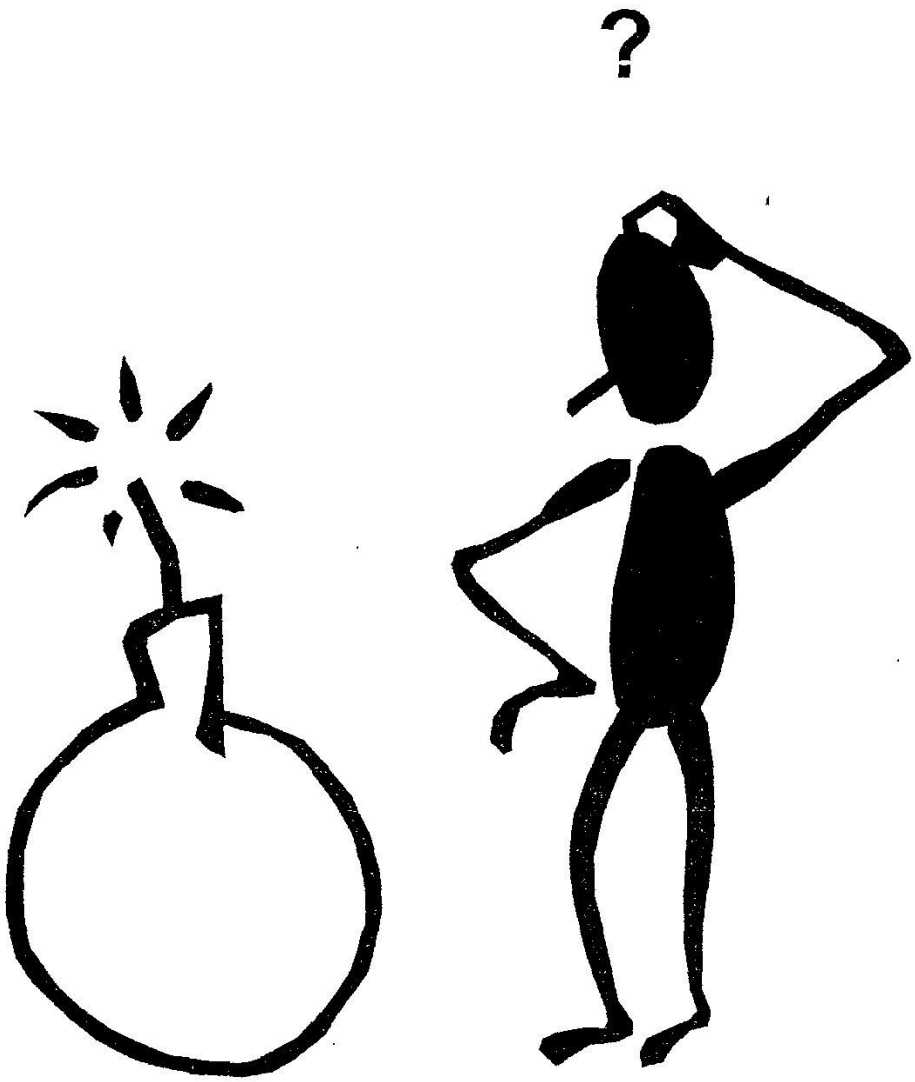
Swammerdam Institute for Life Sciences

(SILS)

Rules for working at Microbiology and Molecular Biology.



SILS/Microbiology/Molecular Biology and Microbial Food Safety,
Science Park 904, 1098 XH Amsterdam

Versie 4.1

Contents

1 General

Room	Room name
D3.122	Multicultivator room
D3.123	Fermentor lab
D3.124	Synechocystis-Yeast-DNA lab
D3.125	Microbiological kitchen
D3.126	Centrifuge room
D3.126a	Weighing and chemical stock room
D3.127	ML-II lab
D3.133	Bacillus lab
D3.140	HPLC/FPLC room
D3.142	-80 storage room
D3.142a	Microscope room
D3.143	Protein and QPCR lab
D3.144	ML-II lab

Offices: C3.258e, C3.262, C3.263, C3.264, C3.268 and C3.269

Opening hours of the building:

See FNWI Safety Guidelines: (https://medewerker.uva.nl/fnwi/az/item/gebouwen-arbo-en-milieu-fnwi.html?f=fnwi)

1.1 New people

» New people must be registered at the secretariat.
» New people must be introduced to Dennis Rijnsburger by their supervisor.
» New people will be introduced to all colleagues by their supervisor.
» All new people need to get the rules from Dennis Rijnsburger.
» A desk and a lab table will be appointed.
» The supervisor is obliged to show the new colleague around the lab. The supervisor or one of the lab assistants or technicians can answer all questions about procedures and equipment.

