

3. Investigate DNA Extraction

CIE Biology Jones
pp 111-122



G11 Biology 2017-2018

Learning Objective

Investigate the possibility of isolating DNA from plant tissue

Success Criteria

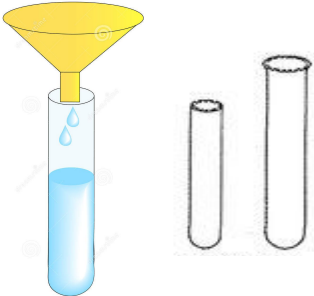


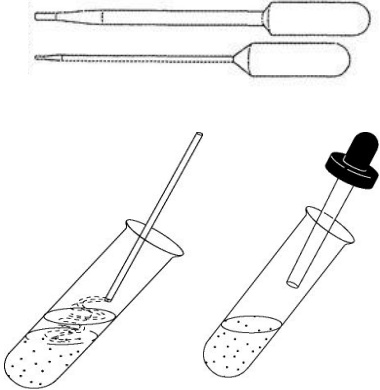
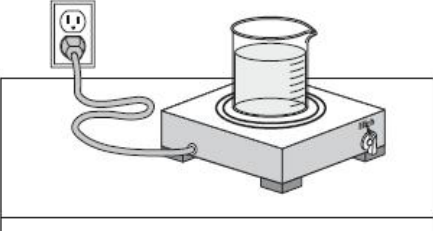

1. Students carry out research on possibility of DNA extraction from plant tissues.
2. Students will create at least one reasonable comment on each of the following topics:
 - a. Sources of error in the procedure
 - b. How to improve procedure
 - c. Describe the quality of the experimental results

Terminology

English	Kazakh
Homogenate	Гомогенді
Filtrate	Сүзу
Precipitate	Сұйықтықтардың
Disruptive	бөлінуі
Extract, extraction	Бұзу
Isolation	Экстракция, бөліп алу
Lysis	Изоляция
Enzyme	Лизис
spoiled	Фермент

Add revision of lipids forming micelles.... And other stuff...pictures
videos

Equipment

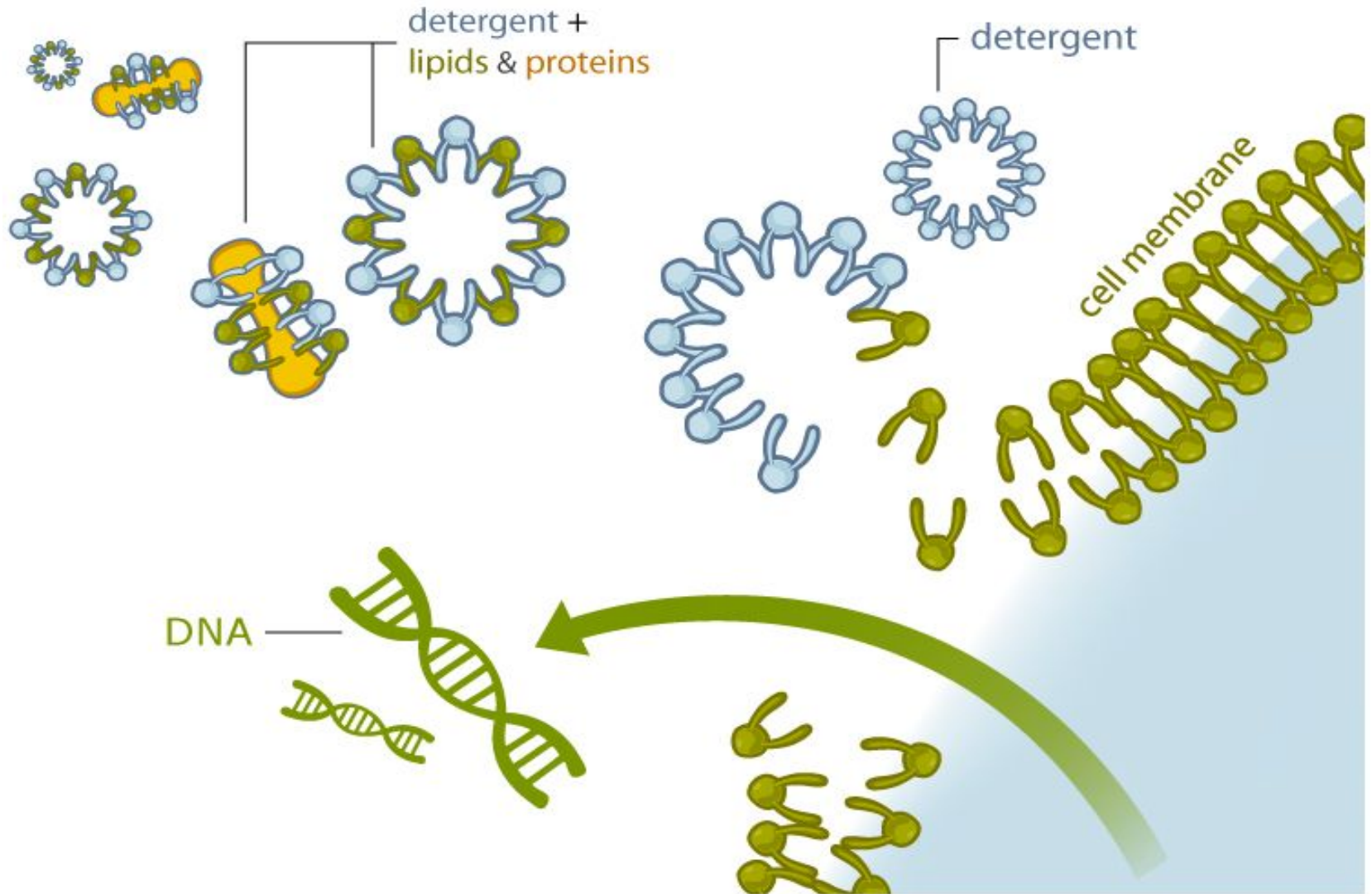
Funnel and test tube	Mortar and pestle	Digital scale
		
