[redacted]

17/20 2005 Midterm. 34/40

1. Given the following DNA sequence;

5' AAACATAATCCGCCCTTAACATATCAGAAGCCAGACATTAATGACCCTACGCCTGCTGG 3'

(2 marks)

a) Design the 10-mer primers that could be used to amplify the sequence using PCR.

Primer for Sense Strand → 5' CCAGCAGGCG 3'

Primer for Anti-Sense Strand → 5' AAACATAATC 3'

(2 marks)

b) What is the difference between the two primers that may make PCR problematic and what would you do to cope with this problem?

The primer for the sense strand has a higher GC content than that for the anti-sense strand. This means the sense strand primer will require a higher annealing temperature vs. the other primer at which to complimentarily anneal to the template strand. To overcome this problem, you may want to make the anti-sense strand primer longer so that it to will ~~require~~ a higher annealing temp or choose a annealing temp that is midway b/w the annealing temps.

2. The following DNA sequence is a part of a gene in a plasmid that you would like to mutate and study (assume this sequence is in frame).

mutate

TTA|GCA|GCG|ATA|AGC|TTT|AAT|GCG|GTA|G

Leu Val Ala Ile Ser Phe Asn Ala Val

(2 marks)

a) Design the most appropriate 11mer oligo-nucleotide to mutate the Ser in the sequence to an Arg

ATA|AGA|TTT|AA

(1 mark)

b) Does your mutation change any restriction sites on the gene?

Yes ~~HW~~ Hind III can no longer cut at this sequence

7

[redacted] Pst I → CTGCA↓G

2005

Midterm

3. Given the following fragment of DNA: Total Bp = 49

TTCGGCGTGACTGCA↓GAAGATGCCTGCA↓GAGAGATCCGATTAGACCTAC

15 bp (A) 13 bp (B) 21 Bp (C)

(3 marks)

a) Digest the DNA fragment with PstI and run the digestion product on an agarose gel. Give the nucleotide sequence of the fragments and number the fragments in the order they would appear from the top to bottom of the gel.

[illegible] ←

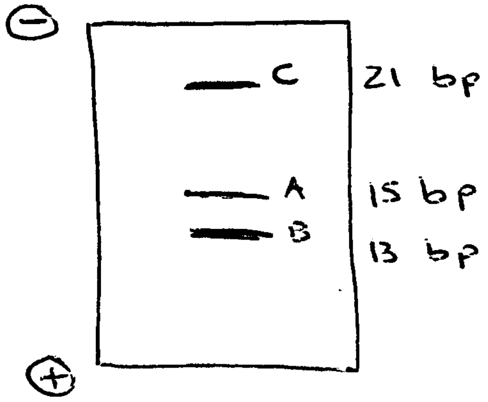
letter

Fragment # A → 5' TTCGGCGTGACTGCA 3' 15 BP long.

[Fragment # B → 5' GAAGATGCCTGCA 3' 13BP long]

Fragment # C → 5' GAGAGATCCGATTAGACCTAC 21 BP long

when run on a Gel.



order of fragments from top to bottom of gel : C, A, B.

2½

arks)

b) Draw a representation of the sequencing gel that would arise from the dideoxy sequencing of the second largest fragment (use a tetranucleotide as the 5' - labeled primer). Number the bands on your gel diagram sequentially from the largest to the smallest DNA fragment.

