



Lecture 11:

Finding the allele 1: DNA extraction

Course 410

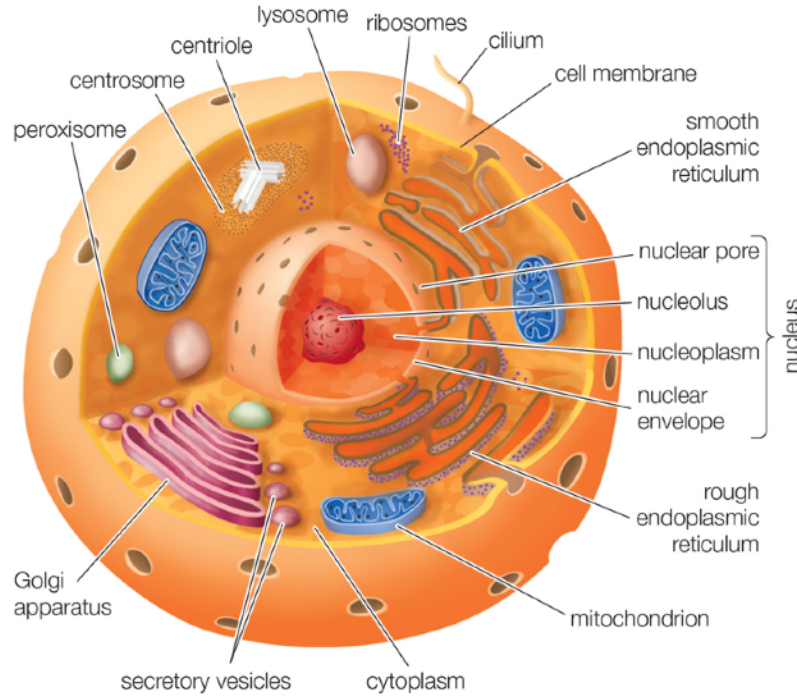
Molecular Evolution



Where is the allele located?

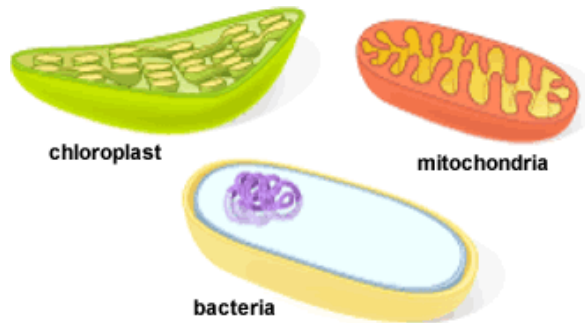
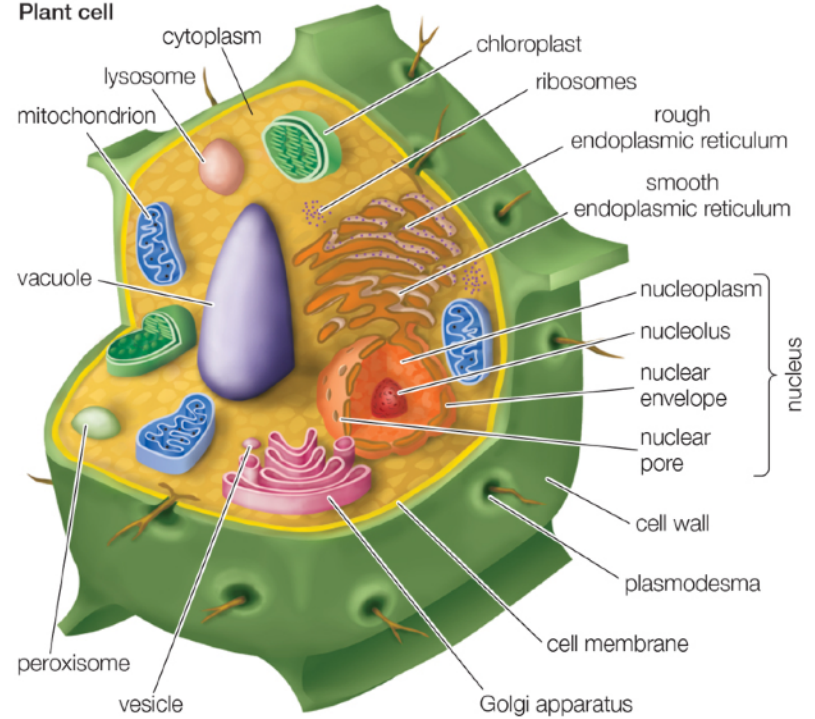
Typical animal cell and plant cell


