Techniques in Molecular Genetics

2013 edition

H.E. Schellhorn

Day 1

- Introduction
  - Why are we here?
    - Overview
    - Teaching Coordinator, Teaching Assistants
    - Changes for 2013
    - Techniques
  - Use of a Pipetman
  - Streak a culture, Make some media
  - DNA Management Software

Course Rationale

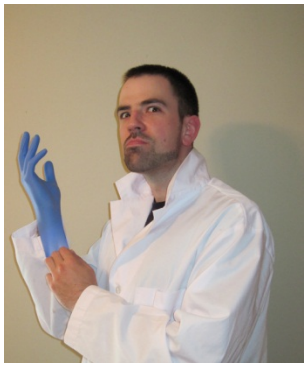
This course is primarily aimed at students who are starting to work in molecular biology research mainly Biology and Molecular Biology student who have completed third year and are working in the Biology department during the summer. The formal part of the course, consisting of two weeks of laboratory/lecture, runs the first two weeks of May.

The objective is to provide participants with formal instruction in the scientific process including laboratory techniques that they need to accomplish their research objectives. By combining theory with practice, much duplication in instruction among labs will be eliminated.

Instructors

Dr. H.E. Schellhorn

Ms. Alison Cowie



Jas Wasniewski

Sarah Chaing

Sharmila Sathiasothy

Significant Changes for 2013

- Addition of Realtime PCR talk and Assignment

Grading Scheme

Quiz I	20%	(May 7th, days 1-4)
Quiz II	30%	(May 14th, days 1-9)
Overnight	10%	
Rotation Assignments	10%	
Course performance	10%	
Lab notebook	20%	

Use and understand the principle of the following laboratory equipment/tools…

- Laboratory notebook.
- Centrifuge.
- Spectrophotometer.
- Image analysis.
- Scanner.
- Scintillation counter.
- Autoclave.
- pH meter.
- PCR cycler.
- Transilluminator.
- Balance.
- Analytical balance.
- Sonicator.
- Gel dryer.
- Computer.
- Web tools.
- Spreadsheets

Potential Overnight assignments..

- Use Refworks to write a short essay…
- Write an SOP/AUP
- Read “instructions to authors” and answer a short quiz
- Prepare a table comparing protein methods
- Prepare a table comparing graph types
- Prepare a publication quality graph
- Prepare an order sheet for purchase of chemicals
- Write and submit Primer, DNA sequencing order

Wiki/Website

- Part will be public…some parts will require a login in.
- Include product manuals (PDFs), assay manuals (PDFs) reference tables, calculators and sample spreadsheets.
- Will also include web resource for each technique.

Practical

- Why do experiments fail?
- How to plan experiments
- Where to store samples.

4XX3: Lab Rules and Organization

NO FOOD

Lab coat: General safety, and biosafety level 2 tissue culture work

Safety equipment: Fire, Eye wash, shower

Safety goggles: for acid/base handling, fume hood for HCl, SDS, BME

Gloves: for handling of acrylamide, acid, basis, ethidium bromide
biosafety level 2 tissue culture work

Clean balances!!!

Waste: biological waste vs non-biological waste

Reasoning

- Scientific method
- The falsifiable hypothesis-Popper
- What makes a good (powerful) hypothesis?
- Induction/deduction Reasoning (specific to the general)
- Predictive models
- Theory/Proven fact
- Cause vs correlation
- Reductionist/holistic
- Science/Magic
- Orthogonality
- Conjecture vs plausible explanation

Ethics

- Plagiarism—degrees of plagiarism, recent examinations of the problem
- Fraud
- Accreditation
- Misunderstanding

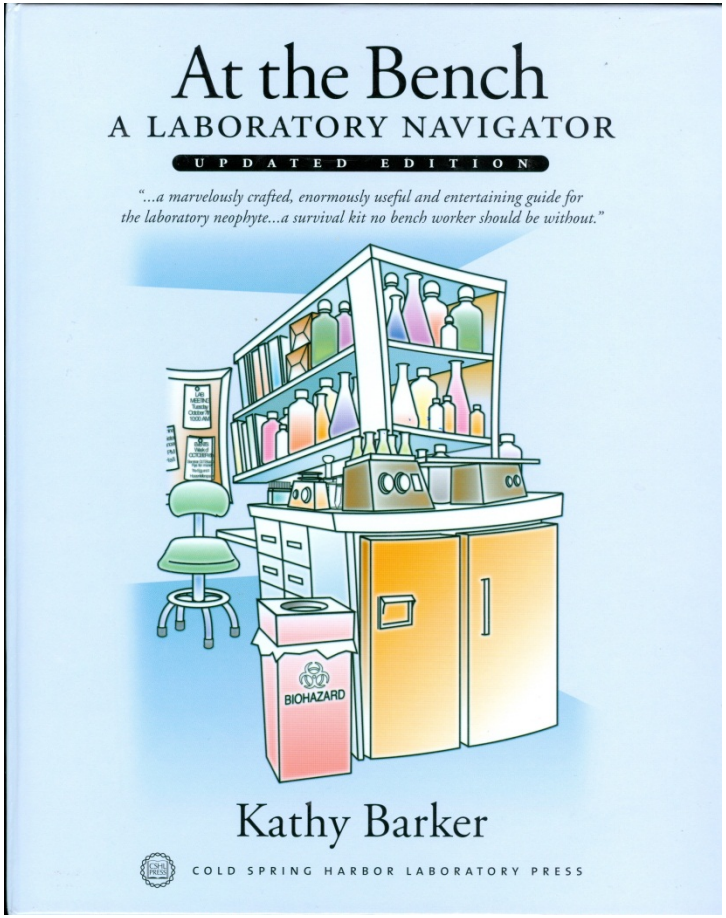
The 10 most common mistakes made in laboratory research. (HES)

