Polymerase Chain Reaction: Don't assume

Background

Since the first description of the technique [1], the polymerase chain reaction (PCR) has become an indispensible tool with applications in virtually all biological, biomedical and biotechnological areas of science [2-6].

As many readers will be aware there has been considerable debate over the content of the new Higher Biology. However, one thing is certain; PCR will be one of the topics covered when the Arrangements Document is published in November 2010. So, what resource might one use to support teaching and learning? A number of animations and digital images are available [7] but we believe that there is no substitute for practical work.

We have previously described [8] the development of a PCR protocol suitable for use in secondary schools and colleges. More recently our colleagues at the *National Centre for Biotechnology Education* (*NCBE*) have refined the protocol [9] and made it available in the form of a self-contained kit. The current (July 2010) cost of the full kit from *NCBE* is £140 [10]. The PCR kit, as marketed by *NCBE*, allows students to extract chloroplast DNA from plants and identify possible evolutionary relationships between different species. What the protocol, as written, does not demonstrate is that increasing the number of amplification cycles during PCR will increase the amount of DNA present for staining in the electrophoresis stage. Clearly for PCR to work this increase in the amount of DNA present must be happening but we thought it would be both interesting and useful to have a protocol which could be utilised to show that this is the case. So, there is one key aim of the experiment we describe here – *viz* to show that an increase in the number of PCR cycles leads to an increase in the amount of DNA which can be observed.

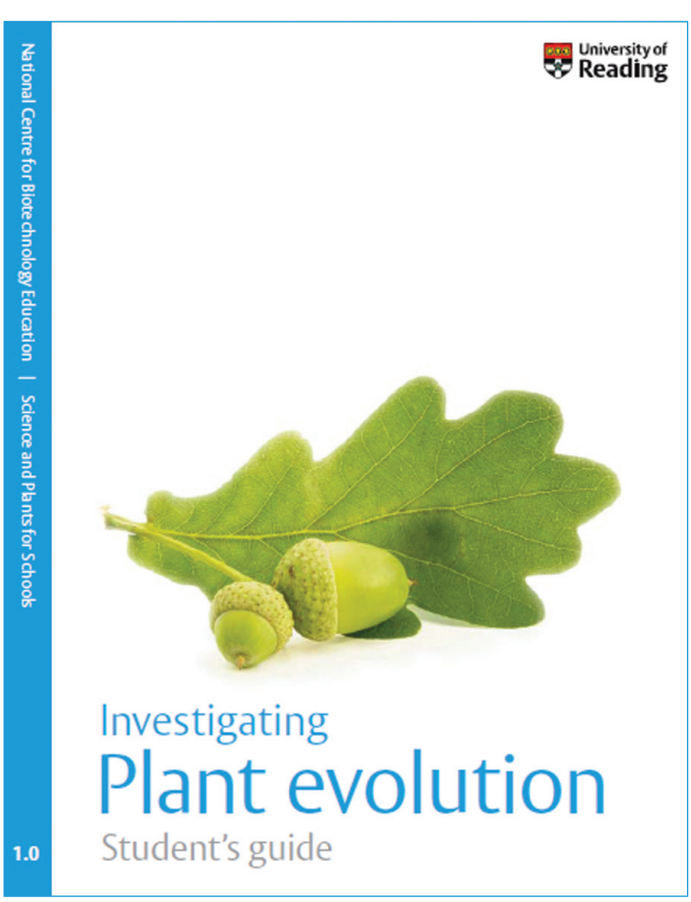

Figure 1 *- See the Student's Guide for full information.*

Methodology

We utilised the *Investigating Plant Evolution* kit from *NCBE* for this practical. The DNA that is amplified is a highly variable non-coding region of chloroplast DNA (cpDNA). In addition to the items in the kit you will need to supply the following:

1. some fresh plant material – any soft, thin leaf will do (we used spinach and red chard)

2. a thermal cycler or three water baths maintained at 94°C, 55°C & 72°C

3. apparatus for gel electrophoresis

It is not our intention here to rewrite the method involved since it is more than adequately covered in the Student Guide booklet accompanying the kit (Figure 1) [9]. We believe that the learning experience for your students will be enhanced if the following are emphasised:

1. the PCR bead contains (i) equal amounts of dATP, dTTP, dCTP, dGTP, (ii) the heat-stable enzyme *Taq* polymerase, (iii) buffer, and (iv) $MgCl_2$

2. the primers provided have been synthesised so as to be complementary to the 5' end of the targeted cpDNA

3. the three steps in a PCR cycle involve:

   (i) denaturation (94°C)
   (ii) annealing (55°C)
   (iii) extension (72°C)

Clear diagrams explaining each step of the PCR process are given in the Student Guide accompanying the kit [9]. Although thermal cyclers are beyond most school budgets the protocol works equally well when performed manually using water baths (we are aware of a UK-based supplier that markets 'PCR' water-baths which incorporate 3 chambers into a single housing [11]). Since it is unlikely that your students will undertake PCR on many occasions during their period in school or college one could make a strong case to suggest that the learning process will be enhanced if the amplification is done manually. If you have emphasised what happens at each temperature the students will be able to envisage the step each time they transfer the tubes from one water bath to the next.

