Guidelines for Keeping a Laboratory Record

Dr. David R. Caprette
Rice University
Department of Biochemistry and Cell Biology

If you have build a perfect demonstration do not remove all traces of the scaffolding by which you have raised it.
Clark Maxwell

The following is a general description of how to keep a proper laboratory notebook. Requirements for different teaching, research, clinical, or industrial labs will most likely vary. Some institutions/labs will require less stringent record keeping, others will hold you to a very strict protocol. A well kept notebook provides a reliable reference for writing up materials and methods and results for a study. It is a legally valid record that preserves your rights or those of an employer or academic investigator to your discoveries. A comprehensive notebook permits one to reproduce any part of a methodology completely and accurately.

Outline of procedures

Examples

Choosing a notebook

For most purposes you may select a *bound* notebook, quadrille-ruled. A teaching lab may require tear-out duplicate pages for making carbon copies. An engineering or industrial research/development lab will likely require a specific type notebook with prenumbered pages and places for date and investigator's and supervisor's signatures on each page. Pads of tear-out graph paper or spiral bound notebooks without pre-

numbered pages are not acceptable. It must be impossible to tear out a page without leaving evidence. It is safest to select something that is clearly labeled as a *laboratory* notebook.

Preparing the Notebook

Please use a ball point pen for all entries, so that the marks will not smear nor will they be erasable.

Put your name, a telephone number and/or address, and project name or course number on the outside front cover of the record. Put that same information on the first page inside, or on the inside front cover. If your notebook does not include a prelabeled table of contents section, then reserve the next several pages for a table of contents by labeling the top of each page as *Table of Contents* and numbering each page. If your notebook does not have prenumbered pages, you may wish to use lower case Roman numerals, as in a standard publication. Next, number the next several pages with Arabic numerals in sequence, and you are ready to begin recording data.

What to enter

Above all, it is critical that you enter all procedures and data directly into your notebook in a timely manner, that is, while you are conducting the actual work. Your entries must be sufficiently detailed so that you or someone else could conduct any procedure with only the notebook as a guide. Few students (and not that many researchers for that matter) record sufficiently detailed and organized information. The most logical organization of notebook entries is chronological. If a proper chronological record is kept and co-signed by a coworker or supervisior, it is a legally valid record. Such a record is necessary if you or your employer are to keep your rights to your discoveries.

Depending on requirements set by a teacher, superivsor, company, or whatever, you may not have to confine your notebook entries to lab notes only. On the other hand a student might record your class lecture notes, lab lecture notes, ideas, questions, library research notes, and notes that are part of any pre-lab preparation. The bare minimum entries for an academic lab course, for each lab study, should include title of the lab study; introduction and objectives; detailed procedures and data (recorded in the lab itself); summary.

We usually record a lot more information in a laboratory notebook than we would report in a research paper. For example, in a published article we don't report centrifuge type, rpm, rotor type, or which machine was used. However, if a procedure is unsuccessful you may want to check to see that you used the correct rpm or correct rotor. Perhaps the centrifuge itself was miscalibrated. You would need to know which machine you used. In a research paper one does not report which person performed which tasks, because such information is useless to a third party. However in the notebook it is important to note who was responsible for what procedure. Again, you may need such information to troubleshoot your experiments.

Making entries

Someone else may need to consult your notebook sometime, so please make your entries clear and legible.

When you make your first entries of the day, start by entering the date, writing out the month or abbreviation for the month (e.g., 5 Apr '04, or April 5, 2004, but not 4/5/04). The use of numerals only can cause confusion. For example, in Europe the day comes before the month. Thus April 5, 2004 would be written as 5/4/04. When you start each new page of a notebook enter the date next to the page number. Each page should be

numbered and dated consistently. Most of us use the upper right corner of each page for date and page number.

Depending on how your notebook is designed you may choose whether or not to use the backs of pages. If you leave them blank, put a corner-to-corner line through them to void all blank spaces. Some people use the backs for rough calculations, then void remaining blank space. You might also decide to save space (and trees) and use both sides of each page. Obviously you cannot use both sides with notebooks that are designed to make duplicate copies. In situations where you turn in duplicate copies to a supervisor, you obviously must start each new set of entries on a new page.

Write a title for each and every new set of entries. Distinct sets of entries should be separated by using informative headings and by leaving a single space or two between individual sets of entries. Specific information can be more readily located that way. For a new laboratory study, write down a *very brief* introduction to the study, and list the objectives. If you have a specific hypothesis, write it down. The object is to make it completely clear what you intend to do.

