Acknowledgments

Biology 20L is the 4th version of an introductory lab course offered by the Biology Department at UCSC. The course was developed, and the original version of the laboratory manual was written in 1995 by Dr. Gary Silberstein. Gary's direct involvement with the course ended the following year with his return to full-time research, though we continue to consult with him. The present version of the manual incorporates additions and changes contributed by course instructors, staff and students. Even though the course will inevitably continue to change, I cannot envision a time when the letter and spirit of the manual will not reflect Gary's original contributions.

The authorship of what follows should be attributed to "Gary Silberstein et al." We have avoided the tedious task of identifying the specific author or authors of each exercise, even those that are attributable primarily to one individual.

We all owe our thanks to the members of the Biology Department preparation staff, who provide technical support for the course. We are indebted to them for their competent work and for their thoughtful suggestions.
Preface

The following is taken from Gary Silberstein's preface to the original version of this manual.

“In the summer of 1992, while revising laboratories for our Introductory Biology Series, I was struck by the fact that the student exercises bore no resemblance to my experience in a working biology laboratory. I began to wonder why an introductory laboratory course couldn’t teach, along with traditional materials, some practical, technical and analytical skills. Extending this query to my colleagues, I conducted a formal survey of our research faculty, which returned 100% support for the concept, as well as a list of basic laboratory skills deemed essential for beginning biology majors.

The year following my survey found me writing a laboratory manual for the Howard Hughes Summer Institute in Molecular and Cell Biology, an eight-week, eight-hour-per-day intensive research project for twenty top-ranked sophomore and junior undergraduates selected from all over the United States. I expected this excellent group to “hit the ground running.” Instead, they puzzled over the basics of making solutions, operating automatic pipettes, and following simple protocols. The concept of the Basic Skills element of this manual was born as we spent the first week-plus teaching basics.

The Institute revealed even more about undergraduate writing. Clearly written experimental results and their analysis proved as difficult for the Institute’s select student as it does for other beginners. Without clear guides, I found that even the best floundered. To remedy this I created a detailed protocol for constructing reports. Its descendant appears in this manual.”

We hope you will agree that the course reflects these concerns and addresses them productively.

The idea that laboratory instruction should move students away from learning what others have discovered, and towards the ability to discover for themselves, did not originate with Gary. An interesting account of an extreme example of the so-called "investigative" style of laboratory instruction, which has become mythologized in its retelling among biologists, is offered in the following paper (posted on the course WWW site):

How Should A Young Person Study Botany?
W. J. Beal
Course Description

Purpose of the Course

Biology is a science of experimentation, not dry theory. Over the past three decades, fundamental discoveries in molecular and cell biology have transformed the biology laboratory with experimental tools of great diversity and sophistication that are now considered essential to nearly all advanced work in academic and industrial biology. This laboratory course focuses on some of these tools and the critical thinking that it takes to understand and use them.

The purpose of the course is to develop the intellectual and technical skills that are necessary to design, implement, analyze, and communicate basic experiments in a variety of fields of modern biology. Why give skills such prominence? First, without an understanding of the experimental theories, designs, and techniques, biological concepts are difficult and sometimes impossible to understand. Secondly, core skills are useful in their own right. They are necessary for a higher level of understanding and performance in advanced laboratory courses and academic research projects as well as providing important practical background for jobs in academic and industrial biology.

Goals

The goals of the Laboratory are to teach:

1. practical, professional laboratory skills centered on accurately executing written protocols and properly using laboratory tools, chemicals, etc.;
2. record-keeping and documentation of laboratory work (laboratory notebook);
3. communication of experiments and results (reports);
4. experimental theory and design.

Lectures

There is a weekly, seventy-minute lecture for the entire class in which the theoretical and practical aspects of the following week’s experiments will be discussed. This is intended to give you strong background several days before lab. It will minimize the need for in-lab lectures and maximize time “at the bench.” You will get a lot more out of the lectures if you have read the following week’s labs in advance. Attendance of lectures is required and occasional quizzes will be given during lecture.
Follow-up Laboratories

A typical complaint in most introductory laboratory courses is the hectic pace and lack of time to repeat failed experiments or to refine or extend interesting results. Our schedule includes two Follow-Up Sessions during which you can conduct troubleshooting protocols to investigate failed experiments. If your instructor feels that you are doing well you will be allowed to design a “next step” or follow-up experiment within the limits of our preparation resources. The virtue of the follow-up idea from your point of view is that it approaches real research by emphasizing troubleshooting and the “next-small-step” nature of experimentation.

