INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Plant Molecular Biology

34th International Biology Olympiad

3-10 July 2023, United Arab Emirates University

Practical Exam

Plant Molecular Biology

Total points: 100

Duration: 90 minutes

General Instructions:

In this practical exam, you have 90 minutes to complete **TWO tasks.**

You can perform the experiments in any order.

- **Task 1: Restriction digestion of DNA, and its analysis (65 points)**
- **Task 2: Plasmolysis of onion cells and its analysis (35 points)**

During task 1 there is time for incubation and gel run. Use this time to carry out any task of your choice.

Important Information:

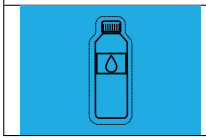
Write your answers in the answer sheet. Only answers given in the answer sheet will be evaluated.

Make sure that you have received all the materials and equipment listed, including a graph paper. If any of these items are missing, please raise your card immediately.

During experiments, ensure that you wear gloves, and handle the equipment and samples carefully.

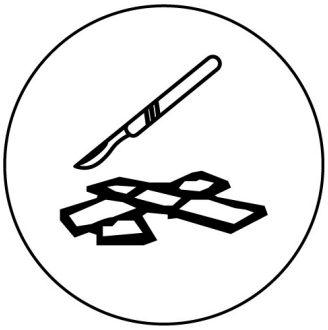
Any spilled solutions, samples or equipment damaged by you will not be replaced, apart from gloves.

Use the following cards to ask for water/washroom/incubation/photography/queries.

Drinking water	Washroom	Sample incubation	Photographic documentation	Other queries
				

- **IMPORTANT:** Two waste bins have been provided on the desk

- DISCARD COVERSLIPS, SLIDES AND SCALPEL IN THE WASTEBIN MARKED



- DISCARD BIOLOGICAL MATERIAL SOLUTIONS ETC. IN THE WASTEBIN MARKED

Stop answering as soon as you hear the whistle at the end of the exam.

No paper, materials or equipment should be taken out of the laboratory.

Good luck!

Materials provided for task 1:

1. 50 µl of linear DNA (50 ng/µl) labelled **DNA**; on ice.
2. 30 µl of HindIII restriction enzyme labelled **H3,** on ice.
3. 30 µl of Kpn I restriction enzyme labelled **KI,** on ice.
4. 20 µl of 10X buffer for restriction digestion labelled **BUF,** on ice.
5. 100 µl of nuclease free water labelled **W**; on ice.
6. 30 µl of 6X loading dye labelled **LD,** on ice.
7. 2 – 20 µl Micropipette
8. 1 box of yellow tips.
9. 4 x 1.5 ml microfuge tubes labelled as 1, 2, 3, and 4; on stand (labelled with desk number)
10. Agarose gel electrophoresis system with incorporated power supply
11. Agarose gel containing DNA binding stain (already placed in the electrophoresis system).
12. Photograph of digestion pattern corresponding to Figure 1 and 3 in the write up.
13. Ruler

Materials for task 2:

1. Scales of peeled onion (in plastic bag)
2. Slides (in plastic bag)
3. Coverslips (in small petridishes)
4. Fine forceps (x 2)
5. Fine needle (x 1)
6. Scalpel (x 1)
7. Brush (x 1)
8. A cardboard cup containing 15 ml tubes each with approximately 6 ml of:
   - Solution isotonic to onion cells (labelled **IS**)
   - Deionized water (labelled **W**)
   - 20% NaCl solution (labelled **20N**)
   - Solution labelled **A**
   - Solution labelled **B**
   - Solution labelled **C**
   - Plastic droppers (x 10)
9. Microscope
10. Photograph of onion epidermal cells corresponding to Figure 4.

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Q1-4

English (Official)

Material common to both tasks:

1. Orange card with sign of thermometer (temp. flag).
2. Green card with sign of camera (photo flag).
3. Red card with sign of "**?"** (general query).
4. Yellow card with a sign for washroom (toilet break).
5. Blue card with a sign of water bottle (drinking water).
6. Digital Clock.
7. Tissue paper.
8. Waste bins (x 2).
9. Disposable gloves.

Task 1. Restriction digestion of DNA and its analysis

INTRODUCTION

Digestion of DNA with restriction enzymes creates fragments of different sizes. The DNA fragments generated by digestion can be resolved based on their size, by agarose gel electrophoresis.

First, you are required to set up a restriction digestion of linear DNA (provided) with enzymes HindIII (H3) and KpnI (KI), and analyze the resulting DNA fragments by agarose gel electrophoresis. The DNA fragments will be visualized using GelGreen stain (non-hazardous), which fluoresces under blue light (non UV) when bound to DNA. As an analytical task, you will have to assess the size of some of the DNA fragments.

In a second analytical task, you will be analyzing a photograph of restriction digestion profile of a plasmid (vector) originally constructed for carrying out plant transformation. Scientists routinely check whether vectors are assembled correctly by carrying out restriction digestions, which could then be followed by sequencing of different parts of the vector.

Part 1:

1.1. Restriction digestion of DNA (32 Points)

PROCEDURE

1. Set up the restriction digestion reactions in the 1.5 ml microfuge tubes labeled 1, 2, 3, and 4. Add the components into each tube serially, as mentioned in Table 1.1.

S. No.	Component	Tube 1	Tube 2	Tube 3	Tube 4
	Volume (µl)				
1	Nuclease free water (W)	13	8	8	3
2	Linear DNA (DNA)	5	5	5	5
3	Restriction Buffer (BUF)	2	2	2	2
4	Hind III (H3)	0	5	0	5
5	Kpn I (KI)	0	0	5	5
	Total volume (µl)	20	20	20	20

Table 1.1

2. Make sure that the components are mixed well by using the micropipette.

3. Place the tubes in the microfuge stand (check whether the number on the rack corresponds to your table number).

4. Call the scientific volunteer immediately, by raising the 'temp flag'. The scientific volunteer will place the tubes in an incubator at 37^0C for 20 minutes. Record the start and stop time.

