Montgomery College – Rockville Campus – BIOL 150
Lab Review Guide for Exam 2

Lab Practical 2: Exercises 4B, 8, 14 & 15

Exercise 4B: Enzymes and Bioinformatics.

Q4.1. What are enzymes and what are they made of?

Q4.2. What is the importance of enzymes in living system?

a. Do you think high fever for long period of time would alter cellular function? Explain your answer.

Q4.3. Every enzyme has its specific substrate. What determines this specificity of an enzyme?

Q4.4 Enzymes increase the rate of a reaction by:

A) decreasing the activation energy needed to start the reaction
B) providing the activation energy to the reaction
C) A and B
D) neither A nor B

Q4.5. If you keep an enzyme at $0^{o}C$ for an extended period of time and then bring back to its optimum temperature, would you expect the enzyme to be active? Explain.

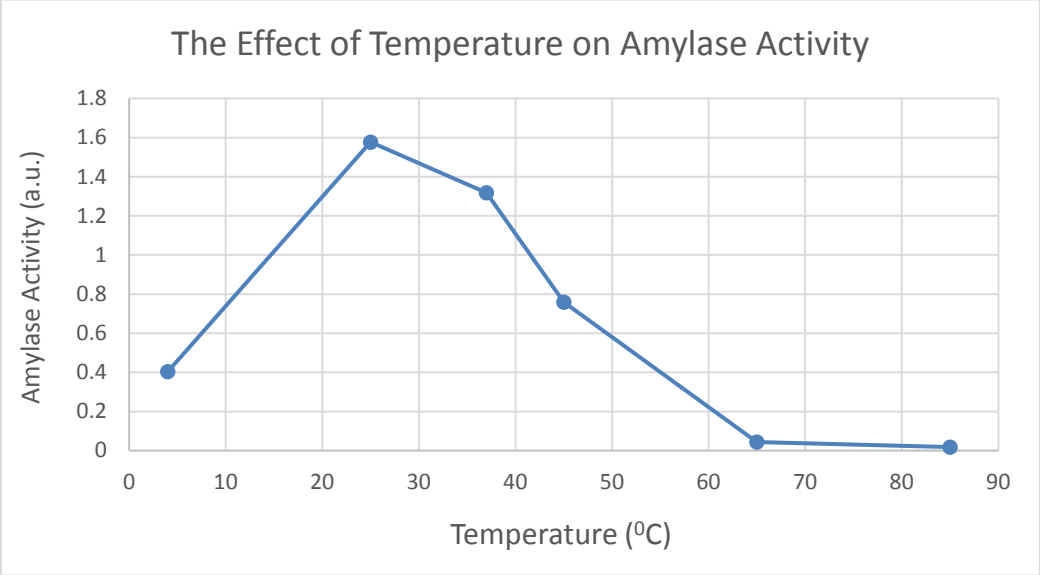
Q4.6. List three factors that can denature a protein/enzyme.

Q4.7. What is the optimum pH of an enzyme?

a. Why is the pH of the environment of an enzymatic reaction important?
b. Do all enzymes have the same optimal pH?

Q4.8. List and describe the steps of the scientific method that you used to design your Amylase experiment(s).

Q4.9. Look at the graph on the right and fill the table below for ***human amylase***:



Temperature	Structure	Function
4 °C		
85 °C		

Word bank: Denatures, Unfolds, Correct 3D Structure, Properly folded, Active, Inactive, Activation energy(E_A), Heat from Environment, Transition State, Enzyme activity.

Now, you can do the same for fungal and bacterial amylases.

Q4.10. Suppose 1ml of amylase enzyme was added to 5 ml of 2% starch. The solution was kept at 45°C for 15 min.

a. Do you think this mixture would give positive a Benedict's assay? Explain your answer.

b. Do you think this mixture would produce a color change if heated with DNS (3, 5-dinitrosalicylic acid)? Explain your answer.

c. Why did we use DNS assay instead of Benedicts test in the enzyme lab?

d. You decided to perform a Biuret assay on the enzymatic solution. You observe that the solution tested positive. Why?

Q4.11. Be able to calculate dilution factors and know how to convert units from ml to µl.

Q4.12. To construct a standard curve for estimation of maltose, you performed the DNS assay. The volume of standard maltose (5mg/ml) solution used is in Column 2 and the final volume of the solution per tube (after the DNS assay) was 10.0 ml. Calculate the final concentration of maltose in each tube (**Hint: use $C_1V_1=C_2V_2$**) and enter it in Column 3. The absorbance of each tube with varying concentrations of maltose (mg/ml) was recorded in Column 4 of the following table. Use the data in the table below to prepare a maltose standard curve on the graph paper supplied to you. Remember to use TAILS.