Animal cell



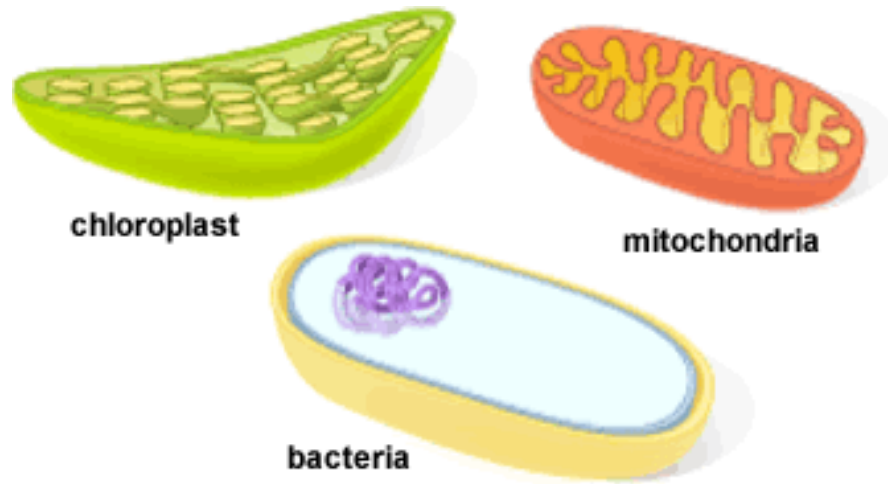
© 2010 Encyclopædia Britannica, Inc.

Plant cell



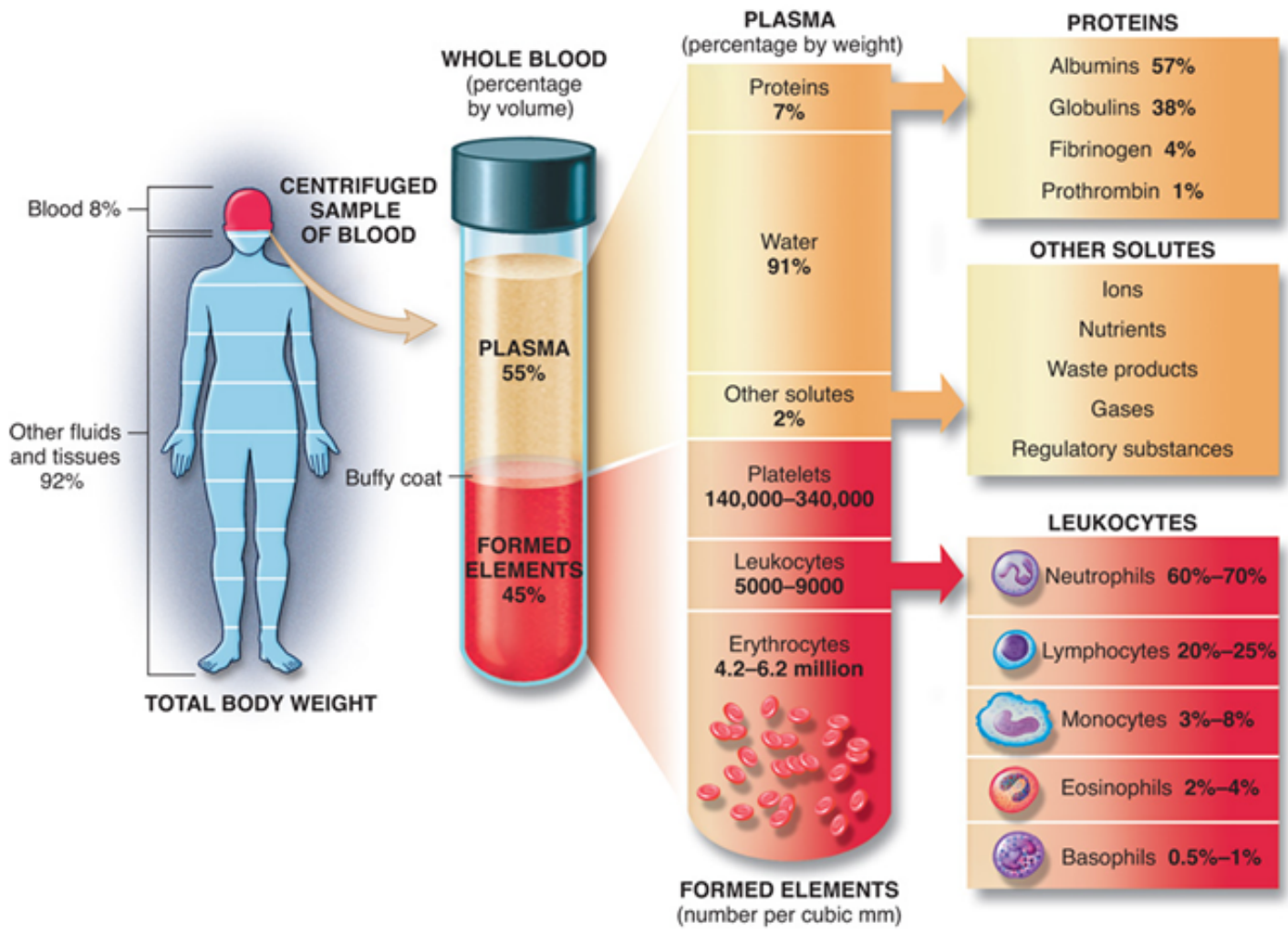
- 
- In an animal cell, DNA is found in the **nucleus** and **mitochondria**.
 - In a plant cell, DNA is found in the **nucleus**, **mitochondria**, and **chloroplast**.
 - In bacteria, DNA is found in **plasmids** and bacterial **chromosome**.

