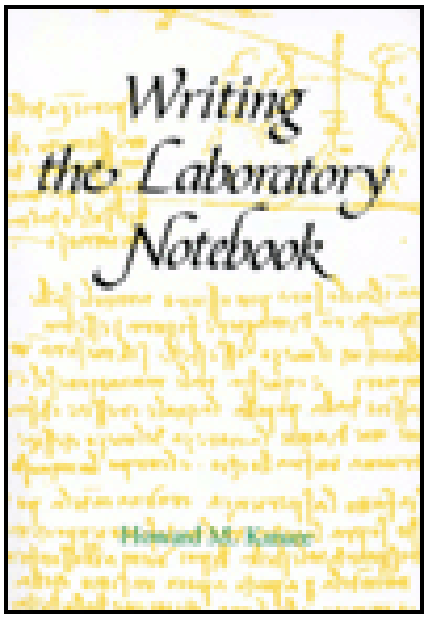
Good Laboratory Notebook Practices

Lab Notebooks

“It’s a notebook, not a neat book”



Main References:

- *Writing the Laboratory Notebook, Howard M. Kanare, American Chemical Society, Washington, D.C. 1985, ISBN: 0841209332.*

- **Good Laboratory Notebook Practices by Lucy H. Senter** https://www.research.msstate.edu/rresources/pdf/seminar/Senter_Lab_Notebooks.ppt

More References

- **GLP Recordkeeping**
  http://users.stlcc.edu/departments/fvbio/Lab_Practices_GLP_STLCC.htm
- **Good Laboratory Notebook Practice**
  http://www.mddionline.com/article/good-laboratory-notebook-practice-0
- **Laboratory Notebook Guidelines**
  http://www.bookfactory.com/special_info/lab_notebook_guidelines_A4.html
- **Advice on keeping a laboratory notebook**
  http://www.swarthmore.edu/NatSci/cpurrin1/notebookadvice.htm
- **Guidelines for Keeping a Laboratory Record**
  http://www.ruf.rice.edu/~bioslabs/tools/notebook/notebook.html#entry
- **Good Laboratory Notebook Practices by Lucy H. Senter**
  https://www.research.msstate.edu/rresources/pdf/seminar/Senter_Lab_Notebooks.ppt

Notebook is a legal document

Your data may have to be explained, defended, reconstructed or repeated without your assistance, so others must be able to understand what you did.

