

LEE LAB LAB MANUAL VERSION 1.1**Contents**

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Welcome to the Lee Lab. I am very pleased you are joining my lab and I have every expectation that your tenure here will be a productive and enjoyable one.

My goals for the lab are straightforward

- Learn lots of new stuff
- Do good science
- Work hard
- Make the lab a fun place for you and other potential recruits

This manual is intended as a guide to working in my lab, what my expectations are, what are the general responsibilities; hopefully it will answer many of the questions you may have.

I. LEE LAB ADDRESS:

Department of Pharmacology
The University of Michigan Medical School
MSRB3, Room 2200
1150 West Medical Center Drive
Ann Arbor, MI 48109

Lab phone: 734-647-5557

LAB MEMBERS CONTACT INFORMATION

Angel Lee

Principle Investigator:

work: 734-647-6004 home: 734-477-0233 Cell: 734-775-8502

Email: awmlee@umich.edu

Emergency contact: David States, work: 734-615-9287 Cell: 734-250-0030

Home address: 2254 Belmont Road,
Ann Arbor, MI 48104

Heather Grifka

Laboratory technician

Cell: 1-734-417-0268

Email: hgrifka@umich.edu

Emergency contact: Duane Grifka, cell: 810-691-2022

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Apt 3
Ann Arbor, MI 48104

Soojie Yu

Laboratory technician

Home: 1-734-944-2415, Cell: 1-734-945-8890

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Emergency contact: Yun J Yu, home: 734-944-2415 work: 734-485-1923

Home address: 1299 Wedgewood Circle
Saline, MI 48176

Reda Jaber

Undergraduate research assistant

Cell: 313-443-3859

Email: redjaber@yahoo.com

Emergency contact: Ryan Jaber (brother), 313-802-0622

II. FOR NEW LAB MEMBERS

1. **ID badges** - contact Denise Gakle (1st floor office)
2. **Lab key** (one key opens all of the doors except for the tissue culture room) - contact Denise Gakle
3. **email address** – contact Dar-Wei Liao (1st floor office); you will be assigned a username and password.
4. **Library account** – contact Taubman library to set it up using your email username and password. This will give you online access to library resources from home.
5. **Safety Training by OSHA** –sign up online for a training session. Familiarize yourself with where the lab safety manual and chemical hygiene plan are kept. Familiarize yourself with where the Material Data Safety Sheets are kept.
6. **Radiation Safety Training by OSHA** – this is a separate training session from the one above. You will need to be certified prior to usage of radioactive materials. Sign up on line.
7. **Animal Work Training by ULAM** - if your work requires it you will need to sign up for 4 courses offered by ULAM before you can access the animal rooms and animals.
8. **Computers**
 - Lab G4 – this is the lab’s fastest computer and is to be shared by all.
 - Lee Instrument PC – this is ONLY to be used for instrumentation: luminometer and Kodak imager.
 - Microscope PC – to be used only for capturing digital images
 - **Computers are intended for data analysis and research-related work. Please don’t abuse this privilege.**
 - ***Absolutely NO ONE downloads/installs computer programs without prior approval. There can exist many incompatibilities that end up in time wasted.***
9. **Phone Policy**
 1. Phones are intended to be used for lab-related matters. The lab gets billed for all long distance calls. Always use the 1-800 number if available. If you need to make personal long distance calls during work, please get a phone card.

2. Please try to restrain yourself from holding long conversations on your cell phones. It is disruptive to others in the lab as well as disruptive to your own work.
3. While I understand the need to be in contact with your families for emergency situations, please exercise judgment if the phone call is not of an emergent nature. If you are in the middle of a procedure, I expect that you will take a phone message and call back at a time when it is not disruptive to lab work.

10. Emergency contact:

Notify me for all lab emergencies (fires, freezer meltdown, accidents etc.)

Contact information for all lab members are to be made available to all in the lab.

III. LAB POLICIES

Attendance and vacations

In general, the lab does not operate on a 9 to 5 schedule since we are a research lab. There will be mandated hours for attendance at specific lab activities, in particular weekly lab meetings.

If you will be unexpectedly absent from the lab, it is required that you call in to inform someone of this absence. You are expected to call in each of the days you will be absent and to make arrangements for lab work to be continued in your absence.

