

Forest Molecular Genetics

Laboratory

Safety manual

2015

For all staff, students and visitors working
in

the Forest Molecular Genetics Laboratory.

(Compiled by Elna Cowley for FMG)

This copy belongs to:

Name:.....

1. General

Safety is an issue that cannot be over-emphasized and must never be compromised in the Forest Molecular Genetics Laboratory (FMG). Many of the chemicals that are used are *hazardous* e.g. ethidium bromide and acrylamide (which are *carcinogens*), *acids* and *flammables* such as ethanol.

Safety procedures that must be followed at all times in the FMG Laboratory are outlined below. A list of emergency phone numbers is posted near the telephone in each of the labs.

1.1 Contact Details:

- Prof. Zander Myburg (012-420 XXX) is the Group Leader of the Forest Molecular Genetics Laboratory and Mrs. Elna Cowley (012-420 XXX) is the Lab Manager (Room 6-30)
- Mrs. Adrene Laubscher is the 6th floor Safety Officer (012-420 XXX) Room 6-11
- The University's 24 hour emergency number is 012-420 XXX or 083 654 XXX
- All accidents / incidents must be reported to the lab manger (Mrs. Elna Cowley), the Group Leader (Prof. Zander Myburg) and the 6th floor Safety Officer (Mrs. Adrene Laubscher)

1.2 Protective clothing and footwear

- Always wear appropriate safety clothing. Lab coats and closed shoes are compulsory.
- Wear safety glasses and safety gloves when appropriate, e.g. when preparing an agarose gel.
- Always wear gloves in the gel loading areas.
- Lab coats and gloves should not be worn in the passages, tearoom and toilets. Take care not to touch door handles when wearing gloves.

1.3 Food / Beverages

- No food or beverage is to be consumed in the laboratory.
- No food, beverages or empty containers are to be placed on any of the benches, or in the laboratory refrigerators or freezers.
- Coffee mugs are **not** allowed inside the laboratory.

1.4 Safety Shower & Emergency Eyewash stations

- Safety Shower is still being organized
- Eyewash bottles are located in every lab, above the basin. Use the bottle for the initial rinse, then follow with the pipe attach to the water tap. Rinse the affected eye / area for at least 20 minutes

1.5 Fire Extinguisher

- Fire extinguishers are located next to the doors of each laboratory.
- There are fire hoses located next to the lifts.

1.6 Spill Kits

- Spill kits are located on the bench above the computer in Lab X and above the fume hood in Lab Z. Instructions on how to use the spill kits are included inside the spill kit containers.
- All FMG Group members should know how to use these kits.

1.7 First Aid

- A first aid cabinet / box is located in Lab Z and Lab X. This kit only contains medical supplies to be used in emergencies, such as when somebody is burned or cut in the laboratory. All other treatment **must** be arranged through the UP emergency number 420 XXX or XXX.

2. Emergency Procedures

2.1 Evacuation

- When there is a fire drill or evacuation, the safety officer on the floor will instruct you to evacuate the building. Meet the Lab Manager on the grass in front of the Fabi building.

2.2 Major Chemical Spill

In the event of a major chemical spill, **contain the area affected** by the spill using the laboratory spill kit, **signpost the spill** with a notice (to alert other possible visitors to the area during your absence of the danger) and **evacuate the laboratory**. Notify Mrs. Elna Cowley (Lab. Manager) and the 6th Floor Safety Officer (Mrs. Adrene Laubscher) on 012-420 XXX and the UP Risk Management Office on 012-420 XXX.

2.3 Injuries

2.3.1 Serious Bleeding

Apply direct pressure to the point of bleeding with your gloved hand. Elevate and support the source of bleeding. Apply the pad of a sterile dressing and bandage firmly. All other treatment **must** be arranged through the UP emergency number 012-420 XXXX.

2.3.2 Extensive Burns

Flush the burn with large amounts of water for at least 20 minutes. Cover injuries with sterile gauze, towel or sheet. Do not try to remove clothing sticking to the burn. Leave the neck and head

uncovered. All other treatment **must** be arranged through the UP emergency number 012-420 XXXX or 083 654 XXX.

2.3.3 Minor Burns

Cool the burnt area under cold running water. Cover with burns dressing. All other treatment **must** be arranged through the UP emergency number 012-420 XXXX or 083 654 XXX.

2.3.4 Eye Injuries

Chemicals splashed in the eye:

- Eyewash bottles are located in every lab, above the basin. Use the bottle for the initial rinse, then follow with the pipe attach to the water tap. Rinse the affected eye / area for at least 20 minutes
- Cover the eye with a sterile eye pad. All other treatment must be arranged through the UP emergency number 012-420 XXXX or 083 654 XXX.

