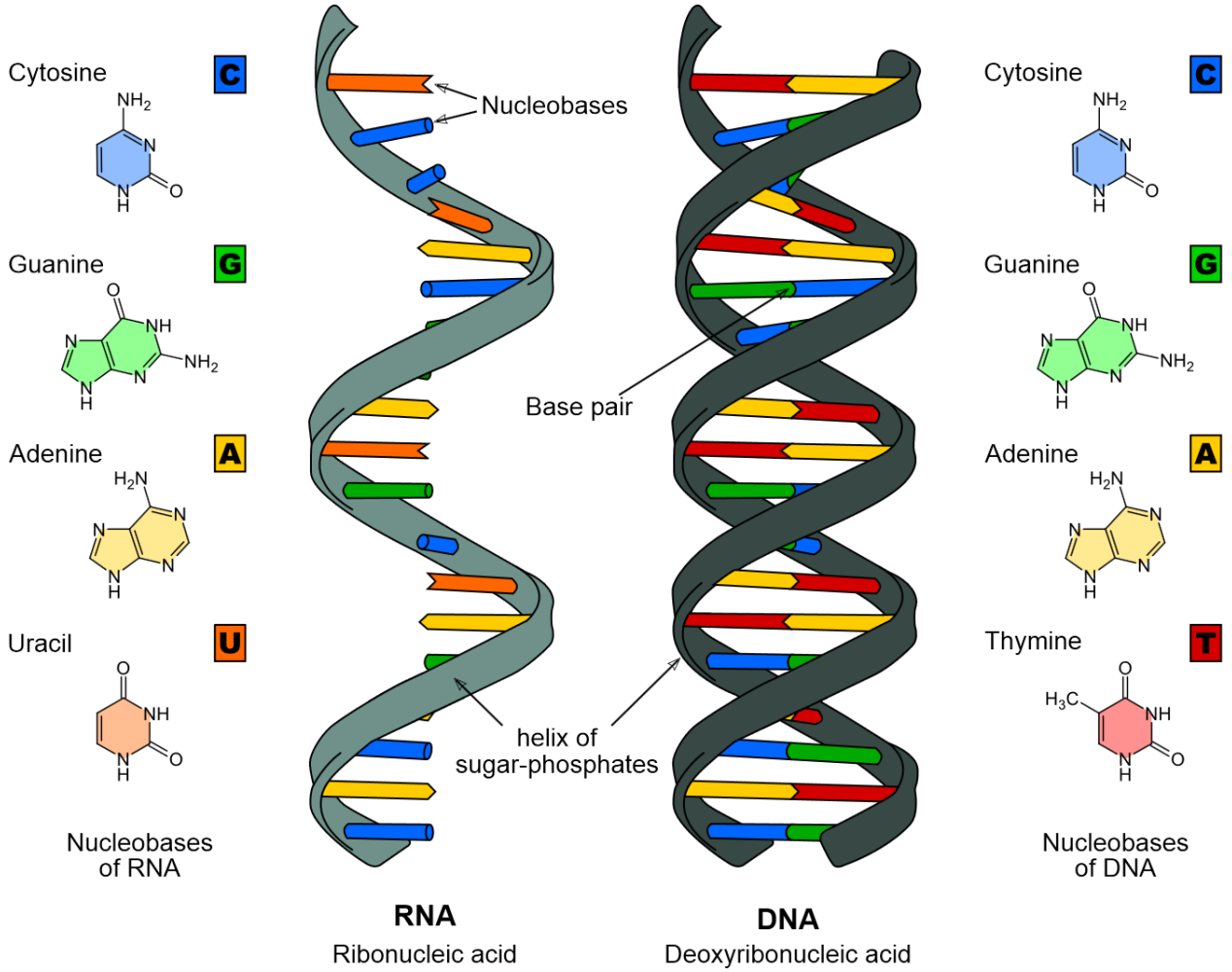
DNA-RNA EXTRACTION

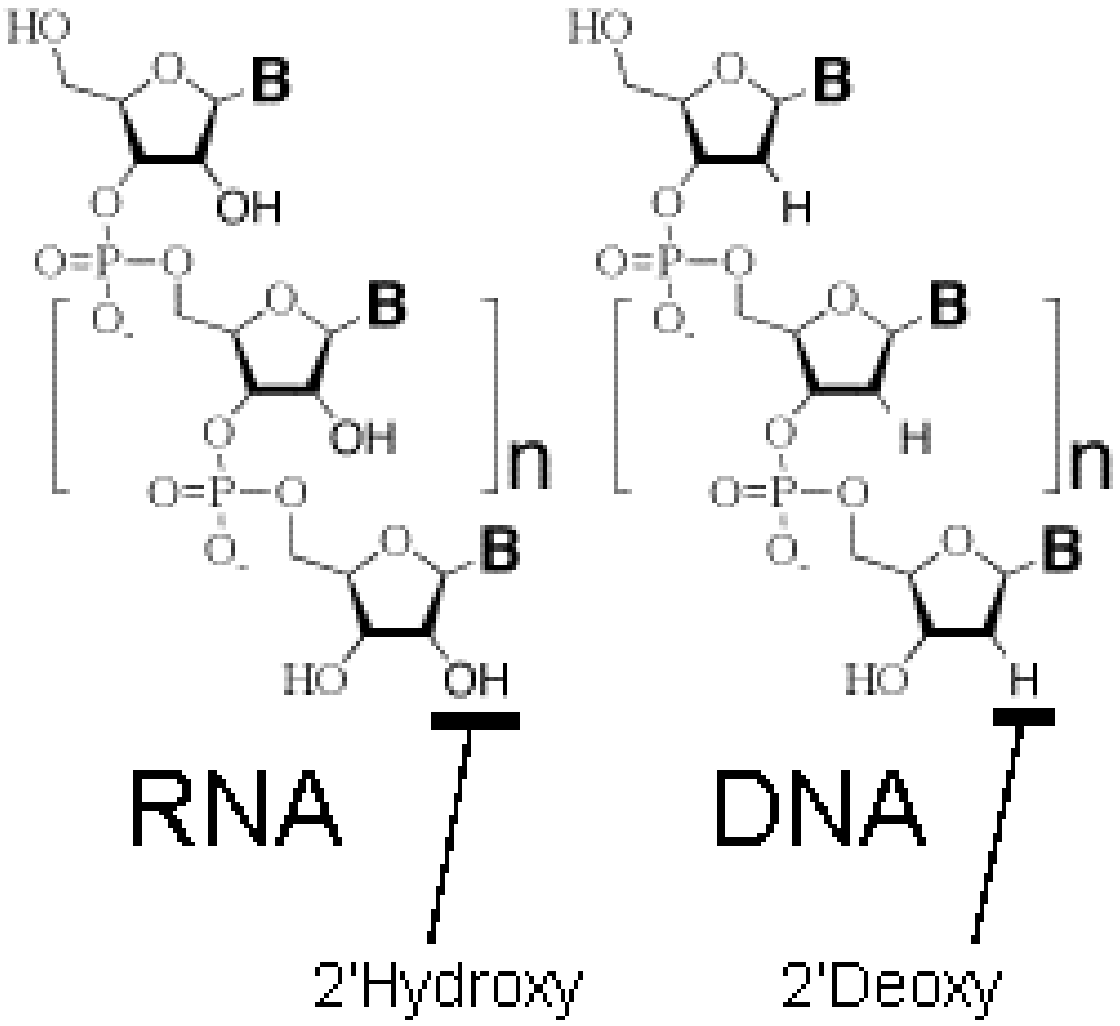
Dr. Amira A. T. AL-Hosary

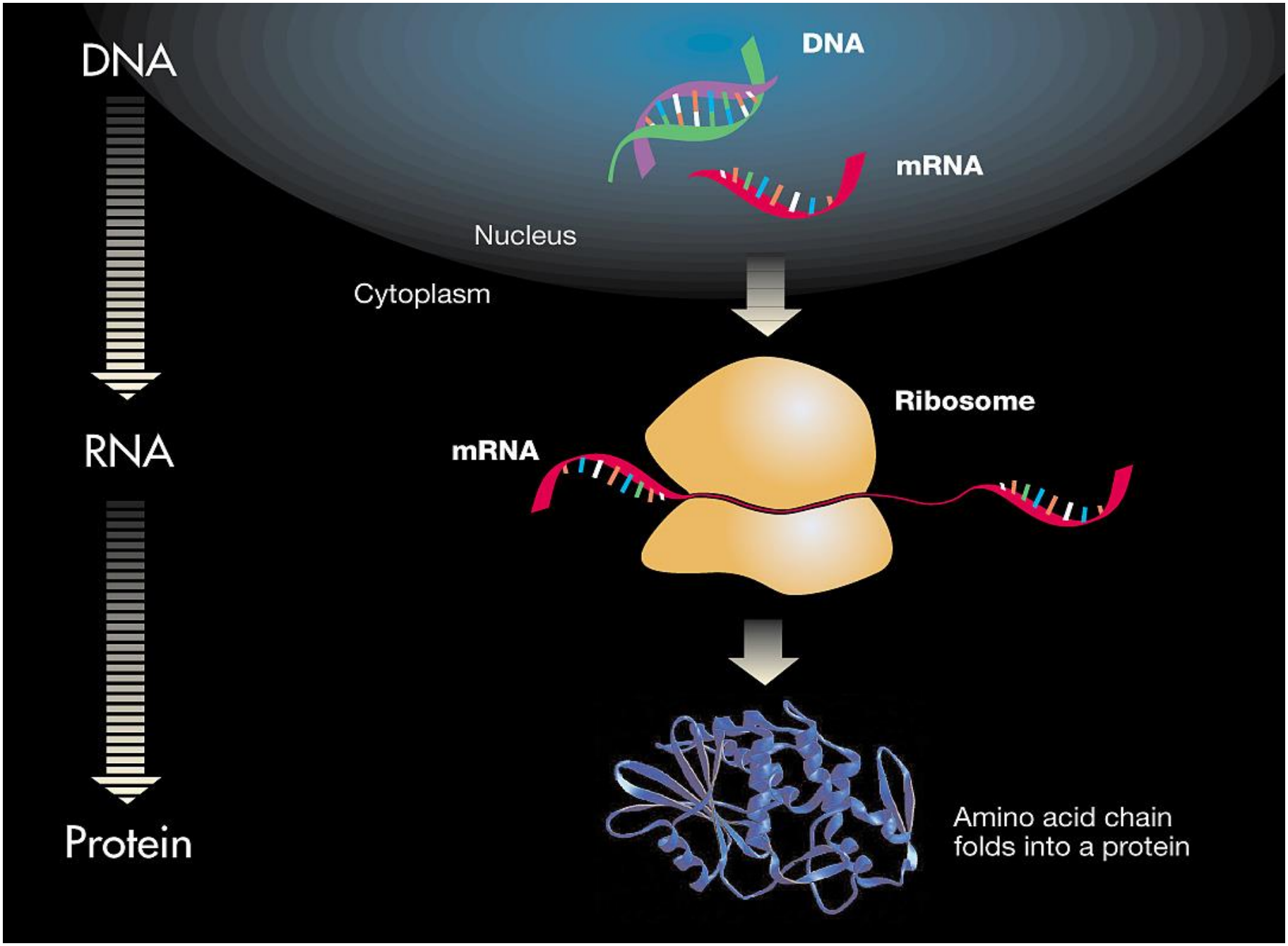
Lecturer of infectious diseases, Faculty of Veterinary Medicine, Assiut University, Egypt

Nucleic Acids (DNA & RNA)



DNA and RNA Breaks (Nucleotides)




DNA
RNA
Protein
DNA
mRNA
Nucleus
Cytoplasm
Ribosome
mRNA
Amino acid chain
folds into a protein

I. **DNA (chromosomal and Extra-chromosomal (plasmid)).**

II. **Cellular "total" RNA**

Messenger RNA (mRNA): 1-5%, *Serves as a template for protein synthesis*

Ribosomal RNA (rRNA): >80%, *Structural component of ribosomes*

Transfer RNA (tRNA): 10-15%, *Translates mRNA information into the appropriate amino acid*

Definition

DNA Extraction = DNA isolation

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

The first isolation of DNA was done in 1869 by Friedrich Miescher.

Miescher isolated various phosphate-rich chemicals, he called it nuclein (now nucleic acids), from the nuclei of white blood cells in 1869 in Felix Hoppe-Seyler's laboratory at the University of Tübingen, Germany.

The significance of the discovery, first published in 1871, was not at first apparent, and it was Albrecht Kossel who made the initial inquiries into its chemical structure.

Later, Friedrich Miescher raised the idea that the nucleic acids could be involved in heredity.

Types of samples

Samples

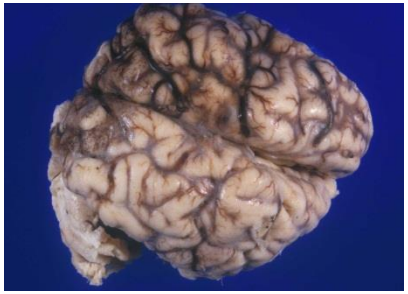
Blood (citrate, EDTA or heparin)

