



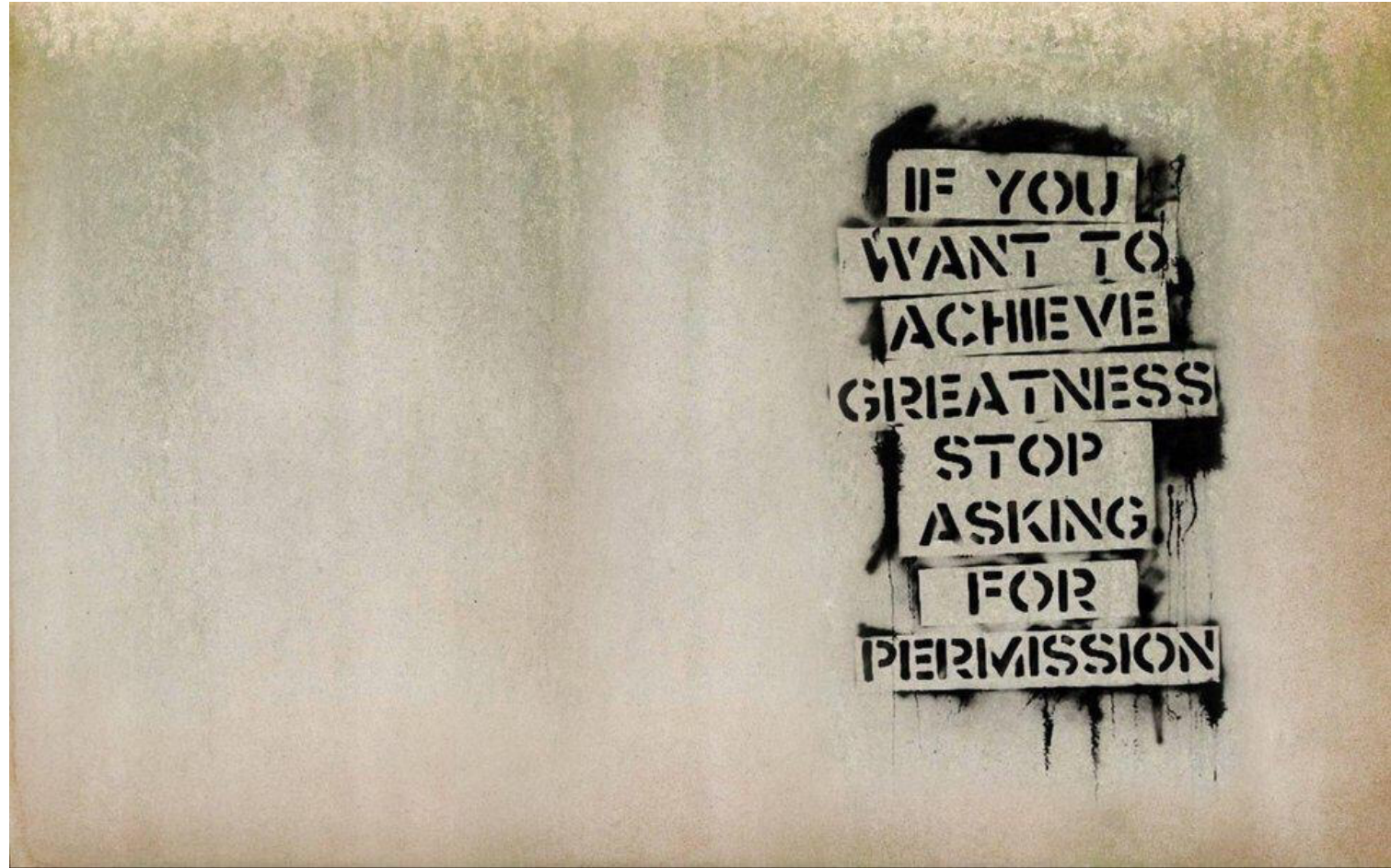
# Lecture 5.1

## Molecular techniques

### I. DNA extraction

Course 485

# Lessons for life



# AIMS

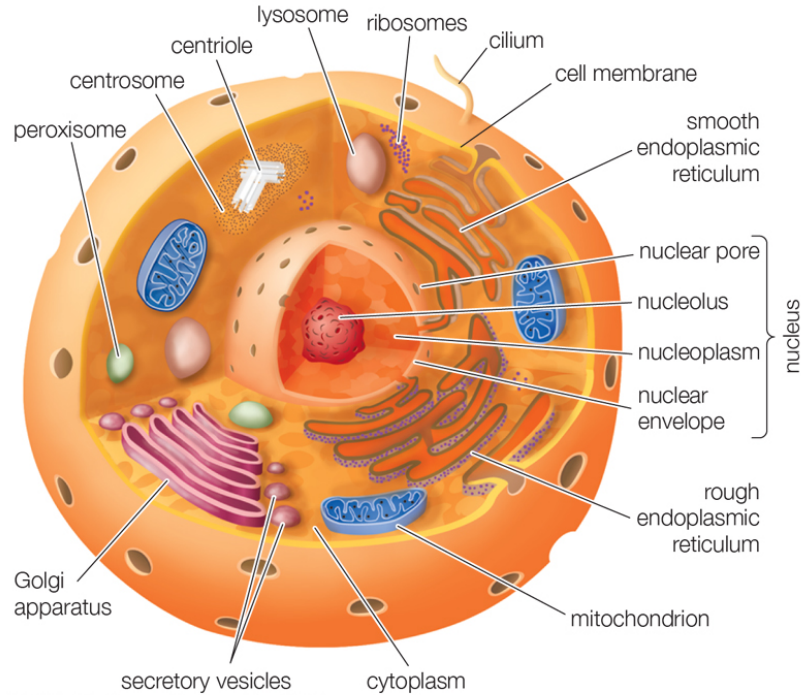
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- Understand the sources of DNA in different organisms.
- Understand the cellular structure that needs to be disrupted to extract DNA.
- Understand the process of DNA isolation and the different methods employed.
- Understand the reasons for using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

