

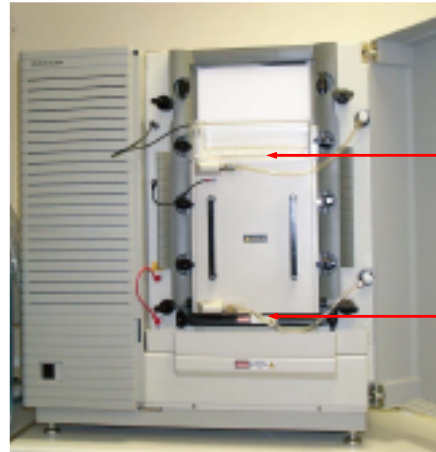
When the PCR is complete, we next need to determine the size of our amplified DNA fragment. This is likely to vary between individuals depending on the number of repeat units within the microsatellite.

Because DNA molecules always carry a slight negative charge, we can separate fragments of different sizes using a technique known as gel electrophoresis.

To do this we prepare a very thin polyacrylamide gel held between two glass plates. We introduce our sample at one end of this gel and apply a powerful (3000V) electric field to draw the DNA through the gel.

If we think of the gel as a dense matrix of cross-linked molecules like coconut matting it is possible to imagine how this matrix would retard the progress of a long DNA molecule as it tries to weave through the gel. Clearly the longer the molecule the more it will be retarded.

In fact, so precise is this relationship that we are able to discriminate between DNA fragments that differ in size by only one base.



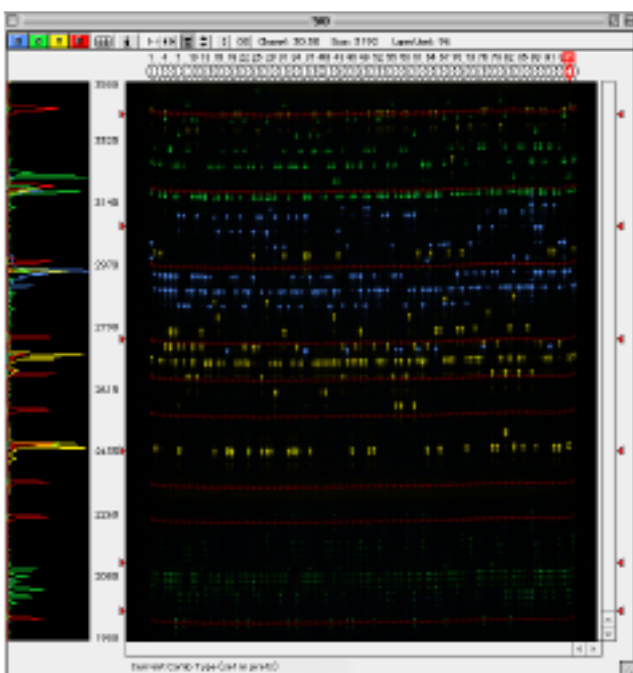
← samples introduced at the top of a gel...

....are detected by a camera at the bottom

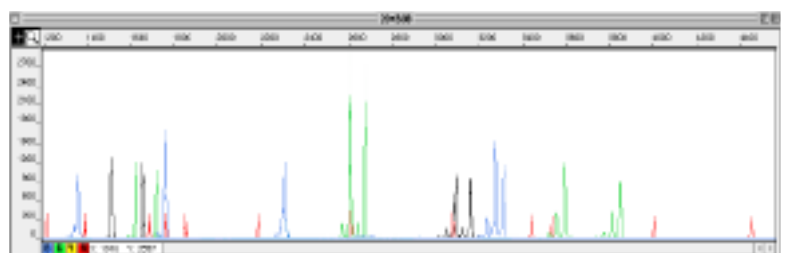
The LNS sequencer ready for action

It is possible to detect these fragments by using specially 'labelled' primers in the PCR. These labels are fluorescent dyes that a camera, positioned towards the end of the gel, can detect as they pass by. Very small fragments, say 50 bases long reach the end of the gel in about 30mins, longer fragments may take several hours.

As the camera detects a dye passing, it passes this information to a computer that deduces the size of the attached fragment from the time taken to pass through the gel.



composite image showing the fragments present in 96 samples



detailed electropherogram of one sample: the red peaks represent the fragments of known size used to estimate the sizes of the unknown fragments

There are a number of dyes available; each is a different colour, so it is possible to size more than one microsatellite at a time, by labelling each with a different colour. In addition, we add to each sample several fragments of known length, labelled with a different colour to the PCR products; the computer uses these to calibrate the size estimation.