1.2 Location for rules, schedules, protocols and user manuals

You can find all the rules, schedules, protocols and user manuals on the P drive. For students; ask you supervisor.
P:\\public.uva.nl\fnwi-public\Microbiology_MMP_MBMFS_Photanol

Laboratories

To prevent chaos, everyone working in the lab has to comply with the following rules:

2.1 General

All laboratory spaces are at least ML-I, so **it is required** to wear a lab coat closed at all times (a new/clean lab coat can be obtained at the “distribution center, B0.132”)
Please wear gloves where necessary to protect yourself or the experiment you are doing. However, it is NOT ALLOWED to move from lab to lab wearing two gloves (You should NEVER touch door handles, lab furniture, glassware, etc. wearing gloves).
Tables for general use and equipment like pH-meters, microscopes, autoclaves, centrifuges etc. **MUST ALWAYS BE LEFT CLEAN AND TIDY AFTER USE**. Do not hesitate to point that out to someone else if necessary.
In windowsills only baskets with drying clean labware are allowed (remove for window cleaning).
One is supposed to keep one's own lab table and sink clean and to tidy them at least once a week, e.g. Friday afternoon or Monday morning. This is important for the prevention of accidents and infections.
On a regular basis, the lab heads organize a cleaning day for the lab. It is not allowed to perform any experiments until all tasks are fulfilled that day. You will get an email when this cleaning day is.
A part of a shelf in the cold room (4°C) is appointed to everyone. Things may only be stored in special black containers and agar plates must be wrapped in plastic bags to prevent growth of molds. Everything placed outside a container will be removed on the next cleaning day. You will get an email when this cleaning day is. See also the rules on the door of the room.
Everything you store or incubate, must be labeled with your full name, date and description. Unlabeled things will be removed and discarded. Students should also mention the name of their supervisor on the label or alternatively, the lab number they are working in.
Several tasks have to be carried out in the lab, e.g. cleaning, sterilizing labware or preparing stock solutions. Sterilizing labware is carried out by our lab assistant Dennis. The other tasks are shared among the researchers. If Dennis is not available for a prolonged period (illness, holidays) his tasks will be assigned to the researchers. It is very important that these tasks are carried out properly, because everyone's research depends on it.
Make sure you know the operating procedure of an apparatus before using it (read it or ask somebody who knows).
For some equipment it is necessary to make a reservation. If you don't do that, the apparatus may be occupied when you need it.
After 17:30 hr. all equipment should be shut down, unless there is a reservation or the apparatus has to stay on permanently.
When you leave the lab at the end of the day, please turn off the lights.

2.2 Weighing- and Chemical stockroom D3.126a

Chemicals are stored in the stockroom and are sorted out by labcode. The type of chemical determines the location within the stockroom. A list of the different categories and the corresponding locations can be found near the door in the weighing and chemical stockroom. Some chemicals can be found in laboratory D3.124.
If you are looking for a chemical, use the program Lab Servant (https://labservant-prd1.ic.uva.nl). This is a webbased program.
Please weigh your chemicals one by one and return them to their location a.s.a.p. Dangerous chemicals (toxic, corrosive, flammable) should be weighed in the fume hood in laboratory D3.124.
Weigh your chemicals carefully and properly and prevent spilling of chemicals.
Clean scale and surroundings after weighing. Remove spilled chemicals with a brush or a (wet) tissue. Put cleaned (washed and dry) spatula back.
Do not leave anything you brought into the weighing room (containers etc.) behind.
Every **FRIDAY** (see scheme on the door) clean the weighing room, clean the balances, wipe everything clean, refresh water bottle(s), empty the water bucket and, if necessary, replace the waste container. After you're finished mark this on the list.

2.3 Rules for handling microorganisms

Below are the official rules, made by the government, known as Annex 9. Included are only the parts for ML-I and ML-II:
ANNEX 9, RELATING TO SECTION 5 AND SECTION 24 OF THE 2013 REGULATION ON GENETICALLY MODIFIED ORGANISMS AND ENVIRONMENTAL MANAGEMENT
Regulations pertaining to the categories of physical containment and the R&D service group (ODG).

