

DNA Extraction



DNA extraction

The problems with isolating DNA, are:

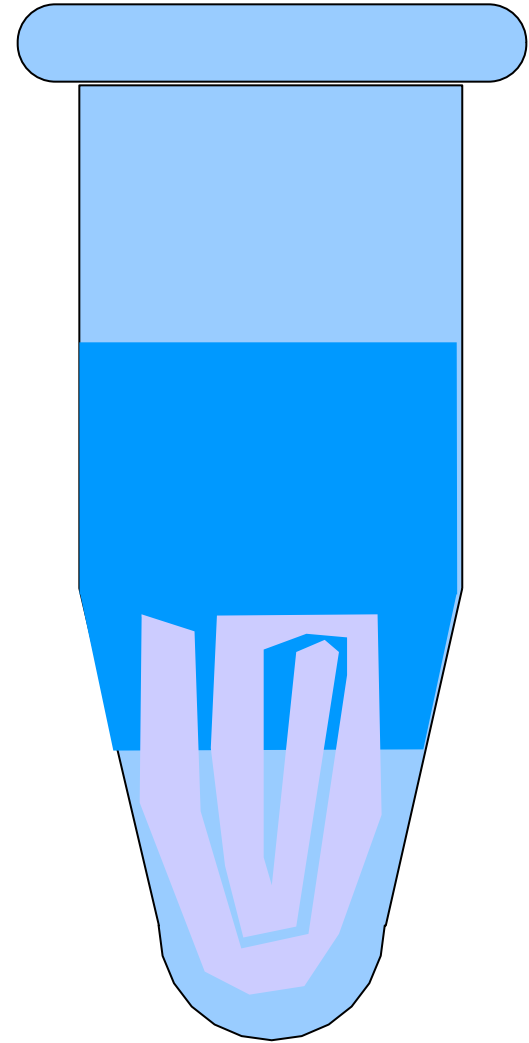
- The DNA is found in nucleus so it is enclosed by two membranes
- the presence of DNAses, which degrade the DNA
- the presence of other macromolecules which co-purify with or polymerase to the DNA during isolation

DNA extraction

- We should prepare lyses buffer:
 1. Tris which is correcting for pH
 2. Chelators such as EDTA to removing cations such as Mg which are required for nuclease activity
 3. Detergents such as sodium dodecylsulphate (SDS) are used to dissolve the cell membranes, inhibit enzyme activities, also dissociates proteins from DNA and make them more accessible to degradation by proteinases used in DNA isolation
 4. NaCl elevate salt concentration

