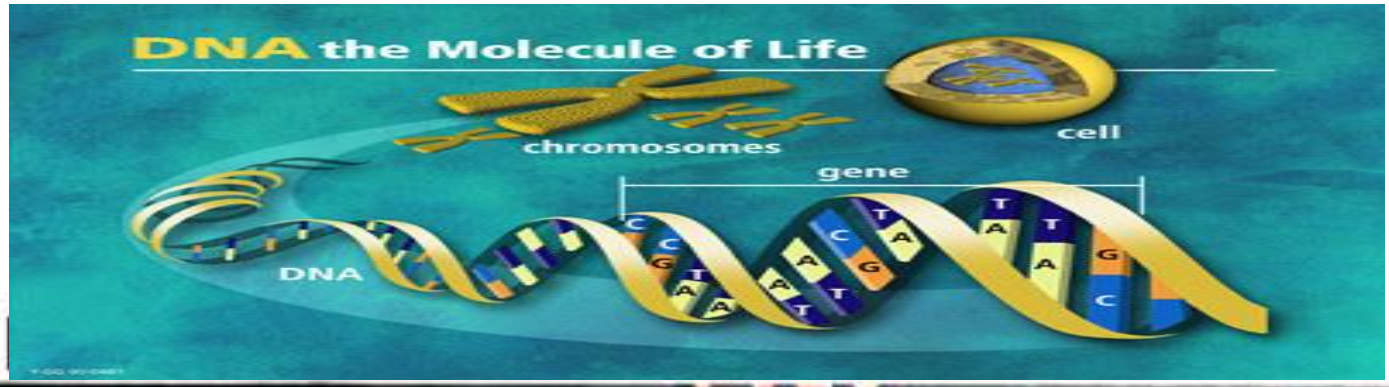
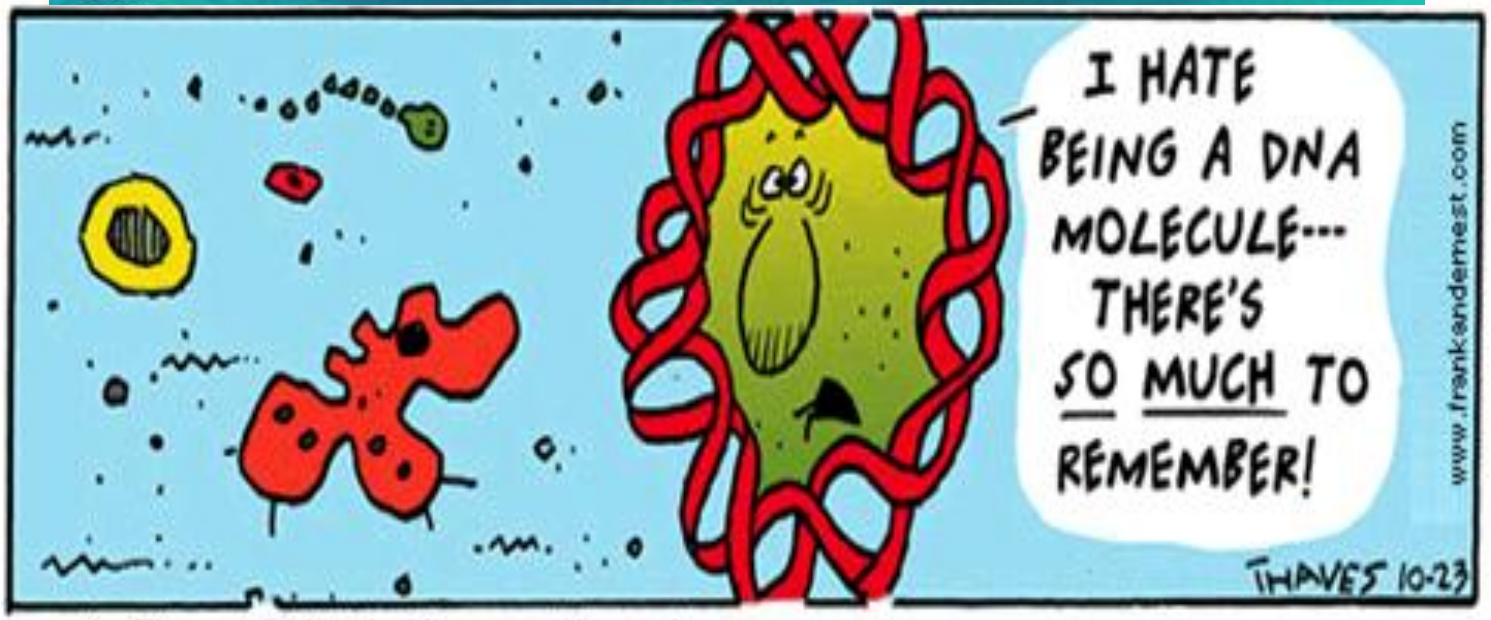
Extraction of DNA



