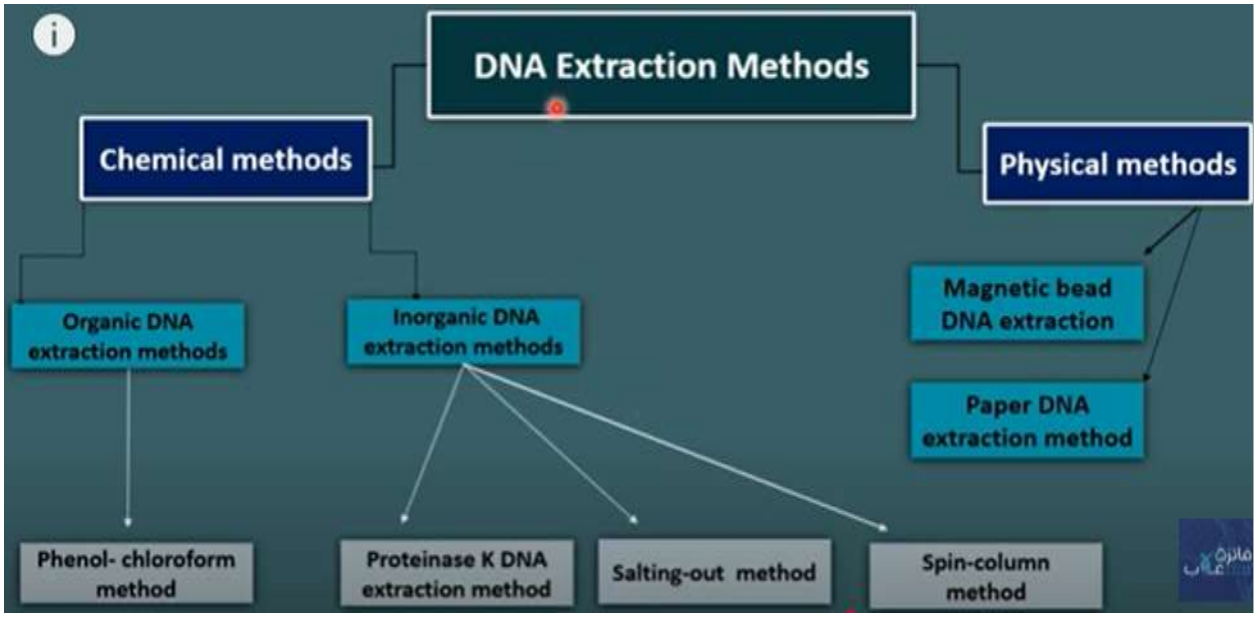
Lab 4

Chromosome preparation



Organic Solvent Solution الاستخلاص بطريقه خليط المذيبات العضوية

A mixture of organic solvents is used after the cell destruction process to get rid of the salts and proteins that separate in the organic solvent layer, while the DNA remains in the aqueous layer. Among the organic solutions used are phenol, chloroform, and isoamyl alcohol.

DNA can be separated based on the solubility of DNA molecules in immiscible solutions, this is called liquid-liquid DNA extraction methods.

This method is known as the phenol-chloroform-isoamyl alcohol (PCH) DNA extraction method, or PCI.

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Although this method is considered one of the best, and the quantity and quality of DNA obtained is very good, it is not recommended due to the harmful effects of phenol and chloroform.

The main advantage of PCI is that it can isolate DNA from almost all types of tissues (it works with animal, plant and bacterial cells) and can isolate RNA when used with guanidine thiocyanate.

This method is unsafe because phenol is volatile and can cause burns. Chloroform can cause fainting. Therefore, it requires training, preparation, and handling of chemicals.

Materials

1-TE : It is used to dissolve and save DNA and can be stored for long periods at temperatures ranging from 4 to -20 degrees Celsius.

2-SDS solution 25% : It is used to denature proteins and dissolve fats (lipid solubilization), thus destroying cell membranes and releasing cell components, including DNA.

3-Organic Solvent Solution : This mixture is used to form two completely isolated layers: the aqueous layer containing the DNA and the organic solvent layer containing the rest of the components.

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4-Sodium chloride solution

5-Loading buffer

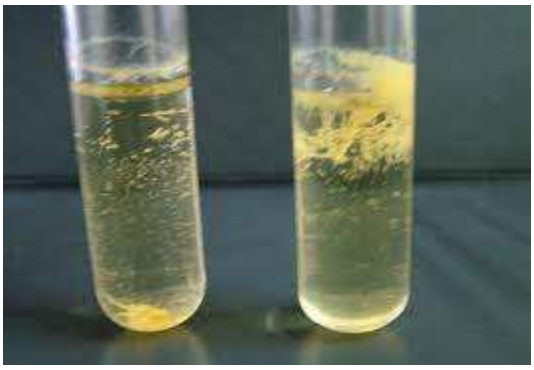
6-TBE

7-Isopropanol is used to precipitate DNA.

8-Ethanol is used to wash DNA.

Procedure

1. Inoculate 10 ml of Brain Heart Infusion Broth with a single, pure, young colony of E. coli, and incubate for 18 hours at 37°C.



2. The bacterial culture is centrifuged at 6000 rpm for half an hour, then the filtrate is discarded and the precipitate is taken.

3. The precipitate was washed with 2 ml of TE buffer and then centrifuged at 6000 rpm for 15 minutes. The washing process was repeated two or three times.

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4. Add 600 microliters of 25% SDS solution and optionally 2 microliters of RNase solution and place in a water bath at 55°C for 15 minutes.

5. Add 2 ml of 5 M sodium chloride solution and stir the tube two or three times. Then leave the tubes at room temperature.

6. Add isoamyl alcohol and gently stir the tubes for half an hour. Then, centrifuge the tubes in a cooled centrifuge at 6000 rpm for half an hour. Repeat this step 2-3 times. Two layers will form: an upper aqueous layer containing the DNA and a lower solvent layer containing the remaining components.

7. The aqueous layer containing DNA is gently drawn off and transferred to clean, sterile tubes. Then, isopropanol alcohol is added to it and the tubes are gently inverted until a visible white network (DNA) appears.

8. The precipitated DNA network is lifted using a Pasteur pipette with a closed, curved end and transferred to sterile Eppendorf tubes and washed with 70% ethanol, cooled to 5°C, two to three times. Then, the tubes are left at room temperature for half an hour to allow the ethanol to evaporate.

9. The DNA precipitate was dissolved by adding 50 microliters of TE buffer and then stored at -20°C until use.

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Disadvantages

-Large quantities of toxic substances (phenol and chloroform) are used, which must be disposed of using biologically sound methods.

-The DNA extracted using this method is of low purity.

Advantages

- Economically inexpensive (cheap).

- High concentrations of DNA can be obtained.