Mon June 20, 2016
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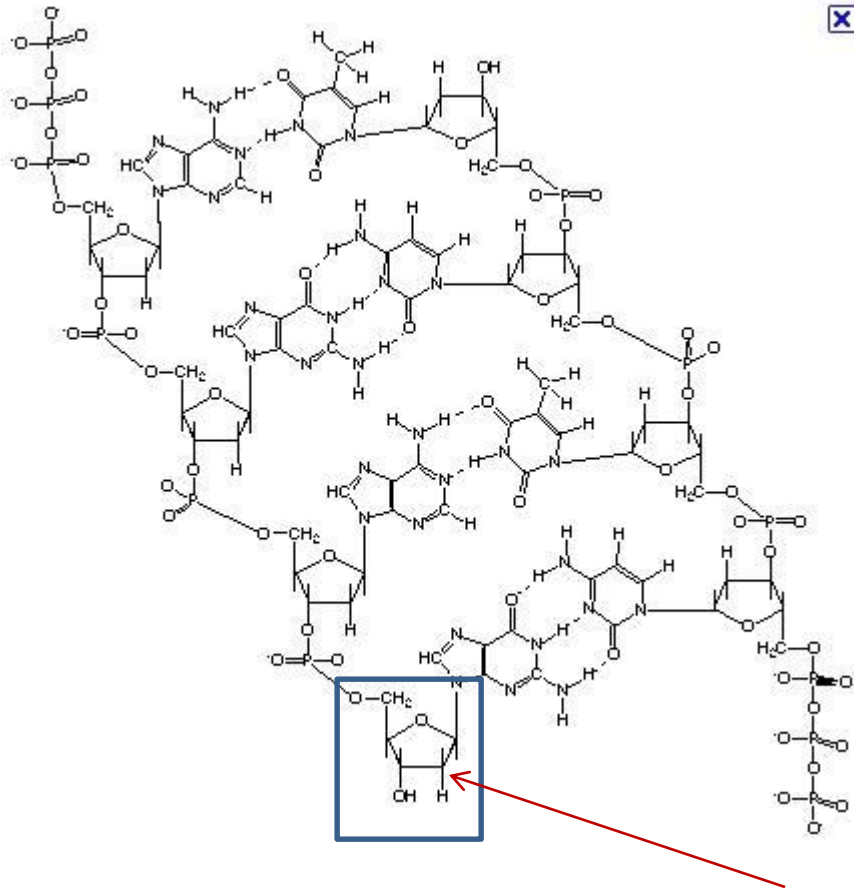
DNA extraction from grinding cells- what is happening at each step

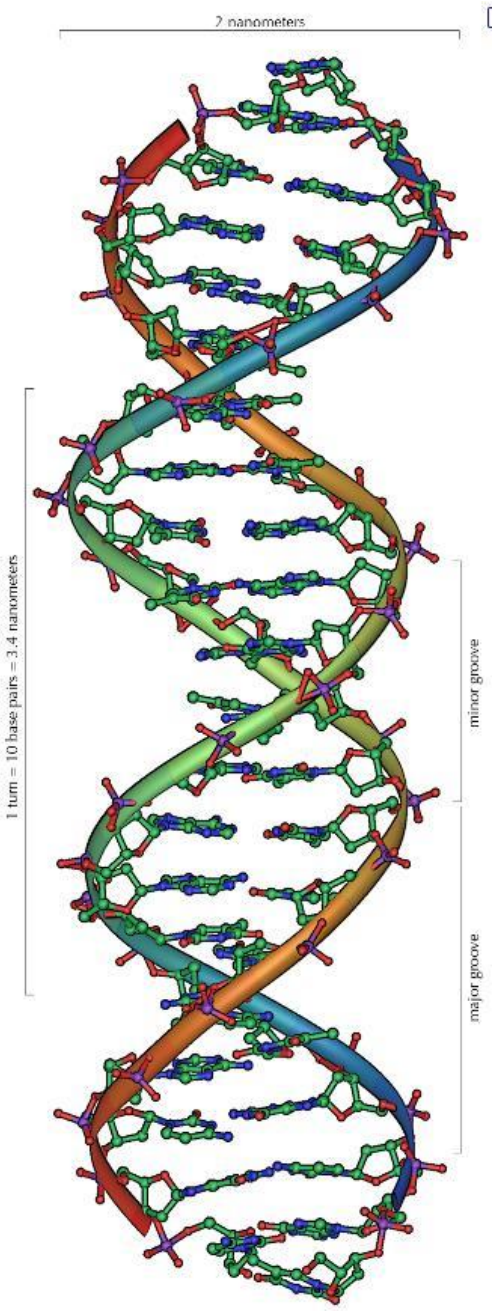
Living organisms have cells

- The directions for what a cell does are in the DNA, so ‘all’ cells have DNA.
  - Red blood cells in mammals are oxygen-carrying packages – no DNA is included.
  - Viruses get other cells to do the work, some have RNA instead of DNA
- Some cells have two sets of nested containers – the inner part is the nucleus with the DNA

