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?

Protein catalysts which speed up reactions. They are made of amino acids.

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

We would not be able to function, our reactions would not happen to a significant extend.

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

High fever would denature enzymes and probably destroy their function (by significantly altering their structure).

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

The active site shape and chemical interaction with substrates (with amino acids at the active site).

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°C for an extended period of time and then bring back to its optimum temperature, would you expect the enzyme to be active? Explain.

Yes, it would be active – its structure has not been altered and with the ideal temperature the enzyme can catalyze the reaction.

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

Temperature, pH, salt, can denature an enzyme/protein.

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

The ideal pH is the pH of the environment in which an enzyme is found.

a. Why is the pH of the environment of an enzymatic reaction important?

The activity of an enzyme is dependent on pH (structure is affected by pH). An enzymatic reaction requires an active enzyme in order to take place.

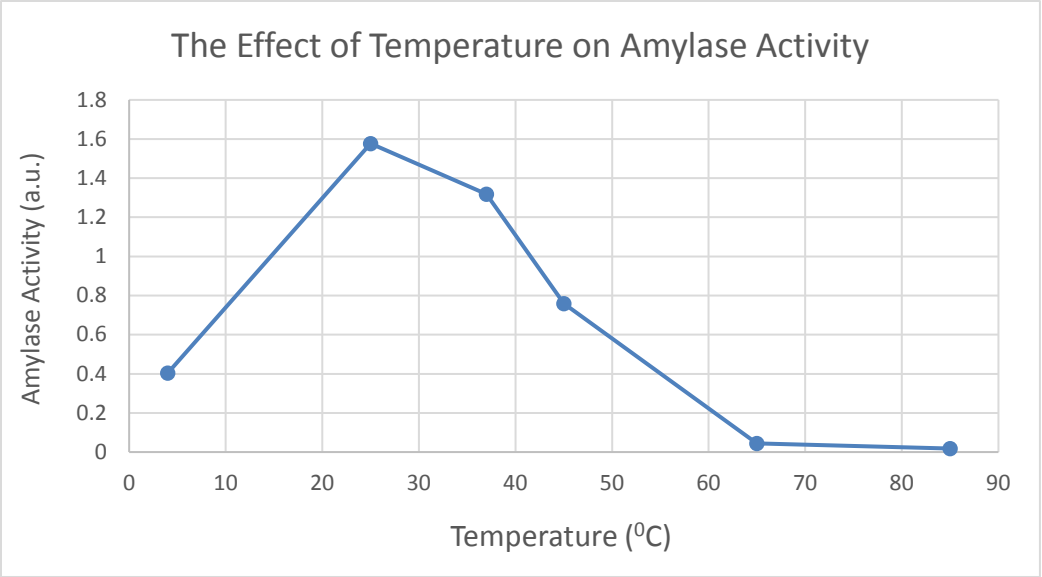
b. Do all enzymes have the same optimal pH?

No, enzymes' optimal pH is the one in which they are found.

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

- Question: e.g. "How does pH affect enzyme activity?", "How does temperature affect enzyme activity?"
- Hypothesis: a possible explanation or answer to the question we had. It has to be falsifiable and testable, e.g. "human amylase would be most active at 37 C"
- Prediction: "If we put human amylase and starch at various temperatures, it will break starch down faster at 37 C".
- Experiment: you test your hypothesis. Think of our **independent variable**, e.g. temperature of reaction. **Dependent variable**: maltose produced (absorbance measurements) which can tell us how much activity the enzyme had. Experimental groups and Control groups: e.g. negative control was missing the enzyme. We used the controls as our blanks in the spectrophotometer.
- Conclusions: is our hypothesis supported or not?

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



Temperature	Structure	Function
4 ºC	Properly folded	active
85 ºC	Unfolds, denatures	inactive

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.

Fungal: similar to the human enzyme. Active at low temps and inactive at high temps.

Bacterial: active at low (low activity) temps but most active at 85 C as it comes from a thermophilic bacterium.

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

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

Yes, the product of starch hydrolysis is maltose, which is a reducing sugar and would give a positive Benedict's test (contains a carbonyl group).

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

DNS would change color because it becomes reduced by maltose.

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

Benedict's is a qualitative test and the DNS assay is quantitative (we can measure the absorbance of light by the reduced DNS).

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

Biuret test identifies peptide bonds in proteins – enzymes are proteins.

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

1000 ul = 1 ml

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.

