Laboratory Handbook

Department of Ecology and Evolution

English
Location of Laboratories

4th floor:
4321-4322 Big lab room for Goudet, Salamin, Guisan, Yuko and Pannell groups, Person in charge: Dessi (Dessislava.SavovaBianchi@unil.ch, room 4320), Illumina seq (Luca Fumagalli)

4312-4314: Lab Ian Sanders

4324 Dry room, John Pannell

4313 Incubators, bacteria, fungi, freezers, Ian sanders

4316 Autoclave, MilliQ water, TissueLyser

4317 Lab of Ian Sanders

4306 Growth room

3rd floor:
3123.1 COSEC (coordinator of security) Luca Fumagalli

3116 Lab for DNA extraction and Qiagen extraction with robot and vertical laminar floor

3125 Lab of ant’s tracking

3128 Lab of Tanja Schwander’s group

3207 Lab of Claus Wedekind’s group

3120 Cold room

3118 Autoclave for waste / freezer -80°C (C7)

3117 White room, material for field work (field centrifuge, liquid nitrogen tank)

3113 Chemical stock room

3108 Eco-ethology lab (microbalance and microscopy)

3218 Conservation biology lab (low copy DNA lab)

2nd floor:
2108 Small molecular biology lab

2109 Big molecular biology lab room

2110 GC, spectrophotometer, horizontal laminar flow, Speedvac, Washmachine…), RNA libraries lab

2104 PCR machines and freezers

2104.1 Ethidium bromide room, freezers
2210 Microsatellite detection (Consolée Aletti)
2210.1 Tecan, qPCR 96 well machine and PCR-free hood

1st floor:
1112 Ecophysiology lab primarily for Christe, Roulin, and company (ELISA, Western blot, respirometry, antioxidant analysis, RNA extraction…)
1110 Franck Chalard atelier
1110 Armindo Texeira atelier
1101-1109 animalerie
1117-1206 Kawecki’s labs
1000 recycling room and waste disposal: (paper, aluminium, Styrofoam)
1118 Liquid nitrogen room

Outside the building **Recycling and waste disposal** (glass, PET, plastics, metal, electronic) + cabinet chemical waste
Overview

Since there are many people working in the labs, it is necessary to follow some common rules and courtesies so that everyone can work together in harmony.

For the group using the lab of the second floor (Chapuisat, Christe, Fumagalli, Kawecki, Keller, Perrin, Roulin, Schwander, Wedekind), Anne-Lyse (anne-lyse.ducrest@unil.ch) is in charge of the general lab organization. Catherine, Christine L.M., Nadège, Françoise, Céline Simon, Céline Stoffel, Zoé and Roberto are the technicians to whom you can approach with your questions or concerns.

For the group using the lab of the common fourth floor (Alexander, Goudet, Guisan, Pannel, Salamin and Yuko), Dessi (Dessislava.SavovaBianchi@unil.ch) is in charge of the lab organization.

For the group of I. Sanders, Réjane (Rejane.Seiler@unil.ch) is in charge.

In general each piece of the common equipment has one or more persons who are in charge of it (see Responsibility list). Please feel free to contact this person(s) on how to use it and if it needs to be repaired. However, this person is not responsible for keeping the equipment clean, but rather, each user is asked to clean the common equipment after each use if necessary.

To get access to the lab you need to follow the following steps:

1) Before starting in the lab, you should imperatively contact Anne-Lyse, Dessi or Réjane depending on your group’s lab affiliation.
2) You should add the procedure sheet to your lab book and read the security file and sign the security sheet and give it to Anne-Lyse (mailbox).
3) Follow the general information on the smooth running in the lab (Anne-Lyse, Dessi, Réjane).
4) Just before starting follow the different posts explaining the different methods you will be using (see sheet in your lab book).
5) Only when you have done each step you could start lab work. If you do not follow this procedure, you will be excluded of the labs.
6) If you do not respect the rules, you can be excluded from the labs.
Smooth Running of the Laboratory