1. Failing to promptly write up experiments and write out protocol before hand.
2. Failing to include the proper controls.
3. Not preparing enough material.
4. Failing to store properly store material promptly.
5. Allowing a distraction to screw up the addition of a key reagent.
6. Improper (usually insufficient) mixing or agitation. esp. frozen reagents.
7. Not discussing results with your supervisor/colleague before proceeding to the next step.
8. Not checking the accuracy of pipettor/pH meter (or other instrument....) before assay.
9. Calculation error made in making up reagent (factors of ten/failure to take into account water of hydration in calculation).
10. Calculation error made in determination of results.
11. Not labelling tubes/dishes etc.

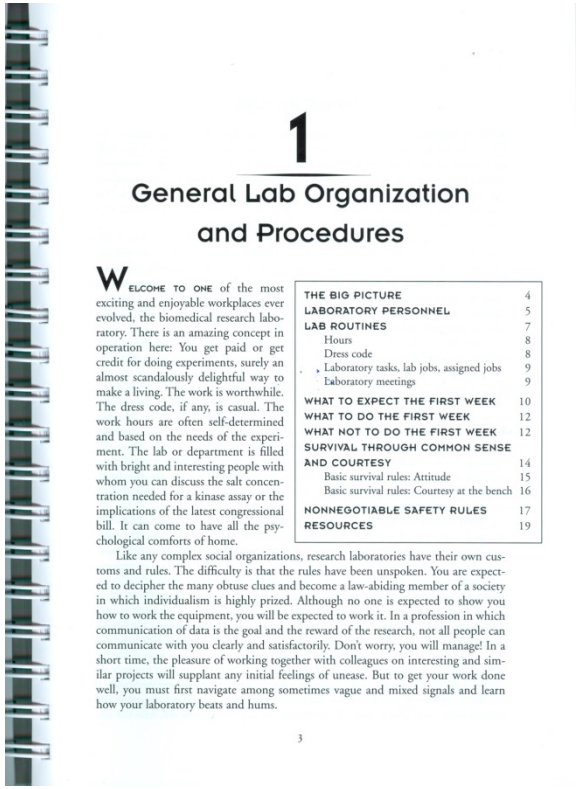
Documentation

- keep a copy of all protocols
- must be able to document **primary** literature references
- must include details of all experiments
- must have original protocol and your own protocol that is sufficient to allow another person to reproduce the experiment

Recommended book



There are very few books that described the laboratory environment for new researchers. "At the Bench" is probably one of the best and I recommend that you either buy or, perhaps preferably, ask you supervisor to but it for the lab.



1

General Lab Organization and Procedures

WELCOME TO ONE of the most exciting and enjoyable workplaces ever evolved, the biomedical research laboratory. There is an amazing concept in operation here: You get paid or get credit for doing experiments, surely an almost scandalously delightful way to make a living. The work is worthwhile. The dress code, if any, is casual. The work hours are often self-determined and based on the needs of the experiment. The lab or department is filled with bright and interesting people with whom you can discuss the salt concentration needed for a kinase assay or the implications of the latest congressional bill. It can come to have all the psychological comforts of home.

Like any complex social organizations, research laboratories have their own customs and rules. The difficulty is that the rules have been unspoken. You are expected to decipher the many obtuse clues and become a law-abiding member of a society in which individualism is highly prized. Although no one is expected to show you how to work the equipment, you will be expected to work it. In a profession in which communication of data is the goal and the reward of the research, not all people can communicate with you clearly and satisfactorily. Don't worry, you will manage! In a short time, the pleasure of working together with colleagues on interesting and similar projects will supplant any initial feelings of unease. But to get your work done well, you must first navigate among sometimes vague and mixed signals and learn how your laboratory beats and hums.

Presentations

- Basic area - why is it of general interest?
- Background relevant observations
- Hypothesis being tested - make a clear statement
- Describe basic experimental approach including methodology and alternative methodology
- Present results - describe the results and compare, when appropriate, to your own work and to literature results

Dissemination of Results

before computers

- rough results presented first and results for publication drafted later

today

- publication can be made directly from raw data

For the student:

- standardized instruction
- familiarity with departmental equipment
- appreciation for expectation of performance
- coupling of theoretical with practical training
- can concentrate on practical material without the distraction of other courses

For the professor

- reduction in need for instruction
- increased productivity of student during the summer..

Supervisor Expectations…

- Read the literature
- Ask questions (be inquisitive..)
- Keep regular hours (at least until you publish a paper..)
- Give lab talks (with enthusiasm..)
- Produce final tables/figures
- Organize data—cross references to computer files
- Properly store samples (archival if necessary)
- Properly dispose of samples.
- ***Be Intellectually Engaged!!!!***

Supervisor Expectations…

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- ***Be Intellectually Engaged!!!!***

Student Expectations…

- Reference Letter
- Valuable Experience
- A Paycheck..