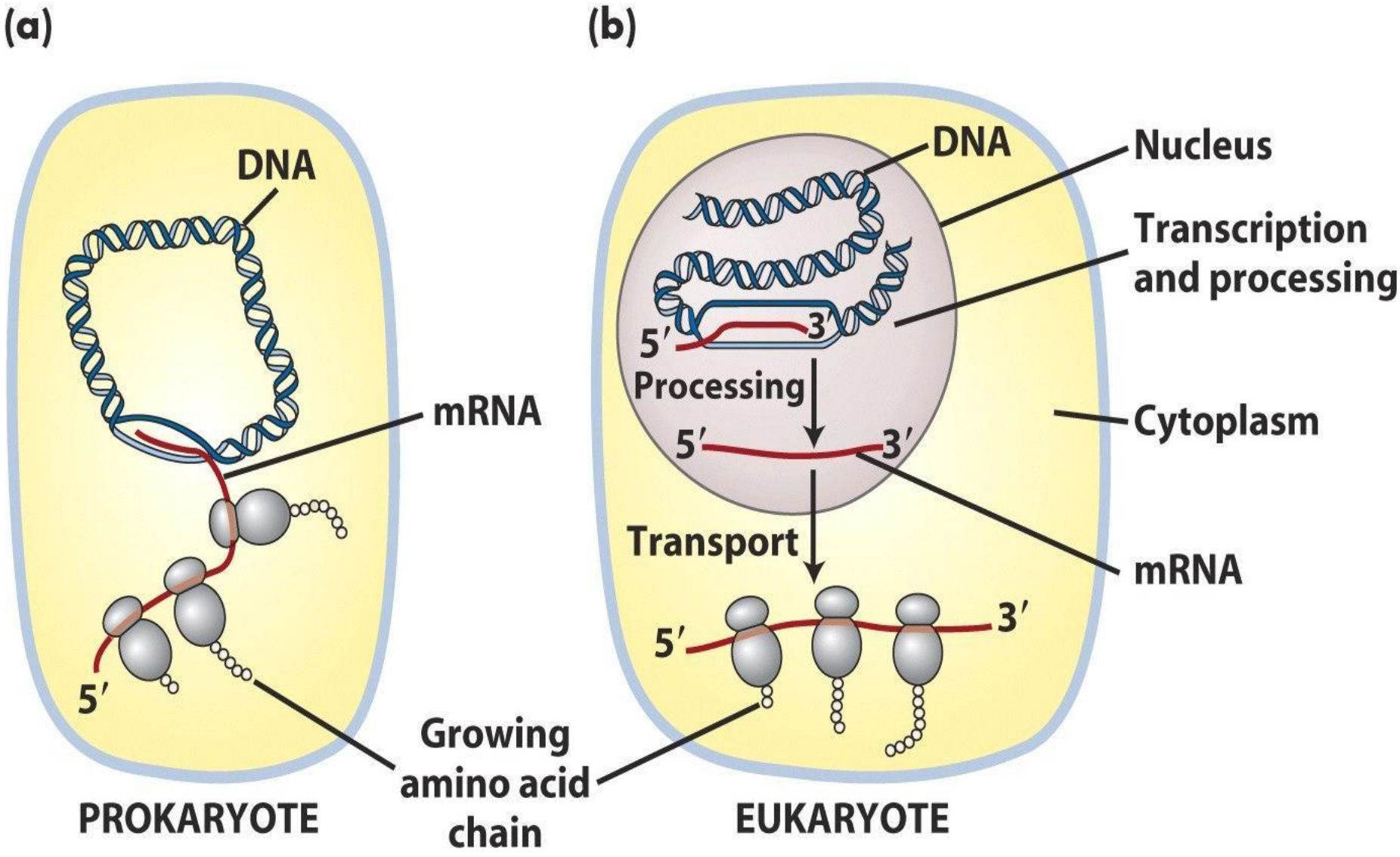


Aims

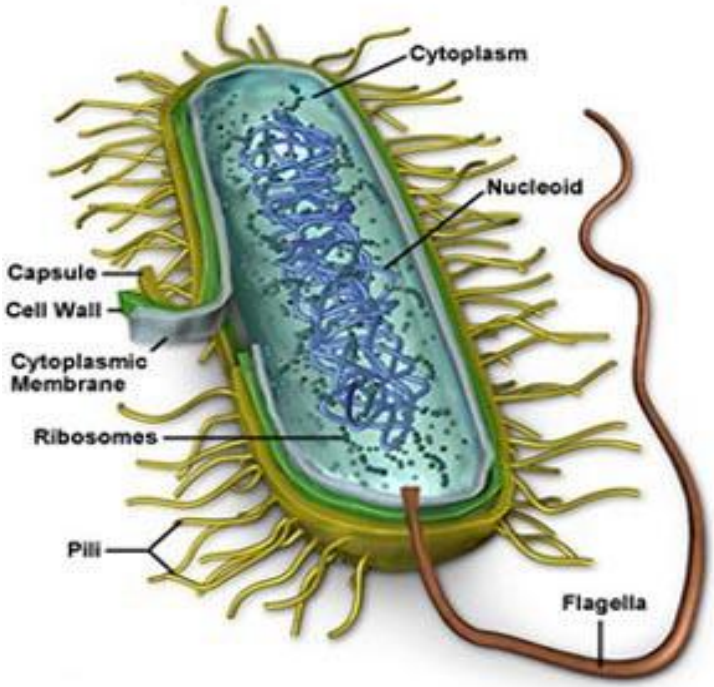
To be able to understand the principles and applications Of

- DNA extraction
- Polymerase Chain Reaction