Follow-up laboratory periods are not “free-time.” You are expected to use the entire four hours to either troubleshoot failed experiments or carry out a short investigation that extends earlier work.

Student Responsibilities

A. Materials you should bring to all laboratory sessions:

1. this manual;
2. three-ring, loose-leaf notebook;
3. clipboard (cheap at Bookstore—get one!);
4. 3-hole punched paper (spiral bound notebooks containing 3-hole-paper);
5. dividers with see-through tabs for 3-hole binder;
6. color pencils (6 colors);
7. Macintosh or PC floppy disk;
8. graph paper (see Appendix);
9. metric ruler.

B. Attendance

Laboratory and Lectures are required. Absences will be allowed for illnesses and family emergency only. Unexcused absences will affect your grade and evaluation.

C. Pre-Lab Preparation

Before coming to lab, you are expected to review protocols and prepare data sheets and tables appropriate for the day’s work. These are to be brought to the lab in the laboratory notebook. Your instructor may check your notebook at the beginning of lab to see if you are prepared.
D. Web Site and e-mail

You will be expected to consult the course web site for supplementary readings and data. You should also read your e-mail regularly. Instructors may communicate information to you by e-mail.

E. Assigned Reading

For each exercise there is some assigned reading, often in Biology, by N. A. Campbell. There may also be assignments on hard copy reserve or the course web site. These assignments represent the minimum reading. Additional sources will help you create discussions that are more meaningful.

F. Written assignments.

It is course policy that all assignments must represent individual work. Any evidence of “sharing” will result in zero credit for both participants.

G. Laboratory reports.

These reports will comprise the heart of your work and will be the primary means by which we evaluate your skill and acumen. Their goal is to teach you how to report experimental observations in an accurate and concise fashion. The details of what we expect in a report are included at strategic places in this book. The details of how to prepare a laboratory report are described in the next section. Your instructor may add to or modify these. All assignments are due on the date requested by the Instructor. Late submissions will be penalized.

H. Laboratory notebook.

Your laboratory notebook will be critiqued early in the course, probably while you are in the lab. While comments from Instructors may seem informal, pay close heed to suggestions. Include in the appropriate sections of your lab book answers to all problems and questions posed in the manual. Your notebook will be collected at the end of the quarter and graded. Details for setting up and keeping a notebook are in the next section.
Grading

Final grades and narrative evaluations will be determined by the individual instructors based on the following elements:

- Attendance
- Laboratory notebooks and written reports
- Quizzes (administered during lab or lecture)
- Final Exam
- Subjective evaluation of your participation by Instructor

The final element is a partially subjective assessment of how well-prepared you are coming into the laboratory and how carefully you carry out the work. Time-management, the nature of the questions you ask, decorum, interest, enthusiasm all contribute to this.
DOCUMENTING AND COMMUNICATING YOUR WORK

In this course we distinguish between laboratory notes ("datasets") and formal written reports. You will create a set of notes for each and every exercise. Several exercises will be expanded into full formal reports. Your instructor will determine which exercises are to be submitted as full reports and establish due dates for the reports in their section. Also, they may have special directions for writing reports that expand or are different from the following general guidelines.

I. Laboratory Notebooks

The philosophy of the laboratory notebook.

Keeping accurate records of laboratory work is a fundamental laboratory technique as important as enzyme assays or bacterial plating. Careful laboratory note-taking is crucial to successful lab work for three reasons: First, when things go wrong, as they inevitably do, a detailed record of what was done is mandatory for troubleshooting the problem. Second, and most obvious, your notes are the basis for communicating your results either in writing or orally. Third, and perhaps not so obvious, in all industrial laboratories, your notebook is the property of your employer and is a legal document. Often after you leave a project, other workers will use what you have written to carry on the work. For these reasons, write your notes legibly, with care, and concern for your own immediate needs. At the same time, write as though others will use your notes.

Loose-leaf Notebooks.

The loose-leaf notebook, with sections delimited by dividers, allows for the flexible, expandable, and orderly organization of your work. It is used by many professionals and is a powerful tool for keeping large amounts of data and notes logically arranged. You will use only loose-leaf notebooks in this course.

