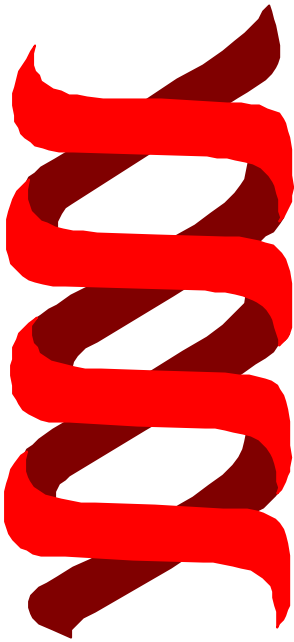


DNA FINGERPRINTING & JAPANESE KNOTWEED

DNA FINGERPRINTING AND JAPANESE KNOTWEED



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:

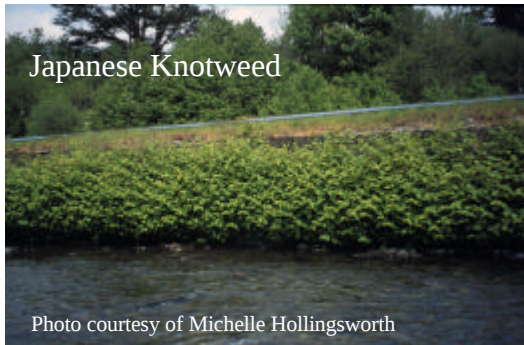
www.protist.biology.washington.edu/fingerprint/dnaintro.html

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.

The family history of Japanese Knotweed

Japanese Knotweed (*Fallopia japonica*) is a plant native to Japan, Taiwan and Northern China. It was introduced into the UK in the 1850s for use as an ornamental garden plant. However it quickly escaped from cultivation and rapidly started to colonise areas such as canal sides, road verges, cemeteries, streams and river banks. Its growth in urban areas has caused significant damage as its shoots are able to push through asphalt and damage pavements and car parks. Also the height to which Japanese Knotweed can grow (3 metres) can cause visibility problems on roadsides. Once this plant has established itself it is extremely difficult to eradicate and hundreds of thousands of pounds are spent each year on the control of this weed. In 1981 it became a criminal offence to knowingly plant Japanese Knotweed in the wild.

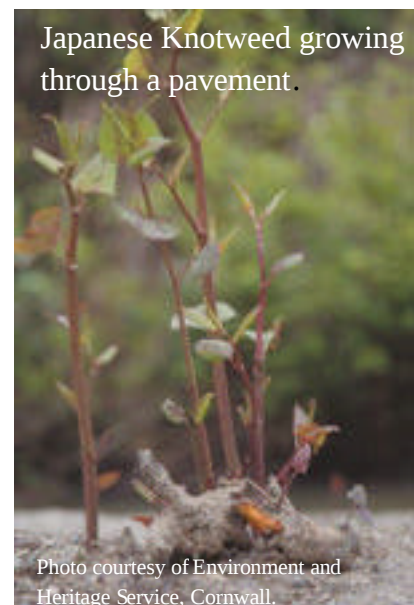
So how does this plant manage to propagate itself so efficiently? In Britain, Japanese Knotweed plants are all female plants (they have both male and female



