The DNA laboratory at Natural History Museum, UiO

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For deliveries: Give the lab mobile phone number (93299849) to the courier and inform the lab manager that you are expecting a package delivered there.

DNA lab web pages

This is where relevant lab info can be found (list over users and people associated to the lab, bench fee, working hours sheet, guidelines, reports, protocols, etc.).

The DNA lab is also on Facebook (https://www.facebook.com/groups/NHMOslo.DNAlab/).

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Guidelines for users of the DNA laboratory at NHM

Before starting

Supervisors/project leaders are obliged to inform the lab manager on new users coming to the lab at least two weeks in advance. New users must apply for lab access by filling in the application form. New users will have to undergo an introduction period (usually 3 days) before they are allowed to work in the lab. During this introduction they will also be given a lab book and instructed on how to use it.

Before the actual analyses can start in the DNA lab, your samples have to be registered in the DNA Bank database (Corema). This is most easily achieved by filling in the relevant import template with all relevant information for your samples, and then sending the file to the DNA Bank technical curator for import to the database. Please ask the DNA Bank technical curator for advice and instructions. If you have already registered vouchers for the DNA samples in some other collection, it should be possible to export most of the necessary information from that collection database. Contact the administrator of the relevant collection or database for further help on this. After the data have been successfully imported to Corema, you will receive a list of DNA Bank accession numbers for all your samples.

Any sample tubes or references to your samples should include these accession numbers!

The DNA Bank will assist you with labelling of sample tubes, ensuring that you always will have clearly labelled samples to work with in the lab or in other contexts. Derivatives of these samples, e.g. DNA extracts, will have to be labelled by the user, until these are deposited in the DNA Bank.

Guest researchers bringing collected material from outside Norway to the DNA lab are encouraged to sign a material transfer agreement (MTA). The MTA can be found using this link.

It is required to have a lab safety course before starting the lab work. As a minimum requirement, the course HMS0503 must be completed. This is an online course that can be fulfilled in ~1 hour.

A checklist of all requirements before starting the lab work has been made and can be found here.

Lab reservations

All lab work must be booked using BookitLab. This is mandatory for all lab users. Your bench fee ("leieseted" cost) will automatically be calculated based on your BookitLab reservations. In order to make lab reservations, you will need a Feide user account. Consult your project leader to obtain such Feide account. Costs for using the lab are found here.

Ordering of lab reagents and equipment

Standard lab equipment such as plastic consumables, pipettes, centrifuges and vortexes are purchased by the lab. Reagents for fragment analyzer, (standard) MinION sequencing, ddPCR and Macrogen sequencing labels are also purchased by the lab, but the lab users must report usage.

Other reagents (e.g. PCR reagents, kits), and services (e.g. external sequencing, shipments) must be ordered using project funding. This can be performed by completing this ordering form.

E-mail

As of December 1st 2013, all users of the lab must subscribe to the e-mail group "mol-lab-users" (https://sympa.uio.no/nhm.uio.no/info/mol-lab-users). Send an e-mail to a technician and you will be added to the list. All important e-mails will be sent to this group. When you finish with your lab work you can follow the link above and press unsubscribe.

Introduction

Lab rules and routines exist for a reason. It is important that you understand and are aware of why things are done in a certain way or another. If you do not understand, or think that there is something that does not make sense, just ask.

All lab users will be given 3 days of introduction and lab training. This will be given by the lab technicians. **This means that lab training will NOT be given by supervisors/researchers/other students unless agreed otherwise with the lab manager!** New lab users can use the lab independently after the training period and after approval by the lab technicians.

Read through protocols and make sure you understand what you are doing before starting. This is the only way you will learn something as well as be able to have a more active participation in the lab work. Do not imitate other user's habits, the fact that another user does things in a certain way does not mean it is ok.

Work in a clean, tidy and focused way. Aim to become as systematic as possible. Remember that you are working with quite advanced techniques that require precision, concentration and discipline. We share the lab with other students and researchers and you have to keep this in mind when working. Clean after you, don't use up ingredients without saying so/filling up with new, empty the tip trash when you are finished working. Don't generate sources of contamination that can affect others by being sloppy and dirty.

