INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Viewing exam as superuser/admin

PRACTICAL EXAM 1– PLANT ANATOMY, BIOSYSTEMATICS AND EVOLUTION

INTRODUCTION

Max. total points 100
Exam duration 90 minutes
six questions

Land plants constitute a monophyletic lineage of Eucaryotes, that has been extremely successful in adapting to terrestrial habitats. Land plants structure terrestrial ecosystems physically and provide the framework for ecological interactions between all other land-living organisms. In this exam, we take a closer look at the evolution of the key characters, that led to the dominance of land plants on Earth.

Purpose of exam: Plant Identification , morphological description and reconstruction of ancestral relationships

The exam consists of five parts.

Identification of specimens (5 points)
Morphological description (36 points)
Morphological variation and character matrix coding (29 points)
Mapping character evolution on a phylogenetic tree (21 points)
Evolution of key characters in land plants (9 points)

We suggest you read the entire exam file before you begin the lab work.

MATERIALS & EQUIPMENT

In order to do your lab work, you need the materials listed below. Please, ensure that these materials are available to you. **If anything is missing, contact the exam personnel by raising your pink card immediately – and no later than 15 minutes after the beginning of the exam. Please handle all the materials carefully as they will be used by all your teammates..**

5 Herbarium sheets (H1–H5). (Please note that the information included in the lables is not important for identifying the specimens to species). IMPORTANT: PLEASE DO NOT WRITE ON THE NEWSPAPER PAGES PROTECTING THE PLANTS - IF YOU DO IT WILL EXPEL YOU FROM THE EXAM.
5 Photos of live plants in nature (P1–P5)
2 Collections of live plants (F1–F2)
4 Alcohol-preserved collections of selected plant parts (A1–A4)
4 Anatomical sections mounted on microscope slides (M1–M4)

1 petri dish
1 forceps
1 teasing needle
1 click-on macro-lens for the tablet
1 touch pen for the tablet
1 microscope

Others
1 sheet with pictures of the included materials ,

1. IDENTIFICATION OF SPECIMENS (5 POINTS)

The materials (see above) belong to eight species of land plants, listed below in alphabetical and numbered order:

1 Allium ursinum (Wild garlic)
2 Equisetum arvense (Common horsetail)
3 Lycopodium annotinum (Bristly club-moss)
4 Pinus sylvestris (Scots pine)
5 Pisum sativum (Garden pea)
6 Polypodium vulgare (Fern)
7 Polytrichum commune (Common hair moss)
8 Selaginella kraussiana (African clubmoss)

These 8 species represent five major evolutionary lineages of land plants: Mosses (1 species), Lycophytes (2 species), Ferns and their allies (2 species), Gymnosperms (1 species) and Angiosperms (2 species). Notice that Selaginella kraussiana will be used throughout this exam as an exemplar species which signifies that some of the answers have already been provided

Q. 1 Identification of specimens

Identify each species on the five herbarium sheets as one of the eight species listed above.

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
H1	○	○	○	○	○	○	○	○
H2	○	○	○	○	○	○	○	○
H3	○	○	○	○	○	○	○	○
H4	○	○	○	○	○	○	○	○
H5	○	○	○	○	○	○	○	○

Identify each species on the five photographs (P1-P5) as one of the eight species listed above.

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
P1	○	○	○	○	○	○	○	○
P2	○	○	○	○	○	○	○	○
P3	○	○	○	○	○	○	○	○
P4	○	○	○	○	○	○	○	○
P5	○	○	○	○	○	○	○	○

Identify each species on the two live plants (F1-F2) as one of the eight species listed above.

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
F1	○	○	○	○	○	○	○	○
F2	○	○	○	○	○	○	○	○

Identify each species on the four alcohol-preserved specimens (A1-A4) as one of the eight species listed above.

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
A1	○	○	○	○	○	○	○	○
A2	○	○	○	○	○	○	○	○
A3	○	○	○	○	○	○	○	○

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
A4								

Identify each species on the four anatomical sections (M1-M4) as one of the eight species listed above.

	A.URS	E.ARV	L.ANN	P.SYL	P.SAT	P.VUL	P.COM	S.KRA
M1								
M2								
M3								
M4								

2. MORPHOLOGICAL DESCRIPTION (36 POINTS)

Locate the position of the nine morphological structures (listed below) on your materials and document your identification photographically (An example is shown in Fig. 2.1).

Morphological structure
Anther
Operculum
Sorus
Sporophyll
Microphyll
Sporangium
Sepal
Seed
Pollen grain

Protocol
1. Mount the click-on macro-lens for the tablet onto the camera lens of your tablet.
2. Choose one specimen from the materials, in which the relevant structure is represented.
3. Photograph plant specimen. Photograph only one specimen of each structure.
4. Indicate with an arrow on the photo the position of the structure (see Fig. 2.1 for an example).
5. Repeat this procedure (steps 2–5) for the remaining morphological structures.

Figure 2.1: Example of how to mark a structure with an arrow: Calyptra on Polytrichum commune (step 4 in the protocol above)

Each uploaded photo with an arrow placed at the correct structure earns you 3 points. An additional point is awarded for clarity of the photos. The structure should be, 1) fully represented, 2) fill up the picture frame and, 3) in focus.

Drag-and-drop the blue arrow. The arrow point has to be exactly at the structure in question.

Q. 2 Photos of 9 morphological structures (27 points) and clarity of structure (9 points)

Anther photo – 3 points

Operculum photo – 3 points

Sorus photo – 3 points

Sporophyll photo – 3 points

Microphyll photo – 3 points

Sporangium photo – 3 points

Sepal photo – 3 points

Seed photo – 3 points

Pollen grain photo – 3 points

Additional Points due to "missing" pictures (Pictures that failed to upload without further attempt).

3. MORPHOLOGICAL VARIATION AND CHARACTER MATRIX CODING (29 POINTS)

As a first step in the character analysis, we want you to describe the morphological variation across the following eight species in a way that will allow for mapping character evolution on a phylogenetic tree:

1 Allium ursinum
2 Equisetum arvense
3 Lycopodium annotinum
4 Pinus sylvestris
5 Pisum sativum
6 Polypodium vulgare
7 Polytrichum commune
8 Selaginella kraussiana

In Table 3.1. below you will find 9 morphological characters of key importance for the evolution of the Land Plants listed. Each character is broken down into two states. Notice that the assignment of character states implies an evolutionary direction, where '0' indicates the least derived (older) condition and '1' the derived (newer) condition:

Table 3.1. Definition of character states (0 or 1) for 9 characters.

	State 0	State 1
Character 1	Dominated by gametophyte phase	Dominated by sporophyte phase
Character 2	Stem without roots	Stem with roots
Character 3	Stem without vascular tissue	Stem with vascular tissue
Character 4	Female gametophyte released from sporophyte	Female gametophyte retained on sporophyte
Character 5	One sporangium per sporophyte	More than one sporangium per sporophyte
Character 6	Sporophyll with either one sporangium or sporophyll absent.	Sporophyll with more than one sporangium
Character 7	Homosporous, i.e. with only one kind of spore	Heterosporous, i.e. with megaspores and microspores
Character 8	Male gametes motile	Male gametes not motile
Character 9	Without double fertilization	With double fertilization

Q. 3 Character states for the eight study species (max 29 points)

The next step in the character analysis is describing the morphological variation in a table called a character matrix.
The character matrix below is constructed to describe the variation of the 9 above-mentioned key characters across the eight species, that we focus on in this exercise.

Code the matrix by filling out the empty cells. For any given combination of a species and a character assign either the state '0' or the state '1' to the cell. We have already coded Selaginella kraussiana and additional 5 cells for you in fig 3.1
By tapping in a cell, you choose a state (1 or 0). First tap gives you '1', second tap gives you '0', and third tap deletes the content of the cell. NB: Please, be patient - a short delay when shifting between states may occur!

0–29 correct cell values earn you no points, wrong or no value give 0 point
Every correct cell value > 29 correct cells earns you 1 point. Max. 29 points for 58 correct cell values.

1 = 1

0 = 0

	1	2	3	4	5	6	7	8	9
Allium ursinum									
Equisetum arvense									
Lycopodium annotinum									
Pinus sylvestris									
Pisum sativum									
Polypodium vulgare									
Polytrichum commune									
Selaginella kraussiana									

Character number:

Plants:	1	2	3	4	5	6	7	8	9
Allium ursinum						1			
Equisetum arvense						1			
Lycopodium annotinum									
Pinus sylvestris						1		1	
Pisum sativum						1			
Polypodium vulgare									
Polytrichum commune									
Selaginella kraussiana	1	1	1	0	1	0	1	0	0

Figure 3.1: Cell values provided to Question 4.

4. MAPPING CHARACTER EVOLUTION ON A PHYLOGENETIC TREE (21 POINTS)

A phylogenetic tree is a hypothesis about the ancestral relationships among a set of study organisms (Fig. 4.1).

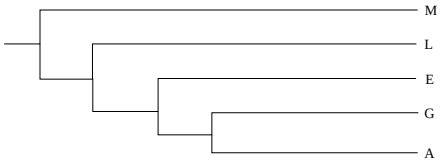


Figure 4.1: A phylogenetic tree showing the hypothetical relationships among the five major lineages of land plants. G = Gymnosperms; L = Lycophytes; A = Angiosperms; M = Mosses; E = Ferns and their allies (Monilophytes).

In this next step, we map character evolution on a given phylogenetic tree such as the one shown in Fig. 4.2. The tree represents a worldwide consensus between different hypotheses about the evolution of land plants.

