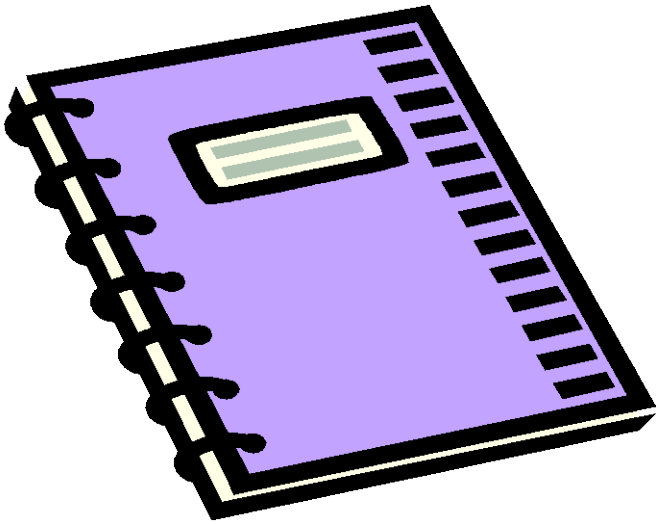
College of the Canyons: Introduction to Biotechnology: Custom Labs



Lab Notebook

Version 7-20-12

- **Lab notebooks are used to document all ideas stemming from lab research (either academic or industrial).**
- **Lab notebooks provide a daily record of procedures performed, data gathered, and determined results for each lab.**
- **They also help provide legal proof of research activities (this would be especially important for cutting edge research).**
- **Lab notebooks are to be completed as the lab is being performed.**
- **In addition to documenting key lab activities, this lab book will serve as a tool to help assess student ability to write and think critically about lab exercises. Subsequently, the content and format is slightly novel to facilitate the educational process.**
- **The notebooks will be collected and graded periodically throughout the semester (see syllabus).**

For more information on the College of the Canyons' Introduction to Biotechnology Course, contact, Jim Wolf, Professor of Biology/Biotechnology at (661)362-3092 or email: jim.wolf@canyons.edu

The following lab protocol can be reproduced for educational purposes only. It has been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references.

I. Background:

Laboratory Notebook

In a biotechnology course, one of the primary objectives is to make the laboratory setting as realistic as possible. In research laboratories (either academic or industrial) the lab notebook is the source for all of the ideas stemming from research efforts. While the exact nature of a notebook is subject to lab specifics, individual idiosyncrasies, and familiarity with the subject matter, all good lab books have certain things in common. The following is a list of these attributes. Note that these attributes apply to notebooks in a research setting. Since the academic setting is substantially different from the research setting (i.e. in research, experiments are often repeated dozens to hundreds of times, while in a education setting, it is rare to repeat an experiment more than once) you should be aware of these differences. You should balance your lab notebook with an additional three ring binder-full of Standard Operating Procedures (SOPs) handed out in lab. The lab notebook and SOP binder *may (at my discretion)* be used during exams or quizzes or as a resource for reports. Consider the following "education specific" lab book idiosyncrasies in light of the implied detail of a complete research lab book (as seen on last pages of this handout.)

Education Lab book Idiosyncrasies:

1. Your lab book and SOP binder shall contain only materials relevant to lab. You must leave your lab notebook in class at all times, and you should keep you SOP binder with you at all times. No lecture notes, Xeroxed lecture related materials or other material clearly related to lecture shall be permitted in these books.
2. The detail in the lab book is largely up to your discretion (although a general format should be followed). Grading emphasis will be placed on completeness (see below.) Additional materials, while welcome; will not result in extra credit.
3. Strive to write the notebook in the following listed format, and you can use SOP's to provide additional detail (especially in the materials and methods section.)
4. Your lab notebook will have a spiral binding or solid spine (no loose leaf) and will have **carbon copy pages that are easily removed.**
5. FINAL NOTE: KEEP YOUR LAB NOTEBOOK CURRENT! WRITE YOU INTRODUCTION TO THE LAB DURING THE LAB AND ENTER ALL DATA DIRECTLY INTO THE LAB NOTEBOOK. WRITE YOUR CONCLUSIONS AT THE END OF THE FINAL LAB PERIOD. **AGAIN**… KEEP CURRENT, AS YOU WILL BE SUBSTANTIALY PENALIZED IF YOU LET YOUR NOTEBOOK LAPSE.

Typical Components of A lab Note Book:

These criteria will be used in grading your notebook.

1. In front of the notebook, put identifying information including: Name, course, room number, Professor's name (PH # and other contact information as well.)
2. Table of contents: **Keep Current**!
3. Title, date, and room # and/or collaborating signatures/name of lab partners should be on each page of the lab notebook.
4. Introduction: For each project includes a brief introduction; 4-6 sentences per paragraphs and 1-2 paragraphs per subject. .