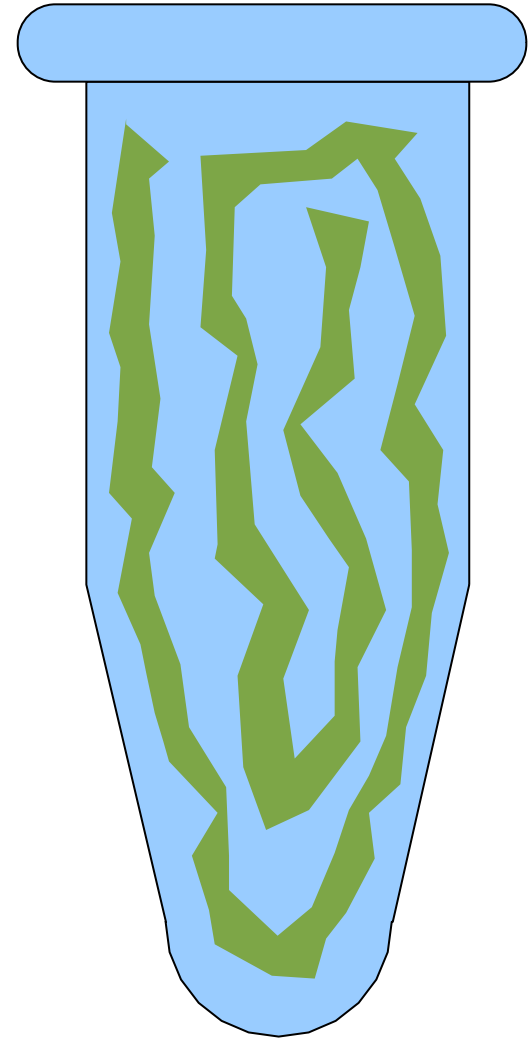
DNA Isolation

dissolving cells in lyses buffer



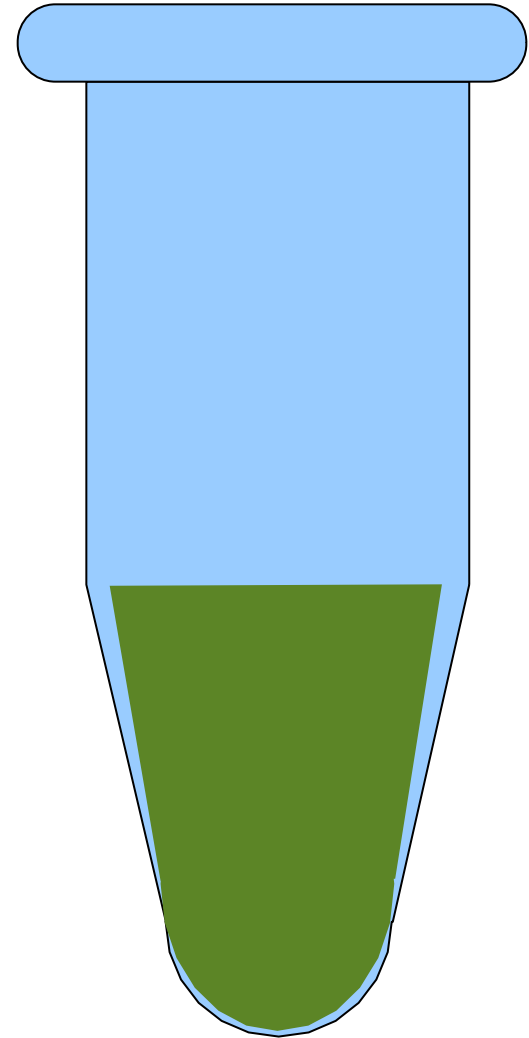
DNA Isolation

Dissolved in lyses
buffer shaking



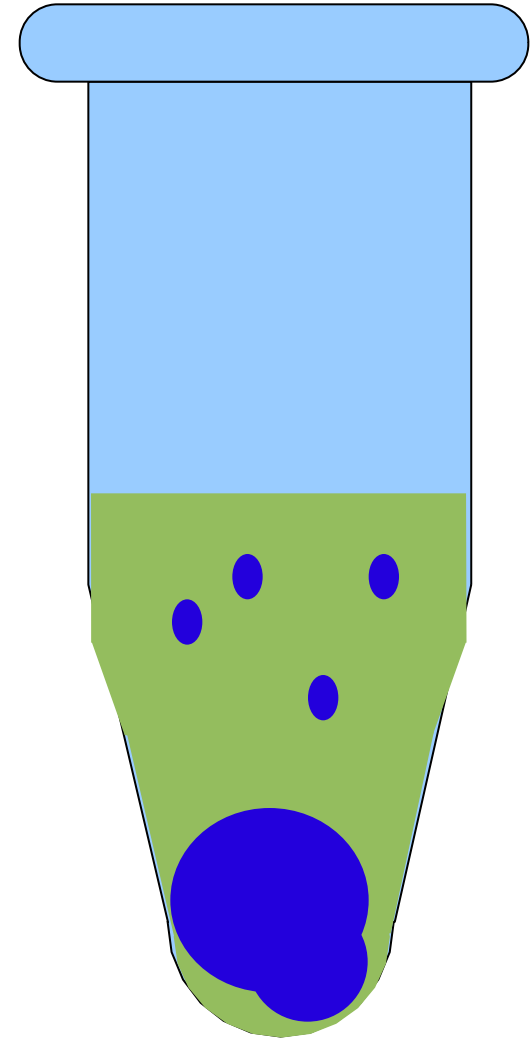
DNA Isolation

Cell start to be lysed
and the buffer become
more dense



DNA Isolation

**Separate phases by
Centrifugation
Cell debris will appear
And some proteins are
still in buffer**

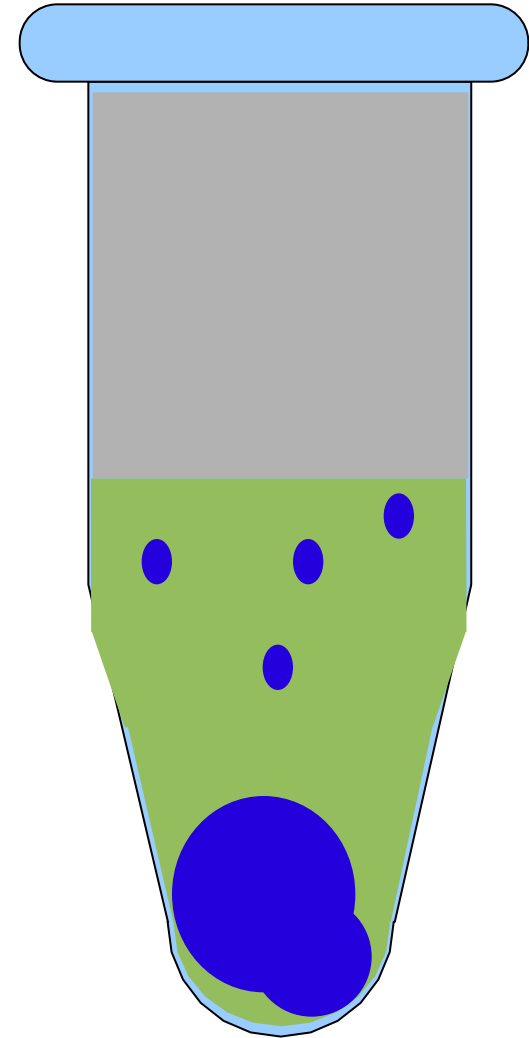


DNA Isolation

- To remove the small proteins, organic solvents used
- Organic solvent denatured these proteins and make it precipitated
- Organic solvent include:
 1. Neutral phenol 25ml
 2. Chloroform 24ml
 3. Isoamyl alcohol 1ml