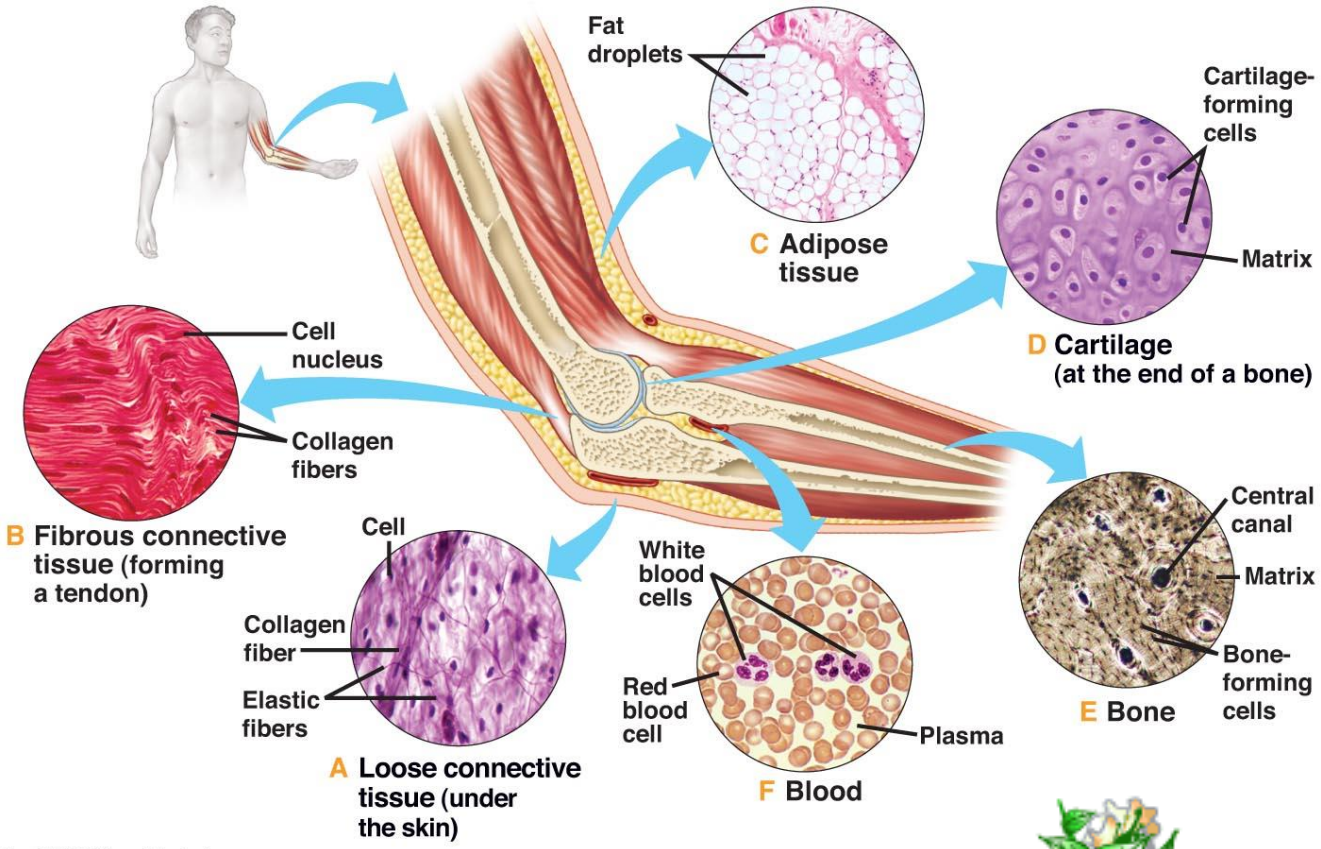
Blood spotted on filter paper

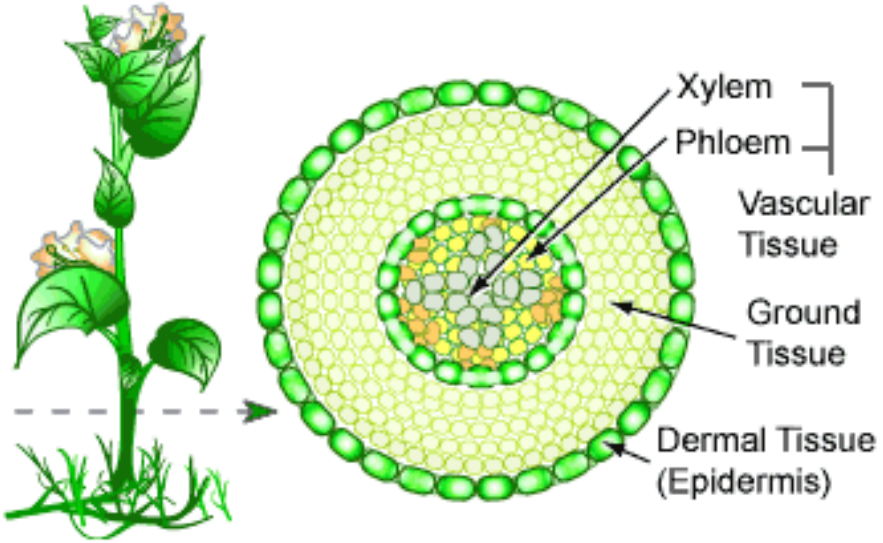
Insects

Plant

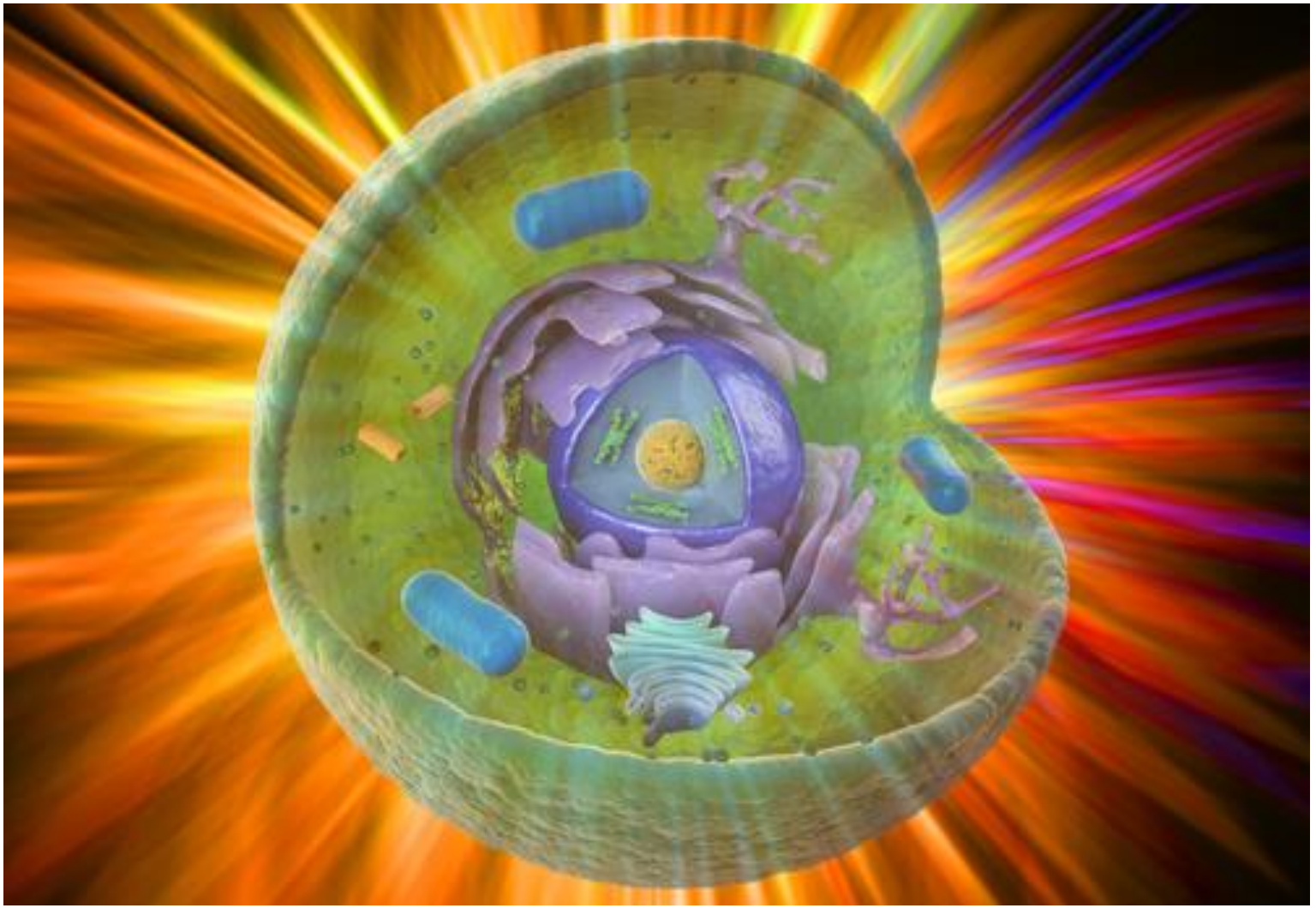
Tissue







DNA Extraction



Procedure of DNA extraction
Step I

Cell Lysis

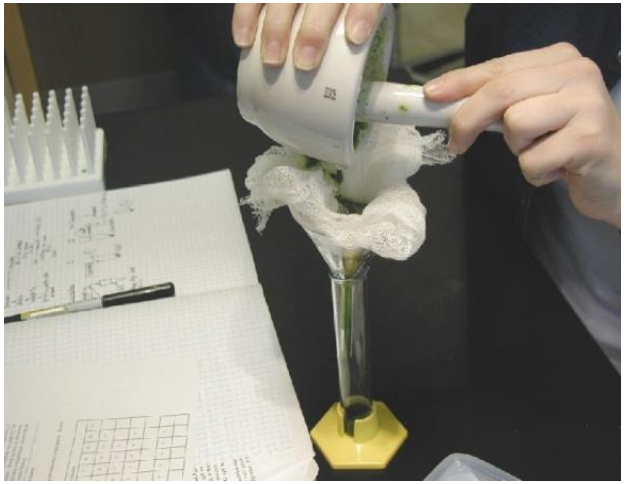
Breaking the cell to expose the **DNA**.

This is commonly achieved by:

1- chemical method.

