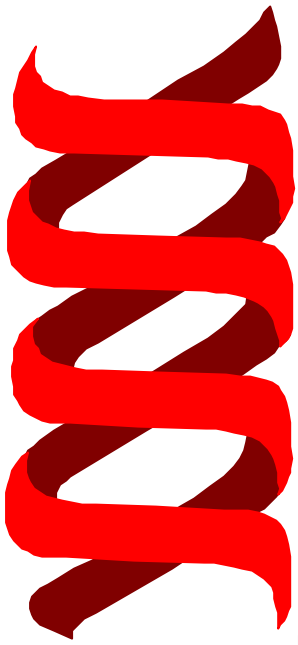
DNA FINGERPRINTING

&

CONSERVATION OF BRITISH ORCHIDS

DNA FINGERPRINTING AND CONSERVATION OF BRITISH ORCHIDS



DNA, or deoxyribonucleic acid, is found in all living organisms. DNA is a long chain of nucleotides, the order of which differs from organism to organism. In complex organisms such as humans and other mammals, each individual (except for identical twins) has unique DNA. Differences in DNA make one individual different from the next – for example, one person might have DNA containing genes for blue eyes, while another has DNA containing genes for brown eyes.

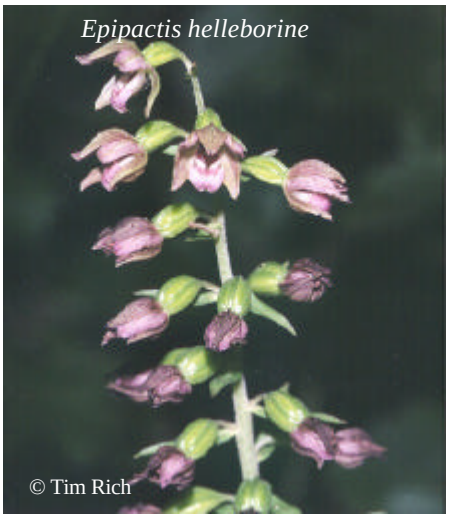
DNA fingerprinting is a scientific technique that can provide us with information about an organism's DNA. In DNA fingerprinting, DNA is firstly cut into smaller pieces by enzymes called restriction endonucleases which recognise specific sequences of bases within the DNA molecule. As DNA from each organism is different, these restriction endonucleases will cut the DNA from each individual at different places and produce fragments of different lengths. Gel electrophoresis is then used to separate the DNA fragments. To do this, the pieces of DNA are placed in a gel, and an electric current is applied to the gel. The electric current makes the DNA fragments move through the gel, with the negatively charged DNA moving towards the positive electrode. Smaller fragments move more easily through the gel and so travel faster than larger ones. The DNA fragments create many different bands on the gel and form a banding pattern representative of an individual. The banding patterns from different DNA samples can then be compared to see if the DNA came from the same or related individuals.

For more information on DNA fingerprinting and its applications in a forensics context, go to:
www.protist biology.washington.edu/fingerprint/dnaintro.html

You might have heard of the use of DNA fingerprinting to identify criminals, test for paternity and diagnose genetic diseases. But DNA fingerprinting can also be an invaluable tool to scientists who study plants and animals, and conservationists trying to save endangered plants and animals. DNA fingerprinting can be used to explore genetic diversity, determine new species, and understand movement of organisms within their environment, to name just a few uses. Today you will learn how to use DNA fingerprinting to better understand the natural world.

British orchids and conservation action plans

The UK is one of 153 nations that have signed up to a 'Convention on Biological Diversity'. As a result of this, numerous Biodiversity Action Plans were created to ensure that the biodiversity of species and their habitats we enjoy in this country are carefully conserved. However, have you ever asked yourself how do conservationists know ***what*** to conserve? How do they tell what is rare and what is common? The first step is to recognise and define individual species. The situation is made even more complex by taxonomic controversies. These occur when there is disagreement as to whether a particular plant is a distinct species or whether it is simply a variant of an existing species. Many botanists and conservationists are now using molecular techniques to solve these taxonomic controversies.



Scientists at the Royal Botanic Gardens Edinburgh (RBGE), in collaboration with others from the Universities of Glasgow and Newcastle, have been using DNA profiling technologies in order to help them define and recognise different species. Their research has involved a British orchid called *Epipactis youngiana.* This orchid, which is found on mine spoil heaps in Northumberland and Glasgow, was first described in the 1980s. It was thought to be a new unique species, therefore it was immediately given full conservation status. Dr P Hollingsworth (RBGE) and colleagues have been trying to work out whether *E. youngiana* is indeed a rare species in its own right or whether it is a variant of a more common orchid species, *Epipactis helleborine*. It is vital that these kinds of questions are answered because resources are limited and rare species must be given the correct conservation priority. You can read more about this research and other research undertaken by the Royal Botanic Garden Edinburgh at:

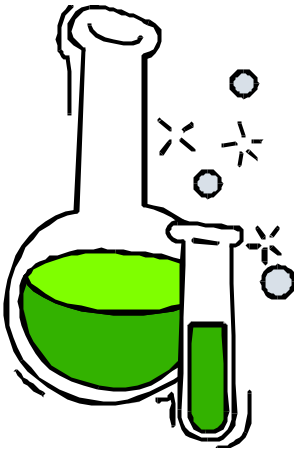
www.rbge.org.uk/rgbe/web/science/research/conservation/congen.jsp

Today you will use a simplified version of DNA Fingerprinting to investigate whether *E. youngiana* is indeed a distinct and rare species worthy of full conservation status or whether it is simply a variant of the more common *E. helleborine*. *E. youngiana* looks very different from *E. helleborine* therefore your initial observations would suggest that they are different species. You must now examine the DNA profiles of these two plants and decide for yourself whether they are one species or two.

The table below gives you more information about each of the DNA samples you will be using:

DNA Sample	Plant sample was collected from	Place of Collection
G1	*E. helleborine*	Glasgow
G2	*E youngiana*	Glasgow
G3	*E. youngiana*	Glasgow
N4	*E. helleborine*	Newcastle
N5	*E youngiana*	Newcastle
N6	*E. youngiana*	Newcastle

STUDENT GUIDE



Materials
Per individual or group
EcoR1/Pst1 enzyme mix (ENZ)
Pipette tips
P20 micropipette
Microtubes
Marker pen
Disposal jar
Foam microtube rack
Ice container
Loading dye (LD)

To be shared
DNA from G1
DNA from G2
DNA from G3
DNA from N4
DNA from N5
DNA from N6
HindIII DNA markers (M)
Water bath at 37°C
Agarose gel electrophoresis tanks
Power supply
TAE Electrophoresis buffer
Water

Safety

Electrical hazard from electrophoresis tank.

DNA Stain can mark clothes and be an irritant.

Eating and drinking are not allowed in the lab.

Methods

1. Make sure your enzyme mix is kept on ice.
2. You have been provided with labelled microtubes each containing 10µl DNA from the different locations. Label each tube with your initials.

G1: Glasgow 1 G2: Glasgow 2 G3: Glasgow 3

N4: Newcastle 4 N5: Newcastle 5 N6: Newcastle 6

3. Using a separate tip for each sample, pipette 10µl enzyme mix (ENZ) into the bottom of each tube.
4. Close the cap. Mix the enzyme and DNA by flicking the tubes gently.
5. Incubate for 45 minutes at 37°C.

The DNA is being cut into fragments by the restriction endonucleases.

6. Using a separate tip, add 5µl Loading Dye (LD) to each tube.

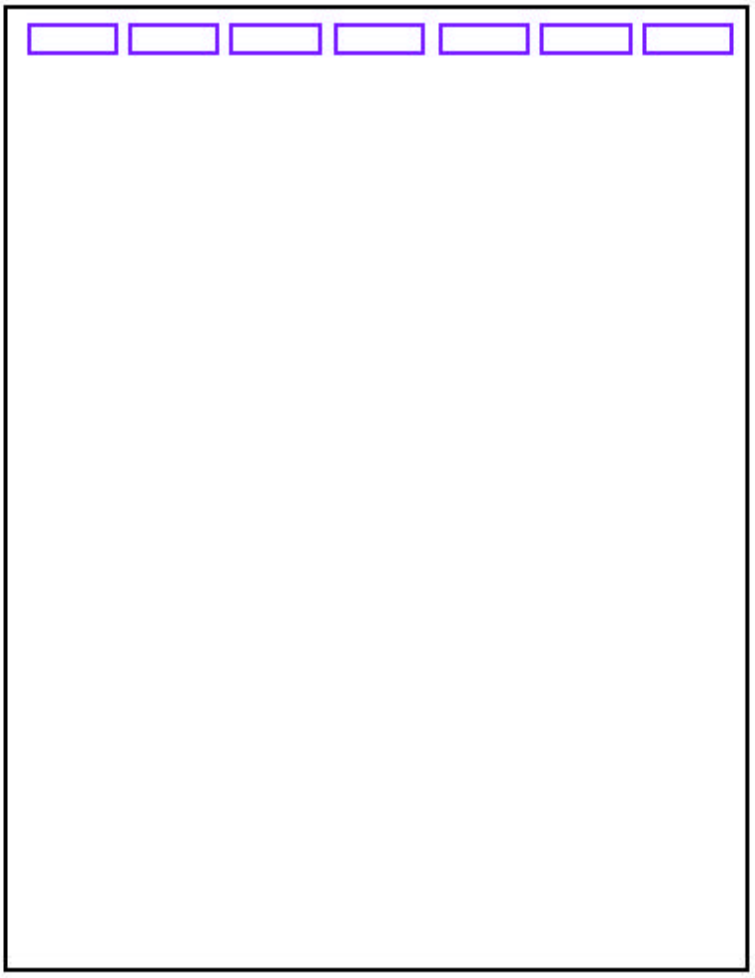
   The Loading Dye is dense so it helps the DNA to sink into the wells. It also contains a mixture of Dyes to monitor progress of the electrophoresis: a faster moving dye which will move with DNA fragments of ~500 base pairs and a slower moving dye which will move with DNA fragments of approximately 5 kilo base pairs.
7. Load 10µl of the DNA size marker (M) into the well on lane 1.
8. Load 20µl of G1, G2, G3, N4, N5 and N6 into the wells on lanes 2-7 respectively.
9. Close the electrophoresis tank, run at 100V for 30 minutes.

The negatively charged fragments of DNA will separate according to size.

10. Turn off the power.
11. Carefully, transfer the gel to a staining tray.
12. Cover the gel with 100x Fast Blast™ DNA stain and leave for 3 mins.
13. Pour off the stain, rinse the gel with tap water and cover with distilled water to destain the gel, changing the water occasionally.
14. Observe the banding pattern. When bands are clearly visible drain off the water and place the gel in a plastic bag. The gel will last for some weeks and longer if stored in a fridge.
15. Draw the pattern of bands you see (next page).

RESULTS

Below, draw the pattern of bands you see on your gel.



Analysis Questions:

(a) From your results do you think that *E. youngiana* is a different species from *E. helleborine* or is it a variant of it?

(b) Why are the DNA fingerprints from DNA samples 1, 2 and 3 different from the fingerprints from samples 4, 5 and 6?

(c) Can you think of other uses of DNA Fingerprinting that could help scientists research ecology or biodiversity of plants and animals?

TEACHER/TECHNICAL GUIDE

This scenario is designed to be used with the BIO-RAD DNA Fingerprinting Kit (Catalogue Number 166-0007-EDU). The instruction manual that comes with this kit contains excellent technical and teacher materials. We refer you to those materials for instructions on preparing the agarose gels, enzyme mix, aliquoting of DNA samples etc. Particular care should be taken however, to ensure that:

1) the lyophilised DNA samples and enzyme mix are thoroughly hydrated.
2) the enzymic digestion is carefully carried out, i.e. that the enzyme is well mixed with the DNA sample and that the incubation is carried out for the full 45 minutes at the correct temperature.

In the BIO-RAD DNA Fingerprinting scenario each DNA sample stands for a different suspect, here (British Orchid Conservation) each DNA sample stands for a different orchid DNA sample collected from mine spoil heaps in either Glasgow or Newcastle. The picture below shows the results you could expect from this DNA Fingerprinting practical. To achieve this result you must use the combinations of DNA samples from the BIO RAD kit shown in the table below. So, for example, DNA samples 1,2 and 3 all show a similar fingerprint. This suggests that whilst the *E. youngiana* looks different from *E. helleborine* they share a similar genetic background. Therefore *E. youngiana* is actually a variant of *E. helleborine* rather than a distinct species in itself. The DNA fingerprints for DNA samples 4,5 and 6 also support this hypothesis. This fingerprint is different to that from DNA samples 1,2 and 3 because it is from the Newcastle population rather than the Glasgow population. These distinct populations are separated by distance.

It should be noted that the Green and Violet DNA samples (Crime Scene and Suspect 3) are exactly the same and that is why they are interchangeable. Also not all the BIO-RAD kit DNA samples are used in this practical. The unused DNA samples can be stored (as directed in the instruction manual) and used at a later date.

Picture 1 - Results of gel electrophoresis

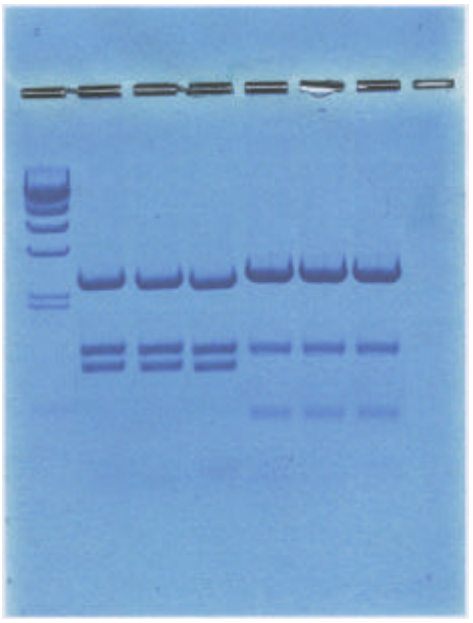


Table 1 - Showing DNA samples to use for each location to set up orchid conservation scenario.

Bidiversity usage – Rare Orchids scenario	**Colour Coding of DNA sample in BIO-RAD kit**	**BIO-RAD Usage – Forensic scenario**	**Location on Gel**
G1	Orange	Suspect 2	Lane 2
G2	Orange	Suspect 2	Lane 3
G3	Orange	Suspect 2	Lane 4
N4	Red	Suspect 4	Lane 5
N5	Red	Suspect 4	Lane 6
N6	Red	Suspect 4	Lane 7

Answers to Analysis Questions

(a) From your results do you think that *E. youngiana* is a different species from *E. helleborine* or is it a variant of it? Would you suggest that *E. youngiana* keeps its full conservation status?

Answer: As the *E. youngiana* from Glasgow shows the same DNA fingerprint as the *E. helleborine* from Glasgow (and likewise with the Orchid samples from Newcastle) it seems that *E.youngiana* is not a different species from *E.helleborine*. These results support the hypothesis that *E. youngiana* is a variant of the more common *E. helleborine* species. It would therefore seem sensible to reassess the full conservation status of *E. youngiana*.

(b) Why are the DNA fingerprints from DNA samples 1,2 and 3 different from the fingerprints from samples 4, 5 and 6.

Answer: DNA samples 1,2 and 3 are from Glasgow whereas DNA samples 4,5 and 6 are from Newcastle. Due to the separation by distance the populations of orchids have therefore become more genetically distinct.

(c) Can you think of other uses of DNA Fingerprinting that could help scientists research ecology or biodiversity of plants and animals?

Answer: Please refer to other biodiversity scenarios provided as part of this pack for other examples. Students should be able to come up with examples of their own.