DNA Extraction

- **DNA Extraction:** is an isolated process of DNA from various sources

- **The aim:** is to separate DNA present in the nucleus of the cell from other cellular components.

Application of DNA isolation

- **It is needed for genetic analysis which used for:**

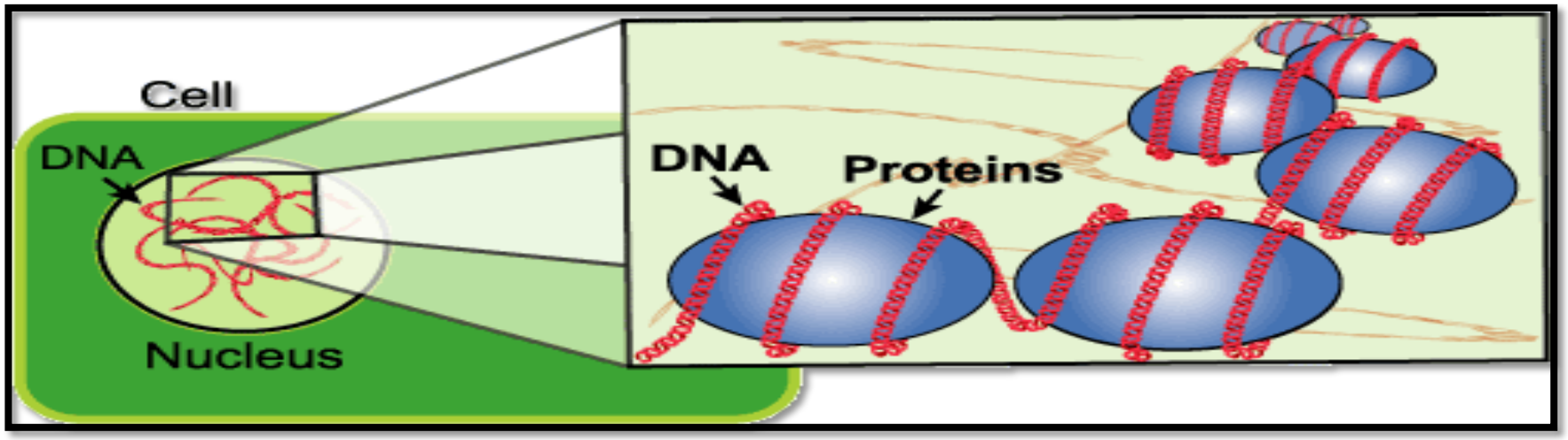
- **1- Scientific:** use DNA in number of Applications , such as introduction of DNA into cells & animals or plants for diagnostic purposes (gene clonining)
- **2- Medicine:** is the most common. To identify point sources for hospital and community-based outbreaks and to predict virulence of microorganisms
- **3- Forensic science:** needs to recover DNA for identification of individuals , (for example accident , or war victims , and paternity determination).

- **Many different methods and technologies are available for the isolation of genomic DNA.**
- **All methods involve:**

➢ **disruption and lyses of the starting material followed by**

Removal of proteins and other contaminants and finally

Recovery of the DNA



Sample Collection

A- Source: Sample can be isolated from any living or dead organism

Common sources for DNA isolation include:

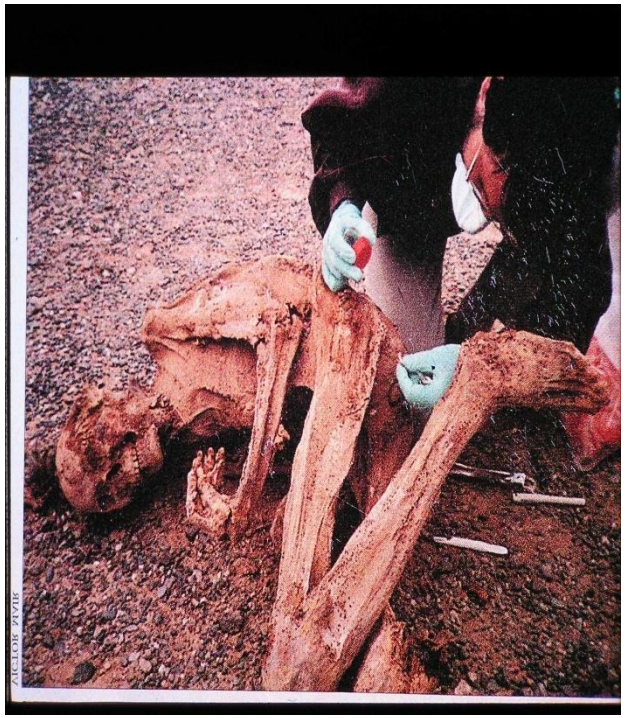
- **Whole blood**
- **Buffy coat**
- **Bone material**
- **Buccal cells**
- **Cultured cells**
- **Amniocytes or amniotic fluid**
- **Sputum, urine, CSF, or other body fluids**

