Hatfull Lab Notebook Guidelines

"A basic test of the quality of the notebook is that, if necessary, the outside reader could replicate the whole study and reach the same results.... to allow an outside reader to follow the thoughts, logic, and decision-making processes used by the researcher in implementing a specific protocol... It should articulate 'What do the results mean and what do I need to do now?' "
Adapted from Hanauer et al, 2009

What your lab notebook IS:

Your lab notebook is a record of all the work that you perform in the lab.

Protocols, notes etc. may be copied, but original data must stay in the lab!

Your notebook is the property of the lab.

There needs to be sufficient **detail** in your notebook, **organized** and **legible** for the following purposes:

1. So that YOU can repeat an experiment exactly as before, troubleshoot an experiment that hasn't worked, or write up the experiments for a manuscript to be submitted for publication. You may need to do these things years after the entry, so you can't count on remembering details!
2. So that the PI or ANOTHER LAB MEMBER can repeat an experiment exactly as before, troubleshoot an experiment that hasn't worked, or write up the experiments for a manuscript to be submitted for publication. You may have graduated by the time this happens! It is SAD to see a student's work go to waste and a chance at publication lost because no one else can understand it.
3. As a legal record of when the work was done and what were the results. This is important for patents or if there are any charges of fraud (extremely rare, but exceedingly serious when it happens).

What your lab notebook is NOT:

A lab notebook is not a lab report! The major difference is that your notebook entries must be written **as you do the work.** Although it needs to be organized, neat and legible, it is not expected to be "polished." **Take notes early and often.**

If you must grab a paper towel to take some notes during an experiment, you tape the paper towel into the notebook, you do not recopy it. You can add information after the fact, but it must always be dated.

Components of each lab notebook entry:

- Title (at top of page). In phagehunting work, be sure to include what phage you are working on.
- Objective(s): what you plan to do
- Materials:
- Procedures: can include methods, protocols, and adaptations of protocols, instruments (and their settings). This is where you record what you do different from a standard protocol.
- Results:
- Conclusions:
- Next Steps: what do you plan to do next. What results do you have to record tomorrow?

- Not all components will be needed every day. You may substitute the following when necessary:
- Inferences: when you don't have enough data for a conclusion, but your data is pointing there!
- Impressions: put on paper any impressions you have of your work. Was it a good hands kind of day?
- Summary: did you meet your objectives? What do you expect next?
- Next Steps: what do you plan to do next. What results do you have to record tomorrow?

General Notes:

- Write with a ballpoint (not erasable) pen in your notebooks.
- Number every page with a page number or experiment number, but be consistent and keep a table of contents. Date every entry.
- When crossing out a mistake, make only a single line through the mistake. NEVER use whiteout to correct mistakes. The mistake must be visible and legible. Initial and date changes.
- Write carefully and legibly. The notebook is not yours- it is for your whole lab, including people who may need to repeat or publish your experiments after you leave the lab.

- Periodically, write a summary of your work, with a description of what is working, what is abandoned, and what you are to do next.

A stellar notebook will have:

- Table of contents and/or an index
- Dividers or tabs for easy navigation between sections
- Flow charts describing experiments
- For every day's work, start with your objectives for the day. Record what you do as you do it, end with a summary of what you did and what your next step is.
- An experiment name on every page of your notebook. If you repeat an experiment many times (especially if you are making modifications as you go), you made want number them. If you are working with multiple phages, you cannot use the word phage in place of the name of the phage. label what you are doing with precision!
- Every experiment have an experiment number or descriptor. Stored samples are to be labeled with identical experimental numbers or descriptors. Record in your notebook how things are labeled and where they are stored.
- IT IS BETTER TO ERR ON THE SIDE OF TOO MUCH DETAIL THAN NOT ENOUGH DETAIL

Adapted from Dr. N. Kaufmann, written for Undergraduate Researchers at University of Pittsburgh, prepared with the help of Dr. Oke, Dr. Chapman, S. Seguin, AY2008/2009 Fellows

Sample lab notebook entry: The following is a series of two excerpts from the notebook of Emilee Shine (Undergraduate Researcher in the lab of Dr. G. F. Hatfull, from May 2011 – present).

1. Initial lab experiment (p.123-124) to determine actual gene start. Page 124 is a great example of Purpose.
2. Explanation of results of that same experiment (p.131). This explains Emilee's thinking **as** she interprets her results. Note that she has identified how she has labeled the product of this experiment and where/how she stored it.

