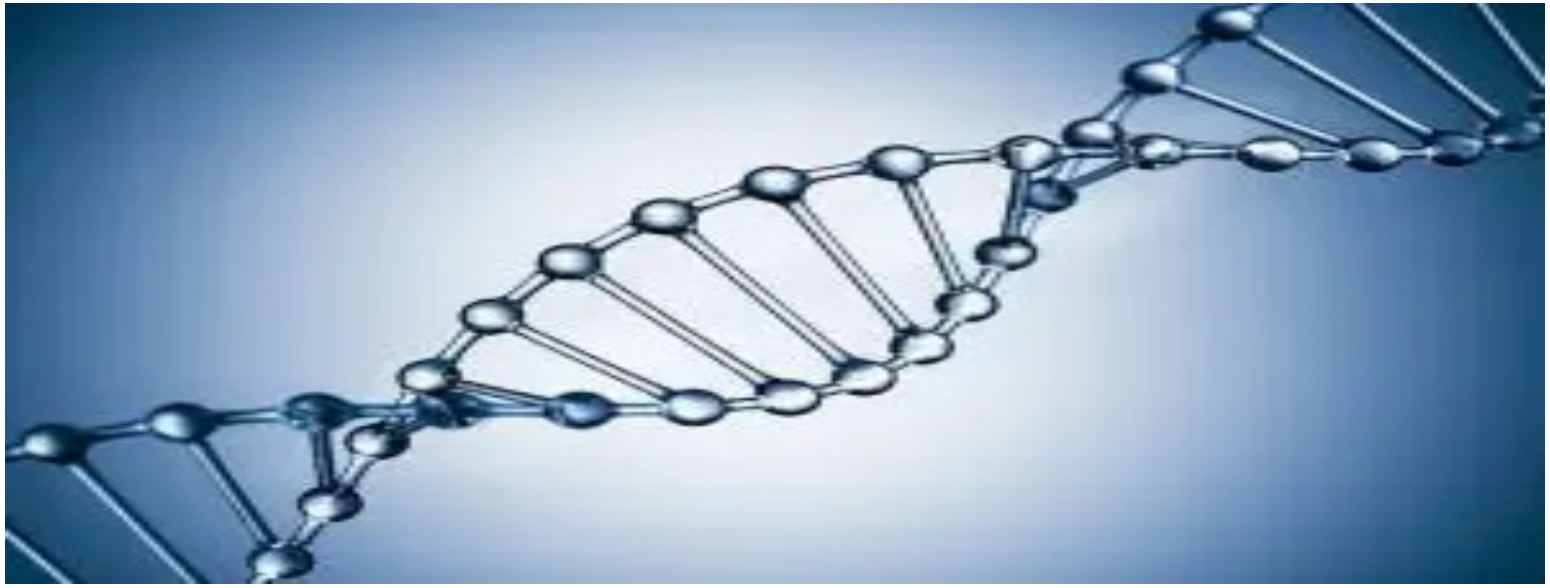
NEAR EAST UNIVERSITY
DESAM RESEARCH INSTITUTE

Nucleic Acid Isolation Techniques



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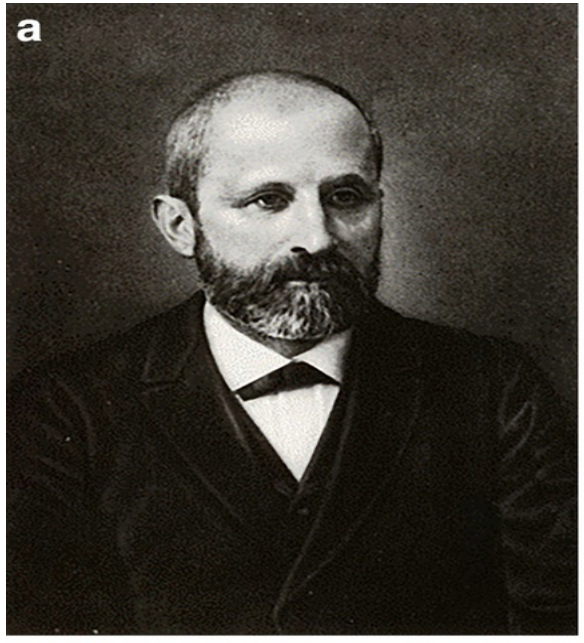
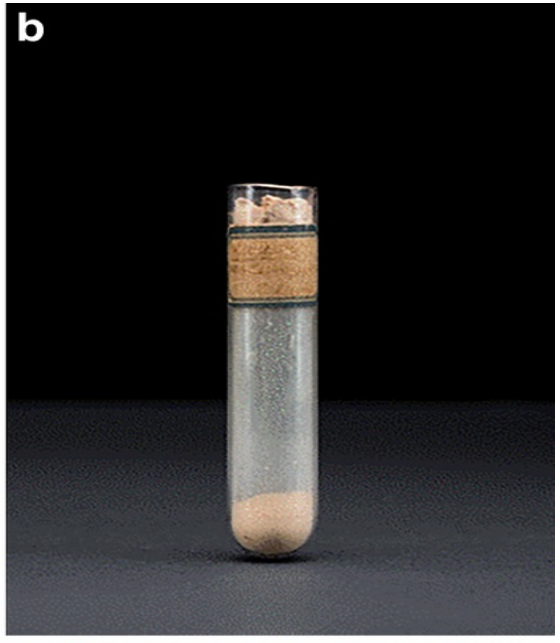
History





- Swiss physician Friedrich Miescher
- 1869
- ‘Nuclein’

