

# S1 Introduction to work with Gene modified organisms of risk group 1

# Genetic technology

According to Gentech law (GenTG)  
& Gentechnik-Sicherheitsverordnung (GenTSV):

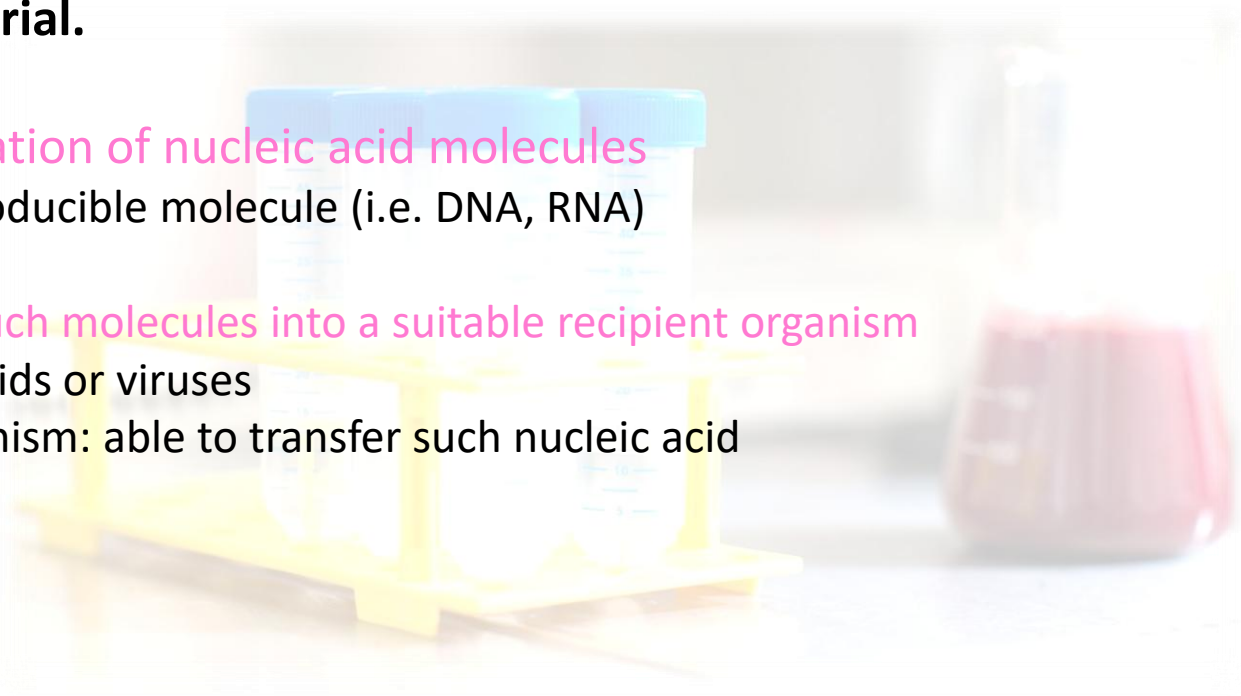
**Genetic technology is a method for the targeted modification of genetic material.**

**In vitro combination of nucleic acid molecules**

- To a new reproducible molecule (i.e. DNA, RNA)

**Introduction of such molecules into a suitable recipient organism**

- vectors: plasmids or viruses
- recipient organism: able to transfer such nucleic acid



# Safety levels

## Safety level 1:

no risk for health and environment

## Safety level 2:

low risk for health and environment

## Safety level 3: not at DRFZ

moderate risk for health and environment

## Safety level 4: not at DRFZ

high risk for health and environment



# At DRFZ we work with:

Gentechnically modified organisms (GMO):

## Cells (Anlage 368-00)

- *Project leader: Ute Hoffmann*
  - In vitro gene modification of cells (e.g. mouse cells) or bacteria (e.g. E. coli K12) of risk group 1 (cloning, transfection) with vectors of risk group 1
  - **Please provide Formblatt Z until 15th of February to Ute Hoffmann**
  - Analysis of cells from GMO mice
  - **Attention:** Gene modification of human cells from blood mostly S2
- **All organisms have to be documented in Formblatt Z and send to Ute latest by February 15th yearly**

## GMO mice (Anlage 114-97)

- *Project leader: Ute Hoffmann*
- Breeding and experimental usage of knock out, knock in or tg mice
- Please provide „Abschlussbeurteilung“ before starting new breedings, importing or crossing of strains to Theres Manthey

# S2 Introduction to work with

Untested human material

Infectious pathogens

Gene modified organisms

# What does S2 mean?

## S2: Safety level 2 according to gene-technic safety regulations

Gentechnik-Sicherheitsverordnung GenTSV

- for work with gene modified organisms of risk group 2

### ➤ low risk for health and environment

## S2: Protection level 2 according to biological agents regulations

Biostoffverordnung BioStoffV

- for directed activities with pathogenic micro-organisms of risk group 2
- for non-directed activities with non-tested human material of risk group 3\*\*

### ➤ low risk for health and environment



# S2 work carried out at the DRFZ

## Human samples with unknown infectious status

Human samples not tested for infectious micro-organisms

i.e. HAV, HBV, HCV, HIV

risk group 3\*\* (Biostoffverordnung): micro-organisms not transferred by aerosols

Undirected work with these samples

belongs to protection level 2 (Biostoffverordnung)

Project leader

Ute Hoffmann

Examples of samples

- Blood from buffy coats or filters without certificate
- Blood from colleagues
- Other body fluids such as synovial fluid, lung lavage, urin, saliva etc.
- Solid tissues as biopsies e.g. gut, skin, bone, cartilage, lung, tonsils
- Stool samples



# S2 work carried out at the DRFZ

## Human samples with unknown infectious status

### Risks

#### Absorption

- by inhalation of aerosols
- via the skin especially in case of injuries or chronically pre-damaged skin
- via eye or mucosa in case of splashing