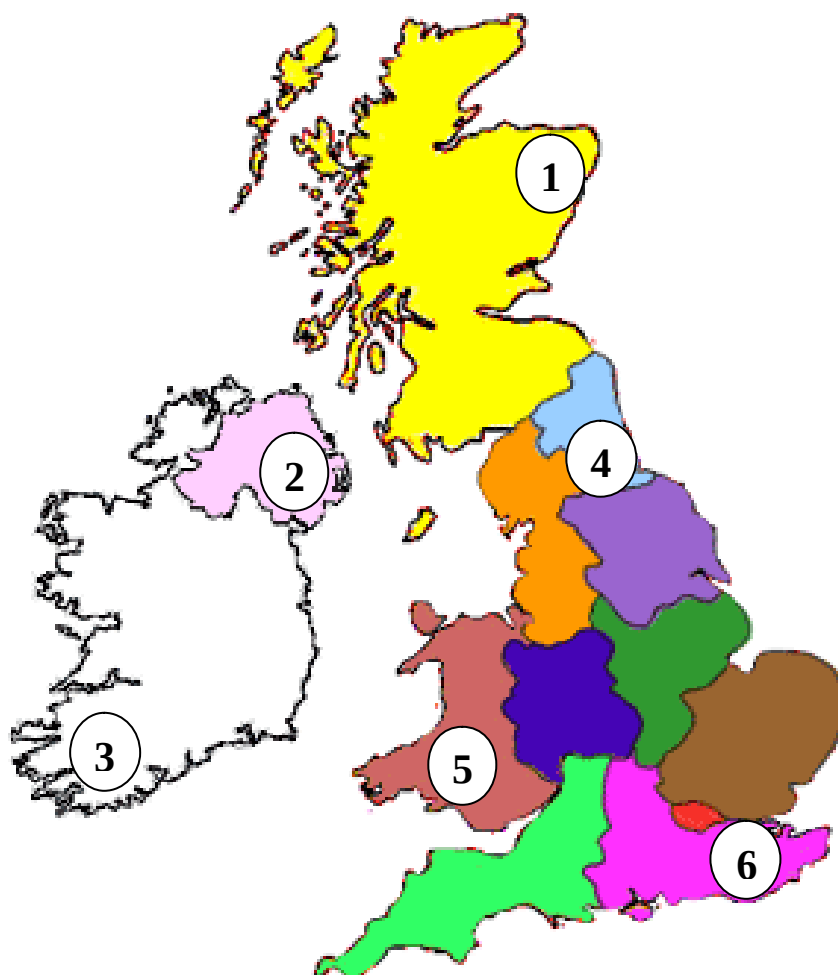
sex organs but the male parts are sterile, in effect making them females). Therefore Japanese Knotweed cannot reproduce by standard sexual reproduction and, in Britain, seems incapable of producing seed. However, Japanese Knotweed has a vigorous and fast growing root system and is capable

of regenerating a whole plant from a tiny fragment of root. It is this capability that is thought to explain how this plant has become such a problematic invasive weed. As sexual reproduction is one means by which genetic variation can be maintained, the lack of sexual reproduction in this plant must impact the genetic diversity of Japanese Knotweed in this country.

Dr M Hollingsworth from the Royal Botanic Garden in Edinburgh has been researching the genetic diversity in Japanese Knotweed. She has been using DNA profiling technologies to investigate whether the Japanese Knotweed in Britain is multiclonal or monoclonal. If it is multiclonal, that would mean that there are many different populations of Japanese Knotweed in Britain. Within each population the plants would be genetically similar. Between populations, however, there would be genetic differences. If it is monoclonal then **all** the Japanese Knotweed in Britain is genetically identical i.e. the plants are all clones of one original plant! For more information about this research download the Leicester University Bulletin, May 1999 (www.le.ac.uk/bulletin). The article is on page 10.



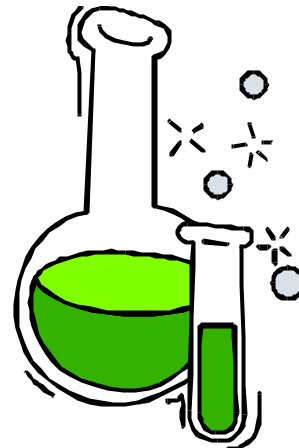
Picture 1 – Map showing distribution of Japanese Knotweed in the British Isles and where the DNA samples were collected from.



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 containing 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.

