INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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IBO 2018, Tehran, Iran

Practical Exam "Biochemistry & Molecular Biology"

Student Code:



IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Biochemistry & Molecular Biology

Total Points: **100**

Duration: **90 minutes**

General information

-Protocol instructions and Answer sheet will be provided in your cabins within an envelope.

- Total points: 100

- Exam time: 90 minutes

- Please write your student code into the box on the upper left side of the title page.

-Write all answers on the **answer sheet**, which is provided as separate sheet. Points will not be given to answers written on the question paper**.**

- In order to show a flag, place it in the **flag stand** (=small tube) located on the left wall of your desk.

-Please ensure that all the materials and equipments are present in your cabin. If anything is missing, you must report it **within five minutes** after start whistle by showing your yellow flag in the flag. Report of item(s) missing after the five minutes will not be considered.

-In case of emergency, put your yellow flag in the flag stand.

-Additional materials will not be provided in any case of material loss during the experiments.

-We suggest you familiarize yourself with the experiments before starting by reading the entire protocol before starting.

-Stop answering **immediately** when the stop whistle is blown at the end of the exam. Put the entire protocol along with the answer sheet in the envelope. Lab assistants will collect the envelopes.

-In case you have placed your green flag in the flag stand, but picture of you gel has not been taken by the time that the stop whistle blows, stand up next to your cabin and wait until an assistant comes and takes the picture.

Good luck

Write the indicated number in the tables below.

1	

7	

PRACTICAL EXAM OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Materials: CHECK AND REPORT ITEMS MISSING WITHIN FIVE MINUTES AFTER WHISTLE

A. Biological

1. 500 µl bacterial lysate (labeled “Bacterial lysate BL”; in box)

2. 500 µl BSA solution, 1 mg/ml (labeled “BSA”; in box)

3. 30 µl plasmid DNA, 250 ng/µl (labeled “plasmid DNA”; in box)

4. 10 µl DNase (labeled “DNase, 0.015 Units/µl”) **on ice**

5. 20 µl DNA size marker (labeled “DNA size marker”; in box)

B. Non-Biological

1. Waste bucket (labeled “Waste bucket”)

2. 1 ml Lysis Buffer (labeled “Lysis buffer LB”; in box)

3. 2 ml Buffer A (distributed in two tubes, each labeled “Buffer A”; in box)

4. 1 ml Buffer B (labeled “Buffer B”; in box)

5. 3 ml phosphate buffered saline (distributed in two tubes, each labeled “PBS”; in box)

6. 8 µl DNase reaction buffer (labeled “DNase buffer”) (**on ice**)

7. 50 µl gel loading dye (labeled “Loading dye”; in box)

8. 26 X 1.5 ml tubes (in box)

9. 4.2 ml Bradford reagent (in 15 ml tube) (labeled “Bradford reagent”)

10. Affinity chromatography column (labeled “Column”; placed on column holder rack)

11. Micropipette Stopper Tip (labeled “MST")

12. Column holder rack (labeled “Column holder rack”)

13. Tube rack for fraction collection (labeled “Tube rack”)

14. 2-20 µl Micropipette

15. 20-200 µl Micropipette

16. 100-1000 µl Micropipette

17. Yellow tips for 2-20 µl and 20-200 µl Micropipettes

18. Blue tips for 100-1000 µl Micropipette

19. 96 well plate (with student’s name)

20. Aluminum foil

21. Agarose gel electrophoresis system with incorporated power supply

22. Agarose gel containing DNA-binding stain (already placed in electrophoresis system)

23. Disposable gloves

24. Goggles

25. Water proof pen marker

26. Three flags, colored red, green, and yellow

27. Flag stand (=small tube) located on the left wall of your desk

28. Your name tag (placed on shelf)

We suggest that you familiarize yourself with the experiment by reading the entire text below before starting the experiment

Introduction

In this experiment, you will test the ability of a protein named Pep (that is positively charged under the experimental conditions) to interact with DNA. You will be supplied with the DNA to be tested, but you must purify the protein Pep from a crude bacterial lysate. The bacteria had previously been transformed with a plasmid expression vector into which the Pep encoding gene with a histidine tag had been cloned. Purification will be done by affinity chromatography. The histidine tag has affinity for and binds to nickel which is attached to the resin in the columns. After binding, the protein can be detached from the resin by changes of buffers used in the chromatography protocol. Eluted fractions will be collected in several tubes. You will determine the protein concentration in two of the fractions by the Bradford method. This is a colorimetric assay in which attachment of Comassie brilliant blue to protein results in increased absorbance at wavelength of 595 nm. By using a standard curve derived by assay of a bovine serum albumin (BSA) protein solution of known concentration, the protein concentration of the fractions can be determined. Subsequently, you will test the ability of the Pep protein in one of the fractions to interact with DNA by performing a gel retardation assay. In this assay, interaction of DNA with protein retards the migration of the DNA on agarose gels during electrophoresis.

