

Unit 4 Biology

Revision Booklet 11

Topics

Manipulating DNA: tools and techniques

Restriction Enzymes

Probes

Polymerase Chain Reaction

Gel Electrophoresis

DNA Profiling

Name:

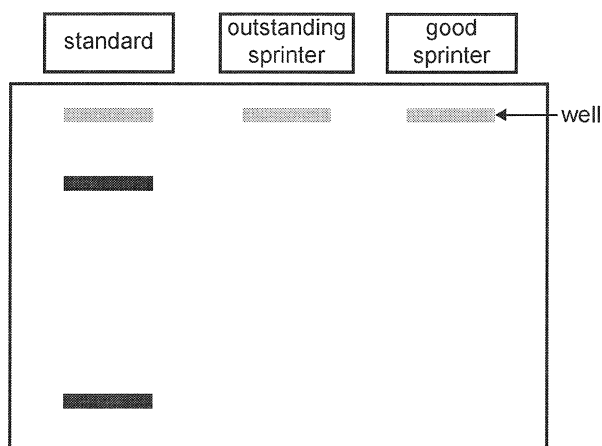
Scientists investigating the performance of athletes found that one gene contributing to the performance of sprinters is the ACTN3 gene. There are two alleles of the gene, the 577R allele and the 577X allele.

The 577X allele codes for a very short protein fragment in muscle fibres due to a stop codon mutation.

The table below summarises the athletic potential for the three possible genotypes for the ACTN3 gene.

ACTN3 genotype	Athletic potential
577R / 577R	outstanding sprinter
577R / 577X	good sprinter or long-distance runner
577X / 577X	very good long-distance runner

A scientist tested sprinters to see if they possessed the 577R allele. Samples were obtained from athletes' muscle fibres. A standard containing proteins of the same lengths as the proteins coded for by both alleles 577X and 577R was used as a comparison. The standard and the samples were exposed to gel electrophoresis. In gel electrophoresis, protein molecules separate according to size and charge in the same way as DNA molecules. The result for the standard is shown below.



- b. On the diagram of the gel above, draw the bands expected for an outstanding sprinter and for a good sprinter.

Explain why you have placed the bands in these positions.

3 marks

Question 6

DNA sequencing is often performed to help produce phylogenetic trees or to classify organisms. Sections of nuclear DNA from similar organisms are sequenced and compared for similarities.

- a. Name and describe another DNA method that is used to determine how closely related two species are.

Name _____

Brief description _____

2 marks

Scientists use a specific mitochondrial gene called *cox1* in comparative studies in fish. The *cox1* gene lacks introns and is only 654 bp long, making it economical and easy to sequence. The sequenced gene appears like a 'barcode' found on grocery products, for example



A worldwide database has over 45 000 *cox1* gene sequences obtained from different kinds of fish.

Illegal fishing occurs in Australian waters. Often only a small portion, such as a fin, is kept by the fishermen and the remainder is thrown overboard.

- b. How would the barcode database be used to identify the fish species that had been caught by the fishermen? Provide an example of a situation (other than illegal fishing) when humans would want to identify the fish species.

2 marks

Scientists using the *cox1* gene have seen that even within the same species of fish, differences within the DNA sequence can occur.

The scientists believe this is often due to the redundancy in the genetic code.

- c. Explain what redundancy in the genetic code means.

1 mark

As the database grows it is hoped that at least five samples for each species will be collected and analysed. The species samples will be taken from as many different locations as possible.

- d. Why is it important to sequence multiple samples from the same species?

1 mark

Total 6 marks

SECTION B – continued
TURN OVER

Question 8

DNA includes sections that are called short tandem repeats (STR). Mutations in STRs occur, on average, every 500 generations.

Different numbers of these repeats have no obvious effect on the individual.

- a. What is the likely reason for this?

