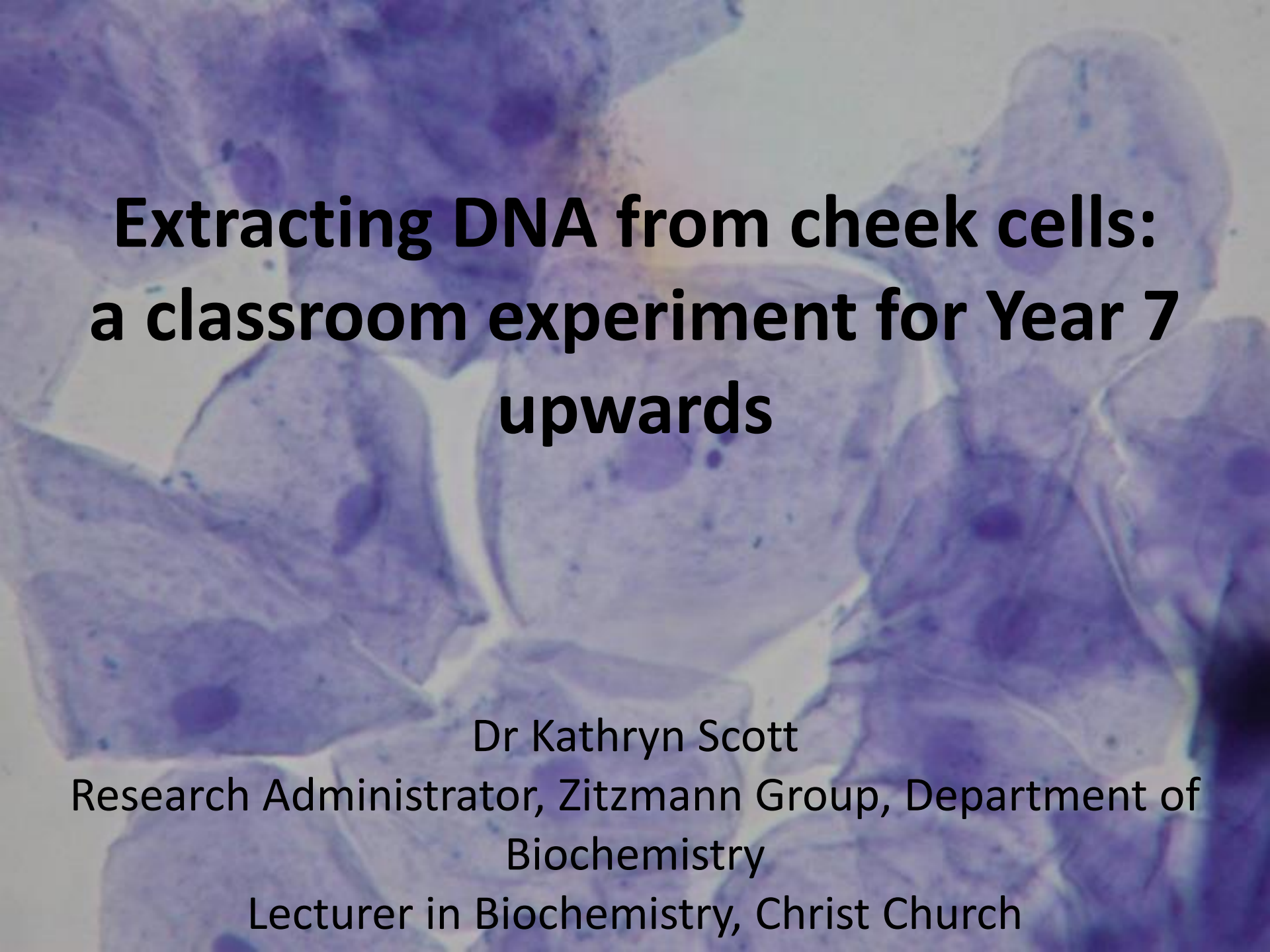

Extracting DNA from cheek cells: a classroom experiment for Year 7 upwards

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Extracting Human DNA in the Classroom

- Buccal (cheek cells) can be harvested painlessly and in sufficient quantity to visualise DNA extracted in a simple 4-step protocol

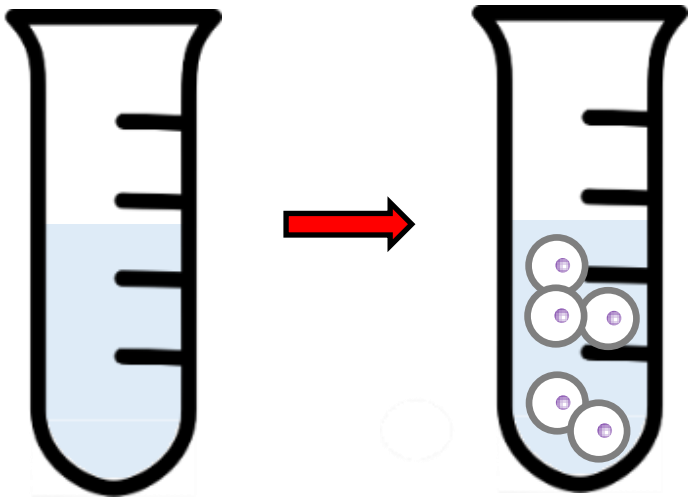
- We will be carrying out an optimised DNA extraction and discussing 'kitchen chemistry' alternatives to the materials used

- DNA extraction based on:

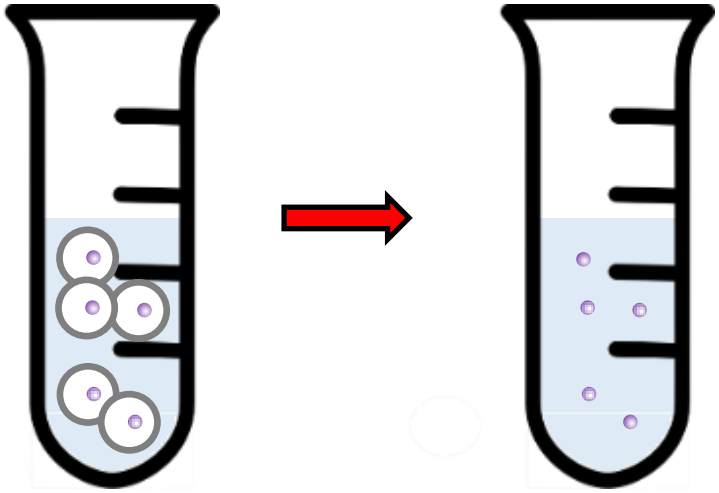
  R.P. Hearn & K.E. Arblaster. DNA Extraction Techniques for Use in Education (2010) *Biochem Mol Biol Edu* **38(3)** 161-166