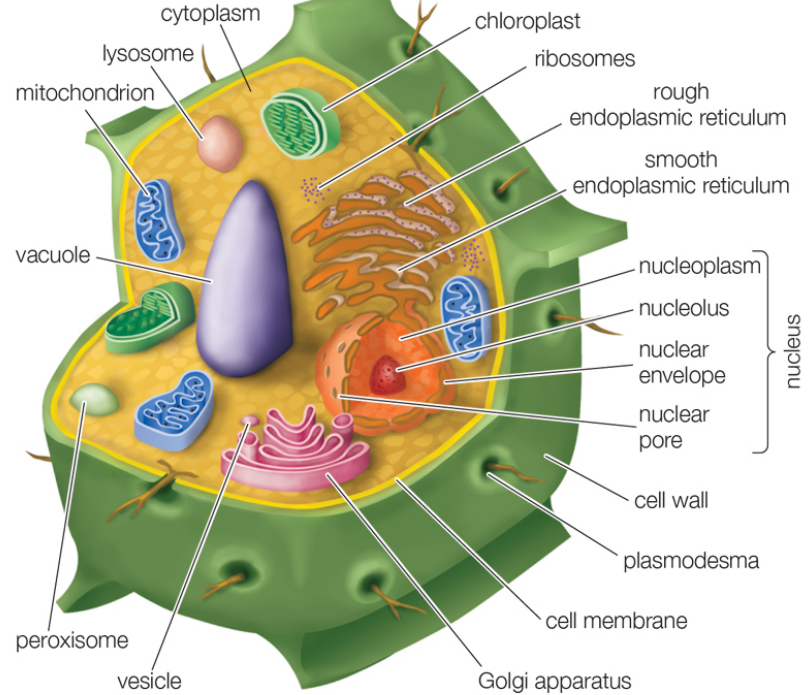
# Cell Structure

## Typical animal cell and plant cell

### Animal cell



### Plant cell



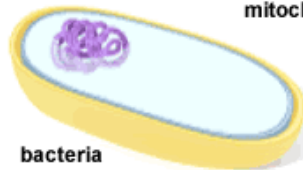
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chloroplast



mitochondria



bacteria

# Cells and DNA

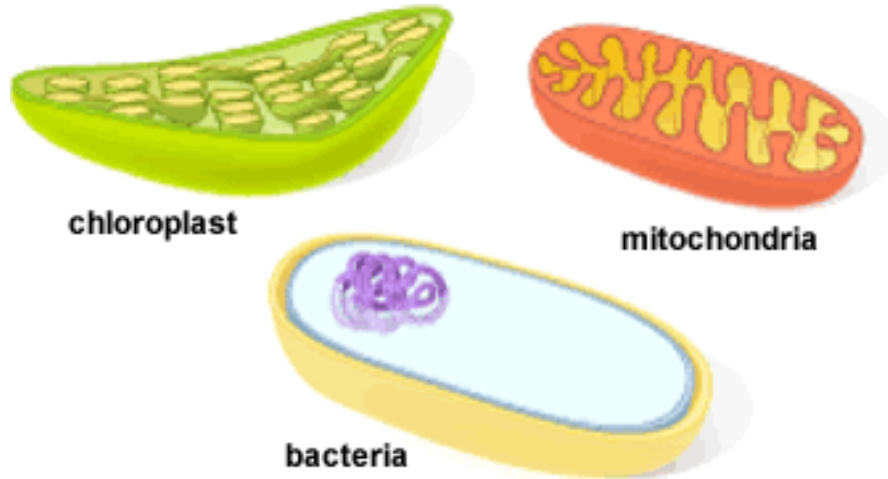
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## Where Do we get the DNA from?

- 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

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**In humans, where can we get DNA from?**

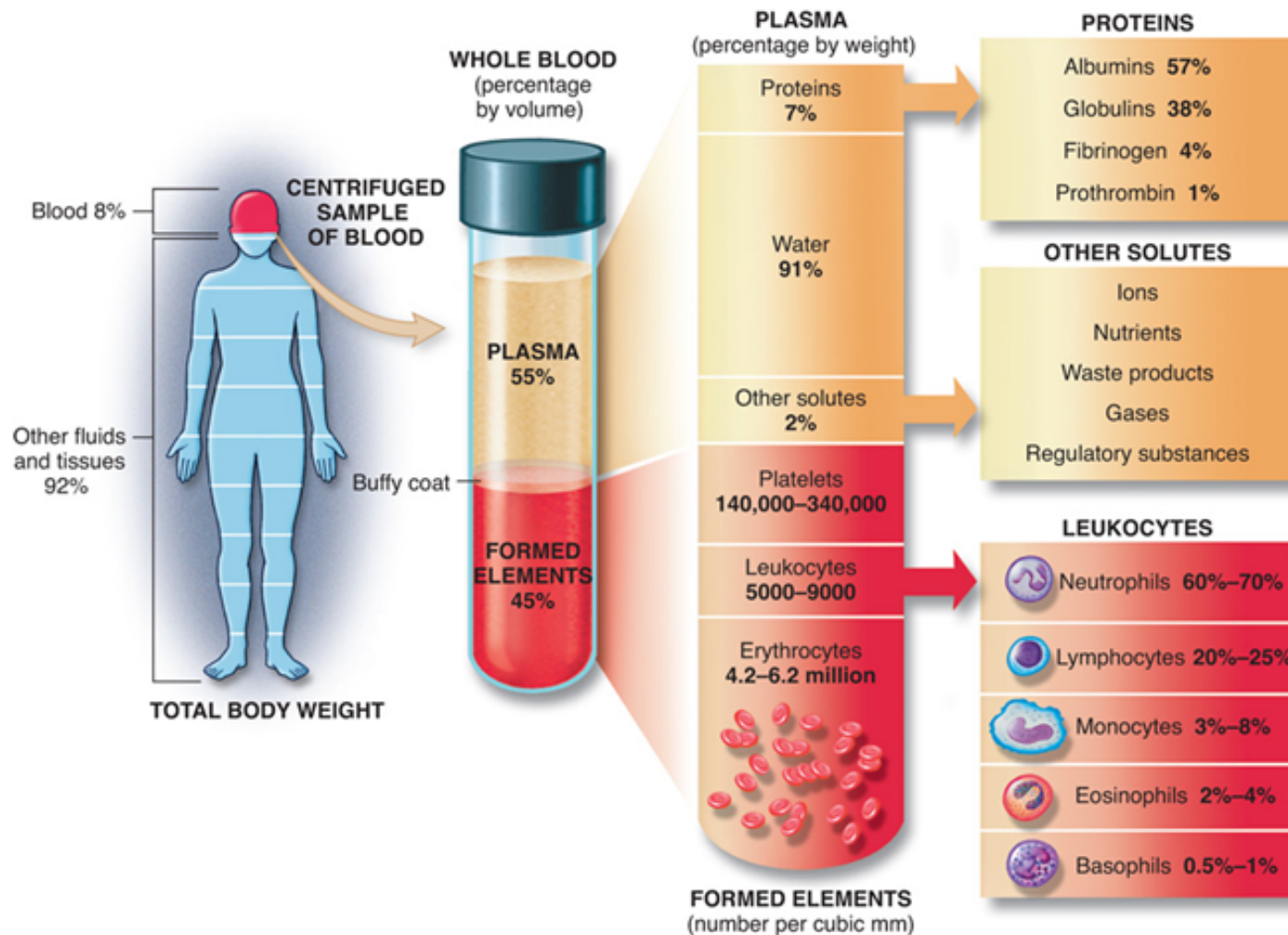
# Cell Structure

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- 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).