- Each person is assigned a bench space. See seating chart. If there is a problem, please see Anne-Lyse (anne-lyse.ducrest@unil.ch), Dessi (Dessislava.SavovaBianchi@unil.ch) or Réjane (Rejane.Seiler@unil.ch).
- Each group has its own set of pipettes. For pipette problems please see Nadège Remollino (Nadège.remollino@unil.ch).
- **Write your name and date** on all your PCR plates, boxes, and racks that are stored in the refrigerators and freezers, so that if necessary, the owner can be quickly identified, for the long term storage, see explanation below.
- Respect the common equipment. **Never use an instrument before having followed an instruction.**
- For both the standard and multichannel pipettes, make sure that you use the correct pre- and post-PCR pipettes to prevent contamination (if differentiated).
- Clean the centrifuges and all other materials and equipments after utilization.
- Inform Françoise Dolivo (francoise.dolivo@unil.ch), Dessi or Réjane when you take the next-to-last pack, bag, or box of a consumable item which are in common.
- If you need lab coat, you should ask Nadège Remollino (Nadège.remollino@unil.ch), there is a basket in the big lab where to put your dirty one, the cleaning will be organized by Nadège depending on the amount.
- If you use the autoclaved boxes of tips from the drying oven, you must re-fill the tip boxes (using **clean gloves** to avoid RNAse, endotoxine autoclaving resistant) and put them in the white tray for autoclaving. Please do not forget to stick a **small** piece of autoclave tape (striped) onto the tip box. When the tray is full, please autoclave the tip boxes, and afterwards, place them in the drying oven.

**Waste disposal:** *Recycling* – Aluminum, excess tip boxes, glass, paper and cardboard into the appropriate recycling bucket or box. When the bucket or box is full, take it to the recycling room on the first floor or outside at the first floor.

- **Bacterial** (and other biological) **solid waste** are discarded into biological waste bags placed into a white box. When the bag is full up to the upper part of the box, place your waste into a new bag and close it with the clip. **Bacterial** (and other biological) **liquid waste** need to be autoclaved (in the waste autoclave on the 3rd floor).

- **Chemical waste:** *Organic compounds* (phenol, chloroform, …) need to be discarded into appropriately labeled plastic containers (chlore and other halogen containing or not containing solvants), thiocyanate guanidinium waste (e.g: QG Qiagen) found in the chemical fume hoods (if particular ask Catherine), **never add bleach to chemicals, or mix the chemicals.** Other **non-hazardous** lab waste can be discarded into the standard lab garbage cans, which will then be incinerated. When a container is full, it should be put under the fume hood in the lab and correctly annotated (name of the chemicals, date, CAS number, concentration if known).
• **Water system**: Water with different qualities are available in the labs:
  In tape water there are a lot of particles, microorganisms, colloids, inorganic and organic substances and dissolved gases.

  a) *Deionized water = demineralization water or ion exchange water (DI)*: is purified by passing through ion-exchange resins which exchange hydrogen and hydroxide ion for dissolved minerals, which then recombine to form water.

  b) *Reversed osmosis water (RO)*: this process removes bacteria, pyrogens and inorganic solids above 200 Da through external pressure applied to the more concentrated side of an osmotic membrane. Usually the water was previously partially filtered, demineralized and recirculates and thus it is a water of high quality (*Type II water*: >5-10 Mohm/cm, conductivity: 0.1 uS/cm, <50 total organic carbon TOC/ppb part per million).

  c) *MiliQ water*: is obtained by combination of different purification system filtration, reversed osmosed, deonization and UV treatment. This allows to obtain pure water of *type 1+. That is with a resistivity of 18.2 (Mohm/cm), conductivity 0.056uS/cm, < 5 TOC/ppb) at 25°C. This water is RNase free. Don’t touch the water outlet, avoid any projections of solid or liquid chemicals, never connect a pipe! **Always come with a clean (without powders) recipient to collect MiliQ water.**

Where to find what:
- MiliQ water: room 2208, 3116, 4316. Pay attention that in 3116, the water is not recommended for RNA purification, because of low turn over.
- RO water: Helix tank in 2208 close to the MiliQ water.
- RO water: RIOS tank in 2210, pay attention you should not take water when the wash machine is running.
- Osmosis water circuit in the corridor at the third floor and in room 3118: circulating water of a conductivity of 8 uS/cm (Type IV) produced at the Unil (Yvan Marendaz). This water is for moisten chambers, incubators.
- DI water: in forms of water bottle ordered by Catherine ([Catherine.Berney@unil.ch](mailto:Catherine.Berney@unil.ch)) at Renggli are found in 2109 close to the sink and balance. Another bottle is found under the autoclave of the second floor as a cooling system. The water does not circulate, thus may contain a lot of microorganisms. Thus use this water for rinsing your glassware and not for your buffer preparation.

