Data analysis task

Health & Medicine

Data Analysis Task- Molecular (Southern Blotting Using the restriction map list the size of fragment(s) generated from digestion of the entire wildtype allele shown in Figure 1 with

a) EcoRI- 1. 5, 1. 2 Kbp

b) HindIII- 1. 75, 0. 3, 0. 95 Kbp

c) EcoRI + HindIII- 1. 0, 0. 65, 0. 55, 0. 3 Kbp

2) Explain why there are only two bands on the Southern blot of EcoRI digested genomic DNA from the RR individual?

-The two bands represent individual restriction fragments. It can therefore be implied that the enzyme cut the sample into two fragments only. This also implies that the R gene has only one restriction point for the enzyme

3) Using the information given for each restriction enzyme digest (Figure 1 and 2), explain the alteration in the R-gene DNA of the mutant r allele?

-the size of the gene is relatively the same as that of the R. This is by quick inference from the total weight of the bases. It though, has different restriction sites for each of the enzymes. This implies a mutation in the base sequence, most likely a deletion.

For the EcoRI enzyme: The 1. 5kbp band has been replaced by a 1. 25kbp band. This could imply that 0. 25kbp has been deleted from this region in the R to create the r allele.

For the HindIII enzyme: The 0. 3kbp band has been replaced by a 0. 05kbp band. Like above, the simplest explanation would be that 0. 25kbp has been deleted to form the r mutant

In the combined EcoRI+HindIII: The 0. 3kbp band has disappeared and a new 0. 05kbp band has been created. As in above there may have been a deletion of 0. 25kbp.

4) What are the possible outcomes to the primary sequence of the translated protein?

The protein may end up being modified to produce a less active analogue or useless protein. It thus forms a less coloured product, thus pink.

5) Why are there more bands in DNA from the carrier (Rr) than in the homozygous wild type (RR) or patient - what do the fainter bands represent?

-there are more bands due to the increased number of restriction sites present. Both of the genes contribute fragments to the gel that have been sectioned by the enzyme. These portions have complementary bases to the probe, thus forming more bands. Simply, there is a copy of R and r portions in the heterozygote. The lighter bands represent homozygote state. That is, there is less DNA to map. This can only imply that half the DNA is present.

6) Describe and explain what the resulting autoradiograph would look like if you performed this blot, but:

a) Samples were run without digesting the DNA with restriction enzymes.

-this would lead to a thick smear, as the cDNA would bind to the numerous complementary portions that would be available.

b) The DNA separated by gel electrophoresis was transferred to nitrocellulose or nylon without prior treatment with alkali.

-the alkali is needed to denature the DNA into single open strands. It thus would not give a result.

c) The nitrocellulose membrane was not blocked during pre-hybridisation.

-blocking is done to prevent background hybridisation signal. Thus if not done the result would include bands of unwanted DNA

d) The blot was processed without washing at high stringency.

-there would be numerous DNA ladders and mixed smears due to mixing of

DNA from many external sources and also from unwanted portions.

7) When the blot was probed at high stringency with a probe made with DNA that flanks the R gene (as seen in Figure 1) a smear typical of thousands of hybridising bands was seen. How could this be explained?

-DNA that flanks the R gene has complementary portions for all the bases. It is thus not specific to any sequence. It therefore gives a smear characteristic of all the hybridized portions.

8) The turnaround time for Southern Blot analysis is typically around 10 days. Now that you have identified the region in which the mutation lies explain with detail how a faster test could be designed?

Now that the portion has been identified, a faster test would only need one to cut the DNA at the specific location. After that has been done, the cut portion is denatured, pre hybridized and transferred to a gel block. Alternatively, it could be run through PCR and analysed using an electronic DNA analyser.