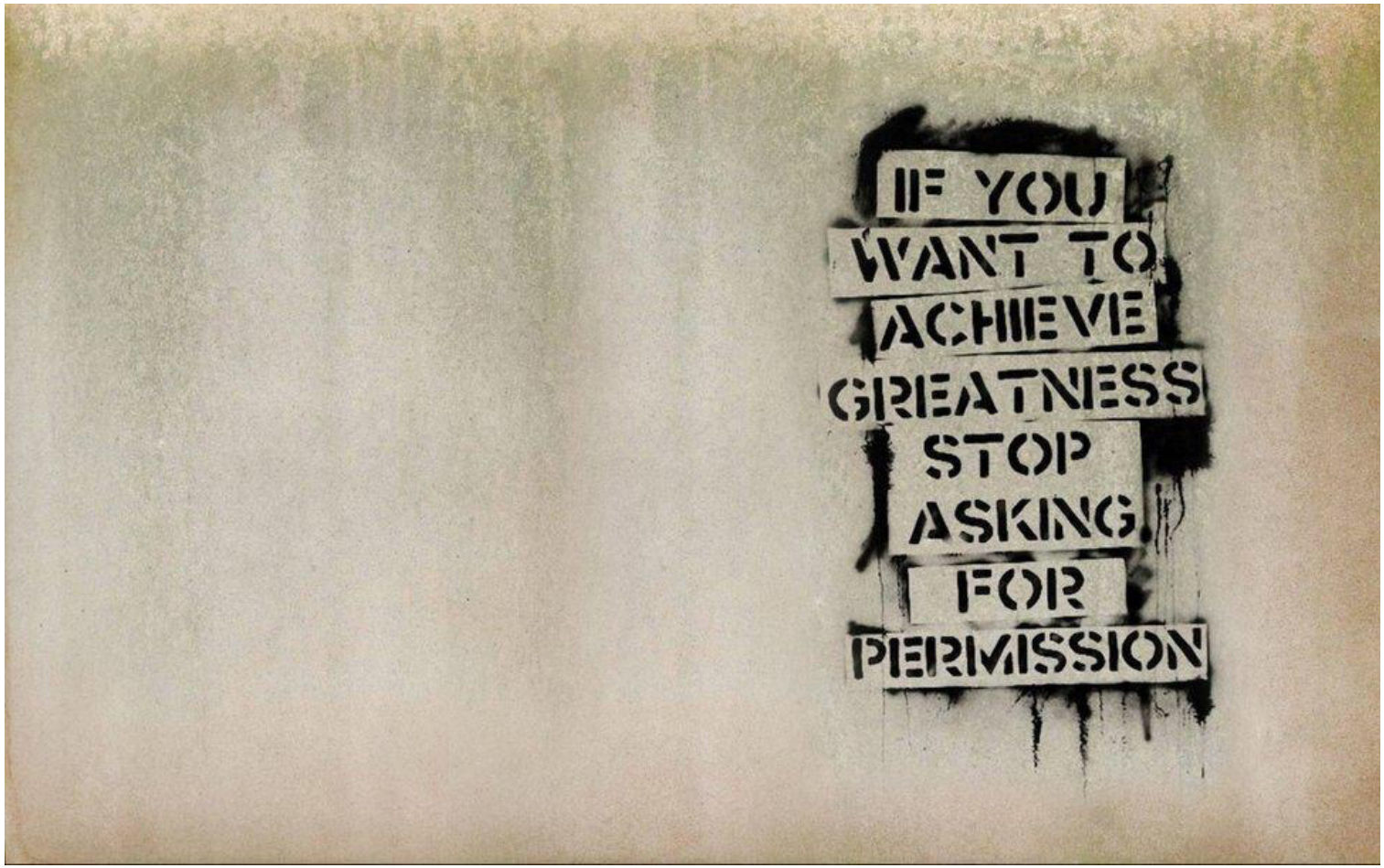
Molecular techniques

I. DNA extraction

Course 281

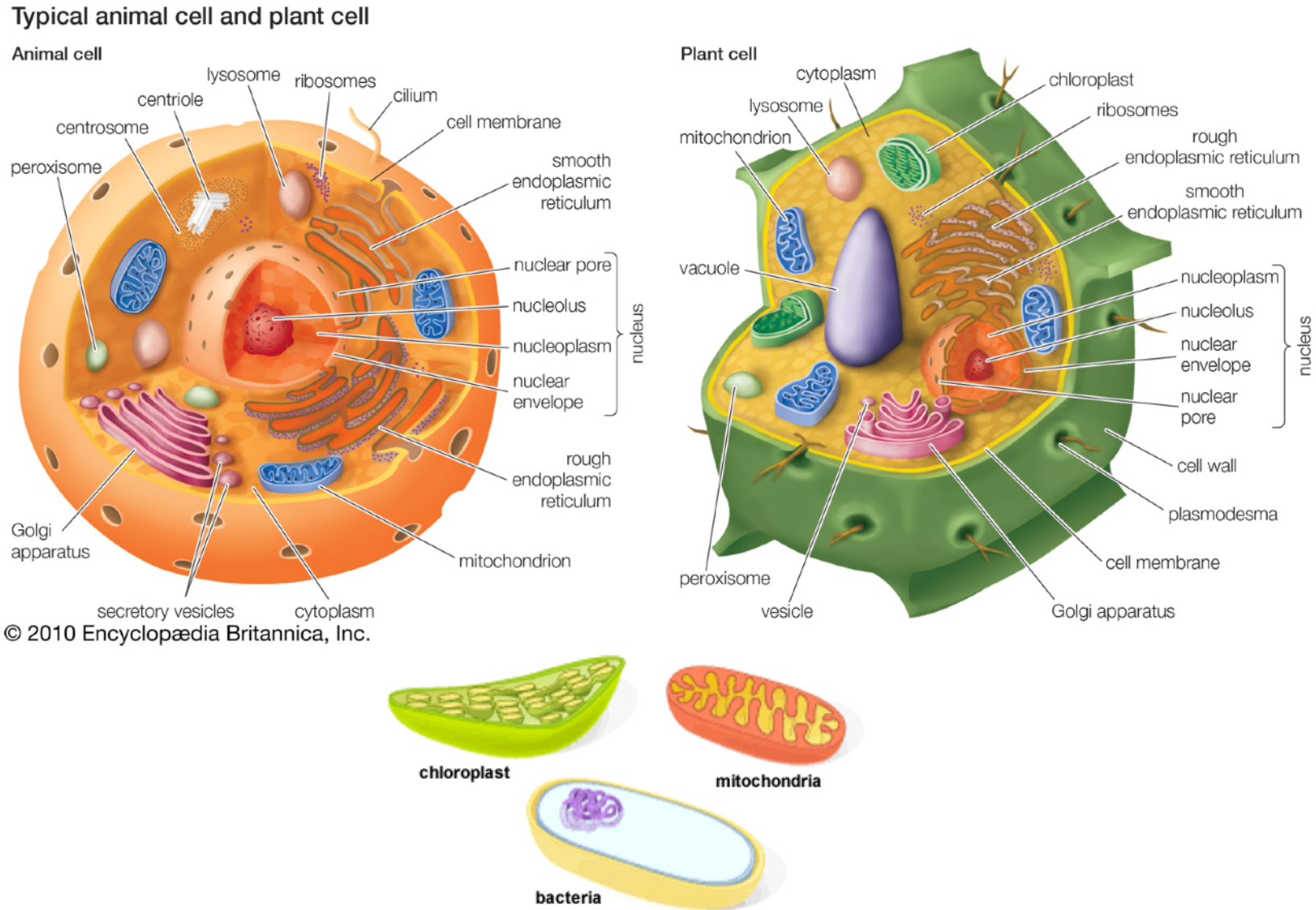
Lessons for life



AIMS

- Understand the sources of DNA in different organisms.
- Understand the cellular structure that needs to be disrupted to extract DNA.
- Understand the process of DNA isolation and the different methods employed.
- Understand the reasons for using specific chemicals in DNA extraction and what each does.

Cell Structure



Cells and DNA

Where Do we get the DNA from?

