

TEGlab

LAB USER MANUAL

Version 3.0

updated 16.1.2014

(EMERGENCY INSTRUCTIONS ON THE LAST PAGE!!)

TEGlab members and phone numbers

The area code for all phone numbers is 02

Key personnel

Ville Aukee, Laboratory manager	333 7083
Tatjana Saarinen, Laboratory master	333 5548
Raija Rouhiainen, Secretary	333 5575

For additions and/or corrections to the manual, contact ville.aukee@utu.fi

(EMERGENCY INSTRUCTIONS ON THE LAST PAGE!!)

LAB RESPONSIBILITIES

COMMON CHEMICALS, ORDERS	TATJANA
SEQUENCING, ABI (MAINTENANCE, ORDERS)	TATJANA
ETHANOL	CHRISTIINA
DISHWASHING, AUTOCLAVING	JOHANNA
GMO WASTE (NOTIFY)	JOHANNA
HAZARZOUS WASTE	TATJANA
BALANCE- AND PH-AREA	TATJANA
PRE-PCR	SHARED
CLONING AREA	USER CLEANS
RNA ROOM	ERICA
GEL ROOM	TATJANA
ION TORRENT PREP	MERI

Responsibilitites include supervising the tidiness of the assigned area, reporting of problems or malfunctions to TEGlab manager, advising in use of equipment and space, etc. However, lab users are always expected to clean after themselves!!

(EMERGENCY INSTRUCTIONS ON THE LAST PAGE!!)

First things to do when starting in the lab

- Read this manual
- Introduce yourself to Ville, room 7011
- Join the TEGLab mailing list. Go to <https://lists.utu.fi/mailman/listinfo/teqlab> and fill in the form
- Fill in the TEGlab user license form (ask Ville)

Some rules of the lab:

- Attend the regular TEGlab meetings (about every first Wednesday of each month) and learn about new things or discuss issues.
- You must have filled out the lab user license before starting
- Be sure you have an assigned bench where to work.
- Subscribe to TEGlab mailing list (see above for instructions).
- Keep you bench place clean, and be tidy elsewhere too.
- Be safe. A laboratory can be a dangerous place.
- Inform Ville at least 2 working days before vacating your bench
 - o clear out your bench & freezer storage

COMMON SPACE AND EQUIPMENT

If needed, ask Ville for specific instruction manuals for each piece of equipment. Certain people will also be able to help you with operation of certain equipment (refer to the lab user license)

If something breaks down or is otherwise out of order, contact Ville. When ABI is in question, you may also contact Tatjana.

Pre-PCR lab (7114)

Each bench section should have one vortex and minicentrifuge. DO NOT move any machines without consulting Ville first. Please DO NOT shift items or equipment between pre and post-PCR sections. Ask Ville about possible freezer space in this section of the lab.

DNA-extraction hood (in 7114)

There's a vacuum pump and a manifold for DNA extraction in this hood. Consult Katja or Kristiina in the use of these.

Ultrapure water

The main ultrapure water system is in machine room 7114 (pre-PCR). The backup clean water machine is in dish washing room 7109 (RO-Water).

See instructions for the simplest way to operate the water machine:

How to use PureLab Ultra machine

Manual Dispense

Step 1 • Product Quality = When you see only this:



PRESS THE POWER BUTTON

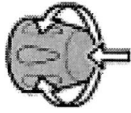
ONCE AND WAIT UNTIL THE SCREEN CHANGES (ABOUT 30 SEC. – look for value

18.2Ω)

1. ENSURE PURELAB Ultra is in recirculation

Step 2 • Dispense

1. PRESS Dispense Controller once.



2. ROTATE Dispense Controller clockwise.

If the Dispense Controller is not rotated within 15seconds the unit will revert to recirculation mode.

3. ADJUST the flow from the unit by rotating the Dispense Controller as necessary.

Note: To prevent accidentally leaving the unit on continuous droplet dispense the PURELAB Ultra will only dispense for a maximum of 4 minutes from the last turn of the Dispense Controller. For large volumes it is recommended that the Volumetric Dispense mode is used.

4. PRESS Dispense Controller once to stop dispensing and return to recirculation mode.

Post PCR room (7116)

This room contains the PCR machines, and some bench place to prepare post-PCR samples. Note that the PCR machines have a reservation system. Keep as few personal PCR-programs as possible in the machines. DELETE all programs which you are not using anymore! The fancy looking BioRad PCR machines are not for common use.