Splinters of glass or metal in the eye:

- Cover both eyes to prevent eye movement. Use a shield to ensure no pressure is placed on the injured eye. All other treatment **must** be arranged through the UP emergency number 012-420 XXXX or 083 654 XXX.

2.3.5 Chemical Injuries (spillage on skin and clothes)

Wash off IMMEDIATELY with large amounts of cold water (at least 10 minutes). Organic materials can be absorbed through the skin and in these cases follow the cold water washing by a thorough washing with warm water and soap. Contaminated clothing should be removed as soon as possible and thoroughly washed. All other treatment **must** be arranged through the UP emergency number 012-420 XXXX or 083 654 XXX.

3. General Procedures

3.1 Planning

Familiarize yourself with, and practice procedures ahead of time to reduce the risk of accidents due to haste or unforeseen circumstances.

3.2 Manual Handling

Always consider the risk of manual handling before you lift or move large or heavy items (greater than 10 kg). If possible, subdivide items into smaller lots before moving them. If you have any doubts about your ability to move an item, get someone else to assist you. Do not risk back

injuries by carrying heavy items up the stairs, rather use the lift. Trolleys are also available for moving heavy goods.

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3.3 Labeling

Label your personal items on the bench and in refrigerators/freezers: Label all solutions, racks and boxes with:

- Your name, date and journal no. with page number e.g. 55p100
- Full chemical name and optional abbreviation of each component within
- Molarity and pH
- Hazard symbol, if appropriate

4. Hazardous Materials

4.1 General Chemical Use

4.1.1 Material Safety Data Sheets

All hazardous chemicals stored in the Laboratory are documented with a Materials Safety Data Sheet (MSDS) and, where appropriate, additional instructions regarding safety, use and disposal. These are held in the Lab Safety and MSDS File located in Lab Z and in Lab X. Ensure that you are familiar with these documents before you use a particular chemical. If there is no MSDS for a chemical you want to use, obtain one from the supplier or the web and put it in the lab MSDS file prior to use. www.merck-chemicals.co.za

4.1.2 Acids

- Strong acids (e.g. HCl and H₂SO₄) must always be used in the fume hood.
- When using strong acids, you must wear a lab coat and safety glasses.
- Refer to the appropriate MSDS/Protocol Sheet for details on the use, safety hazards and disposal of these chemicals.

4.1.3 Handling liquid nitrogen

- Avoid wearing disposable gloves when handling liquid nitrogen as liquid trapped between skin and the glove can cause severe burns. Rather use leather gloves provided in the laboratory.
- Ensure that all vessels used for transfer and transport are freely vented to avoid the possibility of pressure build-up and explosion.

4.1.4 Preparation of agarose gels containing Ethidium bromide

- Take extreme care when handling Ethidium Bromide as this is a known carcinogen and a Teratogen.

- Always use the rubber holder provided to handle bottles containing boiling agarose to reduce the risk of burns or of dropping the bottle.
- Never overfull the bottle when melting a gel. The bottle should never be more than half full. Never close the lid tightly as this can cause a pressure buildup inside the bottle.
- Only allow negative pressure to be exerted on the bottle while preparing the gel i.e. running a cold tap over the bottle. Never shake a bottle containing molten agarose.

4.1.5 Preparation of poly acrylamide gels

- Polyacrylamide gels contain highly toxic and unstable chemicals in liquid form. Preparation and pouring of gels should therefore be done with extreme care and only in room 6-28 (Li-Cor room)
- Double gloves and lab coats should be worn at all times when preparing polyacrylamide gels.

4.1.6 Working with phenol and chloroform

- Always work in a fume hood (switched on!) when handling phenol or chloroform.
- Wear a lab coat and gloves.
- Liquid waste should be decanted into a dedicated waste bottle which should be clearly marked.

5. Waste Disposal

All users must be aware of the appropriate means of disposal of different types of waste. Please make sure you do not to endanger yourself or others in the process of waste handling.

5.1 Dispose of biological waste properly

- Autoclave all liquid media that contain bacterial cells or viruses before disposing down the drain.
- Solid media (agar, tips, tubes etc that were exposed to bacterial cells, and also agarose and polyacrylamide gels) should be disposed of in the biological waste containers, that will be removed and incinerated. Please note that the biowaste boxes should not weigh more than 15kg. The boxes are weighed on site before removal. The rule of thumb is to fill the boxes only 2/3 or 3/4 depending on the type of content.
- Please see attach document: **Laboratory chemical disposal procedure.**

5.2 Toxic and chemical waste must be disposed of properly

- Tips and gels containing EtBr or Gel Red should be disposed of in the biological waste containers.
- Buffer that was used to run agarose gels should be disposed of into the Liquid Waste drum containing activated charcoal.