We will use a character mapping procedure called deleted transformation (DELTRAN). Proceed according to the following protocol:

1. Characters are only allowed to change forward from '0' to '1'.
2. Minimize the number of times that a character changes on the tree (principle of parsimony)
3. If it is impossible to restrict a change in a given character to just a single branch, then let the character change more than once (parallel evolution).
4. Indicate on the tree on which branch a given character changes state. Notice that changes of characters evolving in parallel (= changing state several times) is indicated with two vertical bars (||), whereas a unique character state change (= changing a single time) is indicated with a single vertical bar (|). Finally, indicate the number of the character that changes its state from 0 to 1 such as shown on the tree below.

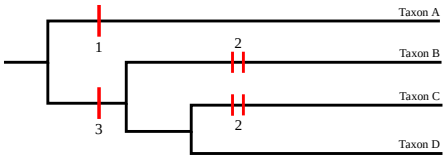
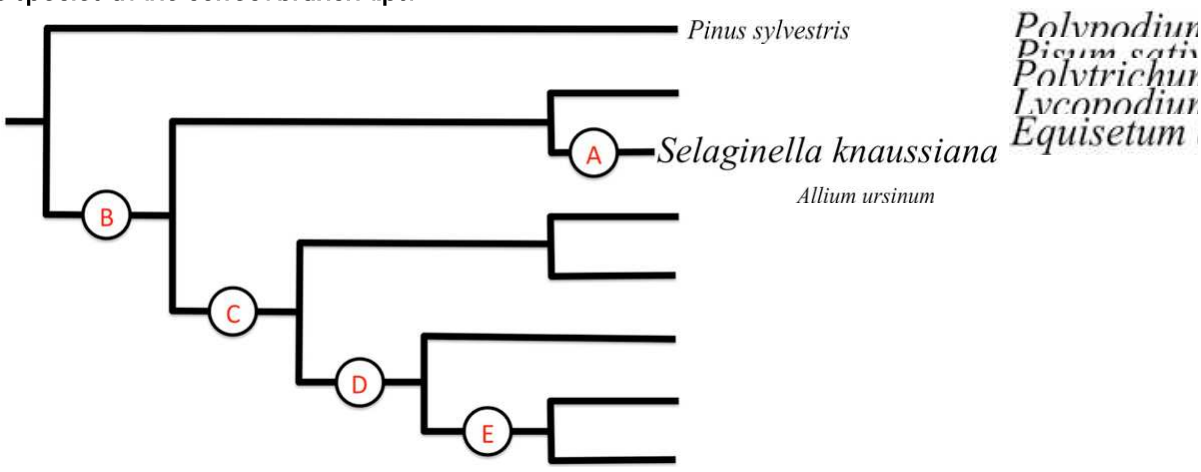


Figure 4.2: A hypothetical phylogeny of four taxa A–D.

Q. 4 Phylogenetical relationships of the eight study species (21 points)

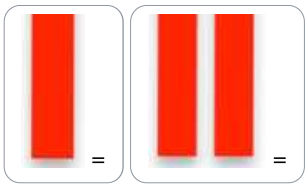
Place the species at the correct branch tips.



Q. 5

In the table below you find the branches A-E of the phylogeny above in the rows and the key characters 1-9 in the columns. By tapping on the cells you indicate on which branches the character state changes occur, according to the DELTRAN principle described above. One tap gives you a unique character change, two taps a parallel character change. A third tap will delete the content of the cell. Notice that the character state changes may be unevenly distributed over the phylogenetic tree and consequently some cells will have to be left empty.

0-15 correct cells give you 0 point.
16-40 correct cells give you 8 points
41–45 correct cells give you 16 points



	1	2	3	4	5	6	7	8	9
Branch A									
Branch B									
Branch C									
Branch D									
Branch E									

5. EVOLUTION OF KEY CHARACTERS IN LAND PLANTS (9 POINTS)

Based on reconstruction of character evolution similar to what you have just been through, botanists discuss the drivers of early land plant evolution. Two hypotheses are widely accepted:

Hypothesis H1: Early evolution in land plants reflects an increasing independence of water for completion of reproduction

Hypothesis H2: Evolution in land plants reflects an increasing ecophysiological ability to cope with dessication.

Notice that the hypotheses are not necessarily mutually exclusive

Q. 6 Choice of evolutionary hypotheses (max. 9 points)

Based on part 4, indicate below for each character state change, whether this supports H1, H2, both or none.
Each correct answer in a cell earns you 1 point.

✓ = Support	— = No support

	H1	H2
Change in character 1		
Change in character 2		
Change in character 3		
Change in character 4		
Change in character 5		
Change in character 6		
Change in character 7		
Change in character 8		
Change in character 9		

END

INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Viewing exam as superuser/admin

PRACTICAL EXAM 2 MOLECULAR BIOLOGY

GENERAL INFORMATION

Max. total points 100
Exam duration 90 minutes
15 questions

The exam consists of three parts.

Part 1. Restriction enzyme map of plasmid (48 points)
Part 2. PCR-based genotyping of yeast mutants (37 points)
Part 3. Amino acid auxotrophy of mutant yeast (15 points)

We suggest you read the entire exam file before you begin the lab work. In order to accomplish the entire exam, you have to run the three parts in parallel (see Figure below).

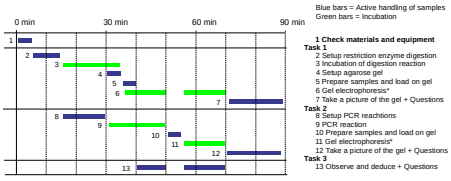


Figure 0.1: Suggested time table for completion of the three parts of the exam.

1 Check materials and equipment

Task 1

2 Setup restriction enzyme digestion
3 Incubation of digestion reaction
4 Setup agarose gel
5 Prepare samples and load on gel
6 Gel electrophoresis*
7 Take a picture of the gel + Questions

Task 2

8 Setup PCR reactions
9 PCR reaction (in thermal cycler)
10 Prepare samples and load on gel

11 Gel electrophoresis*
12 Take a picture of the gel + Questions

Task 3
13 Observe and deduce + Ouestions

*Use the same gel, but different wells for the samples

INTRODUCTION

MATERIALS AND EQUIPMENT

In order to do your lab work, you need the materials A-V listed below. Please, ensure that these materials are available to you. **If anything is missing, contact the exam personnel by raising your pink card immediately – and no later than 15 minutes after the beginning of the exam.**

Please notice

1. All liquids are provided in 2X excess amount of what is needed in order to do the analyses. Additional materials (including tubes and agarose gel) cannot be provided in case of spillage or errors during the set-up of experiments.
2. Remember, in order to collect liquids at the bottom of a tube, use the technique that you were shown on Monday.
3. Remember, one of the purposes of the exam is to test your lab work skills.
4. Tubes labeled with a blue line on the label should be **kept on ice at all times.**
5. All solutions are frozen so you will have to **thaw and mix them** before use.

MATERIAL ANNOTATION	QUANTITY	MATERIAL	USED IN PART
A	1	micropipette 1-10 µL	1, 2
B	1	micropipette 20-200 µL	1, 2
C	1	box of pipette tips 1-10 µL	1, 2
D	1	box of pipette tips 20-200 µL	1, 2
E	1	rack for PCR tubes	1, 2
F	1	rack for 1.5 ml tubes	1, 2
G	5	1.5 ml tubes (in envelope)	2

H	2	strips of 0.2 ml PCR tubes (in envelope). Do not break the tubes apart	1, 2
I	1	White bag with agarose gel with 12 wells. (Do not open the bag until you are ready to load the gel)	1, 2
J	1	OneRun electrophoresis system with running buffer	1, 2
K	1	marker pen	1, 2
L	1	stopwatch/timer	1, 2
M	1	paper tissue	1, 2
N	1	set of plastic gloves (handed out in the waiting room)	1, 2
O	2	zip-lock bags (in envelope)	1
P	1	Booklet with photos of yeast grown on eight different media	3
Q	1	Chart of biochemical reactions for formation of various amino acids (on the wall of the workspace)	3
R	1	1 Kb Plus DNA ladder (in envelope)	1, 2
S	1	Pink card for contat with exam personnel	1, 2, 3
T	2	Small ID tags	1,2
U	1	Big ID tags	1
V	1	Water 540 µL	1,2

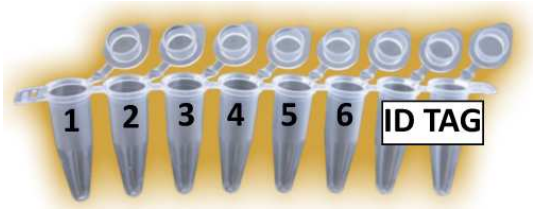


Figure 0.2: Material H: Tubes and ID label filled out with country code and student number.

Ice bucket containing 1.5 ml tubes with a set of liquids.

LIQUID ANNOTATION	QUANTITY	LABEL	VOLUME (µL)	CONTENT	USED IN PART
1	1	dNTPs	60	dATP, dGTP, dCTP and dTTP mix	2
2	1	DNApol buffer	140	DNA polymerase buffer (5X)	2
3	1	Loading buffer	100	Loading buffer for agarose gel electrophoresis	1, 2
4	1	Prime A	30	Primer pair A	2
5	1	Prime B	30	Primer pair B	2
6	1	Prime C	30	Primer pair C	2
7	1	Prime D	30	Primer pair D	2
8	1	Prime E	30	Primer pair E	2
9	1	DNApol	14	DNA polymerase	2
10	1	1 Kb ladder	12	DNA ladder for agarose gel electrophoresis	1, 2

11	1	Buffer 1	8	Buffer 1 (10X)	1
12	1	Buffer 2	8	Buffer 2 (10X)	1
13	1	Buffer 3	8	Buffer 3 (10X)	1
14	1	TemplateWT	6	Template DNA wild type	2
15	1	Template mutant	6	Template DNA mutant	2
16	1	Plasmid tube 1	6	Plasmid tube 1	1
17	1	Plasmid tube 2	6	Plasmid tube 2	1
18	1	ApaI	6	ApaI restriction enzyme	1
19	1	EcoRI	6	EcoRI restriction enzyme	1
20	1	SmaI	6	SmaI restriction enzyme	1

1. RESTRICTION ENZYME MAP OF PLASMID (48 POINTS)

An experiment was designed to determine the subcellular localization of protein Alb in Saccharomyces cerevisiae. The experimental strategy required that the gene alb (999bp) was fused with the sequences encoding two different fluorescent proteins. This was achieved by cloning the gene into two plasmid backbones (pX and pZ) resulting in plasmid pX:alb and pZ:alb.

