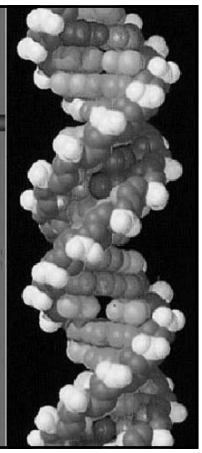
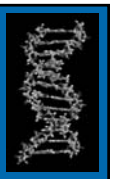
PCR training course

DNA
Extraction

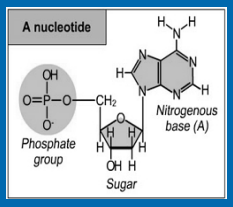
Prof.Dr. / Hamdy M. El-Aref

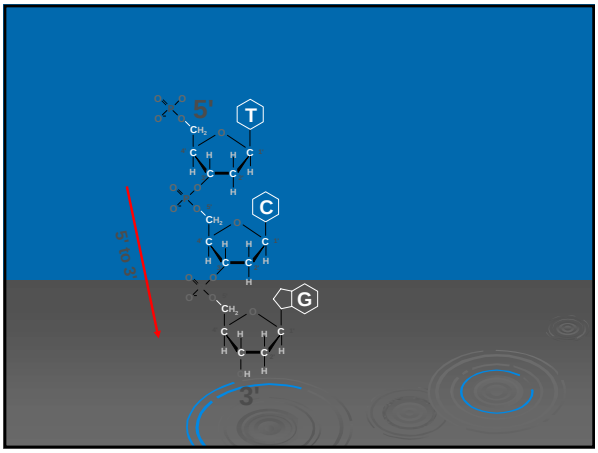


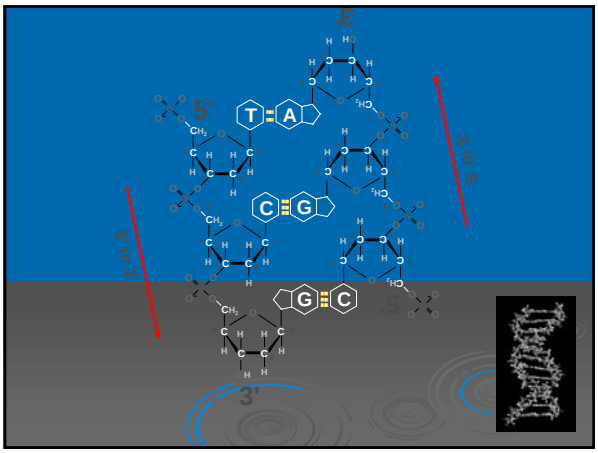
What is DNA

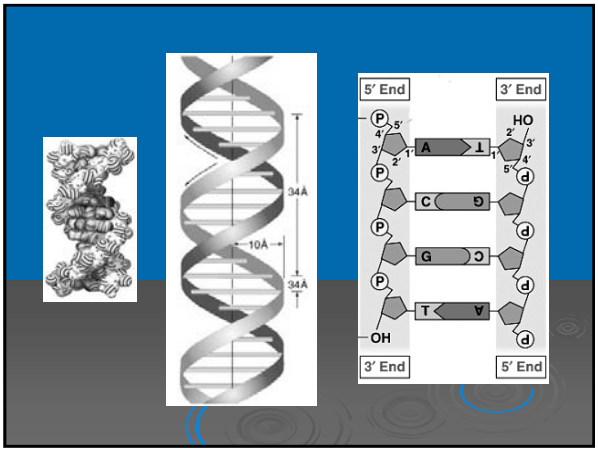


- DNA is the genetic material.
- Is a double helix.
- Made up of subunits called nucleotides
- Nucleotide made of:
  1. Phosphate group
  2. 5-carbon sugar
  3. Nitrogenous base

