INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

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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).

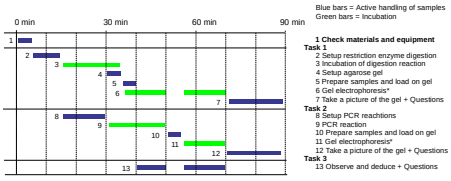


Figure 0.1: Suggested time table for completion of the three parts of the exam.

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

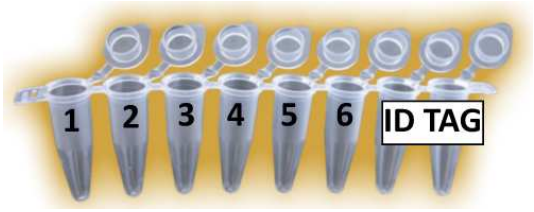


Figure 0.2: Material H: Tubes and ID label filled out with country code and student number.

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)

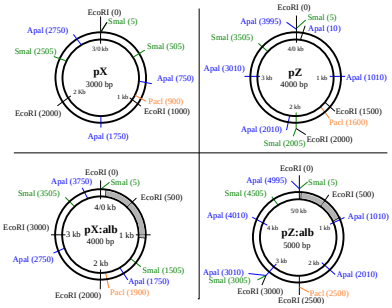
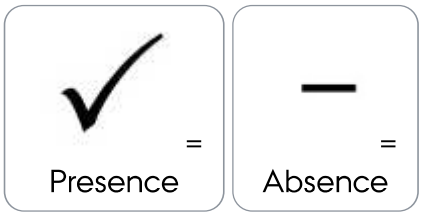


Figure 1.1: Restriction digestion map for ‘pX’, ‘pZ’, ‘pX:alb’ and ‘pZ:alb’.

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.

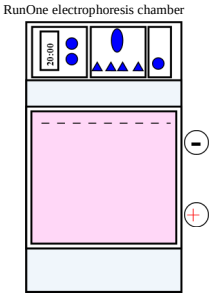


Figure 1.2: RunOne electrophoresis chamber (left) and posssible orientations of the agarose gel.

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

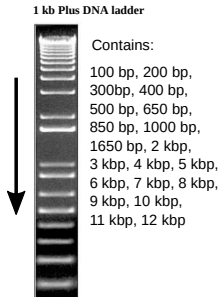
Q. 6 DIGESTION OF PLASMID DNA BY RESTRICTION ENZYME

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	○	○

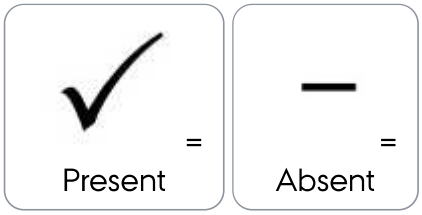
You will now be provided with a standardized gel picture showing results obtained for the restriction enzyme you chose (Question 3). Use this picture to answer Questions 7 and 8. NOTE YOU WILL ONLY HAVE ACCESS TO THIS PICTURE ONCE YOU HAVE DOCUMENTED YOUR GEL.

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

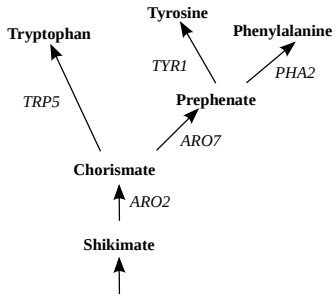


Figure 2.1: Biosynthetic pathway for the formation of aromatic amino acids (from upper left: Tryptophan, Tyrosine, Phenylalanine, Prephenate, Chorismate and Shikimate) and genes encoding the individual enzymes in the pathways.

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.

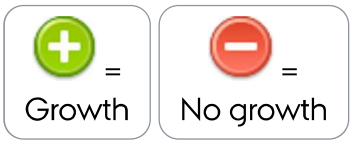
Table 3.1. Growth media

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					

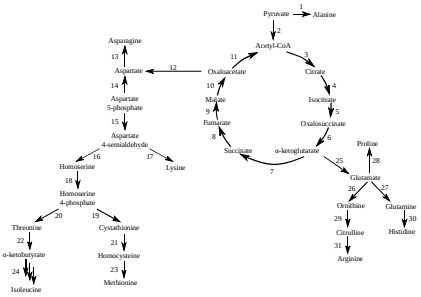


Figure 3.1: Simplified model for amino acid biosynthesis in yeast.

END