

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 β -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).

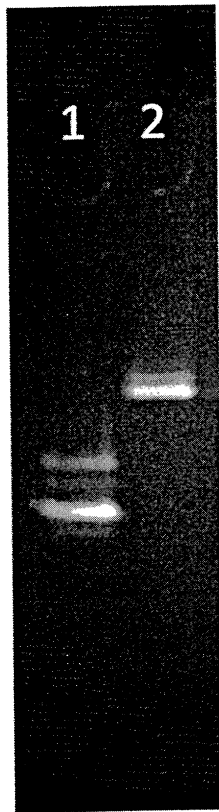


Figure 1: Native PAGE of two samples visualized using a blue-light transilluminator. In lane 1 the sample contains a green fluorescent protein (eGFP) and in sample 2, a red fluorescent protein (DsRed).

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)

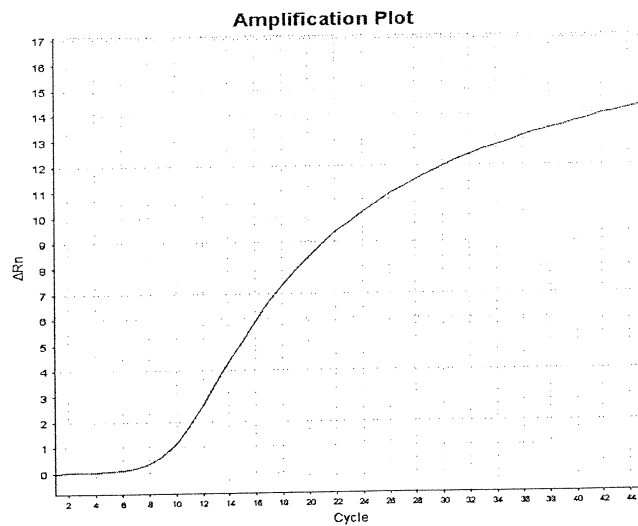


Figure 2: Amplification plot from a real-time PCR analysis of a gene from a virus.

- 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?

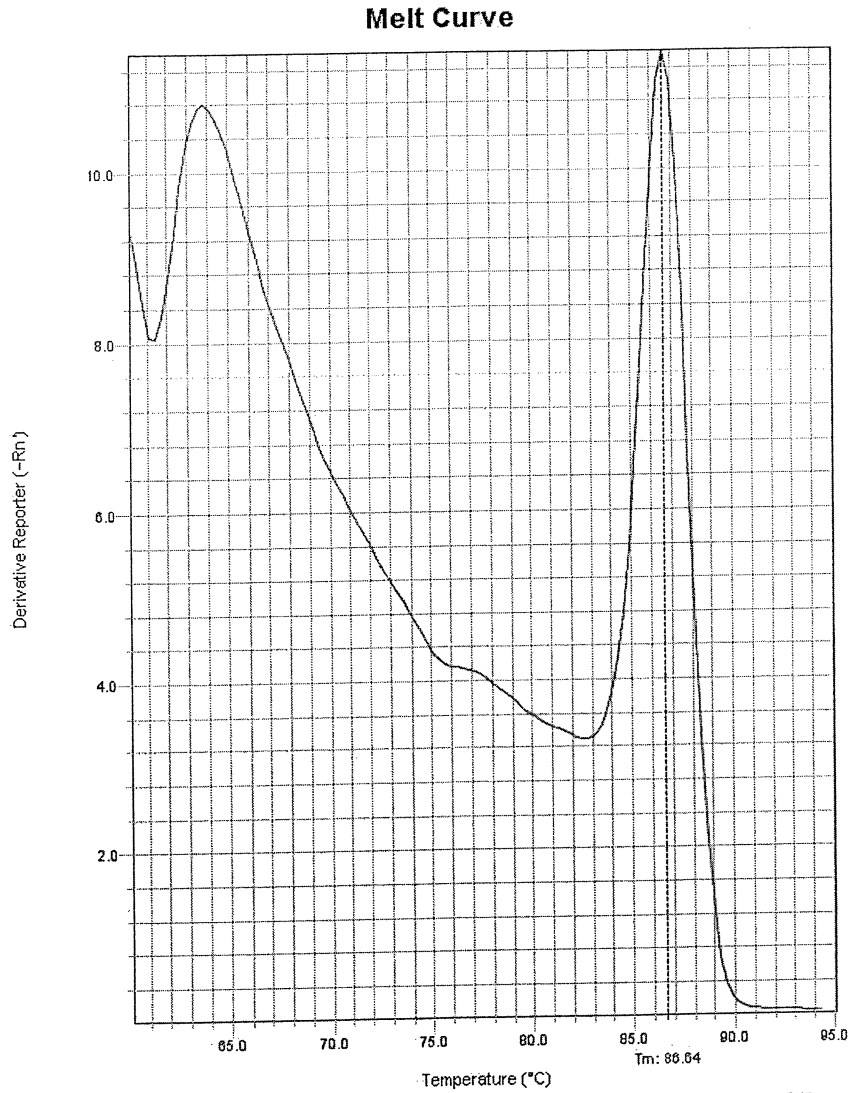


Figure 3: Melt curve obtained after the amplification of a PCR product.