IonTorrent-related material is also located here (the benches next to the PCR machines) .

This room also contains **the Electrophoresis laboratory:**

Agarose electrophoresis apparatuses, UV-tables, Dark Reader, and the GelDoc imaging system are here.

SAFETY NOTES: Some still use Ethidium bromide, so always wear **gloves** when you are working here and dispose them when you leave the room.

TEGlab encourages people to use safer dyes, such as Midori Green (provided in the fridge) With the UV-tables use BOTH goggles and protection mask if you are working **in direct UV exposure**. If you want cut bands from your gel, use the Dark Reader. Don't cut gels on the Gel Imaging system!

Cold rooms (7113 & 7071):

Shelf space in cold room is allocated according to the needs of the groups. If you need storage place there, contact Ville.

RNA laboratory (7066):

Clean (DNA and RNase free) space for working with RNA samples. DO NOT move any pipette sets from here or to here. Also new pipette sets should be autoclaved before placing here. Erica is in charge of this area. A

TissueLyser for preparing samples is also here (ask PnP research group for permission to use).

ABI Room (7075):

Keep this laboratory's door closed because of air conditioning.

Do not run the sequencer during thunder storms; the laser might get damaged.

The lab has ABI Prism 3130xl Genetic Analyzer (16 capillary) sequencing machine. You can leave your ready sequencing samples to Tatjana or, once you have received proper training, operate the machine yourself. NOTE: Use ONLY SEMI-SKIRTED 96 Detection Plates in this machine otherwise there is risk of capillary damage.

This room also contains Thermo centrifuge, which is able to spin truly fast rpm at low temperature. Consult Mikko Nieminen.

This room also contains the Iontorrent + OT2, which are "next generation" sequencing machinery. Meri Lindqvist will tell you more about the possibilities of using it.

This room also contains 4 deep freezers (1 is an emergency freezer). Ask Ville for space.

Sequencing and Fragment Analysis

Tatjana Saarinen is in charge of preparing (adding formamide & size standard in fragment analysis) and running sequencing- and fragment analysis samples.

Preparation and handling of EtBr agarose gels

TEGlab encourages people to use safer gel dyes, like Midori Green, which is ours to use in the gel lab fridge. But if you insist on Et.Br.:

The ethidium bromide (EtBr) used for staining of agarose gels is mutagenic: it can directly incorporate into DNA and change its structure. EtBr is harmful if inhaled, swallowed or in skin contact. It is soluble in water and thus can be removed from the skin by rinsing with water and mild detergent. When using EtBr in the lab you must 1) prevent its evaporation, 2) prevent contact with your skin by always using gloves when handling items possibly contaminated with EtBr, 3) protect others by not spreading EtBr anywhere outside the apparatus directly involved in the gel run.

Always dissolve the agarose in the microwave first, and let the agarose cool off (in a Erlenmeyer bottle under running water or using magnetic stirrer) before adding one drop of EtBr 2,5µl/50ml gel solution (stock solution's concentration is 10mg/ml) pipette stock solution inside the fume hood. Cast the gel and rinse off the tube or bottle. **Discard your gloves when you leave** the electrophoresis laboratory. After the gel run DO NOT leave power

packs *on* and DISCONNECT cables from the electrophoresis apparatus. When using the BioRad gel imaging system or the UV-tables, make sure your gel is dry and that you clean the light tables well. Don't use wet gloves when typing.

Washing room (7109)

A dishwasher, an autoclave and a backup clean water machine are in this room. Johanna takes care of operations here. She collects the dishes from the laboratories. If you have something special to wash or to autoclave, talk with her.

Teaching laboratory (7119)

Ethanol storage, 2 scales and a pH-meter are here.

ADDITIONAL NOTES

Ethanol (Christina Nokkala)

Ethanol is stored in an assigned locked cupboard in teaching laboratory 7119. Keys are in Christiina Nokkala's possession. Ask her for ethanol (or Ville if she is not around)

Fridges, freezers and the cold room

The lab has plenty of freezer space, due to the fact we have our own freezer room (7111). Defrosting of standing freezers will be organized at least once a year, Ville will inform you about this on the email list.

Dishwashing (Johanna, room 7109)

Used lab-ware should be rinsed and taken into the basins placed near the sinks in every laboratory. Johanna collects them and does the actual dishwashing. If you have something special to wash or autoclave discuss with her.

Keys to corridor

Raija the secretary can provide you with keys. Or if you already have university's electronic key notify number of your key to her to get access activated.

pH-meter (room 7119)

Always consult Tatjana before using the pH-meter for the first time. Instruction sheets in Finnish and in English are next to the machine on the wall. The pH-meter is always turned on; if it is turned off it has to be recalibrated. Calibration solutions are next to the pH-meter.

Pipettes (Tatjana Saarinen, room 7058)

Tatjana Saarinen takes care of all common pipettes (marked TEGLab). She also calibrates and replaces broken parts to them. If you have some problems or you are not sure of some pipette's calibration, contact her. NOTE: there are also pipettes which are only for certain groups to use (clearly marked). Ask permission to take such a pipette.

Postal service

In the top shelf of the mail pigeon holes, you can find a locker for university internal mail (sisäinen posti) and outgoing mail (ulkoinen posti).

When expecting a special delivery such as -20°C or < +4°C package, give a forewarning to secretary and/or laboratory master. You may also make an announcement on the teglab-mailing list.

Fume hoods and hazardous waste

Hazardous waste is collected in bottles in the fume hoods. Phenol, chloroform, iso-amylalcohol and isopropanol are poured into the collecting bottles assigned to this, and dirty pipette tips and eppendorf tubes used with these chemicals should be in a separate bottle. If there is no bottle for that chemical you are using ask suitable bottles from Johanna.

Any waste containing bacteria should be placed to a RISKIPAK box (yellow plastic bag).

It is essential that you clean up after yourself after using the fume hoods.

LIST OF COMMON EQUIPMENT

Sequencing:

- ABI Prism 3130xl genetic sequencer / 16 capillaries + analysis software GeneMapper 4.0
- IonTorrent + OneTouch

PCR:

- 2 x Eppendorf Mastercycler Gradient (96-well)
- 1 x MJ Research PTC 100 (96-well)
- 3 x Applied Biosystems 2720 Thermal Cycler (96-well)
- 1 x Finnzymes Piko

Electrophoresis:

- lots of small and large apparatuses

Gel documentation / pictures / manipulation

- BioRad GelDoc System

Cloning:

- fully equipped cloning laboratory (7069). Talk to Ville First.

Cell culture:

- incubators, fully equipped cell culture laboratory

Spectrofotometry:

- NanoDrop ND-100
- Agilent 8453 + Dell processor

DNA extraction:

- 2 x Eppendorf Vacuum Manifold, salt extraction

Cold storage:

- HERA Deep Freeze -85 °C
- 3 x deep freezers
- -20 °C freezers
- -20 freezer room
- refrigerators
- +4 cold room x 2

in addition:

- laminars, fume hoods, large and small centrifuges, heat blocks, ovens, analysis- and cup scales, ice machine, UV-tables, DarkReader, incubators, autoclaves, PureLab Ultra Water-system, microscopes (light- and preparation), RNA-lab, TissueLyser, etc.

LIST OF CHEMICALS FOUND IN THE LAB

If you use see that any chemical is running low, please inform Tatjana Saarinen. It can take up to a month for certain chemicals to arrive, so let her know well in advance. All the chemicals are in the teaching laboratory, in a locked room. If you're looking for something special, best to ask Tatjana first (room 7058). Phenol, chloroform, isopropanol etc. are stored in the fume hoods or in a fume cabinet in teaching lab. Updated list of the common chemicals is in: \\Utu.fi\\verkkolevyt\\Biologian genetiikka\\Kemikaalilista. Detailed information about each chemical's toxicity, safety and handling information etc. can be seen in ChemDAT – database also in our network drive. Path: \\Utu.fi\\verkkolevyt\\Biologian genetiikka\\Kemikaalilista\\Chemical Data Sheets.

There are also the safety notions in paper form in the pre –PCR lab.

Unfortunately those are in Finnish only.