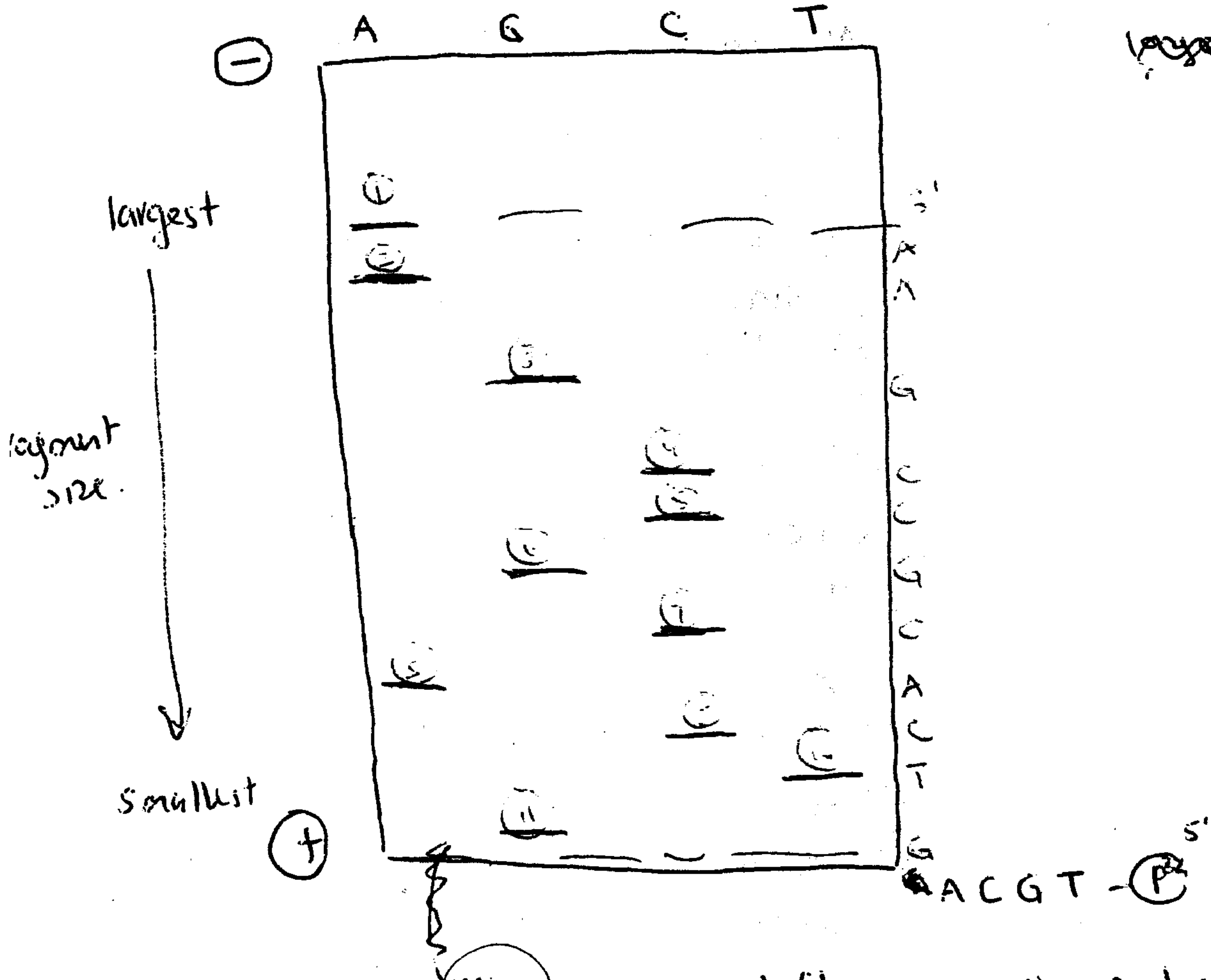
2nd largest fragment → ~~TTCGGCGCGTGACTGA~~

5' TTCGGCGTGACTGCA 3'

Primer → 5' TGCA 3'

5' (P)32-TGCAGTCACGCCGAA 3'

↑ order ~~[illegible]~~ synthesized via Sanger.



Note: (may) see some left over primer bands at bottom in each well ... not likely b/c probably all used up in rxn.

(4/4)

marks)

4. You have a patient who is producing abnormally low quantities of a certain protein. The gene for this protein is normal but you have found another gene in your patient that binds to DNA and has the partial sequence shown below and compared to a normal individual. Position 334 is located in an α-helix of the homeodomain (a DNA binding domain) of this protein. Analysis of the structures of other proteins of this family shows that position 334 makes contact with the DNA backbone in the major groove of the double helix. Speculate on how this defect may account for the disease.

*protein likely an enhancer of DNA synthesis for a gene for the protein that is abn. prod.)

				*		
Codon Number	331	332	333	334	335	336
				lys		
Normal	CAA	AAA	GAG	AAA	AGA	ATG
Patient	CAA	AAA	GAG	GAA	AGA	ATG
				glu.		

