DNA EXTERACTION

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Definition

DNA Extraction = DNA isolation

It is a process used for purification (Deoxyribonucleic acid) DNA from sample using combination of physical and chemical methods.

Types of samples

Samples

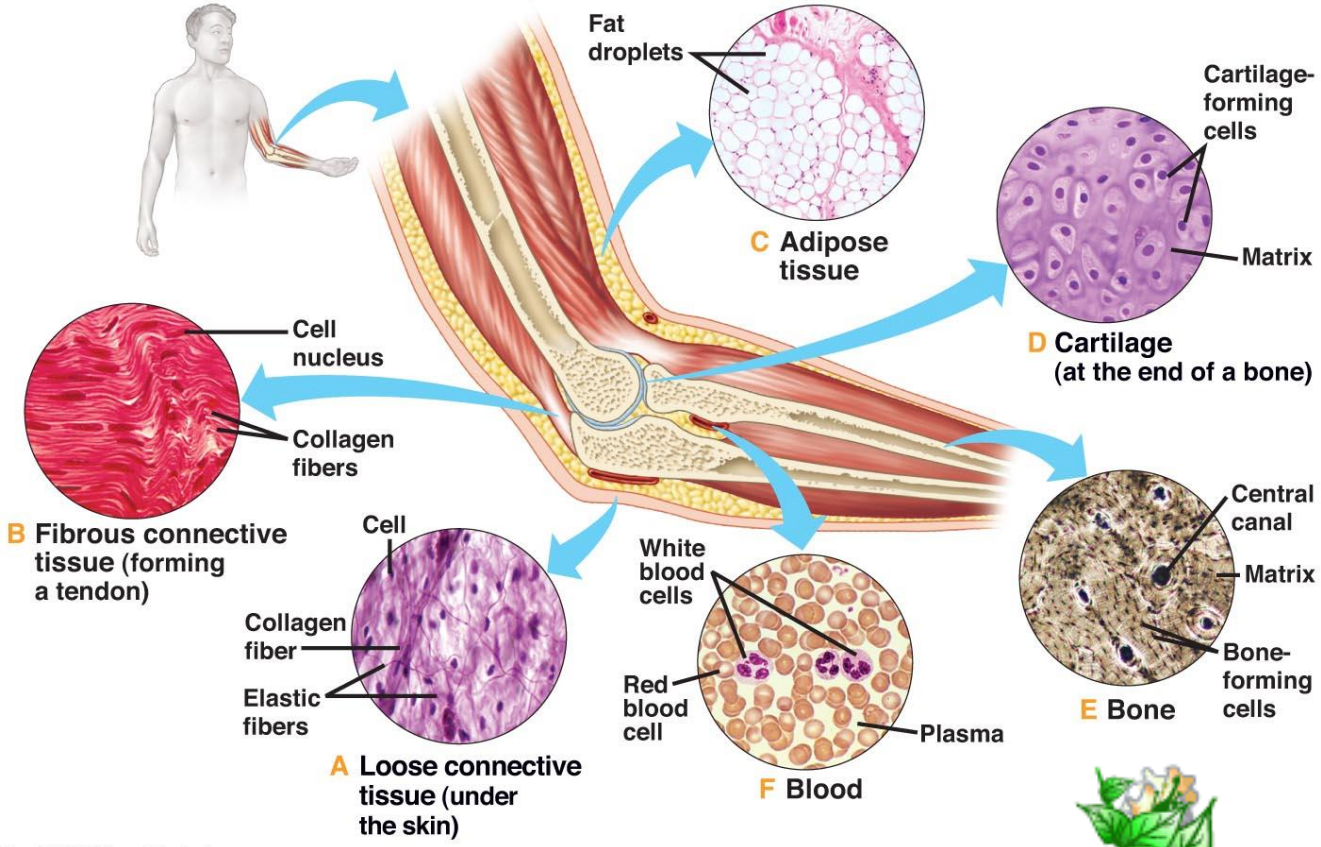
Blood (citrate, EDTA or heparin)