Nucleic Acid Extraction

- **Nucleic acid extraction (NAE);**

  Extraction process of nucleic acid from various sources

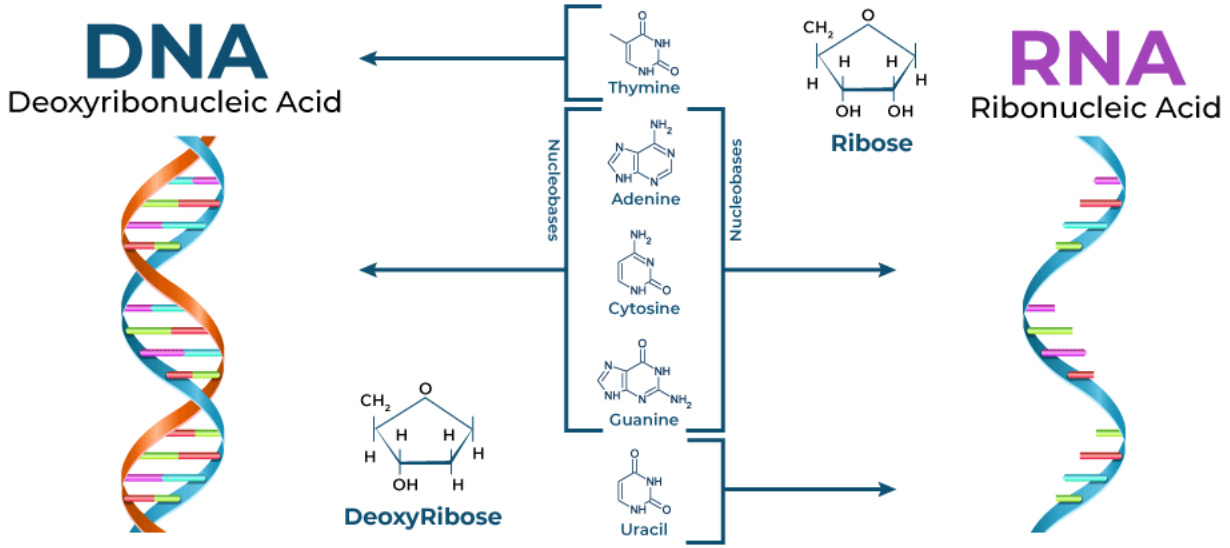
- **Aim of the NAE;**
  - Separate the nucleic acid present in the nucleus of the cell from other cellular components

Applications

- **Molecular Biology Research**
- **Clinical Diagnostics**
- **Biotechnology and Industrial Applications**
- **Microbial Ecology and Environmental Studies**
- **Forensic Analysis**
- **Basic Science and Education**

DNA vs RNA

- Double stranded
- Long, thin strands
- Relatively stable
- Replicates its own

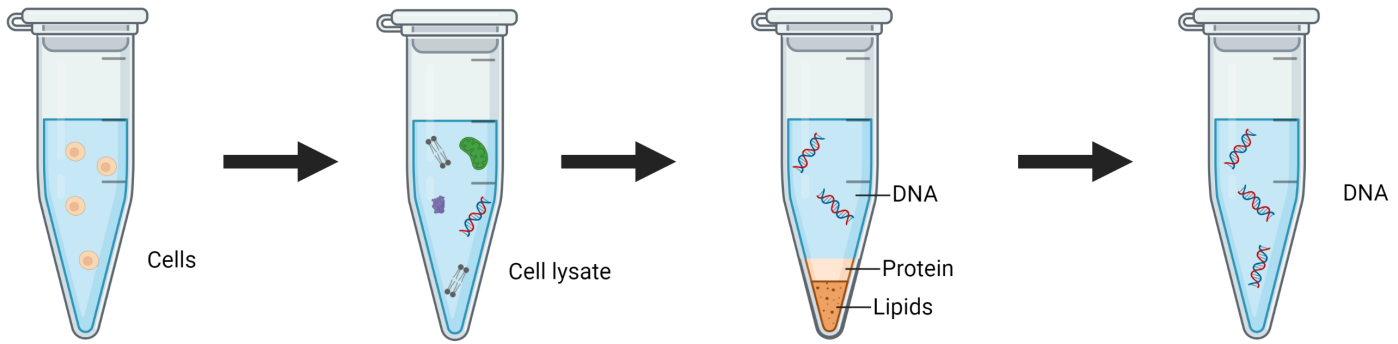


- Single stranded
- Small
- Unstable
- Easily degraded
- Does not replicates its own

Principle of DNA Extraction

Three major process of the extraction steps

Lysis → Precipitation → Purification



Lysis

- Achieved by **physical** or **chemical** methods

- **Main aim;**

  disrupt the cell wall and/or cellular membranes

Lysis

Chemical Lysis

- Lytic enzymes
- Chaotropic agents
- Different types of detergents

Mechanical Lysis

- Grinding
- Shearing
- Bead beating
- Shocking

Precipitation agents are added to the DNA solution, reducing the solubility of DNA and causing it to aggregate

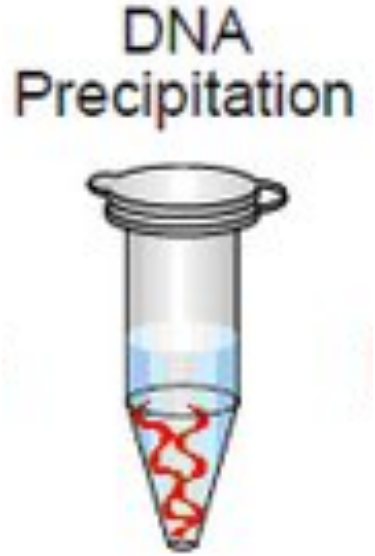
relies on the solubility properties of DNA in different solutions

causing DNA molecules to clump together and precipitate out of solution

involves the addition of a precipitation agent to the DNA solution

utilized to separate DNA from other cellular components

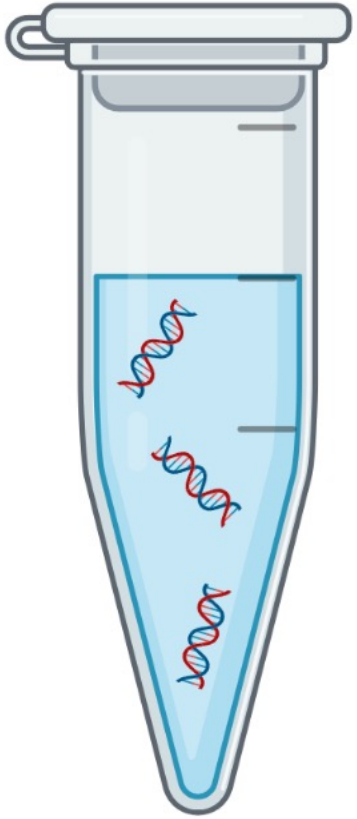
a crucial part of DNA extraction



Purification

DNA washed with ethanol or other buffers

DNA is eluted and stored



DNA

Consideration Factors

- Choosing suitable method is important for quality and quantity
- Other factors;
  - Time
  - Cost
  - Potential toxicities
  - Yield
  - Laboratory equipment
  - Expertise requirements