  - Original optimised protocol requires a centrifugation step

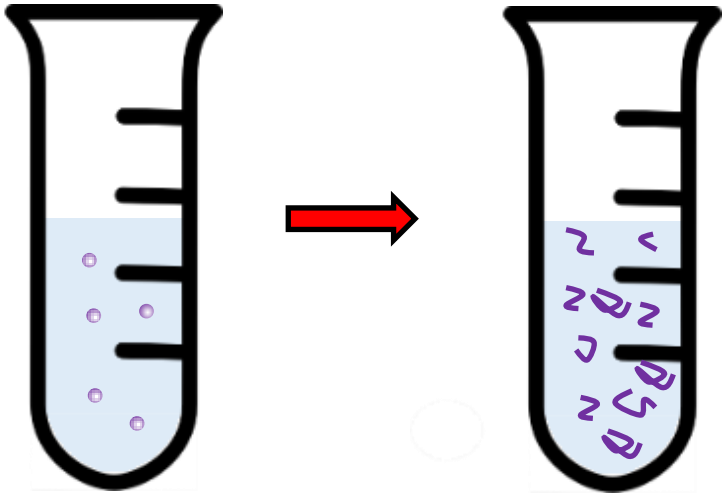
The Steps in DNA Extraction



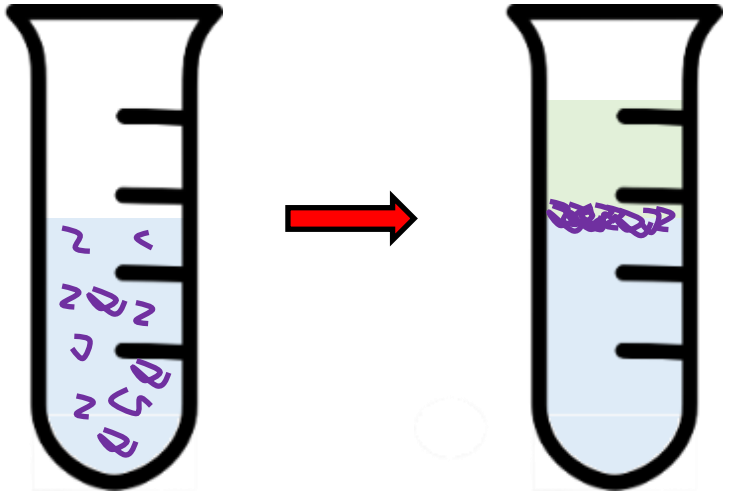
1. Cell Harvesting



2. Cell Lysis



3. Protein Digestion



4. DNA Precipitation

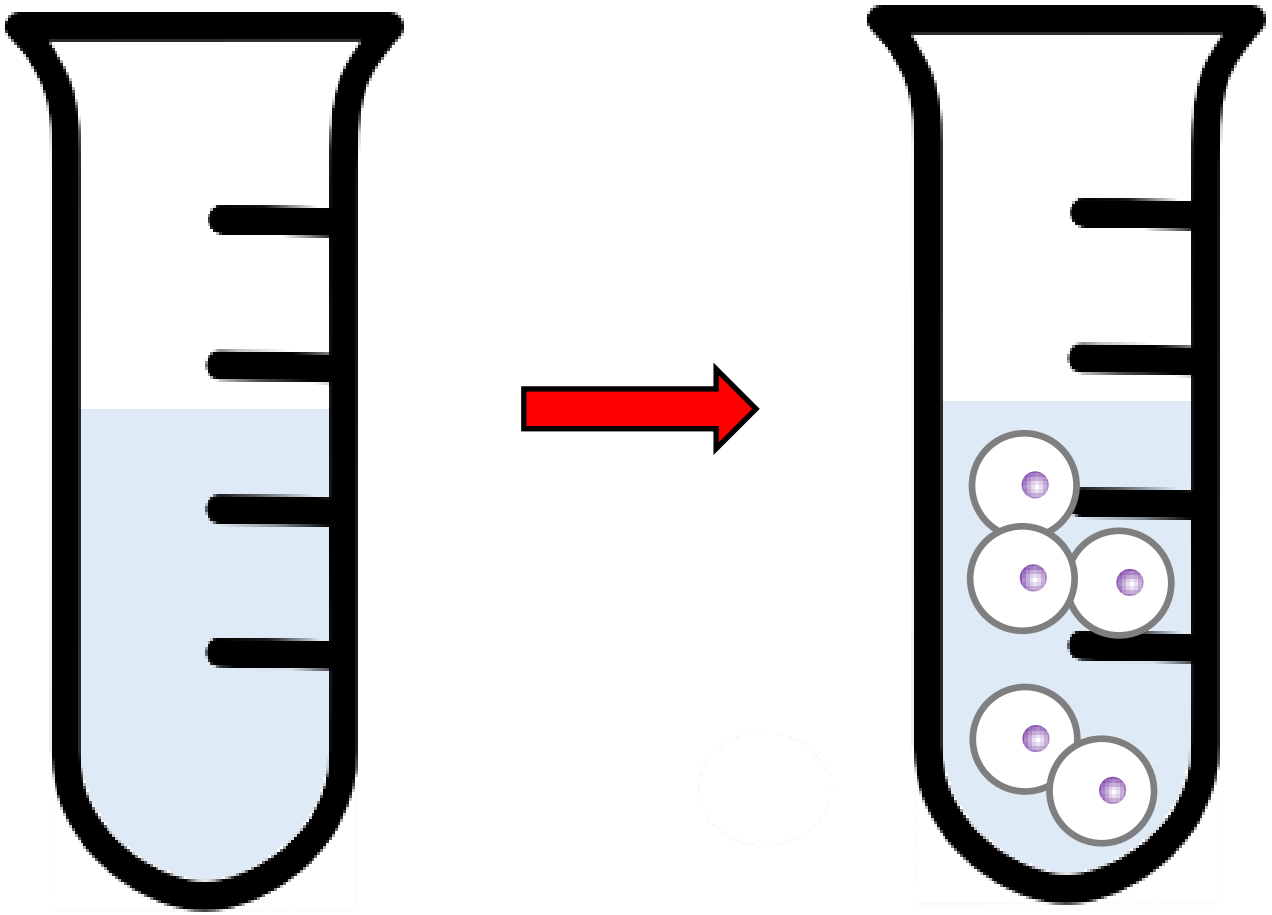
Objectives

- Basic level students will
  - Know that DNA is found in the nucleus of cells
  - Learn how to extract DNA from cells and describe the purpose of the key steps of cell lysis, protein degradation and DNA precipitation
  - Observe the appearance of human DNA
- More advanced students will also
  - Learn why buccal cells are a good choice for this experiment
  - Understand the role of SDS and EDTA in cell lysis
  - Understand the role of salt and alcohol in DNA precipitation

Risk Assessment

- Biological samples should only be handled by the person from whom they are taken
- Lysis buffer is an emetic and may cause irritation if in contact with skin or eyes
- Protease solution may cause irritation if in contact with skin or eyes
- Isopropyl alcohol is toxic if consumed and if absorbed through the skin

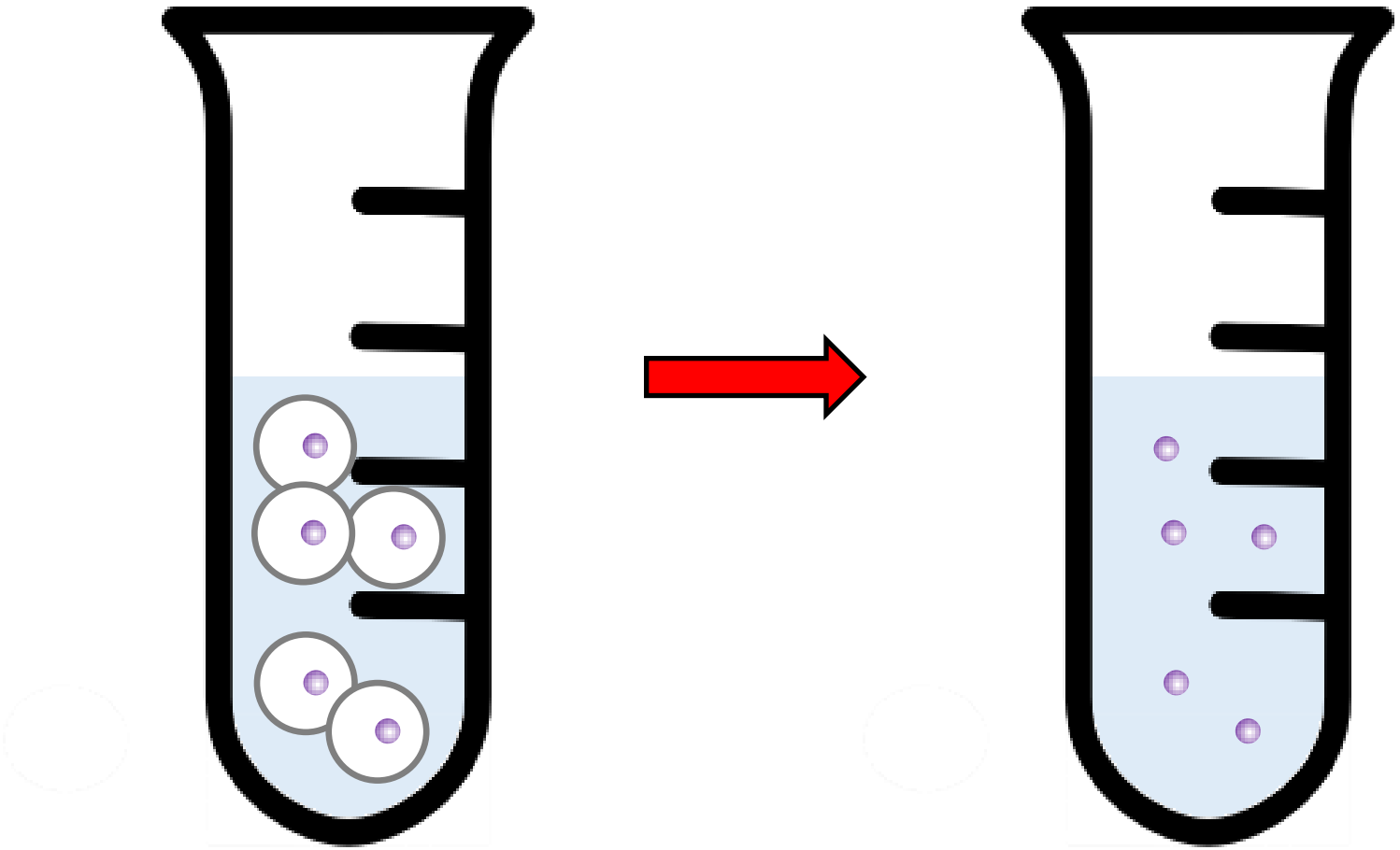
Step 1 – Cell Harvesting



Step 1 – Harvesting Cells

- Pipette 3 ml water into your drinking cup
- Gently chew the inside of your mouth for 30 seconds
  - **Gently** – blood doesn't help
- Take the water from your tube into your mouth and move it around for 30 seconds
  - Don't swallow the water
- Carefully spit the water back into your cup

