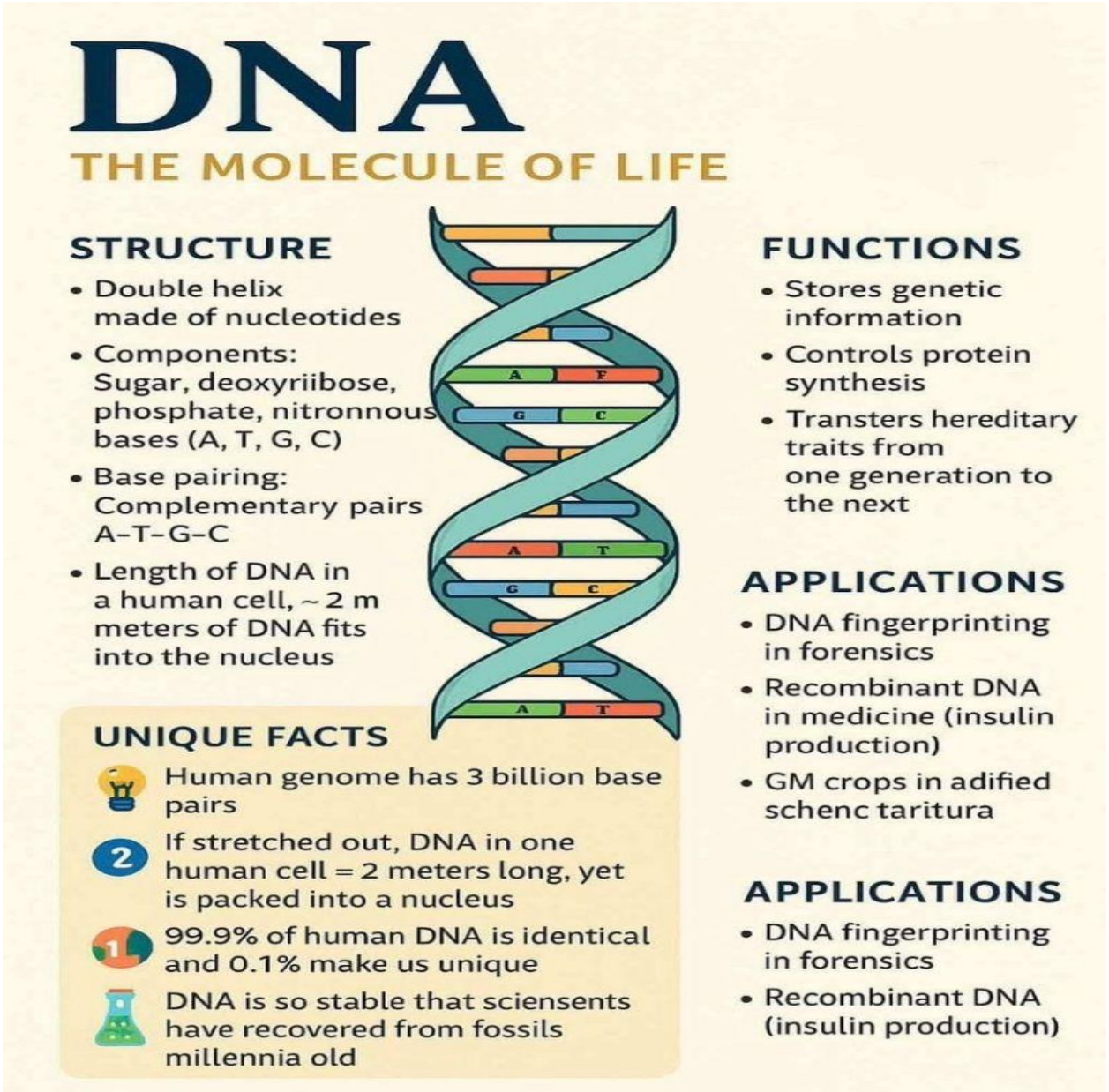
Molecular Biology Laboratory

Lab 5: Chromosomal DNA extraction from bacteria

DNA: The Miracle That Transformed the World!