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



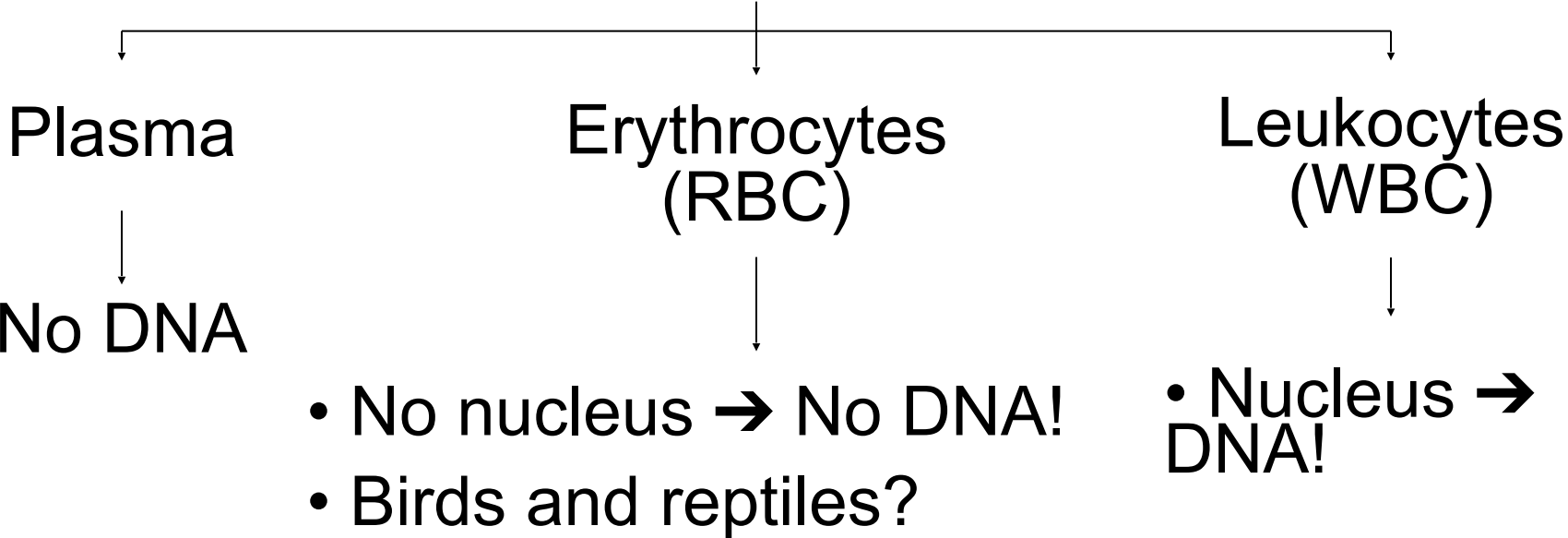


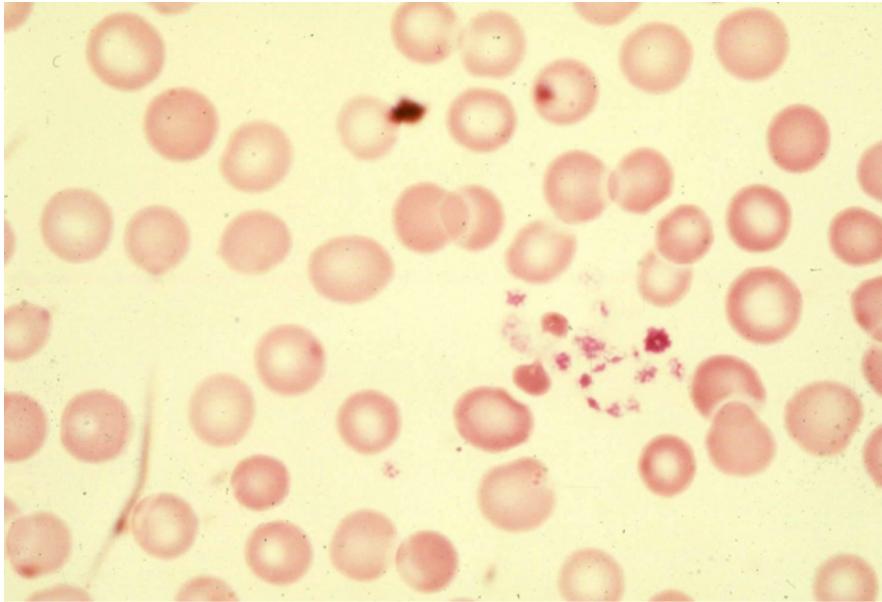
In humans, where can we get DNA from?