Nucleic Acid Extraction Methods

- Phenol-Chloroform Extraction
- Chelex-100 Extraction
- Alkaline Extraction
- Cetyltrimethylammonium Bromide (CTAB) Extraction
- Salting Out Method
- Ethidium Bromide (EtBr)-Cesium Chloride (CsCl) Gradient Centrifugation
- Silica Matrices
- Spin-Column Extraction
- Magnetic Beads
- Anion Exchange Resin Based Extraction
- Filter Paper Based
- Automated System

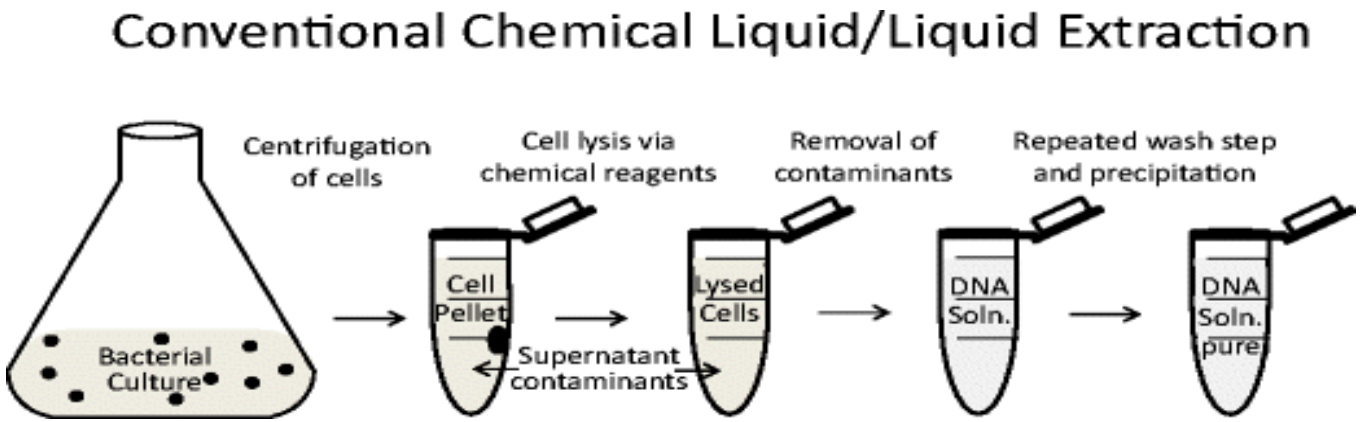
Conventional Methods

- **Advantages**

- Cost-effectiveness
- High yield
- No specialized equipment required

Disadvantages

- Labour intensive
- Risk of contamination
- Variable reproducibility
- Exposure to hazardous chemical



New Methods

Advantages

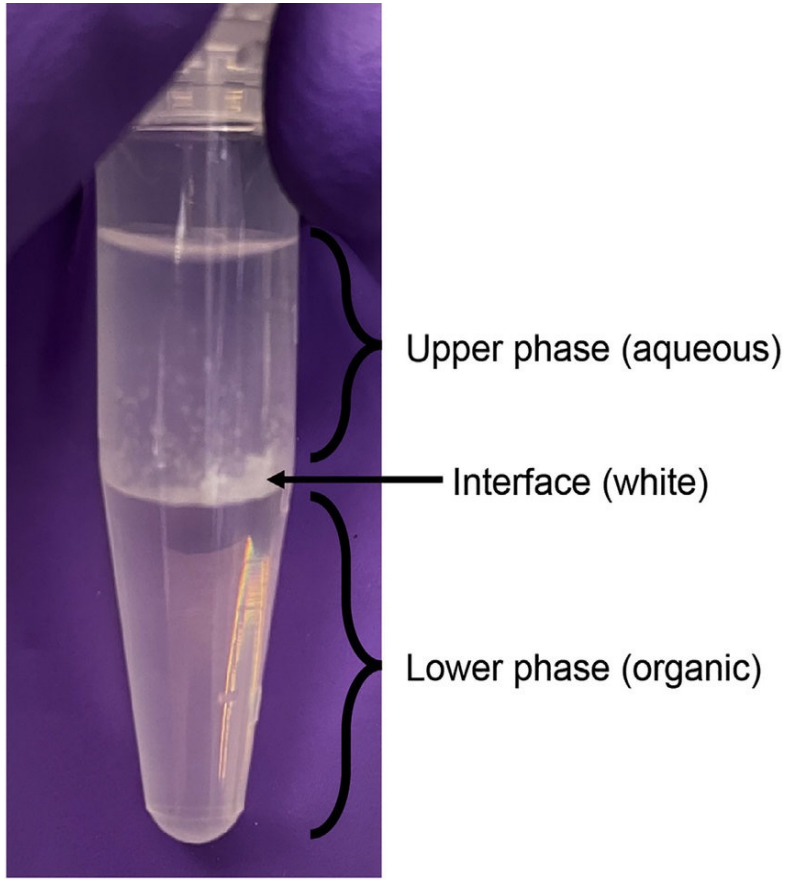
- Ease of use
- Time saving
- Reduced risk of contamination
- Scalability
- Versatility

Disadvantages

- Cost
- Sample size requirements
- Manufacturer dependence
- Complexity of equipment