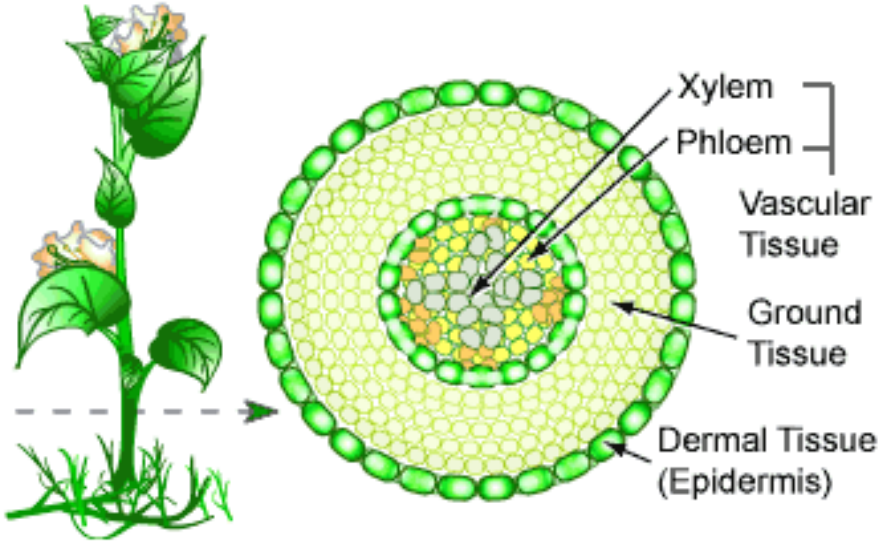
Blood spotted on filter paper

Insects

Plant

Tissue





Procedure of DNA extraction
Step I

Cell Lysis

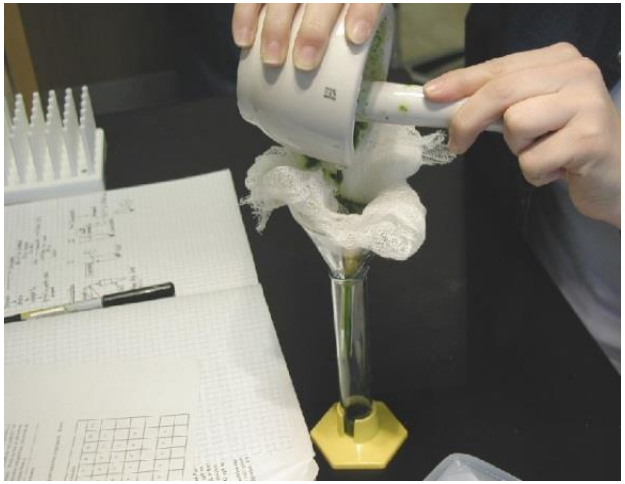
Breaking the cell to expose the **DNA**.

This is commonly achieved by:

1- chemical method.

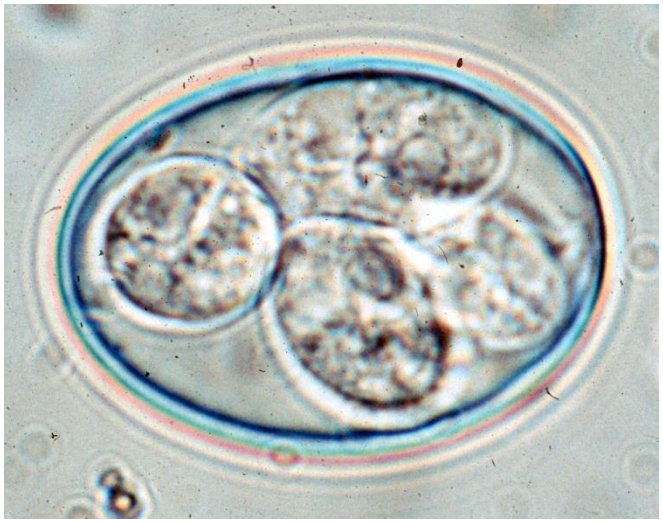
2- physical methods like grinding, blending or sonication the sample. and