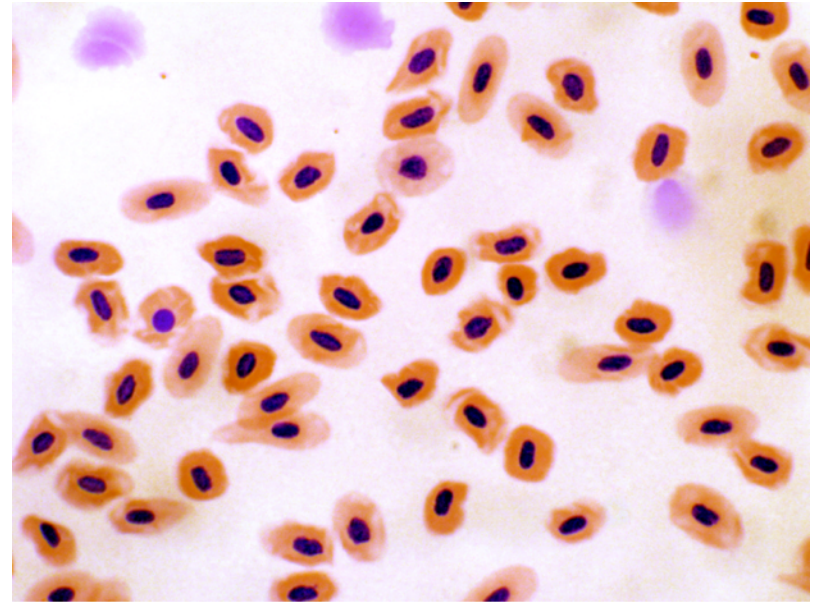


Blood is a connective tissue that is rich in proteins




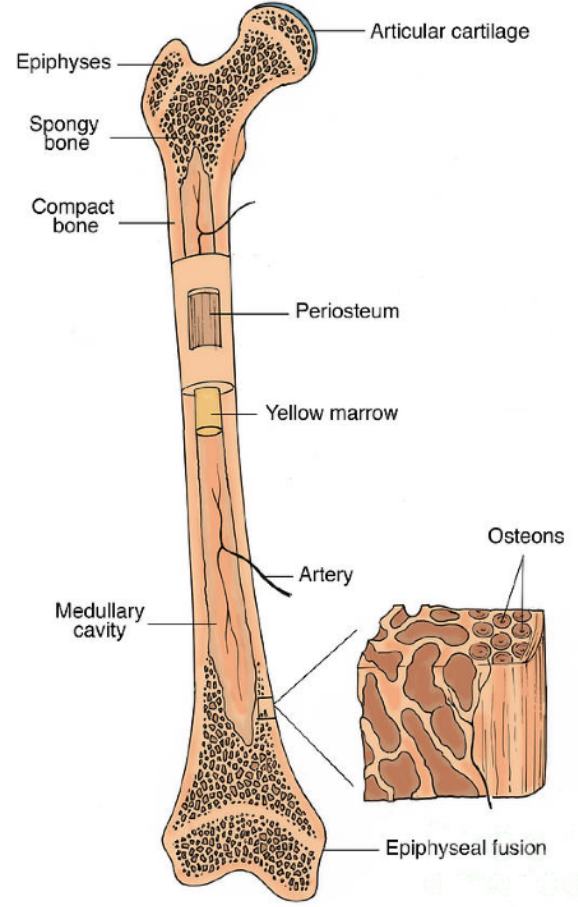
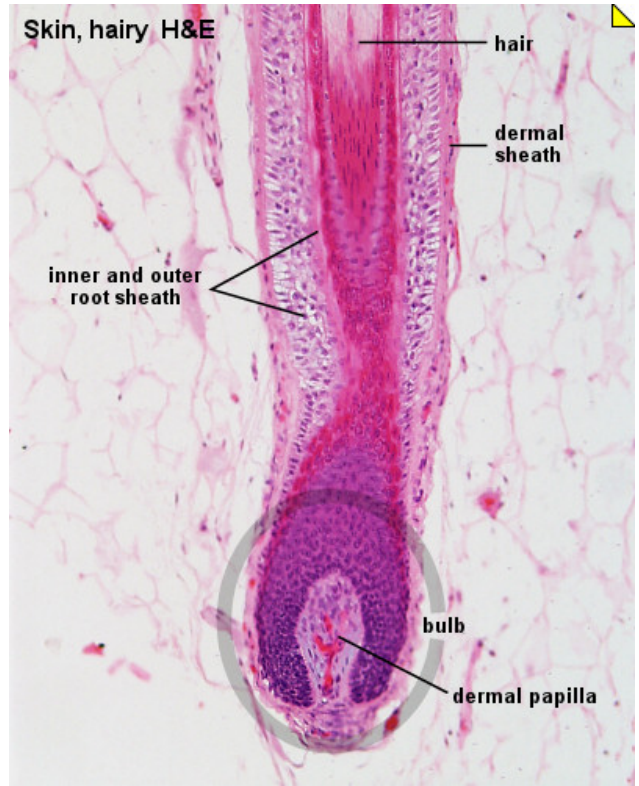
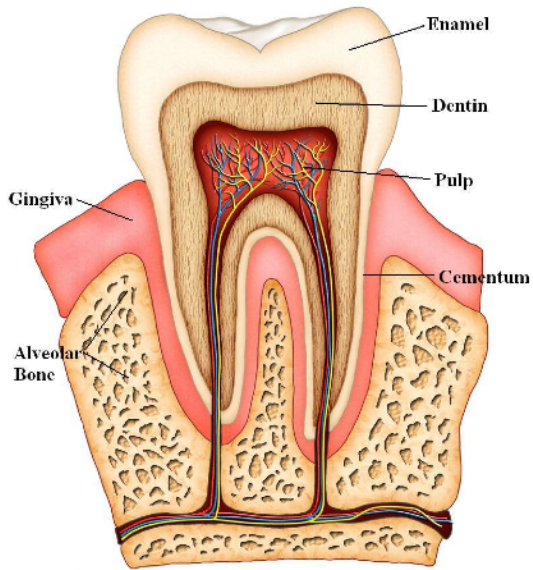


