BIO 405L. Cellular and molecular biology laboratory

DNA extraction

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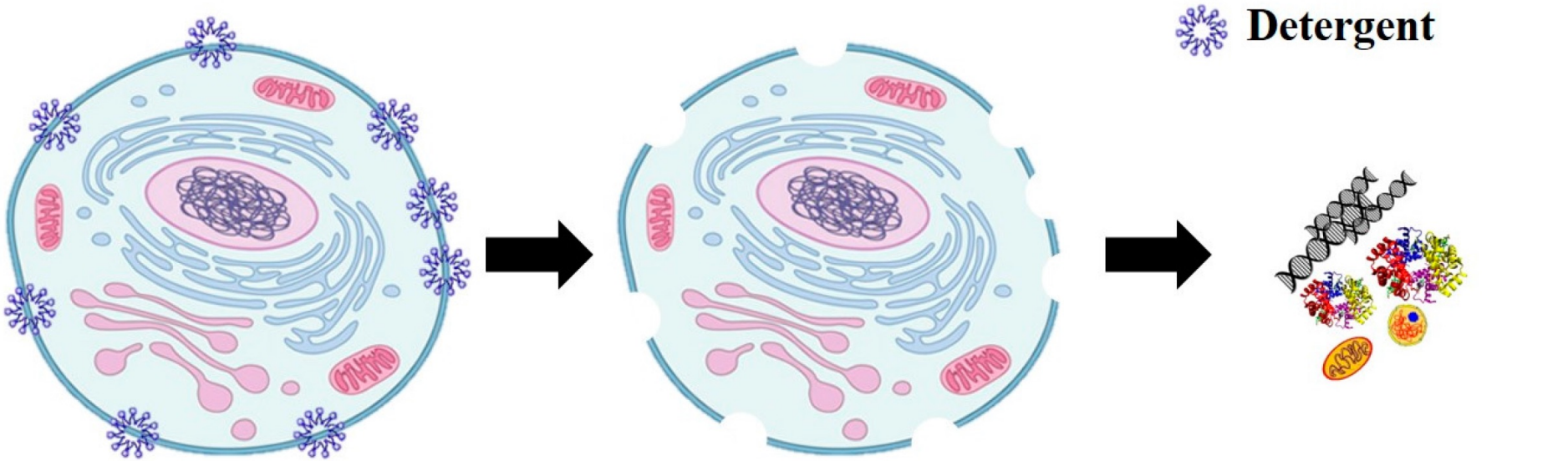
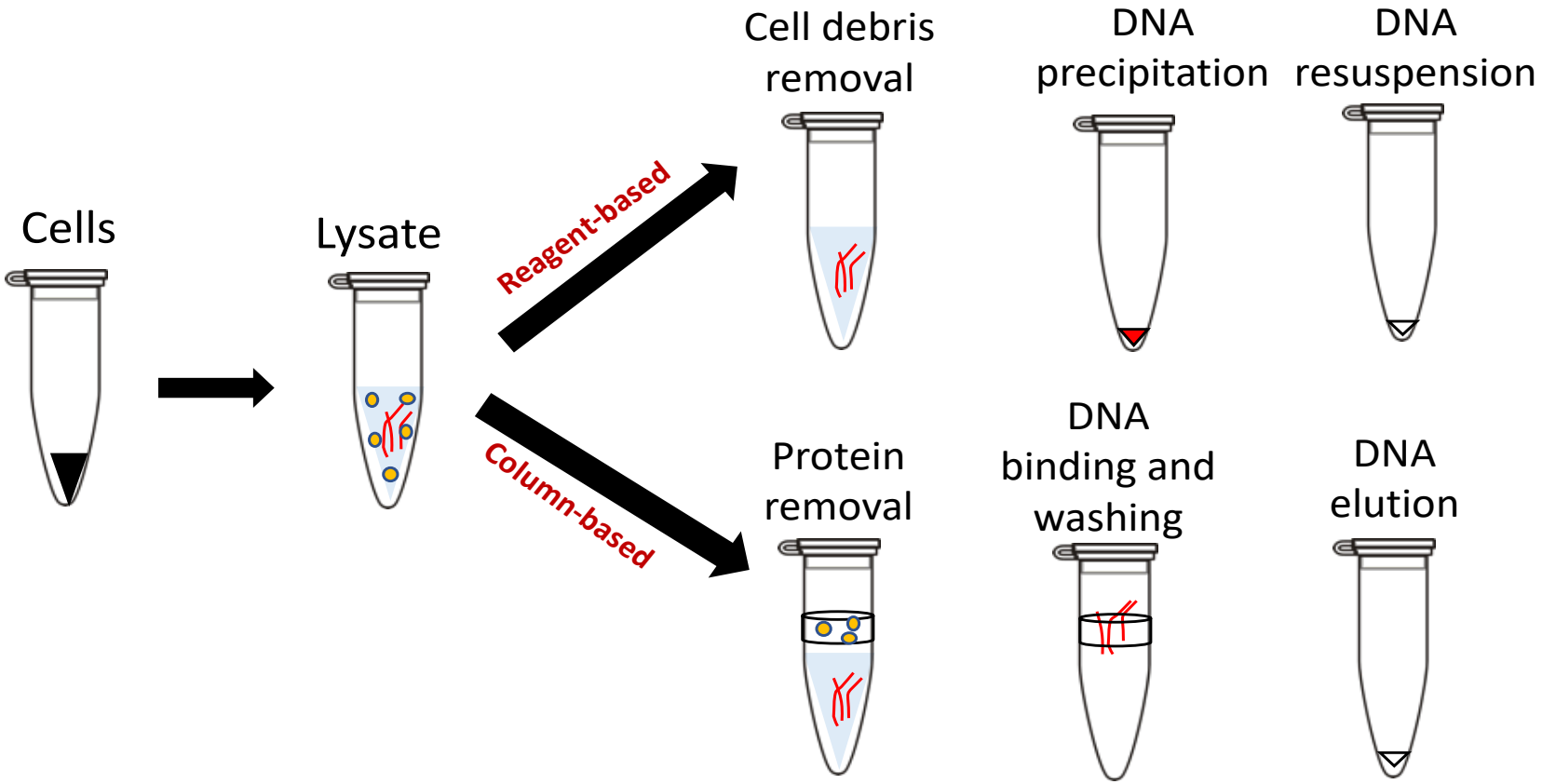