Grinding of the samples

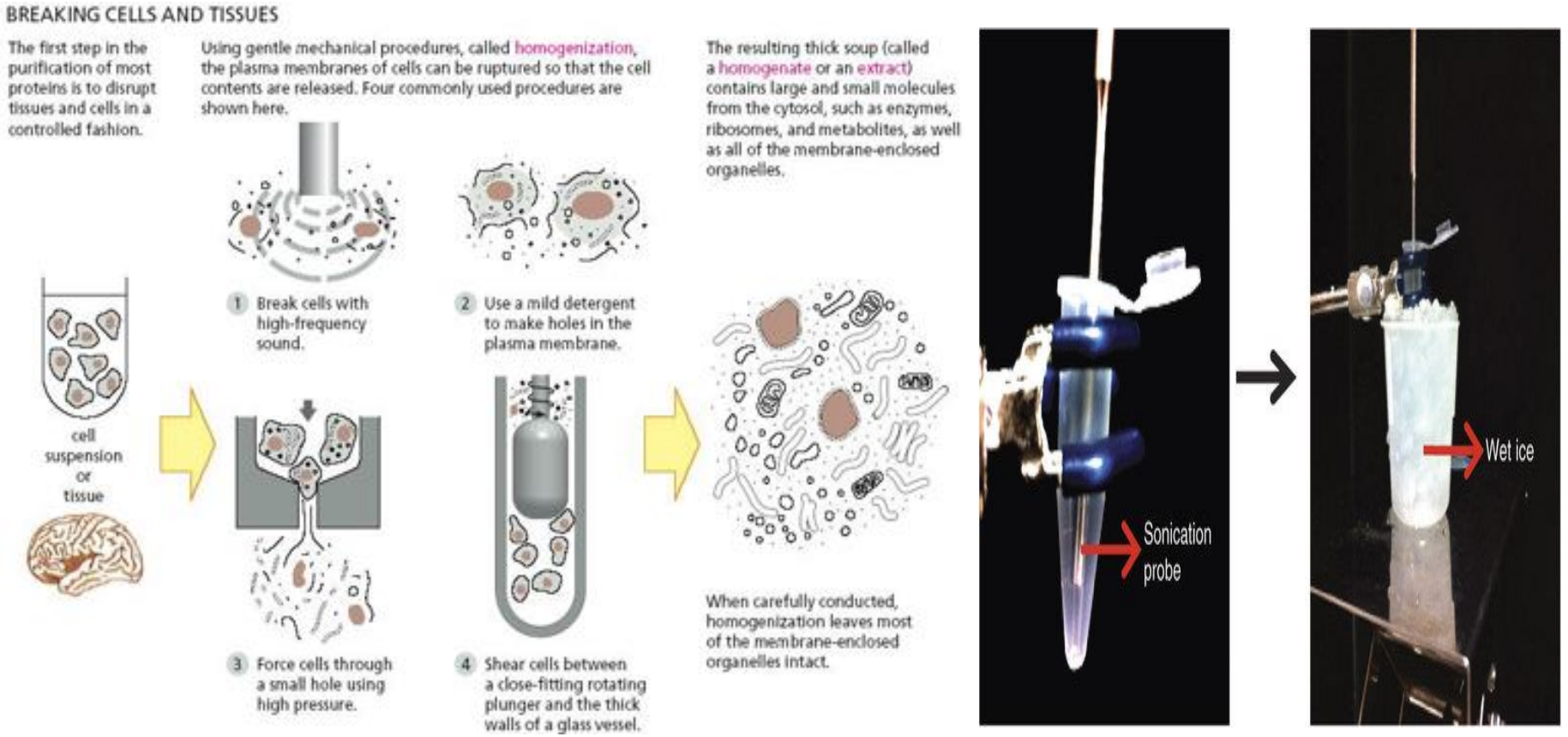


Blending of the samples

Blending of the samples with Silica beads

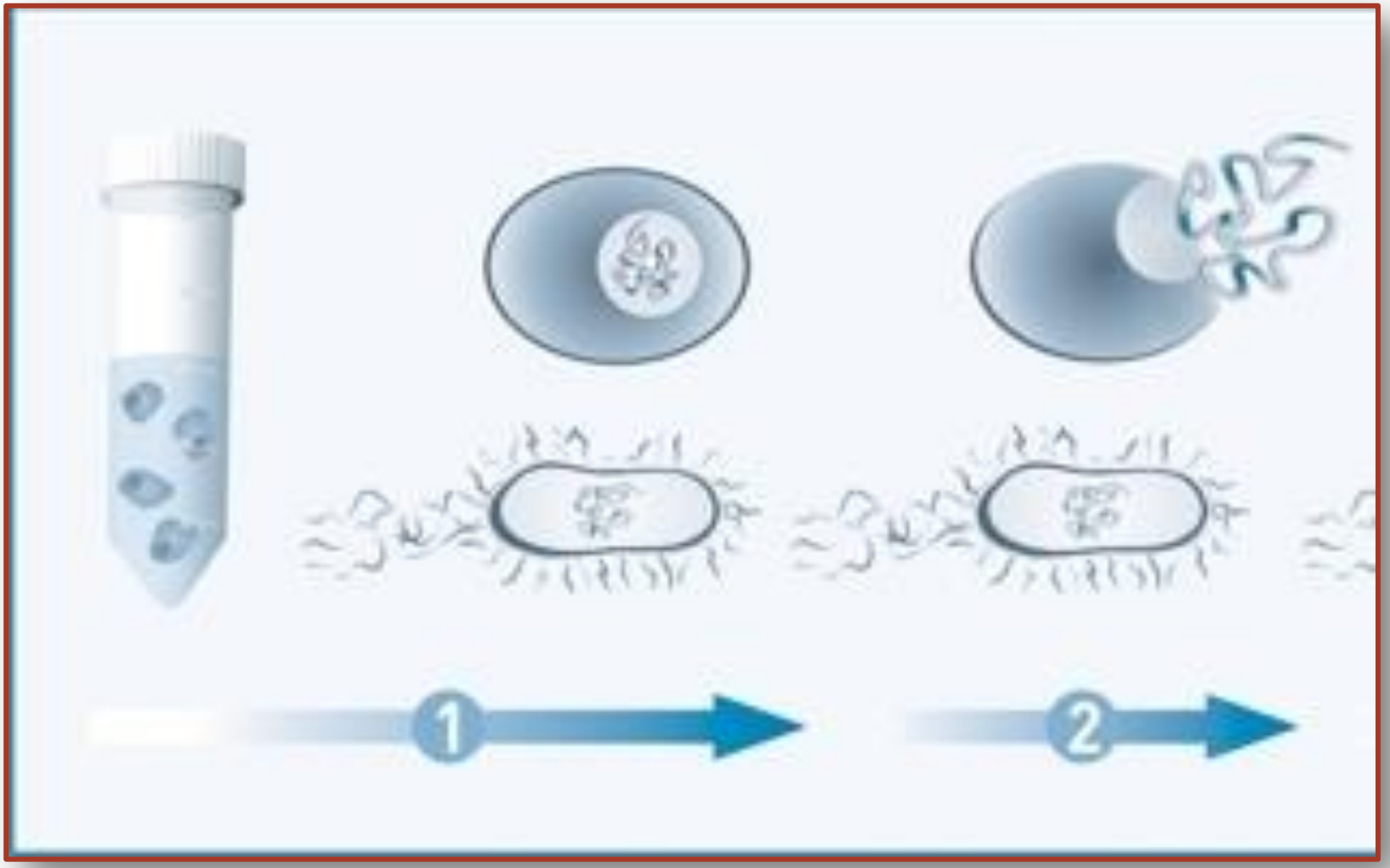


sonication of the sample



It means subject (a biological sample) to ultrasonic vibration so as to fragment the cells, macromolecules, and membranes.

Cell lysis



Procedure of DNA extraction
Step II

Removing membrane lipids, proteins and RNA

by adding detergent, surfactants, protease and Rnase.

DNA purification

Ethanol precipitation: by ice cold ethanol or isopropanol. *The DNA is insoluble in these alcohols*, so it will aggregate together, giving a pellet upon centrifugation.