- All chemical waste has to be disposed into different waste containers (NO MIXING).
- All waste containers must be clearly labelled.
- Phenol and chloroform waste must be disposed of in the appropriate containers in the laminar flow in Lab 6-20 (Lab Z).
- Mrs. Elna Cowley must be notified for removal of waste.

5.3 Sharps

- Sharps e.g. needles, blades, broken glass etc. must be disposed of in the Sharps waste containers.

5.4 Chemicals

Chemicals are to be disposed of according to the procedures specified in the MSDS / Protocol Sheets. If you are using a protocol that generates waste that is not suitable for the waste containers in the fumehoods, you must formulate a proposal that specifies the type and amount of waste, and the methods of storage and disposal. Any such waste must be disposed of promptly. Ensure waste has the appropriate labels and hazard diamonds.

5.5 Removal of full Biohazard waste boxes

- Biohazard waste boxes must be lined with two bags.
- Biohazard waste boxes must not be overfilled.
- When the box is full, all bags that are inside the box must be sealed with a knot.
- The lid must then be taped down in a crisscross manner.
- Full boxes (not more than 2) are then loaded on to the lab trolley.
- Using the service elevator take the boxes to the basement (Level 1) and place them in the big wire cage.
- If the service elevator is not working: Use the front elevators to move the boxes next to the EXIT door in Dept. of Plant pathology on the ground level.

6. Equipment

6.1 General

The equipment routinely used in the Forest Molecular Genetics Laboratory is generally expensive to repair.

For this reason:

- No equipment may be removed from the lab without prior permission from **Prof Zander Myburg or Mrs. Elna Cowley.**

- No item of equipment in the Forest Molecular Genetics Laboratory may be used unless instruction in its use and authorization has been provided by the **group leader** or **laboratory manager**.
- You should not hesitate to **ask** if you do not know, or cannot remember how to use equipment.

6.2 Tagging of faulty equipment

If a piece of equipment is faulty and/or unsafe for use, place a “Danger/Out of Order” note in a visible location on the equipment, and inform the lab manager.

6.3 Listed below are notes and tips to assist with safe use of laboratory equipment:

6.3.1 Agarose gel apparatus

- Ethidium Bromide is a known carcinogen , therefore you must exercise caution when placing gels into the tank and removing them from the tank. Always wear double gloves.
- Verify that the correct coloured leads are connected to the appropriate terminals both at the tank end and the power pack before switching on current. Never switch on the power if there is moisture on the bench top.
- Handle gel holders and combs with care. Always rinse, dry and replace holders & combs in the drawers after use.
- Please note that there are designated EtBr areas in the lab.

6.3.2 Autoclave

- Do NOT use the department autoclave without prior training.
- Do not autoclave something if you are unsure if it is autoclavable.

6.3.3 Balance

- Balances must be cleaned after use by wiping with a cloth dampened with water.
- Gloves must be worn when weighing out chemicals, and if necessary an appropriate mask.
- Always read the labels on chemical bottles and containers carefully before use.
- Spatulas must be washed and dried immediately and placed back where they belong.
- Any inadvertent spills that occur while making up a solution must be cleaned up immediately.

6.3.4 Fume hood

- All experiments with volatiles must be done in a fume hood.
- Lab Z is equipped with one fume hood.
- The fan and light must be turned on whenever the fume hood is in use.
- Laboratory coats, gloves and safety glasses must be worn when working at the fume hood

using phenol or chloroform (e.g. RNA extractions).

- All samples left in the fume hood must be clearly labeled with your name, the date, description, and any relevant safety warning.
- Waste reagent containers located in the fumehoods are to be used *only* for those chemicals listed on the outside of the container. *Do not put anything else in these containers.*

6.3.4.1 Tips for safe fume hood use

- Ensure that the fume hood is running correctly.
- Keep solutions/sources at least 15 cm inside the hood.
- Keep the hood as empty as possible – do not block the rear of the unit, this impedes air flow.
- Position yourself so that the glass of the sash is between you and the substance you are protecting yourself from.
- Remove all the personal items (waste and otherwise) from the fume hood as soon as possible as clutter inside a fume hood reduces its efficiency.

6.3.5 Centrifuges

- Ensure that the centrifuge is correctly balanced.
- Ensure that the rotation speed does not exceed the safety limit for the rotor buckets used.
- Ensure that both the inner and outside lids are closed properly before use.
- Do not open the centrifuge while the rotor is still turning.
- After every centrifugation the rotors must be wiped with damp (dH₂O) paper toweling to clean.

7. FMG GMO Facility Information

October 2013 – October 2016

Registration number: 39.2/ University of Pretoria – (11/023)

You are only allowed to work with a GMO (plant or bacterial) in the following FMG registered facilities.