Why not use bound notebooks? People obviously do and they work, but their inflexible structure can create serious organizational problems. Working on unrelated elements of a large experiment, e.g., DNA assays, bacterial transformations, etc., at the same time is common. In bound books, information must be entered sequentially. Unless you correctly guess the number of pages you'll need ahead of time, entries that logically belong together become fragmented with interspersed, unrelated material.
Setting up your laboratory notebook

1. Label dividers with the name of the laboratory exercise.
2. Remove the pages for each exercise from this bundle and place them behind the appropriate divider.
3. Place all laboratory notes (protocols, data, write-ups, graphs) and relevant lecture notes in back of the printed pages in the appropriate section.

Entry of information

1. NO REWRITING OF LABORATORY NOTES! All information must be written on 8.5 x 11" pages at the time that an element of an experiment is performed, e.g., when a solution is made, a gel set up, a reaction run, etc. Never write things down on scraps of paper or paper towels! All photographs, semi-log plots and other relevant notes can be taped on loose-leaf pages and placed in the book.

2. Daily Entries: A lab notebook is not a lab report. Therefore each section does not need a lengthy introduction, a materials and methods section, or conclusions. Professionals typically use a “working” format consisting of the following sections:

   Date/number: Date and number each page! Restart page numbering at #1 within each section. Also remember to make the most use of the unique set of six numbers in the date, e.g., 06/28/93, to key your test tubes, photographs, graphs, and anything else, to your notebook work. Sometimes this can be a life-saver! No kidding . . .

   Title/Purpose: A one-sentence statement of why you are doing something is usually efficient. E.g., “Restriction digest to test for inserts in the plasmid, pGEM7Zf+.” Longer is permissible, but try to keep it short.

3. What to Enter?

   Lengthy procedures. We all follow documented procedures when performing experiments. Yours are in this manual. An excellent technique for gaining an understanding of an experiment is to transform the text of the procedure as given in this Manual into a diagrammatic representation of the work to be performed, adding your own comments.

   Almost invariably at the bench you will do something different from the written procedure that may affect the outcome of the experiment. I recently saw a very nice technique for keeping track of such things that also incorporates the “transcription” concept. The student drew a line down the center of a page. On the left side were the steps of the procedure written out. The space to the right
of each step was used for notes that were entered as the step was executed. This technique may work for you.

“Simple” procedures/”little” details. In preparing restriction digests, solutions, weighing, etc., record in detail exactly what you did. Referring to a general procedure in this manual will not suffice. You will quickly find out that these details enable you to troubleshoot problems and you will get the hang of what to enter and what to leave out.

Data. Numbers, tables, calculations, observations, photographs, drawings.

Results. Write as much or as little as you need in order to remind yourself a month or a year from now what happened. For any day’s work, this may not be much. It can even be a single sentence.

Tip: In the space above the Title/Purpose, write one sentence describing your results. This is very handy when you thumb through the book wondering how an experiment came out. This statement may only be a recording of the yield of some purification, colony number for a transformation, etc. Whatever is pertinent and may be useful in getting an overview of how a week or so of work went.

Conclusions: If you have any, write them down.
II. Formal Laboratory Reports

Structure of a laboratory report.

Reports must be organized in a standard scientific publication format in the following order:

Title
Introduction “why is experiment being done?”
Methods and materials “what did you do?”
Results “what did you observe?”
Discussion “what do your observations mean” and “what next?”
References

Figures and Tables

There are two keys to successful writing: first, knowing your audience; second, a plan of attack.

Audience: Who are you are writing the report for? Do not make the mistake of thinking, "my instructor knows all about this. S/he doesn't need to hear this again.” Imagine your audience to be a colleague (perhaps a fellow student) who knows general biology but is not familiar with this specific work and will want to repeat it. You will want to explain why you did the experiments and exactly how. It is safe to assume that your reader will need considerable background. Don't skimp.

Plan of attack/protocol: without a plan of attack, writing a research report can be a nightmare. Here is a suggested mode of operation.

Step 1. Title

What is the title of the report? This is not trivial!