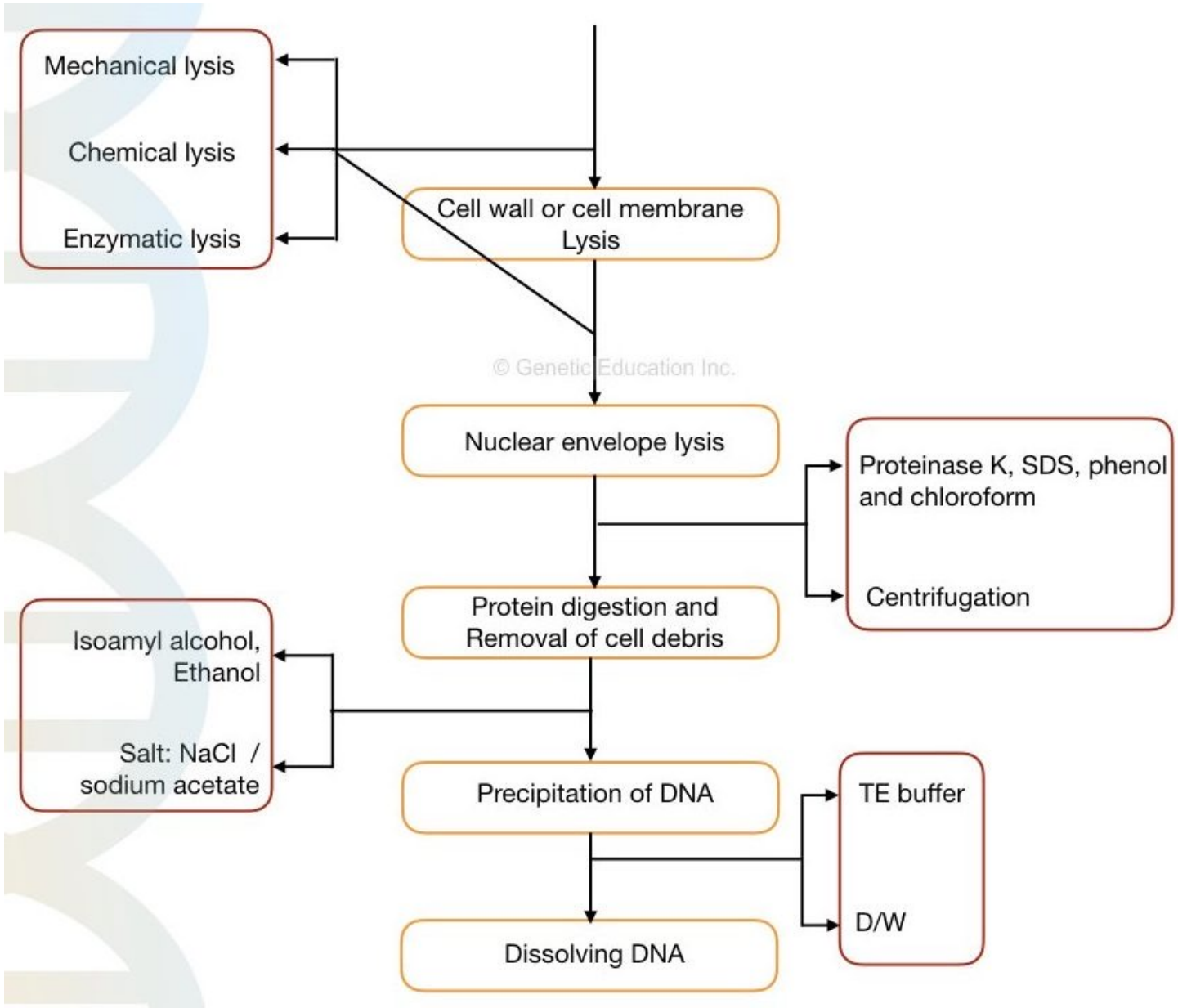
DNA extraction is a process to purify DNA from a biological sample using a combination of physical, mechanical, enzymatic and chemical methods.

