

**Genetics Laboratory:
Problems in Genetic Analysis**
Third Edition*

**University of Richmond
Fall 1999**

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*Please report all errors, however minor, to one of the authors!

Genetics Laboratory: Problems in Genetic Analysis

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Laboratory exercises

Lab 1: Serial dilution of a bacterial culture

Lab 2: Isolation and characterization of plasmid and chromosomal DNA

Lab 3: Genetic analysis of mutant *Drosophila melanogaster*

Lab 4: Recombination and complementation of mutants of bacteriophage T4

Lab 5: Properties and mutagenesis of genes of the *lac* operon

Lab 6: The *Agrobacterium tumefaciens* genome project

Bio 213L Genetics Lab (Fall, 1999)
Calendar of Events

Week		Lab Exercise	Assgt.Due
1	Aug 31-Sep 3	Lab 1: Serial dilution of a bacterial culture	Dilution problems
2	Sep 7-10	Lab 2: Isolation and characterization of plasmid and chromosomal DNA <i>Isolate DNA</i>	
3	Sep 14-17	Lab 2: Isolation and characterization of plasmid and chromosomal DNA <i>Digest DNA and run electrophoresis</i> Assigned paper for Exam I: Discuss	Lab 1 report
4	Sep 21-24	Lab 3: Genetic analysis of mutant <i>Drosophila melanogaster</i> (continues until Week 12) Lab 2: <i>Discuss results</i>	
5	Sep 28-Oct 1	Lab 4: Recombination and complementation to map genes of bacteriophage T4 <i>Titer bacteriophage T4</i>	Lab 2 report
6	Oct 5-8	Lab 4: Recombination and complementation to map genes of bacteriophage T4 <i>Perform T4 crosses</i>	
7	Oct 12-15	NO LAB (Fall Break)	
8	Oct 19-22	Lab 4: Discuss results	
9	Oct 26-29	Lab 5: Regulation of the <i>lac</i> operon	Lab 4 report
10	Nov 2-5	Lab 5: Discuss results Lab 6: The <i>Agrobacterium tumefaciens</i> genome project <i>Group discussion</i>	Lab 3 Progress report
11	Nov 9-12	Lab 6: The <i>Agrobacterium tumefaciens</i> genome project <i>Individual discussions</i>	Lab 5 report
12	Nov 16-19	Lab 6: The <i>Agrobacterium tumefaciens</i> genome project <i>Individual discussions</i>	
13	Nov 23-26	THANKSGIVING BREAK	
14	Nov 29	1 st UR Symposium on <i>Agrobacterium</i> genomics	Lab 6 poster
14	Nov 30- Dec 3	Poster presentations (encore performance)	Lab 6 poster
15	Dec 6		Lab 3 report

I. Administrative matters

I.A. Grading

This lab is an integral part of a four-credit course. It is not a one-credit addendum to a three-credit lecture course. The material in the lab will appear in class and in exams just as material from the class will appear in the lab. As with exams in class, all assignments will be graded 90-100 A, 80-89 B, etc. There will be no curve at the end of the semester. This way, you can always calculate where you stand at any moment. The lab grades will contribute 30% directly to the overall grade in the course. The lab contribution will be calculated as a weighted average, using the weights for each assignment shown below. Each assignment is due during the normal lab period in the week listed.

Assignment	Weight	Week due
Weekly quizzes (5x8 weeks)	40	Sep 1 - Nov 6
Lab 1 - Dilution problems	5	Aug 31-Sep 1-3
Report	20	Sep 14-17
Lab 2 - Report	50	Sep 28-Oct 1
Lab 3 - Progress report	25	Nov 2-5
Final report	75	Dec 6
Lab 4 - Report	50	Oct 26-29
Lab 5 - Report	25	Nov 9-12
Lab 6 – Poster	50	Nov 29 AND Nov 30-Dec 3
TOTAL	340	

Lateness policy

The maximum score obtainable on any given assignment is:

$$50\% + (50\% \times 2^{-d/5})$$

where **d** is the number of days late. Besides providing an example of the common biological process of exponential decay, this formula encourages you to turn in assignments on time, which makes life easier on all of us, but also encourages you to turn in assignments sometime if you miss a deadline. A report that would have elicited a score of 95 if turned in on time will become an 89 if turned in one day late or a 79 if turned in three days late, but even three weeks late, you can still salvage a 50, which is much better than 0. But 0 is what you get for anything handed in after December 6.

