WELCOME IN THE MOLECULAR LAB

- The lab is spread over **3 adjacent rooms on the 4th floor**:
  Room 7-3A: pre-PCR and fume hood
  Room 7-9A: electrophoresis and laminar flow (LAF)
  Room 7-9B: post-PCR, sequencer and thermocyclers

- The normal **working hours** of the institute also apply to the lab (7h30 – 18h30).
  Exceptionally, staff members or visitors who are already familiar with the lab are also allowed into the lab in the weekend, if they have the permission of their host or promoter. Students are not allowed to work in the lab outside the normal working hours, unless they are working together with their host or promoter.

- **The molecular lab is a place with chemical and toxic products**, so for your own **safety** it is best to wear a lab coat and gloves all the time. Ask your host or promoter for a lab coat if you don't have one! The following is strictly not permitted in the lab: food and drink / coats / bags and backpacks / books and literature.

- **The molecular lab is a place where biological material is handled** (tissues, DNA, bacterial cultures). All lab users must do everything possible to **avoid contamination**. Therefore it is necessary that everybody wears gloves and that everybody always works neatly. Every lab user has his/her work space and this must be cleaned regularly. Your host or promoter will supply you with a set of pipettes and tips and provide you with a space in the freezer and in the fridge, where you can keep your material and store your samples and products.

- The **general lab material** (tips, gloves, glassware, tubes, general chemical products…) can be used by everyone. Karin Breugelmans is in charge of the purchase of general lab material and products. Make sure you contact her when a product or material is nearly finished. **Specific products** (enzymes, kits, primers, abs. ethanol, cryoboxes ...) have to be bought by your own research group as needed. Your host or promoter will show you where your products are stored and will tell you who does the ordering for your research group.

- To use the **space in the fridges and freezers** as economically as possible, it is very important that all stored tubes are labelled and put in boxes that also have a label with at least your name on it. Ask your host or promoter for storage boxes. In the lab there is a **common fridge** for products and solutions. Everything in this fridge must be labelled with the name of the user. For solutions, the label also has to contain the name of the product and the date of preparation.

- **The smooth operation of a clean lab is in everybody's interest**, for good results as well as for safety. Therefore it is necessary that every lab user takes into account the needs of all other lab users. Everyone, and this includes all visitors and students, is responsible for the smooth operation of the lab. People who work in the lab for a longer period of time, are given some **extra responsibility (i.e. allocated a specifictask).** On

the door of the lab, a list is posted with who is responsible for which task. Please direct any questions/comments on a particular task to the relevant person.

- **Airco:** The pre and post-PCR lab have air conditioning. The air conditioner will be switched on when temperatures are high (i.e. during warm summer weather). When the air conditioning is switched on, the doors of these rooms are to be closed at all times. The airco will be maintained at a constant temperature for the rest of the summer. Please do not change the temperature of the airco during that time.

- **Balance:** Please clean the balance after every use. Clean and dry the used spatulas and recipients and put them back in the right place.

- The clean **glassware** is at your disposal in the cupboard in the lab. After use, rinse with demineralised water and put on the rack to dry (when not dirty) or on top of the dishwasher (when dishwashing is necessary). Never use tab water to rinse, because Brussels' tab water has a lot of chalk in it. **Use demineralised water only**.

- **Autoclave:** Everybody has to sterilise their own tips, filled goblets with tubes, etc. in the autoclave. For the sterilisation of fluids, make sure that the lid is never entirely closed. Before filling the autoclave with tips and bottles, make sure to check the water level. The heating device must be completely under water, but the water should not touch the basket. **Only use demineralised water in the autoclave**.

- **DNA extractions:** Extractions should always be done very carefully, so as to avoid contamination. Clean the work space very carefully before and after you have finished your extractions. In 2009 a special room became available for the extraction of ancient DNA. Ask Gontran for information if you want to use it.