Imagine a molecule that, with its incredible coding, evolves life from a single cell to billions! Stretching 2 m in every cell and weaving its genetic magic, it reveals hereditary secrets and crafts life-saving drugs like insulin. It even unlocks mysteries from ancient fossils! You are part of this wonder—ready to shape history with your 0.1% genetic uniqueness?



Isolating the genetic material (DNA) from cells (bacterial, viral, plant or animal) involves three basic steps:

1- Rupturing of cell membrane to release the cellular components and DNA
2- Separation of the nucleic acids from other cellular components
3- Purification of nucleic acids.

The method of isolation of genomic DNA from a bacterium comprises following steps:

1. Bacterial culture growth and harvest.

2. Cell wall rupture and cell extract preparation.

3. DNA Purification from the cell extract.

4. Concentration of DNA solution.

What is the purpose of extracting DNA from cells?

Extracting DNA from cells has multiple purposes in research, diagnostics, and industry, such as the classification of species through 16S rRNA and whole-genome sequencing. It is also fundamental in molecular cloning and genetic engineering, where bacterial DNA is manipulated to create recombinant DNA to express proteins in host cells. DNA extraction also allows the identification of antibiotic resistance genes, helping to track and combat the spread of resistant strains. Additionally, bacterial DNA is used to study gene function and regulation, providing insights into bacterial physiology, metabolism, and adaptation. In biotechnology, purified DNA allows engineering bacteria for applications such as biosensors, biofuels, and the production of biomolecules. It also facilitates evolutionary and comparative genomics studies, shedding light on genetic variation, evolution, and horizontal gene transfer among bacteria. Other applications include vaccine development by identifying virulence factors and antigens, which can

be targeted for preventing bacterial infections. In forensic microbiology, DNA helps trace contamination sources and analyze microbial evidence.

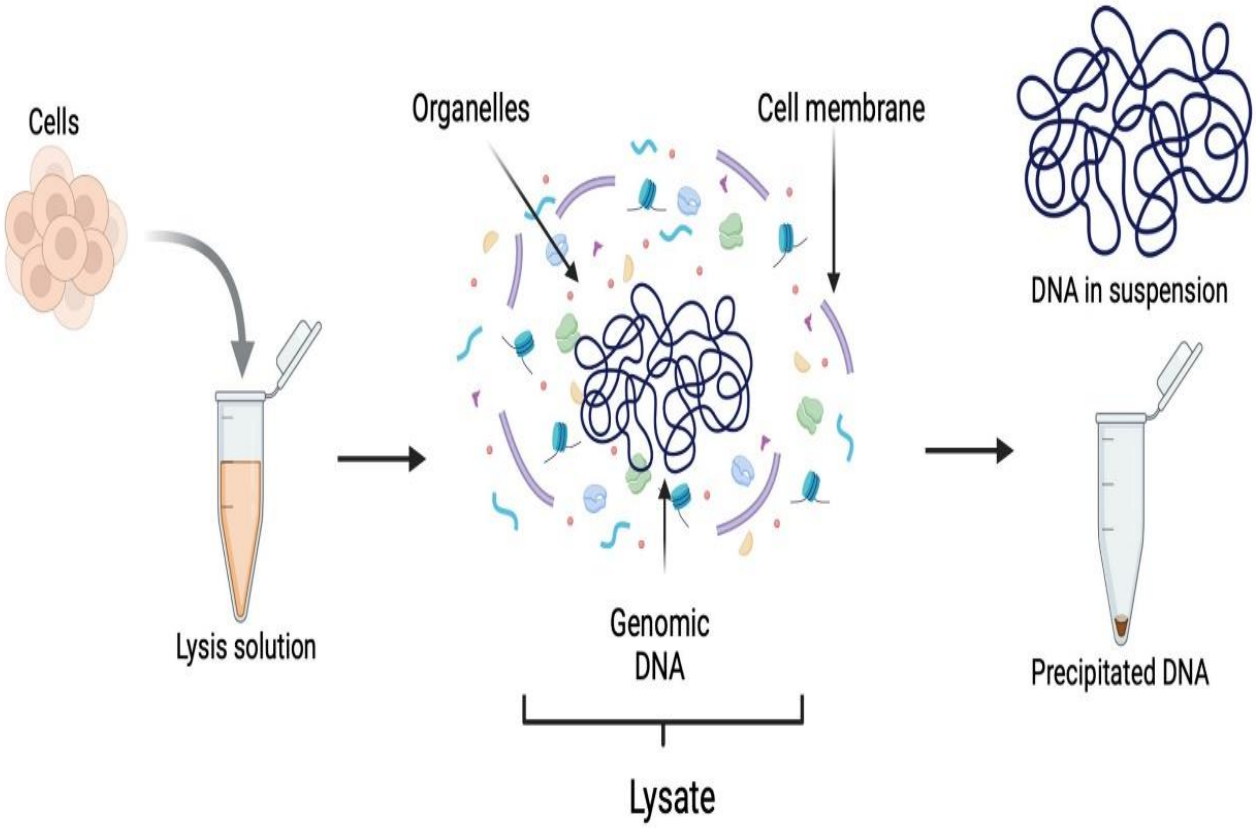
What are the steps, reagents and materials to extract DNA?