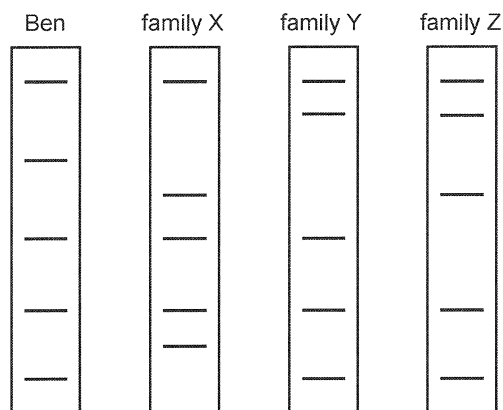
1 mark

A young man, Ben, wants to find out more about his genetic ancestry. He sends a sample of cells, obtained from a swab of his mouth, to a laboratory. On receipt of the sample, the laboratory treats the cells to release the DNA to enable identification of STR markers.

- b. Name the process used to produce many copies of the STR markers.

1 mark

Each of the STR markers produced is labelled with a dye and subjected to gel electrophoresis. Five of Ben's STR markers were compared with three family groups who have the same surname as him. The following gels resulted.



- c. Explain which family is Ben's most recent common ancestor.

2 marks

Total 4 marks

Question 5

Some people prefer to eat Wagyu cattle because of the high level of marbling (fat) in the meat. Four separate DNA markers are used to test for marbling in an animal. Tested cattle are scored on a scale of zero to eight, eight indicating the highest degree of marbling.

- a. What does the use of four markers suggest about the inheritance of this characteristic?

1 mark

A Wagyu breeder discovered a small number of individuals in her elite herd that were suffering from Chediak-Higashi Syndrome (CHS). CHS is an autosomal recessive condition that can affect species other than cattle. The breeder required further information.

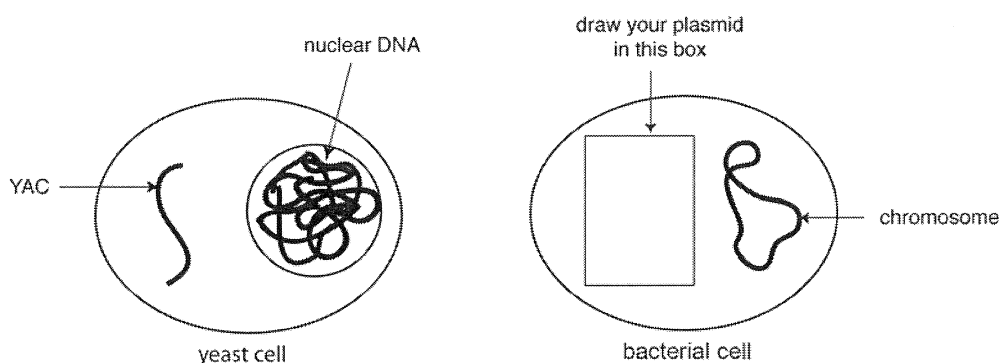
Gene probing was used to target *CHSI*, the allele responsible for the condition. The genetic probes for the Wagyu CHS locus were derived from human alleles.

- b. Given that the gene probe for a human works for the Wagyu, what can you infer about the chemical code for this allele?

1 mark

The Wagyu *CHSI* allele was isolated and given a fluorescent tag. It was introduced into a yeast cell as a large, independent, cytoplasmic chromosomal segment called a Yeast Artificial Chromosome (YAC). In addition to the allelic DNA, a YAC includes a centromere and a replication sequence. The yeast cells are then incubated in the presence of growth stimulants and given time to replicate.

This procedure is similar to genetic engineering of bacterial plasmids, however the YAC is able to contain much larger pieces of DNA than a plasmid.



- c. i. In the bacterial cell **above**, draw a plasmid in the **blank box**.
- ii. Bacterial plasmids lack a centromere. Why are YACs made with a centromere?
- iii. What term describes the process of copying a gene?

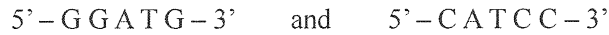
1 + 1 + 1 = 3 marks

A test was developed to identify each of the normal and mutant alleles. Two cows were chosen for testing.