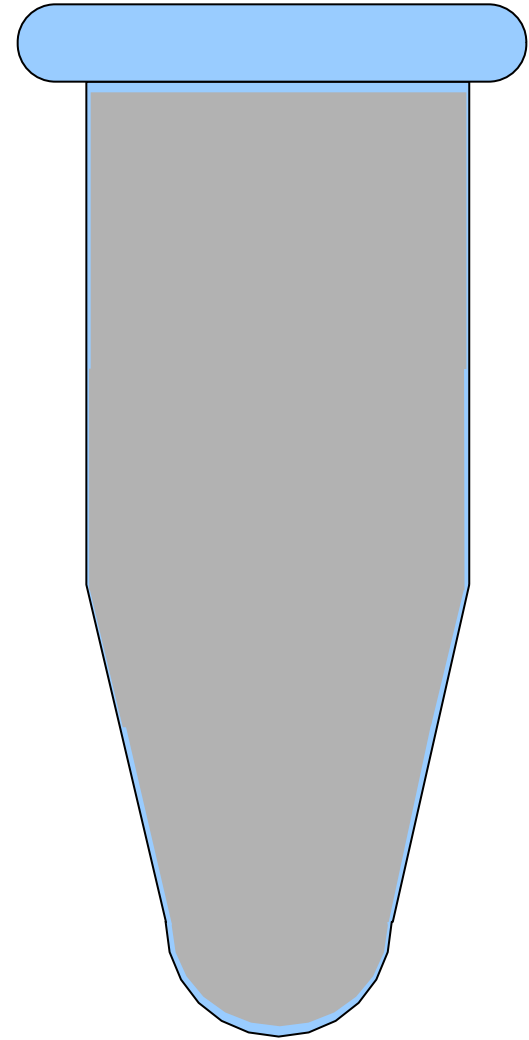
DNA Isolation

The organic solvent
Were added in an
equal volume



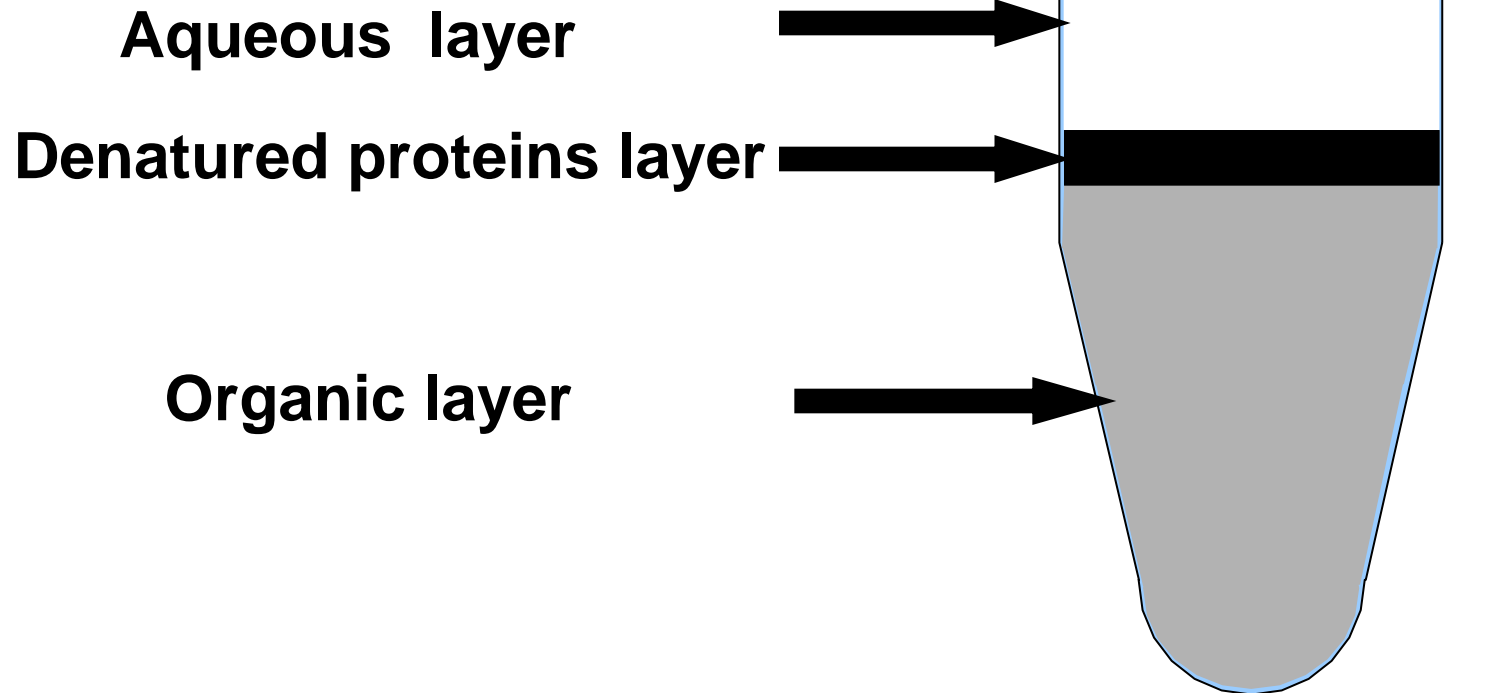
DNA Isolation

Mix it!

