Solutions to Recombinant DNA Problems

1) One important part of this problem is to remember that:
   - When you cut **linear** DNA, you get one more DNA fragment than the number of cuts. For example, a single cut results in two pieces. Picture cutting a straight piece of string; if you cut it once, you get two pieces.
   - When you cut **circular** DNA, you get the same number of fragments as the number of cuts. For example, a single cut results in one piece. Picture cutting a loop of string; if you cut it once, you get only one piece.

a) One way to work this out is – remember that this is a circular DNA:
   1. Since you get only one full-length fragment with A and the DNA is circular, A must cut only once. This gives the partial map:

   ![Diagram of circular DNA cut by A](image)

   - 12kbp
   - A

   2. Since you get only one full-length fragment with A and the DNA is circular, A must cut only once. This gives the partial map:

   ![Diagram of circular DNA cut by A and B](image)

   - 12kbp
   - A
   - B

   3. The only remaining question is the relationship between the A and B sites. Since the “double digest” of both A and B gives 4 and 8 kbp fragments, the A and B sites must be both 4 and 8 kbp apart. How is this possible? Since the DNA is a circle, the distance from A to B is 4kbp going one way and 8kbp going the other way. This gives the complete map:

   ![Diagram of circular DNA cut by A and B](image)

   - 4kbp
   - A
   - 8kbp
   - B

b) It is best to do this piece-by-piece – remember that this is a linear DNA:
   1. Since you get two fragments from treatment with A, A must cut only once and the partial map must be:

   ![Diagram of linear DNA cut by A](image)

   - 10kbp
   - 2kbp
   - A
2. Since you get two fragments with B, B must also cut only once and the partial map must be:

```
      9kbp     3kbp
      |         |
   ____|_______
```

3. Putting them together leaves two possible arrangements that are consistent with the single-enzyme digests:

I) where the A and B sites are on the same end of the molecule:

```
      9kbp     1kbp     2kbp
      |         |         |
   ____|____|____
```

   treating this with A and B would give 9, 2, and 1 kbp pieces

II) where the A and B sites are on different ends of the molecule:

```
      3kbp     7 kbp     2kbp
      |         |         |
   ____|____|____
```

   treating this with A and B would give 7, 3, and 2 kbp pieces

the results are only consistent with version I – so that’s the correct map.

2) a) Sequences (ii), (iv), (v), and (vi) are DNA palindromes.

   b) i) ATAT ii) GCCGGC iii) TATATA

   iv) CGTACG v) TGCGCA vi) CGGCCGCCG

3) a) upper strand with annealed primer (spaces added for clarity; there would not be any gaps in the real DNA):

```
5' -TCCGATAAAT GCCCGCGTGA GTGC GGCAAC GGC GaGGgAT CGGTCCCATG-3'  
   |          |          |          |         |         |
3' -GCCCTA GCCACGGTA-5'
```

lower strand with annealed primer:

```
3' -AGGCTATTTA CGGGCGCAT CACGCGTTTG CCGCGCCCTA GCCACGGGTAC-5'  
   |          |          |          |          |          |
5' -T GCCCGGGC GTA GTGC-3'
```
b) Newly replicated sequences are underlined.

upper strand:

5′-TCCGATAAAT GCCCCCGCTA GTGCCGCACAC GGCACGGGAT CGGTGCCATG-3′

3′-AGGCTATTTTA CACGCCGGGT GCGGCCTTA GCCACGGTA-5′

lower strand:

3′-AGGCTATTTTA CACGCGCCCTA GCCACGGTA-5′

5′-T GCCCGCCCTA GTGCCGCACAC GGCACGGGAT CGGTGCCATG-3′

c) original template strands with annealed primers:

upper strand:

5′-TCCGATAAAT GCCCCCGCTA GTGCCGCACAC GGCACGGGAT CGGTGCCATG-3′

3′-GCCCTA GCCACGGTA-5′

lower strand:

3′-AGGCTATTTTA CACGCGCCCTA GCCACGGTA-5′

5′-T GCCCGCCCTA GTGCCGCACAC GGCACGGGAT CGGTGCCATG-3′

primers annealed to strands polymerized in first round:

5′-T GCCCGCCCTA GTGC-3′

3′-AGGCTATTTTA CACGCGCCCTA GCCACGGTA-5′

3′-GCCCTA GCCACGGTA-5′

5′-T GCCCGCCCTA GTGC-3′

Page 3 of 5
d) Newly replicated sequences are **underlined**.

- from original template strands:

**upper strand:**

5' -TCCGATAAAT GCCCCCGTA GTGCGGCAAC GGCUGCAGAT CGGTGCCATG-3'

3' -AGGCTATTTA CGGGCGGCAT CACGCCGTTG CGCGCCCTAT GCCACGGTA-5'

**lower strand:**

3' -AGGCTATTTA CGGGCGGCAT CACGCCGTTG CGCGCCCTAT GCCACGGTAC-5'

5' -T GCCGCGCCGTA GTGCCGCAAC GGCUGCAGAT CGGTGCCATG-3'

- from strands polymerized in first round:

5' -T GCCGCGCCGTA GTGCCGCAAC GGCUGCAGAT CGGTGCCATG-3'

3' -AGGCTATTTA CGGGCGGCAT CACGCCGTTG CGCGCCCTAT GCCACGGTA-5'

3' - A CGGGCGGCAT CACGCCGTTG CGCGCCCTAT GCCACGGTA-5'

5' -T GCCGCGCCGTA GTGCCGCAAC GGCUGCAGAT CGGTGCCATG-3'

e) \(2^{30} \times 10^{-14} \text{ M} = 1.07 \times 10^{-5}\) moles per liter

f) 40 bp

predominant product:

3' -ACGGCGGCCAT CACGCCGTTG CGCGCCCTAT GCCACGGTA-5'

5' -TGCCGCGCCGTA GTGCCGCAAC GGCUGCAGAT CGGTGCCATG-3'

note that it is only the region containing the primers.
4) a) Oligo #2 pairs with nucleotides 20-28 on the bottom strand
Oligo #3 pairs with nucleotides 18-10 on the top strand
Oligo #4 pairs with nucleotides 50-42 on the top strand

b) i) No PCR product will be produced because both primers pair with the bottom strand of DNA.

   ii) A 50 bp PCR product will be produced with the following sequence:
       
       5’ ACGTT...........CAGCA  3’
       
       3’ TGCAA...........GTCGT  5’

   iii) No PCR product will be produced because the following structures will result after
       the first round of polymerization:

       ![Diagram of polymerization](image.png)

   iv) A 31 bp PCR product will be produced with the following sequence:
       
       5’ ATTGC...........CAGCA  3’
       
       3’ TAACG...........GTCGT  5’

c) Since the PCR product contains the oligos, it will have the sequence of the oligo and not the
   template at any mismatches. Therefore, the PCR product will be:

   5’ ACGGTGACATGGGCATCG  3’
   
   3’ TGCCACTGTACCCGTAGC  5’