Vacations – you are expected to provide me with ample notice if you plan to take a vacation. In general I will be flexible about the time, however there will be instances when I will not be able to accommodate your requests, in particular, in the two months before a grant deadline, when it will be disastrous for key personnel to be absent. Therefore if there is an upcoming grant deadline (which I will let the lab know well in advance), I expect all lab personnel to be on hand for the two months preceding the deadline unless it is an unexpected family or medical emergency. In that situation, I expect that you will take the time necessary to get your affairs in order and to return to the lab as soon as possible and not to leave matters open-ended.

Otherwise, I expect at least 6 weeks' notice for a week of vacation time and longer notice if you are planning to take off for more than a week. However if there are major experiments scheduled on prearranged dates with lots of ensuing work (such as establishment of stable cell lines after FACS sort) you will need to work around that to minimize the disruption to your project and the burden imposed on your colleagues.

Time card (for Research Assistants)

This should be filled out in accordance with departmental policies.

Lab jobs

Lab jobs are to be rotated, see later. Timely fulfillment of lab job responsibilities is a part of your performance assessment.

Stock items

We share common buffers. Let the person responsible for making that item know when the reagent is running low. Where appropriate, remove a previously aliquoted vial for your own use, rather than going in and out of common vials.

IV. LAB NOTEBOOK

It is a nonnegotiable rule of the lab that every person in the lab must keep a clear and detailed laboratory notebook. The business of the lab is results and the communication of those results, and the lab notebook is the all-important documentation of each person's research. There are dozens of reasons to keep a clear and detailed lab notebook and only one – laziness – for not.

Unless you specifically ask for permission you will not remove a lab notebook from the lab (even if it is to bring it to the library) under any circumstances.

The lab notebook belongs to the lab and is to be left behind when the lab member leaves.

Rules:

1. Each lab notebook is to have a Table of Contents at the front of the notebook documenting the title of the experiments recorded in that notebook. Examples,

Optimization of retroviral transduction with pVPack into 293T

Cloning of the 3' UTR of mouse Gab2
2. Each experiment is to be entered into the lab notebook in chronological order. However it is useful to organize your lab notes into subsections, since most of the time you will have several experiments running in parallel not all related to the same objective.
3. Each sheet in your note book should have a date and page number for that day.
4. All primary data (computer printouts, plots, autoradiographs) should be permanently affixed in the notebook (punch holes with the 3-hole puncher and insert into the relevant parts of the notebook).
5. Format for each experiment: (1) Objective (2) Methods (3) Results (4) Summary – conclusions and future plans. Pitfalls. It is not sufficient simply to have the data in the notebook. What are the interpretations?
6. Every lab procedure is to be recorded, no matter how trivial it may seem. Examples: transformations should be recorded, preparation and freezing of cell stocks.
7. Outline new experiments (flow charts), particularly important for complicated subcloning projects

SAMPLE PAGE FROM LAB NOTEBOOK

DATE _____

Objective: Test new PCR primers for Gab1, Gab2 and Gab3
 Methods: rt-pcr

Template: 1st strand cDNA synthesis from RNA prepared on _____
 For exponentially growing 32D cells in WEHI

Primers from paper on Gab3 [cite reference]

[enter here the recipe for the master mix, the primer stocks, template dilutions etc.]

PCR conditions

File _____ Denature at _____ x _____ sec
 Anneal at _____ x _____ sec
 Extend at _____ x _____ sec
 # cycles _____

File _____

And so on.

Results [paste in gel picture with documentation]

Summary

1. strong band for Gab2 and b-actin
2. several bands for Gab1, one in correct position. Nonspecific priming probably because we were altering the PCR conditions when we discovered that the program was for a thermal cycle and not step cycle
3. no Gab3 band observed. Why? Problem with primers, PCR conditions or is our 32D not the same as Rohrschneider.
Possible steps:.....
4. 3' UTR – very faint smear. Possible problems – oligodT primer used was too short. Solution:

V. PRESENTATIONS, FIGURE PREPARATION AND DATA ANALYSIS

My lab has designated software programs for data analysis, preparation of figures and seminar presentations. Please make sure you are aware of what these are and how to use them.

Under no circumstances are you to download onto any of the lab computers programs that have not been approved by me. Additionally, no illegally obtained copies of software are to be downloaded or to be used for any part of data preparation related to this lab.

