DNA probes (pg 545)

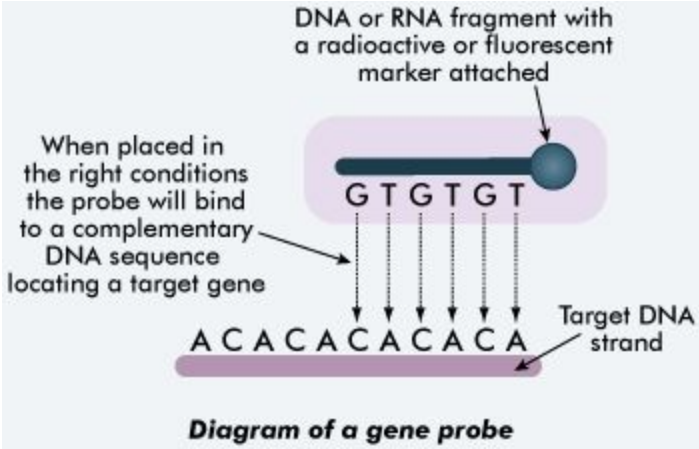
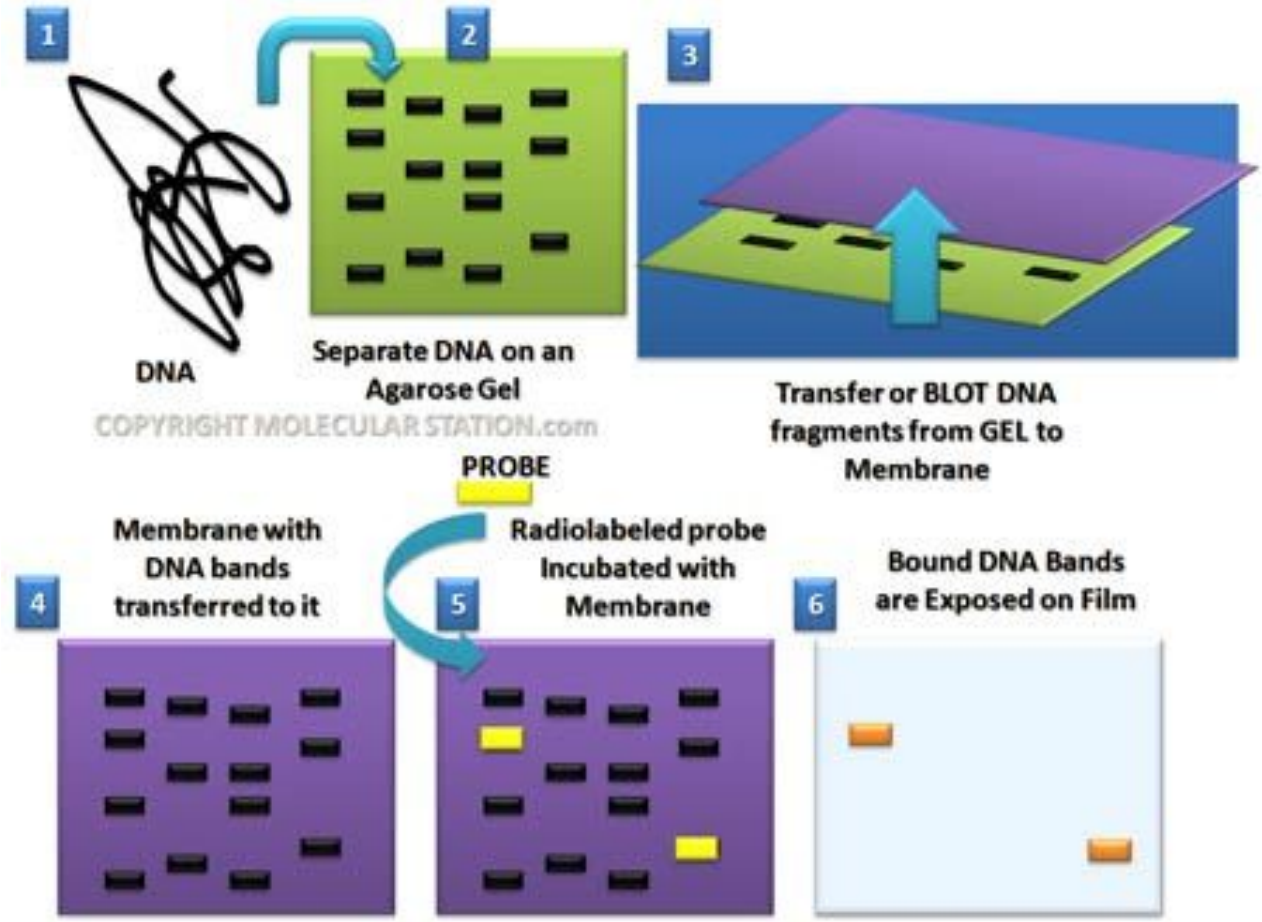