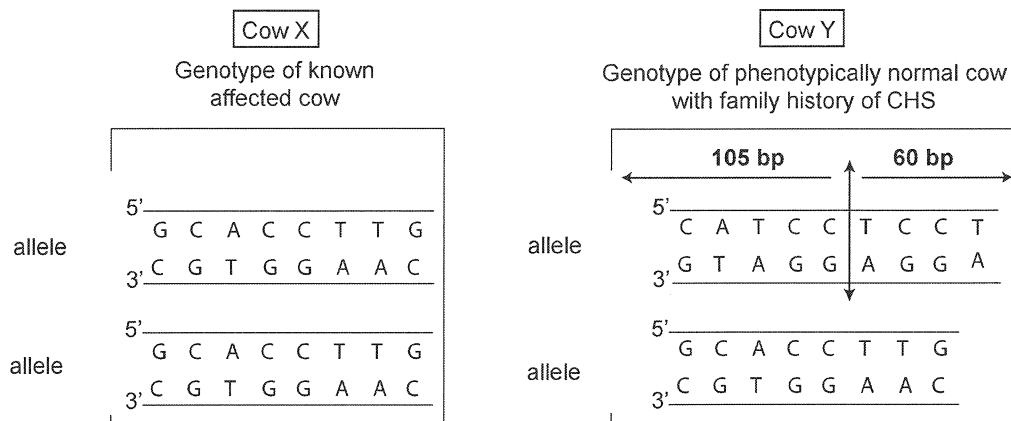
Cow X – a cow known to have the autosomal recessive disease CHS

Cow Y – a phenotypically normal cow with a family history of CHS

The CHS locus was isolated from each, amplified and then treated with *FokI* restriction enzyme which recognises the nucleotide sequences

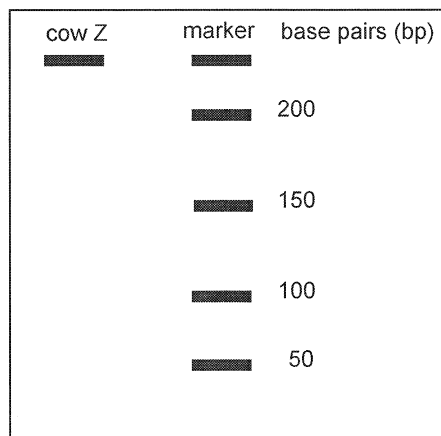


The genotypes at the CHS locus for the two cows are shown in the following figure.



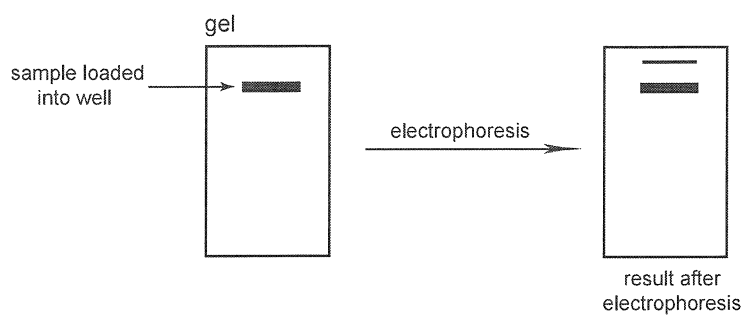
- d. i. Explain whether Cow Y is heterozygous or homozygous at the CHS locus.

- ii. On the electrophoretic gel diagram below, draw in the band(s) that would accurately show a profile for an unaffected cow Z with no history of CHS in the family.



1 + 2 = 3 marks

A farmer suspected that one of his cows was a CHS carrier. He sent a sample of the cow's hair follicles for testing. A technician ran a gel of DNA sequences from the hair follicles and obtained the following result.



- e. What mistake must the technician have made in his procedures to obtain this result?

1 mark

Total 9 marks

Question 7

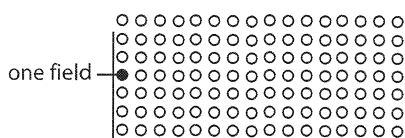
Many techniques in molecular biology require the use of probes.

a. What is a probe?

1 mark

DNA microarray technology, also known as DNA chip technology, allows screening to detect mutations.

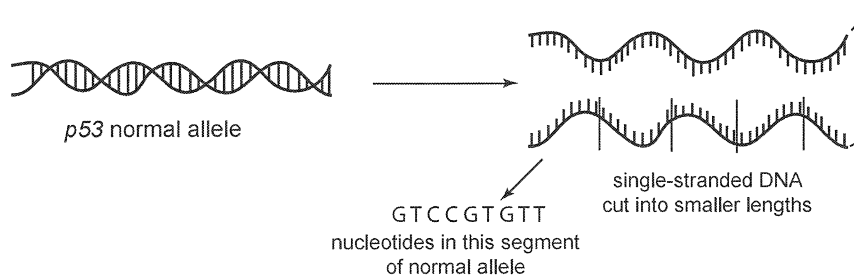
A DNA chip is made of glass and can contain thousands of fields. Each field is like a tiny well in which reactions can occur.



