

DNA FINGERPRINTING & REFORESTATION PROJECTS

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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 FAIROAK Project

The flora of northern Europe has changed dramatically during the last 10,000 years. As climatic conditions have become more temperate since the end of the last ice age, European flora suited to a temperate climate have been able to migrate northwards from areas where species have survived because local conditions are favourable (*refugia*) in southern Europe.

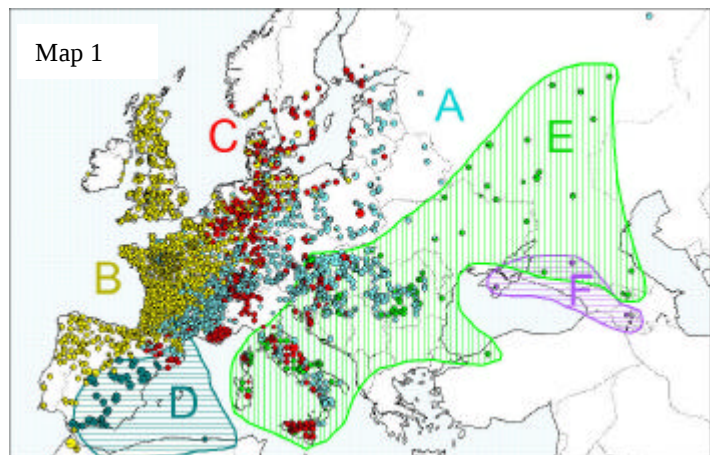
Oak species represent a major component of the European forest resource. They supply quality wood, stabilise forests and enhance biological richness in forest ecosystems. Despite the woodland culture (*silvicultural*) and economic importance of oaks, little was known of their genetic diversity. This has hampered genetic improvement programmes, decisions about seed transfers and choice of sources (*provenances*) from plantations. The more the species is used to convert agricultural lands to forest (*afforest*) and enrich existing forest currently in monoculture, the more information about genetic diversity will become important.

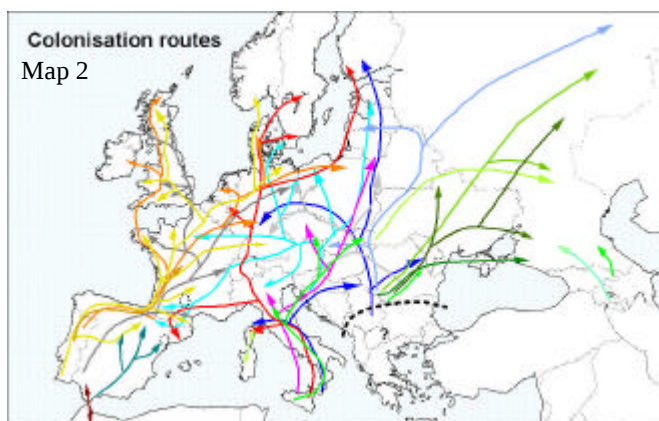
The FairOak project was set up in 1994 and aimed to create a map of the oak (*Quercus petraea* and *Quercus robur*) genetic resources available across Europe, based on a systematic sampling of oak populations across the continent. Working collaboratively, scientists from a large number of institutions across Europe monitored the chloroplast DNA (cpDNA) in 2673 populations (12,714 trees) ranging from the Mediterranean in the south, to Scotland and Scandinavia in the north. Using DNA Fingerprinting techniques, (restriction and electrophoresis of cpDNA) they have been able to identify differences in the cpDNA of trees of the same species across Europe. By comparing the cpDNA of trees in different areas, they have worked out which trees share the same genotype.

Evolution and conservation relevance

Geographically mapping the data in this way gives information on where genetic diversity is concentrated. This is solid, scientific information which forestry or environmental agencies can use as a basis for development of conservation policies.

Six major lineages have been identified from the 12,714 trees that were analysed. From Map 1, you will see that these six lines (A-E) have quite separate geographical distributions along a longitudinal gradient and that the southern European regions (Iberian and Italian peninsula, Balkan regions) are clearly hot spots of diversity.