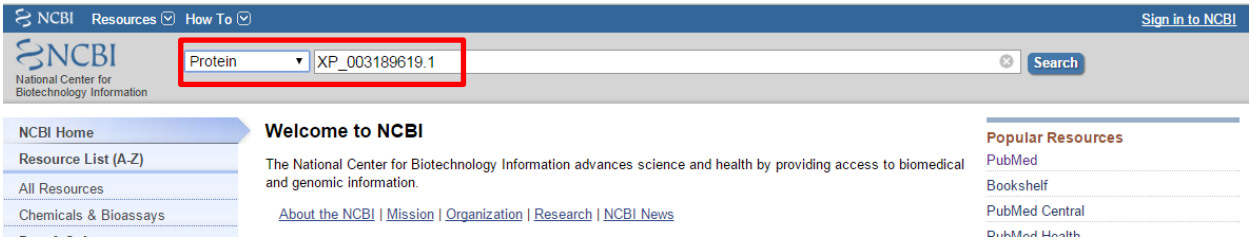
Tube	Maltose solution (ml)	Concentration of maltose (mg/ml)	Absorbance
1	0.0		0.0
2	0.2		0.3
3	0.4		0.6
4	0.6		0.8
5	0.8		1.2
Unknown sample			0.7

Spectrophotometer instructions video:
https://www.youtube.com/watch?v=kVC3D0pYkR4

Q4.13. Draw the maltose standard curve on the graph paper below and use it to find the concentration of maltose (in mg/ml) for the Unknown sample in the table. Show your work on the curve.

DNA Transcription and Protein Assembly Video:
https://www.youtube.com/watch?v=nHM4UUVHPQM

The following screenshots are from the NCBI website. You need to remember how we get the information for a specific protein.



Shown below is the output from NCBI after you entered the accession number for your protein (e.g. XP_003189619.1).

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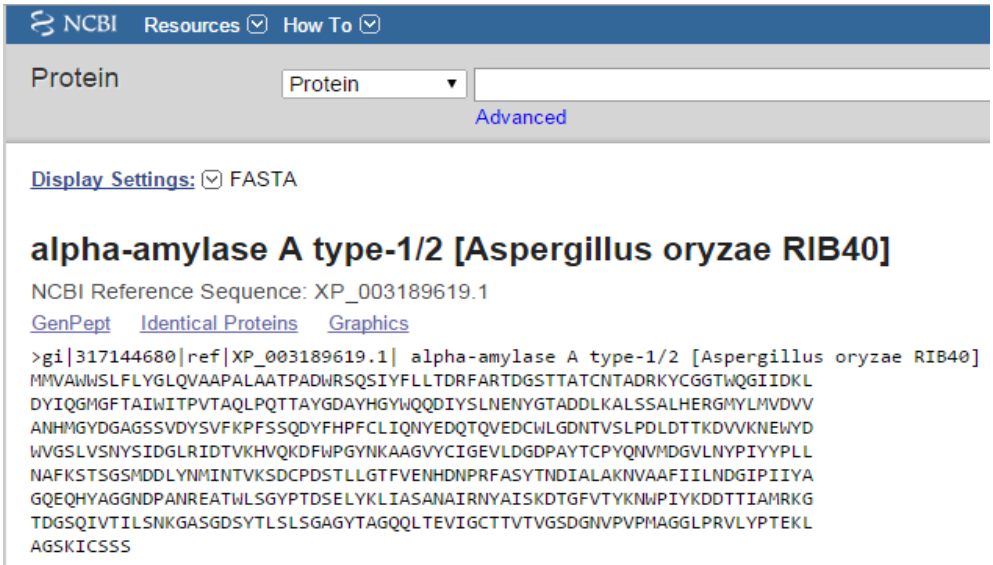
Q4.14. How can you obtain the FASTA sequence? Why do we need the FASTA sequence?

This is the FASTA sequence format that you need to get information from other software like PEPSTAT.



Q4.15. Fill in the following table with information from the PEPSTAT website. You have a picture from the PEPSTAT web page below the table, in the next page.

Amylase	Aminoacids	Polar aminoacids	% polar aminoacids	Non-polar amoinoacids	% Non-polar aminoacids
Fungal					

This is the output from PEPSTAT to analyze aminoacid sequence data. You should remember how to get this information.