Record everything you do in the lab, even if you are following a published procedure. For example, if you started by obtaining a quantity of tissue from an instructor, then write down that you obtained tissue, describe it, note how much, what condition, etc. How much you write down is up to you, but any *relevant* information should be there. For example, it doesn't matter much if you received a chunk of liver in a red ice bucket or a black one. However, it *does* matter that the material was on ice. **If you change a protocol in any way or decide between alternative methods, then the correct information must be recorded in the notebook.** For example, a protocol for tissue fractionation may recommend centrifugation at 9400 x g, but we may decide to use 12,000 x g in the lab. The correct g force must be noted.

If you make a mistake, put a line through the mistake and write the new information next to it. *Never* erase or obliterate an entry. When you finish a page, put a corner-to corner line through any blank parts that could still be used for data entry. Every bit of every page must be legible and filled, either with information or with a mark that voids the section (see examples).

The summary

When you have finished a project, summarize what you have accomplished. You don't have to draw conclusions, just indicate what sort of data or observations you collected, samples you saved (and where and how you saved them), or any other relevant information that wraps up the study. For a continuing study keep the summary extremely brief. In fact, if the notes are well organized and it is obvious where the study left off, you need write nothing more than "To be continued..." Summaries help maintain continuity. They indicate where the work left off and how it might resume.

Organization

Doing two things at once?

What if you are conducting two long procedures at once, each with long waiting periods? For example, suppose you are conducting a protein assay and preparing a gel for your samples out in the laboratory. Back in the cell culture room, you are harvesting and processing tissues for primary culture. Both procedures involve waiting periods, yet you will complete both tasks by the end of the day.

Simply use your best judgment. You could divide each page into columns and keep your two records side-by-side. You might date two consecutive pages, keeping both records separately. In either case, when you leave the laboratory for the day cross out any unused parts of a page that precede the last entry.

Continuation pages

What if you need more than one page for a project? With continuing research, that will always be the case. Proper use of continuation notes makes it possible to follow your path through a long experiment or series of experiments without having to leaf through every page of your notebook.

For example, let's say you labeled some protein samples with the radioisotope S-35, ran a gel, and placed the gel in a film cassette in order to produce an autoradiograph. During the two days your film is in the freezer, you devote all of your time to a cloning project that is part of an unrelated study. After you put your film cassette in the freezer, simply write *Continued, page* ___, then enter the date and title of your other project, and continue to record information.

When you resume work on the protein samples, enter the date, write *Continued from page* ___, and enter your autoradiography results. This way, everything you do in the laboratory is recorded chronologically, yet someone interested in following your progress could start from the beginning and follow every procedure on just that one study, from start to finish.

Are things getting too sloppy?

Perhaps your data records are scattered throughout the notebook, and you would like to summarize them. Go ahead. You may re-enter tables or figures any time you wish to organize your work a bit better. To prevent confusion over duplication of data you may put a line through a table or figure you intend to re-draw, initial and date the change, and note the page on which the re-organized data can be found. Just don't obscure any of the original entry.

Repeated procedures

So far you have been advised to record each step you perform in the laboratory, regardless of whether the procedure is published somewhere. However, once you carry out a procedure, you can refer to that part of your notebook, and only note changes you make. For example, the first time you prepare a sequencing gel you should write down the exact formulation, how you mix the gel, how long you let it cure, etc. The next time, just refer to the name of the procedure and the appropriate page(s) of your notebook.

Loose materials

Suppose you enter raw data into a computer and have a printout with 400 pieces of data. Or, suppose you generate a graph using a software program. You might even have a silver-stained gel that you wish to refer to frequently, or a fluoroescence photomicrograph that sums up your results nicely. Some investigators prefer to attach such materials to the notebook itself, but too many such items make a sloppy notebook and can stress the binding. Loose data should be kept in a separate folder or notebook, with location noted in the book.

Table of Contents

Record all entries in the table of contents as you go along. You can organize it anyway you like but it is advisable to include multiple levels in a table of contents, that is, indicate where a new study starts and include subheadings for specific parts of a study, methods, sets of data, etc. The idea is to enable someone (such a supervisor, grader, or yourself a year from now) to find anything quickly. List each set of entries with dates and page numbers. If you are seriously anal-retentive, you might record every experiment in chronological order, then use the remaining blank space to cross reference the contents experiment by experiment.