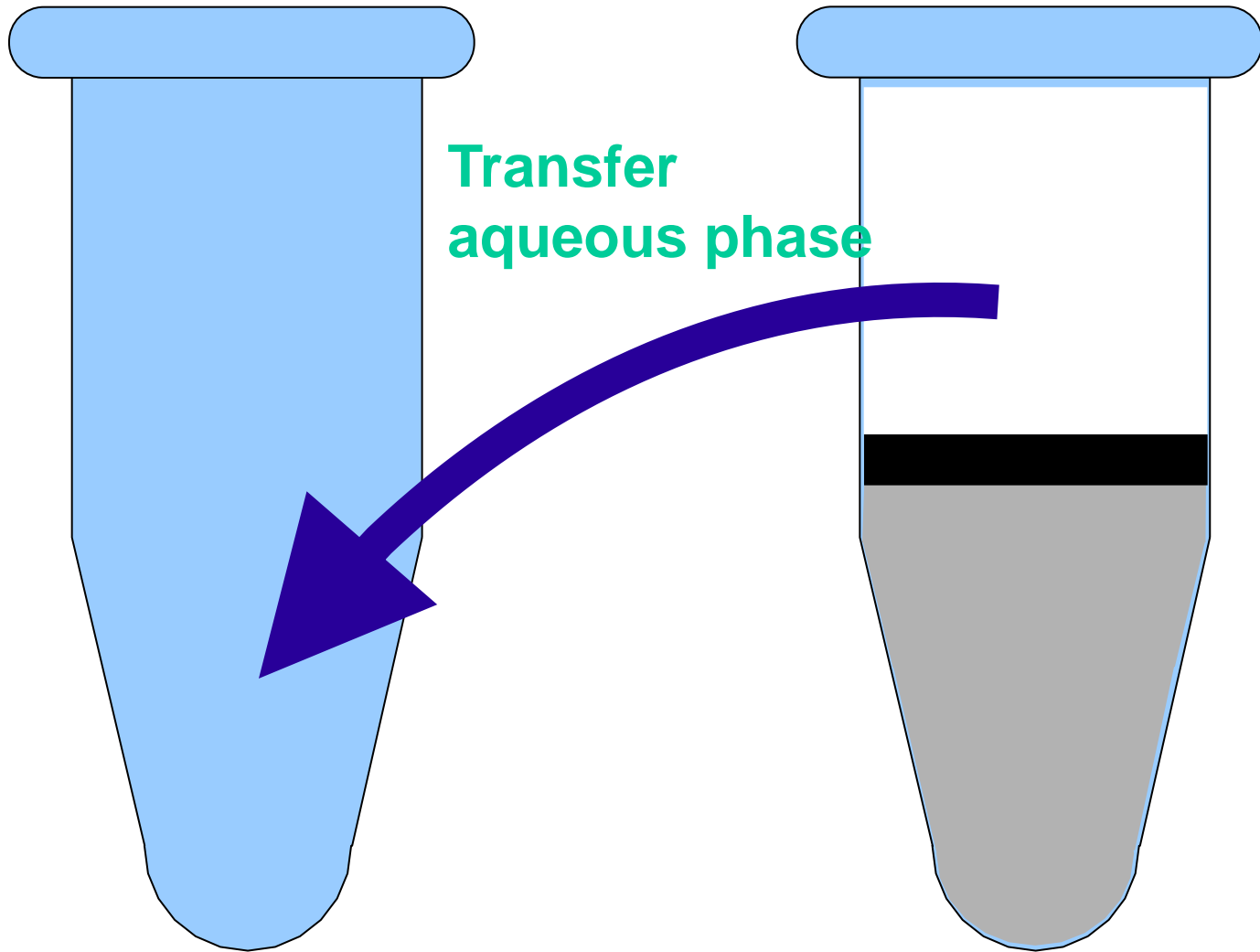


DNA Isolation

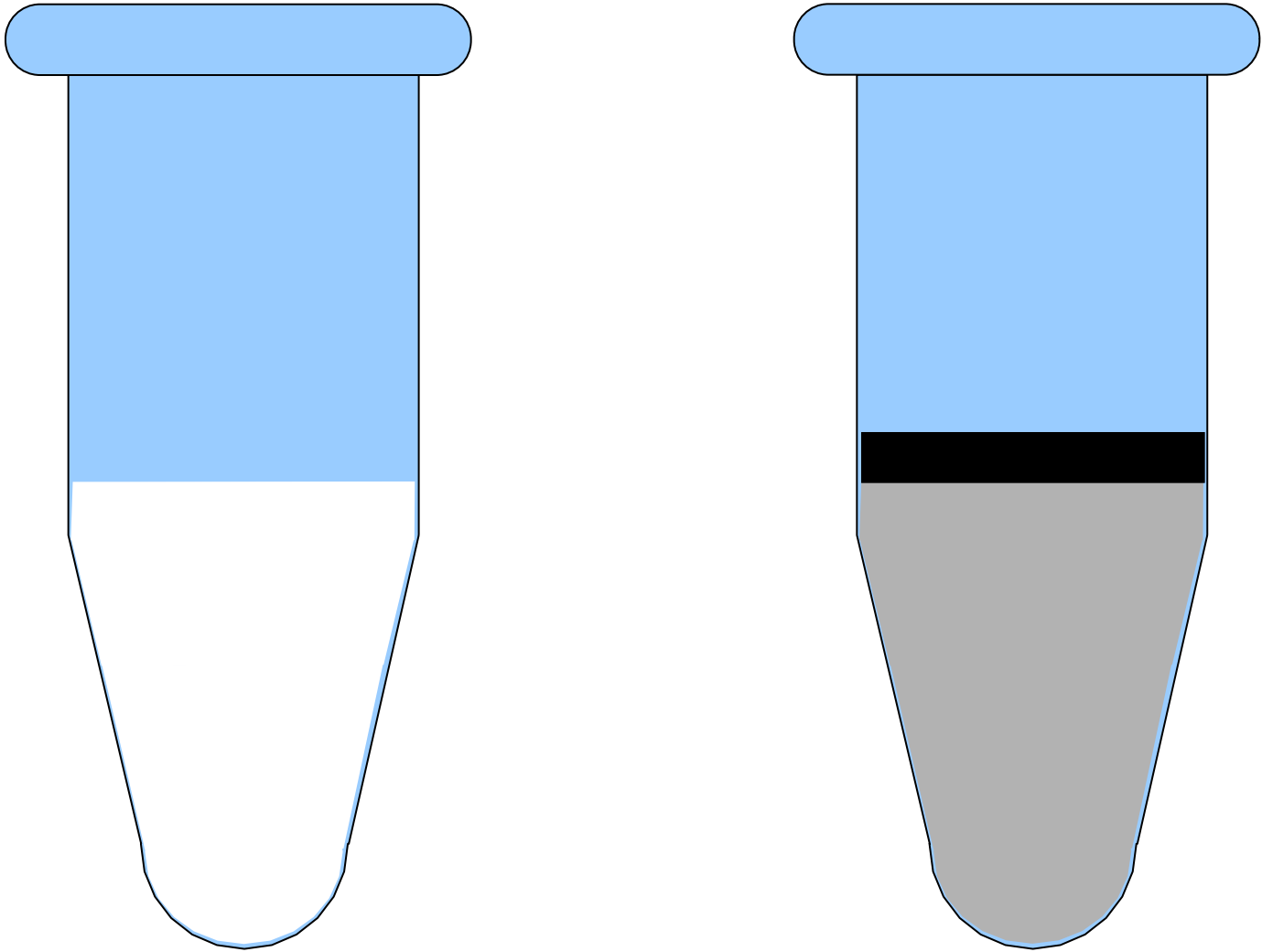
Separate phases by
