

Leaving Cert Biology

Experiments

Isolate DNA from a Plant Tissue



Materials/Equipment

Onion (10 cm ³)	Plastic syringe
Washing up liquid (not the concentrated type) cm ³)	2 Beakers (250
Blender	Boiling tube
Distilled water	Test tube rack
Sharp Knife	Graduated
cylinder (100 cm ³)	
Table Salt (3 g)	Retort stand
Chopping board	Glass
rod/wooden skewer/wire loop	
Protease enzyme e.g. trypsin (1%)	Electronic
Balance	
Coffee filter paper	Lae funnel
Ethanol at freezer temperature	Weigh boat
Droppers	Glass stirrer
Water bath (60 °C)	Plastic Syringe
Spatula	Disposable
gloves	
Ice – water bath	Thermometer

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 3 g of table salt to 10 cm³ of washing-up liquid in the beaker and make up to 100 cm³ with distilled water.
3. Chop the onion into small pieces.
4. Add the chopped onion to the beaker with the salty washing-up liquid solution and stir.
5. Put the beaker in the water bath at 60 °C for exactly 15 minutes.
6. Cool the mixture by standing the beaker in the ice-water bath for 5 minutes, stirring frequently.
7. Pour the mixture into the blender and blend it for no more than 3 seconds.
8. Carefully filter the mixture into the second beaker.
9. Transfer about 10 cm³ of this filtrate into the boiling tube.
10. Add 2 – 3 drops of protease to the filtrate and mix gently.
11. Trickle about 10 cm³ of the ethanol, straight from the freezer, down the side of the boiling tube, to form a layer on top of the filtrate. Leave the tube for a few minutes without disturbing it.
12. Observe any changes that take place at the interface of the alcohol and the filtrate.
13. Using the glass rod, gently draw the DNA out from the alcohol.
14. Record the result.

Result

Conclusion/Comment

SKILL ATTAINMENT

ISOLATE DNA FROM A PLANT TISSUE

Following instructions

Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus

Use the electronic balance
Set and maintain the water bath at the correct temperature
Use the thermometer
Use the timer
Use the blender for the correct length of time
Filter the mixture into a clean beaker
Transfer the filtrate to the boiling tube
Add the correct amount of protease to the filtrate
Trickle the ethanol down the side of the boiling tube
Draw out the DNA

Observation

Accurately read the temperature of the water bath
Observe two distinct layers of liquid after the addition of the alcohol Notice a change in viscosity at the interface of the two liquids Notice cloudy matter appearing at the interface of the two liquids Observe the appearance of the DNA

Recording

Write up the procedure
Record the result

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

DNA is isolated from cells by taking advantage of the different chemical and physical properties of the molecules and structures within the cell.

Reasons for the main steps in the activity:

Chopping the onion

The physical chopping breaks the cell walls and allows the cytoplasm to leak out.

Adding the washing-up liquid

Breaks down the lipids in the phospholipid bilayer and causes the protein in the membranes to break apart. This results in the release of the nuclear material from the cell.

Adding the salt

Once the cell is destroyed, the ion levels within the cell change. The proteins in the membranes, which have been exposed by the detergent, are now positively charged. These naturally attract the negatively charged phosphate groups in the DNA. This causes a problem in extracting the DNA. The salt is added to minimise the attractive forces between the DNA and the protein by shielding the DNA molecules, causing them to clump together.

Heating the mixture to 60 °C for exactly fifteen minutes

Causes DNases, released from the lysosomes, to be broken down. After fifteen minutes DNA itself will be broken down.

Cooling the mixture

Decreases the rate of the chemical reactions, slowing the action of any remaining enzymes before they destroy the DNA.

Blending

Further destroys cell walls and membranes. Causes DNA to be released. Blending for more than three seconds shears the fragile DNA strands.

Adding protease

Breaks down the proteins associated with DNA.

Filtering

Strains all the large cellular debris out of the mixture. DNA passes through the filter with the liquid.

Using cold ethanol

Ethanol forms a layer on top of the onion filtrate. The alcohol tends to draw the water out of the DNA molecule, making it less dense. It is now found at the interface of the two liquids. DNA is insoluble in freezing cold ethanol but soluble in alcohol at room temperature.

Testing for DNA

Currently, there is no satisfactory method of testing for DNA available for use in second level laboratories.

Advance preparation

- Set up the water bath.
- Place the ethanol in a freezer *at least* 24 hours before carrying out this activity.

Helpful hints

- Use a medium-sized onion.
- Some alternatives to onions are: tomatoes, peas, peaches, nectarines and kiwi fruits.
- Use a cheap brand of washing-up liquid. However, it is important not to use the bacteriocidal washing-up liquid as this contains an enzyme which causes the breakdown of DNA.
- It is vital to blend **for no more than three seconds**. If the DNA appears fluffy, it was sheared in the extraction process. It should appear as thin threads.
- Since the ethanol must be very cold for this procedure to work, it must be kept in a freezer overnight. Dispense the ethanol into small bottles, one for each student group. Remove the bottles directly from the freezer, just before the students use them.
- Industrial methylated spirits may be used as a cheaper alternative to ethanol.
- If the DNA remains at the interface, place the boiling tube back into the ice bath.
- Other sources of protease include fresh pineapple juice, contact lens protein removal tablets and meat tenderiser.

