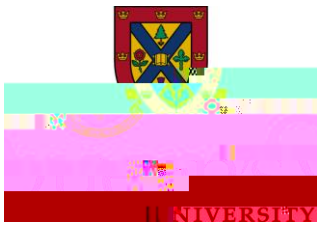
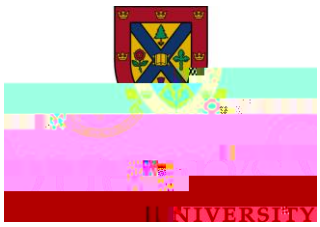


Second generation LV is a three-plasmid packaging system where HIV-specific accessory genes (except for Rev and Tat) have been removed from the Packaging plasmid, reducing the chance of RCR formation. (Fig 1)

Third generation LV is a four-plasmid system, where the packaging plasmid is split into two plasmids: One encoding structural (gag) and replication (pol) genes and another (helper plasmid) encoding the regulatory protein Rev. The requirement for Tat is eliminated through the addition of a chimeric 5'LTR fused to a heterologous promoter on the transfer plasmid, meaning that expression of the transgene is no longer dependent on Tat transactivation. With these modifications, there is a significantly reduced capacity of RCR formation. (Fig 1)



leading to additional or modified biosafety requirements or to classification as **RG2+/RG3**.

The appropriate level of risk must be determined by completing and submitting with the Biohazard permit application both a Pathogen and Local Risk Assessment. Decisions about containment should take into account a range of parameters/considerations including:

a) the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
b) the nature of the transgene (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
c) the vector titer and the total amount of vector,
d) the inherent biological containment of the animal host, if relevant, • negative RCR testing.

The table below can be used to assist the PI on determining the appropriate Risk Group for the LVV in use.

Deletion of viral accessory genes
Deletion of *tat* regulatory gene

LVVs - CL2+

CL2 admin controls plus:

Regular monitoring and control of the containment zone airflow direction.

6.3 Personal Protective Equipment

The following personal protective equipment MUST be worn when working with Lentiviral vectors:

- Gloves (consider double gloving for RG2+). Gloves will be worn during all cell culture manipulation and double gloving will be encouraged since micro-holes may be present in gloves and so that the outer pair can be removed before moving from the biosafety cabinet to prevent the spread of contamination to 6t contamination to 6t

- Aseptic techniques and procedures
- Personal protective equipment (e.g., lab coats, goggles, glove selection)
- Signage and labels
-

10. Revision History

Revised	Date	Changes
[redacted] Queen's Biohazard Committee (IBC)	06-24/2024	**Initial release**