



**GUIDELINES
MOLECULAR NEUROBIOLOGY
LABORATORY**

GUIDELINES FOR THE USE OF THE MOLECULAR NEUROBIOLOGY LABORATORY

Edition October 2007

Updates will be posted at the facility's website.

Contact: Luis Quiñones "lquinone@neuro.upr.edu"

About the Molecular Biology Laboratory

The molecular neurobiology laboratory is a shared facility sponsored by the RCMC program at the University of Puerto Rico.

It is the purpose of the facility to enable researchers from the Institute of Neurobiology, and from other UPR dependencies, to investigate molecular aspects of their projects without having to establish molecular laboratories of their own. To this end, the facility provides space, equipment, basic reagents, limited technical support, and consultation services that are needed for standard molecular experimentation.

At present there are 7 workbenches available (**appendix C, photo no. 1**). The laboratory is well-equipped for all types of standard molecular biology techniques involving DNA, RNA and protein. A list of the available equipment can be found at the facility's website.

Special pieces of equipment include: a dedicated clean room for the assembly of PCR reactions and RT-PCR experiments and a real-time thermocycler (**appendix C, photo no. 5**). We have a Milli-Q Synthesis A10 water purification system for the generation of high purity water (**appendix C, photo no. 3**). No experiments with radioisotopes are possible at this time.

In addition, we provide a number of standard reagents, buffers, bacteriological media and plates, and disposable plastic materials that are essential for basic molecular biological experiments. See the appendix for a list of the materials.

All special reagents, such as modifying enzymes, polymerases, kits, special vectors, DNA and RNA standards, fluorescent probes, primers and oligonucleotides have to be provided by the users.

It is understood that users of the facility are familiar with standard molecular biological concepts and techniques. We are not a general training facility. We will however introduce users to properly using the available equipment and we will assist users with trouble-shooting techniques and procedures.

All documents concerning the use of the facility can be downloaded from the facility's website which can be accessed at www.neurobio.upr.clu.edu or at rcmi.rcm.upr.edu .

Instrument manuals and a limited number of books and printed pdf files are available in the bookshelf in the office area of the laboratory.

General molecular biology literature available in the laboratory

Sambrook et al. (1989) Molecular cloning. A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York

Ausubel et al. (2000) Current Protocols in Molecular Biology. John Wiley & Sons, Inc. New York, Chichester, Brisbane, Toronto, Singapore

These two publications are frequently updated and represent the "gospel" of molecular biological technology. In addition, get a free subscription to BioTechniques. It is a treasure chest of special recipes. To subscribe, go to www.BioTechniques.com.

Where to find us and when

The laboratory occupies several rooms at the basement level of the south wing of the Institute of Neurobiology. The address is:

Institute of Neurobiology, UPR-MSB
201 Blvd. Del Valle
San Juan, Puerto Rico 00901

Official laboratory hours are from 8 am to 4 pm Monday through Friday. On most days the laboratory is open until at least 7 pm. In particular cases, and following approval by the facility's overseeing committee, registered users may get a key and work at any time.

Whom to contact

For questions regarding the RCMI program at the University of Puerto Rico:

Dr. Emma Fernández-Repollet
Director, RCMI Program
efernandez@rcm.upr.edu
787-763-9401

For questions about the current grant period for the Molecular Neurobiology Laboratory:

Dr. Joshua Rosenthal
Grant Director, Molecular Neurobiology Laboratory
jrosenthal@neuro.upr.edu
Tel. (787) 758-2525 Ext. 7000
(787) 721-4149 Ext. 272
Fax: (787) 725-3804

For technical and scientific questions regarding everyday use of the laboratory:

Luis Quiñones
Laboratory Technician
lquinone@neurobio.upr.clu.edu
787-724-1543

For applications to use the facility and for complaints contact the overseeing committee:

