

### The Exam:

FRIDAY, February 28, 2003, 9:30-10:55am, Walker Memorial Open notes, but NO old test/bible material and no books. BRING a calculator.

### **Readings for the Exam:**

41-53, 73-74, 77-97, 117-138, 750-773, 781-804, 813-839

## Do the PSETS!

### **Break Down Your Studying:**

Proteins and Amino Acids Nucleic Acids Protein Purification DNA Replication Transcription Translation \*\* Use the key terms handed out at last nights review session! Make sure you know what they all are.

## QUESTION 1

- A. What is meant by saying the Genetic code is non-overlapping, has no commas, and is degenerate?
- B. What affect does this have on the size of the genetic code?
- C. Why don't we use a punctuated coding method?
- D. Why does the amount of radiolabeled protein product level off in an in vitro transcription extract after adding DNAse? Why does adding RNA rescue this activity?
- E. Consider the DNA fragment CAGCAGCAGCAG. In order to proceed with the Khorana method of RNA synthesis, what DNA oligo(s) would you need?
- F. Considering part C, what protein products would you expect to obtain using these fragments in the Khorana approach?

## QUESTION 2 (Gumport 27-12)

Suppose that a bacterial mutant is found to replicate its DNA at a very low rate. Upon analysis, it is found to have normal levels of activity of DNA Pol I & III, DNA Gyrase (Helicase), and DNA Ligase. It also makes normal amounts of the WT SSBs used to keep the strands apart. The sequence of the oriC region of its chromosome is found to wild type. What two defects might account for the abnormally low rate of DNA replication in this mutatant? Explain briefly.



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## QUESTION 3 (Exam 1, 2001)

Consider the use of isoelectric focusing to separate the three polypeptides, A, B, and C.

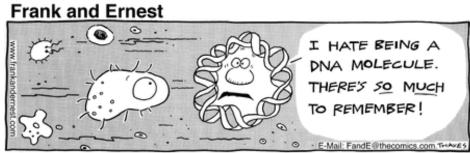
- A = Ala-Gly-His-Pro-Gln-Thr-Val
- B = Ile-Leu-Cys-Tyr-Asp-Glu-Ala
- C = Lys-Arg-Pro
- A. Write the sequence of these three polypeptides using the one-letter symbols
- B. Predict which residues of these polypeptides will be predominantly charged when these polypeptides are focused at their respective isolelectric points circle these residues in the three sequences that you have written in part (a). For the residues that you circled, indicate whether the charge would be positive or negative.
- C. Sketch a simple diagram of the separation of these three polypeptides by isoelectric focusing. Indicate which part of the gel (or paper) is high pH, and which is low pH. Indicate the polarity of the electric field. Indicate the relative positions of the three polypeptides. Please do <u>not</u> calculate the precise pl's of these polypeptides, show only their positions relative to the pH gradient, the electric field and each other.
- D. Draw the chemical structure of the predominant species of peptide C when it is focused at its iso electric point.

### **QUESTION 4**

(Removed for being inaccurate, my apologies)

## **QUESTION 5**

- A. A mutant tRNA<sup>Ala</sup> synthatase can load both Glycine and Alanine. Is this disastrous? What about a tRNA<sup>Leu</sup> synthatase can load both Leucine and Asparagine.
- B. If we radiolabel all of the oxygen atoms in the Asparagine with heavy isotopes, what would be the fate of all the oxygen atoms? Where would they reside? Draw structures indicating this!
- C. You decide you really like labeling things and radiolabel the phosphates on ATP. You add them into your cell free translation system. Where do you expect all of the radioactivity to be once you filter?
- D. You add G<sub>s</sub>TP to your cell free system instead of GTP. G<sub>s</sub>TP is a GTP analogue which hydrolyzes very slowly. What advantages and disadvantages does this have? What if we use G<sub>f</sub>TP, which is a GTP analogue that hydrolyzes very fast?



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