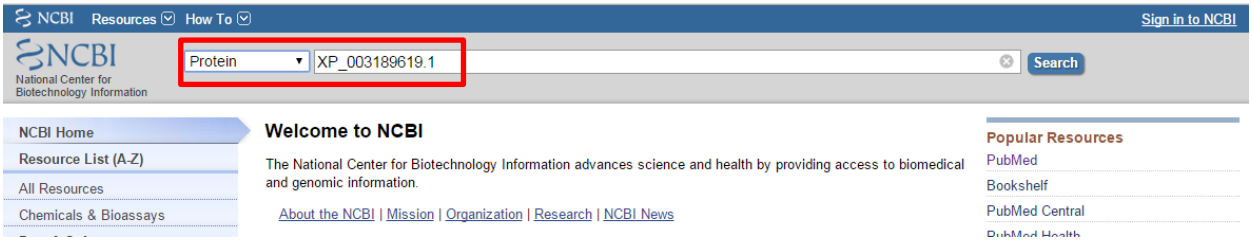
Tube	Maltose solution (ml)	Concentration of maltose (mg/ml)	Absorbance
1	0.0	0	0.0
2	0.2	0.1	0.3
3	0.4	0.2	0.6
4	0.6	0.3	0.8
5	0.8	0.4	1.2
Unknown sample		0.24	0.7

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.



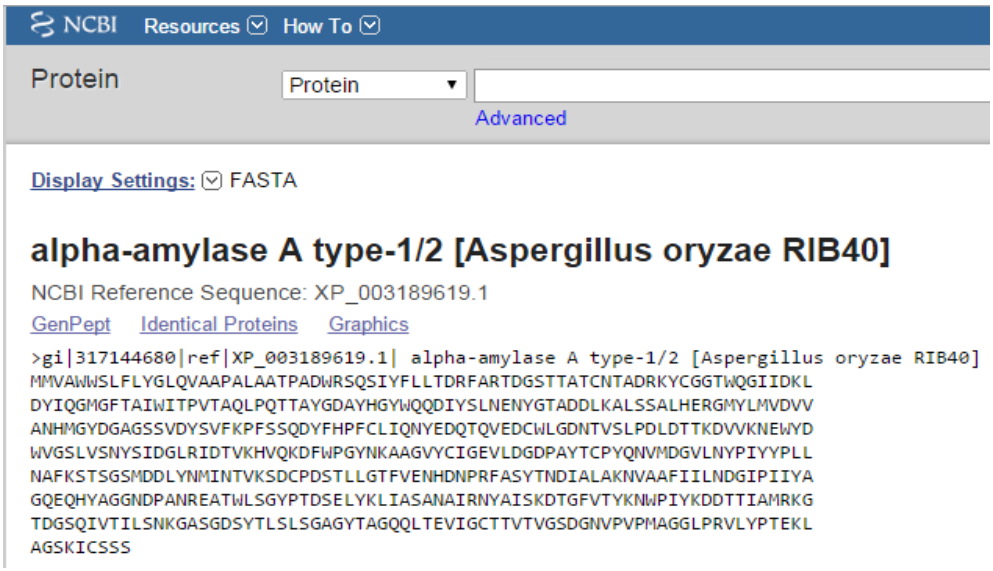
Shown below is the output from NCBI after you entered the accession number for your protein (e.g. XP_003189619.1).

]

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

Put the NCBI accession number in the search box of the NCBI website, remember to choose « protein » in the drop down menu on the left. Click search. Click on « FASTA » in the resulting page.

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	478	212	44	266	56

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

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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
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Q4.16. The following table sumarizes the overall results obtained by various groups this semester for the 3 day enzyme lab (Ex 4B). 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°C**	**85°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

The amino acids outside the active site affect the overall shape of an enzyme and perhaps preference of certain temperature, pH, salt conditions, etc.

Exercise 8: Mitosis

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

G1: cell growth, preparing for DNA synthesis (make nucleotides, make the enzymes needed for DNA replication), RNA synthesis, protein synthesis, organelle synthesis, membrane synthesis, DNA repair

S: DNA replication, centrosome is duplicated

G2: cell growth, DNA repair, make components for mitosis/meiosis

M: cell division (nucleus divides, cytoplasm divides = cytokinesis).

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

Mitosis: division of the nucleus

Cytokinesis: division of the cytoplasm

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

Plant: builds a cell plate (Golgi vesicles carrying components of the cell membrane and cell wall arrive in the middle of the cell and fuse together to form the cell plate)

Animal: makes a cleavage furrow (actin microtubules make a ring around the cytoplasm and the ring contracts and pinches the cytoplasm off).