EMBOSS Pepstats

Input form | Web services | Help & Documentation

Tools > Sequence Statistics > EMBOSS Pepstats

Results for job emboss_pepstats-I20141114-165809-0951-35565916-es

Summary | Submission Details

Download output file

PEPSTATS of fungal from 1 to 478

Molecular weight = 52489.35 Residues = 478
Average Residue Weight = 109.810 Charge = -20.5
Isoelectric Point = 4.2897
A280 Molar Extinction Coefficients = 105660 (reduced) 106160 (cystine bridges)
A280 Extinction Coefficients 1mg/ml = 2.013 (reduced) 2.023 (cystine bridges)
Probability of expression in inclusion bodies = 0.586

Residue	Number	Mole%	DayhoffStat
A = Ala	37	7.741	0.900
B = Asx	0	0.000	0.000
C = Cys	9	1.883	0.649
D = Asp	42	8.787	1.598
E = Glu	12	2.510	0.418
F = Phe	14	2.929	0.814
G = Gly	42	8.787	1.046
H = His	7	1.464	0.732
I = Ile	28	5.858	1.302
J = ---	0	0.000	0.000
K = Lys	20	4.184	0.634
L = Leu	33	6.904	0.933
M = Met	9	1.883	1.108
N = Asn	26	5.439	1.265
O = ---	0	0.000	0.000
P = Pro	21	4.393	0.845
Q = Gln	19	3.975	1.019
R = Arg	10	2.092	0.427
S = Ser	36	7.531	1.076
T = Thr	40	8.368	1.372
U = ---	0	0.000	0.000
V = Val	29	6.067	0.919
W = Trp	10	2.092	1.609
X = Xaa	0	0.000	0.000
Y = Tyr	34	7.113	2.092
Z = Glx	0	0.000	0.000

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	164	34.310
Small	(A+B+C+D+G+N+P+S+T+V)	282	58.996
Aliphatic	(A+I+L+V)	127	26.569
Aromatic	(F+H+W+Y)	65	13.598
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	266	55.649
Polar	(D+E+H+K+N+Q+R+S+T+Z)	212	44.351
Charged	(B+D+E+H+K+R+Z)	91	19.038
Basic	(H+K+R)	37	7.741
Acidic	(B+D+E+Z)	54	11.297

Q4.16. The following table sumarizes the overall results obtained by various groups this semester for the 3 day enzyme lab (Ex 3B). Analyze the data and explain how amino acids outside the active site contribute to the function of the enzyme.

	Amylases		
	Human	**Fungal**	**Bacterial**
Substrate	Starch	Starch	Starch
Product	Maltose	Maltose	Maltose
Optimal temperature	**37 °C**	**45^{0}C**	**85^{0}C**
Optimal pH	**7**	**5**	**7**
Total number of amino acids	**496**	**478**	**686**
Amino acid sequence similarity	20% - 30%	20% - 30%	20% - 30%
3 amino acids at the active site (D197, E233 & D300)	Conserved	Conserved	Conserved

Exercise 8: Mitosis

Q8.1. List the 4 stages of the cell cycle and describe the primary cellular activity in each stage.

Q8.2. What is the difference between mitosis and cytokinesis?

a. How do plant and animal cells differ in the execution of cytokinesis?

i. Why don't plant cells undergo cytokinesis in the same manner as animal cells?

The cell wall is rigid and cannot be squeezed and constrict in the middle.

Q8.3. List the different phases of Mitosis and briefly describe each phase.

a. Know the number of chromosomes and the physical state of chromosomes in each stage.

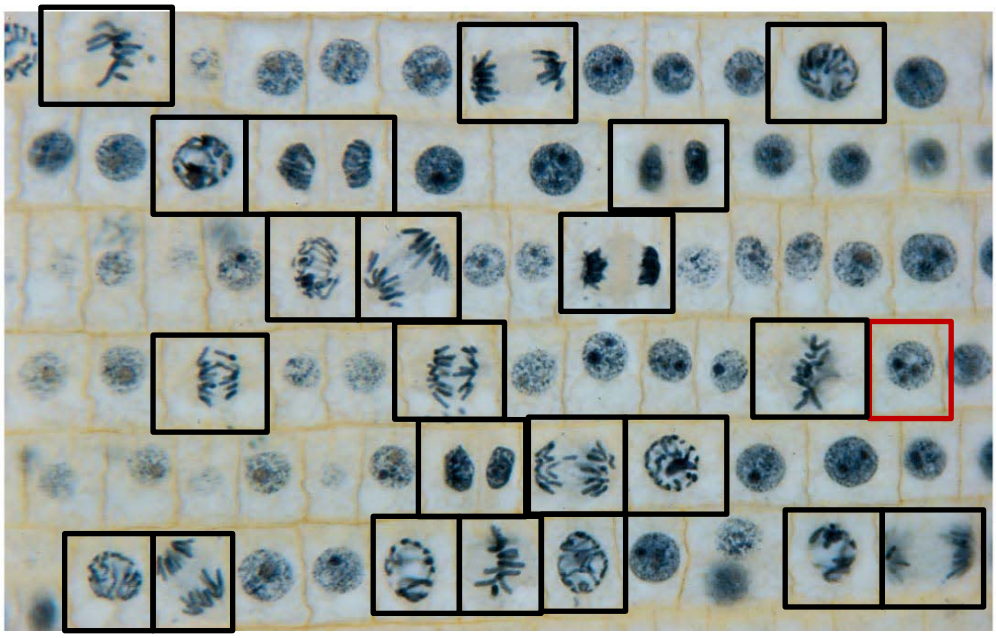
i. If a human cell has 46 chromosomes at G_1 phase, how many chromosomes will it have at G_2, prophase, metaphase, anaphase, telophase, and after cytokinesis?