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

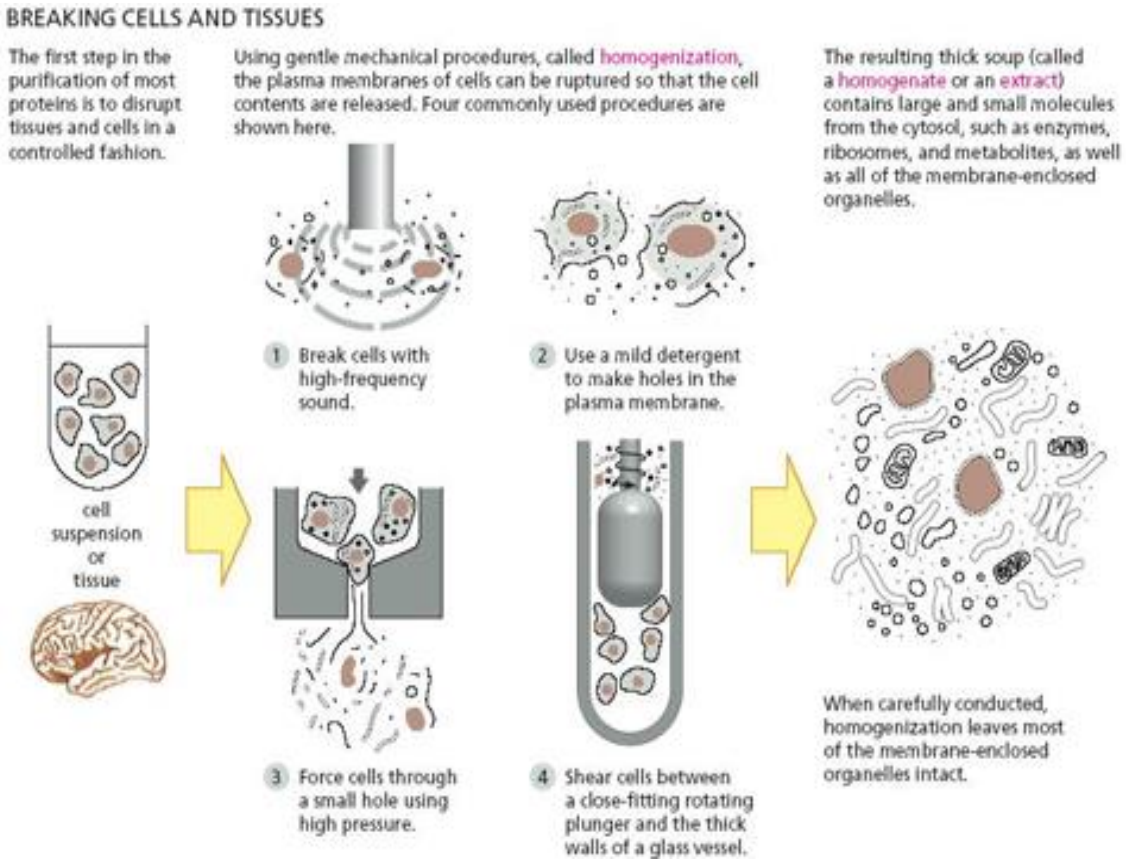
Grinding of the samples

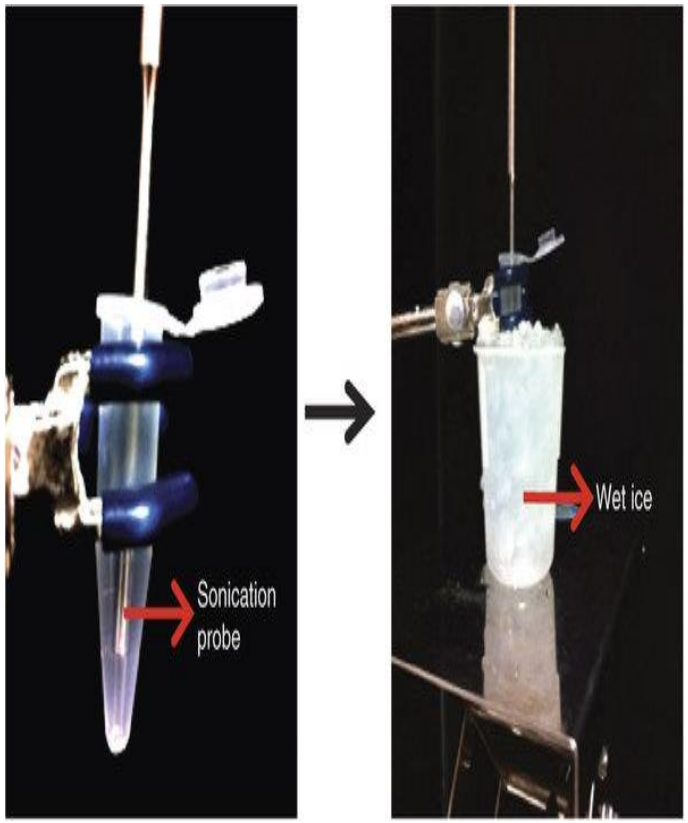


Blending of the samples

Blending of the samples with Silica beads

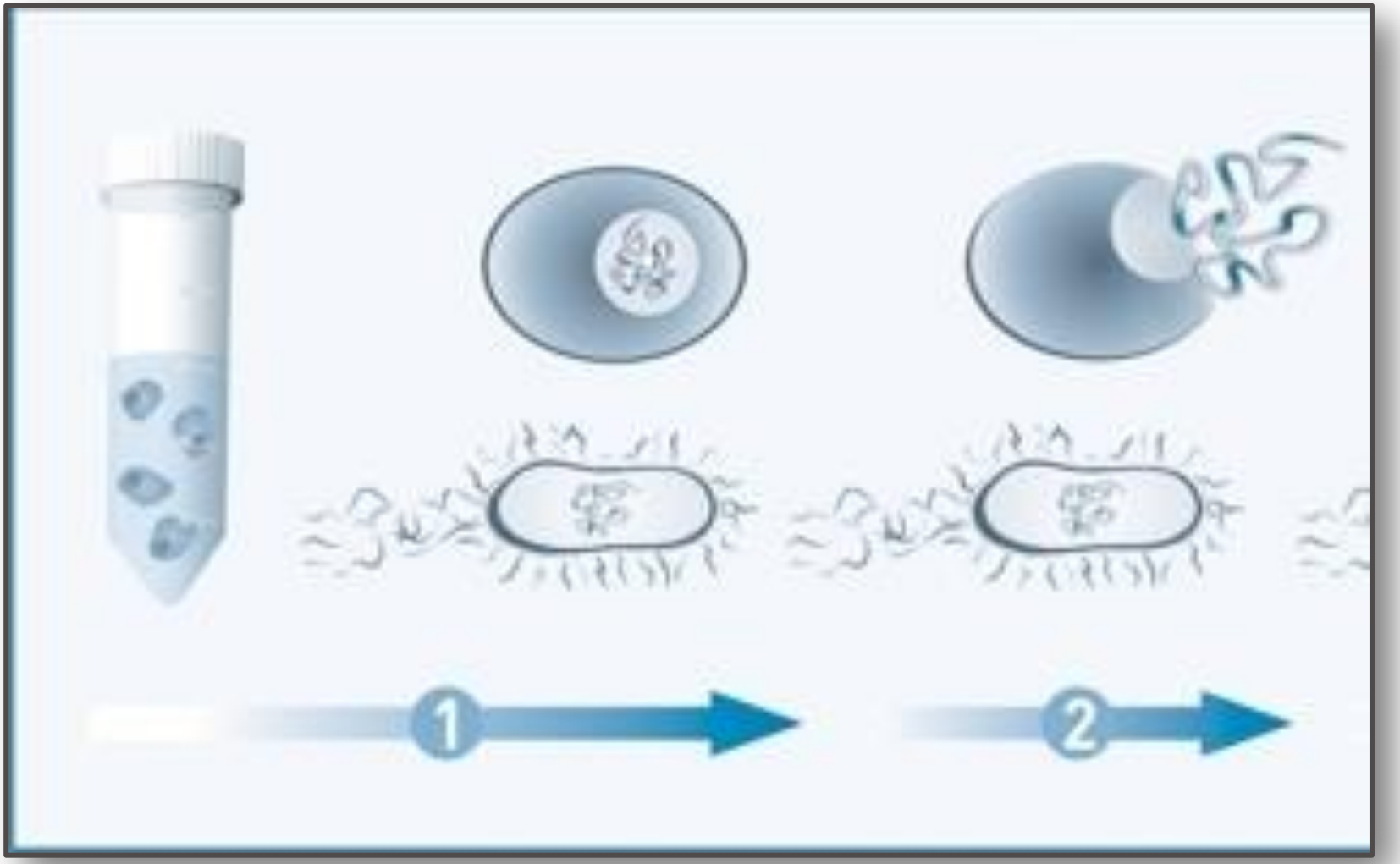
Sonication of the sample





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.

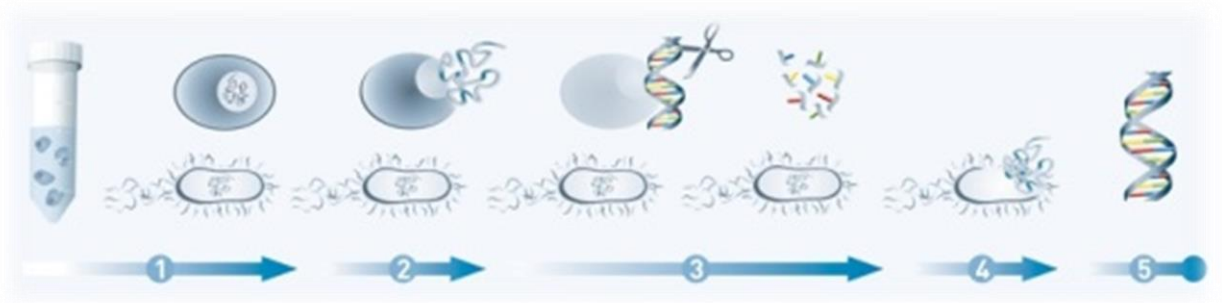
After centrifugation of the sample denatured proteins stay in organic phase while aqueous phase containing nucleic acid is mixed with the chloroform that removes phenol residues from solution.