#### Infection

- with hepatitis A (HAV), B (HBV) and C (HCV) virus
- with human immunodeficiency virus (HIV)

#### Allergenic and toxic potential

**All samples are potentially infectious !**

TRBA 100 (Technische Regeln für biologische Arbeitsstoffe)





# S2 work carried out at the DRFZ

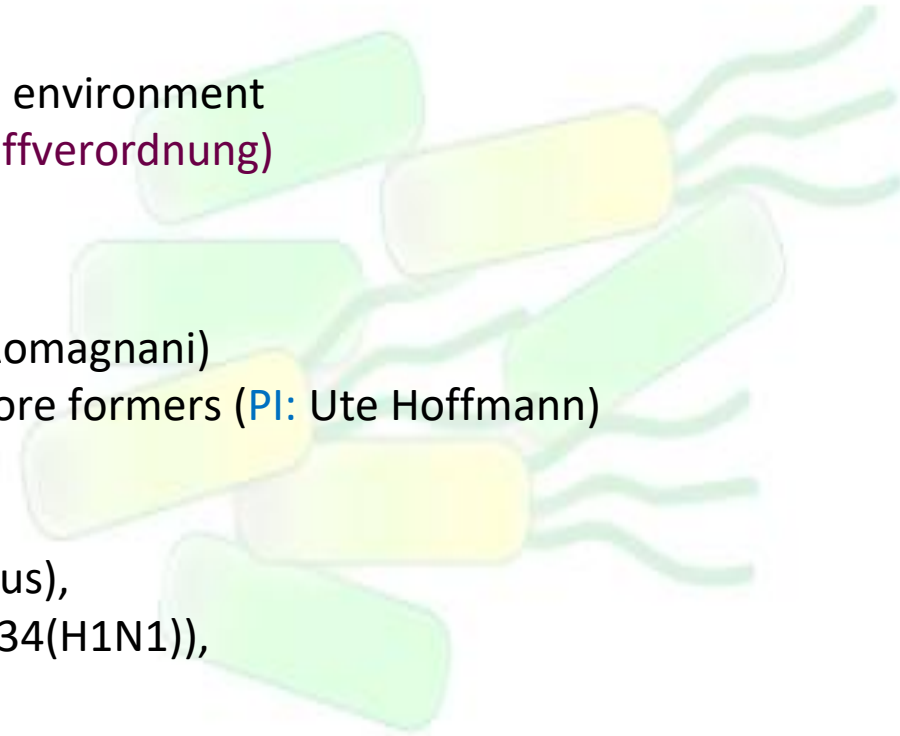
## Infectious pathogens

### Micro-organisms as virus or bacteria

- can cause human disease
- might be hazardous to employees and environment
- belong to **risk group 2 (low risk, Biostoffverordnung)**

### Pathogens currently used at DRFZ

- Pseudomonas aeruginosa (**PI:** Chiara Romagnani)
- S2 intestinal commensals including spore formers (**PI:** Ute Hoffmann)
- Helicobacter hepaticus,  
Citrobacter rodentium,  
LCMV (lymphocytic choriomengitis virus),  
Influenza A Virus (A/Puerto Rico/8/1934(H1N1)),  
Vacciniavirus  
Vesikuläres Stomatitis Virus  
(**PI:** Ahmed Hegazy and Max Löhning)



# S2 work carried out at the DRFZ

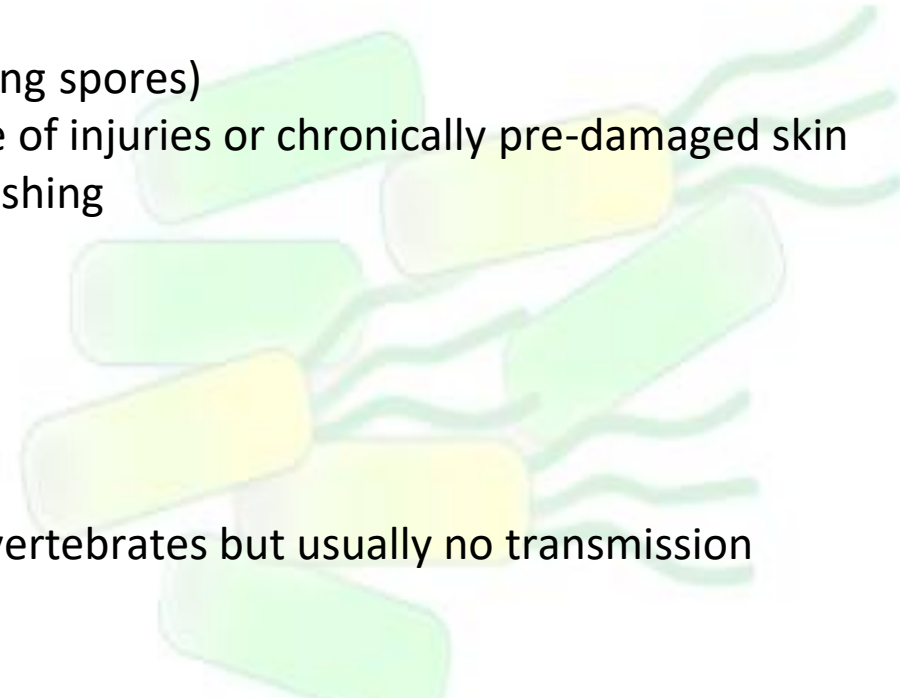
## Infectious pathogens

### Risks

- Absorption
  - by inhalation of aerosols (including spores)
  - via the skin especially in the case of injuries or chronically pre-damaged skin
  - via eye or mucosa in case of splashing
- Infection
- Allergenic or toxic potential

Facultative pathogenic for humans and vertebrates but usually no transmission between the two host groups

Please contact the project leader before you start with your work for detailed introduction! This is mandatory!



