Biological/Genetic laboratory safety instructions Annual Renewal 2024

07/02/2024 Ingo Thievessen

Fabry Lab: 02.041-43 (S1), 02.045-49 (S2), 02.070-71 (S2)

Franze Lab: All S1 laboratory rooms

- Before you start working in above laboratories, you have to familiarize yourself with the rules and regulations of the lab and uphold these at all times. For that, we have (1) a general lab instruction manual at the door in every lab, (2) lab-specific instruction manuals for equipment and chemicals, and (3) a powerpoint-presentation on lab instructions on our website (bio.physik.fau.de/methods/protocols-and-instruction-videos/) in German.

- When you enter one of the laboratories, please note the signs posted outside and inside on the door and the wall. There you will find specific instructions ("*Betriebsanweisung*") describing potential dangers when working in a laboratory, or working with chemicals, or devices. If you have questions about these safety instructions or technical issues, please come to see Ingo, Wolfgang or Ben. Do not attempt managing by yourself, if you are not sure about possible safety issues! For issues concerning lab order and cleanliness, please contact the room responsible person (label outside on the door).

Safety standard 1 (S1) laboratories:

02.041 room responsible: Thabi; hood, incubator microscope, rheometer, respiratory device, 3D printers.
02.042 room responsible: Johannes; magnetic tweezer, laser setup, etc.
02.043 room responsible: Lovis; stopped flow device, flow cytometer, server, laser setup.

Safety standard 2 (S2) laboratories:

02.045 room responsible: Astrid/Ingo; bacteria lab, chemical hood, shaker, incubator, ultrasound device.
02.046 room responsible: Tina/Lars; main lab, cell culture hoods, chemical hood, incubators.
02.048 room responsible: Ingo; confocal microscope, microscope #4.
02.049 room responsible: David; microscopes #2 and #3, marine biology lab.
02.070 room responsible: Astrid/Wolfgang; millipore device; dishwasher, autoclave, hot oven.

02.071 room responsible: Astrid/Wolfgang; -80C freezer, liquid nitrogen tanks.

Lab order & cleanliness:

Room responsible person

Operating instructions, safety etc.:

Ingo, Wolfgang or Ben

General lab rules - I

(a) No eating, no drinking, no smoking, no putting on makeup.

(b) Wear gloves and eye/ear protection, when needed (Liquid N₂, ultrasound device etc.). Wearing a **lab coat** is mandatory in all laboratory rooms.

(c) All doors must be closed and can only be opened with a chip, which you get from Heike. The windows must be closed at all times. If there is no other way to escape the S1/S2 laboratory rooms, *e.g.* in case of fire, open the windows and leave the building via the emergency ladder outside the building facing Gebbertstraße.

(d) In case of fire alarm, please leave the laboratory immediately via the **staircase (not lift**) or the outside fire stairs at the end of each hallway. If this is not possible, open the window and leave the building using the emergency ladder outside the building facing Gebbertstraße. After leaving the building, **do not leave the area!** Please gather outside the building on the **assembly area** (parking lot, no assembly sign!) and await instructions from the police, fire fighters etc. Only then you can enter the building safely again.

(e) If you cause a fire, stay calm, set off fire alarm, and report fire. Attempt to extinguish fire without selfendangerment. If you are not absolutely sure, do not attempt to extinguish any fire yourself and leave the place.

(f) If you hurt yourself, use the first aid kits, which are located in every room marked by a green cross in the cupboards above the sink near the door, or in the office hallway. Please let Wolfgang, Ingo or Ben know. Please do not hesitate to see a doctor at the clinic if you think it could be necessary! Serious injuries have to reported to the FAU public health officer. In any case, note the incident on an accident report sheet (attached to the first aid kits) for insurance reasons in the case of possible health issues related to the incident!

General lab rules - II

(g) There are **emergency showers** above the doors in S2-rooms 02.045-49 and **eye showers** in all labs. In case you need them after chemical spilling over yourself or setting yourself on fire etc., **use them immediately**!

(h) When you take out samples from the **liquid** N₂ tanks (room 02.071), please wear **protective gear**: lab coat, helmet with visor and thick blue gloves (located on the shelf above the tanks).

(i) When you brake glass, spill chemicals or bacteria or cell medium on the bench or on the floor, make sure that you clean it up properly with detergent / alcohol and remove any glass debris in the glass container under the bench in room 02.046. Wear lab coat and gloves! If you are not sure about how to clean or decontaminate, please contact Wolfgang, Ingo, or Ben.