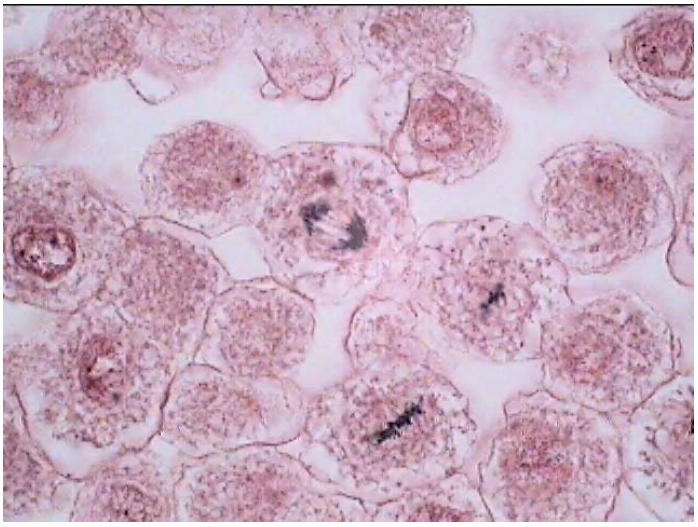
Q8.4. Why are the onion root tip and the whitefish blastula useful tissues for the study of cell division?

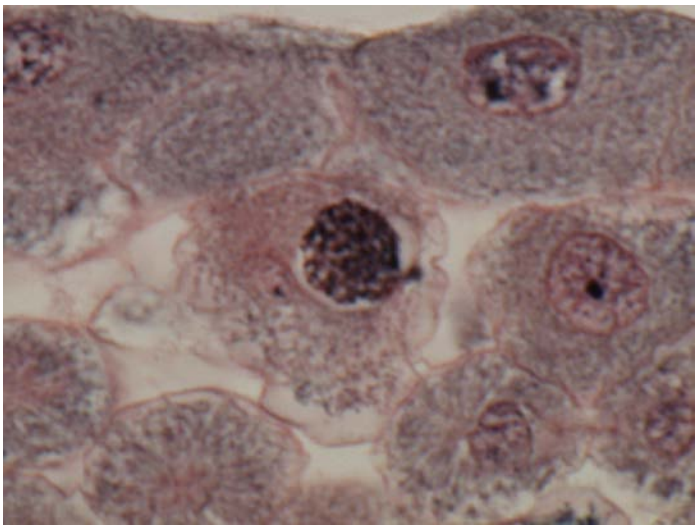
Q8.5. What are the genetic consequences of mitotic cell division for the resulting daughter cells?

Q8.6. List several important values or attributes that mitotic cell division provides to multicellular life. In other words, in what ways is mitotic cell division useful for life?

Very important: You should be able to identify the different stages (shown under microscope) of cell cycle/mitosis in an onion root tip and whitefish blastula. The Ackerman Learning Center (SW 105) has slides and microscopes. If you are not confident, you may spent more time on the slides and study them at your convenience.







Exercise 14 -- Analysis of DNA Using Restriction Enzyme and Electrophoresis:

Q14.1. What are nucleases and what are they made of?

a. How does an endonuclease differ from an exonuclease?

b. What is a restriction endonuclease?

Q14.2. What were the 2 restriction endonucleases (RE) that we used in our lab?

a. Do they produce sticky ends or blunt ends when they cut the DNA molecules?

b. What is a palindromic DNA sequence?

Q14.3. How does the number of restriction sites relate to the number of fragments produced for linear DNA or circular DNA?

Q15.4. What is electrophoresis?

a. What does agarose gel electrophoresis allow us to do?

b. What is the chemical nature of agarose?

c. What factors affect the migration rate of DNA through an agarose gel?

Q14.5. For DNA molecules of equal sizes, how do the different shapes (conformation) of DNA differ in terms of distance traveled through an agarose gel?

Q14.6. In your DNA electrophoresis experiment, why did you run a DNA ladder (lane 5) and undigested pAMP DNA (lane 4)?

Q14.7. Write some practical applications for use of restriction endonuclease.

