DNA Extraction

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Learning outcomes

❑ **At the end of this session, students should be able to:**

1. Discuss different **methods** for DNA extraction
2. Recognize the main **steps in the DNA extraction** protocol and the chemistry involved in each step.

Introduction

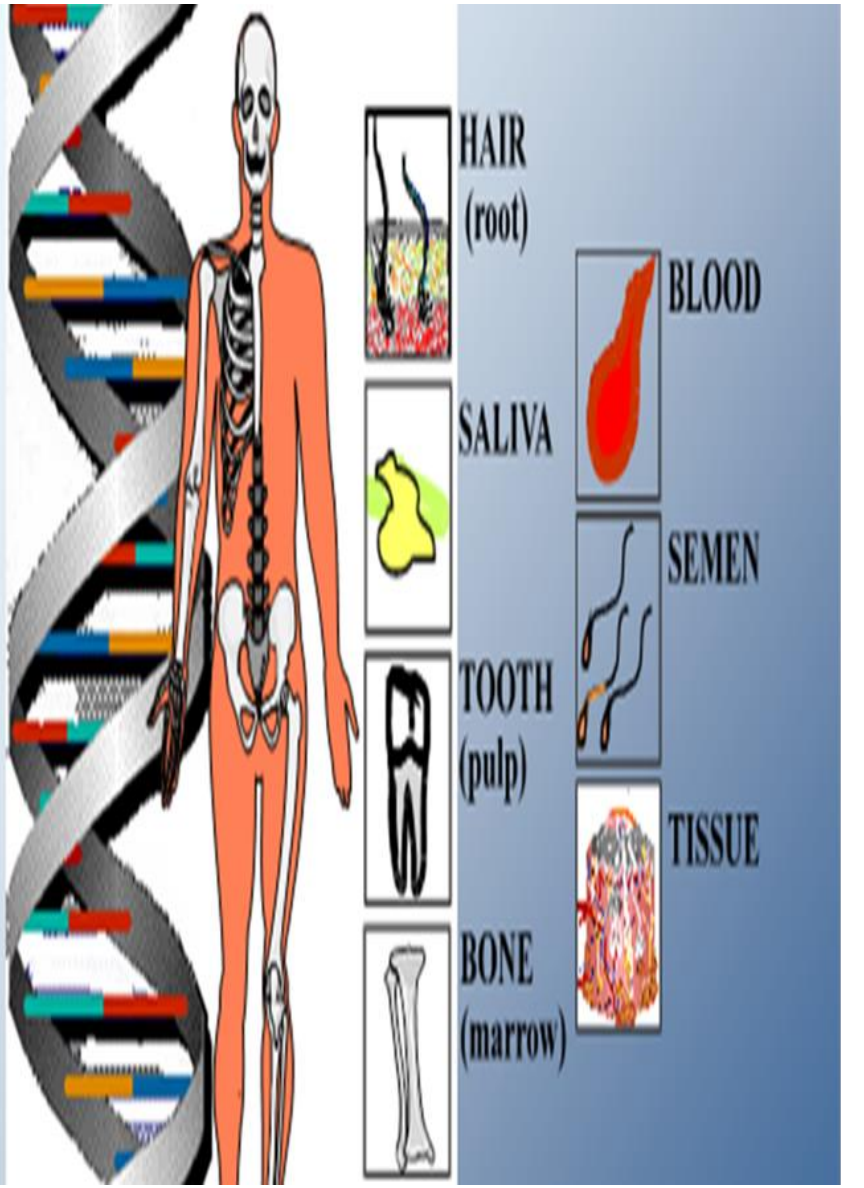
❑ **DNA, also known as deoxyribonucleic acid:**

- The fundamental molecule found in all living things
- Carries the genetic information in the cell
- Contains instructions for our body cells to perform their specific functions

DNA Extraction

- DNA extraction is a routine procedure to isolate & Purify DNA from biological samples for subsequent **molecular or forensic analysis.**