For a teaching lab you might list each and every set of entries made in your notebook, in chronological order, including complete and informative titles. Examples of sets of entries include an introduction, a summary, a set of procedures for a specific preparation, a complete data set, calculations for diluting samples or preparing assay standards, etc. A grader should be able to find any specific entry quickly, without flipping through pages.

Notebook Checklist

As you record your activities in the laboratory, ask yourself, "Did I..."

- Keep up with the table of contents?
- Date each page?
- Number each page consecutively?
- Use continuation notes when necessary?
- Properly void **all** blank pages or portions of pages (front and back)?
- Enter all information **directly** into the notebook?
- Properly introduce **and** summarize each experiment?
- Include complete details of all first-time procedures?
- Include calculations?

Appendix One

Examples of Notebook Pages and Entries

Here are examples of a title page and specific notebook entries. The work may be ancient history but as an example of recordkeeping this mateiral may be far more effective than general guidelines in delivering the message.

Cover and title page

Laboratory Record

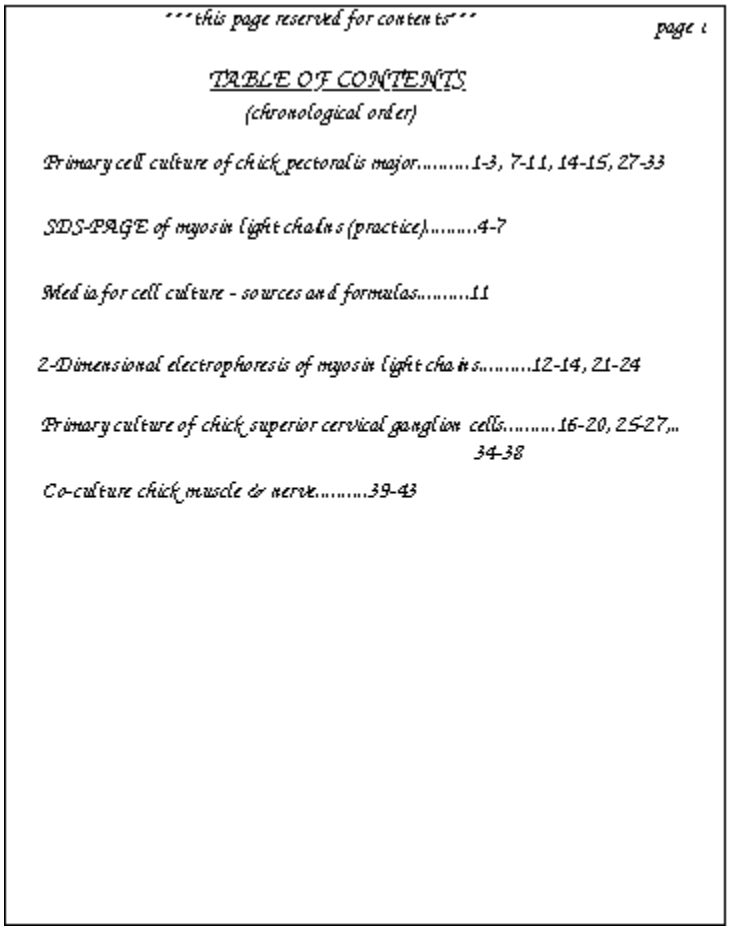
Patterns of Myosin Light Chain Isoenzymes in Developing Chick Skeletal Muscle

David R. Caprette
Department of Biochemistry & Cell Biology
Rice University
211A Anderson Biology Lab
6100 S. Main
Houston, Texas 77005

(555) 555-5555

Table of Contents

Reserve several front pages for contents by indicating their purpose at the top of the page. Do not leave completely blank pages in the front of the notebook, for future tables, since blank pages could also be used to add entries out of order. If pages are not pre-numbered, it may be convenient to use small case Roman numerals to number the contents pages. Record contents in chronological order.