Mammals RBC

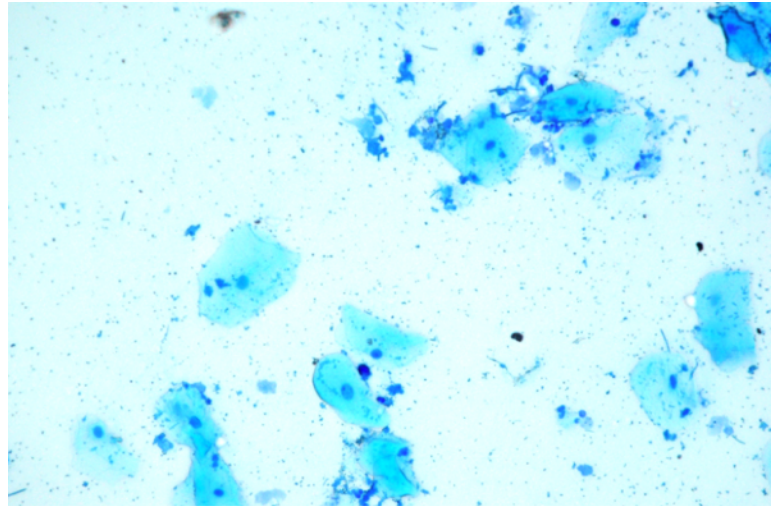


Birds RBC

- 
- 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?



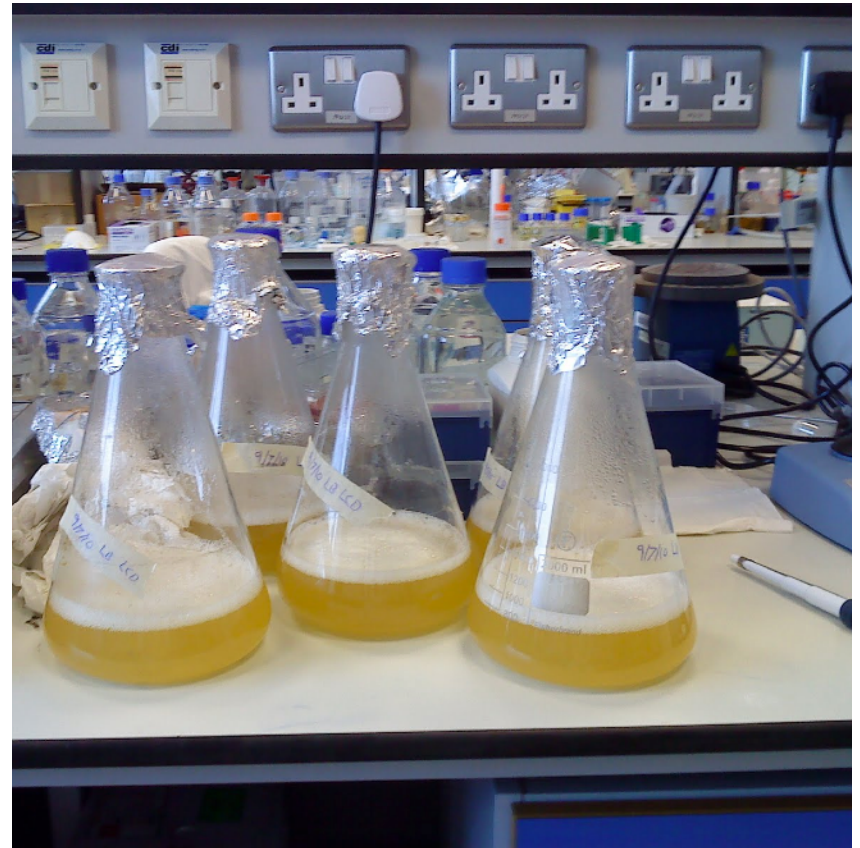
- Saliva samples contain cells (mostly epithelial) and can be a good source of DNA.



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



plate



Flask

Plants contain living cells and non-living cells



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

Animals are not different from humans!



Not every animal tissue has DNA!



How to get DNA out of the cell?