centrifugation



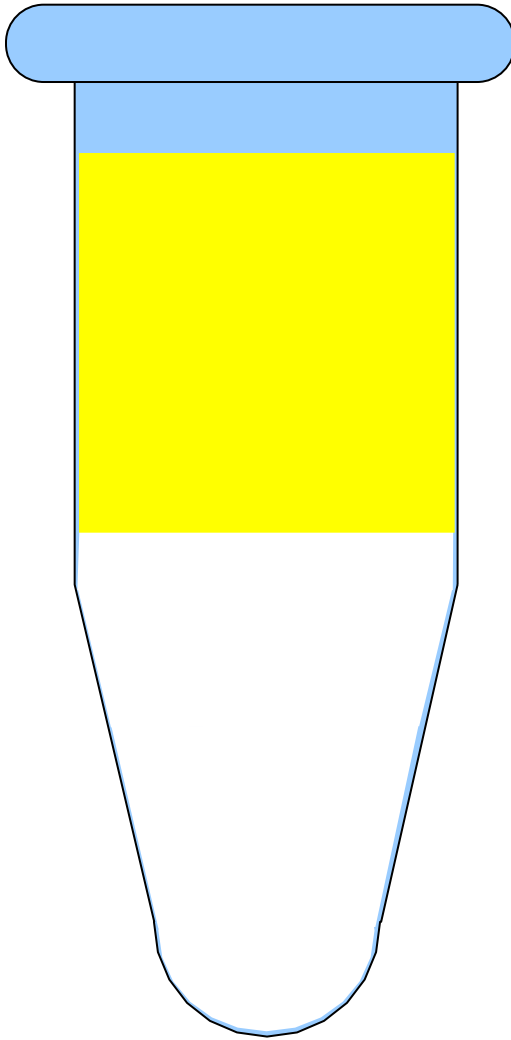
DNA Isolation



DNA Isolation



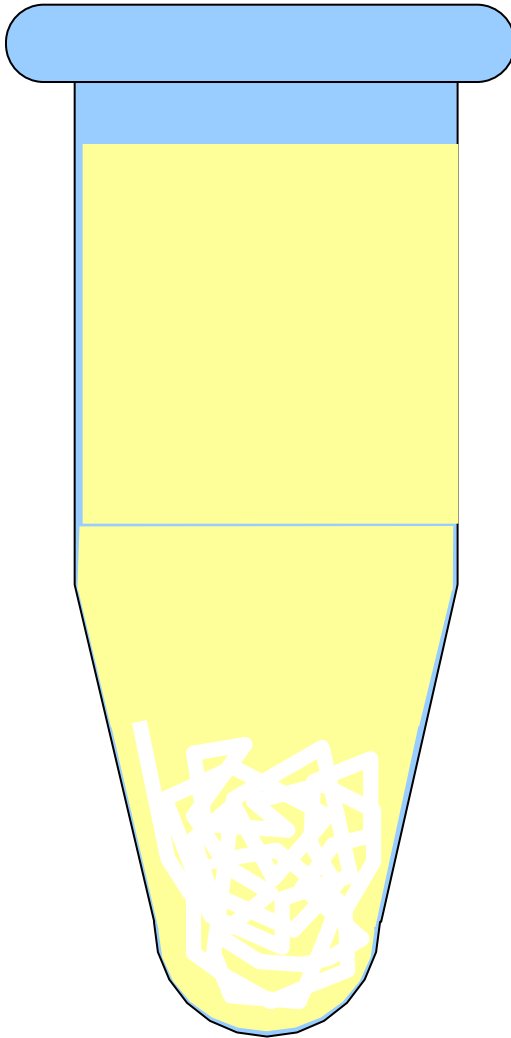