"'this page reserved for contents"' *page i*

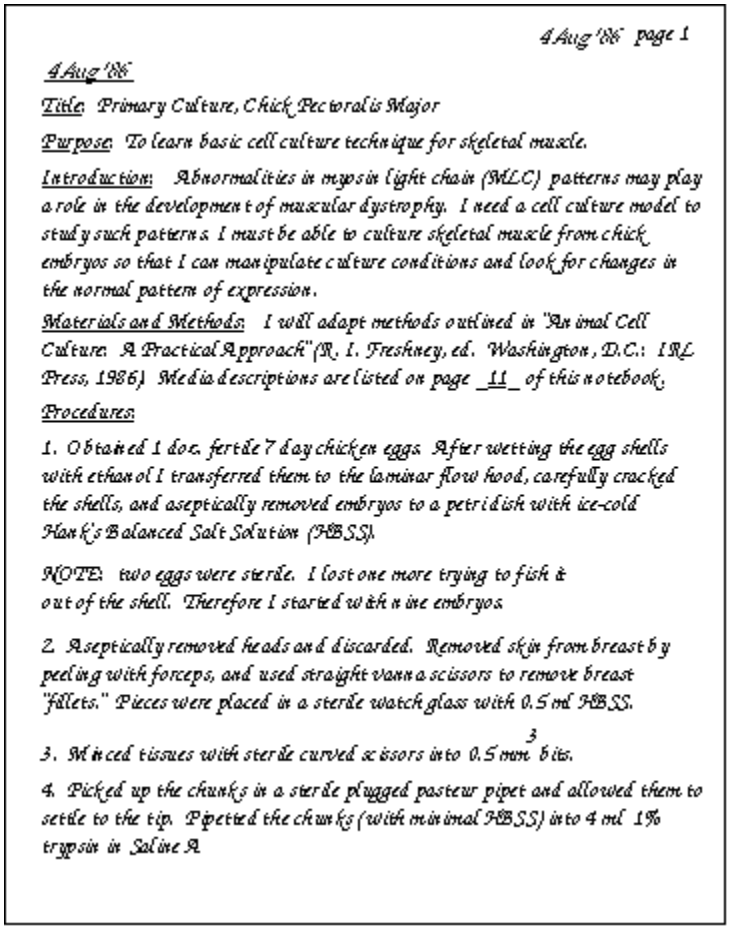
TABLE OF CONTENTS

(chronological order)

Page 1

Always date and number every page in a consistent manner (e.g., at the top right, or bottom center, etc.). Start every experiment with a title and a brief introduction. Follow with "procedures," "materials and methods," or whatever you choose as the title for your laboratory work.

Document procedures within a reasonable time of doing them - during busy times your memory can fail you. Even if a procedure is outlined in a paper or published laboratory manual, record all of your procedures the first time you do them. Record **HOW** each procedure is done, even such mundane procedures as pipetting, calibrating a spectophotometer, or finding cells in a microscope. That is the only way you will be able to catch possible mistakes later, modify your procedures, or report exactly what you did. Do all of your calculations and take all notes right there in the notebook. Never do calculations or record observations on 'lab stationery' (paper towels). Loose papers can be lost easily.



4 Aug '86 page 1

4 Aug '86

Title: Primary Culture, Chick Pectoralis Major

Purpose: To learn basic cell culture technique for skeletal muscle.

Introduction: Abnormalities in myosin light chain (MLC) patterns may play a role in the development of muscular dystrophy. I need a cell culture model to study such patterns. I must be able to culture skeletal muscle from chick embryos so that I can manipulate culture conditions and look for changes in the normal pattern of expression.

Materials and Methods: I will adapt methods outlined in "Animal Cell Culture: A Practical Approach" (R. I. Freshney, ed. Washington, D.C.: IRL Press, 1986) Media descriptions are listed on page _11_ of this notebook.

Procedures:

1. Obtained 1 doz. fertile 7 day chicken eggs. After wetting the egg shells with ethanol I transferred them to the laminar flow hood, carefully cracked the shells, and aseptically removed embryos to a petri dish with ice-cold Hank's Balanced Salt Solution (HBSS).

NOTE: two eggs were sterile. I lost one more trying to fish it out of the shell. Therefore I started with nine embryos.

2. Aseptically removed heads and discarded. Removed skin from breast by peeling with forceps, and used straight vanna scissors to remove breast "fillets." Pieces were placed in a sterile watch glass with 0.5 ml HBSS.