A. DATA ANALYSIS

Graphing (line graphs, bar graphs)

Deltagraph

Statistical analysis

Excel

B. FIGURE PREPARATION FOR MANUSCRIPT

Graphics for Assembly of figures

Canvas (we have both Mac and PC versions)

Document (text)

Microsoft Word

C. PRESENTATIONS

Seminars

Powerpoint or Canvas

D. IMAGING

Scans of images

Adobe Photoshop

If you produce figures in other than Canvas, you will need to convert them to Canvas in an acceptable format. Canvas is the program I use for grant preparation. There are no exceptions to this rule.

Additionally, do not use Excel to produce bar graphs and line graphs for publication. Deltagraph produces superior plots. If you use Excel I will ask you to convert them to Deltagraph before any manuscript is submitted.

As I work from home and can only access the Lee Lab G4, please make sure that figures, data files, presentations and manuscripts are on the G4 and up-to-date. If you work at home and have files on your home computer, make sure these are downloaded to the Lab G4 (make sure your computer is not infected). If such files are not on the G4, it means that they don't exist for me and will result in delays in manuscript submissions, graduation etc.

VI. LAB JOBS

Equipment maintenance

1. Each piece of equipment in the lab will be assigned a “champion”. The champion must know how to operate the piece of equipment in question, recognize problems or impending problems and learn what to do about repairs.
2. Pipettors – each lab member has a set of pipetman that he/she is responsible for maintaining (periodic calibration, replacement of minor parts such as gaskets, O-rings). Professional maintenance is grossly overpriced as many of the services can be performed by ourselves (such as calibration and parts replacement).
3. Common pipettors (PCR, tissue culture, radioactive) – will be assigned a champion.

Equipment	Champion	maintenance
Tissue culture room (hood, incubators, Sorvall centrifuge, water bath)	W. Liu	(1) CO2 tanks, (2) tissue culture hood water in humidifying pans (change every 2 weeks), (3) waste, (4) change water in water bath (5) supplies stocking (6) change brushes
Liquid nitrogen tanks	W. Liu	Make sure that Cryogenics is filling on schedule. Periodically (once a month) monitor level of LN2 in tank. Update fill date sheet on wall.
Fume hood	W. Liu	Call for repairs, monitor air flow
Power supplies (6 in total)	W. Liu	Make sure they are functional at all times.
Microwave	W. Liu	Keep clean
Recirculating water bath	W. Liu	Maintain level of 50% water/50% methanol
Water baths (5 in total, one in cold room)	Reda	(1) maintain water level – never let them go dry (2) Once a month change the water in the 3 water baths) – clean, rinse out and fill w/ dH2O; (3) once every few months, change water in the large shaking water bath
PCR machines	W. Liu	Know how to run diagnostics, routine maintenance
Computers	W. Liu & K. Kaur	(1) Know how to run Norton utilities, (2) boot from Norton, (3) periodic check with Norton antivirus, (4) interface with Dar-Weia
HP Laser printer	W. Liu & K. Kaur	Maintenance, order new toner cartridge when needed(BEFORE you change cartridge, remove cartridge and shake several times to redistribute the tone, frequently that will significantly prolong the life of the cartridge)

Bench top centrifuges (4 in total)	K. Kaur	Monitor performance; pay attention to the noise they are generating, change liners periodically
Freezers (-20 x 2, -80, cold box)	K. Kaur	(1) monitor temp levels (2) organize a team effort defrost (3) keep -80 as frost free as possible (and humming)
Stirrers/heater	K. Kaur	Keep enamel surface clean (scream at the person who leaves messes)
Common pipettors	all	Routine maintenance
Balances	all	Keep clean (scream at the person leaving messes), calibrate
Eppendorf general purpose centrifuge	K. Kaur	Know how to operate and troubleshoot. Keep clean.
Microscopes	A. Lee & K. Kaur	Alignment, bulb replacements
96-well plate reader	A. Lee	
Turner luminometer	A. Lee	
Sonicator	A. Lee	
HPLC	A. Lee	
Cytospin	A. Lee	
Spectrophotometer	K. Kaur	

LAB JOBS

These assignments will continue throughout the duration of your tenure in the lab unless some one complains or when new members join.

1. Common buffers –

These are to be used by everyone in the lab so please pay extra attention to their preparation.