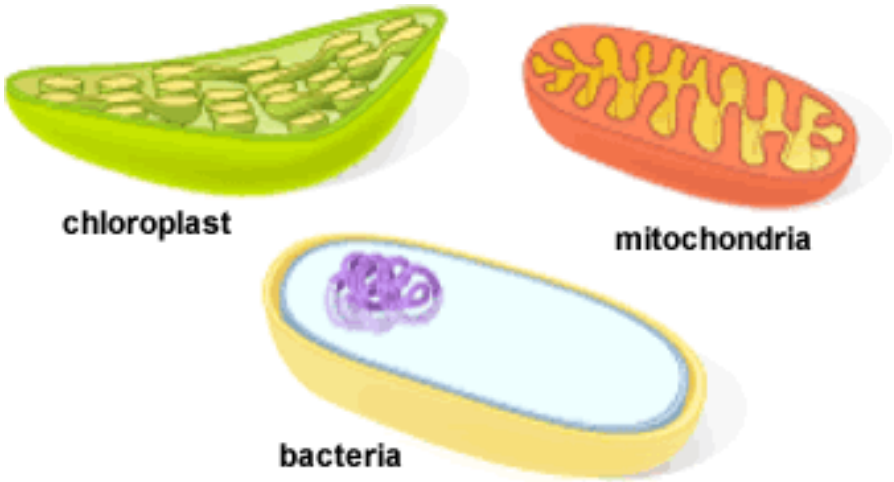
- In an animal cell, DNA is found in the **nucleus** and **mitochondria**.

- In a plant cell, DNA is found in the **nucleus**, **mitochondria**, and **chloroplast**.

- In bacteria, DNA is found in **plasmids** and bacterial **chromosome**.

Cells and DNA

- The mitochondria and chloroplast resembles a bacteria in genome structure.
- Knowing the DNA source you are targeting is essential.



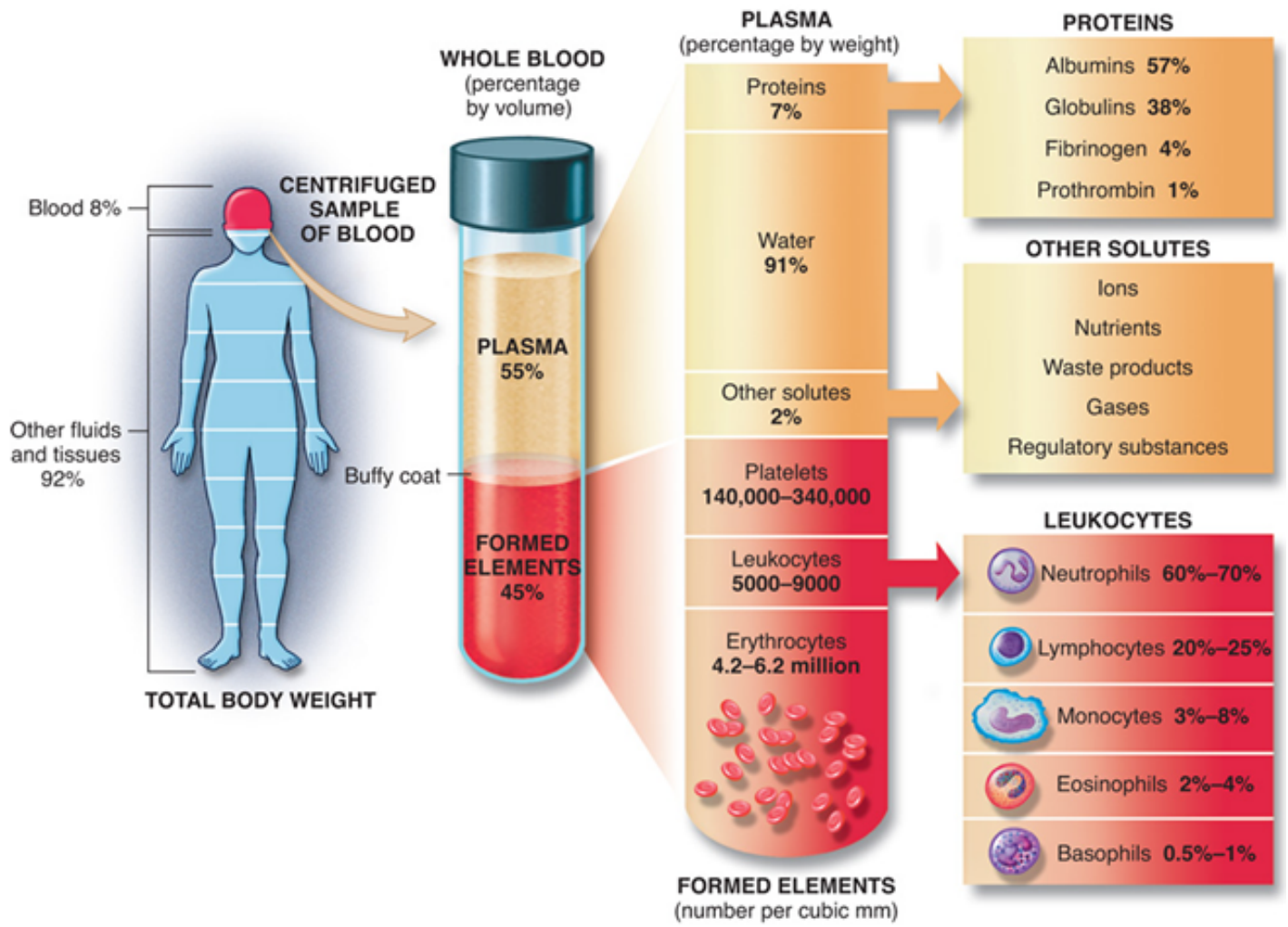
Cells and DNA

In humans, where can we get DNA from?