DNA microarray technology has been used to survey the *p53* gene because a mutation of this gene is present in about 60% of all cancers. The position of a mutation in the *p53* gene of a patient, Patient X, who has breast cancer, can be determined.

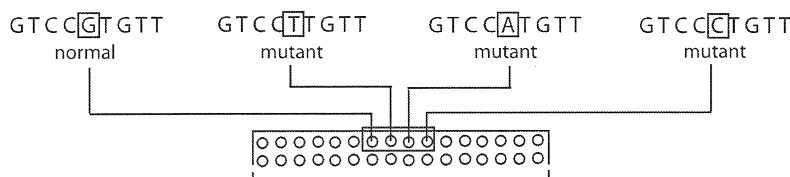
Steps in the screening procedure are outlined below.

Step 1 Treat a normal allele of *p53* to break it down into nucleotide sequences.



Step 2 Each segment must be tested, one nucleotide at a time. Tests for the first segment of the allele are outlined below.

Consider nucleotide five [G] in the normal sequence. Sequences of this section are manufactured so that all possible mutants of base five are formed. Each of these sequences is placed in a different field.



Step 3 Two solutions are added to each of the fields.
These solutions contain

Solution I Complementary normal strand, labelled with green fluorescent dye

Solution II Complementary strand from DNA of person with breast cancer, labelled with red fluorescent dye.

Step 4 Allow time for hybridisation of strands and then wash the DNA chip to remove excess dyes.

Step 5 Examine fields under UV light to distinguish colours remaining. Interpret results.

The results for Patient X are shown below.

	GTCC G TGTT normal	GTCC T TGTT mutant	GTCC A TGTT mutant	GTCC C TGTT mutant
Colour observed after hybridisation and washing	↓ green	↓ No colour appears (black)	↓ red	↓ No colour appears (black)

b. What is the function of each of the two different fluorescent dyes used?

2 marks

c. What does hybridisation mean?

1 mark

d. What mutation resulted in Patient X having breast cancer?

1 mark

A daughter of Patient X was also tested for the first segment of the allele.

e. Would you expect the result of her test to be green, red or black? Explain your answer.

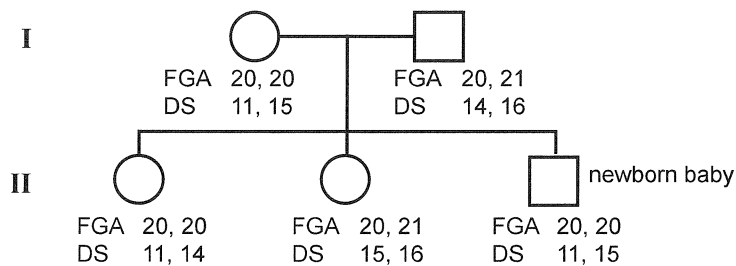
1 mark

Total 6 marks

Question 2

Some gene loci have several alternative alleles. With these gene loci, the DNA profile of an individual may be given in terms of the relative sizes of the alleles. For example, gene locus THO has three alleles that are called THO 7, THO 9 and THO 10.

In a maternity hospital, a mother claimed that she had accidentally been given the wrong baby boy. A DNA analysis of two gene loci, locus FGA and locus DS, was carried out on members of the family and the results are included in the following pedigree. Assume that no mutation involving these two loci has occurred in the family.



- a. Does the data support the mother's claim that she has been given the wrong baby boy? Give reasons to support your answer.

2 marks

Many trials involving the technique of gene therapy have been carried out in humans, with varying success.

- b. What is the aim of gene therapy?

1 mark

The technique of gene therapy also involves the use of vectors.

- c. Name one agent that could act as a vector in gene therapy.

1 mark

In 1999, during a gene therapy trial for a particular enzyme deficiency, a young man died from multiple organ failures four days after starting the treatment. He was in excellent health before participating in the trial.