3. Minced tissues with sterile curved scissors into 0.5 mm^3 bits.

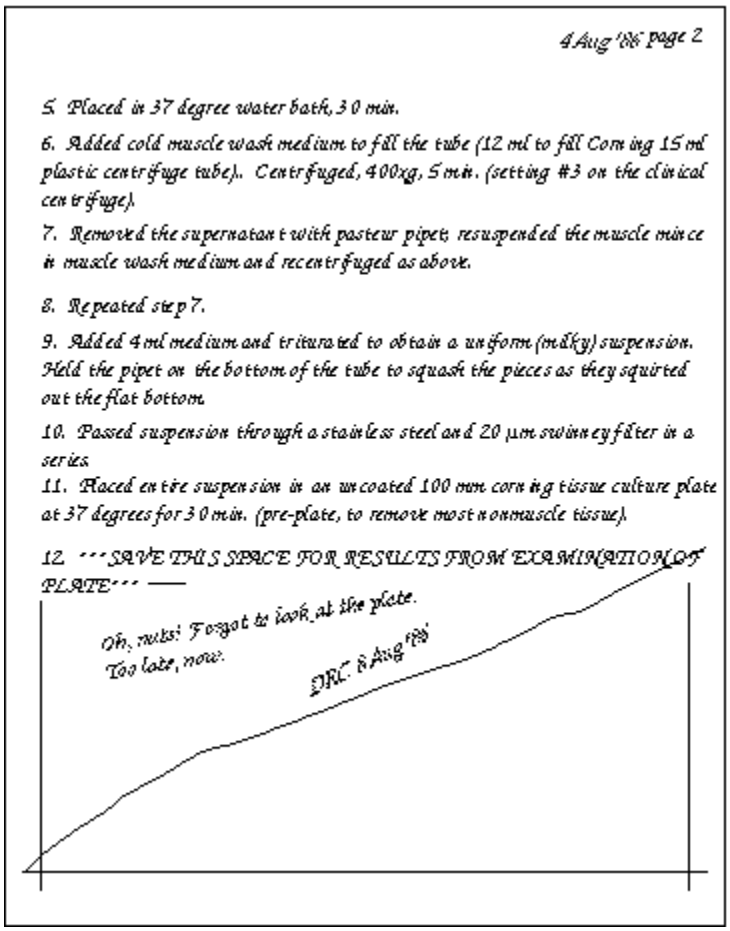
4. Picked up the chunks in a sterile plugged pasteur pipet and allowed them to settle to the tip. Pipetted the chunks (with minimal HBSS) into 4 ml 1% trypsin in Saline A

Page 2

Include the date at the top of the page even if it is the same day as on the previous page.Never mark anything out so as to obscure what was written, no matter how sloppy or 'dumb' it looks. Writing disparaging remarks about your insructor or supervisor is a bad idea. You will be in trouble if you obscure the remark, but could be in even more trouble if you leave it in!

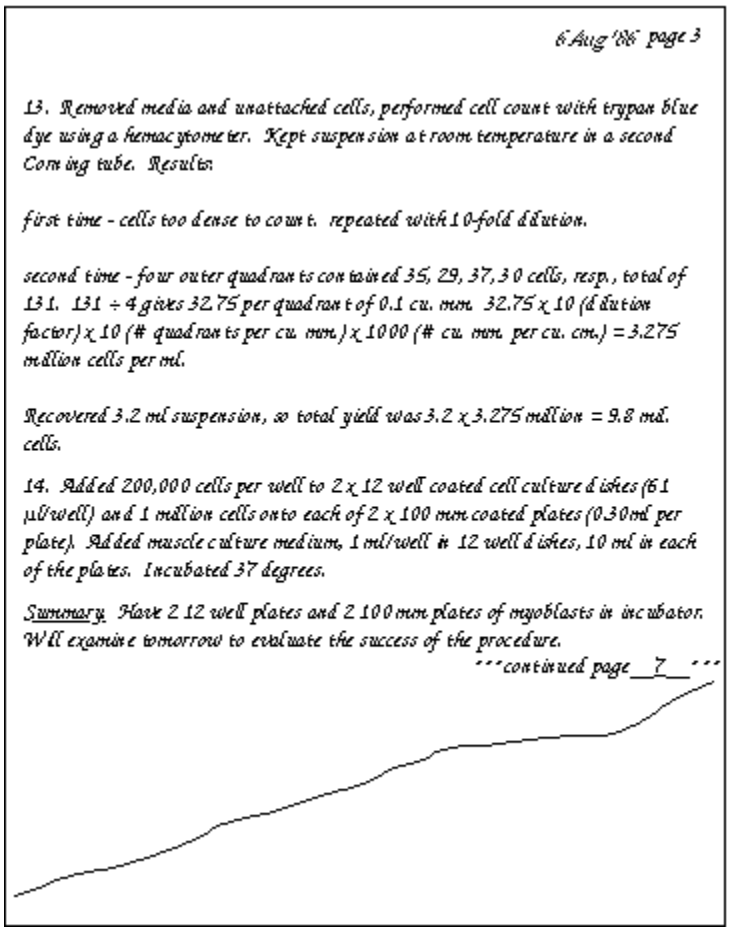
Any significant amount of blank space must be voided by drawing a corner-to-corner line through the space. This 'rule' is there to ensure that your notebook is a legal record of lab activities. If you fail to void a blank space, you could go back and change something, that is, you could falsify your record.