Sample Collection

B. Sample age:

May be fresh or has been stored . Stored sample can come from:

- ❖ Archived tissue samples ,
- ❖Frozen blood or tissue (biopsy material) ,
- ❖ Exhumed bones or tissues &
- ❖ Ancient human sample.
- ❖Dried blood spots



Extraction of DNA

Key Steps

- Lysis of the cells
- Removal of contaminants includes

1. Proteins
2. RNA
3. Other macromolecules

- Concentration of purified DNA

• Summary of DNA extraction

- **There are three basic & two optional steps in a DNA extraction** :

1- **Cell lysis ,** to expose the DNA within .

2- **removing membrane lipids** by adding a detergents or surfactants .

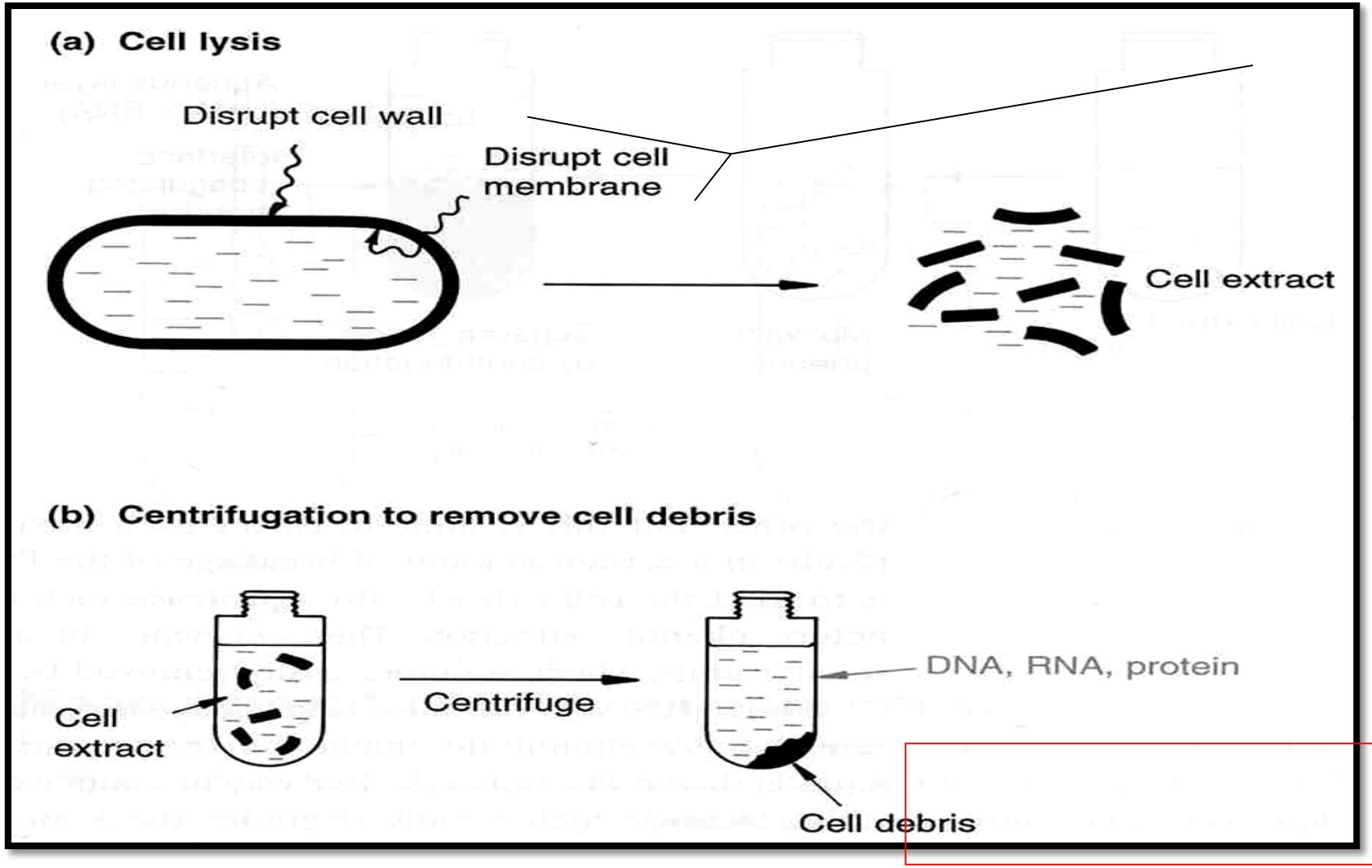
3- **removing proteins** by adding a protease .