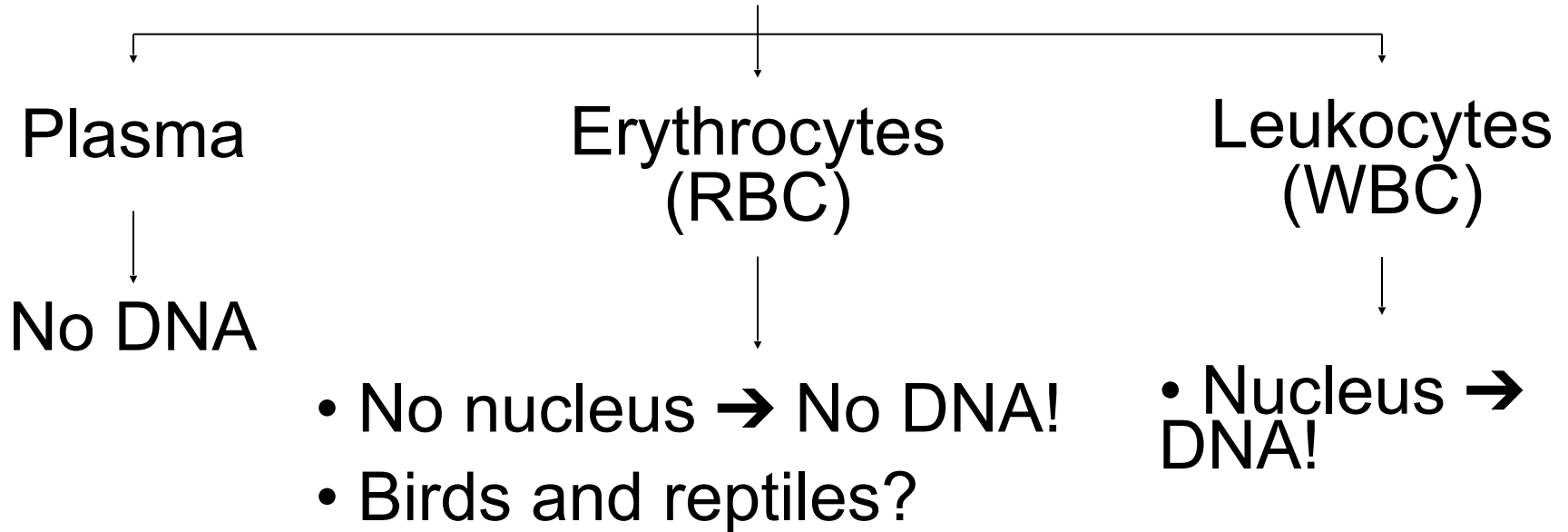


# Human DNA sources

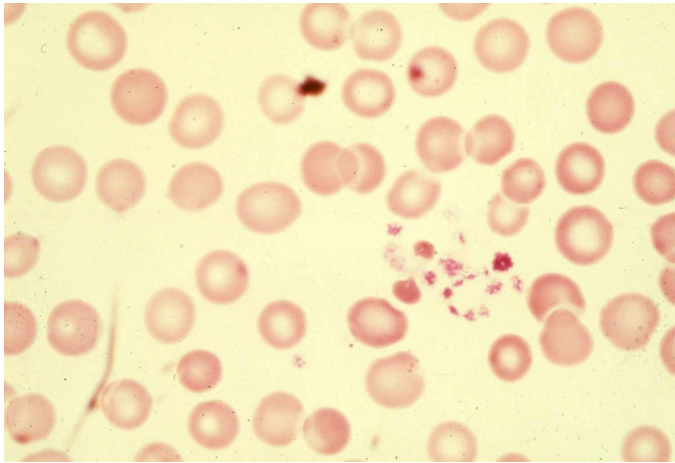


# Human DNA sources

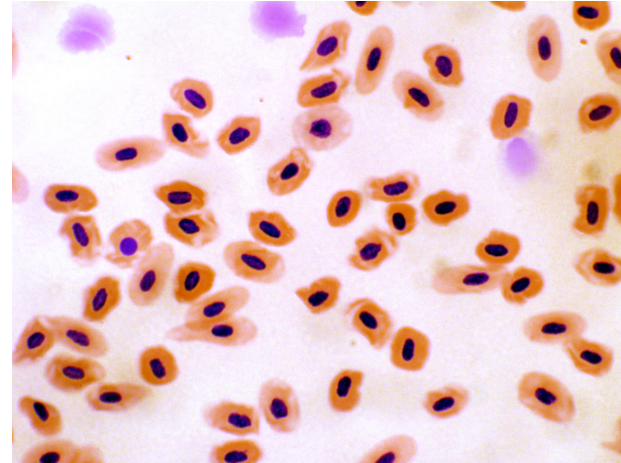
**Blood is a connective tissue that is rich in proteins**