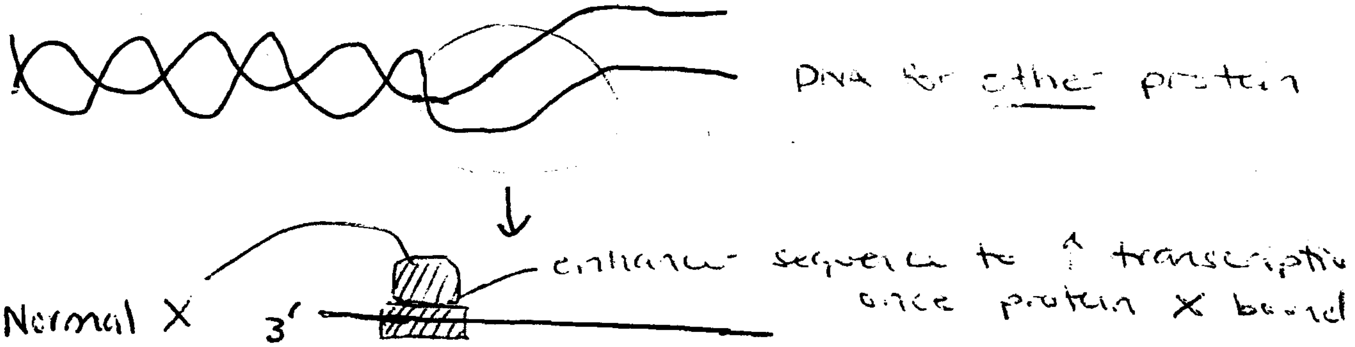
The patients gene for the DNA-Binding Protein (we'll call it X) contains a mutation at codon 334 such that the amino acid glutamine is coded for instead of the amino acid lysine at this position in the protein. Since the patient is producing abnormally low quantities of the other protein as compared to a person who does not have this mutation, it can be said that this mutation in Gene X is responsible (ie. the mutation does have a negative effect on protein X function. it is not a silent mutation).

Since we know that protein X is a DNA-Binding Protein ~~that~~ whose DNA-Binding Domain is found at position 334, we can speculate that changing the amino acid at this position results in protein that can no longer bind to the DNA sequence of the other protein. Normally, when protein X binds to the gene for the other protein (at a specific DNA ~~transcription~~ enhancer region), it would enhance or increase the transcription of that gene ~~so~~ that the protein would be produced in increased amount. However, (over

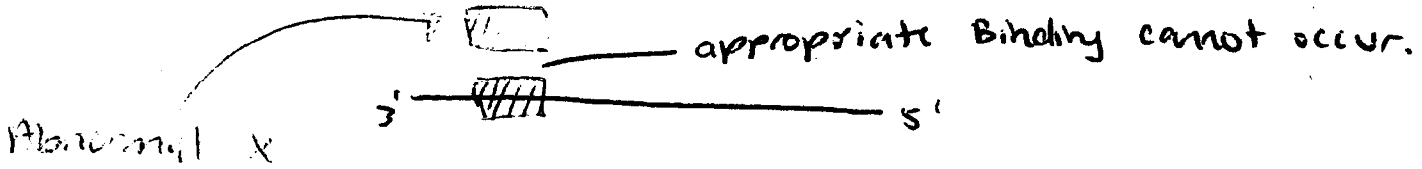
2

Since the amino acid glutamine has substituted the amino acid lysine in the DNA-Binding Domain the conformation or properties of the bind-site of the protein has changed such that the DNA-Binding Domain can no longer bind to the specific DNA sequence on the other protein's gene that would enhance it's transcription.

The conformational change in the DNA-Binding domain of protein X can be attributed to the differences in the sidechains of the amino acids that has been substituted. The lysine must have been an integral role in contributing to appropriate folding or the charge of the active sit of the protein so that the DNA-Binding Domain of the protein would function. The glutamine, whose side chain properties are different from the lysine, cannot contribute to the ~~DNA~~ bind site structure and charge in the same way ∴ ineffective DNA Binding.



↳ interacts to ↑ transcription.



↳ cannot interact w/ the enhancer sequence so transcription of gene not enhanced.