Practical 6 - R(a) Extraction of DNA from Fruit and Vegetables

This practical focuses on **– Using complex apparatus and procedures**.

Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Describe a method that can be used to extract DNA from plant tissue.
- Explain using theory from A2, the main stages used to extract DNA from plant tissue.

Safety Information

	You should wear eye protection throughout this practical.
	Ethanol is **highly flammable**. There should be no flames in the same room.
 	Methylated spirit (IMS) is **highly flammable**. There should be no flames in the same room.
	Protease enzymes such as Bromelain and Papain are all **harmful**.
The general safety precautions for working with DNA, such as those provided by NCBE, should be followed.	

Background information

- In forensic science DNA is extracted to obtain DNA for genetic fingerprinting, in genetic engineering it may be used for modifying plants and animals and in medicine it may be used to research inherited diseases and develop cures or gene therapy
- Initially tissue is broken up mechanically. It is important that the tissue is broken down as finely as possible
- Detergent is used to disrupt the cell membranes and nuclear membranes.
- The cell fragments are separated by filtration.
- DNA is separated from the extract.
- A protease enzyme is used to remove soluble proteins.
- DNA is precipitated using ice-cold ethanol.

You will extract DNA from fruit or vegetables using a method which has been adapted from a method that is used in laboratories all over the world.

Method

Mechanical break up of plant tissue

1 In a large beaker, mix 3 g of table salt with 10 cm^3 of washing up liquid.

2 Add 90 cm^3 of water so you have 100 cm^3 all together.

3 Add 50 g of chopped fruit or vegetables.

4 Place the beaker in a water bath at 60 °C for **exactly** 15 minutes.

5 After 15 minutes, place the beaker into an ice bath for 5 minutes, stirring frequently.

6 Filter the mixture through a coffee or large filter paper in a filter funnel; place a clean beaker underneath the funnel to collect the filtrate. Do not over fill the funnel or the filtrate will be contaminated by foam.

Separation of DNA – step 5 should be done slowly and carefully so the ethanol forms a layer on top of the filtrate/protease mix.

1 Use a measuring cylinder to measure 10 cm^3 of filtrate.

2 Pour the 10 cm^3 of filtrate into a boiling tube and add 2-3 drops of protease enzyme using a teat pipette.

3 Shake the boiling tube to mix the contents.

4 Use a measuring cylinder to measure 6 cm^3 of ice-cold ethanol.

5 Slowly and carefully pour the ethanol into the boiling tube containing the filtrate/protease mix.

6 Leave the boiling tube in a rack for a few minutes without disturbance.

7 After a few minutes you will see a white substance floating out into the ethanol – this is the DNA!

Review considerations

1 The washing-up liquid breaks down the membranes. Why is it necessary to breakdown the nuclear membrane?

2 In step 4 a temperature of 60 °C is used to denature DNAases. Why is it important to denature DNAases?

3 Why is the mixture filtered?

4 Describe the action of the protease enzyme.

Lesson Plan

Extraction of DNA from Fruit or Vegetables

Context

A practical set in the context of 9700 Syllabus – a simplified method of extracting DNA from fruit or vegetables.

It is anticipated that students will have completed an AS practical course and so they will have good basic practical skills. It is also assumed that they will have reviewed work completed in AS on the structure of a plant cell and the action of enzymes.

Key aims of lesson

This practical is designed to develop practical skills and relate work completed in AS to new situations.

Intended learning outcomes

By the end of this practical and by answering the questions the student should be able to:

- Describe a method that can be used to extract DNA from plant tissue.
- Explain the main stages used to extract DNA.

Resources required

White board or flipchart and suitable pens or chalkboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	**Teacher / Student Activities**
End of previous lesson	**Preparation** – students to review the structure of a plant cell and the action of enzymes
0-5	**Introduction** to the aims, intended outcomes and background information - teacher led oral presentation
5-10	**Context** - review plant structure, action of enzymes and location of DNA – teacher led questioning, student responses/discussion
10-15	**Introduction to method** - teacher to go through method
15-50	**Carrying out the practical** - students carry out the practical work. At the end the teacher compares the amount of DNA each student or group has extracted. Students tidy away apparatus as soon as they have finished.
50-60	**Drawing together the threads** – students to complete questions. Teacher-led check of answers through questioning, student response/discussion.

Useful Information

- It is best to use soft fruit or vegetables. Frozen peas and onion give good results. If hard fruit or vegetables are used the mixture will need to be blended for 5 seconds before filtering. Fruit with a skin will need to be peeled and the skin discarded before weighing. Fish eggs can also be used
- Different types of fruit and vegetables can be compared.
- Students should consider that:

  1 Cells and cell membranes have to be broken to release the DNA from the nucleus.

  2 DNAase must be denatured as it is an enzyme that breaks down DNA,

  3 Filtration separates the cell wall material from the DNA and soluble proteins.

  4 Protease enzymes break the peptide bond between the amino acids in the polypeptide chain. The protein binds to the active site of the enzyme lowering the activation energy. The peptide bonds break and peptides are produced.

Technical Information

Extraction of DNA from Fruit and Vegetables

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.
It is convenient to weigh out the salt so that each student or group has 3g. A measuring cylinder should be used to measure the washing-up liquid.

1 Approximately 50g of fruit or vegetable should be used. For example frozen peas or onion.

The fruit or vegetables should be pre-chopped very finely.

2 Top pan balance and spatula

3 1 10 cm^3 measuring cylinder (this will need to be washed before being reused)

4 1 100 cm^3 measuring cylinder

5 2 500 cm^3 beakers

6 1 water bath at 60 °C

7 1 ice bath

8 1 coffee filter or filter paper and funnel

9 1 boiling tube and rack

10 1 teat pipette

11 3 g table salt or 3 g sodium chloride

12 Washing up liquid

13 2 cm^3 protease solution (Novo Neutrase available from *NCBE). Other proteases, such as 2% solutions of papain or bromelain, may be used. These are less effective so the tubes may need to be left longer before DNA separates.

14 Ice cold 95% ethanol (place the ethanol in a freezer overnight). Ice cold methylated spirits (IMS) can be used as an alternative.

Safety Precautions/Risks.

Ethanol = F 

Methylated spirits (IMS) = F, H  

Proteases as per individual product, ie Bromelain = H, Papain = H 

General safety precautions for working with DNA can be obtained from NCBE. www.ncbe.reading.ac.uk
(http://www.ncbe.reading.ac.uk/NCBE/SAFETY/dnasafety1.html)

A risk assessment should be carried out as a matter of course.