Minicolumn purification: DNA may bind (adsorption) to the solid phase (silica or other) depending on the pH and the salt content of the buffer.

Procedure of DNA extraction
Step III

DNA purification :

Phenol–chloroform extraction

In which phenol denatures proteins in the sample.

After centrifugation of the sample denaturated proteins stay in organic phase while aqueous phase containing nucleic acid is mixed with the chloroform that removes phenol residues from solution.

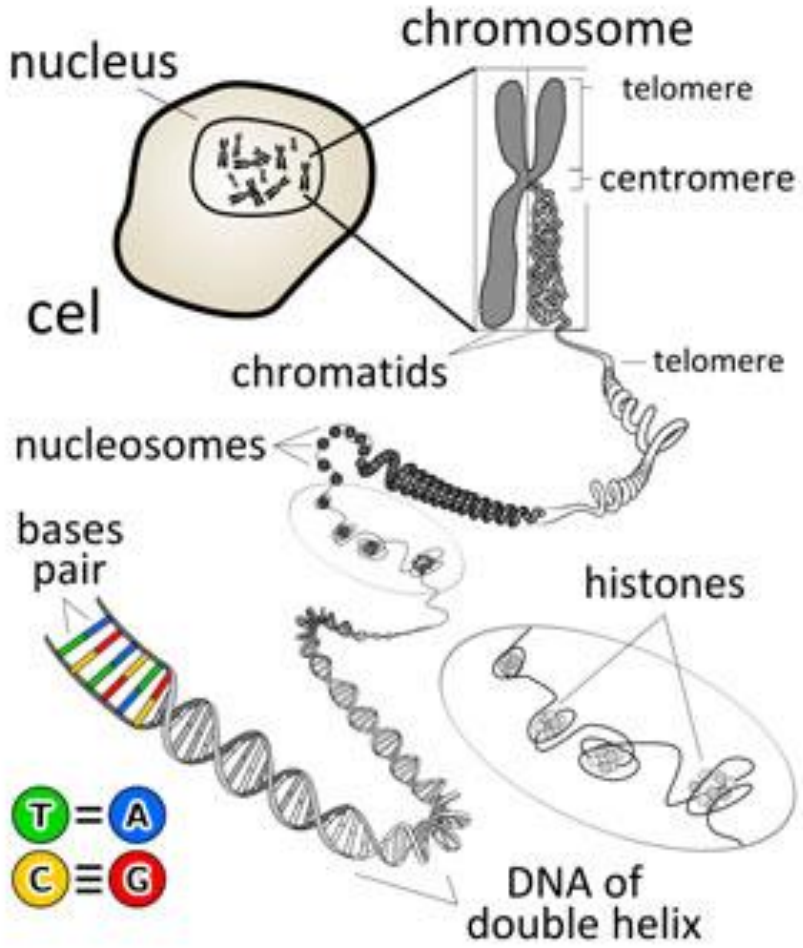
Mg^{2+} and Ca^{2+}, which prevents enzymes like Dnase from degrading the DNA.