Diagram of a gene probe

- short stretch of nucleotides
- complementary to a stretch of DNA to be detected
- useful to detect mutant alleles (e.g Cystic Fibrosis, Cancer genes)
- used in DNA fingerprinting
- attached to a fluorescent or radioactive probe

Using DNA probes (pg 546)



1. Sequence the DNA to be detected, and design a probe to part of the DNA (or use a genetic library)

2. Take some cells from the person being tested

3. Extract DNA from the cells

4. Break the DNA into fragments using RE (avoiding the allele of interest)

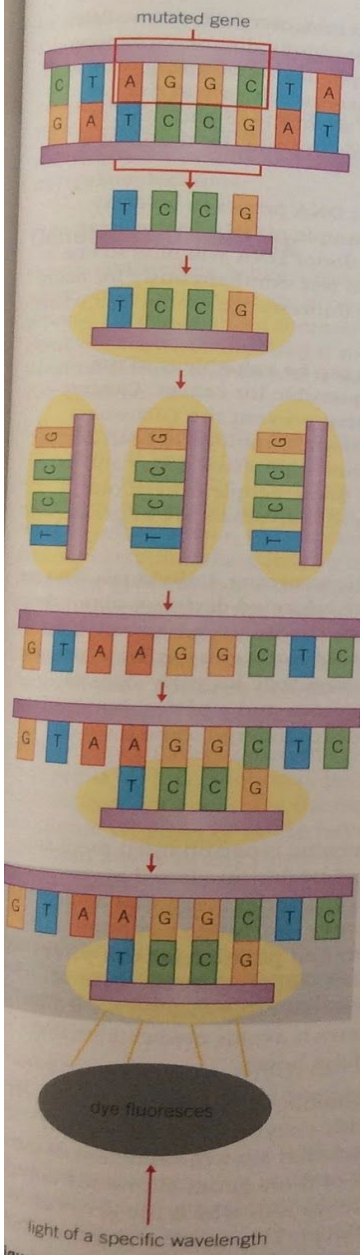
5. Separate the DNA on an agar gel

6. Transfer the DNA to a nylon membrane (Southern Blot)

7. Heat the membrane (or use mild alkali) to denature the strands

8. Add probe 9. Wash off excess probe

10. Visualise the bands using either UV light or an X-ray film



1 The sequence of nucleotides on the mutated gene is determined by DNA sequencing. Genetic libraries now store the DNA sequences of many of the genes responsible for common genetic diseases.

2 Fragment of DNA with complementary bases to the mutant allele of the gene is produced.

3 DNA probe is formed by fluorescently labelling the DNA fragment.

4 PCR techniques are used to produce multiple copies of the DNA probe.

5 Probe is added to single-stranded DNA fragments from the person being screened.

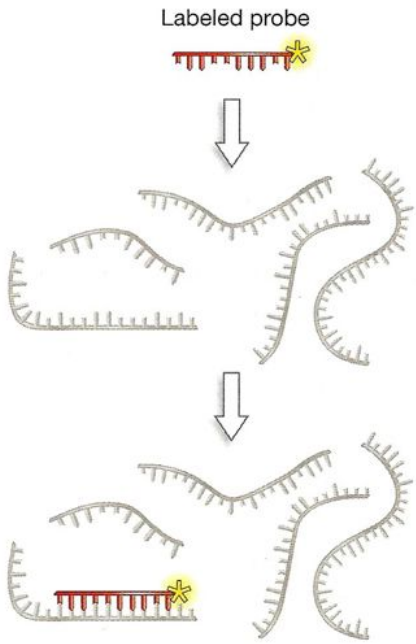
6 If the donor has the mutated gene, some donor DNA fragments will have a base sequence that is complementary to the probe and the probe will bind to its complementary bases on the donor DNA.

7 These DNA fragments will now be labelled with the probe and can be distinguished from the rest of the DNA fragments.

8 If complementary fragments are present, the DNA probe will be taken up and the dye will fluoresce – this is detected by a special microscope. If complementary fragments are not present, the DNA probe will not fluoresce.