# S2 work carried out at the DRFZ

## Gene modified organisms (GVO):

### Gene technical experiments:

Transfer of *nucleic acids* (i.e. plasmids, vectors) from *donor* to *recipient* with **low risk for health and environment** (same as infectious pathogens)

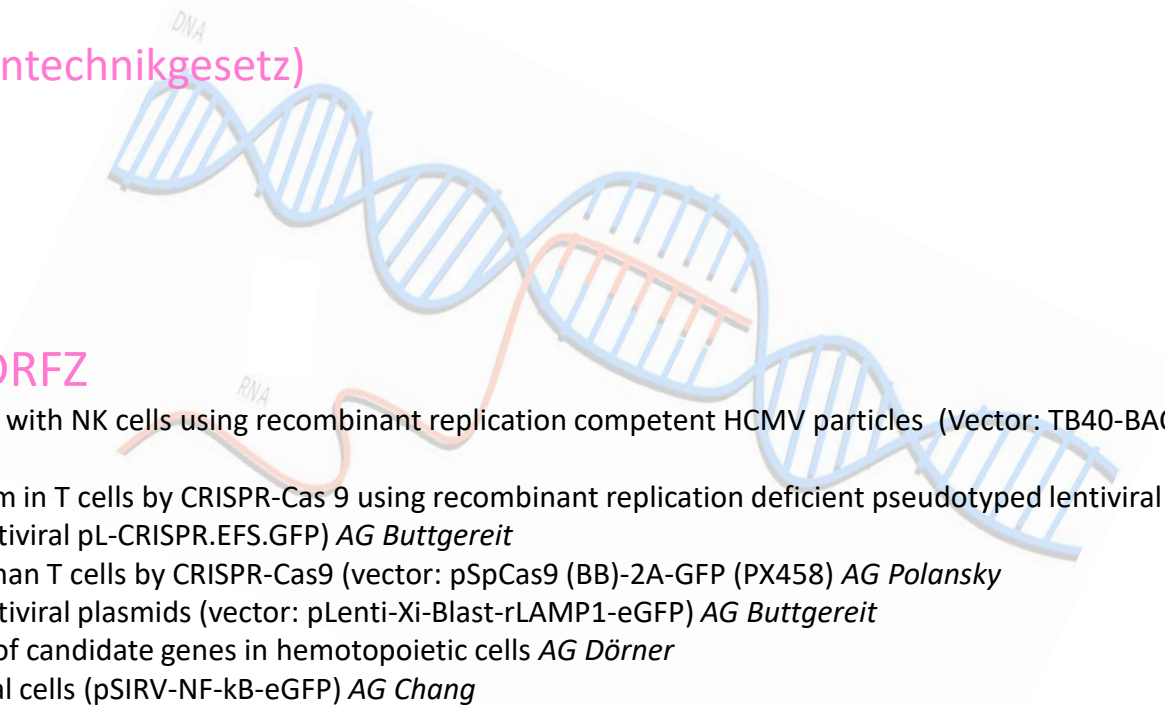
Belong to **risk group 2 (Gentechnikgesetz)**