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.

Prophase: we see the nucleus and see the chromosomes as thick strings inside

Prometaphase:

Metaphase: no nucleus, chromosomes lined up

Anaphase: chromatids segregate and move towards opposite poles

Telophase: chromosomes have arrived at the poles and forming nuclear membranes around them – cytokinesis has started

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

Prophase: diploid number of chromosomes but each one is made of 2 chromatids (duplicated)

Metaphase: same as prophase

Anaphase: 2x the diploid number (92) and they are made of one molecule of DNA (one chromatid)

Telophase/Cytokinesis: each of the 2 daughter cells will have a diploid number of chromosomes (each chromosome is one molecule of DNA)

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?

G1: 46, one chromatid each

G2: 46, two chromatids each (duplicated)

Prophase: 46 duplicated

Metaphase: 46 duplicated

Anaphase: 92 unduplicated

Telophase: 2 nuclei, 46 unduplicated in each of them

Cytokinesis: 46 unduplicated chromosomes in each of the 2 daughter cells

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

Onion root tip is a growing tissue as it is looking for nutrients and water in the soil.

The whitefish blastula: stage of embryonic development.

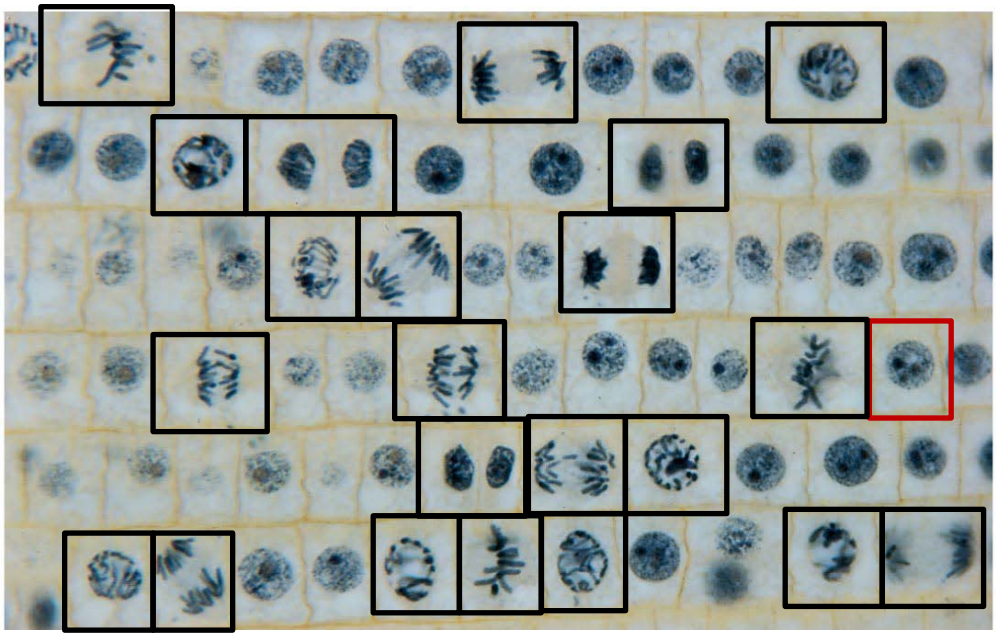
Q8.5. What are the genetic consequences of mitotic cell division for the resulting daughter cells?

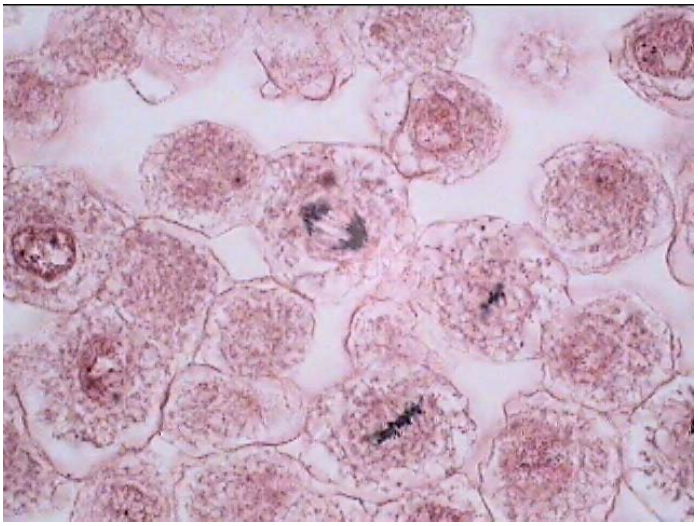
Daughter cells are identical to the parent cell (both in the number and genetic composition of the chromosomes).