DNA extraction from cells involves isolating and purifying DNA from various types of tissues or cells. The process generally includes two main steps:

1- disruption of the cell membrane (and the cell wall, if present, as in plant cells) to release the DNA.

2- purification, which involves separating the DNA from other cellular components and debris.

This process is called cell lysis and the resulted fluid is known as lysate (Figure 1).



Procedure:

1) Grow bacteria in 3-4 ml of brain heart infusion broth for overnight at 37C°.

2) Centrifuge 1-1.5mL of bacterial culture in Eppendorf tube for 5min and remove the supernatant

3) Suspend sediment by 1.5mL of the lysis buffer consisting of:

400Mm Tris-Hcl, 60Mm EDTA, 150mM NaCl, 1% SDS PH = 8. And leave the mixture at room temperature for 10min to analyze the cell walls.

4) Add 150 µl of potassium acetate solution consisting of:

5M potassium acetate 60mL, Glacial acetic acid 11.5mL, D.W. 28.5mL

5) Shake the tubes by vortex 10sec.

6) Centrifuge for 1min\10000xg.

7) Transfer the upper aqueous phase to another tube and add a similar size of a mixture phenol: chloroform (1:5) at pH = 8 and shake well the contents of the tube.

8) Centrifuge for 1min\10000xg.

9) Transfer supernatant to another tube and add a similar volume of Isopropanol and shake well

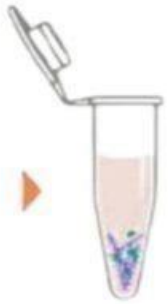
10) Centrifuge for 2min\10000xg, and neglect the supernatant.

11) Wash the sediment by 1 mL of alcohol Ethanol 70%.

12) Centrifuge for 1min\10000xg, neglect the supernatant and placed in an inverted-on filter paper to dry completely.

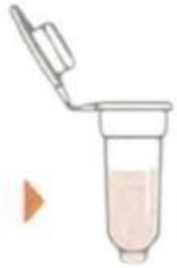
13) Dissolve the sediment by)10-20 µl (from TE buffer and keep freezing (-20C°).

DNA EXTRACTION



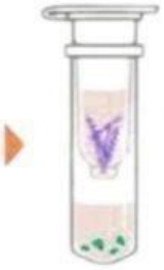
1) LYSIS

Lysis buffer contains chemicals that lyse the cell to release cellular contents out.



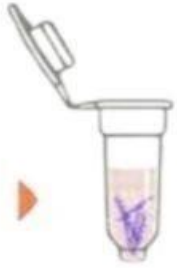
2) BIND

A binding buffer helps DNA to bind with the silica membrane in the column.



