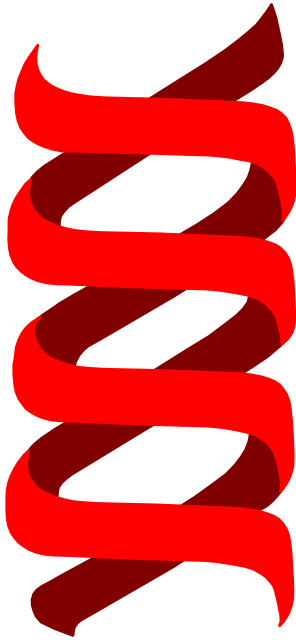


DNA FINGERPRINTING AND OTTER POPULATIONS



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.

DNA Fingerprinting and otters

Otters are found in Britain and throughout the world. Otter numbers have declined throughout much of its home range. In the UK, otters are a protected species and measures have been taken to conserve otter habitat.

To better assess the otter population of Britain, an understanding of their behaviour and numbers is necessary. However, otters are typically shy and wary of people, making studying them in the wild particularly difficult. One way of determining the presence of otters in an area is by searching for their faeces. Recently, scientists have been using DNA fingerprinting on otter faeces to get a better



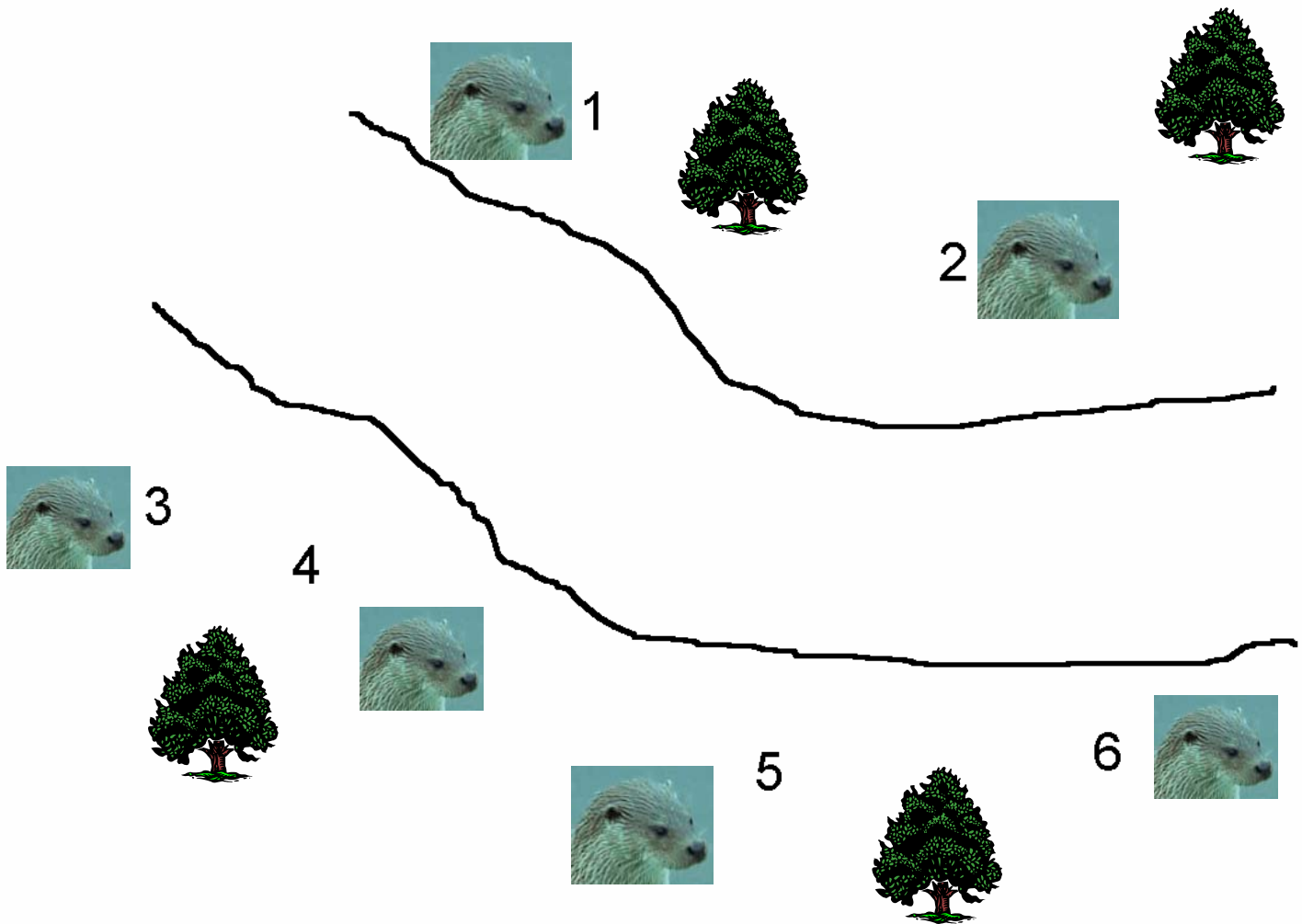
Photo courtesy of J. McMinn
www.ottersite.btinternet.co.uk/index.htm

understanding of the number of otters in an area. They compare the DNA from different faecal samples to see if they are from the same individual. The number of different DNA fingerprints found corresponds to the minimum number of otters in an area, while the spatial distribution of the faeces along with the DNA profile gives information about the movement of individual otters. This technique is currently being used by Dr. John Dallas and his group at the University of Aberdeen to monitor otter populations in England. For more information on their project, go to:

www.abdn.ac.uk/zoology/molecol/emg.htm. For more information on otters and their conservation in the UK, go to www.waterpolicyteam.org.