Procedure of DNA extraction
Step IIII

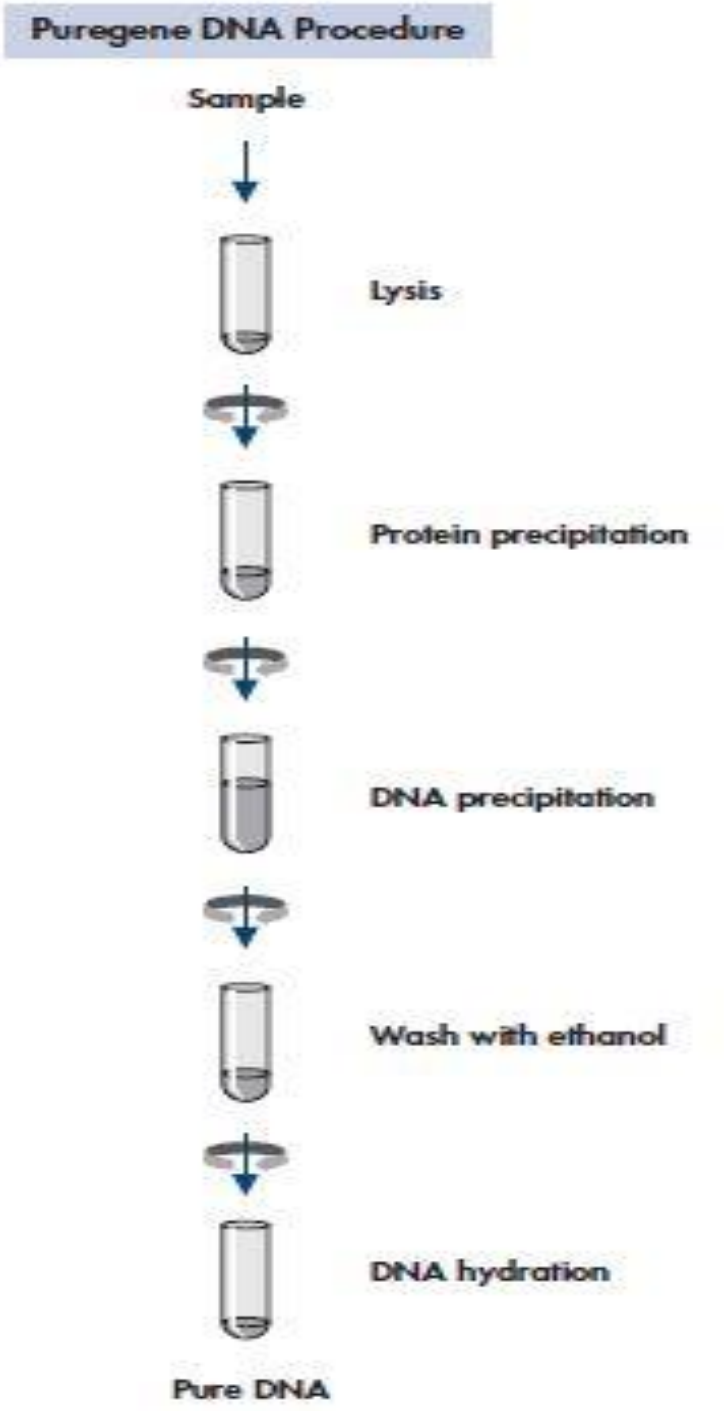
DNA Hydration:

After isolation, the DNA is dissolved in slightly alkaline buffer, usually in the hydration or elusion buffer or in ultra-pure water.

