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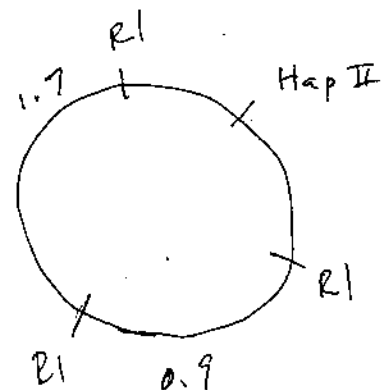
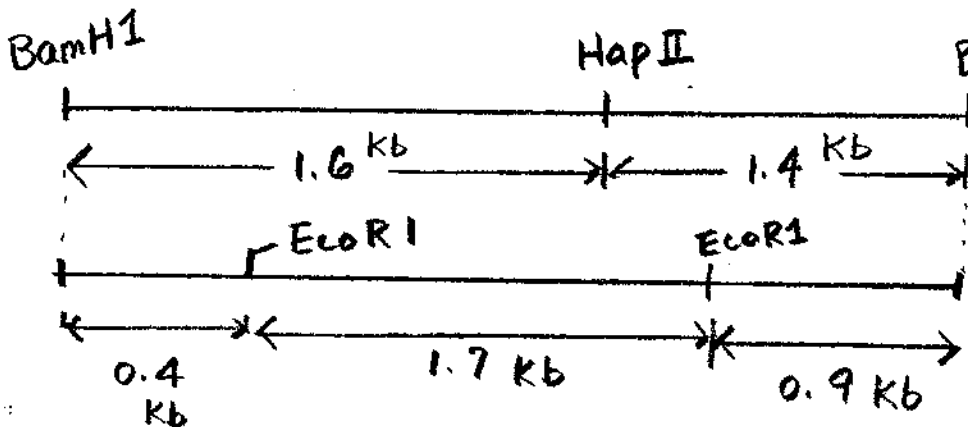
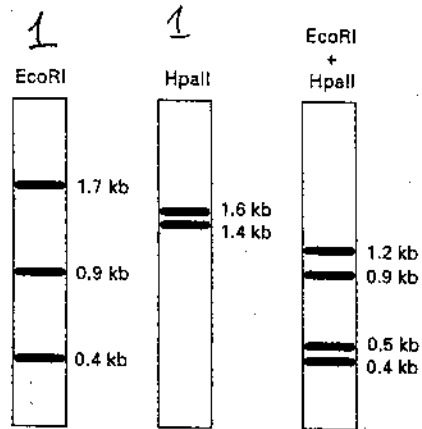
E. Wobble pairing occurs between the first position in the anti-codon and the third position in the codon. Is this correct or false? If false, correct the mistakes. (3)

correct

F. Describe steps require energy during translation elongation. (3)

- conformational change of ribosome upon binding to charged tRNA (aa-tRNA) at A site
- Translocation of ribosome after peptide bond formation (EF2 GTP → EF2 GDP)

2. You wish to make a restriction map of a 3.0kb BamHI restriction fragment. You digest three samples of the fragment with EcoRI, HpaII and a mixture of EcoRI and HpaII. You then separate the fragments by gel electrophoresis and visualize the DNA bands. From these results, prepare positions of the EcoRI and HpaII recognition sites and the distances in kilobases (kb) between them. (8)



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3. One strand of a section of DNA isolated from *E. coli* reads:  
5' GTAGCCTACCCATAGG 3'

- a. Suppose that an mRNA is transcribed from this DNA using the complementary strand as a template. What will the sequence of the mRNA in this region be? (3)

5' GUAGCCUACCCAUAGG 3'

- b. How many different peptides could potentially be made from this sequence of RNA? Would the same peptides be made if the other strand of the DNA served as the template for transcription? (4)

3 different peptides

no.