DNA isolation

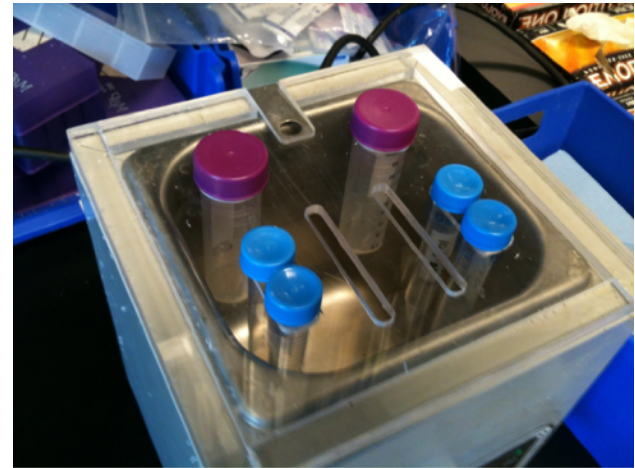
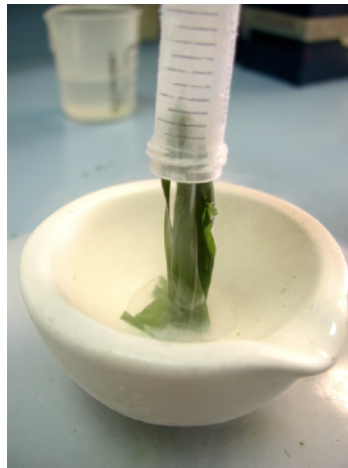
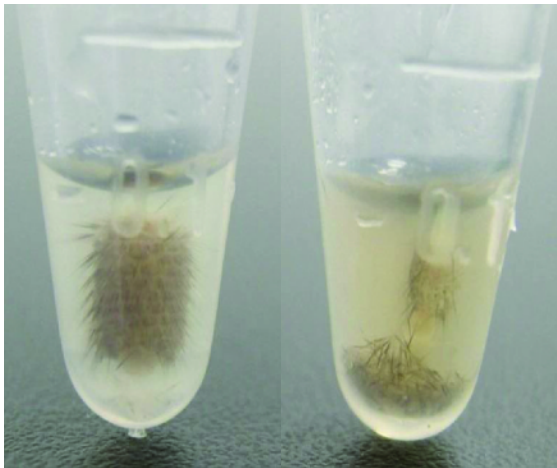
1. Cell and tissue disruption
2. Lysis of cell membrane
3. Separating DNA from the rest of the cell content.

1. Cell and tissue disruption

Enzymatic
(proteinase K)

Grinding
(liquid N)

Boiling
(Alkali)