9.1 Physical containment, working regulations and procedures for activities in laboratories, plant growth chambers, greenhouses and animal cages
9.1.1 Laboratories
9.1.1.1 ML-I workspace
9.1.1.1.1 Regulations on design and equipment of ML-I workspace

a. **The workspace consists of a permanent structure, of which the work surfaces, floors, walls and doors are finished with a non-absorbent material, and in which the work surfaces are resistant to water, acids, alkalis, solvents, disinfectants and decontamination reagents and easy to clean.**
b. **The workspace is accessed through a door that:**
    i. **clearly marks it as an ML-I area;**
    ii. **displays the names and telephone numbers of at least one person responsible for the workspace and the biological safety officer.**

Equipment

c. **There is an autoclave on site.**
d. **A washbasin and a soap dispenser or other facility with which the hands can be disinfected by means of a validated method are present in the workspace.**
e. **The workspace has separate coat hooks for work clothes.**

Other

f. **All equipment is in good working order.**

9.1.1.1.2 Working regulations for ML-I

General

a. **The workspace is to be kept clean and tidy.**
b. **Access to the workspace is restricted.**
c. **It is forbidden to eat, drink or smoke in the workspace, to store any food or drink and to apply cosmetics; contact lenses and hand-face contact should be avoided and cutlery and drinking vessels are not to be brought in.**
d. **Mouth pipetting is prohibited.**
e. **The workspace will be kept free from vermin.**
f. **Following any contamination, all contaminated surfaces will be disinfected immediately.**
g. **Any clothes contaminated by spills of or accidents with genetically manipulated organisms are to be sterilised or disinfected before washing.**
h. **All personal belongings, including any clothes not worn, are to be stored outside the workspace.**
i. **ML-I working regulations are to be observed during any work involving non-genetically manipulated organisms.**
j. **Subject to the written consent of the biological safety officer, the workspace may be used for work involving non-genetically -manipulated organisms, provided the workspace is not being used for work involving genetically manipulated organisms. This will be indicated on the entrance door. The staff concerned will be informed in advance.**
k. **Genetically manipulated organisms originating from ML-I may be stored in ODG, subject to the regulations as specified under 'ODG'.**

During work

l. **During any and all work activities, the doors and windows of the workspace are to be kept closed.**
m. **During any and all work activities, the formation and dispersion of aerosols is to be avoided.**
n. **It is forbidden to wear wristwatches or any jewellery on hands or arms.**
o. **Fitting protective clothing is to be worn. This clothing is to be left in the workspace after completion of the work activities.**

End of work

p. **The work surfaces are to be disinfected when the work activities are completed and at the end of every working day.**
q. **On leaving the workspace, hands are to be washed with soap or disinfected by means of an alternative validated method.**

Waste and contaminated material

r. **All biological waste is to be collected in sealable break-resistant, leakproof containers or equivalent packaging. The waste must be inactivated before it leaves the establishment or be taken to a waste incinerator plant for immediate incineration.**
s. **Any material that has come into contact with genetically manipulated organisms must be inactivated or disinfected before it is washed, reused or disposed of as waste.**
t. **Waste containing genetically manipulated organisms originating from ML-I may be stored in ODG, subject to the working regulations applicable for the containment level at which the waste was produced.**

Other

u. **The maximum amount of culture fluid to be cultured is ten litres, unless a bioreactor is used.**

v. No plants or animals, modified or non-modified, that are not part of an experiment are allowed in the workspace.
w. No animals or plants may be kept in the workspace.

9.1.1.1.3 Additional regulations for specific cases

9.1.1.1.3.1 For activities involving the use of a bioreactor

a. The effective capacity of the bioreactor does not exceed 100 litres.
b. The bioreactor is constructed in such a way that the dispersion of genetically manipulated organisms is restricted.
c. Sampling from the bioreactor, the addition of materials to the bioreactor and the transfer of materials to another system occur in such a way that prevents the formation and dispersion of aerosols and the contamination of external surfaces;
d. The contents of the bioreactor will only be discharged after any genetically manipulated organisms contained have been inactivated according to a validated method.

9.1.1.3 ML-II workspace (in theory not applicable to Photanol)

9.1.1.3.1 Regulations on design and equipment of ML-II workspace

<u>Workspace</u>

a. The workspace consists of a permanent structure, of which the work surfaces, floors, walls and doors are finished with a non-absorbent material, and in which the work surfaces are resistant to water, acids, alkalis, solvents, disinfectants and decontamination reagents and easy to clean.
b. The workspace is accessed through a lockable door that:
i. clearly marks it as an ML-II area;
ii. has a biohazard sign; and
iii. displays the names and telephone numbers of at least one person responsible for the workspace and the biological safety officer.
c. Any windows in the workspace cannot be opened.

<u>Equipment</u>

d. There is an autoclave in the building.
e. A washbasin and a soap dispenser or other facility with which hands can be disinfected by means of a validated method are present in the workspace. These facilities can be operated without using the hands.
f. The workspace has coat hooks for work clothes.
g. There is a class II safety cabinet in the workspace.

<u>Other</u>

h. All equipment is in good working order.

9.1.1.3.2 Working regulations for ML-II

<u>General</u>

a. The workspace is to be kept clean and tidy.
b. It is forbidden to eat, drink or smoke in the workspace, to store any food or drink and to apply cosmetics; contact lenses and hand-face contact should be avoided and cutlery and drinking vessels are not to be brought in;
c. The entrance door to the workspace is to be locked at all times when there are no staff in the workspace.
d. Unauthorized persons are prohibited from entering the workspace.
e. Mouth pipetting is prohibited.
f. The workspace will be kept free from vermin.
i. Following any contamination, all contaminated surfaces will be disinfected immediately.
g. Work clothes are to be sterilised before washing.
h. All personal belongings, including any clothes not worn, are to be stored outside the workspace.
i. ML-II working regulations are to be observed for all work involving genetically manipulated organisms classified as ML-I or work involving non-genetically modified organisms.
j. Subject to the written consent of the biological safety officer, the workspace may be used exclusively for ML-I work in compliance with the regulations specified under 9.1.1.1 or

exclusively for work involving non-genetically manipulated organisms. This will be indicated on the entrance door. The staff concerned will be informed in advance.

k. *Genetically manipulated organisms originating from ML-II may be stored in ODG, subject to the regulations as specified under 'ODG'.*

<u>During work</u>

l. *During any and all work activities, the doors of the workspace are to be kept closed.*
m. *Any work during which aerosols could form or uncovered work involving aerogenic microorganisms must take place in a class II safety cabinet;*
n. *It is forbidden to wear wristwatches or any jewellery on hands or arms.*
o. *Fitting protective clothing is to be worn. This clothing is to be left in the workspace after completion of the work activities.*

<u>End of work</u>

p. *The work surfaces are to be disinfected when the work activities are completed and at the end of every working day.*
q. *On leaving the workspace, hands are to be washed with soap or disinfected by means of an alternative validated method.*

<u>Waste and contaminated material</u>

r. *All biological waste is to be collected in sealable break-resistant, leakproof containers or equivalent packaging. The waste must be inactivated before it leaves the establishment or be taken to a waste incinerator plant for immediate incineration.*
s. *Any material that has come into contact with genetically manipulated organisms must be inactivated before it is washed, reused or disposed of as waste.*
t. *Waste containing genetically manipulated organisms originating from ML-II may be stored in ODG, subject to the working regulations applicable for the containment level at which the waste was produced.*

<u>Other</u>

u. *The maximum amount of culture fluid to be cultured is 10 litres, unless a bioreactor is used.*

9.1.1.1.3 Additional regulations for specific cases

9.1.1.1.3 For activities involving the use of a bioreactor

a. *The effective capacity of the bioreactor does not exceed 100 litres and the air vent in the bioreactor has a built-in hydrophobic absolute filter or equivalent.*
b. *The bioreactor is constructed in such a way that the dispersion of genetically manipulated organisms is greatly restricted.*
c. *Sampling from the bioreactor, the addition of materials to the bioreactor and the transfer of materials to another system shall occur in such a way that prevents the formation and dispersion of aerosols and the contamination of external surfaces.*
d. *The contents of the bioreactor will only be discharged after any genetically manipulated organisms contained have been inactivated according to a validated method.*

9.1.1.3.3.9 For activities with an aerogenic genetically modified virus

a. *Gloves must be worn during the work.*
b. *Uncovered work must take place in a class II safety cabinet.*

9.1.1.3.3.10 For activities involving genetically manipulated organisms which are infectious through broken skin

a. *During the work, gloves are to be worn that extend over the sleeves of the work clothes.*
b. *Uncovered work must take place in a class II safety cabinet.*

9.1.1.3.3.11 For activities involving genetically manipulated organisms that are harmful to pregnant women or the embryo/fetus

a. *Pregnant staff are excluded from participating in the work.*

9.1.1.3.3.12 For activities involving genetically manipulated organisms which could be spread via utensils (fomites)

a. ***Gloves must be worn during the work.***
b. ***Uncovered work must take place in a class II safety cabinet.***

9.1.1.3.3.12 For activities involving genetically manipulated organisms which could be spread by orofecal transmission

a. ***Gloves must be worn during the work.***

Always regards microorganisms as potentially dangerous. This means:

All the strains/plasmids we are working with, must be documented and stored in the general lab stock. So if you receive new strains or plasmids or construct them yourself make 4 stocks: 2 of them you should give to Wei Du (for MMP) or Belinda Koenders (if not available Richard de Boer) (for MBMFS) for the strain collection, and the other 2 you should use for working stocks. Nobody is allowed to touch the strains in the collection. If you need a strain, prepare the media or plate for it and ask Wei for MMP and Belinda or Richard for MBMFS to inoculate. For more information, ask Wei or Belinda.
All lab ware containing microorganisms or nutrients for microorganisms should be kept closed during transportation and storage in order to prevent spreading of the microorganisms and/or growth of other organisms.
Microorganisms, including genetically modified organisms, and including organisms with an antibiotic resistance gene should be killed before disposal via de sewer system. See section 2.3.1.
Inoculated agar plates and used pipettes should be put in the blue bin with yellow lid. Should you have any questions, ask your general lab assistant Dennis.
Clean up spilled culture fluid ASAP and clean the table and floor with 70% alcohol or diluted bleach.
Stick to VMT rules (Safe Microbiological Techniques) and use a flame when necessary.
When you use the vacuum system, a collecting bottle with disinfectant should be used and this bottle should be emptied regularly.

2.3.1 Waste protocol

For the Synechocystis-Yeast-DNA lab D3.124:

Waste protocol for EVERYONE:
Empty small erlenmeyer flasks at the moment you put them in the waste basket, under the fume hood into a big collecting erlenmeyer flask and put some water in the small ones.
This way, most cells are removed already and the pH will not be extreme.

Every **MONDAY and THURSDAY** morning (or the evening before):

1. fill and start the dishwashers yourself with all the dirty glassware from the lab, found under the sinks. Use program #2
2. Make sure that also the caps are recycled in time, and place them in a white bucket on the "dirty glassware" bench.
3. Once it is washed, bring the glassware back from the kitchen to the lab.

WHENEVER NECESSARY (full gray container) but at least on **TUESDAY** morning:

1. Collect and autoclave the bio-waste with program #6.
2. When finished dispose the content of the erlenmeyer flasks in the sink, brush, rinse and put it in the dishwashers, start program#4. Put the stoppers in the"normal"waste bin.
3. When finished, put on new stoppers and place the erlenmeyer flasks on the autoclave table in a gray box. Also the tubes should be emptied, washed, placed with clean caps on the autoclave table.
4. Biowaste is: all the erlenmeyer flasks and bottles/tubes under the hood.

For the Bacillis lab D3.133:

Waste protocol for EVERYONE:
Empty small erlenmeyer flasks at the moment you put them in the waste basket into a big collecting erlenmeyer flask and put some water in the small ones.
This way most cells are removed already and the pH will not be extreme.
In case of Bacillus bleach should also be added to these big and small erlenmeyer flasks!!

Every **TUESDAY and FRIDAY** morning (or the evening before):

1. Fill and start the dishwashers yourself with all the dirty glassware from the lab, found under the plate readers. Use program #2
2. Make sure that also the caps are recycled in time, and place them in a white bucket on the "dirty glassware" bench.
3. Once it is washed, bring the glassware back from the kitchen to the lab.

WHENEVER NECESSARY (full gray container) but at least on **WEDNESDAY** morning:

1. Collect and autoclave the bio-waste with program #6.
2. When finished, dispose the content of the collection erlenmeyer flasks in the sink, brush, rinse and put it in the dishwashers, start program#4. Put the stoppers in the"normal"waste bin.
3. When the dishwasher is finished put on new stoppers and place the erlenmeyer flasks on the autoclave table in a gray box. Also the tubes should be emptied, washed, placed with clean caps on the autoclave table.
4. Biowaste is: all the erlenmeyer flasks and bottles/tubes in the grey container next to the 30/37°C incubator.

Also See section 3 (Biological waste) for detailed description of waste disposal.

2.4 Fermenter laboratory D3.123

In the fermenter laboratory a number of utilities are present for the cultivation of microorganisms. Please live up to the following rules:

The table for assembling/disassembling fermenters (right from the sink) and the table for preparing media (also right of the sink) must be left clean and emptied after use.
Please put everything that is not used anymore back where it belongs. Dirty parts should be cleaned and dried first. Broken parts must be repaired or thrown away.
The erlenmeyer flasks for additions to culture media should be cleaned and put back on the shelf immediately after use (including magnetic stirrer, tubing and a dry filter).
Unused cultures should be disposed of according to the rules (see 2.3) as soon as possible. The fermenter setup should be cleaned, reassembled and left ready-to-use. Remove all unnecessary junk.
Defective equipment and parts must be put on the correct spot with a clear description of the problem after notifying one of the lab assistants. Make sure you do this a.s.a.p. after finding the problem.
Equipment that is not in use and functions properly, should be put back into storage close to the used setup.

2.5. Lab heads

Every lab has one or more responsible persons ("lab heads") who on a regular basis have a look at equipment in the lab, such as centrifuges, pH meters, thermo blocks, fridge, etc.
If there is misuse, they should point that out to the respective person or the whole lab.
Also they should arrange and organize regular cleaning efforts, freezer-thawing, etc.
If you experience problems about cleanliness, safety or there like in the lab address the following persons and see if the problem can be solved on a "lab basis".

Room	Room name	Labhead
D3.12 2	Multicultivator room	Joeri Jongbloets
D3.12 3	Fermentor lab	Sabrina Botton/Tania Darphorn
D3.12 4	Synechocystis-Yeast-DNA lab	Wei Du/Margarita van der Deen
D3.12 5	Microbiological kitchen	Dennis Rijnsburger
D3.12 6	Centrifuge room	Dennis Rijnsburger
D3.12 6a	Weighing and chemical stock room	Dennis Rijnsburger
D3.12 7	ML-II lab	Richard de Boer
D3.13 3	Bacillus lab	Tania Darphorn/Wishwas Abhyankar
D3.14 0	HPLC/FPLC room	Eugenie Troia
D3.14 2	-80 storage room	Belinda Koenders
D3.14 2a	Microscope room	Richard de Boer
D3.14 3	Protein and QPCR lab	Belinda Koenders
D3.14 4	ML-II lab	Belinda Koenders

2.5.1 Sensitive equipment and responsible persons

There is a limited number of sensitive equipment in our labs. Better care taking of these machines includes, so called, responsible persons. Some people volunteered to take care of some of this equipment. If you do not know how to handle a certain machine or equipment this people must be the first to address for help and give an introduction to the respective piece of equipment. Naturally more people use certain machines and also know the procedures. Also there are short "safety sheets" next to this machines to give a good guideline what to do and what not to do.

Sensitive Equipment, Sensitive Machines	Responsible Person 2019
HPLC: Shimadzu (UvA)	Hugo
HPLC: Shimadzu (Photanol)	Eugenie

HPLC: Triathlon and RI detector	?
Agilent HPLC and Fluorescence detector	?
Absorption Plate Readers D3.133	Juan/Tania/Belinda
Fluorescent Plate Readers (FluoroStar)	Margarita
Spectrostar Plate reader	Anja
Autoclaves	Dennis
Washing Machines	Dennis
Photobioreactors	Hugo
Chemostat Equipment (Control Units, Pumps, Valves, …)	Sabrina
Infors reactors	Gabriël
Multicultivators	Wei/Joeri
Lab Strain Collection	Wei MMP/Belinda MBMFS
PhastGel System (Pharmacia)	?
FPLC and fraction collector (Amersham)	Max
Spectrophotometers FPLC lab (Agilent/HP)	?
Oxygraph (Ocean Optics)	?
Controlled Batch Equipment	Tania
Q-PCR (Applied Biosystems)	Richard
Microscope bacillus lab	Juan
Light incubators	Aniek
pH meter FPLC/HPLC lab	?
pH meter D3.133	Tania
pH meter D3.124	Margarita
pH meter D3.143	Belinda
Denovix	Margarita
PCR machines	Margarita
Fluorescence microscope Zeiss	Richard
Anaerobe cabinet	Niels

2.6 Ordering goods and chemicals

All stocked chemicals can be found on Lab Servant (https://labservant-prd1.ic.uva.nl/index.php/login)

2.6.1 Stocks

2.6.1.1 General consumables

Several plasticware and such qualify as general consumables and are present in larger amounts in the labs. More specifically, they are located in 5 different cupboards, one of which is in the kitchen, two are in the chemostat lab, one in the big lab, and one in the bacillus lab. Each cupboard contains different consumables such as gloves, pipette tips, cuvettes, inoculation loops, syringes and so forth. Lists of those general consumables are present on these cupboards.

The turnover of these consumables is quite high; hence, the re-ordering has to be efficient. The rule is that whenever you open the SECOND TO LAST package of a certain consumable, fill it in on the “Consumable re-ordering” file located on the kitchen (D3.125) computer. The information you need: your name, the article description and the article number. Fill it in and close the file, so that Dennis can have a look at this file in its most updated version.

Some other (plastic) consumables are distributed all over the lab in different places. Those are mostly not so popular, but if something is going to be finished, soon re-ordering is done via Dennis as well. Hence,

when stocks of something runs out (usually when the last big box is opened up) please inform Dennis or send an e-mail to d.rijnsburger@uva.nl.

2.6.1.2 Chemicals

Stocks of chemicals are in the bunker (B0.***) or in the “kluis” (D-3.126-kluis). So some popular chemicals might be re-stocked by Dennis faster than others might. The rules for re-ordering chemicals are as follows: When a chemical is going to be finished soon fill it in on the “Chemical re-ordering” file located on the kitchen (D3.125) computer. The information you need: your name, the lab code, the CAS-nr and the barcode. Fill it in and close the file, so that Dennis can have a look at this file in its most updated version. When a chemical is re-ordered by Dennis, he will place a green sticker on the soon-to-be-finished bottle. If you finish a chemical, bring the empty bottle to the kitchen and place it into the grey tray next to the computer (D3.125). Check if the respective chemical has been re-ordered, if not, fill it in. If you need a completely new chemical or something different from what we already have in stock tell Dennis or send an e-mail to d.rijnsburger@uva.nl. You are NOT allowed to use **Lab Servant** for ordering chemicals! Dennis will order all chemicals.
The turnover of some goods and chemicals is rather high and the stock is limited due to a limited storage capacity. Therefore, it is very important to order things on time. Finished=finished!
See also www. uva.nl/profile/d.rijnsburger for everything about ordering (Primers, enzymes in the Thermo Scientific freezer, etc.)

2.6.1.3 Enzymes

Enzymes are sensitive, please treat them accordingly. They should be kept cold at ALL TIMES to ensure their quality.

Restriction enzymes are located (as working stocks) in the freezer in the bacillus lab (D3.133). There are quite a few regular restriction enzymes and a box of FastDigest Enzymes (Thermo Scientific). A list of all the enzymes, including their alternative Thermo name is on the freezer. Note that some enzymes are quite expensive, whereas others are not. We also do not have a stock for all enzymes (also see the list). Many of these enzymes are aliquoted (to ensure the quality, everything with a volume above 150ul is aliquoted).
Some rules for the restriction enzymes

- You can only pipet the enzymes next to the freezer, so DO NOT TAKE THEM TO YOUR BENCH. The ice block (ORANGE holder) will keep the enzyme cold, but not cold enough to retain its activity if it outside the freezer for 5 minutes every time (which is the time you will need to walk with it). Everyone depends on the enzymes to retain their activity, so treat them carefully.
- If they are about to be finished soon, please first check the box with aliquots. Otherwise, notify Dennis or send an e-mail to d.rijnsburger@uva.nl and get a new one from him from the Thermo Scientific freezer (in the kitchen). YOU SHOULD ALIQUOT IF YOU TAKE OUT A NEW ENZYME.
- We only have stock of the most common enzymes, see the list. If one of the not stocked enzymes run out and you need it, please make sure to tell Dennis in time.
- DO NOT leave bags of novel enzymes in the freezer. Please place the enzyme and the extra buffers in the appropriate box (for every set of buffers there should be a box on the higher shelf).

Aliquots of Polymerases (My Taq and Herculase), T4 Ligase and dNTP’s are kept by Mara and Max. They are taking care that they are present and orders them. Please ask them for an aliquot.

DNA loading dye and DNA ladders are kept by Adam, please ask him for new when it runs out on the DNA bench in D3.124.

Kits for PCR or DNA isolation are in the cupboard in the bacillus lab (D3.133). On top of the cupboard there is a backup. As soon as this backup is opened, inform Théo about this. He is taking care that they are present and orders them.

2.6.2 Orders for repairs, etc.

Repairs, courier services etc. are handled by Dennis

2.7 Cleaning Glassware (and re-usable plastic, etc.)

Rinse with water, remove writing, tape, etc.
All dirty glassware must be collected in boxes on the floor (under the sink) in the labs.
Caps: rinse and put into separate buckets
Every lab room has its own cleaning day (see scheme beside the dishwasher in the kitchen).
The general procedure is as follows: If it is your turn bring the dirty glassware (on the planned day) to the kitchen and place it in the dish washer, start it, program 2. Once finished collect the cleaned glassware and bring it back to your lab, re-filling the cup boards.

2.8 Tapping liquid N_2

In the kitchen you can tap liquid N_2.
When you are tapping liquid N_2 wear SAFETY GLOVES AND GOGGLES!!! This is mandatory.

2.9 DNA gel electrophoresis Midori Green and ethidium bromide

There is a general place for DNA gel electrophoresis. Please make sure that the place is kept clean of spills at all times. Make sure to wash/rinse all equipment after use (including combs and gel pouring equipment).

DNA gels are stained with **Midori green**, mix 1 ul of Midori in 20 ml of agar before to running. The gel is made on the DNA gel electrophoresis table.
REMEMBER: Midori Green is not toxic & carcinogenic, but because of safety precaution we treat it as such to protect yourself and others. The following rules apply when working with it:

- The Midori areas in our lab (D3.124) are: 1) the 50ml Erlenmeyer flask used for pouring the gel; 2) the Eppendorf tube within Midori Green; 3) the inside of the electrophoresis machines; 4) the inside of the photography machine; 5) the transilluminator next to Margarita's bench in the corner of the lab Nowhere else!
- You must wear **one BLUE glove** if you touch anything inside of the Midori area.
- Do not touch anything outside of the Midori area with **BLUE glove**.
- Do not remove anything from the Midori area and do not put anything in it.
- Dispose of any contaminated material (gels, tissues, **BLUE glove**) in the chemical waste. TAE buffer with Midori should be disposed in the Red waste. Clean treys and combs with water (dispose the used paper in the chemical waste bin), and also the photography machine after use.