What are you going to write about? The title helps you to define what data are relevant and what is irrelevant as well as suggesting ways to structure arguments. Sample titles: “A comparison of the Biuret assay with a UV absorbance method for protein determination” or “The effect of temperature and pH on transformation efficiency of competent Escherichia coli strain DH5α”. Note that these titles are very specific; not “A study of different protein assay methods” or “A study of transformation efficiency.”
Step 2. Methods

The benefit of writing the methods section before the results is that it will focus your thinking on the specifics of the process. With the methods fully presented you are primed and ready for redirecting your attention fully to the later sections.

Describe exactly what you did using the past tense, not why you did it. Include sufficient details (concentrations, times, volumes) so that someone else could repeat your experiment and confirm your results. Assume that your audience is an experienced laboratory worker who can sort out minor details (what size tube?, which micropipette?) for themselves and needs only the general procedure to follow. Of course, this requires mature judgement on your part in deciding which details of your method are essential and which are incidental or trivial.

Most methods can be stated very concisely unless details are crucial to understanding the experiment. In the latter category, you must carefully describe detection methods: “protein concentrations were determined using the Biuret reaction”.

A special category of methods, calculations, is recognized in this laboratory. For all quantitative work involving calculations, include your calculations. If they are extensive, place them in a special appendix.

Step 3. Results

Review your results and summarize each section/study/experiment. (Here is where the “top of the page” summary comes in handy.)

Prepare figures (graphs, photographs, drawings, etc.) and tables to present key findings. They will appear together in a separate section at the end of the report, but are essential for writing this section.

Write figure and table titles and legends. Details about the specific experiment or technique should be included in the legend, e.g., reaction mixtures, gel concentration.

Irrelevant information/data What would be “irrelevant” data? The details of how you made a solution would be irrelevant. (unless it is a lab about solutions and dilutions!) What pipette size you used. The details of how an enzymatic reaction is set up. (this belongs in the methods or in a figure legend.) Most RAW data, e.g., Tables of spectrophotometer readings, are not presented. If you feel you must include raw data, put it in an appendix at the very end of the report. What to include is tricky to know at first.
List the points as you would present them in oral argument. (Try talking it out to your partner: “Here’s what we did and why we did it. Here’s what happened,” etc.)

Arrange figures and tables in the same order as you have arranged the points of the argument. This is their tentative order of appearance in the text. Number each figure and table. Note that figures and tables always have their own numerical sequence, e.g., Fig. 1, 2, 3; Table 1, 2, 3. Graphs, drawings, maps and sample calculations are numbered as figures.

Logic flow There is, or should be, an inherent logic to laboratory work. Experiments address questions. Your job is to arrange what you did in small steps to lead the reader to the answer(s). Have no illusions about this; even apparently simple studies can be very tricky to present.

Writing results. With figures and tables in front of you, analyze them in order, writing a sentence or a paragraph describing the specific result(s) and referring to the numbered illustrations, etc. Let your reader know when it is time for them to consult a given figure. This is signaled by the in section of “(Fig. X)” at the appropriate position in the text.

The four most common mistakes students make when “writing-up” results are:

1. Asking the data to speak for you.

"I think I was naive and dumb because my view was results speak for themselves."

   Hilary Rodham Clinton
   (on how she has been perceived by the American people)

As Hilary found out to her dismay, no result speaks for itself. You must describe for your reader the important points about the result(s). Simply referring to a table or graph (e.g., “Figure 2 shows the rate for the enzyme”) without careful description, will not do. In effect you are saying “Here it is, I don’t have a clue what is important here and what’s not. You figure it out.”

Specific comments are best; for example “Enzyme rates were linear over a ten-minute period, after which the reaction velocity decreased, reaching zero by fifteen minutes.”

2. Missing the point completely.

“Enzyme optical density readings changed from 0.020 to 0.550 for the 1/100 dilution and 0.20 to 0.780 for the 1/200 dilution.” Here you are asking the reader to do calculations for you and figure out what the important point is.
3. Superfluous generalities and non sequiturs.

“The reaction worked because the color changed.”

4. Including conclusions.

Conclusions—that is, what you think your results mean—must not appear here in the results. The word “because” almost always indicates that what you are about to say belongs somewhere other than in the Results section.