4- **removing RNA by** adding an Rnase.

5- **precipitating the DNA** with alcohol- usually ice cold ethanol. In these alcohols , DNA strand will aggregate together, giving a pellet upon centrifugation . This step also removes alcohol- soluble salt.

Extraction of Genomic DNA

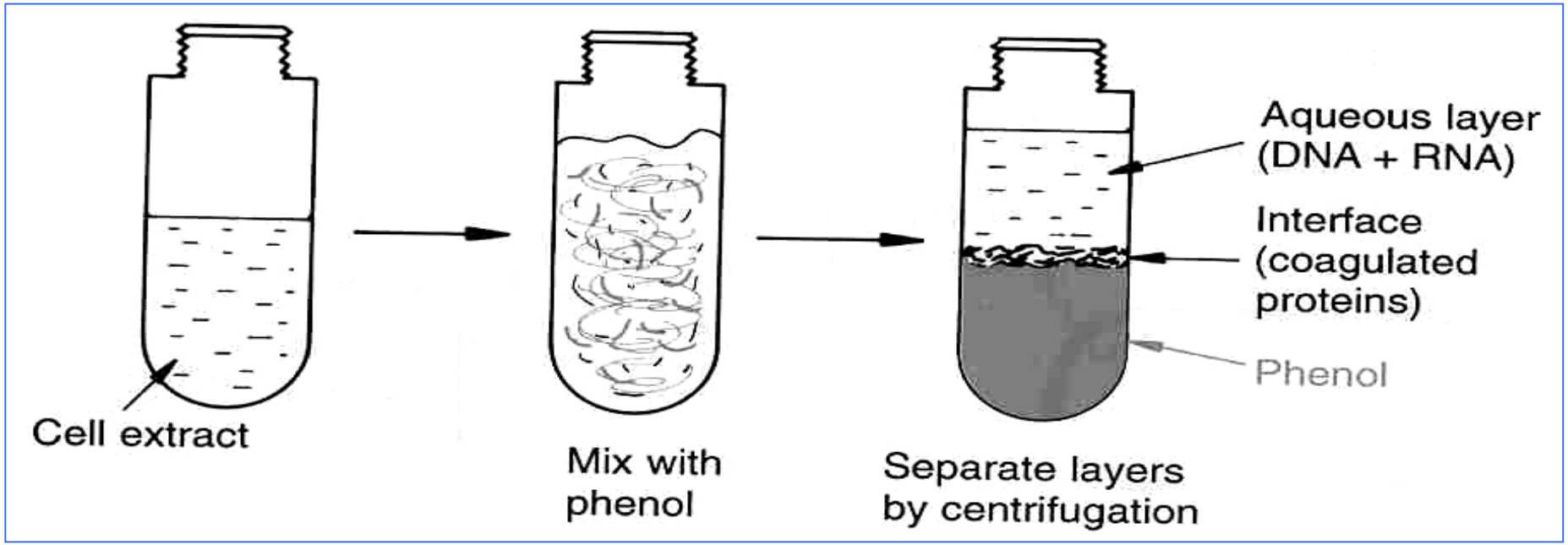
Bacterial genomic DNA prep: cell extract