- d. Suggest one event that could have occurred within the young man as a result of him receiving gene therapy that resulted in his death.

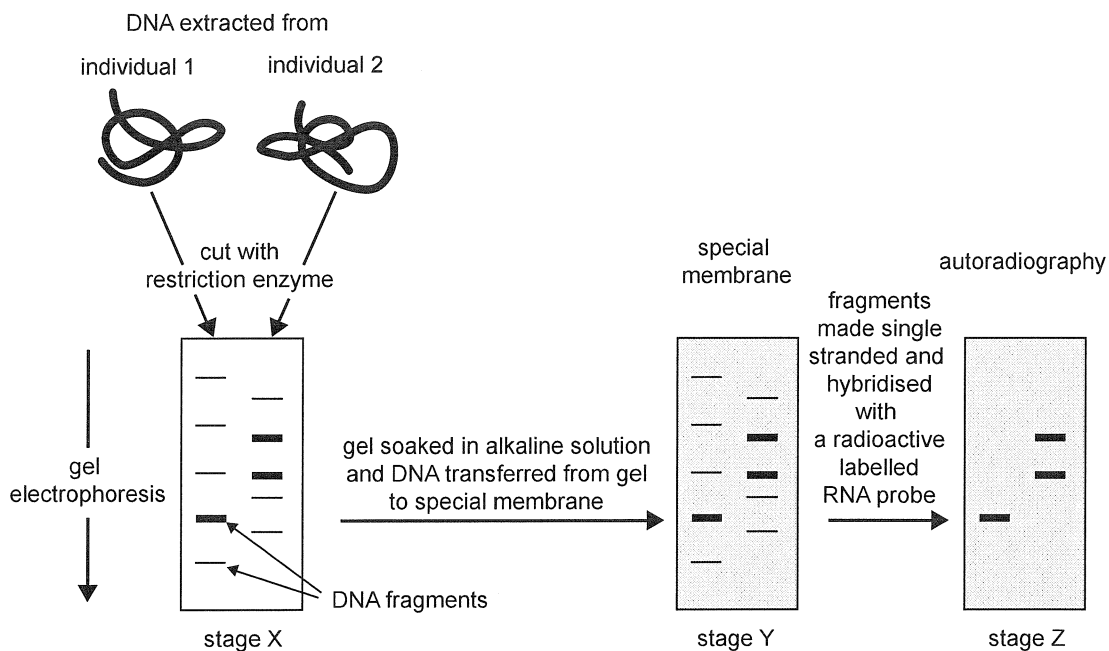
1 mark

Total 5 marks

SECTION B – continued

Question 6

RFLP (Restriction Fragment Length Polymorphism) analysis is commonly used to determine genetic variation between individuals. The procedure is summarised below.



In this procedure, scientists select a particular restriction enzyme from an available range.

- a. Explain the reason for their choice.

1 mark

Electrophoresis uses electrical current to sort DNA fragments.

- b. i. Describe one characteristic of this sorting process.

- ii. Explain why the DNA of each individual produces a different pattern of fragments after gel electrophoresis, even when the same restriction enzyme is used.

1 + 1 = 2 marks

Examine stages Y and Z.

- c. Describe, at the molecular level, what is meant by the term 'hybridised'. Why is it necessary to carry out hybridisation?

2 marks

Total 5 marks

SECTION B – continued
TURN OVER

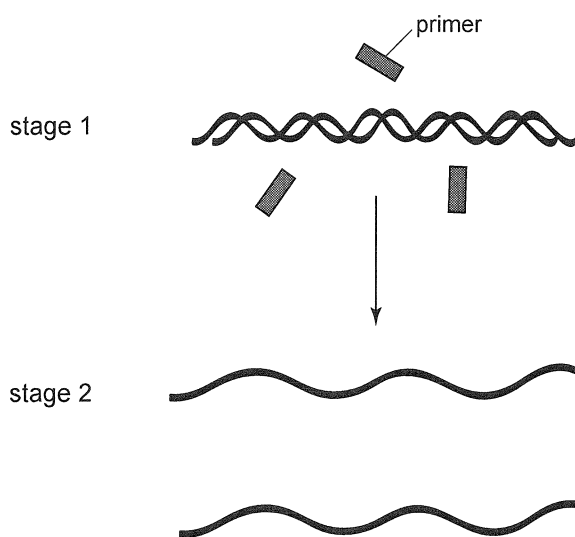
Question 2

Victoria Police forensic scientists conduct DNA profiling using samples taken from crime scenes. Traces of DNA of less than 1 nanogram can be amplified and then profiled.

