

extracting the pea (dna)

This is a crude but effective method of isolating DNA and RNA from peas. First, the tissue is broken up mechanically. Household detergent is used to degrade both the cell membranes and those surrounding the nuclei. Cell fragments are separated by filtration; the nucleic acids and soluble proteins remain. Protease is used to partly degrade soluble proteins, then the nucleic acids are precipitated into ice cold ethanol. The pea DNA can be run on an electrophoresis gel if it is first dried then dissolved in TE buffer.

Aim

To isolate nucleic acids (DNA and RNA) from peas.

Preparation

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

Timing

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

Materials and equipment

Needed by each person or group

- Peas, about 50 g (frozen ones are suitable, but thaw them first)
- Washing-up liquid, 10 cm³ (use a watery type, not the thicker, concentrated variety)
- Table salt, 3 g
- Distilled water, 90 cm³
- Very cold ethanol, about 10 cm³, straight from the freezer (industrial methylated spirit, IMS, is suitable, but please see the safety note on this page)
- *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)
- 1 cm³ plastic syringe (without a needle)
- Large plastic funnel
- 250 cm³ beakers, 2
- Boiling tube or plastic graduated tube
- Glass rod with a flattened end or a spoon for stirring

Procedure

1. Dissolve the salt in 90 cm³ of distilled water. Add the washing-up liquid and mix gently.
2. Mash the peas using a glass rod or a spoon. Add the pea pulp 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.
4. Cool the mixture by placing the beaker in an ice water bath for 5 minutes, stirring frequently.
5. **OPTIONAL:** Pour the mixture into a blender and blend it for *no more than 5 seconds*.
6. Filter the mixture into a second beaker. Ensure that any foam on top of the liquid does not contaminate the filtrate.
7. **OPTIONAL:** Add 2–3 drops of protease to about 10 cm³ of the pea extract in a boiling tube and mix well.
8. Very carefully pour ice cold ethanol down the side of the boiling tube, to form a layer on top of the pea extract.
9. Leave the tube, undisturbed, for a few minutes. Nucleic acids (DNA and RNA) will precipitate into the upper (ethanol) layer.

Safety

Ethanol in freezers

Most freezers are not spark-proof. There have been instances of explosions when flammable vapours *e.g.*, from ethanol have accumulated within a freezer and been ignited by a spark. If you do not have a spark-proof freezer, you **MUST** ensure that any ethanol is placed in a sealed, vapour-tight plastic container. If you don't have access to a freezer, put some ice in an insulated container, then stand the bottle of ethanol in it for several hours before use.



RESOURCES

Marmur, J. (1961) A procedure for the isolation of DNA from micro-organisms *Journal of Molecular Biology* **3** (2) 208–218.

This paper describes the original DNA extraction method on which this, and many similar procedures, are based. It is listed here purely for historical interest.

Investigating plant DNA by Dean Madden [Ed.] (1995) National Centre for Biotechnology Education, The University of Reading.

This is a guide from an NCBE practical kit, explaining how to extract DNA from a variety of plant tissues and how to 'run' it on an electrophoresis gel.

Millar, R. (1996) DNA from bacterial cells [Edited by J. Richardson]

dna from other things

The method described above can be adapted for use with other plant or animal cells, for example, onions or fish sperm. In fact, DNA was first isolated from salmon eggs and sperm by Freidrich Miescher in 1897. Working before the days of freezers, Meisher started in the lab at 5 am to ensure that his ethanol supply was cold enough.

Materials and equipment

- Fish sperm ('milk'), cut from a fresh or frozen fish (*e.g.*, from cod, salmon or trout)
- Household detergent
- Saturated solution of table salt (about 13% by mass)
- Cold ethanol (IMS)
- Gauze
- Teaspoon
- Mortar and pestle
- Filter funnel
- 250 cm³ beaker
- 100 cm³ measuring cylinder



Procedure

1. Grind a teaspoonful of fish sperm.
2. Add 50 cm³ of water and stir.
3. Filter through several layers of gauze.
4. Add a few drops of detergent and 50 cm³ of saturated salt solution. Stir again.
5. Gently pour cold ethanol onto the filtered solution. The DNA precipitates at the border between the water and ethanol.

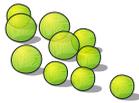
This method was kindly supplied by Dr Elisabeth Strömberg of the Mathematics and Natural Sciences Resource Centre (M/N/RC), University of Göteborg.



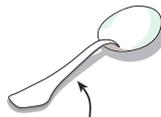
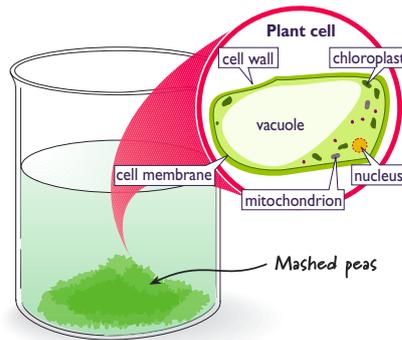
- 1** Add 3 g of table salt to 90 cm³ water. Stir or shake well to dissolve the salt. Gently stir in 10 cm³ of washing-up liquid.