- Electrophoresis

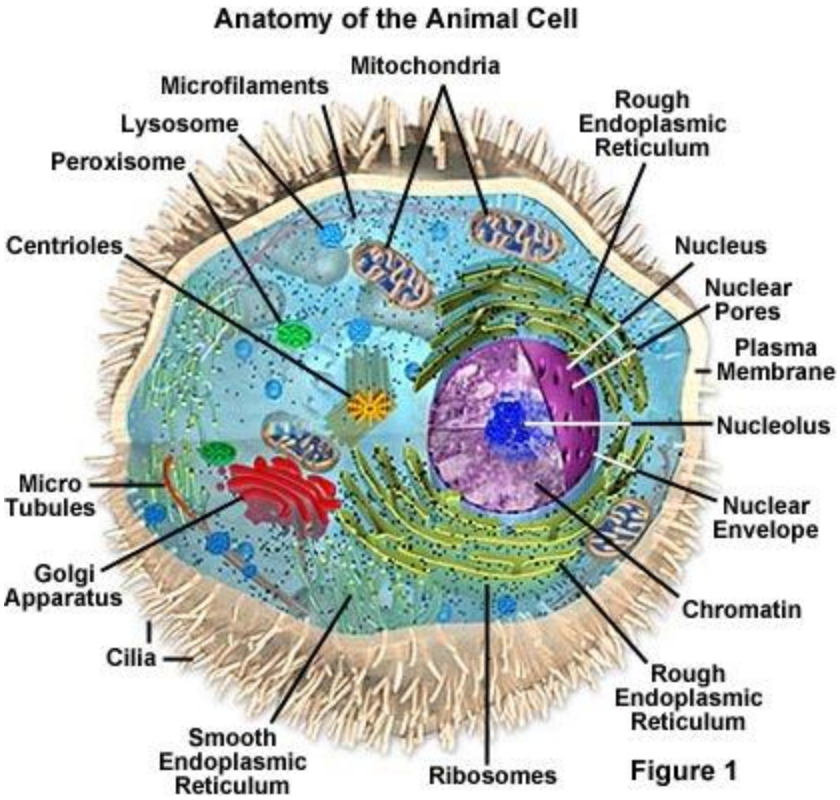
Basic knowledge



Basic structure of the cell



Prokaryotic cell



Eukaryotic cell

What are the Most Commonly used DNA Extraction Procedures ?