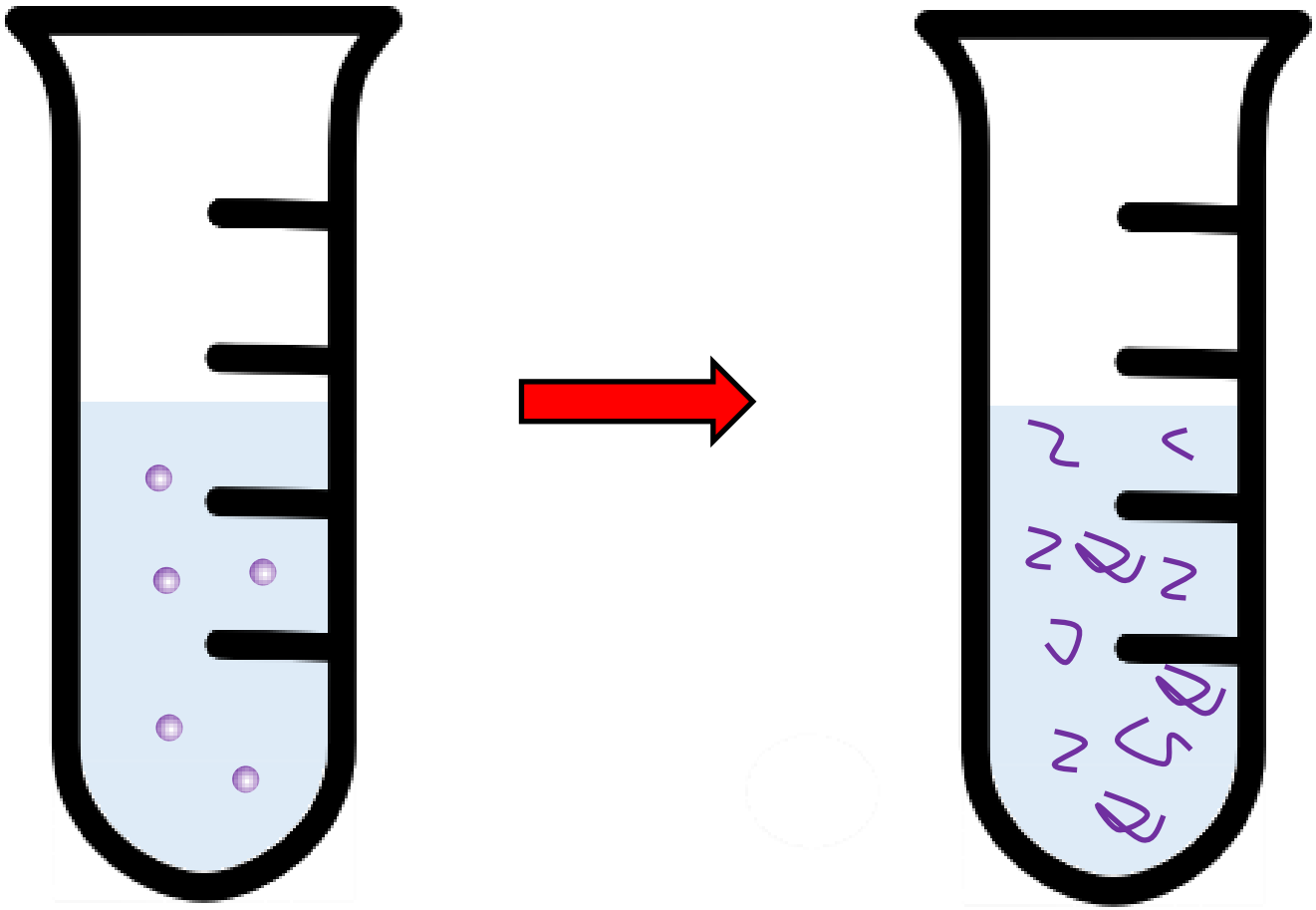
Step 2 – Cell Lysis



Step 2 – Cell Lysis

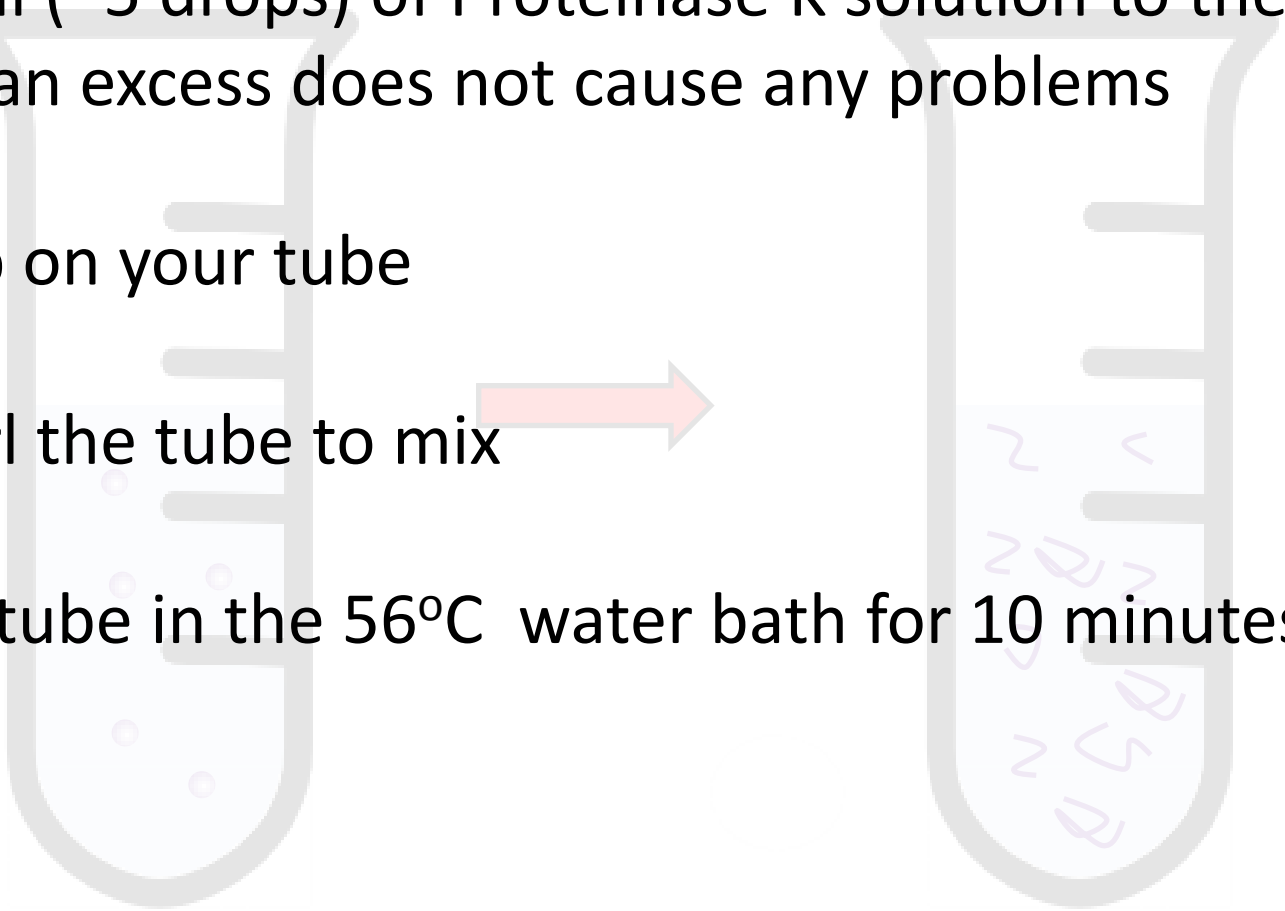
- Add 2 ml of lysis buffer to the test tube you will be using for DNA extraction
- Pour the contents of your cup into the test tube
- Put the cap on your tube
- Gently swirl the tube to mix
  - Shaking shears the DNA leading to short strands at the end of the experiment