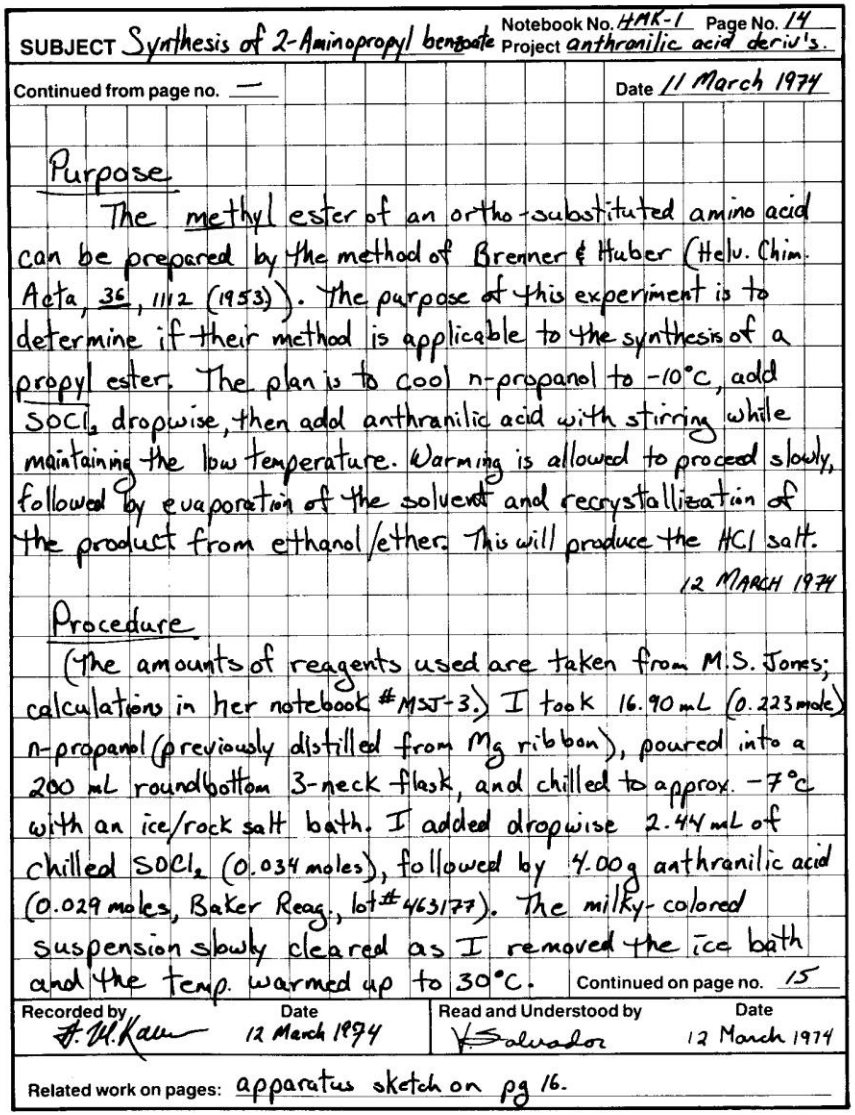
Bad Record-Keeping

LeMonnier, French astronomer who gets no credit for the first sightings of the planet *Uranus*. His notes were so bad that he thought it was a comet. Discovery of *Uranus* is instead awarded to Herschel.

Gordon Gould had many ideas related to the production and use of lasers. He foresaw that they could cut steel or ignite fusion reactions. His notes were witnessed by a candystore notary instead of a colleague. He had undocumented meetings with the "maser people." Years and years of legal proceedings were required to get him *some* of the credit he deserved.

Types of Documentation

- Notebook—factual details of experiments, including thought experiments, ideas, inventions, etc.
- Logbook—for example, a list of measurements made on the NMR, GPC, Balance, etc.
- Diary (Journal)—What you were feeling, a personal record, opinions—stuff that is less factual than the notebook. Depending on the situation, this *might* be appropriate to place in the notebook but be careful to delineate fact from opinion.



SUBJECT Synthesis of 2-Aminopropyl benzoate Notebook No. HMK-1 Page No. 14
Project anthranilic acid deriv's.

Continued from page no. —

Date 11 March 1974

Purpose

The methyl ester of an ortho-substituted amino acid can be prepared by the method of Brenner & Huber (Helv. Chim. Acta, 36, 1112 (1953)). The purpose of this experiment is to determine if their method is applicable to the synthesis of a propyl ester. The plan is to cool n-propanol to -10°C, add $SOCl_2$ dropwise, then add anthranilic acid with stirring while maintaining the low temperature. Warming is allowed to proceed slowly, followed by evaporation of the solvent and recrystallization of the product from ethanol/ether. This will produce the HCl salt.

12 MARCH 1974

Procedure

(The amounts of reagents used are taken from M.S. Jones; calculations in her notebook #MSJ-3.) I took 16.90 mL (0.223 mole) n-propanol (previously distilled from Mg ribbon), poured into a 200 mL roundbottom 3-neck flask, and chilled to approx. -7°C with an ice/rock salt bath. I added dropwise 2.44 mL of chilled $SOCl_2$ (0.034 moles), followed by 4.00 g anthranilic acid (0.029 moles, Baker Reag., lot# 463177). The milky-colored suspension slowly cleared as I removed the ice bath and the temp. warmed up to 30°C. Continued on page no. 15

Recorded by H. M. Kane Date 12 March 1974

Read and Understood by V. Salvador Date 12 March 1974

Related work on pages: apparatus sketch on pg 16.

Figure 1.1 A page from a properly kept notebook.

Notebook Properties

- Written as the work is performed
- Dated and signed by author
- Each section has a clear, descriptive heading
- The writing is legible and grammatically correct
- Active voice in first person:
  "I chose these two components…"
- Read by witness and signed/dated
- Do Not write over; cross and write above

Notebook Properties

Paper has to be very good quality.

Notebook should be bound.

No spiral notebooks! No loose-leaf!

Page layout easy to graph, date, sign, etc.

It is better to glue or tape that original paper snippet into the lab book than it is to copy the result.

Table of contents!

What to write with?

No pencils. Erasures are a definite no-no!

No aqueous-based pens (e.g., most felt-tips).

Best bet for general use: black, ballpoint pen.

No white-out!! Just one strike through.

Explain and initial errors.

