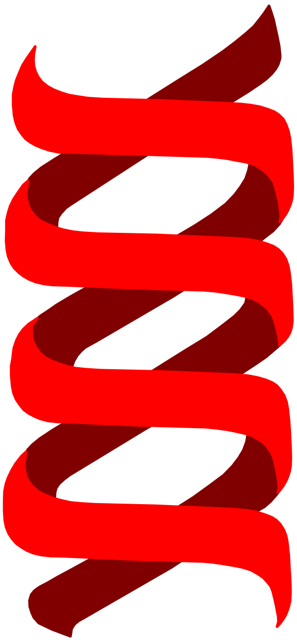
# DNA FINGERPRINTING AND HEDGEHOG RE-COLONISATION OF EUROPE



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.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.

## Where did our hedgehogs come from?

DNA fingerprinting can be used to determine how closely related different populations of a species are. If two populations have been separated for a long time we would expect that their genetic make-ups would differ. The longer the two populations have been apart, the more we would expect their DNA to differ. Scientists can use their understanding of DNA to understand where animals or plants in a certain area came from.

Photo courtesy of jwarnick.com/hedgies

Recently, this technique has been used to find out how plants and animals re-colonised Europe after the last ice age. As most of Northern Europe was completely covered in snow and ice during the last ice age, many animals could not survive there. But after the ice age ended, Northern Europe was once again hospitable to many animals, including hedgehogs. Hedgehogs survived the ice age in more southerly, warmer areas of Europe, such as Italy, Spain and Greece. It is possible to use DNA fingerprinting to compare the DNA of hedgehogs in Northern Europe to the DNA of hedgehogs in Southern Europe to better understand the routes by which hedgehogs have re-colonised Northern Europe. While scientists often use a more technical procedure, called DNA sequencing, to determine the exact DNA code for an area of the hedgehog genome, you will be doing DNA fingerprinting today. For more information on DNA sequencing, go to: www.ornl.gov/TechResources/Human_Genome/faq/seqfacts.html. Recently, Professor Godfray Hewitt has been using genetic techniques to understand post-glacial re-colonisation of many organisms, including hedgehogs. For more information on his work, go to: www.uea.ac.uk/bio/GH.html.

Today you will use DNA fingerprinting to better understand the re-colonisation of Northern Europe by hedgehogs after the last ice age. You will be given DNA from hedgehogs from throughout Europe. It is your job to compare the DNA fingerprints of the hedgehogs and to work out routes of re-colonisation after the ice age. The map below shows the locations of hedgehogs from which DNA samples were taken. Remember you are looking for genetic similarity that shows the routes (from Spain, Italy or Greece) taken by hedgehogs to re-colonise Europe after the ice age.

Picture 1 – Map showing locations where DNA samples from hedgehogs were taken:

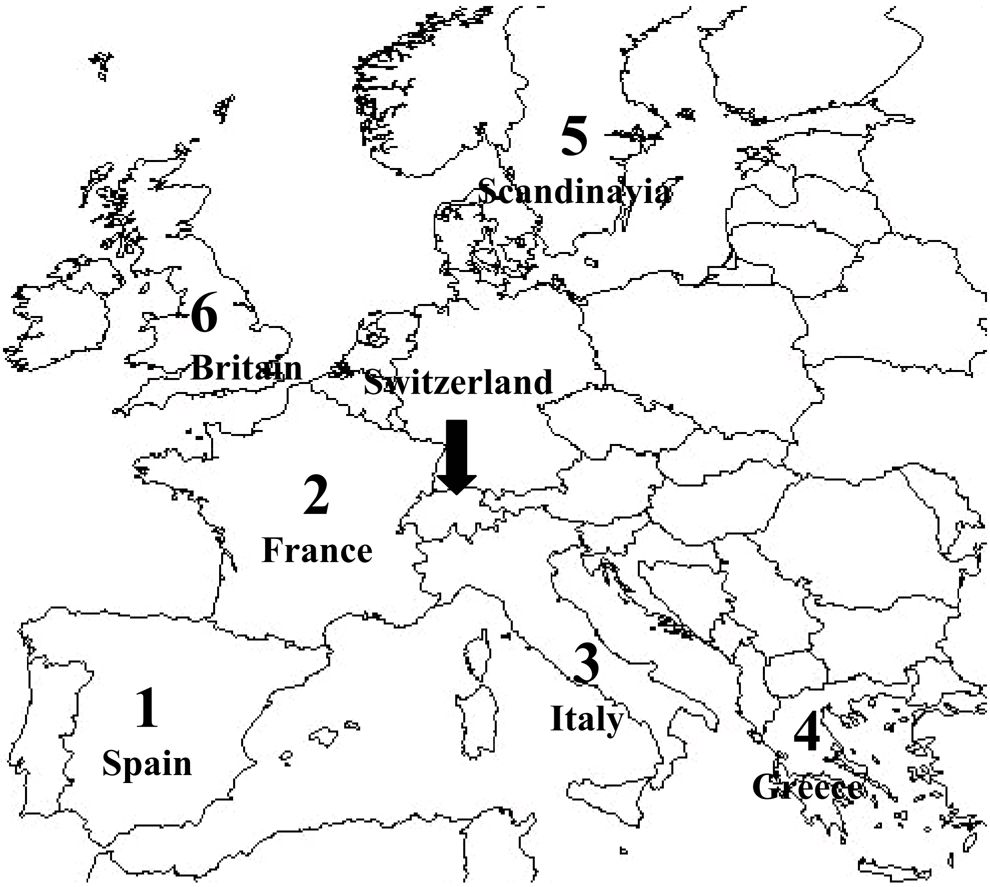
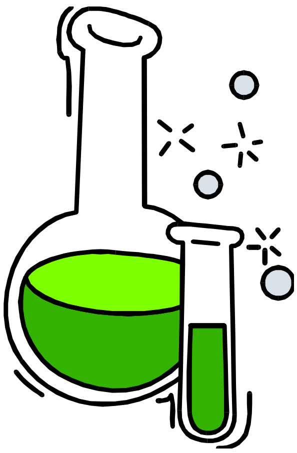



