NAME ___________ *KEY* ___________

# Final Exam

## Applied Molecular Genetics
## BIOC 571a

**December 4, 2001**

***Please limit your answers to the space provided.***

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

1. What are you measuring in real time using the "GeneAmp Real-Time" quantitative PCR assay? Why is it necessary to confirm the specificy of PCR amplification conditions using gel electrophoresis before scaling this system up for high throughput diagnostic assays?

**GeneAmp real time PCR involves the use of two standard PCR primers that flank a target sequence to be amplified. An oligonucleotide probe is designed that is complementary to the target sequence and contains two fluorochromes; a reporter molecule covalently linked to the oligo that fluoresces, and an attached quencher molecule that blocks reporter fluorescence in intact probe molecules. The amount of reporter fluorescence is measured in real time (at each cycle) and is proportional to the amount of PCR product because Taq polymerase degrades the annealed oligo probe and the linked quencher fluorochrome during each cycle, thus releasing the reporter molecule and causing fluorescence. It is critical that the probe oligo have a higher Tm than the PCR primers so that it is annealed (and degraded) during each extension cycle.**

**It is critical to work out all of the PCR amplification conditions, primer designs and oligo probe specifications by gel electrophoresis (or hplc) prior to high throughput applications because the GeneAmp assay does not permit direct verification of individual products based on size.**

2. What skills are required to take advantage of the "power of yeast genetics" to investigate gene function of a human orthologous gene, as compared to using yeast as a molecular genetic tool to identify interacting proteins in a two-hybrid screen?

**Investigating the function of a human orthologous gene in yeast requires a working knowledge of yeast genetics in order to take full advantage of the "power of yeast genetics." For example, if the gene has a lethal phenotype in yeast when deleted, or is deleterious when over expressed, the researcher would want to perform tetrad analysis and select for second site repressors of the phenotype, respectively. Moreover, many gene products function as components of large protein complexes and it is often necessary to construct complex genetic backgrounds using various yeast strains, some of which harbor conditional mutations that require sophisticated genetic selection and screening strategies to take full advantage of.**

**In contrast, using a yeast system for two-hybrid screening only requires that the researcher be able to clone genes into recombinant plasmids by standard bacterial plasmid methodologies and transform yeast cells using straightforward protocols.**

3. What would explain the lack of gene expression of an integrated transgene in a founder mouse that tested positive by DNA analysis through several generations? How is a genetically modified mouse created by DNA injection of fertilized eggs different than a mouse that was derived from reconstitution of a blastocyst using stably transfected embryonic stem cells?

**The lack of gene expression from a founder transgenic mouse would likely be due to DNA integration into an area of the genome that does not permit proper gene expression even though it is carried through the germline.**

**Transgenic mice created by DNA injection into fertilized eggs results in random DNA integration into the genome. These types of transgenic models are of limited usefulness because they do not mimic natural genetic mutations. In contrast, transgenic mice derived from stably transfected ES cells and blastocyst reconstitution are designed to contain DNA integrated at homologous sites in the genome. These genetic models create "knock-out" or "knock-in" genotypes.**

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. Describe the "molecular genetic" strategy that makes it possible to use proteomic methods to identify proteins that associate in vivo with a target (bait) protein? Why might this proteomic approach be better than the yeast two hybrid method to study protein interactions?

**Applications of mass spectrometry to protein identification using small samples sizes (ng amounts of protein) has made it possible to investigate protein interactions through the use of co-immunoprecipitation techniques. In this strategy, the target gene of interest (bait) is modified using standard molecular genetic methods (PCR cloning and DNA sequencing) to add a heterologous peptide sequence to the amino or carboxy terminal ends that is recognized by a high affinity antibody. Typical peptide-antibody combinations are the FLAG, HA or c-myc tags. The modified bait protein is transfected into an appropriate cell type and immunoprecipitated using methods that preserve non-covalent protein-protein interactions. The co-immunoprecipitated proteins are identified by mass spectrometry by directly analyzing the precipitate or by excising individual bands from an SDS PAGE gel.**

**This proteomic approach to in vivo protein complexes is better than the yeast two-hybrid strategy in that the bait protein is expressed in the appropriate cell type, the protein is full-length with only a minor modification, and what you see is what you get with regard to associated proteins (positive results are not dependent on an indirect transcriptional activation assay).**

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 DpN1? 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 DpN1 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.**