“It’s a notebook, not a neat book.”—R. Cueto

Employer Checklist

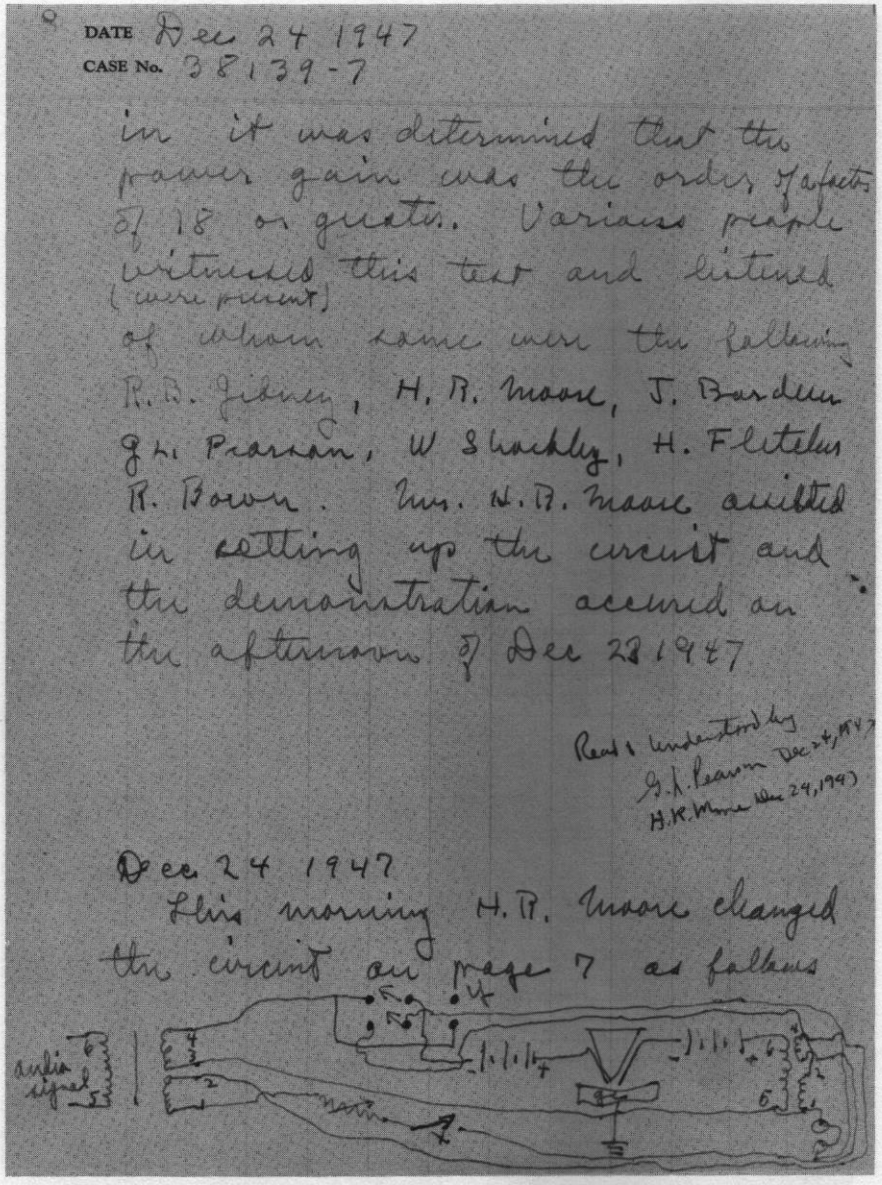
- Black, ballpoint pen used?
- Legible handwriting?
- Table of contents up-to-date?
- Entries signed/dated (October 13, 2002 better than 10/13/02)
- Clear headings saying what this page is about?
- Written in first person?
- Complete sentences?
- Could the work be followed by another scientist? (avoids jargon?)

Employer Checklist

- Is the researcher correctly "thinking in the notebook"—i.e., ideas and plans and observations integrated and written down.
- Are entries witnessed appropriately?
- Is the notebook stored safely when not in use?

What goes in the notebook?

- Plans
- Realities (deviations from the plan)
- Observations
- Sketches and photographs
- “Links” to the notebooks of others in your group
- “Links” to instrument logbooks and data on disks
- Ideas: a notebook is a repository of creativity
- E-mails from collaborators (tape or paste them in)
- Plot-as-you-go graphs: do it!
- Summaries of papers you have read.
- Hints, concerns and tips you may get from science



DATE Dec 24 1947
CASE No. 38139-7

in it was determined that the power gain was the order of factors of 18 or greater. Various people witnessed this test and listened (were present) of whom some were the following R. B. Gibney, H. R. Moore, J. Bardeen G. L. Pearson, W Shockley, H. Fletcher R. Bown. Mrs. H. R. Moore assisted in setting up the circuit and the demonstration occured on the afternoon of Dec 23 1947

Read & understood by
G. L. Pearson Dec 24, 1947
H. R. Moore Dec 24, 1947

Dec 24 1947
This morning H. R. Moore changed the circuit on page 7 as follows

audio signal

Figure B.5

First transistor amplifier, AT&T Bell Labs
(Walter H. Brattain)
Dec. 24, 1947

Recordkeeping Guidelines

- Complete the title page when the notebook is issued
  - All persons recording in the notebook must also sign the title page and give an example of initials used
- Table of contents
  - Record only the first page number of each multi-page experiment.