- **Qubit:** For measuring DNA concentrations we have the Qubit system. Please do not calibrate this device every time. If you have doubts about your measurements first take some measurements with the standards supplied with the Qubit. If you still have problems please direct your questions to Carl or Frederik.
  See addendum **'Protocol recalibration Qubit'**

- **Pre-PCR** (room 7-3A): This is the place where we 'make' the PCR reactions and where we dilute and aliquot our PCR reagents.
  Post-PCR (room 7-9B): In this lab we do all the manipulations **after** the PCR amplification, such as loading gels, purification of PCR and sequencing reactions.
  Pipettors, tips and reagents should never be transferred from the post-PCR to the pre-PCR lab. Do not enter the pre-PCR lab with gloves that you used in the other labs.
  More info: http://www.hpa-standardmethods.org.uk/documents/qsop/pdf/qsop38.pdf

- **Thermocyclers:** There is a booking list for the use of the PCR machines. Please stick to the time schedule on these papers. Do not book 2 slots in a row to do only 1 PCR (you can book 2 slots when you are actually doing 2 PCRs). At the end of your PCR programme you can put the 'soak-temperature' at 20°C. Lower temperatures (e.g. 4°C) are an unnecessary pressure for the machine. Don't forget to **STOP the PCR program** when you take out your samples. Switch off the machine when you are finished and

after you have ascertained from the booking list that the machine will not be used for the rest of the day.

- **Agarose gel electrophoresis** is done in TBE-buffer. The TBE-buffer is used 4 times. The fourth person to use the buffer must discard the buffer after use, rinse the tank with demineralised water and return it to the bench. The next person to use the same tank, must fill the tank again with new 1x TBE-buffer. Put your name on the paper when you use the electrophoresis tanks.

  For the visualisation of DNA-fragments on the agarose gels, Midori Green is used.
  See addendum **'How to work with Midori Green?',** to make and handle agarose gels.

- **Formamide** is used to dissolve your samples before putting them on the sequencer for analysis. It is a **very toxic** product and can therefore **only be used under the fume hood**. Used plates with formamide are collected in the fume hood, so that they can be removed as toxic waste. *See Material Safety Data Sheet (MSDS) at the fume hood for more information.*

- **AB3130*xl* sequencer:** Every manipulation of the sequencer should be noted in the log book; the number of runs you do, as well as the maintenance work you do (changing the buffer, polymer and capillaries ...). Do not forget to book the sequencer in advance on http://teamup.com/ks3737e1b059bbb47e/ . The polymer contains polyacrylamid, *see Material Safety Data Sheet (MSDS) at the sequencer for more information.*

- **The sequencer PC** is intended to run the sequencer and save the data generated by the sequencer. It should also be used to transfer the images of your agarose gels from the flash card to the computer (the flash card is not to leave the lab). After generating agarose images, please transfer them immediately from the flash card to the computer and erase the images immediately from the flash card. The flash card has a limited capacity, and everybody would like to have space to save their images. On this PC, **every lab user has one folder** in which you can save data. Transfer your data to your own computer through the network as soon as possible. **The sequencer PC is not a mass storage device**, but a transitional station to transfer your data.

Wearing gloves is necessary in the lab, but please do not use them in the offices or to pick up the phone.

If you have finished your lab work for the day, you have to clean your lab bench and the space you used in the fridge or freezer. Check with your host or promoter what should be kept and where it should be stored, and what can be thrown away. The lab benches you have used, have to be cleaned and your tip boxes have to be re-filled. Make sure that your data are saved properly, so your folder on the sequencer PC can be removed.
And finally, a farewell drink or a piece of cake is always appreciated by the staff members...!

In case of accidents: If an accident happens and someone is injured, seek help immediately. The most relevant phone numbers are given below. Individuals suffering minor

accidents (small cuts and bruises, etc.) should obtain first aid from their supervisors or the nearest available persons.

If **equipment is damaged**, please report this immediately to the supervisor and Karin Breugelmans promptly. Only in this way can we ensure proper and speedy repair or replacement of damaged equipment.

In the event of major **fire**, please evacuate the building immediately and seek professional help (see phone number below). In the event of a small fire, use the nearest fire extinguisher to put out the flame and/or seek the assistance of your supervisor or any nearby colleague.

Some useful phone numbers:

LAB pre-PCR …… …….....	288
LAB electrophoresis …….....	287
LAB sequencer …….....	416
Freshwater Biology: Isa Schön / Zohra Elouaazizi …….....	312 / 313
Entomology: Frederik Henderickx.....	137
Vertebrates: Erik Verheyen …….....	286
Invertebrates: Karin Breugelmans / Thierry Backeljau ….....	420 / 339
Safety (W. Swalus) …….....	561
Poison Centre …….....	0-070 245 245
Ambulance or Fire department…….....	0-112
Doctor on duty …….....	0-02 479 18 18

HOW TO WORK WITH MIDORI GREEN?

