PRACTICAL BIOTECHNOLOGY

DNA your onions?

ADAPTED FROM A SOURCEBOOK OF BIOTECHNOLOGY ACTIVITIES

ISOLATION OF DNA from cells is the first step in many investigations in molecular biology. The method described here is an adaptation of the 'Marmur preparation' which is used in laboratories throughout the world. First, tissue is broken up mechanically. Detergent helps to degrade both the cell membranes and those surrounding the nuclei. Cell fragments are separated by filtration; the DNA and soluble proteins remain. An enzyme removes the protein, then the DNA is precipitated using ice-cold ethanol.

Materials

For preparing the onion tissue extract:

Liquidizer
Sharp vegetable knife and chopping board
Water bath set at 60°C
Ice, in a jug
Thermometer
250 cm^3 beakers, 2
Coffee filter paper (do not use laboratory filter paper)
Onion, about 100 g
Washing-up liquid, 10 cm^3 (do not use the thicker, concentrated type)
Sodium chloride, 3 g
Distilled water, 100 cm^3

For separating the DNA from the onion extract:

Slender glass rod
10 cm^3 syringe for measuring out liquids
Boiling tube
Rack or small beaker for holding boiling tube
Onion extract (see below), 6 cm^3
Protease enzyme, Novo Nordisk *Neutrase*®, 4 drops (available from the NCBE)
Ice cold 95% ethanol, 9 cm^3
NOTE: it is important to use this concentration of ethanol. Place it in a freezer in a plastic bottle overnight to ensure that it is *ice cold*.

Practical details

Prepare the onion extract as follows:

1. Add the sodium chloride to the washing-up liquid. Make up to 100 cm^3 with distilled water.
2. Cut the onion into small pieces roughly 5 mm square. Place the pieces in a beaker, then pour on the detergent / salt solution.
3. Stir the mixture and maintain it at 60°C in a water bath for exactly 15 minutes. *This treatment causes the onion cell membranes to break down. The detergent forms complexes surrounding the membrane phospholipids and proteins, causing them to precipitate out of solution. In addition, sodium ions from the salt shield the negatively charged phosphate groups of the DNA molecules causing them to coalesce. At 60°C, DNAse enzymes, which would otherwise start to cut the DNA into fragments, are partially denatured.*
4. Cool the mixture in an ice water bath for 5 minutes, stirring frequently. *This slows the breakdown of 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 the second beaker. Ensure that the foam on the surface of the liquid does not contaminate the filtrate.
   The filtrate contains soluble proteins and DNA.

NOTE: *The filtrate may be prepared before a lesson and stored in a refrigerator for 1–2 days if desired.*

Separating DNA from the onion extract:

1. Add the enzyme to the onion tissue extract in a boiling tube and mix well. *The enzyme will degrade the proteins associated with the DNA.*
2. Form a layer of ice cold ethanol on top of the onion extract / enzyme mixture by pouring it slowly down the side of the boiling tube. Leave the tube for 2–3 minutes without disturbing it. *DNA is insoluble in ice-cold ethanol. Bubbles will form and whilst other compounds in the mixture dissolve, the DNA will precipitate.*
3. Gently rotate the glass rod in the liquid, at the interface of the alcohol and detergent mixture. Take care not to mix the layers too much or to break up the fragile DNA. The white web of mucus-like DNA can be drawn from the tube with a Pasteur pipette and resuspended in 4% sodium chloride solution.

Safety

This protocol presents no particular safety hazards, although care should be exercised when chopping the onion or using a liquidizer.

Further activities

1. The DNA may be stained e.g. using aceto-orcein, and examined under a microscope.
2. The acidic nature of the DNA may be confirmed using Universal indicator solution.
3. Certain animal tissues (e.g. cod roe, liver, calf thymus i.e. sweetbread) or microorganisms may be treated in a similar way to extract their DNA.

IMPORTANT NOTE

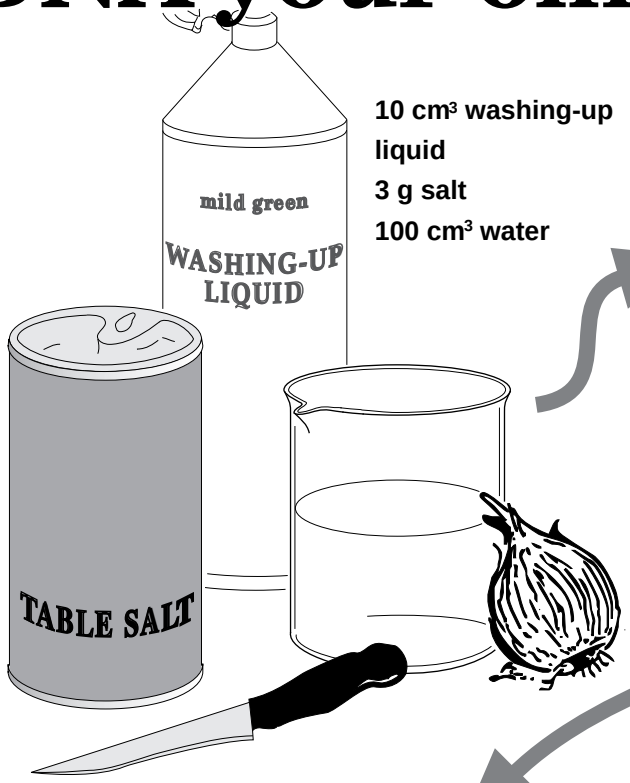
Nucleic acids prepared in this way will not be very pure. The essential purpose of the technique described is to demonstrate the major principles involved in the extraction of DNA from tissue.