### Project leader

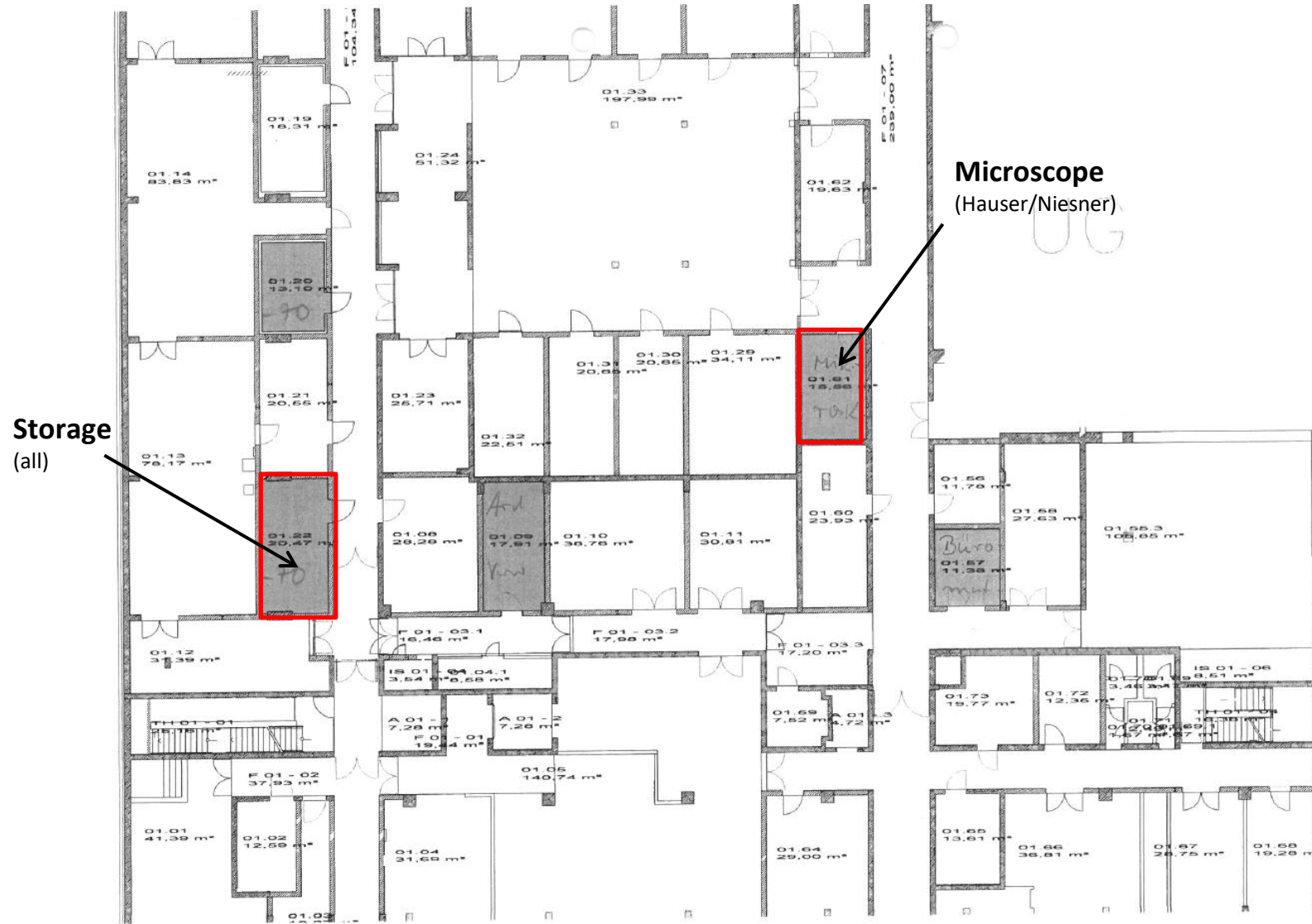
Ute Hoffmann

### Current S2 projects at DRFZ

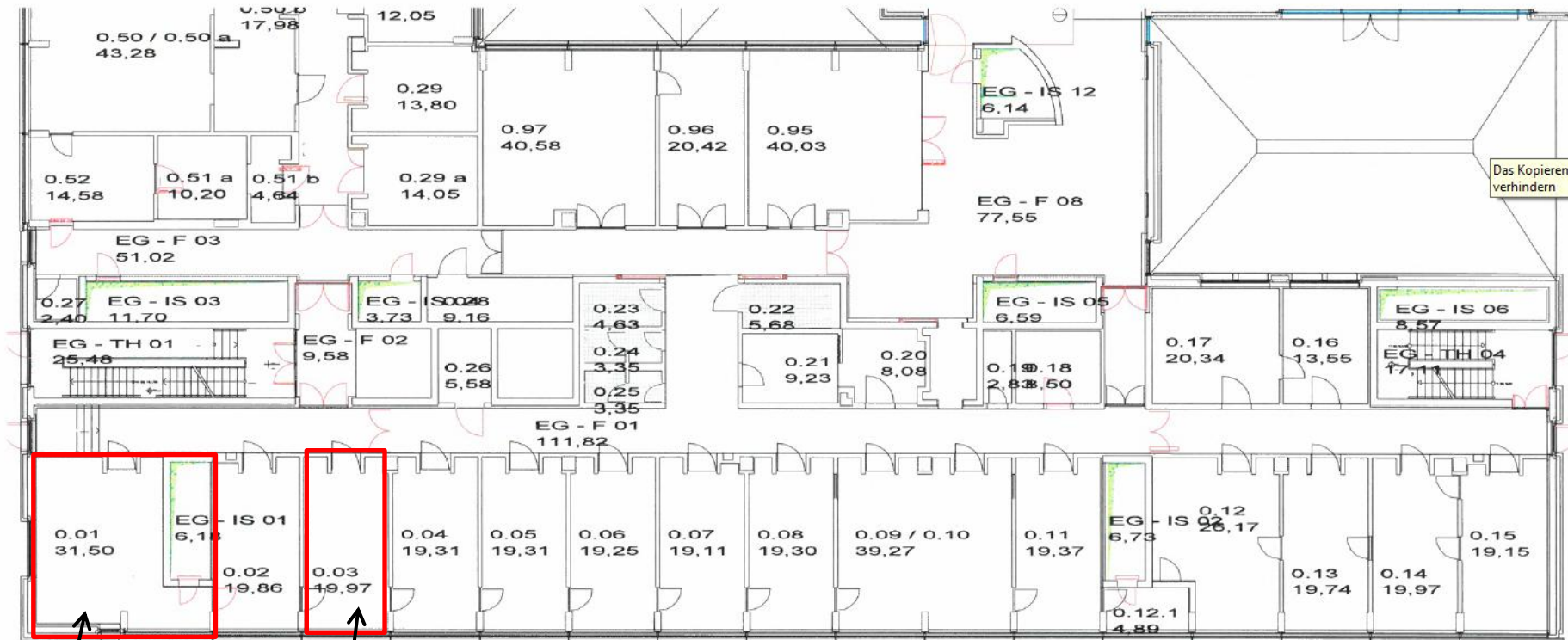
- Interaction of HCMV-infected cells with NK cells using recombinant replication competent HCMV particles (Vector: TB40-BAC4) *AG Romagnani*
- Switch of genes of circadian rhythm in T cells by CRISPR-Cas 9 using recombinant replication deficient pseudotyped lentiviral virus particles (transfer vector: lentiviral pL-CRISPR.EFS.GFP) *AG Buttgereit*
- Knock out of genes in primary human T cells by CRISPR-Cas9 (vector: pSpCas9 (BB)-2A-GFP (PX458) *AG Polansky*
- Infection of cartilage cells with lentiviral plasmids (vector: pLenti-Xi-Blast-rLAMP1-eGFP) *AG Buttgereit*
- CRISPR/Cas9-based manipulation of candidate genes in hemotopoietic cells *AG Dörner*
- Retroviral transduction of intestinal cells (pSIRV-NF-kB-eGFP) *AG Chang*
- Lentiviral transduction of NK cells *AG Romagnani*
- Gamma retroviral T cell receptor transduction into primary human T cells to investigate the specificity of the T cell receptor *AG Hiepe*
- CRISPR/Cas9-based manipulation of different genes involved in signalling pathways in peripheral blood mononuclear cells (PBMZ) and specific immune cell populations of patients with autoimmune diseases and healthy individuals *AG Mashreghi*