Micropipette, dropper	Water bath	Graduated cylinder – volume mL
		

Introduction

- In the DNA isolation procedure, cell walls (plants) and cell membranes are broken down by tissue homogenization (via mashing or blending). The detergent, sodium laurel sulfate (SDS), solubilizes phospholipids in the cell and nuclear membranes. Mashing, heat, and detergent facilitate cell lysis. A filtration step may be included to remove solid components from those dissolved in the DNA lysis buffer. The addition of alcohol precipitates the DNA, enabling DNA to be isolated from other solution components.

Notes:

1. Soap action: In this figure, detergent disrupts the cell membrane phospholipids releasing membrane proteins and liberating DNA into the solution.



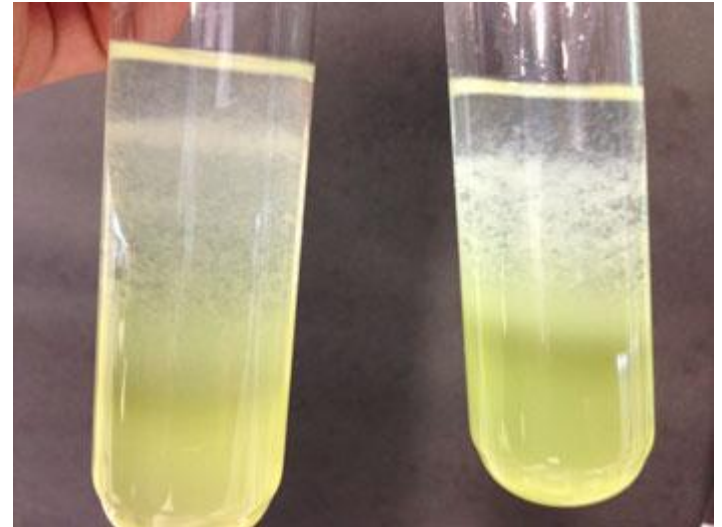
6. DNA is not soluble in alcohol.
When ethanol is added, the DNA *precipitates* where the water and ethanol meet.

