National Exams December 2015

04-Bio-B10, Analytical Biochemistry

3 hours duration

NOTES:

1. If doubt exists as to the interpretation of any question, the candidate is urged to submit with the answer paper, a clear statement of any assumptions made.

2. This is a CLOSED BOOK EXAM.
   Any non-communicating calculator is permitted.

3. FIVE (5) questions constitute a complete exam paper.
   The first five questions as they appear in the answer book will be marked.

4. Each question is of equal value.

5. Most questions require an answer in essay format. Clarity and organization of the answer are important.

Q	Mark
1	/20
2	/20
3	/20
4	/20
5	/20
Total	/100

Marking Scheme:

Question 1: (20 marks total) Polyacrylamide Gel Electrophoresis (PAGE)

a. (6 marks)
   i. (3 marks)
   ii. (3 marks)
b. (6 marks)
c. (4 marks)
d. (4 marks)

Question 2: (20 marks) Polymerase Chain Reaction (PCR)

a. (4 marks)
b. (4 marks)
c. (4 marks)
d. (4 marks)
e. (4 marks)

Question 3: (20 marks total) Flow Cytometry

a. (5 marks)
b. (5 marks)
c. (10 marks)

Question 4: (20 marks total) Affinity Chromatography

a. (5 marks)
b. (5 marks)
c. (3 marks)
d. (7 marks)

Question 5: (20 marks total) Fourier Transform Infrared (FTIR) Spectroscopy

a. (10 marks)
b. (3 marks)
c. (7 marks)

Question 6: (20 Marks Total) Atomic Force Microscopy (AFM)

a. (6 marks)
b. (4 marks)
c. (2 marks)
d. (2 marks)
e. (6 marks)

Question 1: (20 marks total) Polyacrylamide Gel Electrophoresis (PAGE)

A technician has decided to run two protein samples side by side (Figure 1) using a native PAGE approach (without sodium dodecyl sulfate or a reducing agent like b-mercaptoethanol). In the first sample, the technician expects to find a green fluorescent protein variant (eGFP, MW= 32.7 kDa). In the second, the technician expects to find the red fluorescent protein, DsRed (MW= 27.6 kDa).

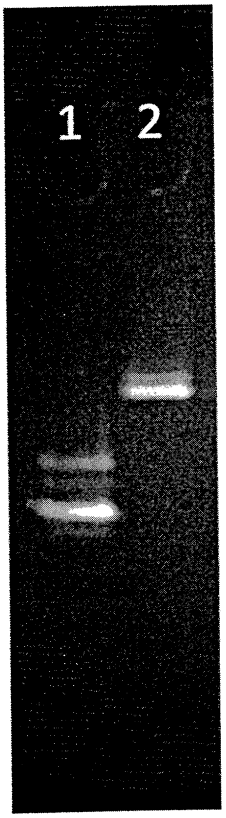

Figure 1: Native PAGE of two samples visualized using a blue-light transilluminator. In lane 1 the sample contains a green fluorescent protein (eGFP) and in sample 2, a red fluorescent protein (DsRed).

a) (6 marks total) In a variation of the gel presented above, SDS and b-mercaptoethanol are added in the sample preparation.

i. (3 marks) Why is SDS added to samples and the gel?

ii. (3 marks) Why is b-mercaptoethanol sometimes added to samples prior to loading on an SDS-PAGE gel?

b) (6 marks total) Given the two proteins being analyzed, give a reason why the technician decided to run a native PAGE gel. Make sure to justify your answer.

c) (4 marks total) Explain why one might see multiple fluorescent bands in Lanes 1 and 2 in the gel in Figure 1.

d) (4 marks total) Given the molecular weights of the two proteins, are the protein bands where you would expect them to be? Why? Justify your answer.

