

ABE Test III  
200 points  
Open book and open Notes  
April 23, 2004

Reading knowledge (40 points)

1. PCR denotes (check one). (50 points)

- polymerase chain reaction
- a type of protein assay
- an optical detection method

cDNA is obtained by

- Eco I
- reverse transcription of mRNA
- reverse transcription of DNA

Both PCR and reverse transcription require

- a primer
- low pH
- low temperatures
- dideoxy nucleotides

One of the following enzymes is a Type II restriction enzyme that gives blunt ends

- Taq I
- Hind II
- Hind III

The following is an example of a selectable marker

- pBR 322
- Ampicillin resistance
- Pvu II

Western blot refers to technique for determining size of fragments of

- DNA
- Protein
- Polysaccharides

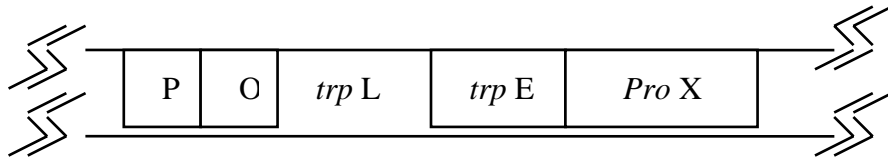
Eco RI denotes a

- restriction enzyme
- reverse transcriptase
- Ligase

A plasmid is

- chromosomal DNA
- circular DNA
- an inclusion body

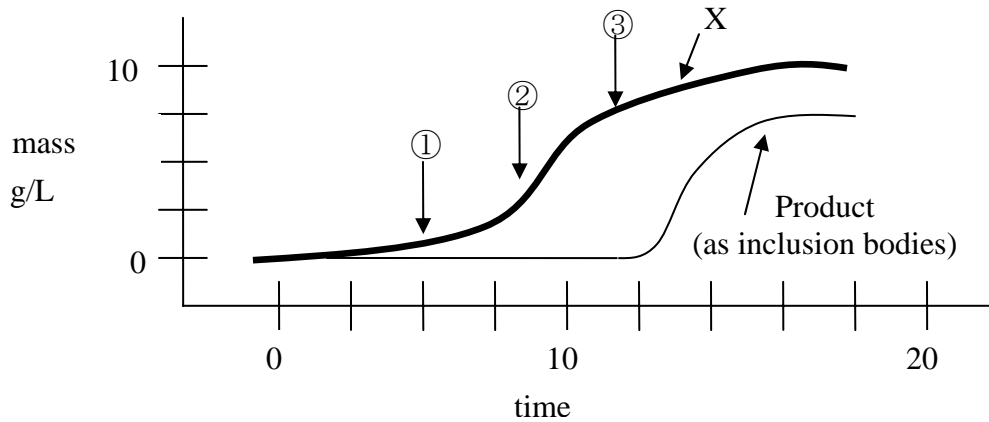
2. A gene that codes for a valuable protein is engineered into a plasmid consisting of regulatory regions and the *trp E* gene of the *trp* operon. The *trp* operon, when turned on, initiates a sequence of events that ultimately leads to the biosynthesis of tryptophan. The “hybrid” operon consists of:



This operon, when recombined with the plasmid is introduced into *E. coli*.

- (a) The transformed *E. coli* is grown in a medium containing tryptophan, but protein corresponding to the *Pro X* gene is *not* formed. Explain. (20 points)
- (b) At the end of the fermentation, the tryptophan is depleted from the medium, and a protein containing a sequence of amino acids corresponding to *trp E* and protein X is detected. Explain. (20 points)

3. The growth of *E. coli* that has been transformed with the Trp E operon coupled to proinsulin is carried out in a batch fermentation. At the end of the fermentation tryptophan is cut off in the media and product is formed in inclusion bodies that quickly accumulate, killing the cells. (40 points)



Answer the following questions

1. The tryptophan is cut off at point

\_\_\_\_\_ ①  
 \_\_\_\_\_ ②  
 \_\_\_\_\_ ③

2. When the tryptophan is cut off, the cell begins to manufacture pro-insulin at point

\_\_\_\_\_ ①  
 \_\_\_\_\_ ②  
 \_\_\_\_\_ ③

3. The cell forms an inclusion body that contains primarily

\_\_\_\_\_ DNA  
 \_\_\_\_\_ pro-insulin  
 \_\_\_\_\_ lipids

4. The product accumulates because

\_\_\_\_\_ the cells are near stationary phase  
 \_\_\_\_\_ the cells have reached a critical cell count  
 \_\_\_\_\_ a change in fermentation condition has induced its production

5. The product of the fermentation is

\_\_\_\_\_ intracellular  
 \_\_\_\_\_ extracellular

4. A size exclusion chromatogram is given in Figure 1 for a three component mixture. The components are:

- (a) \_\_\_\_\_ Blue Dextran (mw = 2,000 kD)  
\_\_\_\_\_ NaCl (mw = 0.06 kD)  
\_\_\_\_\_ BSA (mw = 66 kD)

Fill in the corresponding peak numbers (①, ② or ③) in the spaces given above.

(b) Separation of these compounds is likely:

- a. rate controlled
- b. diffusion controlled
- c. convection controlled
- d. charge controlled

(c) The same three component mixture is injected into a different size exclusion chromatography column with pores that exclude molecules larger than 50 kD. Please sketch below what you would expect the elution results to be. Please label the peaks.

5. A recombinant protein product is generated by a transformed bacterium, and is accumulated inside the cell after an inducer is added. After fermentation, the intracellular product must be recovered in an active conformation and purified from reagents that were used during the fermentation, and any subsequent recovery steps. (40 points)

The product is to be purified by the following separation methods.

- \_\_\_\_\_ size exclusion LC
- \_\_\_\_\_ centrifugation
- \_\_\_\_\_ crystallization
- \_\_\_\_\_ diafiltration
- \_\_\_\_\_ cell lysis

- (a) Which step is likely to come first (mark with a "1" above)?
- (b) Organize these unit operations into a probable sequence of steps.

- (c) If there is a 3% yield loss at each step, give the overall anticipated yield loss for the sequence that you show in step b of this problem.