More information about the safety notes of all chemicals can be found from

[ICSCs pages](#)

Agar bacteriological

Agar Corn Meal

Agarose low EEO

iso-Amylalkoholi

Ammonium Sulfate

Ammonium persulphate

Ammoniumheksafluoridifosfaat

Ferriammoniumsulfat

3-Aminopropyl-triaethoxysilan 98%

Ampicillin Sodium Salt

Aktivkohle

Alcian Blue 8 GX

Acridine orange

Azure B-certified

Acrylamide

Bis-Acrylamide

Albumin

Acetone

Acetic Acid 99-100% (Etikkahappo)

Boric Acid (Boorihappo)

Bariumhydroxid-Octahydrat

Bromophenol Blue

Blotting Grade Blocker

Casein trypsin peptone (tryptone)

Calcium Chloride anhydrous

Calcium Chloride-Dihydrate

Citric acid monohydrate

sitruunahappo-1-hydraatti

Certistain

CTAB Cetyltrimethyl ammonium bromide

Chloroform

Chromosulfuric acid

Carmin for microscopy

Colchicine

Canada Balsam in Xylol Neutral

trans-1,2-**D**iamino-cyclohexane-N,N,N,N-tetraacetic acid

Dithio-oxamide (rubeanic acid)

Deoxycholic acid

Sodium Salt(3d,12d-Dihydroxy-5b-Cholan-24-oic acid)

N,N-Dimethyl-Formamide

Dimethyl Sulfoxide

EDTA Dinatriumsuola (TITRIPLEX)

Entellan

Ethylene Glycol-bis(2-aminoethylether)-

N,N,N,N-tetraacetic acid

Ethylenediaminetetraacetic acid

Ethylene Glycol

TriEthanolaminhydrochlorid

Ether (Diethyl Ether) Eetteri

Etan-1,2-Diol 40% in vatten

Etaani-1,2-Diol 40% vesiliuos
(Glyoxal 40 wt.% solution in water)

Eosin yellow

Exsikkatorfett

Formamide

Formamide Ultra pure grade

Formic Acid 98%

Formaldehyde solution

Fuchsin, Basic D(+)-

Glucose (Dextrose, Corn sugar)

D(+) Glucose anhydrous

Glycine

Gelatine

Glycerin(etwa 87%) (Glycerol)

L- Glutamic acid

D(+)Galactose

Hepes

Hydrochloric acid 36-38% (Suolahappo)

Hydrochloric acid 2,0 N (2,0 M)

Hydrochloric acid 0,1 N (0,1 M)

Jod doppelt sublimiert

Kaliumchlorid (Potassium chloride)

Kaliumdihydrogenphosphat

(Potassium Dihydrogen Phosphate)

Kaliumcarbonat(Potassium carbonate)

di-Kaliumhydrogenphosphat-Trihydrat

Kaliumdisulfit (Potassium disulfite)

Kalium Chrom(III) Sulfat

(Potassium Chromium(III) Sulfate)

Kaliumnitrat

Kaliumacetat (Potassium Acetate)

Kaliumhydroxid

Kaliumjodid neutral

Kuparisulfaatti

Koboltti-II-kloridi heksahydraatti

Koboltti-II-Nitraatti

Kamferia

Ksyleenisyanoli (Xylene cyanol F.F)

DL- **L**actic acid sodium salt

Lactic acid about 90% (Maitohappo)

Litiumkloridi (Lithium chloride)

Methyl-4-hydroxybenzoat (Nipagin M)

Magnesiumsulfat- Heptahydrat

Magnesiumacetat Tetrahydrat

Magnesiumchlorid

L(-) Malic acid

Maleic acid (Maleiinihappo)

D(+)- Mannose

Mangan (II)-chlorid-tetrahydrat

Methyl green

Methyl α -D-Mannopyranoside

N,N,N,N-tetramethylethylen-diamin

(Temed)

Methylene blue trihydrate (Metyleenisini)

MOPS

(3-(N-Morpholino)propane-sulfonic acid)

Milk (Non-Fat Dry Milk)

Mowiol

Methanol

Natriumhydroxid (Sodium Hydroxide)

tri- Natriumcitrat-Dihydrat

Natriumfosfaatti (Sodium phosphate)

anhydrous

Natriumhydrogenphosphat

(Sodium Hydrogen Phosphate Anhydrous)

di- Natriumhydrogen-phosphat Dihydrat

Natriumchlorid

Natriumhydrogen-carbonat

Natrium carbonat anhydrous

Natriumdihydrogen-phosphat-Monohydrat

Natriumacetat-Trihydrat

Natriumsulfit

Natriumiodid

Natriumasetaatianhydraatti

(Sodium acetate anhydrous)

di- Natriumtetraborat-Decahydrat

Natriumnitrat

Natriumthiosulfat-Pentahydrat

Natriumborhydrid

Natriumcacodylat

Naphthol Green B

Orcein

Phenol

Phenol. Saturated solution

Paraformaldehyd

Pepton aus Casein

n- Propyl Gallate anhydrous

1- Propanol

2- Propanol (Isopropyylalkoholi)