Materials

- lab page from HES first labbook
- At the bench
- Gilson Guide to Pipetting
- Protocol sheet
- Handouts

Thesis

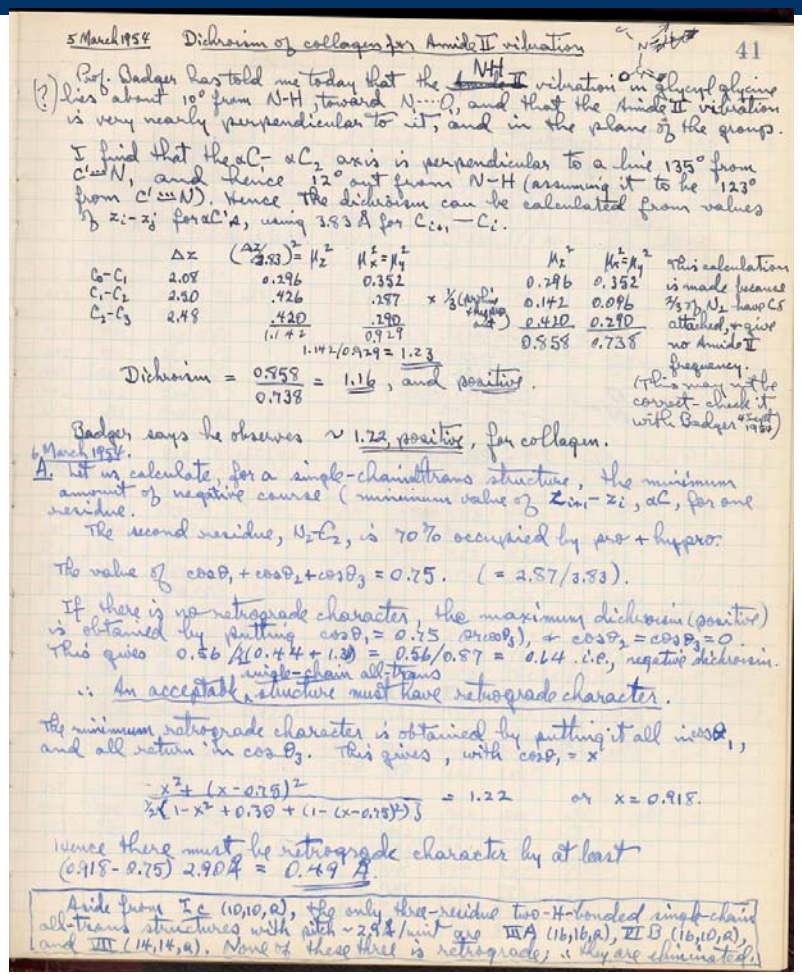
- Use “Table of Contents” function in Word
- Use Reference Manager or equivalent (Refworks)
- Protocols (Standard Operating Procedure (SOP)
- Appendices (raw data)

- Handouts
- Chapter 1
- Top ten list
- Protocol sample
- Flowcharts
- Sample lab book
- Merck index
- Organization of data in spreadsheet
- Software tools

Laboratory notebook

1. Title: e.g. Lab #1 - Microscopy and Examination of Living and Stained Cultures
2. Introduction: Briefly state why this experiment was conducted in your own words – do not copy the lab manual.
3. Objective: Briefly state what you are attempting to determine 4. Materials and Methods: If same as the manual then refer to the lab manual. If different from the manual state the differences. For your own understanding use flow charts to illustrate procedures.
5. Results: If possible, use table(s) and/or figure(s) to present raw data. **Provide brief descriptions of what the data mean.**
6. Discussion: Briefly discuss what you can conclude from your results. Sometimes experiments fail either because of an unanticipated variable or because of experimental error. If your results deviate from expectations, identify possible sources of error, provide alternative hypotheses, and suggest improvements for future experiments.
7. Questions: Briefly answer the questions given at the end of each lab.
8. Fill in the table of contents to permit easy orientation.
9. Sign and date each page. Signature of lab partners or advisors must be included. However, this is an essential procedure in government and industry labs.
10. Answer the Lab Rotation questions on the pages indicated.

Linus Pauling (Nobel Laureate) maintained labnotes….



5 March 1954 Dichroism of collagen for Amide II vibration 41

(?) Prof. Badger has told me today that the NH Amide II vibration in glycyl glycine lies about 10° from N-H toward N···O, and that the Amide II vibration is very nearly perpendicular to it, and in the plane of the group.

I find that the $\alpha C_i - \alpha C_{i+2}$ axis is perpendicular to a line 135° from $C'_i \cdots N_{i+1}$, and hence 12° out from N-H (assuming it to be 123° from $C'_i \cdots N_{i+1}$). Hence the dichroism can be calculated from values of $z_i - z_j$ for αC_i's, using 3.83 Å for $C_{i+1} - C_i$.

	Δz	$(\Delta z/3.83)^2 = \mu_z^2$	$\mu_x^2 = \mu_y^2$		μ_z^2	$\mu_x^2 = \mu_y^2$
$C_0 - C_1$	2.08	0.296	0.352		0.296	0.352
$C_1 - C_2$	2.50	.426	.287	× ⅓ (angle between hydrogen bonds)	0.142	0.096
$C_2 - C_3$	2.48	.420	.290		0.420	0.290
		1.142	0.929		0.858	0.738

1.142/0.929 = 1.23

This calculation is made because ⅔ of N_2 loop CS attached, gives no Amide II frequency. (This may not be correct - check it with Badger 9 Sept 1980)

Dichroism = $\frac{0.858}{0.738}$ = 1.16, and positive.

Badger says he observes ~ 1.22, positive, for collagen.

6 March 1954.

A. Let us calculate, for a single-chain trans structure, the minimum amount of negative course (minimum value of $\sum z_{i+1} - z_i$, αC, for one residue.