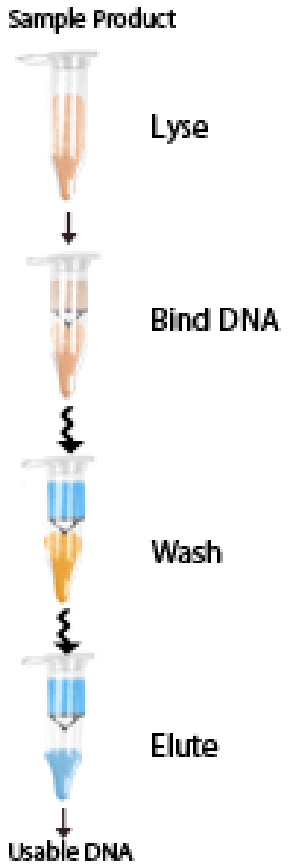

1
2
3
4
5


chromosome
nucleus
telomere
centromere
cel
chromatids
telomere
nucleosomes
bases
pair
histones
T = A
C ≡ G
DNA of
double helix

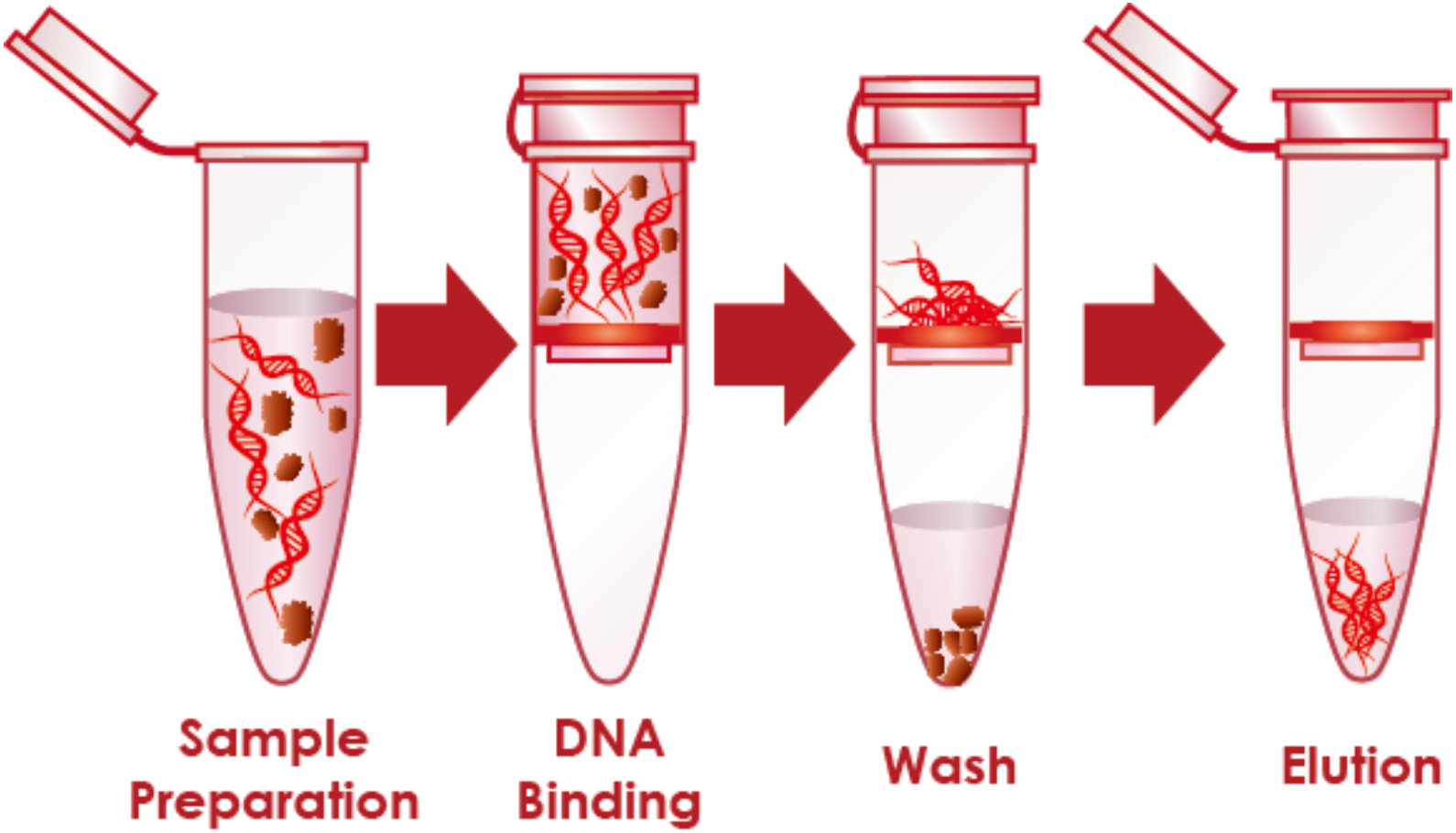
DNA Extraction



DNA Extraction Kit



DNA Extraction steps