Reda Jaber

1. Protein electrophoresis buffers
 - Reservoir buffer
 - Acrylamide
 - 4X lower gel
 - 4X upper gel buffers
2. Transfer buffer – 10X Tris-Glycine
3. ECL buffers
 - 10X TBST
 - TBS
4. Monthly radiation surveys; disposal of radioactive waste
5. 50 X TAE
6. 10 X TBE
7. Ampicillin stocks
8. Agar plates
9. Disposal of biohazard waste

W. Liu

1. Protease inhibitor reagents
 - Aprotinin
 - Leupeptin
 - Pepstatin
 - PMSF

- Vanadate
- Benzamide
- DTT

2. Tips and Tubes – make sure we have adequate supply of racked and autoclaved tips and tubes. During the academic year supervise the workstudy student to do this.

2. Glassware

During the academic year, I employ a workstudy student to wash dishes.

At other times you will be responsible for washing out your own glassware. This should not a big chore since we use primarily disposables.

Distinguish between the different “classes” of glassware in the lab: TC – tissue culture only; “RNA”- RNA work only.

If you use detergent to wash the glassware make very very sure that all the soap is rinsed out.

Give the dishes a final rinse with deionized water and then put them in the oven to dry.

Put away the glassware in a timely fashion.

3. Racking tips and tubes during term breaks

The months of Dec-Jan (3-4 weeks), May, June, July and August – we will not have a work study student to do the tip racking. During these months, you will rotate on a monthly basis to perform this task and ensure that the lab has adequate supplies.

4. Everyone’s responsibility

Before you leave for the day, make a sweep of your bench area, the –20 and –80 freezer areas as well as the tissue culture area (benchtop, hood, incubator) to make sure that no valuable reagents have been left out.

We had an unfortunate incidence when a box of our valuable reagents (modifying enzymes) was left out for 9-10 hrs (value of reagents is over \$1800 if we have to replace items). This necessitated the time consuming task of testing of each reagent.

VII. ORDERING

Ordering procedures

1. If you find that certain supplies or reagent are running low, put the item on the board, together with the **catalog number**.
2. Online Orders will be placed only by me.
3. Orders requiring POs will be filled out by you. After authorization from me you will need to give the order to Ingrid. When you get the PO you will need to call the vendor. Date the order form and record confirmation number.
4. All orders will require authorization from me.
5. Determine if an order can be placed through the core. If it can, then place the order through the core (location in MSRB2) – no shipping cost and usually a discount. If not, proceed to obtain a PO from Ingrid – this can take 1-2 days so plan in advance
6. You will be responsible for tracking orders that you placed.
7. Packages that arrive must be signed and dated, the packing slip to be placed in the appropriate location. All perishables must be stored immediately. Data sheets must be dated and filed.

Computer files

We have Excel files for all chemicals ordered in the lab in the Lee Lab G4. Look for the file in Lee Lab Budget. NEVER MODIFY.

Reagents storage location

1. room temp items are stored in chemical cabinet, dessicator or on the shelf above the balance
2. 4 deg items are stored in the cold box (either in dessicator or outside). Many of our antibodies are stored in the cold box
3. -20 items are in the -20 freezer in the lab area.

When in doubt where an item is stored, look up the item in the catalog to determine storage conditions.

What to do when you get a package

1. if you receive a package for the lab, please sign your name and most importantly, date the packing slip.
2. if it is a chemical or some other reagent, make sure that you write the date on the reagent bottle, vial or whatever it is that you got. This is particularly important for perishable reagents which have expiration dates. This will avoid having to go back to the paperwork to find that piece of information.
3. specification sheets (data sheets) for reagents - this should also be dated and filed in the Chemical reagent notebook, under the appropriate section. If you don't know where this is, ask me. **DON'T JUST LEAVE IT ON YOUR DESK, IT WILL SIMPLY GET SUCKED INTO THE GREAT JUNK PILE BEYOND.**
4. Instruction manual for kits - this should also get filed in a LAB PROTOCOL note book (shelved with the common lab stuff). Don't file it with your notes because nobody else will be able to find it. If you must have your own copy, either download it from the web or else make a copy.

Please help the lab function efficiently by doing this. Many of our reagents are shared and if one person doesn't do his/her part, it will affect the entire lab.