Cell Structure

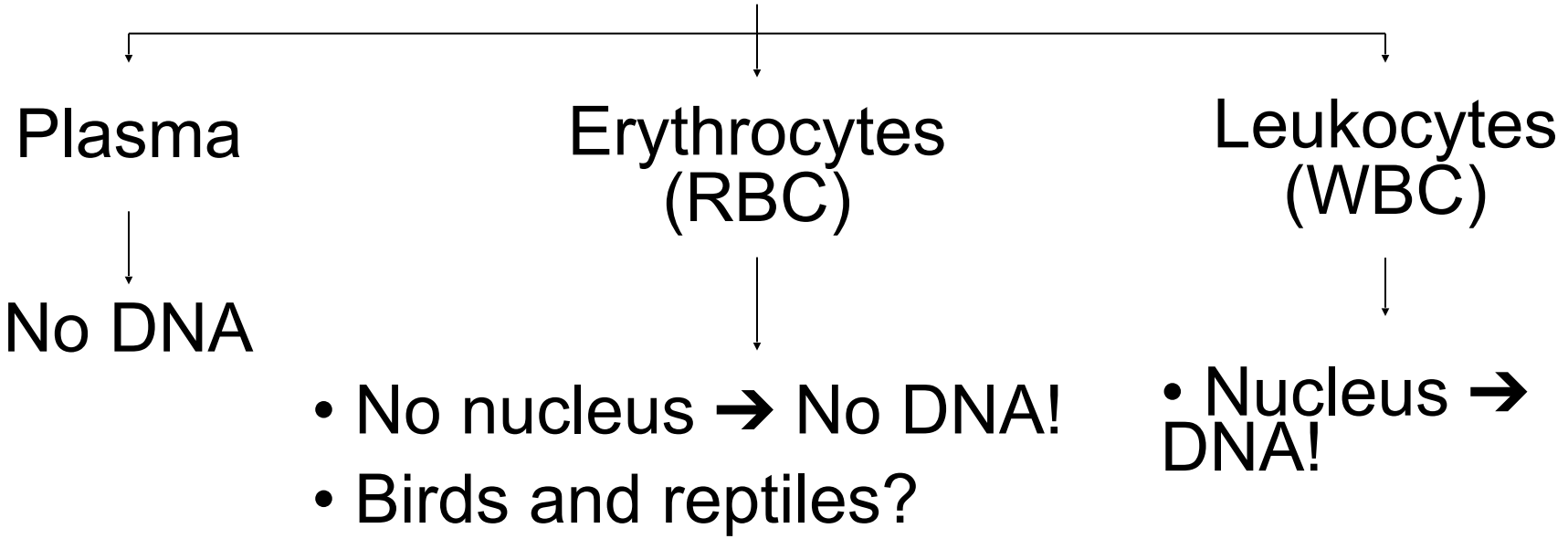
- All living cells contain hereditary information coded in DNA (some exceptions! **What are they?**).
- **DNA in human cells is found in the nucleus and mitochondria**.
- The DNA of the mitochondria resembles that of bacteria.
- The DNA in the nucleus is packaged into 22 pairs of autosomal chromosomes and one pair of sex chromosomes (XX or XY – some exceptions).

Human DNA sources

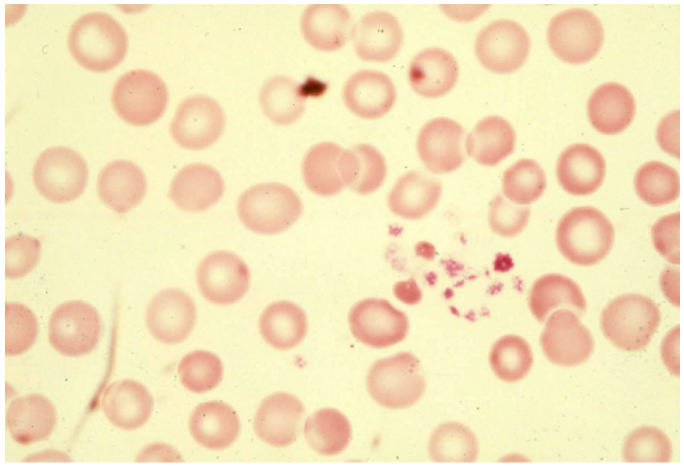


Human DNA sources

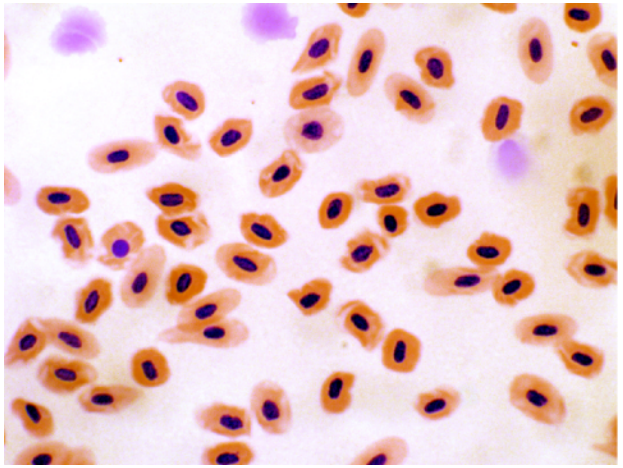
Blood is a connective tissue that is rich in proteins



