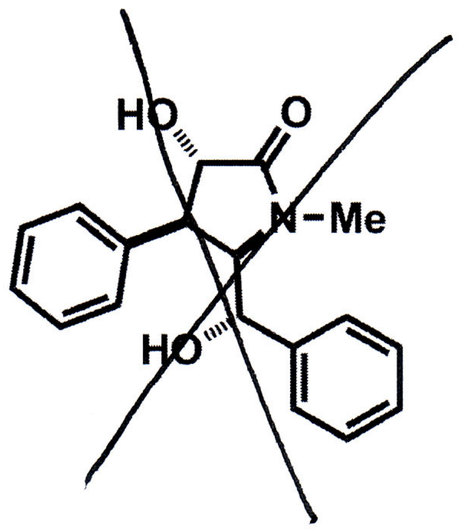
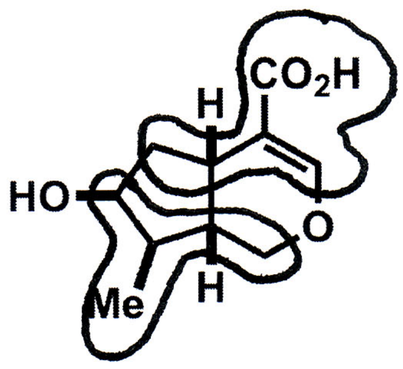
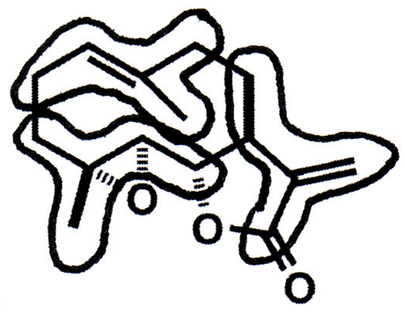
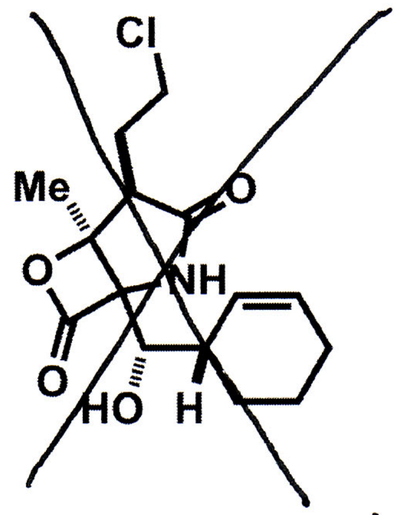
1. In the following set of molecules, identify the terpenes and circle the isoprenyl subunits. Draw an "X" over the structures that are not terpenes (12 points).



Not 5n carbons!