Start time:

Stop time:

5. After 20 minutes, raise the 'temp flag' to ask the scientific volunteer to get your tubes from the incubator.

6. Add 4µl of loading dye (LD) into each of the 4 tubes. Mix by using the micropipette.

7. Get ready to load the gel. Leave the gel electrophoresis apparatus turned off at this time. In the upper right corner, there are two buttons for high-level or low-level blue illumination. Switch on the low-level blue illumination. This will enable you to see the wells clearly. Be careful **not to** press the power button. Do not move the gel electrophoresis apparatus.

8. Leave the first well blank. Load 20 µl of each sample from tube numbers 1 to 4 consecutively into 4 separate adjacent wells of the electrophoresis gel (left to right, when positive pole is closer to you).

NOTE: The gel is covered with electrophoresis buffer; so, load the samples very gently inside each well to prevent spillover while loading.

9. After you have finished loading the samples, place the orange-colored photo hood onto the apparatus. Switch off the low-level illumination.

10. Run the gel for 40 minutes. Start the electrophoresis unit by pressing the power button on the apparatus' lower right corner. Record the start and stop time.

To keep track of the time, you can note the start time and calculate the expected stop time.

Start time:

Stop time:

11. Photo hoods should not be removed during the run. Stop the run by pressing the power button.

12. Press the button for high-level lighting. Due to the DNA binding stain in the gel, you will be able to see DNA through the hole on top of the photo hood.

13. Raise the 'photo flag' to call the scientific volunteer. The scientific volunteer will take the photograph.

14. Check and approve the photograph. It will be printed and given to you with your student code written on it. Submit the photograph with the answer sheet.

Q.1.1.1 Attach the photograph of the gel	32.0pt

Part 2:

1.2 DNA fragment analysis: Sizing digested DNA (24 Points)

The picture in **Figure 1** represents the profile of fragments (**Tubes 1 to 4**) observed following a digestion with the same combination of restriction enzymes (in the same order) as in part 1. Reaction time and enzyme activity were sufficient to allow for a complete digestion of the DNA. To determine the sizes of different fragments, the gel was run for a longer period of time. Fragments smaller than approximately 1000 base pairs (bp) are not observed in the gel. For the analysis, a photograph of the same gel has been provided in addition to figure 1.

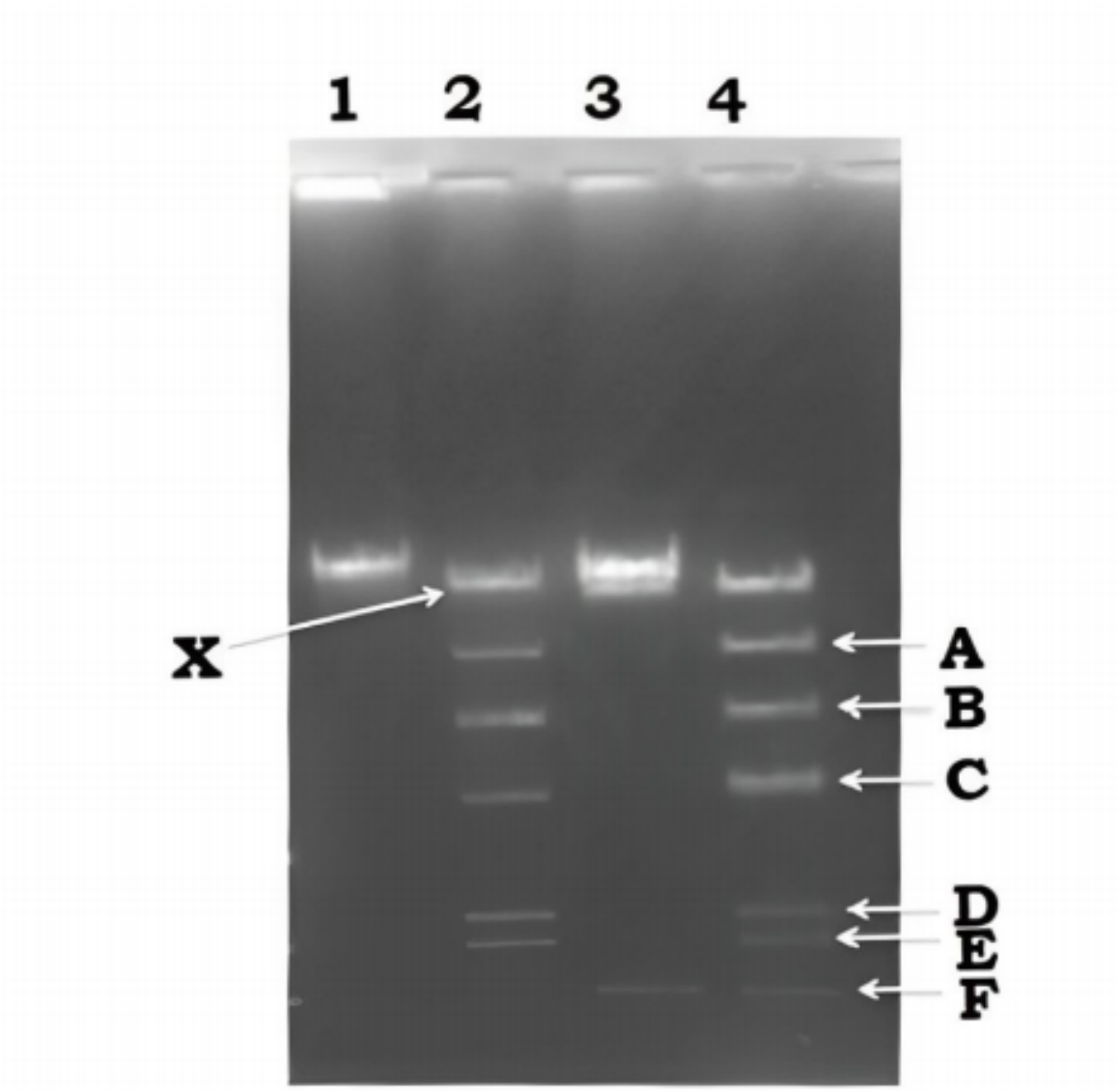

Figure 1. Digestion profiles of linear DNA run on agarose gel.