Human DNA sources



Mammals RBC

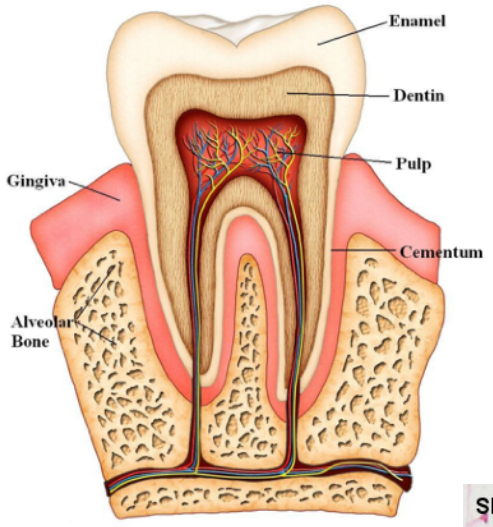


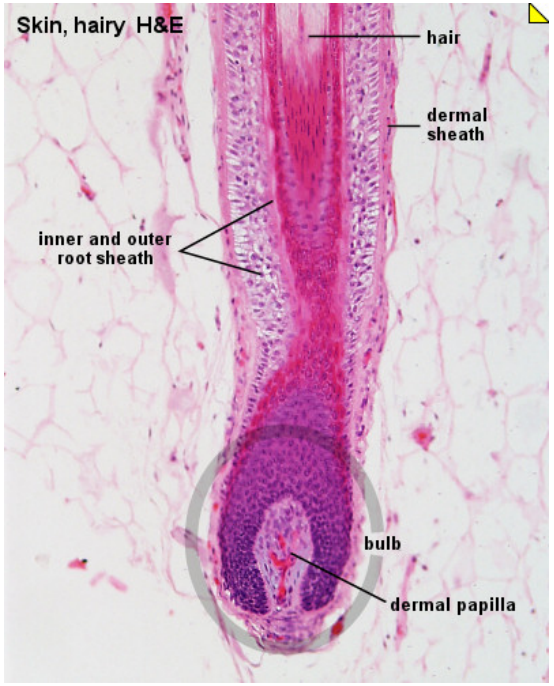
Birds RBC

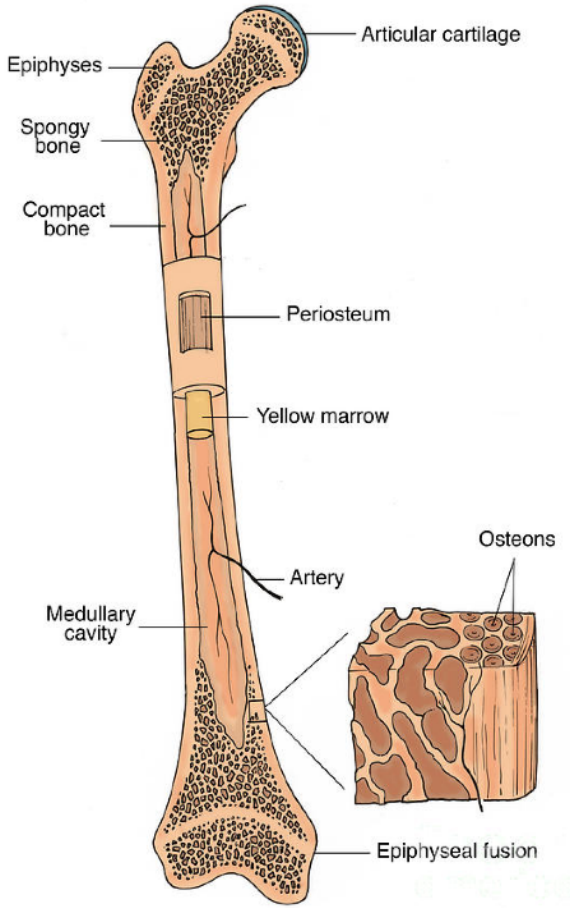
Human DNA sources

- We can get DNA only from living cells.
- Can we get DNA from trimmed hair?
- Can we get DNA from teeth enamels?
- What about bone?

Human DNA sources

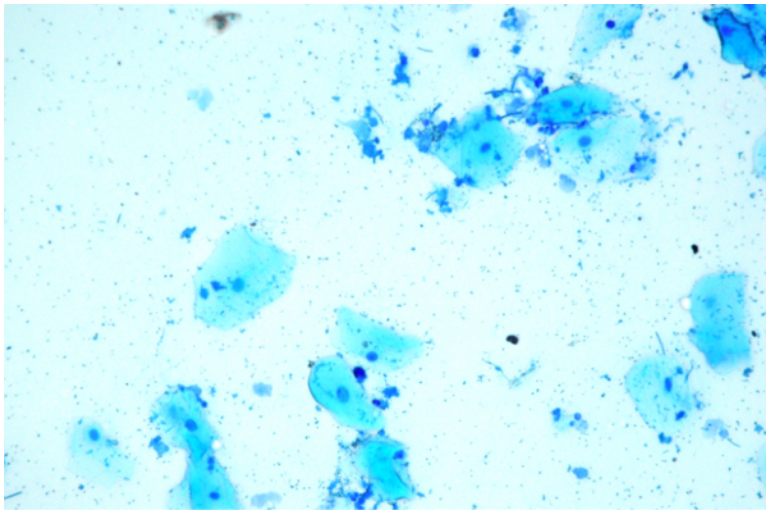






Human DNA sources

- Easy to collect and DNA is easy to extract.
- Saliva samples contain cells (mostly epithelial) and can be a good source of DNA.
- Check 23andme.com



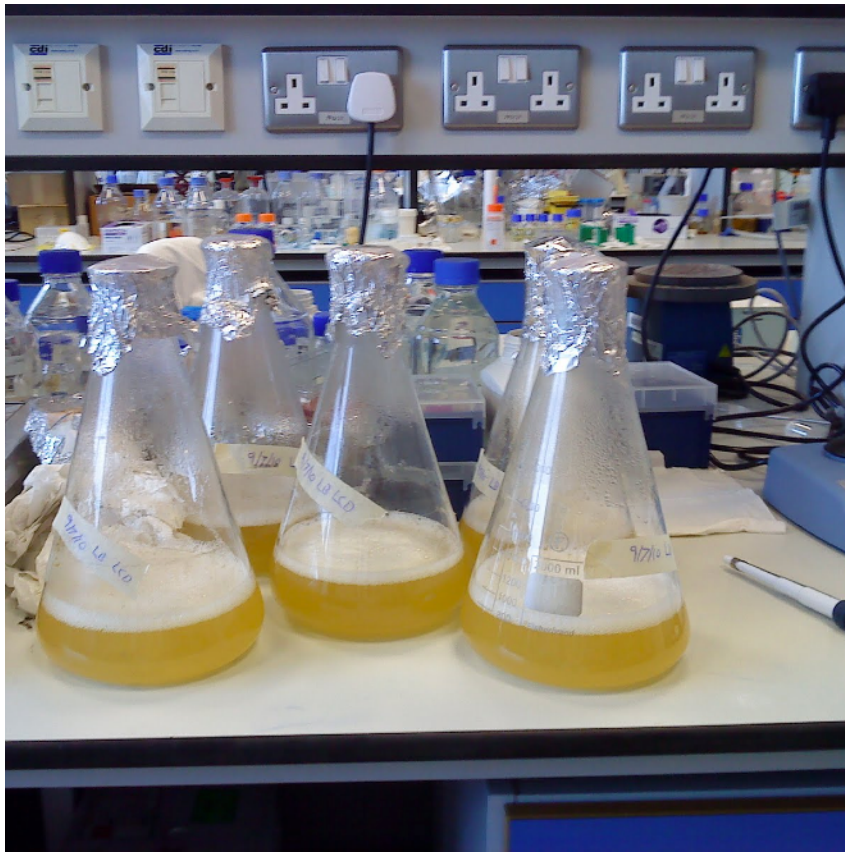
Cells and DNA

What about other organisms?

Bacterial DNA

We can grow bacteria in the lab and extract chromosomal DNA and plasmid DNA.

plate



Flask

Plant DNA

Plants contain living cells and non-living cells

Plant leaves are good source of DNA.
Other tissue may be hard to get DNA from.

Animal DNA

Animals are not different from humans!

Not every animal tissue has DNA!

Getting DNA

How to get DNA out of the cell?