ADDITIONAL INFORMATION

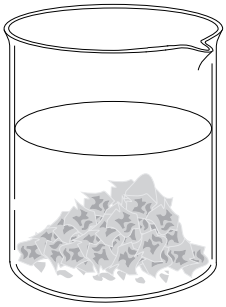
This work is adapted from *A Sourcebook of Biotechnology Activities* by Alison Rasmussen and Robert Matheson (1990) National Association of Biology Teachers / North Carolina Biotechnology Center.
ISBN: 0 941212 09 2.

The full publication is available from the NABT, 11250 Roger Bacon Drive #19, Reston, Virginia 22090, USA.

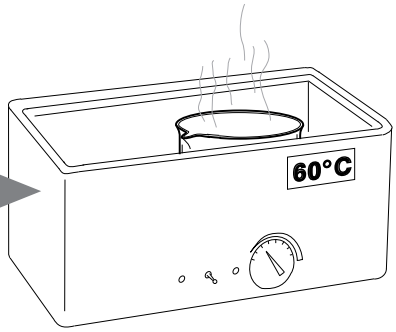
DNA your onions?



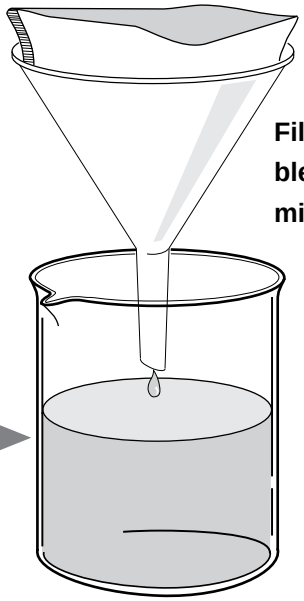
10 cm³ washing-up liquid
3 g salt
100 cm³ water



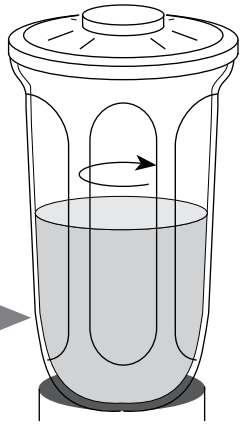
Add the salty washing-up liquid solution to the whole chopped onion



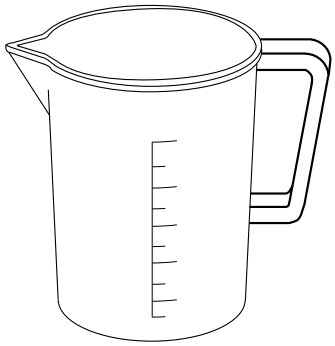
Place in water bath at 60°C for 15 minutes



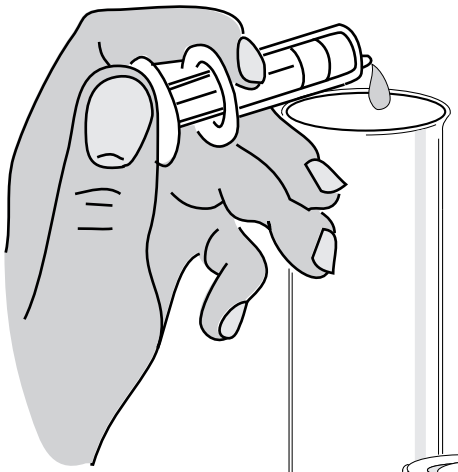
Filter the blended mixture

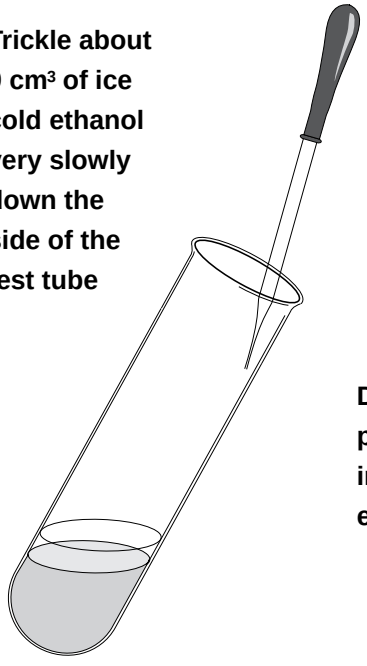


Blend in a liquidizer for 5 seconds only

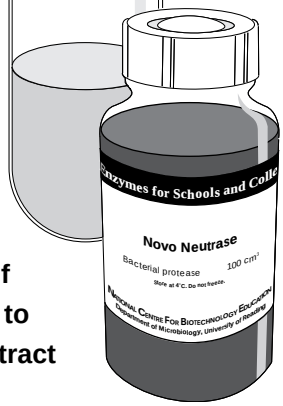


Cool the mixture by standing it in jug of ice for a few minutes

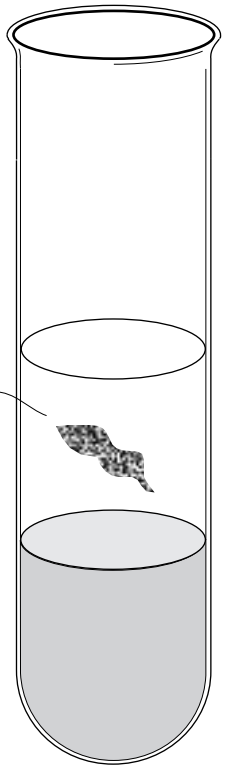




Trickle about 9 cm³ of ice cold ethanol very slowly down the side of the test tube



Add 2—3 drops of protease enzyme to 6 cm³ of onion extract



DNA

DNA precipitates into the ethanol layer