BIOLOGY 324 **Name** _____
TAKE HOME FINAL - 11 March 2000
DUE back in by 5:00pm, March 16th.

(1) *Required* 30 point question: Polymerase Chain Reaction

- You are trying to PCR a gene from the human genome, which has a haploid genome size of 3 x 10^9 base pairs. Shown below are the conditions for the PCR reaction.

- PCR conditions: 50 µl reaction with the following **final** concentrations or amounts.
  - 1X PCR buffer
  - 5 mM $MgCl_2$
  - 250 µM dNTPs
  - 2.5 µM forward primer (T_m=72°C)
  - 2.5 µM reverse primer (T_m= 58°C)
  - 30 ng of human genomic DNA
  - 0.5 U Taq

- Assume the PCR reaction was set up correctly in that the appropriate amounts of the components were added together on ice without any contamination.

- The PCR reaction was initiated by placing the PCR tube in the machine, turning on the machine, and setting it for the following conditions:
  - 95°C - 1 minute
  - 53°C - 1 minute
  - 72°C - 1 minute

These conditions were repeated for 30 cycles and then the samples were held at 4°C.

- However, the PCR reaction failed utterly.

(A) Identify <u>three</u> possible major problems with this reaction and how these problems could be fixed.

(B) Identify <u>three</u> other parameters which may help optimize the PCR reaction.

Your answers may **NOT** include: trying a new DNA preparation, repeating the reaction "as is", mixing it more thoroughly, buying a better *Taq* polymerase, trying a new PCR machine or adjusting the lengths of times for the cycles. Be sure to justify your answers in detail.

(2) *Required* 10 point question: PCR Primer Design

● Shown below is a DNA sequence. Identify a putative forward and reverse PCR primer which will amplify the central region. The primers should be 15-mers in length.

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5' CTAATACGACTCACTATAGGGCAAGCAGTGGTAACAACGCAGAGTACGCGGGAGTCGAGTTCCAGACAGCCACACGTGTT 3'
3' GATTATGCTGAGTGATATCCCGTTCGTCACCATTGTTGCGTCTCATGCGCCCTCAGCTCAAGGTCTGTCGGTGTGCACAA 5'
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Forward primer sequence 5' to 3' _____

Reverse primer sequence 5' to 3' _____

(3) *Required* 20 point question: DNA Sequence Analysis

● Using the text file CLM also sent you containing DNA sequence data in FASTA format, you are to trim off the vector sequence and initial bacterial specific PCR primer sequence (either the 49-68F or 1510-1492R primers). You can assume that the rest of the data are fine as far a quality control is concerned.

(A) Using the basic BLAST search, determine the most similar sequence in all of GenBank and record the highest score (bits).

(B) Now, using the RDP online analysis feature of Sequence Match, determine the most similar sequence in the RDP database based on its S_{ab} or similarity value and record this value.

(C) Determine the orientation of the SSU rDNA gene fragment relative to the pTA vector AND determine which primer (i.e., M13F or M13R) was used to generate these sequence data.

NOW YOU GET TO CHOOSE 2 OF THE FOLLOWING 3 QUESTIONS:

(4) 20 point question: Molecular Biological Protocol Conceptualization

● Diagram a flow chart to outline an approach you would use to get from a biological sample as starting material to the sequence data for a specific gene of interest (make sure to use at least some of the molecular biological protocols we have addressed during the course). The level of detail should include a step-by-step methodological approach where someone with your background knowledge could perform each of the specific protocols. In other words, you do NOT have to describe the steps in a protocol, but rather the logical order of protocols themselves and how one protocol would fit into the next.

For example, after α-complementation ➜ putative-positive clones were screened by the ???? method, etc.

(5) 20 point question: PCR Primer Design Program

● DSL will email each of you a DNA sequence and the respective website addresses. Your task will be to use the *Primer 3 program* site at MIT to design primers to amplify this sequence. These primers should have the following characteristics:

(A) Length of 28-34 bases
(B) T_m of 62-65°C
(C) No more than 3/6 of the 3' bases being G's or C's

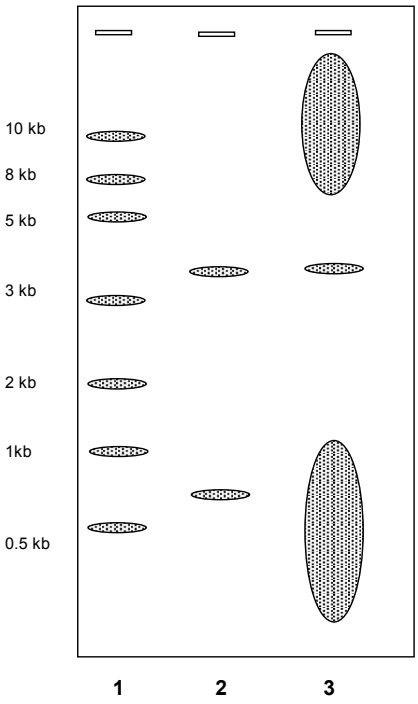
After selecting a putative primer set, go to the *Williamstone Primer* site and use the Oligo calculator to determine the probability of primers hybridizing to themselves or together as primer dimers.

Copy and paste the relevant primer sets from the each of the MIT and Williamstone sites. Include printouts of the data and highlight the relevant primer set, so that I may be clearly able to order the primers if I needed to do so.

(6) 20 point question: Plasmid miniprep by Alkaline Lysis

- You and your lab mate have independently isolated plasmid DNA by alkaline lysis, cut the plasmid DNA with *Eco*R1 and *Hind*III, (which should drop out an insert), separated the fragments by gel electrophoresis, and stained the gel with EtBr. The results are shown below.

- Lane 1 is DNA marker lane, lane 2 is your sample, and lane 3 is your lab mate's sample (gasp!).



(A) Did you have an insert in your plasmid? What about your lab mate? Explain your answer.

(B) Based upon your understanding of the alkaline lysis protocol and your lab notebook, give useful advice to your lab mate about how to improve his/her results. Be as specific as possible. You will not receive full credit for irrelevant or poor advice.

(C) If you were to use the spectrophotometer to estimate the $OD_{260/280}$ ratio of the plasmid minipreps used in lane 2 and 3, what would you expect the results to be prior to the restriction digestion?