TABLE OF CONTENTS

(chronological order)

Recordkeeping Guidelines

- Each recorded lab should have the following parts:
  - Objective or purpose of the lab
  - Plan, outline or flow diagram of lab
  - Step by step procedure
  - Raw data
  - Results, including graphs, tables, figures, photos and/or drawings

Recordkeeping Guidelines

- Each recorded lab should have the following parts:
  - Conclusion: include the biological and chemical concepts involved, whether the objective was met, any problems encountered, and suggestions for future experiments

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

5. Placed in 37 degree water bath, 30 min.

6. Added cold muscle wash medium to fill the tube (12 ml to fill Corning 15 ml plastic centrifuge tube). Centrifuged, 400xg, 5 min. (setting #3 on the clinical centrifuge).

7. Removed the supernatant with pasteur pipet; resuspended the muscle mince in muscle wash medium and recentrifuged as above.

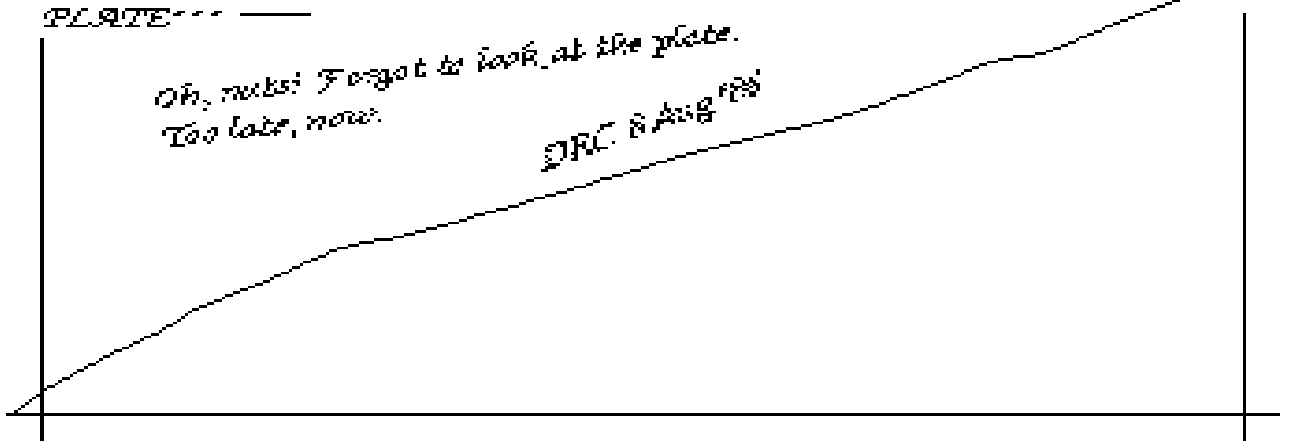
8. Repeated step 7.

9. Added 4 ml medium and triturated to obtain a uniform (milky) suspension. Held the pipet on the bottom of the tube to squash the pieces as they squirted out the flat bottom.

10. Passed suspension through a stainless steel and 20 µm swinney filter in a series.

11. Placed entire suspension in an uncoated 100 mm corning tissue culture plate at 37 degrees for 30 min. (pre-plate, to remove most nonmuscle tissue).

12. ---SAVE THIS SPACE FOR RESULTS FROM EXAMINATION OF PLATE--- ——



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 (0.30ml per plate). Added muscle culture medium, 1 ml/well in 12 well dishes, 10 ml in each of the plates. Incubated 37 degrees.

<u>Summary</u> 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 ***

8. Poured de-gassed resolving gel mix into 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

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 on to 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

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continued from page 7

Primary Culture, chick pectoralis major

Since all cultures were contaminated, decided to re-do all procedures <u>exactly</u> 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.

<u>Results</u> 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.

<u>Summary</u> 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.