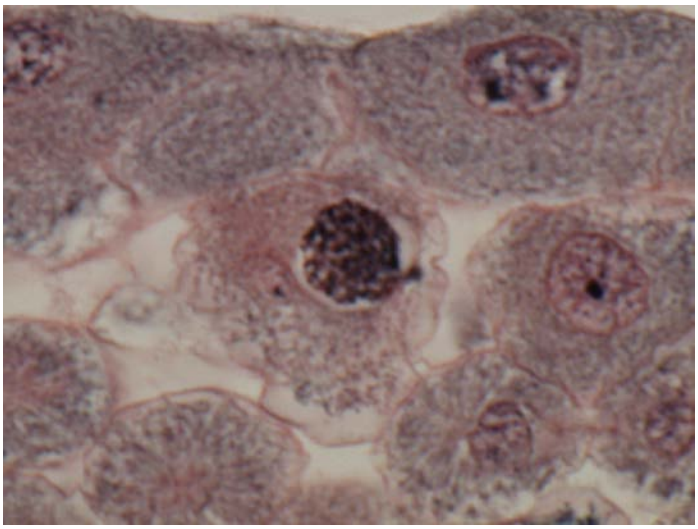
Q8.6. List several important values or attributes that mitotic cell division provides to multicellular life. In other words, in what ways is mitotic cell division useful for life?

ii. Asexual reproduction
iii. Tissue repair
iv. Development of the embryo
v. Growth of an organism

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 Math-Science Center (ground floor of Maclin Tower) 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?

Nucleases are enzymes that cut DNA – made of amino acids.

a. How does an endonuclease differ from an exonuclease?

Endonucleases: cut in the middle of a strand of DNA
Exonucleases: cut at the end of a strand of DNA

b. What is a restriction endonuclease?
They are bacterial enzymes. Bacteria use them to destroy foreign DNA that enters the cell (bacteriophages inject their DNA into bacteria; transformation of plasmid DNA or linear DNA into bacteria).

They recognize palindromic sequences and specifically cut them.

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

We used EcoRI and HindIII.

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

Both produce sticky ends.

b. What is a palindromic DNA sequence?

Reads identical 5' to 3' on each strand.

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

Circular DNA: number of sites is equal to the number of fragments you generate.

Linear DNA: number of fragments = number of sites + 1

Q14.4. What is electrophoresis?

Separating fragments of DNA in a gel matrix using voltage.

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

Separate fragments of DNA in an agarose gel in order to identify them.

b. What is the chemical nature of agarose?

Polysaccharide which comes from red algae (sea weed).

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

Size of fragments

(Charge: all negatively charged)

Shape of the molecule: supercoiled versus relaxed circles versus linear

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?

Circular plasmids have different forms; supercoiled (runs really fast), linear (runs slower- can identify by comparing size to a ladder), nicked circle (slowest: one strand is cut = relaxed circle)

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

To identify the size of fragments (Ladder is a mixture of known size fragments) – by lining up our unknown fragments with the ladder fragments.

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

Cloning: putting a gene of one organism into a plasmid and inserting it into a different organism's cells we can have a protein expressed inside these cells. (e.g. a protein from jelly fish which fluoresces green was expressed in bacteria).

Make insulin or other hormones.

Express genes to make proteins that you can purify and use in other medical treatments.

Gene therapy: replace a nonfunctional gene with a functional one.

In agriculture to replace or introduce genes that give useful characteristics to their crops.

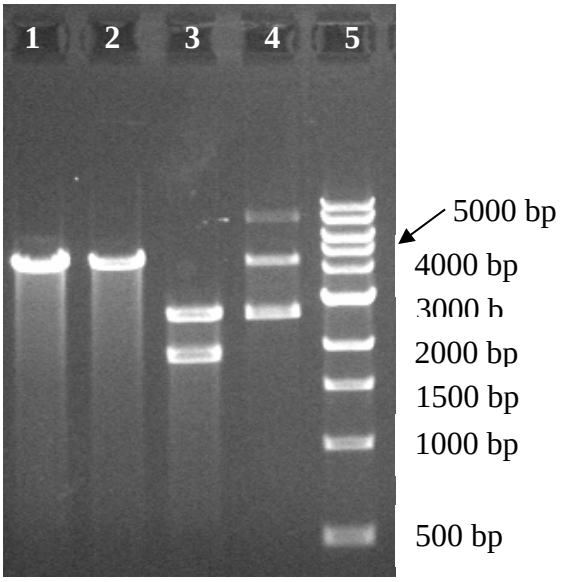
Q14.8. Which enzyme does ***not*** make sticky ends?