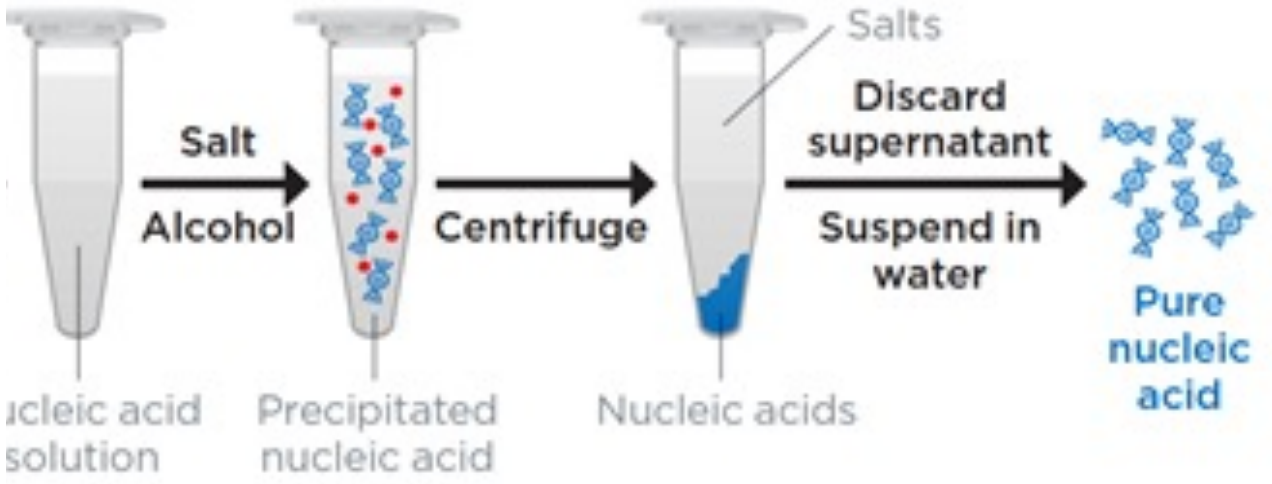


Lysis methods

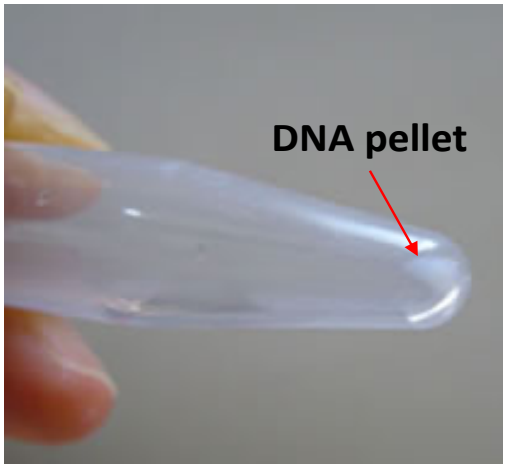
Method	Description	Advantages	Disadvantages
Freeze-Thaw	Uses repeated freezing and thawing cycles to disrupt cell membranes through ice crystal formation and osmotic shock.	Simple, cost-effective, no special reagents required.	Time-consuming; may cause enzyme denaturation.
Chemical	Employs chemicals (e.g., detergents, chaotropic agents) to solubilize cell membranes and denature proteins, allowing release of intracellular contents.	Effective for a wide range of cells; can be tailored to specific needs.	May require neutralization; can interfere with downstream assays.
Enzymatic	Uses enzymes (e.g., lysozyme, protease) to break down cell walls and membranes, often used for gentle lysis and preserving functional proteins.	Gentle on proteins and nucleic acids, preserving activity.	Specific to certain cell types; costly enzymes may be required.
Bead-Beating	Mechanical method using beads that physically grind and shear cells open, effective for tough cell walls such as bacteria, fungi, and plant cells.	Highly effective for hard-to-lyse cells; can process multiple samples simultaneously.	Requires specialized equipment; can cause sample heating and protein denaturation.
Sonication	Utilizes high-frequency sound waves to create cavitation bubbles that shear cells apart, effective for a wide range of cell types including bacteria and yeast.	Efficient and fast; scalable for small to large volumes.	May cause sample heating; requires specialized equipment and optimization.


Cells
Lysate
Reagent-based
Column-based
Cell debris removal
Protein removal
DNA precipitation
DNA binding and washing
DNA resuspension
DNA elution

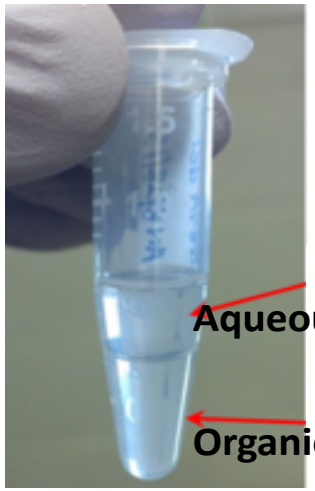

Mechanical lysis
Chemical lysis
Enzymatic lysis
Cell wall or cell membrane Lysis
© Genetic Education Inc.
Nuclear envelope lysis
Proteinase K, SDS, phenol and chloroform
Centrifugation
Protein digestion and Removal of cell debris
Isoamyl alcohol, Ethanol
Salt: NaCl / sodium acetate
Precipitation of DNA
TE buffer
D/W
Dissolving DNA


Salts
Salt
Alcohol
Centrifuge
Discard supernatant
Suspend in water
Pure nucleic acid
ucleic acid solution
Precipitated nucleic acid
Nucleic acids