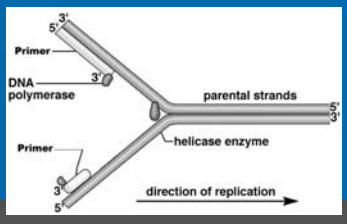


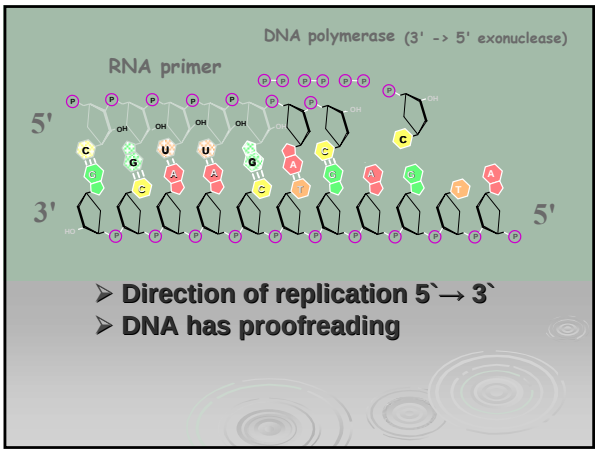




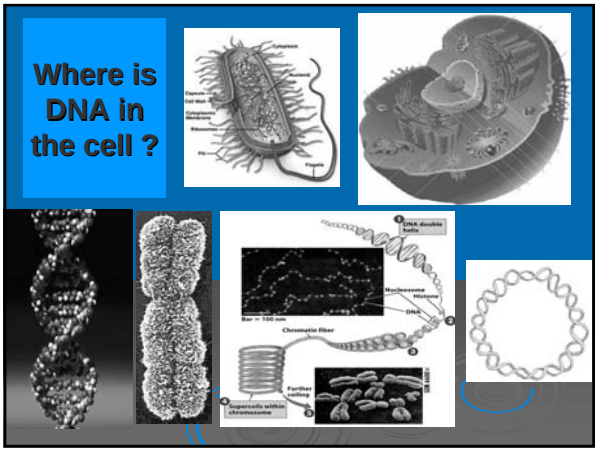


DNA replication start with primer





- Direction of replication 5`→ 3`
- DNA has proofreading



DNA extraction

Most DNA extraction protocols consist of 5 parts:

1- A technique to **lyses the cells** gently and solubilize the DNA

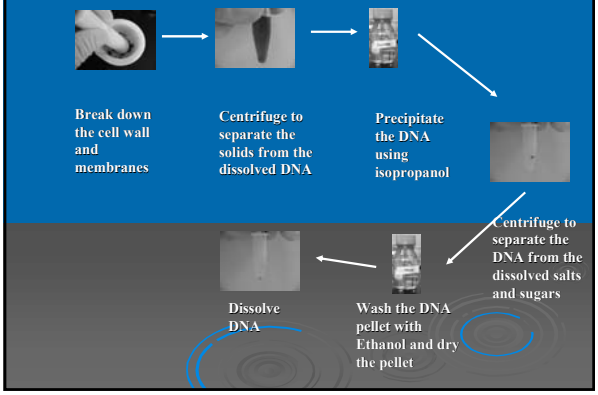
2- DNA degrading enzymes must be deactivated

3- Enzymatic or chemical methods to remove proteins, lipids, RNA, or macromolecules.

4- DNA must be precipitated in alcohol solution

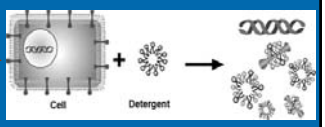
5- Washing : DNA must be precipitated in alcohol solution, then washed, dried, and resuspended in buffer or sterile water.

Overview of DNA Extraction

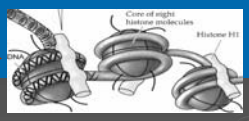


Cell Lyses

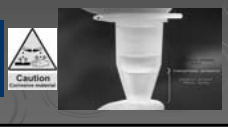
CTAP (or SDS)
Remove lipids
Denature proteins



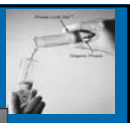
Proteinase K (65 °C)
•Digest protein
•Inactivate DNAse
•Remove Histones



Chloroform/isoamyl alcohol *(phenol)*
•Remove proteins from DNA and RNA



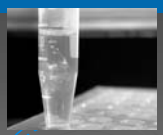
DNA precipitation and washing



Precipitation:
2-3 volumes cold ethanol

Washing: ethanol 70 %

Elution :
- TE buffer
- Water



DNA spooling with glass

DNA extraction from different samples

SAMPLE PREPARATION

- **Blood:** 500µl in 1ml water, centrifuge at 5000rpm/2min and the pellet resuspended in 200µl of TE buffer.
- **Sera:** stored at -20°C.
- **Cells/Bacteria :** Cells collected by centrifugation 7500rpm/10 min and resuspended in 200µl of TE buffer.
- **Tissues:** 30mg of mammalian tissue or 50-100mg of plant tissue grounded in liquid nitrogen with mortar and pestle. The powder resuspended in 200µl of TE buffer.