General

The laboratory consists of eight rooms at the Zoological Museum. A map over the distribution of these rooms can be found on the whiteboard by the entrance.

1. Extraction room — 003, DNA/RNA isolation.
2. Sensi-lab room — 012A, for extraction and pre-PCR work with old/sensible material.
3. Library preparation — 012B, for Next Gen library preparations

4.	Pre-PCR room	011B, for PCR reactions setup. **NO** PCR products allowed here.
5.	Machine room	011A, Storage freezers can be found here as well as the locations for order deliveries.
6.	Post-PCR room	009, where all post-PCR steps are done (further PCR setup, product purifications, etc.)
7.	PCR-machines	007
8.	Sequencing room	005, the room is designated Ion Torrent, MinION sequencing and plate reading in ddPCR experiments. The laf bench is for work in sterile condition.
9.	Gel room	005A, for gel electrophoresis.

Lab instruments and equipment must never be used for the first time without having been instructed by a lab technician. For further use of the instrument, consult the manual. Manuals can be found in folders in the same room as the machine is in.

Lab meetings will be held as required, and active users of the lab are encouraged to participate.

For setting up AFLP reactions and other sensitive reactions in the post-PCR lab, use pipette sets strictly reserved for these activities. These are found on the bench closest to the door. Do not use these pipettes for any other activity.

Some general lab rules

- Only work alone in the lab between 07:00 – 18:00
- Remove lab coats and gloves when leaving the laboratory
- Remember to risk assess your lab work
- Preparedness boards can be found in the rotunde
- Wash your hands when leaving the lab
- Always react to alarms, i.e. fume hoods and fire
- Report about building related problems

Storage of samples

All DNA samples intended for long-term storage must be transferred to the DNA bank (see “before starting”). The DNA lab is NOT a facility for long-term storage of DNA samples. There are several reasons. Most importantly, the samples need to be organized so they are traceable and can be found (together with metadata) decades from now. Accordingly, strictly organised museum DNA bank freezers are the solution, and not unregistered poorly labelled tubes in overfilled freezer racks in the machine room.

The freezers/fridges in the machine room /Pre-PCR are intended for DNA samples from ongoing projects and relevant reagents/chemicals only. The freezers/fridges in the Post-PCR area are only for short-term storage of amplicons (until they have been sequenced) and relevant chemicals. Amplicons

can be stored for 3 months unless agreed otherwise. Guest researchers bringing material not to be registered in Corema must bring their material when they leave.

Leftover material from former students/visitors and from finished projects will be disposed of without further notice. It is the project leader's responsibility to ensure that the project material is stored according to the lab guidelines.

After having extracted DNA from the samples, you should take out an aliquot of DNA extract for your own immediate analyses. Try to take enough for the intended analyses, without emptying the stock unduly (if possible). It will, however, of course be possible to obtain new aliquots from the DNA stock if necessary. The remainder of the extract (DNA stock) should be included in the DNA Bank as soon as possible:

- Obtain plates for long-term storage of DNA (Micronic) from the DNA Bank technical curator
- Transfer the DNA stock to the Micronic tubes
- **Make sure to keep track of which samples goes into which well position!**
- Place the plate(s) in the rack labelled DNA Bank Temp. storage in freezer Lab03 in the machine room next to the Pre-PCR lab (room 011A)
- Download the DNA extracts template file from the NHM Wiki for Best Practice manuals (https://wiki.uio.no/nhm/skf/best-practices/index.php/DNA_Bank), fill in as much information as you have and send it to the DNA Bank for registration in the database.

The samples will be moved to a -80 °C storage by DNA Bank personnel after they have been registered in the DNA Bank.
After the extractions are finished, also the tissue samples should be handed over to the DNA Bank. As for the DNA stocks, it is of course possible to obtain access to these samples whenever that might be desired, but handing them over to the DNA Bank as soon as possible ensures that no samples will be accidently lost or otherwise become unavailable to you or others.

Storage of reagents/chemicals/kits

The DNA lab is responsible for the chemicals purchased for use in the DNA lab. Chemical risk guidelines require us to discard expired chemicals following regulated procedures. As many chemicals have longer shelf lives than their expiry dates, and we want to minimize waste and loss, we have had a pragmatic approach to this. To bring our practice more in line with general rules, all chemicals and reagents that are expired more than 2 years will be taken care of by the lab. These can either be redistributed for direct use or discarded. Storing of chemicals outside designated labs is not allowed.