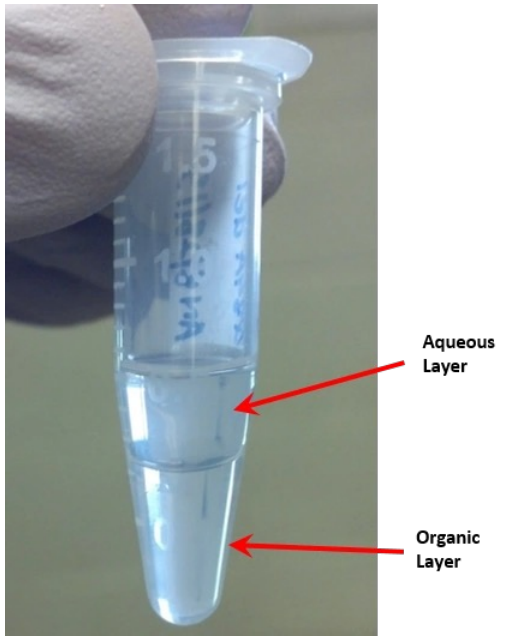
Phenol-Chloroform Method

- a classic method used for the purification of nucleic acids, particularly DNA, from biological samples
- relies on the differential solubility of nucleic acids, proteins, lipids, and other cellular components by using the phenol and chloroform
- Phenol denatures proteins, allowing DNA to partition into the aqueous phase, while chloroform removes residual phenol
- Requires careful handling due to the toxicity of phenol and chloroform



Phenol-Chloroform Method

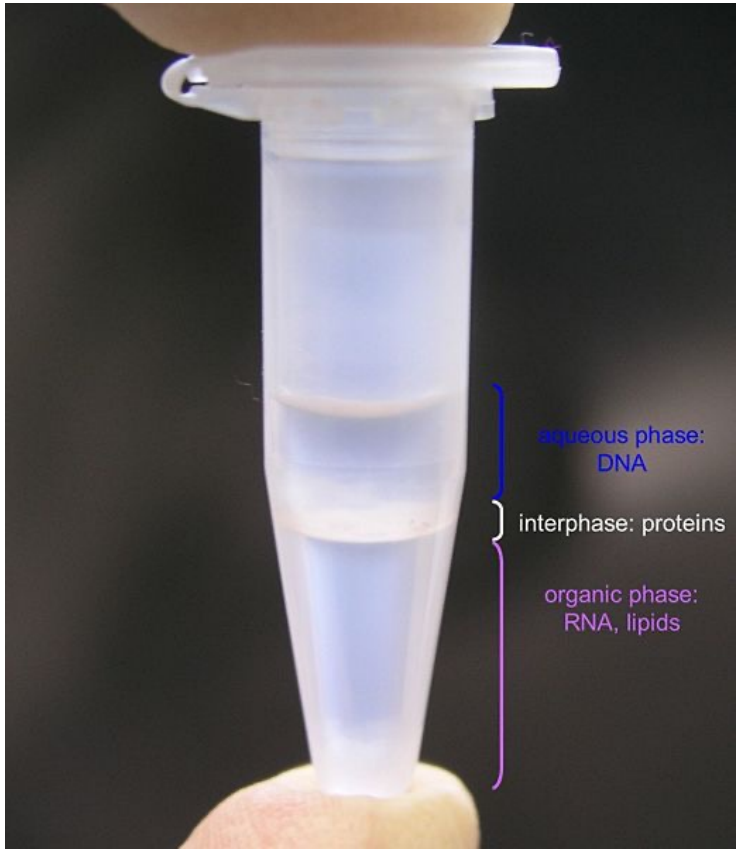
- Phenol is a strong denaturant that disrupts cellular membranes and denatures proteins, including nucleases
- Chloroform is used to separate the aqueous and organic phases, facilitating the removal of proteins and lipids
- DNA preferentially partitions into the aqueous phase, while proteins and lipids remain in the organic phase



Phenol-Chloroform Method

- **Advantages**
  - •Effective Removal of Contaminants
  - •Versatility
  - •Minimal Equipment Requirements

- **Disadvantages**
  - Toxicity
  - Potential DNA Degradation
  - Additional Purification Steps
  - Time consuming



Phenol-chloroform extraction

1. To 100ul eluted DNA, add 0.5 ul of 20% SDS (*this may not be necessary as there are no cells) and 100 ul of phenol-chloroform.
2. Vortex well.
3. Centrifuge at room temperature for 5 min, at full speed (14,000 rpm).
4. Pipette the aqueous phase (upper phase, aprox 80 ul, it is better to leave some DNA than to pipette phenol) to a new labeled tube.
5. Discard original tube.

Safety information

The tube contains phenol-chloroform.

6. Proceed to ethanol precipitation.

7 Add 1/10 volume Na acetate 3M pH 4.8 or 5.2 (ie 8ul for 80 ul DNA solution)
add 2 volumes ETOH 100% (storage -20°C) = 176 ul

Total volume: 264ul, possible with 264 ng of starting DNA - otherwise, you must add a carrier: tRNA, glycogen or linear acrylamide.