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Q1-8

English (Official)

The size of DNA fragments generated by restriction digestion, can be determined by comparing their migration on agarose gels, with those of DNA fragments of known sizes (molecular weight or size markers).

Following gel electrophoresis, the migration distance of each fragment of the size marker is measured. The rate at which a DNA fragment travels during electrophoresis is inversely proportional to the log_{10} of its length in base pairs.

The distance migrated can be plotted against the log_{10} of fragment length. The graph generated by the size marker will contain a region represented by a straight line. The size of the unknown fragments can then be determined using this plot.

In this exercise you are required to calculate the size (bp) of the DNA fragments C and E.

1. The distance migrated (cm) by fragments A to F has been given in Column II of Table 1.2
2. Table 1.2 (Column III) provides the size of DNA fragments (A, B, D, and F).
3. Calculate the log_{10} value of the size of fragments A, B, D and F. Record the log_{10} values, rounded to 2 decimal places, in column IV.
4. Plot a graph of distance migrated against log_{10} size in the graph paper provided along with the answer booklet.
5. Use an appropriate range for the X and Y axes.
6. The plot should be scaled appropriately.
7. Choose the suitable roman numbers (I to VI) from Table 1.2 to label the X and Y axes.
8. Plot a line of best-fit.
9. Based on the best-fit line, record the log_{10} size, to two decimal places of fragments C and E in column V of Table 1.2.
10. Calculate the sizes of fragments C and E and record in column VI of Table1.2 rounded to the nearest whole number.

Q.1.2.1 Plot the graph	6pt

Q.1.2.2 Fill table 1.2 14pt

Table 1.2

I	II	III	IV	V	VI
DNA fragment	**Distance migrated (cm)**	**Size (bp)**	**Log_{10} size (off to 2 decimal)**	**Log_{10} size for fragment C and E (from graph)**	**Size (bp) fragments C and E**
A	5.3	9416		×	×
B	6.0	6557		×	×
C	6.9	×	×		
D	8.2	2322		×	×
E	8.5	×	×		
F	9.1	1514		×	×

Q.1.2.3 Mark a cross (X) in the appropriate column. 4.0pt

Statement	True	False
Kpn I digestion, generates 3 DNA bands.		
There are three sites targeted by Kpn I in the linear DNA provided		
All sites targeted by Kpn I are present in the DNA band marked 'X' in lane 2.		
If band B contains 2 pmoles of DNA strands, band E will also contain 2 pmoles of DNA strands.		

Part 3:

1.3 Generating a restriction map. (9 points)

Figure 2 represents a partial restriction map of the plasmid vector (pACE2023) used for plant transformation. pACE2023 is 16500 base pairs (bp) in size.

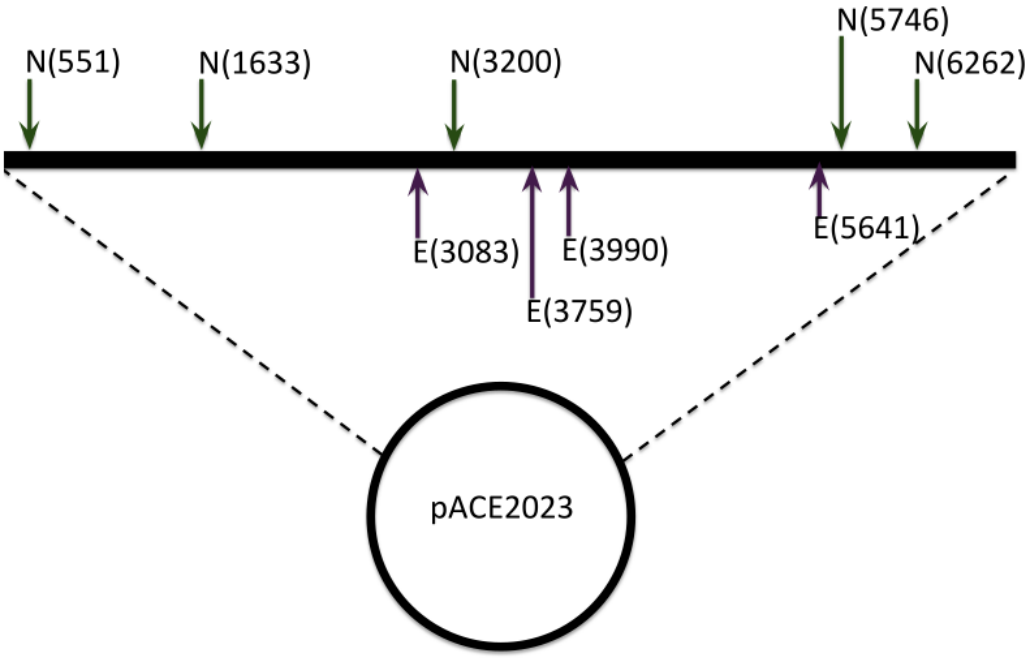


Figure 2: Map of pACE2023. The positions of different restriction enzyme targeting sites have been shown. N= NcoI; E= EcoRV. The numbers in brackets represent the position of that restriction site (in base-pairs).

All sites for NcoI and EcoRV have been shown.

pACE2023 was digested with different restriction enzymes, the result of which is presented in Figure 3.

