Ficha de exercícios 7 – BMC – Módulo de Biologia Molecular

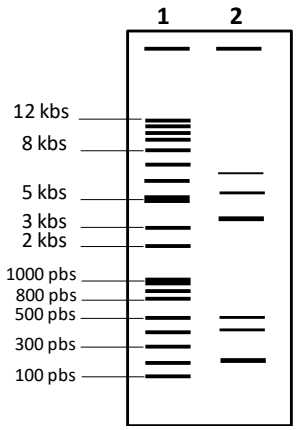
Problem 1. What would be the effect on the PCR reaction if any of the following circumstances arose:

1) there are no primers in the reaction
2) there are no dNTPs in the reaction
3) there is no Taq polymerase in the reaction?

a) PCR would proceed normally
b) Non-specific PCR of random templates will occur
c) The reaction will cease after a few cycles
d) The PCR reaction will not commence

Problem 2. What would the generally expected effect on the PCR reaction be of adjustments that increase the temperature of the annealing phase and reduce the length of the elongation phase?
a) Precision and yield will be reduced
b) Precision will be reduced, but yield will be increased
c) Precision will be increased, but yield will be reduced
d) Precision and yield will be increased

Problem 3.
You have amplified by PCR a *Saccharomyces cerevisae* (Genomic GC content 38.3%) DNA sequence of 3500 pbs to clone the gene of a-amylase in an expression vector using as host the strain *E. coli* DH5a.



1: molecular weight marker
2: PCR amplification sample

The PCR program used was:
95ºC-5´
95ºC-30´´
50ºC-1´
72ºC-3´
x 30 ciclos
72ºC-10´

a. Identify the function of each step of the PCR program and which compounds in the reaction mixture are being used or modified in each step.

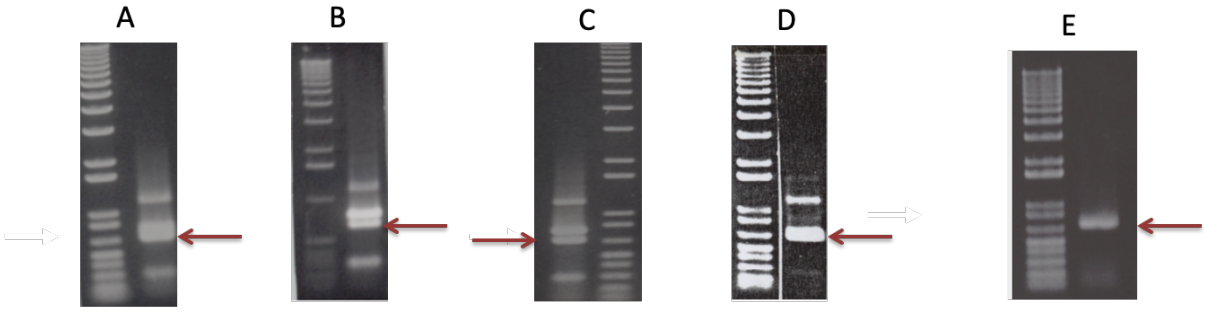
Tº	Time	Step	Compounds used/modified
95ºC	5 min		
95ºC	30 s		
50ºC	1 min		
72ºC	3 min		
72ºC	10 min		

b. The amplification product was analized by agarose gel electrophoresis. Identify the band that corresponds to the fragment we are trying to amplify. Explain why the other bands appear.

c. Propose one alteration to the PCR program that will reduce or prevent the amplification of the unwanted bands.

d. You want to amplify the DNA containing the a-amylase homolog of *Streptomyces coelicolor* (Genomic GC content 72%) using the same pair of primers. How would you change the PCR program?

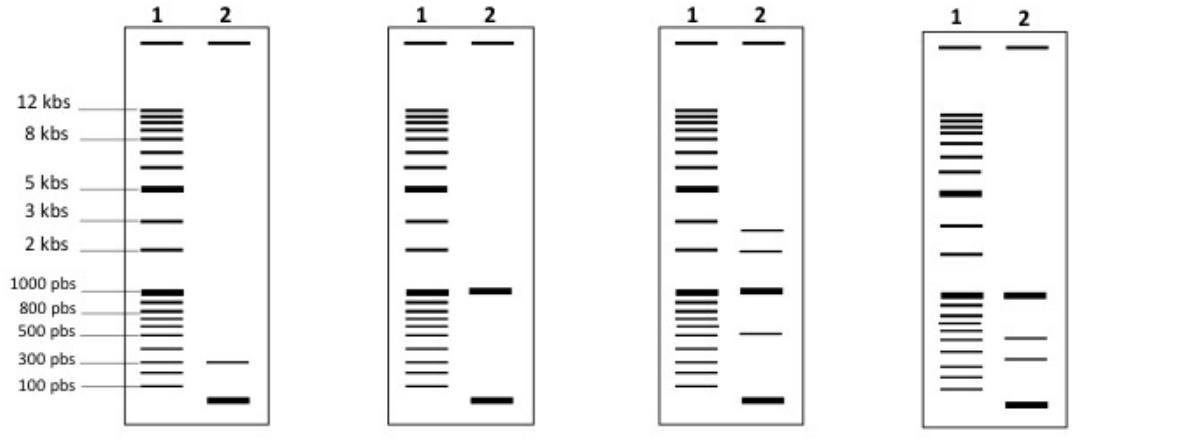
Problem 4.
You want to amplify by PCR a 0.65 Kb DNA sequence from the genome of *E. coli*. The desired DNA band is identified by an arrow.



