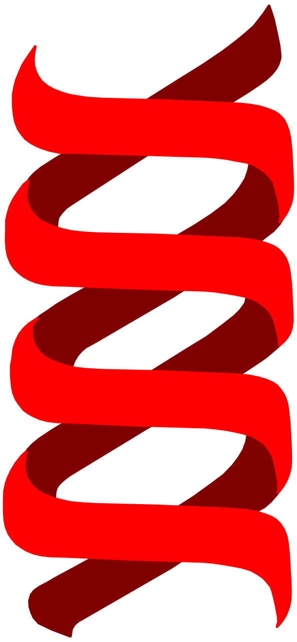
DNA FINGERPRINTING AND ORANGUTAN PARENTAGE



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 and paternity testing

DNA fingerprinting is often used to determine the father of a baby, this is called 'paternity testing'. Scientists are often interested in understanding which individuals in a population of plants or animals are the parents of which offspring. This can answer questions such as: Is there a social hierarchy within a group of animals? Do the animals mate with more than one individual? Will they wander far to find a mate? By asking these questions, we can improve our general knowledge of the animals around us. In addition, understanding reproductive strategies, and the amount of genetic variation in offspring, can be useful to conservationists.

To understand how DNA fingerprinting can be used to assign parentage, we first have to understand where an offspring's DNA comes from. All mammals have two sets of all genes, one from the mother and one from the father. As each animal's genetic make-up is half maternal and half paternal, with enough DNA information we can determine the parents of an individual.

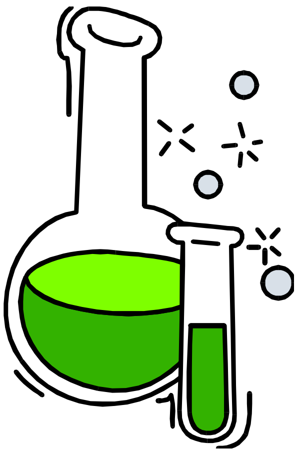
Who is the father?

Orangutans are an endangered species of ape that live in Southeast Asia. Their numbers have dwindled due to habitat loss and poaching, but there are currently efforts to save the orangutans. Orangutans are highly intelligent and are able to make and use tools and solve complex problems. To learn more about orangutans, and orangutan conservation, go to: www.pbs.org/wnet/nature/orangutans/.

DNA fingerprinting has been used by Dr. Benoit Goossens at Cardiff University to better understand mating and genetic variation in orangutans and black rhinoceri, two endangered species. To learn more about his research, go to:
www.cf.ac.uk/biosi/research/biodiversity/staff/benoit.html.

Today you will use DNA fingerprinting techniques to determine which male orangutan is the father of a baby orangutan. You will have DNA from the baby orangutan and several possible father orangutans. It is your job to determine which is the father by carrying out a restriction digest on the DNA samples, running this DNA on gels as described in the methods below, and drawing conclusions from the results.

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 Baby orangutan
DNA from Male a
DNA from Male b
DNA from Male c
DNA from Male d
DNA from Male e
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 orangutans. Label each tube with your initials.

1: Baby orangutan	2: Male a	3: Male b
4: Male c	5: Male d	6: Male e

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.