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**Which water to use for which application**

<table>
<thead>
<tr>
<th>Humidification of incubators,</th>
<th>Dionized and distilled water</th>
<th>Circulating osmosis water</th>
<th>Reversed osmosed water</th>
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<td>Glassware washing</td>
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### Chemical fume hood

- There are two chemical fume hoods on the 2nd floor, and one on each of the 1st, 3rd, and 4th floors.
- All the chemicals, wastes, and contaminated materials (e.g., with phenol, chloroform) should be sorted and separated (in the hood), there are a lot of bottles ready for use. If there is a new, potentially hazardous product, you should read and ask Françoise, Catherine how to store it and to dispose of it.
- Do not mix the chemical waste, label carefully the waste with the name of the chemical, concentration, purity, CAS name if known.
- Keep the hoods clean and organized and as empty as possible. Wipe with technical ethanol if necessary. Remove all non-contaminated material when you are finished.

### Chemicals and equipment websites

- Françoise ([Francoise.Dolivo@unil.ch](mailto:Francoise.Dolivo@unil.ch)) listed all chemicals in a website available to everyone ([http://www2.unil.ch/chemdmf/](http://www2.unil.ch/chemdmf/)) then choose chemdmf. Alibaba is a webside where you will find all the equipment we have in the DEE. Consolée Aletti is in charge of the website.
- Then you log with the SWITCHaai authentication with your usual unil login and password and choose the database: chemdmf or Alibaba, then you choose dee-g as login. Then you choose “Substances available”. Then you can search a chemical.
- If you finished a product or almost finish it, you should choose “product finished” (end of the description page of the product, down), in order to send a mail to Françoise, so that she will order it.
- The database should be used to check the location of a product, if you change the location of a product, It is important to tell Françoise.
• If you order a new chemicals or a new instrument, it is imperative to tell Françoise (chemdmf) or Consolée (Alibaba), so that she can enter the new product in the database.
• You can also get information on a product with the QR code on the sticker of the product or instruments, it will link you to the database.
• Chemicals are stored in a large cabinet (corridor 2108-2109) and in the fume hoods, in the small cabinets under to the hood (mainly solvents) in room 2108 and 2109, in the 3rd floor chemical stock room (3113), and in the refrigerators of the big and small lab rooms and extraction lab (3116).
• Before ordering a chemical, please check that we do not already have it (in the cabinets or chemical stock room). Do not order in excess, especially if only a small quantity of a particular chemical will be used.
• Hazardous chemicals must be used in the chemical fume hood.
• Avoid contamination of the powder stock that is wear clean gloves, do not use spatula in mol biol powder.
• Pure and technical ethanol (and other solvents) are stored in small quantities in the lab. They can be ordered at the magasin BB web page in advance.