8 Vortex.

9 Put on dry ice for 30 min. or overnight at -20°C

10 Centrifuge at 4°C for 30 min at 14,000 rpm.

11 Discard the supernatant.

12 Wash with 500ul EtOH 70% (storage 4°C)

13 Centrifuge at 4°C for 5 min at 14,000 rpm.

14 Discard the supernatant

15 Quick spin

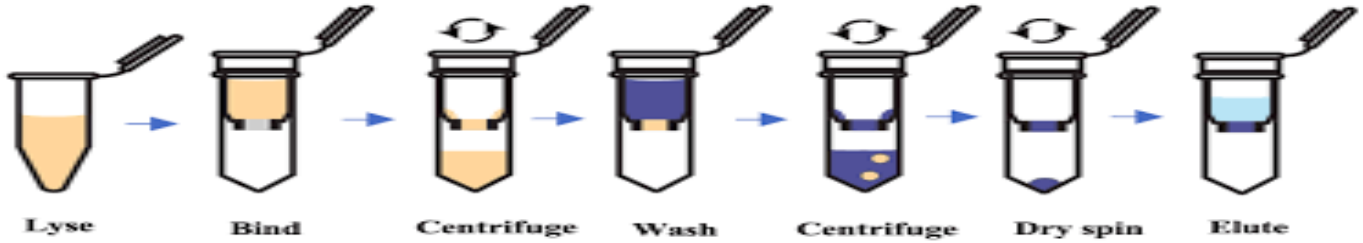
16 Pipette out the last drop of EtOH

17 Speed Vac for 3' or 5-7 min at room temperature.

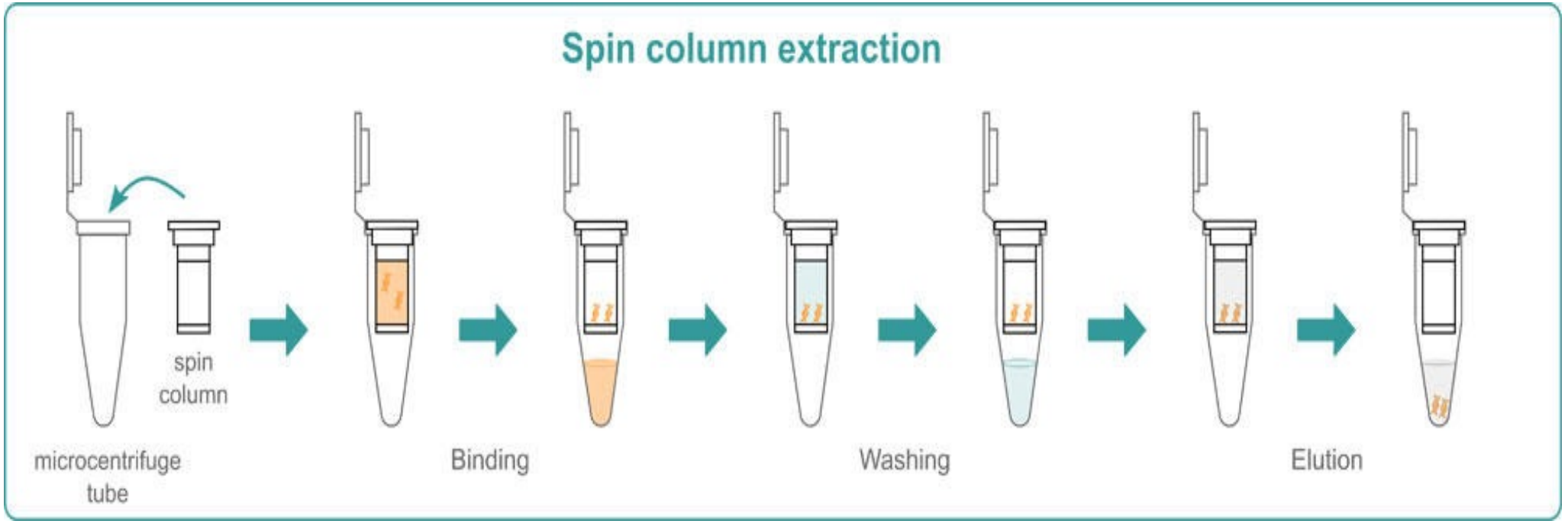
18 Resuspend in 20ul of water ot Tris 10 mM pH 7.5 or 8.0

Silica Binding Extraction (Silica Column-based Extraction)

- Binding elements of spin column system is usually composed of glass particles or powder, silica matrices and ion exchange carriers
- NA binding optimized with specific buffer solutions and extremely precise pH and salt concentrations
- Chaotropic agents disrupt hydrogen bonding and hydrophobic interactions, allowing nucleic acids to bind to the silica surface
- Contaminants such as proteins, salts, and cellular debris are removed by washing, while nucleic acids remain bound to the silica



Silica Binding Extraction (Silica Column-based Extraction)



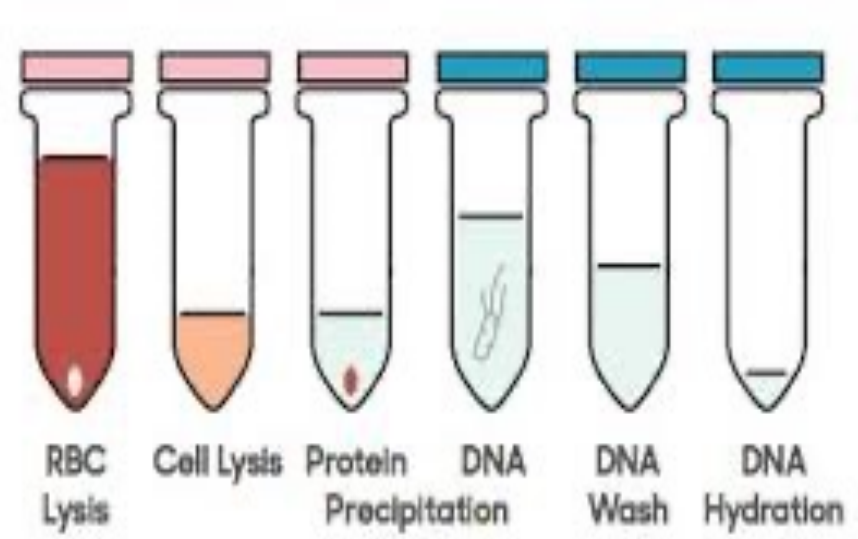
Silica Binding Extraction (Silica Column-based Extraction)

Advantages

- High yield and purity
- Automation and High Throughput
- Versality

Disadvantages

- Chaotropic agents
- Equipment requirements



Mammalian Cells and Blood Lysate Protocol

1. Set a water bath or heat block at 55°C.
2. Add 20 µl Proteinase K to a sterile microcentrifuge tube.
3. Process cells or blood samples:
   - For adherent cells (up to 5 x 10^6 cells), remove the growth medium and harvest cells by trypisinization or a method of choice. Resuspend cells in 200 µl PBS.
   - For suspension cells (up to 5 x 10^6 cells), harvest cells by centrifugation. Remove the growth medium. Resuspend cells in 200 µl PBS.
   - To a sterile microcentrifuge tube, add up to 200 µl fresh or frozen blood sample (if using <200 µl blood sample, adjust the sample volume to 200 µl using PBS). To process blood samples >200 µl and up to 1 ml, scale up all reagent volumes accordingly.