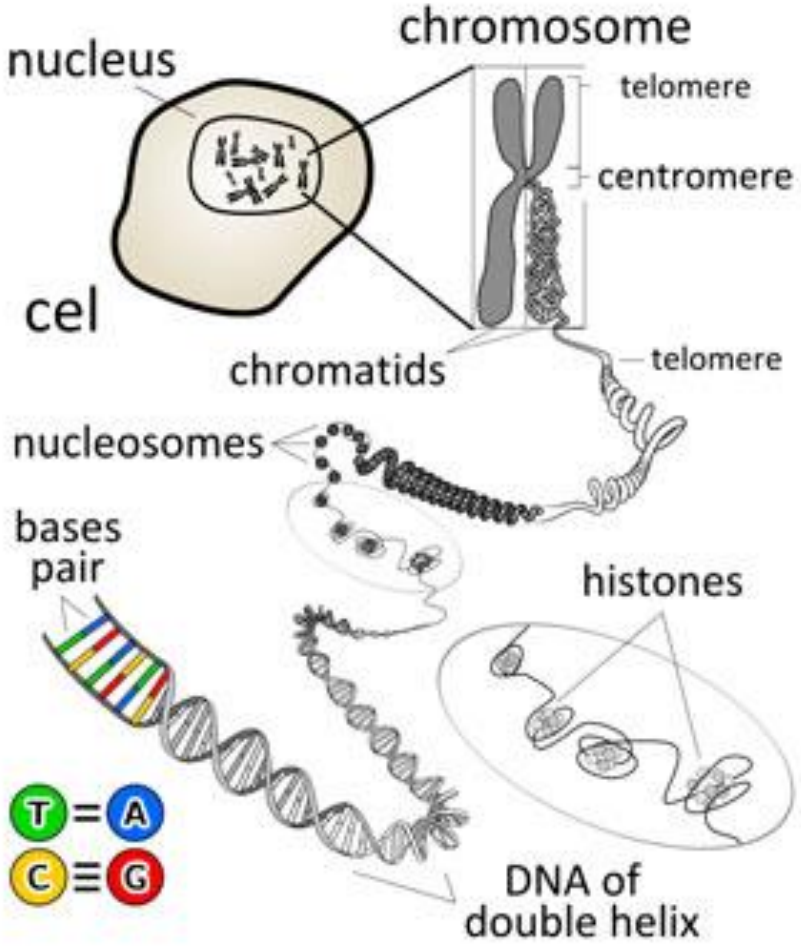
Mg^{2+} and Ca^{2+}, which prevents enzymes like Dnase from degrading the DNA.

Procedure of DNA extraction
Step IIII

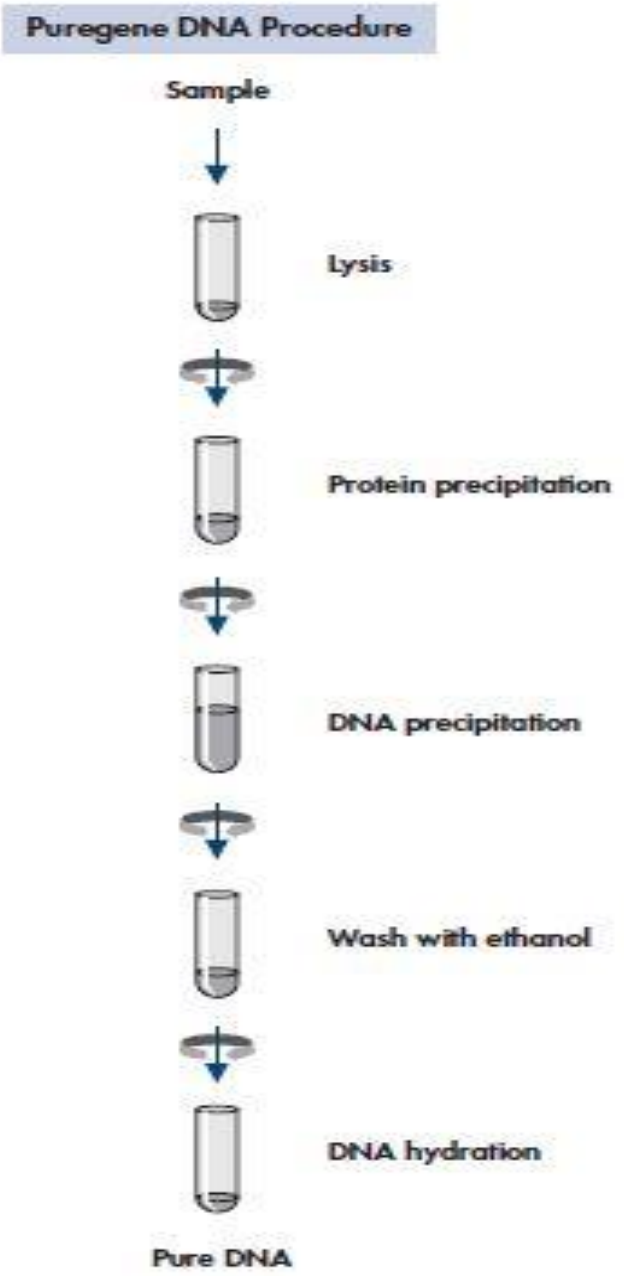
DNA Hydration:

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

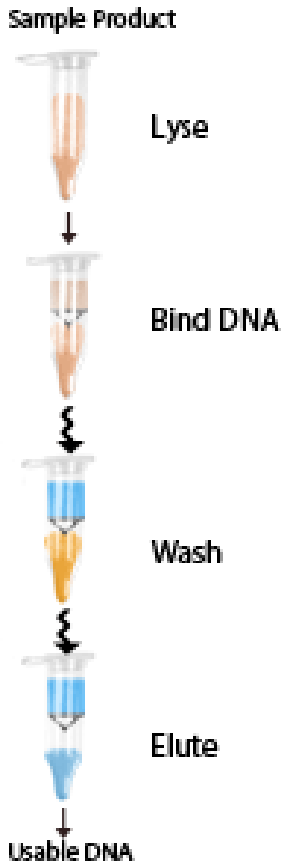

1
2
3
4
5


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

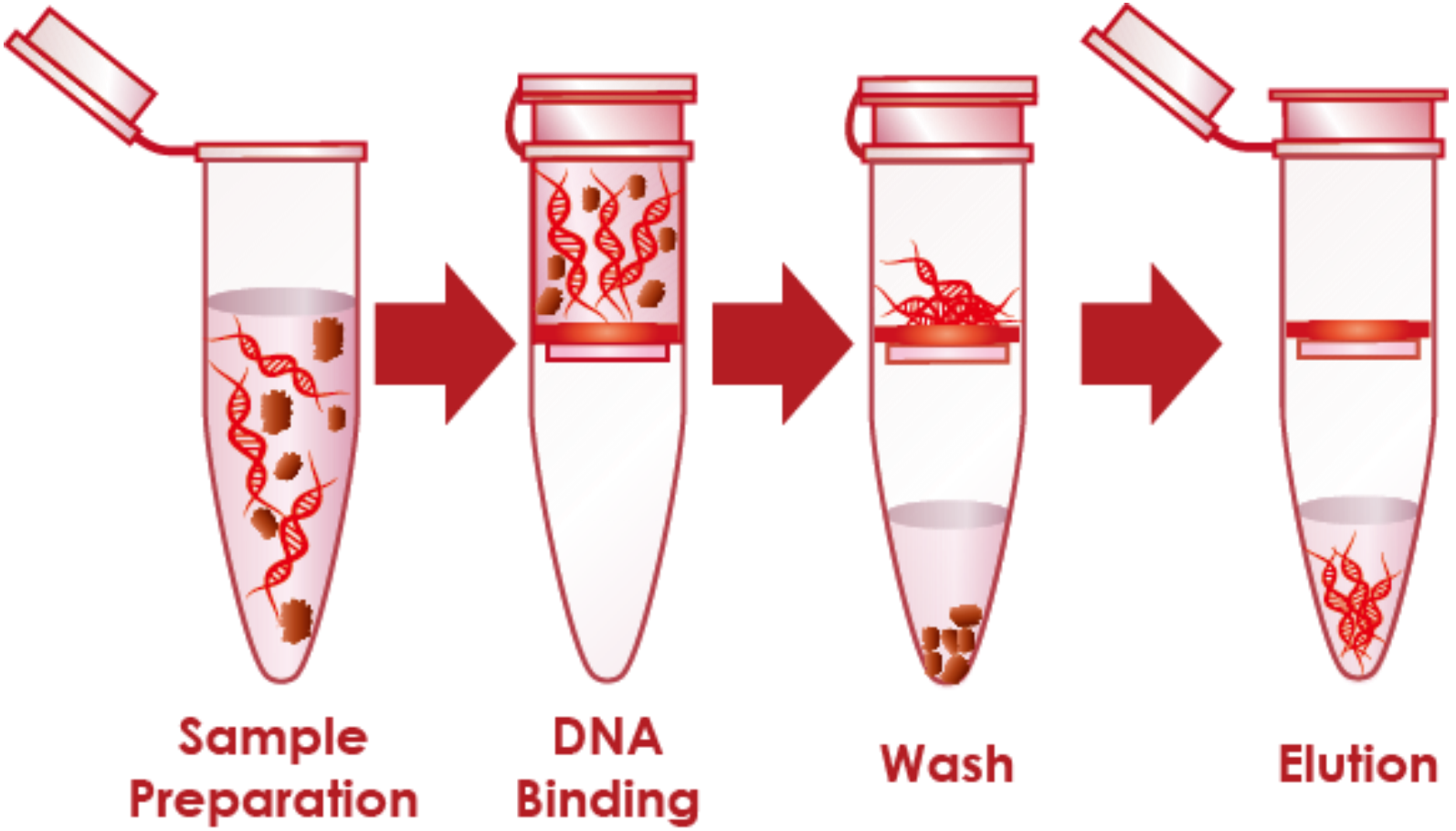
DNA Extraction



DNA Extraction Kit



DNA Extraction steps

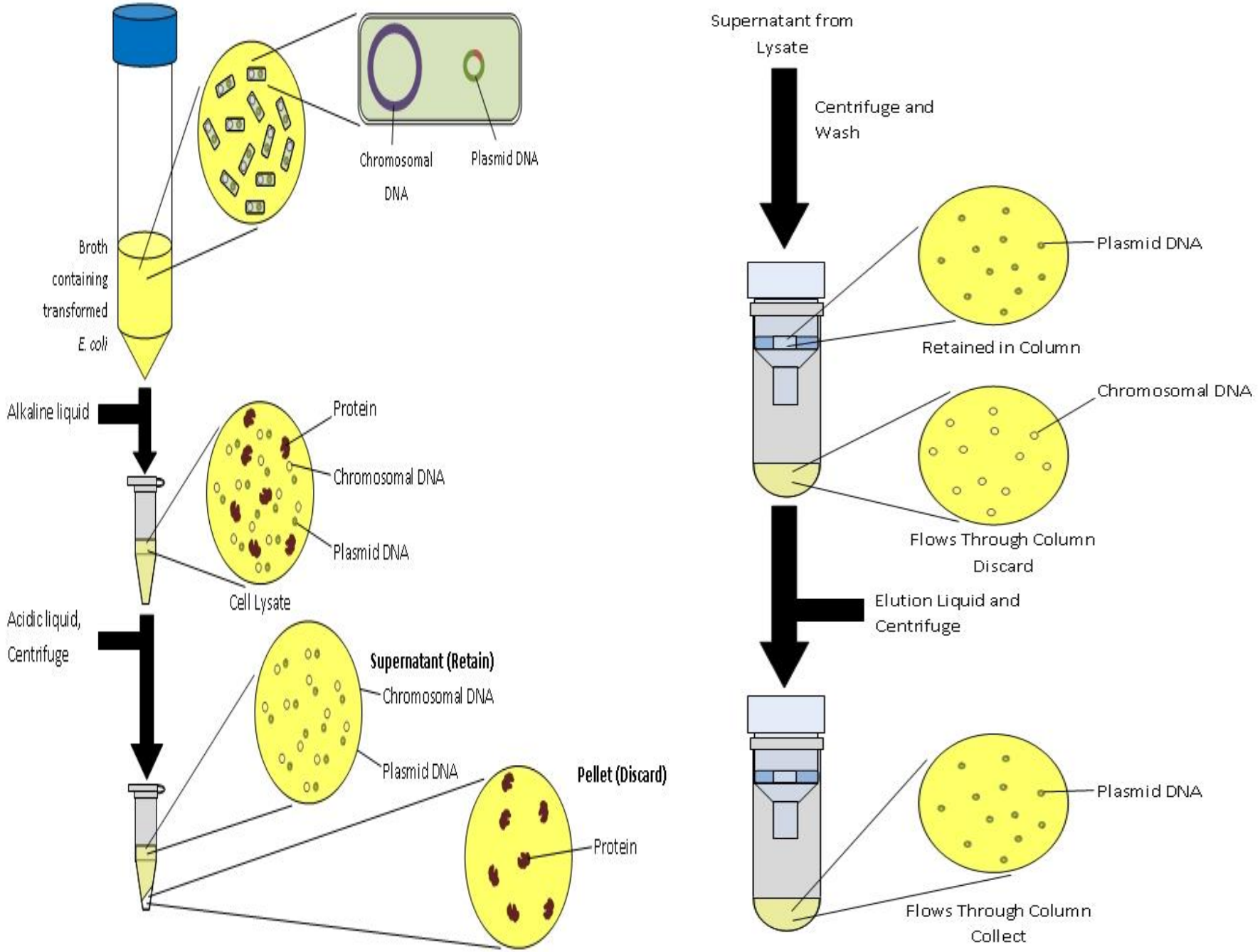


Extera-chromosomal DNA

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.

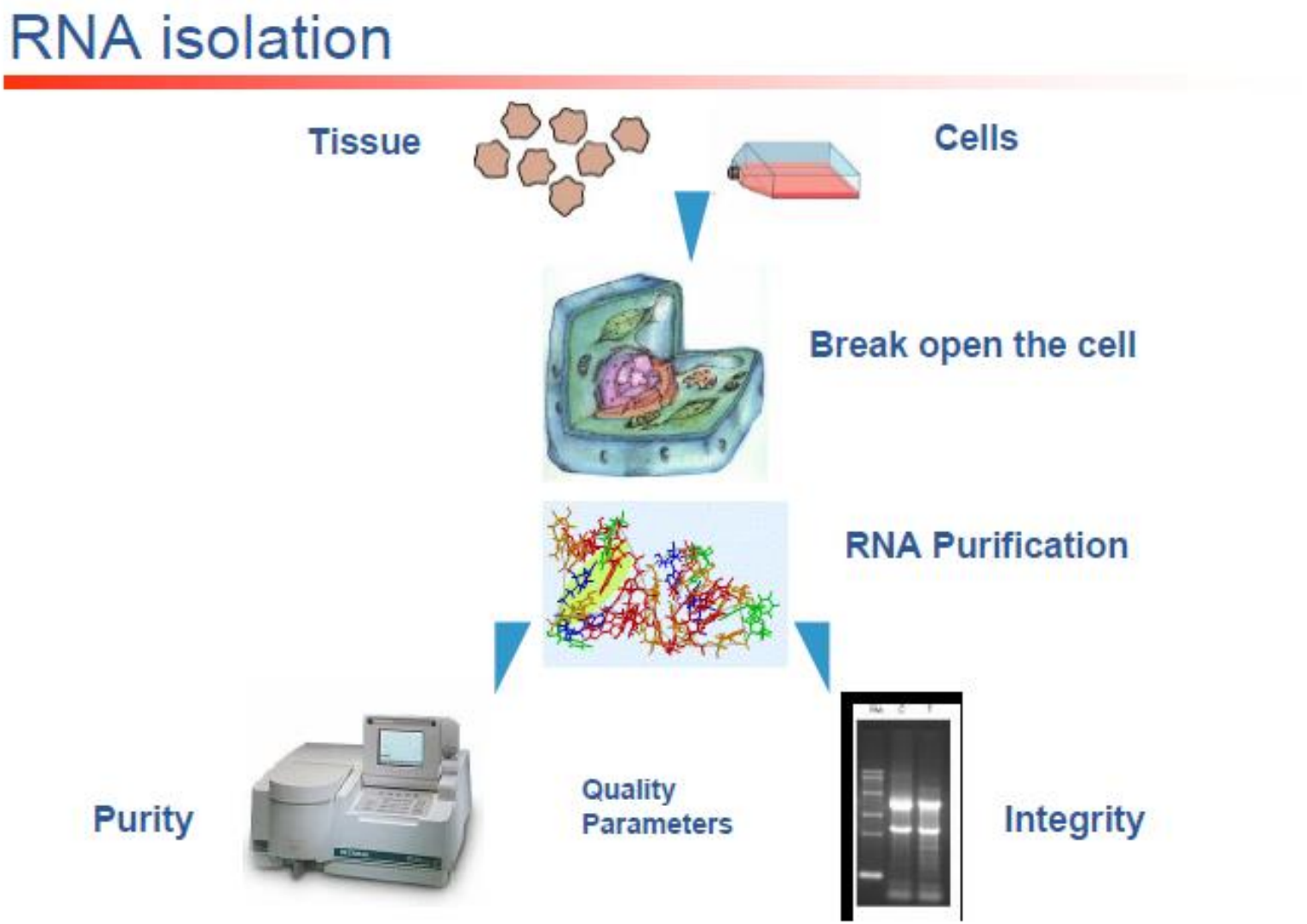
DNA absorbs **UV** light at 260 and 280 nano-metres, and aromatic proteins absorb UV light at 280 nm; a pure sample of DNA has a ratio of 1.8 at 260/280 and is relatively free from protein contamination.

