# Team Based Independent Project (TBIP)

**General Outline** for all three projects: The enzyme lysozyme, ELISA protocols, and a variety of plasmids will be prepared for future use by a potential client. A variety of preparation, quantification and assaying techniques and technologies will be used to generate high quality products and procedures. Techniques will be closely monitored for efficacy and corresponding documentation prepared.

The final results and assessment will take the form of a detailed lab notebook and preparation of accompanying documentation (specifically SOPs). SOPs for the various protocols relating to your TBIP, as well as QA and QC issues will be addressed in the context of a critique by an external agency (i.e. me!) To put this more simply, you will run a lab based on your independent project. You will essentially be the instructor and provide all relevant literature, technical expertise and guidance that a project of this caliber merits. You will present your TBIP to the class, and be critiqued and applauded accordingly. This is a capstone endeavor and should be taken very seriously.

This effort will be team based in that the deliverables can be worked on collaboratively. Be sure you understand what your partners are doing, and I recommend you edit each others work closely. Realize that you are responsible for knowing all the facets of the lab. As for the actual execution of the lab, each team member should do it separately to ensure that you a familiar with the technique and increase the chances for meaningful and accurate data.

**Deliverable Specifics:**

**1. Notebook** should be used to record all experiments conducted. SOPs can be alluded to for many of the procedures, but be prepared to give extra detail as related to new or unique parts of your experiment.

**2. SOP for lab.** While many portions of the lab you will conduct will have standardized SOPs, you will need to make a master SOP. This SOP will include reference to the already prepared SOP's as well as the way in which these individual SOPS link together. During the planning stages of the experiment you will clarify that nature of this master SOP.

**3. Quality Assurance/Quality Control:** In addition to the master SOP you will prepare QA/QC sheets that will address issues of lab quality and assurance. Reference to Seidman and conversations with me will help clarify the scope of these documents. For the sake of brevity, these will be no more than one page long each.

**4. Technical prep sheets:** Using a standardized Excel spreadsheet technical prep sheet for the entire lab will be prepared. These will be prepared as the lab commences and be placed onto the computer to permit editing and clarification. Issues such as supplies, equipment, reagents, amounts, preparation and storage will all be addressed.

**5. <u>Lab Execution</u>** Using all of the lab resources previously prepared and provided, an entire lab period will be allotted to your teams' projects. You will be responsible for presenting a brief lecture explaining the underlying theory and practice. You will also need to prepare all of the needed supplies and handouts (with Abbie and my help) to conduct as much of the lab as is feasible/possible. If your lab has time components (i.e. it takes a day to grow out cells) we will need to plan accordingly to ensure that these supplies are available "ala cooking show." What I mean by this is that in cooking shows, some items are prepared ahead of time so that the host, can reach inside the oven, and magically a cooked pie appears….I think you get the analogy.

# Specific Comments for ELISA Lab

**Overview**: Two ELISA SOPs will be provided and executed. A method of verifying pipeting technique will be used with the computer software in order to establish familiarity with the techniques and software (a methylene standard curve technique will be used.) Once these SOPs are confidently executed, the plate well reader (and associated computer software) will be used to assess the data. In addition to executing the experiment, adequate controls (positive and negative) as well as adequate replicates (i.e. repeating the experiment) should be planned. As a final note (and time permitting) additional experiments should be conducted to look at various factors affecting the accuracy of the technique. For instance, the reagents may be warmed to see the effects of temperature on the process. There are many wrinkles on this idea, and I encourage you to be creative.

**Literature**: American River SOP, Cal Poly SLO SOP, Pasadena College SOP on standard curves, ELISA plate well reader software manual, ELISA chapter from biochem book, Siedman Chapters on light measurement.

**Supplies and equipment**: ELISA plate well reader and computer, Sigma Chemicals, Cal Poly SLO chemicals, light filters, pipets, ELISA plates, etc….

**Deliverable**: See above for overview and the following comments will help. For the purpose of the one day lab, when you are running the lab for the benefit of the whole class, you should try to conduct both labs (American River and Cal Poly SLO, although we can discuss this as time permits.) The students should do the IgG lab themselves and you should demonstrate the effects of the variables you investigated using the modified biotinylated albumin protocol from American River. A demonstration of the standard curve preparation should be conducted as well. Relevant prepared paperwork (SOPs, QC, QC, tech prep, etc) should be prepared and Xeroxed so that all students will have copies for BOTH their files and to edit/return to you. This editing will occur both in class as the labs are being executed and as a take home assignment to be edited by the class and returned to you for final consideration and/or editing.