# Human DNA sources



Mammals RBC



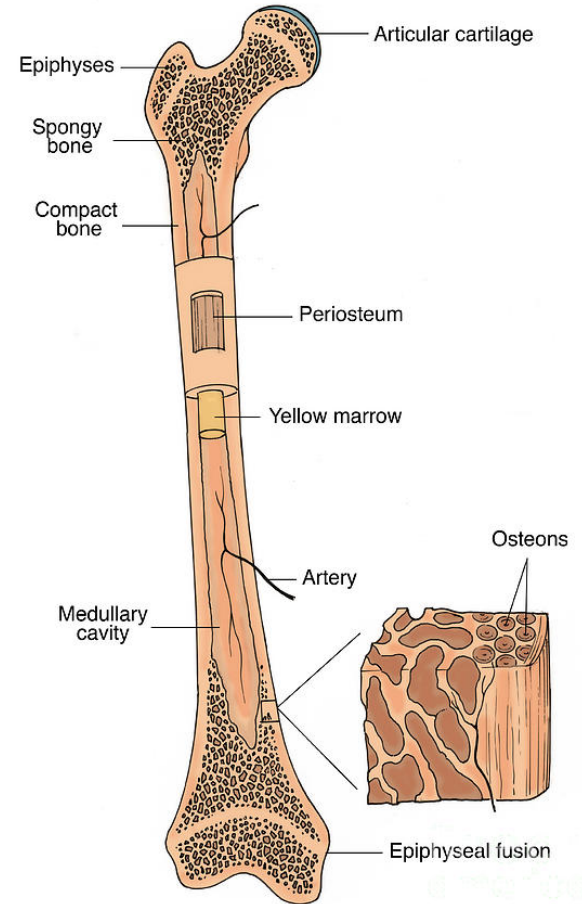
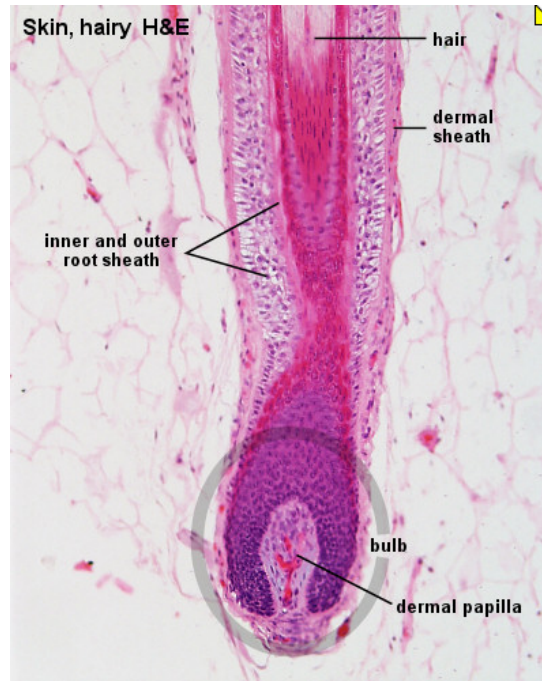
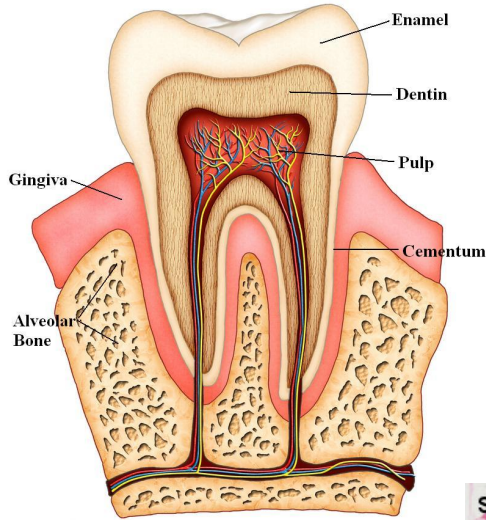
Birds RBC

# Human DNA sources

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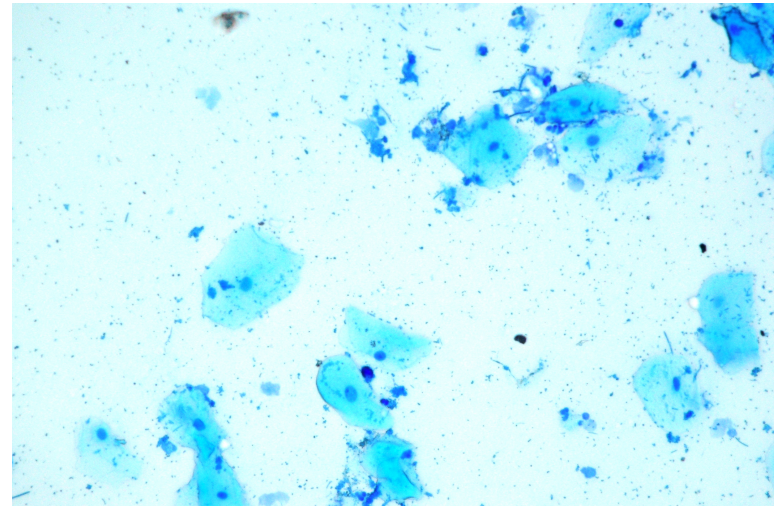
- We can get DNA only from living cells.
- Can we get DNA from trimmed hair?
- Can we get DNA from teeth enamels?
- What about bone?

# 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](http://23andme.com)



# Cells and DNA

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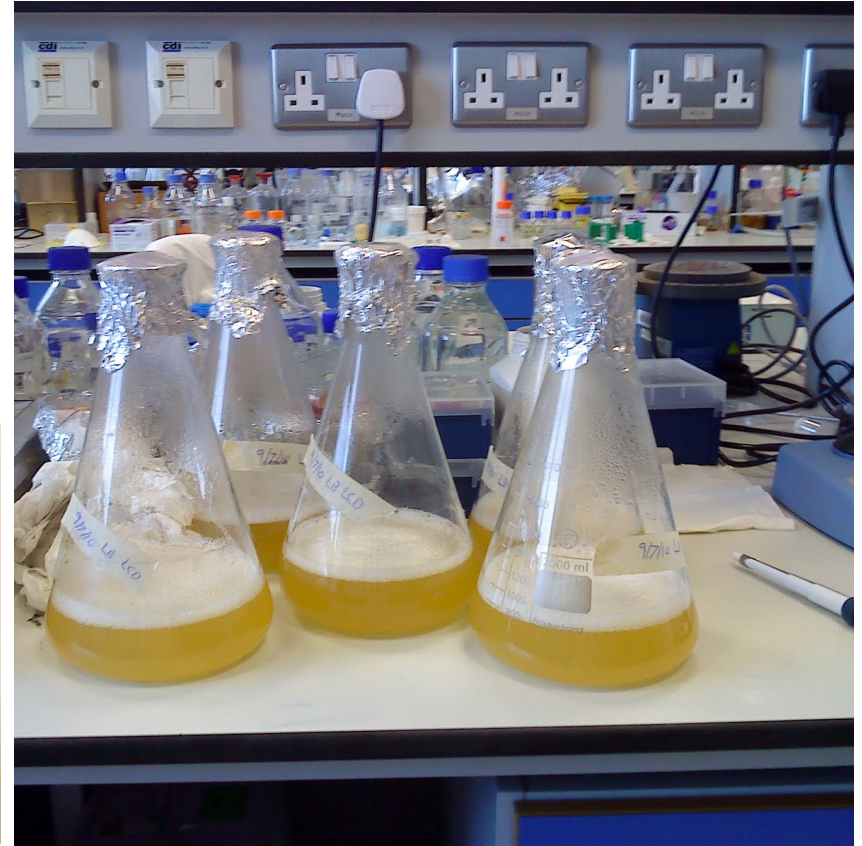
**What about other organisms?**

# 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

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**How to get DNA out of the cell?**