The second residue, $N_2 C_2$, is 70% occupied by pro + hyppro:

The value of $\cos\theta_1 + \cos\theta_2 + \cos\theta_3 = 0.75$. (= 2.87/3.83).

If there is no retrograde character, the maximum dichroism (positive) is obtained by putting $\cos\theta_1 = 0.75$ (or $\cos\theta_3$), & $\cos\theta_2 = \cos\theta_3 = 0$. This gives 0.56 / ½(0.44 + 1.00) = 0.56/0.87 = 0.64 i.e., negative dichroism.

∴ An acceptable single-chain all-trans structure must have retrograde character.

The minimum retrograde character is obtained by putting it all into θ_1, and all return in $\cos\theta_3$. This gives, with $\cos\theta_1 = x$

$$\frac{x^2 + (x - 0.75)^2}{\frac{1}{2}\{1 - x^2 + 0.30 + (1 - (x - 0.75)^2)\}} = 1.22 \quad \text{or} \quad x = 0.918.$$

Hence there must be retrograde character by at least (0.918 - 0.75) 2.904 = 0.49 Å.

Aside from I c (10,10,a), the only three-residue two-H-bonded single-chain all-trans structures with pitch ~ 2.9 Å/unit are III A (16,16,a), III B (16,10,a), and III (14,14,a). None of these three is retrograde; ∴ they are eliminated.

http://osulibrary.oregonstate.edu/specialcollections/rnb/

As with many scientists, Linus Pauling utilized bound notebooks to keep track of the details of his research as it unfolded. A testament to the remarkable length and diversity of Dr. Pauling's career, the Pauling Papers holdings include forty-six research notebooks spanning the years of 1922 to 1994 and covering any number of the scientific fields in which Dr. Pauling involved himself. In this regard, the notebooks contain many of Pauling's laboratory calculations and experimental data, as well as scientific conclusions, ideas for further research and numerous autobiographical musings.

Research Notebook 01
1922

Research Notebook 02
1922-1923, 1932, 1934, 1936, 1973, 1985

Research Notebook 03
1923-1925

Research Notebook 04
1923-1924, 1928-1930

Research Notebook 05
1924, 1929, 1933, 1935

Research Notebook 06
1929-1930, 1934

Research Notebook 07
1930, 1932-1934, 1936

Research Notebook 08
1930-1931, 1933, 1935-1936

Research Notebook 09
1932, 1934-1938, 1940-1941

Research Notebook 10
1933-1934, 1936, 1938

Research Notebook 13
1935-1936, 1938-1939

Research Notebook 14
1936-1939, 1949, 1952

Research Notebook 15
1935, 1937, 1968

Research Notebook 16
1935-1956

Research Notebook 17
1939-1941, 1971, 1988

Research Notebook 18
1936, 1938, 1955, 1967-1969, 1971-1975, 1978

Research Notebook 19
1941-1942, 1944, 1949, 1955-1956, 1959-1960, 1965, 1967, 1976-1978, 1980-1981

