**Molecular Techniques Exam I**

**Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Type out your answers in this word document.

1) Agarose Gel Electrophoresis

1. Describe the factors that one must consider when running an agarose gel for analysis vs isolating DNA from an enzyme digest.
2. What is the depth of gel and how much buffer should be used when preparing an agarose gel?
3. What are the most common problems encountered with agarose gels.

2) Restriction Digest

1. Create a protocol for a standard restriction digest with a total volume of 50 µl digest. Pick two enzymes from NEB and use the information from that site to create your protocol. Your plasmid is at 0.3 µg / µl.
2. Now you wish to do an 'overdigestion' in which you will put the maximum allowable enzyme in the digest. (Indicate how many Units will be in the digest and how you would set this digest up differently.)
3. What does “star activity” mean and why should this be a consideration in a restriction digest?

3) DNA Calculations

Monica purified the pBIT plasmid DNA by alkaline lysis followed by anion exchange chromatography and resuspended the final DNA pellet in a total volume of 500 μl TE buffer. She used the Nanodrop (as you did in lab) and obtained the following absorbance readings: *Show your math and circle the answer. Units must be correct!*

A230 = 0.16 A260 = 2.9 A280 = 2.2

1. What is the concentration of DNA in her sample? (Be sure to include the appropriate units!)
2. What volume of pBIT plasmid DNA would she need to pipet to obtain 50 ng of plasmid to use in a ligation? Explain thoroughly including dilutions that need to be done, if necessary.
3. She wants to set up a ligation reaction with 50 ng of vector DNA. If the vector is 4 kb and the insert is 900 bp, how many nanograms of insert DNA would she need in order to obtain a 1:3 vector to insert ratio?
4. Is the DNA pure? If yes, how do you know? If not, what is the most likely contaminant?

4) Nucleotide Basics

You were given a microfuge tube of plasmid DNA, a tube of RNA and a tube of chromosomal DNA. In a simple mistake, you have forgotten to label the tubes and didn't notice this until after you ran the sample through a mini-prep silica column (Qiagen).

1. Which of the tubes will bind to the silica?
2. When will the DNA or RNA elute if they bind (assume a standard procedure we used in class) and propose a simple experiment to tell which is which.

5) Bacterial Strains in Molec Bio

Kumar is planning to prepare plasmid DNA to clone and asked you to get a competent cell out of the freezer for him to use. You find lots of types of competent cells and have things narrowed down to a BL21 (DE3), a DH5alpha, and JM109.

1. What are the reasons for using EACH of these and which should you bring to Kumar?
2. How is a competent cell prepared?
3. And, oh, by the way... what is the purpose of the 'recovery' step in transformation? When does it happen in the protocol?

6) Software and Molec Bio - Using SnapGene create a plasmid map for watermelon MDH without the precoursor. Use the information from our labs to create your map. Include all the pertinent information on the plasmid map.

7) Restriction Enzyme Cloning - You have to clone a 1.2 kb insert from a plasmid pTrouble (it is made up-don’t bother looking for it) into pET30b using EcoRI and KpnI. Assume that the cut will leave the reading frame intact. Describe the workflow starting with each plasmid purified at 1 mg/ml DNA. Refer to chapter 2 in your book and your notes for reference material.

8) Classic DNA Sequencing Chemistry

1. How does the chemistry of Sanger sequencing differ from Maxam-Gilbert sequencing?
2. How does the early radioactive Sanger method differ from the modern approach using four colored dyes in a dideoxy sequencing method (be specific and more detailed that the notes alone)?

9) NextGen Sequencing

a) How does NextGen sequencing differ from a standard modern four colored sequencing in throughput?

b) Describe either Illumina or another mechanism (step by step) for a NextGen sequencing method.

c) The following series of DNA sequences were obtained from 60 base reads on an Illumina NexSeq instrument in a shotgun sequencing experiment.  Sequences were obtained in both directions.  They were generated in a large run containing numerous other genes, but these were all identifiable by a common index sequence originally contained in the primers. Using BLAST and any other tools you care to, define the forward and reverse primer sequences and the index sequence, and assemble and align these reads into a contig.  Finally, identify the gene from which they were derived.