1. Lysis:

- Detergents
- Organic solvent
- Proteases (lysozyme)
- Heat

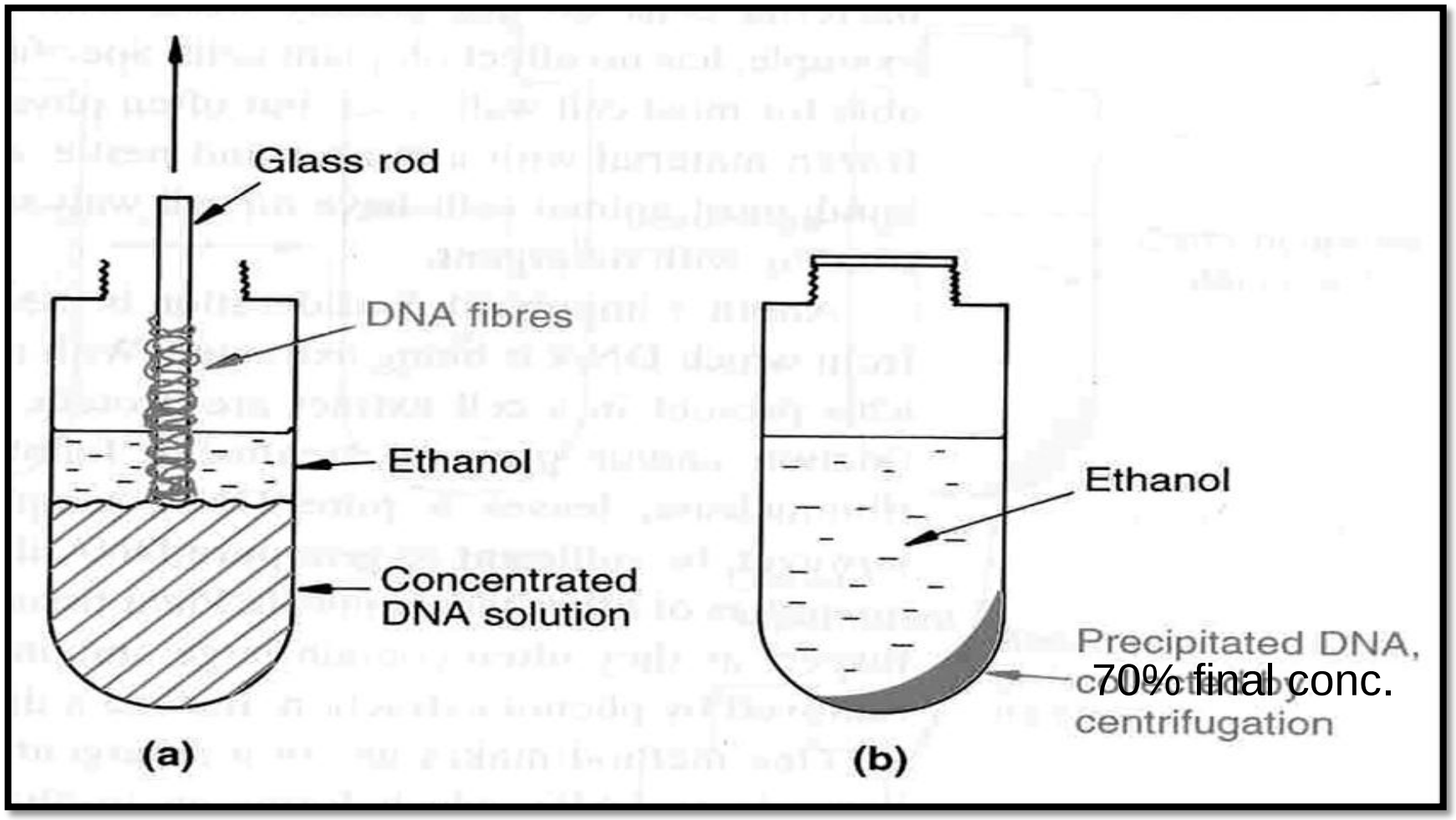
Genomic DNA prep: removing proteins and RNA



2. Need to mix gently! (to avoid shearing breakage of the genomic DNA)

3. Add the enzyme RNase to degrade RNA in the aqueous layer

4. Two ways to concentrate the genomic DNA



70% final conc.

