

Operating charter of the molecular biology and in vitro culture platform - Teams DEP and GEE - Laboratory ESE UMR8079 - Building IDEEV

27 octobre 2023

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1 Introduction

This document is intended for all those who work in the rooms of the building dedicated to the in vitro culture and molecular biology experiments of the DEP and GEE teams. It is available on the ESE laboratory intranet site ([here](#)) : **TECHNIQUE \Fonctionnement général \Laboratoire BM-CIV – Equipes DEP et GEE**

This document must be distributed to all new arrivals **by their manager**.

Before handling, the newcomer reads the present document and signs it in a register book kept by the DEP-GEE shared platform manager : Alodie Snirc (office 2320). This constitutes acceptance of the entire document as of the date mentioned. The newcomer's e-mail address is required so that he/she can be added to the BM-CIV mailing list to attend fortnightly meetings and to be kept up to date with various information relating to the life of the technical platform.

Everyone must feel **RESPONSIBLE** for their own safety, but also for that of others. It is everyone's responsibility to be aware of the laboratory's operating rules and to respect them, in the interests of the community as a whole. Do not hesitate to contact the various managers mentioned in this document, who are ready to answer any questions or requests within their area of competence.

You work under the responsibility of a team leader. As a trainer, his job is to advise you on good laboratory practice. When in doubt, you ask him for advice, and then ask the laboratory referents ([sous-section 2.2](#)) or the competent Health and Safety people at the ESE.

1.1 General rules and good lab practice

Initial training for new arrivals, both permanent and non-permanent, is mandatory. It is provided by the manager(s) of the host research team, or by a member of the team who has already received training and is designated by the manager(s). The "referents" named in the list in the following chapter ([sous-section 2.2](#)) are responsible for ensuring compliance with and, if necessary, re-explaining the laboratory and equipment operating instructions given to you by your trainer. You should contact them if you are unsure of anything.

The basic rules are as follows :

- windows in work areas must be closed during work. It is forbidden to drink, eat, smoke, use cosmetics or store food in work areas,
- n you're the last to leave a room, turn off the lights,
- read the information displayed in handling rooms, on equipment and on chemical products (labels and risk symbols) and follow instructions and protocols,
- wear gloves for all experiments (except when using alcohol combined with a heat source. The risk of burns is high),

- where toxic or carcinogenic materials or micro-organisms are used, gloves, laboratory coats or other protective clothing or accessories must be worn in the workplace. In concrete terms, this means that :
 - * gloves contaminated with toxic or carcinogenic materials or micro-organisms must be placed in the recycling containers provided for this purpose and replaced immediately ;
 - * **when using gloves, care must be taken not to spread dangerous organisms or substances by making telephone calls, opening doors of any kind, using taps, etc. ;**
 - * protective clothing must be removed before leaving work areas ;
 - * gloves must not be worn outside laboratories.
- dirty lab coats should be deposited in the dedicated laundry bin (located in the laboratory rooms specific to the DEP and GEE teams).
- mouth pipettes may not be used ; pipetting aids should be used,
- hypodermic needles and syringes should be kept to an absolute minimum ; after use, they should be disposed of in an appropriate manner (yellow container with red lid),
- during all handling operations, care must be taken to avoid generating aerosols whenever possible,
- wash hands thoroughly after work and before leaving the work area (wash hands before eating, drinking coffee, cake, chocolate, using the telephone, etc.),
- work areas must be perfectly clean and tidy. Workstations must be disinfected according to the hygiene concept before and after use. Workbenches must contain only the equipment and materials actually used. Reserves are to be stored only in areas or cupboards designated for this purpose.
- room cleaning is the responsibility of the users. The person in charge of the room organizes a cleaning schedule to keep the floors clean and ensures that it is carried out correctly.
- when using microorganisms, workers must be given specific oral information (based on their knowledge) before starting work, in order to identify their pathogenic potential,
- For any use of genetically modified organisms (GMOs), a declaration of use or an application for approval for use must first be submitted to the French Ministry of Research’s Commission du Génie Génétique. Each new project involving GMOs must be the subject of a declaration of use if the classification is level 1, or of an approval for use if the classification is level 2 or higher. Declarations of use and approvals are valid for 5 years. Since January 2022, prior approval must be obtained from the facility hosting the GMOs. For further details :
<https://www.enseignementsup-recherche.gouv.fr/fr/nouvelle-reglementation-ogm-demarches-et-documents-en-vigueur-compter-de-janvier-2022-82564>
- the deposit and use of personal items (e.g. bags, cell phones, etc.) in the work area should be kept to a minimum,