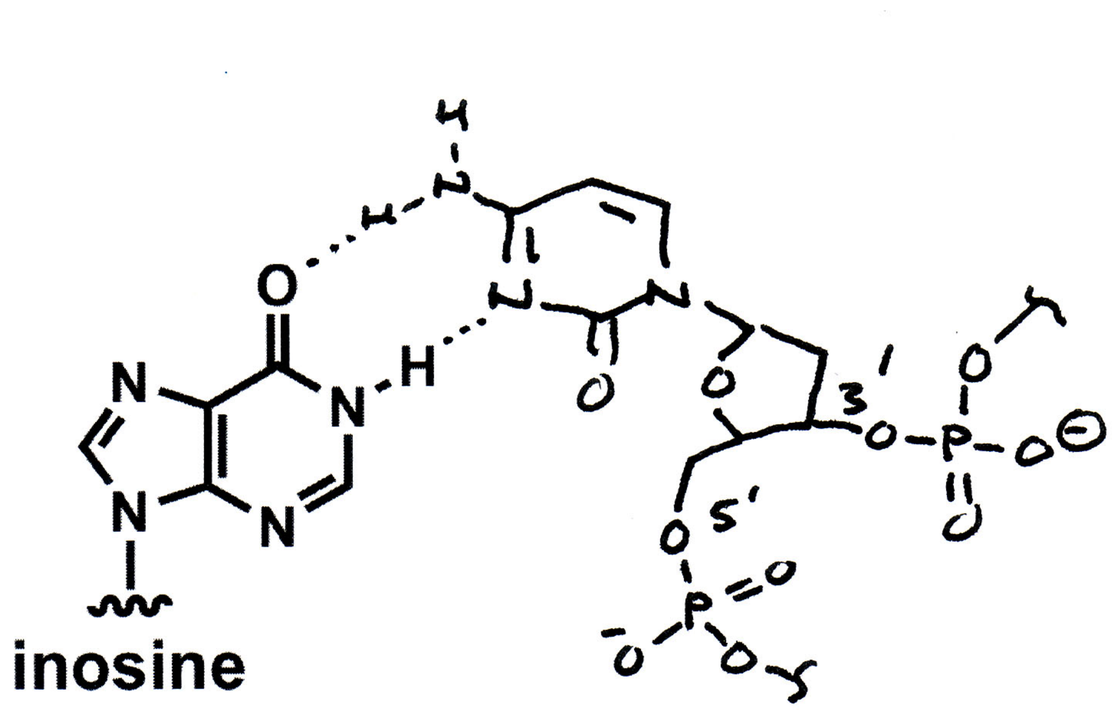




Wrong connectivity.

2. In some tRNA molecules, specific adenosines are deaminated to form the base inosine. This changes the inherent base-pairing properties in these positions of the sequence. Using the Watson-Crick-Franklin base pairing rules, draw the *full structure* of the RNA nucleotide that would have the most favorable interaction with inosine (you do not have to complete the inosine structure). Also clearly indicate the hydrogen bonding interactions and specify the 5' and 3' positions (8 points).

Pairs w/ C!



3. The following DNA segment is found at the 5'-end of a palindromic sequence that is 8 base pairs in length. Complete the sequence and indicate where it would be cut by a restriction endonuclease to leave a 3'-overhang of 4 bases (8 points).

5'-GCTTAA|GC-3'
3'-CG|AATTCG-5'

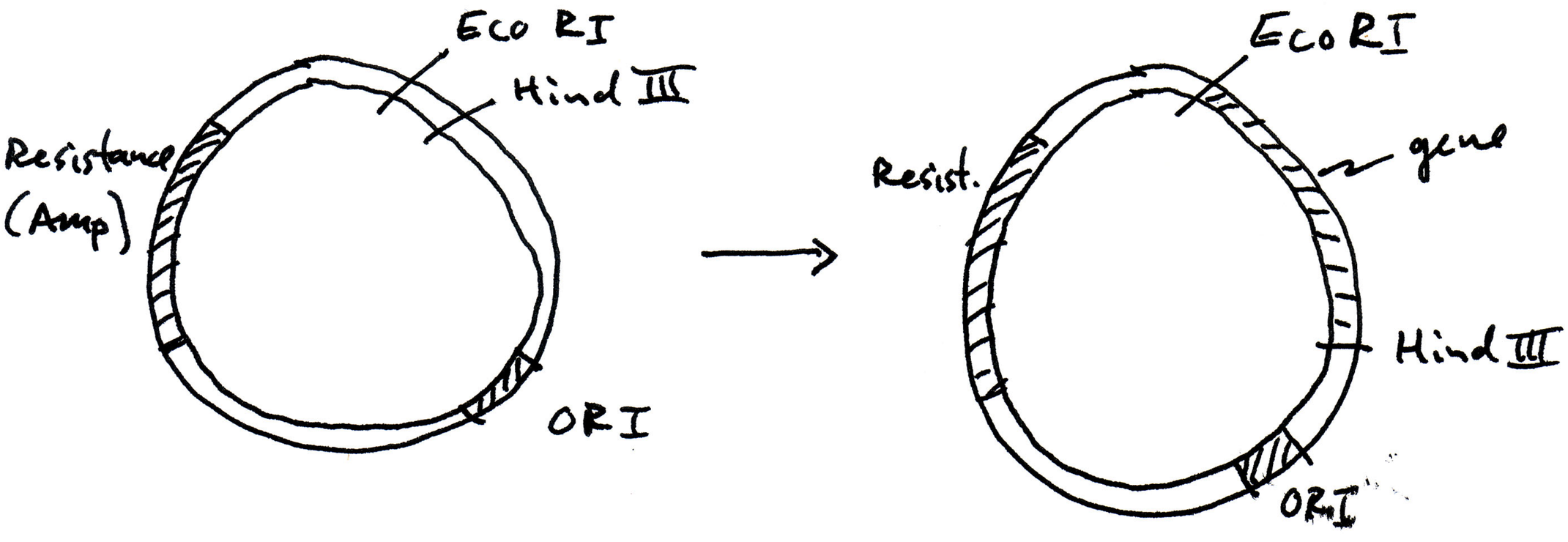
4. Suppose you are interested in producing a protein found in a species of photosynthetic bacteria. You do not have access to the DNA of the original organism, but you know the sequence of the gene of interest. It is 250 bp in length.