# Getting DNA



A new way to get DNA ☺

# DNA isolation

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

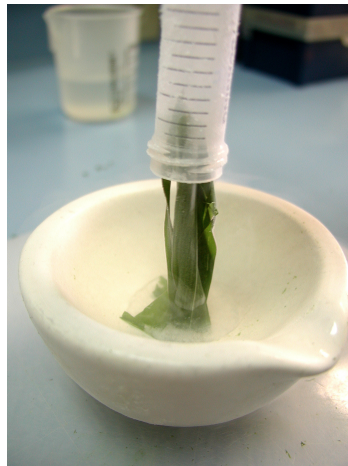
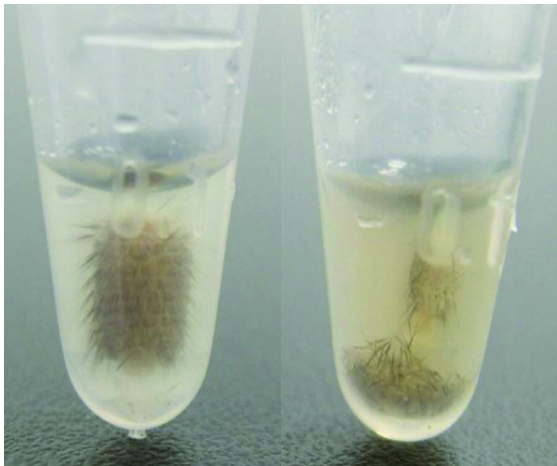
# DNA isolation

## 1. Cell and tissue disruption

Enzymatic  
(proteinase K)

Grinding  
(liquid N)

Boiling  
(Alkali)



# DNA isolation

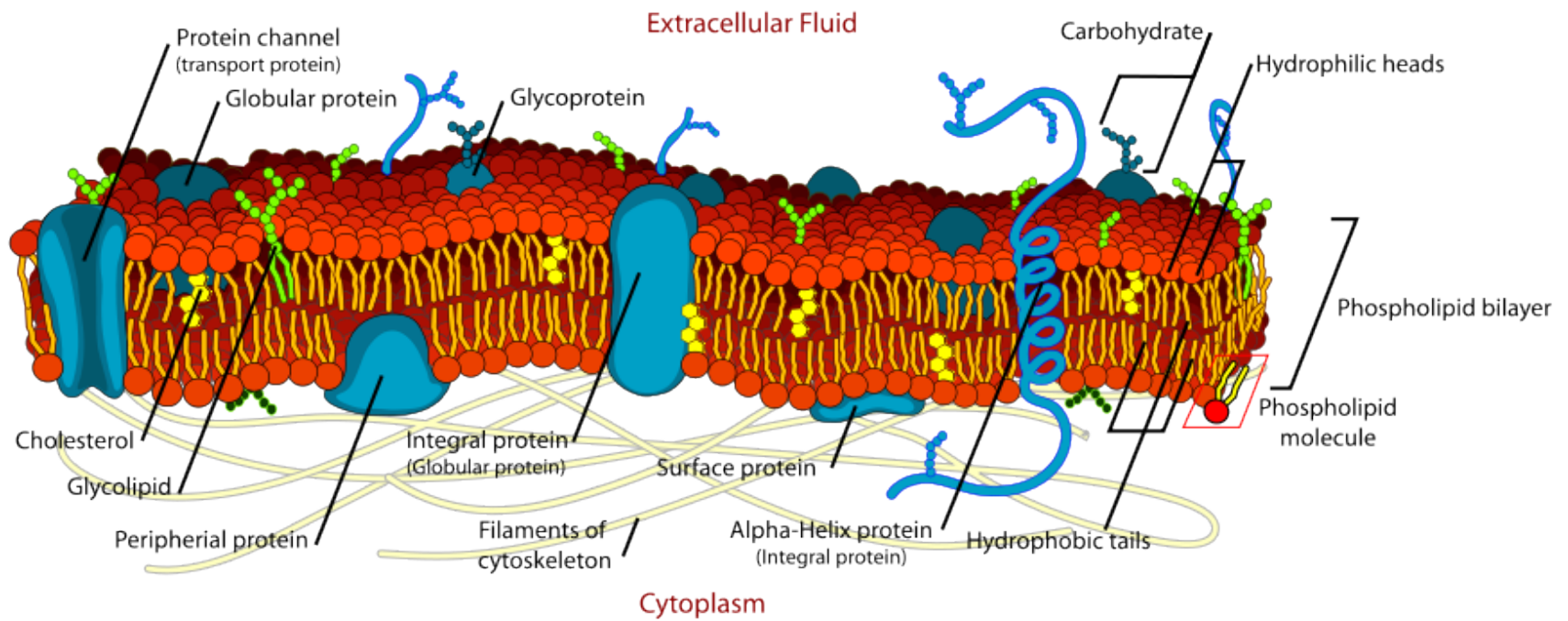
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## 2. Lysis of cell membrane