Ethidium Bromide Room

• Attention: EVERYTHING should be considered CONTAMINATED BY ETHIDIUM BROMIDE. Therefore, do not touch anything without nitrile gloves.
• Everything that enters the rooms does not leave (except you and me, of course)
• Do not leave the room without throwing the gloves in the trash can (within the room).
• All the material used to cool the gels should be washed with soap and the rinsed in distilled water, never used alcohol on the gel boxes or trays, they will crack.
• If the 1x TBE running buffer container (10 L) is empty, you need to re-fill it. The 10x TBE recipe is posted on the wall close to the hood in 2109 behind the balance (EDTA pH 8.0 is ready as a 40 ml solution). The components are found in the cabinet underneath the balance. Make sure everything is mixed and dissolved in a beaker (containing about 800 mL volume of RO water (in room 2110, 2108)), then fill up the container and fill up to the upper lane.
• When your gel is done, turn off the power supply and clean the plastic cover and cover the gel box. Please wrap the cables. Rinse plastic with distilled water if necessary. Clean up.
• If the electrodes of the gel box are encrusted or running buffer has been used too much then the gel box needs to be cleaned. Empty the used buffer into the liquid waste container E2 with filter. Let the gel tank soak with tape water for one night and wash the gel box with soap and rinse with distilled water.
• To avoid bad running because of buffer degradation, the buffer in the gel box should be used 5 times than discarded (notch on the gel tank cover).
• After taking your gel photo, wash and wipe the UV illuminator with 70% ethanol. Do not remove the timer of the gel documentation system, it is to switch off the camera overnight.
• Refill the tip boxes before they are completely empty – *play fair*.
• When the trash can is almost full, remove and tie the trash bag. Place the bag into a cardboard box and then put the box in the back of the room.
• To remove EthBr from your Erlenmeyer used to pour a gel, please rinse it with a small amount of water and discard the water and rest of agarose in the big Erlenmeyer without a filter (E1). When E1 is full pour it in E2 filter to remove agarose remainings. When E2 is full, it can be added to E3 with a spoon of active charcoal (black) and mix. Then pour E3 on the active charcoal filter E4. Discard the filtrated solution of E4 into the sink and open tape water to dilute TBE salts.
• If you need to cut bands out of your gel, *protect yourself*, especially your face and *eyes* with the **UV mask** and take only the tubes you need inside the BET room.
• If you wish to work without EtBr, you can go at the fourth floor. They use the GelRed. Please first ask Dessi Dessislava.SavovaBianchi@unil.ch.

### Ordering reagents and supplies

**A) Consumables**
To work in the lab, **each user has to come with its own tips, tubes, PCR plates, distritips**. For each group a cupboard has been attributed to put his consumables’ stock. The cupboard will be controlled with UNIL Cards. The common consumables are the gloves, the powders, small consumables such as seringes, filters, glasses and the consumables for the BET labs and the Qubit.

**B) Catalogues**
Online catalogues are found on the server: [http://srvbiovm6.unil.ch/cata/](http://srvbiovm6.unil.ch/cata/). You need two accounts: one for the catalogue of the **DEE** and one the **Magasin BB**. An account can be created by a person having administrative privileges: Catherine, Christine L.M., Nadège, Consolée, Dessi or Anne-Lyse. For the Magasin BB, you can ask Christophe Jeanmonod, Catherine.

Once an account has been created, you can access it using your login and password.

**Always order via the catalogues, so that incoming parcels would be easily distributed.**

First step, you should search for the article in the catalogue. Most of the products have been entered already.

There are 2 types of orders:
- online catalogue DEE for consumables to order outside.
- online catalogue Magasin BB for consumables that are in stock in the buildings : Magasin BB.

Always check **first** whether something is available in the catalogue of the **magasin BB** with the search tool before ordering it outside (Catalogue DEE) for the following two reasons: 1) it will be free of charge, 2) the price is negociated for a large amount (lower price).

Pay attention when you search to misspelling, search English and French version, it is easier when you enter only part of a word.
C) **Ordering**

a) **Ordering from the Magasin BB stock center** (magasin BB online catalogue) in Biophore:

You can find the list using the online catalogue: [http://srvbiovm6.unil.ch/cata/Magasin BB](http://srvbiovm6.unil.ch/cata/Magasin BB). It contains consumable for your group and the common stock for the lab. For the small common stock of the lab, once per week, Françoise, Dessi and Céline Simon (extraction lab) will place an order for reagents and supplies that are in short supply in the lab – **however, you must tell them which supplies are needed as they are not omniscient**. These supplies will be delivered on at the mezzanine in a cupboard (the key is located in the room of Françoise), or in the local “post” that opens with the unil card. If you need something for a person of your group **with SNF grant, do not forget to indicate his name for the order**. Christophe Jenamomod [christophe.jeanmonod@unil.ch](mailto:christophe.jeanmonod@unil.ch) will send you a mail, when it is ready in the cupboard, fridge or freezer.