PROTOCOL

A. Purification of Pep protein from bacterial lysate by affinity chromatography

1. Take note of the chromatography column that is already placed in the hole of the column holder rack. Also take note that it fits into the hole tightly. Avoid having to remove it from the hole during the course of the experiment. The column is sealed at the bottom and the resin in the column is covered with a small volume of ethanol. Look carefully at the contents of the column to correctly detect the border between resin and the overlying liquid.

2. Open the red cover on top of the column, remove the micropipette stopper tip (MST) at the bottom of the column, and quickly place tube #1 in the tube rack under the column in order to collect the eluting ethanol. Collection should continue just until there is no ethanol left above the resin. Three to four drops will be collected.

3. Quickly, but gently add 500 µl lysis buffer to the column without disrupting the resin within the column in order to equilibrate the column with LB.

4. Collect the eluting drops in tube #2 just until there is no buffer left above the resin. (Approximately 500 µl will be collected.)

5. Quickly, but gently add 500 µl bacterial lysate onto the column and start collecting drops in tube #3. Be sure that all the BL has entered the column. (Approximately 500 µl will be collected.)

6. Quickly, but gently add 500 µl wash buffer A and start collecting drops in tube #4. As the volume of the buffer above the resin decreases to about 100-200 µl, add 300 µl additional buffer A and continue collection of drops in the same tube #4. Continue with addition of 300 µl three more times (i.e. column should be washed with a total volume of 1.7 ml buffer A). Collection can be continued in tube #5. (Approximately 1.7 ml will be collected in total in tubes # 4 and 5.)

7. Quickly, but gently add 500 µl buffer B to start detachment of Pep molecules bound to the resin in the column. Start collecting drops in tubes #6, 7, and 8. Three drops should be collected in each tube. As the volume of the buffer above the resin decreases to about 200 µl, add 200 µl additional buffer B and continue collection of the eluent just until three drops have been collected in tube #8.

8. Quickly seal the bottom of the column with micropipette stopper tip (MST) by inserting the bottom of column into the wide mouth of the tip.

B. BRADFORD PROTEIN ASSAY (THIS TASK HAS TWO PARTS)

Part 1

Prepare BSA (= the standard protein) dilutions as shown in Table 1:

Table 1

Tube #	9	10	11	12	13	14	15
BSA (1 mg/ml)	0 µl	20 µl	40 µl	60 µl	80 µl	100 µl	120 µl
PBS	200 µl	180 µl	160 µl	140 µl	120 µl	100 µl	80 µl

2. Prepare dilutions of tubes #7 and #8 in tubes #16 and #17 as shown in Table 2:

Table 2

Tube #	16	17
Buffer B eluent fractions	40 µl of Tube 7	40 µl of Tube 8
PBS	20 µl	20 µl

3. Mix contents of each of the tubes (tube 9 through tube 17).

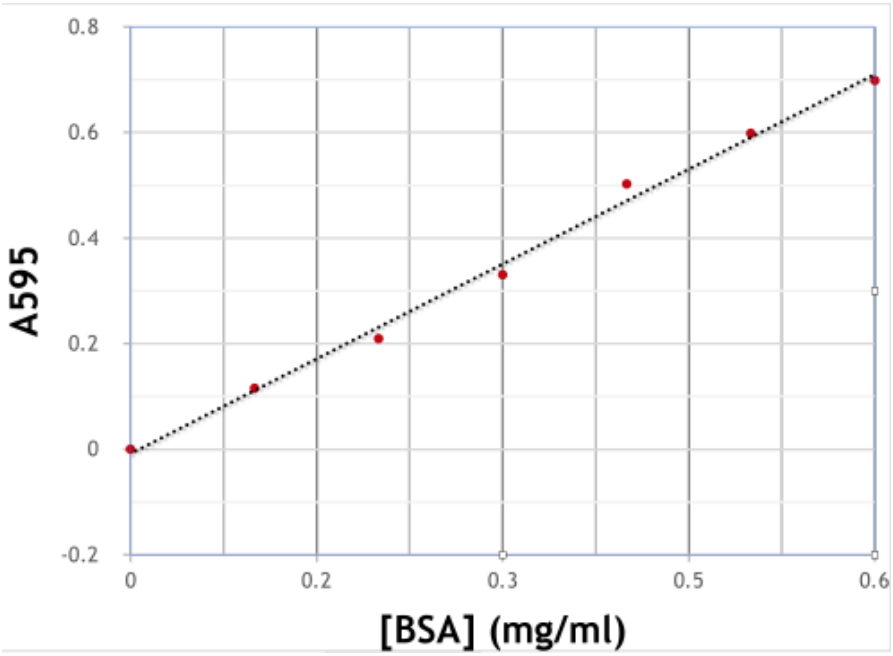
4. Each sample will be assayed in duplicate. For this purpose, add 10 µl of tubes #9-15 into wells of B1-B7, and then again to wells of D1-D7 of your 96 well plate. Subsequently, add 10 µl of tubes #16 into B9 and D9, and 10 µl of tube #17 to wells B11 and D11. Add 190 µl of the Bradford reagent into each of the wells to which samples had been added. Mix gently with micropipette tip and take care not to create bubbles. Bubbles would interfere with absorbance measurements.