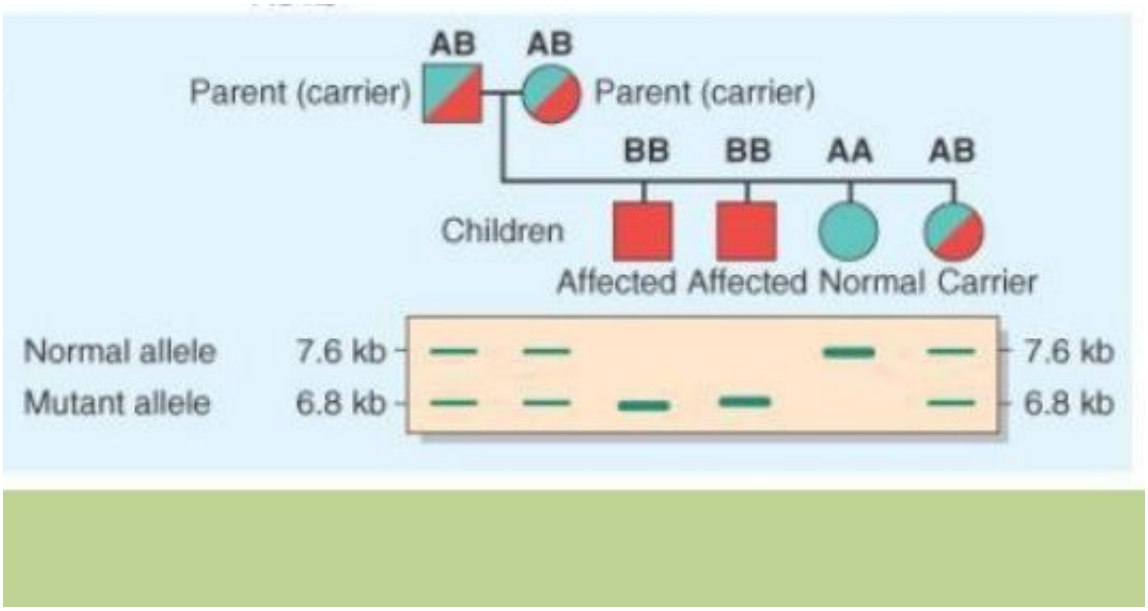
gure 1 *Summary of the process to locate a specific allele of a gene*

DNA hybridisation (pg 545)



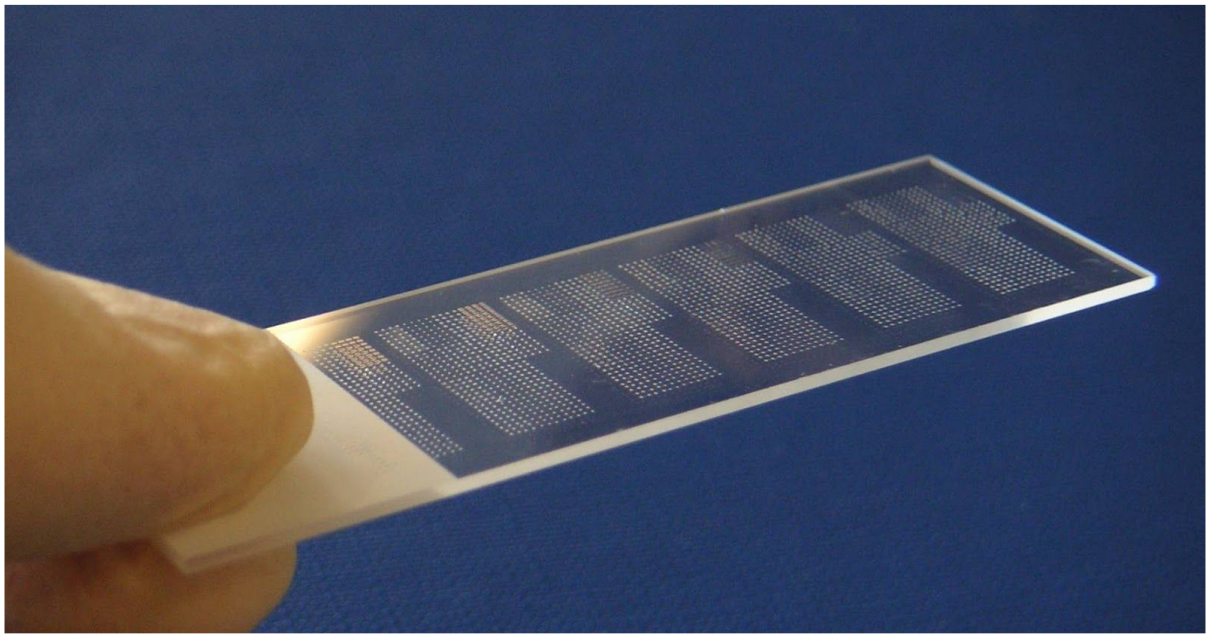
- in order to bind to the probe, the DNA strand must have exposed bases
- ds DNA is heated to separate the two strands
- probe is added to the membrane/solution
- the solution is cooled - H-bonds reform, and some of the probe attaches to its complementary DNA