I.B. Attendance policy

Since you may report only data that you've gathered with your own hands (except where noted in the lab manual), it is incumbent upon your hands to be in lab. If you find that you are unable to attend your normal lab, you should find a lab that you can attend and contact the instructor of that lab. We will make every effort to accommodate your needs based on legitimate reasons, however, we do not consider travel plans for fall break to fall into that category. Fall break is in October. It is now August. **MAKE SURE YOUR PLANS DO NOT CONFLICT WITH LAB!**

There are some labs where you will be asked to come back outside of scheduled lab hours to take care of bacterial plates or flies. There is no avoiding this, since real organisms live without regard to our artificial schedules. However, we will attempt to balance the time you spend outside with shorter hours within lab periods.

I.C. Honor Code

Since most of the lab work in this course is performed in groups, there may arise some question as to what work is expected of you individually and what work may be done as a group.

Work that you are encouraged to do as a group

1. Plan the experiment
2. Perform the experiment
3. Compile results of the experiment
4. Discuss the results and what they mean

Work that you are expected to accomplish by yourself:

5. Perform experiments that are assigned to you. This includes viewing plates outside of class
6. Analyze the data. This includes making necessary calculations and composing appropriate graphs and tables.
7. Conceive an outline for the report
8. Write the report
 - Figures or tables are to be composed by you, not by the group or any other person
 - You may not use previous years' reports. By the way, we change the lab every year and it is exasperating to run across a report that uses a protocol that we are not using this year!
 - All writing must be your own. You may not copy or paraphrase from any other work, including the lab manual, regardless of whether you provide a reference.

II. LAB REPORTS

II.A. Why do them?

All of the lab component of your grade will be based on lab reports. This is appropriate, for once you leave the academic setting, you will seldom be asked to sit for exams, but you will time and again be asked to explain your work to others. In science, the rewards go to those who are most adept in reporting their work. They get the publications -- the currency of science -- and they write the proposals that convince others to support their research. Whether or not you take on science as a career, your professional success will often depend upon your ability to tell a compelling story about what you have done. We wish to give you every opportunity to increase your skills in this area.

The purpose of a lab report (and for that matter, the purpose of a scientific article) is to present a body of experimental work: Why was it done? How was it done? What was the direct outcome of the experiment? What does it all mean? (And what does it all not mean?) Perhaps this statement of purpose seems obvious -- we hope it will by the semester's end -- but if you truly absorb its significance, then writing a report should be no more than fleshing out the simple answers to these questions.

II.B. The components of a lab report

The first lab report will be composed of short answers to questions posed in the lab manual. These questions are actually those that might occur to you if you were writing a full lab report. Treat the lab, therefore, as an exercise in how to do it. All other lab reports will be written out, with each of the sections described below.

INTRODUCTION: Why was the experiment done?

- A. Present an issue of clear scientific interest to the reader
- B. Connect by logical argument this big issue to a specific question that you addressed in the lab
- C. Clearly state the specific question your experiment addressed.
- D. Along the way you may need to explain certain concepts central to understanding the experiment you performed

We have tried to imbed in each lab exercise a scientific question around which you can organize a lab report. Sometimes it's a stretch -- after all, scientific questions don't often fit into week-sized time slots -- but please do your best to enter into the spirit of the exercises and use them as practice for the more realistic situations you will surely confront later.

This scientific question should be the organizing principle for your report, and as such, it needs to be clearly stated. Stating the primary question is the primary goal of the INTRODUCTION. The INTRODUCTION should be seen as a logical progression towards a clear statement of the question.

A secondary purpose of the INTRODUCTION is to explain concepts necessary to understand the experiment you performed. Sometimes, to avoid disrupting the narrative, you may want to defer discussion of a concept to the MATERIALS AND METHODS section. This is especially advisable if the concept relates exclusively to methodology. Remember that your purpose is to explain your experiment, not to write a treatise on some aspect of genetics, so confine your discussion to what is necessary to satisfy a naïve reader interested in your work.