- equipment or materials contaminated by potentially pathogenic microorganisms must be autoclaved or disinfected before cleaning,
- waste containing pathogens and/or GMOs must be collected in accordance with the disposal concept and inactivated by autoclaving or disinfection,
- if potentially pathogenic and/or toxic material is spilled, the contaminated area must be immediately closed off and disinfected and/or cleaned in accordance with current safety standards. Significant safety incidents occurring in the laboratory must be reported to the prevention officers,
- first-aid instructions for accidents involving pathogenic organisms and/or toxic products must be readily available in the work area. All accidents must be reported to the appropriate superiors and prevention officers,
- you must contact your supervisor or the person in charge of the laboratory if you have any problems, or if you are using any of the equipment, products or materials provided in the laboratory for the first time, to report a malfunction or for any other information.

1.2 ESE and Common Services internal regulations

In addition to this document, your supervisor must provide you with the laboratory's internal regulations, which are available on the ESE intranet site ([here](#)). It contains another section on health and safety, which you should also read. Don't hesitate to ask your manager for it.

Mail from prevention officer (AP) dated August 4, 2022 : *Empty containers for laboratory chemical waste in the broad sense (PCL, DASRI, etc.) are available in room 2341, together with labels for affixing to them. Key access only for the moment. Full containers are to be taken down to room 0111 on the ground floor; do not put them in 2341. Room 0111 is in the corridor to the left of the central staircase when the freight elevators are in the back. Remember to fill in the self-adhesive labels with the type of product, the team and the unit, as room 0111 is shared by all three UMRs, and if we have any questions, we'll know who to ask. We have the equivalent of a cupboard and a half, i.e. 3 doors, which are labelled ESE in this room. Get in touch with the APs when the storage capacity of 0111 starts to run out.*

All questions relating to health and safety should be addressed to the two health and safety officers (AP) : Paola Bertolino and Christophe Hanot. In particular, they are responsible for managing and disposing of chemical and biological waste.

In addition, the laboratory has a so-called *commun* service at ESE level. It includes a laundry with autoclave and a weighing room. Its operation is also summarized in another document available on the intranet : **Gestion Ressources \Fonctionnement général \Services communs**.

It is important to read it to find out which days the autoclave is in operation.

1.3 Laboratory notebook

The laboratory notebook is a comprehensive tool for all aspects of research. It is a daily tool for all those who carry out research work (researchers, engineers, technicians, PhD students, trainees, etc.). It enables them to record all their work from day to day : it records the progress of scientific experimentation, from idea to conclusion. The laboratory notebook is first and foremost a means of ensuring the traceability of research work, a recognized component of good research practice.

Within the unit, new laboratory notebooks can be requested from Patricia Le Thuaut (office 2021).

2 Operation of the DEP-GEE shared technical platform

The DEP and GEE research teams share their resources (human and financial) in the management of their molecular biology and in vitro culture laboratories. This translates into the pooling of certain experimental rooms and scientific equipment. Generally speaking, each shared laboratory has bench-top furniture which is primarily used to store accessories for the equipment in these rooms. Cabinets that are left empty can be dedicated to the specific storage needs of the user teams, provided they are clearly and legibly identified to avoid the long-term abandonment and accumulation of equipment.

2.1 Organization of DEP - GEE common rooms

2.1.1 Media and buffer preparation laboratory (room 2308)

This laboratory contains a range of equipment and chemicals which are either shared between the 2 teams (DEP-GEE label) or are specific to one or other of the teams. This differentiation must be respected if we are to work in harmony.

The equipment shared between DEP and GEE is as follows :

- a pH meter whose electrode must be constantly immersed in a KCl medium
- two weighing scales
- a heated magnetic stirrer to homogenize your buffers
- a microwave
- two chemical storage cabinets (one red, the other yellow). The chemicals inside are either shared or specific (see label on container)
- a cabinet (the one on the left) containing shared glassware (DEP-GEE)
- set of pipettes and a pipetboy
- a refrigerator

The equipment belonging specifically to one of the two teams is :

- a small chemical cabinet (powder type) on a stand = GEE-champi team
- a glassware cupboard (the one on the right) where each team has a dedicated space for storing its own glassware

After handling, the user cleans his glassware and weighing utensils or any other items (hand-washing at the sink or with the dishwasher in the laundry (room 2003), where there is also a drying oven) and makes sure to clean the equipment properly to keep the area clean. The user must not forget to retrieve his clean, dry dishes from the laundry and restock them in the glassware cupboards for subsequent users.

2.1.2 Media casting laboratory (room 2340)

This laboratory is mainly used for casting culture media. A horizontal laminar flow hood (Optigel 18, ADSLaminaire) is available for this purpose. Switch on the hood at least 15 min before use to establish a sterile working environment.

