Biol 213L: Genetics Lab (Fall 2000)

Comments on Lab 1: Isolation and Characterization of Plasmid DNA. . .

I found myself saying many of the same things multiple times. It was therefore efficient to set down my comments here and refer to them in your reports (the circled letter/number combinations). This practice also enabled me to comment more fully than time would otherwise permit.

I did not comment much on your data and how it was presented, since the format was pretty much given to you. In future reports, however, this will be an area I will look at most closely. Be sure that you have absorbed the models of Tables and Figures given in the lab manual. The short lesson is this: Tables and Figures should be virtually self-sufficient. The reader should be able to make sense out of them without recourse to the text. This requires extensive legends or footnotes.

Look also to the format of Lab 1 for direction as to how to format future lab reports. Note the focus on a scientific question. Note also that paragraphs should have a unifying point and should not be too long.

The grade given for the report does not include the dilution problems. They will be handed back separately.

General remarks

G1. Don't spell out units. Use abbreviations instead: µl, not microliter. You can get the µ symbol by either of two methods: (A) Hold down the Alt key and type in 230 using the numeric key pad (not the numbers at the top of the keyboard), (B) In MS-Word, click Insert, click Symbol, choose GreekMathSymbols as the font, and click on µ in the third row.

G2. Use proper tense. Results should be given in past tense. Generally recognized facts should be given in present tense. If you give results in present tense you are implying they are God-given truth.

G3. Make tables comprehensible. This means giving every one a title and footnotes to explain each quantity listed. The tables in the manual are intended to be examples.

G4. Don't copy blindly. If you don't understand what something means, don't put it into your report. The little numbers in the headers of Tables 1 through 3 in the manual referred to footnotes at the bottom of the tables. It's pointless to put in the numbers without also putting in the footnotes.

G4. Use superscripts. 10^9 is OK for e-mails, but it looks really cheesy in print. Use 10^9 instead. You can get an exponent in MS-Word by clicking on the x^2 tool, or if you don't see that, clicking on Format, then Font, then Superscript.

G5. Mass (vt?). Somebody help me out. Did I miss the invention of a word recently? Until this week, I had never heard of the word "mass" used as a verb. Can you really mass an object? This week I've read it in multiple reports. What's going on? Just in case there IS now a verb "mass" in the popular vocabulary, I presume that its use should be restricted to measuring mass (e.g. by measuring inertia), not weight (e.g. by measuring force on a scale).

Introduction

I2. Choose an appropriate example. In choosing an example, look beyond the needs of the sentence to the needs of the **Introduction** as a whole. The ultimate question posed by the **Introduction** will be more compelling if you can provide an example here that relates to dilution. It would be most helpful if you can find an example that the reader can recognize. For example, you might bring up the counting of fecal bacteria in samples of hamburger meat.

I8. End the section with a scientific question, like:

Serial dilution was used to solve the problem of what volumes of two pure cultures were used to form a mixture of the two.

rather than a didactic goal, like:

My goal was to understand how to do dilutions.

The latter may be true, but throughout the semester you should adopt the view that you are focusing on a scientific question. This makes the report closer to what you will write later in life (perhaps with nonscientific questions), and it makes report writing a whole lot easier nto have a tangible goal to wrap the words around.

Materials and Methods

M6. Precision and accuracy. The precision of the micropipetter could best be assessed from the standard deviation of the 10 measurements or some other evaluation of the variability. The accuracy could best be assessed by a comparison of the average value obtained to the predicted value of 1 g.

M12. Tables should be numbered consecutively. The first table mentioned in the text should be Table 1, the second Table 2, and so forth. If the manual gives different numbers they must be changed in order to accommodate the order given in your report.

M17. Help with 3rd paragraph. Paragraph 3 caused lots of problems, and I confess it is a difficult paragraph to write. Don't rely on verbal descriptions of formulas. It is much easier on the reader to see a nice concise formula. *Please look at my sample lab report* to see how you could have described the formulas more fully.

M18. Ratio of colonies = ratio of volumes?? There is no reason why the ratio of number of colonies on a plate should equal the ratio of volumes used to make the original mixture. What IS reasonable is that the ratio of numbers of colonies on a plate equals the ratio of number of cells in each of the mixtures in the experiment. The ratio of the volumes is the same only if you assume the initial concentrations of the pure cultures to be equal to each other (please look at my sample lab report to see how this follows). This is an important assumption underlying the method. You should recognize that this assumption need not be true.

M19. Write out formulas. Some of you gave verbal formulas, like:

(Volume of JC158 taken from pure culture) = (number of red colonies) / (number of total colonies)

I think it is easier to read and use formulas with defined symbols than complex phrases. As a reader, I would prefer to see:

$$V_{JC} = V_{Tot} \cdot (N_{JC} / N_{Tot})$$

Where V_{JC} is the volume of JC158 taken from the pure culture, V_{Tot} is the volume of the original suspension (200 µl), N_{JC} is the number of red colonies on a plate, and N_{Tot} is the total number of colonies on that plate.