Step 3 – Protein Digestion

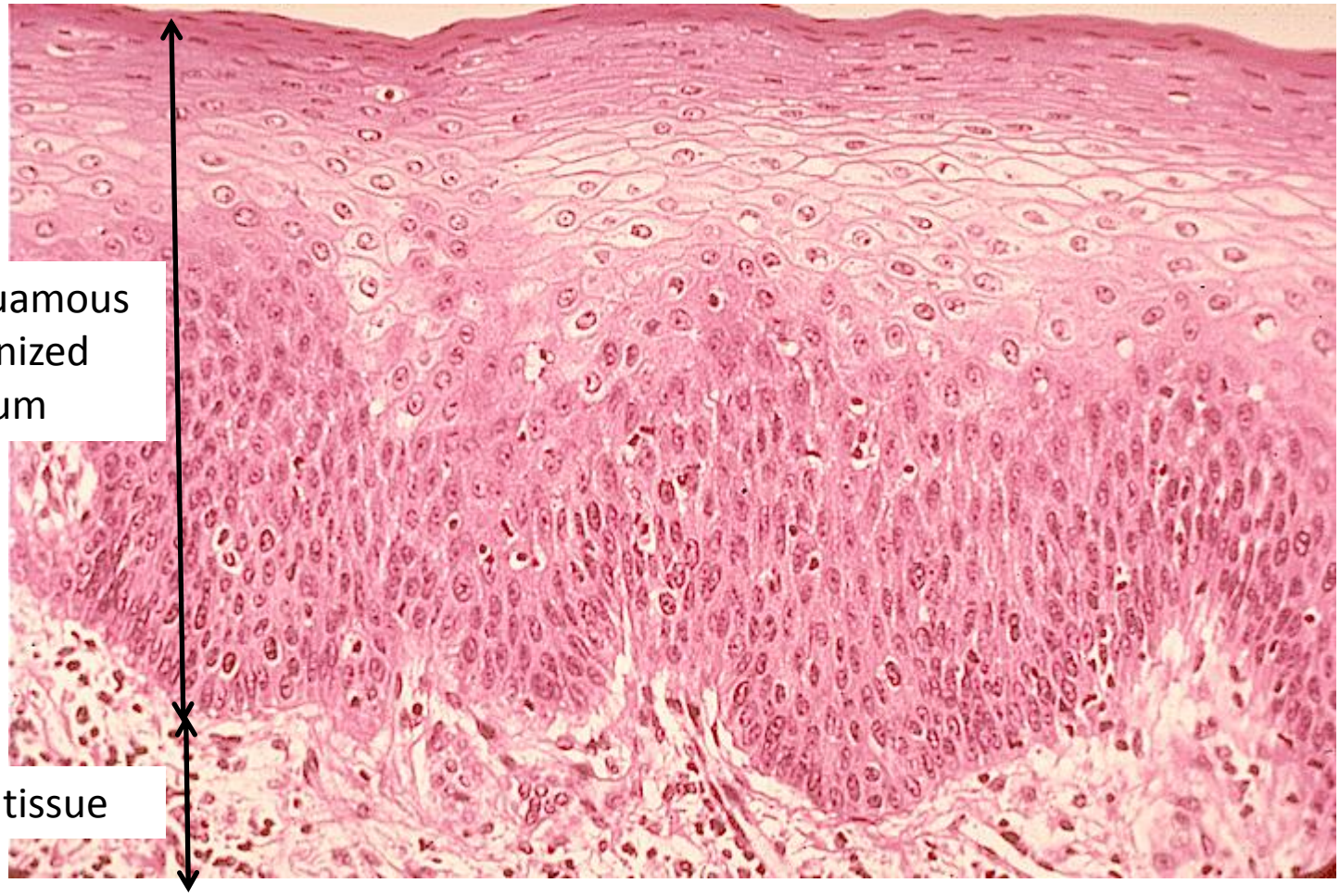


Step 3 – Protein Digestion

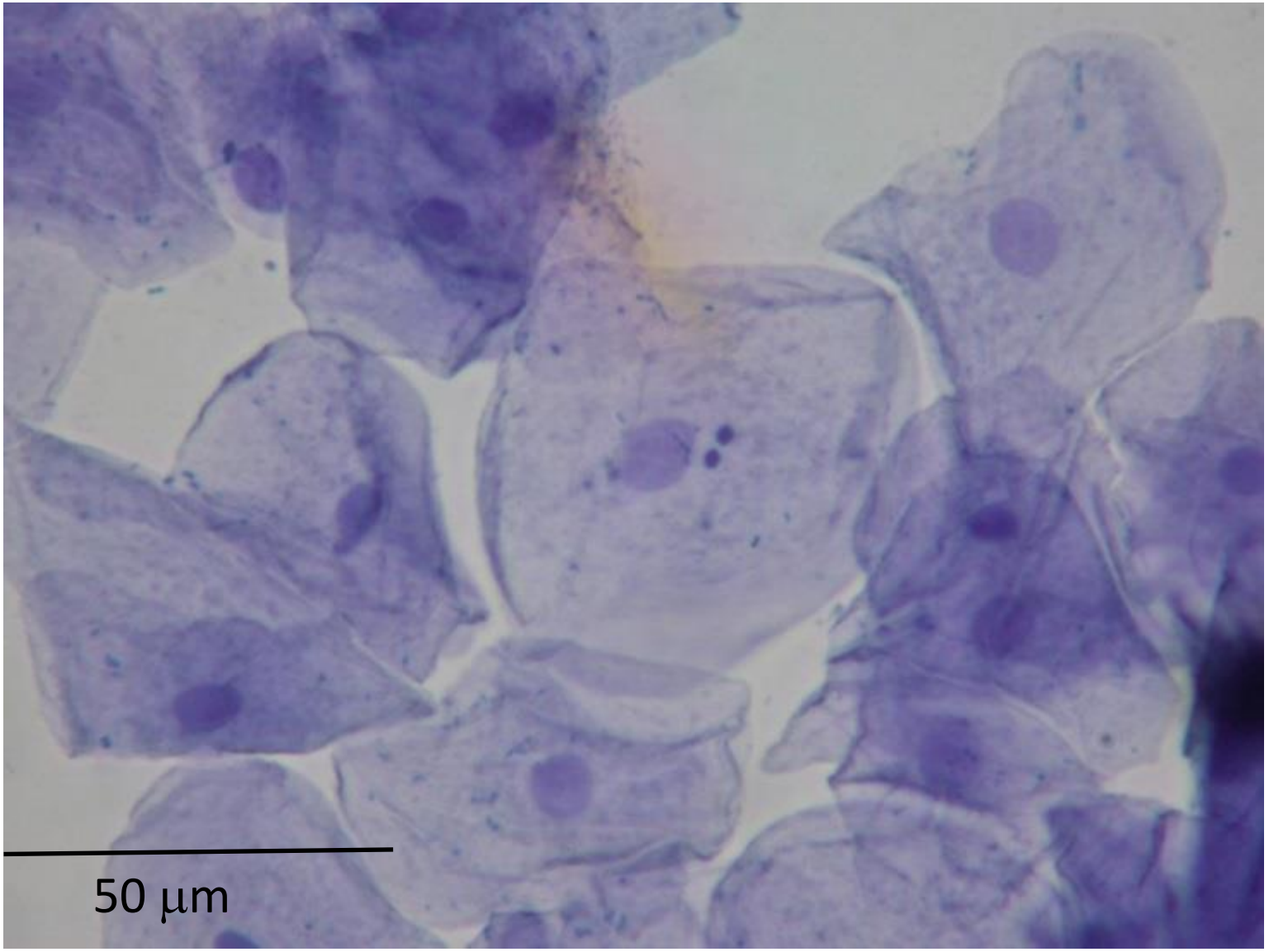
- Add 0.25 ml (~5 drops) of Proteinase K solution to the tube
  - Adding an excess does not cause any problems
- Put the cap on your tube
- Gently swirl the tube to mix
- Place your tube in the 56^{o}C water bath for 10 minutes



Buccal Cells Provide An Excellent Source of DNA



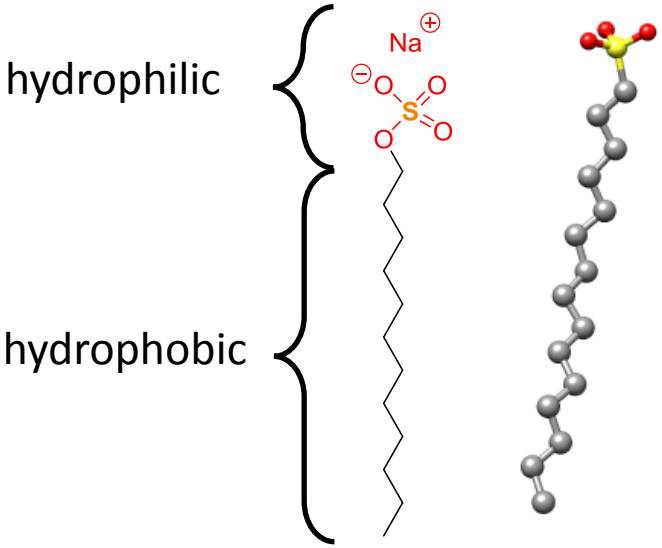
Buccal Cells Provide An Excellent Source of DNA



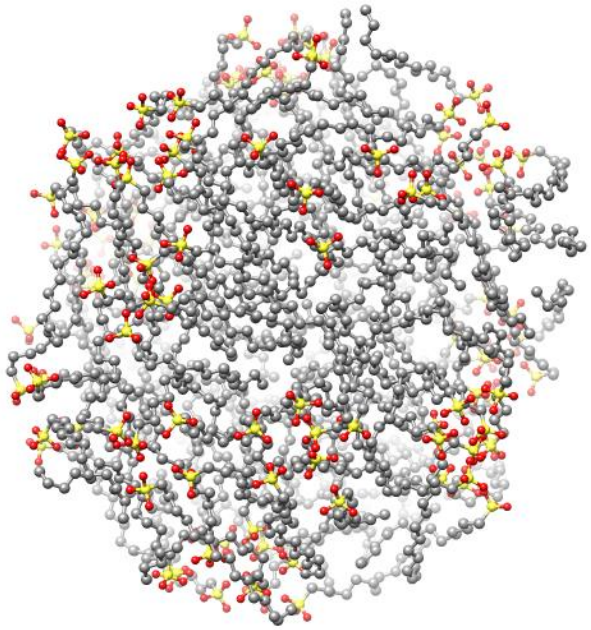
Cell Lysis Buffer

- 50 mM Tris pH 8.0
  - Buffering for DNA stability and optimal enzyme activity
- 1 % Sodium dodecyl sulfate (SDS)
- 1 mM Ethylenediaminetetraacetic acid

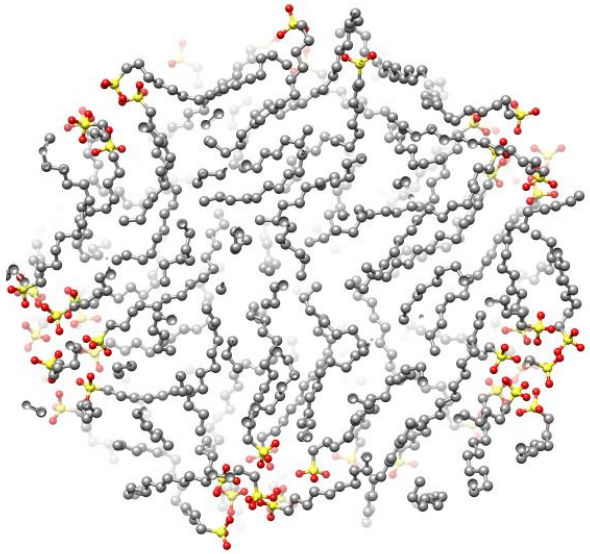
Cell Lysis – The Structure of SDS Micelles



Sodium Dodecyl Sulfate (SDS)