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

The method utilized may be sample dependant or researcher preferences

Task

DNA extraction

- Down stream applications
- Sample
- Search for simple technique

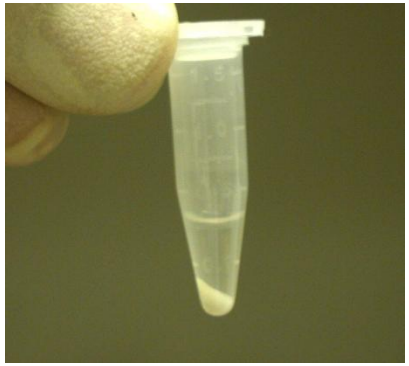
Big picture

PCR from bacteria

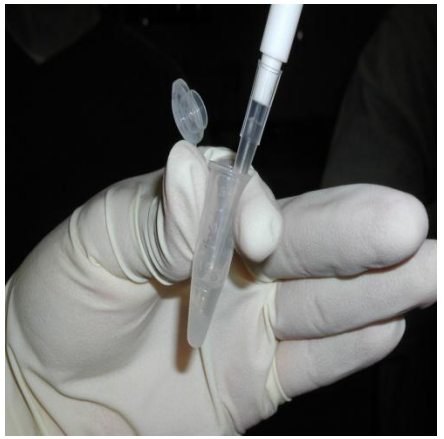
Freshly cultured bacterial colonies

1 colony in 100µl water, heated at 95°C for 5- 10 minutes.

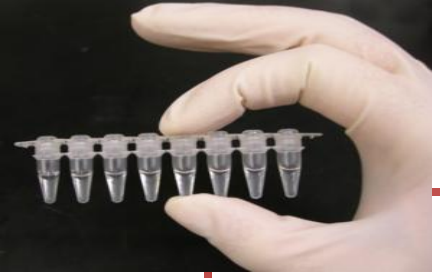
centrifugation



1- 5 ul for PCR



low quality DNA but good enough for routine analyses.



Direct PCR from tissues and plants

- Cells

10ul of cell culture 100 °C in water in PCR machine for 5 minutes

- Tissues

(100 % formamide, heat 95 and 72°C 30 times prior to PCR. Panaccio et al., 2003

- 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

ORGANIC EXTRACTION

PROCEDURE

- Cell Lysis Buffer - lyse cell membrane, nuclei are intact, pellet nuclei.
- Resuspend nuclei, add Sodium Dodecly Sulfate (SDS), Proteinase K. Lyse nuclear membrane and digest protein.
- DNA released into solution is extracted with phenol-chloroform to remove proteinaceous material.
- DNA is precipitated from the aqueous layer by the additional of ice cold 95% ethanol and salt
- Precipitated DNA is washed with 70% ethanol, dried under vacuum and resuspended in TE buffer.

ORGANIC EXTRACTION

REAGENTS

- Cell Lysis Buffer - Non-ionic detergent, Salt, Buffer, EDTA designed to lyse outer cell membrane of blood and epithelial cells, but will not break down nuclear membrane.
- EDTA (Ethylenediaminetetraacetic disodium salt) is a chelating agent of divalent cations such as Mg2+. Mg2+is a cofactor for Dnase nucleases. If the Mg2+is bound up by EDTA, nucleases are inactivated.

ORGANIC EXTRACTION

REAGENTS

- Proteinase K - it is usual to remove most of the protein by digesting with proteolytic enzymes such as Pronase or proteinase K, which are active against a broad spectrum of native proteins, before extracting with organic solvents. Protienase K is approximately 10 fold more active on denatured protein. Proteins can be denatured by SDS or by heat.

