Biological Laboratory Safety, Workplace Safety and Fire Protection

for the S1 and S2 laboratories at the

Flow Cytometry Core Facility

- BMZII -

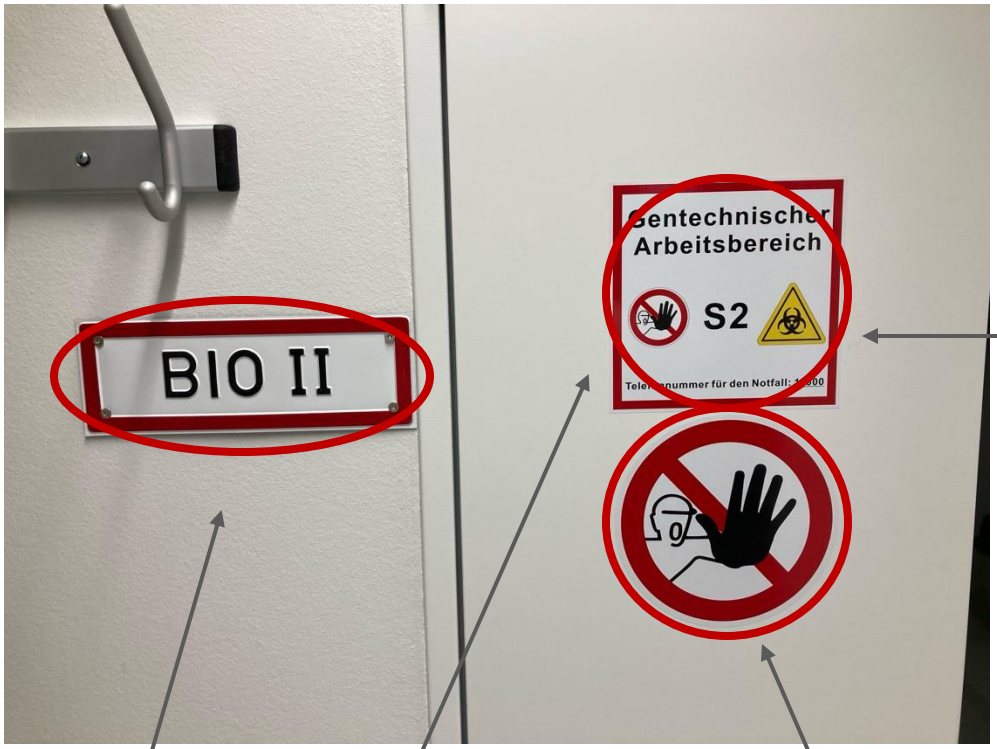
Responsible staff

- **Project leaders** for genetic engineering in the IMMEI and FCCF
  - Prof Christian Kurts
  - Dr Janine Becker-Gotot (deputy)
  - Dr Johanna Breuer (deputy)
  - Prof Veronika Lukacs-Kornek (deputy)
  - Dr Selina Jorch (deputy)

- yearly instructions/visits from authorities
- contact, if you want to use new organisms
- report accidents
- …..

- **BBS** (Biological Safety Officer)
  - Prof Martin Schlee

Security level 2



Biohazard

BIO II

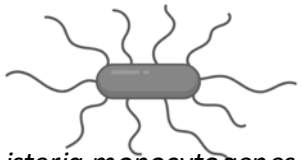
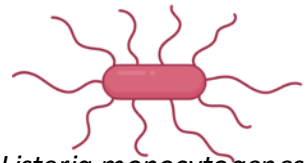
S2

Genetic Engineering Area of safety level 2

No unauthorized access
→ yearly safety instruction necessary (**confirmed in writing**)

Genetically modified organism (GMO)

- **Genetically modified organisms (GMOs)** are organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally
- GMOs are living organisms capable of reproduction

unmodified	**genetically modified**
L-lab status / risk group (RG)	S-lab status / biosafety level (BSL)
 **L1**	 **S1**
Escherichia coli strain K12	*Escherichia coli* strain **K12eGFP**
 **L2**	 **S2**
Listeria monocytogenes	*Listeria monocytogenes* **RFP**