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.
   a. Just under 2000 bp (1800-1900)

b. Determine if pAMP is a linear or a circular DNA molecule.
   Circular as we can see several bands in the undigested lane (supecoiled, linear, relaxed circle forms).



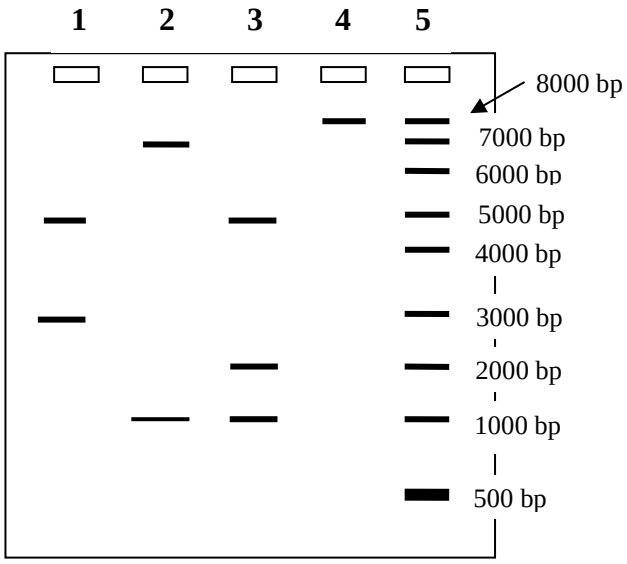
Lane 1: pAMP DNA + EcoRI
Lane 2: pAMP DNA + HindIII
Lane 3: pAMP DNA + EcoRI + HindIII.
Lane 4: pAMP DNA
Lane 5: DNA markers (ladder)

Lonza Flash Gel video:

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

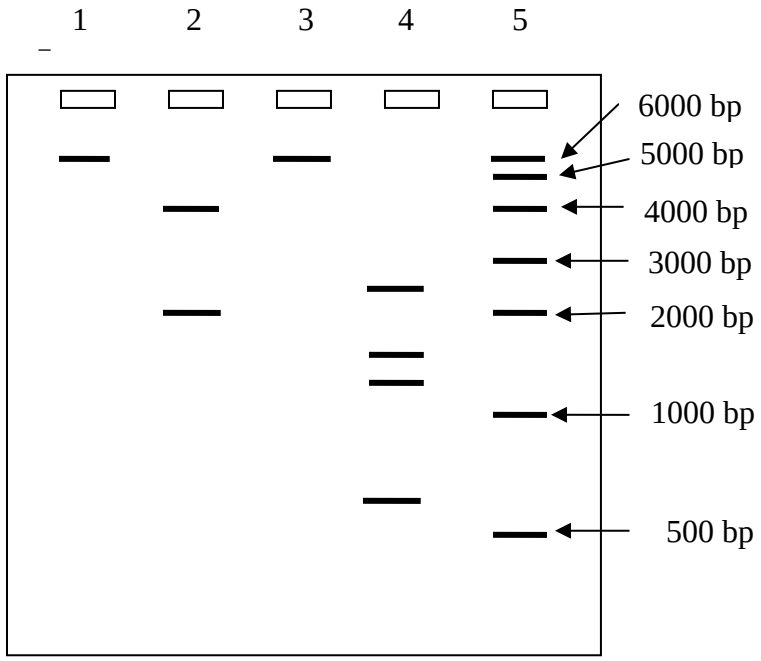
Q14.10. The following electrophoresis gel pattern was obtained from digestion of a DNA molecule by different restriction enzymes as described in the box. **Analyze the gel and determine if the DNA is a linear or a circular DNA molecule.**