(a) Considering that you can obtain any DNA sequence up to 50 bases in length from a commercial supplier, show how you would construct the entire gene *and* introduce a pair of restriction sites (labeled as Eco RI and Hind III) that could be used to incorporate it into a plasmid (10 points).

Use overlapping 50-base segments:

① Anneal segments
② Add 5'- phosphates w/ DNA kinase + ATP (OK to leave out)
③ Seal w/ DNA Ligase
④ Rescue & add rest. sites w/ PCR:

(b) In the space below, provide a sketch of a suitable plasmid that could be used to clone the gene you made in part (a). Be sure to include all of the required components. Next, sketch what it would look like after the incorporation of your gene (10 points).



(c) Now suppose you would like to make a mutant by changing a single base pair in the center of your gene. Provide a sketch and a brief experimental outline that show how you would do this (10 points).

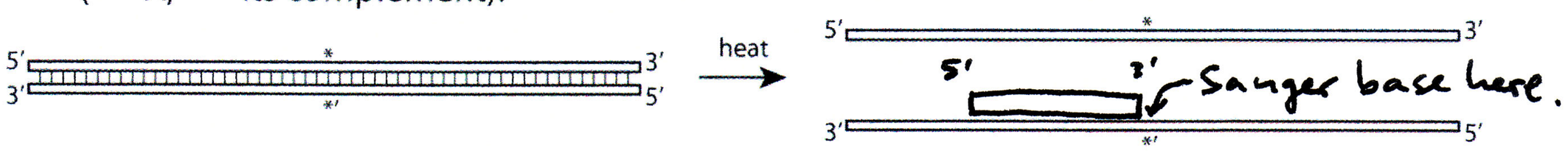
1. Design ~~^~~ overlapping primers that contain mutation
2. Run PCR to extend primers
3. Digest original DNA w/ DPN I
4. Transform into E. coli & grow colonies
5. Select colonies & sequence to confirm mutation.

- Other methods can also be used for full credit.

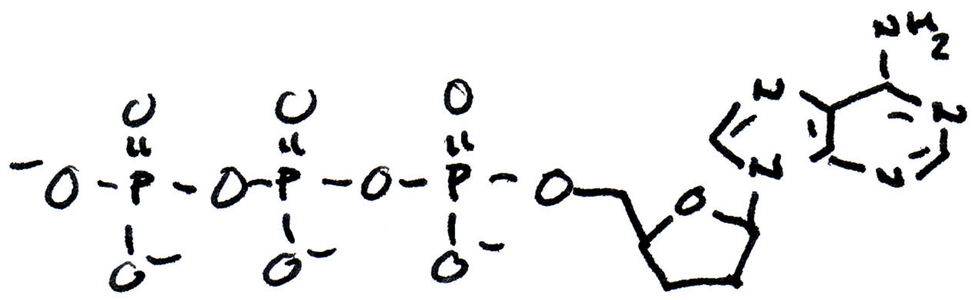
5. Much of the genetic variation between two individuals comes from single nucleotide differences that occur in specific locations in the genome. These Single Nucleotide Polymorphisms, or "SNPs", can change an amino acid residue of an encoded protein or alter the behavior of interon splicing. Given a DNA sample, the ability to identify SNPs is very important for disease screening and forensic analysis. This question examines a number of methods that can be used to detect them.