Biosafety levels

ZBKS database
zbks-online.de

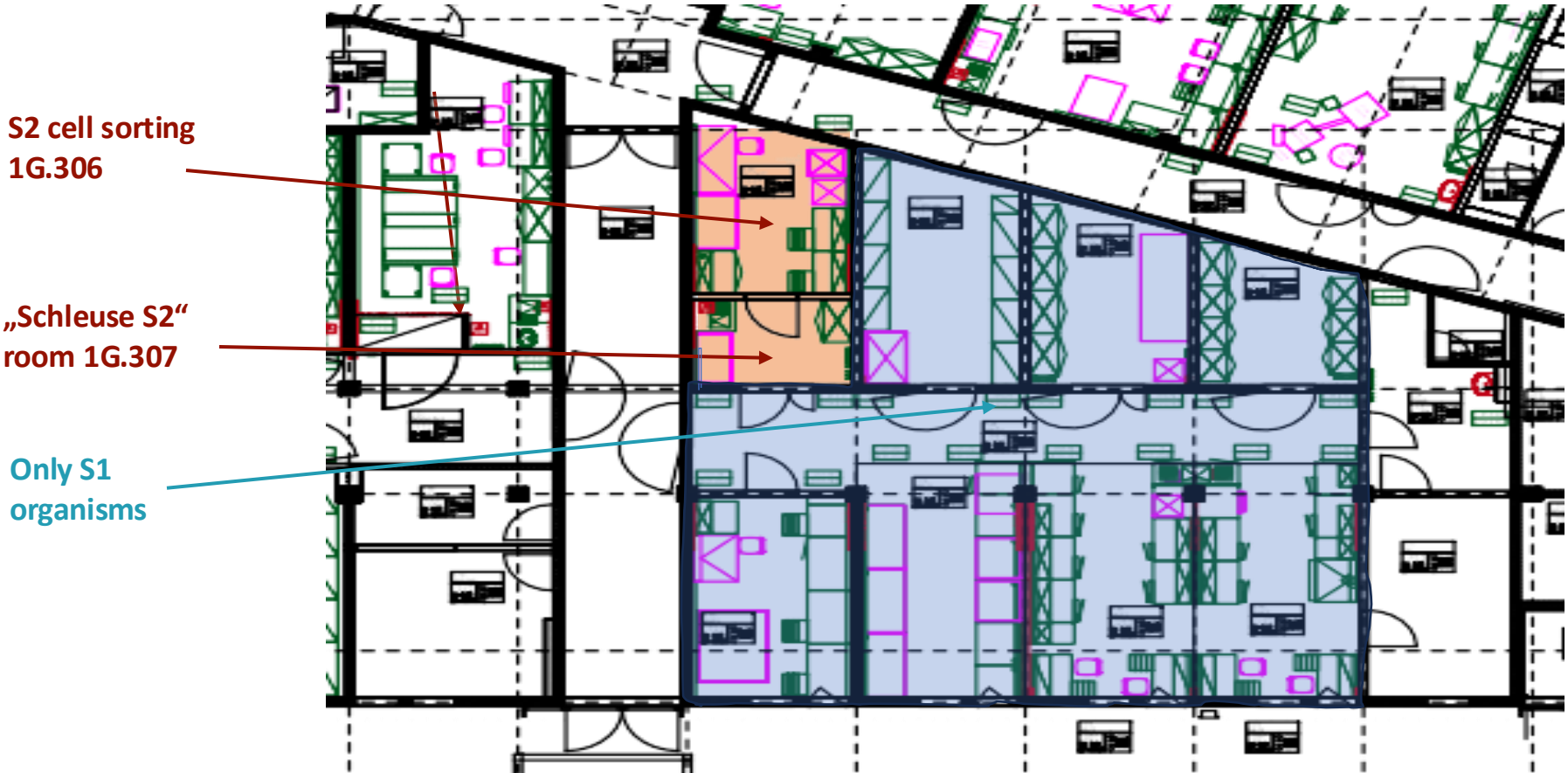
S1	**NO RISK** well-characterized agents not known to cause disease in healthy humans	e.g. E.coli K12
S2	**LOW RISK** may cause serious infection, but effective treatment and preventive measures are available; the risk of spread of infection is limited	e.g. HBV, influenza
S3	Biological agents capable of causing serious diseases in humans. Medium possibility of spread. Prophylactic and curative measures are available!	e.g. HIV
S4	Biological agents capable of causing serious diseases in humans. Medium possibility of spread. Prophylactic and curative measures are NOT available!	e.g. Ebola virus

- Classification refers to healthy adults! (i.e. not to immunosuppressed persons or children)
- Changes in health status (e.g. medication, pregnancy, etc.) must be reported.