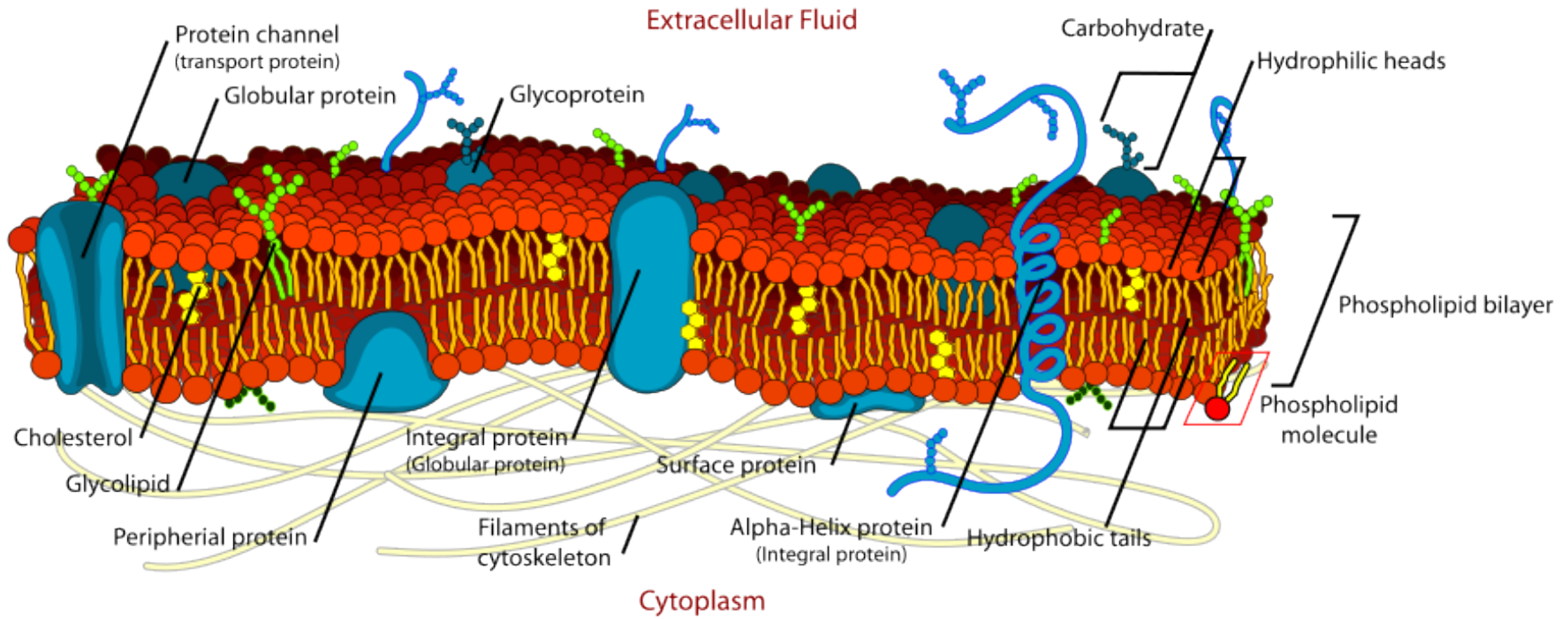




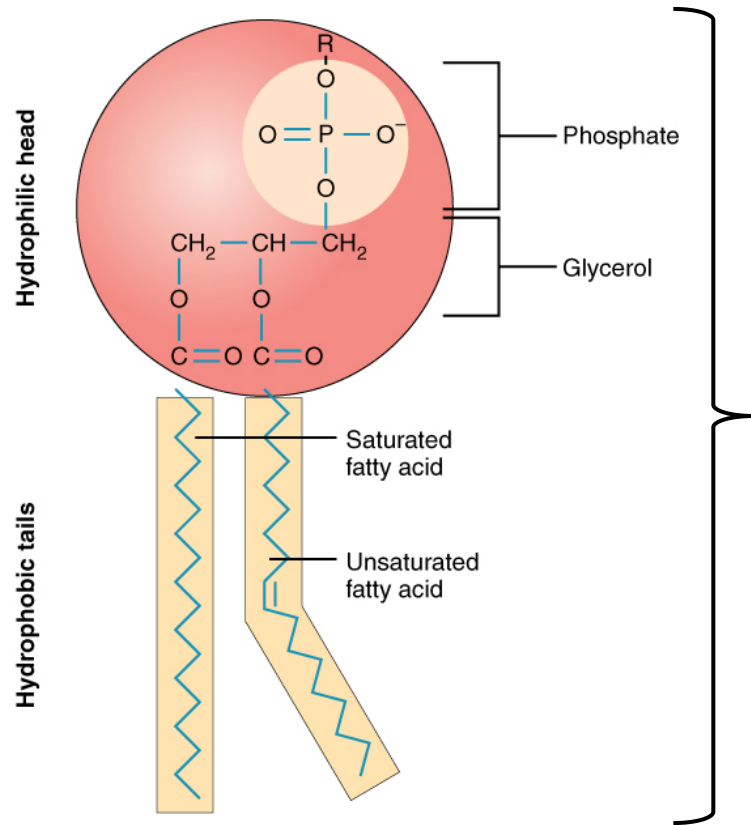
2. Lysis of cell membrane

What is the cell membrane?

What does the cell membrane contain?



- Hydrophilic ?
- Hydrophobic?



Phospholipid molecule



2. Lysis of cell membrane

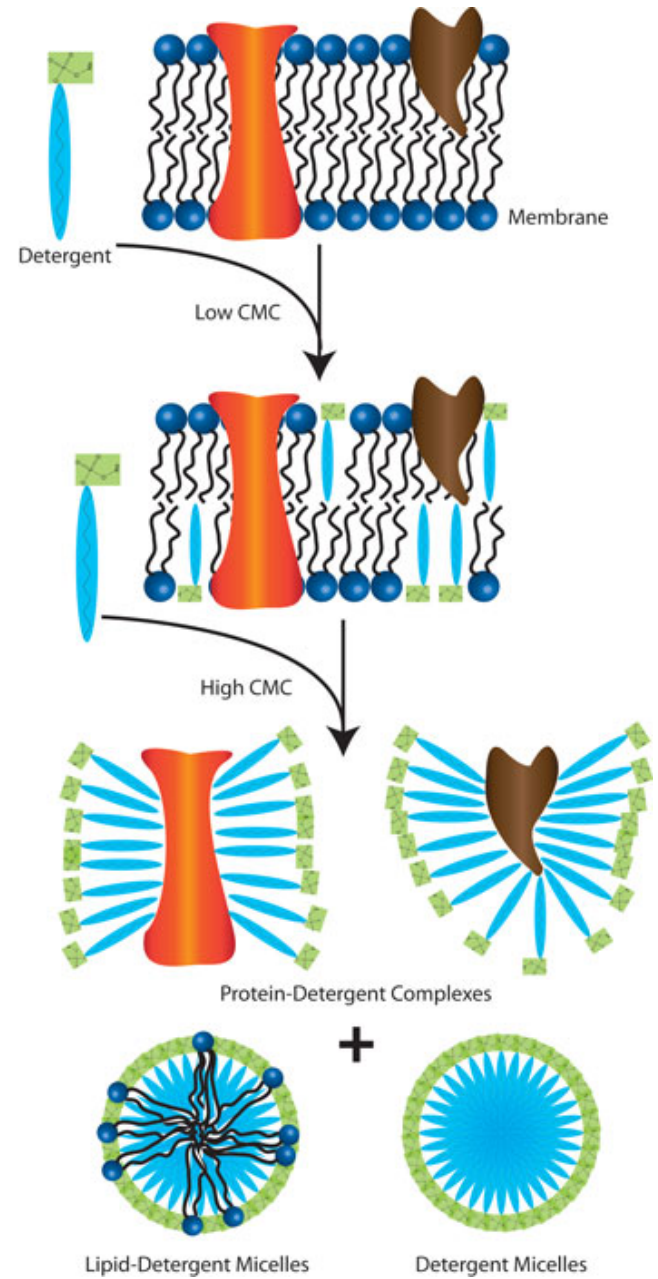
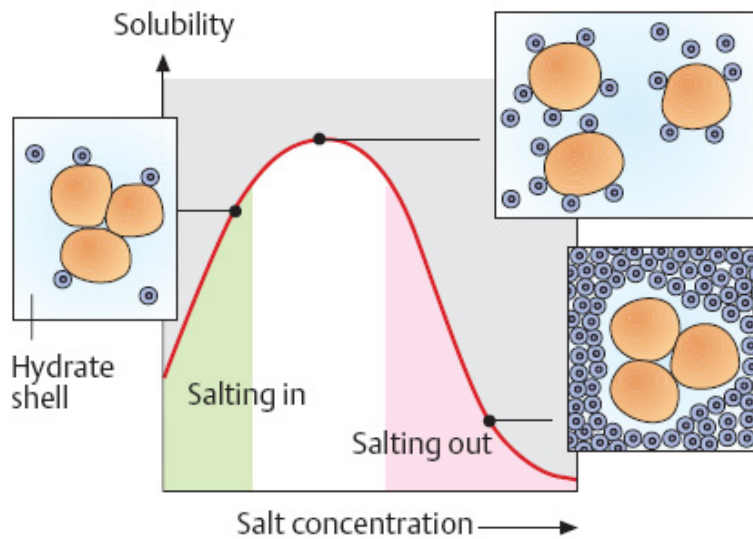
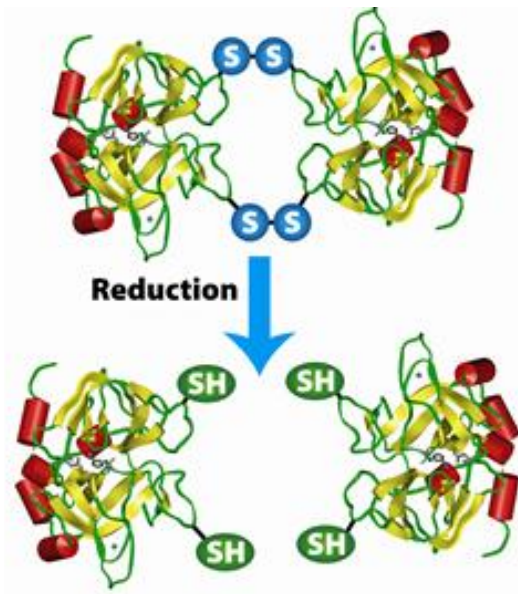
- **Cell membrane contains:**
 - Phospholipid bilayer.
 - Proteins.
 - Other molecules.
- We need to open the cell membrane through **cell lysis**.
- We need to remove the proteins and other molecules.



