

**Bioc471a/571a Homework 3 - Due at the start of class September 11**

1. (2 pts) What does it mean to "titer a phage stock"? If your objective is to purify as much phage DNA as possible, why does it matter how much phage stock you add to the bacterial liquid culture, doesn't it all just mix around and lead to 100% infection eventually?

**Titering a phage stock refers to determining the number of infectious particles per volume of stock, usually in the range of  $10^9$  to  $10^{12}$  particles/ml. This is done by counting the number of plaques produced from a dilution of the phage stock, assuming that one plaque=one infectious particle.**

**You do not want to add so much phage that all of the cells get infected in the first round of infection, otherwise the total yield will be low. It is a much better idea to optimize the ratio of phage to bacterial cells so that the infection progresses during the incubation time. In this way the number of host cells will be increasing during the early rounds so that later rounds will have enough host cells to support viral production.**

2. (2 pts.) What two genetic strategies have been developed to increase the percent of phage particles containing recombinant molecules? Are these genetic selections or a genetic screens? Explain.

**One strategy involves disruption of the  $\lambda$  CI gene which has the net result of inhibiting lysogeny and favoring the lysis pathway by phage containing recombinant genomes. The second involves replacement of the red/gam genes with genomic DNA such that the lysis pathway is favored.**

**These two genetic strategies would be considered genetic selections because only one of the possible outcomes (lysis) produces viable progeny (infectious particles).**

3. (2 pts.) Briefly describe the key components of the pET bacterial expression system. Using  $^{35}\text{S}$ -methionine labeling media (contains radioactive methionine as the source of this amino acid), it has been observed that within 4 hours of adding IPTG to the culture, over 90% of the newly synthesized proteins in cell extracts are derived from the insert coding sequence in the pET vector. What explains the dramatic decrease in the synthesis of E. coli proteins during the induction period?

**The key components of the pET expression system are 1) the pET plasmid expression vector containing the T7 RNA polymerase promoter recognition sequence upstream of the recombinant gene coding sequence, 2) cells that express the T7 RNA polymerase from a lac operon in the E. coli genome (BL21 cells), and 3) T7 lysozyme expression plasmid (pLys) making lysozyme which inhibits T7 RNA polymerase that would otherwise lead to low level expression of the recombinant protein (lac repressor is not a perfect off switch). Note that the pET expression plasmid contains a ColE1 origin of replication which is compatible with the p15A origin contained on the pLys plasmid.**

**The dramatic decrease in E. coli protein synthesis is due to the high efficiency and specificity of the T7 RNA polymerase in exclusive transcription of the pET expression cassette. The rNTP pool, ribosomes and amino acids are all directed toward protein synthesis from the pET insert representing the most abundant mRNA in the cell. Because of this shift away from E. coli gene expression, the pET gene induction and synthesis of recombinant protein peaks at about 6 hours due to depletion of host factors required to maintain pET expression.**

4. (2 pts.) What are two explanations for a recombinant genomic phage that repeatedly hybridizes to your probe with only a faint signal throughout the plaque purification process? What would explain >200 intense hybridizing phage plaques from a cDNA library screening using an uncharacterized probe?

**Two reasons for faint signals of a specific phage insert in a genomic library screen is 1) short region of overlap between the probe and the insert so less radioactivity is hybridized compared to inserts with extended overlap, and 2) decreased percent identity due to hybridization to an orthologous gene in the genome (related sequence), this would also result in less radioactivity due to unstable hybrids.**

**The explanation for >200 intense signals using an uncharacterized probe to screen a cDNA library would be that the probe contains a repetitive sequence element that hybridize to a large number of cDNAs in the library. This would represent "noise" in the experiment that drowns out the "signal" you are looking for. The unique region in the probe does hybridize to a unique cDNA in the library but you cannot identify it because it is of equal intensity to the other 200 plaques.**

5. (2 pts.) What is the advantage of using site directed mutagenesis rather than deletion analysis to study protein structure and function? Why is alanine a good amino acid to use for replacement in a protein coding sequence? What other amino acid would be a good choice for "scanning" based on its chemical structure?

**Site-directed mutagenesis allows you to create amino acid substitutions that maintain the open reading frame. Deletions will be out of frame 2/3 of the time and therefore not direct the synthesis of the desired protein.**

**Alanine is a good substitution because the R group is a CH<sub>3</sub> which is small and [essentially] inert. Another good choice would be glycine which only has a H for an R group. Glycine is not used as much because it can sometimes have too much freedom of rotation depending on the location in the primary sequence. This can ultimately influence the protein structure more than alanine would in the same position (glycine residues are often found in turns).**