Deoxy-ribonucleic acid

- Also ribonucleic acid….

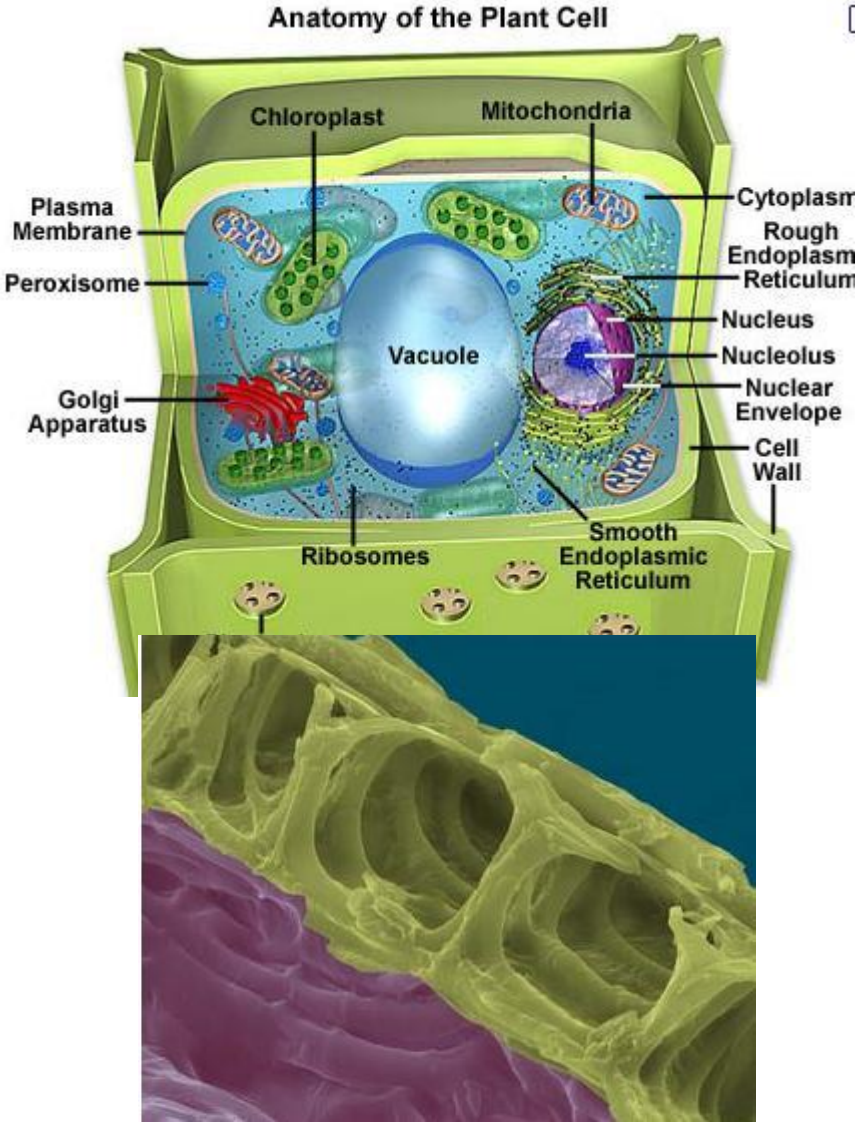


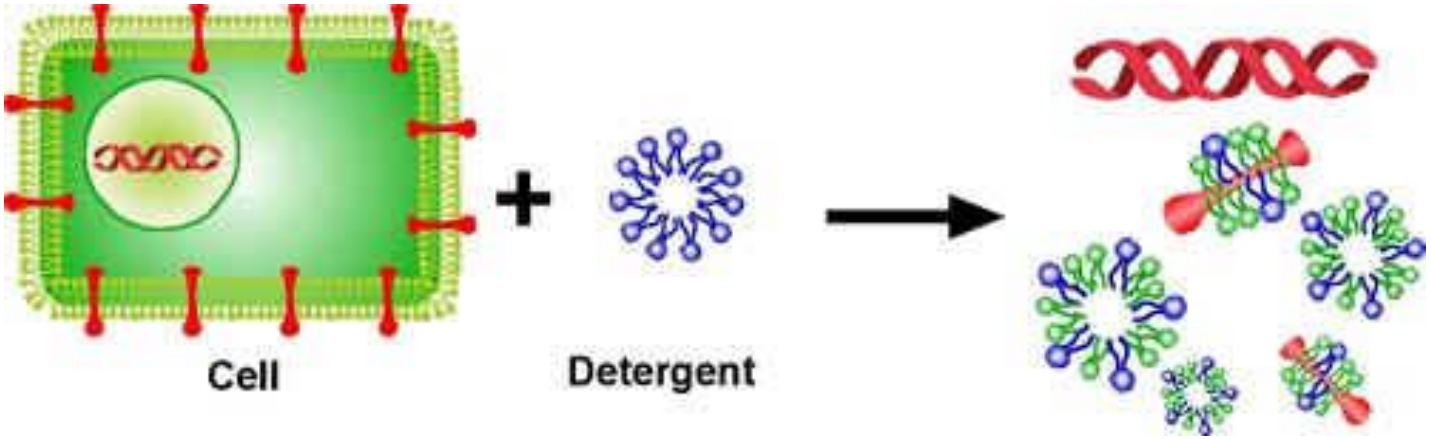


http://familypedia.wikia.com/wiki/DNA

Cell structure is based on separating the inside from the outside.