The room also features a fume cupboard connected to a small chemical storage cabinet. The key on the cabinet must never be removed, as it is required to open it. All chemicals stored in the cabinet must be identified with the team's name.

An orbital shaker incubator (Inova 40R, New Brunswick Scientific) is available to perform your cultures at the required temperature and shaking. You need to reserve your time slots using the schedule provided, indicating your set temperature. Two trays are available, depending on the type of consumables or glassware used for your cultures :

- a flat tray with a non-slip grip
- a perforated metal tray to provide modularity for screwing on different types of accessories : microtube rack or for 15 and 50 mL falcon-type tubes, and handles for attaching Erlenmeyer flasks of different sizes (250 mL, 500 mL...).

Trays can be interchanged and accessories modularized using the screwdrivers and wrenches available in the under-bench cabinet. These tools must remain in their original place. A microwave oven is available for melting media stored in 1L bottles.

The room has only one type of waste garbage can, intended for waste that has not come into contact with biological material (absorbent paper, gloves, plastic packaging).

For all activities involving the handling of biological materials with varying levels of pathogenicity (C1 or C2), **each team has a microbiological safety post in its own laboratories**. The biological waste generated is stored in specific garbage cans corresponding to bags labelled *autoclave*. They must not be filled with more than half the bag. They must undergo a sterilization cycle (known as decontamination) by autoclaving, and then be disposed of either as household waste or as DASRI, depending on the level of biological risk to the organism. Any objects that could potentially pierce the bag (tips, spreaders, inoculators, etc.) should be disposed of in intermediate containers such as plastic bottles, before being placed in the autoclave bag. A stock of plastic bottles is available in room 2340, and anyone can help by bringing in their empty bottle.

Each person who generates biological waste for decontamination must participate in its disposal after the autoclaving cycle. To do so, they register in pairs on the schedule opposite the laundry room (room 2003). In general, decontamination is launched after the Tuesday morning and Friday morning autoclaves, but this can be adapted according to the quantity to be processed. ([sous-section 3.3](#)).

2.1.3 Nucleic acid extraction laboratory & Quality control (room 2140)

This laboratory is available to users for nucleic acid (DNA/RNA) extraction and assay by spectrometry and fluorescence. All workstations and equipment must be cleaned with 70% ethanol after use. This room has two separate work areas :

Extraction area : This workspace takes up 3/4 of the room, and includes a range of extraction equipment. Three water baths are available (2 with demineralized water and 1 with heating blocks). A rotary mini-incubator purchased by the field team (GEE) is available, subject to authorization. Four different centrifuge models are available ([sous-sous-section 2.4.4](#)). Instructions for use are available in the room (in a cardboard box in the cupboard under the bench, where the ball mill accessories are located). However, your supervisor must show you how to operate the machine before you use it for the first time. **Always switch off equipment after use.**

A reservation schedule has been set up to avoid overlapping between the different people who work in this room. Make sure you register in advance to ensure equipment availability. To do this, reserve the equipment you need by indicating your initials on the schedule. Legendarily, match your initials with your full name so that people who need to interact with you regarding a piece of equipment can easily recognize you.

A fume hood is present in this room to carry out all steps involving the use of toxic products or solvents requiring air extraction. The fume hood must be left empty and cleaned after use. Any waste generated (solid or liquid) must be dealt with appropriately and promptly, to avoid waste being left unattended, which can produce continuous releases in the room. Please contact your local waste prevention officer for further information. The fume hood is connected to a small chemical storage cabinet. The key on the cabinet must never be removed, as it is required to open it. You can store the products you need for your manipulations in this small cabinet, provided you have identified them with the name of your team.

No individual materials are to be stored on the benches outside the working hours, but only in dedicated under-bench cabinets.

Boxes of gloves and paper towels are available in this room. **Replace when empty!** Gloves are kept in room 2335 and paper towels are managed by Patricia Le Thuaut (office 2021).

After your extractions, a quality control station for your nucleic acids is located in the same room.

Quality control area : The Synergy HT plate reader from BIOTEK can be used for a wide range of experiments involving absorbance or fluorescence measurements (spectrofluorometer). The device can measure absorbances between 1 and 1000 nm. It has 4 filters for excitation (360/40 ; 485/20 ; 530/25 ;645/40 nm) and emission (460/40 ; 528/20 ; 590/35 ; 680/30 nm) of fluorescence. It is connected to a laptop where each team has its own working session. On the desktop, PDF documents on the operation of the reader and the Gen5 software that drives the machine are accessible. This makes it possible to create specific protocols for experiments that go beyond simple nucleic acid assays.

