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DNA from 'caviar'

Simple extraction of DNA from fish eggs

Aim

This simple practical procedure allows the isolation of impure DNA from 'caviar' or fish eggs. The result is a pellet of thread-like material, which includes DNA but will still be contaminated with lipids, carbohydrates and proteins.

Nevertheless, samples prepared in this way are sufficiently clean to be 'run' on an electrophoresis gel, producing a 'smear' of DNA and RNA fragments of different sizes when stained.

Equipment and materials

Needed by each person or group

- 20–30 g caviar (about 2 heaped teaspoonsful) *e.g.*, roe from capelin (*Mallotus villosus*) or lumpsucker (*Cyclopteris lumpus*).
  Note: such roe is sold under the *Abba*® brand name. The yellow or 'natural' variety works best.
- 15 ml washing-up liquid *e.g., Fairy Liquid*, diluted 1:10 with distilled water
- 1 teaspoon (about 6 g) of table salt
- 2 ml ethanol. This must be ice-cold and at least 80% pure; store it in a freezer before use, *but please see the safety note below.*
- 3–4 drops of protease, *e.g., Novozymes Neutrase*®
- Glass rod
- Coffee filter paper
- Funnel
- Small test tube
- Dropper or pipette for dispensing the enzyme
- Pasteur pipette, the tip of which has been melted and curved to form a small hook
- Microcentrifuge tube (1.5 ml) in which to store the extracted DNA

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Procedure

1 Add the caviar and salt to a mortar, then crush the eggs using a pestle. *The shells of the eggs have to be broken. Proteins are precipitated by the salt.*

2 Add the washing-up liquid solution to the mortar. The liquid should cover the caviar completely. *The detergent dissolves lipids from the membranes of the roe.*

3 Add 3–4 drops of protease to the mixture and stir vigorously. *The enzyme will partially degrade any soluble proteins.*

Fig. 1

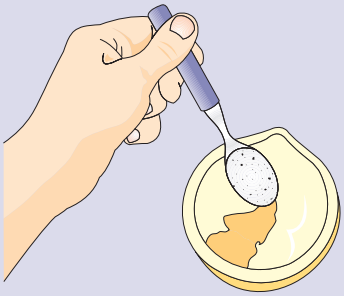


Fig. 2

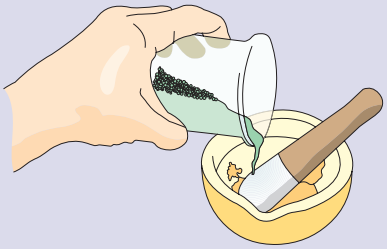


Fig. 3

4 Filter the mixture through the coffee filter and collect the filtrate in a clean test tube.

5 Add the ice-cold ethanol by carefully pouring it along the wall of the tube or use a pipette and add it at the bottom of the test tube. *DNA precipitates as long threads in cold ethanol and can be found at the interface between the detergent solution and the ethanol.*

6 Collect the DNA with the help of a Pasteur pipette with a hooked tip. *The DNA may be transferred to a microcentrifuge tube and stored, frozen, for later use e.g., for gel electrophoresis or staining of the DNA.*

Fig. 4

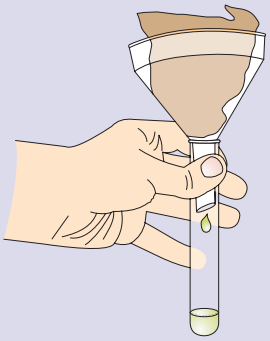


Fig. 5

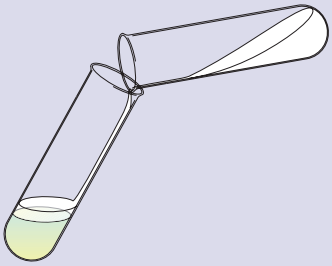
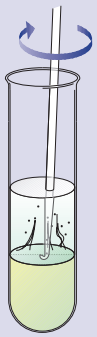


Fig. 6



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* [1].

Preparation

The ethanol must be thoroughly chilled before use.

Timing

This activity takes about an hour.

Troubleshooting

Some washing-up liquids are not suitable for this work. They may extract the lipids so poorly from the cell membranes that DNA fails to be exposed. We have found that *Fairy Liquid* gives good results. The type of caviar used is also important; the yellow ('natural') type works well. Caviar spread that comes in tubes is not suitable for this method, as the fish eggs are combined with other ingredients and are too small to crush properly. It is important to crush the fish roe thoroughly to rupture the egg membranes.

If a protease is not used the method is also less effective because proteins remain in the solution.

Further investigations

To electrophorese the DNA extract, simply dissolve some of it in about 0.5 ml of bromophenol blue loading dye, then load about 20 µl into a well in a 1% agarose gel. Staining with Azure A solution after electrohoresis will reveal the nucleic acids (RNA shows up a lighter pink colour) [2].

Variations of this extraction procedure can be used for other food items, *e.g.*, sperm (milt or soft roe) from fish, calf thymus (sweetbread) or fruits and vegetables like peas or onions [3].

Some fruits, *e.g.*, strawberries and kiwi fruit that reportedly give large amounts of 'DNA', are actually yielding pectin. Soft roe or sweetbreads will give the best result but they may be difficult to obtain.

IMPORTANT!
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* in the UK:
http://www.ncbe.reading.ac.uk

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.

References

1 Delpech, R. and Madden, D. (2001) 'Working with DNA'. In *Topics in Safety* (Third Edition) pp. 99–105. Hatfield: Association for Science Education. ISBN: 0 8635 7316 9.
This chapter may be downloaded from here.

2 *Illuminating DNA* by Dean Madden (2000) Reading: National Centre for Biotechnology Education. ISBN: 0 7049 1370 4.
This comprehensive practical guide can be downloaded from: http://www.ncbe.reading.ac.uk

3 *Investigating plant DNA* by Dean Madden [Ed.] (1995) Reading: National Centre for Biotechnology Education.
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. It may be downloaded from: http://www.ncbe.reading.ac.uk

Further reading

Millar, R. (1996) DNA from bacterial cells *SSERC Bulletin* **189**, p. 6–8.
This article describes the micro-scale extraction of DNA from bacteria as an alternative to Gram staining.