# Safety level 2 rooms at the DRFZ Basement



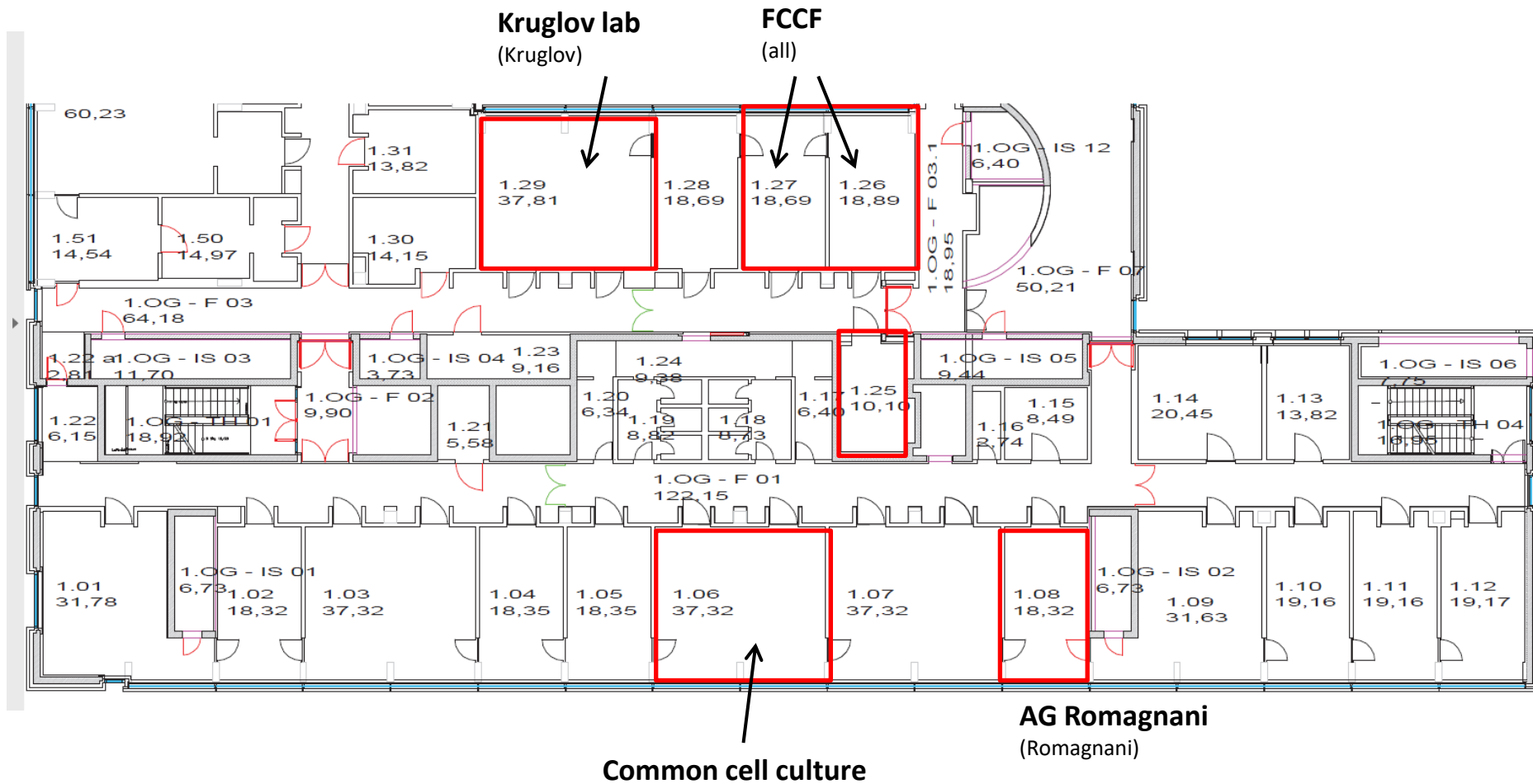
# Ground Floor



**S2 cell culture**  
(AG Latz)

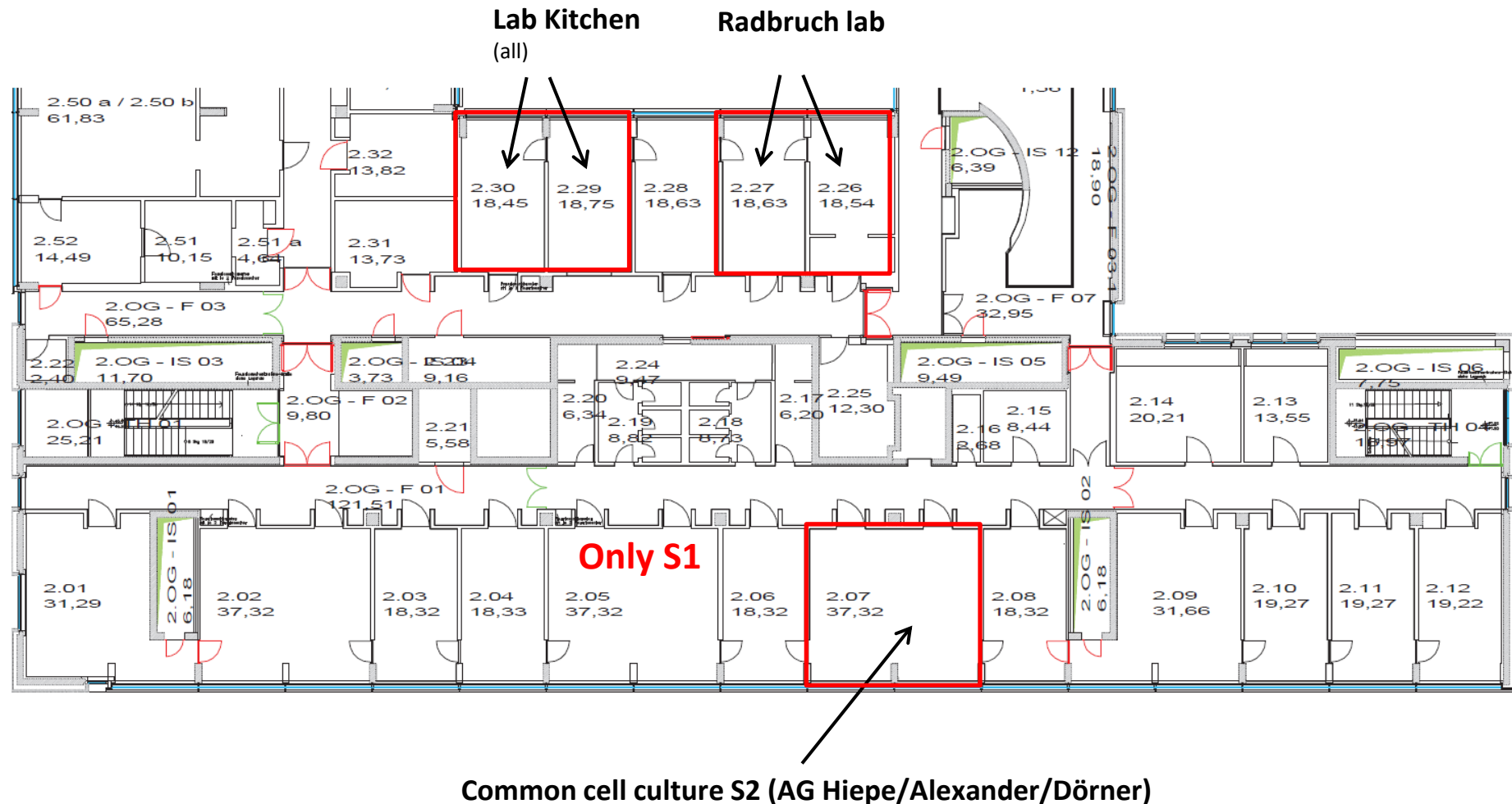
**Devices i.e. Kryotom**  
(alluser)