(j) Please observe and follow all instructions regarding the use and disposal of chemicals, medium and substances (material safety data sheets folders, 02.046). Do not dispose of them in the sink.

(k) When you work in the lab, please make sure that bench & workplace are clean and not contaminated.

(I) Please mark all your bottles and chemical containers with your **name, date, content and safety pictograms** before storing them on the shelf or in the fridge/freezer. Pictograms can be found in 02.046 next to the waterbath.

(m) When using ultrasound devices (stick, 02.045; or bath, 02.046), make sure to wear ear plugs and make everyone in the lab aware that you will use ultrasound.

General lab rules - III

(n) All biological trash needs to be deposited in the autoclave trash bins placed throughout the labs. Please do not use Sekusept for regular biological inactivation!

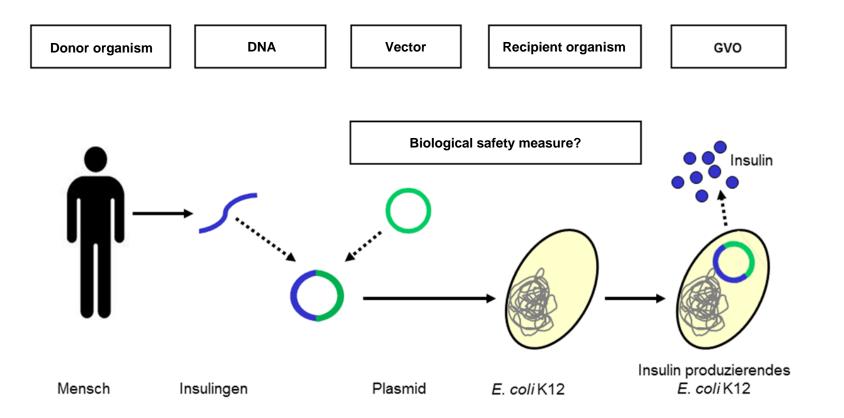
(o) All used glassware, Millipore water as well as biological trash from the lab need to be autoclaved (S2 room 02.070).

Do you have any questions?



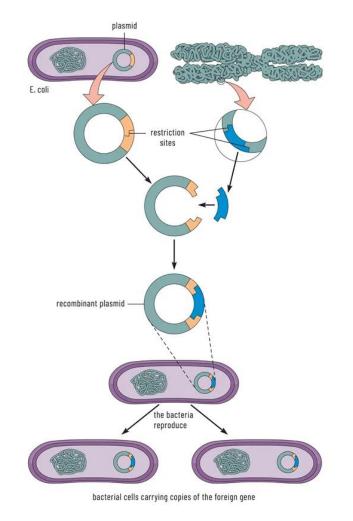
Genetic laboratory safety

Biological research uses **microorganisms** and **viruses** to **genetically modify organisms** such as eukaryotic cell cultures, invertebrates and other model organisms.