Dr. Joshua Rosenthal; jrosenthal@neuro.upr.edu; 787-721-4149 Ext. 272
Dr. Maria Sosa; msosa@rcm.upr.edu; 787-724-2158 (office); 787-723-8410 (lab)
Dr. Jonathan Blackburn; jmblagbu@neuro.upr.edu; 787-724-2184

Materials provided by the facility

All recyclable and disposable glass- and plasticware:

- Dishes
- Beakers
- Bottles
- Flasks
- Graded Cylinders
- Centrifuge tubes and adapters
 - Polypropylene and polycarbonate for Beckman and Sorvall centrifuges
 - Large and small green caps for low-speed centrifuge
- Bacterial incubation tubes, polycarbonate, disposable
- Eppendorf tubes and centrifuge adapters
- Eppendorf tubes, amber, for storage of fluorescent probes
- Regular PCR tubes
- Smartcycler tubes
- Cryogenic tubes
- Small volume storage tubes with color-coded caps
- Pipette tips
 - 10, 20, 100, 200, 1000 μ L
 - with and without aerosol protection
 - autoclaved or not autoclaved
 - Gel-loading tips
- Transfer pipettes
- Multiwell dishes
- Petri dishes

Parafilm
Adhesive tape
Aluminum foil
Saran wrap
Whatman filter paper
Hybridization bags
Latex gloves, L, M, S,

All bacteriological plates and media
LB, NZY
Ampicillin, Kanamycin
Reagents for blue-white selection
All regular salts, solvents and reagents
Gel running and transfer buffers

Maintenance materials for PCR machines
Maintenance materials for Milli-Q system
pH meter standard solutions, pH 4, 7, 10
Quarz fluorescent cells for Quantech fluorometer

GENERAL RULES:

To ensure successful use for all, certain rules have to be set and enforced, if necessary. We all are at times inclined to think that our own research is the most important, but this understandable self-indulgence must not lead to negatively influencing the equally important research of others.

Therefore:

Use common sense and respect when interacting with the other users of the laboratory.

Admission to the molecular neurobiology laboratory

New users have to submit an application to the overseeing committee, stating briefly the project and the required space, procedures and instruments. For each new user a **user registration form** has to be submitted by the responsible principal investigator. **The form is attached in the Appendix A.**

Lab bench and storage space for reagents are assigned on a first-come first-served basis, as long as space is available. Preference is given to resident scientists from the Institute of Neurobiology. Use of specific equipment by guests may be arranged according to instrument availability.

Failure to comply with the lab rules may lead to revocation of admission to the facility by the supervisory committee.

On termination of a project the user has to clean up his bench and storage space before he can be signed out. If he or she had been provided with a key, it must be returned.

IMPORTANT: On publication of data obtained using the molecular neurobiology facility the following sentence should be included in the acknowledgement:

“This investigation was supported, in part, by a ‘Research Centers in Minority Institutions’ award, G12RR-03051, from the National Center for Research Resources, National Institutes of Health.”

Your cooperation in this respect is vital to the success of the program and the support of this facility.

Copies of publications acknowledging support to the RCMi Program should be sent to our office to include the reference in our progress report:

RCMI PROGRAM
OFFICE 621-A
6th FLOOR
UPR MEDICAL SCIENCE CAMPUS

Tel: (787) 763-9401
Fax: (787) 758-5206
E-mail: efernandez@rcm.upr.edu

Rules of engagement

You are the master of your lab bench. What you do on your bench is your business alone, as long as lab safety and the work of others are not impaired by your activities.

Stick to your bench space and do not invade the space of your bench neighbors, however tempting a certain free spot may look.

IMPORTANT: Never take other user's reagents, kits and tools without asking their permission first. Most people are willing to help you out if you ask. Also, do not remove common use reagents or supplies from the premises of the facility, nor keep them solely in your bench/work area, where others may not be able to find them or access them.

Even if you prefer creative chaos on your own bench, keep shared areas clean and organized. This means, clean up after you are done with your work (e.g. gel boxes, etc.).