In labs doing proprietary research (confidential- such as industrial research for profit) a properly maintained notebook is essential. You and a supervisor sign and date each page, and an independent person checks it periodically, say, once per week. The notebook is a legal document that can protect your rights to your work. For example, if you 'beat' another lab to a patentable discovery by just one day, your notebook will be the legal proof you need.



Page 3

In this example, the project was not complete. I wrote a very brief summary, all the same. Following the last entry I wrote ***continued, page ____**** because I knew that the project would be interrupted chronologically. Later, when the project was resumed, I filled in the blank with the number of the page on which I recorded the very next set of information. This way, you never need to reserve space for further entries. Record everything you do, even for different projects, in chronological order.



6 Aug '86 page 3

13. Removed media and unattached cells, performed cell count with trypan blue dye using a hemacytometer. Kept suspension at room temperature in a second Corning tube. Results:

first time - cells too dense to count. repeated with 10-fold dilution.

second time - four outer quadrants contained 35, 29, 37, 30 cells, resp., total of 131. 131 ÷ 4 gives 32.75 per quadrant of 0.1 cu. mm. 32.75 x 10 (dilution factor) x 10 (# quadrants per cu. mm.) x 1000 (# cu. mm. per cu. cm.) = 3.275 million cells per ml.

Recovered 3.2 ml suspension, so total yield was 3.2 x 3.275 million = 9.8 mil. cells.

14. Added 200,000 cells per well to 2 x 12 well coated cell culture dishes (61 µl/well) and 1 million cells onto each of 2 x 100 mm coated plates (0.30 ml per plate). Added muscle culture medium, 1 ml/well in 12 well dishes, 10 ml in each of the plates. Incubated 37 degrees.

Summary. Have 2 12 well plates and 2 100 mm plates of myoblasts in incubator. Will examine tomorrow to evaluate the success of the procedure.

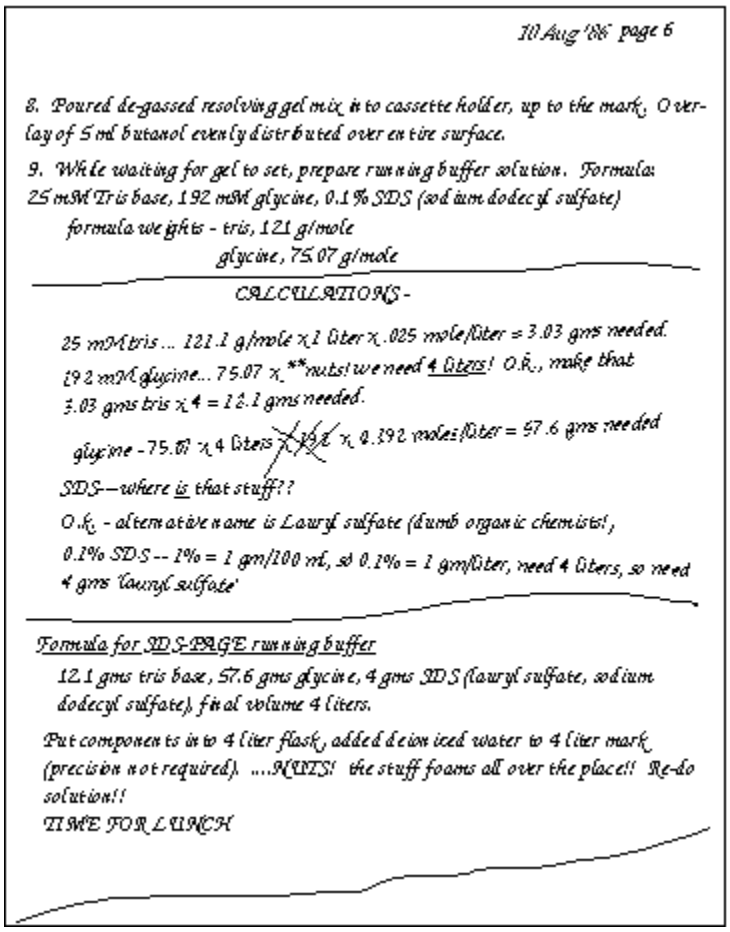
continued page__7__

Page 6

When you make calculations, such as when you prepare a solution for the first time, write down all of your raw calculations. Even if you make mistakes, your progress is recorded permanently. Raw calculations may make your notebook appear a bit 'sloppy,' however if you don't include them you can't go back and correct mistakes.