Research Notebook 20
1950, 1955-1960

Research Notebook 21
1958

Research Notebook 24
1953, 1956, 1962, 1963, 1967, 1968, 1969, 1970, 1973

Research Notebook 25
1958, 1964-1966

Research Notebook 26
1955, 1964-1969, 1974-1976, 1980-1982, 1987, 1990-1991

Research Notebook 27
1952-1954, 1960-1961, 1964, 1971-1972

Research Notebook 28
1951, 1953-1957, 1972-1975

Research Notebook 29
1957, 1986-1988

Research Notebook 30
1954-1958

Research Notebook 31
1966-1971, 1974-1976, 1980

Research Notebook 32
1965-1968, 1974

Research Notebook 35b
1938-1939, 1946, 1955, 1968, 1986-1988

Research Notebook 36
1980-1981, 1986-1987

Research Notebook 37
1971, 1983

Research Notebook 38
1980-1981, 1983, 1985, 1989

Research Notebook 39
1980-1981

Research Notebook 40
1988-1989

Research Notebook 41
1989-1990

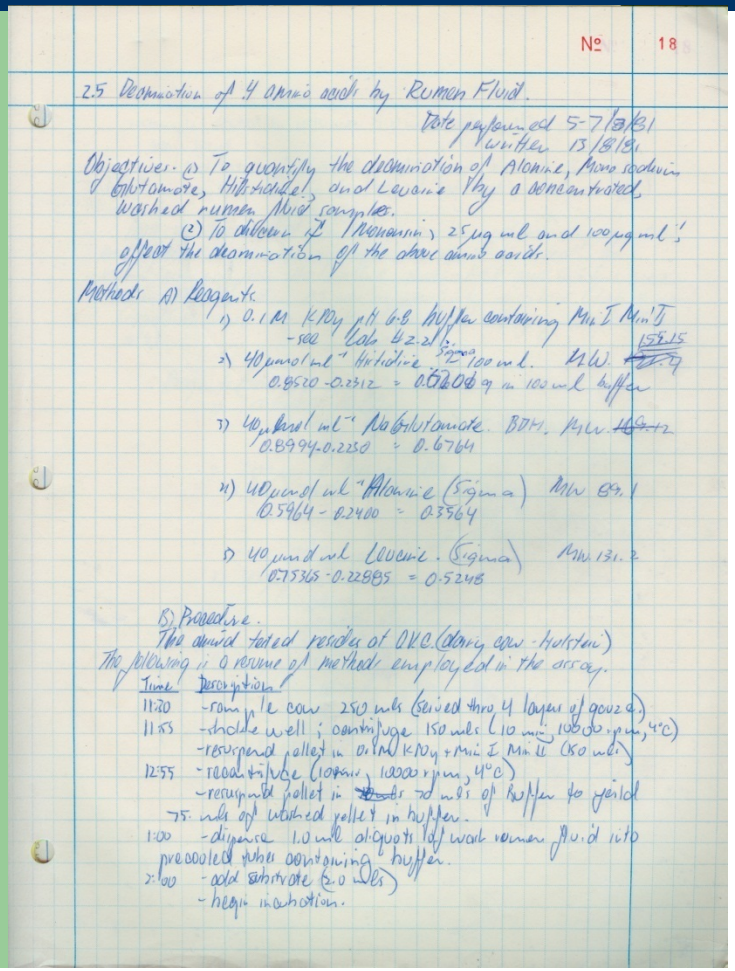
Research Notebook 42
1990

Research Notebook 43
1990-1991

Research Notebook 44
1991-1994

http://osulibrary.oregonstate.edu/specialcollections/rnb/

My first lab book….



№ 18

2.5 Deamination of 4 amino acids by Rumen Fluid.

Date performed 5-7/3/91
written 13/8/91

Objectives: (1) To quantify the deamination of Alanine, Monosodium Glutamate, Histidine, and Leucine by a concentrated washed rumen fluid sample.
(2) To determine if Neomycin, 25 μg/ml and 100 μg/ml, affect the deamination of the above amino acids.

Methods A) Reagents

1) 0.1 M KPO4 pH 6.8 buffer containing Mn I Mn II
- see Lab Book 2.2.1

2) 40 μmol/ml "Histidine" Sigma 100 ml. MW 155.15
0.8520 - 0.2312 = 0.6208 g in 100 ml buffer

3) 40 μmol/ml "MSG Glutamate. BDH. MW 169.2
0.8994 - 0.2230 = 0.6764

4) 40 μmol/ml "Alanine (Sigma) MW 89.1
0.5964 - 0.2400 = 0.3564

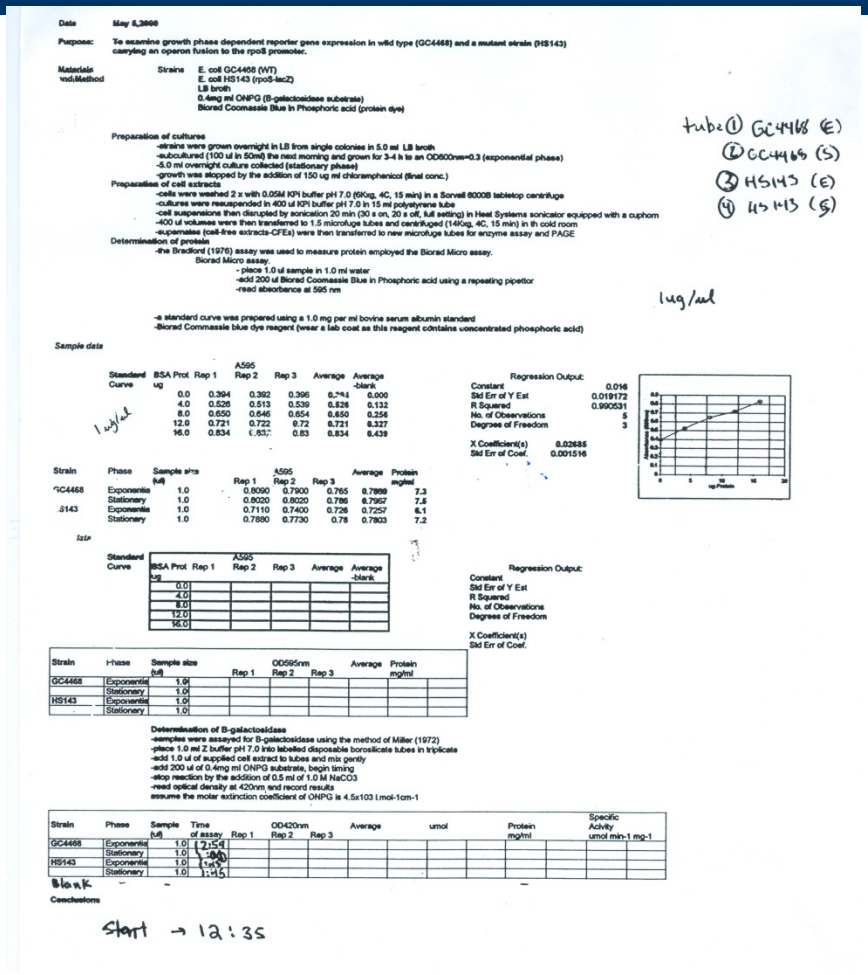
5) 40 μmol/ml Leucine. (Sigma) MW 131.2
0.75365 - 0.22885 = 0.5248

B) Procedure.
The animal tested received 0.K.C (dairy cow - Holstein)
The following is a resume of methods employed in the assay.

Time	Description
11:20	- rumen/le cow 250 mls (strained thro 4 layers of gauze)
11:55	- shake well; centrifuge 150 mls (10 min, 10000 rpm, 4°C)
	- resuspend pellet in 0.1M KPO4 + Mn I Mn II (50 mls)
12:55	- recentrifuge (10000 rpm, 4°C)
	- resuspended pellet in ~~Buffer~~ 20 mls of Buffer to yield
75:	mls of washed pellet in buffer.
1:00	- dispense 1.0 ml aliquots of work rumen fluid into pre-cooled tubes containing buffer.
2:00	- add substrate (2.0 mls)
	- begin incubation.

