

NAME \_\_\_\_\_ **KEY**

# **Final Exam**

## **Applied Molecular Genetics BIOC 471a**

**December 4, 2001**

*Please limit your answers to the space provided.*

**The following questions are worth 10 points each.**

1. What parameter would you change first if your PCR reaction gave too many products? What would you do if the PCR reaction gave very little, if any, of the correct product?

If there are too many products in a PCR reaction then it suggests that primer annealing is non-specific. The first parameter to modify would be to increase the annealing temperature which will increase the specificity of the primers.

If no product was observed it might suggest that the annealing temperature was too high. This can be tested by lowering the annealing temperature 3-5 degrees. However, another possibility is that the primers are not working, in that case, new primers would need to be designed.

2. What two properties of GFP make it a useful "molecular tag" in molecular genetic applications? How and why was the natural *A. victoria* GFP protein gene sequence "humanized"?

The two unique properties of GFP are its ability to *autofluoresce* following excitation with ultra-violet light (no substrates required) and the *stability of the GFP protein structure* which permits the creation of functional gene fusions between GFP and numerous other proteins.

The natural GFP protein was "humanized" by site-directed mutagenesis to convert invertebrate (Jellyfish) codons to more common vertebrate codons (e.g., mammalian cell lines). The "humanized" GFP gene permits higher translation rates because rare tRNAs were not required for protein synthesis.

3. When using ES cells to generate genetically modified mice, does the presence of a chimeric agouti coat color in a first generation pup signify that you have established a founder mouse? Explain.

The presence of agouti coat color in the first generation of genetically modified mice constructed with embryonic stem (ES) cells only tells you that the mouse contains some cells derived from the ES cell line (made from an agouti mouse). It does not signify that the chimeric mouse is a "founder mouse" which requires that all cells in the animal contain DNA from the original ES cell line. This can only happen in the second generation in which chimeric mice containing genetically modified germ cells are bred to an albino mouse and produce genetically modified gametic cells. The founder mice are identified initially by the presence of a uniform agoutic coat color.

4. Describe the primary difference between a cDNA microarray printed in a core service laboratory, (e.g., Arizona Cancer Center), and the commercially-available Affymetrix oligonucleotide chip. List one advantage and one disadvantage of each system in the context of laboratory research.

A cDNA microarray is made by covalently attaching 3' biased cDNA fragments to glass slides using a simple robotic printing device. The double stranded cDNA is denature prior to hybridization with fluorescently labeled cDNA synthesized in vitro using oligo dT primers (3' biased probed).

In contrast, the commercially available Affymetrix oligonucleotide chip is made by photolithography methods in which short single strand oligonucleotides ~25 residues long are covalently attached to a solid surface. By varying the sequence at the center nucleotide (position 13), it is possible to control for hybridization to specific sequences.

An advantage of the cDNA array is that it is relatively inexpensive to produce and can be applied to any organism. A disadvantage is that it cannot be used for DNA sequencing because the cDNA sequences are too long to be specific under normal hybridization conditions.

**An advantage of the Affymetrix oligonucleotide array is that it can be designed in such a way to permit a broad range of applications including DNA sequencing, and because of its robust QC (quality control) attributes, it is ideal for high throughput applications, such as clinical studies using patient samples. A big disadvantage of the Affymetrix system for laboratory research is the limited number of organisms it has been adapted for and the high cost of each array.**

5. What is the meant by the term proteomics? What are two key applications of proteomics to the study of functional genomics?

**Proteomics refers to the application of biochemical methods developed for protein science to investigate the entire repertoire of proteins in an organism or cell (the proteome). Often time proteomics is used as a high throughput discovery or diagnostic tool. Many of the proteomic applications in use today rely on mass spectrometry. Two key applications of proteomics to the study of functional genomics are:**

- 1) **Expression proteomics which involves identifying proteins that are up- or down-regulated using 2-dimensional gels, usually done by comparing two protein samples such as cell extracts from cells grown under two different conditions.**
- 2) **Functional Proteomics which is based on identifying proteins that are part of large complexes, for example, proteins that interact in vivo with a tagged bait protein that can be purified by immunoprecipitation. These experiments use a technique called co-immunoprecipitation.**

6. **"What's that HERV Doing in My Genome?"**

What are three ways an endogenous retrovirus (ERV) could contribute to protein evolution in an organism?