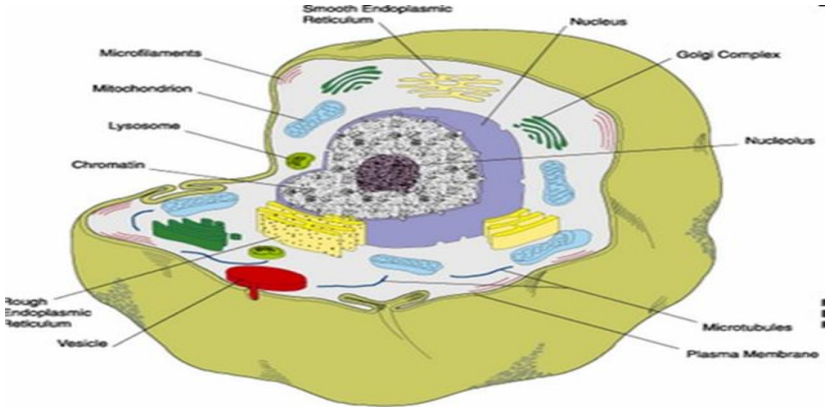
- DNA can be extracted from **whole blood , skin, hair, nails, buccal (cheek) swabs, saliva, semen, muscle tissue, bone marrow, etc.**



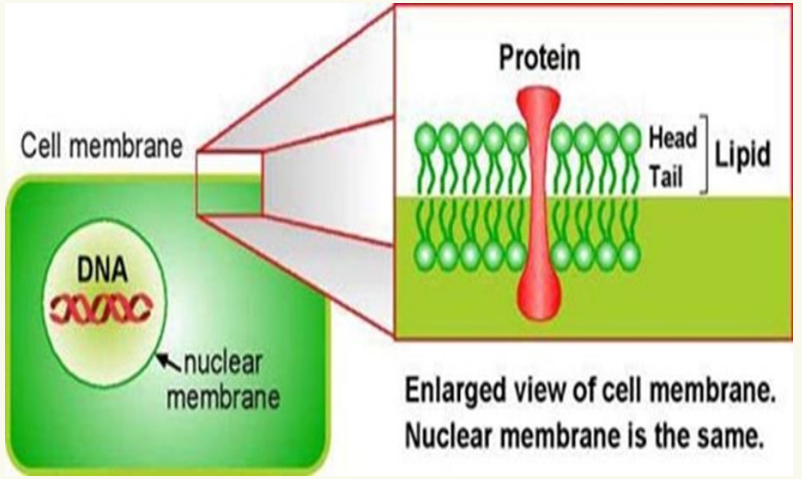
Importance of DNA Extraction

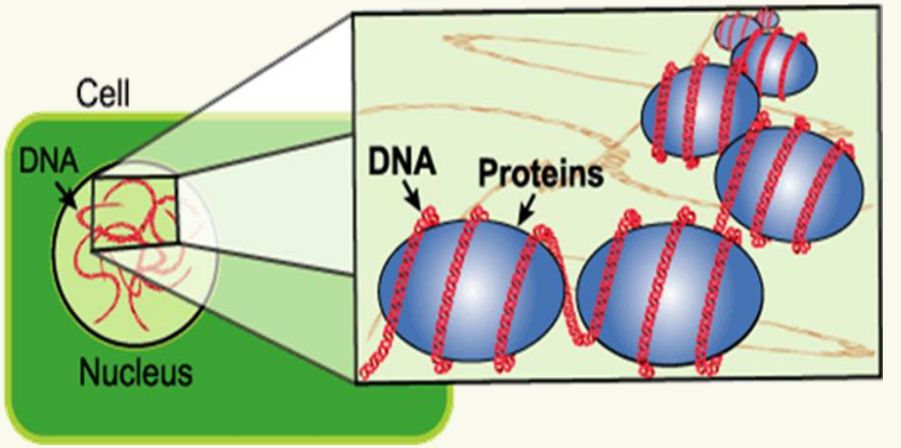
Medical studies	• **Diagnosis of diseases:** Detection of genetic disorders in a patient
Forensic studies	• Criminology/Paternity testing: **DNA fingerprinting to identify individuals.**
Agricultural studies	• Plant and animal breeding