The map of geographical distribution of cpDNA polymorphisms was then compared to the map of fossil pollen deposits, in order to reconstruct the history of oak colonisation of Europe from the last ice age (postglacial recolonisation – see

Map 2). Because oaks are carrier species (species which provide habitat for other organisms) in European forest ecosystems, the identification of their postglacial recolonisation routes is likely to provide useful information on the distribution and diversity of other plant and animal species. Furthermore, the patterns of recolonisation that were identified in the FAIROAK project can be considered as indicators of future migration movements of oaks as a result of climatic changes.

The six lineages observed match the migration pathways that were followed by the oaks during postglacial recolonisation.

Technical and economic benefits

The synthetic maps that have been constructed in the FAIROAK project are based on populations of native origin. These results can be used to trace the geographical origin of trees within a forest and assist in their identification.

It is now possible to identify autochthonous (native) trees from allochthonous (introduced) individuals of the same species. This information is highly valuable for ecological studies into oak decline and dieback, as sensitivity to disease may be greater in individuals that are not native to that particular area.

European regulations recommend that reforestation projects should use stock which is of local origin. Oak trees from northern provenances flush (burst their buds) later than more southern ones, making seed transfer between north and south risky. Knowing where different oak genotypes are native permits the genotyping of seed lots to check their origin.

Further information about the FAIROAK project can be found at:

<http://www.pierroton.inra.fr/Fairoak/>

Today you will use a simplified version of DNA Fingerprinting to determine which oak seed you must use in a reforestation project. You have been given one



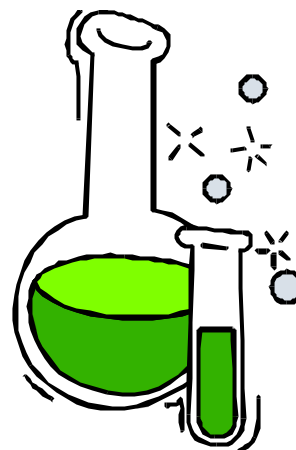
sample of DNA from the seed of a tree which grows in a local wood, and five samples of DNA from seeds of the same type of tree but which grow in different geographical areas. You must now carry out restriction digest of these DNA samples, gel electrophoresis and staining of the resultant gels. By comparing the banding patterns, you will be able to

select the seed whose DNA matches that of the local seed.

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 Area 1
 DNA from Area 2
 DNA from Area 3
 DNA from Area 4
 DNA from Area 5
 DNA from Area 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 coloured microtubes, each containing 10µl DNA from the areas shown below. Label each tube with your initials.
- | | | |
|--------------|----------|------------|
| green: Local | blue: A1 | orange: A2 |
| violet: A3 | red: A4 | yellow: A5 |

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.

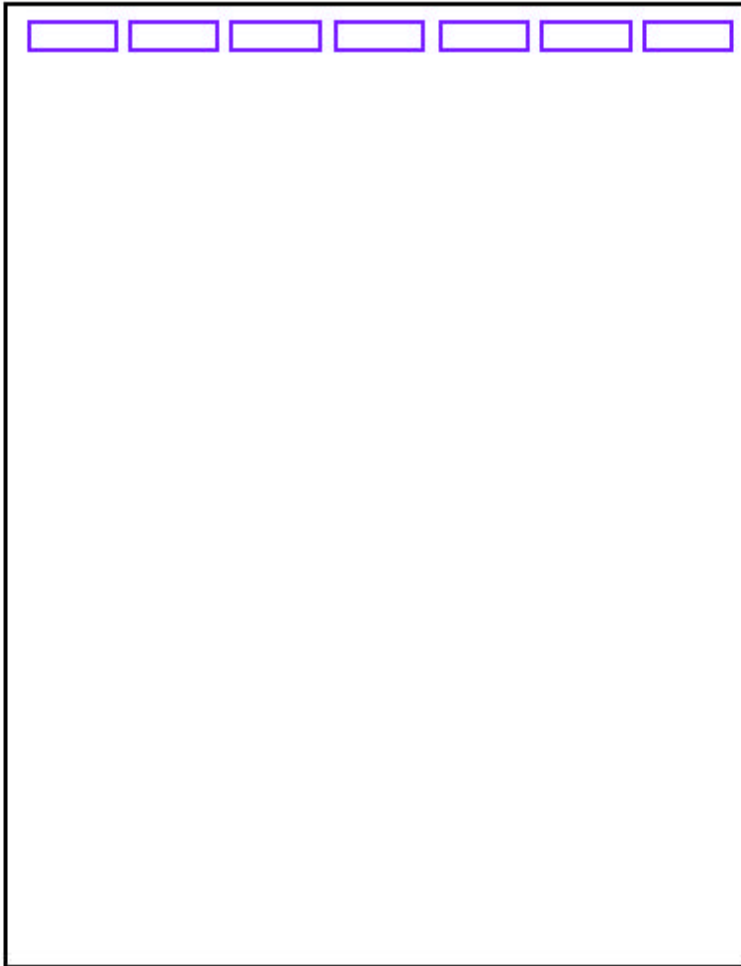
7. Load 10µl of the DNA size marker (M) into the well on lane 1.
8. Load 20µl of L, A1, A2, A3, A4 and A5 into the wells on lanes 2-7 respectively.
9. Close the electrophoresis tank, run at 100V for 30 minutes.