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



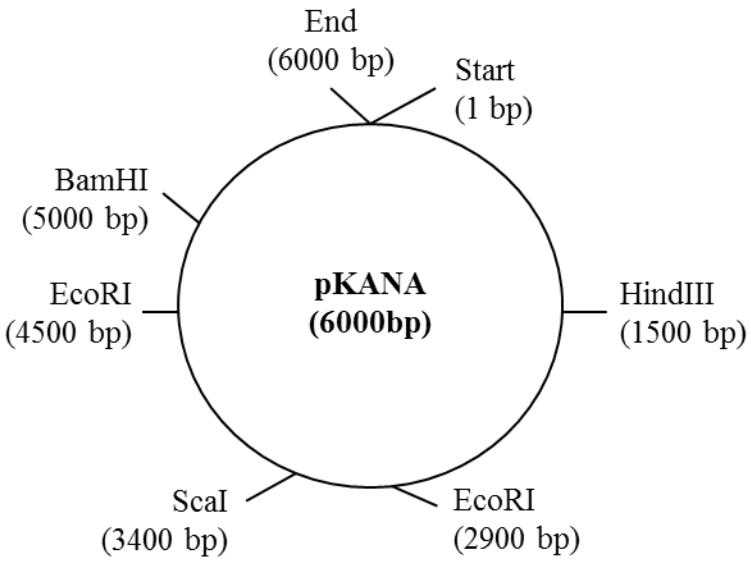
Lane 1: DNA + EcoRI
Lane 2: DNA + HindIII
Lane 3: DNA + EcoRI + HindIII
Lane 4: DNA only
Lane 5: DNA ladder

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.

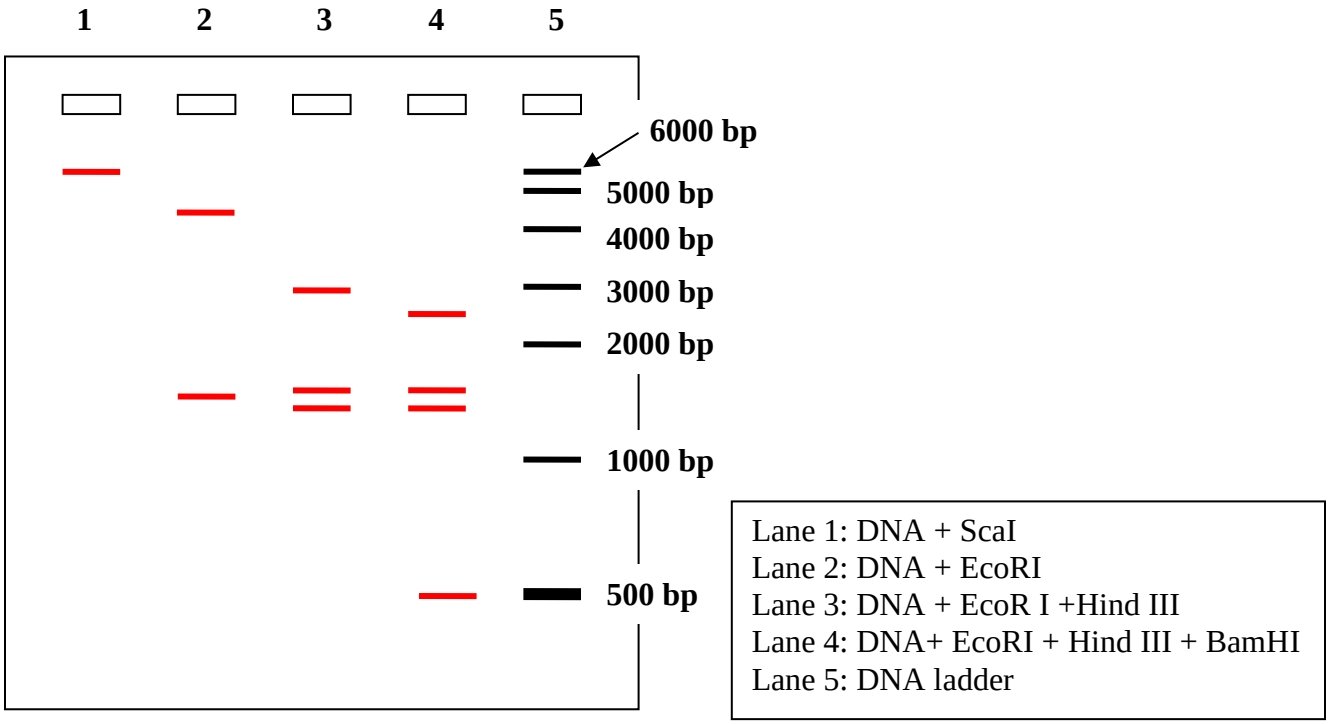


a. How many fragments would be produced if the plasmid is digested with EcoRI and what size would this/these fragment(s) be?
2: 1600 + 4400

b. How many fragments would be produced using EcoRI + HindIII + ScaI and what size would this/these fragment(s) be?
4: 500 + 1100 + 1400 + 3000

c. How many fragments would be produced using ScaI and what size would this/these fragment(s) be?
1: 6000 bp

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.