Genetic Screening (pg 546)



- allows person to make informed decisions about lifestyle and future treatment
- might get themselves checked more regularly
- might choose preventative surgery
- decide on the best course of treatment (personalised medicine)

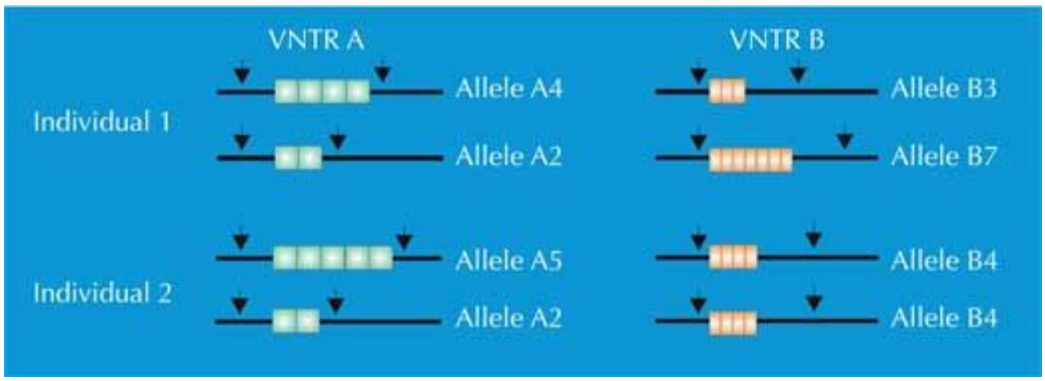
DNA array (pg 548)

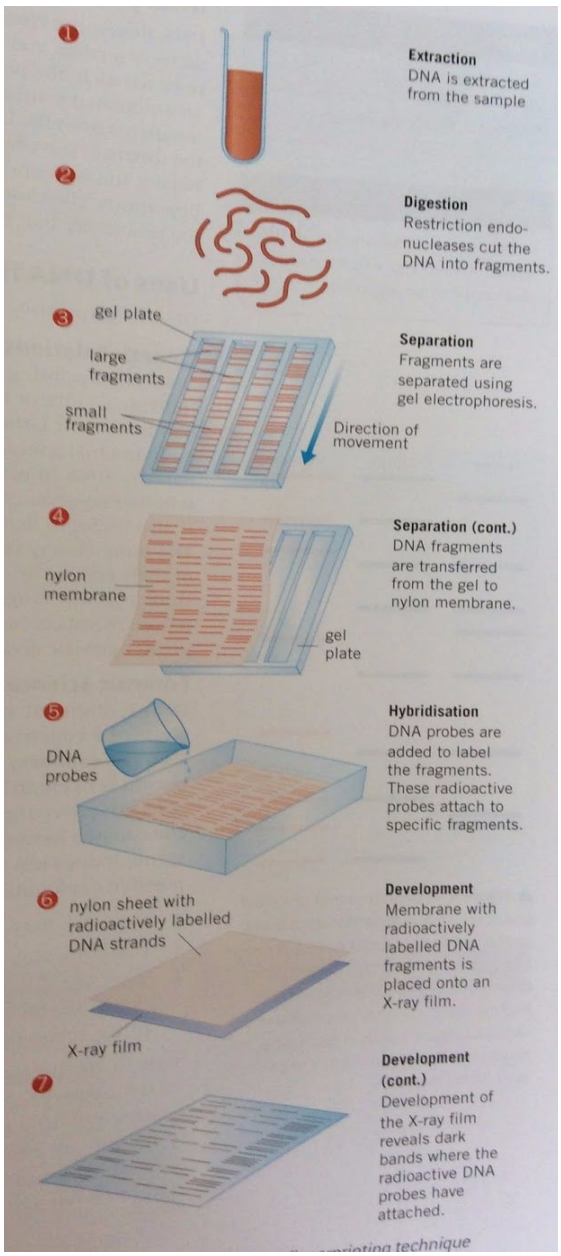


- Several 100 DNA probes are attached to a glass slide
- Patient’s DNA is fragmented, and denatured by heat or alkali
- added to the array
- read by DNA array scanner
- if probe bind to DNA from the patient, a square will light up on the array and is detected by the scanner
- quick and easy way to scan a patient’s DNA for well known conditions