3) WASH

Washing buffer usually contains ethanol or isopropanol which selectively removes only cell debris, proteins and other cellular molecules excluding DNA



4) ELUTE

Elution buffer is a high pH solution that detaches DNA from the silica matrix, dissolves it and elutes it

5) DNA

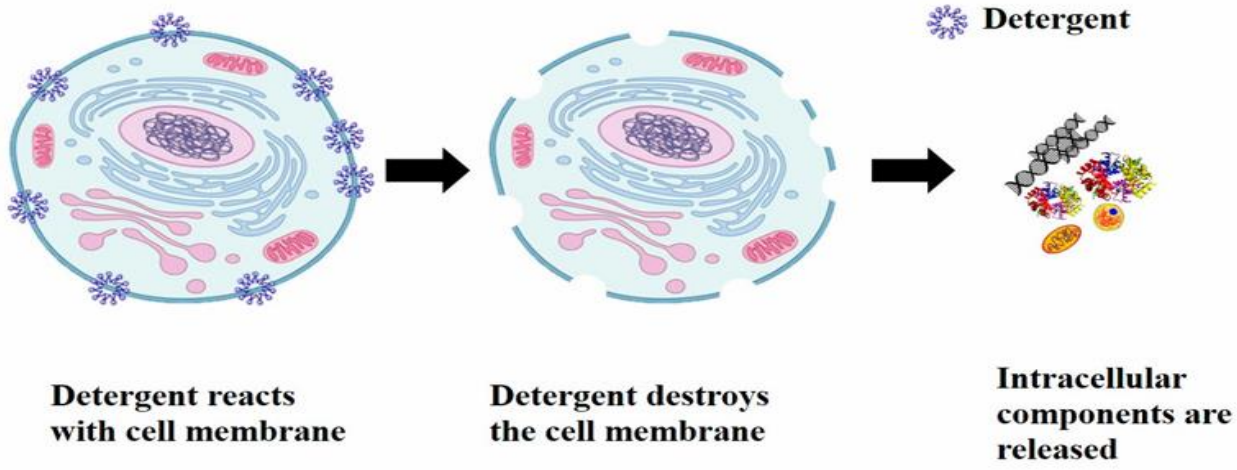
Elution buffer is a TE solution of pH 9.0. is used to elute DNA

Briefly, steps to DNA Extraction:

1. Break the cells to open and expose the DNA.

2. Remove membrane lipids by adding detergent.

3. Precipitate DNA with an alcohol — usually ethanol or Isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-soluble salt.



Extraction DNA by rapid boiling:

1) Grow bacteria overnight at 37C°, on Brain heart infusion agar.

2) Suspend few colonies of bacteria in 1 mL distilled water on Eppendorf tube, and shake well by vortex.

3) Boil in a water bath for (10 min) and centrifuge directly, for 13000 rpm/5min.

4) separate the supernatant and put in another clean Eppendorf tube and then centrifuge tubes quickly (6000) rpm/ 2min, remove the supernatant and melts the pellet with a solution of the TE buffer.

Benefits of additives:

1- TE buffer: Disrupting the bacterial cell walls to contain EDTA, which works on:

- Helps to capture ions Mg++ & Ca++ from the cellular walls which provide stability of the cell wall.
- Stop the effectiveness of enzymes Nuclease such DNase (destroys the DNA).
- Helps disengagement nuclear protein complex.
- Dissolving the DNA sample.
- Acts as a buffering agent to maintain a stable pH (~ 8.0), which protects DNA from degradation during extraction.

2- Sarkosyl or SDS: Ionic detergent works:

- Denaturation proteins which acting on breaking peptide bonds and thus disengagement amino acids.
- Remove the fat which found in cell walls.

3- Chloroform: Phenol

- Denaturation proteins.
- Eliminates the remaining phenol atoms in the DNA preparations (as phenol effect the solubility the DNA in water).
- Inhibit the effectiveness of the enzyme RNase.

4- Isopropanol: precipitation DNA.

5- Ethanol 70%: used to wash the DNA sample which helps to reduce dry sediment by alcohol.

6- Enzymes (e.g., Proteinase K, RNase, Lysozyme)

- Proteinase K digests proteins, including those bound to DNA, aiding in their removal.
- RNase degrades RNA, ensuring RNA-free extracted material.
- Lysozyme breaks down bacterial cell walls, especially in gram-positive bacteria, to access DNA.

7- Detergents (e.g., SDS, Triton X-100)

- Disrupt cell membranes by solubilizing lipids and proteins, lysing cells and releasing contents, including DNA.
- Denature proteins to ease their removal during purification