Overview of DNA Extraction



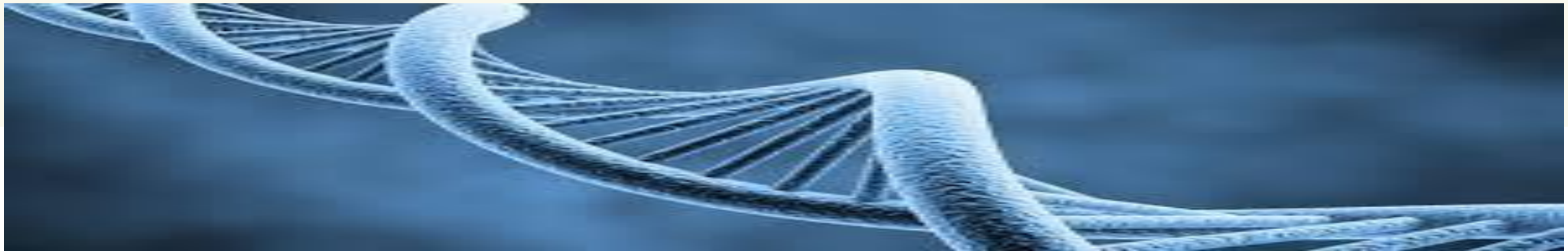
- DNA is located inside the nucleus protected within a **nuclear** membrane which is surrounded by a **cell membrane**





Methods of DNA Extraction

- There are many types of methods to isolate DNA depending on the type of the sample and the purpose from extraction
  - **Non-Organic (Proteinase K and Salting out)**
  - **Organic (Phenol–chloroform extraction)**
  - **Adsorption method (Spin column purification)**
  - **Magnetic Beads based Isolation**



1. Non-Organic DNA Extraction

❑ Principle:

Cells are lysed → proteins are **digested** by **Proteinase K** → proteins are **precipitated** using **high salt** → DNA remains in solution and is then **precipitated** with **alcohol**.

❑ Steps:

- Cell lysis (detergent breaks membranes)
- Add **Proteinase K** → digests histones and proteins
- Add high salt (e.g., NaCl) → proteins precipitate
- Centrifugation → remove protein pellet
- DNA precipitation using ethanol/isopropanol

1. Non-Organic DNA Extraction

❑ Advantages:

Good for routine lab work

- Safe (no toxic organic solvents **as phenol or chloroform.**)
- Simple and inexpensive

❑ Disadvantages:

- May have **protein contamination**
- **DNA purity** is **lower** than organic methods

2. Organic DNA Extraction method (Phenol-Chloroform Extraction)

❑ Principle:

Uses **organic solvents** to **separate DNA** (/RNA) **from proteins and lipids** based on density & solubility differences .

❑ Steps:

1. Cell lysis
2. Add phenol–chloroform
3. Centrifugation → separates into 3 layers:
   - ➢ **Upper aqueous phase (clear layer)** → RNA
   - ➢ **Interphase (White/Cloudy layer)**→ DNA
   - ➢ **Lower organic phase (Pink layer)** → Proteins lipids+ phenol, chloroform
1. Collect aqueous layer
2. Precipitate DNA with alcohol

2. Organic DNA Extraction method (Phenol-Chloroform Extraction)

❑ Advantages:

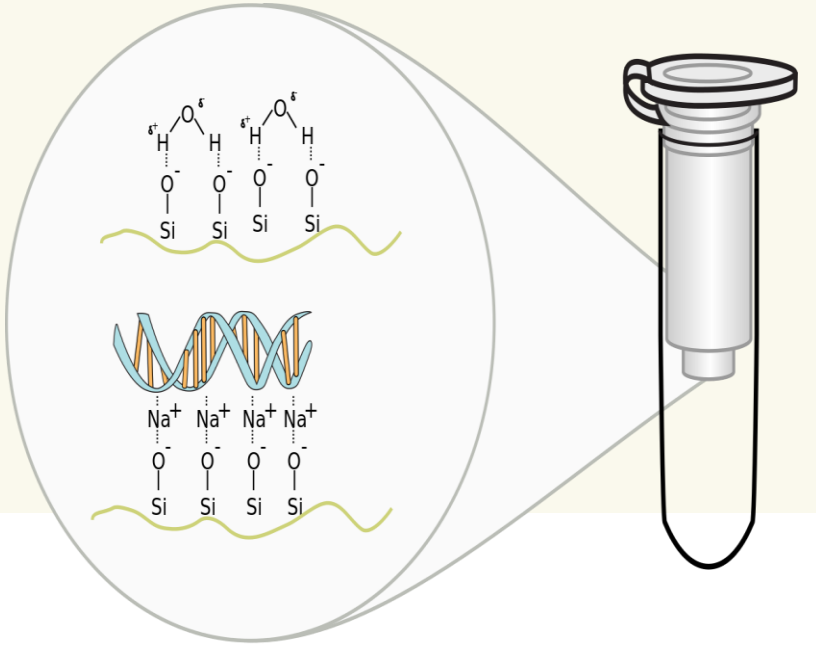
- High yield (concentration) of DNA
- Inexpensive
- Effective removal of proteins and lipids (High purity)

❑ Disadvantages:

- Uses toxic and hazardous chemicals
- Time-consuming
- Requires careful handling

3. Adsorption method (Spin column purification method)

- The most common method for extracting DNA from cells
- Principle : based on DNA binding to silica surfaces in the presence of certain salts and under certain pH conditions.