S1-/S2 area

- FCCF Basement BMZ II -

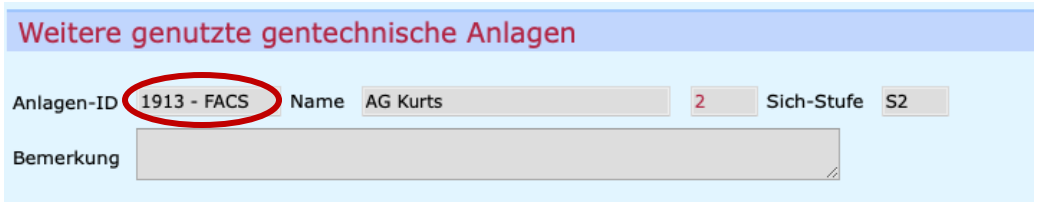
- **S2 area:** cell sorting & cell culture room & “Schleuse” (autoclaving of S2 waste)
- **no** work with S2 organisms in the other rooms !!!



FCCF

- **Sorting of S2 samples** (BD FACSAria Fusion Cell Sorter)
  - announce S2 sort in advance
  - projects with S2 GMOs must be registered with the authorities beforehand

- **Document** the use of GMOs (S1 &S2) in the FCCF in the GenTech Explorer (**ID 1913**)
  (via the function "other genetic engineering facilities used)
  - including cell lines, plasmids



- **Waste**
  - Do not throw samples containing chemicals that can form dangerous vapors (e.g. Phenol, bleach solutions…) into the autoclaving waste
  - dispose them in the chemical waste

Documentation of GMOs

- notification / registration / approval -

Performance of **new/additional** genetic engineering work:

- **S1:** can be performed without further notification to the authority
  obligatory: documentation of structure / construction / characteristics of GMOs!

- **S2: must be notified** to the authority before the intended start of the work (also e.g. new fluorescence markers)
  obligatory: documentation of structure / construction / characteristics of GMOs!

Documentation of GMOs

- requirement of records -

- You are **obliged to record** precisely your experiments involving GMOs: production, handling, release, and decontamination

- Lab book
- List of all S1 and S2 organisms
  (production, receipt from other labs, storage…)
- List of all Cell lines
- List of all genetically modified mouse strains, which are housed in the iFET
  (first description/publication, “Abschlussbeurteilung”)

electronic documentation in **GenTech Explorer**

- Records must be stored for 10 years (S1 work) or for 30 years (S2 work)

Protective Clothing

Always wear:

- lab coat / laboratory uniforms
  (also at the documentation desk;
  hang lab coats on coat hooks & not over the chairs)

locker room
01/347

- closed shoes
- long trousers

- safety glasses
  (in case there is a chance of a small splash to the eyes, such as when opening a bottle or tube)

- gloves
  (suitable for respective hazardous materials, e.g. acid-proof gloves)

- face mask
  (work with lentivirus, work with vaccinia virus, that is not performed under the hood, and in the animal rooms)

→ store your street jackets at the left side at the entrance & your bags or similar items in the locker room

Gloves

- Always select gloves according to the work you are performing; i.e. with or without use of desinfectants

white Peha-soft Nitrile:

- suitable for applications as pure cell culture work
- disinfection with Ethanol, Bacillol and Sensiva Desinfectant must not be carried out beforehand → protective function will be destroyed

Nitril 3000 X-Long blue:

can be used **up to 8 hours without perforation** due to usage of Ethanol, Bacillol and Sensiva Desinfectant

Dermatril P:

can be used **up to 30 min without perforation**

General lab rules

S1-/S2-facilities

- **Inform** yourself: always be informed about potential danger, **before** you start working
- **Strictly forbidden** within laboratories
  - eating, drinking, smoking
  - storage of food or drinks
    (don't store food/drinks at the documentation desks)

- **Avoid working alone** in the lab (let someone know you are working alone, and make arrangements to call and check in periodically)
  → **UKB security service: 66000** (number also on the whiteboard)
- Keep your working space tidy
- No absorbing material on work surfaces!
- Cleaning of working area and equipment after experiments
- Do not store anything directly on the ground, use dollys

Save handling of GMOs

S1-/S2-facilities

- Keep the **doors and windows shut at all times** while handling GMOs!
- **Avoid aerosols formation** (work under the hood)

- **Avoid sharp instruments** (scalpels, needles, razor blades etc.) whenever possible – **No recapping!**
- **Transport** of GMOs between rooms/outside of the S1/S2 facility only in **unbreakable tightly closed devices** (labeling!)

Work with biosafety level 2 organisms

- **S2 organisms:**
  - Adeno (e.g. AdenoOVA, injected into mice → S1)
  - Influenza (e.g. IFV-eGFP)
  - Vaccinia (e.g. VV-OVA)
  - Listeria (e.g. *Lm*-OVA)
  - Legionella (e.g. mCherry)
  - Uropathogenic E.coli (e.g. *E.coli*-eGFP)
  - Lymphocytic choriomeningitis virus (LCMV; e.g. LCMV-OVA)
  - Candida albicans
  - Pseudomonas aeruginosa
  - Salmonella typhimurium
  - Staphylococcus aureus
  - Shigella flexneri
  - Lentiviral particles
- (AG Wilhelm):
  - Trichuris muris
  - Nippostrongylus brasiliensi
  - Citrobacter rodentium
  - Klebsiella pneumoniae
  - Heligmosomoides polygyrus (bakeri)

Work with biosafety level 2 organisms

- **S2 cell cultures** & “Spülküche” (autoclaving of S2 waste)
- Changes in health status (e.g. medication, pregnancy, etc.) must be reported
- **No entrance for pregnant women and immunocompromised individuals!** (additionally see works physician (“Betriebsarzt”) for risk assessment)

Work with biosafety level 2 organisms

- **Lab coats** (laundry container for S2 lab coats within the lab)
- **Change lab coat & gloves after leaving the S2!**


- **All waste** generated in the **S2 area must be autoclaved**
  (don't throw falcon tubes with liquids into the waste; leave the tubes open)


- Disinfection of equipment! (Descosept)
- Do not take chemicals out of the S2
- Biocontainment for centrifuges

- use (autoclavable) plastic flasks for the cultivation of S2 bacteria
  (e.g. for S.aureus)

Waste disposal

- always autoclave GMO containing waste
- in the S1 area, autoclave all contaminated waste
- **all waste** generated in the **S2 area must be autoclaved** before it leaves the institute

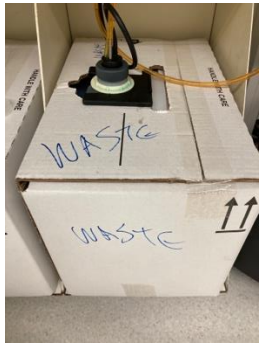
  → Do not autoclave samples containing chemicals that can form dangerous vapors (e.g. phenol, trizol, chloroform etc.)

- infectious human material (e.g. blood samples)
- glass waste (without autoclaving bag!)

- general note: do not fill up the garbage to the absolute upper edge!

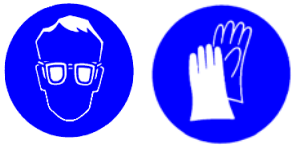
Waste disposal

- Liquid waste from flow cytometers S1-

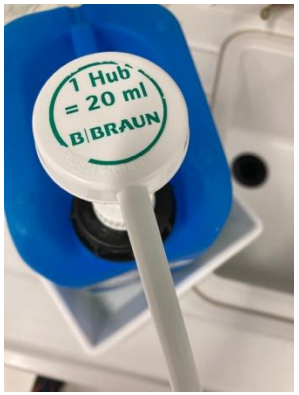


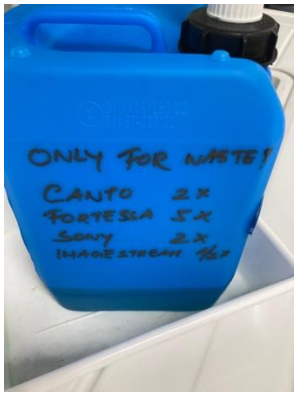
Dispose all liquid waste into the laboratory sink.