In particular, it can be used to quantify nucleic acids in absorbance on the Nanodrop principle, with up to 16 samples run simultaneously ; with the Take3 plate. Due to its high cost (€3,000), please take good care of it. The plate is housed in a black case in the first drawer of the under-bench cabinet near the unit. The simplified protocol for absorbance quantification is available in paper format in the same drawer.

It is also possible to perform fluorescence quantifications per 96-sample plate on this instrument. A default protocol is available in each session, but it is necessary to understand how the software works in order to use it correctly.

The Qubit4 is available for low-flow fluorescence assays. This device was purchased by BIOM-DEP-GEE as part of the 2022 pooling agreement. The purchase of consumables (tubes and assay kits) is the responsibility of those who need them. A simplified protocol for reagent preparation is provided with the unit. Please refer to your assay kit's protocol for correct sample dosing. Depending on your needs, assay kits are available for double-stranded or single-stranded DNA, RNA or proteins. Find out more about the capabilities of Qubit (<https://www.thermofisher.com/fr/fr/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit.html>)

2.1.4 PCR Laboratory & Electrophoresis (room 2201)

This room is physically divided into two parts : the first houses all the thermocyclers, and the second is the electrophoresis laboratory.

1. PCR Laboratory - 2201A

This room is equipped with a fleet of 7 peltier thermocyclers with heated covers, 3 of which have the temperature gradient option (sticker indicating this option). Each instrument is accompanied by a schedule on which you must register to use it. Booking is compulsory, and you must respect the time and duration of your reservation. During busy periods, don't hesitate to book your slots in advance to ensure machine availability. If you need a lot of machines, make sure you don't penalize other users.

Instructions for use for each type of thermocycler are available in the first drawer of

the under-bench cabinet. However, you must be accompanied by your supervisor when using either type of thermal cycler for the first time.

Your PCR plates and tubes must be sealed before entering the room, and must remain sealed until they leave the room. Plates and/or tubes must be perfectly identifiable by your initials written in indelible black marker.

When programming your temperature cycles, name your program using your initials first, so that it's easy to identify. Machine programs are cleaned once a year. We ask that you regularly delete unused programs (e.g. when students leave), taking care not to delete all machine programs.

Thermocyclers must not be moved, and you must switch off the machine you have been using when you remove your PCR. The lid must be folded back to prevent airborne dust from settling inside the plates. At the end of the PCR cycle, and if you cannot directly recover your PCR products (for example, when, exceptionally, you run a PCR at the end of the day and only recover it the following morning), it is possible to introduce a step that maintains the PCR products at a given temperature. To optimize thermal cycler life, this temperature should not be lower than 10°C.

RT-PCR or qPCR : The laboratory is equipped with an RT-PCR Step-one Plus (Applied Biosystems) for absolute or relative quantification of DNA or gene expression. For all applications, please contact Fanny Hartmann, who is responsible for this equipment.

2. Electrophoresis lab - 2201B

This room is used for all experiments linked to PCR product revelation, gel cutting, etc. In terms of hygiene and safety, this is one of the most sensitive rooms. **The use of nucleic acid intercalant (in this case, gelRed) must be confined to this room, because of its potentially CMR (carcinogenic, mutagenic and toxic to reproduction) properties.**

This room is divided into several areas : a gel preparation area, a migration area with electrophoresis tanks, and a gel image capture area. It is imperative to have a lab coat dedicated to the gel room, which will not leave this area. In addition, the gloves used in this room must be removed on leaving the lab.

Equipment and bench space are shared. Each user is responsible for the cleanliness of his or her bench and equipment. This means that they must ensure that the equipment is clean immediately after use :

- the cleanliness of the bench,
- cleaning and rinsing of equipment (Erlen, test tubes, etc.) and removable parts of the tank, storage of equipment and products (tanks, cone boxes, bench garbage cans, scissors, markers, etc.),

- restocking of any small equipment or products used (gloves, absorbent paper, Kleenex, blue charge, TAE 0.5X, deionized water, cone boxes, scotch tape, etc.),

Each user uses the blue charge that he or she has prepared or purchased in advance. It must never be left permanently on the gel room benches. It must be easily identifiable (type of loading solution + your initials or team).

TAE 0.5X containers are replenished from TAE 50X stock solution bottles, taking care to use the oldest ones first. The current bottle remains on the shelf in the immediate proximity of the TAE 0.5X container. Empty stock solution bottles should be rinsed, the autoclave indicator removed, and returned to the laundry room (2003)

In addition to the standard electrophoresis tanks (with different formats, in the under-bench cabinets), we have a Pippin Pulse, which is a FIGE (Field Inverted Gel Electrophoresis) pulsed-field electrophoresis system from Sage Science. It is mainly used for quality control of large DNA fragments or native DNA upstream of MinION sequencing. Gels are 14×12 cm SeaKem Gold agarose (very expensive, please note) or equivalent. Migration buffers are either TBE or KBB from Sage Science. Deposits must be at least 200 ng of DNA.