VIII. Meetings

Required attendance: weekly lab meetings. Lab presentations are pre-assigned. If you are unable to do it, make sure you swap with someone ahead of time.

Suggested attendance:

1. Departmental seminars: (almost) every Wednesday at noon - check the departmental web page for notification
2. Student seminars: (almost) every Friday. Required for graduate students. Suggested for others. Make sure we all attend our own graduate students' presentations
3. Seminars at large - I will inform you of any interesting seminar. You can also locate them by going to the departmental web site.

IX. Common lab protocols

Most of these are available on the Lab G4. To access lab computers:

1. Go to Chooser and click on Apple Share
2. Access Lee Lab G4
3. User name: awmlee Password: mrmoh

Download the desired protocols and modify your own copies, not the archival lab copy!

For recipes of common buffers-

There is an index card holder next to the first desk as you enter from the side of the shaking incubator.

X. Distribution of Lab Reagents

Distribution

All requests for lab reagents (including plasmids, antibodies, cell lines and other valuable and expensive reagents) should be referred to me.

Third party reagents

The lab has many plasmids, antibodies, growth factors (CSF-1), cell lines that have come from other laboratories or institutions under proprietary conditions (i.e. I have to sign a material transfer agreement which is a legally binding document). *Hence these reagents cannot be distributed to third parties, not even in small quantities.*

Reagents that have been generated in our lab

In general, I am willing to distribute reagents produced in our lab that have been described in publications. However there may be instances where the possibility of a patent exists. Again all requests, however informal (e.g. from other members of the department) must be directed towards me.

Commercially available reagents

Inexpensive chemicals (generally these would be chemicals stored in the room temperature chemical cabinet) - I don't mind members from other labs borrowing occasionally. However, this practice must be terminated if it becomes a common occurrence.

Disposable plasticware - if some one wants to borrow these items (in small quantities), make sure they replace what they borrow. We do not support other labs' research endeavors.

Other reagents – unless there is an established collaboration, reagents such as growth factors, antibodies, signaling reagents etc. are not to be borrowed. My policy is that my research funds support the research ongoing in my lab and not other labs. “Borrowing” reflects poor planning, laziness and bad lab practices. Under no circumstance is isotope to be transferred to another lab- this is against NRC regulations.

Likewise, except for the most inexpensive chemicals that we only need a very small amount of, no lab member should make a habit of borrowing consumables from other laboratories.

XI. Lending of Lab Equipment

I am usually willing to help other labs out in cases of emergencies. However no lab equipment should leave our lab area. For example, if some one wants to borrow a gel box, they can run their gel in our gel box in our area. Should lab equipment be damaged by members of other laboratories, they will be held responsible for repairs.

Specialized lab equipment

HPLC, fluorescence microscope, electroporator will not be available to other labs.

Other specialized equipment

ELISA plate reader, PCR machine, luminometer, spectrophotometer - individuals from other labs may use them once in a while (defined as once in several months) but only if it doesn't inconvenience any of our lab members. In any case, no one from other labs should leave their data on our computers (they will be deleted) and they should use their own supplies (cuvettes, tubes, plates etc.). Should any person be found to abuse our instruments (not checking with us, leaving it turned on, being messy etc.) he/she will have the privilege forfeited.

General equipment

Micro Centrifuges, speed-vac, shaking incubator- absolutely no organics or radioactivity!!!!

The policy is the same as for "other specialized equipment".

Freezers (-80 deg)

I brought my -80 freezer with me from WashU and is not a departmental freezer. There are departmental freezers (I have chosen to maintain our own). We cannot accommodate other individuals' requests for usage of our -80 freezer due to access and space issues.

LN2 containers

These units belong to our lab. There are departmental LN2 containers for labs (I have chosen to maintain our own). We cannot accommodate other individuals' requests to store items in our LN2 tanks. I spend over a thousand dollars in LN2 costs every year to keep them filled (plus many phone calls to maintain that service) and we need the space for our own reagents.

Tissue Culture Incubators and Hood

Unless it is an emergency, our incubators will not be available to other labs. We do a large amount of cell culture, many of them long term and we do primary cell cultures. Should labs need to borrow some space for a short (a couple of days at

most), make sure their cultures are not infected with bacteria, yeast and other fungi before taking up residence in our space.

Our tissue culture hood is not available at any time to other labs.