alcohol

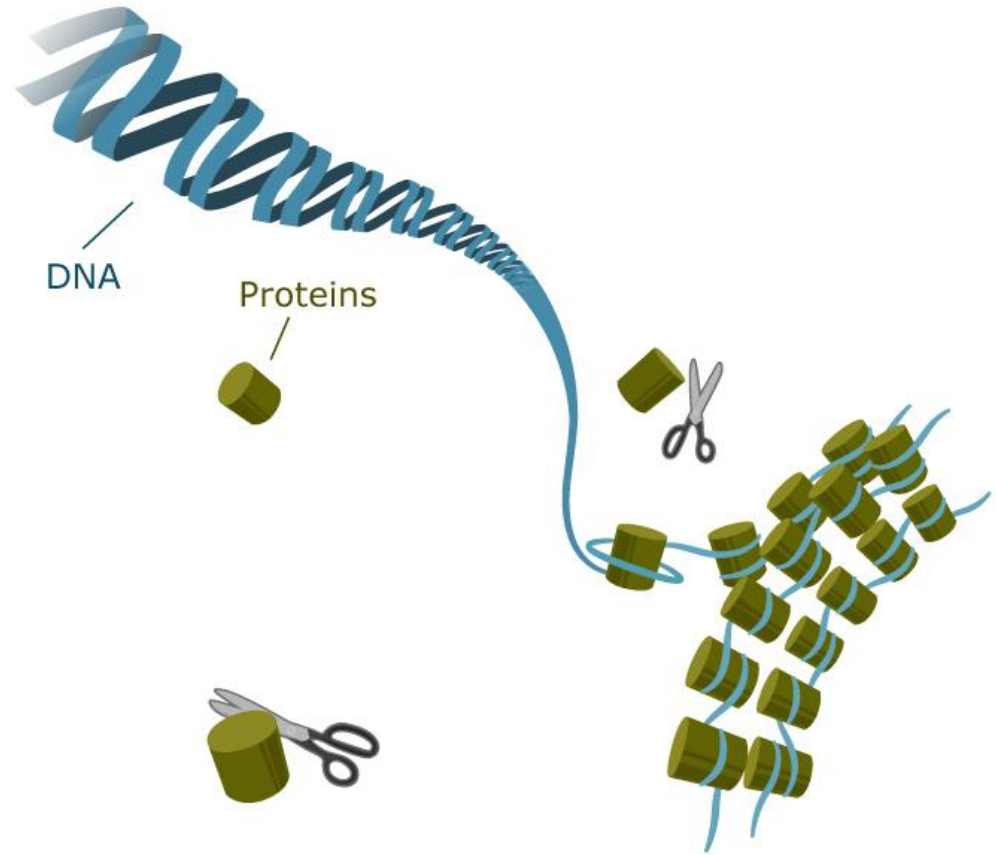
DNA coming out of
water solution into the
alcohol layer

2. DNA is highly soluble in water
because the phosphate group of each
nucleotide carries a negative charge
and associates electrostatically with
polar water molecules. DNA is
hydrophilic.

water

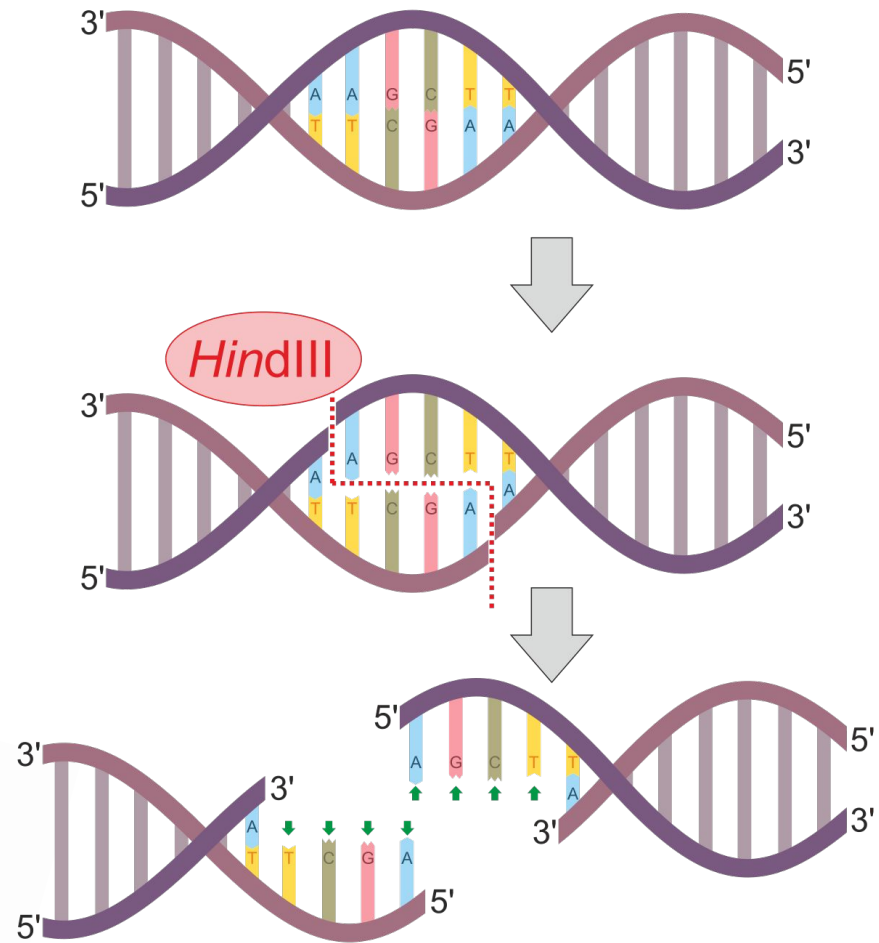
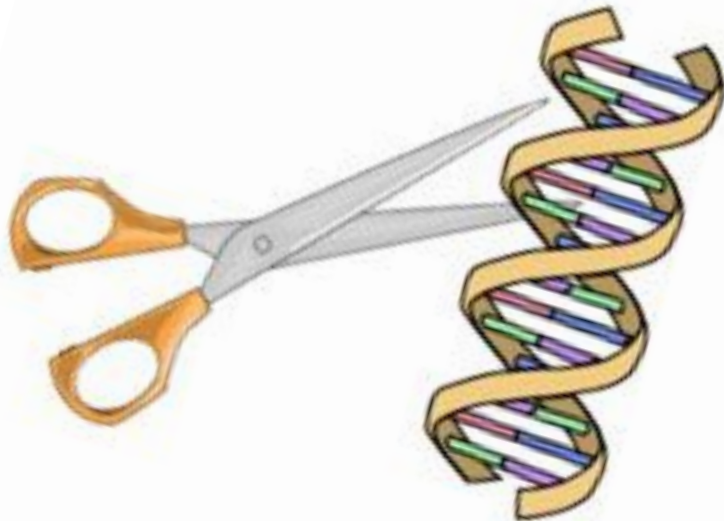