5. Place a lid on the 96 well plate and wrap the plate with aluminium foil to prevent exposure to light. Incubation in the dark should continue for 5 minutes. (You may start Part 2 of this task during the 5 minute incubation.)

6. After completion of the 5 minute incubation, placed your red flag in your flag stand. A lab assistant will take your coded plate to a Spectrophotometer station and have absorbance of all your wells read at wavelength of 595 nm. The absorbance readings will be used to score your performance (45 POINTS).

Part 2 NOTE: All data presented in Part 2 of the Bradford assay pertain to experiments performed earlier by an examiner.

1. The Bradford assay described in Part 1 was earlier performed by an examiner, and the absorbance readings were used to draw a standard curve provided below.



2. Using the standard curve and the A_{595} (absorbance at 595 nm) of tubes #16 and #17 obtained by the examiner and shown below, calculate protein concentration of fractions in the examiner's tubes #7 and #8. Write the protein concentrations of tubes #7 and #8 (rounded to two decimal places) **on the answer sheet (5 POINTS)**.

Tube 16:	Well B9: 0.32	Well D9: 0.34
Tube 17:	Well B11: 0.41	Well D11: 0.43

C. GEL RETARDATION ASSAY

This assay will be performed with contents of **your** tube #8 (= tube containing drops you collected in the third tube after addition of buffer B).

1. Dilute contents of tube #8 as follows:
- Mix 10 µl of contents of tube #8 and 40 µl of PBS in tube #18. .

2. Tubes #19-25 should be prepared as described below in Table 3. Add components in said order (from top to bottom):

Table 3

Tube #	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Plasmid DNA	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
Pep, tube #18	0	3 µl	7 µl	0	0	0	0
Pep, tube #8	0	0	0	4 µl	7 µl	0	7 µl
PBS	13 µl	10 µl	6 µl	9 µl	6 µl	9 µl	2 µl
DNase buffer	0	0	0	0	0	2 µl	2 µl
DNase (0.015 U/ µl)	0	0	0	0	0	2 µl	2 µl

3. Three minutes after addition of DNase to tubes 24 and 25, add 3 µl gel loading dye to each of tubes #19-25 and mix.

4. The gel electrophoresis apparatus will be off at this time. Be careful not to press the power button or any other button on the apparatus. Load 15 µl of each of tubes #19-25 consecutively into 7 adjacent wells (from left to right when positive pole is closer to you) of the agarose gel in the electrophoresis apparatus. In the eighth well add 15 µl of DNA size marker which already contains gel loading dye. NOTE: The gel is covered with electrophoresis buffer, therefore add the 15 µl aliquots very gently to the bottom of each well to prevent spill over while loading.

NOTE: One well of the gel has been left empty.

5. After completion of loading, insert the orange colored photo hood onto the apparatus. Press the power button on the lower right surface of the apparatus to start the electrophoresis. Record time of start of electrophoresis. The two buttons on the upper right are for illumination with high level or low level blue lighting. Press the button for high level lighting. This will enable you to visualize migration of the DNA in the gel real time during electrophoresis because the DNA binding stain is in the gel. Migration should be visualized through the hole on top of the photo hood. The photo hood should not be removed.

6. Disconnect the electricity 15 minutes after start of electrophoresis by pressing the power button. Place your green flag in your flag stand to attract attention of lab assistant. He/she will take photo of the gel through the hole of the photo hood (35 POINTS). You may proceed to the theory questions below during the 15 minute interval.