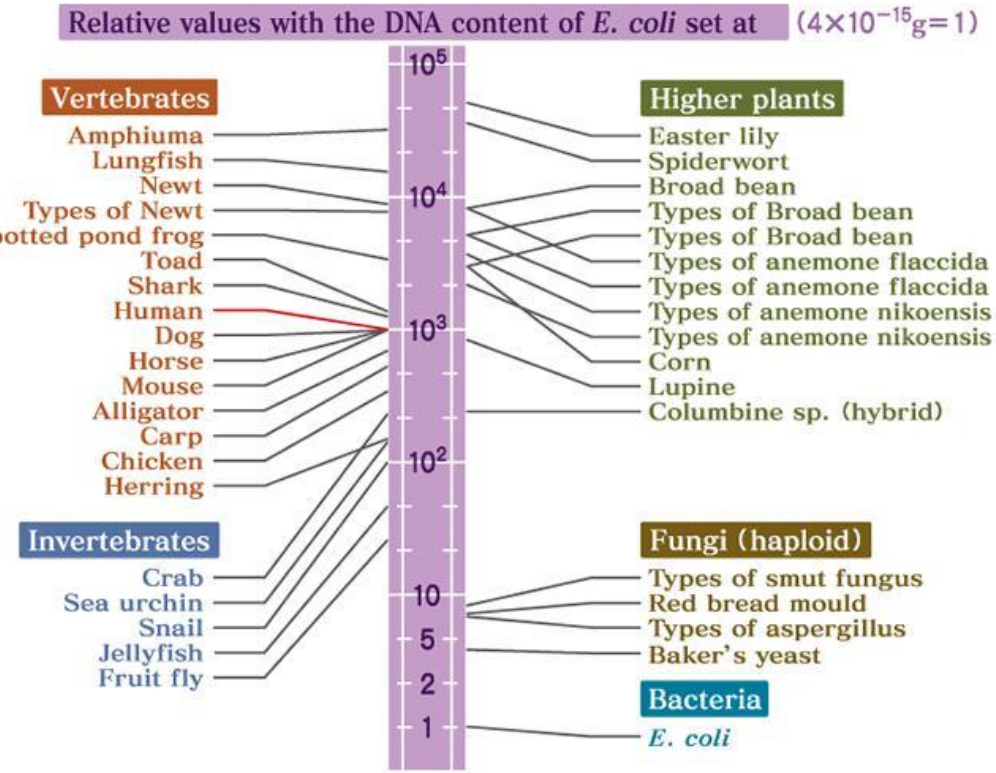
- Membranes and cell walls form the barriers for cells.
- To get out the DNA you have to break open the cell.
  - Soap will dissolve membranes
  - Cell walls have to be crushed
    - Things inside the cells tend to get crushed as well.