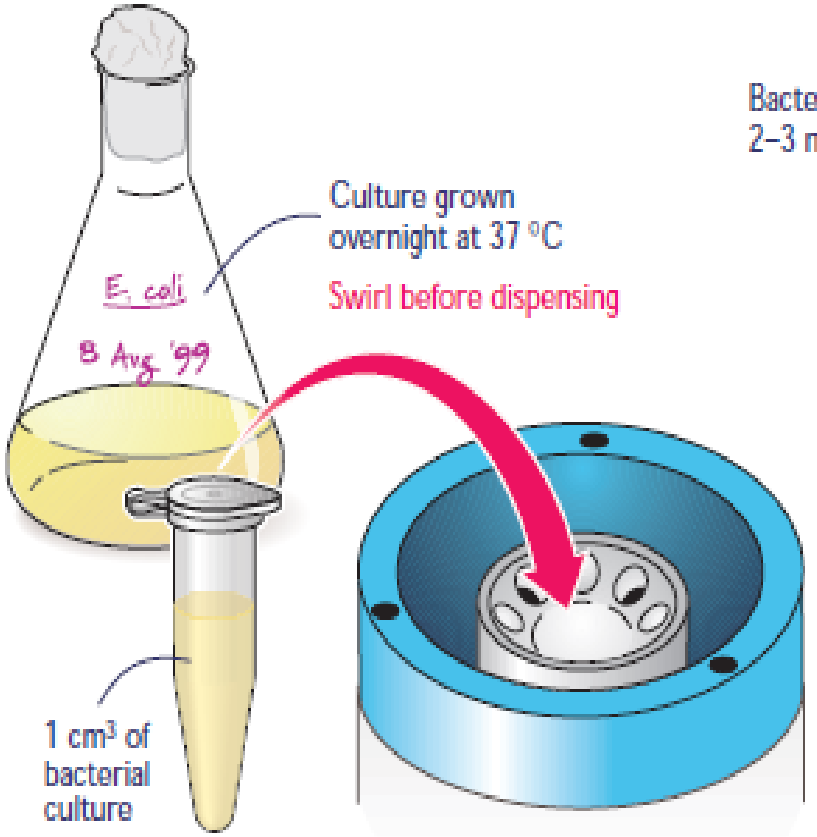
“spooling” Ethanol precipitation

Extraction of plasmid DNA

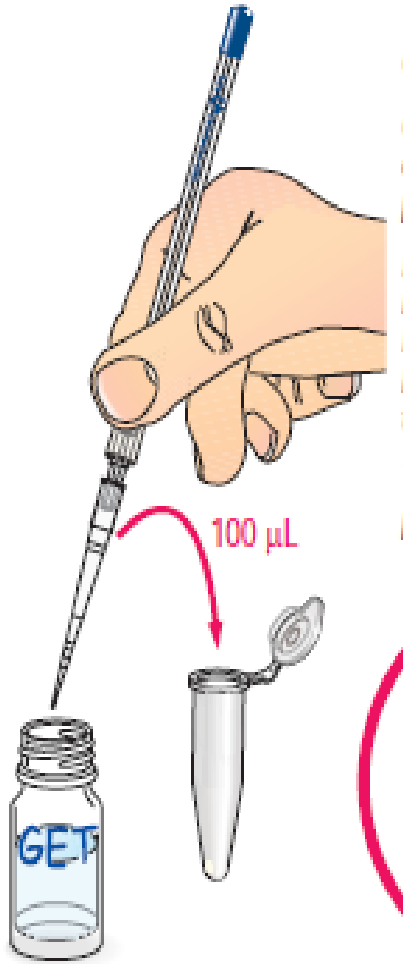
1 Spin 1 cm³ of bacterial culture for 1 minute at 8 000 x g, or until a pellet is formed.