Items (bottles, containers, kits, etc.) that you store in general storage areas (e.g. all freezers and refrigerators are shared) have to be properly labeled with content and with your name. Don't expect others to be able to decipher your artistic scribble. Try to write legibly. Keep in mind that freezers and refrigerators need periodic thawing and cleaning. At times like that it comes handy if you can identify your precious samples.

Procedure in case of problems involving lab user's behavior

In a shared laboratory there are always points of friction between lab users. That is normal and results from different temperaments and work habits. Most of these minor conflicts can easily be addressed and managed. Most of the time they are settled between the individual users and don't need intervention.

If a user complains to Luis Quiñones about another user of the lab, they will talk to the respective person and try to solve the conflict.

In case a conflict cannot be resolved by talking to the involved users, a meeting with the concerned PIs will be held.

In cases of severe or repeated disruption of lab security or other disturbances affecting work conditions in the lab, a written report will be submitted to the overseeing

committee. This committee will then meet and decide whether access to the facility will be revoked. The committee's decision will be final.

SPECIFIC GUIDELINES

Safety

Some of the reagents we use in molecular biology are potentially harmful to your health. Make sure you understand the hazards involved. An orange binder with *Material Safety Data Sheets* can be found in the catalog shelf. If you are uncertain, ask staff personnel. Wear gloves and protective clothing whenever necessary.

Accidents happen to everybody! If one happens to you, don't cover it up! Clean up by yourself or, if a hazardous substance was involved, call staff personnel for help. If an accident resulted in damage of an instrument or a piece of equipment, notify staff personnel immediately so that replacement and/or repair can be initiated. **A problem report sheet can be found in the appendix B.** This sheet is also to be used to report damage or malfunction of a piece of equipment.

If an accident results in personal injury, always notify staff personnel or, in case we are not available, call others for help.

A first aid kit and a list with emergency telephone numbers is located at the lab technician's desk, next to the telephone.

We hope you will never need them, but familiarize yourself with the location of fire extinguishers, the emergency shower (you don't need to test it) and the eye-wash installation.

Literature on lab safety available in the laboratory:

Stricoff, R.S. & Walters, D.B. (1995) Handbook of Laboratory Health and Safety. 2nd edition. John Wiley & Sons, Inc. New York, Chichester, Brisbane, Toronto, Singapore

*A binder with **material safety data sheets** of reagents used in the lab.*

*Also, keep in mind that most suppliers of reagents have websites, where **material safety data sheets** can be inspected and downloaded.*

Food and drink

No food and drinks are allowed at the lab benches and in the refrigerators and freezers at any time. A special small FOOD ONLY-labeled refrigerator and a coffee maker are located in the office area and in the lunch room.

Sharps and waste disposal

Needles, razor blades, broken glass and other sharp or pointed objects are to be disposed into the provided containers. Never throw them into the general waste baskets.

Potentially hazardous reagents (mostly organic solvents, phenol, paraformaldehyde solutions, etc.) have to be collected in the provided labeled bottles under the sink at the 104 lab.

Bio-hazardous materials such as used bacteriological plates, media and bacterial cultures, must be autoclaved before they can be disposed of in the waste.

Radioactivity

Although principal investigators, lab staff and users of the facility may hold personal licenses for handling radioisotopes, the molecular biology facility as such is not licensed for use of radioisotopes at this time.

Therefore there is only one applicable rule:

NO radioisotopes are to be stored or used in the laboratory at any time!

Use of the FORMA -70°C freezer

The FORMA -70°C freezer (**appendix C, photo no. 6**) is a delicate and expensive piece of equipment. It is meant exclusively for storage and is definitely not a cold experimentation area. Don't abuse it as a rapid freezing device. It should be opened only as long as it takes to identify and take out stored items. Leaving the door ajar for extended times may destroy other peoples reagents, such as competent cells, or may even interfere with proper function of the freezer.

If you need more time to sort out cells or reagents, do it out of the freezer in a polystyrene box with dry ice.

Literature on cryopreservation available in the laboratory

Brockbank et al. (2001) Cryopreservation Manual. Published on the FORMA Scientific website.

