Safety guidelines for working with Lentiviral vectors

Lentiviral Vectors

Lentiviruses (lenti- is slow in Latin) are genus of slow viruses of the Retrovidae family, characterized by a long incubation period. Lentiviruses, including the HIV (Human), SIV (simian,) FIV (feline), are all pathogenic to humans. Lentiviruses can deliver significant amount of genetic information into the DNA of the host cell, and can target both dividing and non-dividing cells (unlike Retroviruses that infect only dividing cells). The native envelop of Lentivirus based vectors (the HIV-1) contains the Gp120 glycoprotein which is recognized by the CD4 receptor on the target cells (Lymphocytes). In order to broaden the range of target cells the native envelop was replaced (pseudotyped) by vesicular stomatitis virus G (VSVG).

All these unique features render Lentiviruses as the most efficient method for gene delivery. They are used to replace a damaged/missing gene in gene therapy, and in basic science are used to introduce or silence genes in a wide verity of models. It is an excellent method replacing non-efficient transfections, used for in vivo labeling, transgenic mice, high throughput screening and more.

The attractive features of Lentiviruses, especially with HIV-1 based Lentivirus vectors, raises Biosafety issues. Laboratory workers handling Lentivirus are the risk group. Lentivirus penetration may occur:

- Through the skin - via puncture or absorption (scratches, cuts, dermatitis, or other lesions)
- Through mucous membrane - eyes, nose, and mouth.

The goal of the safety and environment unit is to bring about a safe working environment. This goal can be achieved by your comprehensive implementation of our "Biosafety guidance and Safety protocol" provided herein.

Biosafety Guidance & Safety Protocol for Research

The basic principle is to avoid contamination with the recombinant virus, which can spread through droplets or aerosol. Therefore, all the work should be contained within a BSC (Bio Safety Cabinet, hood) in a Lentivirus approved room. According to the decision of the Institutional Biological Committee, which was approved by the institutional safety committee, Lentivirus work will be carried out within the facilities of each department or of a group and is under the responsibility of the principal investigator. This room can also serve as general tissue-culture room.

We recommend using the third-generation plasmids which allow the production of replication-deficient virus, for example Invitrogen Virapower system (recommended by the NIH). This system incorporates several safety features as follows:

- Only 3 viral genes are used in vector system (gag, pol on one plasmid, and rev on another). Tat is not expressed in the system.
- HIV-1 Env is replaced by the VSV-G gene.
- The genes encoding structural and other essential genes are separated onto 4 plasmids (3 plasmids and Vector).
- The vector is “self-inactivating” due to a deletion in the 3' LTR (U3).

• Users of 2nd generation vectors must check that the Lenti vector is not culturable, every new production.

Working procedure with Lenti Virus:

1. The allocated tissue-culture room should be equipped for work with lentiviral vectors (see item 2). The room must be approved by the Biosafety officer.
2. Obligatory equipment in the allocated tissue-culture room BSL2+:
    - BSC Class II
    - Centrifuge and sealed safety centrifuge tubes
    - Microscope
    - Incubator
    - Biosafety waste disposal equipment.
    - A set of micro pipettes.
3. Signs should be posted on the entrance stating:
    - The use of Lentivirus
    - Restricted entrance for authorized personnel only.
4. Before starting your work:
    - Wear a laboratory-coat (preferably disposable) and 2 pairs of gloves.
    - The gloves should cover the wrists.
    - Prepare decontamination solutions as specified under decontamination.
    - Prepare biohazard bag for solid infectious waste inside the BSC and another biohazard bag in the biohazard bucket.
    - Use of sharps is not recommended, if you cannot avoid using sharps prepare a separate bucket for sharp disposal.
    - Use only screw capped tubes for freezing (Do not overfill the tubes – 3/4 only).
5. Disposable equipment (pipettes, flasks/plates) must be decontaminated before discarding it to the biohazard bag (details under decontamination).

6. Use tips and pipettes containing filter. Collect the used tips in a screw-cup plastic bottle (cleaned used plastic medium bottle), dispose into the biohazard bag after you have closed the bottle.
7. Shut the tissue-culture door while working with Lentivirus (collection and infection).
8. No other vectors are allowed in BSC during the work with Lentivirus production.
9. Do not leave virus-containing solutions unattended in the hood or in the centrifuge.
10. All disposable personal protective equipment (gloves and disposable lab-coat) should be discarded in a biohazard bag located inside the approved room.
11. You must not leave the Lentivirus approved room to any other room wearing the disposable lab-coat. You may leave the room wearing a clean fabric lab-coat and gloves.
12. Cells for virus packaging and for infection should preferably be grown in flasks (not in plates).
13. Plates/flasks should be placed in the incubator on a tray such as a sterile plate cover, bigger than your lentivirus containing plate.
14. Pay special attention to avoid aerosols and splashes.
15. Do not touch anything outside the BSC with contaminated gloves. Replace the gloves upon leaving the BSC and throw the gloves to the biohazard bag (inside the BSC). Wear new gloves for opening the incubator or using other facility in the room.
16. To visualize the cells under microscope, take the following steps:
    - Take the flask/plate to the microscope on the designated tray
    - Before leaving the microscope clean the stage with 70% ethanol
17. Taking viruses out from the virus room: Close the disposable tube (screw cup) containing the virus with parafilm, decontaminate the tube with 70% ethanol, put in a small biohazard bag, close the bag, decontaminate with 70% ethanol, put in a second small biohazard bag, close the bag, decontaminate and only then be taken out from the virus room. Alternatively, for freezing, decontaminate the tube with 70% ethanol, put in a leakproof and closed Box, decontaminate with 70% ethanol, then put in a second small biohazard bag.
18. Taking infected cells out from the virus room - Taking out cells that undergone viral transfection, for performing any process that includes the use of equipment placed outside the virus room, or laboratory work on devices such as FACS, microscopy, will be possible under 2 conditions:
    18.1 At least 48 hours after viral transfection.
    18.2 At least 4 medium / cell replacements (for 2nd generation Lenti virus, 3rd generation Lenti virus at least 3 rinses) were performed (gently to avoid losing cells). This rinsing ensures that the number of viruses remaining on the flask is less than the dose required for infection. Decontaminate the flask with 70% ethanol.
19. Biohazard bags used in the hood must be closed (not hermetically sealed) and transferred to the biohazard bag placed in the biohazard bucket.

20. Decontaminate small volume liquid waste, up to 1 ml, with 0.5% Sodium Hypochlorite, collect the decontaminated liquid waste in a screw-cup plastic bottle (cleaned used plastic medium bottle), closed the bottle and dispose into the biohazard bag.
21. Collect larger liquid waste (up to 500 ml) in a screw-cup plastic bottle (cleaned used plastic medium bottle) and dispose into the biohazard bag.
22. At the end of each session, wipe the hood, the incubator handle and the equipment used (microscope, centrifuge, etc.) with 70% Ethanol.
23. After removing the gloves, wash hands with soap and water.
24. Centrifugation must be carried out in aerosol sealed tubes in a centrifuge located in the approved room.
25. If you have to use an ultracentrifuge (located in another room) you should follow these instructions:
    - Post a clear sign notifying the use of Lentivirus, as well as information specifying the length of your run, your name and group, your mobile phone number.
    - Balance the plastic tubes by adding an exact volume of medium containing virus inside the BSC of the approved room.
    - When filling inside the BSC in the virus room, do not exceed 75% of the tubes volume.
    - Gently insert the plastic tube into the metal bucket, paying attention to avoid splashes.
    - Cover the metal bucket using the provided metal screw and O-ring.
    - Spray the exterior of the metal bucket and covers with 70% Ethanol, change your glove and the rest of your personal disposable protection, leaving them in the Lentivirus approved room. You may leave the room wearing a clean fabric lab-coat to access departmental ultracentrifuge room.
    - Take the sealed tubes in a leakproof sealed box, to the centrifuge using the provided stand and place them inside a carrier such as a cooler.
    - After centrifugation open the lentivirus containing tubes only inside the BSC in the Lentivirus approved room. Clean the metal centrifuge buckets and covers (inside and outside) with 70% Ethanol in the BSC. The ultracentrifuge and the rotor should be decontaminated using 70% Ethanol even if you did not notice a spill.
    - Clear guidelines for cleaning spill inside the centrifuge should be posted nearby.

Decontamination

In general, decontamination is done using Sodium Hypochlorite. The stock is 11% Sodium Hypochlorite solution.

- Keep in the hood a bottle of freshly prepared 0.5 % Sodium Hypochlorite (it is good for one week only).
- Prepare 70% Ethanol sprayer.

Liquid waste decontamination

- Decontaminate small volume liquid waste, up to 1 ml, with 0.5% Sodium Hypochlorite, collect the decontaminated liquid waste in a screw-cup plastic bottle (cleaned used plastic medium bottle), closed the bottle and dispose into the biohazard bag.
- Collect larger liquid waste (up to 500 ml) in a screw-cup plastic bottle (cleaned used plastic medium bottle) and dispose into the biohazard bag.

Decontaminating a small volume spill

Cover the spill with paper towel and gently pour on top **1% Sodium Hypochlorite**. Collect the paper towel to the biohazard bag.

Decontaminating a large volume spill

Cover your shoes with disposable shoe covers and wear a face shield to prevent splashing during the decontamination procedure. Cover the spill with paper towel and gently pour on top **1% Sodium Hypochlorite**. Collect the paper into a biohazard bag then disinfect the area once more with **1% sodium hypochlorite** solution. Collect the paper towel to the biohazard bag. **If necessary, call 2222 for assistance**.

Decontaminating a splash:

Cover the splash with paper towel, spray on top with either 1% Sodium Hypochlorite or 70% Ethanol sprayer. Collect the paper towel to the biohazard bag. Discard all decontaminated solid waste in the biohazard bag.

Biological waste is collected in the laboratory by the worker. Petri dishes, flasks, pipettes, tips, contaminated with biohazard materials are disposed in a plastic bag carrying the biohazard sign, inside the orange bin.

Table 1. Biosafety level work practice requirements.

Work Practices	BSL – 1	BSL – 2	BSL – 2+	BSL – 3
Public access	Not recommended	Limit access to lab while BSL – 2 work is being conducted	Restricted	Not permitted
Bench-top work	Permitted	Permitted only for low risk procedures	Not permitted for biohazardous materials	Not permitted for biohazardous materials
Decontamination	Daily and following any spill	Daily and following any spill	Daily; immediately following work with biohazardous materials, and following any spill	Daily; immediately following work with biohazardous materials, and following any spill
Eating, drinking applying lip balm, etc.	Permitted only in designated clean areas	Permitted only in approved and designated clean areas	Not permitted at any time; Food or drink may not be brought into or through lab	Not permitted at any time; Food or drink may not be brought into or through lab
Lab coats	Required	Required	Required (disposable preferable)	Required (disposable required)
Personal Protective Equipment	Based on risk assessment	Required: Wear appropriate combinations of special protective clothing for all activities with biohazardous materials	Required: Wear appropriate combinations of special protective clothing for all activities with biohazardous materials	Required: Wear appropriate combinations of special protective clothing plus NIOSH N95 respirators or equivalent for all activities with biohazardous materials
Biological Safety Cabinet (BSC)	Not required	Required for all aerosol generated processes*	Required for all work with biohazardous agents	Required for all work with biohazardous agents
Storage Equipment	Biohazard signs required on centrifuges, incubators, and freezers	Biohazard signs required, all equipment must be labeled with contents	Biohazard signs required, all equipment must be labeled with contents	Biohazard signs required, all equipment must be labeled with contents

Physical containment	Decontaminate equipment immediately after use	Use physical containment devices during procedures that have a high potential to create aerosols* when using biohazardous material; Decontaminate immediately after use	Use physical containment devices (centrifuge safety cup, sealed centrifuge rotor) for all activities using biohazardous material; Open containers in a BSC; Decontaminate immediately after use	Use physical containment devices (centrifuge safety cup, sealed centrifuge rotor) for all activities using biohazardous material; Open containers in a BSC; Decontaminate immediately after use
Hand-washing facilities	Required	Required (foot, elbow, or electronic activation required)	Required (foot, elbow, or electronic activation required)	Required (foot, elbow, or electronic activation required)
Pipetting	Only mechanical device	Only mechanical device	Only mechanical device	Only mechanical device
HEPA-filtered vacuum lines	Recommended	Required	Required	Required

*Procedures include but not limited to: centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of biohazardous materials after above procedures.

Table 2. Examples for pathogens and their Biosafety level and Risk group classifications.

RISK	GROUP	BSL	EXAMPLES
1	Agents that are Not associated with disease in healthy adult humans.	**BSL1**	• *Escherichia coli*; K12 derivatives (DH5α, JH109, pBluescript, psi2) • Baculovirus
2	Agents that are associated with human disease hazard = percutaneous injury, ingestion, mucous membrane exposure	**BSL2**	• Adenovirus • All human and non-human primate blood-contaminated specimens • All human and non-human primate cell lines • Herpes Simplex Virus
2+		**BSL2+**	• **Lentiviral vectors** • **Pseudo typed Rabies Virus**
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	**BSL3**	• *M. tuberculosis* • Concentrated Lentivirus or Lentiviral vectors **with high likelihood of aerosol formation**. • Respiratory illness or airborne transmissibility - **no current use of this risk group classification at the Technion**