2 **3** Pour the supernatant into disinfectant. Use a micropipette to remove as much liquid as possible from the pellet.

4 Add 100 µL of ice-cold GET buffer to the pellet. Cap the tube and resuspend the cells well by tapping the tube vigorously until no lumps remain.



Remember to balance the centrifuge!

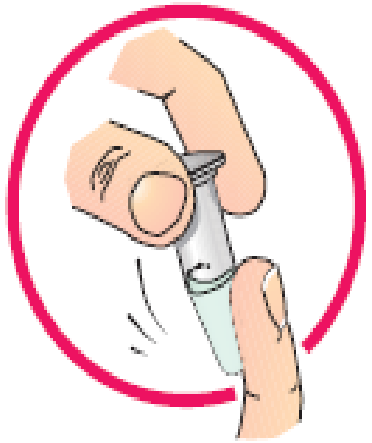


Glucose/EDTA/Tris

Glucose maintains the osmostic pressure and prevents lysis of the cells.

EDTA weakens the cell membrane and chelates Mg^{2+} ions that are needed by DNases, protecting the DNA from breakdown.

Tris buffers the solution at pH8.



5 Add 200 µL of SDS + NaOH solution. Mix well by inverting the capped tube. Leave for 5 minutes on ice.

Sodium Dodecyl Sulphate + Sodium hydroxide

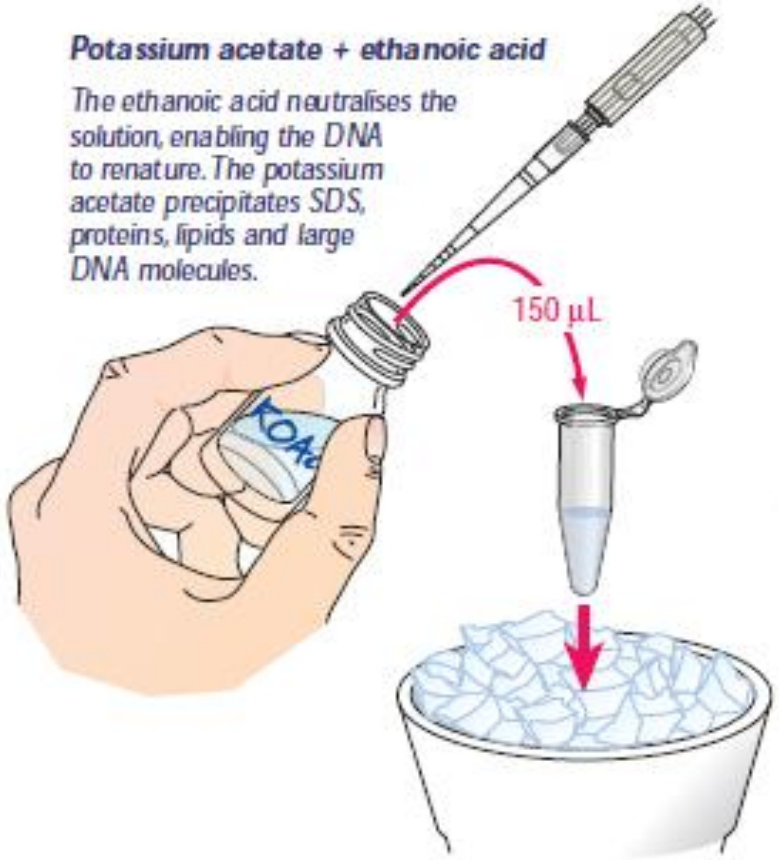
The SDS dissolves the cell membrane lipids and degrades the cellular proteins.

The NaOH splits the double-stranded DNA into single strands.

6 Add 150 µL of *ice-cold* KOAc solution. Mix well. *A white precipitate should appear.* Stand the tube on ice for 5 minutes.