Steps of DNA Extraction

4 steps are used to isolate and purify the DNA from the rest of the cell:

(1) **Lysis:** of cells

(2) **Binding:** DNA adsorption to silica-gel membrane

(3)**2 Wash steps:** Removal of contaminants

(4) **Elution:** Pure and concentrated DNA

Components of DNA Extraction Kit

1. Proteinase K (optional).
2. Lysis Solution.
3. Wash Buffer I, II (concentrated)
4. Elution Buffer
5. Spin Columns
6. Collection Tubes

Protocol of DNA Extraction from blood

1. Cell lysis:

- Break open cellular and nuclear membranes and release nuclear contents
- Steps:
  - Pipette 300µL of whole blood in an Eppendorf, add 20 µL of Proteinase K
  - Add Lysis buffer, then mix by pipetting.
  - Incubate sample at 56°C for 15 minutes in a heat block or water bath with frequent mixing.

Protocol of DNA Extraction from blood

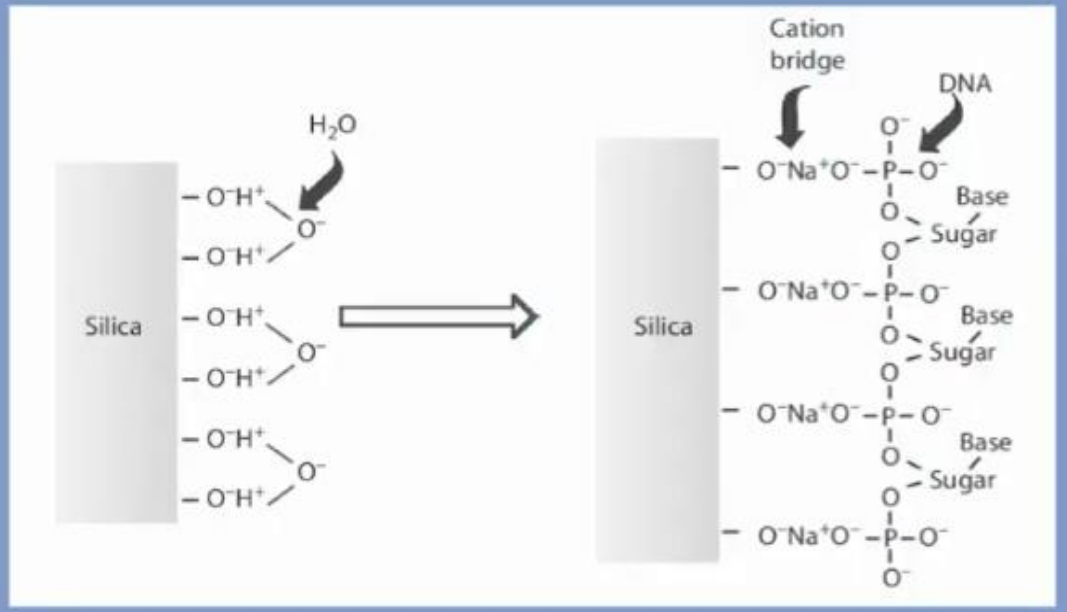
2.DNA binding to silica membrane:

- DNA must be separated from the cell's debris
- DNA binds silica In high salt, protein does not bind (flows through column)
- Steps:

- Add ice cold absolute ethanol and mix by pipetting
- Transfer the prepared mixture to a spin column with collection tube.
- Centrifuge, replace the collection tube by a new one

Protocol of DNA Extraction

Binding



Silica & DNA are negative

Ethanol dehydrates both

Cation bridge is formed

DNA binds to silica membrane

Protocol of DNA Extraction from blood

3.Membrane washing:

- Most protein has washed through, but some might be left. Wash to remove proteins, and any impurities
- Steps:

- Add wash buffer to the spin column and centrifuge (repeated 2 times "Wash buffer 1&2")
- Discard the flow-through & centrifuge the empty spin to dry it from residual ethanol.