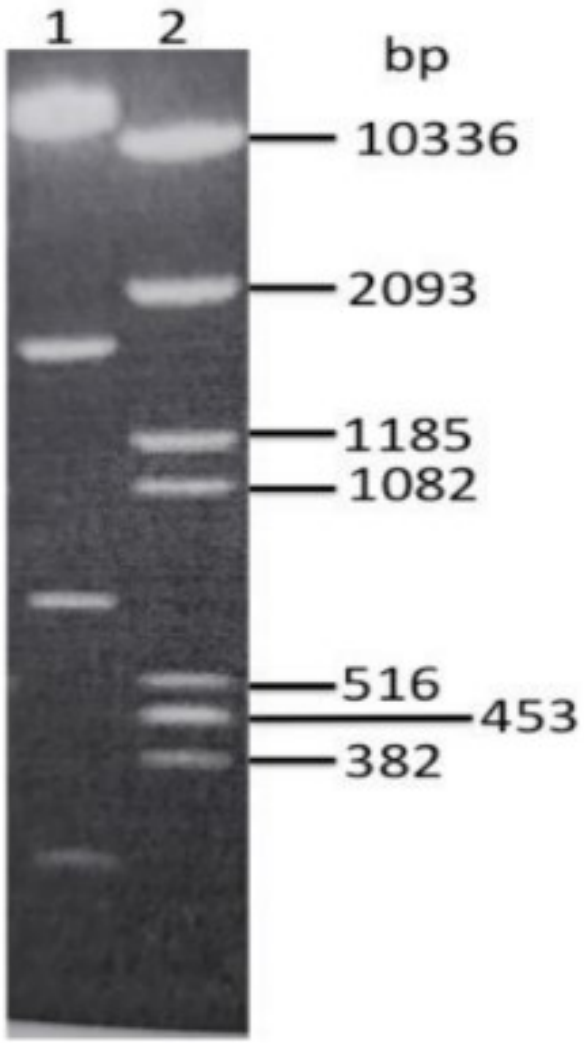


Figure 3: Digestion profile of pACE2023. Lane 1: vector digested with EcoRV (E); lane 2: vector double digested with both HindIII (H) and NcoI (N).

The size of DNA bands in lane 2 is indicated.

The intensity of fluorescence of the 453 bp fragment is double that of the 516 bp fragment.

Answer the following questions:

Q.1.3.1 What is the size (in base pairs) of the largest fragment obtained upon digestion with EcoRV?	1.5pt

Q.1.3.2 How many Hind III targeted sites are present in pACE2023?	2.5pt

Q.1.3.3 Identify the location of the Hind III site(s) in pACE2023 by marking a cross (X) in the appropriate cell(s) (one cross for each site) in the table. Mark a circle in the remaining cell(s). 5.0pt

Outside 551 and 6262 region	Between 551 and 1633	Between 1633 and 3200	Between 3200 and 5746	Between 5746 and 6262

Task 2. Plasmolysis of onion cells – an analysis

INTRODUCTION

Water potential is defined as the difference of free energy present in the water of a system when compared to free energy of pure water at constant temperature and pressure. It influences the direction of movement of water in plants. The symbol for water potential is

ψ (psi).

Potential is measured in units of pressure, usually in megapascals (MPa).

In living cells, water potential can be calculated by the equation

$\psi = \psi p + \psi s$

where,

1. ψp represents the pressure potential (turgor pressure, i.e., pressure of protoplast against cell wall).
2. ψs is osmotic potential or solute potential; the effect that solutes have on water potential. Pure water contains no solutes and has a ψs of 0.0 MPa.

In this experiment, you will observe the processes of plasmolysis and deplasmolysis in epidermal cells of onion. Based on this, you are required to identify the percentage of NaCl in the three given solutions labelled A, B and C.

Part 1:

2.1 The first task involves mounting onion epidermal cells in a solution that will not affect their turgidity. Carry out the following steps:

1. Choose the appropriate solution for the experiment. Record the solution used in **Table 2.1.**
2. Place 2-3 drops of the solution on a slide.
3. Choose an onion scale from the middle layers. With the help of the scalpel, cut out a piece of the onion scale of approximately 1cm X 1 cm.
4. With the help of forceps peel off the epidermal layer from the inner side of the scale.
5. Spread the epidermal layer in the solution on the slide.
6. Make sure that the peel is a flat single layer of cells and is completely immersed. Keep it immersed with the help of a brush.
7. Carefully place a coverslip over the peel so that there are no air bubbles.
8. Wipe off the extra fluid from outside the coverslip with tissue paper.
9. Switch on the microscope, using the switch on the upper left. This switch is marked with I,II and III. Switch to III for best illumination. Do not touch the wheel marked 8 at the bottom left.
10. Observe the slide at 100X magnification. Note: The magnification power of the eyepiece is 10X. Choose the appropriate objective lens to achieve a total magnification of 100X.
11. Record the magnification power of the objective lens you choose in **Table 2.1.**
12. Focus on a field in the slide that is an appropriate representation of your observation.
13. Match your observation to the panel in **Figure 4**. A photograph of the same panel has been provided in addition to **Figure 4**.

14. Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.**
15. Raise the 'photo card' to call the scientific volunteer. The scientific volunteer will take photographs of the field you have focused on.
16. Check and approve the photograph. It will be printed and given to you with your student code written on it. Submit the photograph along with the answer sheets.

Q.2.1	Attach the photograph	10.0pt

2.2 In this part, you are required to observe the structure of the epidermal cells in 20% NaCl.

1. Place 2-3 drops of 20% NaCl solution on a slide.
2. Carry out the steps mentioned in 2.1.3 to 2.1.6.
3. Incubate in 20% NaCl for 5 minutes.
4. Observe the preparation at a magnification of 100X.
5. Carry out the steps mentioned in 2.1.12 and 2.1.13
6. Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.**

2.3 In this part, you are required to observe the structure of the epidermal cells after incubation in 20% NaCl for 5 minutes followed by incubation in **Solution A for 5 min.**

1. Carry out the steps as mentioned 2.2.1 to 2.2.3.
2. With the help of a dropper, remove the 20% NaCl solution and immerse the peel in Solution A. Incubate for 5 min.
3. Observe the preparation at a magnification of 100 X.
4. Carry out the steps mentioned in 2.1.12 and 2.1.13
5. Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.**

2.4 Carry out the steps as mentioned above in 2.3 to record your observation when the epidermal peel is incubated in 20% NaCl for 5 minutes, followed by incubation in **Solution B for 5 min.**

Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.**

2.5 Carry out the steps as mentioned above in 2.3 to record your observation when the epidermal peel is incubated in 20% NaCl for 5 minutes, followed by incubation in **Solution C for 5 min.**

Record which one of the panels (4a to i) is closest to your observation by marking a cross (X) in the appropriate cell of **Table 2.1. Mark only one cell.**

