Høgskolen i Telemark

Fakultet for allmennvitenskapelige fag

EXAMINATION

4328/9002 1
Applied Genetics

19.02.2015

Time: 3 hours

Language: English, you may answer in Norwegian or English

Pages : 3

Aids: none

Notes: Part A and part B count 50 % each

Attachments: *none*

The examination results will be made available at StudentWeb

ENGLISH

Part A

1. Why is the integrity (quality) of DNA important?

2. What are microsattelites?

3. What is strand slippage, and why is that important for microsatellite instability?

4. What are the differences between a SNP and a length polymorphism?

5. What is the difference between dNTP and ddNTP and why is that important?

6. Describe the equipment and materials needed to amplify DNA by PCR?

7. How does an Allele-discrimination (AD-assay™) PCR work?

8. Why is it important to use positive and negative controls in PCR?

Part B

Question 1

You are employed at a company offering genetic tests for pets. A customer has sent you 6 DNA samples from different dogs and want you to test for a particular heart disease. This heart disease is caused by a mutation in the HEART gene. The most common mutation is a length mutation (deletion) but single nucleotide mutation may also cause the disease. These are dominant mutations that only require a mutation in one allele (either length mutation or single nucleotide mutation that cause a stop codon) to cause the disease.

The first thing you need to do is a PCR of the HEART gene and you find primers for the region of interest in some articles. The normal length of the PCR product is 300bp but the disease allele is 200 bp. The single nucleotide mutation can be in several places, but all within the amplified region of the gene. The primer sequences are:

Forward- 5`- AGCGCTTGTCGTTGCAAGTA-`3

Reverse – 5`-GCTGCACATCTGCGTACTGA-`3

After PCR you use an acrylamide gel to check the results (figure 1).

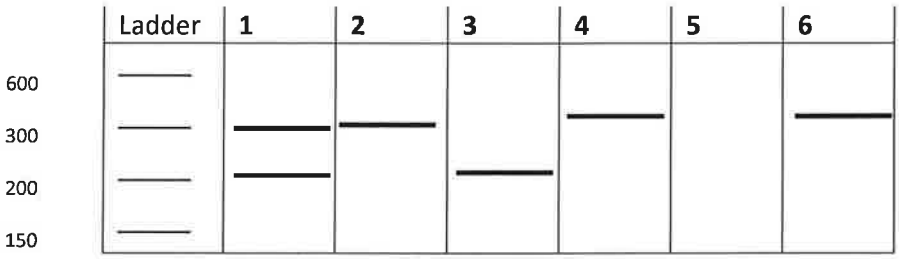

Figure 1. Results from PCR of the HEART gene of DNA from dogs.

a) What would be a good PCR program for this analysis (write all the steps with appropriate temperatures and no of cycles?

b) What informations can you give the owner for each dog based on this result?

c) Which dog (no 1-6) need to have more genetic testing. Describe the methods you would use to get the best information of the HEART gene?

d) You have one negative result (dog no 5). What can you do to check if there is something wrong with the DNA sample?

Question 2

a) What are the advantages of real time PCR over conventional PCR?

b) Describe the physical and biochemical processes underlying the function of a TaqMan probe.

c) In real time PCR, one sometimes encounters 'crossing curves' whose slope is flatter than that of the other curves. What is the cause of crossing curves?

d) What factors determine the melting temperature of a PCR product?