Getting DNA

A new way to get DNA ☺

DNA isolation

1. Cell and tissue disruption
2. Lysis of cell membrane
3. Separating DNA from the rest of the cell content.

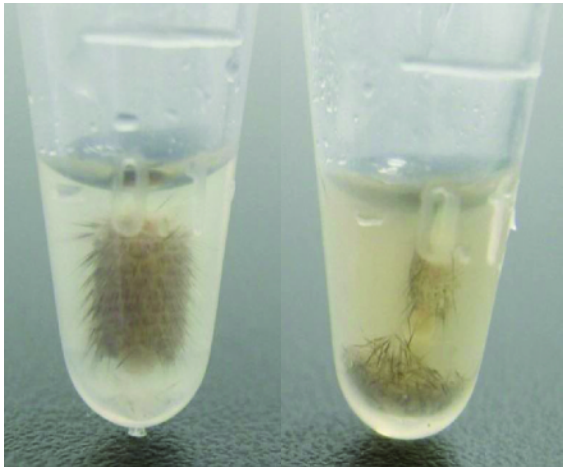
DNA isolation

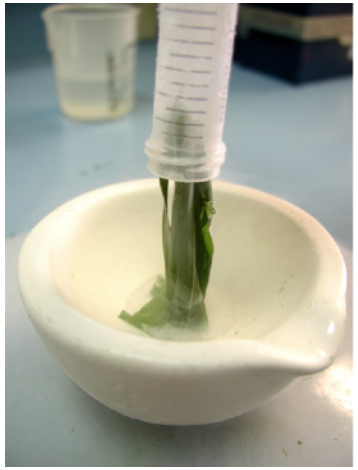
1. Cell and tissue disruption

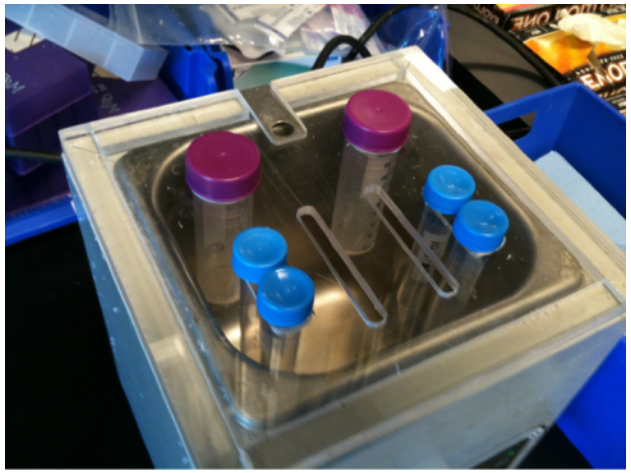
Enzymatic (proteinase K)

Grinding (liquid N)

Boiling (Alkali)





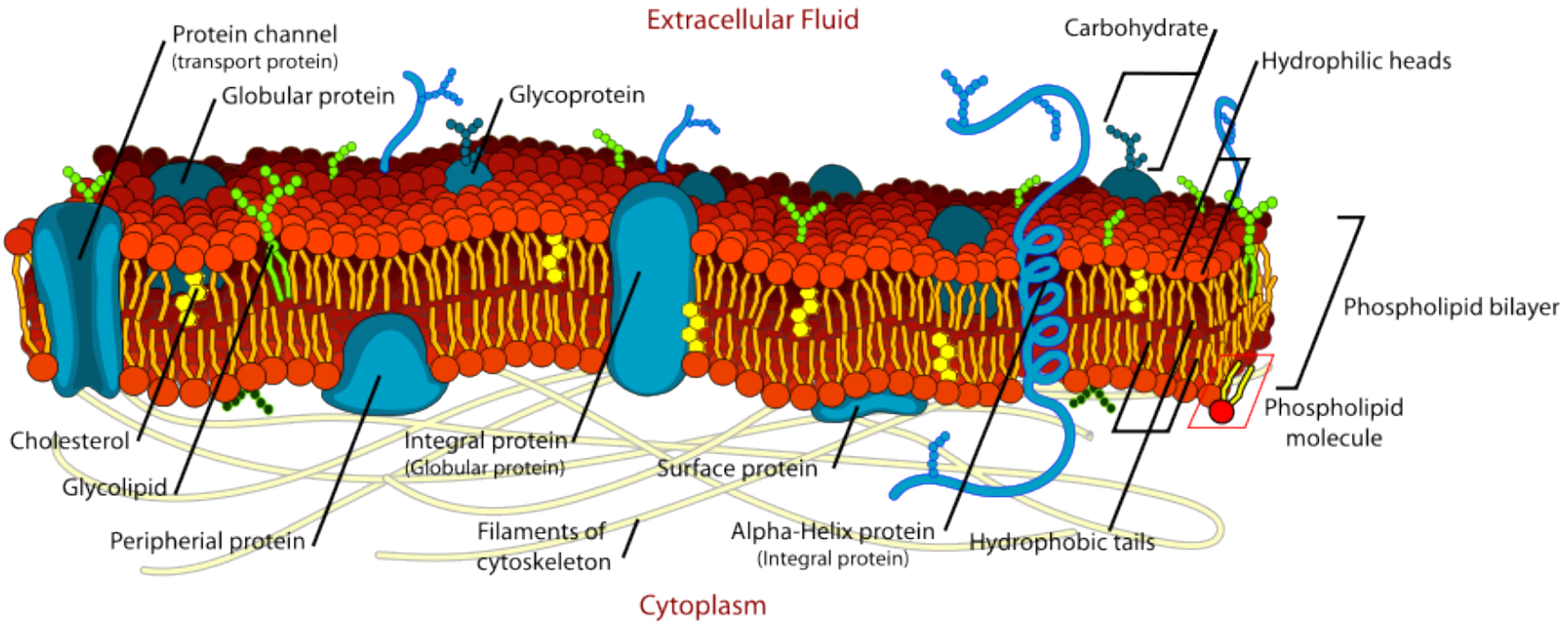


DNA isolation

2. Lysis of cell membrane

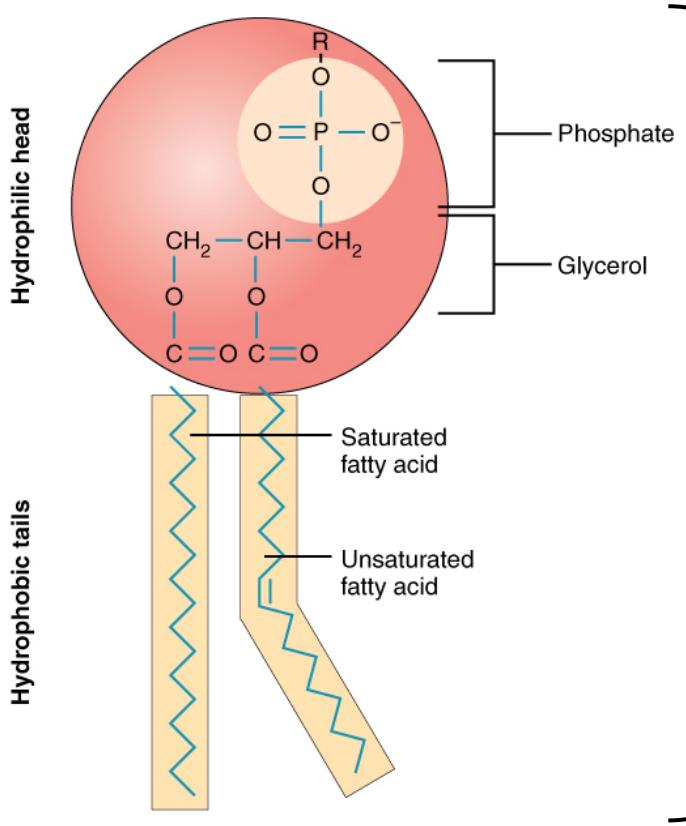
What is the cell membrane?
What does the cell membrane contain?