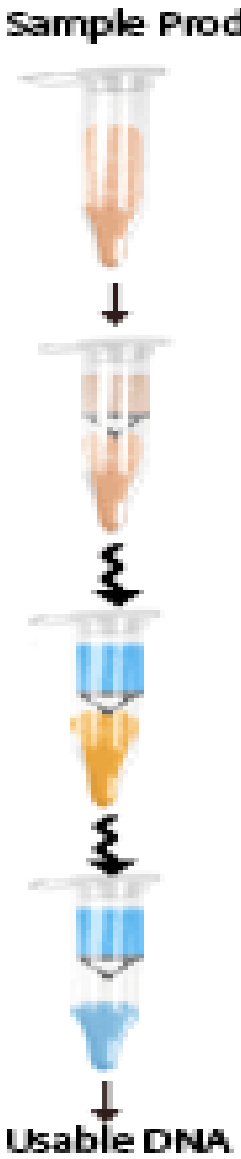
Protocol of DNA Extraction from blood

4. Elution of DNA:

- Elution buffer releases the DNA from the membrane .
  Now the DNA will flow through and be collected.
- Steps:

- Add 50 µl of elution buffer to the center of the spin column membrane, incubate for 2 min at room temperature, and then centrifuge.
- Discard the spin and store DNA at -20°C.

Summary of DNA Extraction Using Spin column purification method



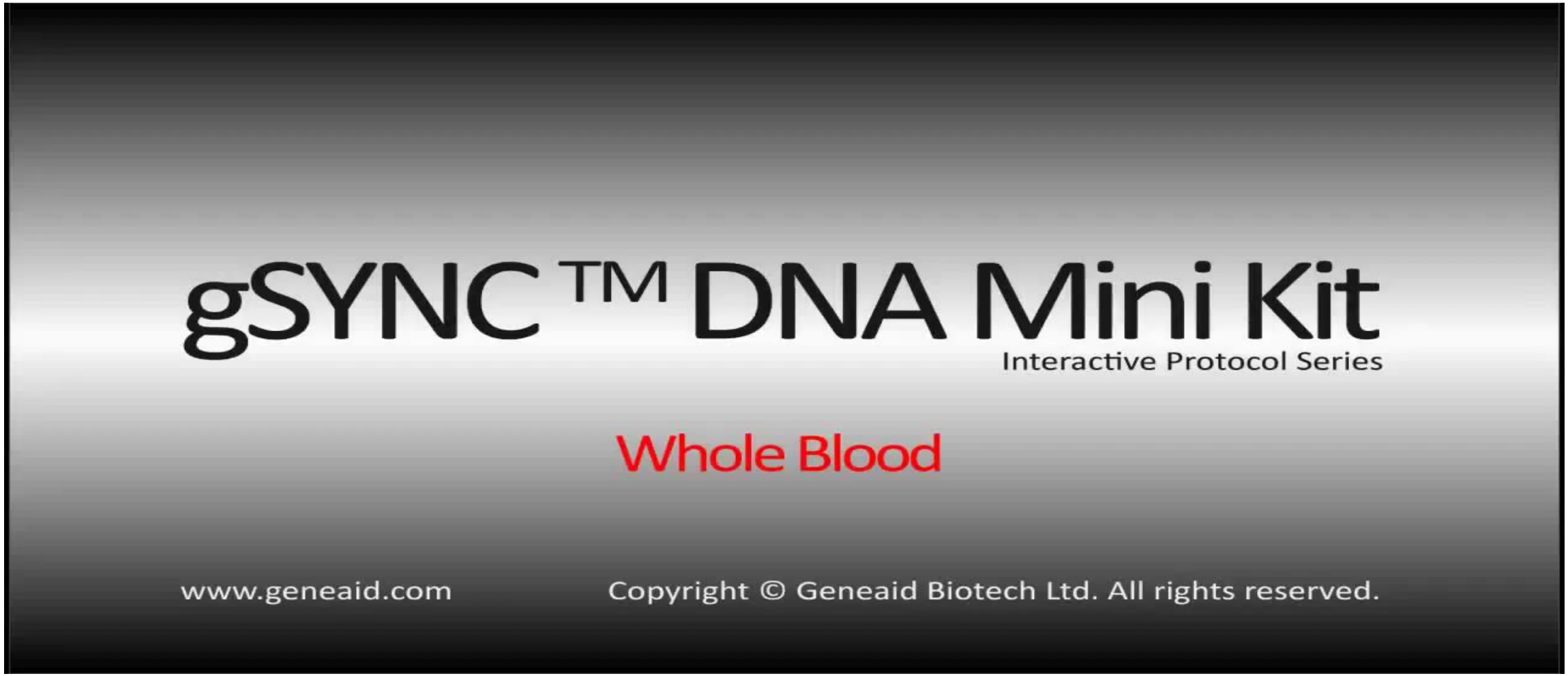
1. Pipette **300µL of whole blood** in an Eppendorf +/-**20µL** proteinase K
2. Add **Lysis buffer** → **mix** by pipetting
3. Incubate sample **at 56°C for 15 minutes** in a heat block or water bath