ORGANIC EXTRACTION

REAGENTS

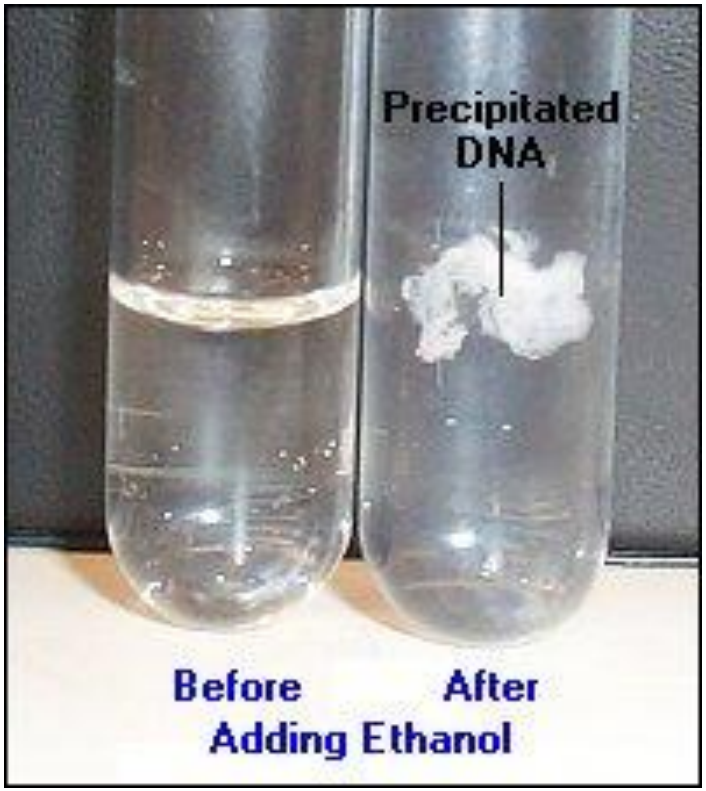
- Phenol/Chlorform - The standard way to remove proteins from nucleic acids solutions is to extract once with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform. This procedure takes advantage of the fact that deproteinization is more efficient when two different organic solvents are used instead of one.
- Also, the final extraction with chloroform removes any lingering traces of phenol from the nucleic acid preparation.
- Phenol is highly corrosive and can cause severe burns.

Concentrating DNA
Alcohol Precipitation

- The four critical variables are the purity of the DNA, its molecular weight, its concentration, and the speed at which it is pelleted.
- DNA a concentrations as low as 20 ng/ml will form a precipitate that can be quantitatively recovered.
- Typically 2 volumes of ice cold ethanol are added to precipitate the DNA.

Concentrating DNA
Alcohol Precipitation

- Solutes that may be trapped in the precipitate may be removed by washing the DNA pellet with a solution of 70% ethanol. To make certain that no DNA is lost during washing, add 70% ethanol until the tube is 2/3 full. Vortex briefly, and recentrifuge. After the 70% ethanol wash, the pellet does not adhere tightly to the wall of thetube, so great care must be taken when removing the supernatant.


Precipitated
DNA
Before
After
Adding Ethanol

Concentrating DNA
Microcon®100 Centrifugal Filter Unit

Qiagen DNA Extraction

Tissue Lysis. Detergents lyse membranes. Proteinase K breaks down proteins.

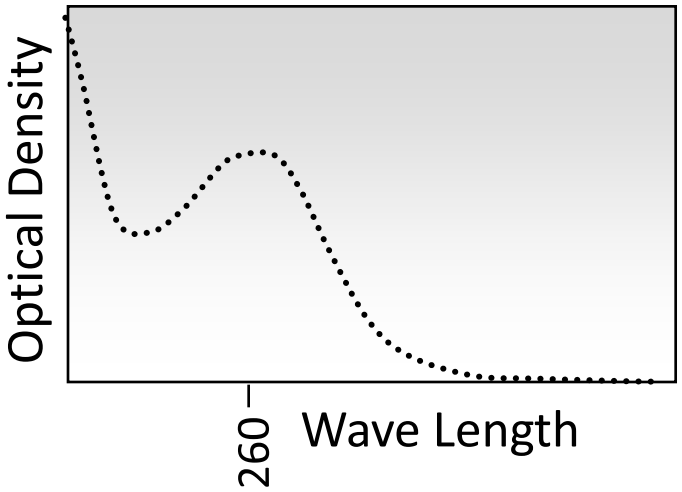
Sample loading onto column. Nucleic acids bind to membrane and other components flow through.

Column washing. Removes any contaminants.