Despite everything you may have learned throughout your life, there is no law that states that you must present a **hypothesis** in the INTRODUCTION. Often times, you have no rational basis on which to form a hypothesis, except after some results have been obtained. Fine. Seek hypotheses as you go along, and tell us about them, but don't rewrite history by pasting them into the INTRODUCTION.

MATERIALS AND METHODS: How was the experiment done?

- A. Describe the kind of experiment that can answer the question you posed

- B. Explain the principle behind the experiment you performed
- C. Relate whatever else we need to know to understand the experiments you did

In scientific papers, the MATERIALS AND METHODS section is used to provide the specialist reader with sufficient information to permit the reproduction of the experiment. This may be the format you're used to from previous courses. In this course, however, the section will have a different purpose: to help the reader (a person like yourself) understand how the experiment works. We've made this change because few of you will write scientific articles, but all of you will find it necessary to present your ideas in a way that's comprehensible to others.

The section should start where the INTRODUCTION left off: with the primary scientific question your report is about. At the beginning, you should present in broad outline a reasonable strategy by which the question may be answered. Then, explain how the strategy was implemented, and help us to understand the technical aspects of the experiments.

It is neither necessary nor desirable to reproduce what is in the Lab Manual. You may simply refer to specific protocols described in the Manual, giving details only when you strayed from the protocol or went beyond it.

RESULTS: What was the outcome of the experiment?

- A. Restate the goal of the experiment and how you hoped to achieve that goal
- B. Describe the results in the form of a coherent story
- C. Refer to figures and tables, showing your results, as visual aids in support of your story

The purpose of the RESULTS section is to tell a story, the subject of which is how you went about answering the primary scientific question you put forth in the INTRODUCTION. The data comprises the raw material for the story, but it cannot stand alone, any more than you can grasp the story of Goldilocks from a table of porridge temperatures. Your creative input is essential to weave together the information you gathered into a narrative that speaks to the scientific question.

A good place for your story to begin is with a reminder of the scientific question and how you hoped to answer it. From there, take the reader through the results you obtained. Their order need not correspond to the order in which you performed the experiments, especially if a different order would make a better story. In guiding your reader, point out specific parts of the data, helping us make sense out of it. Imagine yourself describing your results orally, with slides of your tables and figures. In such a setting you would not think of reciting the data in the table -- your audience wouldn't stand for it! Rather, you would talk about trends, emphasizing the most informative results.

While the story demands that you draw some meaning from your data, you should be careful not to burden the RESULTS with too much interpretation or too many conclusions. They should be reserved as much as possible for the DISCUSSION section. Admittedly, it is often difficult to judge where best to draw the line. One rule of thumb is to permit yourself the most obvious conclusions to move the story along, while reserving anything more complicated for later.

Pay careful attention to how you present your results. Consider what format would best display to the reader the information inherent in the data. Just because you have a color printer and know how to use a spread sheet does not mean that a rainbow pie chart is the best possible means to present measurements of gene expression. Are the raw numbers or percentages more informative? How can a Table be organized so as to make relationships as clear as possible?

When you give quantitative results, consider the accuracy of your measurements. Just because your calculator gives a recombination frequency of 0.000539687 doesn't mean that these digits have any physical

significance. Examine your raw numbers to determine the accuracy of the final calculated quantity. When you supply a number, you imply that each digit is meaningful. Make it be so.

Tables and figures should appear at the end of the report, not embedded within the text. This way (we hope) you will not be tempted to drop a table on the reader without verbal preparation. Presume that the reader is not conscious of the tables or figures until you refer to them by number in the text. Wherever they do appear, tables and figures should make some sense on their own. They should:

1. Be numbered (Table I, Table II, etc; Figure 1, Figure 2, etc)
2. Contain descriptive titles (e.g. "*Induction of lacZ expression in response to lactose*", not "*Spectrophotometric results*"), which, in the case of Tables, should appear on top and, in the case of Figures, on the bottom.
3. Provide clear labels of columns and rows (for Tables) and axes (for graphs)
4. Indicate in footnotes (for Tables) or legends (for Figures) whatever the reader needs to understand what is being presented.
5. Be confined to a single page. It is difficult to read a table split by a page break.