Photo courtesy of jwarnick.com/hedgies

# 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 location 1
DNA from location 2
DNA from location 3
DNA from location 4
DNA from location 5
DNA from location 6
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 of which contains 10µl DNA from the different locations. Label each tube with your initials.

| | | | | | |
|---|---|---|---|---|---|
| L1: | Location 1 | L2: | Location 2 | L3: | Location 3 |
| L4: | Location 4 | L5: | Location 5 | L6: | Location 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.

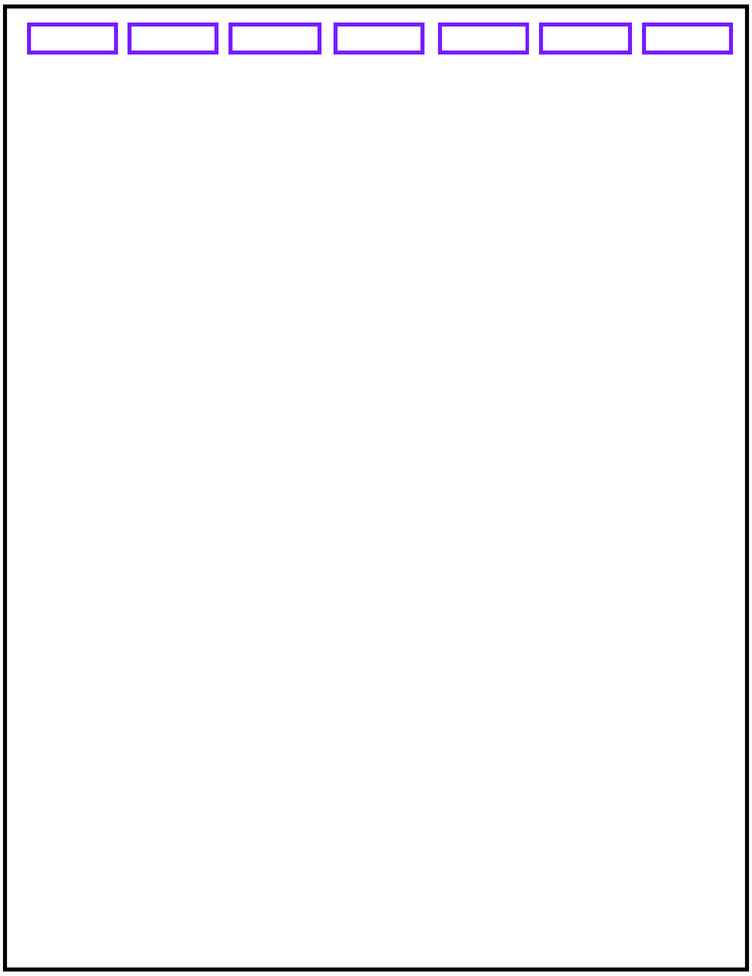
   *The Loading Dye is dense so it helps the DNA to sink into the wells. It also contains a mixture of Dyes to monitor progress of the electrophoresis: a faster moving dye which will move with DNA fragments of ~500 base pairs and a slower moving dye which will move with DNA fragments of approximately 5 kilo base pairs.*
7. Load 10µl of the DNA size marker (M) into the well on lane 1.
8. Load 20µl of L1, L2, L3, L4, L5 and L6 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 where did the hedgehogs in Britain originate?

(b) Hypothesise what results you might get if you carried out DNA fingerprinting on hedgehogs from Switzerland.

(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

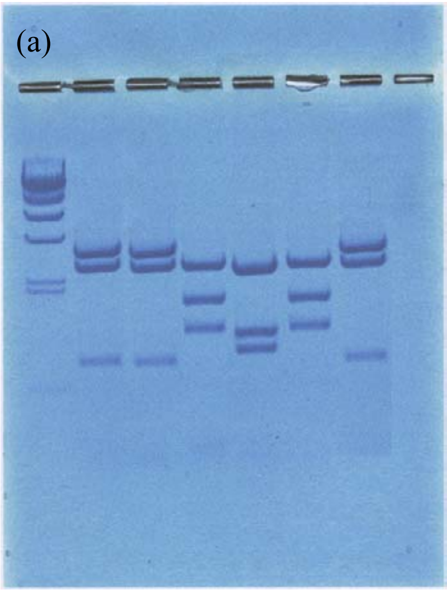
This scenario is designed to be used with the BIO-RAD DNA Fingerprinting Kit (Catalogue Number 166-0007-EDU). The instruction manual that comes with this kit contains excellent technical and teacher materials. We refer you to those materials for instructions on preparing the agarose gels, enzyme mix, 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 (hedgehog re-colonisation scenario) each DNA sample stands for a different hedgehog DNA sample collected from a particular location in Europe. The pictures below show the results of the DNA Fingerprinting from this practical. To achieve this result you must use the combinations of DNA samples shown in the table below. So, for example, the DNA collected from Locations 1, 2 and 6 all have the same DNA Fingerprint and therefore show that the hedgehogs share a common genetic origin. Below is a table telling you which DNA sample from the BIO-RAD Kit you should use to create this scenario.

It should be noted that the Green and Violet DNA samples (Crime Scene and Suspect 3) are exactly the same and so 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 (a) and interpretation of these results in terms of Hedgehog re-colonisation (b). Each coloured circle corresponds to hedgehog populations which show a common genetic origin.



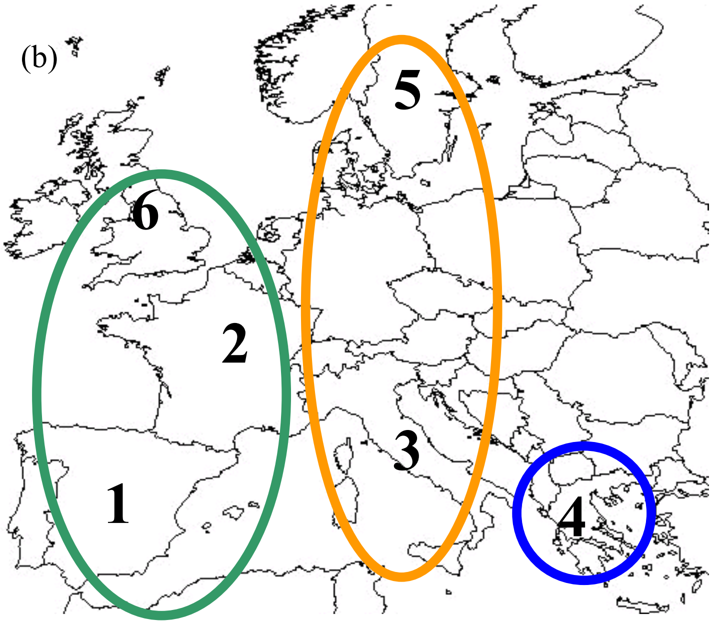




Table 1 - Showing DNA samples to use for each location to set up 'Hedgehog Re-colonisation' Scenario.

| **Biodiversity Usage - Hedgehog Re-colonisation scenario** | **Colour Coding of DNA sample in BIO-RAD kit** | **BIO-RAD Usage – Forensic scenario** | **Location on Gel** |
|---|---|---|---|
| Location 1 | Green or Violet | Crime Scene or Suspect 3 | Lane 2 |
| Location 2 | Green or Violet | Crime Scene or Suspect 3 | Lane 3 |
| Location 3 | Orange | Suspect 2 | Lane 4 |
| Location 4 | Blue | Suspect 1 | Lane 5 |
| Location 5 | Orange | Suspect 2 | Lane 6 |
| Location 6 | Green or Violet | Crime Scene or Suspect 3 | Lane 7 |

**Answers to Analysis Questions**

(a) From where did the hedgehogs in Britain originate?

**Answer: Spain.**

(b) Hypothesise what results you might get if you carried out DNA Fingerprinting on hedgehogs from Switzerland?

**Answer: It would be expected that the DNA Fingerprint would be a combination of the banding patterns from hedgehogs originating in Spain and Italy. This is because these two populations of hedgehog would no longer be separated by distance and genetic mixing could occur.**

(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 example. Students should be able to come up with examples of their own.**