E. coli was transformed with the two ligation mixes. Plasmids from two resulting E.coli transformants were purified, yielding DNA found in the tubes “Plasmid tube 1” and “Plasmid tube 2”. Your task is now to determine which two of the four possible plasmids (pX, pX:alb, pZ or pZ:alb) are contained in each of the two tubes (one in each tube)

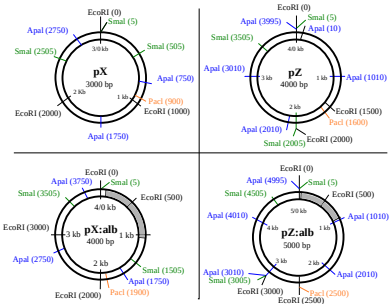
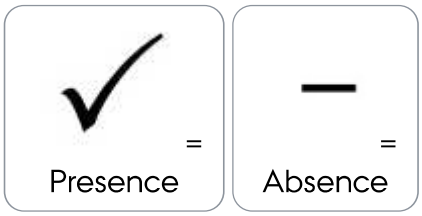


Figure 1.1: Restriction digestion map for ‘pX’, ‘pZ’, ‘pX:alb’ and ‘pZ:alb’.

Q. 1 SIZES OF DNA FRAGMENTS (2 POINTS)

Indicate here by a tag, the presence of expected sizes of DNA fragments obtained by a complete PacI digestion of the four plasmids



	PX	PX:ALB	PZ	PZ:ALB
0.5 kb	☐	☐	☐	☐
1.0 kb	☐	☐	☐	☐
1.5 kb	☐	☐	☐	☐
2.0 kb	☐	☐	☐	☐
2.5 kb	☐	☐	☐	☐
3.0 kb	☐	☐	☐	☐
3.5 kb	☐	☐	☐	☐
4.0 kb	☐	☐	☐	☐
4.5 kb	☐	☐	☐	☐
5.0 kb	☐	☐	☐	☐

Different restriction enzymes require different reaction conditions (Table 1.1)

Table 1.1. Restriction enzymes and their optimal reaction conditions.

Enzyme	% activity in Buffer 1	% activity in Buffer 2	% activity in Buffer 3	Optimal reaction temperature °C
ApaI	25	50	100	25

EcoRI	10	100	10	37
PacI	100	75	10	37
SmaI	0	0	100	25

RESTRICTION ENZYME DIGESTION OF THE TWO PLASMIDS

Q. 2 CHOICE OF RESTRICTION ENZYME (4 POINTS)

You have three restriction enzymes available, ApaI, EcoRI, and SmaI. Tap the one of these three enzymes that will allow you to distinguish between the four possible plasmids. Please note, that DNA fragments with a size smaller than 100 bp will result in very faint bands and that their size cannot be determined accurately.

1. APAI
2. ECORI
3. SMAI

Q. 3 DIGESTION SOLUTIONS (4 POINTS)

Restriction enzyme digestion of the two plasmids

Protocol

1. Label a strip of 0.2 mL PCR-tubes with S1, S2, S3 and S4 respectively.
2. Design your digestion experiment to identify the two plasmids using one of the enzymes for all digestions. Include a control with uncut plasmid DNA. The total volume for each reaction should be 10 µL. A typical restriction enzyme digestion includes the following: 2 µL plasmid DNA, 1 µL of restriction enzyme, 1 µL Buffer and water to a final volume of 10 µL.

Indicate with integers, what you add to each of the four tubes (all amounts are in µL). Write '0' if you do not want to add this specific ingredient.

	S1 (µL)	(S2 (µL) (Control)	S3 (µL)	S4 (µL) (control)
Plasmid tube 1				
Plasmid tube 2				
ApaI				
EcoRI				
SmaI				
10 x Buffer 1				
10 x Buffer 2				
10 x Buffer 3				
Water				
Total volume				

Q. 4 REACTION CONDITIONS FOR DIGESTION (2 POINTS)

Protocol continued

3. Mix the necessary components for the restriction digestion reactions as you specified in Question 2.
4. Set the volume on the pipette to 5 microliter and mix thoroughly by pipetting up and down 5-10 times in each tube. Avoid creating bubbles by keeping the pipette tip below the surface of the liquid at all times.
5. Place the strip of 0.2 ml tubes in the small Zip lock bag. Close the bag and stick on label with your ID number to the outside of the bag

Indicate here, the reaction conditions you use for the digestion.

1. 25°C

2. 37°C

Protocol continued

6. Raise your pink card and an Official will bring your samples to an incubator of your choice.
Complete digestion of the plasmid DNA will require 15 minutes of incubation.
7. After 20 min raise your pink card and ask an Official to return your samples. Your samples will NOT be returned unless you ask for it.
8. Check that you have received your own samples.
9. Add 3 µL of loading buffer to each of the samples
10. Mix by pipetting up and down 10 times (avoid making bubbles)

Use the Agarose gel electrophoresis' protocol, and analyze the restriction enzyme digestions by gel electrophoresis.

AGAROSE GEL ELECTROPHORESIS

WARNING: ELECTRICAL HAZARD – Do NOT move the RunOne electrophoresis apparatus once it has been started! Do NOT insert objects through the lamella in the lid of the apparatus when it has been started!

WARNING: Potentially HAZARDOUS COMPOUNDS. ALWAYS wear gloves when handling the agarose gel as it is prestained with a DNA binding dye.

Suggested workflow:

Your samples from Part 1 and/or 2 should be analyzed by agarose gel electrophoresis.
a. Load the samples from Part 1 and run for 15–20 minutes.
b. Stop the apparatus
c. Load the samples from Part 2
d. Restart the apparatus with all the samples and run for an additional 15–20 minutes.

Electrophoresis Protocol

1. Put on rubber gloves.
2. Take the lid off the RunOne electrophoresis chamber by lifting it straight up.

3. Prepare the agarose gel for electrophoresis by unpacking it from the white bag. Remove the transparent lid and carefully lift tray with the gel out of the transparent casing. Handle the gel carefully as it easily breaks. Keep the white bag for later use.
4. Examine the gel for visible damage such as cracks, missing wells or bubbles trapped inside the gel. If you identify such problems report it immediately to the Officials by raising your pink card.
5. Place the gel as shown in Fig. 1.2.

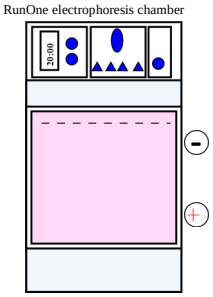


Figure 1.2: RunOne electrophoresis chamber (left) and posssible orientations of the agarose gel.

Electrophoresis protocol continued

7. The gel should be completely submerged in the running buffer. **If this is NOT the case** please raise your pink card and an Official will help you.
8. Load the agarose gel as described in Table 1.2 (samples from Part 1) and Table 1.3 (samples from Part 2). Load 3 µL of 1 Kb ladder, 13 µL sample from Part 1 and 15 µL from Part 2.

Table 1.2. Well 1–5 for Part 1

Well	1	2	3	4	5
Sample	1 Kb ladder	S1	S2 (control)	S3	S4 (control)
Volume (µL)	3	13	13	13	13

Table 1.3. Well 6–12 for Part 2.

Well	6	7	8	9	10	11	12
Sample	1Kb ladder	PCR-1	PCR-2	PCR-3	PCR-4	PCR-5	PCR-6

Volume(µL)	3	15	15	15	15	15	15

Electrophoresis protocol continued

9. Place the lid on the RunOne apparatus (the tab slide down into the power supply box).
10. Check that the voltage is set to 100 V otherwise use to "Voltage select" button.
11. Switch on the power by pushing the "Run/Stop" button and run the gel for 15 to 20 minutes.

Note that you will not be able to see the DNA bands migrating through the gel in real time, but that the blue indicator dye in the loading buffer migrates at the same speed as a 300 bp DNA fragment.
12. After 20 minutes switch off the power by pushing the "Run/Stop" button.
13. Hold up your pink card and an Official will bring you the equipment required for viewing the DNA in the agarose gel and for documenting this.

Document the DNA band pattern in the gel by taking a photo of it using your tablet and the equipment the IBO Official brings you. The Official will assist you setup and photoraph the gel.
14. Put on a pair of gloves.
15. Transfer the agarose gel in its tray onto the light-table.
16. Place the black photo hood on top of the light-table.
17. Place the tablet on top of the photo chamber in a way that the lens is pointing down into the chamber.
18. Capture a picture of your gel.

Q. 5 PHOTOGRAPH YOUR DNA BAND PATTERN

Take three favorable pictures of your DNA gel. These images will be used to judge the success of your PCR and restriction enzyme digestion reactions, loading of a ladder and the validity of the analysis.

Upload the first image of your gel

Upload the second image of your gel

Upload the third image of your gel

20. Once you have documented your gel, please transfer it to the white bag, in which it was delivered

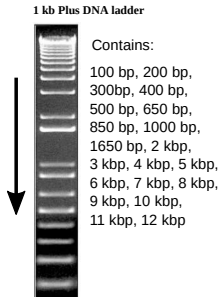
Q. 6 DIGESTION OF PLASMID DNA BY RESTRICTION ENZYME

Indicate here for each sample, if the plasmid DNA has been digested by the added restriction enzyme, or not.

