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:


  • 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°C water bath for 10 minutes
Buccal Cells Provide An Excellent Source of DNA

Stratified squamous non-keratinized epithelium

Connective tissue

50 μm
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

hydrophilic

hydrophobic

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

Cross section from a computer simulation of a pure POPC bilayer
SDS Disrupts Cell Membranes

A concentration of 0.3% - 1% SDS is sufficient to disrupt the membranes of buccal cells.
Cell Lysis – What Does EDTA Do?

Ethylenediaminetetraacetic Acid

[Co(EDTA)]\(^{-}\)
EDTA Inhibits Enzymes such as DNase I

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?

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

Stratified squamous keratinized epithelium

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

Poor DNA yield
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