# 1st Floor

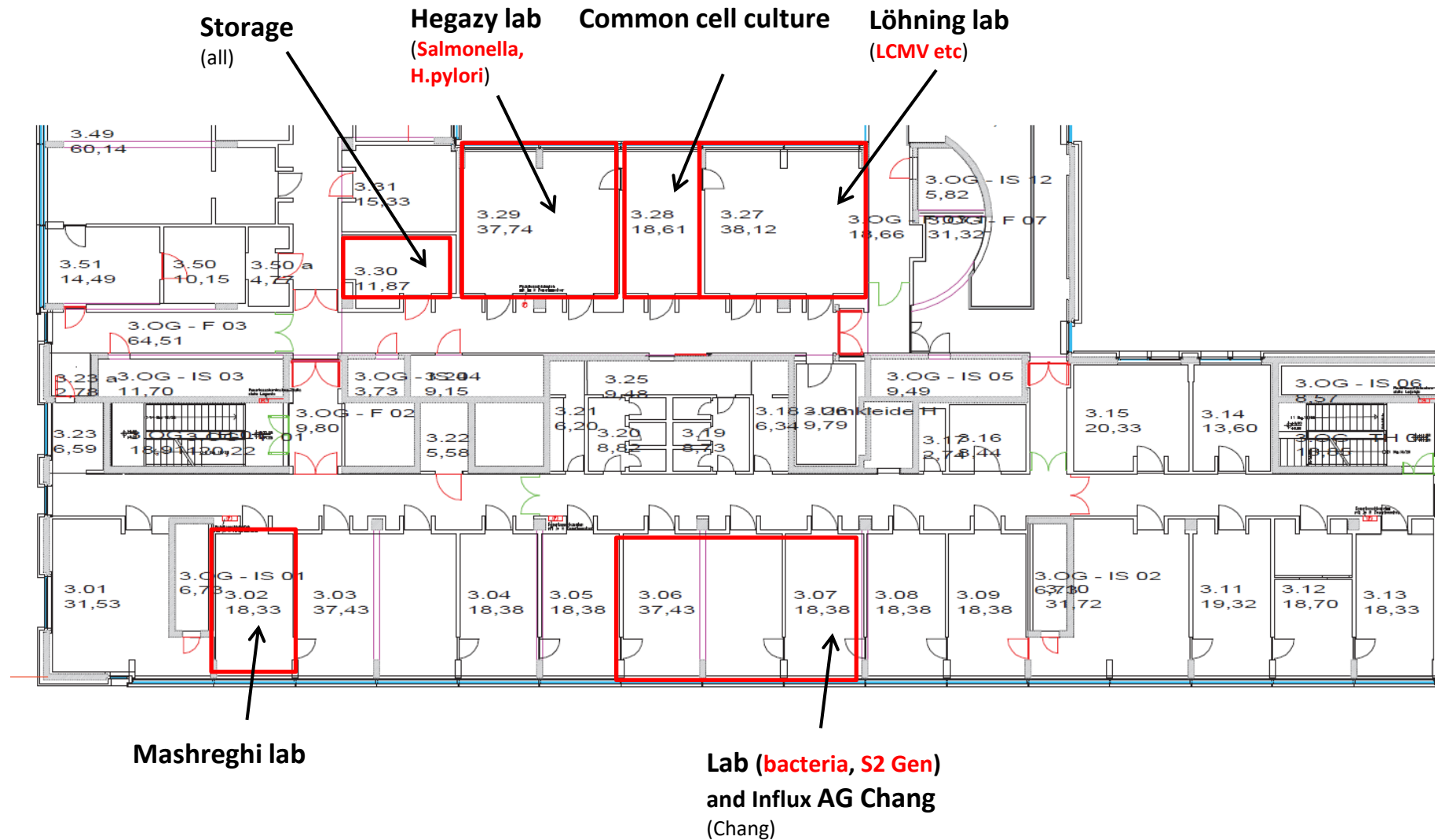




# 2nd Floor



# 3rd Floor





# Safety rules

Work with biological material or gene modified organisms of risk group 2/3\*\*

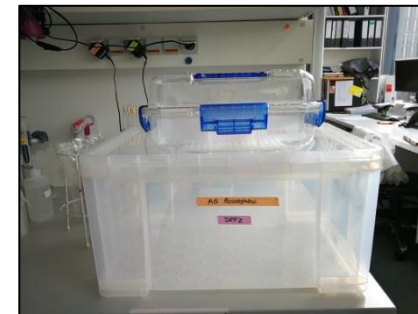
- only allowed in S2 labs

## Access

- **No access to S2 labs without prior introduction by the project leader/group leader!**
  - Ute Hoffmann PI for work with S2 GVOs and untested human material
  - Ahmed, Chiara, Max and Ute for pathogens used in their groups
- Meet the occupational health physician before starting to check vaccination status against Hepatitis A+B

## Transport

- *In house:* in closed, leak-proof, unbreakable falcon tubes or FACS tubes with lid or boxes
- *To other institutes:* in closed, leak-proof, unbreakable tubes etc within another closed, leak-proof, unbreakable box containing absorbent material



# Safety rules

## Clothing

- Closed and robust footwear
- Gloves (**eco Nitril** from **ecoShield**)
- Safety glasses and face mask if necessary

## Lab coats

- Closed lab coats mandatory
- Labelled lab coats only for S2 labs
- Don't use S2 lab coats in S1 labs
- After wearing for 2 weeks:  
put in bin for S2 lab coats (in room 3.30)
- Contamination:  
put immediately into an autoclave bag  
and store in an empty autoclave bin in the lab kitchen



# Safety rules

## Disinfectant

- All labs: B15 2% - 5 min incubation
- Labs with spore forming bacteria: OxyFoamS - 60 min incubation
- Contaminated dishes and instruments: Sekusept aktiv 7% bath - 60 min incubation

## Disinfection

In case of contamination and before you finish working:

- Disinfect the sterile bench or other workplaces by squeezing bottle and paper towel
  - Disinfect contaminated lab dish by dipping it into a bath
  - Exchange B15 2% daily, unused solution is fine for 28d (stock solution in room 0.01, 1.05, 2.29 and 3.30)
  - Disinfection of incubator once a month by wiping with B15 2% or OxyFoamS and then start sterilization program
- In case of contamination same procedure

# Safety rules

## Sterile bench

- Use the sterile bench **for all S2 work** (aerosol formation could be possible i.e. during spilling, plating etc)
- It has to be clean, tidied up, not overcrowded
- Disinfection of work space after finishing your work wiping with **B15 2%**
- Cleaning once a month by using **B15 2%** (work space, below the plates) and also the top wiping with **B15 2%**

## Centrifugation

- Use only tight and closed tubes

## Pipettes and needles

- Single use **plastic pipettes for all human material**
- Use only safety needles and scalpels
- Glass pipettes could be used for work with S2 GVO material



Pipette disposal

# Safety rules

## Waste

### Glass pipettes

- Collect in **Sekusept Aktiv 7%** filled containers
- Bring to lab kitchen when it's full



### Liquid waste

- Collect in closed, unbreakable bins
- Bring to lab kitchen (2.29/30) for autoclaving every evening



### Solid waste

- Collect in S2 labelled autoclave bins (also pipette tips)
- Spore forming bacteria waste: Please label for a different sterilization program
- **Fill them only up to 2/3** and bring them to lab kitchen
- Don't store this waste over weekend



### Sharp items

- Collect in yellow multi-safe bins
- Bring them to lab kitchen



# Safety rules

## Handling of -80°C freezers I

- Wear a lab coat & thermo gloves

## Boxes

- Use ONLY plastic boxes to store samples or reagents (with or w/o grid, available at lab manager lab 2.08)
- label boxes with appropriate tags and permanent marker before storage (mandatory: workgroup, name and Box ID)



## Storage

- store your boxes only in the space dedicated to your workgroup
- storage of lose tubes or bags is NOT ALLOWED

Materials not meeting this criteria will be disposed!

By the end of the year, please reduce and inventory your samples!

## In General

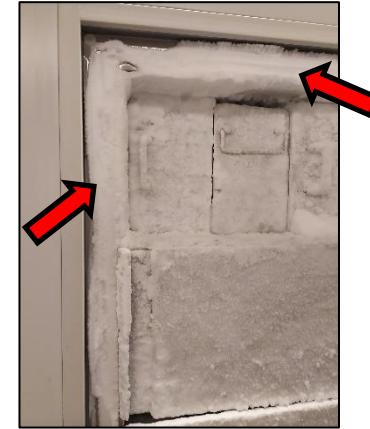
- Refer to senior staff / supervisors in case of questions or un-clarities BEFORE „just trying out“
- Keep proper inventory of your freezer content – at vial and box level – to reduce time to search & find

# Safety rules

## Handling of -80°C freezers II

### Open/Close

- Open the freezer only as short as possible to remove a box or a complete rack
- Make sure that the freezer door is properly closed after use!
- Do **NOT** open the freezer, if the current temperature is already to warm ( $> -70^{\circ}\text{C}$ )
- Remove ice at the door sealing regularly to ensure a proper closure



### In case of technical or mechanical problems

- please inform:  
the lab managers ([labmanager@drfz.de](mailto:labmanager@drfz.de))  
or the in-house technicians ([Betriebstechnik@mpiib-berlin.mpg.de](mailto:Betriebstechnik@mpiib-berlin.mpg.de))

### Act responsibly!

Be aware that all your colleagues rely on proper functionality and use of the freezers.