DNA fingerprinting (pg 550)

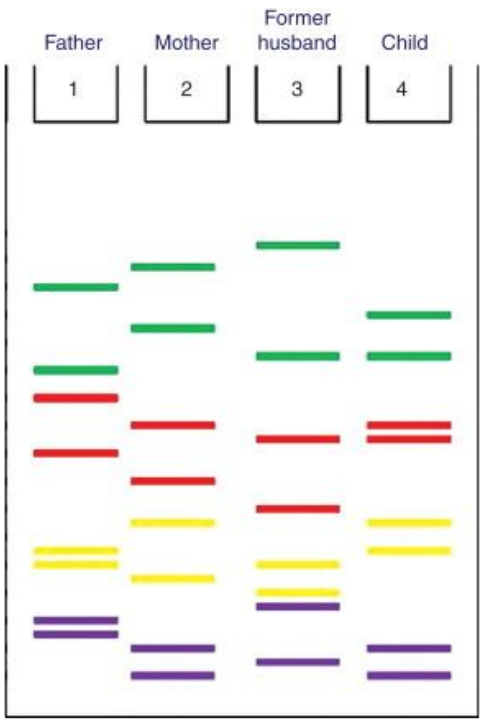
- uses variable number tandem repeates (VNTRs)
- stretches of DNA ~10 bp long
- that occur in the introns (which rarely mutate)
- and occur in all individuals
- the number of times this sequence repeats is different for each individual
- because we inherit one allele from each parent
- only monozygotic twins have identical VNTR repeats



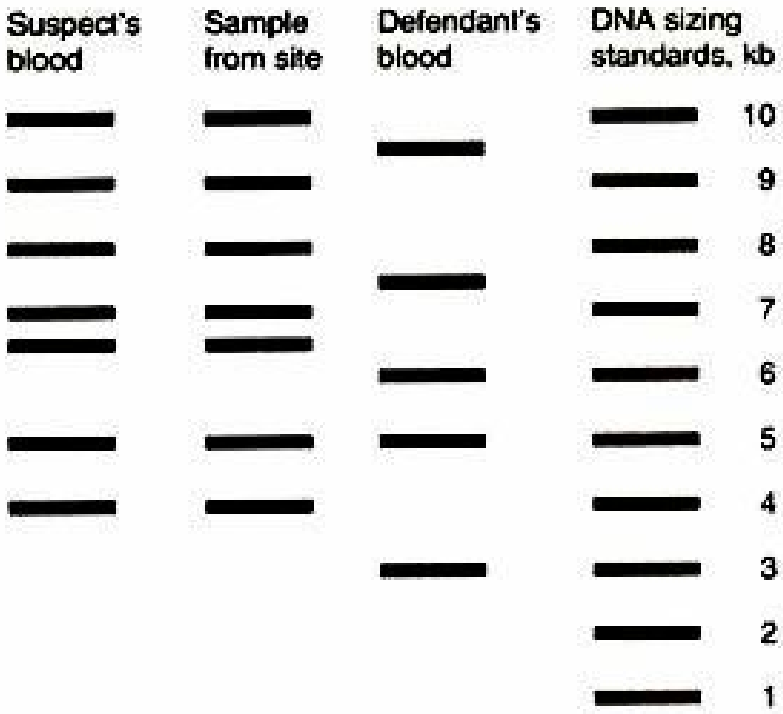

1
Extraction
DNA is extracted from the sample
2
Digestion
Restriction endonucleases cut the DNA into fragments.
3
gel plate
large fragments
small fragments
Direction of movement
Separation
Fragments are separated using gel electrophoresis.
4
nylon membrane
gel plate
Separation (cont.)
DNA fragments are transferred from the gel to nylon membrane.
5
DNA probes
Hybridisation
DNA probes are added to label the fragments. These radioactive probes attach to specific fragments.
6
nylon sheet with radioactively labelled DNA strands
X-ray film
Development
Membrane with radioactively labelled DNA fragments is placed onto an X-ray film.
7
Development (cont.)
Development of the X-ray film reveals dark bands where the radioactive DNA probes have attached.

Paternity Test

- a child inherits half its alleles from the mother, and half from the father
- any bands in the child's fingerprint, that are not inherited from the mother, must ALL come from the person who is the father of the child



Forensics


Suspect's blood
Sample from site
Defendant's blood
DNA sizing standards, kb
10
9
8
7
6
5
4
3
2
1