DNA Isolation



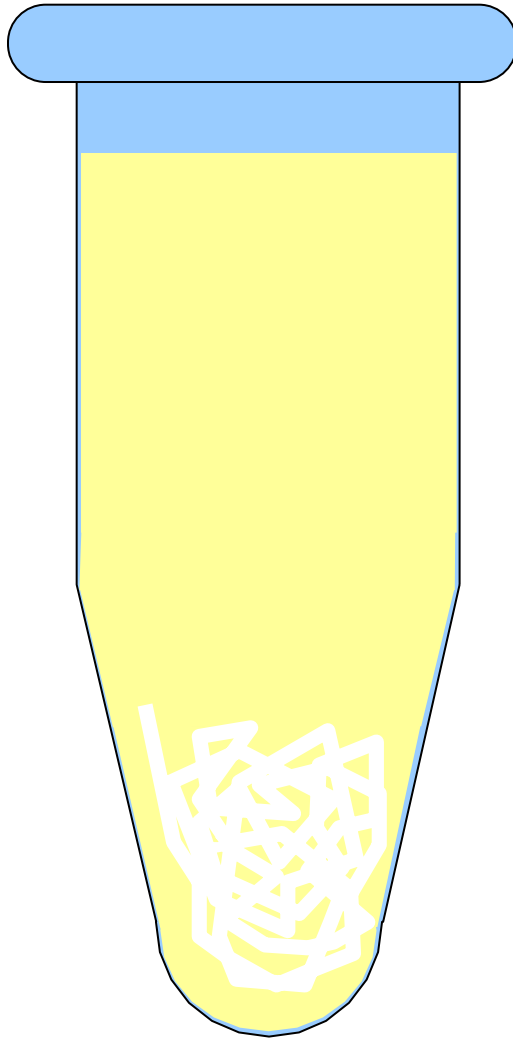
Add iso-propanol
Mix gently to
precipitate DNA