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

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.
Unpack, solubilize cells.

B. Addition of cell lysis solution.

Lyses the cells and releases their contents. (alkaline solution)

C. Addition of neutralization solution.

Neutralize the alkaline pH so that chromosomal DNA and proteins will precipitate.

D. Centrifugation after addition of neutralization solution.

Separate chromosomal DNA/proteins from soluble plasmid DNA (supernatant)

E. Collection of supernatant (the clear fluid).

Contains plasmid DNA

F. Addition of DNA purification resin to the supernatant

Bind plasmid DNA to beads due to attraction of negatively charged DNA to positively

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

Wash off anything not bound to beads (everything else but plasmid DNA)

H. Washing the column by column wash solution.

Wash off any salts or anything that is nonspecifically binding to the beads.

I. Spinning the mini-column after finishing vacuuming.

Remove any traces of the alcohol wash.

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

Elute (detach) plasmid DNA from the beads.

K. Spinning the column after addition of water.

Get plasmid DNA in the tube.

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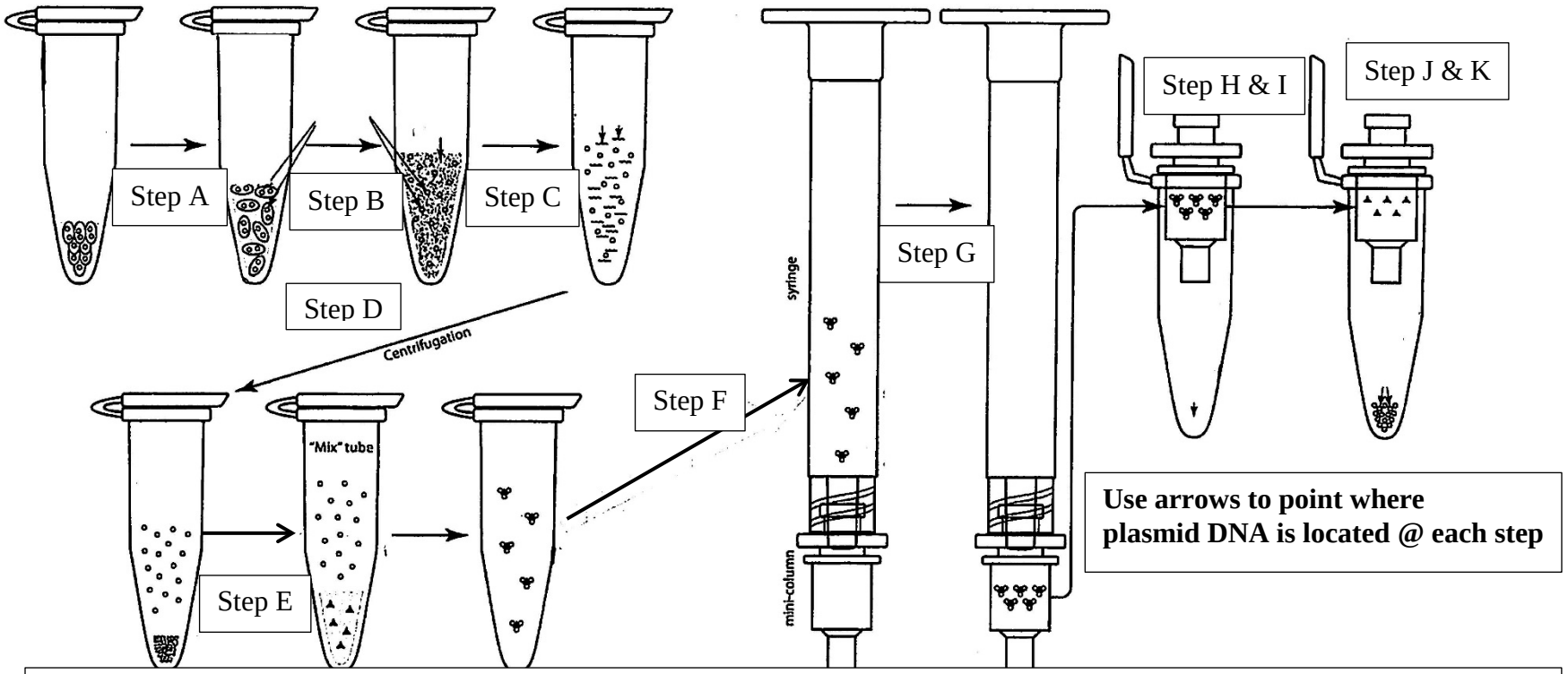
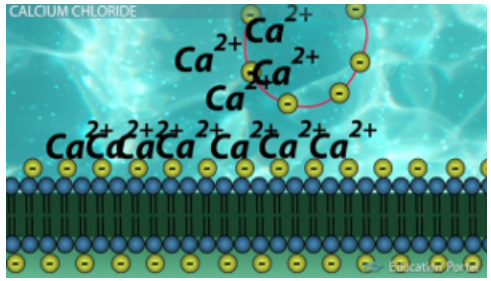
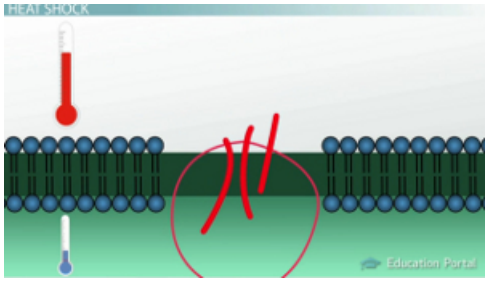


At Step A __resuspension buffer__ was added to __pellet____
At Step B __lysis buffer_ was added to __pellet_____
At Step C __neutralization buffer was added to ___pellet_____
At Step E _supernatant_ was added to _____resin_____
At Step J ___water____ was added to ___the resin on the mini column_____
In figure draw and arrow to show where the plasmid DNA is found at that step.
Centrifugation @ step D was done to __separate chromosomal DNA/proteins from plasmid _____
Step I was done to _remove last traces of wash_____
Step K was done to _collect plasmid DNA in tube_____





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?

Plasmid DNA from E. coli bacteria

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

pGLO carries a gene which expresses an enzyme (beta lactamase) which digests ampicillin.

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

Plate A had colonies (E. coli + plasmid+ampicillin+arabinose): if the plasmid did not get into the bacteria, they would all die on ampicillin. Also, the colonies fluoresce green if placed on UV light box as they produce the GFP protein (arabinose sugar added to the plate allows the AraC protein to bind the promoter in front of GFP and help the RNA polymerase transcribe the GFP gene).

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

Plate B: (E. coli + H2O, ampicillin) – control for ampicillin: make sure ampicillin works and kills E. coli

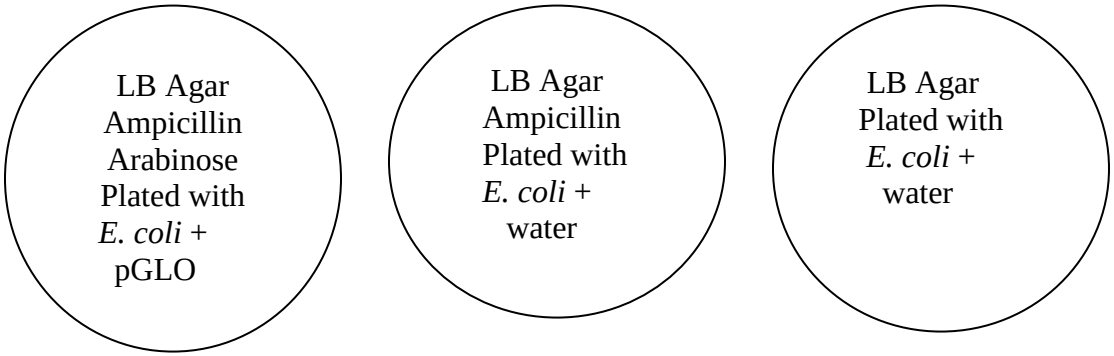
Plate C: (E. coli + H2O, just LB agar) – control for healthy bacteria: bacteria grows

a. Why did we use these and each control tell us when we compared them to plate A?
On plate A cells are growing because they are transformed with the plasmid.

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

Bacteria that carry this plasmid can destroy the ampicillin.

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



Colonies (fluorescent) No growth Dense growth (lawn of bacteria)

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