Step 4. Discussion

Discuss what happened and what it means with respect to the original goals of the study. Keep the figures and tables right in front of you and, referring to the results (e.g, “... as shown by gel analysis [Fig. 34, lane 2] ...”), Keep in mind the difference between results and discussion is that results point out what observations and data are important while the discussion explains what it means.

Replication, Repeatability, and Experimental Error

A long-standing and deplorable tradition among undergraduates is to attribute unexpected experimental data to "experimental error", and leave it at that. This facile dismissal is next to meaningless. Let's consider “experimental error” more carefully.

We can distinguish 2 types of error, spurious error and systematic error. Spurious errors are bloopers, just one of those things, momentary lapses of concentration. They are spontaneous accidents.

Systematic errors arise from a more systemic, entrenched, and consistent disorder in the procedure or materials.

To clarify this distinction lets consider examples from the bacterial dilution and plating exercise. If you forget to mix one tube in the dilution series, that is an example of a spurious error. An example of a systematic error would be preparing LB + Ampicillin agar plates from an outdated and defective stock of the antibiotic.

The distinction between spurious and systematic error is somewhat arbitrary, but nevertheless useful. The key issue would be the likelihood that the same error would occur again in the same way if you did the experiment more than once.

Good scientific practice demands that investigators be aggressive in seeking to expose both spurious and systematic error. Spurious errors can be detected by replicating an experiment. If you do a procedure twice (i.e. replicate it) you are unlikely to repeat the same spurious error twice. With respect to our example, it is unlikely that you would forget to mix the same tube twice in two dilution series.
Systematic errors are more difficult to detect. For this, science sets the standard of independent **repeatability** by a **different investigator**.

Replication and repetition of experiments is inevitably constrained by logistic issues such as the cost of reagents and materials, preparation time, etc. Some 20L exercises are repeated by all groups in the lab doing the same experiment. Even so, this could not reveal systematic errors introduced by preparation of class materials.

Replication and repetition, while being efficient approaches to **detecting errors**, frequently do not reveal the **source of the error**, much less the **remedy**. In discussing the results you should always strive to evaluate the specific source of errors, their significance for interpretation of data, and appropriate remedies. Well thought out recommendations for troubleshooting make excellent fodder for discussions.

**Step 5. Introduction**

This is the place to explain why you are doing an experiment. What is its purpose or rationale?

We frequently find that introductions to student lab reports are confined to broad generalities. It is fine to begin by providing a broad perspective to the work. Indeed, this offers a rare opportunity to bring your personal perspective to bear. But your **Introduction** must lead the reader progressively from the general to a specific.

Foremost, you need to give a clear statement of any hypothesis you are testing. "The purpose of this experiment was to explore the concept of limiting nutrients." NO! That is the purpose of innumerable experiments and not unique to one. Rather, "The purpose of this experiment was to test the hypothesis that organisms require chemical elements in the same molar ratio as they occur in biomass." This was the specific hypothesis you used to make a specific prediction. The introduction can also describe the specific experimental system you will use to test the hypothesis.

It is excellent practice to state in the introduction any predictions about what might be found, e.g.: "Increasing the nitrogen concentration should cause the growth yield of the cultures to increase only until the ratio of nitrogen to carbon in the culture equals the nitrogen to carbon ratio in the cells." Predictions alert the reader to what findings (results, data) you think will be important and they foreshadow the **Discussion** by providing a clear basis for drawing conclusions.

**WARNING! PITFALL!** The educational "purpose" of a lab as it is written in a Lab Manual is almost never the same as the purpose for doing an experiment. Therefore, never simply parrot back the Purpose written in this manual. Below is an example of the educational purpose and educational purpose of a typical exercise:
**Our Educational Purpose:** "The purpose of this exercise is to teach you some of the basic characteristics of enzymatic reactions by investigating reaction kinetics."

**Your Experimental Purpose:** "The purpose of the experiment was to determine reaction conditions that give an apparent maximum velocity for an amylase preparation and then use these conditions to investigate enzyme induction."

**Step 6. Sweat the details**

Good writing should continually engage the reader. Use complete sentences and complete thoughts. Check for formatting and spelling. Number pages before submitting the report.

A few of the finer points:

- Numbers never begin with a decimal point. Always use the format "0.425", not ".425".

- Multiple nouns that modify other nouns are hyphenated as in “white-eyed”, “plasmid-encoded.”

- The axes in a graph should always be fully labeled, including units.