It is more important that notebooks be complete than neat (although that latter is nice too…).

How spreadsheet can be adapted to notebooks…



Date May 8,2008

Purpose: To examine growth phase dependent reporter gene expression in wild type (GC4468) and a mutant strain (HS143) carrying an operon fusion to the rpoS promoter.

Materials and Method

Strains
E. coli GC4468 (WT)
E. coli HS143 (rpoS-lacZ)
LB broth
0.4mg ml ONPG (B-galactosidase substrate)
Biorad Coomassie Blue in Phosphoric acid (protein dye)

tube① GC4468 (E)
② GC4468 (S)
③ HS143 (E)
④ HS143 (S)

Preparation of cultures
-strains were grown overnight in LB from single colonies in 5.0 ml LB broth
-subcultured (100 ul in 50ml) the next morning and grown for 3-4 h to an OD600nm=0.3 (exponential phase)
-5.0 ml overnight culture collected (stationary phase)
-growth was stopped by the addition of 150 ug ml chloramphenicol (final conc.)

Preparation of cell extracts
-cells were washed 2 x with 0.05M KPi buffer pH 7.0 (5Kxg, 4C, 15 min) in a Sorvall 6000B tabletop centrifuge
-cultures were resuspended in 400 ul KPi buffer pH 7.0 in 15 ml polystyrene tube
-cell suspensions then disrupted by sonication 20 min (30 s on, 20 s off, full setting) in Heat Systems sonicator equipped with a cuphorn
-400 ul volumes were then transferred to 1.5 microfuge tubes and centrifuged (14Kxg, 4C, 15 min) in th cold room
-supernates (cell-free extracts-CFEs) were then transferred to new microfuge tubes for enzyme assay and PAGE

Determination of protein
-the Bradford (1976) assay was used to measure protein employed the Biorad Micro assay.
Biorad Micro assay.
- place 1.0 ul sample in 1.0 ml water
-add 200 ul Biorad Coomassie Blue in Phosphoric acid using a repeating pipettor
-read absorbance at 595 nm

1ug/ul

-a standard curve was prepared using a 1.0 mg per ml bovine serum albumin standard
-Biorad Commassie blue dye reagent (wear a lab coat as this reagent contains concentrated phosphoric acid)

Sample data

Standard Curve ug	BSA Prot ug	Rep 1	A595 Rep 2	Rep 3	Average	Average -blank
	0.0	0.394	0.392	0.396	0.741	0.000
	4.0	0.526	0.513	0.539	0.526	0.132
	8.0	0.650	0.646	0.654	0.650	0.256
	12.0	0.721	0.722	0.72	0.721	0.327
	16.0	0.834	0.837	0.83	0.834	0.439

1 ug/ul

Regression Output:

Constant	0.016
Std Err of Y Est	0.019172
R Squared	0.990531
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	0.02585
Std Err of Coef.	0.001516

Strain	Phase	Sample size (ul)	Rep 1	A595 Rep 2	Rep 3	Average	Protein mg/ml
GC4468	Exponential	1.0	0.8090	0.7900	0.765	0.7880	7.3
	Stationary	1.0	0.8020	0.8020	0.786	0.7967	7.6
HS143	Exponential	1.0	0.7110	0.7400	0.726	0.7257	6.1
	Stationary	1.0	0.7880	0.7730	0.78	0.7803	7.2

late

Standard Curve ug	BSA Prot ug	Rep 1	A595 Rep 2	Rep 3	Average	Average -blank
	0.0					
	4.0					
	8.0					
	12.0					
	16.0					

Regression Output:

Constant	
Std Err of Y Est	
R Squared	
No. of Observations	
Degrees of Freedom	
X Coefficient(s)	
Std Err of Coef.	

Strain	Phase	Sample size (ul)	Rep 1	OD595nm Rep 2	Rep 3	Average	Protein mg/ml
GC4468	Exponential	1.0					
	Stationary	1.0					
HS143	Exponential	1.0					
	Stationary	1.0					

Determination of B-galactosidase
-samples were assayed for B-galactosidase using the method of Miller (1972)
-place 1.0 ml Z buffer pH 7.0 into labelled disposable borosilicate tubes in triplicate
-add 1.0 ul of supplied cell extract to tubes and mix gently
-add 200 ul of 0.4mg ml ONPG substrate, begin timing
-stop reaction by the addition of 0.5 ml of 1.0 M NaCO3
-read optical density at 420nm and record results
assume the molar extinction coefficient of ONPG is 4.5x103 l.mol-1cm-1

Strain	Phase	Sample (ul)	Time of assay	Rep 1	OD420nm Rep 2	Rep 3	Average	umol	Protein mg/ml	Specific Activity umol min-1 mg-1
GC4468	Exponential	1.0	12:54							
	Stationary	1.0	1:00							
HS143	Exponential	1.0	1:05							
	Stationary	1.0	1:15							
Blank										