- 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

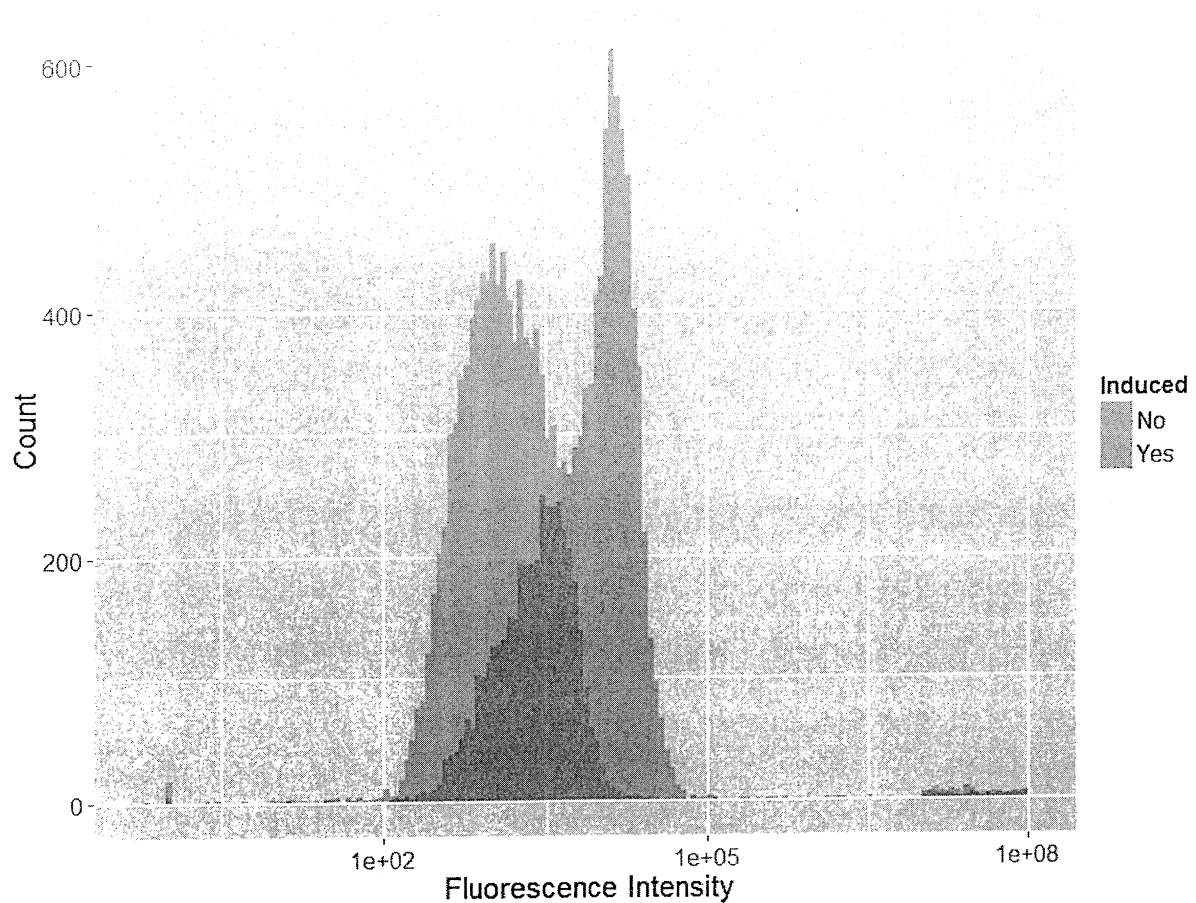


Figure 4: Histogram of fluorescent intensity for two samples (histograms are overlaid one on top of the other). Each sample contained 10000 events (whole cells). The sample represented in pink contained cells cultured in the absence of an inducer. The sample represented in blue contained cells cultured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

a) (5 marks) Figure 4 describes populations of whole cells. Describe how one could discriminate between whole cells and cellular debris using flow cytometry.

b) (5marks) How is the fluorescence of a cell measured?

c) (10 marks) How effective was the induction if the results presented in Figure 4 were taken 5 hours after the inducer (or equivalent time for the non-induced system) was added to the culture? Explain.

Question 4: (20 marks total) Affinity Chromatography

a) (5 marks) In your own words, explain the driving principle of affinity chromatography.

b) (5marks) Antibodies play a large role in affinity chromatography. Draw and label an IgG antibody and its major features.

c) (3marks) Lectins can be immobilized on a solid support for affinity chromatography applications. What type(s) of molecule is targeted if lectins are used?

d) (7 marks) What are the necessary steps in affinity chromatography to recover a purified product? Use a sketch to support your answer.

Question 5: (20 marks total)

a) (10 marks) Sketch a block diagram of an FTIR spectrometer.

b) (3 marks) What are the units most commonly used to identify bands in FTIR spectroscopy?

c) (7 marks) In your own words, what is a Michelson interferometer?

Question 6: (20 marks total) Atomic Force Microscopy (AFM)

a) (6 marks) What are the 3 most commonly used modes of operation of an atomic force microscope?

b) (4marks) Explain the major differences between the 3 modes of operation of an atomic force microscope.

c) (2 marks) What type of resolution (size scale) can be expected on the height (z-coordinate of sample analyzed by AFM)?

d) (2 marks) What type of resolution (size scale) can be expected on lateral features of the surface?

e) (6 marks) What advantages exist for looking at surface features using AFM over Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM)?