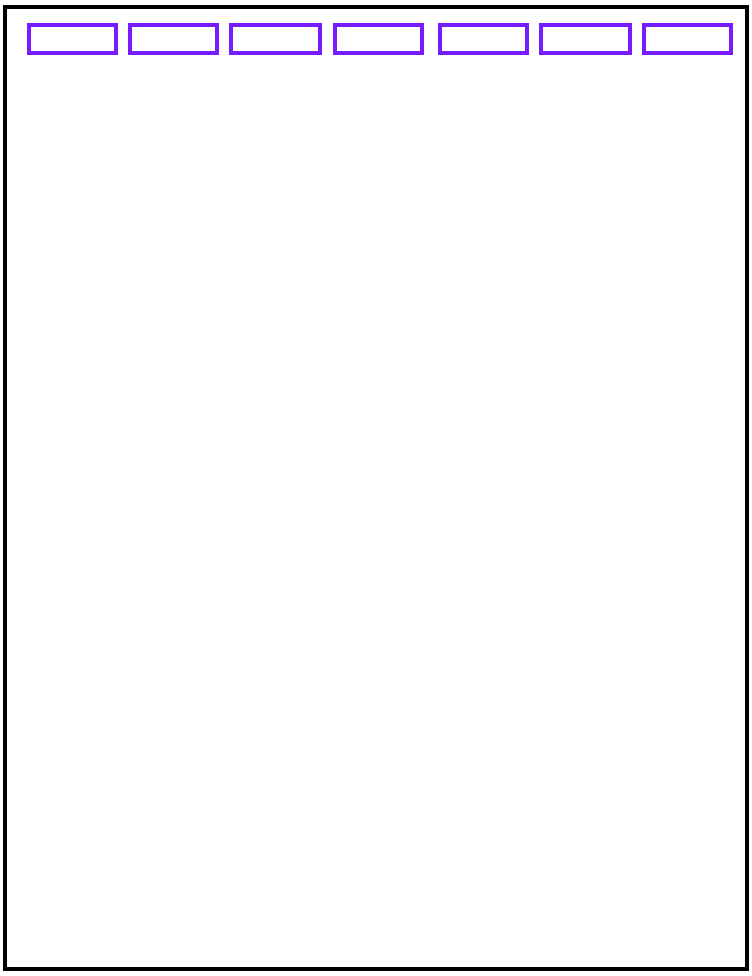
7. Load 10µl of the DNA size marker (M) into the well on lane 1.
8. Load 20µl of 1, 2, 3, 4, 5 and 6 into the wells on lanes 2-7 respectively.
9. Close the electrophoresis tank, run at 100V for 30 minutes.

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) Who is the father of the baby orangutan?

(b) How can this kind of research aid conservationists?

(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 (orangutan parentage scenario) each DNA sample stands for an individual orangutan. The picture below shows the results you would expect from the DNA Fingerprinting practical outlined here. TO achieve this result you must use the combinations of DNA samples from the BIO-RAD Kit shown in the table below.

Picture 1 - Results of gel electrophoresis.

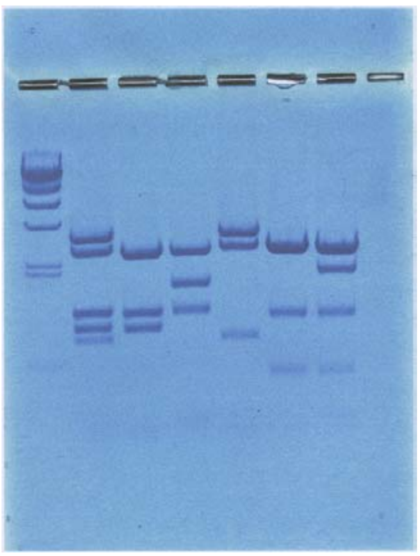


Table 1 - Showing DNA samples to use for each location to set up 'Orangutan parentage' Scenario.

Biodiversity Usage – Orangutan parentage scenario	**Colour Coding of DNA sample in BIO-RAD kit**	**BIO-RAD Usage – Forensic scenario**	**Location on Gel**
Baby orangutan	Green	Crime Scene	Lane 2
Maternal DNA	Blue	Suspect 1	Lane 3
Male a	Orange	Suspect 2	Lane 4
Male b	Violet	Suspect 3	Lane 5
Male c	Red	Suspect 4	Lane 6
Male d	Yellow	Suspect 5	Lane 7

Answers to Analysis Questions:

(a) Who is the father of the baby orangutan?

Answer: Male c

(b) How can this kind of research aid conservationists?

Answer: This kind of research can aid conservationists by informing them about the general biology of orangutan. For example it can be used to determine if there is a social hierarchy amongst the orangutans. If there is a social hierarchy, a dominant male will have fathered most of the offspring. Also, this technique can show if the orangutans are monogamous (one mate) or polygamous (many mates). Conservationists can then use this kind of information to help develop breeding strategies to repopulate endangered species. (There are also numerous other possible answers to this question.)

(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 multiple examples of their own.