Today you will compare DNA from otter faeces found around a river to obtain an estimate of the number of otters that live in that area and understand better the movements of those otters. In the picture on the next page, you can see the river where the otters live. Each number is the location where a sample of otter faeces was collected. DNA has been extracted from the faeces. Your task is to carry out a restriction digest of each of the DNA samples, run the DNA on gels as described below, and draw conclusions about the otters living there from the results of your gel.

Picture 1 – Map of river showing locations where faeces samples were collected.

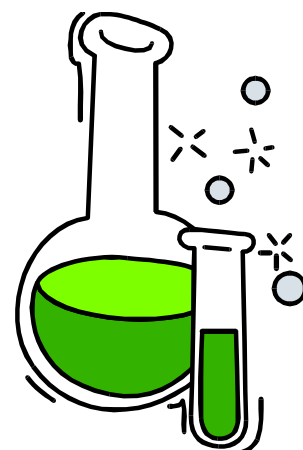


Photos courtesy of J. McMinn, www.ottersite.btinternet.co.uk/index.htm

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 shown in picture 1.
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 BlastTM 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.

A diagram of a gel electrophoresis gel. It consists of a large rectangular area representing the gel, with a black border. At the top of the gel, there are seven small, empty rectangular boxes, each representing a lane. These boxes are outlined in purple and are arranged horizontally, separated by small gaps. The rest of the gel area is blank, intended for drawing the pattern of bands.

Analysis Questions:

- a) How many otters are in the area around the river?

- b) What can you say about the movement of the otters?

- c) Do they wander far?

- d) Are they territorial?

- e) 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 (Otter Population scenario) each DNA sample stands for a different otter faecal sample collected from a particular location. The pictures below show the results of the DNA Fingerprinting. 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, 3 and 4 all have the same DNA Fingerprint and therefore come from the same Otter. The otter has an obvious territory and appears to stay in the northeast section of the river. 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 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 - showing results of gel electrophoresis (a) and interpretation of these results in terms of Otter populations (b). Each coloured circle corresponds with one individual otter.

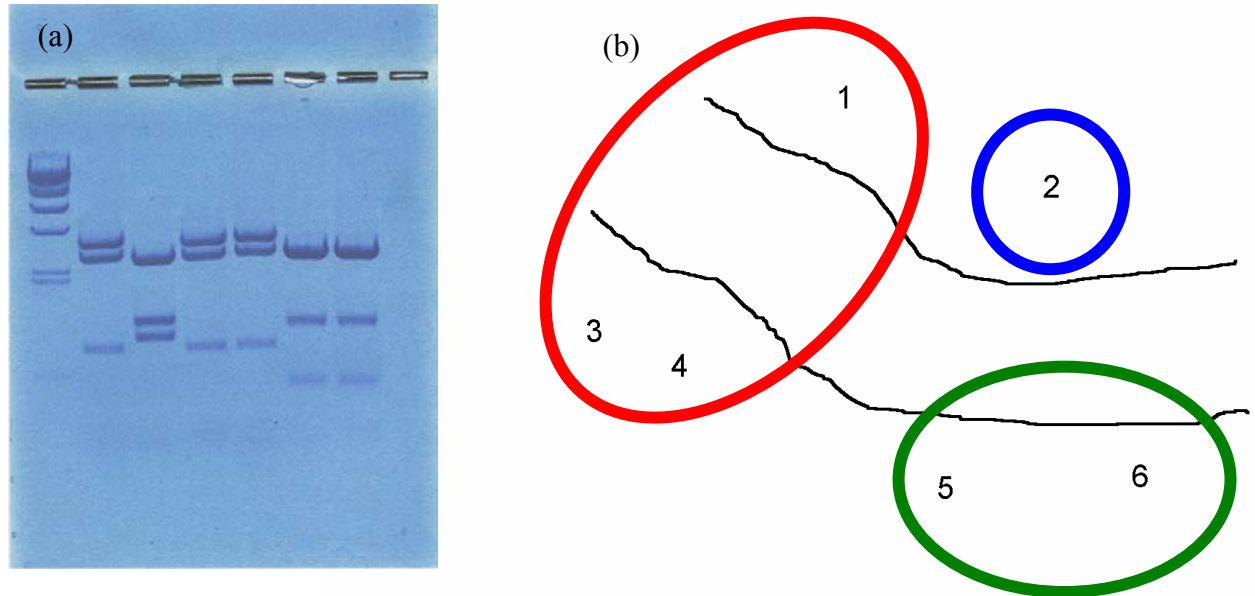


Table 1 - Showing DNA samples to use for each location to set up 'Biodiversity and Otter Population' Scenario.

Biodiversity Usage - Otter Population 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	Blue	Suspect 1	Lane 3
Location 3	Green or Violet	Crime Scene or Suspect 3	Lane 4
Location 4	Green or Violet	Crime Scene or Suspect 3	Lane 5
Location 5	Red	Suspect 4	Lane 6
Location 6	Red	Suspect 4	Lane 7

Answers to Analysis Questions:

a) How many otters are in the area around the river?

Answer: At least 3

b) What can you say about the movement of the otters?

Answer: They do move around. In this study for example, one of the otters (red circle in picture above) has travelled around both sides of the river. Collection of samples further a field could investigate whether this otter travels even further.

c) Why might the otters travel long distances?

Answer: The otters might have to travel large distances to find food or mates.

d) Are they territorial?

Answer: From the results in this DNA Fingerprinting practical, showing that the otters live in discrete locations, it seems that they are territorial.

NB: In reality male otters have distinct territories which they patrol, visiting females within their area.

e) 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 multiple answers of their own.