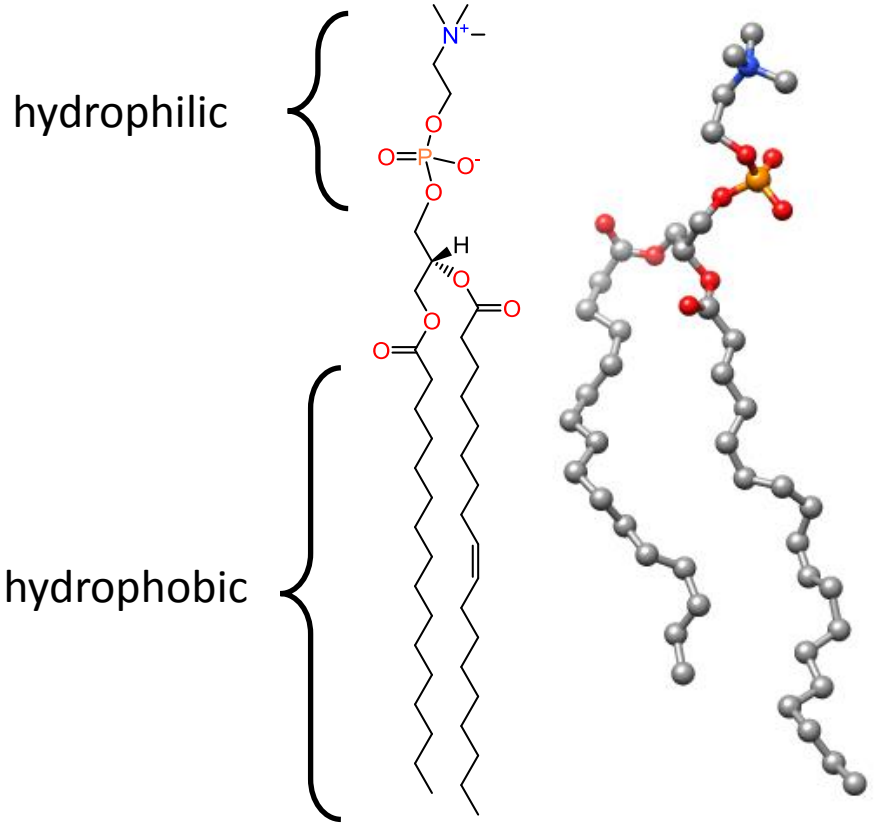


Computer simulation of a Sodium Dodecyl Sulfate Micelle

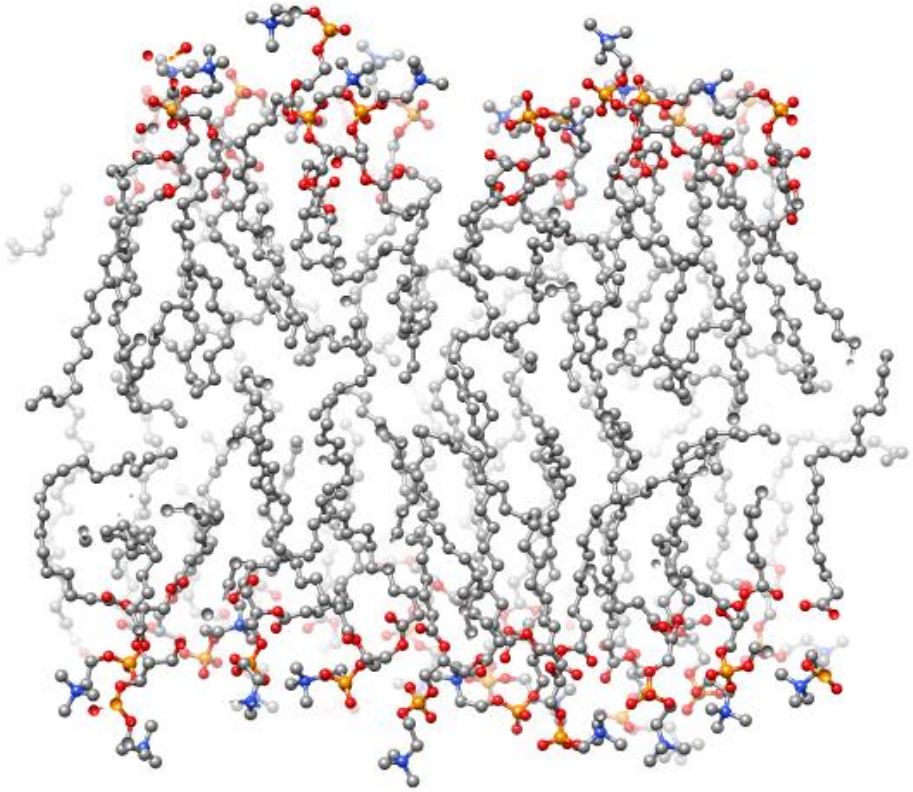


Micelle Cross-section

The Structure of Cell Membranes

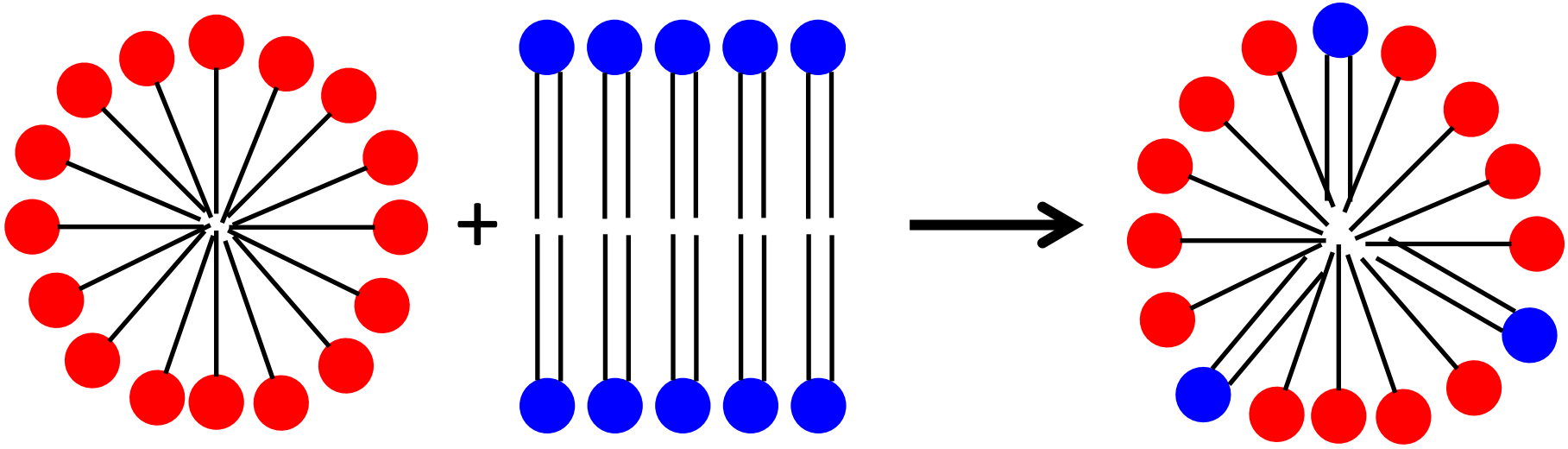


The lipid 1-palmityl-2-oleoyl-phosphatidylcholine (POPC)



