5 Metachromatic leukodystrophy (MLD) is a genetic disease that affects the nervous system.

MLD is caused by mutations in the *ARSA* gene located on chromosome 22. The *ARSA* gene, which is 3150 base pairs (bp) in length, includes 8 exons and is shown in Fig. 5.1.

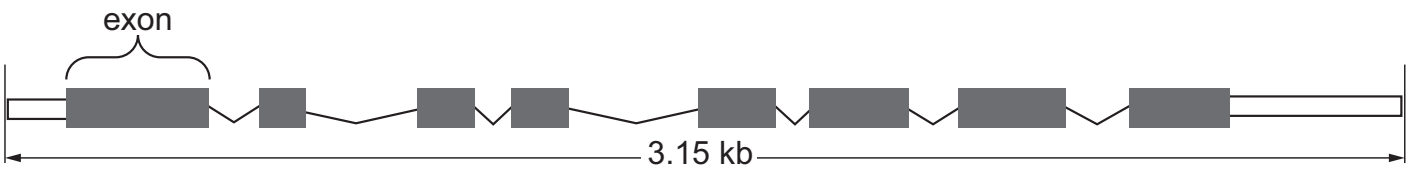

Fig. 5.1

A genetic test using DNA sequencing is available to identify mutations associated with MLD in the *ARSA* gene. The sequencing method can only work if a DNA fragment size is less than 1000 bp.

The test involves a number of different stages:

- Genomic DNA is extracted from a blood sample.
- Polymerase chain reaction (PCR) using 5 pairs of primers selects 5 different DNA fragments of the *ARSA* gene.
- Gel electrophoresis is carried out on the DNA fragments.
- The DNA fragments are sequenced and analysed for mutations.

Table 5.1 shows the length of the DNA fragments and the exons present in each DNA fragment.

Table 5.1

fragment number	DNA fragment length / bp	exons in DNA fragment
1	405	1
2	737	1-2
3	706	2-4
4	860	5-7
5	916	7-8

(a) Genomic DNA and primers are added to the PCR machine.

Name **two** other substances that are added to the PCR machine, **and** explain why each substance is added.

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(b) Gel electrophoresis is carried out on the 5 different DNA fragments produced as a result of PCR. Each DNA fragment is put into a loading well on the gel.

The final lane on the electrophoresis gel contains a DNA ladder of known lengths of DNA.

Fig. 5.2 shows the results of the gel electrophoresis.

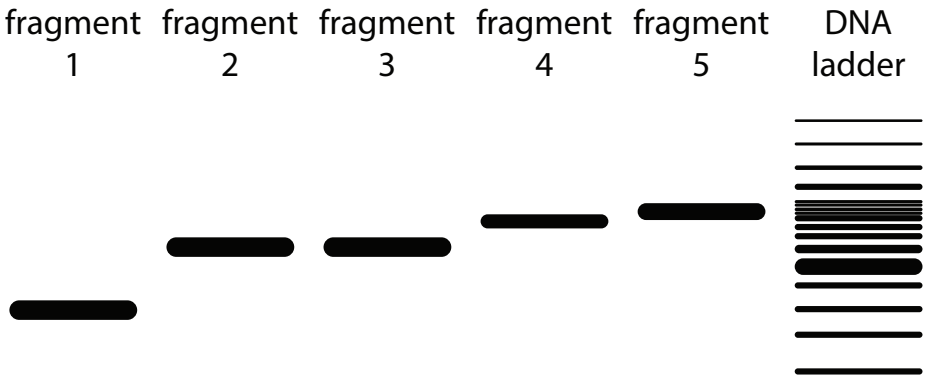

Fig. 5.2

(i) Suggest the role of the DNA ladder.

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(ii) In the genetic test for MLD, PCR and gel electrophoresis are carried out before DNA sequencing.

Suggest **and** explain reasons why PCR and gel electrophoresis are carried out before DNA sequencing.

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(c) Calculate the number of copies of DNA that are obtained if PCR is run for 35 cycles.

Assume that there is only 1 copy of DNA at the start of PCR.

Give your answer in standard form to **two** significant figures.

number of copies .....

[1]

(d) MLD is a rare autosomal recessive disease. It is estimated that there is one case of MLD in every 40 000 births worldwide. Children with MLD have a reduced life expectancy.

- MLD is a degenerative disease that causes severe disability.
- Before 2022, there was no known cure for MLD and the only treatment for symptoms was to provide pain relief.

A new gene therapy treatment known as Libmeldy® became available in a number of countries in 2022. The treatment must start before symptoms are present. Although the treatment may provide a cure for MLD, a single dose is extremely expensive.

Blood samples from newborn babies are screened for a number of rare genetic diseases (neonatal screening) in most countries of the world, but none include screening for MLD.

Discuss the social and ethical considerations of including screening for MLD when carrying out neonatal screening in a country where gene therapy for MLD is available.

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[Total: 14]

8 LibertyLink® soybean is a genetically modified crop. It was first grown in 1996 and used in food products from 1998. It has been grown in 6 countries and used in food products in 21 countries.

Table 8.1 summarises the modifications made to the soybean plant to produce LibertyLink® soybean.