5’CAGCTATGACAGGTCAGGCCACGAAGGGTCCTCCTCCTCAGCCATCTCCTGGTCGTCTTC

5’TCGTTCGACCAGGTCAGGAGTGAACTGCAGCACTTAGCCACCTCCCGCCTGCCAAGCTTC

5’CAGCTATGACAGGTCAGGTCGTCTTCGTAGGAGGGTGGGTTTAGCCTGGGTGTTTTGTGC

5’CAGCTATGACAGGTCATTGTGCTGCACGATTCTGGAGTAGTACGTGTTGAGGCAGAAGAC

5’CAGCTATGACAGGTCACAGAAGACGTCGGCCGTCGCCCTGATGAACCTCTTCTCTTCCTC

5’CAGCTATGACAGGTCACTTCCTCAGTGAAGCTTGGCAGGCGGGAGGTGGCTAAGTGCTGC

5’CAGCTATGACAGGTCAAAGTGCTGCAGTTCACTCCTGTTCCCCACTTTCCACTTCATGGT

5’CAGCTATGACAGGTCACATTTCAGGCATATGACCCAGGGAATGTTGGAAGATTCTTTAAG

5’CAGCTATGACAGGTCACTGGTGTCAGGATAGTCTCCGTTTCTAAAAATGGGGTGACAAAC

5’CAGCTATGACAGGTCACAAACCAGCCCAGGGAGAACTGCAGCATTCGGTCAGCGGCTTCC

5’TCGTTCGACCAGGTCAAGATGTGGAAGCCGGCTGACCGAATGCTGCAGTTCTCCCTGGGC

5’TCGTTCGACCAGGTCACCCATTTTTAGAAACGGAGACTATCCTGACACCATGAAGTGGAA

5’CAGCTATGACAGGTCAGAACCCTCACACACCTTTCAAAGCCTCATTGATGTAGGTTTTGT

5’CAGCTATGACAGGTCAGCGTCCCCCAGGGCGCAGCTCTGTTCATTGCCGTGGAAGGCCAC

5’CAGCTATGACAGGTCATGGTTTTGTGGTTTCCTTCGTTCCTCCGTGTTCGGTTGGTCGCC

5’TCGTTCGACCAGGTCATCTGCCTCAACACGTACTACTCCAGAATCGTGCAGCACAAAACA

5’TCGTTCGACCAGGTCACAGGAGATGGCTGAGGAGGAGGACCCTTCGTGGCCTTCCACGGC

5’TCGTTCGACCAGGTCAGCTGCTGAACTGGATCAAGGAAGAGTATGGTGACATCCCCATTT

5’TCGTTCGACCAGGTCACGAACACGGAGGATACTGATAGGATATTTTACCACAAAACCTAC

5’CAGCTATGACAGGTCATGGTTCATAAATCATCCCGGCAAAACATTTCAGGCATAGACCCA

5’CAGCTATGACAGGTCAGGAAGATTTCTTTAAGGGGAACTGAACCCTCACACACCTTTCAA

10) Sequence Alignment

A question that has long intrigued taxonomists and vertebrate biologists is the evolutionary relationship of the hippopotamus to other vertebrates.  Although they are sometimes called “river hogs,” hippos have often been put in the same taxa as whales.

As the molecular biologist on the team, yours will be a critical piece of evidence in the controversy!  So weigh in: Using the alpha chain of hemoglobin as a reference protein, perform pair-wise alignments between hippo, whale and pig orthologs.  Is hippo hemoglobin more related to that of the pig or of the whale? Does the answer change if you alter the substitution matrix from the default BLOSUM62?

Hemoglobin alpha chain Gen-Bank accession numbers: Whale P18971; Hippo: P19015; Pig: P01973

Hint:  Accession numbers can be copied or pasted into BLAST query boxes just like FASTA sequences, and the software will retrieve the sequences from Gen-Bank.