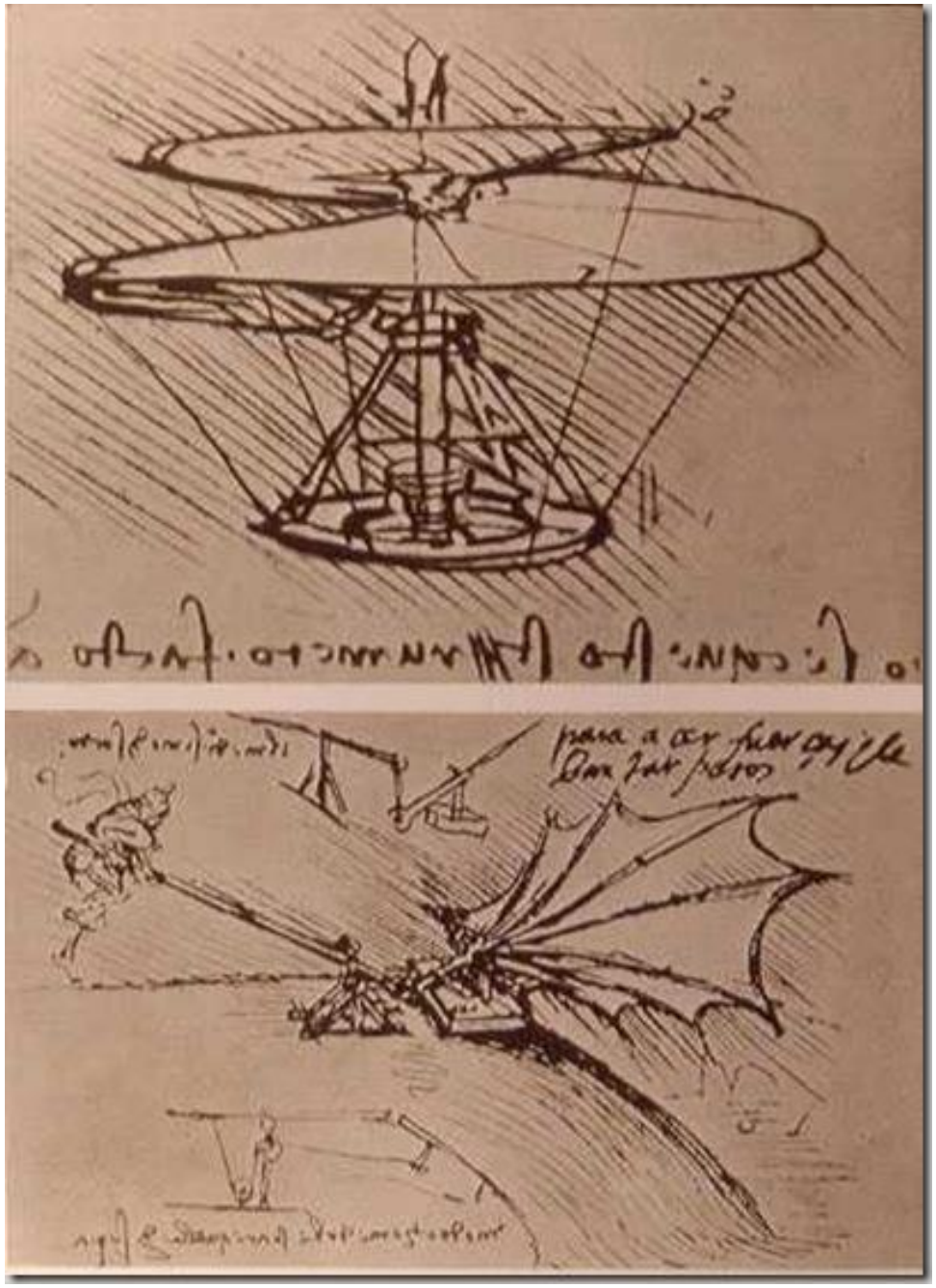
Recordkeeping Guidelines

- Lab notebooks should include *everything* about the work so that another person can read the notebook and know exactly what was done.
- If procedures or other information are copied from a source, the source must be identified in the lab notebook.

What is data?

- Raw data: original of handwritten information or a printout from equipment.
  - Descriptions of observations, procedures, events, for example
- Calculated data: derived from a calculation or statistical evaluation of the raw data.
- Transcribed data: copied raw or calculated data; should indicate “exact copy of original” or where the original data is located.