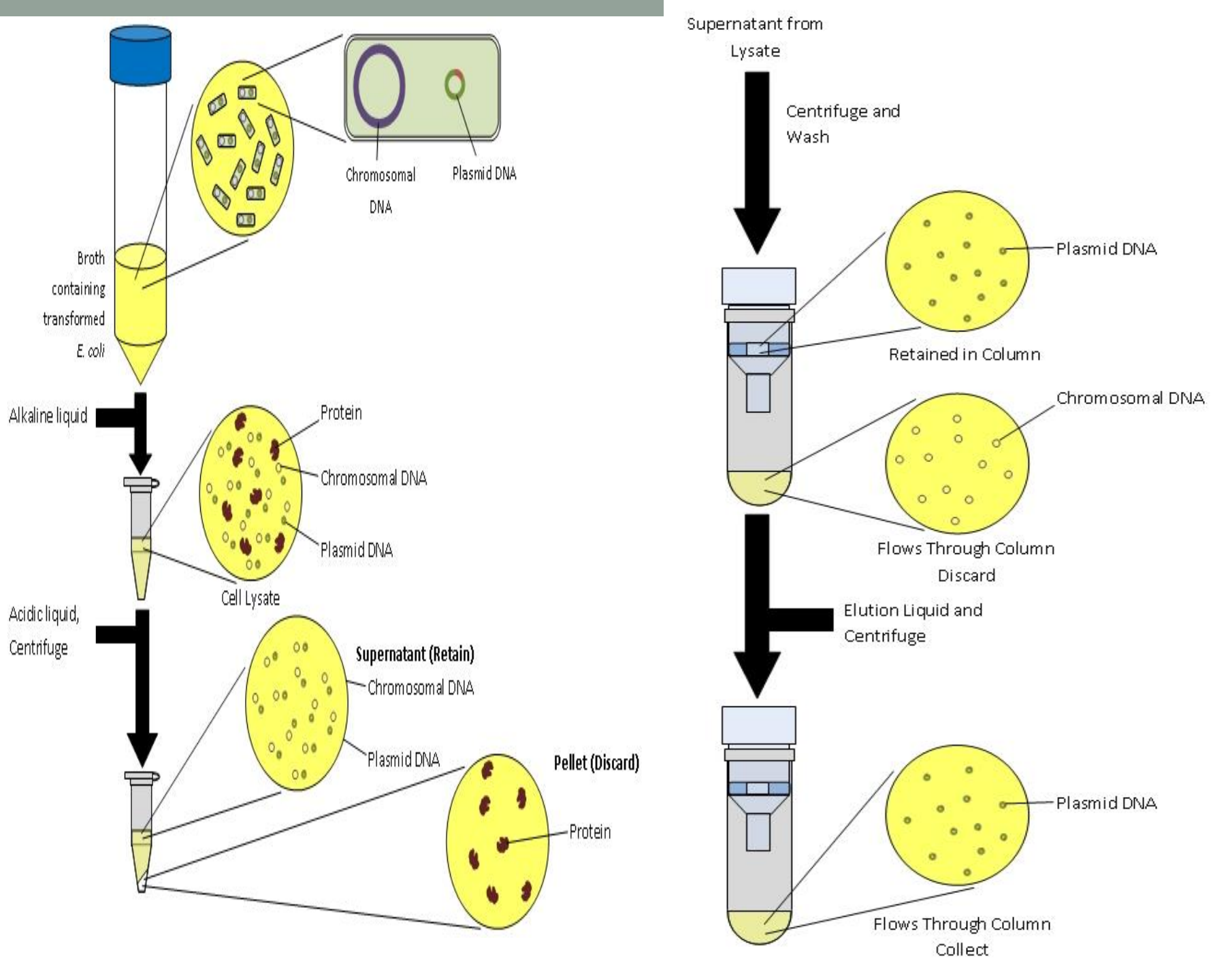


Exterachromosomal DNA

Extraction of the Exterachromosomal DNA

Extrachromosomal DNA is generally easy to isolate.

Plasmids may be easily isolated by cell lysis followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, plasmid DNA can be purified from soluble fraction.