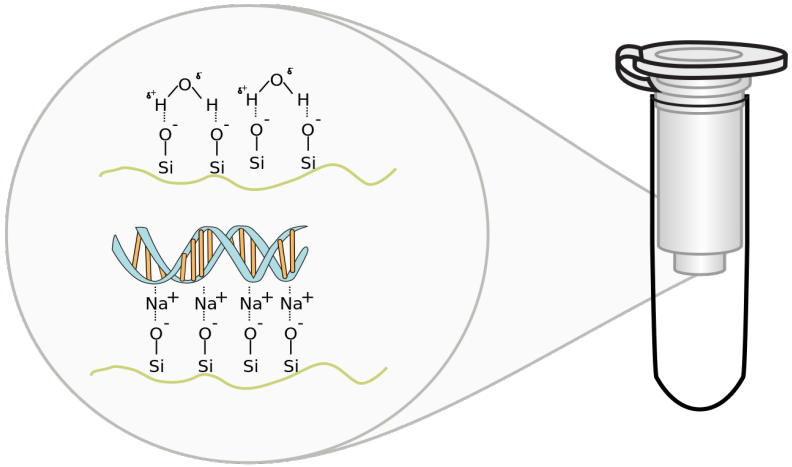
A


DNA pellet

B


Aqueous layer
Organic layer

C



DNA/RNA

contaminants

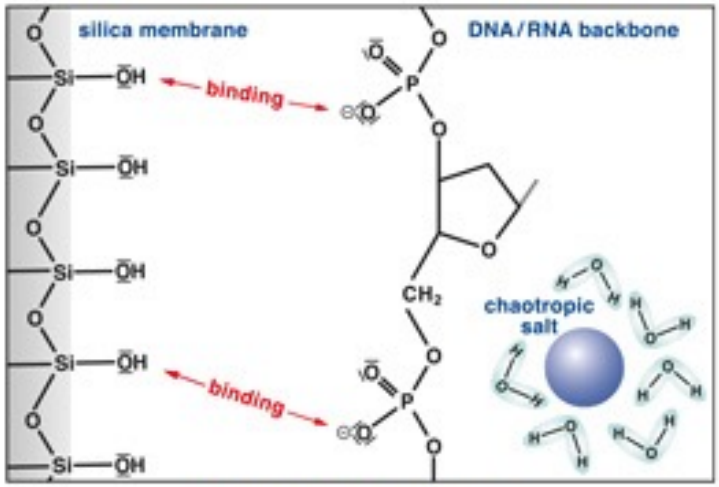
Sample lysis, release of DNA/RNA from cells, tissue, etc.

DNA/RNA is bound to the silica membrane under high-salt conditions

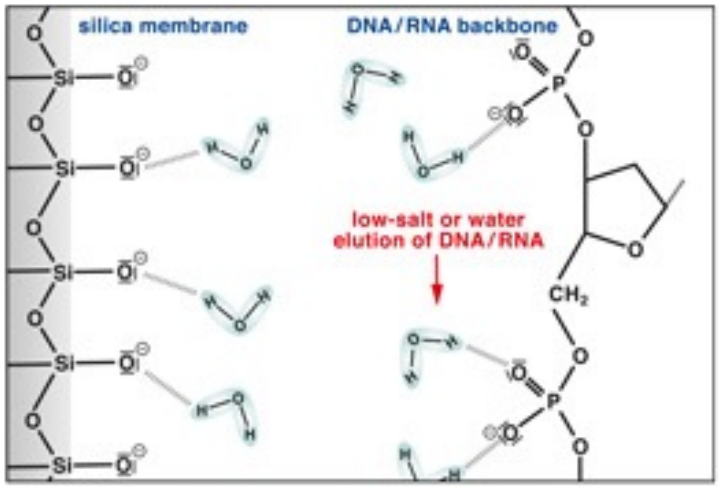