5. Materials and Methods: In many cases these points will be covered in your SOP. Be sure to reference the SOP in this portion of the lab notebook. Be specific in your reference of the SOP (i.e. SOP # 3, HPLC lab, pages 3-5.) and add any addenda noted in lab lecture. This section is at most 1/2 a page long. So, again, do not write out the entire materials and methods section. Just reference the SOP, and any addenda mentioned in the pre-lab lecture. The included example at the end of this lab included a M and M section FYI only.
6. Data and results: Collected directly from experiments, this section should include, but not necessarily be limited to: tables, graphs, procedural details, instruments used, calculations, computer printouts and gel images.
7. Conclusion: Include a brief interpretation of all relevant experimental outcomes. In addition you should answer the final questions listed at the end of the lab handout. This section will be from 1-4 paragraphs long and remember to answer the questions in essay format (no listing or numbering of answers.)

II. Format: (The format of the lab notebook should be as follows):

Introduction: A 3-5 sentence introduction should be sufficient. Remember to BOTH introduce the subject in a general sense, and to say what the specific lab was addressing (this sentence is often called the Hypothesis being tested). Ex: Photosynthesis is the reaction where by plants utilize carbon dioxide and water, in the presence of light and chlorophyll to produce sugars and oxygen. (Put in a 3-5 extra introductory sentences after the first sentence). The purpose of this lab was to investigate the relationship of light intensity to the rates of photosynthesis in the aquatic plant Elodea. (Hypothesis being tested sentence used to close out the introduction).

Materials and Methods: Do not bother writing out all of the procedures in your notebook. Simply allude to the SOP's: "i.e. SOP's, Transformation Protocols, pgs 3-5," unless changes are made. In that case an addenda is necessary.

Data/Results: In this section you state what data you gathered. This may be presented textually, in a graph, table, or diagram. What ever method you choose, again ensure that you are both clear and to the point. Actual numbers, formulas, calculations, graphs or other results should be also included in this section. Be sure to fully label and graphs, gel images, or other data with relevant information. Avoid *explaining* your results or data in this section.

Conclusion: In the conclusion section comment on whether or not the experiment was successful, how it may be improved, and some closing comments on what was deduced. At the end of every lab handout there are several questions for you to answer. These questions should provoke thought and understanding of the labs. Answers to these questions are to be addressed in the conclusion section of the lab notebook and will be graded when the lab notebooks are turned in (see syllabus). Please note that the questions should not be answered directly but should be worded in such a way that the question is answered in your own words. Example:

Question: Why is polarity so important in the HPLC lab?

Entry in lab notebook: Separation of the Kool-Aid dye occurred as a direct result of polarity. The more polar the solvent used, the less of the non-polar dye was pulled through the HPLC column. Therefore, polarity served as the main basis for separation of the different dyes used in grape Kool-Aid. (NOT: Polarity was important in the HPLC lab because…)

And finally: KEEP CURRENT, KEEP CURRENT, KEEP CURRENT…..

III. Example:

Following is an example from a previous BioSci 230 student's lab notebook: This student received full credit for this lab notebook entry. Again, recall that the material and methods section in your lab notebook should be much briefer!

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE
7	Transformation	3-14-01

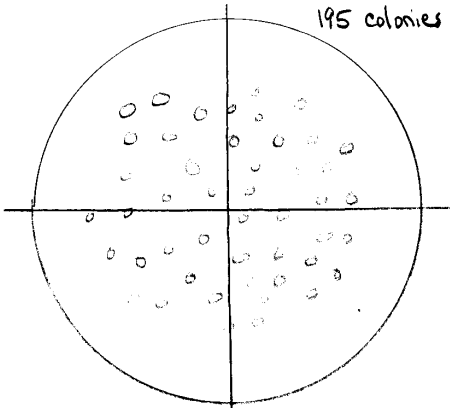
NAME	LAB PARTNER	LOCKER
Shane Ramey	Ron Moat	— Biotech 230

I. Intro: Cells are transformed when new DNA is inserted and thus changes the cell's characteristics and those of its offspring. Transformation rarely happens in nature, but occasionally a cell happens to take up and use a piece of DNA that is adrift in the cell's environment. Usually, however, when this happens it is a fatal event for the cell since the foreign DNA could deny its ability to survive or immediately kill it. Biologists, though, have learned in the last thirty years how to successfully transform bacteria and yeast cells.

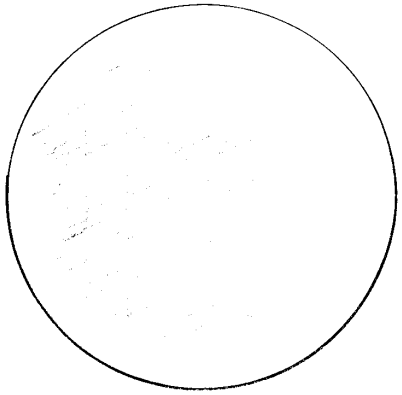
In this lab Escherichia coli (E. coli, a common bacterium in the human large intestine) is transformed from an ampicillin-sensitive E. coli to an ampicillin-resistant cells.