Q.2.2 Fill in Table **2.1** 15.0pt

Table 2.1									
2.1.1. Solution used for task 2.1 =									
2.1.11 The magnification power of the objective selected =									
	Panel								
	4a	4b	4c	4d	4e	4f	4g	4h	4i
2.1.14 (solution chosen by you)									
2.2 (20% NaCl)									
2.3 (20% NaCl followed by solution A)									
2.4 (20% NaCl followed by solution B)									
2.5 (20% NaCl followed by solution C)									

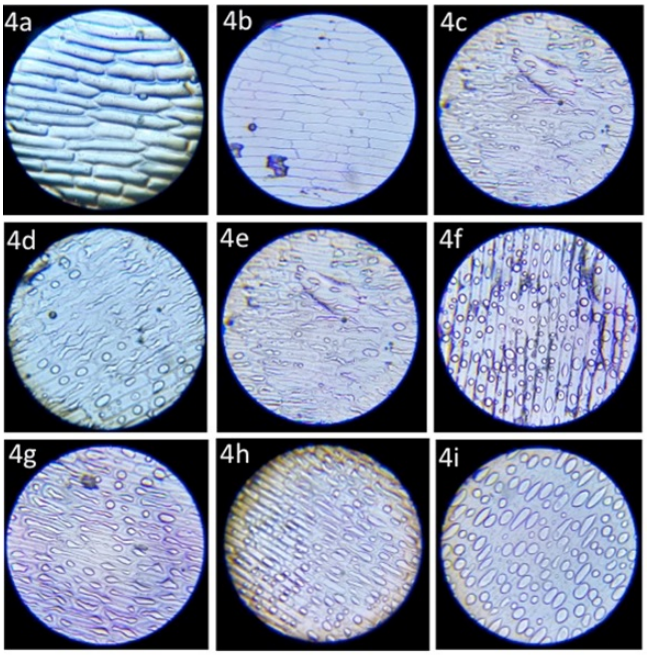


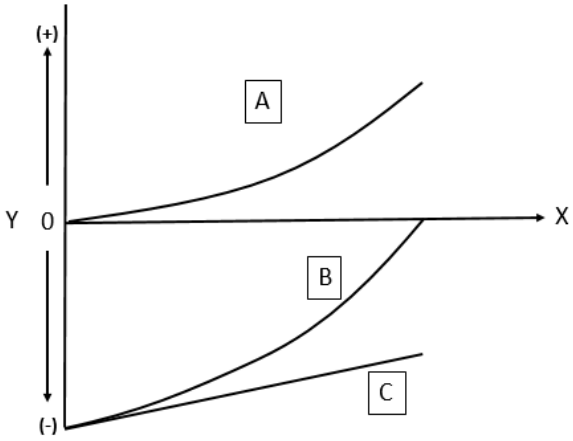
Figure 4

Q.2.3 Based on comparing your observations following treatments with solutions A, B and C, identify the concentration of NaCl in each of these solutions by marking a cross (X) in the appropriate cell in the table. 5.0pt

% of NaCl	Solution A	Solution B	Solution C
0			
5			
15			

Part 2. Answer the following:

The following graph shows how water potential (ψ), pressure potential (ψp) and solute potential (ψs) depend on the water content of a plant cell placed in pure water.



X	water content of cell
Y	Pressure (MPa)

Q.2.4 What are the correct labels for the graph? Identify the potential represented by the curves A, B and C by making a 'X' in the appropriate cell in the table. 3.0pt

	A	B	C
Water potential (ψ)			
Pressure potential (ψp)			
Solute potential (ψs)			

Q.2.5 X and Y are two plant cells adjacent to each other. 1.0pt
Cell X has solute potential of -9 MPa and pressure potential of 4 MPa.
Cell Y has solute potential of -8 MPa and pressure potential of 5 MPa.
Indicate the movement of water between the two cells by marking an $\longrightarrow$ in the proper direction between cells X and Y in the box below. Mark '=' in the box, if there is no movement of water.

X ☐ **Y**

Q.2.6 The solute potential of a plant cell is -8.5 MPa and its pressure potential is 1.5 MPa. The plant cell is placed in a solution with a water potential of -7 MPa. Identify the following statements as True or False by marking a cross (X) in the appropriate cell 1.0pt

Statement	True	False
The water potential in the plant cell will be -7 MPa		
Plasmolysis will be observed in the plant cell		

Plant Molecular Biology

Experiment 1. Restriction digestion of DNA and its analysis

I. Restriction digestion of DNA

A.1.1.1 $(32.0\ \mathrm{pt})$
Attach the photograph of the gel

II. DNA fragment analysis: Sizing digested DNA

A.1.2.1 $(6.0\ \mathrm{pt})$
Plot the graph

A.1.2.2 $(14\ \mathrm{pt})$
NOTE: Do not fill in anything in cells with cross ($\times$) marks.

Table 1.2

I	II	III	IV	V	VI
DNA fragment	**Distance migrated (cm)**	**Size (bp)**	Log_{10} **size off to 2 decimal places**	Log_{10} **size for fragment C and E (from graph)**	**Size (bp) fragments C and E**
A	5.3	9416		$\times$	$\times$
B	6.0	6557		$\times$	$\times$
C	6.9	$\times$	$\times$		
D	8.2	2322		$\times$	$\times$
E	8.5	$\times$	$\times$		
F	9.1	1514		$\times$	$\times$

A.1.2.3 (4.0 pt)

Statement	True	False
Kpn I digestion, generates 3 DNA bands		
There are three sites targeted by Kpn I in the linear DNA provided		
All sites targeted by Kpn I are present in the DNA band marked 'X' in lane 2		
If band B contains 2 pmoles of DNA strands, band E will also contain 2 pmoles of DNA strands		

A.1.3.1 (1.5 pt)

A.1.3.2 (2.5 pt)

A.1.3.3 (5.0 pt)

Outside 551 and 6262 region	Between 551 and 1633	Between 1633 and 3200	Between 3200 and 5746	Between 5746 and 6262

Experiment 2. Plasmolysis of onion cells – an analysis

A.2.1 (10.0 pt)
Attach the photograph

A.2.2 (15.0 pt)