b) **Qiastock, Promega, Life Technologies and Biolabs** stock in Biophore

A certain number of **Qiagen, Promega, Life technologies** and **Biolabs** (enzymes available at the CIG) products are available in the Biophore. It is usually cheaper and quicker than ordering outside. In the departmental online catalogue (DEE), look for the links “Qiastock - 24%”, “Promega sur Place” and “Life Technologies sur place”. Standard non-urgent orders will be delivered 2x per week by Catherine and Consolée. Urgent orders will be delivered by Consolée. For the moment you will find the consumable under the DEE catalogue, but it should change soon to the magasin BB.

c) **External orders: Catalogue DEE**

The external orders should be done through the catalogue DEE. Pay attention to **delivery (shipping and handling (10.- to 100.-) charges)**! Try to minimize by planning ahead – urgent orders usually cost more. Also, group orders from the same vendor or supplier.

Your orders placed using the online DEE catalogue will be faxed each Monday afternoon by Consolée. It is also possible to fax urgent orders the next day, and sometimes even the same day. However, if it is not really urgent, please wait for the normal Monday faxes because multiple orders from different users can be grouped together to save on shipping and handling charges.

The following are considered urgent cases:

1. You absolutely cannot continue your experiments (but maybe you should plan better).
2. You will be able to take advantage of an offer or discount that will expire by the end of the week. Note, Life Technologies (and probably
others) is a little nasty in that orders must arrive within working hours of that day – the date of the fax is not sufficient.

Please always check if an item already exists in the database before entering it. If an item is not found in the departmental online database, it is possible to enter it into the system as a new item. Please ask a technician to do it for you to avoid duplicates and mistakes:

Departmental Services

Microsatellite sequencing (fragment analysis) and library preparations:

Consolée prepares and runs microsatellite plates on Mondays, Tuesdays, Thursdays, and Fridays (Mon and Thurs – 4 plates/day; Tues and Fri – 8 plates). It is possible to enter the names of your samples on an Excel spreadsheet before the run (See with Consolée).

You will need to sign up somewhat in advance (because the service is often full) on the monthly calendar posted on the door of room 2212. Indicate the number of samples that you plan to do and your name and phone number.

For the actual run, bring your samples (= fluorescently marked PCR fragments) to the refrigerator in the lab (room 2212) and transfer them into a 96-well sequencing plate (3 µL, 2 µL or 5 µL per well) and clearly indicate on the plastic cover which wells contain your samples, the volume transferred, your name, your phone number, your group and the ROX (350 or 500 = labeled standard that Consolée should add). You should also write the number of colours you are using. For any other questions ask Consolée Aletti (Consolee.Aletti@unil.ch).

The results of both type of sequencing will be placed on the server srybio1 (DNA sequences under Sequence and microsatellites under ABI3100) 1 day after the sequencing run. Contact Michel.Schuepbach@unil.ch for access to the server.

Roberto Sermier (Roberto.sermier@unil.ch) is in charge of the GeneMapper software used to analyze microsatellites. Please see him for access.

Consolé can also prepare for you libraries (see with her).

Taq and PCR Machines (room 2104)

We have for the DEE a stock of the Taq polymerase: GO Taq (Promega) for an advantageous price (We buy huge amount one per year).

The Taq has to be ordered in the DEE catalogue (http://srvbiovm6.unil.ch/cata/DEE) under

1) GoTaq® G2 DNA Polymerase, 250 units ; Cat # M784X
2) **dNTP’s 25mM aliquots, cat # U1410:** The dNTPs is only for use with the Taq, not for other applications. You can find other dNTPS in “Promega sur place”. You should select as Fournisseur "Consolée Aletti" and as Fabricant: "Promega". Your Taq or dNTP will be placed in the -20°C freezer (local de poste) with your name on the plastic bag and you will receive a mail from Consolée when your order is ready. Please note that there is no delivery on Wednesday. The buffer, Magnesium and Q solution are available in the freezer F2.1 the last down drawer in the big lab 2109.