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.

A diagram of a gel electrophoresis setup. It consists of a large rectangular area representing the gel, enclosed by a black border. At the top of this area, there are seven small, empty rectangular boxes, each outlined in purple, representing the wells where DNA samples are loaded.

Analysis Questions:

- (a) From which area would you select seed for the reforestation project?

- (b) Explain one technical or economic benefit of the FAIROAK project.

- (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 (FAIROAK Project) each DNA sample stands for a different oak seed DNA sample collected from different areas. The picture below shows the results you could expect from this DNA Fingerprinting practical. To achieve this result you must use the combinations of DNA samples shown in the table below.

Picture 1 - Results of gel electrophoresis

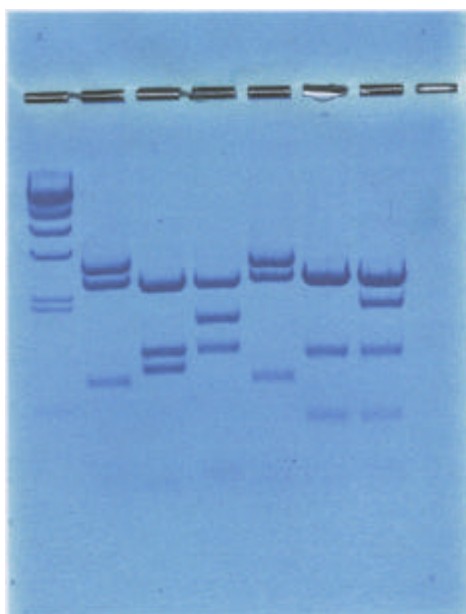


Table 1 - Showing DNA samples to use for each location to set up FAIROAK Project scenario.

Biodiversity usage - FAIROAK project	Colour Coding of DNA sample in BIO-RAD kit	BIO-RAD Usage - Forensic scenario	Location on Gel
Local	Green	Crime Scene	Lane 2
Area 1	Blue	Suspect 1	Lane 3
Area 2	Orange	Suspect 2	Lane 4
Area 3	Violet	Suspect 3	Lane 5
Area 4	Red	Suspect 4	Lane 6
Area 5	Yellow	Suspect 5	Lane 7

Answers to Analysis Questions

(a) From which area would you select seed for the reforestation project?

Answer: Seed from Area 3 should be used for the reforestation project.

(b) Explain one technical or economic benefit of the FAIROAK project.

Answer: When looking at disease or dieback susceptibility in oaks from a particular area, ecologists will now be able to determine whether native or introduced trees are more susceptible to a particular disease. Furthermore they will have the means to repopulate an area with oaks which are less susceptible to disease and dieback. Second, as seeds can now be genotyped and oaks native to a particular area can be selected, reforestation projects are made more efficient.

(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.