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1. Dr. Oliver Smithies is a British-born American geneticist and Nobel laureate who developed electrophoresis in 1995 and co-discovered homologous recombination.

a). Please describe the principles and applications of the several electrophoresis methods such as SDS-PAGE, IEF electrophoresis and so on. You could make a table to compare these approaches (15%).

b). Please describe the homologous recombination and illustrated how transgenic and knock out mice is generated? (10%) How to make tissue/organ specific genetic knock out animal? (5%)

2. Please answer the following questions:

a). Transcription – (5%)

i. Transcription is the process that transfers information from _____ to _____.

ii. In eukaryotic organisms, transcription occurs in the:

a. Nucleus b. Ribosome c. Membrane

b). Translation – (5%)

i. Translation is the process that transfers information from _____ to _____.

ii. In eukaryotic organisms, translation occurs in the:

a. Nucleus b. Ribosome c. Membrane

The following DNA sequence encodes a hypothetical polypeptide called IOM in the hypothetical bacteria *E. Unknown*. Transcription starts at and includes the C/G base pair in bold letter. The underlined T/A base pair indicates the terminator.

5'-TTCCCCTATGGATGGTCATCTACGATGCCCCCATCACTAAAGCTTG-3'
3'-AAGGGGATACCTACCAGTAGATGCTACGGGGGTAGTGATTTCGAAC-5'

c). What are the first 7 bases of the transcribed RNA? Please also label the 5' and 3' ends. (5%)

d). What are the first 4 amino acids of the subsequent polypeptide? Make sure you have labeled the N- and C- termini. (5%)

e). How many total amino acids are encoded in this polypeptide? (5%)

(背面仍有題目,請繼續作答)

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You identify a strain of bacteria containing a mutant tRNA that enabled it to add a tryptophan residue when it recognizes the codon UAG in the mRNA.

f). What is the sequence of the anticodon of the mutant tRNA? Please make sure you have labeled the 5' and 3' ends. (5%)

g). In the presence of the mutant tRNA, the IOM polypeptide would be: (2%)

a. the same length
b. shorter
c. longer

Why?(3%)

3. Which of the following could change gene regulation (select all that apply) (5%)

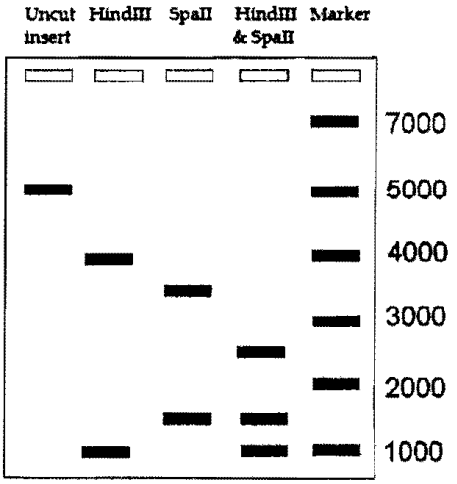
a). Deleting a promoter

b). Moving a yeast culture to a new food source

c). Raising the temperature of a bacterial culture

d). Mutating a repressor gene, such that the resulting protein no longer functions

4. As you would like to create the genomic library (starting with an EcoRI digesting of the DNA), and identify one vector that contains your X-gene. Now you decide to analyze this vector further and therefore cut the vector with EcoRI followed by purification of the genomic insert. Then the insert products were digested with 2 different restriction enzymes SpaII and HindIII. You obtain the following results after electrophoresis:



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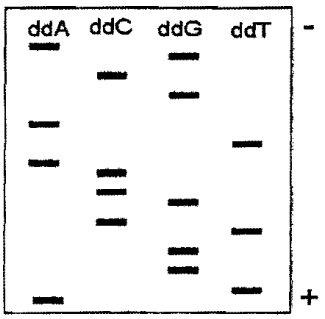
a). Draw a map of the genomic insert indicating restriction sites for the enzymes SpaII and HindIII. EcoRI sites are already shown for you. Be sure to include distances. (10%)

I.....I
EcoRI EcoRI

b). You thus perform a sequencing of the DNA fragment and separate on a gel. Part of the gel image was illustrtaed as follow:



What is the sequence represented on the gel? Be sure to indicate the 5′ and 3′ ends. (10%)

After sequencing the X-gene obtained from your genomic library, you find a 32 base pair insert in the middle of it that does not correspond to the mRNA for the X protein. What is it? (5%)

If you want to express the X-gene in *E. coli.*, given the above results, would you want to use genomic or cDNA library for this experiment? Why? (5%)

參考資料：

The Genetic Code

	U	C	A	G	
U	UUU phe	UCU ser	UAU tyr	UGU cys	U
	UUC phe	UCC ser	UAC tyr	UGC cys	C
	UUA leu	UCA ser	UAA STOP	UGA STOP	A
	UUG leu	UCG ser	UAG STOP	UGG trp	G
C	CUU leu	CCU pro	CAU his	CGU arg	U
	CUC leu	CCC pro	CAC his	CGC arg	C
	CUA leu	CCA pro	CAA gln	CGA arg	A
	CUG leu	CCG pro	CAG gln	CGG arg	G
A	AUU ile	ACU thr	AAU asn	AGU ser	U
	AUC ile	ACC thr	AAC asn	AGC ser	C
	AUA ile	ACA thr	AAA lys	AGA arg	A
	AUG met	ACG thr	AAG lys	AGG arg	G
G	GUU val	GCU ala	GAU asp	GGU gly	U
	GUC val	GCC ala	GAC asp	GGC gly	C
	GUA val	GCA ala	GAA glu	GGA gly	A
	GUG val	GCG ala	GAG glu	GGG gly	G