Name________________*KEY*__________

Bioc471a/571a Homework 2 - Due at the start of class September 4

1. (2 pts) Explain the rationale behind comparing the amino acid sequences of trypsin genes from humans and Drosophila, to that of the Drosophila chymotrypsin gene, in order to design an oligonucleotide probe for screening a mosquito cDNA library. This would be an alternative approach to that of using a Southern blot to identify a DNA fragment to use as a probe as outlined in the Lab Practicum we discussed in class.

The best oligonucleotide probe for this approach will be one (or a pool of probes) that cover the most highly conserved region as this will likely also be conserved in the mosquito genome. By using both paralogous and orthologous gene sequences in the comparison, it increases the chances of finding important regions. In this case, sequences around the catalytic serine (Ser195) are the most conserved.

2. (2 pts.) Using the known cDNA sequence of the mosquito trypsin gene shown in the notes for lecture 3, write the sequence of the BEST possible Drosophila-based oligonucleotide probe of 24 nucleotides that could have been designed (hindsight is always 20-20). Write the sequence in the conventional 5' to 3' direction.

The highest degree of similarity between the fly and mosquito trypsin cDNA sequences would be an oligonucleotide that has only three mismatches out of 24 (87.5% identical):

5' - TGCCAGGGTGACTCCGGTGGCCCA - 3'

3. (2 pts.) Explain why the E. coli K-12 strain used in recombinant DNA methods is not a dangerous pathogen, even though it is a close cousin of the highly virulent E. coli strain 0157:H7.

The E.coli K-12 strain is non-pathogenic, primarily because it does not produce toxin proteins like E. coli strain 0157:H7. Other differences include the inability of E. coli K-12 to live outside of the lab because of genetic deficiencies in host defenses (restriction systems), DNA repair and dependence on nutrient rich culture media.

4. (2 pts.) The E. coli lacZ gene is often used in molecular genetic applications as a marker of cells that are able to activate a cell-specific gene promoter, e.g., the Nodal gene promoter-lacZ fusion gene is expressed in a limited set of mouse heart muscle cells as shown in lecture 4. What protein function is encoded by the lacZ gene and what is it about this protein that makes it a good cell marker in higher eukaryotes (hint: the Drosophila trypsin gene would NOT make a good marker gene for the opposite reason).

The E. coli lacZ gene encodes the enzyme beta-galactosidase which catalyzes the cleavage of lactose into galactose and glucose (side reaction converts lactose to allolactose at a low level). This gene is a good marker in molecular genetic methods because beta-galactosidase activity is NOT found in most cell types (some plants have a similar enzyme) and therefore the lacZ gene product (beta-galactosidase) can be easily identified. Another reason why it is a good reporter gene is that a chromogenic substrate (X-gal) has been developed (although a similar type of trypsin substrate could also be developed).

5. (2 pts.) The enzyme alkaline phosphatase removes the phosphate group from the 5' end of DNA strands. The enzyme ligase can only ligate together DNA strands that contain a 3' OH group and a 5' phosphate group. Explain how dephosphorylation of enzyme digested plasmid vector sequences with alkaline phosphatase reduces the number of colonies containing plasmids without inserts, but does not eliminate the recovery of recombinant plasmids? (hint: something happens inside the E. coli cell using host enzymes).

The in vitro ligation in this case will covalently link together both DNA strands but only at two of the four "nicks" (the two nicks containing a 5' phosphate from insert with 3' hydroxyl from vector). This presumably works because the recombinant molecule is repaired by the host enzymes (DNA repair enzymes). Although this strategy is often used, the latest edition of "Molecular Cloning" suggests that it doesn't work as well as you might think, most likely due to loss of recombinant molecules that are degraded in E. coli (i.e., not repaired). Unfortunately, blue-white screening is not always an option.