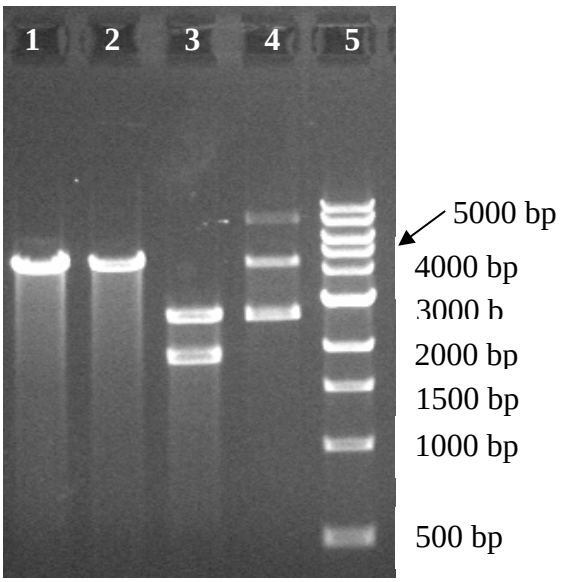
Q14.8. Which enzyme does ***not*** make sticky ends?

Enzyme	Recognition site	Enzyme	Recognition site
*Bam*HI	G↓GATCC CCTAG↑G	*Hin*dIII	A↓AGCTT TTCGA↑A
*Eco*RI	G↓AATTC CTTAA↑G	*Sca*I	AGT↓ACT TCA↑TGA

Q14.9. The restriction enzyme digestion of the pAMP DNA followed by ***conventional*** (not the ones we used) agarose gel electrophoresis in **Ex. 15** yielded the following pattern DNA bands.

a. Estimate the size of the DNA band at the bottom of lane 3 by comparing it with the DNA ladder.

b. Determine if pAMP is a linear or a circular DNA molecule.



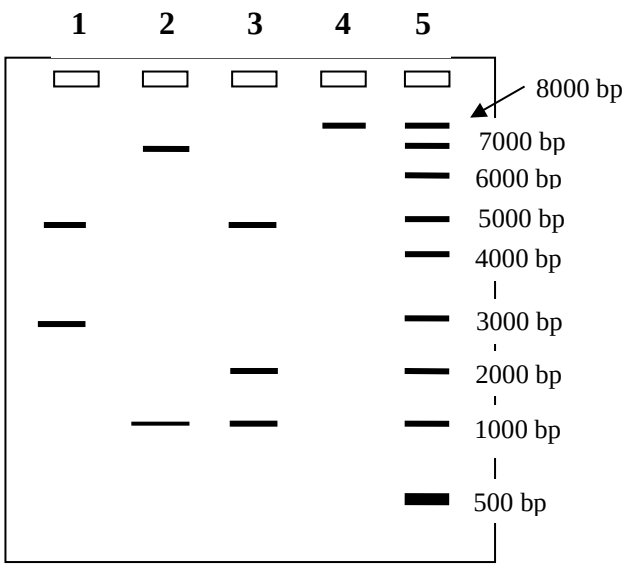
Lane 1: pAMP DNA + EcoRI
Lane 2: pAMP DNA + HindIII
Lane 3: pAMP DNA + EcoRI + HindIII.
Lane 4: pAMP DNA
Lane 5: DNA markers (ladder)

Lonza Flash Gel video:

https://www.youtube.com/watch?v=fzt3kcTKRho

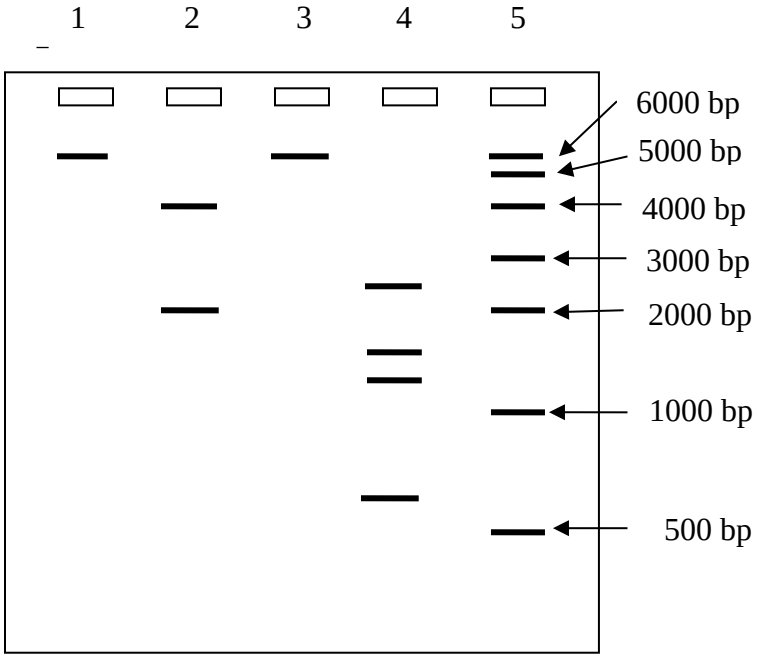
Q14.10. The following electrophoresis gel pattern was obtained from digestion of a DNA molecule by different restriction enzymes as described in the box. **Analyze the gel and determine if the DNA is a linear or a circular DNA molecule.**

The "DNA only" lane has just one band so it has to be linear.