Helicopter

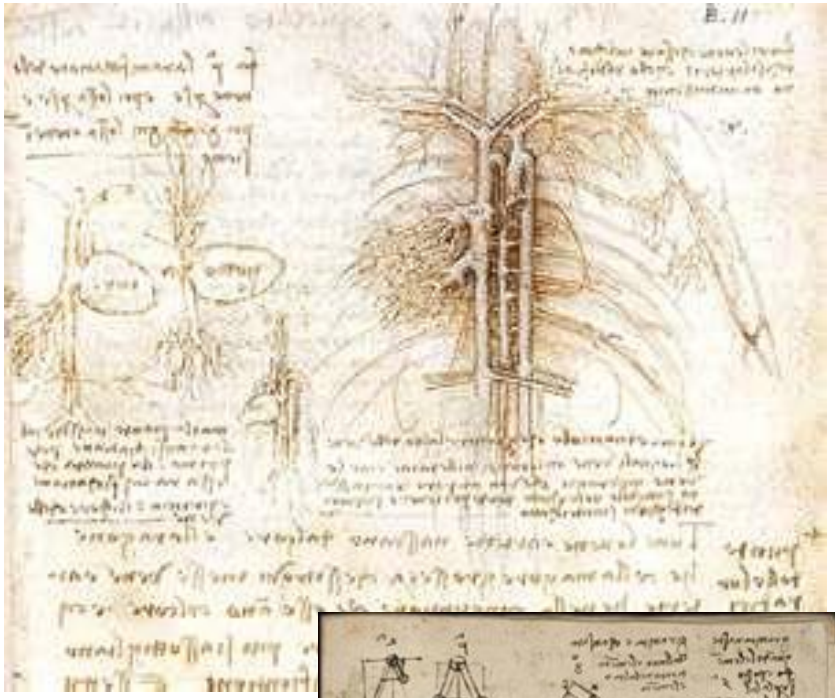


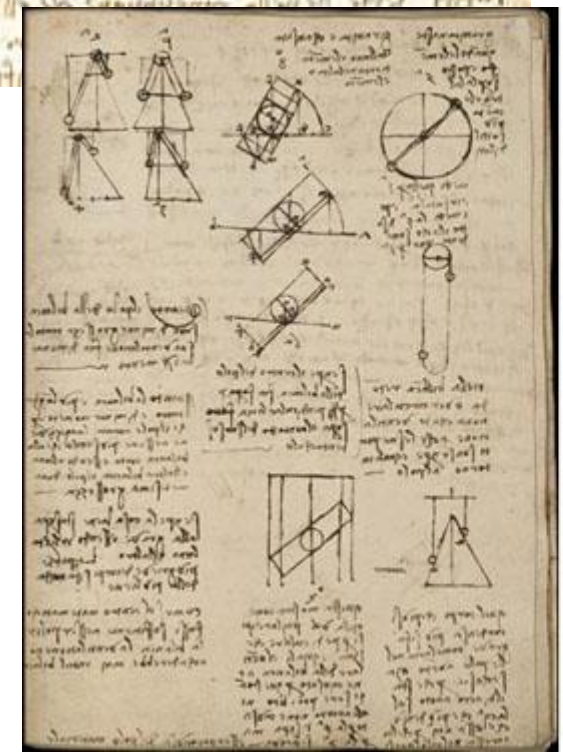
Lifting Wing

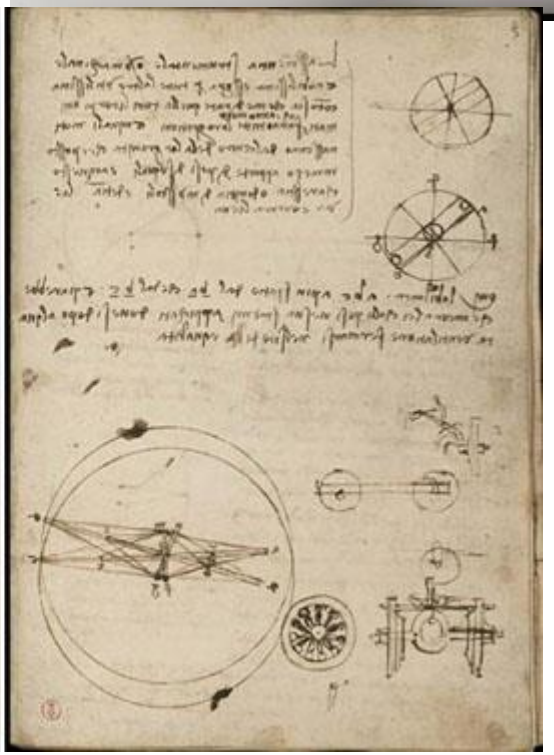
By

Da Vinci

1493







Da Vinci Notes

1500s

What do you Record?

- Objectives, ideas, experimental plans or outlines, preparations, procedures, data, observations, calculations, discussions, conclusions, future plans and potential uses
- What actually happened
- Results

What do you Record?

- Notes of unexpected results or observations
- Deviations to a planned protocol
- All measurements and important test conditions (weights, volumns, temperature, etc.)
- All units.

What do you Record?

- Indicate if the numbers are estimated, rather than measured
- Indicate if the numbers were calculated and provide the equation
- If using Excel, print out the formulas
- Indicate if the number has been rounded or truncated
- Document critical events to prove compliance with SOPs

How do you record the data?

- Directly into the notebook; not on post-its, paper towels, scraps of paper, etc.
- In black or blue, indelible ink; no gel pens
- Make entries only in the ruled areas of the numbered pages
- Unnumbered pages can not be used
- Only one experiment per page
- Attach forms or printouts

What is the procedure for attaching forms and printouts?

- Attach only to numbered pages within the ruled area only
- Taped on at least 2 sides
- Fully exposed, not folded
- Not covering any previously recorded entries
- With hash marks on at least two corners
- Write the notebook and page number on the attachment
- Sign and date along the edge

Who generated the data and where?

- Record the data on the same day it is generated, not after the fact.
- A single page can cover events from more than one day, by the dates must be indicated on each event
- The person making the entry must sign the page.