A	B	C	D	E
95ºC-5´	95ºC-5´	95ºC-5´	95ºC-5´	95ºC-5´
95ºC-30´´	95ºC-30´´	95ºC-30´´	95ºC-30´´	95ºC-30´´
53ºC-1´	53ºC-1´	53ºC-1´	53ºC-1´	53ºC-1´
72ºC-3´	72ºC-3´	72ºC-3´	72ºC-3´	72ºC-3´
72ºC-10´	72ºC-10´	72ºC-10´	72ºC-10´	72ºC-10´

b. Besides altering program temperature, what other parameter have we altered that resulted in the sample analysed in gel E?

Problem 5.
You have amplified by PCR a DNA fragment of 1000 pbs using Taq polymerase (1 kb/min).
You have analysed the amplification product in a agarose gel.
Lane 1: DNA molecular weight ladder;
Lane 2: PCR sample.
Consider the following 4 experimental results and associate to the respective PCR program.



Programa A

94ºC	10´	
94ºC	30´´	30 ciclos
52ºC	30´´	
72ºC	3´	
72ºC	10´	

Programa B

94ºC	5´	
94ºC	30´´	
55ºC	30´´	40 ciclos
72ºC	60´´	
72ºC	10´	

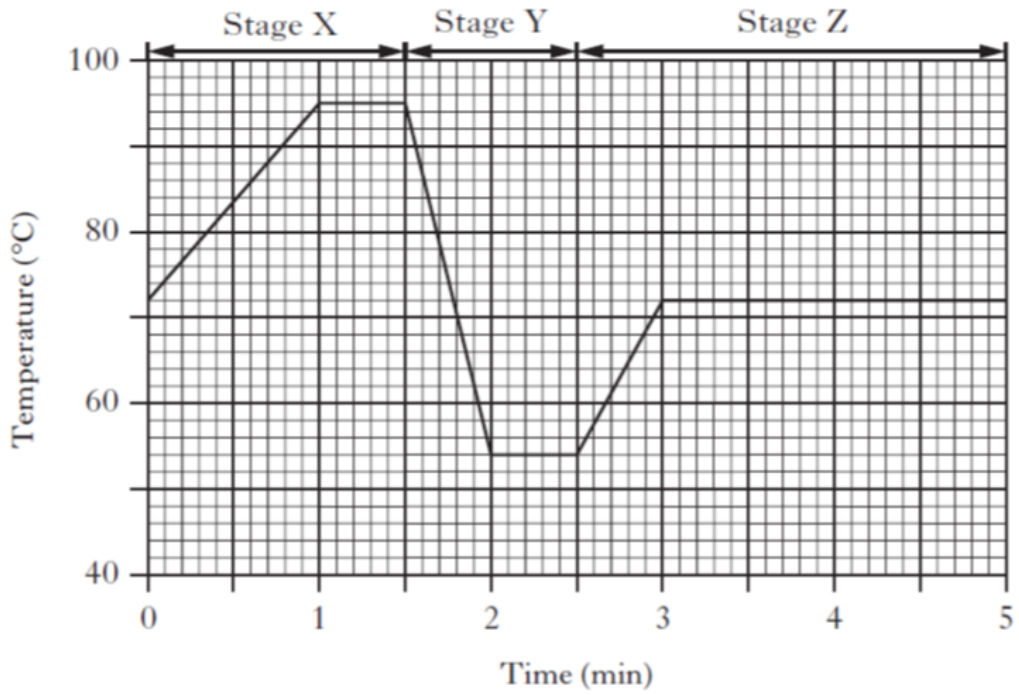
Programa C

94ºC	10´	
94ºC	30´´	
60ºC	30´´	30 ciclos
72ºC	30´´	
72ºC	10´	

Programa D

94ºC	10´	
94ºC	30´´	
62ºC	30´´	40 ciclos
72ºC	60´´	
72ºC	5´	

Problem 6. The graph below shows how the temperature of the DNA in a reaction tube is changed during one PCR cycle.



a) Calculate the maximum change in temperature that the reaction tube experiences during one cycle of PCR.
b) Describe what happens to the DNA during stage X.
c) Short sections of DNA called primers are involved in Stage Y.
State what happens to these primers during Stage Y.
d) Suggest why the temperature is increased during Stage Z.
e) A forensic scientist discovered a tiny spot of blood at a crime scene. A sample taken from this spot contained 100 molecules of DNA. The sample underwent PCR cycles for 40 minutes.
(i) Use the graph to calculate how many molecules of DNA would be present after this time.
(ii) What process would then allow an individual to be identified from the DNA?