**PCR machines**
- You must reserve the PCR machines. Sign-up sheets are in the room. Please sign up only for the amount of time necessary for your PCR run. As a reminder, if you have many samples, there is a 384-well PCR machine available in the 2212.
- There are different types of 96-well plates: PCR plates without edges for the 2nd floor PCR machines, and plates with edges for microsatellite preparation or sequencing reaction to Microsynth.
- **The final temperature should not be lower than 12°C** (saves energy and less stress on the machines).
- If possible do **not let the PCR machines run overnight** for the grey PE 2720 and 9700, the cover stays at 100°C even when the block is at 12°C.
- If you are the last person to leave the lab, please switch off PCR machines with finished runs and placed the plates in the 4°C fridge above the PCR machines.
- The first person to arrive in the morning please take out all the plates from the PCR machines, place them in the refrigerator below the PCR machines, and turn off the PCR machines.
- If your plate needs to remain at particular temperature, please leave a note on the machine.
- If for some reason your PCR machine (reaction) should not be stopped, also please leave a note on the machine.

**The Robots**
- **Qiagen BIOSPRINT: room 3116**
  - The robot is used to extract DNA in 96-well plate format – 96-samples in about 40 min. Roberto Sermier (4246) and Céline Simon (4162) are in charge of the robot. Please tell them, when you take the next-to-last pack, bag, or box of a consumable item.

Each user must indicate in the notebook next to the robot the number of samples and plates. Billing will be based on your usage. Please, use the S-block up to 96 wells, even when a block is used only half in once. Pay attention that you have to come with your distritips, tips, tubes and **proteinase K** (Proteinase K is available in the DEE catalogue in Promega stock, for the buffer to dissolve the powder ask Céline or Roberto).
At the moment we still have kits in stock, so you can use, but please, use the S-block up to 96 wells, even when a block is used only half in once.

- **TECAN : room 2212**

The person in charge of this machine is: Consolée, Céline Stoffel, Roberto and Nadège.

This robot is quite flexible and can do many things including:
1. Filling buffers for Qiagen robot
2. Transfer from tubes to 96- or 384-well plate and vice versa
3. Aliquot from one 96-well plate into other plates
4. Prepare PCR reactions for both 96- and 384-well plates
5. Fast PCR set-up
6. Quantify DNA in plates
7. Hit-picking
8. more…

New scripts can be created based on your needs, ask Roberto.

Each user must indicate in the notebook/binder next to the robot the number of samples and plates used. Billing will be based on your usage.

- **Quantitative PCR machine**

  **ABI7500 : room 2212**

The person in charge is Anne-Lyse.

The qPCR machine measures the number of cycles necessary for the appearance (detection) of PCR products, permitting:
1. Precise quantification of gene expression (i.e., mRNA)
2. Allele discrimination of genes
3. Copy number
4. Libraries quantification

**QuantStudio 6: CIG DAFL**

We have a 384 well plate qPCR machine from (Life Tech) at the GTF. You can use it couple to the Tecan there.

A software to read the plate is installed in the computer of the nanodrop at the second floor.

**Spectrophotometers**

- **Nanodrop: room 2109**

  Quantification of nucleic acids, proteins using only 2 ul of sample.
**Nanodrop with fluorescence: room 2109**
Quantification of nucleic acids with fluorescence in 2µl. A common kit is available for DNA. For RNA each group buys its own kit. You should inscribe in a sheet telling how much you use. Kit for DNA is in common in the lab, for RNA buy your kit. Françoise is in charge of the instrument.

**Qubit instrument** with a kit for DNA quantification and tubes are found on the shelf in room 2110. You should inscribe the number of µl of fluorescent dye you used. Tell Celine Stoffel when you take the next-to-last tubes and when the dye is well advanced.

- **Spectramax: room 2110**

  The person in charge: Anne-Lyse.

  Spectrophotometer can measure in the UV and visible range (from 180 nm to 800 nm) for 96-well plates.