DNA Isolation

Mix gently to
precipitate DNA



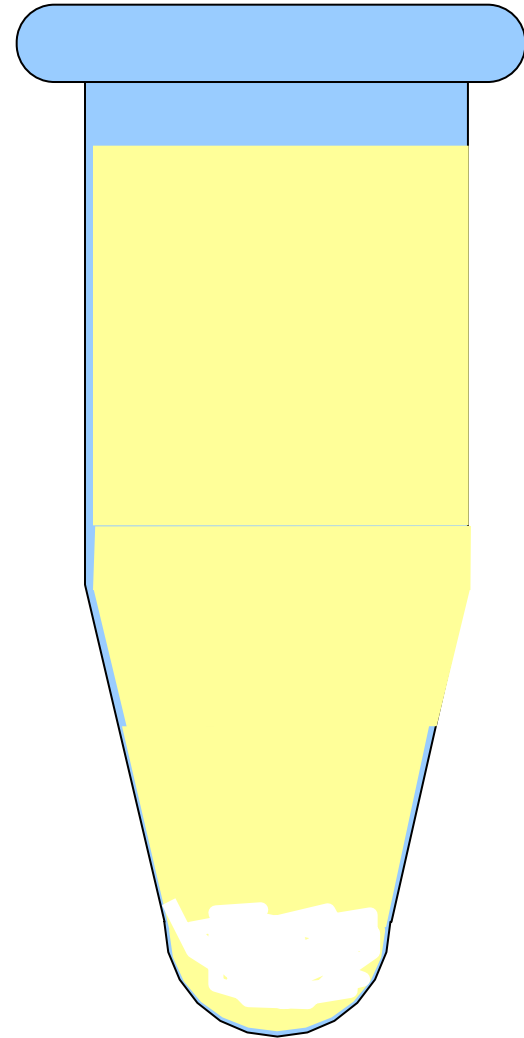
DNA Isolation



centrifugation

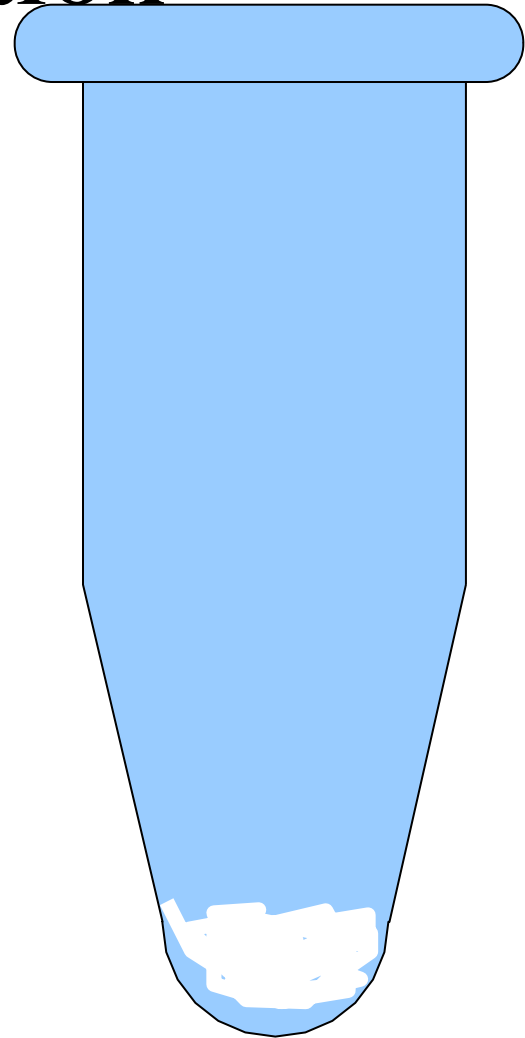
DNA Isolation

DNA pellet formed



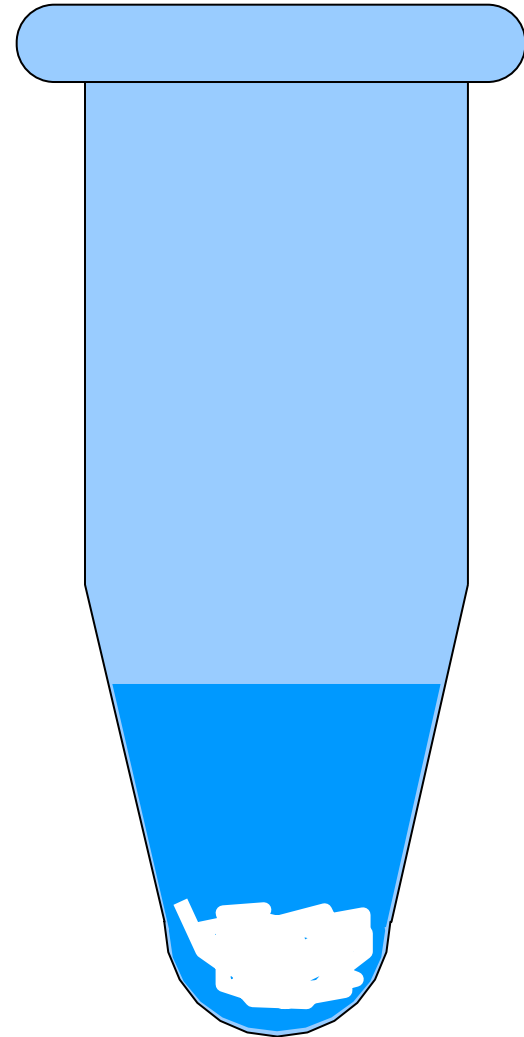
DNA Isolation

Remove buffer