Chromosomal DNA
Plasmid DNA
Broth containing transformed E. coli
Alkaline liquid
Protein
Chromosomal DNA
Plasmid DNA
Cell Lysate
Acidic liquid, Centrifuge
Supernatant (Retain)
Chromosomal DNA
Plasmid DNA
Pellet (Discard)
Protein
Supernatant from Lysate
Centrifuge and Wash
Plasmid DNA
Retained in Column
Chromosomal DNA
Flows Through Column Discard
Elution Liquid and Centrifuge
Plasmid DNA
Flows Through Column Collect

DNA Detecting

By using Spectrophotometer:

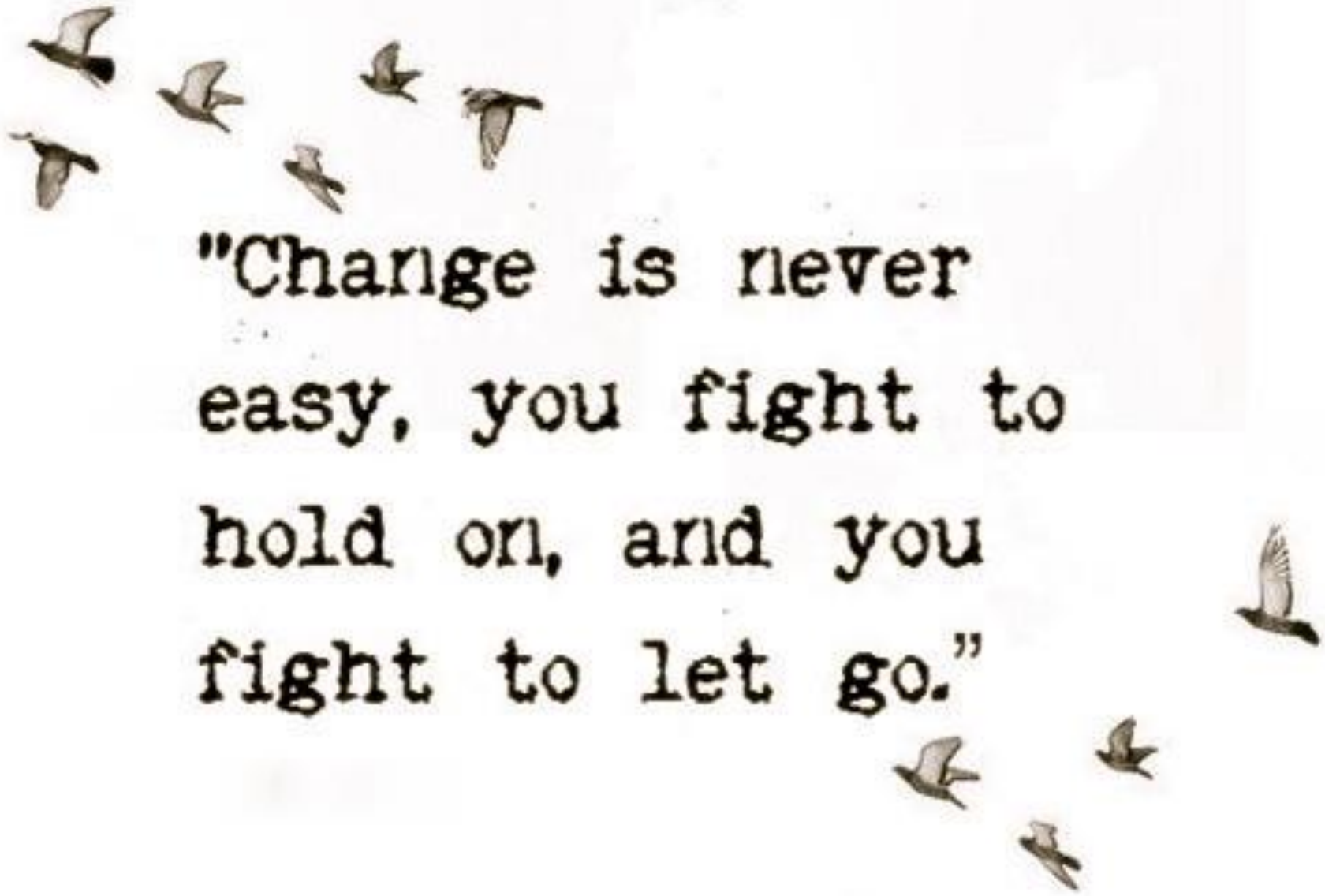
Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm is used as a measure of DNA purity.

DNA absorbs **UV** light at 260 and 280 nanometres, and aromatic proteins absorb UV light at 280 nm; a pure sample of DNA has a ratio of 1.8 at 260/280 and is relatively free from protein contamination.

DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8.

Gel Electrophoresis:

Running it on an agarose gel, staining with ethidium bromide and comparing the intensity of the DNA with a DNA marker of known concentration.


"Change is never
easy, you fight to
hold on, and you
fight to let go."

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