1. Add ice cold **absolute ethanol** and mix by pipetting
2. **Transfer** the mixture to a **spin column** with collection tube→**Centrifuge**
3. **Replace the collection tube** by a new one

1. Add **wash buffer** to the spin column and **centrifuge** →**Discard** the flow-through.
2. Repeat 2 times
3. **Centrifuge the empty spin** to **dry** it from residual **ethanol.**

1. **Add 50 µl of elution buffer** to the center of the spin column membrane, **incubate for 2 min** at room temperature, and then **centrifuge.**
2. **Discard the spin** and store DNA in Eppendorf at **-20°C.**

Protocol of DNA Extraction



https://youtu.be/GW7L5-9qVYU?si=NujcZURMkf7k2VjX

3. Adsorption method (Spin column purification method)

❑ Advantages:

- Fast and user-friendly
- High purity DNA
- Widely used in clinical labs

❑ Disadvantages:

- More **expensive** (kits required)
- **Limited DNA yield** compared to some methods

https://www.youtube.com/watch?v=ZN1bZ6Q_mG4

4. Magnetic Beads Nucleic acid Isolation

Principle:

DNA binds to **magnetic beads coated with silica or** other materials;
a magnet is used to separate DNA .

❑ Steps:

- Cell lysis
- Add magnetic beads → DNA binds
- Apply magnetic field → beads separate
- Wash beads
- Elute purified DNA

4. Magnetic Beads Nucleic acid Isolation

❑ Advantages:

- Very fast,
- High purity,
- No need for centrifugation,
- Easily automated (high-throughput)

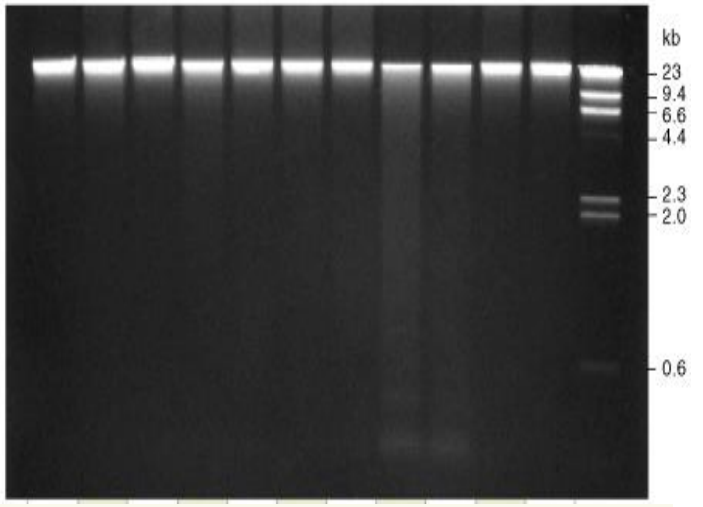
❑ Disadvantages:

- Very expensive
- Requires optimization

https://www.youtube.com/watch?v=SJ6c40lJuK0

Assessment of DNA quality

- The product of DNA extracted will be used in subsequent experiments.
  Poor quality DNA will not perform well in PCR.



- For assessing quality & quantity of extracted DNA:
  1. **Agarose gel electrophoresis:**
     - **(assess DNA quality)**
  2. **NanoDrop spectrophotometry:**
     - **(assess DNA purity and concentration)**


Thank
you