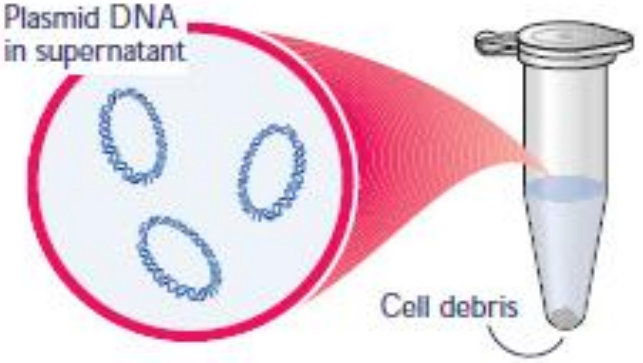
Potassium acetate + ethanoic acid

The ethanoic acid neutralises the solution, enabling the DNA to renature. The potassium acetate precipitates SDS, proteins, lipids and large DNA molecules.



7 Spin down the cell debris for 5 minutes. *The plasmid DNA remains in the supernatant.*

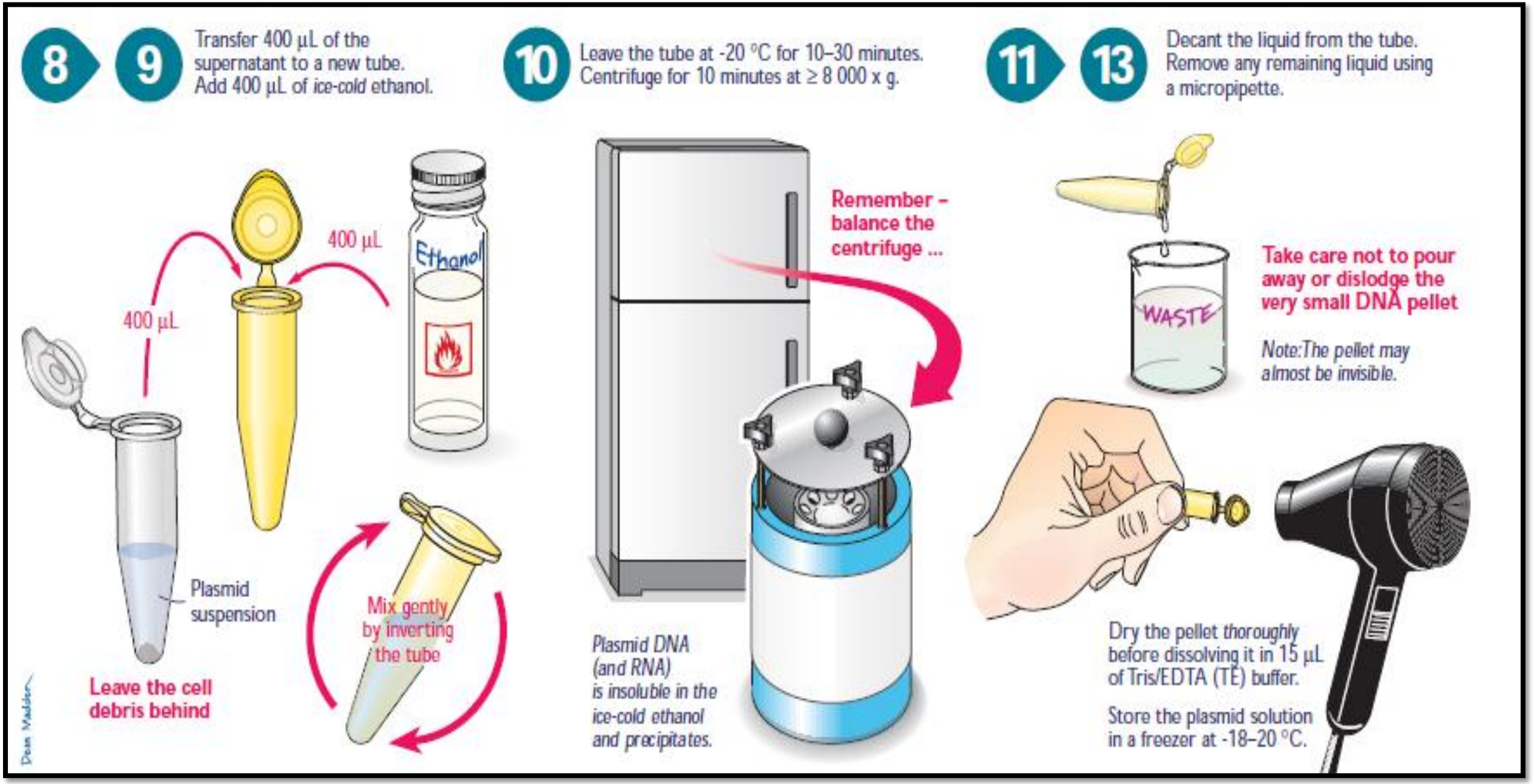
Remember to balance the centrifuge!