Waste management

A stock of buckets/cans is available in the room. If not, please restock for the collective. Remember to label the buckets and cans according to the **indications on the room door**. General stock is held in locked room 2341. When the general stock decreases, inform the AP to restock with the university's health and safety department.

There are 3 types of waste :

- Small white garbage cans on the bench : "GelRed contaminated agarose gels". **label UN2811**
- Large white garbage cans under the bench : "Gloves + GelRed contaminated paper towels" **label UN3175**
- Can on bench : "Used solution TAE 0.5X" **label UN2810**

When the buckets or cans are full, close them with the appropriate lids or caps. Add the appropriate label with ESE - UMR8079 marker. Everyone must participate in the collective by taking the initiative to take the cans and buckets downstairs to room 0111 ([sous-section 1.2](#)). A poster on the door of the electrophoresis room summarizes the instructions.

Gel electrophoresis image capture

The gel capture system is directly connected to the laboratory network. This device generates digital photos which are retrieved in a folder accessible via the ESE network. For first-time use, follow this procedure to connect your computer to the common photo folder. Your computer must first be connected to the ESE network via a fixed

IP address (contact Nicolas Moulounguet to obtain one).

Photo recovery procedure :

- **on mac :**
 - (a) Go to Finder
 - (b) Go then Connect to the server
 - (c) Enter the address smb ://172.24.28.158 then log in with the identifier : **User** et mdp : **esegee**. Choose Genosmart. You'll come across the SYGFN1100 folder containing the photos.
- **on PC :**
 - (a) Right-click on the workstation and select "Connect to a network".
 - (b) Choose a drive other than Z (Y, X or other) and in folder, enter \\172.24.28.158\genosmart. Click on Finish.
 - (c) A logging window appears. Enter the login : **User** and **mdp : esegee**. Remember your login details.
 - (d) You have access to the SYGFN1100 folder.
- **on Linux :**
 - (a) Enter the address smb ://172.24.28.158/genosmart
 - (b) Enter **User** and mdp : **esegee**.

Photo management :

To avoid overloading the host computer with data, please retrieve your photos from your own computer and then delete them from the SYGFN1100 shared folder. Photos remaining in this folder for more than 3 months will be automatically deleted.

2.1.5 Freezer storage room (2330 and 2337)

At the entrance to each of the rooms (on the notice board) is the distribution decided before the move, based on the needs expressed by the teams. Before adding a new freezer, all the teams concerned must be informed so that they can reach an agreement. Each freezer must be identified so that the person responsible can be quickly identified and alerted in the event of a malfunction.

2.1.6 Cold room (2138)

At the entrance to the cold room, you'll find the division of space between the BIOM-PEPA-DEP-GEE teams. Please respect each other's space and keep the cold room clean.

2.1.7 Biological sample storage room (2136)

This room is designed to hold biological samples for storage and culture. It is imperative to identify the owners of the samples. Climatic chambers may be added in the future, if required. Compressed air inlets are used to connect certain equipment (humidity-controlled ovens, etc.).

2.2 Responsibilities of the DEP - GEE shared laboratory

Room	Designation	Name
	Management, financial management, ordering and maintenance	Alodie Snirc
2140	Extraction lab & Quality control of nucleic acids DEP - GEE	Alodie Snirc & Amandine Labat
2201	PCR Lab & Electrophoresis Lab DEP - GEE	Alodie Snirc & Amandine Labat
2340	Prep media Lab DEP - GEE	Alodie Snirc & Amandine Labat
0103	Alcohol storage access	Olivier Chauveau, Alodie Snirc & Amandine Labat

2.3 Financial terms

At the beginning of each year, the overall annual budget is estimated on the basis of the previous year's basic operating costs. This cost includes two main types of expenditure : the purchase of consumables (such as gloves, tubes, PCR plates, Petri dishes, pipette cones, chemicals, etc.), and the purchase and maintenance of equipment. The teams' contribution is calculated on the basis of several indicators reflecting the activity and general consumption (use of thermocyclers, gloves, cones, Petri dishes, etc.) of the 2 user teams. Thus, the estimate of a team's relative activity (expressed as a % of total activity) in year N is used to define its fixed contribution to the budget for year N+1.

All these elements are discussed and validated at a BM meeting in January each year.

All requests for access to the BM-CIV lab must be made to all DEP and GEE team leaders and to the lab manager.