(a) In one technique, termed the "primer extension method", a synthetic DNA primer is prepared to match the bases of DNA that are directly *upstream* of the SNP site (marked by the stars, below). After mixing this strand with the DNA of interest, the sample is heated and then cooled to allow rehybridization. Next, DNA polymerase is added, in addition to one of the Sanger bases that is normally used for sequencing purposes. If it matches the SNP, a single base is added to the primer, which can be detected using gel electrophoresis. Using the diagram below for "Individual A", indicate where you would place the primer to use this technique, and then provide an accurate structural drawing of the Sanger base that would successfully extend it (8 points).

DNA segment from Individual A
(* = A, *′ = its complement):

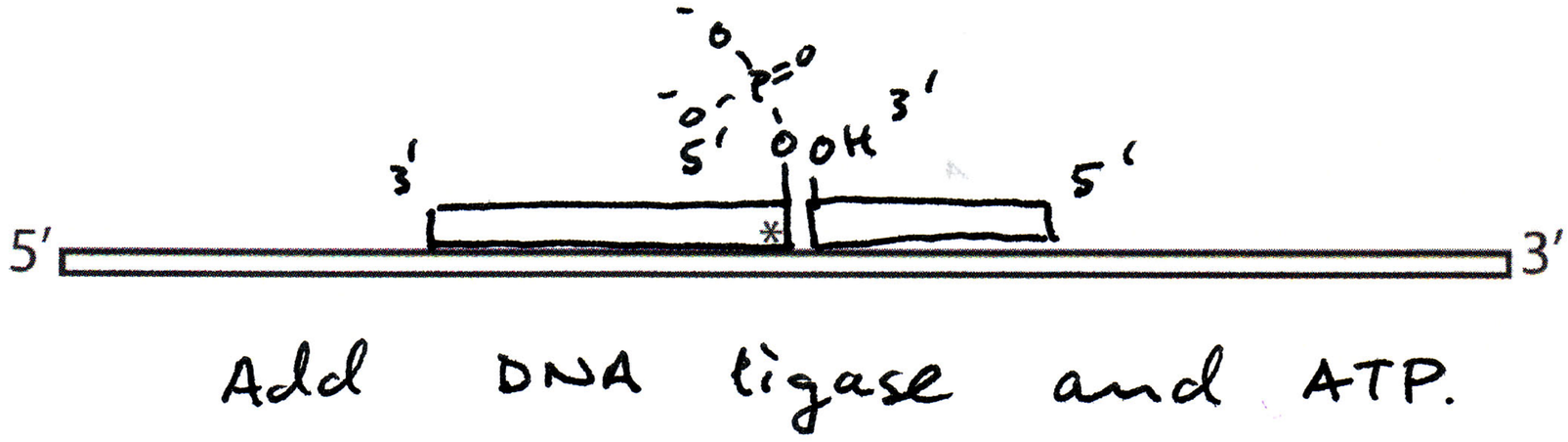


Structure of appropriate Sanger base:

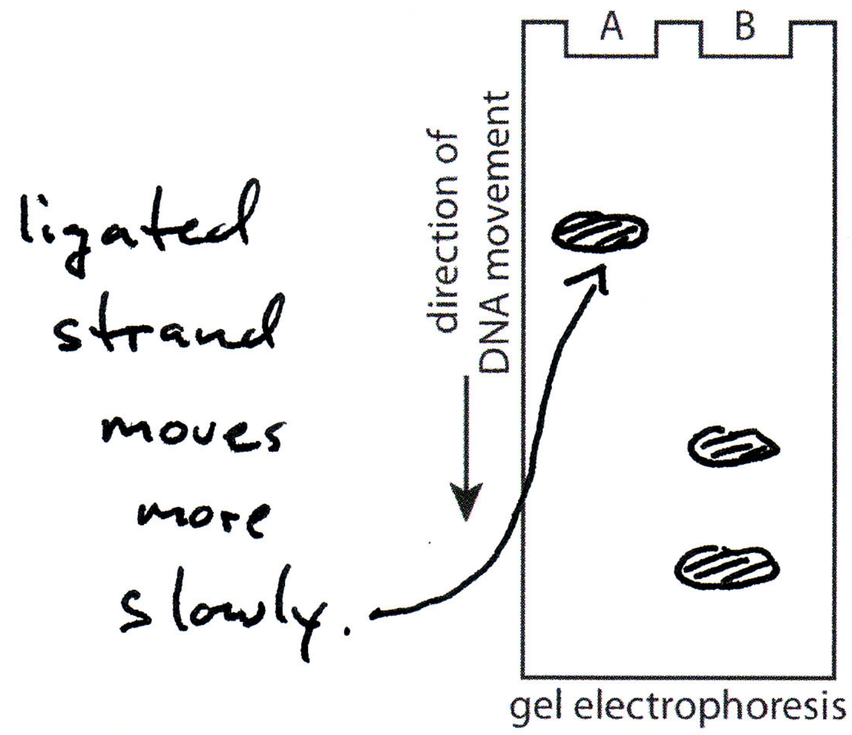


- You can also use a primer on the other strand with ddATT

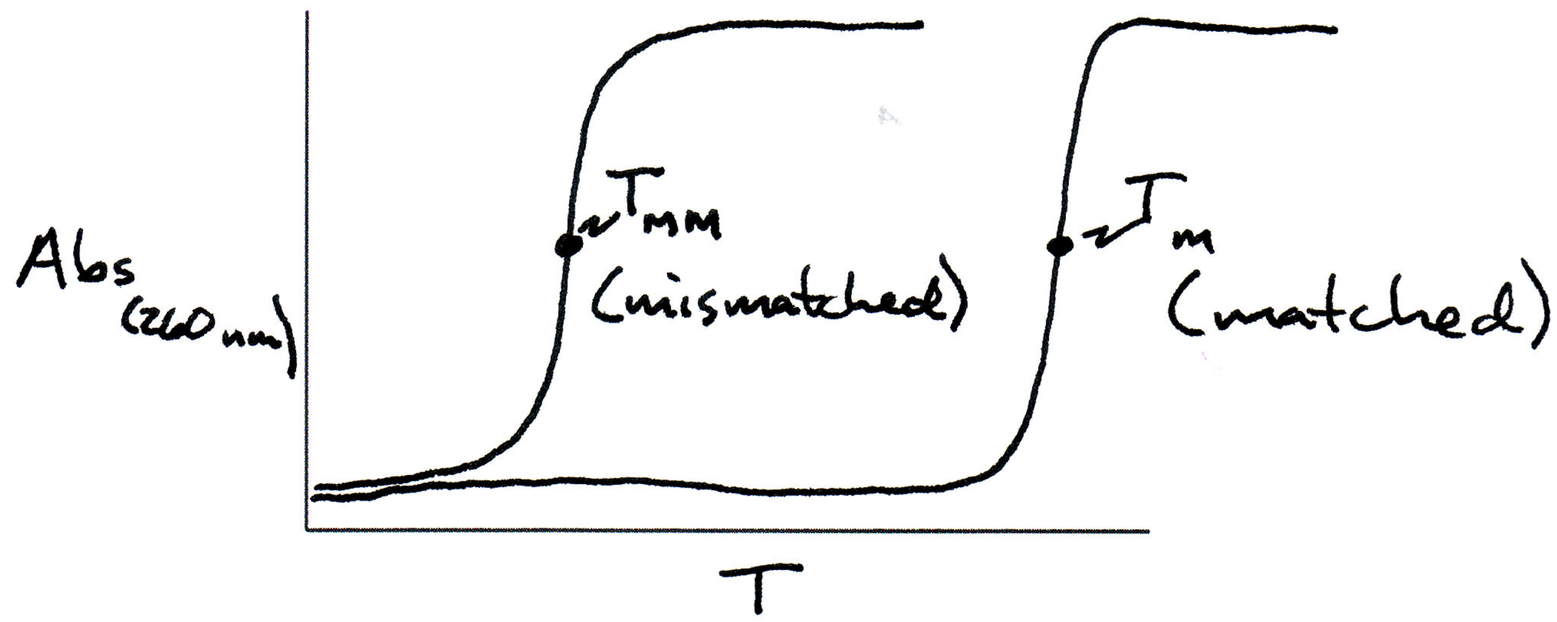
(b) DNA ligation can also be used to identify SNPs. In this method, one piece of synthetic DNA is designed to overlap the SNP site, plus a number of bases that are "upstream" from it. A second synthetic strand is also added, which pairs with the bases that are immediately "downstream" of the SNP site. Given this information, draw the locations of the synthetic strands on the *single stranded* DNA segment shown below. Next, draw the specific chemical functionalities that you would need to have on the ends of the synthetic strands to ligate them together. Finally, specify exactly what you would have to add to join them (6 points).