8 **9** Transfer 400 µL of the supernatant to a new tube. Add 400 µL of *ice-cold* ethanol.

10 Leave the tube at -20 °C for 10–30 minutes. Centrifuge for 10 minutes at ≥ 8 000 x g.

11 **13** Decant the liquid from the tube. Remove any remaining liquid using a micropipette.


8
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11
13
Decant the liquid from the tube. Remove any remaining liquid using a micropipette.
400 µL
400 µL
Ethanol
Plasmid suspension
Leave the cell debris behind
Mix gently by inverting the tube
Remember – balance the centrifuge ...
Plasmid DNA (and RNA) is insoluble in the ice-cold ethanol and precipitates.
WASTE
Take care not to pour away or dislodge the very small DNA pellet
Note: The pellet may almost be invisible.
Dry the pellet thoroughly before dissolving it in 15 µL of Tris/EDTA (TE) buffer.
Store the plasmid solution in a freezer at -18–20 °C.
Dean Madden

phenol/chloroform extraction

DNA purification: phenol/chloroform extraction

(phenol : chloroform : isoamyl alcohol)

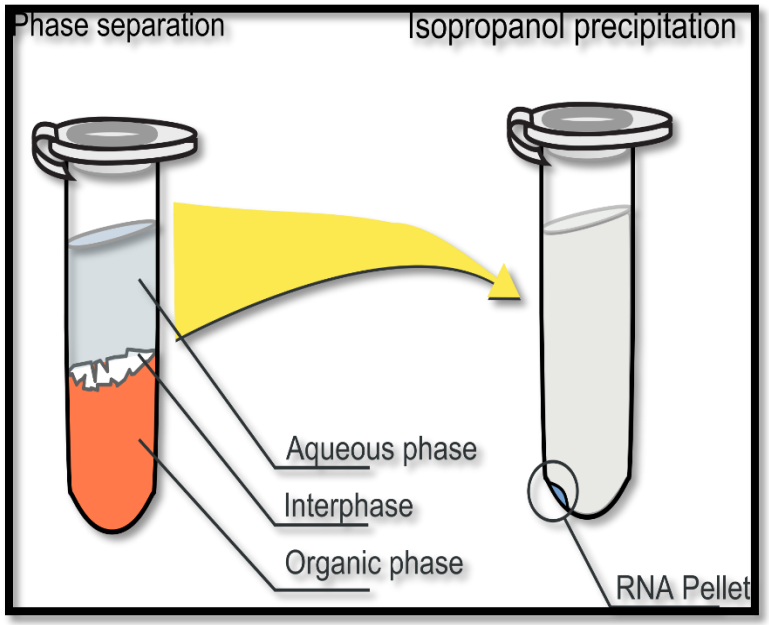
Phenol: denatures proteins, precipitates form at interface between aqueous and organic layer

Chloroform: increases density of organic layer

Isoamyl alcohol: prevents foaming

Phenol extraction

1. Aqueous volume (at least 200 microliters)
2. Add 2 volumes of phenol:chloroform, mix well
3. Spin in centrifuge, move aqueous phase to a new tube
4. Repeat steps 2 and 3 until there is no precipitate at phase interface
5. (extract aqueous layer with 2 volumes of chloroform)



Extraction of DNA from Whole Blood

- رابط تحميل الفديو

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