Questions (15 POINTS in total):

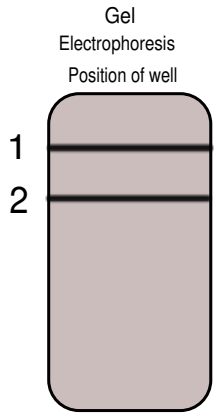
Indicate if each of the following statements is true or false with “X” in the answer sheet.

1. The larger the linearity range of a protein assay, the less one needs to be concerned about the concentration of the sample of interest to be used in the assay (1 **POINT)**.

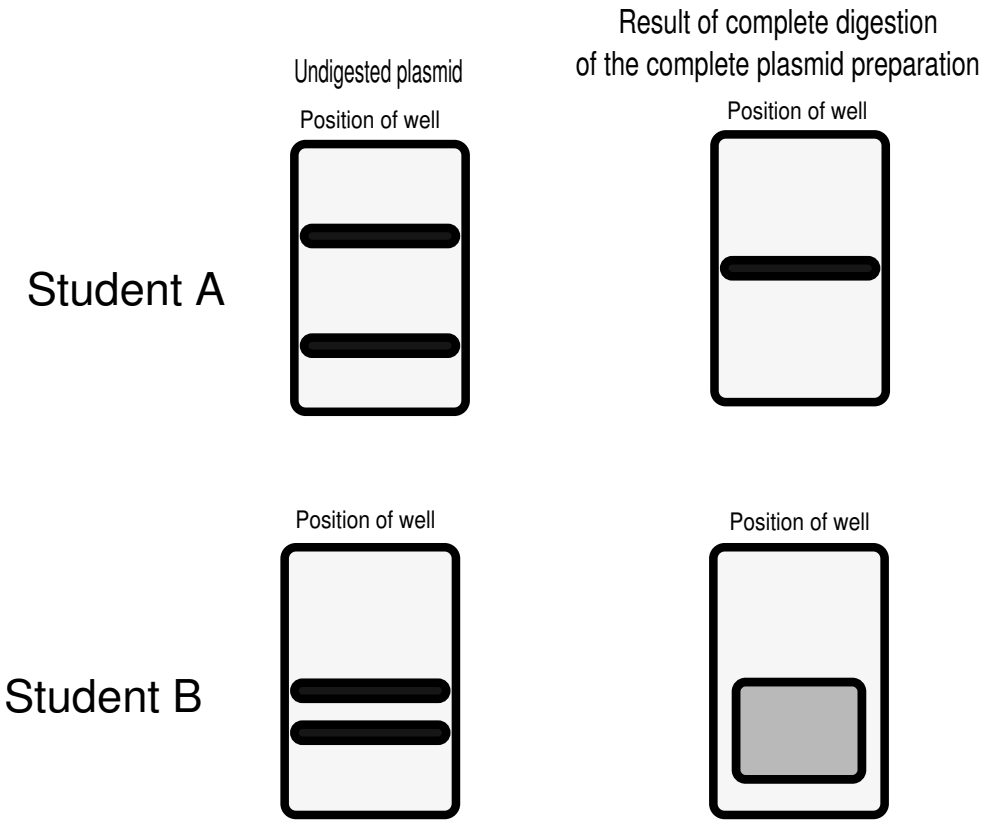
2. The effect of Pep on DNase activity on the plasmid DNA is likely to depend on the sequence of the DNA (1 **POINT)**.

3. Observation of stained DNA in gel regions above the wells would reflect results of interaction of DNA with high concentrations of Pep **(1 POINT)**.

4. Assume the schematic figure shown below represents the electrophoresis pattern of contents of tube 19. The upper band may be circular plasmid DNA in which one phosphodiester bond in one strand has been broken (1 **POINT)**.



5. Student A attempted to purify a plasmid, and student B attempted to purify another plasmid. Neither plasmid had repetitive sequences. After electrophoresis of the plasmids, both students observed two bands as shown below. Based on known size of the plasmids, the upper band was unexpected. Each performed restriction enzyme digestion on the plasmid preparation under conditions of achieving complete digestion, and subsequently electrophoresed the digestion product. Results are shown below. The map of the plasmids showed that each contained only one recognition site for the enzyme used.



Indicate if each of the following statements is true or false **in the answer sheet (4 POINTS)**.