Cross section from a computer simulation of a pure POPC bilayer

SDS Disrupts Cell Membranes



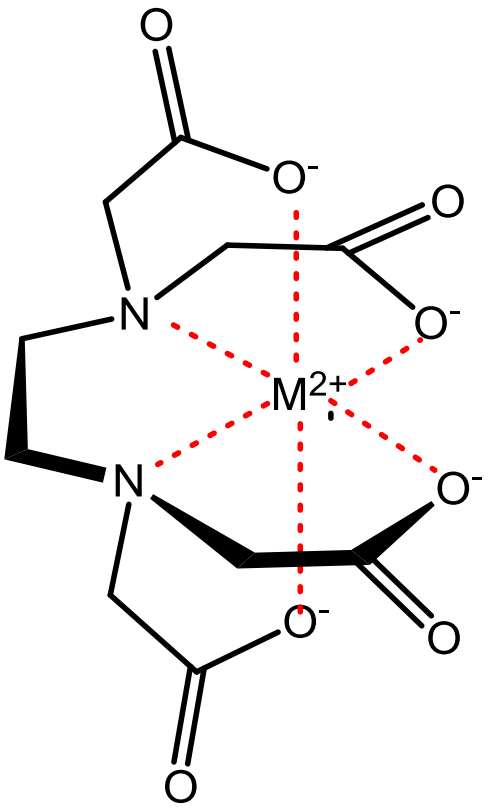
SDS micelle

Lipid bilayer

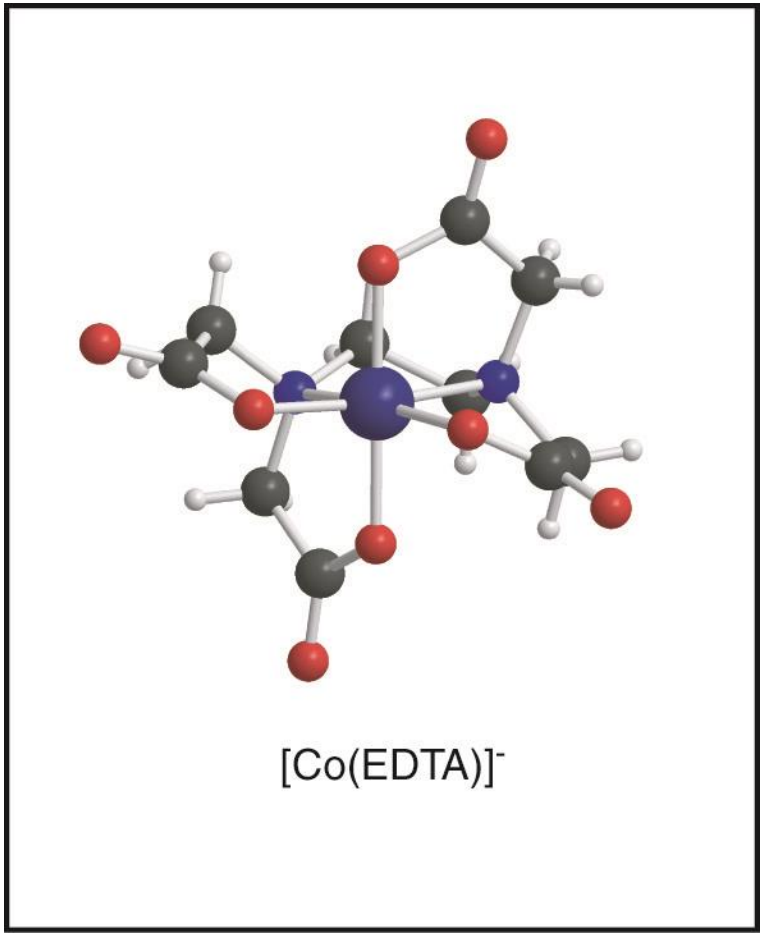
Mixed micelle

A concentration of 0.3% - 1% SDS is sufficient to disrupt the membranes of buccal cells

Cell Lysis – What Does EDTA Do?



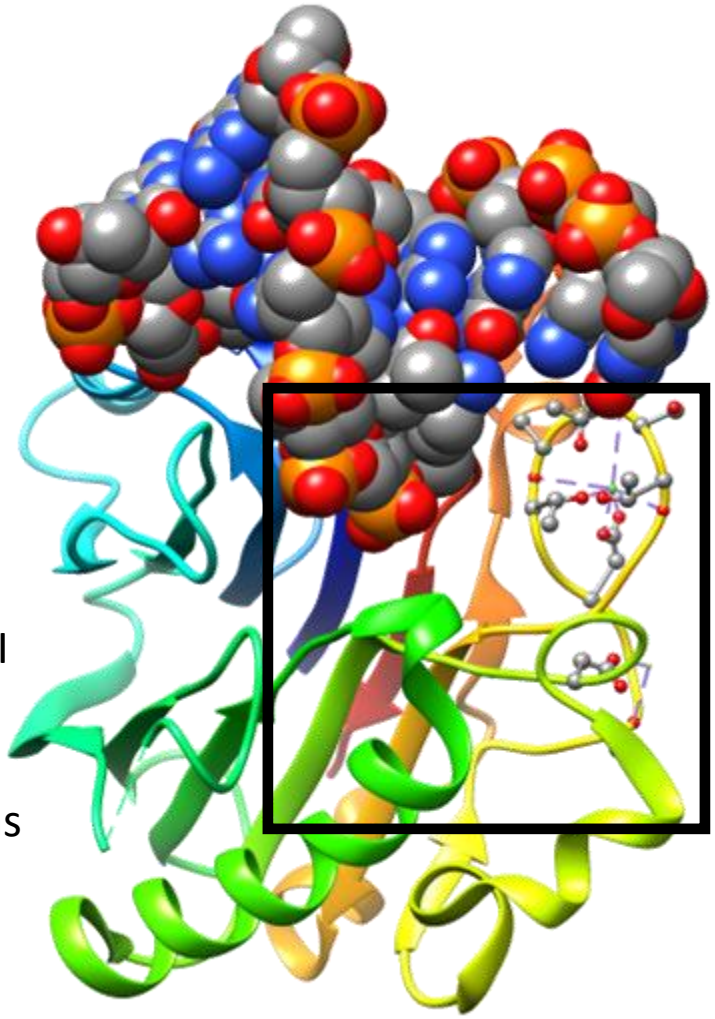
Ethylenediaminetetraacetic Acid

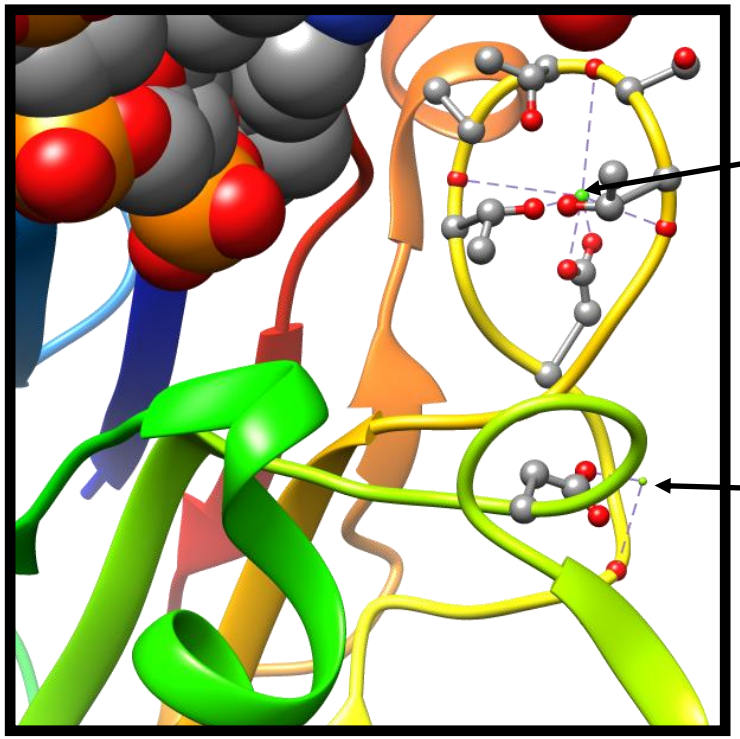


EDTA Inhibits Enzymes such as DNase I

DNA

DNase I from bovine pancreas





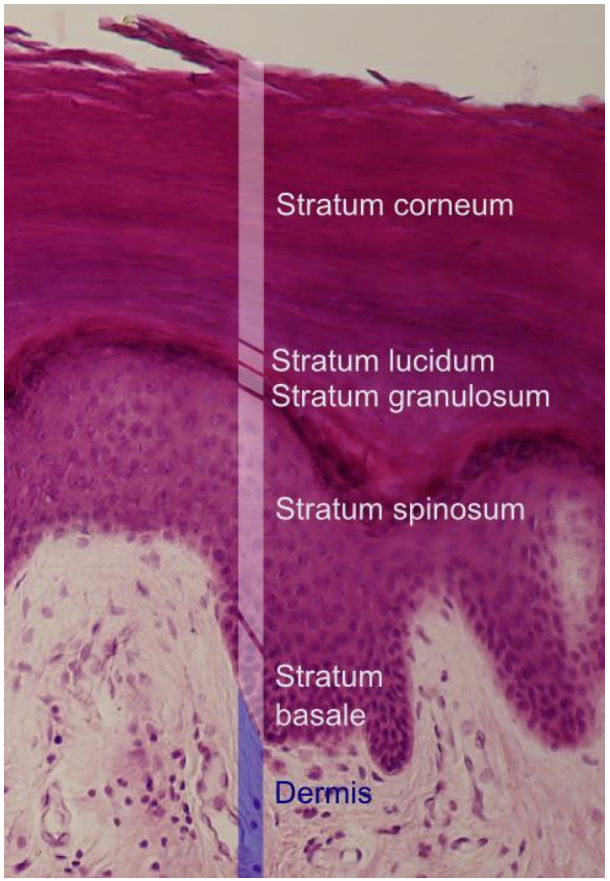
Both Ca^{2+} and Mg^{2+} are essential for DNase I function

DNase enzymes are found in most cells

Discussion Point

- Given that the lysis buffer is very similar in composition to shampoo, why does shampoo not lyse our skin cells

Stratified squamous **keratinized** epithelium



"Epidermal layers" by Mikael Häggström, based on work by Wbensmith - File:WVSOM Meissner's corpuslce.JPG at Wikimedia commons

