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DNA your onions?

A crude method of extracting DNA from onions

Aim

This popular method of isolating DNA requires little more than onions, household detergent and salty water. Onions are the best material to use because their cells contain a relatively large amount of DNA (1C = 415 Mb). They are cheap and available throughout the year, and unlike some plant materials, are highly unlikely to cause allergic reactions.

Classroom methods

The isolation of DNA has become a popular activity in school laboratories over the past 30 years. Although similar practical protocols had been described previously (*e.g.*, Sands, 1970), these were not adopted widely owing to the complexity of the procedures involved and the hazardous nature of several of the solvents required (Falconer and Hayes, 1986).

These early methods for isolating DNA were derived from the work of Julius Marmur (Marmur, 1961), which in turn were developed from the classic work of Oswald Avery, Maclyn McCarty and Colin MacLeod (who first showed that DNA was the genetic material in the 1940s).

Simpler methods of isolating DNA first appeared in American school textbooks in the mid-1980s (*e.g.*, Helms, *et al*, 1986) and subsequently made their way into specialist school biotechnology projects (*e.g.*, Rasmussen and Matheson, 1990). By the early 1990s these methods had crossed the Atlantic, featuring in German and English publications (Bayrhuber, *et al*, 1990; NCBE, 1991).

With the arrival of simple and inexpensive methods, what was an undergraduate or post-16 practical exercise moved down the age range and even into the primary classroom (Assinder, 1998).

Three easy steps

All of the methods of isolating DNA have three similar steps:

- the cells are lysed;
- histones associated with the DNA are denatured;
- the DNA is precipitated in an organic solvent, such as ethanol.

In the method described below, a kitchen blender is used to break open the cell walls. Detergent breaks down the phospholipid membranes

surrounding the nuclei, releasing the DNA. The detergent, combined with heating, degrades the histones associated with the DNA by destroying their secondary and tertiary structures. This allows a protease to hydrolyse the histones to peptides and amino acids. In research, Proteinase K (a protease obtained from the fungus *Engyodontium album*) is often used to hydrolyse proteins. It is active over a wide pH range even in the presence of the detergent SDS (sodium dodecyl sulphate). In the method described here, a cheaper protease obtained from *Bacillus amyloliquefaciens* is used instead.

After the histones have been degraded, the DNA is precipitated in ice-cold ethanol. When it is dissolved in water, the negatively-charged phosphate groups of the DNA are surrounded by a coating of water molecules. Ethanol, added to an aqueous DNA solution, disrupts the electrostatic interactions between the water and the DNA molecules, causing the DNA to precipitate out of solution. Sodium ions (from the salt used in the extraction solution) also disrupt the DNA–water interactions, enhancing the precipitation of the DNA.

For research use, the DNA might be further purified by treatment with phenol and trichloromethane (chloroform) either separately or together. Phenol and to some extent trichloromethane further denature the proteins, and the products of denaturation are soluble in phenol. Sometimes isoamyl alcohol is added to the solvent mixture, which reduces the tendancy of the denatured proteins to foam.

Other sources of DNA

In the search for sweeter-smelling alternatives to onions, several authors (*e.g.*, Smith and Ansell, 2008) have suggested applying the 'onion method' to a variety of fruits, including kiwi fruit, bananas and strawberries. Although these fruits seem to yield copious amounts of DNA, the substance produced is in fact little more than pectin.

Advance preparation

The ethanol used must be ice cold. Place it in a tightly-sealed plastic bottle in a freezer at least 24 hours before you attempt this activity. **Please read the safety note, below.**

If desired, the onion can be chopped up to 12 or so hours in advance.

Equipment and materials

Needed by each person or group

Equipment

- 1 cm^3 plastic syringe (without a needle)
- Plastic funnel
- 250 cm^3 beakers, 2
- Test tubes, 2
- Plastic spoon for stirring the mixture
- Chopping board
- Knife for chopping onion

Materials

- Small onion
- Washing-up liquid, 10 cm^3
- Table salt, 3 g
- Water, 90 cm^3 (tap water is suitable)
- Very cold ethanol, about 10 cm^3, straight from the freezer (industrial denatured alcohol, IDA, is suitable) **Please read the safety note, below.**
- *Novozymes Neutrase®* (a protease), 2–3 drops
- Ice, in a jug with cold water
- Coffee filter paper (do not use laboratory filter paper, as liquid takes too long to pass through it)

Also required (to be shared by the class)

- Water bath, maintained at 60 °C
- Blender or liquidiser

Timing

Isolating the DNA takes approximately 35 minutes, including an incubation period of 15 minutes.

