Bi 430/530 Winter 2008–Problem set 1, 50 points (10 points per question)
Due in class on **Friday, Feb 1st**
Late work will have 5% points deducted for the first day, 10% for the second day, etc. Answers must (as much as possible) be typed.

In working through these questions you may use any source (books, internet, co workers, classmates, etc.), but answers must be in your own words. Show all calculations.

1. Your first assignment in your new job is to make plasmid DNA for cloning purposes. Your supervisor hands you an agar plate with a bacterial strain containing the plasmid, which confers resistance to ampicillin and is 5,436 base pairs long.
    a. What should you do to purify the plasmid? (be brief)
    b. At the end of the plasmid prep, you resuspend the DNA in 50 microliters of 10 mM Tris pH 8.0. You quantify the plasmid by making a 10-fold dilution (10 microliters of plasmid plus 90 microliters water) and measure the absorbance at 260 nm, obtaining a value of 0.448. What is the concentration of the plasmid in micrograms per milliliter?
    c. What is the concentration of the plasmid in nanograms per microliter?
    d. What is the molar concentration of the plasmid, expressed in terms of micromolar (10^{-6} mol/L) and nanomolar (10^{-9} mol/L)?
    e. The protocol you want to use the plasmid for requires 4 picomoles (10^{-12} moles) of plasmid DNA. Do you have enough plasmid DNA to do this protocol?

2. Your lab studies a gene (*mpb1*) important for metabolism of PCBs by bacteria. You isolate a new organism from a PCB-contaminated superfund site that appears to break down PCBs, and you want to see whether this organism also contains an *mpb1* homolog. Describe a method for doing this (note: several different methods may work). Be specific on the details of the method, and the expected outcomes of your experiment.

3. You wish to study the expression of a new gene that appears to be the master signal for maintenance of totipotency (ability to differentiate) for human embryonic stem (ES) cells. A Nobel Prize may be yours if you prove the importance of this gene.

a. Your approach for monitoring expression of this gene is to use the method of quantitative RT-PCR for measuring RNA levels. How will you proceed in this method, and what kind of controls are required to make sure the assay is quantitative?
b. If you expect that mRNA levels are high in non-differentiated ES cells, and are reduced in cells that have already differentiated, how will the RT-PCR data look?
c. You unexpectedly find that mRNA levels for this gene do not change following cell differentiation, so you decide to measure protein levels. What method should you use for measuring protein levels, and how does this method work?

Use the following DNA sequence for both questions 4 and 5:

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5’ttcgacagtccgaagacgttttattattagatcaaatgtcgaaaggtcgttttaa
ttttggaaccgttcgagggctttaccataaagattttcgagtatttggtgttggtat
ggtagagtctcgggcaattactccaaattaaaaccagatgataatggaaagctgaca
gacaggtaccattagctctgatagtgattacattcaatctcctaaggttgatgtata
tcccaaagtgtactcacaaaatgt3’
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(remember: the DNA is double stranded but is represented by only one strand above)

4. Your lab has just obtained the DNA sequence shown above, and you want to use the sequence as a marker for the presence of a specific bacterium in environmental samples, using a PCR assay.
    a. Design a primer pair that will amplify any part of this sequence, indicating the melting temperature (T_m) for each primer (show calculations). Be sure to indicate the endpoints of the PCR product relative to the above sequence.
    b. You perform the PCR but there are lots of bands when you run the reaction on a gel (you expected to see only one band). Suggest two possible reasons for this, and the PCR protocol changes you might make to solve this problem.

5. Is the DNA sequence above likely to be part of an open reading frame? What is the probable function of the open reading frame? Be specific about why you think it is likely to have this function. (hint: you will need to use a computer connected to the Web to answer this question).