July 16, 2012

Testing Charlie Start Codons

Purpose: As mentioned on pg. 120, we only tested if the proposed start codon could be a start, not if it was actually the start. The greatest flaw with that experiment was that the valine alteration was made on a plasmid w/ a stop codon after the 3rd start, therefore the phage was forced to use that start. Now, the question is ~~that~~ could Charlie technically use all 4 proposed starts as starts - which might suggest that we have different lengths of int being made (and therefore, might have different functions). Greg did this on BPs and changed all methionine starts to isoleucine and all valines to a different valine - by knocking out pairs so that only one start was left. He found that he had transformants (integration), but it was at lower transformation efficiencies for some. I'll do the same w/ Charlie - the first two methionine starts I will alter to isoleucine (ATG→ATA) - don't do ATC b/c there's evidence of that being a start codon for Tb (although its really rare) and after use the last 2 valine starts (GTG → GTC) and do this until only one start is available and coelectroporate them.

(A) X 2 X 4
(B) 1 2 3 X
(C) X 2 3 X
(D) X 2 3 X

first round
second round
third round.
start codon intact

Procedure:

1. First round of PCRs:

(A) 1st start knock out (KO)

39.6 µl	dH_2O
5 µl	10X buffer
1 µl	dNTPs
0.8 µl	pGWB82 (~40ng)
1.3 µl	prmES67
1.3 µl	prmES68
1 µl	Pfu Turbo
50 µl	

(B) 2nd start KO

39.4 µl	dH_2O
5 µl	10X buffer
1 µl	dNTPs
0.8 µl	pGWB82 (~40ng)
1.4 µl	prmES69
1.4 µl	prmES70
1 µl	Pfu Turbo
50 µl	

(C) 3rd start KO

40 µl	dH_2O
5 µl	10X buffer
1 µl	dNTPs
0.8 µl	pGWB82
1.1 µl	prmES40
1.1 µl	prmES41
1 µl	Pfu Turbo
50 µl	

(D) 4th start KO

39.8 µl	dH_2O
5 µl	buffer
1 µl	dNTPs
0.8 µl	pGWB82 (~40ng)
1.2 µl	prmES42
1.2 µl	prmES43
1 µl	Pfu

95°C	30 sec.	
95°C	30 sec.	12 cycles.
55°C	1 min	
68°C	6 min	
4°C	hold.	

2. Digest w/ 1 µl of DpnI @ 37°C for 1 hour.
3. Transform into NEB5α and plate on LB/KAN.
4. Pick ~~mutants~~ colonies and extract plasmids.
5. Second round of PCRs ~~on the~~ using the extracted plasmids.

(A) KO 2nd Start.

39.2 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82A (~30 ng)
1.4 µl prmES69
1.4 µl prmES70
1 µl Pfu Turbo
50 µl.

(B) KO 3rd Start

39.8 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82B (~30 ng)
1.1 µl prmES40
1.1 µl prmES41
1 µl Pfu Turbo
50 µl.

(C) KO 4th Start

39.6 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82C (~30 ng)
1.2 µl prmES42
1.2 µl prmES43
1 µl Pfu Turbo
50 µl.

(D) KO 1st Start.

39.4 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82D (~30 ng)
1.3 µl prmES67
1.3 µl prmES68
1 µl pfu turbo
50 µl.

95°C 30 sec
95°C 30 sec
55°C 1 min
68°C 6 min
} 12 cycles.
4°C hold

6. Digest w/ 1 µl of DpnI @ 37°C for 1 hour
7. Transform into NEB5α and plate on LB/KAN. Pick mutants + extract.
8. Third round of PCRs using the extracted plasmids.

(A) KO 3rd Start

39.8 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82A
1.1 µl prmES40
1.1 µl prmES41
1 µl Pfu Turbo
50 µl.

(B) KO 4th Start

39.6 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82B
1.2 µl prmES42
1.2 µl prmES43
1 µl Pfu Turbo
50 µl.

(C) 1st Start KO

39.4 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82C
1.3 µl prmES67
1.3 µl prmES68
1 µl Pfu Turbo
50 µl.

(D) 2nd Start KO

39.2 µl dH_2O
5 µl buffer
1 µl dNTPs
1 µl p6WB82D
1.4 µl prmES69
1.4 µl prmES70
1 µl Pfu Turbo
50 µl.

* same amount of plasmid ~30 ng.
* same cycles as above.

9. Digest w/ 1 µl of DpnI @ 37°C for 1hour.
10. Transform into NEB5α and plate on LB/KAN. Pick mutants and extract.

Continued from page ____ Book Number_____ Continued on page_____

SIGNATURE | DATE (MM/DD/YYYY) | SUPERVISOR | DATE (MM/DD/YYYY)

Samples kept are the final plasmids stored in green freezer box labeled as "SDM A", "SDM B", "SDM C", and "SDM D" w/ ng/µl concentration on side.

July 26, 2012

Sequencing.

Purpose: To check if All Start Codons Are Correctly knocked out.