Results

R1. Assessment vs causation. You assessed whether your micropipetter is accurate/precise. You didn't cause it to be so.

R3. Observations, not conclusions. Stick as close as you can to what you saw without being ridiculous. You didn't see that the volumes were the same – that's your conclusion. You saw (perhaps) that when the micropipetter picked up 1 ml water, all of the water was sucked up without sucking up any air.

R4. Give quantitative results. It isn't enough to say that you tried the procedure lots of times. How many times? This is important because someone could say that the micropipetter produces errors about one every five times, and if you had only tested it three times you weren't likely to have noticed.

R8. Give quantitative results. To say the measurements were "similar" means different things to different people. Better to say that "*... the measurements fell within a range of 0.95 to 0.98"*, or *"... the measurements were all within 1.4% of each other"*, or *"... the measurements had a standard deviation of ± 2%."*

R10. What problem was solved? It should be a scientific problem, such as determining what volumes of pure cultures were used to create the mixture you were given. I hope you learned something from the exercise, but that should not be given as the purpose, unless you want to focus your report on that: evidence that you learned something, possible errors in the learning process, etc (don't).

R13. Speak from your own observations. If you did not look at your plate more than once, or did not consult with your partners, then you have no way of assessing whether the colony counts were consistent over time. Don't rely on hearsay. Maybe I or someone else told you that the color fades away after 48 hours. That constitutes possibly useful advice. To cite a statement, you need a written source, such as an article, text, or lab manual.

R18. Observe plates carefully. An important observation was that on some plates there were clusters of colonies that could not be explained by a random distribution of cells. Most likely, the surface of the plate was still wet for some time after plating, and divided cells had an opportunity to float away from each other. In this way, one plated cell could produce many close by colonies.

R19. Distinguish characteristics of the plate from characteristics of the original mixture. The concentration of cells on one of your plates was something like a hundred cells divided by some volume. What you calculated was the concentration of cells in the original mixture. Similarly, the volumes you calculated were derived from the cell numbers on plates C and D but were not the volumes of the cultures on those plates.

R24. Why cross check? The answer to this question was intended to introduce the paragraph that follows it. Therefore it ought to relate to the sums of the initial volumes. You should see that summing the volumes calculated by the Absolute Number Method is a check on the method's validity. It ought to add up to 200 µl. If it doesn't, you know something went wrong.

R26. Your assumptions cannot be used as evidence. In using the ratio method, you assumed that the volume of the mixture was 200 µl. That's the only reason why the two volumes of the two strains add up to that number. It's therefore inappropriate to use the sum of the two volumes as evidence that your experiment worked!

R28. Anticipated concentration of total cells in mixture. You anticipated that the concentration of cells in the mixture would be $2 \cdot 10^9$ cells/ml. If you don't believe me, look at the right hand column of the table describing your dilutions.

Discussion

D1. Major experimental question. You could have put either: *Was the P1000 accurate and precise?* Or *What volumes of pure cultures were used in making the original mixture of JC158 and AB3517?* I think the second question sounds more major and certainly more interesting.

D5. Assumptions of Absolute Number Method. The shakiest assumption of this method is that the initial concentrations of both pure cultures was $2 \cdot 10^9$ cells/ml. You also assume that nothing serious went wrong with your dilutions. You are not assuming that particles are neither created nor

destroyed, unless you also want to say that you're assuming the law of gravity holds and various other physical laws.

D6. **Assumptions of Ratio Method.** The most obvious assumption of this method is that the ratio of the two strains is constant throughout the dilution series, but shakiest assumption is that the initial concentrations of the two pure cultures were the same.

D7. Did cells die or replicate over the course of the experiment? Surely they did, if you count the experiment beginning the day before you got to lab. Did the cells die/grow during <u>your</u> manipulations? They should not have grown, since you diluted into TM (Tris-Magnesium buffer), a medium with no food. Did they die? If they did, then you would expect plates later in the experiment to have fewer cells than plates earlier. If you plated **D** after **C**, then **D** should have fewer than 10-fold fewer cells. I believe this wasn't true in anyone's experiment. The minute or two between plating is probably not enough time for many cells to die.

D9. Error attributable to pipetting. The error you are trying to explain is the discrepancy between the two methods: Absolute Number and Ratio. The numbers used by both methods are the numbers of colonies on your plates. Since you plated serially diluted cultures, pipetting error could have led to different conclusions by the two methods. Your assessment of the accuracy and precision of pipetting allows you to estimate this error. *See my sample lab report* for an example of how this might be done.