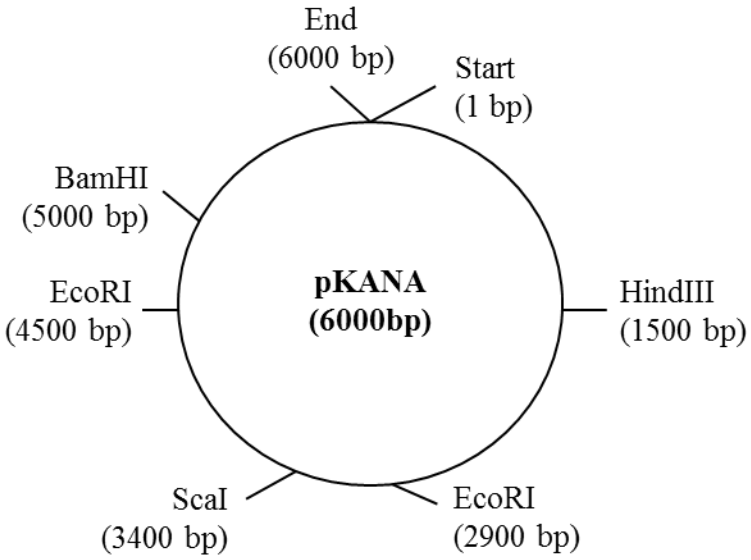
Lane 1: DNA + EcoRI
Lane 2: DNA + HindIII
Lane 3: DNA + EcoRI + HindIII
Lane 4: DNA only
Lane 5: DNA ladder

Q14.11. The following electrophoresis gel pattern was obtained from digestion of a DNA molecule by different restriction enzymes as described in the box. **Analyze the gel and determine if the DNA is a linear or a circular DNA molecule. Draw the restriction map based on the following agarose gel.**



Lane 1: DNA + BamHI
1 fragment; 6000 bp
Lane 2: DNA + HindIII
2 fragments; 4000bp and 2000 bp
Lane 3: DNA + EcoRI
1 fragment; 6000 bp
Lane 4: DNA + BamHI + HindIII + EcoRI
4 fragments; 2500bp, 1500bp, 1300bp, and 700bp
Lane 5: DNA ladder

Q14.12. Examine the restriction map of plasmid pKANA on the right.

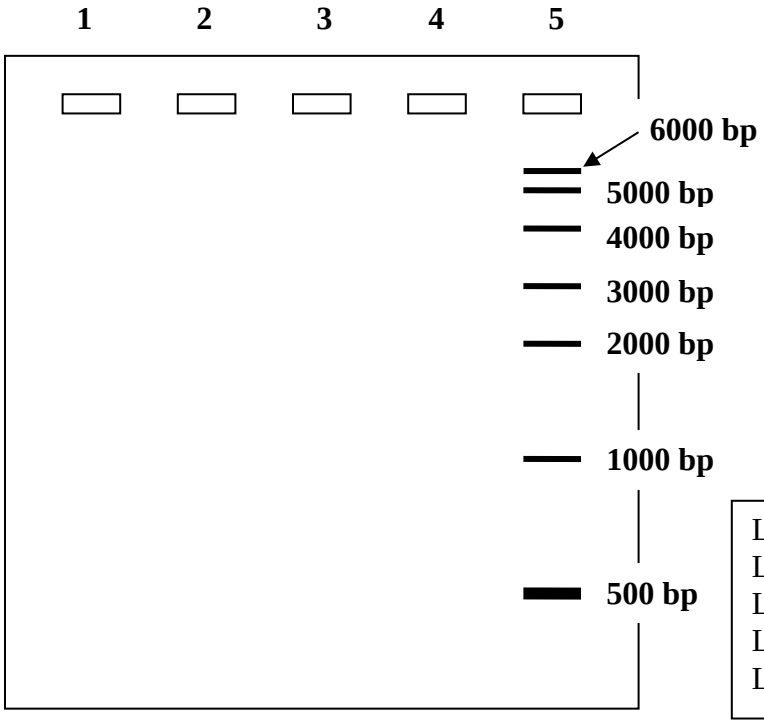


a. How many fragments would be produced if the plasmid is digested with EcoRI and what size would this/these fragment(s) be?

b. How many fragments would be produced using EcoRI + HindIII + ScaI and what size would this/these fragment(s) be?

c. How many fragments would be produced using ScaI and what size would this/these fragment(s) be?