- 1) **Cellular transcripts could use polyadenylation signals located in the viral LTRs leading to increased stability in mRNA.**
- 2) **The integration of ERVs may provide alternate splicing signals for mRNA and thus create novel protein products.**
- 3) **Transcriptional regulation of cellular genes by insertion of a nearby LTR could activate or inactivate gene expression.**

7. **"Prostate Mining"**

What property of a mutagenic primer is required for a PCR-based in vitro mutagenesis strategy to work? Why is the product of the in vitro DNA replication reaction treated with the restriction enzyme DpnI? What is the advantage of using the "PfuTurbo" DNA polymerase as compared to another thermostable DNA polymerase such as Taq?

**The melting temperature ( $T_m$ ) of the mutagenic primer must be high enough to avoid denaturation during PCR temperature cycling.**

**The product is treated with DpnI to degrade the parental strands and select for the newly synthesized mutant DNA. This occurs because the template strand is methylated at specific sites in *E. coli*; the in vitro synthesized DNA is not methylated and survives DpnI digestion.**

**PfuTurbo is used instead of Taq because this enzyme has higher fidelity, and thus, a low error rate.**

8. **"Out, Damned Spot!"**

What would be a possible phenotype in a transgenic mouse that expressed the mutant receptor in all cells? How could you construct the mouse to avoid this problem?

If the mutant receptor is indeed a cause of melanoma, a transgenic mouse embryo carrying the mutation may not be viable. Even if living mice are born, the mutant receptor may be able to disrupt development to an extent that conclusions drawn from experiments using the mice are not valid. In order to overcome this problem it would be necessary to construct a regulatable system such that the gene for the mutant receptor could be controlled and only turned on once development has proceeded normally. For example, the tetracycline regulated promoter system, or a cell-specific promoter that does not cause a lethal phenotype.

9. **"Methuselah Mouse"**

The researchers found that the dFOY (dove) promoter was hyperactive as compared to the mFOY promoter (mice), and that the dFQ (dove) enzyme had an increased catalytic efficiency relative to mFQ (mouse) enzyme. They observed that single mutant dFOY/mFQ and mFOY/dFQ transgenic mice had moderately decreased free radicals, whereas the double mutant dFOY/dFQ mice had greatly reduced free radicals and a marked increase in life span.

What was their proposed molecular mechanism for the dFOY/dFQ genetic synergy?

The researchers propose that both an increase in FOY enzyme, and presence of an highly active FQ enzyme, is required for significant free radical elimination (and extended life span). Therefore, the single transgenic dFOY mice (assume they used homozygous dFOY promoter mice) produced more intermediate product because of the elevated amounts of mFOY enzyme derived from the dFOY promoter. However, the mFQ enzyme could not process the intermediate fast enough. Similarly, even with the dFQ enzyme present (homozygous dFQ transgenic mice), the level of intermediate product generated by the rate-limiting amount of mFOY protein, resulted in an improved free radical clearance. It was only when both dove transgenic genes were functioning together, that the mice could eliminate a large amount of free radicals leading to less oxidative damage and an increased lifespan. Other explanations include that there are different amounts and species of free-radicals produced in mice and the two enzymes act differently in the mice.

**"Why Cancure Can't Cure"**

10. Explain the idea behind the production of chimeric antibodies, specifically, what are "primatized" chimeric antibodies. How are the chimeric antibodies created? What role do you think Genentech (the makers of human growth hormone) has in the partnership with IDEC Pharmaceuticals (the creators of primatized therapeutic antibodies), i.e., what can Genentech do that is of value to IDEC?

Primatized chimeric antibodies are produced by injecting monkeys with human antigens to create a primate derived variable region (the functional end of an antibody). Humans are not immunologically reactive to antibodies containing monkey-derived variable region protein structures, however, humans are very reactive to mouse-derived variable regions.

The chimeric antibodies are created by cDNA cloning methods to isolate gene sequences encoding the specific antibody. The functional monkey-derived variable region sequences are ligated onto constant region human antibody cDNA to create a chimeric antibody gene that is mostly human protein with a minimal amount of monkey protein (variable region). These chimeric cDNAs are then used in protein expression systems to produced large quantities of therapeutic antibody proteins. Genentech likely provides the large scale protein production capability and drug marketing expertise to produce enough therapeutic antibody to be used in the clinic.