Cell membrane



Cell membrane

- Hydrophilic ?
- Hydrophobic?



Phospholipid molecule

DNA isolation

2. Lysis of cell membrane

- **Cell membrane contains:**
  - Phospholipid bilayer.
  - Proteins.
  - Other molecules.
- We need to open the cell membrane through **cell lysis**.
- We need to remove the proteins and other molecules.

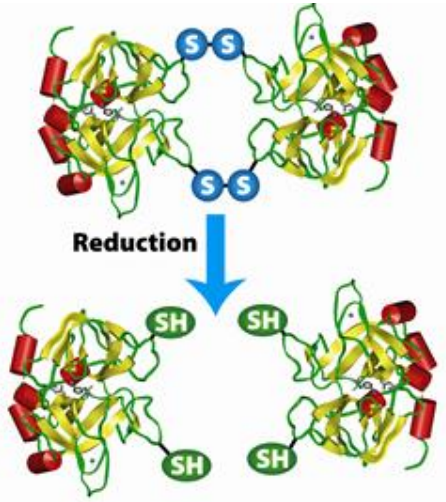
DNA isolation

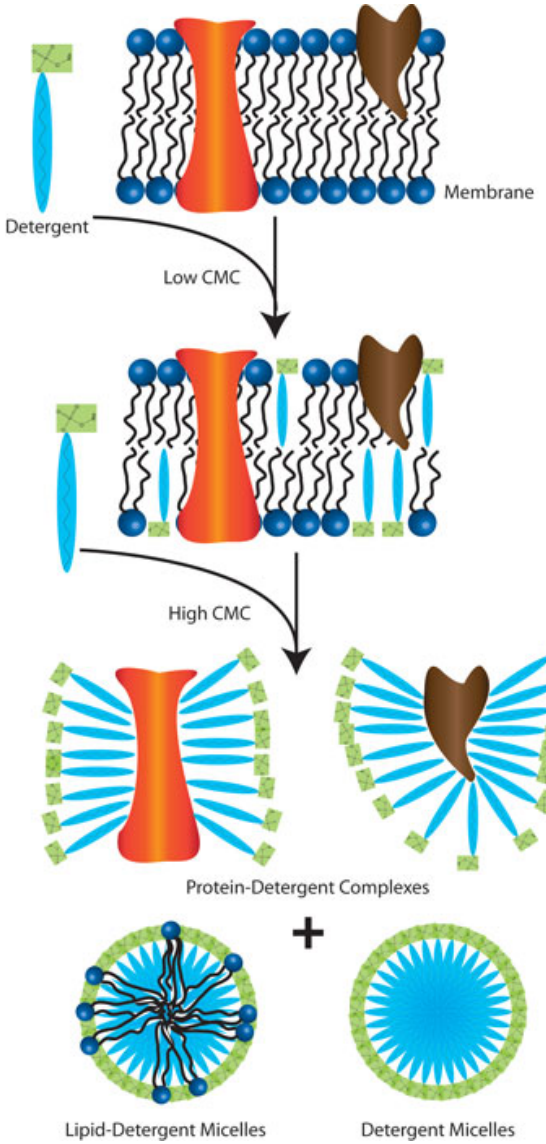
2. Lysis of cell membrane

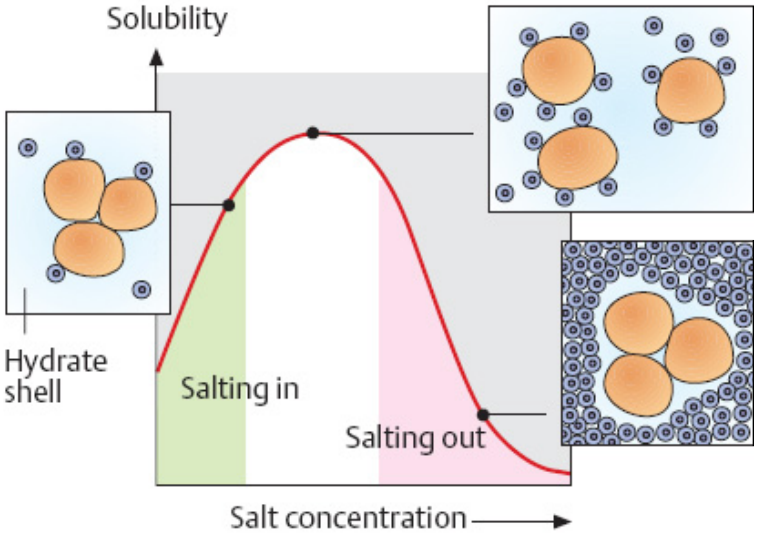
Lysis buffer:

- Detergent (SDS)
- Buffer (Tris-HCl)
- Salt
- Reducing agent (mercaptoethanol)
- Chelating agent (EDTA)