  Utility:
  1. Endpoints: nucleic acids, proteins, ELISA
  2. Spectrums
  3. Kinetics with programmable time points

**Database and long term storage**
All the users should note their boxes for long term storage of DNA, RNA, tissues, bacterial clones… following the sheet stucked on the box cover. Everything should be fulfilled correctly and entered in the database, if it is not done, the boxes will be thrown away in one month.

The cryoboxes will be distributed by Guillaume Fabre: guillaume.fabre@unil.ch and Jerome Wassef: Jerome.Wassef@unil.ch. They will give you the cryoboxxes and an access to places to store your samples at -20°C or -80°C freezers and explain you how to fulfill the excel files for the database.

The person in charge of the database is Roberto Sermier (Roberto.sermier@unil.ch). In each group there is a technician responsible for the database. The database is a filemarker pro file available on the server smb://nas.unil.ch/ that can be searched for samples. Ask the technician if you need something because we are moving from the previous database to this new one, something may be missing.

For the primers, Taq… in use, you do not have to enter them in the database, you are allowed to have boxes with only you name, groups and date in the drawers in the vertical freezers in the lab.
All -80°C and -20°C freezers are equipped with alarm system. When you look for a box in the freezers, do it as quickly as possible, so that the freezers remain open as short as possible. From August 2017 the night alarms go to the UNIL security system be careful!

- **-80°C**

The -80°C freezers are used for precious samples and their long term storage, i.e., tissues, RNA– but not extracted DNA that is better conserved at 4°C or -20°C.

The persons in charge are: Anne-Lyse, Christine LM and Dessi and Réjane (for the 4th floor).

All samples must be stored in cryoboxes, either plastified cardboard boxes or plastic boxes. It is advised that they be rubber-banded. On each box, you should fulfill the sheet on the cover and you should fill the rack record that is found on the door of the freezer and filled the filemarkerpro data base accordingly.

When you open a freezer, you should be quick. It means you check on the sheet where your box is before opening the freezer, you open the door, take the rack, your box and close the door. Close it as soon as possible is important to avoid condensation, and thawing of the other samples. If you cannot open the freezer, put your samples in the C5, which is the rescue freezer.

The C10 at the first floor is the rescue freezer that should be kept for emergency. The C10 and C5 also contain the dry ice stock that can be used and refilled when you receive a parcel, you can also find dry ice in the -80°C of the local of the post.

Never add a lot of samples at room temperature at one time in a freezer (100°C difference). Work on dry ice, when you check your samples.

Wear special blue gloves above the normal gloves, do avoid burning.

- **-20°C**

Chest freezers → long term storage.
Upright freezers → short term storage.

- For both types of freezers (-80°C or -20°C), you must fulfill the sheet on the cover with your name, group name, date, and short summary, rackname and pos of the box. If your boxes are not marked like this, they may become lost after a freezer failure or during the yearly freezer cleaning and be thrown away if not entered in Quartzy.

- Please examine and remove unnecessary samples from time to time because space is limited.
- Leaving person should empty their drawers or racks according to the discussion with their boss on what to keep.

### Refrigerators
For all refrigerators, you can place items that need to be stored at 4°C. If necessary, you can also place racks with your tubes for a very short time (e.g., several hours). In all cases, please write your name, group and date.

**Liquid nitrogen**

Liquid nitrogen has a temperature of -196°C and causes burns by freezing, which can be extremely serious particularly if the nitrogen reaches the cornea. There is also risk of asphyxiation in the case of massive release. This is why it is recommended to store liquid nitrogen in a sufficiently ventilated room. Never take the lift with a bottle of liquid nitrogen.

For sample storage in liquid nitrogen there are tanks coupled to a self-filling system in room 1218. The system should be put in use before, thus ask some weeks in advance to Anne-Lyse or Catherine if you need to use it.

For sample processing, liquid nitrogen can be collected in a proper Dewar at the 5. floor of the DBMV. It means that you need to go when the doors are opened from 8:00 to 12:00 and 14:00 to 18:00 and closed during the weekend. Wear the proper gloves, spectacles and shoes. The first time go with somebody who knows how to do.

Act slowly when you plunge an element in the bath of liquid nitrogen. Never place nitrogen in closed containers, or in a Thermos bottle: instead use special broad opening containers Dewar.