II. Materials/Methods: Initially the E. coli cells must be weakened so that they will more readily accept the foreign DNA. This is performed by adding E. coli colonies to 50mM ice-cold $CaCl_2$ and putting on ice for at least 1 minute. Next, the plasmid containing ampicillin-resistant DNA is introduced to the suspended bacteria. This is accomplished by adding the plasmid to the bacteria and icing for 15 minutes. Then the plasmid must be made to enter the cells by transferring them suddenly from the ice bath to a 42°C water bath for 90 seconds. The cells are allowed to regain their strength by adding sterile Luria broth and incubating in a 37°C water bath for 5 minutes. The cells are plated on solid media with and without added antibiotic and incubated at 37°C for 24 hours. (i.e. SOP's, Transformation (Genetic Engineering) Protocol).

III. Data/Results:



+ plasmid cells on Luria broth agar with ampicillin



- plasmid cells on Luria broth agar without ampicillin

SIGNATURE	DATE	WITNESS'A
Shane Ramey	3-14-01	Ronald A Moat 3-14-01

NOTE: INSERT BACK COVER UNDER COPY SHEET BEFORE WRITING

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE
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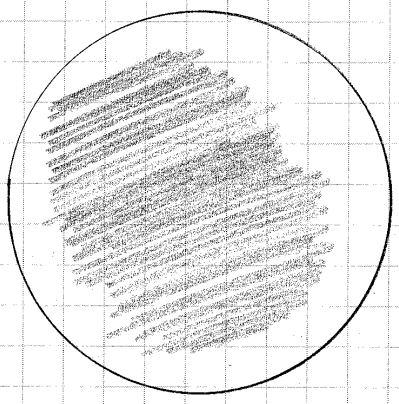
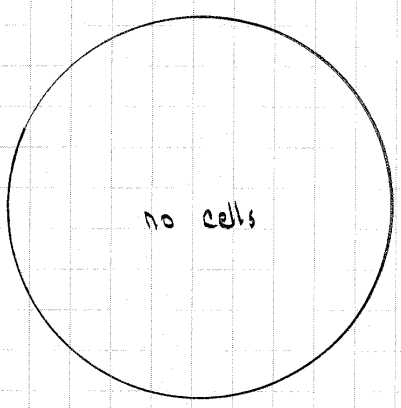
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Shane Ramey	Ron Moat	—	Biotech 220





\+ plasmid cells on Luria broth without ampicillin

\- plasmid cells on luria broth agar with ampicillin

780 colonies of ampicillin resistant bacteria growing on + plasmid agar.

Transformation Efficiency:

$$\frac{0.005\,\mu g}{\mu L} \times \frac{10\,\mu L}{1} \times \frac{100\,\mu L}{500\,\mu L} = 0.01\,\mu g \text{ plasmid DNA transferred to one plate.}$$

$$\frac{780 \text{ colonies}}{0.01\,\mu g} = 78{,}000 \text{ colonies}/\mu g$$ } number of colonies transformed per microgram of plasmid DNA.

IV. Conclusion: Normal *E. coli* are killed by the antibiotic ampicillin, which prevents certain bacteria from making cell walls. However, ampicillin has no effect on *E. coli* cells that have previously taken in a relatively small ring of DNA (plasmid) that contains the code that "tells" the cell how to destroy ampicillin. Any non-transformed bacteria will not gain this resistance and die. Because of this it is easy to visualize which, and how much, bacteria were successfully transformed with the plasmid DNA. Also, bacteria grow very rapidly, are relatively easy to culture and manipulate, and thus are extremely useful for recombinant DNA technology.

Here, one tube of bacteria (the + plasmid) received plasmid and another tube (the - plasmid) does not. $CaCl_2$ is added to each tube which in turn heated. The $CaCl_2$ helps to make the cell membranes more rigid and the heat shock helps to make them more permeable. This may or may not provide the cells to accept the plasmid.

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NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
Shane Ramey	Ron Moat	—	Biotech 330

100 µL of cell suspension from both the + plasmid and - plasmid samples is transferred to two types of media, one with and one without ampicillin. The plates are then incubated and evaluated.

The - plasmid cells act as expected -- they grow well on the ampicillin free plate and do not grow at all on the plate containing ampicillin. A nice lawn of growth is evident on the amp-free plate.

The + plasmid cells also grow into a nice lawn on the amp-free plate. However, some cells form colonies on the plate with ampicillin. A lawn does not exist, but growth is evident. This growth illustrates the fact that some of the original bacteria was successfully transformed with the plasmid.

By counting the amp-resistant colonies on the plate it is determined that 780 of these colonies are growing. With this data it is further determined that 78,000 colonies were transformed per microgram of plasmid DNA.

As stated, the amp-resistant cells did not grow a dense lawn on the plate containing the antibiotic, indicating that a low percentage of the cells were actually transformed with the plasmid. This is due to the fact that plasmid insertion is very limited and results in low amp-resistant cell yield.

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