Elution of DNA. DNA is released from column and ready for use in downstream applications.

Using Spectroscopy to analyze DNA

DNA absorbs UV light with a major peak at 260 nm

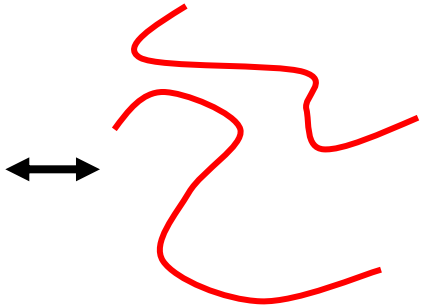


This absorption is useful because it varies with the structure of DNA *(&RNA)*

i.e. extinction coefficient depends on the structure

dsDNA

Low extinction coefficient

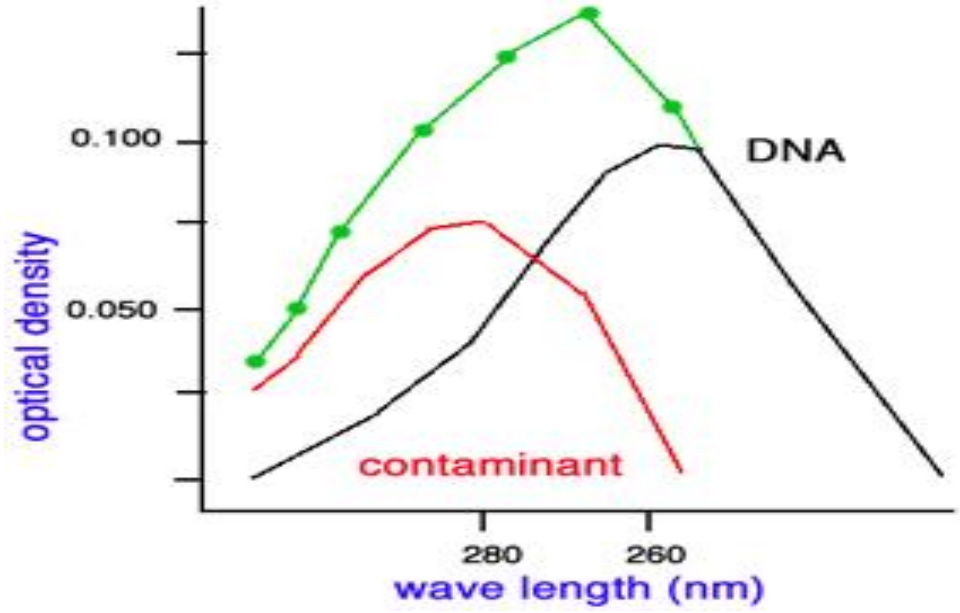


ssDNA

Higher extinction coefficient

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

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)

Storage

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

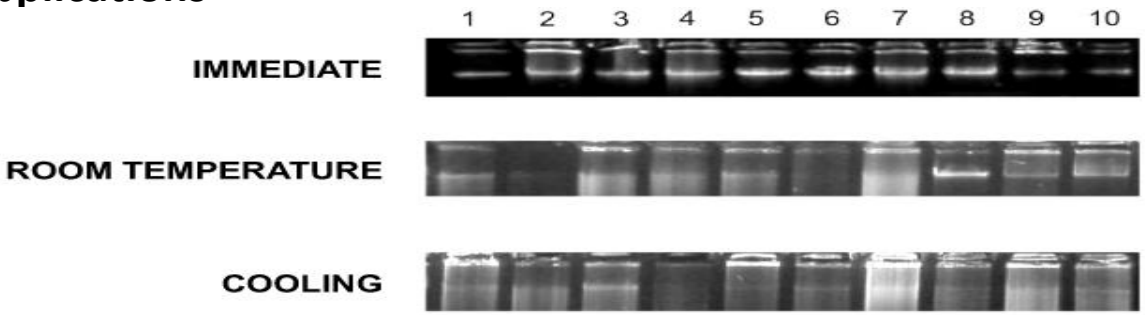


FIGURE 1- Quality of DNA evaluated by agarose gel electrophoresis. When the samples were processed immediately after collection, the DNA bands were strong and clear. When the samples were stored at room temperature or cooled, the quality of DNA decreased and the bands were degraded or absent