Lab hygiene/safety

Eating and drinking is not allowed anywhere in the labs. Wash your hands after having worked in the lab before going back to your office.

Use gloves and coat at all times when in the lab! These are to protect yourself and to avoid contamination of your samples and those of others. Gloves and coats should not be used outside the laboratory. Try to use your pinkie when opening doors and change gloves if you

go from working in post-PCR areas over to pre-PCR areas. Always wear closed shoes.

Used tips are disposed in the yellow plastic waste containers on the benches. These containers are then emptied into "riskavfall" (hazardous waste) marked cardboard boxes with black plastic bags in them. This is a confusing solution because it is not really hazardous waste but plastic that has been in contact with chemicals. The main reason for this is that tips make holes in normal plastic bags making them difficult to handle by the cleaners if disposed of in normal bins. The lab manager will dispose of full boxes as needed.

All users of the lab must clean up after themselves and wash glassware, etc., immediately after use. All plastic racks must be transferred to (1) bleach/deconex bath and (2) water bath (in this order) and thereafter be transferred to the respective locations for air drying.

Always work in a fume hood when handling volatile chemicals, e.g., chloroform and mercaptoethanol. There are two hoods in the lab, designated for this use, one in the extraction room and one in post-PCR (for work with e.g. formamide).

Hazardous chemicals

Gelred is used in the gel room to stain gels. This dye is meant to not be able to cross cell membranes which make it of low toxicity. However, we still keep the same system we had with both EtBr and SYBR dyes (also said to be non-toxic at their time): always wear nitril gloves when working in the electrophoresis room and remove them as soon as you leave the room in order to avoid spreading the chemical to other rooms. You will find safety spectacles in the room to protect against UV light in case of needed. Used gels and other waste that was in contact with the dye are disposed of in the special cardboard containers in the room ("riskavfall"). Do not store gels in the fridge! Pregnant women should not run gels since TBE buffer contains boric acid that can be harmful to the unborn child. The concentration is very low, but there is no need to be exposed to it.

Chloroform extractions can only be performed after dedicated fume hood training with a lab technician.

First aid kit and eye wash bottle are placed in the "rotunden". Another eye wash is placed in the post-pcr room. In addition there is a first aid kit next to the mail shelf on the other end of the hallway (by the elevator). Safety spectacles are placed in the extraction lab, post-PCR lab and gel room.

HMS data sheets are collected in a binder in the post-PCR lab. These data sheets contain important information about handling of chemicals, danger and protection. Users of the DNA laboratory are responsible for knowing the content of these HMS data sheets.

Pregnancy and lab work

Pregnant lab users have to be particularly aware of which chemicals they will be working with and the properties these substances inherently have. **Pregnant women must not be exposed**

to chemical characterized as carcinogenic, mutagenic or harmful to genes or reproductive ability (CMR substances). It is recommended to inform the lab manager about pregnancy.

The employer (supervisor) is required to relocate or find alternative tasks for pregnant woman in order to completely avoid CMR substances (Regulation of the performance of work (in Norwegian), §7-4).

Rooms

Use the specified rooms for the different processes and DO NOT move tools from one room to another, especially not pipettes. This is particularly important in the case of the extraction/isolation and pre-PCR rooms, DO NOT bring things from post-PCR areas rooms into these rooms.

Taking Eppendorf tubes and strips/plates

When taking Eppendorf tubes (1.5 and 2.0 ml) out of jars, pour tubes on the jar lid and pick them from there, **do not** stick your hand into the jar. Same goes for PCR-plates, strips and lids in bags, "pour out" the number of strips/lids needed on to your hands or on a clean surface. If you pour out more than you need, do not use your hands to pick them up and put them back. If you think they might be contaminated, throw them away instead.

Accidents – ALWAYS report to technician

If you have any kinds of accident (contaminate a pipette, break something, realize something is not working etc.) tell the technician and/or the lab leader.

