The PCR and Plant evolution
Teacher’s notes
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)**

130 µL of ready-diluted DNA ruler is provided in a 1.5 mL screw-capped tube. Before use, add 12 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 14 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.

**IMPORTANT:** Please note that the DNA ‘ladder’ or ‘ruler’ supplied in the kit is NOT the same as that described in the accompanying Student’s guide. It is an improved version with more regularly-distributed DNA fragment sizes (see sheet provided with this guide).

Equipment and materials

**Equipment and materials**

Required by each student or working group (there is sufficient material in the kit for 16 amplifications)

**Equipment included in the kit**

- Punch for cutting 2 mm discs
- Pipettes to measure 2, 10, and 100 µL volumes
- Floating microcentrifuge tube holder

**Equipment not included in the kit**

- Pestle
- Pair of forceps
- DNA electrophoresis equipment and power supply
- Microsyringe dispensing units
- Protective gloves (to protect hands from DNA stain)
- Pencil
- Permanent marker pen for marking the tubes and the lanes on the gel tank
- Access to three water baths maintained at 55, 72 and 94 °C, or a thermal cycler (PCR machine)

**Materials included in the kit**

- Microcentrifuge tubes (1.5 mL)
- Micropipette tips
- Whatman® FTA® Plant Card
- FTA® Purification Reagent (~ 350 µL per plant sample)
- TE-1 buffer (~ 350 µL per plant sample)
- ‘Ready-to-go’ PCR beads (1 per plant sample)
- PCR primers and sterile distilled water, for each sample as follows:
  - 10 µL of Primer 1
  - 10 µL of Primer 2
  - ~ 6 µL of sterile distilled water
- 1 kb DNA ladder/ruler (only one is required per gel)
- Bromophenol blue loading dye (~2 µL per sample)
- Electrophoresis (TBE) buffer solution
- Agarose powder (make a 1.5% agarose gel by dissolving the powder in diluted electrophoresis buffer)
- Toluidine blue O (0.04% w/v) in water (for staining DNA)

**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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris-HCl (pH 9.0)</td>
<td>20 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>dNTP mixture</td>
<td>0.2 mM of each dNTP</td>
</tr>
<tr>
<td>Taq polymerase</td>
<td>2.5 units</td>
</tr>
</tbody>
</table>

**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).

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

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

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

Plant classification

Guidance for selecting plant species is provided by the web-based Tree of Life project: 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/

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/
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.

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.

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.

Hot water baths

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.

Electrical supply

The gel electrophoresis equipment was designed to be used with direct current at low voltages (≤36 volts) with dry cell batteries. Under no circumstances should this voltage be exceeded, as the live electrical components are exposed.

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 (Toluidine blue O)

The concentrated DNA stain solution is Toluidine blue O, which when diluted as directed, forms a 0.04% solution in water. 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. [This stain replaces Azure A, which was previously supplied with this kit.]

Acknowledgements

Science and Plants for Schools

Several people contributed to the development of the original practical protocol from which this kit was developed and we wish to thank them all.

Kenny Hamilton from Breadalbane Academy in Aberfeldy did much of the experimental work while on a Royal Society of Edinburgh Teacher Fellowship at the University of Edinburgh. Craig Simpson of the Gene Expression Programme at the Scottish Crop Research Institute provided invaluable advice on the design of the original protocol as well as laboratory facilities. Kath Crawford and Kirsty Menzies of the SAPS Scotland Biotechnology Project and Jan Barfoot of the Scottish Institute for Biotechnology Education provided both subject and technical expertise. Additional support from Professor Mary Bownes’s research group of the University of Edinburgh is gratefully acknowledged.

Paul Beaumont, then Director of SAPS, kindly read through the draft version of the Student’s guide and made many corrections.

University of Reading

Andy Harrison at the National Centre for Biotechnology Education adapted the original SAPS protocol. He developed a simpler and cheaper method of extracting plant DNA and a revised PCR protocol. Andy also did most of the work in converting a laboratory procedure into kit form.

Julie Hawkins in the School of Biological Sciences kindly advised on the section in the Student’s booklet dealing with modern plant classification.
A DNA ‘ladder’ or ‘ruler’ consisting of fragments up to 1.2 kb is included so that the DNA under investigation can be compared with fragments of known sizes.

The fragment sizes are given in base pairs (bp).

The bromophenol blue loading dye moves through the gel as if it were made of 500 bp DNA fragments.

With a six-tooth comb, you should use 2 µg of ‘ruler’ solution per well. With the 4-tooth comb you will need about 3 µg per well.

Note: This DNA ruler is NOT the same as the one shown in the student’s guide. It is an improved version with more regularly-distributed DNA fragment sizes.