Cell
Detergent

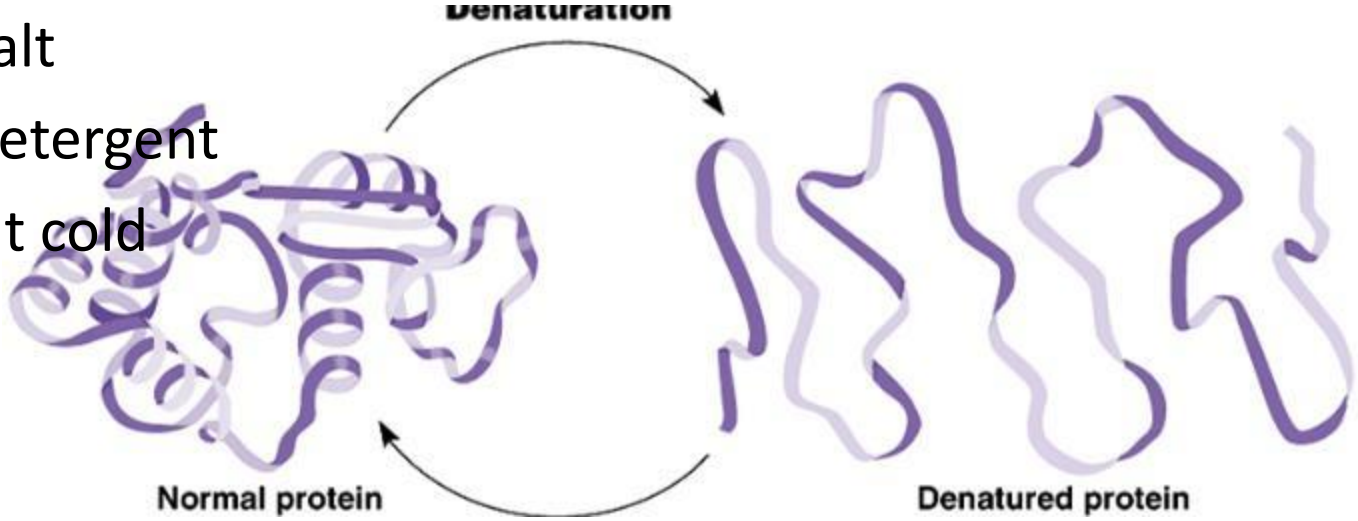
The amount of DNA per cell is very small, so you need some idea about your assay needs.

- How much DNA (the mass) does <u>one</u> plant cell have?
- If I want 10 micrograms of DNA how many plant cells do I need to break open?
- How much leaf tissue is this (what is the mass one leaf cell)?
- What if my efficiency of recovery is only 50% - how much more leaf tissue will I need?



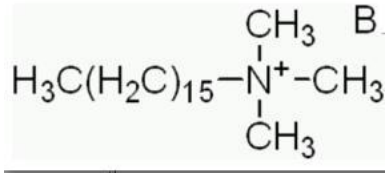
Lysis buffer contains chemicals that protect the DNA while we extract it.

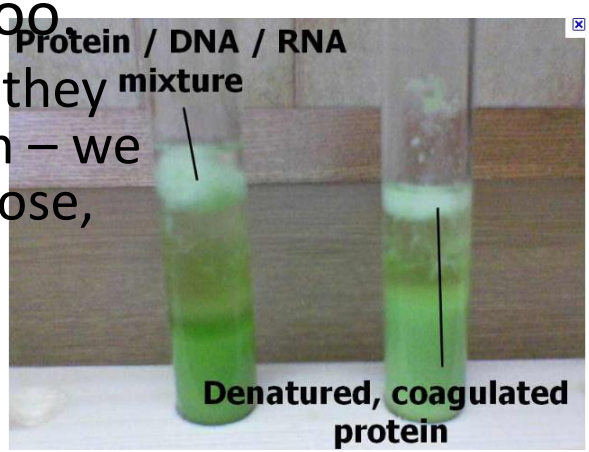
- Cells have active ingredients that can break down DNA – these are chemicals and enzymes.
  - We can stop the processes by keeping the sample cold and blocking or inactivating the enzymes
    - Add salt
    - Add detergent
    - Keep it cold



Plant cells have a lot of carbohydrate and a lot of small chemicals that can react with the DNA.