1,4- Bis (acryloyl) piperazine for electrophoresis

PVP (-K30) Polyvinylpyrrolidone

PVPP Polyvinylpyrrolidone

Polyethylene Glycol

Penicillin-streptomycin solution

Quinacrine Dihydrochloride

SDS (Sodium dodecyl sulfate)

Saccharose

5-Sulfosalisylihappo

D-Sorbitol anhydrous

Silverniträt PA

Starch Potato

Silica gel 60(0,015-0,040 mm)

Silica gel with moisture indicator (blue gel) (for drying)

TRIS (hydroksimetyyli-aminometaani)

Triton X-100

Iso-octyl phenoxy-polyethoxyethanol

α -Terpineol

Trypsin 1:250

Titriplex II

Tween 20

UREA

Xylene

Yeast extract

IN CASE OF AN EMERGENCY

Common emergency telephone number	(0) 112
Police	(0)100 22
Pharmacy HelpDesk	(0) 0800 157 777

Fire extinguishers are located along the corridors.

Emergency shower is on the corridor next to Pre-PCR. Get acquainted where it is before starting to work in laboratory.

FIRST AID –Kits can be found from almost all the labs.

Chemical Safety Instructions are found from the teaching lab chemical storage and from the network drives:

ChemDAT –database , Path: \\utu.fi\verkkolevyt\Biologian
genetiikka\Kemikaalilista\Chemical Data Sheets.

In case of -20 freezer breakdown

1. Check the temperature – If the alarm is beeping, the temperature is above -14°C. If it keeps on rising, start to move the items to other freezers!
2. Use the freezer room (7111) to evacuate. Notify Ville and then everyone by mailing teglab@lists.utu.fi about the incident.

In case of -74°C freezer alarm

Call these people until someone answers and also read the instructions how to proceed.

VILLE AUKEE	050-3204118
SAIJA KILJUNEN	0445382408
MIKKO NIEMINEN	050-5671636
ERICA LEDER	050-3398204
ANTI VASEMÄGI	045-3585410
HEIDI VIITANIEMI	040-7045585
HANNU MÄKINEN	0400-691338
SIIM KAHAR	046-5559427
MATTHIEU BRUNEAUX	044-9197667
MERI LINDQVIST	050-5850694

Instructions for an emergency situation (-75°C Freezer)

-Alarm goes on at -65 °C, normal running temperature is -75 °C. The alarm is connected to a remote system, and people in a security company will see the alarms. They will also start to call people on the list (above).

-Check the alarm codes from the display; see instruction manual

Check that the door is properly closed (Do not open it)

- if not, then close it and wait for a while to see if the temperature returns to -74°C. If yes, then you're good to go home.

Remember: the freezer temperature doesn't change quickly, so monitor it for quite a while (> 15 min) and see which direction it is going.

- if the door is properly closed, but the temperature keeps on increasing, follow this procedure:

Move all the items from the broken freezer into the EMERGENCY FREEZER, which is the 2nd freezer from the door. It should be empty (maybe some containers with dry ice), so all the items will fit. The emergency freezer has been set to -70, so decrease the running temperature to -75.

In case of a GMO related incident

In a situation of unexpected contamination, exposure, or other serious incident occurs, the following procedures will be followed:

1. Prevent additional danger according to possibilities. In a serious case, contact the authorities immediately (tel 112)
2. Perform CPR if someone is hurt
3. Isolate the danger zone
4. inform the people working in the laboratory (genlab@lists.utu.fi, teglab@lists.utu.fi). If necessary, inform the surroundings.
5. Start to eliminate the danger; decontamination of facility, machinery, protective clothing.
6. Repair the damages and find out what happened. Find improvements safety procedures.

Responsible people in a case of GMO incident:

lab manager Ville Aukee, room 7011,

tel. 0503204118

laboratoriomestari Tatjana Saarinen,

room 7085

GMO facilities, class 1:rooms 7069