**What is the cell membrane?**

**What does the cell membrane contain?**

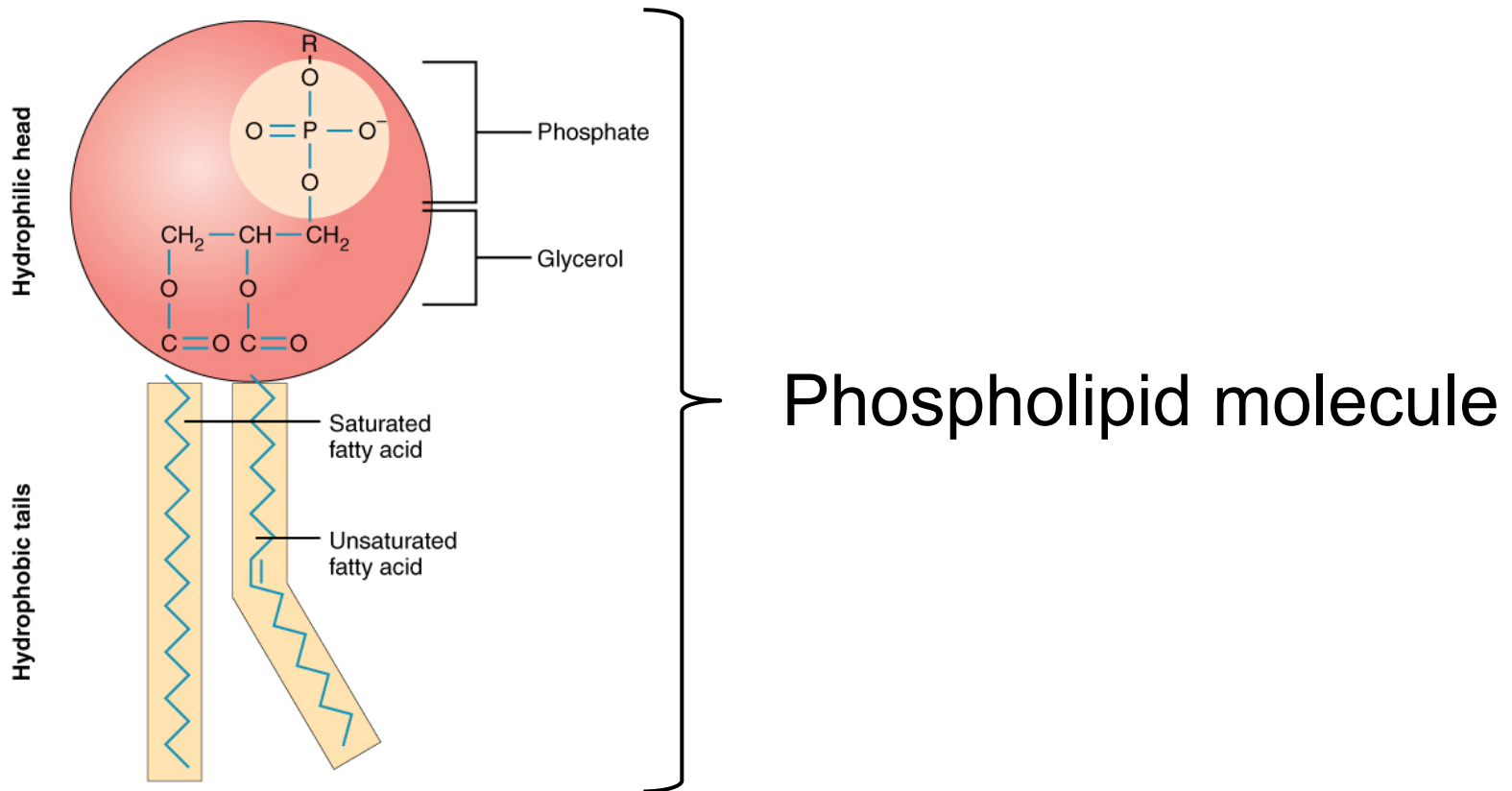
# Cell membrane





# Cell membrane

- Hydrophilic ?
- Hydrophobic?



# 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

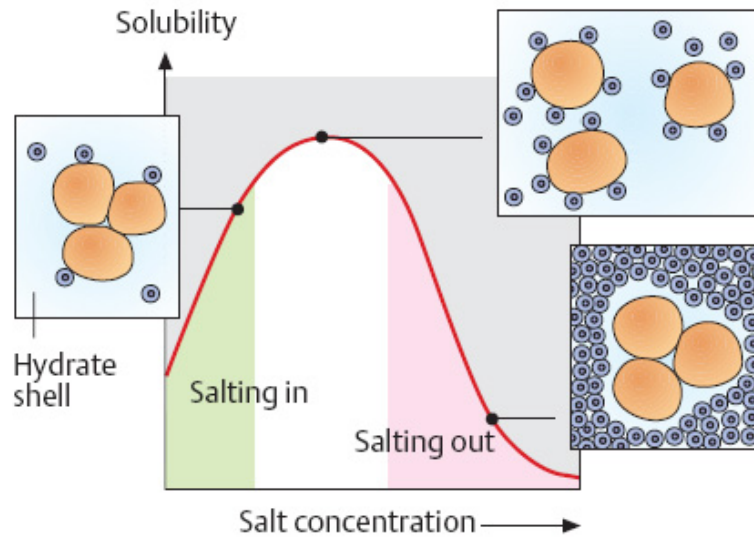
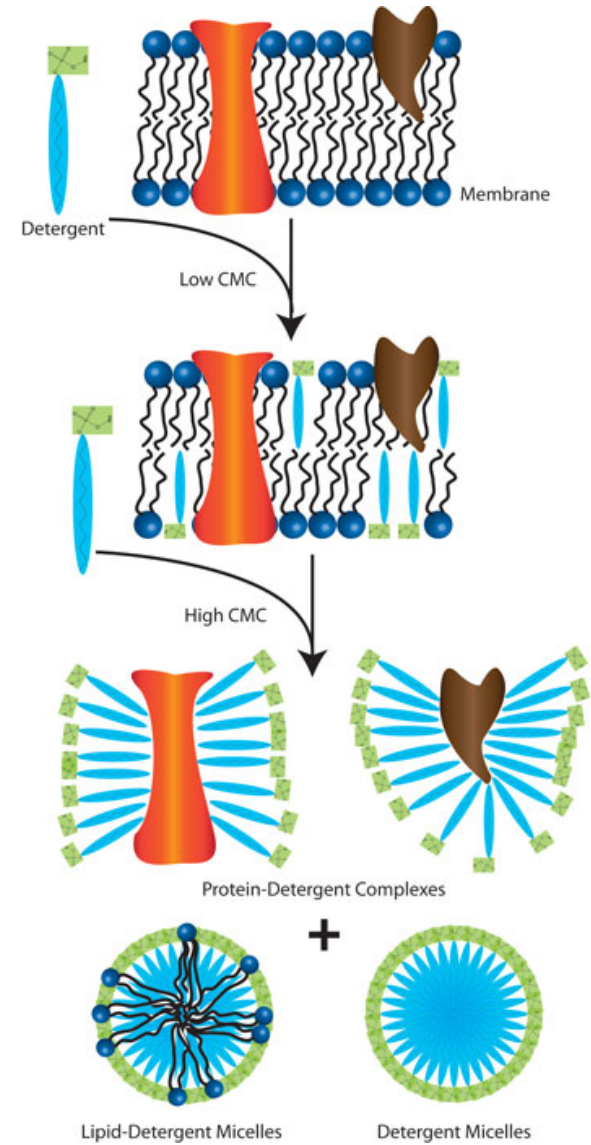
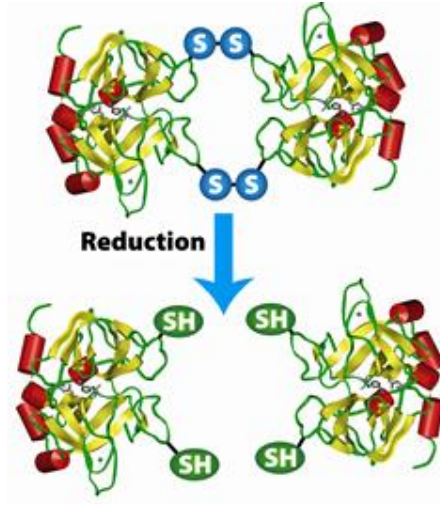
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## 2. Lysis of cell membrane