3. Protease (Meat tenderizer) contains enzymes that will strip away (digest) the histone proteins bound to DNA. The two most common enzymes used in meat tenderizer are the proteases bromelain and papain extracted from pineapple and papaya, respectively.



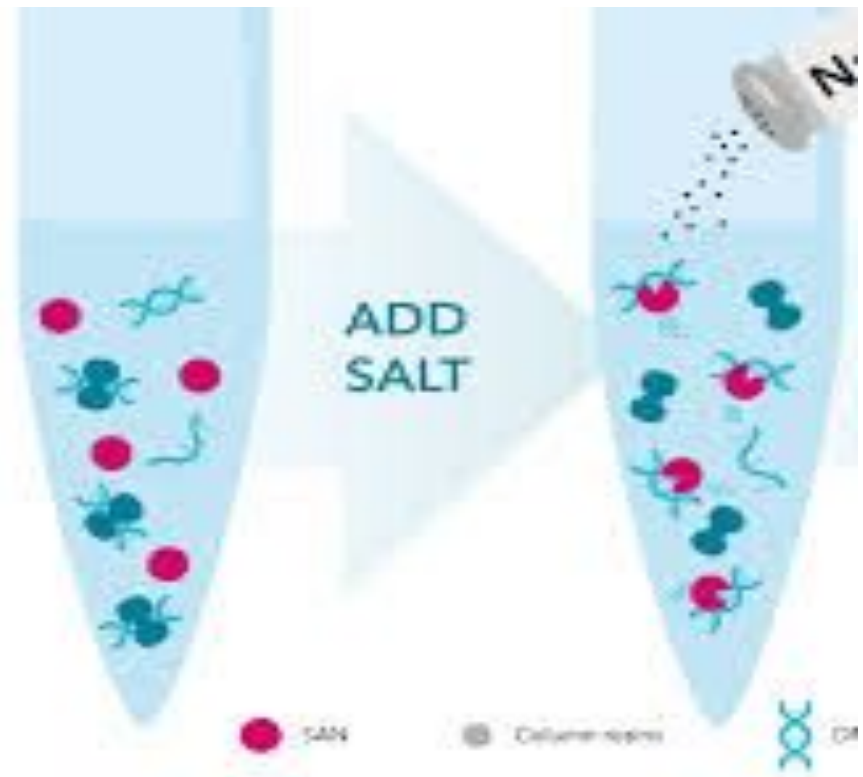
4. Nucleases

1. also known as DNases and RNAses, are denatured at 60°C and thus inactivated thereby protecting DNA from enzymatic degradation.
2. Nuclease enzymes degrade nucleic acids by **breaking the phosphodiester** bond that holds the nucleotides together.
3. **Restriction enzymes** are a good example of endonucleases, which cut within a strand.



5. *Salt (NaCl)* assists in the **denaturation** and removal of **histone proteins and exposes DNA**. Salt also neutralizes the charges on the sugar- phosphate backbone of the DNA molecule. Positively charged sodium ions neutralize the negative charge on the PO_3^- groups on the nucleic acids, making the molecule less hydrophilic, and therefore less soluble in water. **This enables the DNA be precipitated from solution upon addition of alcohol.**

- **The salt binds to the DNA allowing us to see it!**



The DNA in the nucleus of the cell is molded, folded, and protected by proteins.

The meat tenderizer cuts the proteins away from the DNA.

