Problem 1. What would the expected effect be on a PCR reaction if the primers used were shorter than the intended oligonucleotide sequences?

a) The PCR reaction would not commence

b) The PCR reaction would end after one cycle

c) The reaction would generate a single short PCR product

d) The reaction would yield a mixture of non-specific products

Problem 2.

Consider the DNA sequence below.

a. Design primers (16 nts long) that will allow to obtain a PCR product with 400 pbs.
b. Design a PCR program using the Taq enzyme (1000 pbs/min).

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    GGACCGCGGGGCAGGATTGCTCCGGGCTGTTTCATGACTTGTCAGGTGGGATGACTTGGATGGAAAAGTAGAAGGTCATG
  1 +---------+---------+---------+---------+---------+---------+---------+---------
    CCTGGCGCCCCGTCCTAACGAGGCCCGACAAAGTACTGAACAGTCCACCCTACTGAACCTACCTTTTCATCTTCCAGTAC

    GGGTGGCCAACTTGGGCGAGAAAAGGTATATAAAGGTCTCTTGCTCCCATCAACTGCCTCAAAAGTAGGTATTCCAGCAG
 81 +---------+---------+---------+---------+---------+---------+---------+---------
    CCCACCGGTTGAACCCGCTCTTTTCCATATATTTCCAGAGAACGAGGGTAGTTGACGGAGTTTTCATCCATAAGGTCGTC

    ATCAGACAACGTCAGGTGGGAGGACTTGGACGGAAAAGTAGAAGGTCAAGACCAACCTCTTCCAATCCAACCACAAACAA
161 +---------+---------+---------+---------+---------+---------+---------+---------
    TAGTCTGTTGCAGTCCACCCTCCTGAACCTGCCTTTTCATCTTCCAGTTCTGGTTGGAGAAGGTTAGGTTGGTGTTTGTT

    AAAATCAGCCAATATGTCCGACTTCGAGAACAAGAACCCCAACAACGTCCTTGGCGGACACAAGGCCACCCTTCACAACC
241 +---------+---------+---------+---------+---------+---------+---------+---------
    TTTTAGTCGGTTATACAGGCTGAAGCTCTTGTTCTTGGGGTTGTTGCAGGAACCGCCTGTGTTCCGGTGGGAAGTGTTGG

    CTAGTATGTATCCTCCTCAGAGCCTCCAGCTTCCGTCCCTCGTCGACATTTCCTTTTTTTTCATATTACATCCATCCAAG
321 +---------+---------+---------+---------+---------+---------+---------+---------
    GATCATACATAGGAGGAGTCTCGGAGGTCGAAGGCAGGGAGCAGCTGTAAAGGAAAAAAAAGTATAATGTAGGTAGGTTC
```

Problem 3. A researcher was trying to design a PCR experiment. The sequence he wished to amplify is part of a gene *murF* of *Staphylococcus aureus*, that is given below.
5’ GCATGTCAATTGGGCCTATATGGCGTAGCAATTTGGCCGGCTATATGGCCGTAGC 3’
3’ CCTACAGTTAACCCGGATATACCGCATCGTTAAACCGGCCGATATACCGGCATCG 5’
The researcher decided he could only afford to use primers of 6 bases in length.

a) Write down the sequence of the two primers he should order to be made.

b) The scientist carried out the experiment, using appropriate primers of 6 bases in length and a plasmid with the gene *murF* cloned. He then separated the pieces of DNA he had amplified. What technique would he use to separate the amplified pieces of DNA?

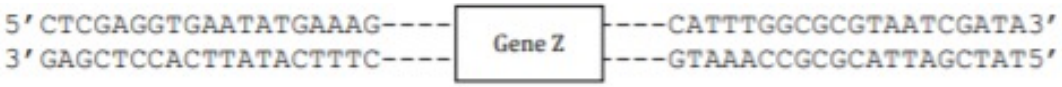
c) The scientist obtained **two** bands of amplified DNA. Explain why at least two bands of amplified DNA would be obtained in the above experiment.

d) What is the size of the 2 bands?

e) When the scientist carried out the experiment using as template genomic DNA from *Staphylococcus aureus*, he counted 8 bands. Explain why he obtained so many more bands.

f) Suggest how he could improve this experiment to obtain more reliable results.

Problem 4. The following is the DNA sequence of Gene Z that you want to amplify by PCR.



a) Which set of primers from the options below, would you use for the PCR reaction?

Primer Fw
1: 5’-GAGCTCCACTTATA-3’
2: 5’-AGGTGAATATGAAA-3’
3: 5’-GAAAGTATAAGTGG-3’
4: 5´-TTCATATTCACCTC-3´

Primer Rv
1: 5’-ATAGCTAATGCGCG-3’
2: 5´-CCGCGCATTAGCTA-3´
3: 5’-TATCGATTACGCGC-3’
4: 5’-TGGCGAGTAATCGATA-3’

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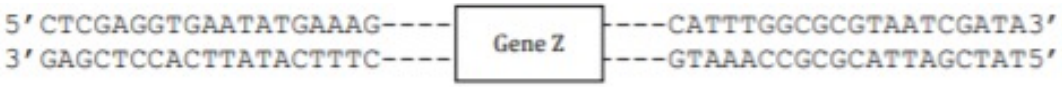

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Primer Rv
1: 5’-ATAGCTAATGCGCG-3’
2: 5´-CCGCGCATTAGCTA-3´
3: 5’-TATCGATTACGCGC-3’
4: 5’-TGGCGAGTAATCGATA-3’

b) What set of primers from the options below, would you use for the PCR reaction? Order them by preference

Set 1: 5’-GGTGAATATGAAAG-3’ and 5’-TATCGATTACGCGC-3’
Set 2: 5’-AGGTGAATATGAAA-3’ and 5’-TATCGATTACGCGC-3’
Set 3: 5’-TCGAGGTGAATATG-3’ and 5’-TATCGATTACGCGC-3’
Set 4: 5’-CTCGAGGTGAATAT-3’ and 5’-TATCGATTACGCGC-3’

Problem 5. What will be the result of a PCR amplification using the following set of primers? Choose from the hypotheses given.

5’ ACTTCGTTCGCCGGGGCTCGATCGATATTTGGAAT 3’

3’ TGAAGCAAGCGGCCCCGAGCTAGCTATAAACCTTA 5’

Primer 1: 5’-GTTC-3’
Primer 2: 5’-GCCC-3’
Primer 3: 5’-TATT-3’
Primer 4: 5´-TAGC-3´
Primer 5: 5´- GGAA-3´
Primer 6: 5 – ATTC-3´

	PCR result
Primers 1+2	
Primers 1+3	
Primers 2+3	
Primers 1+4	
Primers 1+2	

Amplification hypotheses:

1. no amplified product because the primers face in opposite directions.
2. no amplified product because the primers bind to the same strand.
3. A product is amplified.
4. No product because one of the primers does not hybridize.