- You can change how carbohydrates behave by reacting them with other compounds.
  - We will use a detergent called CTAB that makes the carbohydrate mix with chloroform better than with water.
  - The reaction is slow so we use heat and mixing to make sure it is complete.
- A lot of the small chemicals will dissolve in phenol or chloroform, so we add a little phenol too.
  - This will cause proteins to denature so that they won't dissolve in either water or chloroform – we have to add another method to separate those, centrifugation.



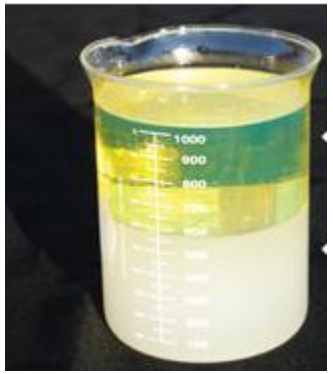


The DNA and RNA stay soluble in the buffer, the goal is to remove everything else.

- What is the difference between something suspended in a liquid and something dissolved in the liquid?
  - Does it sink or float?
  - Mashed up cells are suspended, with some of the inside stuff (cytosol) dissolving if the liquid is water-based (aqueous).
- What is the buffer?
  - Water with some salts and detergent, and also something that keeps the sample from becoming too acidic or basic when the cells break open.
    - TRIS is a very common biological buffer – it balances the acidity to what is common inside cells.

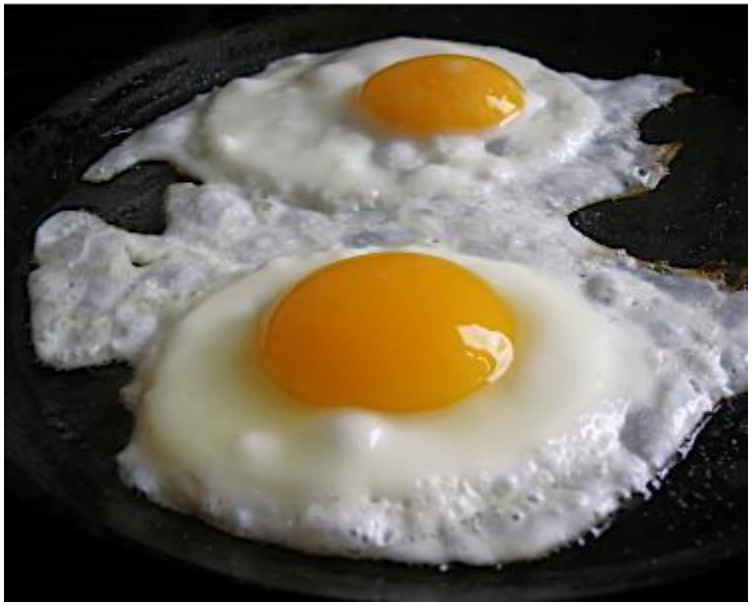
Separating samples using different solubility

- Most biological samples are handled as solutions – in liquids.
- Different parts are MORE soluble in different types of liquids
  - DNA dissolves in water-based liquids
  - Membranes dissolve in oily liquids
    - If you use a detergent you can break up the membrane and the bits + detergent will be soluble in water.
- You can play with differential solubility to separate the parts
  - Oil and water do not mix. If I have something that will dissolve in water and I shake it with oil, it will stay in the water layer.
  - If I put something in the water layer that is more soluble in oil, if I shake oil and water together it will transfer to the oil layer. I have extracted it from the water layer, or phase.



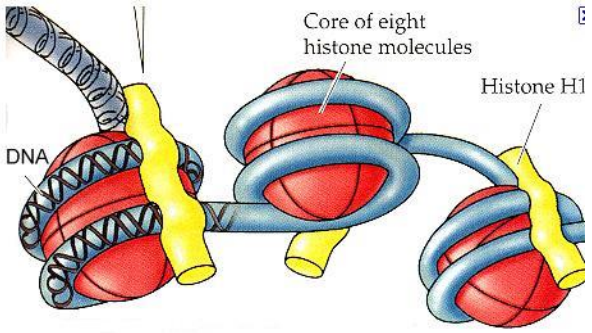
Changing specific properties can also be used in purification methods.