     Note: If you are processing >200 µl blood sample, you need to purchase additional PureLink™ Genomic Lysis/Binding Buffer (catalog no. K1823-02) and Proteinase K (catalog no. 25530-049) available separately. Visit www.invitrogen.com for details.
4. Transfer 200 µl cells or blood in PBS to the tube containing Proteinase K from Step 2.
5. Add 20 µl RNase A to the sample. Mix well by brief vortexing and incubate at room temperature for 2 minutes.
6. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
7. Incubate at 55°C for 10 minutes to promote protein digestion.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
9. Proceed immediately to **Purification Protocol**, next page.

Purification Protocol

The purification procedure is designed for purifying genomic DNA using a spin column-based centrifugation procedure in a total time of **10-15 minutes**.

1. Remove a PureLink™ Spin Column in a Collection Tube from the package.
2. Add the lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol to the spin column.
3. Centrifuge the column at 10,000 × g for 1 minute at room temperature.

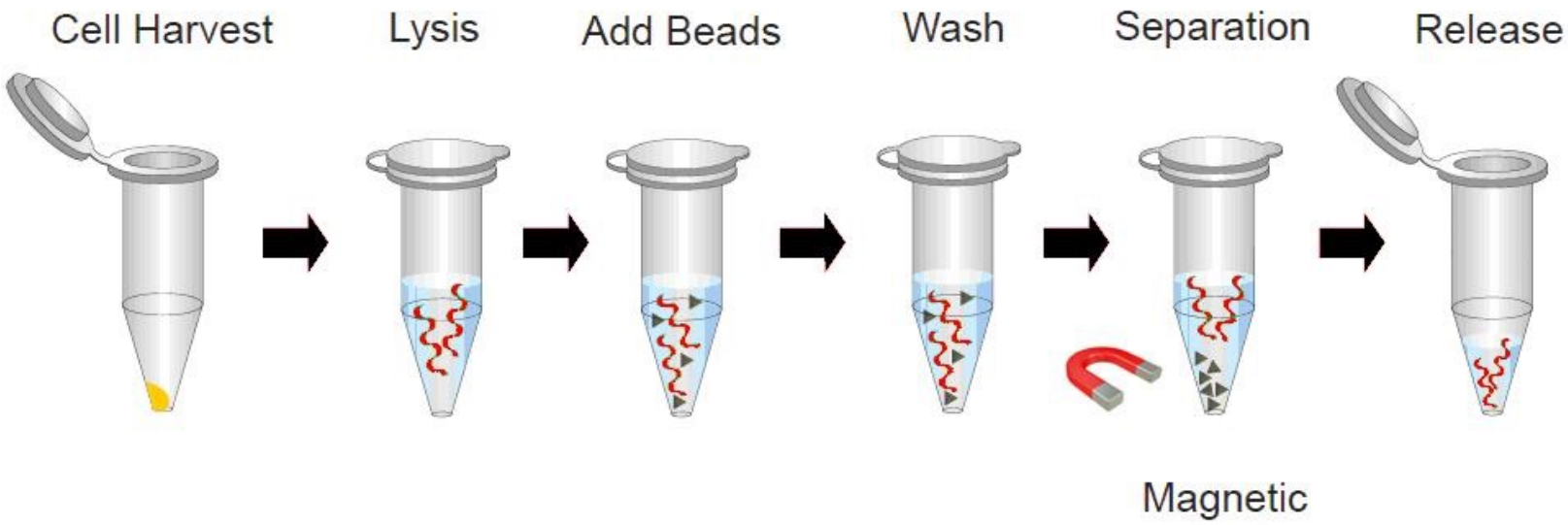
   Note: If you are processing >200 µl starting material such as blood, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink™ Spin Column (above) and centrifuge at 10,000 x g for 1 minute.
4. Discard the collection tube and place the spin column into a clean PureLink™ Collection Tube supplied with the kit.
5. Add 500 µl Wash Buffer 1 prepared with ethanol (page 2) to the column.
6. Centrifuge column at 10,000 × g for 1 minute at room temperature.
7. Discard the collection tube and place the spin column into a clean PureLink™ collection tube supplied with the kit.
8. Add 500 µl Wash Buffer 2 prepared with ethanol (page 2) to the column.
9. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
10. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
11. Add 25-200 µl of PureLink™ Genomic Elution Buffer to the column. Choose the suitable elution volume for your needs.
12. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.

    The tube contains purified genomic DNA.
13. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution.
14. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.

    The tube contains purified DNA. Remove and discard the column.
15. Use DNA for the desired downstream application or store the purified DNA at 4°C (short-term) or -20°C (long-term).

Magnetic-Bead Based Extraction

- This technique involves the use of magnetic beads functionalized with DNA-binding molecules (e.g., silica or paramagnetic particles coated with proprietary surfaces)
- Magnetic nanoparticles coated with a DNA binding antibody or polymer with specific affinity to DNA can be employed to bind DNA to its surfaces
- DNA binds to the beads in the presence of chaotropic salts, allowing efficient capture and washing steps under magnetic field guidance



Magnetic-Bead Based Extraction

- **Advantages**
  - Enables automation, high-throughput processing, and compatibility with various sample volumes and types
  - Require little equipment to perform as it does not depend on centrifugation
  - Does not involve the use of shear forces that could damage the integrity of nucleic acids
  - Take less than 30 minutes to complete

- **Disadvantages**
  - Not cost effective

FTA Paper Cards

- FTA paper cards are filter paper-based cards impregnated with chemicals for nucleic acid preservation and storage
- FTA cards;
  - lyse cells upon sample application, denature and immobilize nucleic acids, and inactivate nucleases, preserving genetic material for downstream analysis
  - contains chemicals (e.g., chaotropic agents) that stabilize and protect nucleic acids
- Biological samples (e.g., blood, saliva, tissue) are applied directly onto FTA cards, eliminating the need for freezing or refrigeration
- Nucleic acids are stable at room temperature for extended periods, making FTA cards ideal for sample collection in remote or resource-limited settings
- FTA cards are suitable for a wide range of sample types, but certain factors (e.g., high protein content) may affect nucleic acid extraction efficiency
- Proper storage conditions (e.g., room temperature, desiccated environment) are crucial for maintaining sample integrity