Interaction between DNA/RNA (hydrate shell is reversibly removed by chaotropic salt) and silica membrane

Contaminants are washed away under high-salt and/or ethanolic conditions to keep the DNA/RNA bound to the membrane

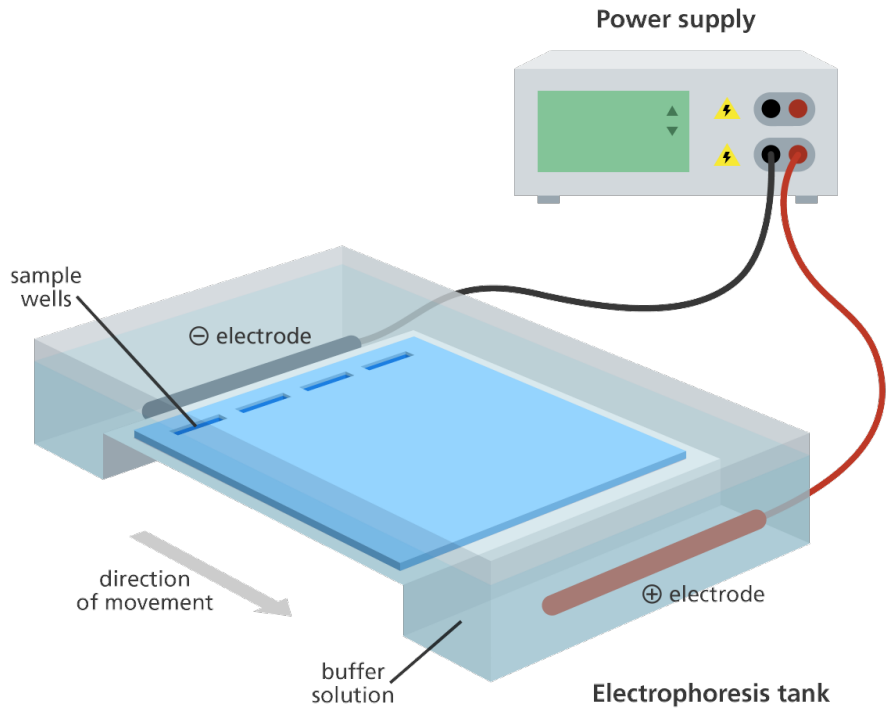
DNA/RNA is eluted in low-salt buffer or water, DNA/RNA is ready to use for downstream applications