********BE CAUTIOUS, HANDLE AS IF IT WAS EtBr********

Always wear a pair of gloves (available in the post PCR lab). Any material that has been in contact with Midori Green should be labelled

1. Weigh **0.6g** agarose, place in a conical flask and add **60ml** of TBE. Swirl to mix. (for a large gel, use 1.2g agarose and 120ml TBE). Only use conical flasks that are labelled with 'Midori Green', they can be found on the rack near the sink in the electrophoresis room.

2. Melt the solution in the microwave for 30s (60s for a large gel). **Make sure the agarose is completely dissolved!** If not, put the solution for another 5s in the microwave. Clean the microwave if agarose solution boils over.

3. Leave a few minutes to cool down (Midori green is sensitive to heat above 60°C). It should be cool enough to hold it in your hands. Meanwhile, prepare the set up of the gel tray by securing the top and bottom with tape. Put the desired comb in the tray.

4. Move to bench with UV illuminator. A dedicated set with pipettes for staining solutions only is available here (labeled with Etbr).

5. Add **2.4 µl** midori green. The Midori is in the fridge (tube in use is stored in the door of the refrigerator) and should be kept in the dark as long as possible. Dispose tips in the adequate bin (foot pedal bin next to the UV). (4.8 µl for large gel)

6. Pour gel in casting tray and leave on the bench until solid.

7. Rinse conical flask with tap water so that agarose is removed.
   ***NB** : In the case you want to prepare a gel in advance, put the gel in a plastic bag and keep it in the fridge and put a date on the plastic bag. It will keep for a few days. Also make sure to put the gel out of the gel chamber so that other people can use the chamber to prepare a gel. If you prepare a big gel but you only need one lane, put in a second lane and cut the gel in two; put the other half in a plastic bag with the date in the fridge and e-mail others that they can use the gel if you won't use the gel yourself, otherwise it is a waste of gel, Midori and effort.*

8. After the agarose is solid, add the DNA ladder (**4 µl**) and samples (**4 µl**). **Do not take the gel to the post-PCR room**. Always add your samples on the bench which also holds the UV illuminator in the electrophoresis room.

9. After completion of electrophoresis, bring the gel in the UV cabinet for visualisation and save the picture.

10. Dispose the gel in the adequate bin (red bin next to the UV and above the bench) and always clean after using the post PCR lab and whenever you empty boxes with tips fill these as soon as possible.

Protocol Recalibration Qubit

1. **First check whether recalibration is needed**.
   Use the current calibration and quantify the DNA concentration of the **2nd size standard (= 10ng/µl)** (comes along with the other Qubit assay reagents).

   - Prepare the Qubit Working Solution by diluting the Qubit reagent 1:200 in Qubit buffer. Prepare 200µl of Working Solution for three replicates (600µl in total).
   - Add 4µl of the 2nd size standard to 196µl of Working Solution.
   - Do this for 3 replicates in total.
   - Vortex each replicate for 2-3 seconds.
   - Incubate for 2 minutes.
   - Insert the tube in the Qubit 2.0 Fluorometer and determine the stock concentrations.

     → **concentrations should be as close as possible to 10 ng/µl.**

2. If recalibration is needed:
   - Contact Carl Vangestel cvangestel@naturalsciences.be or Frederik Hendrickx fhendrickx@naturalsciences.be and they will recalibrate the Fluorometer for you.

     OR
   - Calibrate the Fluorometer yourself following the Qubit Quick Reference Card (attached to the electric wire of the Fluorometer). Next, remeasure the 3 replicates (2nd size standard) to ensure your recalibration has been successful. Afterwards, fill in the Qubit Registration Document (provide **your name, date of recalibration** and **average concentration of 2nd size standard (3 replicates)**). The document can be found on the desk, next to the Qubit.

I hereby acknowledge that I have read the previous pages of laboratory guidelines. As user of the molecular genetic laboratories of the Royal Belgian Institute of Natural Sciences (RBINS), I fully understand these guidelines and agree to abide by them.

Name:

Email:

Position:

School or Institute:

Your supervisor in RBINS:

Starting date: Final date:

…… ……

Date Signature