DNA isolation



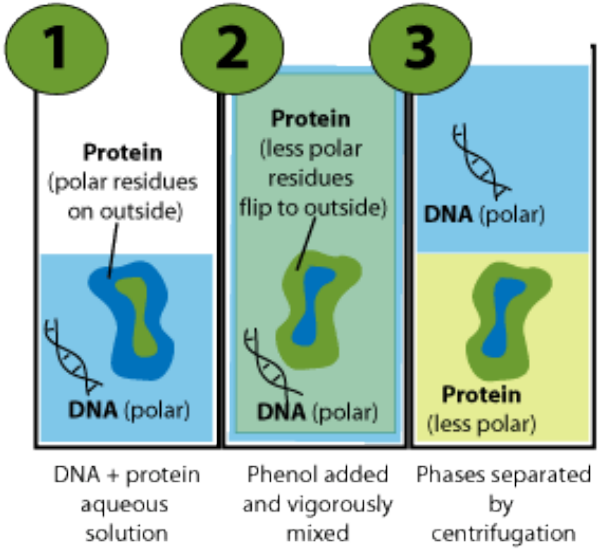




DNA isolation

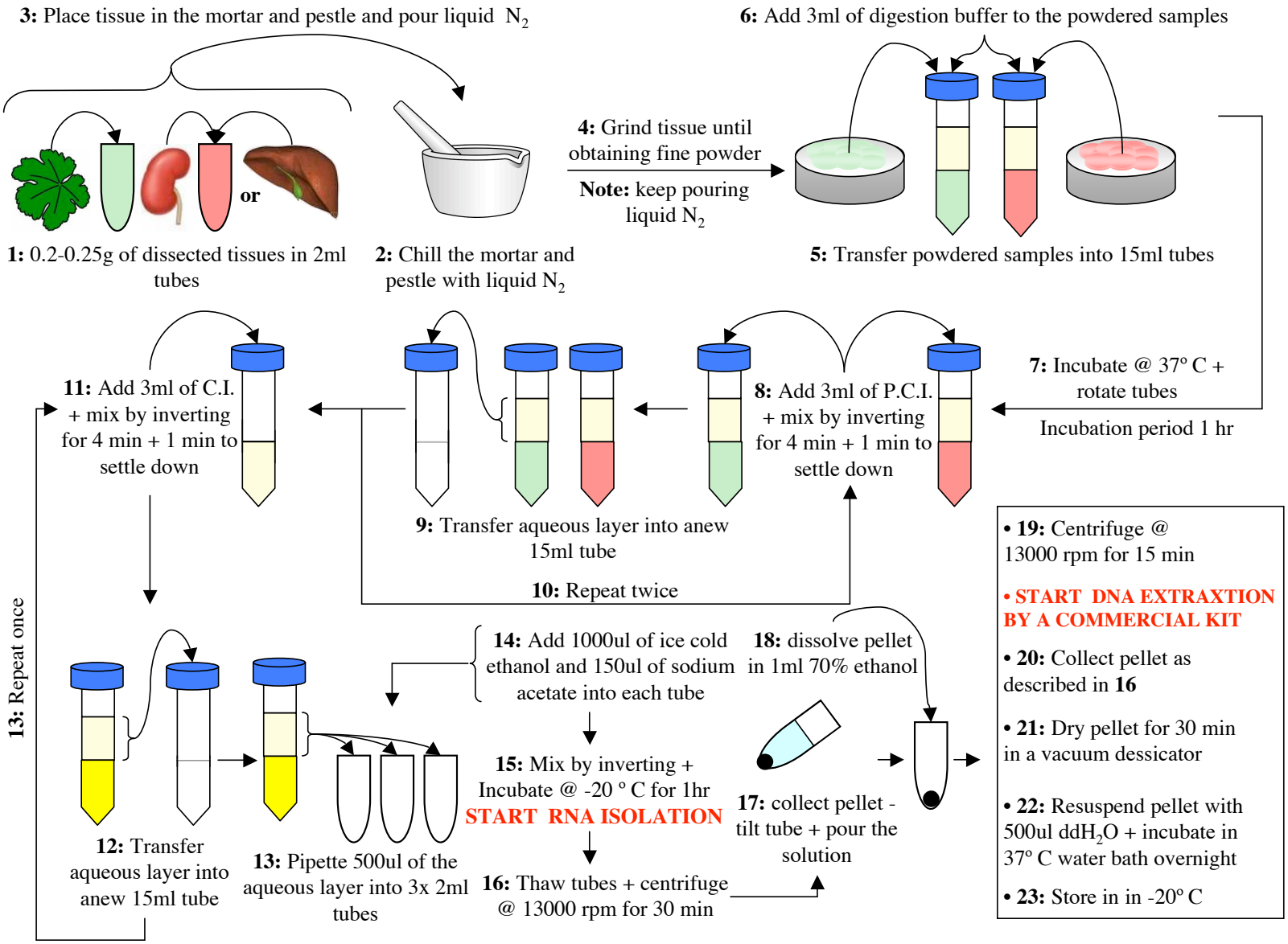
3. Separating DNA from the rest

Organic extraction
(phenol-chloroform extraction)

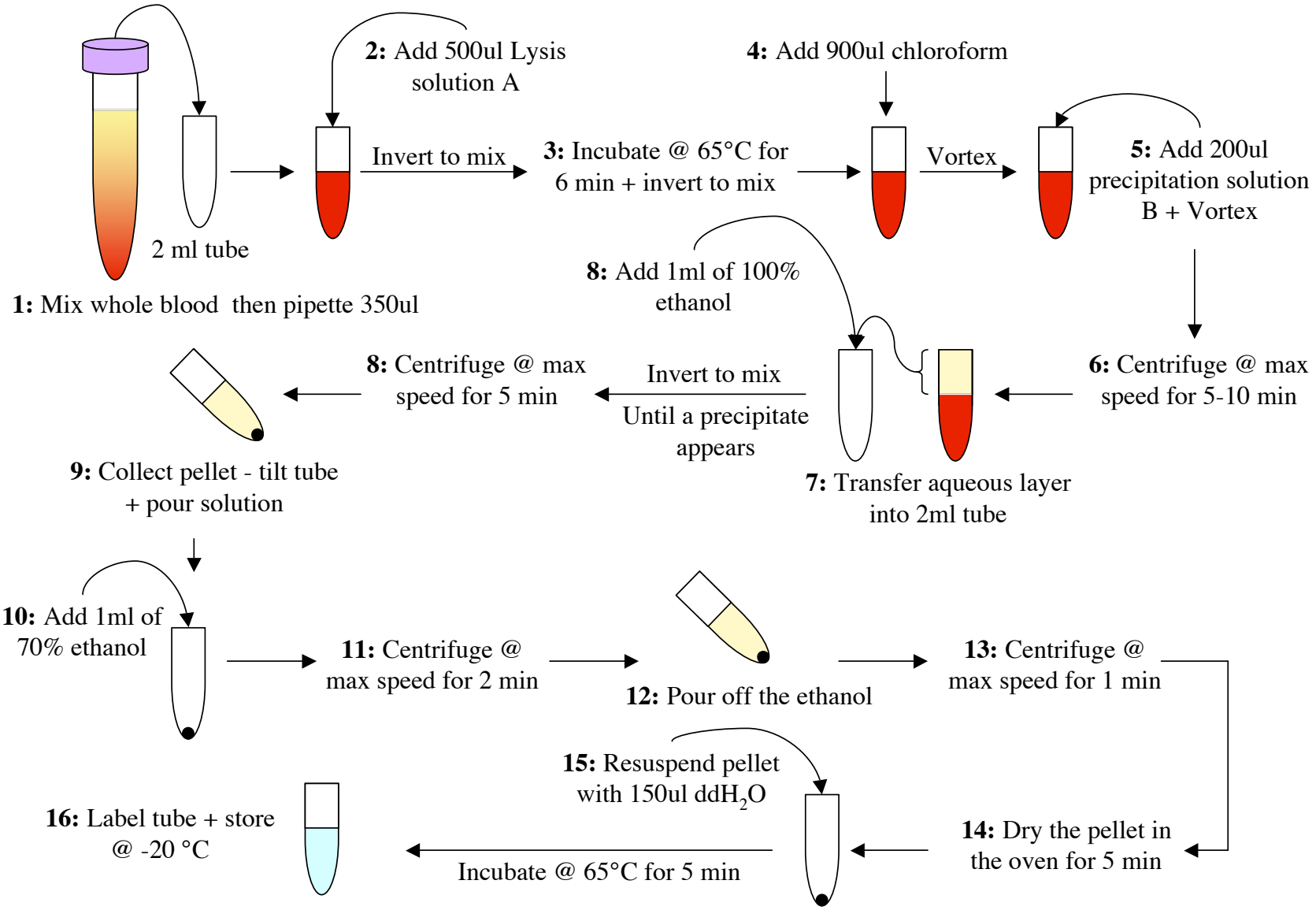


Column extraction
(DNA selective binding)