2.4 Definition of common facilities

The table below lists the main items of shared equipment, for which DEP and GEE share the financial management. This implies regular maintenance and replacement, except for expensive equipment, which will have to be discussed collectively.

Room	Designation
2201	7x Thermocyclers
2201	qPCR (StepOne Plus, AppliedBiosystem)
2140	Spectrofluorimeter (Synergy HT, Biotek)
2140	Qubit4 (Invitrogen)
2140	TissueLyserII (Qiagen)
2140	4x Centrifuges (Deepweel, 50 mL, 15 mL, 5 mL, 2 mL)
2140	2x Water baths et 1x Drying bath
2308/2340	2x Microwaves
2340	Incubator (Inova 40R, New Brunswick Scientific)
2340	1 Horizontal laminar hood (Optigel18, ADSLaminaire)
2308	1 pH-meter, 2x balances et 1x magnetic stirrer

Some of our scientific instrumentation has already been described in previous sections. The remainder is described in detail in the following paragraphs.

2.4.1 Thermocyclers and qPCR

[sous-sous-section 2.1.4](#)

2.4.2 Spectrofluorimeter and Qubit

[sous-sous-section 2.1.3](#)

2.4.3 TissueLyser II

For your first use, you must be trained by your referent or by a person who knows how to use it. This device must always be used **balance** on the same principle as a centrifuge, to avoid breaking the machine.

You can grind biological material in a variety of formats :

- 2 adapters for 2 mL tubes for up to 48 samples.
- 2 adapters for 96 plates for grinding up to 192 samples.
- 2 x 10 mL bowls for grinding larger quantities of the same sample.

Moreover, the addition of beads for grinding can be semi-automated thanks to two dispensing accessories for 3 mm beads or 5 mm beads for 96-well plates. If you don't keep to the sizes specified for each accessory, you're in for a bumpy ride.

2.4.4 Centrifugeuse

Four centrifuges are available in the extraction laboratory ([sous-sous-section 2.1.3](#)) with different kind of rotor :

- a refrigerated centrifuge with a rotor for 8 x 15 mL tubes and a rotor for 6 x 50 mL tubes. When changing the rotor, take care to secure it correctly. For the first time, ask a competent person to show you.
- a Sigma deep-well plate centrifuge, mainly used for DNA extractions in 96-well plates. At the end of use, clean the wells (i.e. where you store your plates) with distilled water to remove any traces of the products used during extraction which may attack the material.
- two refrigerated centrifuges with a rotor for 24 x 1.5 mL / 2 mL tubes (VWR) / 48 x 1.5 mL / 2 mL tubes (Eppendorf) and a rotor for 10 x 5 mL tubes (VWR) / 16 x 5 mL tubes (Eppendorf). Rotors are changed using the T key. Please use the lid to avoid aerosols in the centrifuge. Clean the seal between rotor and lid with water and dry with paper towel.

When you've finished using the equipment, clean it with a paper towel soaked in water, and dry it with a paper towel.

2.4.5 Water-bath

Always check the water level before each use : top it up with demineralized water if necessary. If you notice any impurities in the bain-marie tank or the water it contains, change the water and clean the tank if necessary. Add the blue product (1 mL per liter of water) to extend the life of the water by slowing down the growth of bacteria and fungi. Put a piece of paper with your name on it (any appliance switched on without a piece of paper will be switched off), and don't leave it on any longer than is necessary for your work.

2.4.6 Incubator and laminar flow hood

[sous-sous-section 2.1.2](#)

2.4.7 pH-meter, weighing scales and magnetic stirrer

- pH meter : In case of doubt, don't hesitate to read the instructions on the side of the pH meter, or to ask questions to the right people. The measuring electrode is the most fragile part of the device. It must be kept in its cap, filled with KCl solution, when not measuring, and refilled if the level drops, using the nearby bottle of KCl. Acidic and basic solutions are available for pH adjustment. Calibrated solutions are also available in the under-bench cabinet to ensure that the pH meter is working properly. After use, the electrode must be cleaned with distilled water and immersed in a sufficient level of KCl.
- weighing scales : Weighing cups in various sizes are available, as are weighing spatulas. At the end of each use, you'll need to clean them with 70°C ethanol and deionized water (wash basins available, to be refilled when empty). For weighing, several types

of scales are available in this room. Check that the scales are clean after each use, and clean if necessary. As the scales are balanced, please check that they are properly balanced and, above all, do not move them.

- Magnetic stirrer : Magnetic stirring rods of various sizes are available for preparing your solutions, as well as a stirring retriever. This equipment is inventoried to minimize the risk of it going missing. Cleaning of this equipment is also compulsory (alcohol + water, or washing machine in some cases). All the small equipment made available to the community, such as the weighing spatulas, magnetic bars and magnetic rod (to recover the bars), are essential if this room is to function correctly. This is also a very sensitive piece of equipment, which can easily disappear. Consequently, any unexplained disappearance will result in its non-replacement (it is then up to each team to have its own equipment).

2.4.8 Heat sealer

The heat sealer used to seal PCR plates, and all associated equipment (heat-sealing films, scissors, piece of cloth for final sealing of the film) must not be moved. Heat-sealable films must remain in their plastic pouch before use. Iron-on films must be handled with gloves.

The heat sealer should not be left on all the time, as this will considerably reduce its service life. Switch it on 5 to 10 minutes before use, and place a piece of paper with your name on it on the heat sealer so that it is not switched off by mistake. Switch off the heat sealer as soon as you've finished using it (making sure to ask beforehand if anyone else in the room is using it).

If the heat sealer is switched on in the absence of a nameplate, it must be switched off by the person noticing it.

2.4.9 CodonCode Software

The laboratory has software for analyzing Sanger sequences (cleaning, consensus...) and NGS-type data. The license has not been renewed, but the software is still functional. It is called Codoncode Aligner and can be downloaded from the following link [here](#).

A Mac and PC version is available. However, it does not work under Linux. When you first install it on your computer, you need to select the option «**use server licence**» to access our license. Make sure your computer is connected to the lab's Internet network via Ethernet (not Wi-Fi), and that it has a fixed IP address (if not, contact Nicolas Moulonguet).

The IP address of the host computer is **172.24.28.158**.

2.5 General stock of consumables (2335)

All consumables for the shared platform are located in the **room 2335**. Equipment/consumables specific to each team can be stored in the **room 2331**.

All items removed from stock must be recorded on the stock removal slip located on the metal locker on the right-hand side of the entrance to the room. All consumables are labelled with numbers on the shelves. A summary sheet of all consumables is next to the stock removal sheet. This enables you to check the minimum withdrawal quantity indicated for each type of product. You can directly transfer the product figure to the withdrawal sheet, along with its quantity. This is general stock. You must use this part to replenish your handling parts, which are intermediate storage locations. If you notice an error or a misunderstanding, don't hesitate to inform the labmanager. This system enables us to keep track of our stock on a computerized basis, which is why it's vital that people understand this procedure to avoid errors.

In the case of laboratories used in common by DEP and GEE, consumables (such as gloves) must be entered as "common" with the destination part on the stock withdrawal sheet, instead of being entered by the team.

Basic consumables list :

- **Gloves, paper towels** : Five sizes of nitrile gloves are available in the work rooms (XS to XL). Users are responsible for restocking as soon as a box has been used up. Users are also responsible for replacing the rolls of absorbent paper located near the sinks. For paper towels, the stock is managed by Patricia Le Thuaut (office 2021).
- **Tips and tipbox** : The tip boxes on the benches are not self-service, as they are part of the equipment allocated to the bench. Tip boxes must not be thrown in the garbage can. A plastic recycling procedure has been set up in the laboratory. ([sous-section 3.4](#)).
- **Tubes 0,2 to 5 ml** : The glass jars containing the tubes on the benches are not self-serve, as they are part of the equipment allocated to the bench. When you finish a jar, you must fill it and place it on the autoclave trays waiting in room 2003 (laundry). Remember to put a small piece of tape "autoclave indicator" on the lid of the jar (having removed the old indicator beforehand) and indicating the name of your team to identify the material.
- **PCR plates and strips** : The boxes of PCR plates on the benches are not self-service, as they are part of the equipment allocated to the bench.
- **Deionized water** : Each team chooses whether to purchase sterile deionized water or to prepare autoclaved deionized water : water is drawn from the demineralized water production system, for sinks equipped with one, into a glass bottle which is then autoclaved, and aliquoted into smaller tubes under a laminar flow hood (room 2340).
- **Petri dishes** : Two sizes are available : 94 mm and 60 mm. However, there is still a small stock of 145 mm boxes and 120 x 120 mm square Petri dishes, which will not necessarily be recommended once the stock is finished.
- **Individual materials** : Apart from the common supplies, equipment and products described above, any other supplies, equipment or products are the responsibility of the research team to which you belong (e.g. micro-pipettes, storage racks for tubes, products, reagents and enzymes required for PCR, cloning kits, etc.). It is forbidden

to use this individual equipment without the express authorization of its owner(s).

Any other purchase of consumables is possible if the need becomes recurrent, after discussion and validation at a meeting.