Don't know what I'm talking about? Take a look at the figures and tables in this lab manual for examples.

DISCUSSION: What does it all mean?

- A. Restate the goal of the experiment and the most significant results related to that goal
- B. Review the results as a lawyer would summarize a case, showing how they impinge on the conclusion you have reached
- C. Consider explanations of aberrant results and other matters

The DISCUSSION section places the fewest constraints on your creativity, but there are some guidelines. Your first responsibility is to address the primary scientific question in light of the results you presented, evaluating to what degree you have answered that question. A good strategy is to state the major conclusion you can draw from your results, and its limits, and then to argue for and against that conclusion. In doing so, you should explicitly consider alternative explanations.

Note that in writing a report you will often marshal arguments in the manner of a lawyer, but your client is not a specific hypothesis: it is Truth. You will no doubt on occasion obtain results that do not square with the conclusions you would like to draw. These, too, are worthy of your respect. Note the discordance, and help the reader out with your best thoughts on the matter. Perhaps your conclusions are too simplistic? Try to imagine alternative hypotheses that might account for the aberrant results. What kind of experiment could be performed to decide amongst the competing explanations?

Alternatively, the aberrant result might have come about because of a shortcoming in the protocol (e.g. an erroneous underlying assumption) or an unintended deviation from it (e.g. failure to dilute properly). In this course, there will be no use of the broad term "human error", which serves too often as an excuse to avoid grappling with the true cause of an unexpected result. You should by all means consider whatever causes might have produced your results, but it is of no use simply to give a list of explanations. For each explanation, you must consider -- quantitatively, if possible -- whether the proposed cause is sufficient to produce the results obtained. For example, if you suggest that a strange cell count was obtained because the pipetter might have been used incorrectly, calculate the worst cell count that could have been obtained assuming that the pipetter was used as badly as you can imagine.

Apart from these general considerations, there are many directions a good DISCUSSION can take. A successful experiment often breeds further experiments that go beyond the present conclusions. An unsuccessful experiment breeds experiments to examine why it didn't work.

REFERENCES: Where can the reader find out more?

A reference to some published account should be given for any nonobvious factual statement. The published account may be this Lab Manual, your text book, or a journal article. Your notes are not published. Reasonable people may differ as to what is "nonobvious", and even apart from this, it is difficult to supply guidelines. Please see the sample lab reports in this manual for guidance. Use any format for references you like, so long as each reference includes authors, titles, year, and source. Some examples are provided in this manual. The reference list gives the sources you actually used in writing the report. Never cite a reference that you have not read.

II.C. How to write lab reports

We strongly suggest that you prepare for writing each section of a lab report by writing an outline, as described below. Many students are repelled by outlines, and if you are one of the few that can invent from scratch a series of internally consistent paragraphs, each leading plausibly to the next and comprising a satisfying whole, well, more power to you. The rest of us can use all the help we can get. An outline may help, because it is easier to see the big picture when that picture is described by just a few lines than by a couple of pages of verbiage

Suggested sequence of events in writing a lab report

1. *Compile the data from your group.* It is amazing how often lab partners present different data. Can you imagine what this does to our faith in the accuracy of anything they present?
2. *Analyze your results.* Massage them into meaningful Tables and Figures. It is much easier to see the direction of your lab report when you have all your data in front of you in a coherent form. This step should be done individually.
3. *Discuss your analysis with others in your group.* Brainstorm collectively, trying to think up as many reasonable interpretations as possible.
4. *Decide on a primary scientific question around which to organize the report.* Having a theme from the start makes all efforts at organization much easier. This and further steps should be done individually.
5. *Write the RESULTS section.* Start with an outline, to help you decide the point of each paragraph. If you are describing more than one kind of experiment, consider dividing this section into subsections with descriptive headers.
6. *Reconsider the primary scientific question in light of what you just wrote: does it still fit?*
7. *Write the MATERIALS AND METHODS,* starting again with an outline and using the primary scientific question as the jumping off point. As you go, note what concepts might require introduction earlier in the report. Check that for each result that appears in the RESULTS there is an intelligible explanation of the experiment that produced it.
8. *Write the INTRODUCTION,* from an outline. At the beginning of the outline should be an issue of general interest. At the end should be the primary scientific question. The outline may help you find a series of logical steps connecting one with the other.
9. *Write the DISCUSSION.* This section will probably be the most difficult to outline, since you have the most freedom. By the same token, an outline may be of greatest value here, preventing you from meandering in no particular direction.
10. *Reread what you have written, beginning to end.* Try to read it as a stranger. Does it make sense?