	DIGESTED	NOT DIGESTED
S1	○	○
S2	○	○
S3	○	○
S4	○	○

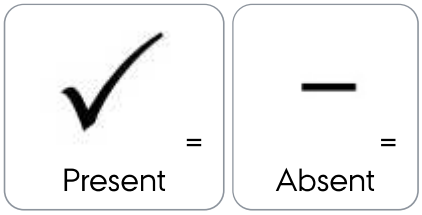
You will now be provided with a standardized gel picture showing results obtained for the restriction enzyme you chose (Question 3). Use this picture to answer Questions 7 and 8. NOTE YOU WILL ONLY HAVE ACCESS TO THIS PICTURE ONCE YOU HAVE DOCUMENTED YOUR GEL.

Use the provided reference for the DNA ladder as a help.



Q. 7 DNA FRAGMENT SIZE(S) OF YOUR RESTRICTION ENZYME DIGESTION

Indicate here, with a tag if a DNA fragment of a given size was present in your restriction enzyme digestion.



	DNA FRAGMENTS FROM DIGESTION OF DNA IN 'PLASMID TUBE 1	DNA FRAGMENTS FROM DIGESTION OF DNA IN 'PLASMID TUBE 2
0.5 kb		
1.0 kb		
1.5 kb		
2.0 kb		
2.5 kb		
3.0 kb		
3.5 kb		
4.0 kb		
4.5 kb		
5.0 kb		
5.5 kb		
6.0 kb		

Q. 8 IDENTIFICATION OF PLASMIDS IN TUBES

Indicate here for each tube which plasmids are present, and which plasmids are absent. Indicate with a '+' in the last column if it was impossible to determine the presence of plasmids for a tube.

	PLASMID PX	PLASMID PX:ALB	PLASMID PZ	PLASMID PZ:ALB	IMPOSSIBLE TO DETERMINE BASED ON THE CHOSEN ENZYME
Plasmid tube 1	☐	☐	☐	☐	☐
Plasmid tube 2	☐	☐	☐	☐	☐

2. PCR BASED GENOTYPING OF YEAST MUTANTS (37 POINTS)

A mutant yeast strain was isolated. This yeast strain, requires tyrosine and phenylalanine in the growth medium to be able to propagate. The ability of the mutant to grow on a medium without tryptophan has not been tested. A back cross of the mutant strain has shown that only a single gene is affected.
Your task is now to determine the genetic basis for the observed auxotrophy. You have five primer pairs available (Table 2.1), each capable of detecting dysfunctional (mutant) alleles of the genes encoding five key enzymes in the biosynthetic pathway for formation of aromatic amino acids (Fig. 2.1).

Table 2.1. The available primer pairs, the genes they amplify and the expected DNA amplicon in the wild type and mutant alleles.

Primer pair	Gene	Size of PCR product (bp) – wild type	Size of PCR product (bp) – mutant
A-forward + A-reverse	TYR1	500	400
B-forward + B-reverse	PHA2	500	250

C-forward + C-reverse	ARO7	500	300
D-forward + D-reverse	TRP5	500	350
E-forward + E-reverse	ARO2	500	300

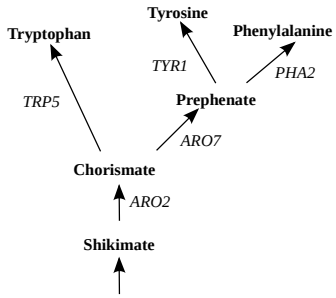


Figure 2.1: Biosynthetic pathway for the formation of aromatic amino acids (from upper left: Tryptophan, Tyrosine, Phenylalanine, Prephenate, Chorismate and Shikimate) and genes encoding the individual enzymes in the pathways.

Q. 9 CHOICE OF PRIMER PAIRS (5 POINTS)

By combining the results from two of the five available primer pairs (Table 2.1) you will be able to determine the genetic basis for the nutritional requirements observed in the mutant yeast strain.

Indicate the combination of two primer pairs (each either A, B, C, D or E from Table 2.1) to be used.

	A	B	C	D	E
First Primer Pair	○	○	○	○	○
Second Primer Pair	○	○	○	○	○

Protocol

1. Make a Master-mix for the PCR reactions in a 1.5 ml tube by mixing: 196 µl Water + 70 µl DNA polymerase buffer (x5) + 35 µl dNTP's + 7 µl DNA polymerase.
2. Mix by pipetting up and down 5-10 times with the pipette set at 100 µl.
3. Find the strip of 0.2 ml PCR tubes and glue on a small label with your ID number.
4. Label the tubes 1 - 6 (DO NOT break the strip of tubes into individual tubes – if you do so, your samples will not be accepted for PCR).
5. Pipette 44 µl of the Master-mix to each of the six PCR tubes.
6. To tubes 1 to 3, add 5 µl of the first Primer pair you have chosen.
7. To tubes 4 to 6, add 5 µl of the second Primer pair you have chosen.
8. To Tube 1 and 4, add 1 µl of wild type template DNA.
9. To Tube 2 and 5, add 1 µl of mutant template DNA.
10. To Tube 3 and 6, add 1 µl of water.
11. Mix the individual reaction by pipetting up and down 5-10 times with the pipette set at 45 µl.
12. Close the lids.

Table 2.2. Three PCR programs (1-3), stored at 10°C.

Program 1	Program 2	Program 3
98°C for 1 min	98°C for 1 min	98°C for 1 min
25 times: 98°C for 15 sec 55°C for 5 sec 72°C for 20 sec	25 times: 72°C for 20 sec 55°C for 5 sec 98°C for 15 sec	25 times: 72°C for 10 sec 98°C for 15 sec 55°C for 5 sec

72°C for 1 min	72°C for 1 min	72°C for 1 min
Store at 10°C	Store at 10°C	Store at 10°C

Q. 10 CHOICE OF PCR PROGRAM (2 POINTS)

Indicate here, which of the three PCR programs (Table 2.2) should be used for successful amplification if the primers have a melting temperature (Tm) of 60 °C and the used DNA polymerase can synthesize DNA at a speed of 25 bp/sec at 72 °C?

1. PCR PROGRAM 1
2. PCR PROGRAM 2
3. PCR PROGRAM 3

IMPORTANT

1. Assure yourself that Questions 9 and 10 are answered as you intended. Once you are happy with the answers, lock them by clicking "LOCK ANSWERS".
Note: after locking, you will not be able to change your answers anymore!
2. Once your answers are locked, raise your pink card and an official will transfer your samples to the thermal cyclers.The PCR program takes 20 minutes to complete. (In the meantime go to Parts 1 or 3 according to the time table in the introduction).
3. After 20 minutes raise your pink card and an official will return your samples. Please note that the sample will NOT be returned unless you ask for it.
4. Upon receiving your samples please check that they are your samples.
5. Prepare the PCR samples for agarose gel electrophoresis by adding 10 µL of loading buffer to each of the samples and mix by pipetting up and down 5 times (avoid making bubbles).
6. Load your samples as described in the Agarose gel electrophoresis section.
7. Documentate the gel by taking a photo (the photo of this gel will be inspected and a maximum of 22 points will be awarded)

You will now be provided with a standardized gel picture based on the primer pairs you previously chose to use for the PCR analysis. use this pciture to answer Questions 11 and 12. NOTE: YOU WILL GET THIS PICTURE FROM THE ASSISTANT RETURNING YOUR GEL.

Q. 11 SIZE OF DNA BANDS (4 POINTS)

Indicate here if a given size of the obtained DNA (bp) products was present '+'. Report your results column-by-column.

	PCR-1	PCR-2	PCR-3	PCR-4	PCR-5	PCR-6
150 bp						
200 bp						
250 bp						
300 bp						
350 bp						
400 bp						
450 bp						
500 bp						
550 bp						

Q. 12 DYSFUNCTIONAL ENZYME STEPS (4 POINTS)

Based on your results, indicate for each of the five possible enzymatic steps shown in Fig. 2.1 if they are functional or dysfunctional, or whether their status can not be determined with the used primers.

	FUNCTIONAL	DYSFUNCTIONAL	NOT POSSIBLE TO DETERMINE
TRP5	○	○	○
TYR1	○	○	○
PHA2	○	○	○
ARO7	○	○	○
ARO2	○	○	○

3. AMINO ACID AUXOTROPHY OF MUTANT YEAST (15 POINTS)

For many years, the haploid fungus Saccharomyces cerevisiae (baker's yeast) has been used as a model organism for elucidating the central metabolism in eukaryotes. Yeast is normally capable of synthesizing all twenty amino acids needed for protein synthesis. Following UV-mutagenesis various amino acid auxotrophic mutants have been isolated. The booklet with eight photos depicts haploid S. cerevisiae strains 1–5 grown on eight different media (A to H). Position 1 = no inoculum, Position 2 = Strain 1; Position 3 = Strain 2; Position 4 = Strain 3; Position 5 = Strain 4; Position 6 = Strain 5. The media composition for plate A to H is given in Table 3.1.
DO NOT MAKE ANY NOTES ON THE PHOTOS.

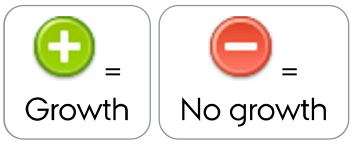
Table 3.1. Growth media

MEDIA	COMPOSITION
A	Rich complex media (yeast extract + peptone + dextrose)
B	Minimal media without any amino acids
C	Minimal media + homocysteine
D	Minimal media + isoleucine

E	Minimal media + threonine
F	Minimal media + methionine + threonine
G	Minimal media + lysine
H	Minimal media + proline

Q. 13 GROWTH/LACK OF GROWTH OF YEAST STRAINS (10 POINTS)

Record here growth or lack of growth for the various strains, use: "+" for growth and "–" for no growth.