What materials and equipment did you use?

- Important materials must be noted:
  - Related to the reconstructability and repeatability of the experiment
  - Variability between batches and lots
  - Be specific: not just “the buffer”; name it.
  - List the purity, concentration, etc
  - List the source, catalogue number, etc
  - Record the recipes

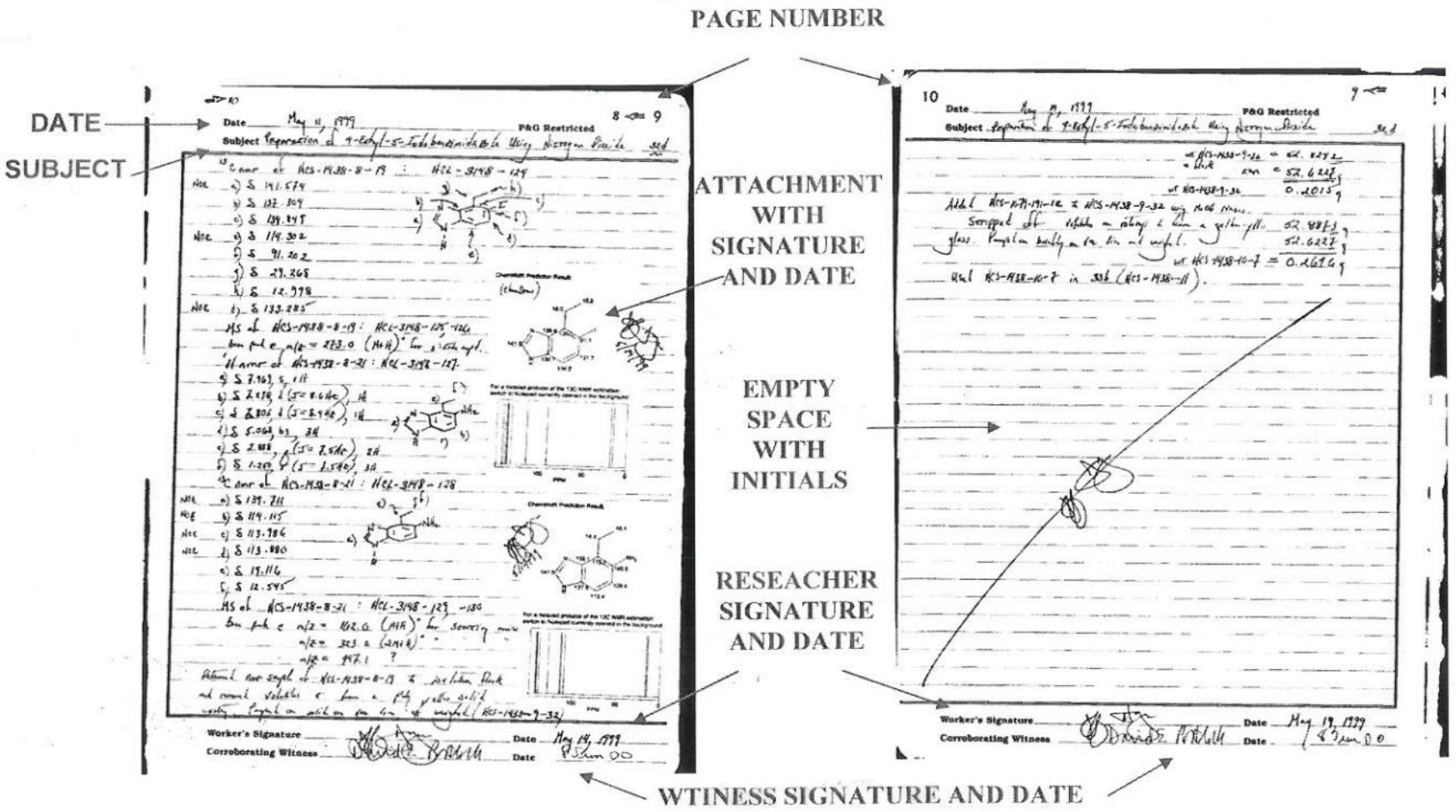
Conclusions: fact vs opinion

- Fact: no reaction was observed; vs Opinion: these two chemicals don't react.
- Fact: Expected results were not obtained; vs. Opinion: No good
- Fact: Under these circumstances, the reaction was unsuccessful; vs. Opinion: failed.

How clear or understandable is your data?

- Legible to others?
- Clear, detailed so someone else in your discipline could understand it and repeat it?
- Include drawings and flow charts to improve clarity?
- Are abbreviations defined and obvious?