RNA Extraction

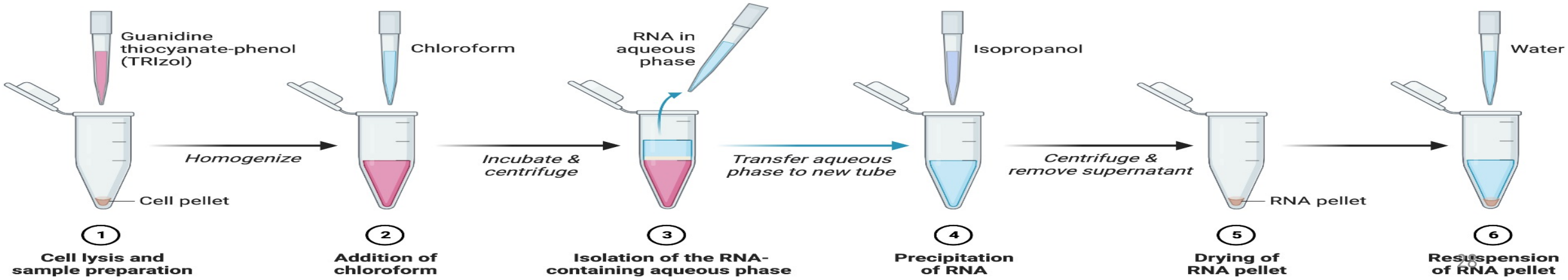
- RNA extraction involves the isolation of RNA molecules from biological samples by disrupting cell membranes, inactivating RNases, and selectively binding RNA

Methods used in RNA isolation;

- Phenol-Chloroform Extraction
- Silica-Based Extraction
- Commercial Kits
- Automated systems

Consider;

- RNA integrity
- RNase contamination (RNases are everywhere!)



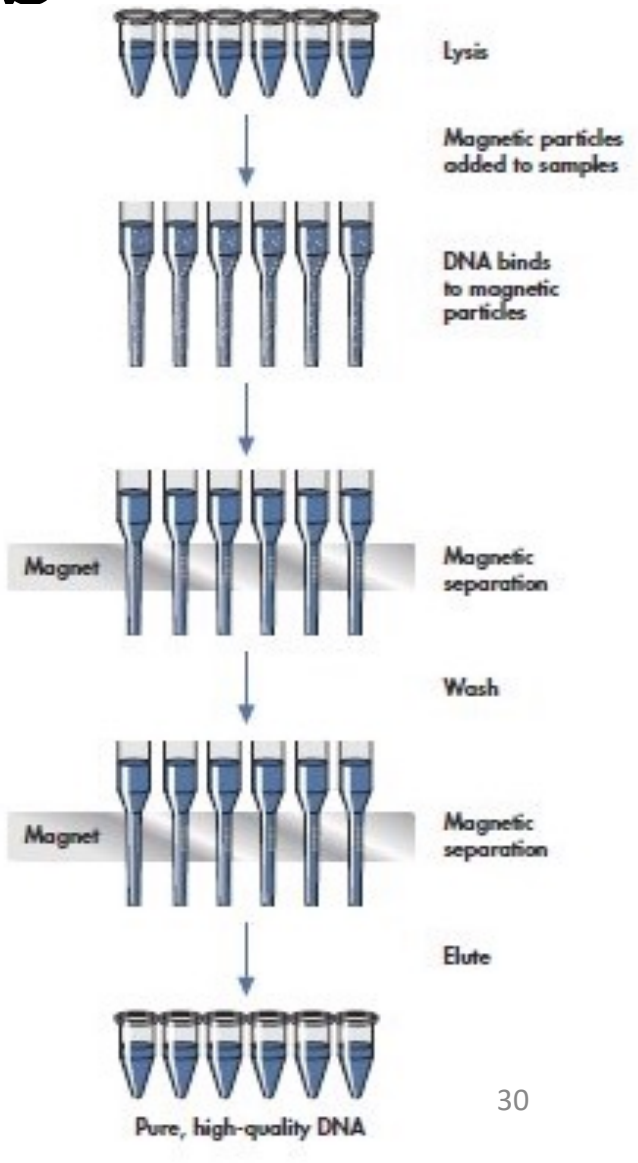
Automated Systems

- **Automated systems**
  - refer to sophisticated instruments or platforms
  - designed to streamline and automate the process of isolating nucleic acids from biological samples

Automated Systems

- Automated systems employ robotic arms, liquid handling systems, and integrated software to perform various steps of the extraction process automatically

- These systems typically follow predefined protocols;
  - sample lysis
  - binding of nucleic acids to a solid phase
  - washing to remove contaminants
  - elution of purified nucleic acids



Automated Systems

Advantages

- Increased Throughput
- Consistency and Reproducibility
- Workflow Standardization
- Fast