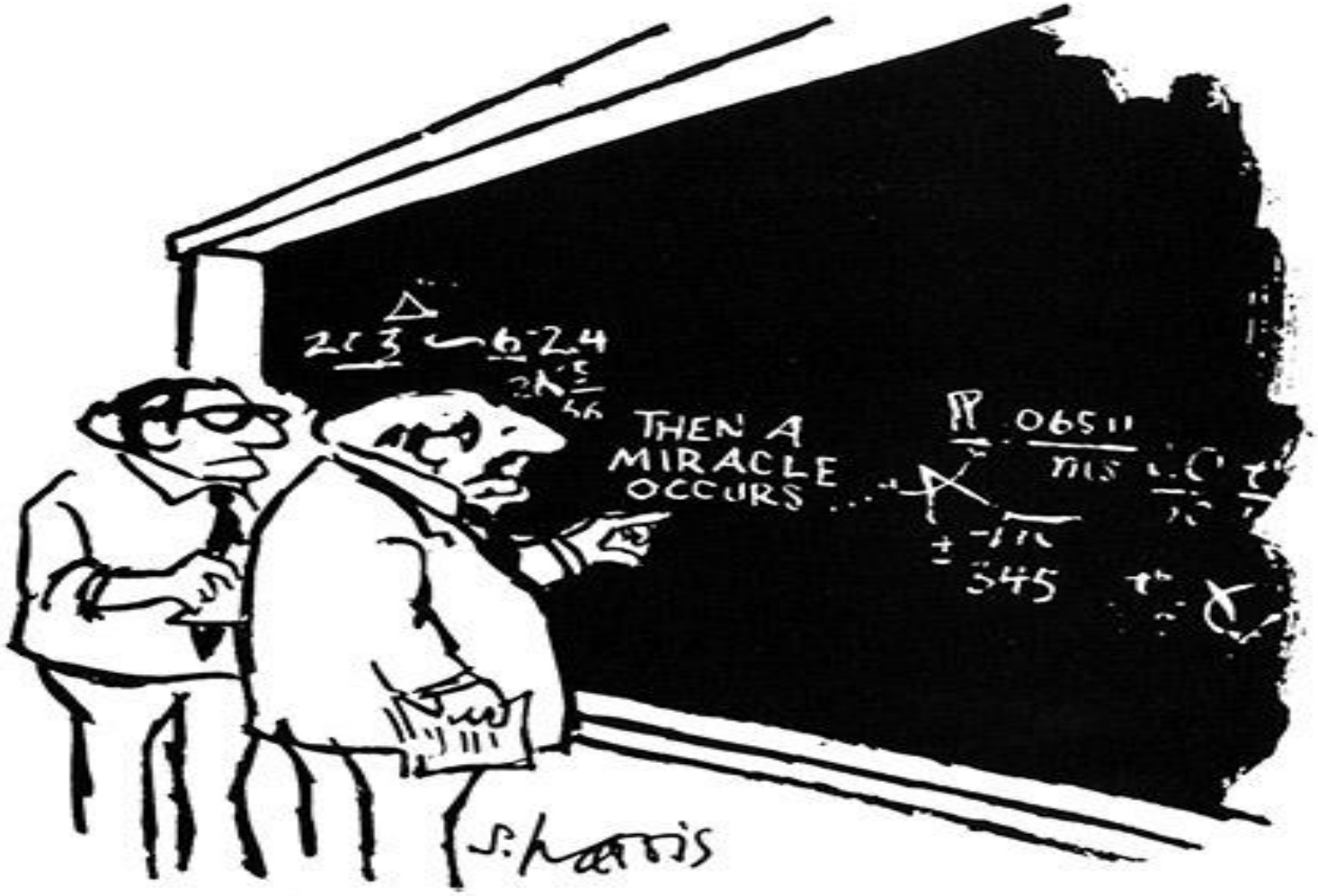
Take home messages

- Planning (DNA extraction is not the goal)
  - Choosing the method is the most important step
  - Don't waste clean thinking on dirty enzymes

    Efraim Racker, Cornell University
  - Don't assume if it is commercial and new from big names it should work fine (read instructions)

Don't raise the fence

Questions



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