Question 2: (20 marks total) Polymerase Chain Reaction (PCR)

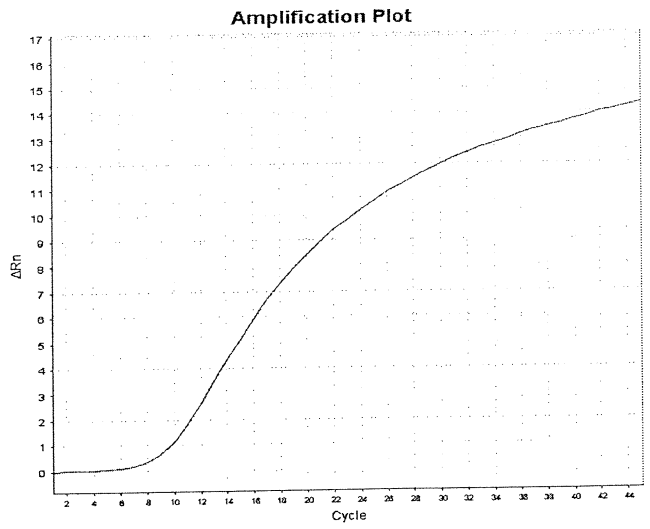

Figure 2: Amplification plot from a real-time PCR analysis of a gene from a virus.

a) (4 marks) Real-time polymerase chain reaction follows the amplification of DNA after each cycle. How is this achieved?

b) (4 marks) In real-time polymerase chain reactions, what is the threshold cycle?

c) (4 marks) Explain the different events that occur in one PCR cycle.

d) (4 marks) A variation on PCR is reverse transcription (RT)-PCR. What occurs in RT-PCR? What is it used for?

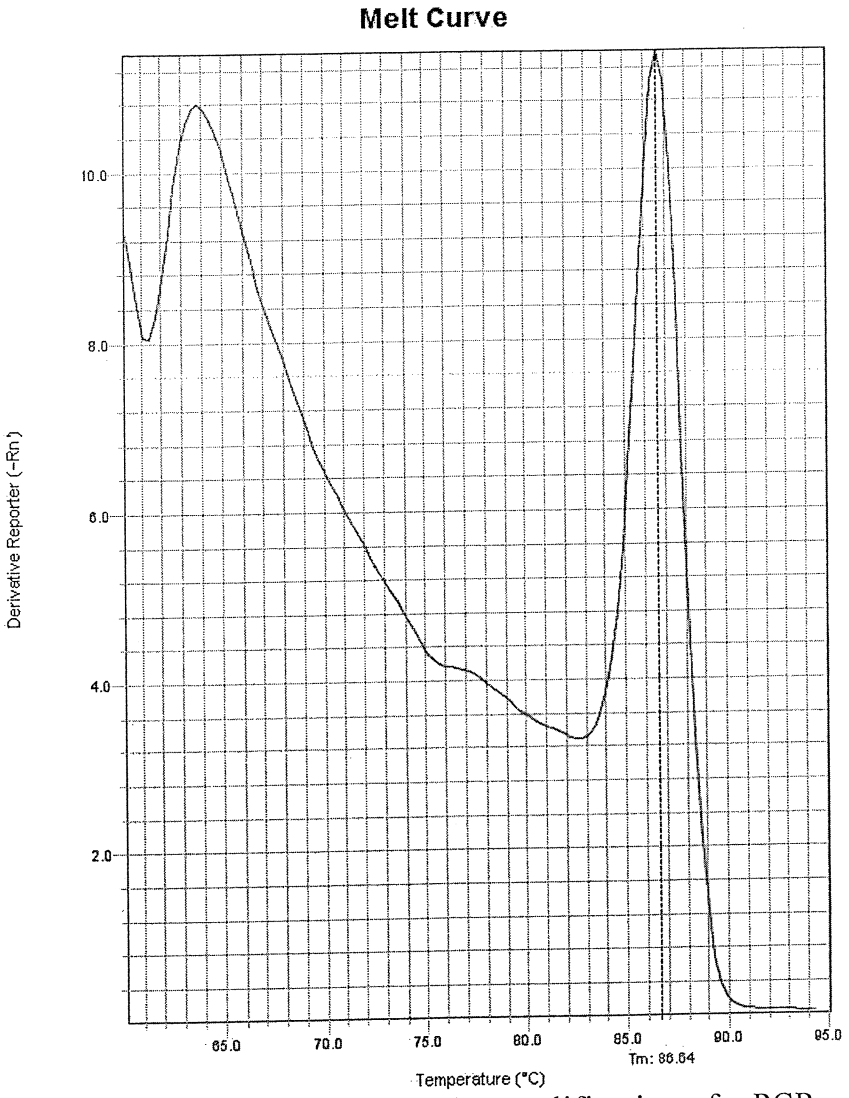

Figure 3: Melt curve obtained after the amplification of a PCR product.

e) (4 marks) At the end of a real-time polymerase chain reaction, it is possible to look at the quality of the amplified product through its melt curve. Based on Figure 3, what can be said of the quality of the expected PCR product (melting temperature 86.64°C). Explain.