Conclusions

Start → 12:35

Reference Texts

- SOPs and AUPs
- Merck Index
- Biochemical Data Book
- Maniatis et al

4XX3 Reference List

1. Anon., in *Handbook of Chemistry and Physics*, R. C. Weast, Ed. (The Chemical Rubber Company, Ohio, 1971).
2. Anon., in *Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench*, J. Roskams, L. Rodger, Eds. (Cold Spring Harbor Laboratory Press, New York, 2002).
3. J. H. Miller, in *A Short Course In Bacterial Genetics*, (Cold Spring Harbor Laboratory Press, 1992).
4. J. Sambrook, D. W. Russell, in *Molecular Cloning: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, New York, 2001), vol. 1.
5. R. F. Schleif, P. C. Wensink, in *Practical Methods in Molecular Biology*, (Springer-Verlag, 1981).
6. R. N. Giere, J. Bickle, R. F. Mauldin, in *Understanding Scientific Reasoning*, (Thomson Wadsworth Co., 2006).
7. K. Barker, in *At the Bench: A Laboratory Navigator*, (Cold Spring Harbor, New York, 2005).
8. Anon., in *Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench*, A. Mellick, L. Rodger, Eds. (Cold Spring Harbor Laboratory Press, 2007), vol. 2.
9. Anon., in *The Merck Index: An Encylcopedia of Chemicals and Drugs*, M. Windholz, Ed. (Merck & Co., Inc., 1976).
10. J. A. Pechenik, in *A Short Guide To Writing About Biology*, (Pearson Education, Inc., 2004).

MolBiol4XX3 Lab Notebook Marking Guide

Criteria	Very Poor	Poor	Good	Very Good	Excellent	Outstanding
Table of contents						
complete titles						
dates						
Signatures on appropriate pages						
Methods						
clearly written so that procedure can be reproduced						
appropriate decimals (I prefer "1.0" rather than "1")						
Complete descriptions of experiments						
Cross referencing where appropriate						
Results						
Figures						
Dated						
Lanes labelled						
Title						
Cross reference to table of composition of samples						
amounts loaded						
Tables						
Completeness						
Title						
Discussion						
Present						
Problems						
Analysis of results						
Comparisons to others						
Conclusions						
summary statement						
Recommendations for improvement						
Overall organization						
OVERALL ASSESSMENT						

Date May 5,2000

Purpose: To examine growth phase dependent reporter gene expression in wild type (GC4468) and a mutant strain (HS143) carrying an operon fusion to the rpoS promoter.

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② GC4468 (S)
③ HS143 (E)
④ HS143 (S)

Preparation of cultures
- -strains were grown overnight in LB from single colonies in 5.0 ml LB broth
- -subcultured (100 ul in 50ml) the next morning and grown for 3-4 h to an OD600nm=0.3 (exponential phase)
- -5.0 ml overnight culture collected (stationary phase)
- -growth was stopped by the addition of 150 ug ml chloramphenicol (final conc.)

Preparation of cell extracts
- -cells were washed 2 x with 0.05M KPi buffer pH 7.0 (6Kxg, 4C, 15 min) in a Sorvall 6000B tabletop centrifuge
- -cultures were resuspended in 400 ul KPi buffer pH 7.0 in 15 ml polystyrene tube
- -cell suspensions then disrupted by sonication 20 min (30 s on, 20 s off, full setting) in Heat Systems sonicator equipped with a cuphorn
- -400 ul volumes were then transferred to 1.5 microfuge tubes and centrifuged (14Kxg, 4C, 15 min) in th cold room
- -supernates (cell-free extracts-CFEs) were then transferred to new microfuge tubes for enzyme assay and PAGE

Determination of protein
- -the Bradford (1976) assay was used to measure protein employed the Biorad Micro assay.

Biorad Micro assay.
- - place 1.0 ul sample in 1.0 ml water
- -add 200 ul Biorad Coomassie Blue in Phosphoric acid using a repeating pipettor
- -read absorbance at 595 nm

1ug/ul

- -a standard curve was prepared using a 1.0 mg per ml bovine serum albumin standard
- -Biorad Commassie blue dye reagent (wear a lab coat as this reagent contains concentrated phosphoric acid)

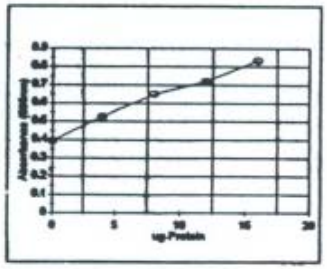
Sample data

Standard Curve	BSA Prot ug	A595 Rep 1	Rep 2	Rep 3	Average	Average -blank
	0.0	0.394	0.392	0.396	0.394	0.000
	4.0	0.526	0.513	0.539	0.526	0.132
	8.0	0.650	0.646	0.654	0.650	0.256
	12.0	0.721	0.722	0.72	0.721	0.327
	16.0	0.834	0.83	0.83	0.834	0.439

1 ug/ul

Regression Output:

Constant	0.016
Std Err of Y Est	0.019172
R Squared	0.990531
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	0.02685
Std Err of Coef.	0.001516



Strain	Phase	Sample size (ul)	A595 Rep 1	Rep 2	Rep 3	Average	Protein mg/ml
GC4468	Exponential	1.0	0.8090	0.7900	0.765	0.7880	7.3
	Stationary	1.0	0.8020	0.8020	0.786	0.7967	7.5
HS143	Exponential	1.0	0.7110	0.7400	0.726	0.7257	6.1
	Stationary	1.0	0.7880	0.7730	0.78	0.7803	7.2

late

Standard Curve	BSA Prot ug	A595 Rep 1	Rep 2	Rep 3	Average	Average -blank
	0.0					
	4.0					
	8.0					
	12.0					
	16.0					

Regression Output:
Constant
Std Err of Y Est
R Squared
No. of Observations
Degrees of Freedom

X Coefficient(s)
Std Err of Coef.

