Equipment and materials

Dispensing the reagents

For students to use

Purification reagent
6.5 mL of the Purification reagent is supplied. Each student or group of students will need 350 µL of this solution, dispensed into a red, 1.5 mL microcentrifuge tube.

TE-1 buffer
6.5 mL of the TE-1 buffer is supplied. Each student or group of students will need 350 µL of this solution, dispensed into a blue, 1.5 mL microcentrifuge tube.

PCR primers and water
Use the 20 µL Volac pipette provided and clean tips to dispense 20 µL of each of Primer 1, Primer 2 and sterile distilled water for each student or group of students. This is more than they need, but these reagents are cheap!

Primer 1 should go in a green 0.5 mL microcentrifuge tube, Primer 2 into a violet tube, and the water into an orange tube. The ‘stock’ solutions are provided in screw-capped tubes with lids of the same colours.

DNA ruler/ladder (1 kb)
25 µL of ‘stock’ DNA ruler is provided in a 1.5 mL screw-capped microcentrifuge tube. Dilute this for students to use by adding 80 µL of sterile distilled water to the tube (use the 20 µL Volac pipette for this). Then add 10 µL of bromophenol blue loading dye (use a microsyringe unit with a white graduated tip). Mix well by drawing the liquid up and down in the tip, then dispense 12 µL into each of 8 yellow 0.5 mL microcentrifuge tubes for students to use.

These diluted solutions can be stored in a freezer for up to a month before use: the undiluted stock can be stored indefinitely in a freezer.

Additional technical details

‘Ready-to-go®’ PCR beads
Each ‘Ready-to-go®’ PCR bead is designed for a single 25 µL reaction. On resuspending to 25 µL, the final component concentrations are:

- Tris-HCl (pH 9.0) 20 mM
- KCl 50 mM
- MgCl₂ 1.5 mM
- dNTP mixture 0.2 mM of each dNTP
- Taq polymerase 2.5 units

DNA extraction reagents
The Whatman FTA® cards contain SDS (a detergent), TE buffer and other proprietary reagents. The Purification Reagent is also proprietary. The TE-1 is simply dilute Tris-EDTA buffer.

Primer sequences
The primers used in the protocol typically span a 400 bp fragment. Their sequences are:

- Primer 1: 5’-CGAAATCGGTAGACGCTACG-3’
- Primer 2: 5’-GGGGATAGAGGGACTTGAAC-3’
Plants to test

We have tested and obtained good results with:

- Nasturtium
- Spinach
- Rocket
- Spring onion
- Watercress*
- Scenedesmus quadricauda
- Chlorella vulgaris
- Red chard
- Sweet and chilli peppers (skin of green varieties)

* Watercress consistently gives a larger PCR product.

Species with tough or fibrous leaves do not work well with this extraction method. In our (limited) tests, none of the following plants gave reliable amplification:

- ‘Cress’ (*Brassica napus* or *Lepidium sativum* seedlings);
- Cabbage and broccoli (both plants are the same species, *Brassica oleracea*);
- Thyme, basil or mint;
- Cucumber or courgette skin;
- Weeping fig (*Ficus benjamina*).

Additional resources

The polymerase chain reaction


An animated simulation of the PCR can be downloaded from Cold Spring Harbor Laboratory’s Dolan DNA Learning Center: [www.dnalc.org/ddnalc/resources/animations.html](http://www.dnalc.org/ddnalc/resources/animations.html)

The Protein Data Bank [www.rcsb.org/pdb] features DNA polymerase in its ‘Molecule of the month’ series.

Timing

This investigation is a complex multi-step process. Careful planning is therefore necessary to fit it into a normal series of school lessons or a single practical session. The whole procedure, including drying the FTA® cards and manual PCR but excluding the electrophoresis, takes about three hours. The duration of the electrophoresis depends upon the equipment and voltage used. As a rough guide, the timing of this protocol is as follows:

- Extracting the DNA onto FTA® cards: 15 minutes
- Drying time: 60 minutes

*OPTIONAL STOP POINT*

- Cleaning the DNA samples: 20 minutes

*OPTIONAL STOP POINT*

- Preparing the PCR reagents: 10 minutes
- PCR manual cycling: 50 minutes
- PCR thermal cycler: ~ 90 minutes

*OPTIONAL STOP POINT*

- Preparing and loading the gel: 30 minutes
- Electrophoresis: 30 minutes – 3½ hours
- Staining the gel: 10 minutes

Plant classification

Guidance for selecting plant species is provided by the web-based Tree of Life project: [http://tolweb.org/](http://tolweb.org/)

The classification produced by the Angiosperm Phylogeny Group is described in:

- APG (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants; APG II. *Botanical Journal of the Linnean Society* 141, 399–436. This paper and additional information is available on-line from: [www.mobot.org/MOBOT/research/APweb/](http://www.mobot.org/MOBOT/research/APweb/)

Molecular structures

The molecular images in the student’s booklet were created using structure data from the Protein Data Bank. The software used to produce the images was Visual Molecular Dynamics. VMD can be downloaded free-of-charge for Macintosh, Windows, Linux and other platforms from: [www.ks.uiuc.edu/Research/vmd/](http://www.ks.uiuc.edu/Research/vmd/)
Safety guidelines

FTA® cards and PCR reagents

FTA® paper is non-toxic to humans and hypo-allergenic. The PCR reagents are also non-toxic. Cleanliness is important to prevent cross-contamination and ensure success, so dirty tubes and tips should not be re-used. Used plastic tubes and tips, which are made of polypropylene, can be disposed of in the normal waste.

Loading dye (Bromophenol blue)

When used as directed, this loading dye presents no hazard. Used loading dye can be washed down the drain.

Electrode tissue

The carbon fibre electrode tissue may release small fibres, which can cause skin irritation if you handle the tissue a lot. Wear protective gloves if you find the tissue unpleasant to handle. The fibres are too large to enter the lungs however, so it is not necessary to wear a face mask. The fibres are soluble in body fluids and are completely biodegradable.

Agarose gel

If a microwave oven is used to melt the agarose gel, ensure that the gel is placed in an unsealed container. A boiling water bath or hotplate may be used instead, but the gel must be swirled as it melts to prevent charring. The use of a Bunsen burner to melt agarose is not recommended.

If you use water baths for the PCR (rather than an automated thermal cycler), take care not to scald yourself when transferring tubes between them. Use forceps or a clothes peg to hold the foam floater and/or wear heat-resistant gloves to protect your hands from the hot water and steam.

Molten agarose can scald and it must be handled with care, especially just after it has been heated in a microwave oven.

Hot water baths

TBE buffer (Tris-Borate-EDTA)

When used as directed, this buffer presents no serious safety hazards. Spent buffer can be washed down the drain.

DNA stain (Azure A)

The concentrated DNA stain solution is flammable and it must be kept away from naked flames. The stain is Azure A, which when diluted as directed, forms a 0.04% solution in 20% ethanol. At this concentration it presents no serious safety hazard, although care should be taken to prevent splashes on the skin or eyes e.g., wear protective gloves and glasses. Used stain may be washed down the drain.

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Science and Plants for Schools

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University of Reading

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