General advice

Part of your grade in this course will be based on your writing. You may think that this is not appropriate: after all, Bio 213 is a course in biology, not English. We believe that it is very appropriate: how you write is intimately connected with how you think. Writing is a means not only to communicate your results to others but to make them coherent to yourself. It is so often the case that the complexities of an

experiment get sorted out only when you try to explain them carefully to others. Please do not mistake "writing" for "mechanics". Some of the best reports I've ever read were written by a person who could barely speak intelligible English, whose sense of grammar was minimal, and whose spelling was creative at best. However, she presented her thoughts so logically that you could see her mind at work, shaping the subject at hand. Give your lab reports the same attention you would give any other piece of art.

Presume your **audience** to consist of people like yourself, but without the benefit of your experience in lab. Your audience should not be your lab instructor: do not presume knowledge you would not have had prior to the experiment.. A lab report should not be treated as a vehicle through which you are evaluated but rather a vehicle by which you explain to others what you have done, helping us along the way as best you can. *Love your reader as yourself!*

Be honest. Write naturally. "Hey, no problem – I'm honest!" Doubtless, you're not dishonest in your writing, but honest writing, with the aim of conveying to the tangible reader the deepest, most pertinent truth you can, is more difficult to attain. More often, our energy flows in the opposite direction, as we worry about what the reader will think about us. Writing in plain language helps. No discipline, not even science, is benefited by stiff, convoluted sentences that seek an effect more than clarity. Many of us were drilled to avoid the use of the words "I" and "we",... and so we should, since the focus of a report should not be on us but on what we did. However, if a pronoun simplifies a sentence, enabling you to explain what you did more clearly, then use it!

Use **short, coherent paragraphs**. By "coherent" we mean that all sentences should address a common theme that unifies the paragraph. Be ruthless in excising material that does not belong.

Use **past tense** for results you obtained or procedures you followed, **present tense** for statements of general truth. Most sentences in the MATERIALS & METHODS and RESULTS sections will be in past tense.

Slang doesn't bother us, so long as it illuminates the intended message (does it?), but **jargon** should be severely avoided, since it is intelligible to only a limited audience. Any term that is not obvious to your chosen audience should be explained or omitted.

III. Potentially Useful Information

III.A. How to prepare for lab

Chance discovery comes only to the prepared mind. We're not just talking about apples falling on heads, ... any connections are more likely when your mind is properly disposed. Since insights are what we're after, it behooves you to arrive in lab prepared. Read the lab exercise beforehand from beginning to end. You shouldn't expect to understand everything – things become a lot clearer when the tubes and plates are in front of you. Also, depending in part on what day of the week your lab falls on, there will be a few occasions when relevant material will be discussed in a class some days after the lab exercise begins. Don't let this throw you off the main course.

Suggested preparations

1. Make a list of what you need to do in lab and in what order you plan to do it. This will be easier to do in some labs than others (very difficult in Lab #1, but give it a try). In particular, note those tasks you need to do as soon as you walk into lab!
2. Note portions of the manual that confuse you. Come back to them after the lab to see what's been cleared up and what is still a problem.
3. Come to lab with at least a basic concept of the principle behind the experiments. If you have problems:
 - a. Figure out the experiments with people in your group (or others in the course)
 - b. Consult with your lab TA or any lab TA. Apart from their experience and general brilliance, they have been imparted secret knowledge about the experiments that they would love to share with you.
 - c. Consult with one of the lab instructors during office hours or by prior appointment.

