Major Exam

BBL231 (Molecular Biology and Genetics)

MM: 35 January 8th, 2021

Duration: 1.5h

Q. 1 4

a) An *E. coli* gene was found to be 70 nm long. What is the maximum number of amino acids that this gene could encode? How many nucleotides in mRNA are required to encode this gene?

b) The sequence of the double stranded DNA is given below:

5′-CAATCATGGACTGCCATGCTTCATATGAATAGTTGACAT-3′
3′-GTTAGTACCTGACGGTACGAAGTATACTTATCAACTGTA-5′

Write the ribonucleotide sequence of the mRNA molecule that is transcribed from the template strand of this DNA.

Q. 2 5

a) For sequencing the *E. coli* genome by sangers sequencing, which one will you choose between shot gun sequencing and primer walking and why?

b) A polypeptide consists of three amino acids, X—Y—Z. Another polypeptide contains segments X and Z but not segment Y. How will you determine if these two polypeptides are produced by translating spliced versions of RNA from a single gene or by translating mRNA from two different genes?

Q. 3 6

Catabolic operons are inducible and anabolic operons are repressible. Justify.

Explain what will happen if a mutation results in inactivation of the following genes or sites in the *E. coli* lactose operon:

(a) regulator, (b) operator, (c) promoter, (d) structural gene *Z*, and (e) structural gene *Y*

Will attenuation of the type that regulates the level of trp transcripts in *E. coli* be likely to occur in eukaryotic organisms? Explain.

Q. 4 Human proteins can now be produced in *E. coli*. Write all the steps beginning from isolating a gene followed by expression of human growth hormone in *E. coli*. 3

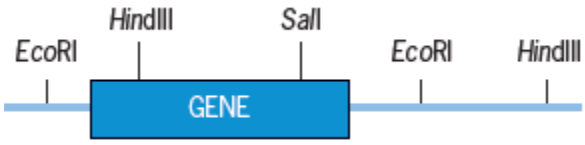
Q. 5 You are studying a circular plasmid DNA molecule of size 10.5 kilobase pairs (kb). When you digest this plasmid with restriction endonucleases *Bam*HI, *Eco*RI, and *Hin*dIII, singly and in all possible combinations, you obtain linear restriction fragments of the following sizes: 3

Enzymes Fragment Sizes (in kb)
*Bam*HI 7.3, 3.2
*Eco*RI 10.5
*Hin*dIII 5.1, 3.4, 2.0
*Bam*HI + *Eco*RI 6.7, 3.2, 0.6
*Bam*HI + *Hin*dIII 4.6, 2.7, 2.0, 0.7, 0.5
*Eco*RI + *Hin*dIII 4.0, 3.4, 2.0, 1.1
*Bam*HI + *Eco*RI + *Hin*dIII 4.0, 2.7, 2.0, 0.7, 0.6, 0.5

Draw a restriction map for the plasmid that fits your data.

Q. 6 5

You are working as part of a research team studying the structure and function of a particular gene. Your job is to clone the gene. A restriction map is available for the region of the chromosome in which the gene is located; the map is shown below. The vector contains unique sites for all these enzymes shown in the map



The sequence is also shown below where the restriction sites are italicized
GACG***GAATTC***GCCAATACCCTTGACAGTTTATGTAAAAGGCCTATAATGTAGAGGAGGTCGCATGC
TTGATCAGCCTTCAGTTGTA***AAGCTT***GCTATTCGCCAAGATCGTGTGGGTTCTGCAAAGAGTGTTGC
TGCTGTTGGTCACGCAGCAAAACAGGTGCCAGGTCGTACACCTGGTAATATTTCAGCG***GTCGAC***AT
TCGCCCAGAATGAGCACTACGGGGGATTACCCCCTAAAAAAAAAAAAATTTGATCATTGA***GAATTC***
CGAGCTAATCAAGCAGGATATACGTTGAACAACCG***AAGCTT***CAGCAG.
Identify the -10, -35, spacer, Ribosome binding site, initiation codon, stop codon, transcriptional terminator in the sequence above. Design PCR primers for cloning this gene for expression. Calculate the melting temperature of the primers and write the PCR program cycle. Write the name of the polymerase that you will use for PCR. Write the restriction sites that you will select for cloning and give justification.

Q. 7 3

(a) Why it is common to observe plasmid multimers in case of rolling circle replicating plasmids? Why DNA primase activity is not required to initiate rolling-circle replication? At which step is it required and why?
b) The *polA* mutants of *E. coli* lack the 3′ → 5′ exonuclease activity of DNA polymerase I. Will the rate of DNA synthesis be altered in these mutants? What effect(s) will these *polA* mutations have on the phenotype of the organism?

Q. 8 6

a) In the environment, a strain of genotype *met bio*$^+$ is present and a strain of genotype *met*$^+$ *bio*, some recombinant genotypes, *met*$^+$ *bio*$^+$ and *met bio*, are formed. How would you determine whether the observed recombination resulted from transformation, conjugation, or transduction?
b) What is cotransduction? How can cotransduction frequencies be used to map genetic markers?
c) Below are the results of an interrupted mating experiment. Draw the map of the *E. coli* chromosome showing these markers
Hfr Strain Markers Donated in order
1 —Z–H–E–R→
2 —O–K–S–R→
3 —K–O–W–I→
4 —Z–T–I–W→
5 —H–Z–T–I→