Main Facility and laboratories Agricultural Sciences Building Forest Molecular Genetics Laboratory 6-20 Forest Molecular Genetics Laboratory 6-27 (FABI II): Arabidopsis Growth Facility Room 1-30.1 and 1-	
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30.1.3 Plant Tissue Culture Facility Room 1-31.1	
Associated laboratories, growth and sample storage rooms Agricultural Sciences Building Cold Room: 6-2 Growth Room: 6-6 Glass wash room: 6-19 Laminar Flow Room: 6-23 Store Room: 6-24 Li-Cor Laboratory: 6-28 BeadXpress Laboratory: 6-31	FABI I Plant Phytotrons: 1-33, 1-34, 1-35, 1-36, 1-36.10, 1-36.11, 1-36.12, 1-36.13, 1-36.16, 1-36.17, 1-36.18, 1-36.19, 1-39, 1-40, 1-41, 1-42, 1-43,
FABI II Growth Rooms: 1-30.1.1, 1-30.1.2, 1.30.1.3.1, 1-31.1.1, 1-31.1.2 Store Room: 1-30.1.3.2	Experimental Farm of the University of Pretoria Phytotron D

General information working with GMOs in the laboratory

1. The use of any food, drink, and application of cosmetics are prohibited in the laboratories at ALL times. Tearooms are provided for these activities.
2. Wearing of protective clothing, eye ware (when appropriate) and closed shoes are required under all circumstances.
3. Protective clothing are removed when leaving the laboratory.
4. Good hygiene is maintained and bench surfaces are impervious to water and resistant to acids, solvents and disinfectants that may be expected in normal use.
5. Bench surfaces and other instruments/equipment are also cleaned at regular intervals and with the appropriate disinfectants.
6. Safety equipment is provided in each laboratory, and the alarm system is fully functional. Laboratory doors are closed when work is in process.
7. Hands are washed as soon as contamination is suspected, when leaving the laboratory and after working with GMMs. Gloves are provided when/where appropriate for protection.
8. Contaminated glassware and other materials awaiting disinfection are stored at a safe place and in an appropriate manner.
9. All disposables and sharps are disposed of in appropriate ways.
10. Emergency kits, disinfectants and spill kits are available to contain and manage spills and emergencies if arise.

Information with regard to GMO waste management:

1. Contaminated glassware and other materials awaiting disinfection are stored in a safe place and in an appropriate manner.
2. Liquid-based waste material is autoclaved (120°C for 20 minutes) or treated with chemical agents (autoclaving: Effectively 100% kill as shown by microbiological testing and incineration: Effectively 100% kill all organisms) prior to disposal.

3. Liquid waste is mainly of a chemical nature. It is discarded in amber 2 litre glass bottles and are collected for disposal by Chemical company.
4. Solid biological material is securely packaged (sealed in containers) and disposed of by incineration via a registered contractor. The take responsibility for the collection and disposal of sealed biohazard boxes, containers and chemical waste . They also supply the correct containers to UP.
5. All laboratories comply in terms of the requested containment levels. Routine Disinfection: Bleach/ 70% Ethanol/ Bronocide/ - work surfaces are cleaned on a daily basis.
6. Sharps are disposed in commercially available containers for sharps and removed by registered contractor.
7. Waste water is drained into a standard sewer.

Forest Molecular Genetics Laboratory Safety Form

Name:

Staff	<input type="checkbox"/>	Student	<input type="checkbox"/>	Visitor	<input type="checkbox"/>
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(Tick (X) appropriate box)

Project Supervisor :

I have read and will abide by the safety procedures outlined in the:

(x)

• Forest Molecular Genetics Laboratory Safety Manual	<input checked="" type="checkbox"/>
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I know the location of:

(x)

<input type="checkbox"/> MSDS sheets	<input checked="" type="checkbox"/>
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<input type="checkbox"/> Forest Molecular Genetics Laboratory Safety Binder (Red)	
<input type="checkbox"/> Fume Hood	
<input type="checkbox"/> Waste Containers	
<input type="checkbox"/> First Aid Cabinet	
<input type="checkbox"/> Fire Extinguisher	
<input type="checkbox"/> "Break Glass" fire alarms	
<input type="checkbox"/> Spill Kits	
<input type="checkbox"/> Lab coats and gloves	
<input type="checkbox"/> Fire Hoses	
<input type="checkbox"/> Main LP gas cut off valve (x2)	
<input type="checkbox"/> Fire Blanket	
<input type="checkbox"/> Sharps waste containers	
I know the fire escape routes in the Natural Sci building	
I understand and will abide by the safety procedures working with GMOs in the laboratory	

Signed:

Date: