UiO : Faculty of Medicine		
Instruction concerning work with Lenti-virus (Containment level 2)		**Edition:1** 23.10.2014 **2** Page : 3 Filing number in ePhorte: 2016/7560
Elaborated: HSE-coordinator Liv Bjørland	**Approved:** Centre Director Kjetil Taskén	**Date:** 30.01.17 and 28.02.17

Information

Lentiviral vector constructs are derived from HIV and are highly efficient vehicles for *in vivo* gene delivery. They have the ability to integrate transgenes into dividing, as well as, non-dividing cells. The two major risks are: 1) the potential generation of replication competent virus; and 2) the potential for oncogenesis through insertional mutagenesis. These risks are largely based upon the vector system used and the transgene insert encoded by the vector.

The handling of LV suspensions, in particular high titer stocks, increases the possible accidental exposure and is considered to be an activity associated with higher risks. The major potential hazard is infection of the researcher by parenteral inoculation (e.g. needle stick accidents). After accidental exposure, LV can potentially integrate in the infected host cell genome. This could not only produce hazards linked with insertional (in)activation or transactivation of genes but might also result in permanent transgene expression with the associated harmful effects based on the gene product expressed. Studies have shown that lentiviruses preferentially integrate in transcriptionally active genes.

Removal of viral genes from the packaging construct is not required for gene transfer but are important for HIV-1 virulence and pathogenicity. The lenti-viral particles produced in these systems are replication-incompetent, but the first and second generation vectors are considered less safe because they contain more original HIV sequences compared with third generation LV, which theoretically decrease the number of recombination events necessary to form RCL.

The *gag*, *pol* and *env* structural genes are common to all retroviruses. HIV-1 contains two regulatory genes, *tat* and *rev*, essential for virus replication. The four accessory genes, *vif*, *vpr*, *vpu* and *nef* dispensable for virus growth *in vitro,* are critical for *in vivo* replication and pathogenesis. These accessory genes (*vif*, *vpr*, *vpu* and *nef*), produced the “second generation” vectors, referred to as the ‘multiply attenuated’ vectors

Elimination of the HIV *tat* gene was a further step “improved biosafety”. The Tat protein is a potent transcriptional activator responsible for the high replication rate of HIV. Separating the overlapping gag-pol structure on two plasmids prevents the formation of functional gag-pol structures, which are essential for vector mobilization. However,both transducibility and production efficiencies are increasingly challenged by the number of plasmids required to produce the full complement of viral genes.

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The risks associated with working with lentiviral vectors must be carefully evaluated. It is imperative that prior to utilizing a lentiviral vector system, a risk assessment must be completed and documented.

Lentiviral vectors may be safely handled using either BSL-2 or BSL-2 enhanced controls depending upon the risk assessment. Cloning an oncogene into LV or enabling shRNA mediated knockdown of tumor suppressor genes clearly remain high risk manipulations requiring that stringent biosafety measures must be implemented.

State-of-the-Art Lentiviral Vectors for Research Use Current Gene Therapy,* 2009, *Vol. 9, No.

Instruction for the lab

- Fill in your credentials in the log book to verify your use of the room.
- Before you leave the room the bench and work place must be disinfected.
- Keep the security-bench tidy – never put more into the bench than you necessarily need. Do not leave the room untidy.
- Sign for accomplished tasks in the log book before you leave the room.
- This lab is used by several groups. Each group has to refill their own equipment.

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1. Always use lab-coat and gloves according to our instructions for protection, and wash your hands after you have finished your work.

2. All samples should be centrifuged with their caps on. If danger of aerosols outside the security-bench, use spectacles and filter-mask P3 for virus.

3. Avoid needles and sharps during your work. Any sharps must be put in plastic tubes before discharged off.

4. Put all contaminated equipment into autoclave-able bags. (Contact the wash- facility for refill of bags)

5. Close the bag and discharge into the box for Lenti when the work is finished and before you leave the room. Notify Liv when the box is full and never continue the work before the box is replaced. All Lenti-work is autoclaved for an hour at 121 degrees before it is sent for incineration.

6. Decontaminate all medium from the Vacu-safe in 2% Virkon overnight before discharging off the solution into the sink. Remember to disinfect the VACU-pump system after each time it is used.

7. Any other biological or contaminated material goes into the yellow boxes.

8. If any spill on the floor, bench or in centrifuge -> soak up as much as possible and handle the paper as contaminated waste (as above). Decontaminate the area for 10 min with 1% Virkon. Decontaminate for 30 min if the contamination is visible. If horizontal surface cover the area with absorbing paper and soak with disinfectant. Rinse, and at last disinfect with 70% of ethanol.

9. Decontamination of the incubator, disinfect with Barricydal/Barrydin and rinse. The replacement for Barricydal is Chemgen HDH_4L . Use 70% ethanol afterwards.

10. Always disinfect with 1% SDS in 70% ethanol when the work is finished and you leave the room.

11. Keep the security-bench tidy – never put more into the bench than you necessarily need.

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