More examples of notebook pages



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[redacted]

* Formatted and Resubmitted PLoS CPP paper with [redacted], discussed additional/future projects

2-Jun-2011

Taking a break from PEM DB generation script for today - will work on figuring out MPI-BLAST on HPC[2] clusters for nematode project.

New genome assembly, 74.94% of RNA-Seq reads map with at least 1 reported alignment → that's up ~2% from the old abyss_n10_k40 assembly (see: 18-May-2011)
- Also in light of Li et.al. Science Express paper (1-Jun-2011), this seems very reasonable and look like the assembly using all data is better than previous assemblies.

Also, need to re-run PHOBOS on this data for updated microsatellite analysis.
generated phobos_new.out > need to perform analysis...

Also, examined PEAKS and Sieve software [redacted] → both available through Thermo and integrate with Proteome Discoverer, both seem to have "cluster" versions but unclear exactly what → PEAKS is for de novo sequence identification as opposed to DB search while Sieve does differential expression analysis (control vs. treatment, etc.) → may want to check for open source alternatives...

Also, the PEAKS (Bioinformatics Solutions) software webpage provides some insight into using multiple identification strategies (multiple algorithms, i.e. X!Tandem, Sequest, MASCOT, etc.) to increase peptide coverage of identified proteins → may want to investigate this for Book Chapter and proteotypic peptide observability followup paper.

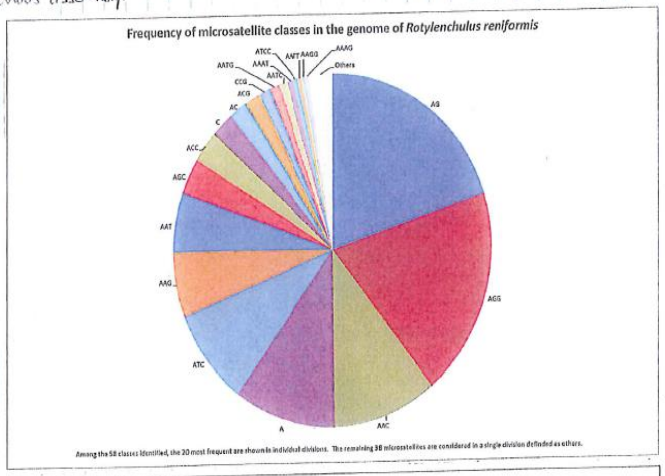
PHOBOS Output on Newest Genome Assembly—
① run PHOBOS, pipe output to file. ② download file, run micro_stats.pl ③ run frequency.pl on micro_stats.pl output file.

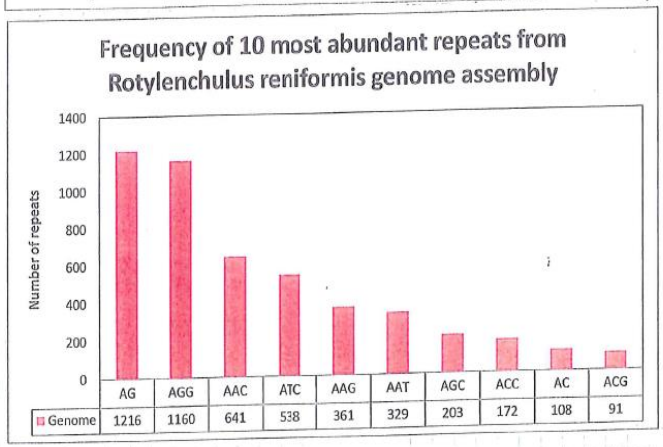
	R. reniformis
Sequence analyzed (bp)	[redacted] 139,677,102
GC Content (in %)	[redacted] 40.16
Number of microsatellite loci	6,055
Average density of loci (no./Mbp)	43.35
Total length of microsatellites (bp)	106,630
Coverage (length in bp/Mbp)	763.40
Genome content (in %)	0.08

41

MOTIF LENGTH	1	2	3	4	5	6
No	771	1,334	3,583	335	29	3
No./Mbp	6.41	11.08	29.77	2.78	0.24	0.02
%	12.73	22.03	59.17	5.53	0.48	0.05
Length (bp)	10,358	26,228	61,226	7,906	814	98
Bp/Mbp	86.05	217.89	508.64	65.68	6.76	0.81

Microsatellite #'s for this assembly seem relatively inline w/ the previous assembly → also [redacted] mentioned that the genome assembly alignment to the reniform ESTs seems comparable to the previous assembly.





Mistakes?

- Never use white-out
- Never erase
- Never write-over
- Never discard or replace attached supplementary data
- Always record a defensible reason for the correction/edit
- Always circle the reason
- Always add your dated initials to the corrected/edited data after the circled reason

Where should the notebook be kept?

- In general, a notebook should be kept in a company or university lab
- Strictly speaking, the lab notebook belongs to the company or university, and should NOT be removed from the premises.