Wear gloves and eye protection!



Provide the corresponding volume of Gigasept AF for each instrument in empty containers.





Waste disposal

- sharp material -

Disposal of sharp material (anything that could injure you) only into sharp safe containers!

Never dispose into autoclave bags!

Do not fill up the sharp safe containers to the absolute upper edge!

Hazardous materials

- What are hazardous materials? -

Substances or mixtures which are

- highly poisonous
- toxic
- harmful to death
- corrosive
- explosive
- highly flammable
- flammable
- carcinogenic, teratogenic or mutagenic
- have other chronically harmful properties

Anyone who works with chemicals must know their properties and the hazards they pose.

Handling of hazardous materials

- Inform yourself about the dangers before handling
- Basically work in a fume cupboard
- **Label:** name of substance, name of the user, corresponding danger symbols 
- Storage only in containers suitable for the hazardous materials
- Keep very toxic, toxic, carcinogenic, teratogenic and mutagenic substances under lock and key
- Proper disposal

Chemicals

- waste disposal -

- **Solid chemical waste** must be disposed of in the blue containers in the storage room (also e.g. plates with crystal violet/blue staining and disposable weighing pans, which were used for weighing chemicals that can form dangerous vapors)

room 1G/311 (storage room)

Open the container only under the fume hood!

Chemicals

- waste disposal -

- **Solid chemical waste** must be disposed of in the blue containers in the storage room (also e.g. plates with crystal violet/blue staining and disposable weighing pans, which were used for weighing chemicals that can form dangerous vapors)

room 1G/311 (storage room)

Open the container only under the fume hood!

- **Liquid chemical waste** must be disposed of in the appropriate canisters/bottles (organic or inorganic) under the chemical fume

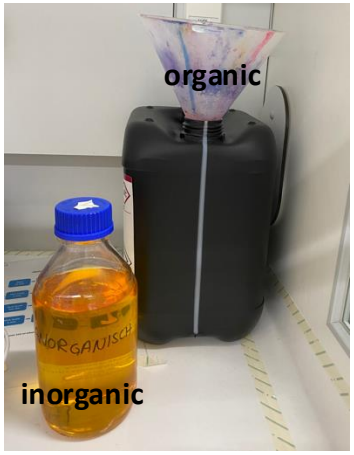
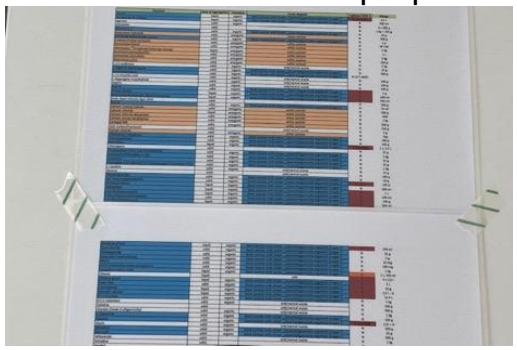


table with chemicals & properties



Decontamination of GMOs

Decontamination with ULTRASOL active

- solution is durable only for 8 hours once dissolved -

- pour powder into 600 ml water
- dissolve / shake
- solution is ready for use **after 15 minutes**
- put ULTRASOL solution on contaminated area
- let sit for **1 hour!** (mark/shield area clearly)
- clean up properly and autoclave everything you used for removal of GMOs from contaminated surface
- prepare bottle with new water and powder package

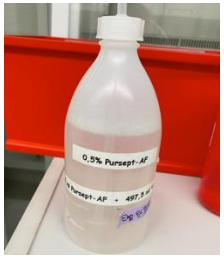
- corrosive effect
- can cause severe eye damage

Hygiene plans

- Disinfectant for surfaces:
  - **Pursept AF 0,5%** (good for 1 month, write date on it, renew if needed, apply for 1 hour)
  - **Bacillol AF** (no preparation necessary, wipe application, apply for 15 min)
  - **Descosept** (no preparation necessary, apply for 3 min)
  - **ULTRASOL active 3%** (good for 1 day, wipe application, apply for 1h); **for lentiviruses!** (Descogen liquid no longer on the VAH-list)
- Ethanol is NOT an official disinfectant (only for cleaning)
- Soak bath for glassware, contaminated racks,…: **gigasept Instru AF 3%** (keep clean, fill up daily, do not let glassware fall dry!)