# Specific Comments for Lysozyme/Spectrophotometer Lab

Overview: Enzyme Purification: Using the technique of gel filtration, the enzyme lysozyme will be isolated from egg white. The gel column will be calibrated with protein standards to aid in predicating which of the column's fractions contain the greatest concentration. Collected fractions will be quantified using standard curve techniques. Assayed fractions will be used to test for lysing action upon lypholized M. luteus cells with a spectrophotometer (with multi-cell attachment.) Additional protein purification steps of dialysis and centrifugation purification will be conducted in effort to optimize enzymatic activity (how to integrate into deliverable. ). Process will be repeated to ensure reproducible results.

**Literature**: Enzyme purification and kinetics lab from Dr. James Schaeffer, Lysozyme purification and assaying lab, Gel filtration CLUES lab, Biochemistry chapter on enzymes, equipment manual for spectrophotometer, Bradfords' BSA protein kit manual

**Supplies and equipment**: Spectrophotometer with multi cell reader and computer printer hook up, gel filtration equipment, Bradfords with albumin standards, M.luteus cells

**Deliverables**: The following comments will help. For the purpose of the one day lab when you are running the lab for the benefit of the whole class, you should conduct the lysozyme purification, M.luteus assay, and protein quantification. The standard curve technique should be explained, but a previously prepared graph should be used. Time permitting the process of calibrating the column should be conducted in lab (if not enough time, a reference graph and technique should be provided.) As you are conducting the lab, strive to get reproducible results, especially with respect to: protein concentration, fraction collection number and M. luteus assay. The mathematics associated with activity levels should be clearly explained and actual data from the experiments used to demonstrate the calculations. Relevant prepared paperwork (SOPs, QC, QC, tech prep, etc) should be prepared and Xeroxed so that all students will have copies for BOTH their files and to edit/return to you. This editing will occur both in class as the labs are being executed, and as a take home assignment to be edited by the class and returned to you for final consideration and/or editing.

# Specific Comments for Plasmid Miniprep/Gel Analysis Lab

**Overview**: Plasmid DNA will be commercially produced for use by other labs. Bacteria containing the plasmid will be grown out, and the plasmid isolated using commercially available kits. Candidate plasmids include: pBLU, pGLO, pGRN, pDRK, pUC18, pBR325 and pAMP. Competent cells for transformation will be prepared in bulk for use both in this lab, as well as surplus for client. Once isolated the plasmid will be quantified using gel imaging densiometry (time permitting and DNA/RNA calculator may be used. Densiometry studies will be conducted using DNA ladders created expressly for the purpose of mass and quantity determinations. RE digests and conformation gels will be

used to verify construct, and transformations conducted to validate efficacy of plasmid. Plasmid concentration will be adjusted to optimize transformation efficacy.

**Literature**: Qiagen mini prep lab manual, Kodak gel imaging equipment and software, transformation SOP from 230, gel imaging chapter from, RNA DNA calculator manual (again time permitting.)

**Supplies and equipment**: Kodak Gel Imaging System, RNA/DNA calculator, Qiagen kit, gel stuff, transformation and conformation supplies (especially competent cells.)

**Deliverables**: The following comments will help. For the purpose of the one day lab when you are running the lab for the benefit of the whole class, you should conduct the DNA quantification using the gel imaging system. The exercise should start with raw product (bacteria containing the plasmid.) Plasmid isolation and conformation gels should be run (note, bacterial grow out plate and over night cultures can be assisted by the biotech staff as needed.) EN digest should be used in conjunction with gel imaging to add a component of molecular weight determination as well as quantification using densiometry. Example petri dishes, to show results of transformation; should be saved from previous labs. Relevant prepared paperwork (SOPs, QC, QC, tech prep, etc) should be prepared and Xeroxed so that all students will have copies for BOTH their files and to edit/return to you. This editing will occur both in class as the labs are being executed, and as a take home assignment to be edited by the class and returned to you for final consideration and/or editing.