Procedure

1 Dissolve the salt in 90 cm^3 of water. Add the washing-up liquid and mix gently.

2 Chop the onion into pieces about 5 mm x 5 mm and add them to a beaker with the salty washing-up liquid solution.

3 Stand the beaker in a water bath at 60 °C for exactly 15 minutes. *The detergent and heat treatment degrades membrane phospholipids and proteins, releasing the DNA. In addition, the positively-charged sodium ions from the salt shield the negatively-charged phosphate groups of the DNA molecules, helping them to precipitate out of solution. At 60 °C, DNase enzymes, which would otherwise start to cut the DNA into fragments, are also denatured.*

Fig. 1

Fig. 2

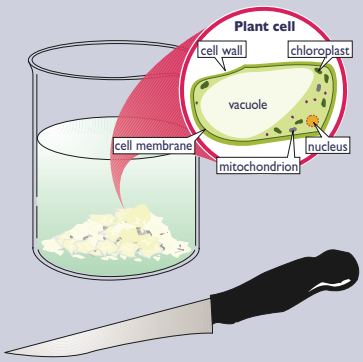
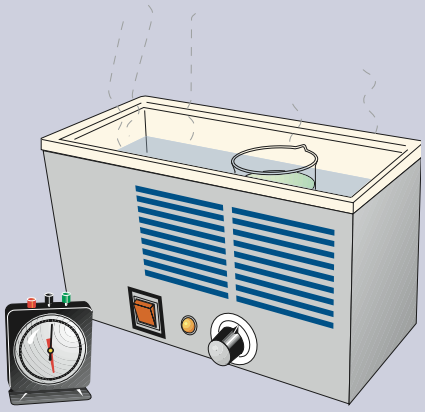


Fig. 3



4 Cool the mixture by placing the beaker in an ice water bath for 5 minutes, stirring frequently. *This slows the breakdown of the DNA which would occur if a high temperature was maintained.*

5 Pour the mixture into a liquidizer and blend for only 5 seconds on high speed. *This degrades the cell walls and membranes further, permitting the release of DNA. Do not blend for too long as this will break up the DNA fibres.*

6 Filter the mixture into a second beaker. Ensure that any foam on top of the liquid does not contaminate the filtrate. *The filtrate contains proteins and DNA.*

Fig. 4

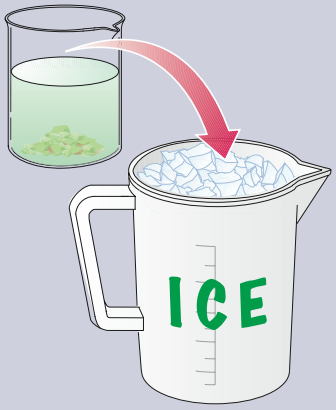
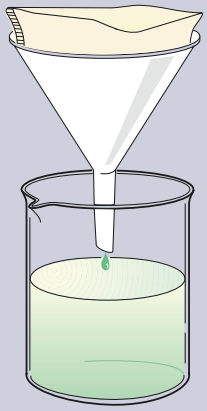


Fig. 5

Fig. 6



7 Add 2–3 drops of protease to about 10cm^3 of the onion extract in a boiling tube and mix well. *The protease will partly degrade the proteins in the preparation.*

8 Very carefully pour ice cold ethanol or IDA down the side of the boiling tube, to form a layer on top of the onion extract.

9 Leave the tube, undisturbed, for a few minutes. Nucleic acids (DNA and RNA) are insoluble in cold ethanol and will precipitate into the upper (ethanol) layer. The DNA is the white material in the clear alcohol layer.

Fig. 7

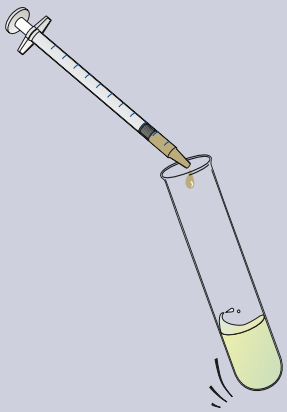
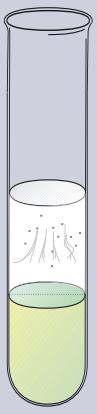


Fig. 8

Fig. 9



Further investigations

A hook for recovering the DNA can be made by briefly heating the tip of a Pasteur pipette in a Bunsen burner flame, then bending the tip round before allowing the glass to cool. To electrophorese the DNA, dissolve some of it in about 0.5 cm^3 of bromophenol blue loading dye, then load about 20 µL into a well in a 1% agarose gel. Staining with 0.04% (w/v) aqueous Toluidine blue O solution after electrophoresis will reveal a smear of DNA fragments.

Variations of this extraction procedure can be used for other food items, *e.g.*, fish sperm (milt or soft roe) or fish eggs (Strömberg, 2001). Several publications refer to the use of calf thymus tissue, but its use in schools is no longer recommended (see Safety note, below).

Safety

Ethanol in freezers

Most freezers are not spark-proof. Consequently, you must ensure that any ethanol placed in a freezer is in a sealed, vapour-tight container. An alternative to using a freezer is to stand the sealed bottle of ethanol in ice for several hours before use. For more information about safety in schools when working with DNA, teachers in the UK should consult Topics in Safety (ASE, 2014).

Use of animal tissue

Since the advent of BSE and vCJD in the United Kingdom, school safety authorities there advise that calf thymus should no longer be used in schools, as there is a risk (albeit small) of accidental exposure to the infectious agent while the extract is being prepared.

Suppliers

Most of the items required for this procedure can be obtained from a supermarket.

Novozymes Neutrase® can be bought in small volumes from the National Centre for Biotechnology Education.

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