(c) Now let's use the method outlined in part (b) to compare two individuals. Assume that the DNA strand overlapping the SNP site was a perfect match for Individual A, but it did not match the site for Individual B. After running the experiment, the samples were loaded onto the gel shown below in the wells labeled 'A' and 'B'. After running the gel, draw what it would look like when the DNA is detected (6 points).

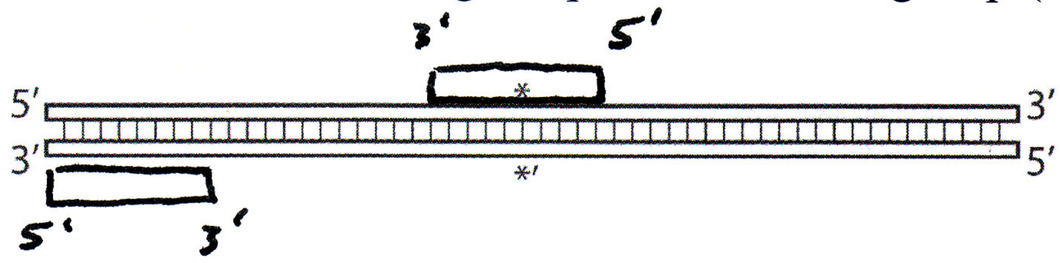


(d) SNPs can also be detected using PCR. In this method, a primer is prepared *such that the SNP site is located in the middle of the overlapping sequence*. One the set of axes shown below, draw a thermal denaturation (or "melting") curve for a 19-base primer that perfectly matches the DNA sample. Label the axes with the appropriate parameters for such an experiment. On the same graph, also draw the thermal denaturation curve that would correspond to a 19-base primer *that does not* match at the SNP site. Clearly label the two curves, and indicate where you would find the "melting temperatures" of each (10 points).



(please provide labels for axes)

(e) Using the DNA drawing provided below, briefly describe how you would run a PCR experiment to determine if the SNP site matched the primer. In your answer, specify what you would need to add to the reaction. Finally, use your answer for part (d) to indicate the temperature that you would need to use during the primer annealing step (8 points).



To run PCR, add
1) thermostable DNA polymerase
2) deoxynucleotide triphosphates
3) Mg^{2+}

Anneal primers to original DNA strands at a temp. between T_m & T_{MM}.

(f) Which of the methods described above would provide the highest degree of sensitivity for SNP detection? Briefly explain your choice (4 points).

PCR would be more sensitive because it amplifies the region containing the SNP.