Document responsible: Siv Lise Bedringaas

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Organizational placement: Department of Clinical Science
University of Bergen

SOP for Working with Gene Modified Organisms (GMOs)

Management and Safety Precautions for Physical Containment Level 2

This SOP should comply with the legislation and local guidelines, provide procedures to prevent the spread of genetically modified microorganisms (GMMs) and genetically modified animals, and prevent health damage to the staff. The SOP will contribute to a good working environment by promoting predictability and security in the work situation.

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1. Overview

1.1 Changes from last version

This is the first version.

1.2 Abbreviations

GMO	Gene modified organism
GMM	Gene modified microorganism
GM-animal	Gene modified animal

1.3 Definitions

GMO	Microorganisms whose genetic composition has been altered using gene or cell technology
Containment of biological factors	Barriers that are applied to prevent biological factors from coming into unintentional contact with people or the environment
Physical containment level 2/ Bio safety level 2	The work that can be conducted at this safety level presents only a moderate potential risk to people and/or the environment, and small-scale work with GM-animals
Accident	Any event involving accidental release of GMM during contained use and which may entail immediate or consequential danger to human and/or animal health and/or the environment

2. Approval

All work that involves GMO must comply with the Norwegian Act relating to the production and use of genetically modified organism and its regulations.

Notification for the specific work must have been submitted and approved by the Norwegian Directorate of Health before working with GMO.

Most of our laboratories are approved for work with GMO at **containment level 1 and 2 / Bio safety level 1 and 2. These laboratories are** labelled with a Biohazard sticker.

Activities covered by the Act is the preparation and use of (list is not exhausted):

- Plasmid- transformed bacteria for amplification of DNA
- DNA-transfer from bacteria to e.g., cells
- DNA- and RNA-transfection of cell culture, even if its transient
- Organisms, cells, or tissue where DNA is edited using modern technologies such as CRISPR/Cas9
- Transfer of genetic material to animals by DNA-vaccination or gene therapy

3. Exposure risks

To reduce exposure as much as possible it is imperative to be aware of the ways one can be exposed and act accordingly. The department/research group should facilitate for e.g. proper protective equipment, personal protective equipment, safety rules and waste handling.

The most probable routes of exposure are:

- **Dermal** via sharps (needle prick etc.). It is prohibited to use any form of sharps!
- **Absorption** through exposed scratches, abrasions on skin or mucous membrane exposure of the eyes, nose, and mouth.
- **Inhalation** via aerosols from e.g., centrifugation or vortex mixers.

4. Risk assessment

- A risk assessment of each project included procedures must be conducted.

The risk assessment must comply by the GMO regulation attachment III and IV cf. § 5.

At the HSE-gateway, you will find a **Risk assessment form** applicable for projects and procedures.

Examples of relevant risks:

Biosafety Considerations and Risk Levels

Biosafety Considerations	Higher Risk	Lower Risk
Vector Design	• Vector packaging functions on two plasmids • Expression of viral genes	• Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	• Oncogene	• Non-oncogene
Vector Generation	• Large scale	• Laboratory scale
Animal Hosts	• Permissive host • Animals engrafted with human cells	• Non-permissive host
Animal Manipulation	• Vector administration (e.g., use of sharps during injection	• Housing and husbandry (no use of sharps)

From: Biosafety Considerations for Research with Lentiviral Vectors.

5. Requirements for work at the containment level 2 - Safety Precautions

5.1 Authorized Personnel

Only authorized personnel can work with GMOs. Staff and students will only be permitted to work with GMOs:

- When their tissue culture and microbiology techniques demonstrate good laboratory practice.
- Once training is completed and documented. (See 9.1)

5.2 Physical Containment

All work with GMO in the laboratory must be performed under conditions of contained use. When working with viral vectors only use the Biosafety cabinet/LAF-bench in the laboratory dedicated for this use.

The door to the laboratory must be closed. (When the door in the GMO room is closed, the air flow is enhanced for better protection due to negative pressure).

It is forbidden to eat or store food in the laboratory.

5.3 Safety

Everyone is responsible for their personal safety, always follow the safety guidelines and take precautions accordingly.

Remember that it is always:

- prohibited to eat, drink, smoke, use cosmetics or store food in the laboratories.
- forbidden to mouth pipette.

Relevant documentation:

- SOP for immediate action and follow-up of puncture and cut injuries in case of exposure to biological factors
- SOP for strakstiltak og oppfølging av stikk og kutt skader ved fare for eksponering av biologiske faktorer

5.4 Personal Protective Equipment

Use a protective coat that protects the front part of the body (yellow coat). In addition, hospital clothing is recommended.

Use nitrile glows (A double set of gloves or "*Touch N Tuff*" is recommended when working with viruses). Remove potentially contaminated gloves and replace them with new gloves before touching anything outside the LAF bench, such as centrifuge, incubator, or fridge.

Protect yourself with a plaster/bandage if you have any wounds or scratches on your hands.

Use plastic shoes cover.

Kits:
A **safety & spill kit** for GMO work is placed in the GMO lab. (See 6.1 8.1 for content overview).
An **emergency kit** is placed in or close to the main laboratories (See appendix 8.2 content overview).

5.5 Documentation

List of mandatory documentation applicable for each research group:

1. GMO Notification/ applications and approvals should be kept together and easily accessible.
2. Risk assessment of projects and new introduced GMOs.
3. Procedures: Information on safety, waste management and work operations must be available.
4. Laboratory journal of all work with GMO and transportation.
5. Registration of all biological factors including GMO in Workplace safety chemical inventory and work performed in exposure register or local logs.
6. In addition, it is recommended to make a safe job analysis (SJA) for every procedure where GMO is used.

6. Working with GMOs

6.1 Virus infection

Retroviruses are enveloped, single-stranded RNA viruses capable of infecting dividing cells. Upon infection, the RNA genome is reverse transcribed and integrates as a DNA provirus into the chromosomal DNA of the infected cell.
Lentiviruses are a group of retroviruses that are capable of infecting non-dividing cells.

A. Before you start the procedure, make sure you have the following:

- Virkon waste container (> 2 l volume): make 1% Virkon solution for decontaminating the plastic. *Be familiar with Virkon instructions and read the safety datasheet!*
- Container for liquid waste.
- 500 ml 1% Virkon solution for cleaning the area (in case of spill)
- Spray bottle of Antibac for disinfecting the area
- Paper towels
- (Chemisorb)
- Bench paper
- Biohazard plastic bags
- Cardboard box for bio waste

The GMO Safety and Spill Kit contains:
  - Lab coat, shoe covers, gloves
  - Masks, safety glasses
  - Cotton swabs
  - Hibi Scrub, Pyrisept or similar for skin disinfection
  - Forceps (to pick up broken glass)
  - Biohazardous waste bags
  - Warning sign if spill occurs

B. Biosafety cabinet/LAF-bench:

- All work with viruses shall be conducted in designated laboratories and LAF-benches.
  - If you need the LAF for a specific time or period, please note it on the reservation form.
- Use white bench paper for all virus work in the dedicated biosafety cabinet.
- Be careful when pipetting to avoid generation of aerosols.
- Use filter tips.
- Vortexing must be carried out in the Biosafety cabinet and the tubes must be corked.
- Keep the Virkon waste container used for disinfection of remaining virus in the cabinet.

C. Incubator:

- Use only designated incubators, if possible.

D. Centrifuge:

- Centrifuge tubes should be prepared and sealed in the LAF-bench. This includes balancing the tubes.

- Spinning infectious material. Always use **parafilm** around the tissue culture dishes during centrifugation.
  - Put a " infectious material" note on the centrifuge.
  - Clean the centrifuge with Virkon and Antibac after use.

E. Transportation:

- If tissue culture dishes are to be transported to a different laboratory, they must be placed in a secondary, closed container.

6.1.1 Waste

- Liquid waste such as medium containing virus, should be removed by suction connected to a vacuum waste-container (reusable or disposable). Use suction to wash the tubing with Virkon solution.
  - When the **reusable** vacuum waste-container is full, dispose the waste into the liquid waste-container and add Virkon. This container will later be put in the box for bio waste lined with a yellow waste bag abeled **"Prøverør/Agarskål"** or **"Cytostatika**" to be incinerated.
  - When the **disposable** waste-container is full, add Virkon to a final concentration of 1% and place it in the box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål" or "Cytostatika" to be incinerated.

- Solid waste such as pipettes, tips, tubes, dishes etc. that have been in contact with virus (medium or other solutions) must be immersed in the Virkon waste container or with Virkon added directly. Incubate for **5-30 minutes** in the cabinet or hood with a note "**Virus contaminants**"!
  - After incubation, Virkon waste must be disposed in a liquid-waste container. This container will later be put in the box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål" or "Cytostatika" to be incinerated later.
  - Tips etc. must be discarded in the box for bio waste labeled "Prøverør/Agarskål" or "Cytostatika"
  - Bench paper and paper towels should be collected in a plastic bag inside the safety cabinet, closed and sprayed with Antibac before deposited in the box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål" or "Cytostatika" and to be incinerated later.

- **Alternatively**, all liquids and solid waste may be autoclaved immediately after work is finished.
  - **DNA fragmentation**: Virkon must be added to the liquid waste if antibiotic resistant genes have been used.

6.2 Cell lines and primary cells

Day to day cell culture work with GMO transfected cell lines or primary cells can be performed in all safety cabinets in our laboratories that are approved for GMO work. This also includes performing transient transfection with transfection agents or electroporation. However, a dedicated safety cabinet is preferable due to risk of contamination of GMO-free cells and waste handling.

6.2.1 Waste disposal

- Liquid waste, such as medium containing viruses, should be removed by suction connected to a vacuum waste-container (reusable or disposable). Use suction to wash the tubing with Virkon solution.
    - When the **reusable** vacuum waste-container is full, dispose the waste into the liquid waste-container and add Virkon. This container will later be put in a box for bio waste labeled "Prøverør/Agarskål" to be incinerated later.
    - When the **disposable** waste-container is full, add Virkon and place it in a box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål" or "Cytostatika".
- Solid waste such as pipettes, tips, tubes, dishes etc. that have been in contact with medium or other solutions containing GMO cells must be placed in the box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål" or "Cytostatika" to be incinerated later.

6.3 Bacterial culture

- Methods for amplification of DNA/RNA using bacterial strains not pathogenic to humans should be conducted in designated areas. The laboratory should facilitate a biosafety cabinet and shaker for growing bacteria. This laboratory should be used when working with live bacteria not sealed in a bottle or tube.
    - Laboratory building: room no. 8205 and 5065
    - Glass building: room no. 6273

- **Exception:** Steps that may be conducted at the main laboratories:

    - The HEAT- transformation step of the DNA (no requirements as long as the cells are contained in closed tubes).
    - Analysis of the transformed clones by PCR.
    - Plasmid purification of lysed bacterial pellet.

6.3.1 Waste disposal

- All bacterial waste should be autoclaved and disposed into a liquid-waste container and put in the in the box for bio waste lined with a yellow waste bag labeled "Prøverør/Agarskål".

- All reusable centrifugation tubes / other equipment should be autoclaved and washed in the dishwasher.

7. Unwanted incidents

7.1 Spill of GMO containing liquids in the biosafety cabinet

When liquids or other substances containing GMO are spilled, the following instructions should be followed. Depending on the amount of the spill, assess the incident with respect to decontamination, evacuating the room and reporting. Everyone should be familiar with our two categories of spill response.

According to the amount and place of the spill, follow one of the three instructions:

7.1.1 Spill in the Biosafety Cabinet

1. Assess severity of the spill, i.e., virus, primary cells, cell lines and volume of liquid.
2. Let the biosafety cabinet continue to operate. Wait at least 5 minutes. (Allows the aerosols to be pulled through the HEPA filter).
3. If necessary, change gloves and put on an extra pair. Remove any contaminated clothing including laboratory coat and gloves and place them in a sealed red biohazard bag to be autoclaved.
4. Use the spill kit for major spills and risk of contamination, and injuries.
5. Decontaminate the surface by gently covering the spill with absorbent paper towels sprayed / soaked in Virkon. Allow sufficient decontamination time (approximately 20 minutes) before clean up.
6. Discard soaked paper towels in a biohazard waste bag. Wipe up residual mess. Clean the surface with Antibac, discard towels in the biohazard bag. Discard the biohazard bag in the box for bio waste with yellow waste bag labeled “Prøverør/Agarskål”.
7. Notify the PI or laboratory manager as to the extent of the spillage.
8. If the spill has escaped the cabinet, proceed as for spill outside the cabinet. (see 7.2).

7.2 Spill of viable GMO containing liquids outside the biosafety cabinet

1. Put on a mask to minimize the chance of inhaling aerosols.
2. Alert anyone in the vicinity.
3. Ascertain extent of the spill – change shoe cover.
4. Evacuate the room and close the door.
5. Remove any contaminated clothing, including laboratory coat and gloves, and place them in a biohazard waste bag to be autoclaved.
6. Wash face and hands; put on clean personal protective equipment.
7. Post a warning sign (there is one in the spill kit).

With Virus: Do not allow anyone to re-enter the area for at least 30 minutes so that aerosols have chance to settle. While you wait, notify the PI or laboratory manager.

Clean-up:

8. Assess the area and degree of the spill and subsequent potential contamination.
9. Soak paper towels in Virkon and gently cover the spill from the perimeter towards the center.
10. Allow at least 30 minutes for the disinfectant to take effect.
11. Carefully absorb the spill and disinfectant solution into dry towels and discard all contaminated material in the box for bio waste with yellow waste bag labeled “Prøverør/Agarskål”.
12. Wipe over spill area and any surfaces that may have become contaminated with Antibac. Discard all contaminated material in the box for bio waste with yellow waste bag labeled “Prøverør/Agarskål”.

7.3 Notification and Reporting of spills and accidents

7.3.1 Any risk of GMO contamination

- Injuries
- Major spill of GMOs in the biosafety cabinet (> 20 ml) and < 10 ml outside the biosafety cabinet
- Outside the contained area (not approved area)

must be reported to:

- The PI
- The HSE department (non-conformities form at https://hjelp.uib.no)

7.3.2 Accidents

See 1.3 for definition,

must be reported to:

- The PI
- Head of the department
- The HSE department (non-conformities form at https://hjelp.uib.no)
- The Norwegian Directorate of Health as soon as practical, GMO-boksen@helsedir.no Phone: 810 20 050

See § 19 for required information.

7.4 Follow up of exposure and injury

The follow-up of retroviral/lentiviral or cell exposure, and laboratory injuries are conducted through the health services at the HR department.

The health services need the results for the blood test and the non-conformity notification to be able to follow up the injuries.

- If you take a blood test ("0"-test) after exposure, and use the documents provided in the SOP "for immediate action and follow-up of puncture and cut injuries in case of exposure to biological factors, you will receive the lab. results and the occupational health service follows up cases with risk of infection transmission.

8. Appendix

8.1 GMO Safety and Spill Kit

The GMO kit contains the following to **be used when spill occurs**.

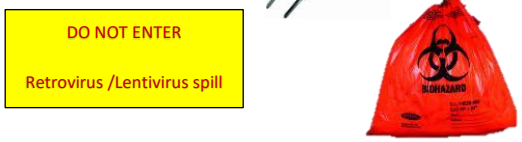
Exception: protective safety glasses and masks might be used during work to improve safety.

Protective clothing:

Extra safety, use with spills:

Skin disinfectants:

Other items:



8.2 First aid and Emergency

Placement: Room 3160

First aid cabinet:

Emergency container:

The lists are not exhaustive.

Make yourself familiar with the content in the cabinet and container, and their locations.

8.3 The application and approval process

- The use and preparation of GMO is strictly regulated by laws and regulations.

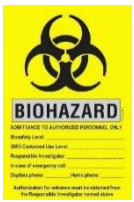
- Activities covered by the Act for the use and preparation of (list is not exhaustive):
  - Plasmid- transformed bacteria for amplification of DNA
  - DNA-transfer from bacteria to e.g., cells
  - DNA- and RNA-transfection of cell culture, even if transient
  - Organisms, cells or tissue where DNA is edited using modern technologies such as CRISPR/Cas9
  - Transfer of genetic material to animals by DNA-vaccination or gene therapy

- The group's principal investigator must ensure that all requirements concerning the laboratories and working conditions, such as safety precautions and HSE, are met. This must be documented in applications submitted to the Norwegian Directorate of Health, and consists of two or more application forms:
  - The laboratories and facilities must be approved for work with gene-modified microorganisms (GMM) or gene-modified animals (GM-animals).
  - The specific work must be approved separately in a notification or application. It is possible to choose between these applications:
    - GMM
    - GMM in combination with animals
    - GM-animals

  Links for application:
  - https://helsedirektoratet.no/genteknologi#genmodifiserte-mikroorganismer
  - https://helsedirektoratet.no/genteknologi#genmodifiserte-dyr

- The Norwegian Directorate of Health must approve the facilities and the use of GMO before the work can be initiated.

- Laboratories that are approved for work with GMO at **containment level 1 and 2 / Bio safety level 1 and 2,** must be labeled with a Biohazard sticker.



8.4 Laws and Regulations

Lov om framstilling og bruk av genmodifiserte organismer m.m. (genteknologiloven)	EN: https://www.regjeringen.no/en/dokumenter/gene-technology-act/id173031/ N: https://lovdata.no/dokument/NL/lov/1993-04-02-38
○ Regulations	https://lovdata.no/dokument/SF/forskrift/2001-12-21-1600 https://lovdata.no/dokument/SF/forskrift/2001-12-21-1602

Forskrift om systematisk helse-, miljø- og sikkerhetsarbeid i virksomheter (Internkontrollforskriften)	https://lovdata.no/dokument/SF/forskrift/1996-12-06-1127?q=internkontrollforskriften
UoB Guidelines, GMO	https://regler.app.uib.no/regler_en/Part-3-Human-Resources-and-HSE/3.2-Health-Safety-and-Environment/3.2.3-HSE-guidelines/Guidelines-for-genetically-modified-organisms-GMOs/
UoB Guidelines, biological factors	https://regler.app.uib.no/regler_en/Part-3-Human-Resources-and-HSE/3.2-Health-Safety-and-Environment/3.2.3-HSE-guidelines/Guidelines-relating-to-biological-risk-factors/

8.5 Links

Norwegian Directory of Health (Helsedirektoratet)	https://helsedirektoratet.no/genteknologi
Application form for GMM	https://helsedirektoratet.no/genteknologi#genmodifiserte-mikroorganismer
Application form for GM-animals	https://helsedirektoratet.no/genteknologi#genmodifiserte-dyr
UoB: Biological factors and genetically modified microorganisms	https://www.uib.no/en/hms-portalen/80327/biological-factors-and-genetically-modified-microorganisms
UoB: Risk assessment form	https://www.uib.no/en/hms-portalen/155112/risk-assessment-biological-factors-and-genetically-modified-microorganisms
UoB: HSE department Contact information:	http://www.uib.no/en/hms-portalen http://www.uib.no/hms-portalen/111462/kontakt-bedriftshelsetjenesten
Biosafety considerations for research with Lentiviral vectors	https://und.edu/public-safety/_files/docs/guidance-on-biosafety-consideration.pdf
Lentiviral Biosafety Manual	https://view.officeapps.live.com/op/view.aspx?src=http%3A%2F%2Fwww.ohsu.edu%2Fxd%2Fabout%2Fservices%2Fintegrity%2Fpolicies%2Fupload%2FTemplate-Lentivirus-Biosafety-Manual.doc

9. Forms

All groups should have forms for training and logs. The attached downloadable forms are examples , feel free to make changes.

9.1 Laboratory Instructions and Training Form for Working with Gene Modified Organisms (GMOs)

Name:	
Starting date for training:	
Lab. Manager:	

Instruction and training include the following topics	**Date**
SAFETY • Risk awareness • Safety precautions • Personal protective equipment • Biosafety chamber / LAF-bench • Spill cleanup	
Good sterile and cell culture techniques • Pipetting • Avoiding aerosols •	
Waste disposal • Virus • Cells • Bacteria	
Unwanted incidents • Cleaning up • Who to report to	

• File a nonconformity report	
Documentation • Journal • Log • Procedures	
Legal documents and local regulations	
All relevant SOPs •	
Completed safety job analysis (SJA) •	
Other:	
•	
•	
Research methods:	
•	
•	
•	

The training has been completed: _____

Signatures: _____ _____

9.2 LOG for working with long-term GMO experiments

Location	**Experiments, examples**	**Date Max 6 months**	**Hours weekly**	**Participants**
lab 1	Cell culture (Knock-out), Transfections with GMO plasmids	01.08.2023 - 22.12.2023	2-3 h	
lab 2	Site-directed mutagenesis, plasmid purification, agarose gel runs		4-5 h	
lab 3	Cell lysis, Protein extraction, SDS-PAGE analysis		2	

9.3 LOG for working with short-term GMO experiments

Location:	**LAF-bench:**

Experiment	**Date**	**Hours**	**Participants**