2. Lysis of cell membrane

Lysis buffer:

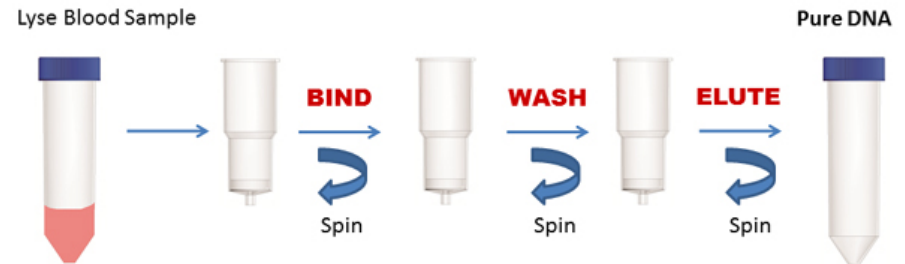
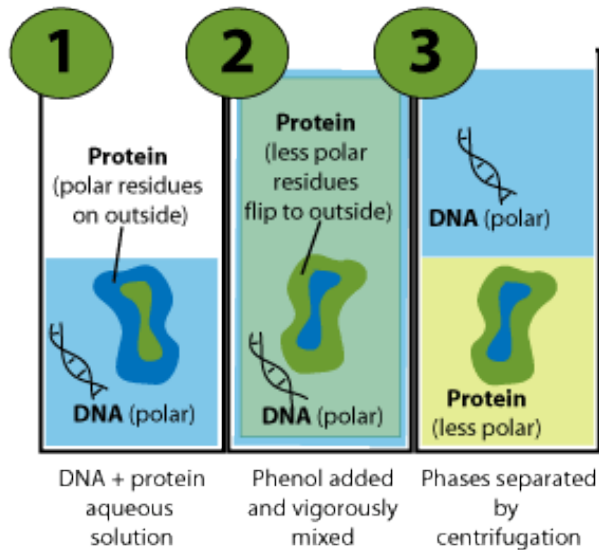
- Detergent (SDS)
- Buffer (Tris-HCl)
- Salt
- Reducing agent
(mercaptoethanol)
- Chelating agent (EDTA)



3. Separating DNA from the rest

Organic extraction
(phenol-chloroform extraction)

Column extraction
(DNA selective binding)





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.



Disclaimer

Figures, photos, and graphs in my lectures are collected using google searches. I do not claim to have personally produced the material (except for some). I do cite only articles or books used. I thank all owners of the visual aid that I use and apologize for not citing each individual item. If anybody finds the inclusion of their material in my lectures a violation of their copy rights, please contact me via email.

hhalhaddad@gmail.com