Disposal of **pursept AF** wipes

= “Wiper Bowl” wipes as well as paper towels on which you use Pursept AF



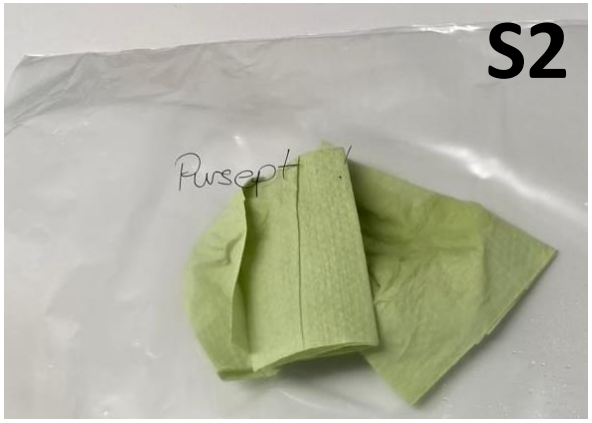
S1:

disposal in normal waste (black bags)

S2:

collect pursept AF wipes in separate waste bags

(Eliane will then distribute the wipes into different bags to avoid a too high concentration during autoclaving)



Personal hygiene

- Always remove gloves before leaving the lab
- Wash hands before leaving laboratory or after handling contaminated material
- Disinfect hands:
  - **S1** area: **desmanol care**

  - **S2** area: **Sterillium Virugard** (also in freezer room)

How to act in case of an emergency

- personal injury -

- cleaning of wounds and disinfection if necessary before applying band aids
- first aiders: Daniela Klaus, Melanie Eichler, Marigona Sutaj, Alisa Fox, Daniela Krauß
- go to the "**Notfallzentrum**" (building 24)
- if serious injury occurred:

  call 0-**112** or UKB Emergency Team **2222**

- **Where are the first Aid facilities?**
  - Eye wash unit (use for at least 10 min!) 
  - Emergency shower 
  - First Aid Kit 
  - Fire extinguisher 
  - Central power switch




- notify the project leader of the accident / injury
- make written note about time of accident and kind of injury ("Unfallbericht")

Behavior in case of fire

- **Keep Calm!**
- **Report the fire**

Push fire alarm button

Emergency call **0-112**

- **Get into safety**

Follow the evacuation alarm - immediately!

Assist helpless people

Close doors and windows

Follow escape routes

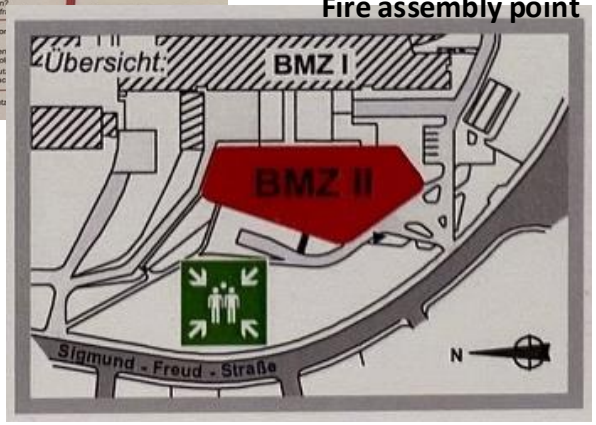
Do not use the elevators

Get to the assembly point & follow instructions

Fire protection

Escape and Rescue Plan

Fire assembly point



Fire Safety Officer: Lucie Delforge, Sandra Rathmann & Janine Becker-Gotot

Key points to remember

1. **INFORM yourself –** know how to work
2. **Follow rules** - Know the do's and dont's regarding safety and mouse work
3. **PREVENT accidents**: Respect the work of others –your own work can affect other people
   e.g., disposal of syringes, scalpels, other sharp material
   e.g., disinfection and cleaning
4. **Disinfect** after working
5. If not sure- **ask** responsible people
6. **PROTECT YOURSELF and** protect **OTHERS**
7. **DOCUMENT** all experiments and work in appropriate forms