Q14.13. Draw the banding pattern for the restriction digestion of pKANA (above) as indicated by the table on the bottom right. The migration of a DNA ladder and its fragment-sizes are identified by the arrows.



Lane 1: DNA + ScaI
Lane 2: DNA + EcoRI
Lane 3: DNA + EcoR I +Hind III
Lane 4: DNA+ EcoRI + Hind III + BamHI
Lane 5: DNA ladder

Exercise 15: Plasmid Purification and Transformation

Answer all questions in pages 319-321 of Lab Exercise 15

Q15. 1 Match the following:

A. LB agar	1. small, circular, extra chromosomal DNA that is self-replicating
B. Glycerol	2. nutrient used to grow bacteria, e.g., *E. coli*
C. Ampicillin	3. chemical used for making cells competent
D. Amp^R gene	4. ability to take up DNA from the surrounding environment
E. $CaCl_2$	5. used as (i) cryopreservative, prevents formation of ice crystals and (ii) osmoprotectant
F. β (Beta) lactamase	6. antibiotic that kills bacteria
G. Plasmid	7. enzyme that breaks down the antibiotic, ampicillin
H. pGLO	8. uptake of DNA from surroundings & change in genetic makeup
I. Competence	9. plasmid that has bla gene (ampicillin resistance gene)
J. Transformation	10. will make β lactamase and grow on LB agar + ampicillin
K. Amp^R *E. coli*	11. cannot grow on LB agar + ampicillin
L. Amp^S *E. coli*	12. codes for the enzyme β lactamase

Q15.2 What was the rationale/logic behind performing each of the following steps in plasmid-DNA purification protocol?

