NEATH PORT TALBOT COLLEGE
COLEG CASTELL NEDD PORT TALBOT

School of Maths & Science
Science Practical

D.N.A. Electrophoresis

◆ Aim

After you practical you should be able to outline the separation of the genetic fragments by electrophoresis and their detection by Fast Blast DNA stain to produce a genetic fingerprint. You should know the main uses for genetic fingerprinting.

◆ Introduction

Competition among eastern mountain gorilla groups for females can be fierce, and levels of aggression among males in neighbouring mountain gorilla groups can be extremely high. There has been a 'murder'; a neighbouring gorilla has been killed by one of the males in the region. The game warden needs to determine which of the five male silverbacks was responsible for the killing. The assailant's hair samples have been recovered from the crime scene. You are required to determine - Who done it? by using the technique of DNA electrophoresis (genetic fingerprinting)

◆ You are provided with
- 1 marker DNA sample
- 1 sample of DNA from crime scene, probably the 'murderers' DNA
- 5 samples of DNA from the 5 suspect gorillas
- Carry out the following procedures 1 – 17, to detect the gorilla that was responsible for the killing

◆Safety

Control Measures

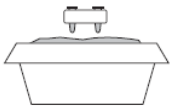
- The wearing of **safety goggles** and a **laboratory coat** at all times will be sufficient to take account of most hazards and significant risks.
- All waste is to be placed in the labelled container immediately after use.
- You are reminded of the need for good laboratory practice in order to maintain a safe working environment.
- Students should wash their hands at the end of the practical in the normal way.

Irritant **1X TAE Buffer solution**

◆ Procedure

1. Place the tube containing the restriction enzyme mix, labelled ENZ, on ice.

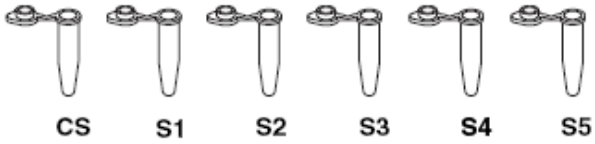
ENZ



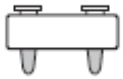
Ice

2. Label one of each coloured micro test tubes as follows:

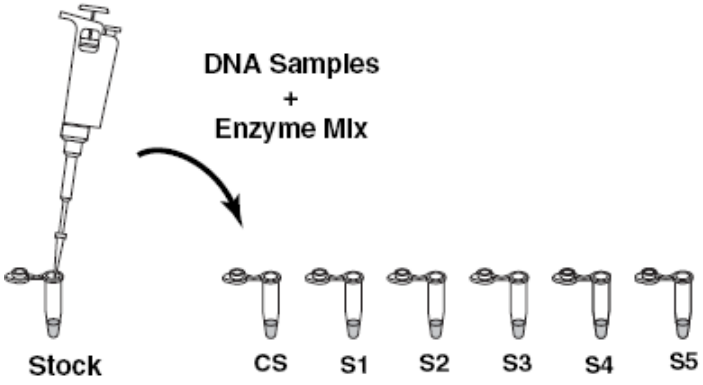
Green tube	CS (crime scene)
Blue tube	S1 (suspect 1)
Orange tube	S2 (suspect 2)
Violet tube	S3 (suspect 3)
Red tube	S4 (suspect 4)
Yellow tube	S5 (suspect 5)



Label the tubes with your name and date. Place the tubes in the foam micro test tube holder.

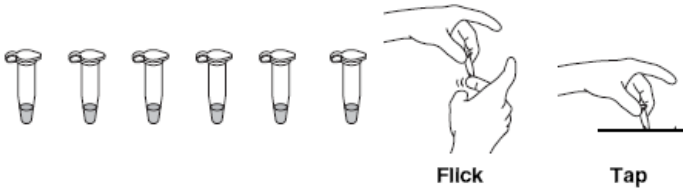


3. Using a fresh tip for each sample, pipette 10 µl of each DNA sample from the stock tubes and transfer to the corresponding coloured micro test tubes. Make sure the sample is transferred to the bottom of the tubes.

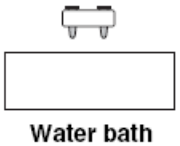


4. Pipette 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipette up and down carefully to mix well.

5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a micro centrifuge is available, pulse spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.



6. Place the tubes in the foam micro tube holder and incubate in a water bath for 45 min at 37°C.

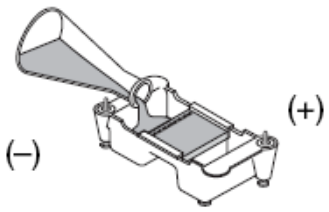


Water bath

7. After the incubation period, remove the tubes from the water bath and if a centrifuge is available, pulse spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube or gently tap on the table top.

8. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.

9. Place the agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer for a Bio-Rad Mini-Sub Cell, horizontal electrophoresis chamber.

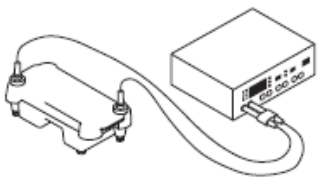


10. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.

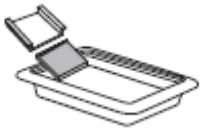
11. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:

    Lane 1: M, DNA size marker, 10 µl
    Lane 2: CS, green tube, 20 µl
    Lane 3: S1, blue tube, 20 µl
    Lane 4: S2, orange tube, 20 µl
    Lane 5: S3, violet tube, 20 µl
    Lane 6: S4, red tube, 20 µl
    Lane 7: S5, yellow tube, 20 µl

12. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid of the horizontal electrophoresis chambers will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black. Turn on the power and electrophorese your samples at 100 V for 50 minutes.

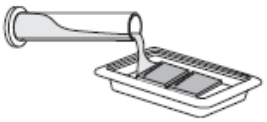


13. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery. Slide the gel into the staining tray.



14. Add 120 ml (or enough to cover the gels) of 100x Fast Blast DNA stain into a staining tray (2 gels per tray). Stain the gels for 2 minutes with gentle agitation. Save the used stain for future use.

15. Transfer the gels into a large washing container and rinse with warm (40–55°C) tap water for approximately 10 seconds.



16. De-stain by washing the gels in warm tap water for 5 minutes each with gentle shaking, for best results repeat de-staining process 3 more times (a total of 4 washes).

17. Carefully remove the gel from the washing container and place on a light coloured background to observe the DNA fragments.

Below: Image of a D.N.A. electrophoresis gel.