Transformation and Enzyme Pre-lab. You must show this completed pre-lab to your GSI at the start of lab! **(Pages 64-90 [focus on 69-76], 91-110 [focus on 91-97) It is due at the end of lab.** *Thus you can use your flow chart during lab.*

Name _____ GSI & Sect # _____ Station # _____

To answer these questions read all of the lab material on constructing recombinant DNA (pages 64-90) even though you are only doing pages 69-76 for lab 3. You also need to read about enzymes (pages 91-110). Write legibly. You will NOT be admitted to lab unless you turn in this completed pre-lab at the start!

1) Define transformation, competent bacteria, ampicillin resistance and "lawn" of bacteria.

2) Fill in the following blanks. Be specific for the experimental conditions for Part 1 of the enzyme lab.

Substrate (_____) + Enzyme (_____) reacts for ____ minutes. The reaction is stopped by the addition of ____. Upon heating reducing units react with the added _____ to produce a brown soluble product. The absorbance of this solution can be determined using a spectrophotometer. A 1μM maltose solution has an Optical Density of 0.4. Therefore a solution with an O. D. of 0.6 would represent a _____ μM Maltose solution.

3) What results would you predict if you added DNS to your reaction tubes (after 5 minutes) and then measured the O.D. of the sample without heating (at or greater than 95˚C)? Explain!

4) Outline the general procedure to measure the optical density of a sample. Include zeroing the transmittance (on/off switch) and the absorbance (with the blank) of the spectrophotometer. A diagram of the spectrophotometer may be helpful.

5) You are given a tube of spit that has already been centrifuged. Outline how you would make the following dilutions: 1) 1/10, 2) 1/100, 3) 1/1,000, 4) 1/10,000 and 5) 1/100,000. You have the following items: test tubes, a dispensette that dispenses 4.5 ml of a phosphate buffer, and a P 1,000 micropipetter. Diagrams might be helpful.

6) Describe how to use a micropipetter – say this out loud pretending you have a micropipetter in your hand (you are on the honor system). Diagram the window for withdrawing 500 μl of solution with a P1000 micropipetter.

Pre-lab continued on the other side.

7) The following three solutions are used in separate transformation reactions (3 separate experiments). Predict transformation results for each realizing that a small number of bacteria get transformed. For each cell in the table indicate the number of colonies (lawn or isolated) and the color (white or blue).

	Type of petri plate	
Solutions	LB only	LB + AMP + X-gal
No plasmid DNA		
PBLU plasmid DNA		
PBLU plasmid DNA with insert in B gal gene		

8) Prepare a flowchart for part I of the enzyme experiment (refer to the flowchart for the photosynthesis lab for an example). Your flowchart should be useful to you. It should be complete enough that you could use your flowchart to do part 1 without the lab manual.

Flowchart - Part I