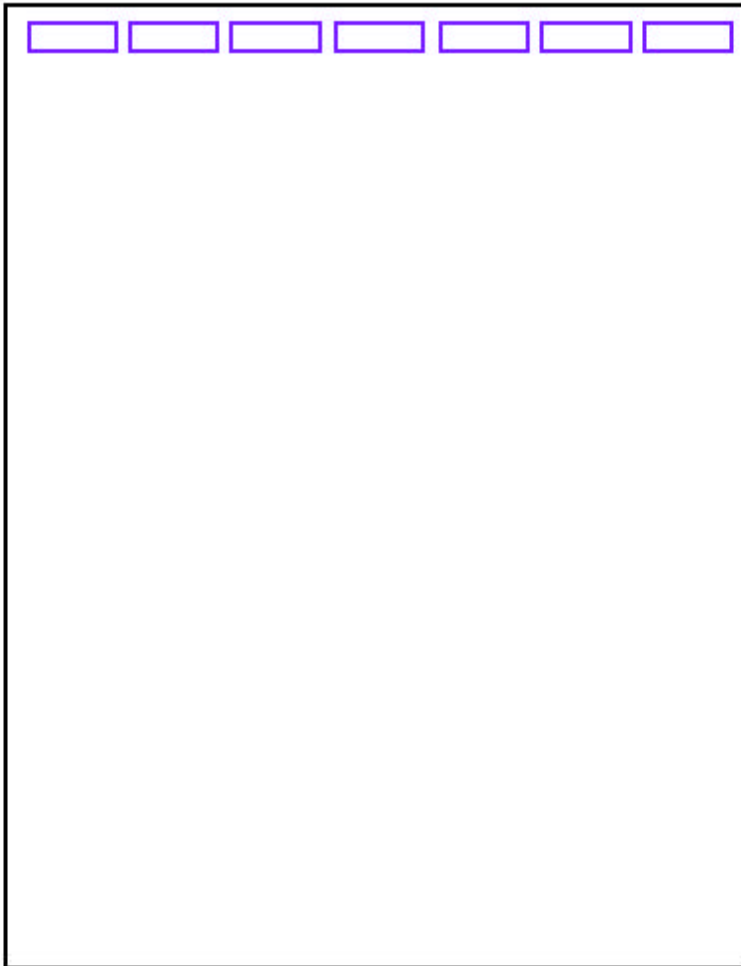
7. Load 10µl of the DNA size marker (M) into the well on lane 1.
8. Load 20µl of L1, L2, L3, L4, L5 and L6 into the wells on lanes 2-7 respectively.
9. Close the electrophoresis tank, run at 100V for 30 minutes.

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 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 or 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 is Japanese Knotweed multiclonal? Explain your answer.
- (b). What disadvantages are there to a plant which propagates in this way?
- (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 (Japanese Knotweed clonality) each DNA sample stands for a different Japanese Knotweed DNA sample collected from various regions in the British Isles. The picture below shows the results of the DNA Fingerprinting. In this scenario all fingerprints are actually the same as Japanese Knotweed plants in Britain are all genetically identical clones. Therefore only one DNA sample from the BIO-RAD kit is actually used. Below is a table telling you which DNA sample from the BIO-RAD Kit you could 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 therefore 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 - Result of gel electrophoresis

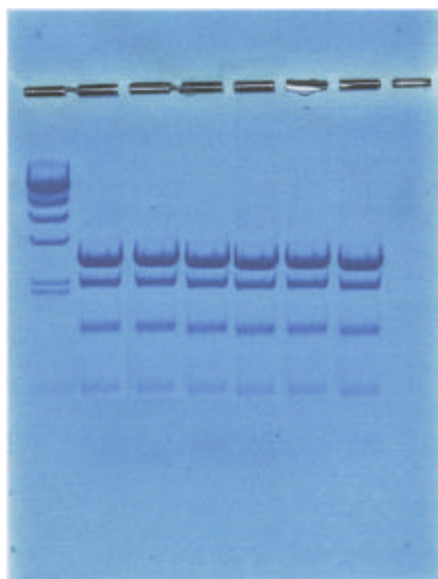


Table 1 - Showing DNA samples to use for each location to set up 'Japanese Knotweed clonality' Scenario.

Biodiversity usage - Japanese Knotweed scenario	Colour Coding of DNA sample in BIO-RAD kit	BIO-RAD Usage - Forensic scenario	Location on Gel
Location 1	Yellow	Suspect 5	Lane 2
Location 2	Yellow	Suspect 5	Lane 3
Location 3	Yellow	Suspect 5	Lane 4
Location 4	Yellow	Suspect 5	Lane 5
Location 5	Yellow	Suspect 5	Lane 6
Location 6	Yellow	Suspect 5	Lane 7

Answers to Analysis Questions

(a) From your results is Japanese Knotweed multiclonal. Explain your answer.

Answer: As all the DNA fingerprints are the same this suggests that all the Japanese Knotweed plants in the British Isles are in fact genetically identical clones. It is not multiclonal.

(b). What disadvantages are there to a plant which propagates in this way?

Answer: Propagating in this way means that no or little genetic variation occurs. This means that the plant cannot evolve or respond to changes in the environment.

Other additional disadvantages for information: Any deleterious mutations which have an effect on the growth and development of the plant cannot be removed. Also, viruses in plants can be eliminated during a cleansing step which occurs as part of the process of sexual reproduction (gametogenesis).

(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.