4. What are the four major types of RNA in a cell? What functions do they serve in the cell? (12)

tRNA — <sup>an appropriate</sup> brings amino acid to ribosome during translation  
based on codon/anti-codon

rRNA — component of ribosome,  
carry out peptide bond formation.

mRNA — codes for protein

micro RNA — known functions include

decrease in specific transcript level  
(mRNA)

" in translation of specific mRNA  
(target)

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5. (22 points)

a. Describe two features of mRNA which enhance translation. (3)

poly A tail

Cap

b. Which DNA sequence would be more difficult to denature? And explain why. (4)

- i. 5' AGCTTTACCCGGGGTAAGG 3' C/G 11  
 ii. 5' GTTAAAACGGCATATAAT 3' 5  
 iii. 5' GCGCAATTGAT 3' 5

(i) contains more GC rich sequence than (ii)  
 and longer than (iii)

Thus,  $T_m$  of (i) is the highest.

c. Describe one critical element that differentiates shuttling vector from other cloning vectors and explain why. (6)

two origins of replication for replicating  
 (ex. yeast and E. coli)  
 in two different organisms.

d. Describe three different methods used to identify the function of a gene and provide one sentence description of the techniques. (9)

- Knockout — Disruption of the gene of interest in embryonic stem cells to make K/O mouse
- RNAi — Disruption of mRNA coding for the gene of interest by expressing miRNA or siRNA (or antisense RNA)
- Transgenic mouse
- Sequence comparison (phylogenetic gene family)

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6. 5' A<sub>1</sub> A<sub>2</sub> G<sub>3</sub> C<sub>4</sub> G<sub>5</sub> A<sub>6</sub> T<sub>7</sub> C<sub>8</sub> C<sub>9</sub> C<sub>10</sub> A<sub>11</sub> C<sub>12</sub> 3' (6)

i. Which nucleotide has a triphosphate group?

A<sub>1</sub>

ii. How many phosphodiester bonds are present in the strand shown?

eleven

iii. Which triphosphate of the nucleotide formed a phosphodiester bond with a 3'-OH group of C<sub>4</sub>

G<sub>5</sub>

7. What is Okazaki fragments and describe Okazaki fragments joining process. (Including enzymes carry out each steps.) (8)

not necessary → (Discontinuous DNA segments synthesized on the lagging strand DNA during DNA replication  
 • Multiple RNA primers are made by primase complementary to the lagging strand DNA  
 → elongation of these primer by a DNA polymerase  
 → {The RNA primer removal} → fill in the gap by DNA pol. δ  
 by RNase H and FEN I

8. What is operon? Describe advantage and disadvantage of operon. (6)

- Several protein-coding <sup>prokaryotic</sup> genes, commonly involved in related functions clustered into a functional region.

and ligation of Okazaki fragments by DNA ligase

- Advantage: Assures production of Multiple proteins involved in related functions

- Disadvantage: If deleterious mutation(s) occurs, possible to lose capacity to perform specific functions as several gene products will be lost simultaneously.

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9. Explain role(s) of the following proteins. (9)

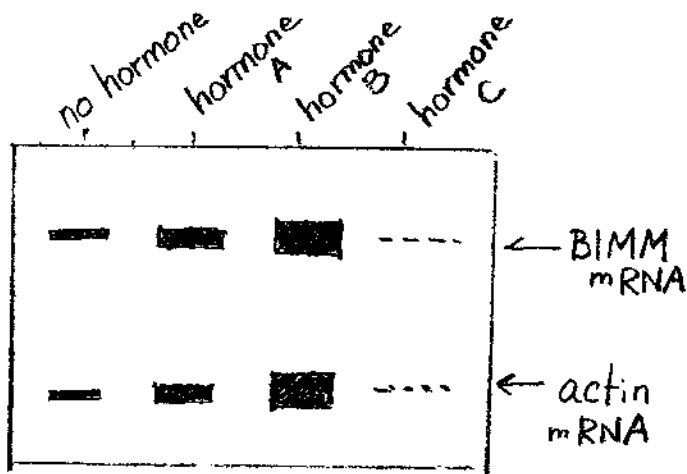
- A. Topoisomerase      release of torsional stress caused by unwinding of duplex DNA during DNA replication
- B. Taq polymerase      DNA polymerase that can withstand high temp. Used for PCR rxn.
- C. Aminoacyl-tRNA synthetase — couples a specific amino acid to the corresponding tRNA

10. Describe why you need two selectable marker genes for gene replacement (knockout) construct for mouse ES cells. (3)

because efficiency of homologous recombination in mammalian cells is very low, the construct needs both positive selection marker for <sup>scoring</sup> integration and negative selection marker genes for eliminating random

11. Your friend performed a northern blot experiment to examine if incubation with specific three different hormones altered expression of BIMM mRNA. The northern blot was also probed at the same time with actin, which does not change with any hormones. Can you choose which one of the hormones is most effective for increase in expression of BIMM RNA? and explain why. (5)

insertion of the K/O construct



You cannot choose specific hormones since actin mRNA level changes and based on the level of actin, all three hormone seem to work similarly.