Results

You can clearly demonstrate that PCR amplifies DNA by dividing your students into three groups:

1. Group 1 whose task is to carry out 20 amplification cycles

2. Group 2 whose task is to carry out 25 amplification cycles

3. Group 3 whose task is to carry out 30 amplification cycles (the normal number of cycles recommended for use with this protocol [9])

   - Lanes 1 and 4 = 20 amplification cycles
   - Lanes 2 and 5 = 25 amplification cycles
   - Lanes 3 and 6 = 30 amplification cycles

We took 3 samples of both spinach and red chard and ran either 20, 25 or 30 PCR amplification cycles. Once these amplifications had been carried out we ran samples of the products using agarose gel electrophoresis followed by staining with Azure A [9]. The results are presented in Figure 2. As can be seen there is an evident increase in the intensity of colour of the dye and this reflects the increase in the amount of DNA present as we move from 20 cycles through to 30 cycles of amplification. This is a nice way of showing that increasing the number of amplifications does indeed increase the amount of DNA present.

We have made no attempt to quantify the intensity of the bands produced - perhaps a good student investigation in the making?

Acknowledgements

We would like to thank Rodger McAndrew who made important contributions to the practical work associated with this article.

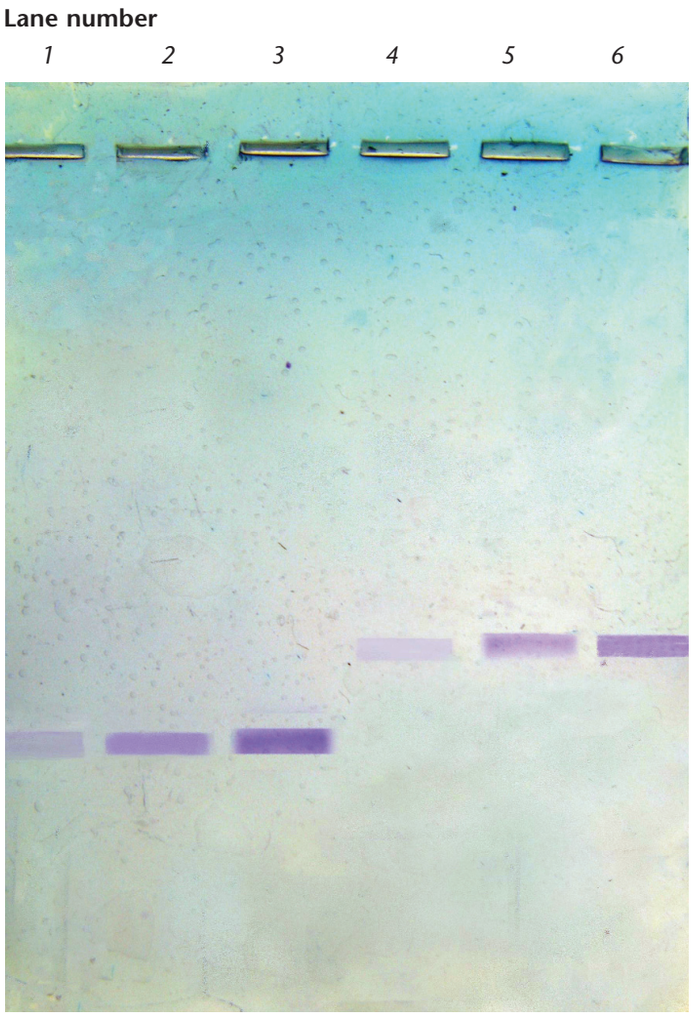

Figure 2 *– Bands of cpDNA, stained with Azure A, obtained following PCR of chloroplast DNA from spinach (lanes 1-3) and red chard (lanes 4-6).*

References

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[9] National Centre for Biotechnology Education (see www.ncbe.reading.ac.uk/) have produced both teacher and student guides and these are available at www.ncbe.reading.ac.uk/ncbe/MATERIALS/DNA/PDF/PlantPCRTG.pdf and www.ncbe.reading.ac.uk/ncbe/MATERIALS/DNA/PDF/PlantPCRSG.pdf respectively (accessed July 26th 2010).
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[11] Edvotek UK (http://edvotek.co.uk/) (accessed July 26th 2010).