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.

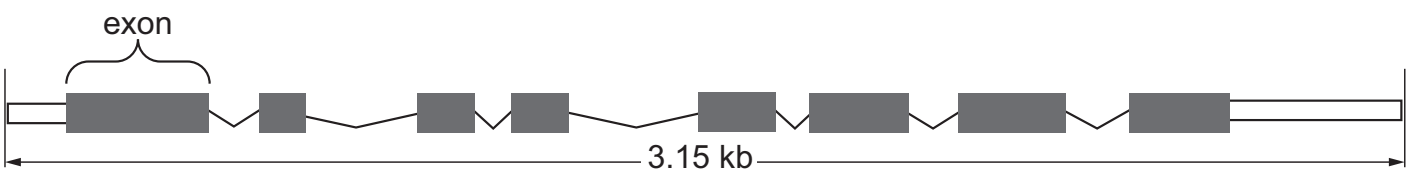

Fig. 5.1

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.

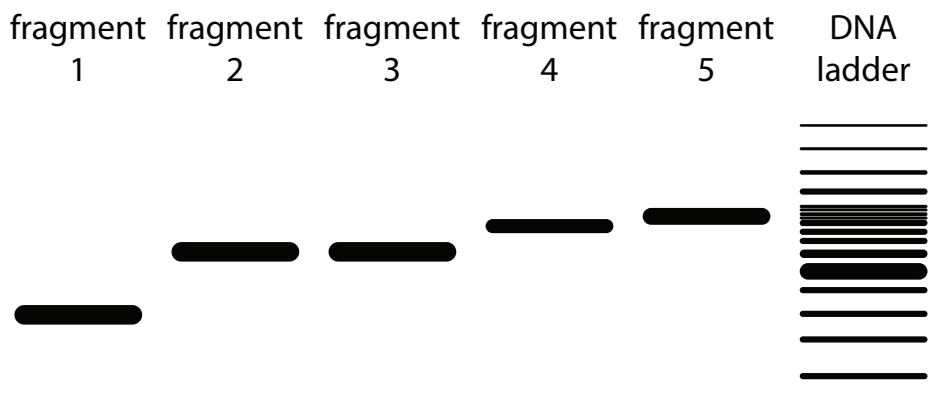

Fig. 5.2

(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]