Question 3: (20 marks total) Flow cytometry

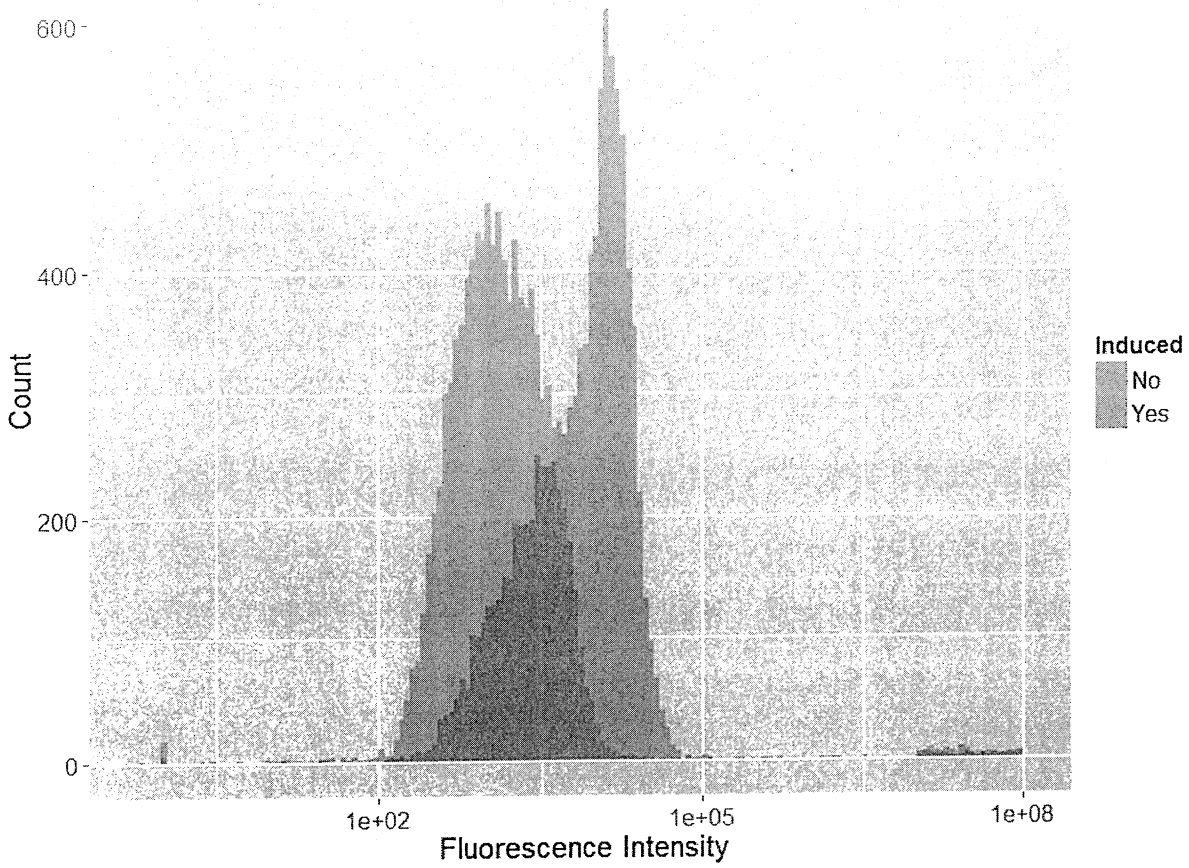

Figure 4: Histogram of fluorescent intensity for two samples (histograms are overlaid one on top of the other). Each sample contained 10000 events (whole cells). The sample represented in pink contained cells cultured in the absence of an inducer. The sample represented in blue contained cells cultured in the presence of an inducer (IPTG).

Engineered cells carrying a fluorescent protein under the control of an inducible promoter were studied using flow cytometry.