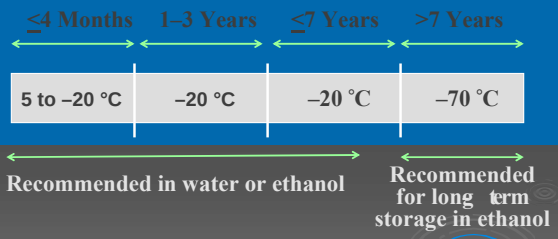
Fermentas method

- **Mix 200µl of sample with 400µl of lysis solution and incubate at 65°C for 5min.**
- **add 600µl of chloroform, mix gently and centrifuge 10,000rpm/2min.**
- **Transfer the upper aqueous phase to a new tube.**
- **add 800µl of precipitation solution, mix gently/2min**
- **centrifuge at 10,000rpm for 2min.**

- **Remove supernatant completely and dissolve DNA pellet in 100µl of 1.2M NaCl solution by gentle vortexing.**
- **Add 300µl of cold ethanol, let the DNA precipitate (10min at- 20°C) and spin down (10,000rpm, 3 4min).**
- **Pour off the ethanol**
- **Wash the pellet once with 70% cold ethanol and dissolve DNA in 100µl of sterile deionized water by gentle vortexing.**

DNA extraction for student training:

Nucleic Acid Storage



Evaluation of Nucleic Acids

(1) spectrophotometrically
- quantity
- quality

DNA	A_{260}	1.0 = ~ 50 µg/ml
	A_{260}/A_{280}	~ 1.8
RNA	A_{260}	1.0 = ~ 40 µg/ml
	A_{260}/A_{280}	~ 0.2

DNA quality and concentration

Example

Experimental sample volume of 100 uL, diluted 100-fold
(1 uL DNA + 99 uL water)

Blank sample volume of 100 uL, diluted 100-fold
(1 uL TE buffer + 99 uL water)

OD260 = 0.185
OD280 = 0.099

Concentration: (0.185)(50 ug/mL)(100) = 925 ug/mL = (0.925 mg/mL)

Quality : OD260/OD280 = 0.177/0.099 = 1.87 (good purity bet. 1.8 - 2.0)

Analyzing DNA samples With gel electrophoresis

Analyzing DNA samples With gel electrophoresis

Analysis of samples:

(A): This sample is fine

(B): This sample is fine

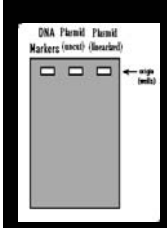
(C) : This sample is fine

(D) : This sample is fine

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

Analyzing DNA samples With gel electrophoresis

- The DNA below is a very high molecular weight, clear, thick band.
- This DNA was extracted in a research lab under optimal conditions
- This DNA is ideal for GE and sequencing.



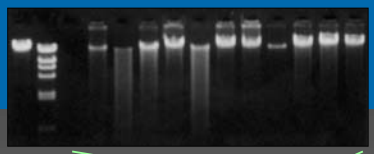
•No RNA contamination
•No DNA degradation

1 kbp and 100 bp ladders

Genomic DNA of 5 species of cereals

DNA Quality from Agarose Gel Electrophoresis

Human Whole Blood DNA

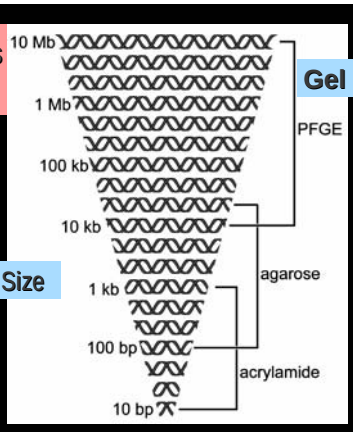


Lambda DNA marker

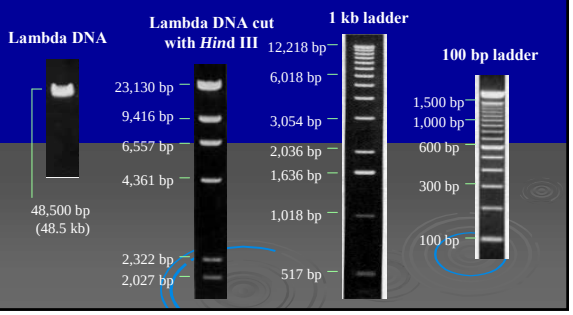
Lambda DNA cut with *Hind* III marker

Whole blood genomic DNA

Electrophoresis of DNA



DNA Size from Agarose Gel Electrophoresis: Compares unknown DNA to known size standards



Troubleshooting Nucleic Acid Preparation Methods

- Problem: No or low nucleic acid yield.
  - Make sure that sample time was allowed for resuspension or rehydration of sample.
  - Repeat isolation from any remaining original sample (adjust procedure for possible low cell number or poorly handled starting material).
  - Concentrate dilute nucleic acid using ethanol precipitation.

Troubleshooting Nucleic Acid Preparation Methods

- Problem: Poor nucleic acid quality
  - If sample is degraded, repeat isolation from remaining original sample, if possible.
  - If sample is contaminated with proteins or other substances, clean it up by re isolating (improvement depends on the extraction procedure used).