When you have a 'recipe' that you anticipate using again, summarize the formula. You may want to include page numbers of all such recipes in a special place in your table of contents (reference materials need not be in chronological order).

Sometimes you just plain have a bad day. Consider that someone else may have a legitimate reason for examining your notebook. Don't put something in there that could embarrass you later. Remember, you can't blot anything out or rip out pages!



10 Aug '86 page 6

8. Poured de-gassed resolving gel mix in to cassette holder, up to the mark. Overlay of 5 ml butanol evenly distributed over entire surface.

9. While waiting for gel to set, prepare running buffer solution. Formula: 25 mM Tris base, 192 mM glycine, 0.1% SDS (sodium dodecyl sulfate)

formula weights - tris, 121 g/mole

glycine, 75.07 g/mole

CALCULATIONS -

25 mM tris ... 121.1 g/mole x 1 liter x .025 mole/liter = 3.03 gms needed.

192 mM glycine... 75.07 x **nuts! we need 4 liters! O.K., make that 3.03 gms tris x 4 = 12.1 gms needed.

glycine - 75.07 x 4 liters x .192 x 0.192 moles/liter = 57.6 gms needed

SDS--where is that stuff??

O.k. - alternative name is Lauryl sulfate (dumb organic chemists!,

0.1% SDS -- 1% = 1 gm/100 ml, so 0.1% = 1 gm/liter, need 4 liters, so need 4 gms 'lauryl sulfate'

Formula for SDS-PAGE running buffer

12.1 gms tris base, 57.6 gms glycine, 4 gms SDS (lauryl sulfate, sodium dodecyl sulfate), final volume 4 liters.

Put components in to 4 liter flask, added deionized water to 4 liter mark (precision not required). ....NUTS! the stuff foams all over the place!! Re-do solution!!

TIME FOR LUNCH

Page 14

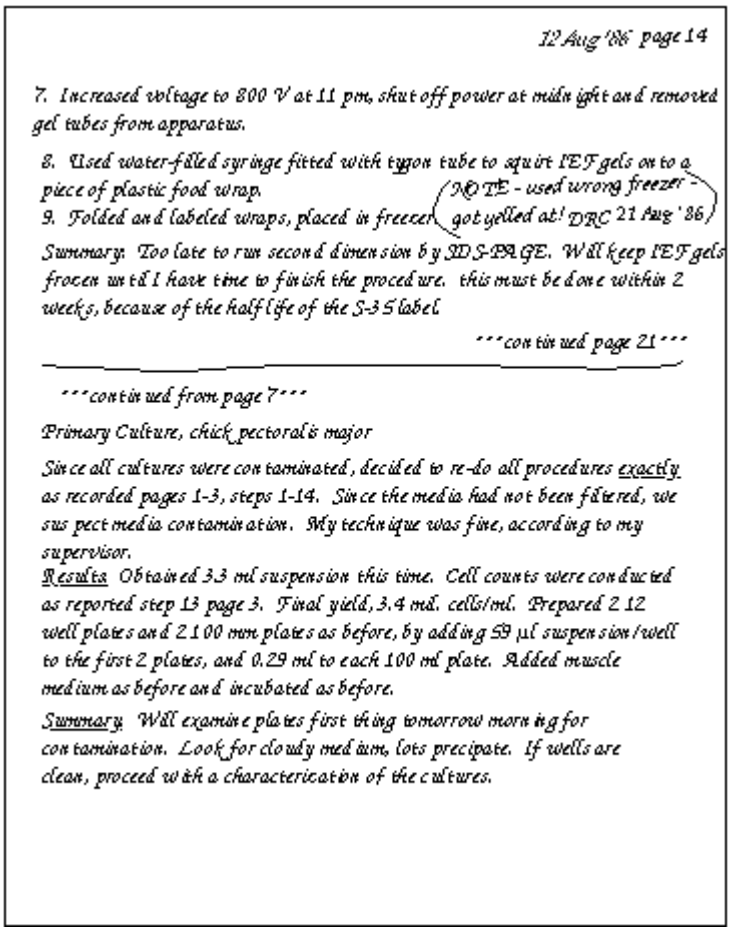
When you really start cranking on a couple of projects, your notebook fills up fast. I used to have about four different things going at once. I didn't simply go outside and sun myself every time I had a one hour wait during a protocol. I worked on something else.