### **Lysis buffer:**

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

# DNA isolation

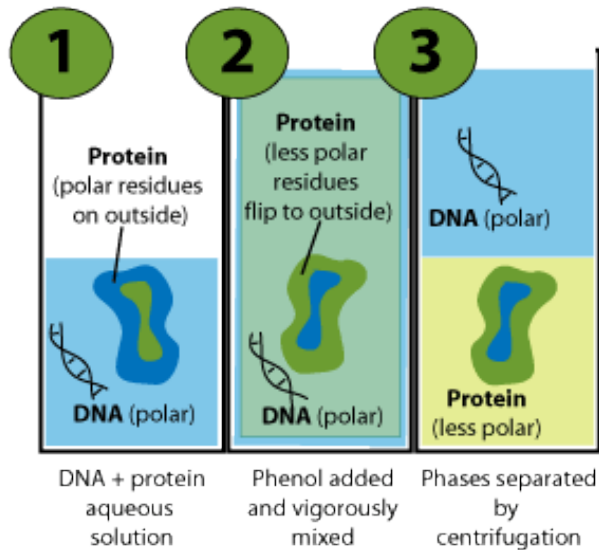


# DNA isolation

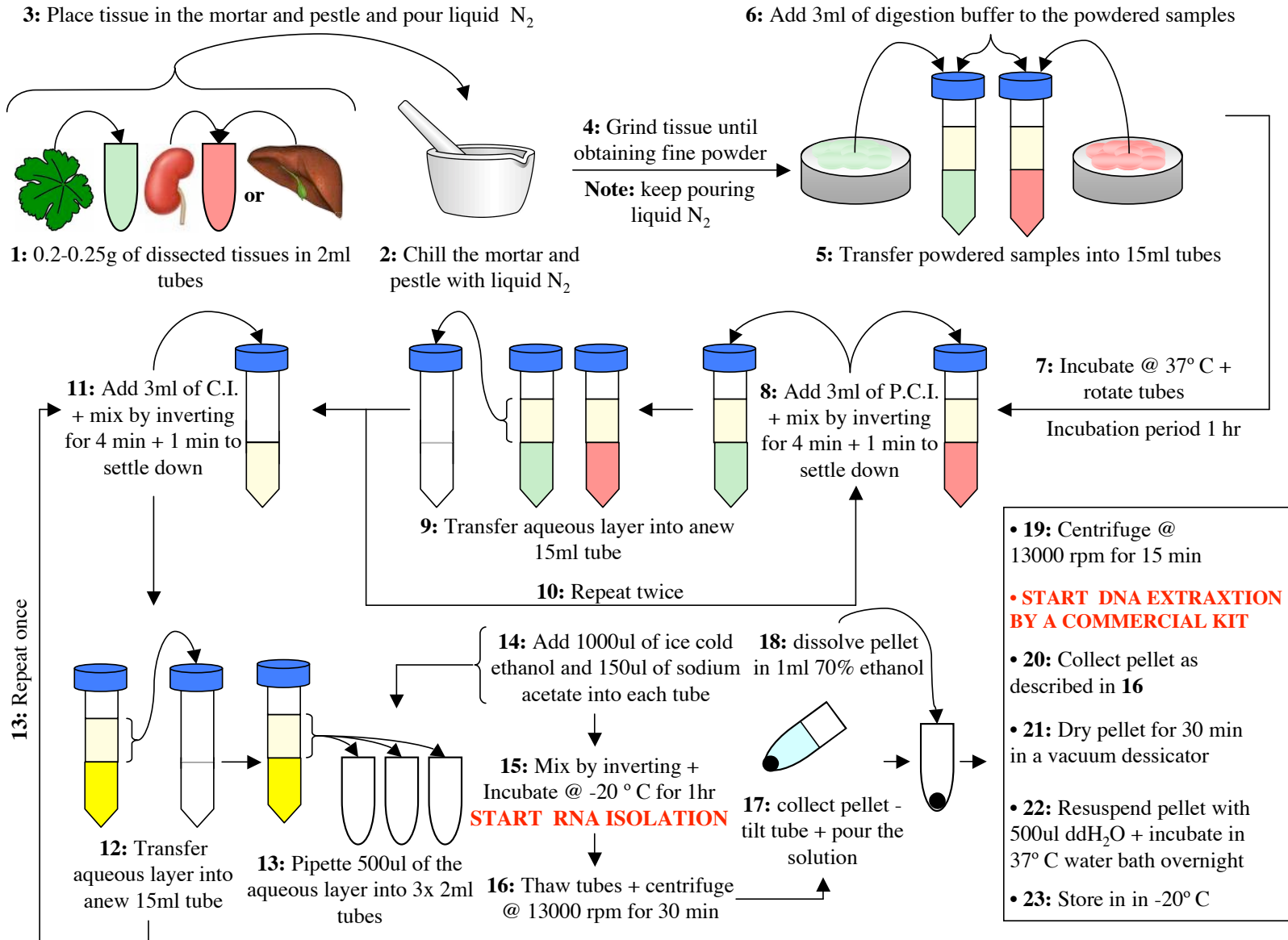
## 3. Separating DNA from the rest

Organic extraction  
(phenol-chloroform extraction)

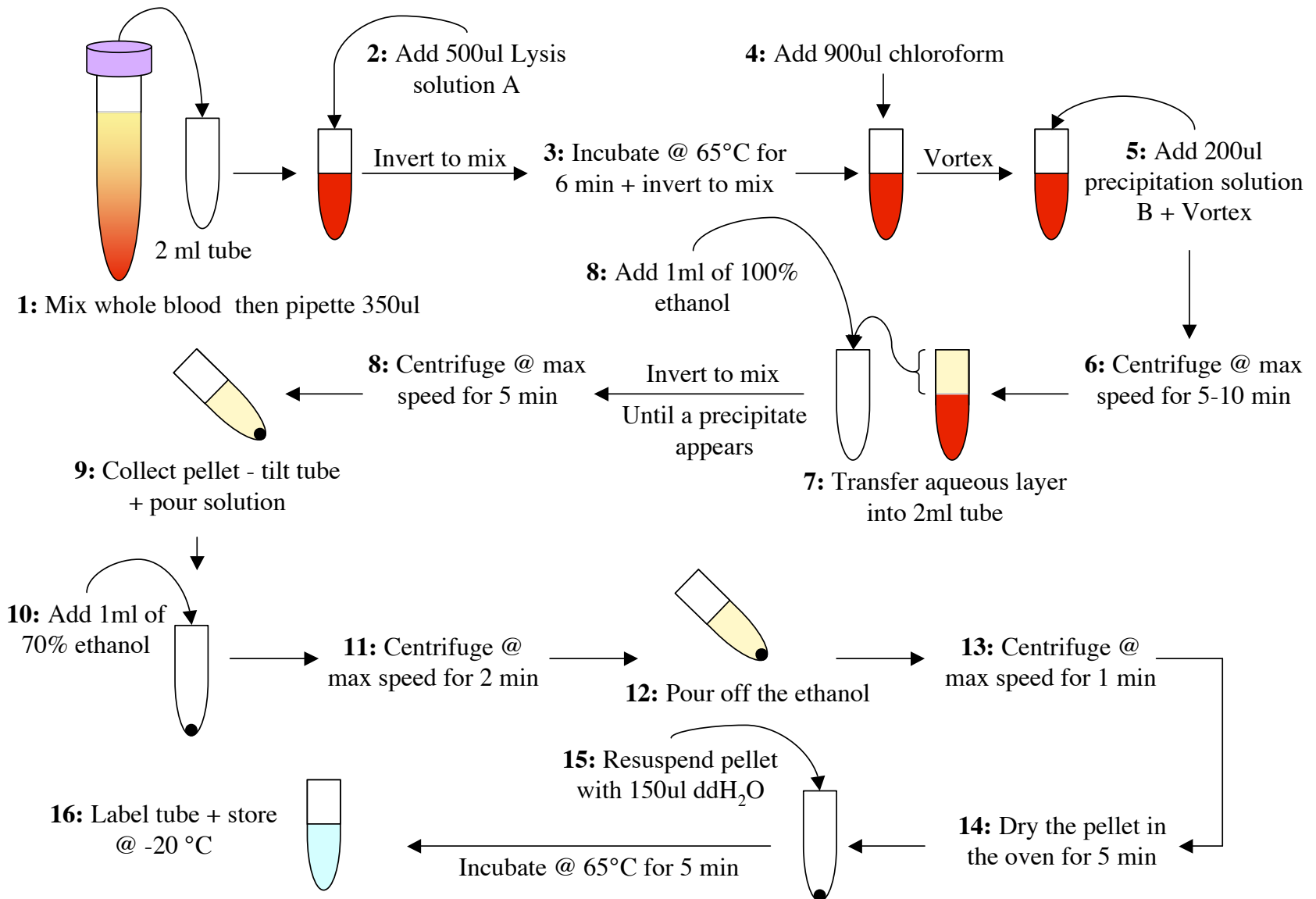
Column extraction  
(DNA selective binding)



