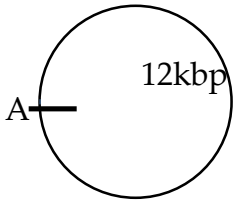
# Solutions to Recombinant DNA Problems

**1)** One important part of this problem is to remember that:

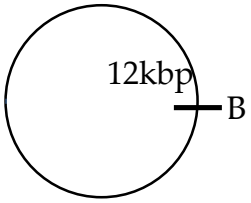
- When you cut <u>linear</u> DNA, you get one more DNA fragment than the number of cuts. For example, a single cut results in two pieces. Picture cutting a straight piece of string; if you cut it once, you get two pieces.
- When you cut <u>circular</u> DNA, you get the same number of fragments as the 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:

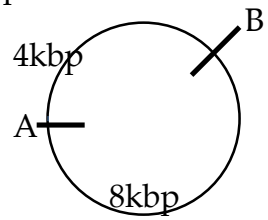
1. Since you get only one full-length fragment with A and the DNA is circular, A must cut only once. This gives the partial map:




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:

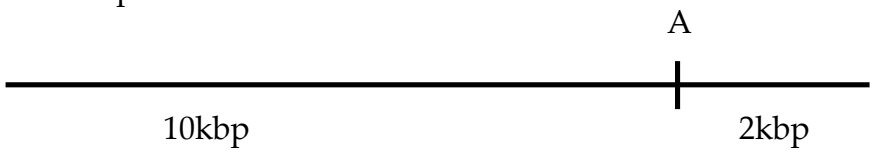



3. The only remaining question is the relationship between the A and B sites. Since the "double digest" of both A and B gives 4 and 8 kbp fragments, the A and B sites must be both 4 and 8 kbp apart. How is this possible? Since the DNA is a circle, the distance from A to B is 4kbp going one way and 8kbp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

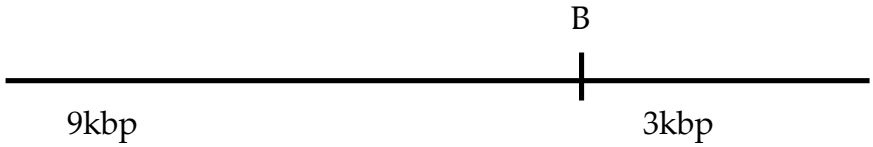



b) It is best to do this piece-by-piece – remember that this is a linear DNA:

1. Since you get two fragments from treatment with A, A must cut only once and the partial map must be:

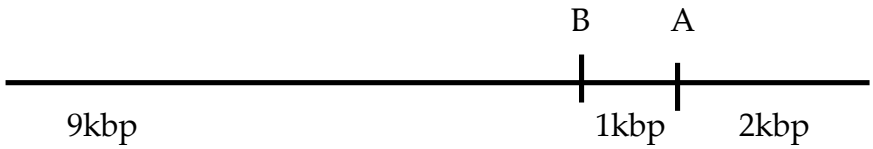


2. Since you get two fragments with B, B must also cut only once and the partial map must be:



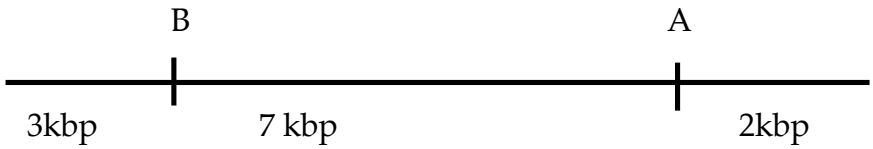

3. Putting them together leaves two possible arrangements that are consistent with the single-enzyme digests:

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




   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:




   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) AT**AT** | ii) GCC**GGC** | iii) TAT**ATA** |
| iv) CGT**ACG** | v) TGC**GCA** | vi) CGGC**GCCG** |

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

```
5'-TCCGATAAAT GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
                                          |||||| |||||||||
                                    3'-GCCCTA GCCACGGTA-5'
```

lower strand with annealed primer:

```
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTAC-5'
            | ||||||||||| ||||
        5'-T GCCCGCCGTA GTGC-3'
```

b) Newly replicated sequences are underlined.

upper strand:

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

lower strand:

```
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTAC-5'
            | |||||||||| |||||||||| |||||||||| ||||||||||
         5'-T GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
```

c) original template strands with annealed primers:

upper strand:

```
5'-TCCGATAAAT GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
                                        |||||| |||||||||
                                   3'- GCCCTA GCCACGGTA-5'
```

lower strand:

```
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTAC-5'
            | |||||||||| ||||
         5'-T GCCCGCCGTA GTGC-3'
```

primers annealed to strands polymerized in first round:

```
         5'-T GCCCGCCGTA GTGC-3'
            | |||||||||| ||||
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTA-5'


                              3'-GCCCTA GCCACGGTA-5'
                                 |||||| |||||||||
5'-T GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
```

d) Newly replicated sequences are underlined.

-from original template strands:

upper strand:

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

lower strand:

```
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTAC-5'
            | |||||||||| |||||||||| |||||||||| ||||||||||
         5'-T GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
```

-from strands polymerized in first round:

```
         5'-T GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCAT-3'
            | |||||||||| |||||||||| |||||||||| |||||||||
3'-AGGCTATTTA CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTA-5'
```

```
  3'- A CGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTA-5'
      | |||||||||| |||||||||| |||||||||| |||||||||
   5'-T GCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCATG-3'
```

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

f) 40 bp

predominant product:

```
     3'-ACGGGCGGCAT CACGCCGTTG CCGCGCCCTA GCCACGGTA-5'
        |||||||||| |||||||||| |||||||||| |||||||||
     5'-TGCCCGCCGTA GTGCGGCAAC GGCGCGGGAT CGGTGCCAT-3'
```

note that it is only the region containing the primers.

**4)**

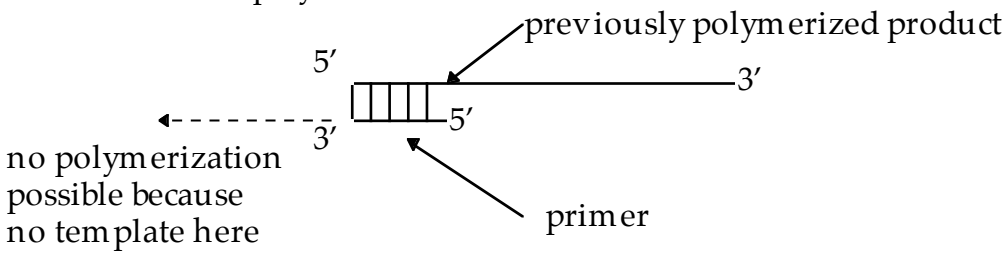
a) Oligo #2 pairs with nucleotides 20-28 on the bottom strand
Oligo #3 pairs with nucleotides 18-10 on the top strand
Oligo #4 pairs with nucleotides 50-42 on the top strand

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:




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′
```