III.B. What items should be at your desk

Each group should have a their own specially marked drawer at their desk, one for each lab section. You should keep a notebook there to maintain a record of what has been done with group projects. That way, for example, one person might go in one day and start a cross needed by the group, noting this in the notebook, and another person might go in later on and see that that cross has already been done and so won't duplicate the effort.

There is one drawer that you share with people from all lab sections who sit at your desk. This drawer contains:

Equipment

Micropipetters: P1000, P200, P20

Pipet pumps

Sharpie (pen that writes on glass)

Microcentrifuge tube rack

Expendables

Autoclaved micropipet tips: one box for P1000 tips, one box for P200/P20 tips

Autoclaved microcentrifuge tubes in sterile container

When a container of tips or tubes is exhausted, place it in the designated area on the bottom shelf, north wall of lab. Replace it with fresh container located nearby on the same shelf.

Matches

Kimwipes (dustless paper towels)

When matches or Kimwipes are exhausted, toss container and ask lab instructor for more.

III.C. Where to find things in the lab

Sterile items (generally on North Wall)

Sterile boxes of pipet tips.....	right lower shelf, North Wall
Sterile containers of microcentrifuge tubes.....	right lower shelf, North Wall
Sterile test tubes (7 ml, 18 ml).....	right lower shelf, North Wall
Sterile toothpicks.....	right lower shelf, North Wall
Sterile pipets (1 ml, 5 ml, 10 ml).....	right upper shelf, North Wall

Other useful non-fly items

Test tube racks.....	left lower shelf, North Wall
Alcohol burners.....	left lower shelf, North Wall
Glass dishes (for alcohol) and glass spreaders.....	left lower shelf, North Wall

Fly-related items (generally on South Wall)

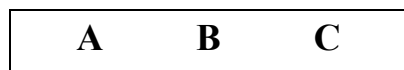
Empty culture vials and flynap vials.....	right lower shelf, South Wall
Fly water.....	right lower shelf, South Wall
Fly food (plus yeast).....	right lower shelf, South Wall
Flynap.....	right lower shelf, South Wall
Index cards.....	right lower shelf, South Wall
Probes.....	right lower shelf, South Wall
Fly culture labels.....	right lower shelf, South Wall
Fly stock cultures.....	left upper shelf, South Wall
Fly experimental cultures.....	left lower & upper shelves, South Wall

Clean up sites

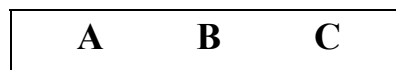
Fly culture vials.....	left bench, North Wall
Flynap vials.....	left bench, North Wall
Glassware.....	right bench, North Wall
Broken glass.....	Southwest Corner
Waste to be autoclaved (orange bag).....	West Wall
Soda can recycling.....	NO FOOD IN LAB!

Groups (and their members)

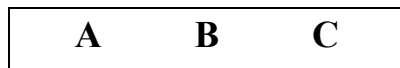
West Wall (back)



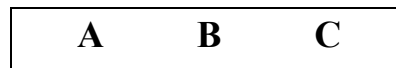
Group 5



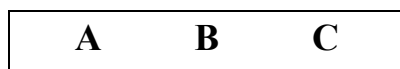
Group 6



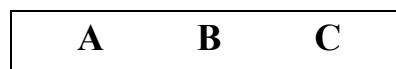
Group 3



Group 4



Group 1



Group 2

East Wall (front)

III.D. Who you gonna call?

Instructors

Sections 1, 2, 4, 5 Section 3

Paula Lessem

E-311

287-6691

PLessem@Richmond.Edu

Brad Goodner

S-201

289-8661

BGoodner@Richmond.Edu

Hours: Tue/Thu 4-5:30 P.M.

...but feel free to nab any one of us regardless of what section you're in.

Lab TA's

Section 1 (Tuesday AM)

Chris Worden (GWorden@Richmond.Edu) [no misprint, "G", not "C"]

Section 2 (Tuesday PM)

Greg Grabowski (GGrabows@Richmond.Edu)

Section 3 (Wednesday PM)

(TBA)

Section 4 (Thursday PM)

(TBA)

Section 5 (Friday PM)

Melissa Hull (MHull@Richmond.Edu)

Next Year's TA's

(Your name here)