# Safety rules

## In case of an unexpected event

### Release of large amounts of micro organisms or GVOs (i.e. spilling)

- Alert colleagues, close the area, inform the project leader

### Waste disposal

- Wear lab coat, glasses, nitril gloves mandatory
- filtering half mask in case of aerosols (FFP2)



### Liquids

- Take up liquids with an B15 2% dry lab tissue
- Transfer to S2 autoclave bins
- Disinfect contaminated area  
(no entering of other colleagues during this time, keep doors and windows closed)

### Contaminated items including lab coats

- Transfer in an extra S2 autoclave bin (except sharp items – multi-safe bin)
- Autoclave immediately



# Safety rules

## Cleaning your hands

### Before you start working

- Washing with **Baktolin** first (2-3 shots, induce foam, dry with paper tissue)
- Desinfect with **Sterillium classic pure** before using gloves (2-3 shots, 30 sec incubation time, drying)
- Use **Prolind Hautschutzcreme sensitive** (blue) – size as large as a cherry stone

### In case of contamination

- Disinfect with **Sterillium classic pure** first
- Washing with **Baktolin** during lab work not required
- Use **Descolind Protect** (red)

### After finishing your work

- Disinfect with **Sterillium classic pure** first
- Wash with **Baktolin**
- Use **Descolind Expert** (blue)



# Before you start and recording of work

## Before new group members start to work with S2 pathogens

- They have to be introduced by the project leaders!
- The Betriebsarzt (occupational health physician) has to be informed

## Before new group members start to work with untested human material

- They have to be introduced by the group leaders!
- The Betriebsarzt has to be informed and vaccination against Hepatitis A+B required

## When you work with S2 GVOs

- All gene technical work and storage has to be recorded in Formblatt Z separate from that for S1!

## When you plan to use new S2 pathogens or S2 GVOs

- Please contact Ute first!
  - S2 gene technical work has to be notified at LAGeSo
  - S2 work with pathogens has to be notified an LAGetSi and Gesundheitsamt

**Attention: All experiments with gene modified infectious pathogens have to be notified at Gesundheitsamt, LAGetSi and LAGeSo! Please contact Ute!**

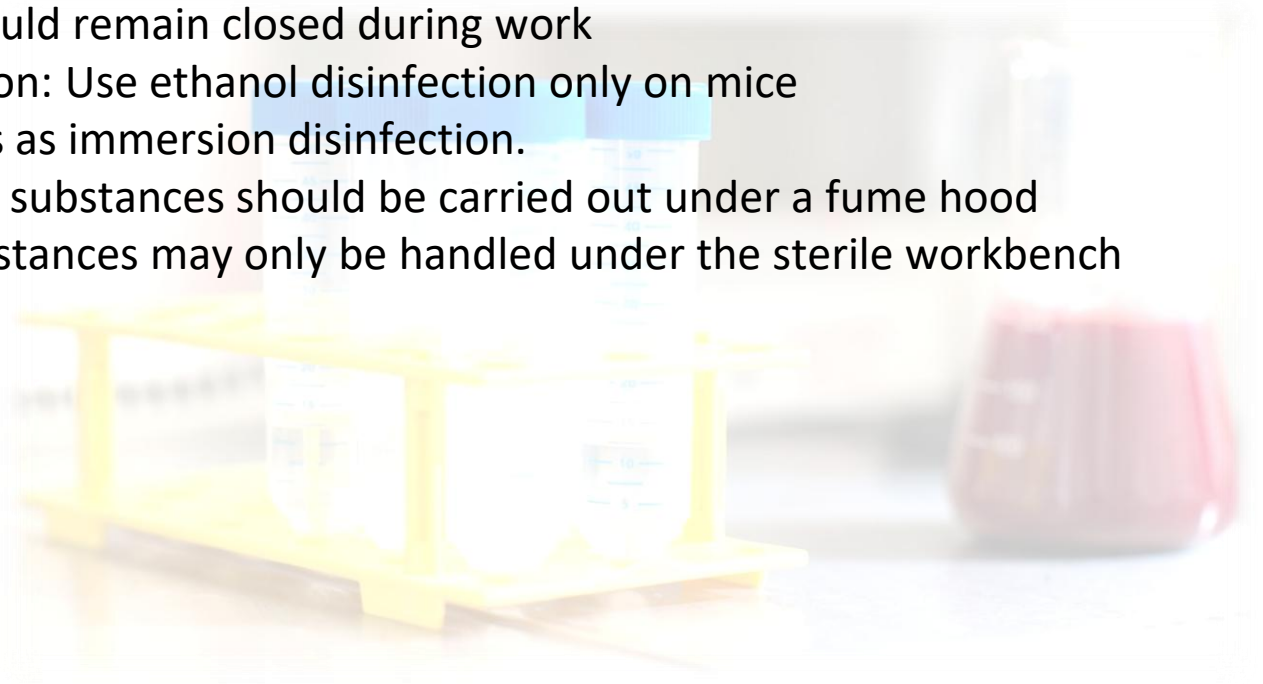
# Reduction of room air exchange rate

## Room air exchange rate (RLT):

- is reduced

## Follow the previous rules even more strictly than before

- Windows and doors should remain closed during work
- Avoid ethanol disinfection: Use ethanol disinfection only on mice and also only in beakers as immersion disinfection.
- All work with hazardous substances should be carried out under a fume hood
- S2 GMOs and S2 biosubstances may only be handled under the sterile workbench



# Final remarks

## In case of an accident/injury

- Please fill out the PDF document (intranet at Job safety – Verbandbuch/First Aid book) and bring it to personnel department

## Working at RFL

- This is a separate facility and you need a separate **mandatory** introduction.
- Please contact: **Timo.Gaber@charite.de**

## In case of considerations and questions

- Don't hesitate to contact Robert and Ute

## Signatures

- Please sign our list **at the lobby**  
**and** cross the material(s) you are working with on this list

Thank you for your attention!