A. Re-suspension of E. coli cells that contain plasmid-DNA.

B. Addition of cell lysis solution.

C. Addition of neutralization solution.

D. Centrifugation after addition of neutralization solution.

E. Collection of supernatant (the clear fluid).

F. Addition of DNA purification resin to the supernatant

G. Passing of the mixture of resin and supernatant through the mini-column by applying the vacuum.

H. Washing the column by column wash solution.

I. Spinning the mini-column after finishing vacuuming.

J. Addition of 60 microliter water to the mini-column.

K. Spinning the column after addition of water.

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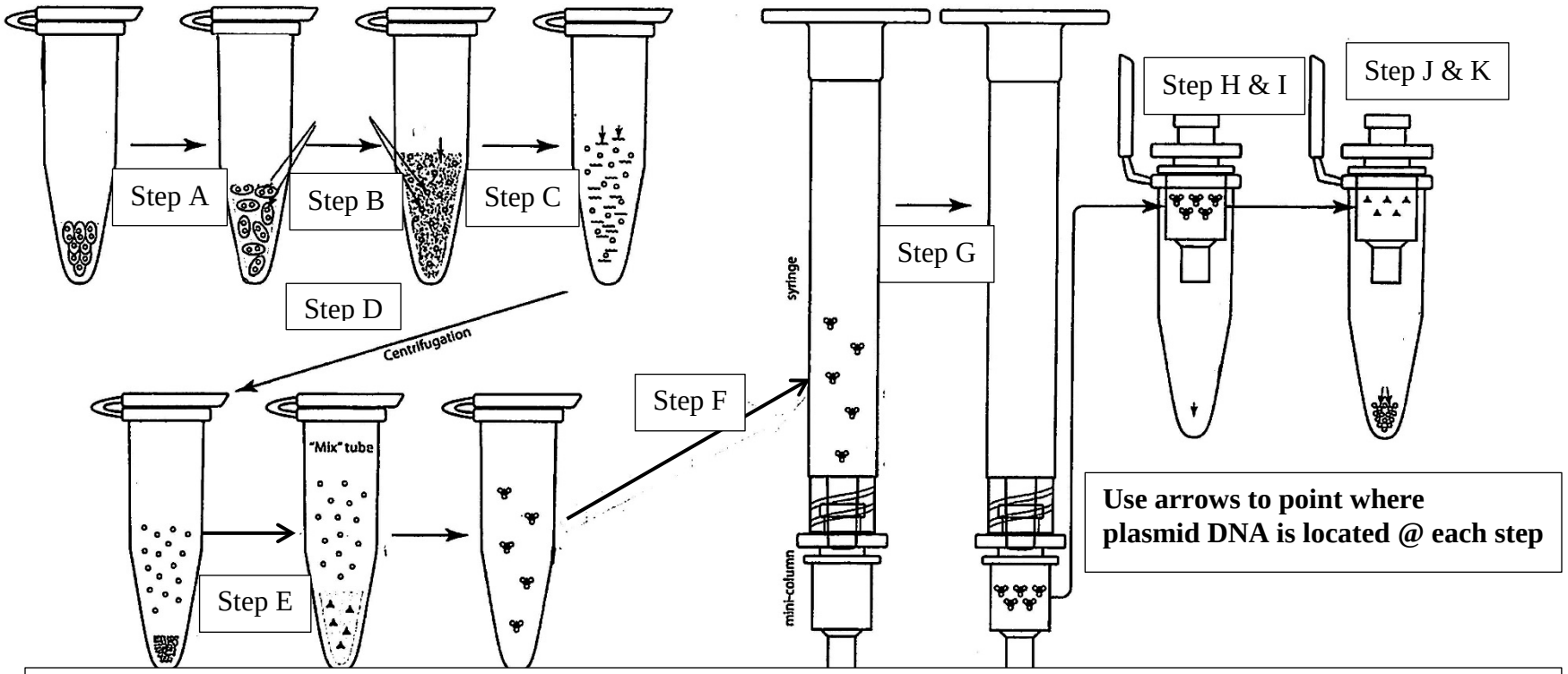
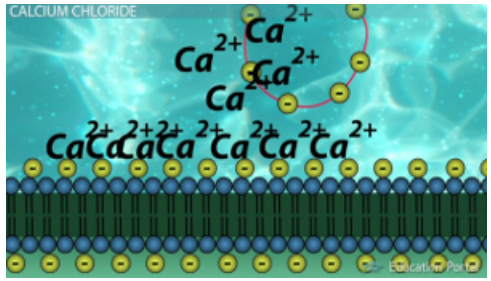
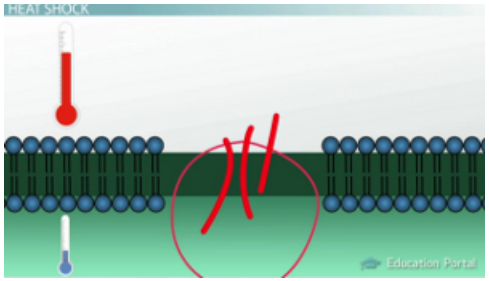


At Step A ____ was added to _____
At Step B __ _ was added to _____
At Step C __ added to _____
At Step E _ _ was added to _____ _____
At Step J _____ was added to ____
In figure draw and arrow to show where the plasmid DNA is found at that step.
Centrifugation @ step D was done to _____
Step I was done to _____
Step K was done to _____





Bacterial Transformation Video (Start @ 1min 20 secs):
http://study.com/academy/lesson/bacterial-transformation-definition-process-and-genetic-engineering-of-e-coli.html

Q15.3. What did you purify in this exercise?

a. What is the link between pGLO and ampicillin resistance?

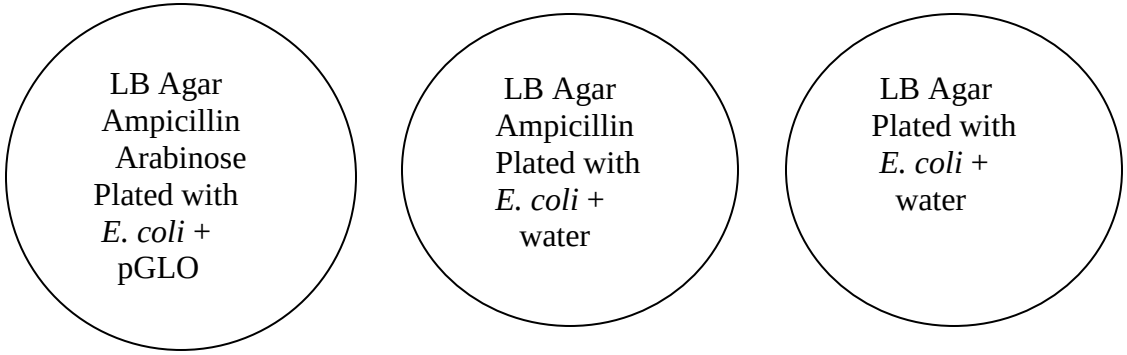
Q15.4. How did we know that our transformation experiment worked?

Q15.5. What were the controls that we used in our transformation experiment (plates B and C)?

a. Why did we use these and each control tell us when we compared them to plate A?

Q15.6. Why do the bacteria survive if they accept pGLO from outside? (similar to Q16.3)

Q15.7. Explain what kind of growth you expect to see on each of these plates. Why would we see this?



Important: You should be able to interpret the results of a transformation experiment