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.

RNA Extraction:



RNA Extraction:

RNA extraction is the purification of RNA from biological samples.

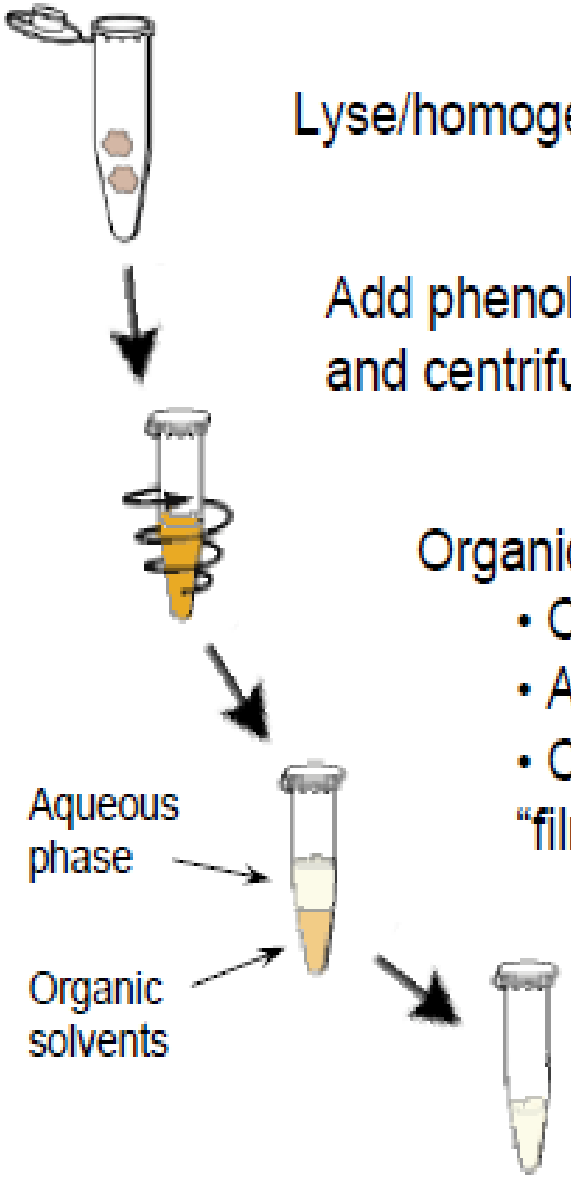
Ribonuclease enzymes in cells and tissues is the main problem because It can rapidly degrade RNA.

Several methods are used to isolate RNA from samples, the most common of these is Guanidinium thiocyanate-phenol-chloroform extraction.

A phenol-chloroform extraction is a liquid-liquid extraction.

A liquid-liquid extraction is a method that separates mixtures of molecules based on the differential solubility.

Organic Extraction of total RNA



Lyse/homogenize cells

Add phenol:chloroform:isoamyl alcohol to lysed sample, and centrifuge

Organic phase separates from aqueous phase

- Organic solvents on bottom
- Aqueous phase on top (contains total RNA)
- Cellular debris and genomic DNA appears as a "film" of debris at the interface of the two solutions

Remove RNA solution to a clean tube; precipitate RNA and wash with ethanol, then resuspend RNA in water

Method:

The extraction of nucleic acids involves adding an equal volume of phenol-chloroform to an aqueous solution of lysed cells or homogenized tissue, mixing the two phases, and allowing the phases to separate by centrifugation.

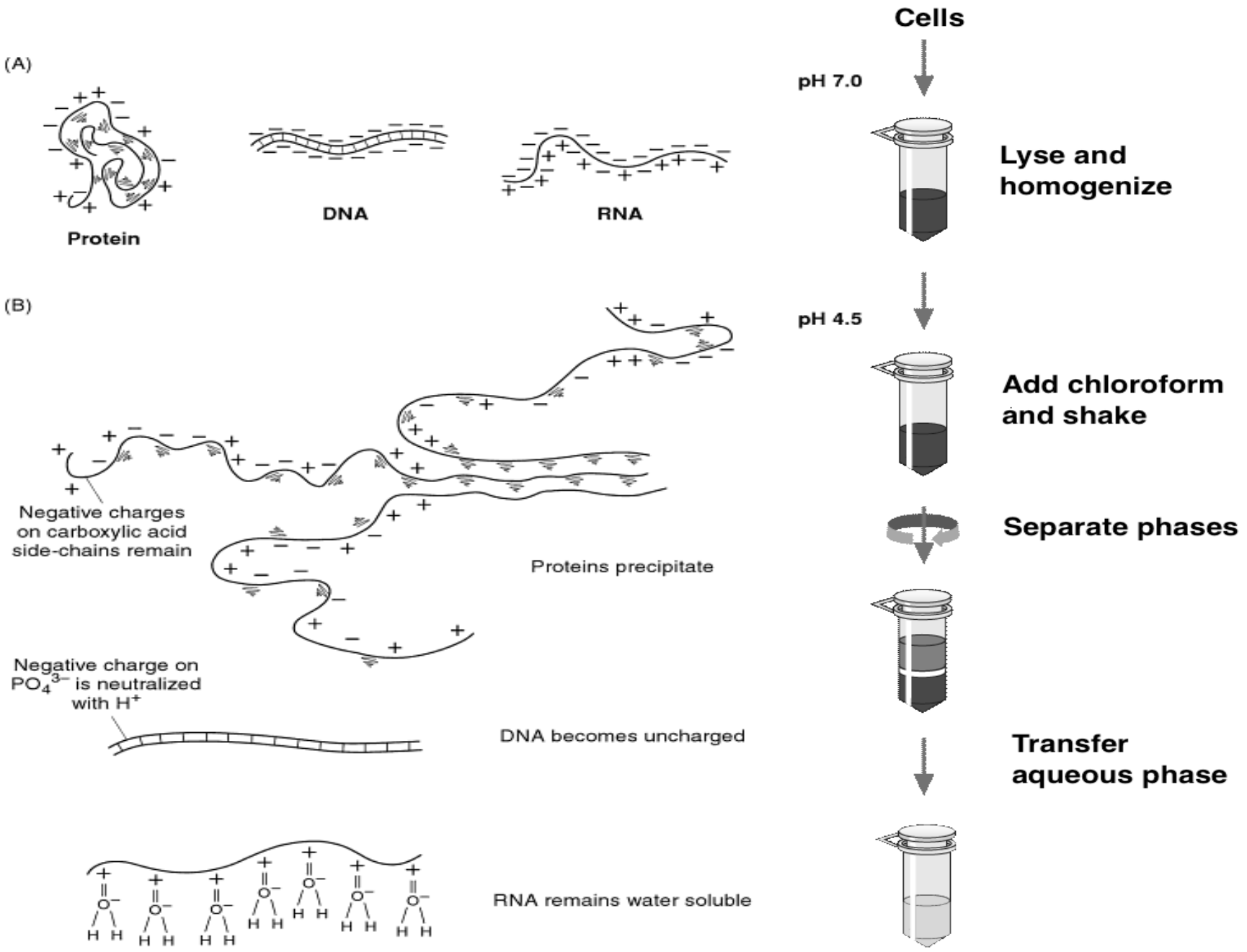
Centrifugation of the mixture yields two phases: the lower organic phase and the upper aqueous phase. Chloroform mixed with phenol is more efficient at denaturing proteins than either reagent is alone.