	STRAIN 1	STRAIN 2	STRAIN 3	STRAIN 4	STRAIN 5
MEDIA A					
MEDIA B					
MEDIA C					
MEDIA D					
MEDIA E					
MEDIA F					
MEDIA G					
MEDIA H					

Q. 14 DYSFUNCTIONAL ENZYME STEP(S) (5 POINTS)

Based on the recorded growth patterns, deduce which enzymatic step(s) that is/are most likely dysfunctional, if any, in the five strains (1–5). For each mutant write the digit (1–31) for the dysfunctional enzymatic step (see Biochemical chart on the wall of the workspace) or 0 if no step is dysfunctional.

	STRAIN 1	STRAIN 2	STRAIN 3	STRAIN 4	STRAIN 5
ENZYME STEP NO. in biochemical chart on the wall					

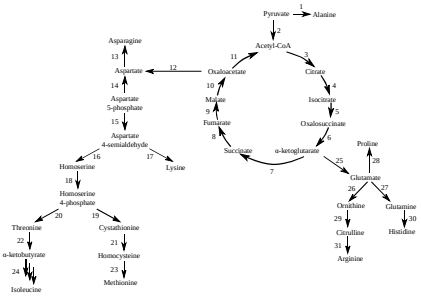


Figure 3.1: Simplified model for amino acid biosynthesis in yeast.

END

INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Viewing exam as superuser/admin

PRACTICAL EXAM 3 - ANIMAL BIOLOGY

Max. total points 99
Exam duration 90 minutes
8 questions

In this exam you will dissect a cod (Gadus morhua). You will be scored on your ability to dissect and identify structures and organs of the fish. In addition, you will be asked about functions of structures. Thus the exam is about functional morphology of an ecologically and economically important fish species.

The exam consists of four parts.

Part 1. Identification of external structures (15 points)
Part 2. Identification of internal structures (37 points)
Part 3. An analysis of the heart (22 points)
Part 4. An analysis of the brain (25 points)

We suggest you read the entire exam file before you begin the lab work. It is important that you get an overview of the exam in order to avoid destroying parts of the fish you might need for later tasks. Please keep in mind that we can only provide one student to each fish :-)

INTRODUCTION

MATERIALS & EQUIPMENT

In order to do your lab work, you need the materials A-J listed below. Please, ensure yourself that all these items are on your table.
If anything is missing, contact the exam personnel immediately – and no later than 15 minutes after start of exam. If you need any assistance during the exam, please raise your pink card..

Material A. 1 cod

Material B. 1 pair of scissors
Material C. 1 tweezer
Material D. 4 pairs of gloves
Material E. Needles
Material F. Number strips (cut off individual numbered tags)
Material G. 1 dissection polystyrene board
Material H. 1 bag for waste
Material I. Cleansing tissues
Material J. 1 touch pen for tablet

NB: Keep the tablet plugged in during the entire exam.

1. IDENTIFICATION OF EXTERNAL STRUCTURES (15 POINTS)

Protocol

1. Place the cod on the polystyrene dissection board with its left side upwards and head towards the left (Fig. 2.1.).
2. Mark the positions, using needles with a numbered tag (Material F), of all 11 external morphological structures listed below.Use the numbers given below for each of the corresponding structures If a structure is present more than once, you only have to mark one of them. To create the tags, mount each numbered tag onto a needle by placing the tag on the polystyrene board and pressing the needle through the tag

External morphological structures

1. Caudal fin
2. Anal fin
3. Pelvic fin
4. Pectoral fin
5. Dorsal fin (only one of these)
6. Operculum
7. Nostril
8. Lateral line
9. Barbel/whisker
10. Anus
11. Urogenital aperture

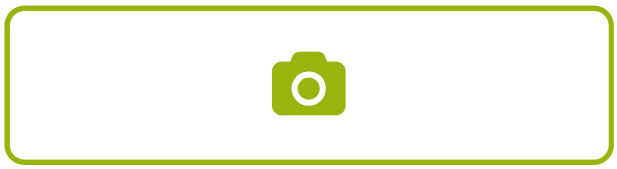
Protocol continued

3. Photograph the cod with all structures 1–11 indicated with numbered needles
Be sure that both the number tag and the structure can be seen in the photo; you may have to stand up while taking the photo. Notice that if a flag is missing or wrongly placed or if a number is not visible, you get 0 point for that particular structure.

Each correctly marked external structure earns you 1 point.

Q. 1 Photo of the cod with all 11 EXTERNAL morphological structures and numbered flags visible (max. 11 points)

Cod photo



Q. 2 Functions of external organs (1 point for each correct statement, max. 4 points)

Indicate if each of the following statements are true or false.

	TRUE	FALSE
In most fish, the lateral line consists of sensitive cells on the surface of the scales	○	○
In the cod the vertebral column bends and extends upwards into the upper lobe of the tail fin	○	○
In most modern fish the lateral line is a system sensitive to motions and vibrations in its surroundings	○	○
During accomodations the lens in a cod's eye changes shape the way it does in a mammalian eye	○	○

2. IDENTIFICATION OF INTERNAL STRUCTURES (37 POINTS)

Protocol

1. First, remove and discard the operculum as well as lateral musculature on the left side of the cod (as in Fig. 2.1).
2. Then separate the organs from the esophagus to the anus, and take them out as a unit; but leave the swim bladder in the fish. You may need to cut through the swim bladder
3. Place the organs on the polystyrene board.
4.Identify, in the dissected organs, the 11 internal structures described below, using needles with numbered tags (Material F),

Figure 2.1: Cod with left-side musculature removed; A numbered tag is placed on the eye to show how a correctly placed tag should look like, i.e. both tag and structure are clearly visible.

Internal morphological structures

1. Primary site for gas exchange
2. Urine-producing organ
3. Gas gland
4. The organ in which pepsinogen is secreted

5. The site where substances from the gall bladder and pancreas are secreted
6. The organ where the main absorption of nutrients takes place
7. Spleen
8. The organ where detoxification of blood takes place
9. Pyloric caeca
10. Swim bladder
11. Gill rakers

Protocol continued

5. Photograph the cod with all structures 1–11 indicated with numbered needles. Be sure that both the number tag and the structure can be seen in the photo. Notice that if a flag is missing or wrongly placed or if a number is not visible, you get 0 point for that particular structure.

Each correctly marked internal structure earns you 3 points.

Q. 3 Photo of the dissected cod with all 11 internal morphological structures and numbered flags visible (max. 33 points)

Cod photo

Q. 4 Functions of internal structures (1 POINT FOR EACH CORRECT STATEMENT, MAX. 4 POINTS)

Indicate if each of the following statements are true or false.

	TRUE	FALSE
Gas is secreted into the swim bladder by diffusion through its entire wall	○	○
The cod lacks a connection between the gas bladder and the alimentary canal	○	○
Catching cod may cause rupture of their swim bladder	○	○
The spleen is part of the immune system of the cod	○	○

3. STRUCTURE OF THE HEART (22 POINTS)

Protocol
1. Remove the heart from the fish and place it on the polystyrene board.
2. Mark the identity, using needles with numbered tags (Material F), of the four structures listed below.

1. Atrium
2. Ventricle
3. Bulbus arteriosus
4. Ventral aorta

Protocol continued
3. Photograph the heart with structures 1–4 indicated with numbered needles. Be sure that both the number tag and the structure can be seen on the photo.
Notice that if a flag is missing or wrongly placed or if a number is not legible/visible, you get 0 point for that particular structure.

Each correctly marked heart structure earns you 5 points.

Q. 5 PHOTO OF THE DISSECTED heart WITH ALL four STRUCTURES AND NUMBERED FLAGS VISIBLE (MAX. 20 POINTS)

Heart photo

Q. 6 FUNCTIONS OF heart STRUCTURES (1 POINT FOR EACH CORRECT STATEMENT, MAX. 2 POINTS)

Indicate if each of the following statements are true or false.

	TRUE	FALSE
The heart of the cod has two atria and one ventricle	○	○
The blood pressure of the cod is mainly created by the ventricle	○	○

4. STRUCTURE OF THE BRAIN (25 POINTS)

Protocol
1. Remove very carefully the central nervous system (brain and spinal cord) (as in Fig. 4.1).
2. Place the central nervous system on the polystyrene board
3. Mark the identity, using needles with numbered tags (Material F), of the seven structures listed below.

1. Tectum opticum (optic tectum)
2. Cerebellum (“little brain”)
3. Medulla spinalis (spinal cord)
4. Telencephalon (containing the olfactory center)
5. Sagittal otolith (sagittal otoliths are the largest of the three ear stones)
6. Optic nerve
7. Muscle(s) controlling eye movements

Protocol continued
4. Photograph the cod with all structures 1–7 indicated with numbered needles. Be sure that both the number tag and the structure can be seen on the photo.
Notice that if a flag is missing or wrongly placed or if a number is not legible/visible, you get 0 point for that particular structure.

Each correctly marked brain and spinal cord structure earns you 3 points.

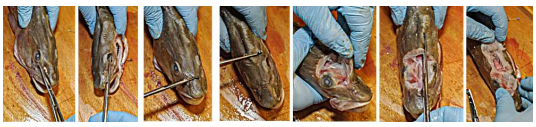
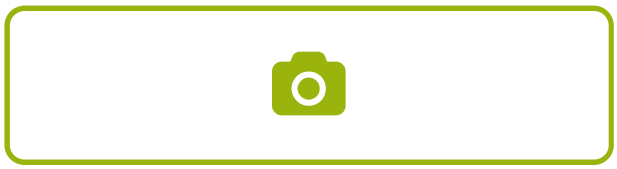


Figure 4.1: Removal of brain and spinal cord.

Q. 7 PHOTO OF THE DISSECTED brain and spinal cord WITH ALL seven STRUCTURES AND NUMBERED FLAGS VISIBLE (MAX. 21 POINTS)

Brain and spinal cord photo



Q. 8 FUNCTIONS OF brain and other STRUCTURES (1 POINT FOR EACH CORRECT STATEMENT, MAX. 4 POINTS)

Indicate if each of the following statements are true or false.