Manual for the FORMA Model 8523 Biofreezer. Manual No. 7028516 Rev. 2

Supplies

If you happen to be the lucky one who opens the last jar or bottle of a general chemical reagent or plastic disposable, please, write the opening date on the jar, then notify staff personnel so that we may reorder in time.

Use of the PCR facility

Use of the PCR facility or any of the thermocyclers requires special authorization and introduction for new users. Incompetent use of thermocyclers or the RNA room may disturb other user's experiments. Contact Luis Quiñones if you want to run PCR reactions.

Users have to provide their own PCR reagents.

The PCR room is an installation designed to keep contaminations out of our tubes for reliable diagnostic and quantitative PCR reactions. Therefore, only use and store PCR reagents in the freezer and use the pipettors, heat block and spinfuges only for the setup of PCR reactions. Always keep bacterial cultures and libraries out of this area. Wear gloves at all times.

The back room is dedicated to RT-PCR, and all equipment there is to be used exclusively for the setup of RT-PCR reactions.

IMPORTANT! Before starting to work in the RNA area, make sure the UV sterilizing lamp is switched off!

Use of the Cepheid SmartCycler

The Cepheid SmartCycler is a computer-controlled thermocycler that allows the detection in real time of one or several PCR products using fluorescent probes (**appendix C, photo no. 5**). Access to the computer and to the SmartCycler software is password-protected.

IMPORTANT! The DELL PC that commands the SmartCycler is not to be used for anything but running this instrument. The proper function of the SmartCycler software depends on specific settings in the computer's operation system. Therefore nobody is allowed to make any changes to the WINDOWS settings on this PC.

Users have to get an introduction to proper use of the instrument and software, and have to open an account. Contact Luis Quiñones for information.

Recommended reading on PCR technology

McPherson et al. (1991) PCR - A Practical Approach. Oxford University Press. Oxford, New York, Tokyo

Get a free subscription to BioTechniques. It is a treasure chest of special recipes, many of them on PCR applications. To subscribe go to www.BioTechniques.com.

Two copies of the Cepheid Smartcycler manual are available on the bookshelf in the office area of the laboratory.

A copy of the HybAid Thermocycler manual is located on the bookshelf in the office area of the laboratory.

A summary on real-time PCR technology will be published soon on the facility's website.

Use of the autoclave

Always make sure that enough, but not too much filtered water is added into the autoclave before starting. Make sure that the pressure is down before attempting to open the kettle. First time users contact Luis Quiñones before autoclaving.

Use of centrifuges

Always double-check that centrifuge rotors are properly balanced! Although unbalanced small table-top centrifuges and spinfuges are not as lethal as an unbalanced ultracentrifuge may become, spindle bearings may get damaged or the machine may jump off the bench.

Special instructions for using the Sorvall centrifuge and for the Eppendorf refrigerated microcentrifuge can be found at the facility's website.

Don't leave the lids of refrigerated centrifuges open for extended times. We live in the tropics after all.

It does not happen often, but centrifuge tubes may break under the mechanical stress. If you are unlucky and produce a spill during a centrifuge spin, clean the rotor and centrifuge kettle thoroughly.

Don't leave rubber or plastic adapters in the rotor when you are done.

Never centrifuge with empty rubber adapters in the rotor. With high G-forces they may get compressed into the bottom of the rotor and you will have a lot of trouble getting them out again.

An introduction into the basics of centrifugation, a G-force table for the SORVALL SS-34 fixed-angle rotor is, and a G-force calculator (Microsoft EXCEL macro, runs on Windows PCs and Apple Macintosh computers) can be found at the facility's website.

Literature on centrifugation available in the laboratory

Basics of Centrifugation.(2001) Published at the website of Kendro Laboratory Products. Provides basic introduction and some bibliography.

Use of the 37°C rotating incubator

Always make sure that your flasks and tubes are properly fixed on the rotating plate. We don't want your *E. coli* cultures all over the place. If you spill, you clean up, thoroughly!