	Label	Template (500 ng)	Primer	Water
1	ES01	0.76 µl SDMA	2.5 µl prmES15	11.74 µl
2	ES02	0.76 µl SDMA	2.5 µl prmES16	11.74 µl.
3	ES03	1 µl SDM B	2.5 µl prmES15	11.5 µl
4	ES04	1 µl SDM B	2.5 µl prmES16	11.5 µl.
5	ES05	1 µl SDM C	2.5 µl prmES15	11.5 µl
6	ES06	1 µl SDM C	2.5 µl prmES16	11.5 µl
7	ES07	0.81 SDM D	2.5 µl prmES15	11.7 µl
8	ES08	0.81 SDM D	2.5 µl prmES16	11.7 µl
9				
10				15 µl.

7/30. **Results:**

Not good news.

For SDM A, all starts should be knocked out except for the 4th one. (So shortest version of int. would have to be made). Unfortunately, the 1st start is not knocked out, so it has 2 possible starts (2nd and 3rd are knocked out correctly)

For SDM B, all starts are knocked out correctly except for the 1st start - which is the way it was supposed to be. So this worked - meaning the longest form of int was the only one that could be made.

For SDM C, the 2nd start was supposed to be the one left intact, but the 3rd one failed to work, so it has 2 possible starts. the 1st and 4th were knocked out correctly. The 1st round of PCRs didn't work.

For SDM D, the 3rd start was supposed to be the only one left intact, but the 1st and the 3rd are left with the 4th and 2nd correctly knocked out - leaving 2 possibilities. The second round of PCR didn't work.

So, only one full set of PCR's (SDMB - 1st start codon) worked correctly. 2nd and 4th starts are always knocked out correctly. it's the 1st (especially) and the 3rd that are messing up. Perhaps I'll just do one more round of PCRs on the remaining ones that didn't work to try and fully knock them out - but I don't know why they didn't in the 1st pla

I noticed that the 1st round didn't work for SDMA and SDMC that used 40ng - perhaps stay away from that amount of template DNA.

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Hatfull Lab Sample Labeling Guidelines

All of the material that you produce in lab must be labeled. The amount of detail that needs to be on the label varies by how long you expect the material to be stored.

1. Material to be stored past the initial procedure for days, weeks, months, or years. For example, Label a 15 mL conical tube that contains a high titer phage lysate with :
   - what is in the tube
   - titer
   - your initials
   - date you produced it
   - page # in your lab notebook that refers to when you made that sample

Example:

Mycobactreiophage Tweety
7×10^{11}
OMG
8.1.2009
p. 87

Make sure your notebook clearly states how the material is labeled and where it is stored.

2. Material that you will only keep for a short time (like dilutions), can be labeled with a little less information. Be sure to include phage name, dilution tube 3, and date. Regularly check your storage boxes for tubes that were kept for short term and discard when appropriate.

DO NOT simply number tubes that you will keep even for short times. Very quickly you end up with a freezer full of numerous tubes labeled #1!

Helpful tips:

- Make sure to use permanent marker to label tubes. Some permanent markers and some label stickers are not permanent on smooth plastic that is being handled, especially in the freezer. Use the label maker when appropriate.
- Some permanent markers are not alcohol proof.
- Common reagents made for all the lab to use should have the initials of maker and date made.
- A disclaimer to discard short term storage. Before discarding a set of tubes from your phage, make sure you have propagated phage from your latest sample. You do not want to discard your best sample of phage.
- MORE DETAIL IS ALWAYS BETTER THAN NOT ENOUGH DETAIL ON A LABEL!

Adapted from Drs. Nancy Kaufmann & Valerie Oke

Hatfull Lab Electronic Data Recording

Organization of your computer files is essential and is your responsibility.

Data is to be stored in 2 places. 1. The GelDoc computer. 2. Your own Electronic Data Storage System (your computer or flash drive).

Expect that you or other lab members will need to return to your data files to publish your work or put together your thesis. Years from when the document was created someone may need to access the data.

1. Save and back up the Raw **File**. This file type contains the most information. With this file type, you can always go back and export a TIFF, etc. but the reverse is not true. You will lose access to information if you do not save the raw data file.

2. Use a systematic method for naming and organizing your files. Describe your naming system in your notebook. For most Phagehunting records, using the phage name is the best way to store the files. You may need to return to a set of files for renaming if you do not have a name at the onset. Establish your naming mechanisms prior to taking pictures whenever possible.

3. In your lab notebook record the name of the file, where the file is saved, and where the file is backed-up.

4. Print out pictures representing the electronic data saved. Tape in any gel photos or other pictures that goes along with the experiment, and clearly label the picture. For instance, show what is in each lane.

Adapted from Drs. Nancy Kaufmann and Beth Roman