

Bioc471a/571a Homework 4 - Due at the start of class September 18

1. (2 pts) How might knowing the complete sequence of *E. coli* K-12 be useful in developing a rapid detection test for pathogenic strains of *E. coli* that have been found in contaminated food products?

By using computer alignment algorithms to compare the genomic sequence of *E. coli* K-12 with that of a pathogenic *E. coli* strain (e.g., *E. coli* 0157:H7), it would be possible to identify DNA sequences that function as strain-specific genomic markers (e.g., PCR primer pairs, RFLPs, etc.). This would permit the design of assays that detect specific pathogenic genomic sequences, as well as assays that detect sequences shared by each strain and could therefore be used as internal controls.

2. (2 pts.) Are molecular markers such as RFLP, STS, or STRP, considered a genotype or a phenotype? Based on the pedigree genotyping data presented in figure 4.8 in the text, what would you conclude if the STRP pattern of one of the offspring were A/A? Explain.

Molecular markers that identify DNA sequences, which is true of RFLP, STS and STRP markers, are considered genotypic markers because expression of the DNA (gene transcription) is not required for them to be identified. Phenotypic markers are based on differences that can be observed without knowledge of the DNA sequence.

The most likely explanation for the A/A genotype in one of the offspring is that the biological father is different than the biological father of the other offspring. A less likely explanation is that some type of rare gene conversion event (or cross-over) happened in the zygote to convert the paternal allele to a "A."

For questions 3 and 4, use the data presented in figure 4.19 in the text (same as in Lecture 8; "Positional mapping of human disease gene").

3. (2 pts.) Is it possible to determine from the PCR results if the S4 or S5 polymorphism is responsible for the disease phenotype? Explain. If this couple were to have another child, what is the probability that the child would be afflicted with the disease?

No, the PCR results only indicate which genotypic markers are most closely associated with the disease phenotype; it could be that the defect is due to either S4 or S5, or neither.

The probability of having a child with an autosomal recessive disease is the same for all pregnancies, 25%, regardless of the number of children already born with or without the disease.

4. (2 pts.) Based on the data in part c of the figure, what is the molecular basis for the disease? How would these data explain the observation that heterozygous individuals are clinically normal?

The best explanation for the disease is that complete loss of ES2 gene expression, presumably due to promoter mutations, is the cause of the disease due to absence of the enzyme.

Heterozygous individuals would be normal because the level of gene expression from one allele is 50% of normal and apparently sufficient to produce enough mRNA to direct the synthesis of the required metabolic enzyme.

5. (2 pts.) Based on the data presented in the second lab practicum in Lecture 8 ("Mapping gene regulatory sequences"), which restriction enzyme segment contains the basal promoter activity required for transcription? Which restriction enzyme segment contains an "enhancer" function that stimulates promoter activity? Explain.

The basal promoter activity is encoded by the ClaI-PstI genomic fragment because this fragment directs a reasonable amount of luciferase activity (~500 units), whereas, deletions from the ClaI site towards the PstI site significantly reduce activity.

The enhancer function in this system is encoded by the KpnI-SmaI fragment because fusion of this fragment to the basal promoter (ClaI-PstI genomic fragment) is sufficient for full gene transcription

Name _____ **KEY**

activity. Enhancer functions are DNA sequences that serve as specific DNA binding sites for regulatory transcription factors.