Table 8.1

name of introduced gene	gene donor organism	gene product	function of gene product
pat	*Streptomyces viridochromogenes*	phosphinothricin *N*-acetyltransferase	stops action of glufosinate, a herbicide

(a) Explain how the modification made to produce LibertyLink® soybean may help to solve the global demand for food.

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(b) **(i)** Name the type of enzyme that could be used to cut out the *pat* gene from *S. viridochromogenes*.

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(ii) Name the enzyme that could be used to join the *pat* gene to a plasmid by forming phosphodiester bonds.

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(c) Suggest reasons why LibertyLink® soybean is used in food products in 21 countries but only grown in 6 countries.

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[Total: 9]

3 Porphyria is a group of rare genetic diseases in which molecules called porphyrins accumulate in the body.

Three examples of porphyria are:

- X-linked protoporphyria
- variegate porphyria
- congenital erythropoietic porphyria (CEP).

(a) X-linked protoporphyria is caused by a mutant allele located on the X chromosome.

Fig. 3.1 shows the pattern of inheritance of X-linked protoporphyria in one family.

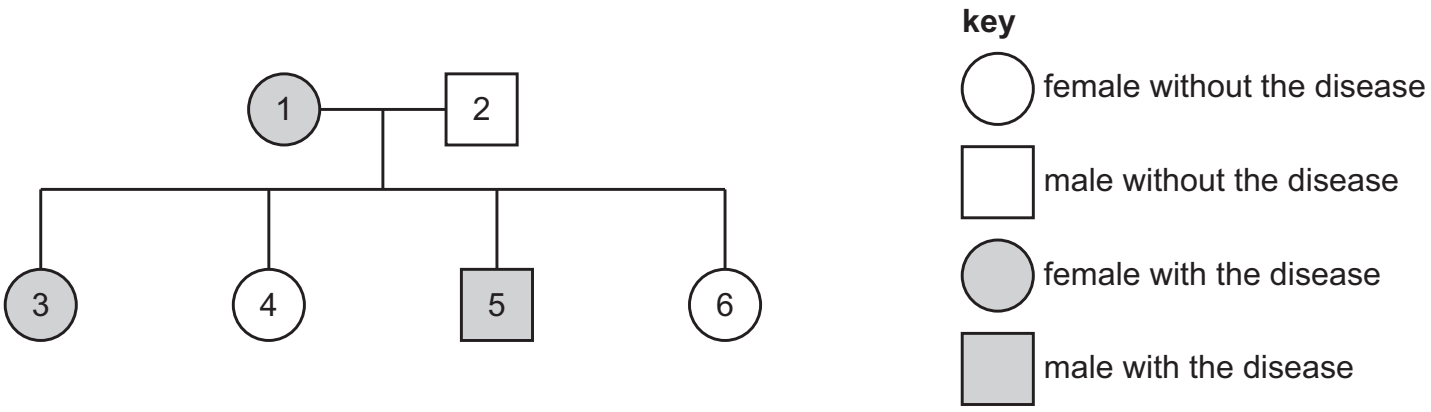

Fig. 3.1

X-linked protoporphyria is described as a sex-linked disease.

Use the information in Fig. 3.1 to make **one** other conclusion about the pattern of inheritance of X-linked protoporphyria.

Give evidence to support your conclusion.

conclusion .....

evidence .....

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[2]

(b) Variegate porphyria occurs in 1 in 100 000 people in Europe.

In the 1600s, approximately 100 Dutch people migrated from Europe to South Africa.

The population of people of Dutch descent in South Africa is now 2.5 million. Variegate porphyria occurs in 1 in 1000 people in this population.

Suggest **and** explain why variegate porphyria is more common among people of Dutch descent in South Africa than among people in Europe.

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(c) Microarrays can be used to detect various forms of porphyria caused by mutant alleles.

Describe how microarrays can be used to detect a disease by analysing gene expression.

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(d) The nucleotide sequences of alleles that cause rare genetic diseases, such as the various forms of porphyria, are stored in a database.

State **one** benefit of having a database of nucleotide sequences for alleles that cause rare diseases.

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(e) Congenital erythropoietic porphyria (CEP) is caused by a substitution mutation in a gene coding for an enzyme.

The mutant CEP allele codes for an enzyme with little or no function.

Scientists used gene editing in the laboratory to correct the nucleotide sequence of the mutant allele in cultured human cells.

The scientists introduced three molecules to the cells:

- an enzyme called Cas9 that causes breaks in DNA strands
- guide RNA (gRNA), attached to Cas9, that is complementary to the mutant CEP allele
- a short length of DNA, known as template DNA, that can replace the section of the allele where the substitution mutation has occurred.

The repair mechanism within the cell allows the template DNA to be inserted so that the mutation is corrected.

Fig. 3.2 shows part of the nucleotide sequence in the mutated CEP allele before and after the gene editing procedure.

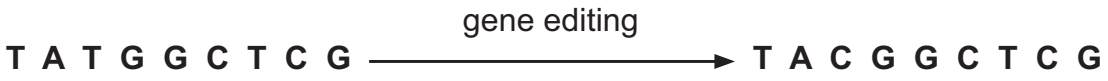

Fig. 3.2

In the future, this gene editing procedure may be used in a person with CEP to prevent the accumulation of porphyrins in the body.

Suggest **and** explain how this gene editing procedure will prevent the accumulation of porphyrins in a person with CEP without damaging other genes.

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