When you switch from one project to another you need to continue to record your activities chronologically. Done properly, it won't be confusing at all. You simply read each passage and follow the continuation notes to follow a single project from start to finish. Magazines do that all the time, to get around ads and to continue long articles. Just be consistent with the titles of your projects.

When you add notes later or correct a mistake, always initial and date your comment. Indicate clearly that this is an additional comment, such as by using a different color ink.

If you follow a previously recorded procedure exactly, you need not rewrite the entire protocol. Just refer to the protocol by page number(s) or by a title. As with projects, be consistent with the names of protocols, formulas, etc.

Sometimes you will want to attach a photograph, sketch, graph, or some other document that is not conveniently written directly in your notebook. Reserve a place for attachment of such document, and you can staple or tape it right there. Just don't hide any of your entries.



12 Aug '86 page 14

7. Increased voltage to 800 V at 11 pm, shut off power at midnight and removed gel tubes from apparatus.

8. Used water-filled syringe fitted with tygon tube to squirt IEF gels onto a piece of plastic food wrap.
9. Folded and labeled wraps, placed in freezer. (NOTE - used wrong freezer - got yelled at! DRC 21 Aug '86)

Summary: Too late to run second dimension by SDS-PAGE. Will keep IEF gels frozen until I have time to finish the procedure. this must be done within 2 weeks, because of the half life of the S-35 label.

continued page 21

continued from page 7

Primary Culture, chick pectoralis major

Since all cultures were contaminated, decided to re-do all procedures exactly as recorded pages 1-3, steps 1-14. Since the media had not been filtered, we suspect media contamination. My technique was fine, according to my supervisor.

Results Obtained 3.3 ml suspension this time. Cell counts were conducted as reported step 13 page 3. Final yield, 3.4 mil. cells/ml. Prepared 2 12 well plates and 2 100 mm plates as before, by adding 59 µl suspension/well to the first 2 plates, and 0.29 ml to each 100 ml plate. Added muscle medium as before and incubated as before.

Summary Will examine plates first thing tomorrow morning for contamination. Look for cloudy medium, lots precipate. If wells are clean, proceed with a characterication of the cultures.

Appendix Two

The Importance of Keeping Accurate Notes

An 'Accidental' Discovery

This story was related to me by a supervisor during my first post-doc. I have no documentation for it, however, it makes a good story even if it is only a story. [David R. Caprette]

In the late nineteenth century, scientists studying the basis for contraction of the heart muscle were hampered by an inability to obtain beating isolated hearts. It was already known that a well-oxygenated heart continues to beat after excision from the subject, therefore no nervous input is needed. However, after a short time hearts removed from animals stopped beating despite rigorous maintenance of temperature and perfusion with an oxygenated physiological saline solution. Electrical stimulation was not successful either. This was especially puzzling, since skeletal muscle can be stimulated to contract for a very long time after its isolation.

A cardiovascular physiologist, Sidney Ringer, was attempting to study isolated hearts and like other physiologists he used a saline solution consisting primarily of sodium, potassium, and chloride ions, with a buffer added to the solution. Like any good scientist he used distilled water to prepare his solutions. Like the other cardiovascular physiologists of the time, he observed that the heart muscle failed to contract after a short time. Then one day, inexplicably, an isolated heart preparation beat vigorously and continued to beat for hours!

It turned out that the lab had run out of distilled water, and rather than remain idle the technician used river water to make up the solutions. River water contains, among other minerals, calcium ions. This accidental discovery led to the finding that heart muscle, unlike skeletal muscle, requires extracellular calcium to contract. If Ringer's technician had not kept a record of how he had proceeded that day the 'mystery' would have remained unexplained, and most likely someone else would eventually have been credited with the discovery.

Keep track of all details of procedures in your notebook, and include any changes to procedure, no matter how seemingly unimportant they are at the time. Not only do you need the details to refine methods and confirm errors, but you may in fact discover something new.