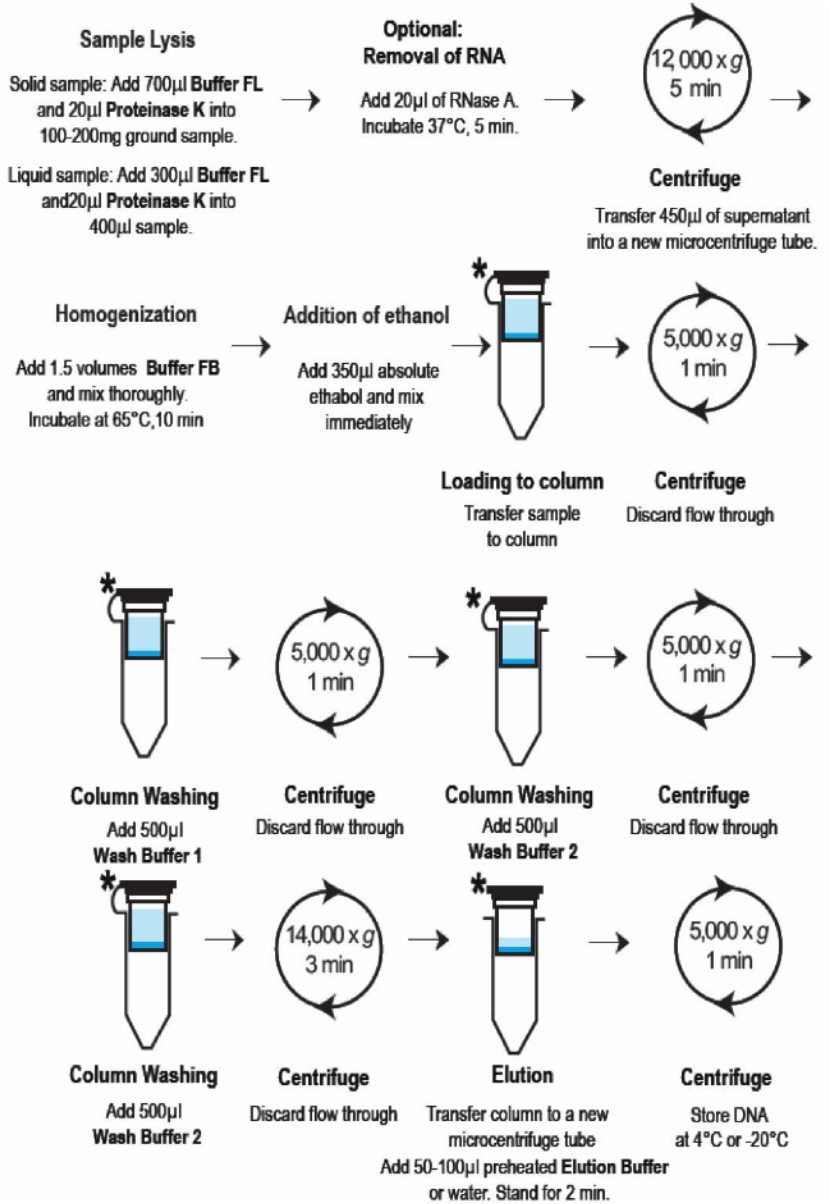
Phenol-chloroform extraction protocol

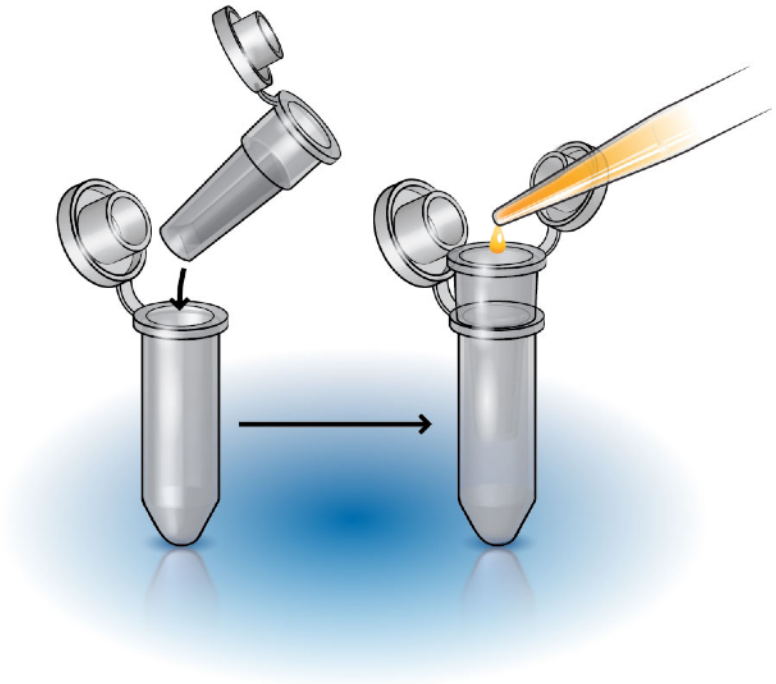


Phenol-chloroform extraction protocol



Column extraction protocol





How it looks?

The secret of life in a speck of jelly!

(Mendel's dwarf)

DNA storage

- DNA is stored in TE buffer to ensure stability and inhibit DNase.
- Long term storage is done using -80 freezers.
- -4 and -20 freezers are used for frequent use of the DNA sample.
- Freezing and thawing a sample may cause damage to DNA.

To know

DNA storage

Genomic DNA

DNA isolation

Detergent

Organic extraction

RBC in mammals

Mitochondrial DNA

Cell and tissue disruption

Phospholipid bilayer

Reducing agent

hydrophilic

Plasmid DNA

WBC DNA

Column extraction

Chloroplast DNA

Cell membrane

salt

Hydrophobic

RBC in birds

Cell lysis

Expectations

- You know the sources of DNA in various organisms.
- You know not all material from a living organism can be a source of DNA.
- You know the process of isolating DNA.
- You know the chemicals and what they do in DNA extraction protocol.
- You know the two major protocols of DNA extraction.
- You know DNA storage methods and stability.

For a smile