Table 2.1									
2.1.1. Solution used for task 2.1									
2.1.11 Magnification power of the objective selected									
	Panel								
	4a	4b	4c	4d	4e	4f	4g	4h	4i
2.1.14 (solution chosen by you)									
2.2 (20% NaCl)									
2.3 (20% NaCl followed by solution A)									
2.4 (20% NaCl followed by solution B)									
2.5 (20% NaCl followed by solution C)									

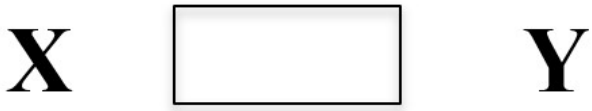
A.2.3 (5.0 pt)

% of NaCl	Solution A	Solution B	Solution C
0			
5			
15			

A.2.4 (3.0 pt)

	A	**B**	**C**
Water potential (ψ)			
Pressure potential (ψp)			
Solute potential (ψs)			

A.2.5 (1.0 pt)



A.2.6 (1.0 pt)

Statement	True	False
The water potential in the plant cell will be - 7 MPa		
Plasmolysis will be observed in the plant cell		

After Sub Jury

ANSWER KEY And MARKING SCHEME

EXPERIMENT 1: Plant Molecular Biology

TASK 1: Restriction digestion of DNA and its analysis – 65 points

Q 1.1.1 Marking scheme for the gel run

Absence of DNA in lane 1 and presence of DNA in lanes 2 to 5 (tests proper loading)
Similar amounts are loaded in each gel with the expected intensity of bands.

2 points per lane – 10 points

If DNA is present but with substantially lower amounts 1 point will be awarded per lane.

Marks for correct digestion pattern –
Lane 2: undigested DNA band;
Lane 3: 5 to 6 fragments;
Lane 4: 2 fragments and
Lane 5: 6 to 7 fragments (tests whether proper digestions were set up and loaded as per instructions):
(No points will be deducted if the first lane has not been kept empty. The pattern of loading from left to right should be UD, H3, KI and H3 + KI)

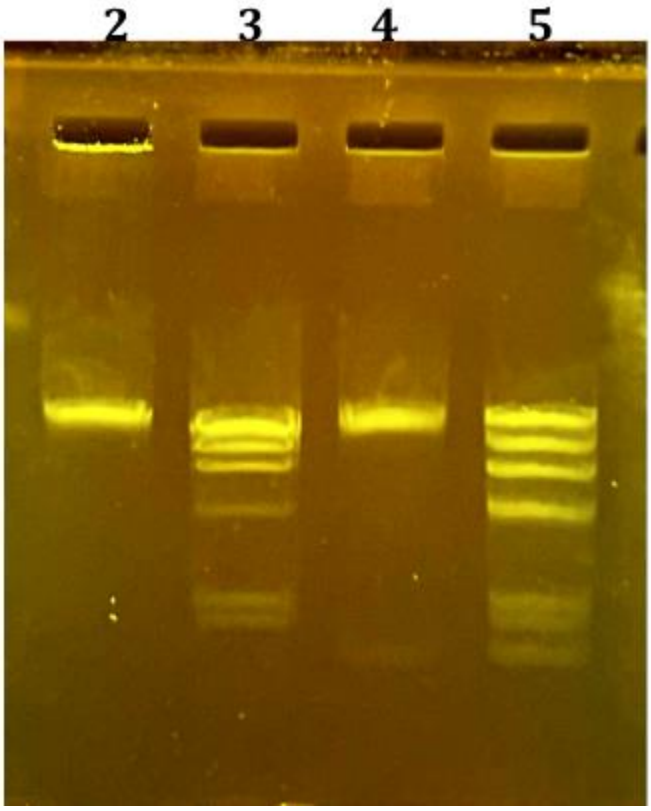
4 points each for lanes 2 to 5 – 16 points.

Resolution of the bands (or rough position of the smallest fragment) – to test whether the gel was actually run for 40 minutes **6 points**

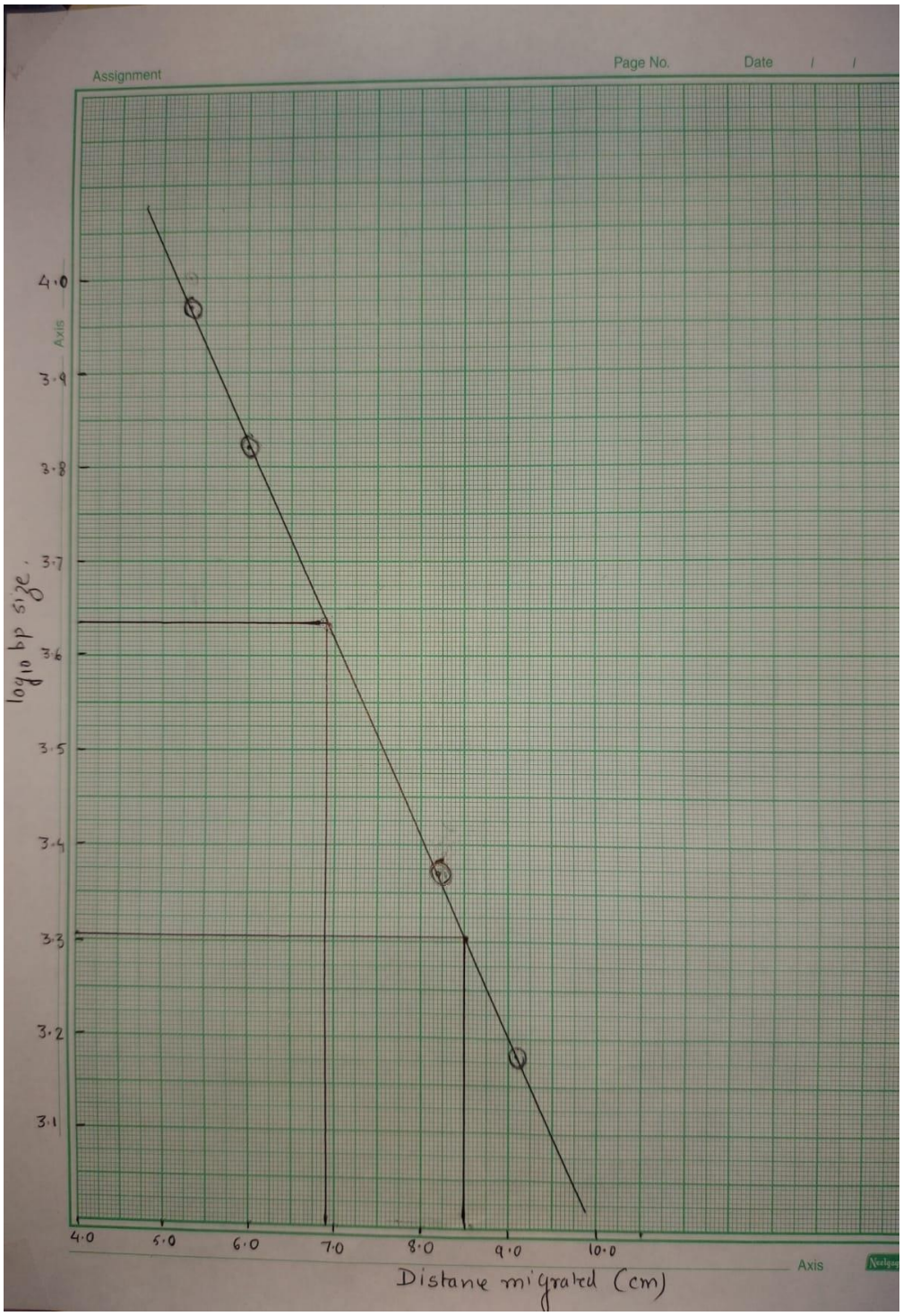
If gel is run at least to 50% of the length 3 points