Always check the thermometer a few minutes after closing the lid, don't rely on the thermostat settings alone. This is important for all temperature-controlled incubators.

If for some reason you want to use the shaking incubator at a temperature different from 37°C, make sure this does not interfere with the other users' work, and ask staff personnel. Put up a big sign indicating the new temperature. Make sure that when you are done, the temperature is again stable at 37°C.

Use of computers

All users are expected to be familiar with the use of personal computers.

The facility currently has 3 computers that are associated with equipment or run software commonly used while conducting molecular biology experiments. These computers are accessible to all authorized users of the facility. A 4th computer, located on the desk of the laboratory technician, is used by the staff of the facility. Since it contains important and sensitive information it is to be used solely by the staff. If a user has a particular reason to use this computer, he or she must ask permission first.

While database searches with sequences can be performed from all computers that are connected to the institute LAN, only the Gateway E series in the facility office area has the DNASTAR program suite for nucleotide and protein sequence analysis installed.

When using any of the lab PCs, always place your files and downloaded sequences in a folder labeled with your name. Don't clutter up the desktop with your stuff. Therefore you may copy files for transfer to your PC or for backup.

Yes, back up your data!!!

Keep in mind that many useful sequence analysis tools are available on the internet, among others at the website of the UPR [high-performance computing facility](#). Some useful web-addresses are listed at the facility's website.

We leave the computers running at all times. So, **DO NOT switch them off when you are done!** They will fall asleep when they get bored.

Telephone use

Telephone calls by users of the facility should be kept short. If this rule is not followed, the privilege of access to the telephone will be revoked.

Use of the gel imaging system

The Nucleo vision gel imaging system (**appendix C, photo no. 2**) allows you to take pictures of your exciting data for documentation and for further processing on your computer. In particular, it lets you analyze the intensity of fluorescent bands or dots for quantitative determination of DNA, RNA and protein samples. First-time users contact Luis Quiñones for an introduction.

When you are done imaging your gels, don't forget your gel on the illuminator plate. Clean and dry the plate. Switch off the UV light!

Don't discard your gels into the sink!

Recommended literature on gel electrophoresis

Sambrook et al. (1989) Molecular cloning. A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York

Ausubel et al. (2000) Current Protocols in Molecular Biology. John Wiley & Sons, Inc. New York, Chichester, Brisbane, Toronto, Singapore

These two collections both cover the basics of gel analysis of DNA, RNA and protein using gel electrophoresis.

To understand the Nucleovision software, a copy of the manual is located on the bookshelf in the office area of the laboratory.

Approval of Guidelines:

Approved by: _____

Dr. Emma Fernández-Repollet
Director, RCMI Program

Date: _____

APPENDIX

APPENDIX A

UPR-RCMI - Molecular Neurobiology Laboratory

Principal Investigator.....

User Registration Form

User First Name.....

User Last Name.....

Degree.....

Gender (M/F).....

School.....

Department.....

Title of Project.....

.....

.....

.....

Role in Project

(PI/student/post doc/guest scientist).....

Type of Service Requested.....

.....

.....

Signature

.....

Date

UPR-RCMI - Molecular Neurobiology Laboratory

APPENDIX C

Photo Gallery

October 2002



1. View of workbenches in the main lab



2. Graduate student Dalynes Reyes, awarded by 2nd time with the RCM I Graduate student enrichment award, dissecting the nerve system of the shrimp *Macrobrachium rosenbergii*



3. Lab technician Luis Quiñones drawing "holy" water from the Milli-Q Synthesis system



4. Lab technician Nilsa Rivera in the main lab at the water baths bench



5. The Cepheid Smart Cycler real-time PCR system



6. Biofreezer and incubators in the facility hallway



7. Graduate student Nietzell Vazquez from Anatomy Dept. working on his doctoral thesis at the Institute.



8. Dr. Claudia Colina from University of Venezuela in Caracas is finishing her postdoctoral degree in molecular biology and electrophysiology.