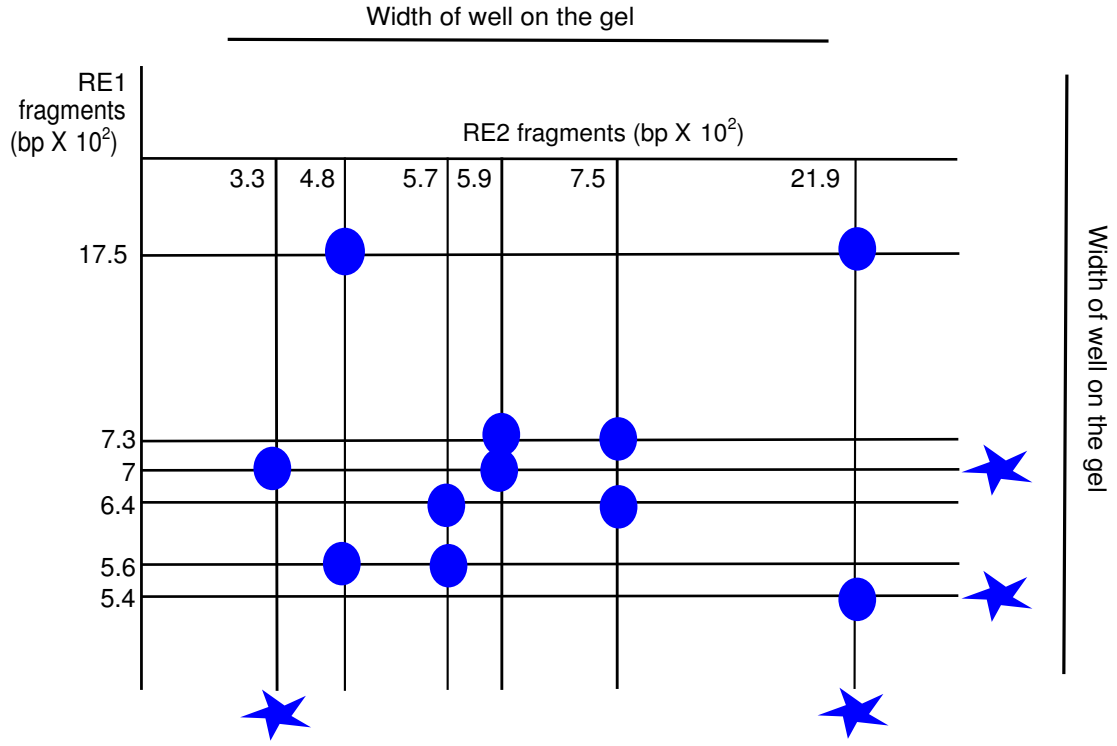
a) The unexpected band of student A may be linearized plasmid DNA.

b) The unexpected band of student A may be circular dimer plasmid DNA.

c) Electrophoresis pattern of partial digestion product of the plasmid preparation of student A with the same restriction enzyme is expected to produce at most three bands.

d) Electrophoresis pattern of partial digestion product of the plasmid preparation of student B with the same restriction enzyme is expected to produce three bands.

6. To determine the restriction enzyme map of two rare restriction enzymes on a linear DNA molecule of interest, RE1 and RE2, the following experiment was performed. First, samples of the DNA molecule were labeled at their 5’ ends and cut separately with RE1 and RE2. The lengths of the fragment products with label are shown in the figure below with **Stars.** Other samples of the DNA in unlabeled form were also cut separately with RE1 and RE2. RE1 digestion products were loaded in a very wide well of a gel and electrophoresed. After electrophoresis, DNA bands on the gel were transferred to a nitrocellulose filter paper by Southern blotting. RE2 digestion products were also loaded in a very wide well of another gel and also electrophoresed. Then DNA bands on this gel were transferred by Southern blotting to the same nitrocellulose filter paper that contained the digestion products of RE1, but transfer was oriented perpendicular relative to the transfer of RE1 restriction fragments. **Circles** show positions of hybridization between RE1 digestion fragments and RE2 digestion fragments.



Indicate if each statement below is true or false (7 **POINTS)**.

a. Complete digestion of the DNA molecule with RE1 and RE2 would produce 11 fragments.

b. The 6.4 $X10^2$ bp and 7.3 $X10^2$ bp DNA fragments produced by digestion with RE1 are adjacent in the undigested DNA molecule.

c. The 5.6 $X10^2$ bp and 4.8 $X10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 are overlapping in the undigested DNA molecule.

d. The 17.5 $X10^2$ bp and 21.9 $X10^2$ bp fragments produced, respectively, by digestion with RE1 and RE2 overlap in the undigested DNA molecule.

e. No single digestion product of one of the restriction enzymes can overlap with three of the digestion products of the other enzyme.