Strain	Phase	Sample size	OD595nm Rep 1	Rep 2	Rep 3	Average	Protein mg/ml

Date May 5,2000

Purpose: To examine growth phase dependent reporter gene expression in wild type (GC4468) and a mutant strain (HS143) carrying an operon fusion to the rpoS promoter.

Materials and Method

Strains
- E. coli GC4468 (WT)
- E. coli HS143 (rpoS-lacZ)

LB broth
0.4mg ml ONPG (B-galactosidase substrate)
Biorad Coomassie Blue in Phosphoric acid (protein dye)

tube① GC4468 (E)
② GC4468 (S)
③ HS143 (E)
④ HS143 (S)

Preparation of cultures
- -strains were grown overnight in LB from single colonies in 5.0 ml LB broth
- -subcultured (100 ul in 50ml) the next morning and grown for 3-4 h to an OD600nm=0.3 (exponential phase)
- -5.0 ml overnight culture collected (stationary phase)
- -growth was stopped by the addition of 150 ug ml chloramphenicol (final conc.)

Preparation of cell extracts
- -cells were washed 2 x with 0.05M KPi buffer pH 7.0 (6Kxg, 4C, 15 min) in a Sorvall 6000B tabletop centrifuge
- -cultures were resuspended in 400 ul KPi buffer pH 7.0 in 15 ml polystyrene tube
- -cell suspensions then disrupted by sonication 20 min (30 s on, 20 s off, full setting) in Heat Systems sonicator equipped with a cuphorn
- -400 ul volumes were then transferred to 1.5 microfuge tubes and centrifuged (14Kxg, 4C, 15 min) in th cold room
- -supernates (cell-free extracts-CFEs) were then transferred to new microfuge tubes for enzyme assay and PAGE

Determination of protein
- -the Bradford (1976) assay was used to measure protein employed the Biorad Micro assay.

Biorad Micro assay.
- - place 1.0 ul sample in 1.0 ml water
- -add 200 ul Biorad Coomassie Blue in Phosphoric acid using a repeating pipettor
- -read absorbance at 595 nm

1ug/ul

- -a standard curve was prepared using a 1.0 mg per ml bovine serum albumin standard
- -Biorad Commassie blue dye reagent (wear a lab coat as this reagent contains concentrated phosphoric acid)

Sample data

Standard Curve	BSA Prot ug	A595 Rep 1	Rep 2	Rep 3	Average	Average -blank
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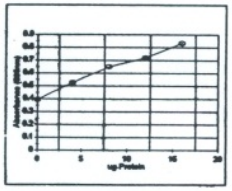
	16.0	0.834	0.83	0.83	0.834	0.439

1 ug/ul

Regression Output:

Constant	0.016
Std Err of Y Est	0.019172
R Squared	0.990531
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	0.02585
Std Err of Coef.	0.001516



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late

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	4.0					
	8.0					
	12.0					
	16.0					

Regression Output:

Constant	
Std Err of Y Est	
R Squared	
No. of Observations	
Degrees of Freedom	
X Coefficient(s)	
Std Err of Coef.	

Strain	Phase	Sample size (ul)	OD595nm Rep 1	Rep 2	Rep 3	Average	Protein mg/ml	
GC4468	Exponential	1.0						
	Stationary	1.0						
HS143	Exponential	1.0						
	Stationary	1.0						

Determination of B-galactosidase
- -samples were assayed for B-galactosidase using the method of Miller (1972)
- -place 1.0 ml Z buffer pH 7.0 into labelled disposable borosilicate tubes in triplicate
- -add 1.0 ul of supplied cell extract to tubes and mix gently
- -add 200 ul of 0.4mg ml ONPG substrate, begin timing
- -stop reaction by the addition of 0.5 ml of 1.0 M NaCO3
- -read optical density at 420nm and record results

assume the molar extinction coefficient of ONPG is 4.5x103 l.mol-1cm-1

Strain	Phase	Sample (ul)	Time of assay	OD420nm Rep 1	Rep 2	Rep 3	Average	umol	Protein mg/ml	Specific Activity umol min-1 mg-1
GC4468	Exponential	1.0	12:59							
	Stationary	1.0	1:00							
HS143	Exponential	1.0	1:03							
	Stationary	1.0	1:05							

Blank

Conclusions

Start → 12:35

Standard Curve

BSA Prot ug	A595 Rep 1	Rep 2	Rep 3	Average	Average -blank
0.0					
4.0					
8.0					
12.0					
16.0					

Regression Output:
Constant
Std Err of Y Est
R Squared
No. of Obeervations
Degrees of Freedom

X Coefficient(s)
Std Err of Coef.

Strain	Phase	Sample size (ul)		OD595nm Rep 1	Rep 2	Rep 3	Average	Protein mg/ml	
GC4468	Exponentia	1.0							
	Stationary	1.0							
HS143	Exponentia	1.0							
	Stationary	1.0							

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-add 1.0 ul of supplied cell extract to tubes and mix gently
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-stop reaction by the addition of 0.5 ml of 1.0 M NaCO3
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assume the molar extinction coefficient of ONPG is 4.5x103 l.mol-1cm-1

Strain	Phase	Sample (ul)	Time of assay	OD420nm Rep 1	Rep 2	Rep 3	Average	umol		Protein mg/ml		Specific Activity umol min-1 mg-1	
GC4468	Exponentia	1.0	12:59										
	Stationary	1.0	1:00										
HS143	Exponentia	1.0	1:15										
	Stationary	1.0	1:45										
Blank													

Conclusions

Start → 12:35