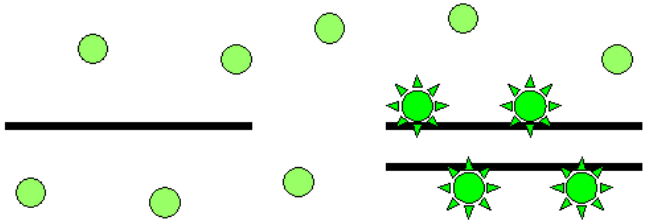


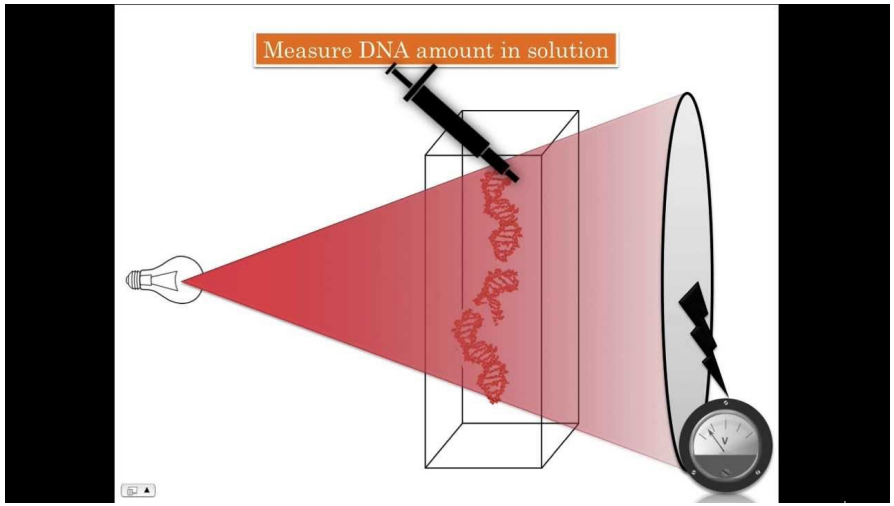
Principle of binding



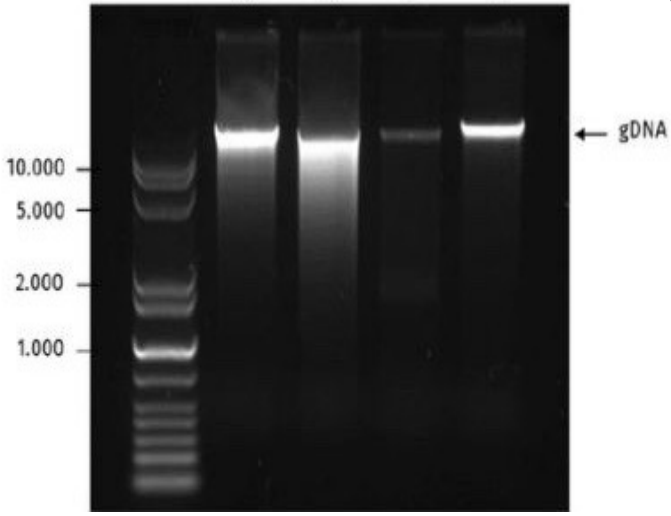
Principle of elution


Power supply
sample wells
⊖ electrode
direction of movement
⊕ electrode
buffer solution
Electrophoresis tank

