Chapter 5:

Recombinant DNA Problems

1) Restriction Mapping
Draw restriction maps for parts (a) and (b).

a) A 12 kbp (kilobase-pair) circular DNA is digested by restriction enzymes, A and/or B:

Enzyme(s)	Fragment size(s) (in kilobase-pairs)
A	12
B	12
A + B	4, 8

b) A 12 kbp linear DNA is digested by restriction enzymes A and/or B:

Enzyme(s)	Fragment size(s) (in kilobase-pairs)
A	10, 2
B	9, 3
A + B	9, 2, 1

2) Restriction Enzyme Cutting Sites

Most restriction enzymes cut at sequences known as "DNA palindromes". These are different from normal palindromes (phrases or words that are identical when read forwards or backwards, for example "radar"); DNA palindromes read identically (when read 5' to 3') on both strands of DNA. For example, the restriction enzyme EcoRI cuts at 5' GAATTC 3' which looks like this as a double-stranded molecule:

```
5'-GAATTC-3'
   ||||||
3'-CTTAAG-5'
```

Notice that both strands read 5' GAATTC 3'. An example of a non-palindromic sequence is:

```
5'-CAGGAC-3'
   ||||||
3'-GTCCTG-5'
```

Notice that the two strands have different 5' ---> 3' sequences.

a) Which of the following sequences are DNA palindromes (sequences are written in 5' ---> 3' order)?

i) GACT ii) GGGCCC iii) GGGAAA

iv) GATC v) ATATAT vi) GTCGAC

b) Add nucleotides to the 3' ends of each of the following sequences to make them DNA palindromes (you may not change the given nucleotides, only add to the 3' end.)

i) AT ii) GCC iii) TAT

iv) CGT v) TGC vi) CGGC

3) Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a method for rapidly replicating a target sequence of DNA. You are given the following linear double-stranded DNA molecule as a template and two single-stranded primers for replicating a target sequence:
50 bp double-stranded DNA template sequence (gaps are for clarity; real DNA would be two continuous strands):

```
5'-TCCGATAAAT GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
   |||||||||| |||||||||| |||||||||| |||||||||| ||||||||||
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTAC-5'
```

primer sequences:

```
5'-TGCCCGCCGTAGTGC-3'

5'-ATGGCACCGATCCCG-3'
```

a) Draw out what the 50 bp strands would look like after they have been denatured, or separated, using heat and then hybridized with the primers. Be sure to include the primers, in the correct orientation, with the sequences with which they form hydrogen bonds.

b) Next, you use DNA polymerase to extend, or replicate, the DNA from the primer to the end of the template sequence. Add these sequences to your drawings of the template strands annealed to the primers. Underline these sequences to show that they are newly replicated.

c) You decide to do another round of replication. Draw the template and replicated strands after they have been heat-denatured and hybridized to more primers.

d) Draw in the replicated sequences polymerized in this second round and underline them.

e) Using a PCR machine which automatically changes the temperature of your tubes through many rounds of replication, you decided to replicate the DNA for 30 rounds. The initial concentration of your template is 10^{-14} moles per liter. Realizing that you double the amount of target DNA each round, what is the final concentration of target DNA?

f) How long (in base pairs) is the predominant product and its sequence after these 30 rounds?

4) Polymerase Chain Reaction II

The Polymerase Chain Reaction (PCR) is the process of amplifying a piece of DNA; it is based upon the process of DNA replication that occurs in all cells. The reaction requires a small amount of DNA, two appropriate oligonucleotides, a mixture of "dNTPs" (dATP, dGTP, dCTP, and dTTP; "d" stands for "deoxy" as in deoxyribose), buffer, and a thermostable polymerase called Taq polymerase. The reaction mixture is placed in a tube and put in a thermal cycler, a machine which can cycle the temperature of the tube (and consequently its contents). It is alternately like an oven and then like a refrigerator.

You have the following piece of template DNA and a number of oligonucleotides (a.k.a. oligos); short pieces of single-stranded DNA. These are shown below:

```
   1         10         20         30         40         50         60
   |    +    |    +    |    +    |    +    |    +    |    +    |
5' ACGTTGACATGGGCATCGAATTGCCCAACTGCAGGTCCTGCTATGCAGCAGATTACGATC 3'
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3' TGCAACTGTACCCGTAGCTTAACGGGTTGACGTCCAGGACGATACGTCGTCTAATGCTAG 5'
```

Oligonucleotides:

Oligo #1: 5′ ACGTTGACA 3′
Oligo #2: 5′ ATTGCCCAA 3′
Oligo #3: 5′ CGATGCCCA 3′
Oligo #4: 5′ TGCTGCATA 3′

a) Each of the oligos base-pairs (forms a complete double-stranded DNA duplex with all bases correctly paired) with a particular stretch of one strand of the template DNA shown above. For example, oligo #1 pairs with nucleotides 1 through 9 of the bottom strand of the template.

For each of the remaining oligos, state where and on which strand each pairs.

b) Each of the following pairs of oligonucleotides were reacted with the template DNA, dATP, dGTP, dTTP, dCTP, Taq DNA polymerase, and the appropriate buffers for many cycles in a PCR machine.

For each pair, state whether or not you would expect a PCR product to be produced. If yes, give the length in base-pairs and the first and last 5 base-pairs of the resulting PCR product. If no, explain why a PCR product would not be produced. Note: it may be helpful to draw out the first few cycles of the PCR reaction to see what would happen.

i) 1 + 2

ii) 1 + 4

iii) 2 + 3

iv) 2 + 4

c) You synthesize another oligo, oligo 5: 5′ ACG**G**TGACA 3′ which pairs with nucleotides 1 through 9 of the bottom strand with one mismatch (shown **bold-underlined**). If you react template DNA with oligos 3 and 5, you get an 18 bp PCR product. What is the sequence of that product?