You can use frozen peas for this, but let them thaw first!



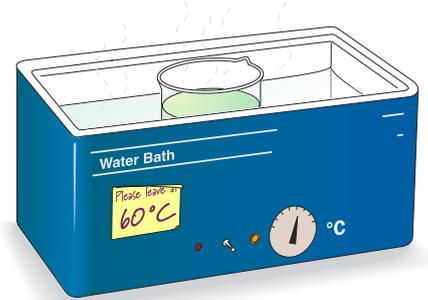
- 2** Add 50 g of peas to a beaker of the salty washing-up liquid solution. Mash the peas up a bit, using a spoon or something similar.



De-luxe pea masher

The washing-up liquid breaks down the cell membranes, releasing DNA from the nucleus, chloroplasts and mitochondria inside each cell.

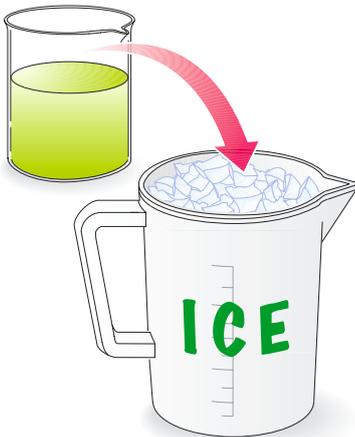
- 3** Stand the beaker in hot water at 60 °C for exactly 15 minutes.



Hot conditions speed up the process and denature DNases that might degrade the DNA ...

... but leave it too long and the DNA is broken up as well.

- 4** Cool the mixture by standing the beaker in a jug of icy water for a few minutes.

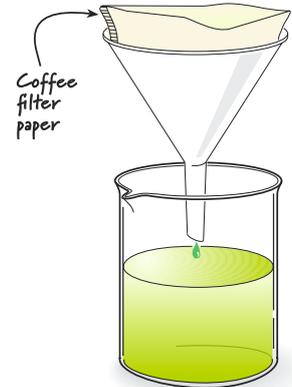


- 5** **OPTIONAL:** Blend the cooled mixture for no more than 5 seconds.

The blender helps to break open the pea cells — but don't blend for too long or the DNA will be sheared.

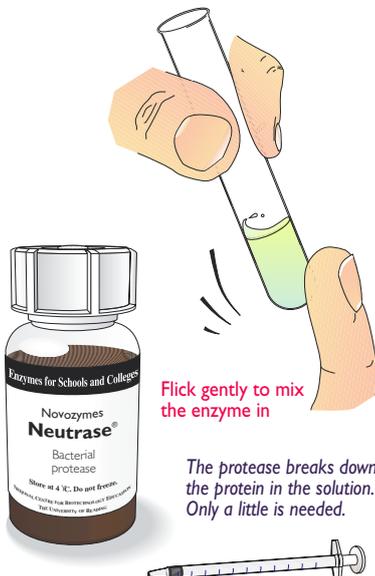


- 6** Filter the pea tissue from the liquid.



This separates the cell wall material from the DNA and proteins, which are now in solution.

- 7** **OPTIONAL:** Add 2–3 drops of protease enzyme to about 10 cm³ of the pea extract.



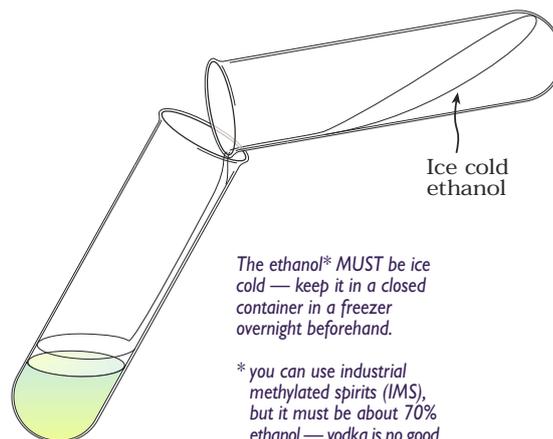
Flick gently to mix the enzyme in

The protease breaks down the protein in the solution. Only a little is needed.

- 8** Very carefully pour an equal volume of ice cold ethanol onto the surface of the pea extract.



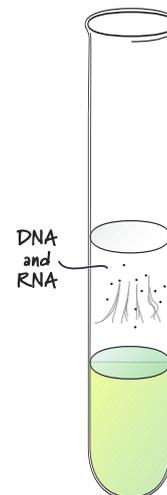
WARNING
Ethanol, if chilled in a freezer, must be stored in a sealed, vapour-tight container.



The ethanol* MUST be ice cold — keep it in a closed container in a freezer overnight beforehand.

* you can use industrial methylated spirits (IMS), but it must be about 70% ethanol — vodka is no good.

- 9** Bubbles of air carry the DNA and RNA into the upper (ethanol) layer. You can scoop it out with a wire loop if you are so inclined.



DNA doesn't dissolve in ethanol — so it comes out of solution in the upper layer.