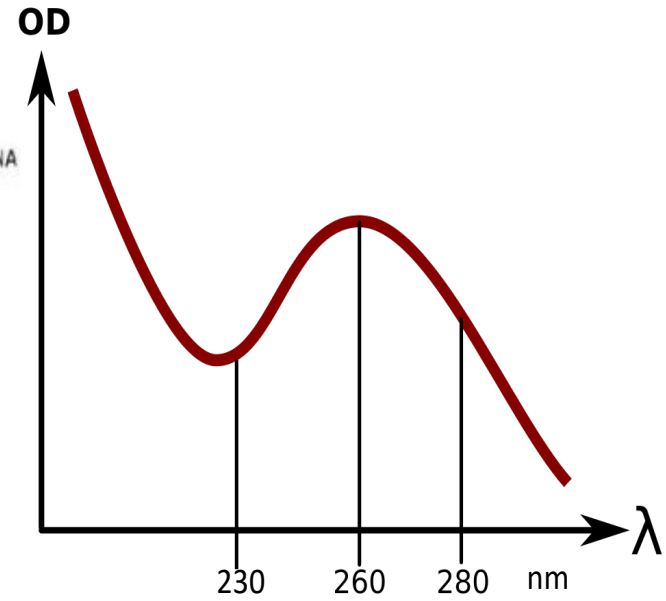




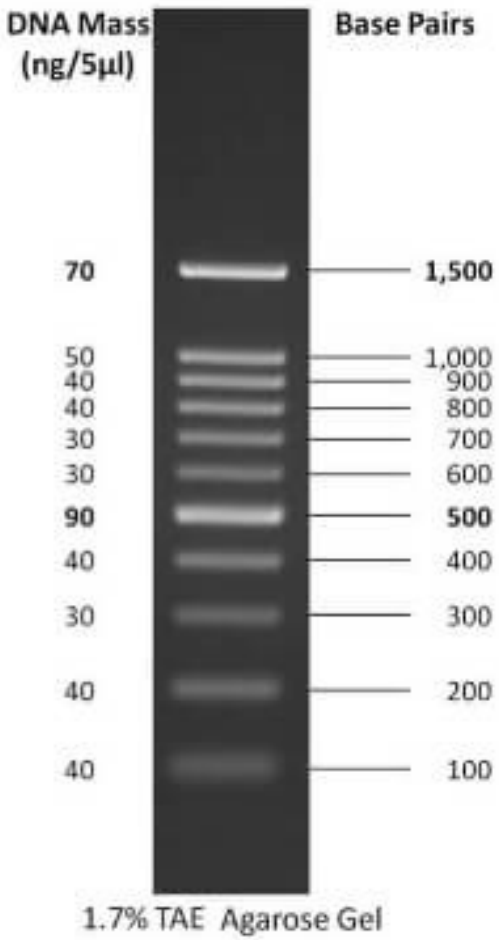
A



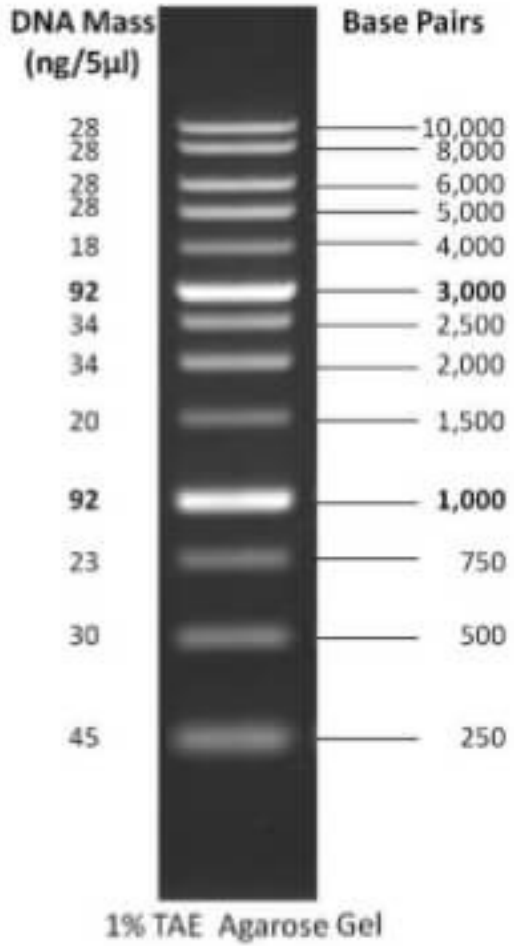
B



260/280 and 260/230 ratios



100 bp DNA ladder



1 kb DNA ladder

Extraction of DNA is often an early step in many diagnostic processes used to detect bacteria and viruses in the environment as well as diagnosing disease and genetic disorders

1. Polymerase chain reaction (PCR) for the amplification of specific genes or gene regions.

2. Sequencing: Portions of, or whole genomes may be sequenced as well as extra chromosomal elements for comparison with existing sequence in the public data base.