Total: 32 points

A reference picture is shared below.



Q. 1.2.1 - Graph



IV/ V

II

PA

1. Correct label of X and Yaxis (as mentioned in the text); X= II and Y = IV/V

1 point for each label = **2 points**

No points deducted if the axis is reversed.

2. Scaling of X axis = 20 mm (or more) corresponding to 1 cm of distance migrated

2 points

(Partial marking: any scale lesser than 20 mm e.g. 10 mm corresponding to 1 cm of distance migrated = 1 point)

3. Scaling of Y axis = 20 mm corresponding to 0.1 of Log_{10} bp

2 points

(Partial marking: any other scale will be given 1 point)

Total = 6 points

Q. 1.2.2

Table 1.2					
I	II	III	IV	V	VI
DNA fragment	Distance migrated (cm)	Size (bp)	Log_{10} size (rounded off to 2 decimal places)	Log_{10} size for fragment C and E (from graph)	Size (bp) of fragments C and E
A	5.3	9416	3.97	[redacted]	[redacted]
B	6.0	6557	3.82	[redacted]	[redacted]
C	6.9	[redacted]	[redacted]	3.64 (Full points for values between 3.62 to 3.66)	4365 (Full marks for correct antilog of value in V)
D	8.2	2322	3.37	[redacted]	[redacted]
E	8.5	[redacted]	[redacted]	3.31 (Full points for values between 3.29 to 3.33)	2042 (Full marks for correct antilog of value in V)
F	9.1	1514	3.18	[redacted]	[redacted]

Marking scheme for Table 1.2

1. Column IV – 1 point for each correct cell

4 points

2. Column V –

i. From the plot the Log_{10} bp value of Fragment C is 3.64. The antilog of this is 4365 which is the size of the fragment. Given a variation of 0.2 on either side the answer can vary between 3.62 and 3.64.

ii. From the plot the Log_{10} bp value of Fragment E is 3.31. The antilog of this is 2027 which is the size of the fragment. Given a variation of 0.2 on either side the answer can vary between 3.29 and 3.33.

3 points for each correct cell = 6 points

3. Column VI –The answer will be based on the correct antilog of the value give in the corresponding cell of column V.

2 points for each correct cell = **4 points.**

Total = 14 points

Q. 1.2.3

1. TRUE (Kpn I digestion, generates 3 bands of DNA - This is based on observation)

2. FALSE (There are three sites targeted by Kpn I in the linear DNA provided - Digestion of the linear DNA with KI shows three fragments and thus there are 2 sites of KI)

3. TRUE (All sites targeted by Kpn I are present in the DNA band marked 'X' in lane 2. On comparing the three digestions it is observed that the sizes of the bands A to E remains unchanged between H3 single digest and H3+KI double digest. None of the fragments are reduced by 1.5 Kb (size of fragment F) in lane3. Fragment X is reduced in size and an additional fragment F is released in the double digest. Thus the 2 KI site lies in fragment X)

4. TRUE (If band B contains 2 pmoles of DNA strands, band E will also contain 2 pmoles of DNA strands.)

1 point for each correct answer = **4 points**

Q. 1.3.1

What is the size of the largest fragment obtained upon digestion with EcoRV?

Answer: **13942 bp (only 1 answer)**

This can be calculated from Figure 2. The size of the smaller fragments totals to 2558 (5641-3083). Thus the largest fragment will be 16500 – 2558 = 13942

1.5 point

Q 1.3.2

How many HindIII targeted sites are present in pACE2023?

Answer: **Three**

2.5 points

Q. 1.3.3

Identify the location of the HindIII site(s) in pACE2023 by marking a 'X' in the appropriate cell(s) (one cross for each site) of the table below. Mark a circle in the remaining cells

Table 3				
Outside 551 and 6262 region	Between 551 and 1633	Between 1633 and 3200	Between 3200 and 5746	Between 5746 and 6262
X	?	X	X	?

1 point for each correct cell = **5 points**

The student has to first interpret that the 453 bp band represents 2 different fragments. This can be deduced in different ways. It is mentioned in the legend to figure 3 that "**The intensity of fluorescence of the 453 bp fragment is double that of the 516 bp fragment.**" Additionally, the given fragment sizes in lane 2 leads to a total size of 16047, which is short of 453 bp from the given size of the plasmid 16.5 Kb.