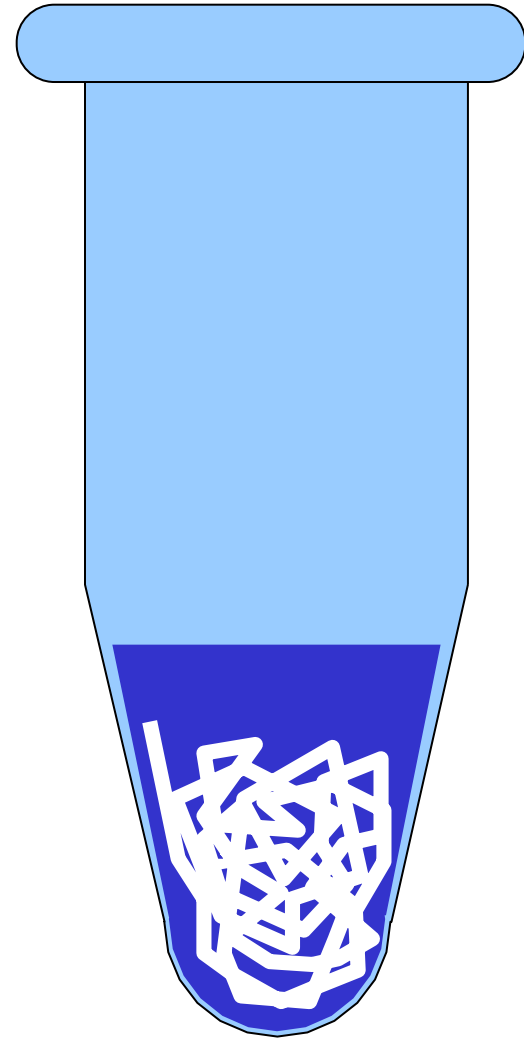
DNA Isolation

Wash with
75% Ethanol
Remove ethanol and
left until dry



DNA Isolation

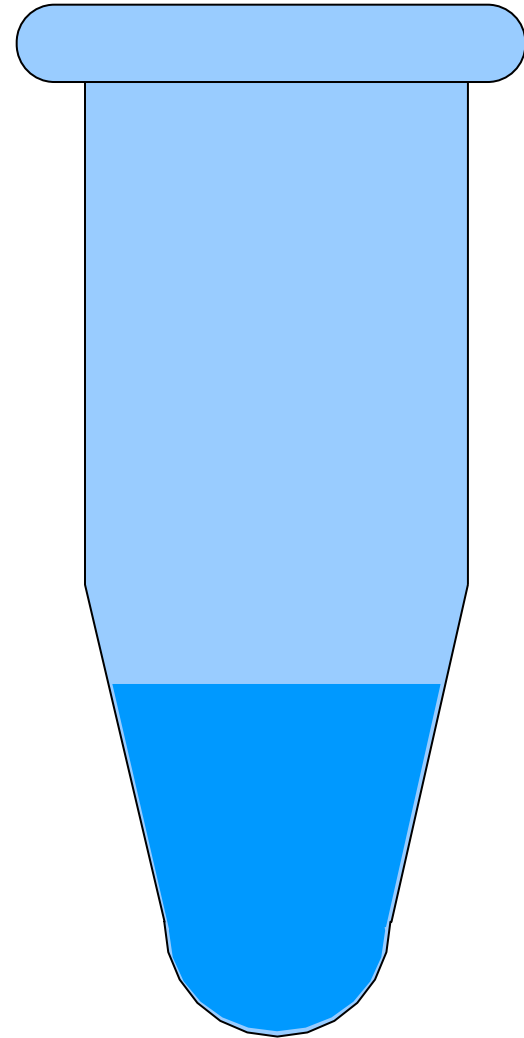
DNA pellet will
dissolved in TE buffer



DNA Isolation

That's it

Let us do it in the
laboratory



Verification of DNA on gel