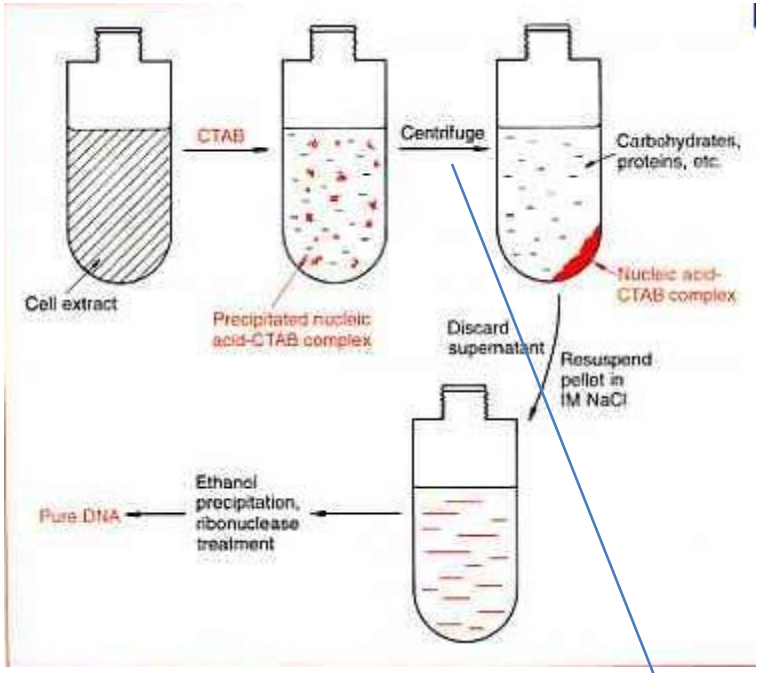
- Some materials change properties depending on their shape.
- Proteins (some) can dissolve in water-based buffers when they are folded up properly but not when they are unfolded
  - Egg white – uncooked it can be shaken up with water to make a solution. Cooked, not so much.
    - The heat 'denatured' the protein so it lost its folded up structure.

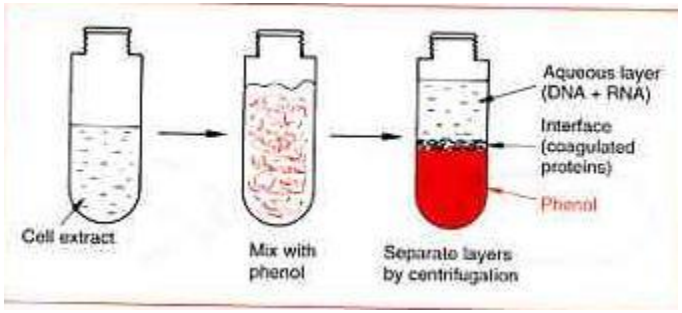


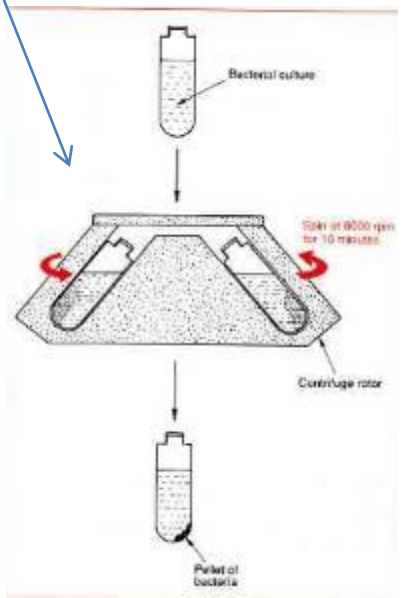
Chromosomes are packaged with proteins that can be hard to remove.

- The six feet of DNA length in your cells has to get wrapped up in order to fit.
  - It is looped around proteins (histones) and these can be hard to remove.
  - There are proteins that break down other proteins (Proteases) – if we add these to the sample the histones are much easier to remove from the DNA.
  - Then use detergent and phenol to denature the protein pieces and phenol and chloroform to extract them away from the DNA.




