BMC – Exercicios

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Conteúdo

Questão 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

RS d)

Questão 2

Consider the DNA sequence below.

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

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

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

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

    CTAGTATGTATCCTCCTCAGAGCCTCCAGCTTCCGTCCCTCGTCGACATTTCCTTTTTTTTTCATATTACATCCATCCAAG
321 +---------+---------+---------+---------+---------+---------+---------+---------
    GATCATACATAGGAGGAGTCTCGGAGGTCGAAGGCAGGGAGCAGCTGTAAAGGAAAAAAAAAGTATAATGTAGGTAGGTTC
```

Q2 a.

Design primers (16 nts long) that will allow to obtain a PCR product with 400 pbs.

A sequencia dada contem exatamente 400 pbs requerindo buscar as 16 primeiras e ultimas bases para os primers

Primer:
```
1 GGAC CGCG GGGC AGGA
```

Reverse:
```
1 AGGC AGGG AGCA GCTG
```

Q2 b.

Design a PCR program using the Taq enzyme (1000 pbs/min).

Melting and Annealing temperature

$$T_m \cong \frac{(4*(13)+2*(3))+(4*(11)+2*(5))}{2} = 56$$

$$T_a \cong T_m - 4 \cong 52$$

n	T/°C	t/min	Phase
1	94	10	Initial Desnaturation
2	94	30	Desnaturation
3	52	30	Annealing
4	72	60	Extension
5	72	10	Final Extension

Questão 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. The researcher decided he could only afford to use primers of 6 bases in length.

```
5’
   1 GCATGT CAATTG GGCCTA TATGGC GTAGCA ATTTGG CCGGCT
  43 ATATGG CCGTAG C
3’
3’
   1 CCTACA GTTAAC CCGGAT ATACCG CATCGT TAAACC GGCCGA
  43 TATACC GGCATC G
5’
```

Q3 a.

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

Para expandir essa sequencia ele deve escolher as 6 primeiras e ultimas bases

Fw:

```
1 GCATGT
```

Bw:

```
1 GCATCG
```

Q3 b.

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

Electrophoresis could be used to separate the strands of DNA

Q3 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.

The backwards primer can attach in two different sites on the gene, generating the different bands

Q3 d.

What is the size of the 2 bands?

55 nts and 29 nts

Q3 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.

Even tho the primers have only three matching sites (2 for the reverse) there is still others similar sites that can be avalible in the right conditions generating the other bands

Q3 f.

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

- Increase the annealing temperature, thus diminishing the possibility of primers bindind to wrong sites.
- Setting the optimal expanding time so that bands of greater size than our sequence cant be expanded.
- and if its not enough consider using a larger primer, there will be a compromise in efficiency in promise of precision.

Questão 4

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

Q4 a.

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

Primer Fw:

a. `1 AGGTG AATAT GAAA`
b. `1 GAGCT CCACT TATA`
c. `1 GAAAG TATAA GTGG`
d. `1 TTCAT ATTCA CCTC`

Primer Rv:

a. `1 CCGCG CATTA GCTA`
b. `1 ATAGC TAATG CGCG`
c. `1 TATCG ATTAC GCGC`
d. `1 TGGCG AGTAA TCGAT A`

RS: Fw: a), Bw: c)

Q4 b.

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

Set 1: `5'-GGTGA ATATG AAAG-3'` and `5'-TATCG ATTAC GCGC-3'`

Set 2: `5'-AGGTG AATAT GAAA-3'` and `5'-TATCG ATTAC GCGC-3'`

Set 3: `5'-TCGAG GTGAA TATG-3'` and `5'-TATCG ATTAC GCGC-3'`

Set 4: `5'-CTCGA GGTGA ATAT-3'` and `5'-TATCG ATTAC GCGC-3'`

RS: 3,4,1,2

1. 5/12 CG, Has as G at the end
2. 4/12 CG, some kind of CG clamp at start
3. 6/12 CG, has at least one CG in the last 4 bases
4. 6/12 CG, Has high CG but no CG clamp at end

Questão 5

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

```
5’ 1 ACTTC GTTCG CCGGG GCTCG ATCGA TATTT GGAAT 3’
3’ 1 TGAAG CAAGC GGCCC CGAGC TAGCT ATAAA CCTTA 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'`

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.

Primers	PCR Result
1+2	3
1+3	2
2+3	1
1+4	4
1+2	3