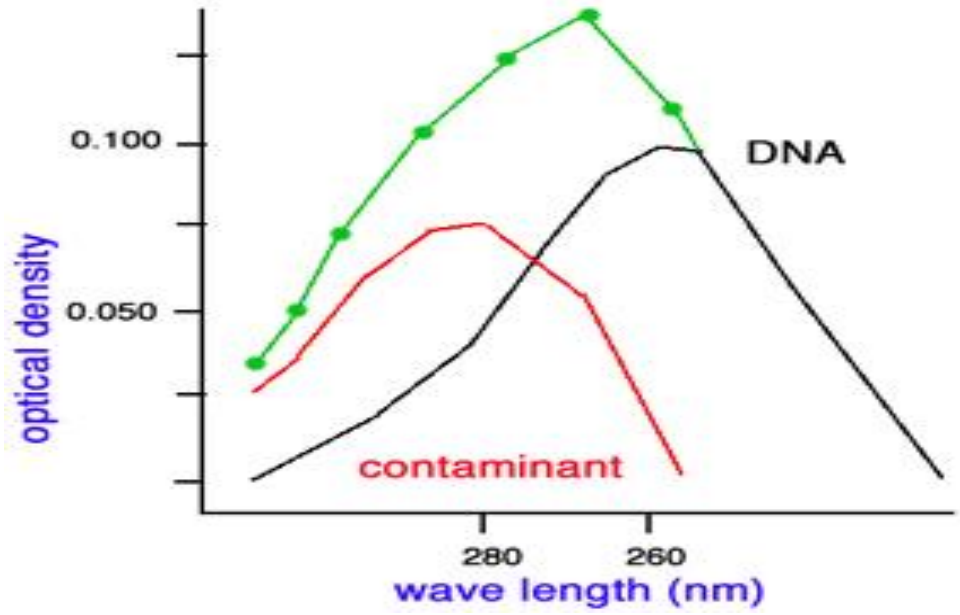


FIGURE 1- Quality of DNA evaluated by agarose gel electrophoresis. When the samples were processed immediately after collection, the DNA bands were strong and clear. When the samples were stored at room temperature or cooled, the quality of DNA decreased and the bands were degraded or absent

Evaluation of Nucleic Acids

- **spectrophotometrically**
  - **quantity**
  - **quality**
- **fluorescent dyes**
  - **gel electrophoresis**



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

DNA quality and concentration

Example

Experimental sample volume of 200 uL, diluted 100-fold
(2 uL DNA + 198 uL water)
Blank sample volume of 200 uL, diluted 100-fold
(2 uL TE buffer + 198 uL water)

OD260 = 0.177
OD280 = 0.096

Concentration
(0.177)(50 ug/mL)(100) = 885 ug/mL (0.885 mg/mL)

Quality
OD260/OD280 = 0.177/0.096 = 1.84 (good purity is between 1.8 - 2.0)

Analyzing DNA samples

With gel electrophoresis

Analysis of samples:

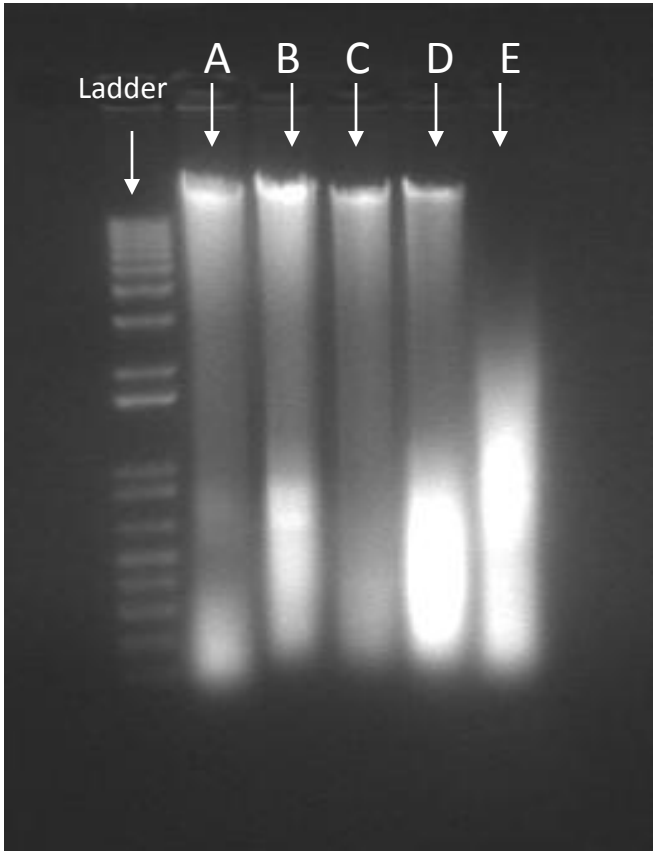
Barley (A): This sample is fine

Corn (B): This sample is fine

Oat (C) : This sample is fine

Rice (D) : This sample is fine

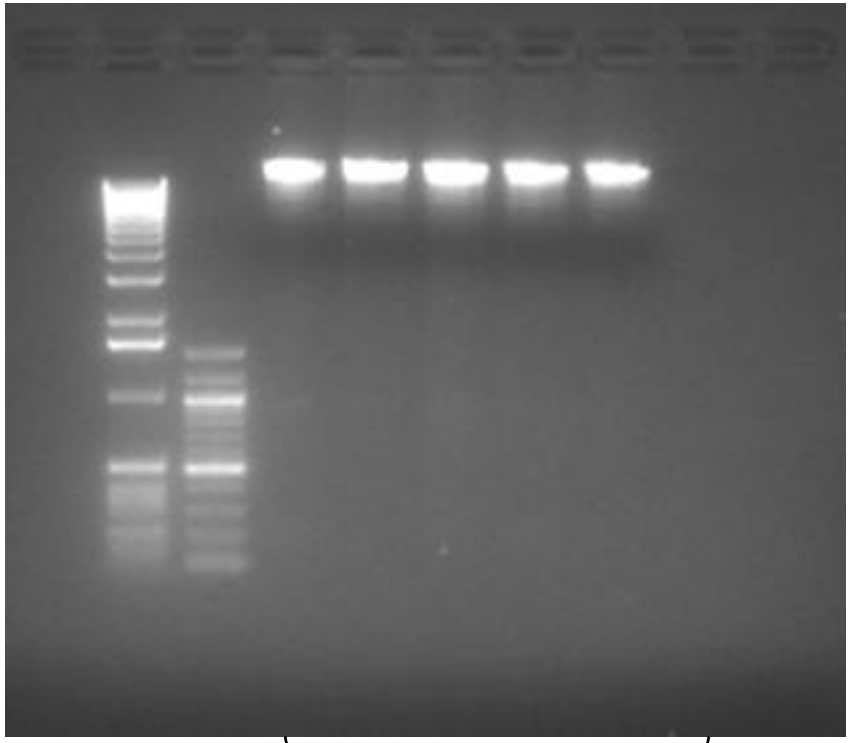
Wheat (E): This sample has severe degradation, but can work for PCR



Analyzing DNA samples

With gel electrophoresis

Below is an agarose gel that has 5 genomic DNA samples from various plants.
Note that 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

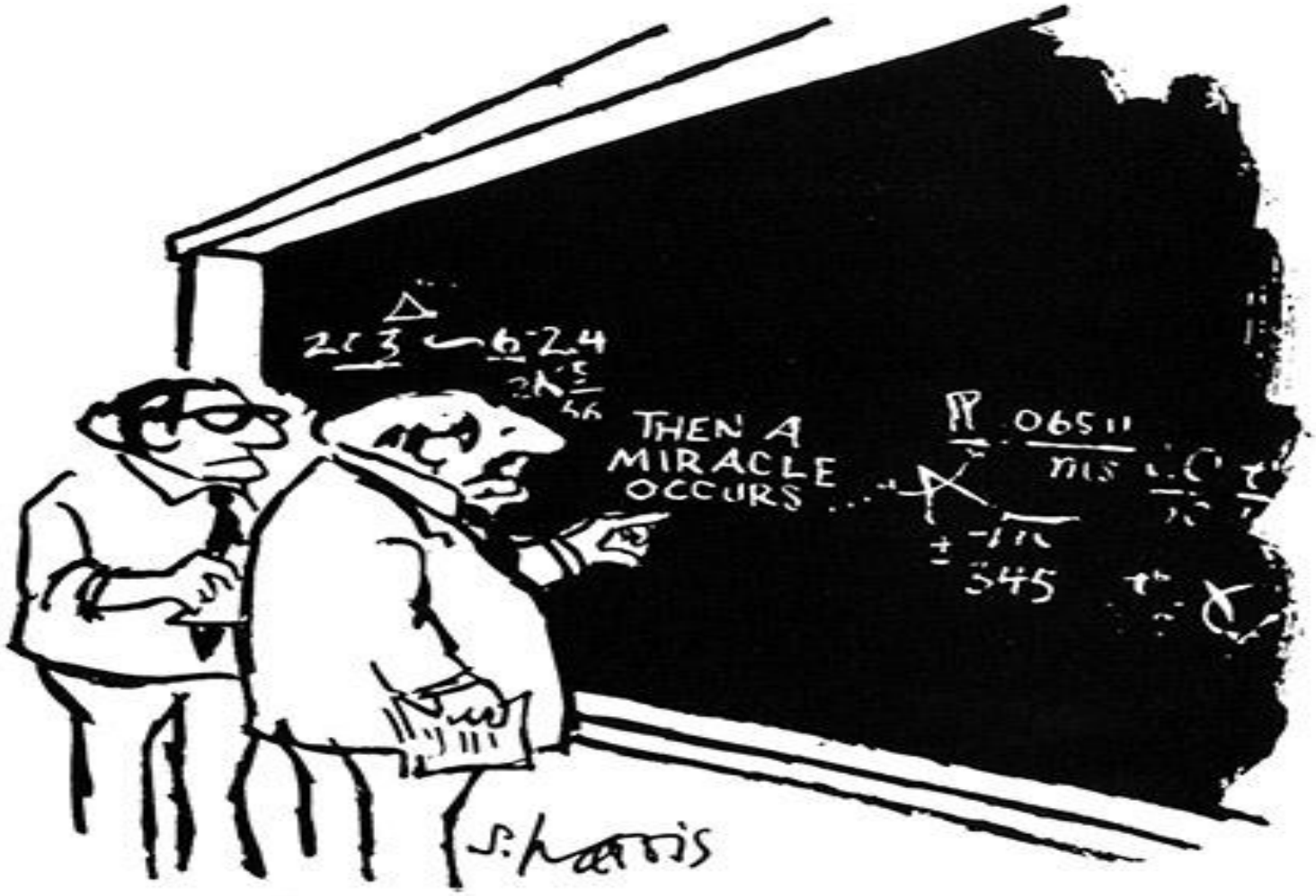


1 kbp and 100 bp ladders

Genomic DNA of 5 species of cereals

- **No RNA contamination**
- **No DNA degradation**

Questions



"I think you should be more explicit here in step two."