Shared responsibilities

Fill tip boxes, clean bench, put used racks in bleach/deconex bath and generally make sure everything looks nice and tidy before leaving a lab after use. Make new dilutions of TBE buffer if needed. Buffer recipes can be found in a folder in the post-PCR lab. Familiarize yourself with the chemicals needed before you start working since several of them need to be handled in the fume hood. Alternatively you can ask the lab tech or manager to make new.

Weekly lab responsibilities: a rota with weekly "lab responsible" will be e-mailed by the lab manager as needed and hang on the whiteboard together with a check list of duties. It can also be found on N:\dna-lab. You as a user have to remember your cleaning week and swap with someone else if needed. The weekly responsible has to regularly check that the labs are in general order (transfer racks from one bath to the next step, full squirt-bottles, and so on) but still, at all times, all users of the lab must clean up after themselves! The lab responsible person's duties are:

(1) Clean the benches in all rooms with deconex (5%), rinse the surface with water, and then with 70% alcohol. Wipe all fridge/freezer and door handles in the lab with ethanol soaked paper.

(2) Change bench paper in the electrophoresis room, wash the area around the UV box and make the room tidy.
(3) Take out racks in the water bath and put to dry. Move racks in chlorine bath over to water bath. Change both chlorine and water baths. Same goes for deconex (5%) and water bath in the extraction room.
(4) Refill plastic bottles with dH_2O, 5% deconex, and 70%.
(5) If you see dirty glassware, put it in the washing machine.
(6) Clean the centrifuges, especially the rotors with deconex and dH_2O.

It is advisable that these tasks are done on Fridays, so everybody can make the benches free. Look after the lab at least 2-3 times during the week. It is possible that some things are not needed, and then you don't need to do them.

Notify whatever might be running low to the lab technicians BEFORE things go empty.

Samples removed from the thermocyclers must be placed in the drawer specifically labeled in the fridge in the post-PCR room or in the fridge in the sequencing room respectively. Always mark your own samples with name and date before storing them in fridges or freezers, unmarked tubes will eventually be thrown away by lab-tech or manager.

The fridges and freezers in the lab are quickly filled up with samples and other items**. It is therefore important that everybody compresses their personal items as much as possible, i.e., frequent cleaning up of old samples and throwing away whatever is not needed.** Storing several strips into one rack or even better: in zip-lock bags (we have drawers where we store these).

Everybody must be particularly careful when using shared solutions in order to avoid contamination. Do not pipet directly from the containers but take out an aliquot in a Falkon-tube for your own use. It is also necessary to be careful with the use of the chemical reagents since these are often very costly.

The DNA-lab has its own doorbell for delivery of packages in the north end of the building as well as a mobile phone that is usually kept by the lab manager. But if you by any chance take a package that was meant for the lab you should take it to the lab immediately. Unpack and freeze/refrigerate if needed and inform the lab-tech or manager on its whereabouts.

All damages and errors on instruments and equipment must immediately be reported to the lab manager.

Use of the thermos cyclers

When you wish to use the thermos cyclers, sign up on the lists next to the instruments on the predefined time slots. Remember to add any potential delays. A 30 minute delay that is not accounted for results in a lost booking and anyone can use the machine.

NGS facilities

The Ion Torrent sequencing and MinION sequencing facilities should only be used by the lab

technicians unless agreed otherwise.

Cloning

Cloning experiments are done in room 005 using the designated cloning facilities. The equipment designated for cloning shall not be removed from 005.

Lab-users conducting cloning experiments have to inform the lab manager in advance about the biological details of the planned experiments (purpose, donor, recipient, vector etc.). They have to demonstrate to the lab manager that they have the respective know-how to conduct cloning experiments.

Pregnant women must not conduct cloning experiments.

And as anywhere else in the lab:
No food or drinks.
Lab coat and gloves at all times.

In case of accidents

Emergency numbers: Fire **110**
Police **112**
Ambulance **113**

Security and alarm centre: 56666
Ullevål University Hospital (Emergencies): 22 11 73 50
Ullevål University Hospital (Ophthalmologist): 22 11 85 45 or 22 11 85 84
The Student Health Centre (Helsetjenesten UiO) 8.00am – 16.00pm: 22 85 31 74
The Emergency Service in Oslo, Storgata 40 (outside office hours): 22 93 22 93

All accidents and unwanted events must be reported to the lab manager.