2.6 Room cleanliness

The manager organizes a cleaning schedule to keep the hall clean. A broom, bucket, mop and cleaning product are available. If this is not the case, please inform the hall manager. Cleaning materials and products must remain in the room. A cleaning procedure is indicated in each room.

Several sinks are available in the handling rooms. This is not a storage area. Sinks must remain perfectly clean and empty.

3 General operation on the ESE scale

3.1 Liquid nitrogen

Liquid nitrogen is centralized at the rear of the IDEEV building for the 3 UMRs. To do this, look for a key on the 1st floor, in one of the GQE labs (room 1138) and note the quantity taken on the notebook (important for financial management). Then go downstairs to room 0117. A protocol with further explanations is posted on the extraction room door. Two Deware vessels are available for transport in the same room. You must be trained to draw nitrogen by your supervisor to avoid any risk of accidents, especially burns.

3.2 Chemical products management

Large quantities of chemicals are stored on the ground floor of the IDEEV building, which has several rooms depending on the type of product (alcohol, solvents, acids, bases, etc.). On the 2nd floor, two rooms are available for storage in secure, ventilated cupboards. Anyone ordering chemical products must identify them (team name or other). He/she assumes full responsibility for their management (storage, use) and disposal (proper disposal of chemical waste). If you have any questions on this subject, please do not hesitate to contact the AP personnel.

The management of absolute ethanol is subject to regulatory control of "incoming" and "outgoing" stocks. Consequently, access to the bunker is **restricted** to some person ([sous-section 2.2](#)). Please contact these people if you need absolute ethanol or access to other products.

3.3 Autoclave (2003)

Autoclave sterilization is available to all unit teams. However, only those with a valid autoclave operating and safety certificate are authorized to handle the horizontal autoclave

(Matachana SC500). A copy of this certificate must be provided to the autoclave manager beforehand. The list of authorized personnel is posted on the entrance door to the room. The autoclave user manual and maintenance log are available in the room. After each use, the sterilization progress ticket supplied by the machine must be pasted into the tracking book located in the same place. The person in charge of the autoclave is responsible for regulatory aspects and maintenance.

The operation is based on the launching of two weekly autoclaves as described below ; the launching of the autoclaves is divided between the staff of the DEP - GEE - DEEM teams. To find out who is running the autoclave, simply consult the monthly diary on the outside wall opposite the laundry.

Storage and retrieval of autoclaving equipment (solid and liquid cycles)

White baskets are available for your autoclaving equipment, as well as a fridge for storing media awaiting sterilization. Autoclave tape is available for equipment that needs to be identifiable. Two types of baskets enable you to sort your materials according to whether they are solid or liquid. **Remember to regularly return autoclaved material from the iron baskets.** The organization of autoclaves follows the rules described in the following sections :

- Tuesday morning = liquid autoclave and Thursday = solid autoclave
- For an autoclave to be launched, at least one solid or liquid basket must be filled.
- if you have an extremely urgent need but the autoclave is not full, **EXCEPTIONALLY**, an arrangement can be made. To do this, check with the person in charge of running the autoclave.

You need to anticipate your needs as far as possible to avoid such emergencies.

After sterilization, the media are generally placed in the oven to maintain this state. Remember to switch off the oven properly to avoid any risk of fire.

A small benchtop autoclave is available for small volumes. Any user can operate it without authorization. The user must first be trained in its use by a competent person.

There are also autoclaves for decontaminating micro-organism waste for disposal. These autoclaves are launched outside conventional cycles, at a temperature of 134°C. In general, a decontamination cycle is launched after a conventional cycle (liquid or solid) if three bags to be disposed of are present in the room. People generating biological waste must participate in its disposal. To do so, they must register on the monthly schedule, preferably in pairs, to take charge of the disposal of decontaminated bags. At the end of the decontamination cycle, the pair is responsible for disposing of either household waste (by placing the decontaminated bags in a black garbage bag and taking it down to the garbage room on the ground floor of the room (0107)), or medical waste, depending on the organization's level of biological risk.

3.4 Recycling empty tip boxes

A cardboard box for empty tip boxes is located in each handling room, and users are responsible for installing it if this is not the case. Empty tip boxes must be placed in an orderly fashion and stored in the cardboard box. Once the cardboard box is full, it must be closed and taken downstairs to room 0104 (-80°C), to await recycling. When ten cartons are full, a collection request can be made to our supplier Dominique Dutscher. Don't hesitate to let Amandine Labat or Alodie Snirc know when the number of cartons reaches the recycling limit.

You'll find a stock of empty cardboard box in room 2331. The format of the Greiner Petri dishes (diameter 94 mm) is the ideal size for the quantities we are authorized to send out. If there are no more boxes of this type available, keep and use boxes of a similar size.