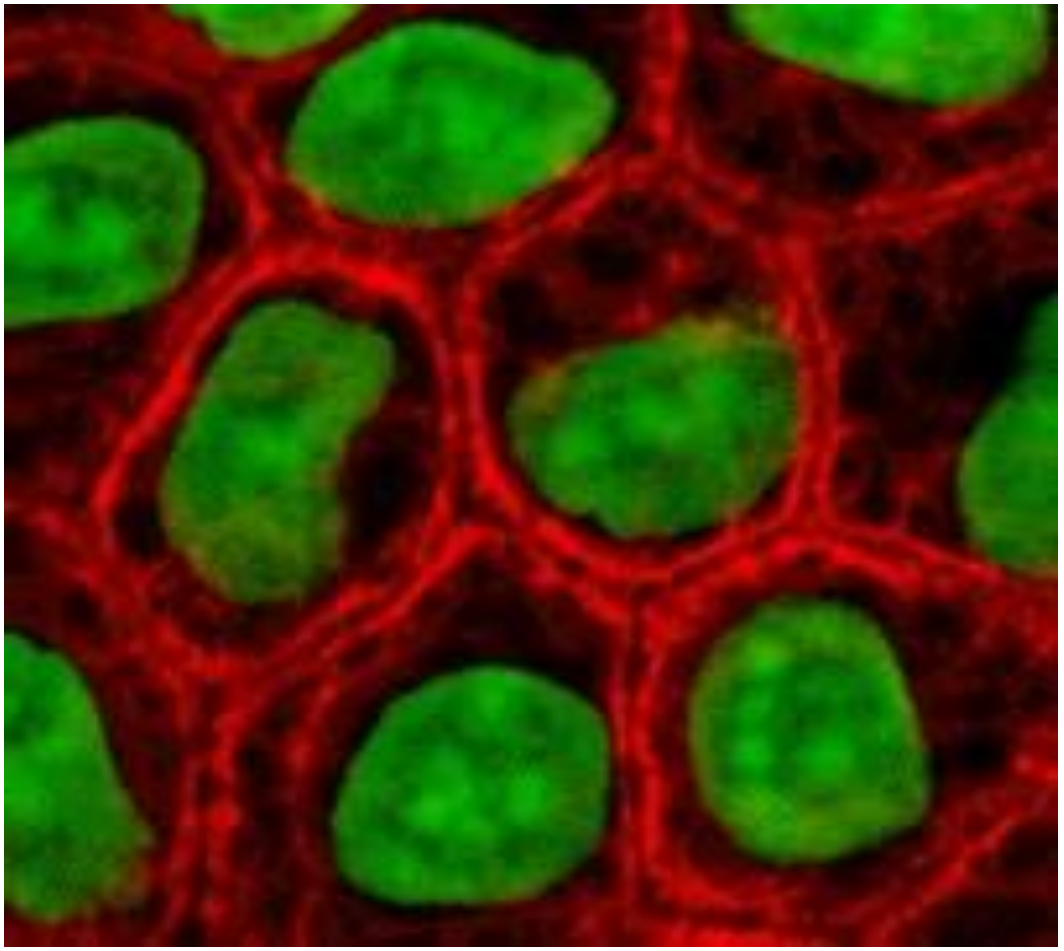
The skin has a protective layer known as the Stratum Corneum. The Stratum Corneum consists of cells that have have lost their nuclei, are embedded in a lipid matrix and are enriched in keratin proteins.

Discussion Point

Keratinized epithelial (skin cells) stained to visualise the DNA (green) and keratin filaments (red)
Note – these cells are from the lower epithelial layers

Keratin has several important roles

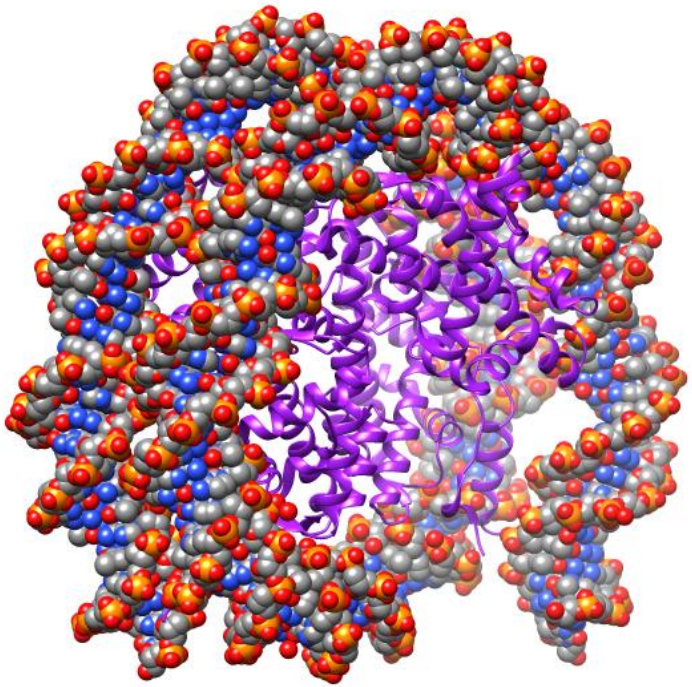
- Strengthens Cells
- Acts like a molecular sponge absorbing water if skin is immersed in water for a long time

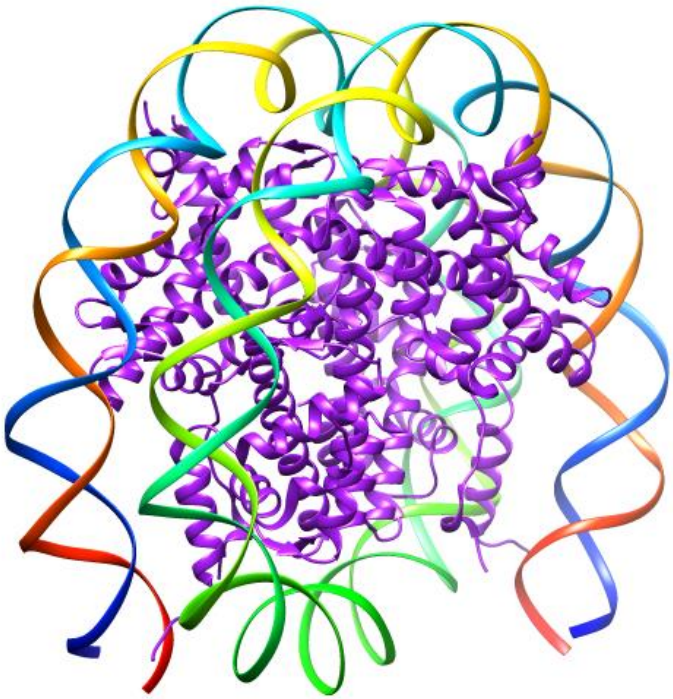


https://commons.wikimedia.org/wiki/File:Epithelial-cells.jpg

Proteinase K Digestion

- Many proteins precipitate under the same conditions as DNA
  - If we digest the proteins into amino acids then only DNA will precipitate



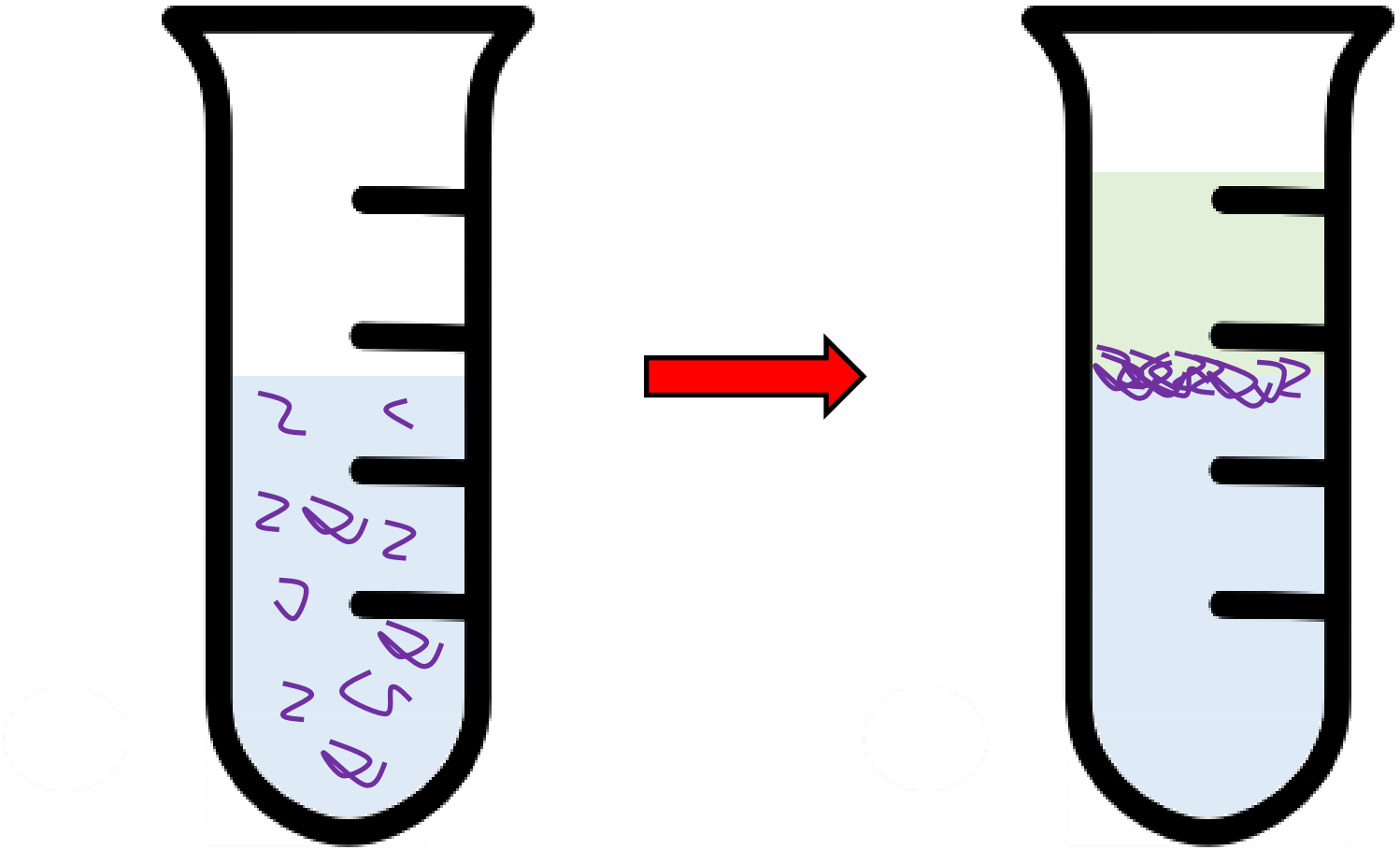


Protein digestion also removes the histone ‘cotton reels’ around which the DNA is wrapped