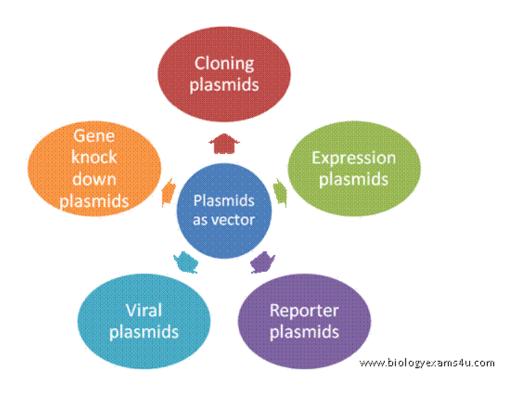


Genetic engineering – workflow principles

1. Isolation and modification of specific donor-derived DNA using Molecular Biology techniques



2. Use of recombinant plasmid-DNA for different purposes:



Genetic engineering – workflow principles

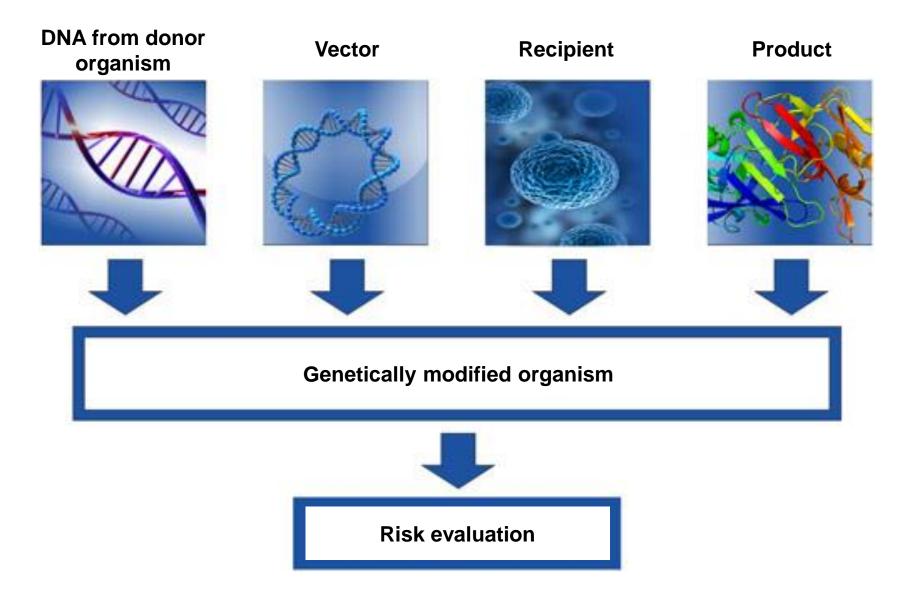
3. Transfer of modified DNA into target system using different types of "vectors":

Bacterial plasmids Viral vectors Envelope Vector "Transfection" of target cell "Transduction" of target cell siRNA/miRNA XXXX Plasmid A293T Cells Nucleus Endosome ransfection Large RNA Complex Transfer Vector Transfect Target Cells Packaging Vector(s) Harvest Lentivirus Reagent Cytoplasm *cDNA, gRNA, or shRNA

Lipofection:

Not very efficient in many cells Mostly used for temporary expression Adenoviruses:Highly infectious, not integrating into genomeLentiviruses:Integrate into target cell genome!

Genetic engineering – risk evaluation



Genetic safety standard levels

S1: "No" risk (*e.g.* majority of cell cultures, E. coli for regular cloning, regular plasmid lipofection etc.)

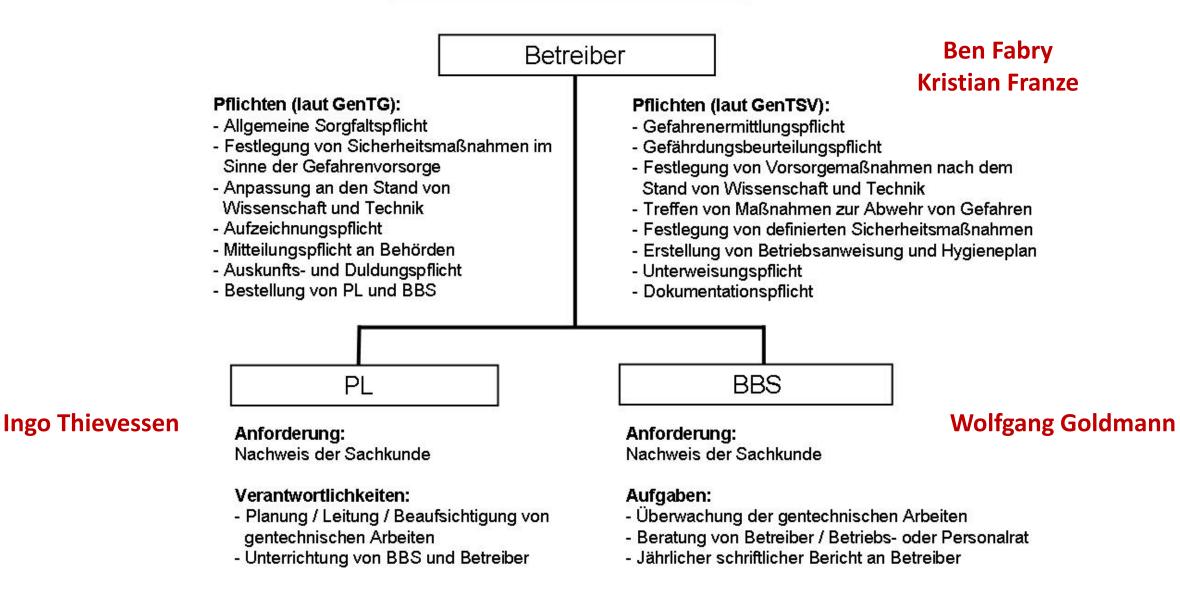
S2: "Low" risk (e.g. most adeno- and lentiviral vectors); Lentiviruses permanently integrate into the genome!

S3: "Moderate" risk (*e.g.* HIV, SARS-Cov2, FSME, West-Nil)

S4: "High" risk (*e.g.* Ebola, Variola, Lassa, Marburg)

...for human health and environment, according to current state of research

Gentechnische Anlage



Biological/Genetic safety lab rules - I

Biological research uses **microorganisms** and **viruses** to **genetically modify organisms** such as eukaryotic cell cultures, invertebrates and other model organisms.

S1: "No" risk (*e.g.* majority of cell cultures, E. coli for regular cloning, regular plasmid lipofection etc.)

S2: "Low" risk (e.g. most adeno- and lentiviral vectors); Lentiviruses permanently integrate into the genome!

S3: "Moderate" risk (*e.g.* HIV, SARS-Cov2, FSME, West-Nil)

S4: "High" risk (e.g. Ebola, Variola, Lassa, Marburg)for human health and environment

Microorganisms or genetically modified organisms (GVOs) of safety level 2 (S2) can cause infections or diseases, or may have sensitizing or toxic effects in humans. Intake may occur through inhalation of aerosols, swallowing, sample contact with the eye or mucous membranes or through existing or accidental skin lesions. Many laboratory activities can cause the formation of aerosols, which may harm researcher or environment.

(a) S2 work may only be performed in rooms 02.045-49 and 02.070-71 and only after separate instruction (Ingo). For all: Be aware that these are S2 rooms!

(b) All work that might be associated with aerosol formation through GVOs must be performed in a sterile workbench (room 02.046), in case of S2 work, only in the bench in the middle bay.

(c) All waste material must be autoclaved.

Biological/Genetic safety lab rules - II

(d) Wear gloves, lab coat, long pants, closed shoes, evtl. protective glasses.

(e) Transportation of samples only in closed, fracture-proof containers.

(f) Centrifugation of viral supernatants only in centrifuge inserts with sealing lids.

(g) Avoid contamination of pens, lab journal, pipettes, door handles, armatures, etc. with gloves when handling infectious material!

(h) Clean safety hood/workspace after finishing using ethanol (lenti-/adenoviruses).

(i) In case of spilling infectious material, warn co-workers, restrict access to contaminated area and inform Ingo, Wolfgang or Ben. Protect yourself when decontaminating the area (e.g. lab coat, gloves etc. **FFP2-mask**!). Soak up infectious liquids with paper, treat surfaces with **Sekusept** for several minutes, wipe it off, collect all waste material, gloves, lab coat etc. and autoclave.

(j) In case of contaminated wounds, disinfect using first aid kit, flush eyes with eye shower, gurgle with $1\% H_2O_2$ (fridge in front of waterbath, room 02.046) in case of contamination in the mouth. Let Ben, Wolfgang or Ingo know about the incident and note it on an accident sheet (first aid kits)!

(k) Consider preventive occupational medical care.

(I) Always take off lab coat and wash hands when leaving S2 rooms.

Do you have any questions?

Laser safety lab rules

Be aware of **direct** and **diffuse** laser radiation, **direct** and **indirect** risks!

LASER CLASSES IN ACCORDANCE WITH EN 60825-1

Class	Description
1	Accessible laser radiation is harmless
1M	Accessible laser radiation is harmless without optical instruments (magnifying glass, telescopes)
2	Accessible laser radiation in the visible spectrum (400 nm to 700 nm), is harmless when exposure to it is brief
2M	Like class 2, without optical instruments (magnifying glass, telescopes)
3R	Laser radiation dangerous for the eye
3B	Laser radiation dangerous for the eye, in some cases also for the skin
4	Laser radiation very dangerous for the eye and dangerous for the skin, danger of fire and explosion



02.043: Cell deformation cytometer, Stopped flow device, FCS/OT

02.042: Cell microrheometer

Laser safety lab rules

(a) Make yourself aware of which type of laser you are workingwith.

(b) Wear appropriate safety googles when necessary (wavelength range, and protection level!). Make sure laser goggles are not scratched, broken or color faded before using.

(c) Protect your skin when working with open class 3B lasers (lab coat/long arm shirt, sun screen etc.)

(d) Be aware of possible diffuse laser radiation! Take off watches, rings etc. when working with open class 3 lasers.

(e) Make sure co-workers in the room are aware that you are working with the laser.

(f) Hang up warning sign on the outside of the door.

(i) Do not open closed laser housings if you are not instructed.

(j) In case of a possible accident, let Ingo, Ben or Wolfgang know, note it in the accidents notebook and see an eye doctor!

Do you have any questions?

Mandatory: All lab users need to sign in the participants list for this safety seminar, displayed in Wolfgang's or Barbara's office today!