	TRUE	FALSE
The telencephalon is relatively smaller in fish than in mammals	○	○
Fish have 12 cranial nerves, the same as in mammals	○	○
Tectum opticum of the cod has two lobes, the left receives information from the left eye and the right from the right eye	○	○
Otoliths are homologous to the inner ear bones of mammals	○	○

END

INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Viewing exam as superuser/admin

PRACTICAL EXAM 4 - BIOCHEMISTRY

Max. total points 100
Exam duration 90 minutes
20 questions

INTRODUCTION

PURPOSE

In this exam you will analyse enzyme kinetics with and without an inhibitor of the enzyme.
The exam consists of two main parts, each of which contains three subparts.

Part 1 (57.5 points)
1.1. Introduction to enzyme kinetics (theory) (0 point)
1.2. Enzyme kinetics experiment of an industrial α-galactosidase using a synthetic substrate analogue pNP-Gal (laboratory work) (Questions 1–2: 40 points)
1.3. Data analysis of enzyme kinetics of α-galactosidase (Questions 4–11: 17.5 points)

Part 2 (42.5 points)
2.1. Introduction to enzyme inhibitors (theory) (Questions 11–13: 2 points)
2.2. Inhibition experiment of α-galactosidase (laboratory work) (Question 14: 27 points)
2.3. Data analysis of inhibition kinetics of α-galactosidase (Questions 15–20: 13.5 points)

Before you begin, we advise you to skim the entire exam to get an overview of the content. Since most points are earned on the lab part, **we recommend you to carry out parts 1.2 and 2.2, before starting with calculations and theoretical questions** (Parts 1.3, 2.1 and 2.3).

MATERIALS & EQUIPMENT

First, verify that you have all items listed below in front of you. **Please raise your pink**

card immediately, if anything is missing – and no later than 15 minutes after the start of the exam.

A. 1 p200 pipette. Use pipette p200 for volume interval 20-200 µL, unless otherwise stated
B. 1 p1000 pipette. Use pipette p1000 for volume interval 201-1000 µL, unless otherwise stated
C. 96 tips for p200 in box. A pipette tip should be discarded after each pipetting, unless otherwise stated
D. 96 tips for p1000 in box. A pipette tip should be discarded after each pipetting, unless otherwise stated
E. > 30 microcentrifuge tubes (1.5 ml)
F. 1 rack for microcentrifuge tubes
G. 2 microtitre plates labelled with your country code + A or B
H. 1 microtitre plate template
I. 1 stopwatch
J. 1 pencil
K. 1 marker
L. 1 calculator
M. 1 ruler
N. Pink card for contact with exam personnel
O. 9 ml 2 M (Molar=mole/Liter) Na_2CO_3 (**Stop**)
P. 6.5 ml 15 mM (milli-molar, milli=10^{-3}) pNP-Gal (**Substrate**)
Q. 15 ml Ultra pure water (**Water**)
R. 5 ml 1mM pNP (**Standard**)
S. 2 ml 0.024 mg/ml (**Enzyme**)
T. 5 ml 0.5 M (**Inhibitor**)
U. One touch pen for the tablet

1.1. INTRODUCTION TO ENZYME KINETICS

α-Galactosidases catalyze the hydrolysis of terminal galactosyl residues in α-galactosides. Typically, the activity of these enzymes is assayed using the synthetic substrate analogue para-nitrophenyl-α-galactoside (pNP-Gal), which is hydrolyzed to galactose (Gal) and para-nitrophenyl (pNP) (Figure 1.1). pNP-Gal is colourless, while the pNP product is yellow and its concentration can be measured quantitatively by determining its absorbance A_{405} at 405 nm using a microtitre plate reader.

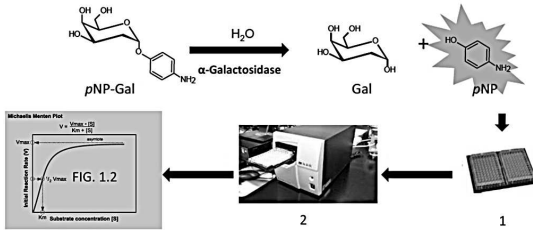


Figure 1.1: Schematic representation of the galactosidase activity assay: pNP is quantified

using a microtitre plate reader (2) that measures the absorbance A at 405 nm. In order to measure enzymatic activity, a standard curve is used to convert absorbance to a product concentration. 1, microtitre plate (Material G).

In Part 1, the dependency of the rate of hydrolysis on the substrate concentration will be investigated. In order to do so, a Michaelis-Menten plot (Figure 1.2), which describes this relationship is available to allow you to estimate the two important parameters V_{max} and K_m (see legend to Fig. 1.2).

The initial reaction rate V_o can be determined from $\Delta[P]/\Delta t$,which is the change in product concentration ([P]) per time (Δt).

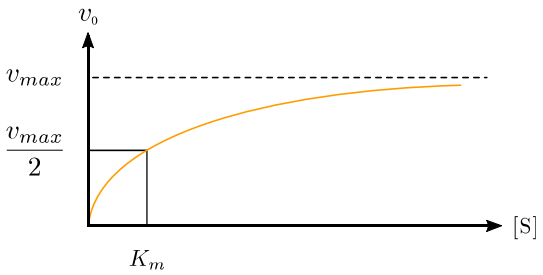


Figure 1.2: Michaelis-Menten plot: Initial reaction rate V_o versus substrate concentration [S]. K_m is the substrate concentration at which the enzyme operates at half its maximum rate, V_{max}, which reflects the saturation of the enzyme active sites with substrate.

Using another plot, the Lineweaver-Burk plot, the parameters V_{max} and K_m can be determined from the Y-axis and the X-axis intercepts, respectively (Figure 1.3). A Lineweaver-Burk plot is generated by plotting the inverse of the initial reaction rate ($1/V_o$) against the inverse of substrate concentration ($1/[S]$).

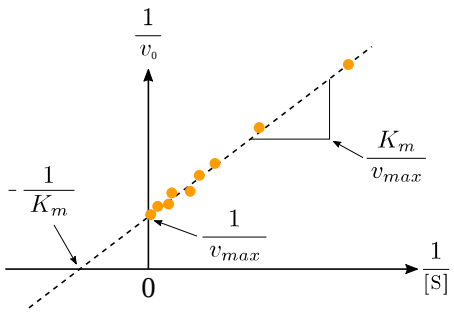


Figure 1.3: Lineweaver-Burk plot: After determining V_o at different substrate concentrations, a Lineweaver-Burk plot is made. A straight line is fitted to the data to determine K_m from the inverse of the intercept of the line with the X-axis, and V_{max} from the inverse of the intercept of the line with the Y-axis. These intercepts are calculated from the equation of the line.

1.2 ENZYME KINETICS EXPERIMENT OF AN INDUSTRIAL ALPHA-GALACTOSIDASE

1.2.1 STANDARD CURVE

Begin by generating the standard curve that will be used to measure the product (pNP) concentration of the enzymatic reactions later on. To generate the standard curve, you will need to dilute the 1 mM pNP standard stock solution (**Standard**) in the stop reagent (**Stop**).

Q. 1 Standard curve dilution scheme

The 1 mM pNP standard stock solution (Standard) will, when needed, be diluted in the stop reagent (Stop). Calculate the volumes of pNP and stop reagent needed to prepare the final standard concentrations in a total volume of 500 µl. Type your calculated values into the table below (Table 1.1).

Tabel 1.1: Dilution scheme to prepare the standard curve

Tube label	St1	St2	St3	St4	St5
[pNP] standard (mM)	0.2	0.4	0.6	0.8	1
Volume of standard stock solution (Standard) (µl)					
Volume of stop reagent (Stop) (µl)					

Preparation of the standard curve
Protocol

a. With your marker, label five 1.5 ml microcentrifuge tubes according to the first row in Table 1.1: from St1 to St5.

b. Transfer the different volumes of the 1 mM pNP standard solution (Standard) to the labelled 1.5 ml tubes according to your calculations in Table 1.1 (use the same pipette tip).

c. Transfer the different volumes of the stop solution (Stop) to the labelled tubes according to your calculations in Table 1.1. The standard solutions are mixed thoroughly by turning the microcentrifuge tubes upside-down 5 times.

d. Transfer 100 µL ultra pure water (Water) into wells A1-A5 and B1-B5 of microtitre plate A (use the same pipette tip, see Fig. 1.4 and/or use the microtitre plate template to help you pipette in the correct wells).

e. Transfer 50 µL of each of the final pNP diluted standard solutions (Table 1.1) into the same microtitre plate wells. Each solution is pipetted in duplicates in two different wells (The subscripts I and II designate replicates of the same solution, Fig. 1.4).

f. Add 100 µL stop reagent (Stop) using a p1000 pipette to each pNP standard, A1-A5 and B1-B5. Mix thoroughly by pipetting the mixture up and down two times.

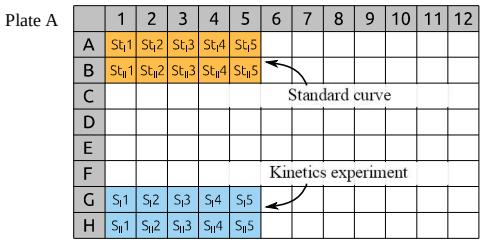


Figure 1.4: Microtitre plate A: St, standards (see Table 1.1); S, reaction mixtures with different substrate concentrations (see Table 1.2 below).

Proceed now with part 1.2.2, where you will set-up the enzymatic reaction mixtures to your microtiter plates.

Important note: the assistants will not accept any microtitre plates in the last 10 minutes of the exam. If you feel that you will not be able to complete part 1.2.2 in time, hand in your plate now by raising the pink card. You results will be shown in Question 2.