Proteinase K Digestion

- Originally extracted from the fungus *Tritirachium album*
- Named due to its ability to cleave Keratin
- Many proteinases only cleave after a specific amino acid
  - This leads to the production of large fragments
  - Proteinase K is relatively non-specific, therefore leaving very small fragments
- Is active over a wide range of temperatures
- Is active in the presence of a wide range of additives including
  - SDS
  - EDTA

Step 4 – DNA Precipitation

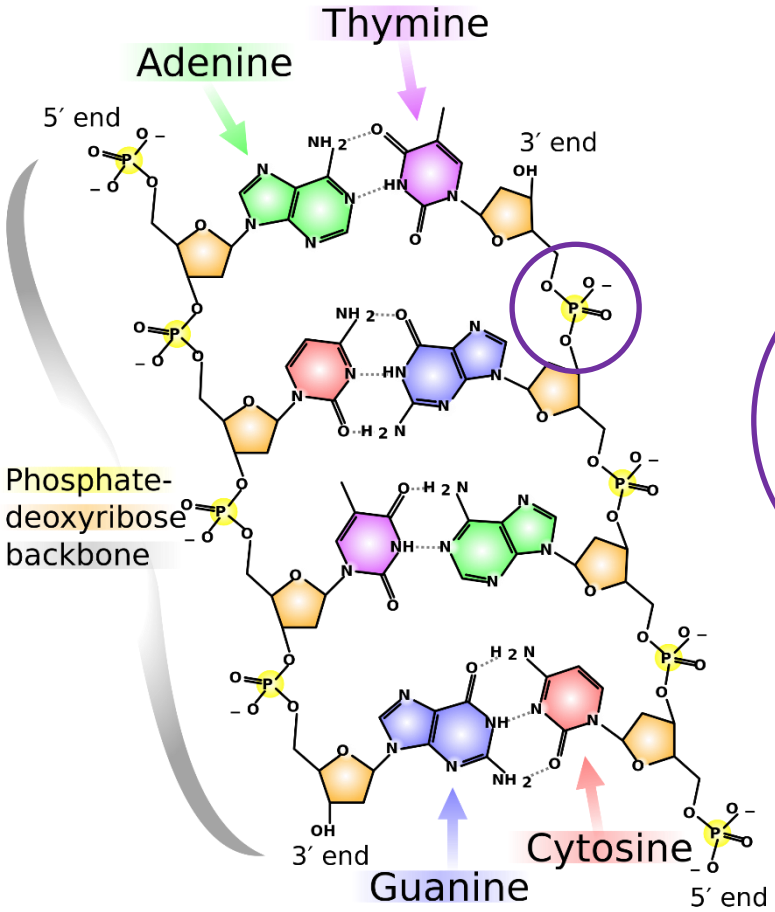


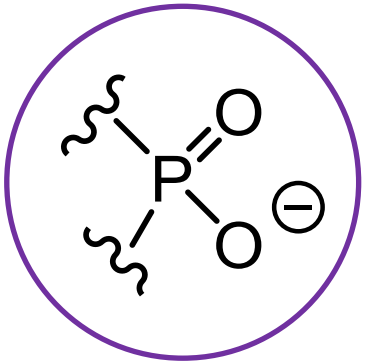
Step 4 – DNA Precipitation

- Add 0.5 ml (~10 drops) of 0.5 M NaCl to your tube
- Swirl your tube gently to mix
- Hold your tube at 45^{o} and carefully pour in 10 ml of cold isopropyl alcohol
- Leave the tube on the desk for 5 minutes
  - It is very important not to shake the tube
- After 5 minutes DNA should have precipitated at the interface between the lysis buffer and the alcohol
  - Swirling so that a vortex forms can aid precipitation
  - Do not shake or invert the tube

DNA Precipitation

- DNA is a highly polar molecule





There is a negatively charged phosphate group joining every base in a DNA chain.

https://commons.wikimedia.org/wiki/File:DNA_chemical_structure.svg

DNA Precipitation

- When DNA molecules and NaCl are dissolved in water the DNA, Na^+ and Cl^- ions will all be surrounded by water molecules
  - Water screens the charges on the DNA and salt ions and prevents them interacting to form strong ionic bonds

- Adding ethanol disrupts the structure of water around the ions, reducing the screening
  - The positively charged Na^+ ions and negatively charged DNA phosphate groups interact to form strong ionic bonds
  - Many ions coming together leads to precipitation

Variations on the Protocol

- The optimised protocol has proven effective in a classroom setting with students as young as Year 5
- Cost per student is still high
    - SDS - £27.50 per 25 g – need 1 g per 100 ml buffer (2ml required per student)
    - EDTA - £14.50 per 100 g – need 29 mg per 100 ml buffer
    - TrisHCl - £37.50 per 100 g – need 0.8 g per 100 ml buffer
    - 100 ml Tris-EDTA buffer pH 8 (10 mM Tris, 1 mM EDTA) - £19.50 (works well)
    - 100 ml 100x Tris-EDTA buffer pH 8 (1 mM Tris, 0.1 mM EDTA) - £18.10
    - ProteinaseK – 10 mg - £23.00

Variations on the Protocol

- Cell harvesting – scraping vs chewing
- Lysis buffer – Tris-EDTA-SDS vs showergel and hand soap
- Enzyme – Proteinase K vs no Enzyme vs contact lens tablets (Subtilisin A)
- Ethanol vs Isopropanol

Variations - Cell Harvesting

- Harvesting sufficient buccal cells is essential for successful DNA extraction

Chewing Cheeks

Scraping Cheeks

- Isotonic vs non-isotonic solutions

Variations – Lysis Buffer

Variations – Lysis Buffer

Tris pH 8.0, 1% SDS,
1 mM EDTA
NO SHAKING

5% Handwash
NO SHAKING

5% Shower Gel
NO SHAKING

Variations Proteinase

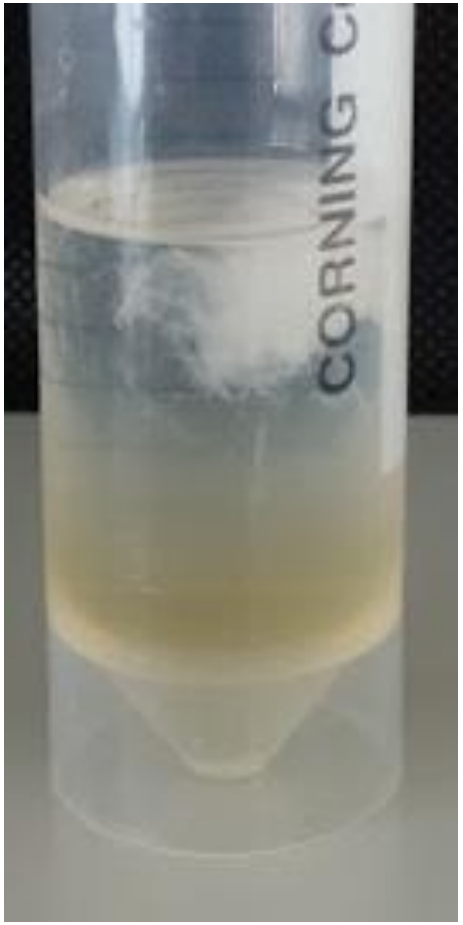
- Proteinase K is active under a wide range of conditions but is only available from specialist manufacturers

- Other proteinases are more readily available
  - Subtilisin A – contact lens cleaner
    - Less expensive than proteinase K ~£10 for a class of 30
    - not compatible with EDTA, reduced activity in SDS, optimal temperature not stated on packaging
  - Meat tenderiser
    - May contain one of a variety of enzymes
    - May be contaminated with DNase (proved to be the case in our experience)

Variations – Protease

Proteinase K
NO SHAKING

Subtilisin A
No EDTA
37°C
NO SHAKING



No Protease
Sample 1
NO SHAKING

No Protease
Sample 2
NO SHAKING

Variations – Isopropanol vs Ethanol

- DNA is less soluble in isopropanol than ethanol
  - therefore a lower volume of isopropanol is required for DNA precipitation

- Isopropanol is much more toxic than ethanol
  - drinking 10 ml of isopropanol could prove fatal
  - Isopropanol is also readily absorbed through the skin

- The benefit of an increase in yield when using isopropanol must be carefully evaluated against the increased risk

Variations – Isopropanol vs Ethanol

Isopropanol
NO SHAKING

Ethanol
NO SHAKING

Pitfalls – Harvesting Sufficient Cells is Vital

DNA from a thorough cell harvest.

Tris pH 8.0, 1% SDS, 1 mM EDTA
Proteinase K
Isopropanol
NO SHAKING

DNA from a second round of cell harvesting immediately after the first.

Tris pH 8.0, 1% SDS, 1 mM EDTA
Proteinase K
Isopropanol
NO SHAKING

Pitfalls – Large sample volume

Proteinase K
AFTER SWIRLING

No Protease
Sample 1
AFTER SWIRLING

No Protease
Sample 2
AFTER SWIRLING

Conclusions

- Human DNA extraction can be carried out in a 45 minute lesson for lower years
  - Upper years benefit from an additional theory lesson

- Upper years can relate the practical to a range of different areas of the curriculum
  - Tissue formation
  - DNA structure and function
  - Enzymes
  - Solubility