- a. Name the process which is used to amplify the DNA.

1 mark

Below is a diagram showing part of this process.



- b. What must be done between stages 1 and 2 to separate the strands of the DNA molecule?

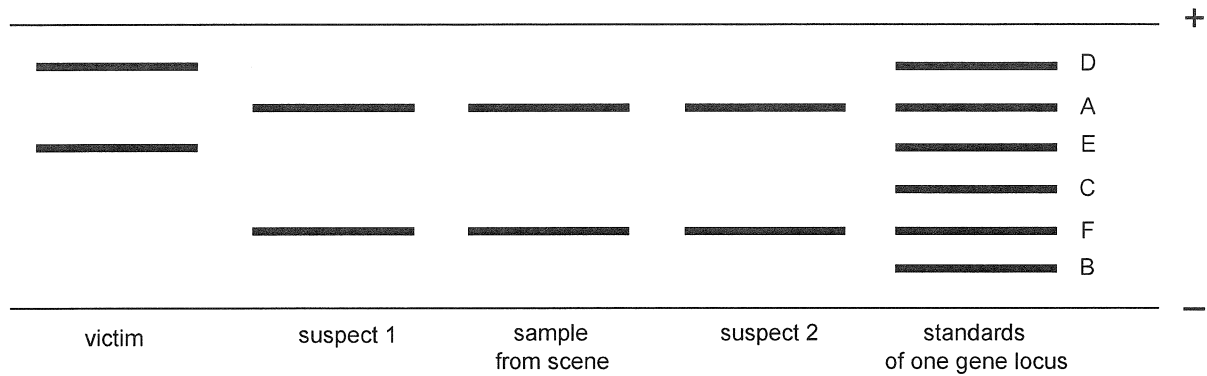
1 mark

- c. Complete and label the diagram at stage 2.

2 marks

Small pieces of DNA of differing length can be compared to determine whether or not a sample could have come from a particular person. In a case, samples of DNA from the victim and the crime scene were compared with samples from two suspects.

The DNA samples were treated with restriction enzymes, amplified and run through gel electrophoresis. The results for one gene locus are shown in the diagram below.



- d. Draw an arrow on the right-hand side of the diagram to indicate the direction of movement of the DNA fragments.

1 mark

- e. What do the standards consist of, and what is their purpose?

2 marks

- f. From these results, give a conclusion which could be drawn about the sample taken from the crime scene.

1 mark

- g. What further action would you recommend to the forensic scientists investigating this case?

1 mark

Total 9 marks

Question 4

- a. Describe the appearance of a bacterial plasmid.

1 mark

A bacterial plasmid was modified in the laboratory so that it contained a gene for an enzyme which provided resistance to the antibiotic tetracycline.

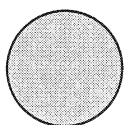
Bacterial cells, which in their natural environment were sensitive to the antibiotic tetracycline, were mixed with the modified plasmid. The bacterial cells were treated so that they could take up the plasmid.

- b. What is the name of the process in which a bacterial cell takes up a plasmid and expresses the genes of the plasmid?

1 mark

The outcome of an experiment is shown below.

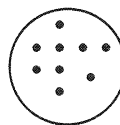
A
bacterial cells only,
spread on agar



B
bacterial cells only,
spread on agar
with tetracycline



C
bacterial cells exposed to
the plasmid, spread on
agar with tetracycline



With respect to the growth of bacteria the results of plates A and C are shown. On plate A there is a continuous growth of bacteria over the surface of the agar. On plate C the colonies are distinguishable from each other.

- c. i. What result would you expect on plate B with respect to the growth of the bacteria?

-
- ii. Explain your answer to c.i.

1 + 1 = 2 marks

- d. Explain why there is a difference in the way the bacteria have grown on plates A and C.

2 marks

Total 6 marks