Disadvantages

- Initial Investment
- Training

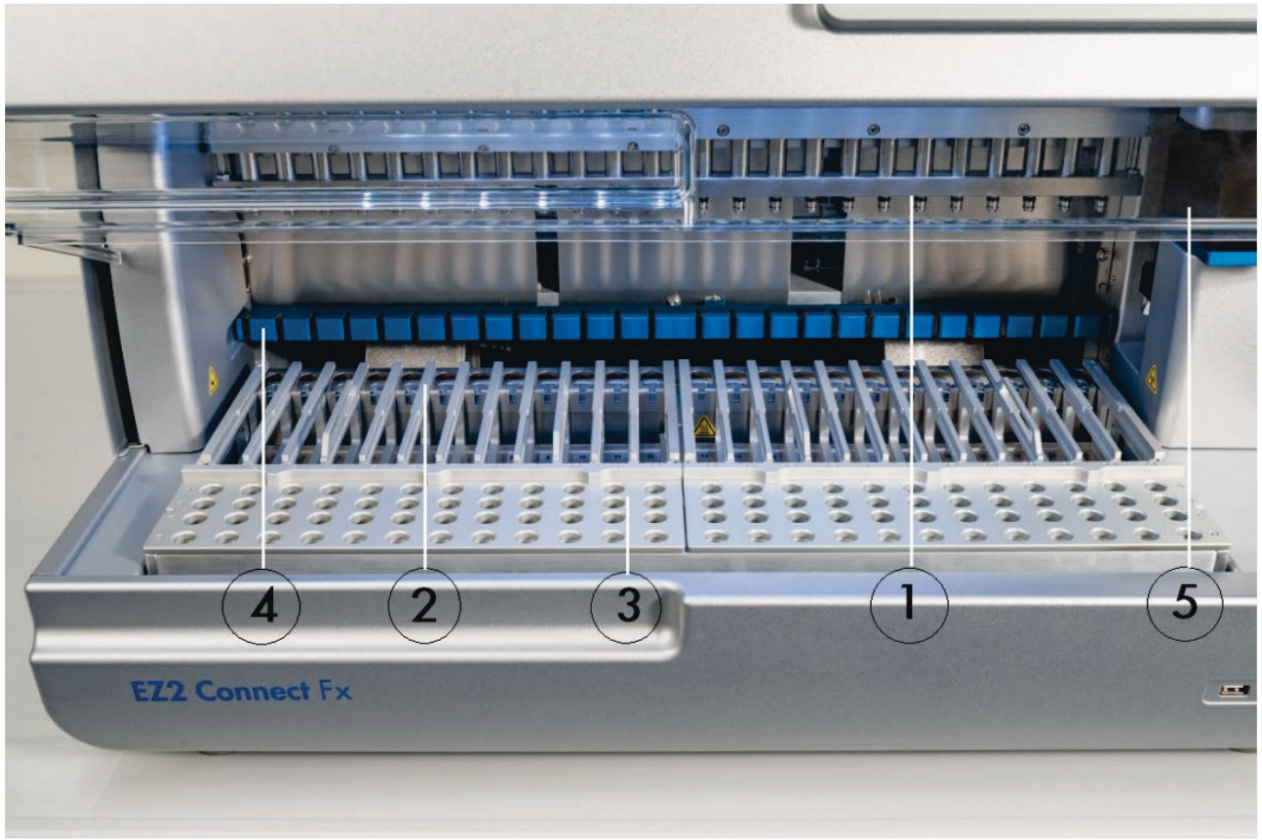


Figure 7. Interior of the EZ2.

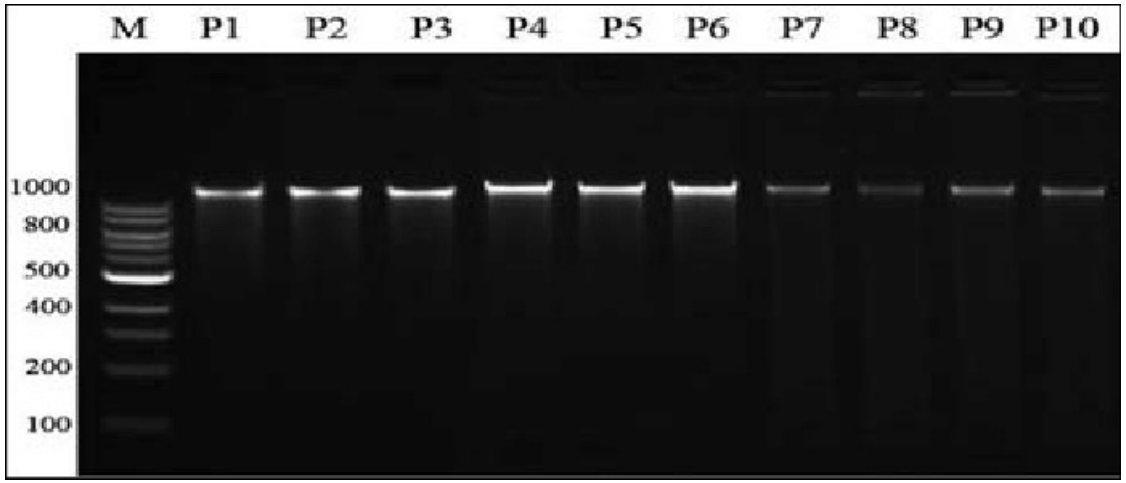
1 Pipettor head
2 Cartridge rack
3 Tip rack
4 Magnet module
5 Camera

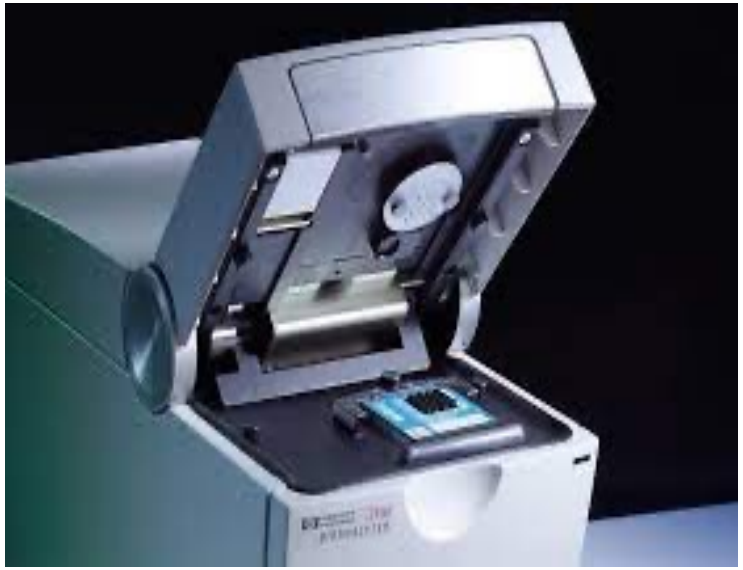
Nucleic Acid Analysis

- DNA quantitation measurement;
  - the process of determining the concentration of DNA in a sample
  - Accurate quantification of DNA is essential for various molecular biology applications

- The choice of DNA quantitation method depends on factors
  - the sample type,
  - the required sensitivity and accuracy,
  - the availability of equipment and reagents

Nucleic Acid Analysis






THANK YOU