The phenol-chloroform combination reduces the partitioning of poly(A)+ mRNA into the organic phase and reduces the formation of insoluble RNA-protein complexes at the interphase

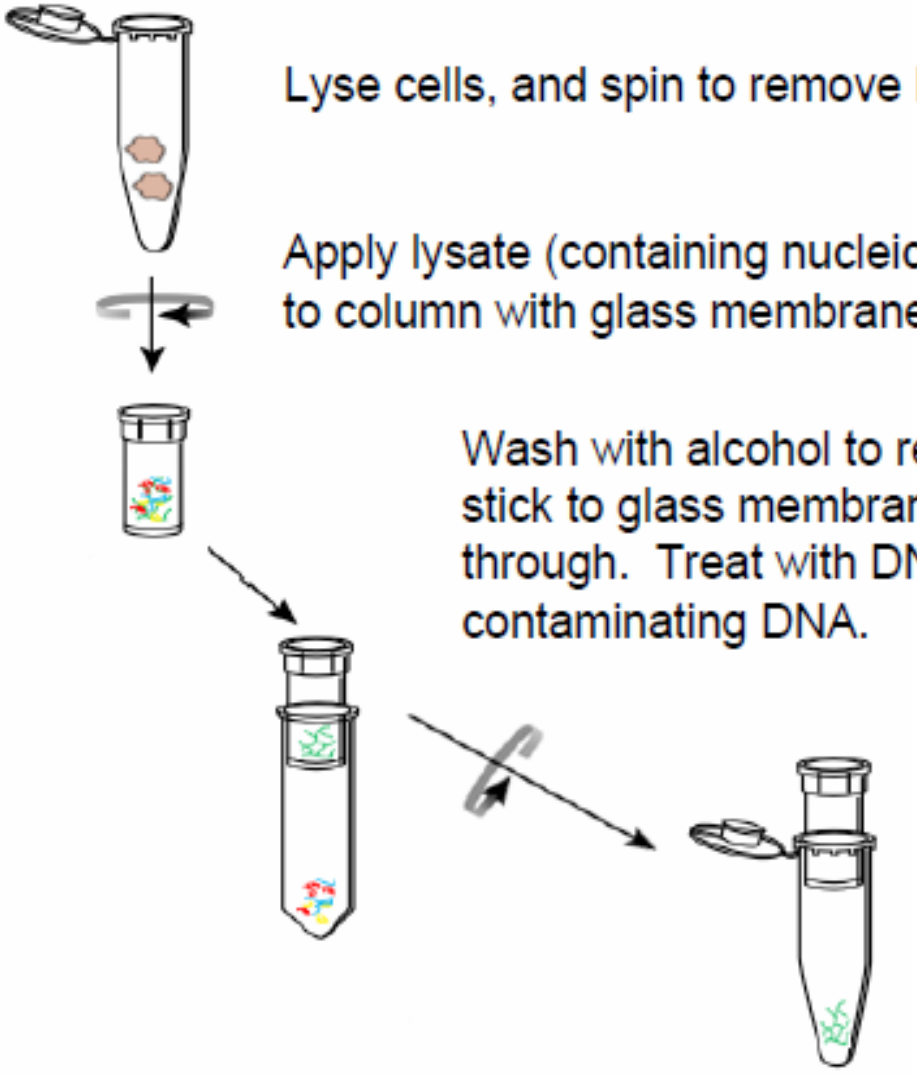
The pH of phenol determines the partitioning of DNA and RNA between the organic phase and the aqueous phase.

At neutral or slightly alkaline pH (pH 7-8), the phosphate diesters in nucleic acids are negatively charged, and thus DNA and RNA both partition into the aqueous phase.

DNA is removed from the aqueous layer with increasing efficiency as the pH is lowered with a maximum efficiency at pH 4.8. At this acidic pH, most proteins and small DNA fragments remain in the organic phase while large DNA and small protein remain at the interphase between organic and inorganic phases.


Cells
(A)
pH 7.0
Protein
DNA
RNA
Lyse and homogenize
(B)
pH 4.5
Add chloroform and shake
Negative charges on carboxylic acid side-chains remain
Separate phases
Proteins precipitate
Negative charge on PO_4^{3-} is neutralized with H^+
DNA becomes uncharged
Transfer aqueous phase
RNA remains water soluble

Affinity purification of total RNA



Lyse cells, and spin to remove large particulates/cell debris

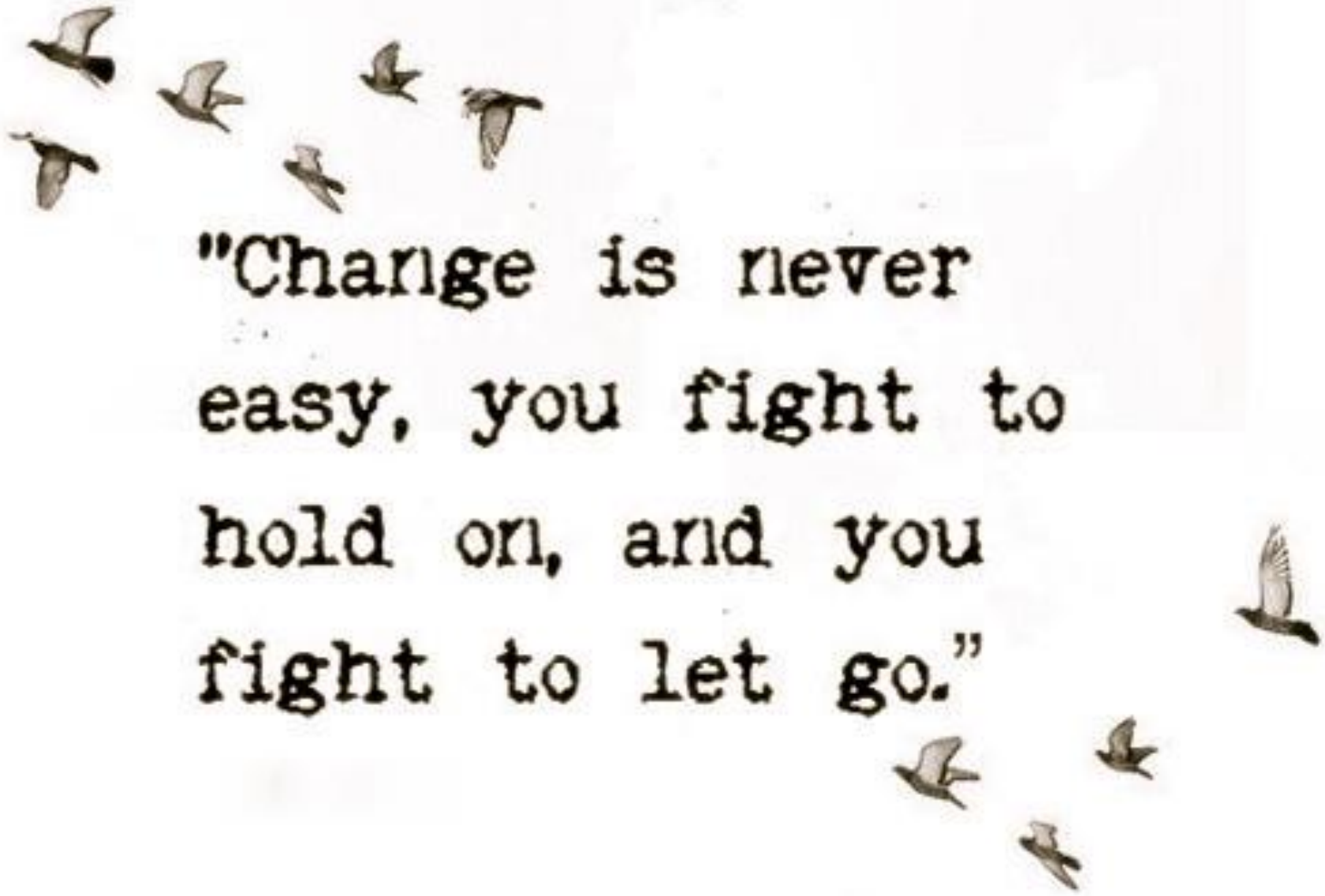
Apply lysate (containing nucleic acids and cellular contaminants) to column with glass membrane

Wash with alcohol to remove contaminants; nucleic acids stick to glass membrane while contaminants wash through. Treat with DNase enzyme to remove contaminating DNA.

Apply water to the column; purified RNA washes off the glass and is collected

Percussion for success in RNA extraction:

1. **Equipment used must be cleane.**
2. **Kept in separate from common lab equipment and treated with chemicals that destroy RNases.**
3. **For the same reason, experimenters take special care not to let their bare skin touch the equipment.**
4. **RNA extraction in liquid nitrogen: commonly using a mortar and pestle (or specialized steel devices known as tissue pulverizers) is also useful in preventing ribonuclease activity.**


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

Amira A L-Hosary
(00201004477501)
amiraelhosary@yahoo.com