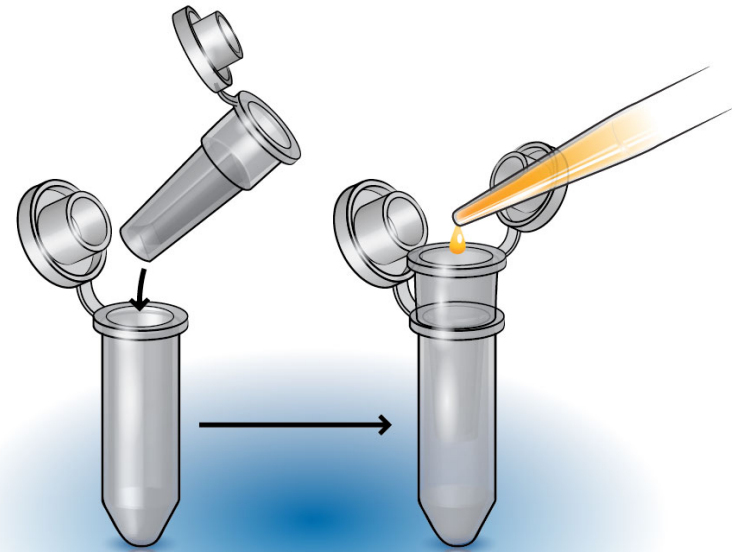
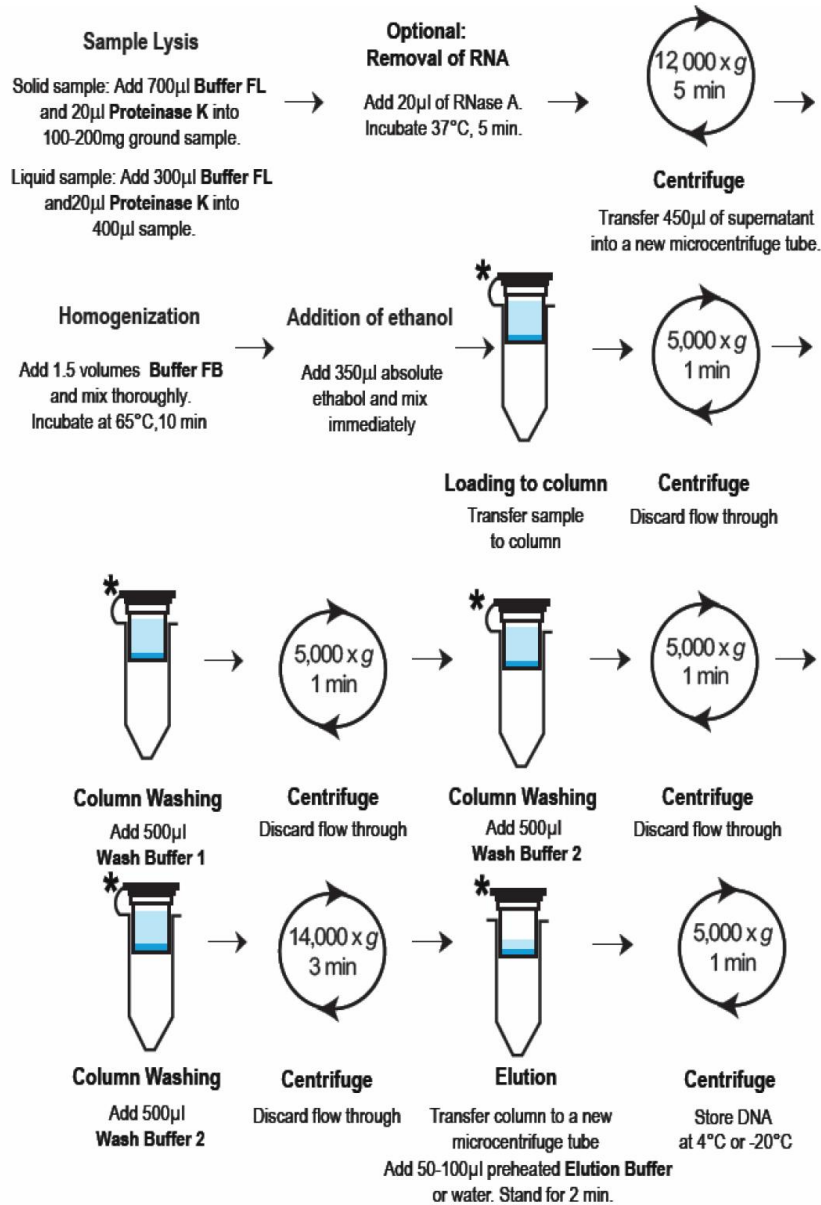
# Phenol-chloroform extraction protocol



# 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

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Genomic DNA      DNA storage      DNA isolation  
Detergent

Organic extraction      RBC in mammals      Mitochondrial DNA

Cell and tissue disruption      Phospholipid bilayer      Reducing agent

Plasmid DNA      hydrophilic      WBC DNA

Cell membrane      Chloroplast DNA      Column extraction

RBC in birds      salt      Hydrophobic

Cell lysis



# Expectations

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- 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

