EXAM 1

Applied Molecular Genetics
BIOC 471

September 26, 2000

Please limit your answers to the space provided.
The following 16 questions are worth 5 points each.

1. What biochemical process in cells is characterized by formation of an RNA-DNA hybrid? Name an applied molecular genetic technique that depends on the formation of RNA-DNA hybrids.

   Transcription requires the formation of RNA-DNA hybrids.

   Northern blots are an example of an RNA-DNA hybrid.

2. What accounts for the sigmoidal shape of DNA denaturation curves?

   Cooperativity of hydrogen bond disruption between complementary strands.

3. What is X-gal and how is it used in blue/white screening?

   X-gal is a chromogenic substrate for the enzyme beta-galactosidase (LacZ). When it is cleaved, it produces a blue color in visible light. X-gal is used to detect the presence of functional LacZ enzyme in cells and cell extracts. Blue/white screening is a strategy using X-gal in which disruption of the gene encoding the alpha subunit of LacZ results in the loss of blue colony formation indicating that the bacteria harbor a recombinant plasmid.

4. Give an example of an antibiotic resistance gene and describe how it is used in applied molecular genetics?

   The ampicillin-resistance gene (amp') encodes the enzyme beta-lactamase which is secreted and hydrolyzes the antibiotic ampicillin. Amp' is used as a dominant selectable marker to detect plasmid replication in transformed E. coli cells.

5. What is the chemical property of biomembranes that prevents exogenous DNA from diffusing into cells? What molecular genetic method has been developed to overcome this barrier?

   Biomembranes are hydrophobic and DNA is negatively charged and hydrated. Chemical transformation and electroporation are two methods used to facilitate DNA entry into cells.

6. Why is infection of E. coli with M13 helper phage required to produce single strand DNA from a phagemid vector? What molecular genetic method utilizes single strand phagemid DNA?

   A phagemid vector contains the M13 origin of replication but does not encode any of the viral proteins required for replication. An M13 helper phage encodes genes for replication but is defective in DNA replication. Infection of a phagemid-containing E. coli cell with M13 helper virus results in replication and packaging of recombinant single strand phagemid DNA. Single strand phagemid DNA can be used for site-specific in vitro mutagenesis and for dideoxy DNA sequencing.
7. What are two explanations for why you would observe intense hybridization signals from ~200 plaques when screening a cDNA library with an uncharacterized probe?

It could be due to the presence of a repetitive DNA sequence element, like Alu repeats, in the non-coding region of the transcript (UTR).

A second explanation would be that the cDNA probe hybridizes to a highly expressed gene that has many cDNA representative clones in the library, for example, actin or ribosomal RNA.

8. What types of genes would you expect to be shared, and to be unique, when comparing the genomes of *E. coli K-12* and *Helicobacter pylori*?

Shared genes would be those involved in metabolism and energy conversion.

Unique *Helicobacter pylori* genes would be those required for toxicity in the host.

9. What is the source of molecular weight (MW) markers in a PFGE Southern blot, and how would they be used to determine the MW of a DNA fragment?

The molecular weight markers for PFGE Southern Blot would be yeast chromosomes as they are easy to manipulate and their sequence (MW) is known.

A MW standard curve would be generated using relative mobility and MW. The relative mobility of DNA fragments identified in the Southern Blot would be used to calculate an estimated MW based on the standard curve.

10. What could be done to decrease the interference of non-specific DNA binding proteins in the EMSA assay?

Add poly dG:dC (or dI:dC) into the EMSA binding reaction to sequester non-specific DNA binding proteins. In most cases, the protein under study will have a higher affinity for the specific binding site contained within the labeled oligo and be less effected by the inclusion of poly dG:dC than non-specific DNA binding proteins.

11. Why is RNaseH activity not included in the first strand cDNA synthesis reaction, but is required for second strand cDNA synthesis, in the Gubler and Hoffman replacement strategy?

RNaseH is an endoribonuclease that recognizes and cleaves RNA-DNA hybrids. Since the extended cDNA strand resulting from first strand synthesis contains an RNA-DNA hybrid, RNaseH would cleave the RNA and thus destroy the template resulting in short cDNA. In contrast, RNaseH is required for second strand synthesis in the replacement strategy in order to create RNA primers for DNA polymerization.
12. How is a blunt-end ligation reaction using adaptor molecules and cDNA set-up to maximize the number of cDNA molecules that become covalently attached to adaptors? What problem does this create and how is it resolved prior to lambda Zap vector ligation?

To maximize adaptor ligation to cDNA, the ratio of adaptor molecules to cDNA is set very high, for example, 10:1 adaptor molecules:cDNA molecules.

High adaptor concentrations in the ligation reaction leads to formation of adaptor dimers which can ligate into the cloning vector just as easily (or more easily since they exist at a high concentration) as the cDNA. This problem is resolved by removing adaptor dimers from the ligation reaction prior to vector ligation, this is done by size-fraction using column chromatography.

13. Would you expect a pool of degenerate oligonucleotide probes to contain any molecules that are 100% complementary to the desired cDNA? Explain.

Yes, because the basis of using degenerate oligonucleotide probes to screen a cDNA library is that all possible codons are accounted for during DNA synthesis. This strategy ensures that at least one combination is a perfect match and thus will hybridize under the reaction conditions.

14. List two criteria that must be met for a protein activity assay to be a viable strategy for cDNA library screening.

The desired gene must be expressed in the cell type used to construct the cDNA library.

A full-length, or at least functional, cDNA clone must exist in the library.

The protein activity must be encoded by a single polypeptide chain (single cDNA).

15. Why are both the Gal4-dependent His3 and LacZ reporter genes used in the yeast two hybrid screening strategy? Which is a genetic screen and which is a genetic selection?

The rate of false positives is very high in the yeast two hybrid screen and therefore it is important to have more than one indicator that a reconstituted transcription function exists in the cell (protein-protein interaction is occurring between the bait protein and target protein).

His3 is a genetic selection and LacZ is a genetic screen.

16. Why can't sodium hydroxide (NaOH) be used to denature RNA in an agarose gel prior that is being used for Northern Blot analysis?

Sodium hydroxide hydrolyzes RNA by catalyzing the formation of a 2' - 3' cyclic phosphate.
17. What would you conclude in the above example if the STRP pattern of one of the offspring were found to be A/A? (10 pts.)

The simplest explanation is that P2 is not the biological father, but rather it is a male that carries the "A" polymorphism.

Another more complicated explanation (and much less likely) is that a sequence duplication took place during meiosis in the P2 paternal sperm creating an "A" gamete that was passed on to the offspring.
18. What is the primary purpose of each of the functional elements in the pYAC4 cloning vector? Write the number corresponding to the best description in the blank. (10 pts.)

2  CEN4  1. chromosome maintenance
4  SUP4  2. chromosome segregation
6  amp\(^r\)  3. origin of replication in yeast
5  URA3  4. genetic screen for YAC inserts
7  ori  5. selectable marker in yeast
8  EcoRI  6. selectable marker in E.coli
3  ARS1  7. plasmid maintenance
1  TEL  8. insert cloning site