Fragments generated by Nco I can be calculated from Figure 2 (Restriction map)

Nco I sites present at	Fragments obtained on Nco I digestion
551	
1633	1082
3200	1567
5746	2546
6262	516
	10789 (16500 – [1082+ 1567+ 2546 + 516])

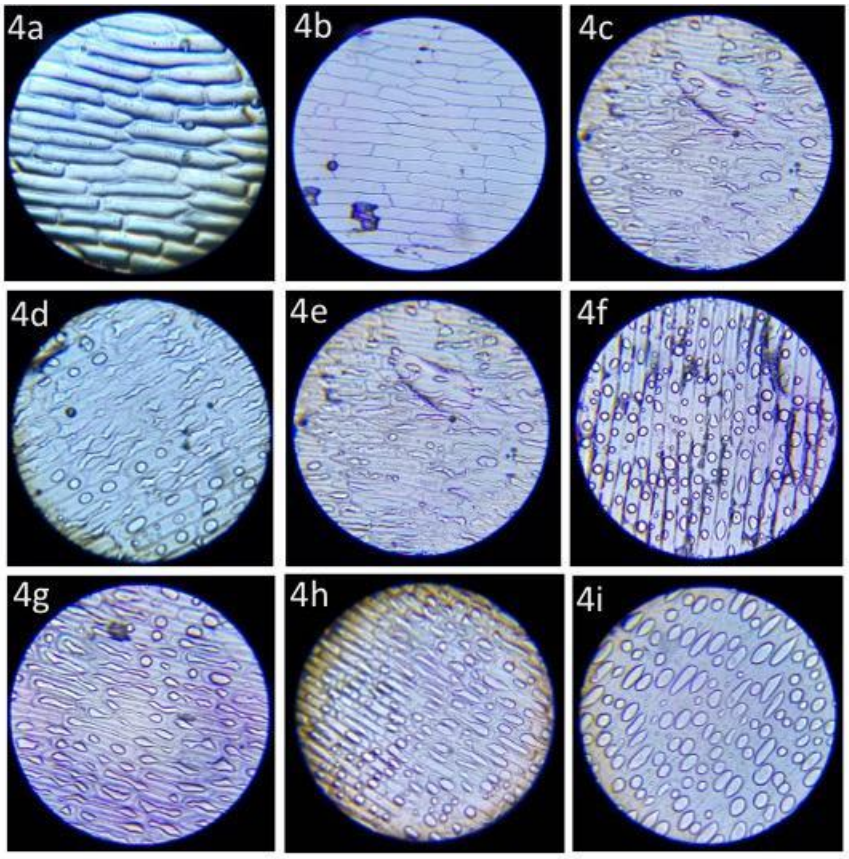
Compare the fragments generated by Nco I alone and double digestion of Nco I and Hind III

Fragments obtained on Nco I digestion	Fragments obtained on double digestion with Nco I and Hind III	Size difference in Nco I digested fragments (column 1) on double digestion	Position of the Hind III site
10789	10336	10789 – 10336 = 453	Outside 551 and 6262
2546	2093	2546 – 2093 = 453	Between 3200 and 5746
1567	1185	1567 – 1185 = 382	Between 1633 and 3200
1082	1082	No change	
516	516	No change	
	453 (X2)		
	382		

Task 2: Plasmolysis of onion cells – an analysis

Q. 2.1 Preparation of onion epidermal cells

Students could choose to prepare the slide in isotonic solution, IS (the correct option) – Panel 4a, or in water (incorrect) Panel 4b. Partial marks will be given in case the student has prepared it in water. The shape of the cells can be used to judge this.



Key:
IS – 4a
20N – 4c, 4d, 4e
20N by A (0%) – 4b
20N by B (15%) – 4e, 4f, 4g
20N by C (5%)- 4h and 4i

.

1. Shape of the cells as in Fig 4a.

4 points
(partial marking: shape of the cells as in fig 4b – 2 points)

2. Prepared as single sheet of cells with no folding etc.

4 points
(partial marking: any other – 2 points)

3. No bubbles

2 points
(partial marking: presence of bubbles – 1 point)
Total points for slide preparation 10 points

Q 2.2: Fill the table

Table 2.1									
2.1.1 Solution used for task I.1 _____ IS _____									
2..1.11	Magnification power of the objective selected = __10____ X								
	Panel								
	4a	4b	4c	4d	4e	4f	4g	4h	4i
2.1.14 (solution chosen by you)	X								
2.2 (20% NaCl)			X	X	X				
2.3 (20% NaCl followed by solution A		X							
2.4 (20% NaCl followed by solution B						X	X		
2.5 (20% NaCl followed by solution C								X	X

The marking scheme takes into account the biological variability that has been observed over several experiments and onion samples. In treatments were

variability has been observed, tick in any one of the marked cells will be given full points

2.2.1= **1.5 points**

2.2.6 = **1.5 points**

2.3 to 2.5 : 3 points for each correct answer = 3 X 4 = **12 points**

Note: The row 2.2. 12 has already been marked with the photograph

Q. 2.3

Table 2.2			
% of NaCl	Solution A	Solution B	Solution C
0	X		
5			X
15		X	

For the complete solution (all 3 crosses) **= 5 points**
(no partial marking)

Q. 2.4

When a plant cell is placed in pure water, water will move inside leading to increase in pressure potential (increasing turgidity) from 0 to + Mpa. Thus, curve A represents pressure potential.

According to the given formula

Water potential = Pressure potential + solute potential

When pressure potential is zero, water potential = solute potential (curves B and C). As water moves in solute potential increases, as a result water potential will also increase and would be more than solute potential. Thus, curve B is water potential and C is solute potential.

Table 2.3.			
	A	**B**	**C**
Water potential (ψ)		×	
Pressure potential (ψp)	×		
Solute potential (ψs)			×

1 point for each correct answer = 1 X 3 = **3 points**

Q. 2.5

X ← **Y**

The water potential for cell X is -5 and that of cell Y is -3. Thus water moves from Y to X

1 point

Q. 2.6

a. TRUE
b. FALSE

Water potential = Pressure potential + solute potential

Thus water potential in the plant cell = -8.5 + 1.5 = 7.0 MPa, which is the same as the water potential of the solution in which the plant cell is placed. Thus there will be no movement of water.

0.5 for each correct answer = **1 point**