If you make a mistake: rectify it. Ask if you're unsure how.
If you see anyone else make a mistake: immediately tell them, have them rectify their mistake(s), and then inform Koen and Wei*. Remember, your safety is on the line as well.

Note: DNA gels are also stained with **Ethidium Bromide**. For more info ask to Wenyang.

3 Waste disposal

There are several categories of waste, which require different disposal procedures: PLEASE MAKE SURE TO DISPOSE YOUR WASTE CORRECTLY. Do not fill the containers more than 80%.

3.1 Biological waste

This covers all cultures, plates, etc. **containing microorganisms**. Several categories of biological waste are collected separately.

3.1.1 Biological solid waste

In square blue container with yellow lid (to be burned): Pasteur pipettes, gloves, eppendorf, tips, broken glass, plates, plastic pipettes, etc. containing microorganisms.

3.1.2 Biological fluid

Sterilized fluid waste may be disposed of down the drain after sterilization! (See also section 2.3.1)

3.2 Chemical waste

3.2.1 Chemical solid waste

In blue round container with black lid.

For example, gloves, tissues, gels, Pasteur pipettes and test tubes contaminated with chemicals without microorganisms.

3.2.2 Chemical fluid waste

In different containers in D.3.124

Color	Waste	For example
Red	Acid anorganic & metals	EDTA
Blue	Halogen rich organic	TCA/ Ethidium bromide
Green	Alkaline inorganic	NAOH/KOH/ cyanide
White	Halogen poor organic	Alcohols/ phenols
Orange	Nitrous acids	Nitric acid

3.3 Glass waste

Only clean bottles, erlenmeyer flasks and volumetric glassware (~ 250 ml) are collected in special glass waste containers for recycling in the hallway near the elevators. All other glass waste must be put into the blue round chemically contaminated waste trash container (black lid).

3.4 Paper, cardboard and plastic (not chemically contaminated)

Collectors for paper, cardboard and plastic can be found in the hallway near the elevators. Remove plastic covers, ring binders and big staples (from cardboard boxes). Cardboard boxes should be made flat and put into the cardboard container. There are office collectors for paper in every room.

Do not hesitate to ask around when you are not sure where to put your waste.

4 Safety in the lab:

It is not allowed to eat, drink or smoke in the lab. Wearing watches and jewelry on arms and hands is prohibited. Wash your hands regularly and prevent physical contact with chemicals and microorganisms. Always wear a lab coat closed and when necessary wear gloves, a mask, safety goggles, etc.
It is not allowed to wear open shoes or high heels.

4.1 Chemicals

Some chemicals are toxic, corrosive, flammable or otherwise dangerous. See also 2.2. Before using chemicals be sure you are aware of the potential dangers and the procedures for clean-up and waste disposal. Information can be found in Lab servant Also see section 3.

4.2 How to act in case of calamities

Spilled chemicals: If you know how to clean up, do it yourself. Otherwise call the local contact for safety matters: Wim de Lange (tel: 5068). See also below for names and phone numbers.
In case of casualties call the meldkamer (tel: 2222).
Fire: call the melkamer (tel: 2222); after that, follow fire instructions on the white paper near the door. There are also several fire guards (BHV) in the building (see below for Microbiology BHV); follow their instructions. When you have extinguished a small fire yourself, check whether the red light somewhere on the ceiling of the lab is flashing. If so, call the meldkamer (tel: 2222) immediately.
Power failure: shut off equipment that may be damaged when the power supply is restored; wait for further instructions through the intercom.
###If there was a calamity, fill in safety notifications form. You can find a link on the homepage from the faculty (https://medewerker.uva.nl/fnwi)

Other calamities: See the white notices that are present all around the buildings.

Local contact for biological safety SILS, Microbiology: Peter Huls (tel: 6203)
First aid ore fire: call the meldkamer (2222).
'BHV' Microbiology:
Dennis Rijnsburger (tel. 7043)
Belinda Koenders (tel: 5078)
Richard de Boer (tel: 6697)

4.3 Contact Persons:

For all urgencies regarding safety, please contact:
Dennis Rijnsburger (tel: 7043 or 06-22153102) or
Richard de Boer (6697)
Wim de Lange for safety (5068)