1.2.2 ENZYME KINETICS EXPERIMENT

Protocol

Prepare the pNP-Gal substrate diluted solutions for the kinetics experiment.

a. Label five 1.5 mL tubes with a marker with S1 through to S5 (Table 1.2).

b. The 15 mM pNP-Gal substrate stock solution (**Substrate**) is diluted with ultra pure water (**Water**) in the labelled 1.5 ml tubes (see Table 1.2 below). The diluted solutions should be mixed thoroughly by turning the tubes upside-down 5 times.

Table 1.2: Substrate dilution scheme for the kinetics assay.

Tube label	S1	S2	S3	S4	S5
Volume (µl) of 15 mM pNP-Gal **(Substrate)**	40	120	240	400	800
Volume (µl) of ultra pure water **(Water)**	960	880	760	600	200

c. Transfer 50 µL of each diluted substrate solution (Table 1.2) and 50 µL ultra pure water (**Water**) into microtitre plate A, wells G1-G5 and H1-H5. (see Figure 1.4 and/or the microtitre plate template).

d. Set the timer at 5 minutes and start it immediately after you pipetted the enzyme

solution to the first well to start the first enzymatic reaction (S_I1) as described below.

e. Pipette 50 µL of the 0.024 mg/ml α-galactosidase enzyme (Enzyme) into wells G1-G5 and H1-H5 starting with S_I1 and $S_{II}1$, and continue in the same order and tempo throughout to $S_{II}5$ to start the enzymatic reactions in each well (hereafter referred to as the "enzymatic reaction mixture"). Ensure good mixing by quickly but gently pipetting 50 µl of the mixture up and down two times in each well.

f. After 5 minutes incubation time add 100 µL 2 M Na_2CO_3 solution (**Stop**) using a p1000 pipette to stop each of the enzymatic reactions in wells G1-G5 and H1-H5 in the same order and tempo as you started them. Mix well by pipetting the mixture up and down two times.

Q. 2 enzyme kinetics experiment

Hand in your microtitre plate containing your samples from parts 1.2.1 and 1.2.2 by raising your pink card. After measurement, the obtained values will be display in the table below automatically.

Note: no microtiter plates will be accepted in the last 10 minutes of the exam!

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

1.3 DATA ANALYSIS OF ENZYME KINETICS

Your task is now to determine the kinetics parameters of substrate hydrolysis by the *α*-galactosidase.
First the standard curve linear function for the product (pNP) should be determined using data from table 1.3
Access to a standard curve enables you to calculate product concentrations in the reaction mixtures, which further allows the determination of the initial reaction rate (V_0) of the enzyme for each substrate concentration.
The standard data set resembling microtitre plate A and shown below (Table 1.3) should be used for the calculation. This will avoid error carry-over penalty from part 1.2. However, your own data will be measured and used in the evaluation of your exam.

Table 1.3: Provided absorbance data for calculations (columns 1-5 in microtiter plate format).

	1	2	3	4	5
A	0.882	1.681	2.473	3.251	3.964

B	0.858	1.657	2.449	3.227	3.940
⋮					
G	0.304	0.728	1.049	1.272	1.512
H	0.307	0.716	1.009	1.234	1.466

Q. 3 Mean Absorbance of standards

Calculate the mean absorbance for each duplicate measurement for the standard curve given in Table 1.3. Enter all answers with three digits after the decimal point.

Table 1.4: Mean absorbance for standard curve

Tube label	St1	St2	St3	St4	St5
[pNP] (mM)	0.2	0.4	0.6	0.8	1
Mean A_{405} nm of duplicates					

Q. 4 standard curve linear function

In the figure below (Figure 1.5), the concentration of pNP (mM) is plotted against the absorbance (Mean A_{405} nm calculated in Question 3).

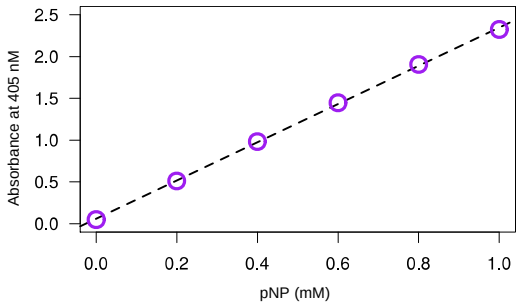


Figure 1.5: Hypothetical pNP product standard curve. The purple circles represent means of measured absorbances and the black dashed line is a linear regression to them.

Determine a and b of the standard curve linear function (see below) mathematically using only the mean absorbances of the two data points St1 and St5. Give a and b with three digits after the decimal point.:

A_{405} (absorbance units at 405 nm) = a·[pNP] (mM) + b, where a is the slope and b is the Y-axis intercept

a (A_{405}/mM)	
b (A_{405})	

The volume of the enzymatic reaction mixture from the experiment in Part 1.2.2 is 150 µl.

Q. 5 Reaction Time

Convert the reaction time given in the protocol into seconds.

Reaction time (seconds)	

Q. 6 Analysis of kinetics data (uninhibited enzyme)

Use the following standard curve equation to calculate the product concentration for each reaction mixture:

A_{405} absorbance = 2.29*[pNP] (mM) + 0.058.

The initial reaction rate V_0 can be determined from Δ[Product]/Δtime, i.e. the change in product concentration per time. Give all numbers with three digits after the decimal point.

Table 1.5: Analysis of uninhibited data

Tube label	S1	S2	S3	S4	S5
Volume of Stock solution (Substrate) (µl) (from table 1.2)	40	120	240	400	800
Volume of ultra pure water (Water) (µl) (from table 1.2)	960	880	760	600	200
Substrate concentration [S] prior to adding into the reaction mixture (mM)					
Substrate concentration [S] in reaction mixture (mM)					
Mean A_{405} absorbance, calculated from Table 1.3					
$[Product_{mean}]$ (mM)					
V_0 (µM/second)					
1/[S] (1/mM)					
$1/V_0$ (second/µM)					

Q. 7 Michaelis-Menten PARAMETERS (graphical estimate)

Shown below (Figure 1.6) is a theoretical Michaelis Menten plot (V_0 versus [S]) resembling the reaction mixtures S1–S5 in Table 1.3.

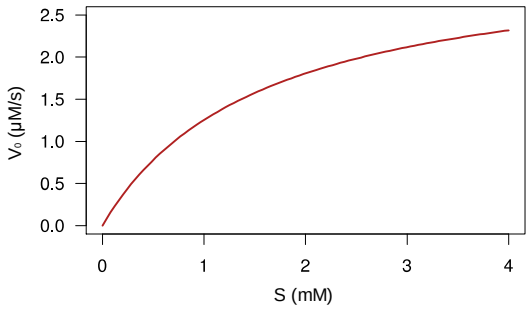


Figure 1.6: Theoretical Michaelis-Menten plot for the kinetics experiment in the absence of inhibitor

Estimate V_{max} and K_m graphically from the Michaelis-Menten plot (Fig. 1.6). Give answers with one digit after the decimal point.

V_{max} (µM/s)	
K_m (mM)	

Q. 8 Lineweaver-Burk linear function

Shown below (Figure 1.7) is the Lineweaver-Burk plot ($1/V_0$ versus $1/[S]$) of the S1–S5 data point in Table 1.3.

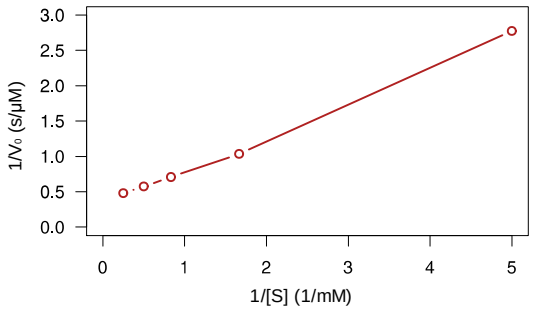


Figure 1.7: Lineweaver-Burk plot of the uninhibted enzyme kinetics data

Determine the linear function of in the Lineweaver-Burk plot (Figure 1.7) in the form shown below mathematically from the two data points for S1 and S5.
Give a and b with three digit after the decimal point.

$1/[V_o] = a \cdot 1/[S] + b$,

a (mM·s/µM)	
b (s/µM)	

Q. 9 determination of Vmax and Km

Using the linear function calculated above (Q. 8), determine K_m and V_{max} mathematically from the intercepts with the axes. Give numbers with three digits after the decimal point (no unit conversions should be done).

V_{max}	
K_m	

Q. 10 Enzyme concentration in reaction mixture

Calculate the enzyme concentration in the reaction mixture in µM from the enzyme stock concentration= 0.024 mg/ml and the enzyme's molar mass (75 000 gram/mole). Give the concentration with three digits after the decimal point.

Enzyme stock (mg/ml)	0.024
[E] (µM) in reaction mixture (micro=10^{-6})	

Q. 11 Turnover rate constant

The catalytic turnover rate constant k_{cat} (reaction rate of 1 enzyme molecule) has the unit 1/second and is calculated as follows:

$$k_{cat} = \frac{v_{max}}{[\mathrm{E}]}$$

Determine k_{cat} . Give number with three digits after the decimal point.

k_{cat} (1/second)	

2.1 INTRODUCTION TO INHIBITORS

Inhibitors are compounds that can specifically bind to enzymes, thereby reducing their activity and resulting in apparent changes in either K_m, V_{max} or both. Change in apparent kinetic parameters can be determined from the Lineweaver–Burk plot of an enzymatic reaction performed in the presence of an inhibitor. Reversible inhibitors can be competitive, non-competitive, or uncompetitive, depending on the mode of binding to their enzyme targets.
The inhibition of enzyme activity and apparent change in kinetic parameters can also be visualised in Michaelis-Menten and Lineweaver-Burk plots (Fig. 2.1).

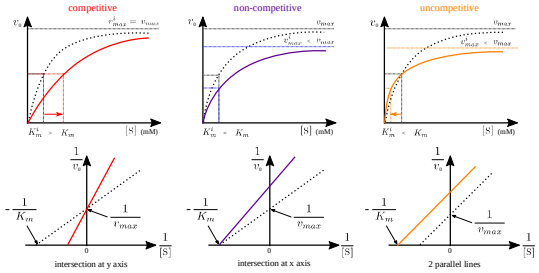


Figure 2.1: Inhibition of enzyme activity in Michaelis-Menten and Lineweaver-Burk plots. Dashed black curves are without an inhibitor and solid curves in the presence of inhibitor. v_0 is

initial reaction rate.

Inhibitors are characterized by their inhibition equilibrium constant K_i, defined as

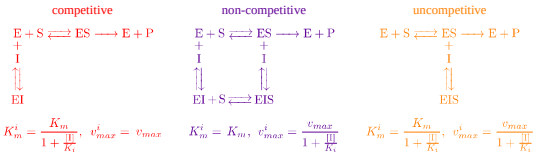
$$K_i = \frac{[\mathrm{I}]\,[\mathrm{E}]}{[\mathrm{EI}]}$$

Where [I], [E] and [EI] are the concentrations of the free inhibitor, free enzyme and enzyme-inhibitor complex, respectively.

For **competitive inhibition**, the apparent K_m in the presence of inhibitor is designated as K_m^i. The chemical equilibrium for substrate (S) and inhibitor (I) binding to the enzyme (E) is shown below. K_m and K_m^i are related according to the equation below:

For **non-competitive inhibition**, the apparent V_{max} in the presence of inhibitor is designated as V_{max}^i. V_{max}^i and V_{max} are related according to the equation below:

For **uncompetitive inhibition**, the apparent K_m and V_{max} in the presence of inhibitor are designated as K_m^i and V_{max}^i, respectively. K_m and V_{max} are related to K_m^i and V_{max}^i according to the equations below:



Equation 2.1: The chemical equilibria for substrate (S) and inhibitor (I) binding to the enzyme (E) is shown in the top part of the figure for different inhibition types. The lower part shows the equations relating the change in apparent kinetic parameters to the inhibitor concentration and to the inhibition equilibrium constant.

Q. 12 factors affecting inhibition

For all inhibition types, the degree of inhibition, i.e. reduction in enzymatic reaction rate, is dependent on: (choose the best of the answers below).

1. INHIBITOR CONCENTRATION [I]
2. SUBSTRATE CONCENTRATION [S]
3. K_I OF THE INHIBITOR
4. CONCENTRATION OF [ES]
5. STATEMENTS 1, 2 AND 3
6. STATEMENTS 1 AND 3

Q. 13 Competitive Inhibition signature

Indicate if the following statement is true or false

	TRUE	FALSE
In competitive inhibition, the increase in substrate concentration [S] reduces or overcomes inhibition.	○	○

2.2. INHIBITION OF ALPHA-GALACTOSIDASE (27 POINTS)

This part is experimentally similar to Part 1b. An inhibition kinetics experiment of α-galactosidase will be conducted in the presence of 50 µL inhibitor, which has a concentration of 0.5 M (mole/Liter).

Protocol

Substrate preparation for inhibition kinetics experiment

a. Prepare the substrate solutions according to Table 2.1, similarly to what you have done in Part 1.2.2. Remember to mix the solutions by turning the tubes upside down 5 times.

Table 2.1: Substrate dilution scheme for kinetic assay.

Tube label	IS1	IS2	IS3	IS4	IS5
Volume (µl) of substrate stock solution (Substrate)	80	160	320	600	840
Volume (µl) of ultra pure water (Water)	920	840	680	400	160

b. Transfer 50 µL inhibitor (Inhibitor) into the microtitre plate B wells A1-A5 and B1-B5 using the same pipette tip. (See Figure 2.2 and/or the microtitre plate template).
c. Transfer 50 µL of each final substrate solution (Table 2.1) to the same well positions (A1-A5 and B1-B5).

Plate B

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS_I1	IS_I2	IS_I3	IS_I4	IS_I5							
B	$IS_{II}1$	$IS_{II}2$	$IS_{II}3$	$IS_{II}4$	$IS_{II}5$							
C												
D												
E												
F												
G												
H												

Figure 2.2: Microtitre plate B: IS, samples are reaction mixtures in the presence of inhibitor at different substrate concentration (see Table 2.1 above).

d. Set the timer at 5 minutes and start it immediately after you start the first enzymatic reaction by adding the enzyme solution to the first well (IS_I1) as described below.
e. Pipette 50 µL of the **α-galactosidase (Enzyme)** into the wells A1-A5 and B1-B5, starting with IS_I1 and $IS_{II}1$, and continue in the same order and tempo throughout to $IS_{II}5$ to start the enzymatic reaction in each well.
Ensure good mixing by quickly but gently pipetting 50 µl of the mixture up and down two times in each well immediately after you pippette the enzyme.
f. After 5 minutes incubation, add 100 µL stop reagent **(Stop)**, using a p1000 pipette, to stop each of the enzymatic reactions in the wells A1-A5 and B1-B5 in the same order and tempo as you started them.
Mix thoroughly by pipetting the mixture up and down two times immediately after you pipette the stop solution.

Q. 14 ENZYME inhibition KINETICS EXPERIMENT

Hand in your microtitre plate containing your samples from part 2.2 by raising your pink card. After measurement, the obtained values will be displayed in the table below automatically. Please use the standard data given below (Table 2.2) for your calculations

Note: no microtitre plates will be accepted in the last 10 minutes of the exam!

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

2.3. DATA ANALYSIS OF INHIBITION KINETICS OF ALPHA-GALACTOSIDASE

In this section you will utilize the theory of Part 2.1 and the supplied inhibition data from Part 2.2 (see Table 2.2 below) to calculate enzyme kinetic parameters in the presence of inhibitor. The Lineweaver-Burk equation for the inhibited data will be compared to the supplied hypothetical Lineweaver-Burk equation for the uninhibited reaction to deduce the type of inhibiton. When you identify the type of inhibition, you will use these two Lineweaver-Burk equations (supplied hypothetical uninhibited and in the presence of inhibitor) to determine the change in the relevant kinetic parameters and to use the relevant equation to determine the inhibition equilibrium constant (K_i).

Table 2.2: Provided absorbance data for inhibition experiment (the first two rows and columns 1-5 in microtiter plate format).

	1	2	3	4	5
A	0.251	0.375	0.507	0.596	0.634

B	0.252	0.380	0.501	0.598	0.635

Q. 15 Analysis of Inhibition kinetics Data

Calculate and fill in the table below. In order to calculate product concentrations in mM, use the standard equation given in Q6:

Absorbance A_{405} = 2.29 * [pNP] (mM) + 0.058

Tube label	IS1	IS2	IS3	IS4	IS5
Volume (µl) of Stock solution (Subtrate) (from table 2.1)	80	160	320	600	840
Volume of (µl) ultra pure water (Water) (from Table 2.1)	920	840	680	400	160
Substrate concentration [S] prior to adding into the reaction mixture (mM)					
Substrate concentration [S] in reaction mixture (mM)					
Mean A_{405} absorbance from table 2.2					
[$Product_{mean}$] (mM)					
V_0 (µM/second)					
1/[S] (1/mM)					
$1/V_0$ (second/µM)					

A Lineweaver-Burk plot is produced based on the inhibition kinetics data IS1-IS5 in table 2.2. The hypothetical Lineweaver-Burk equation of the uninhibited reaction is: 1/[Vo] = 0.363·1/[S] + 0.908, and this line is plotted in Fig. 2.3. This supplied equation should be used for the calculations below, and NOT the equation you have determined in part 1.3 (Fig. 1.7).

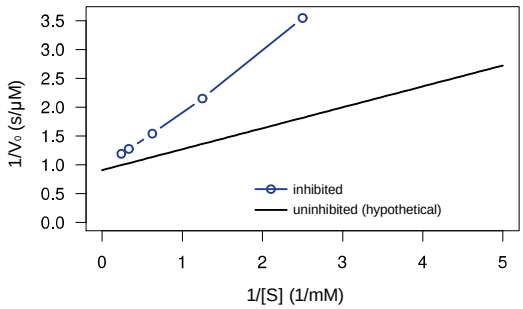


Figure 2.3 Lineweaver-Burk plot of the inhibited data and hypotheical unhibited data.

Q. 16 Lineweaver-Burk linear function (inhibited reaction)

Determine the linear function of the Lineweaver-Burk plot (Fig. 2.3) in the presence of inhibitor in the form shown below mathematically using only the data from IS1 and IS5. Give a and b with three digits after the decimal point.

$1/[Vo] = a \cdot 1/[S] + b$,

a (mM·s/µM)	
b (s/µM)	

Q. 17 apparent Kinetic Parameters with inhibitor

Determine the apparent kinetic parameters in the presence of inhibitor from the Lineweaver-Burk plot of the inhibited reaction. Give the parameters with three digits after the decimal point (no unit conversions should be done in this calculation).

V_{max}^{i}		
K_m^{i}		

Q. 18 Type of Inhibition

What type of inhibition does the inhibitor exert on the α-galactosidase? Choose the most likely type of inhibition based on the size of changes in kinetic parameters in the presence of the inhibitor as compared to the hypothetical data for the uninhibited enzyme

1. COMPETITIVE
2. NON-COMPETITIVE
3. UNCOMPETITIVE

Q. 19 Effect of Substrate concentration

Based on the type of inhibition you have chosen above, how would an increase in substrate concentration influence inhibition? Choose one of the statements below.

1. LESS INHIBITION
2. NO CHANGE
3. MORE INHIBITION

Q. 20 Inhibition Constant

Determine the inhibition constant (K_i) if the concentration of the 50 µL inhibitor added to the reaction mixture is 0.5 M. Give numbers with three digits after the decimal point (no unit conversions should be done in this calculation).

Inhibitor concentration in the reaction mixture (mM)	
K_i (mM)	

END