CTAB
Centrifuge
Carbohydrates, proteins, etc.
Nucleic acid-CTAB complex
Cell extract
Precipitated nucleic acid-CTAB complex
Discard supernatant
Resuspend pellet in 1M NaCl
Ethanol precipitation, ribonuclease treatment
Pure DNA

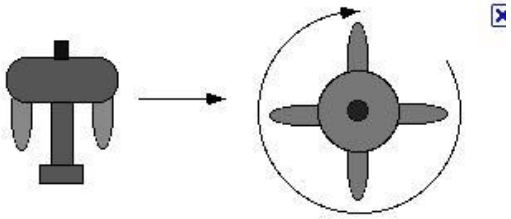

Aqueous layer (DNA + RNA)
Interface (coagulated proteins)
Phenol
Cell extract
Mix with phenol
Separate layers by centrifugation

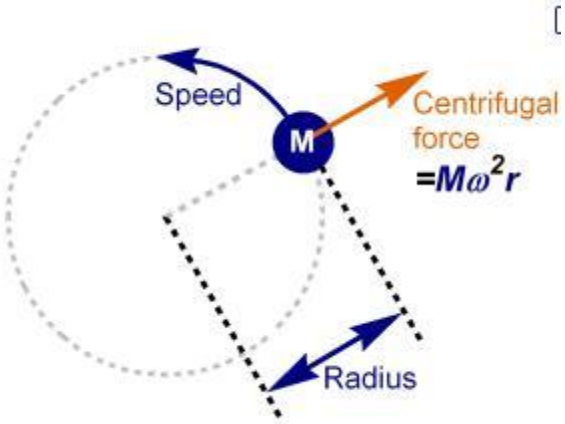

Bacterial culture
Centrifuge rotor
Pellet of bacteria

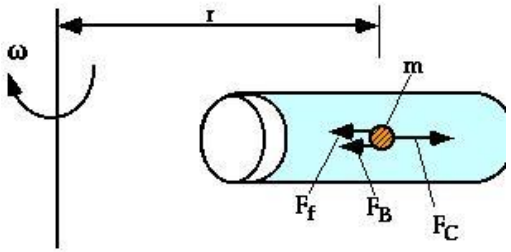
Concentrating the DNA

- The DNA is in solution, in the buffer, but it is very dilute – I want to concentrate it – how?
  - Evaporation
  - Precipitation – make it insoluble.
    - Add an alcohol – DNA does not stay in solution in 70% alcohol. Add 100% alcohol to the buffer until the final solution is 70% alcohol.
    - It will settle out of the solution but I can collect it more quickly if I centrifuge it.

Centrifugation

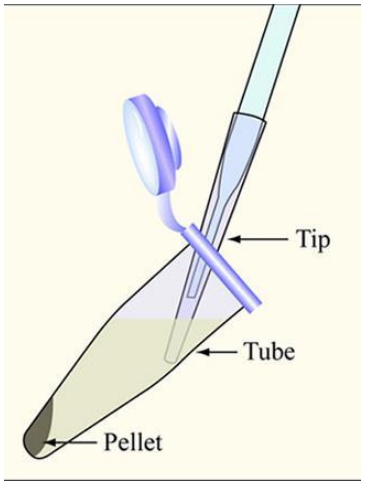






Balance tubes that are directly across from each other!!!

Handling small samples



Re-solubilizing the DNA

- Remove the alcohol from the tube, try not to touch the pellet of DNA.
  - Remove it slowly so the meniscus travels down the tube and not drops are left behind.
  - Let the pellet air dry
  - Add a small volume of buffer to the pellet and let it sit so the DNA goes back into solution, or shake it gently to speed this up.
    - I want to give this enough time that the DNA solution is homogeneous (the same everywhere) – why?

Now I have DNA for my genetic assay

- Actually, I still have to remove the RNA (They purify together). I will use an enzyme to do this, RNAase, and then remove the enzyme, use ethanol to precipitate the DNA one more time, and resuspend it.
- Then I will check the concentration of the DNA with a spectrophotometer, and double-check this using an agarose gel.
- THEN I will be ready to do a genetic assay.

Following a protocol

- Read through all of the directions **before** starting anything.
- Make sure you understand how to do all of the steps
- Make sure you have all of the required solutions, and enough of them'
- Make sure you have the right equipment and supplies (gloves, tips, tubes, etc)
- If something needs to be set to some temperature before use get it started – don't begin until the correct temperature is reached.
- If some of the steps need to be timed be sure you have a timer available.
- If some of the chemicals need to be stored on ice get an ice bucket and ice before taking them out of storage.
- If some material is to be discarded make sure you know if it can go down the sink or needs to be put in a special waste container – and make sure you know where that is.
- If you will not have time to complete the protocol in one day make sure you understand where the allowed stopping times are, and how the sample must be stored (at room temperature in alcohol? In a freezer?).