University of Pittsburgh Institutional Biosafety Committee
Guidance on Biosafety Level Assignment for Lentiviral Vectors

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Background

The use of lentivirus vector systems based on HIV-1 has become a standard laboratory tool used by experienced and novice users alike with ever-increasing availability of commercially-generated expression systems that have increased efficiency of gene expression with greatly reduced biosafety concerns. A description of specific vectors and suppliers is provided at the end of this document; the following is a brief description of the salient features of the available systems relevant to biosafety.

Second generation (2-plasmid) systems are still used, but appears to be used by investigators having the background and knowledge required in virology for specialized manipulations. The second generation vectors separate packaging and gene transfer functions into three distinct plasmids (2 packaging plasmids and one gene plasmid) and lack certain viral accessory genes. These viruses frequently are made to express the vesicular stomatitis virus G (VSV-G) protein in place of viral Env to increase cell tropism.

Third generation systems are more common and are safer by the use of 3 helper plasmids: a VSV-G construct, a Rev construct, along with a Tat-independent gene transfer vector, and a packaging construct (the transgene construct), providing a total of 4 separate plasmids in all. The elimination of the accessory gene Tat is an important component as this protein is essential for replication of wild-type HIV-1. Vector systems that use more than 4 plasmids are available with even higher levels of biosafety. For simplicity, vectors that utilize 4 or more plasmids will be referred to here as third generation.

Both 2nd and 3rd generation vectors are generally self-inactivating by virtue of promoter disabling mutations engineered into the U3 region of the 3’ long terminal repeat. These deletions provide an additional level of safety as vectors should not be able to generate full-length vector RNA after viral integration.

NIH opinion

The Recombinant DNA Advisory Committee (RAC) of the NIH Office of Biotechnology Activities issued a report in December 2006 that reviewed biosafety issues relating to lentivirus vectors. This report advised that reduced biosafety level containment was appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that 1) separated vector and packaging functions onto multiple plasmids, 2) were produced at laboratory scale quantities, and 3) lacked expression of oncogenic transgenes.

The RAC specifically recommended that 4-plasmid systems that met specific criteria could be used at BSL-2 and ABSL-2 without the need to assay for replication competent virus (RCV). The NIH

report did not make specific recommendations relating to 3-plasmid vector systems, preferring to leave this decision to the local institutional biosafety committees.

IBC recommendation

The University of Pittsburgh IBC has adopted the NIH recommendations. As such, the IBC strongly recommends that investigators use 4-plasmid (3rd generation) lentivirus vectors from commercial vendors when at all possible.

Specific criteria for determination of lentivirus vector use: **see Table 1**

- *Oncogenic transgenes*

Lentivirus vectors that incorporate transgenes with oncogenic potential must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

- *Expression of toxins*

Lentivirus vectors that incorporate transgenes that express toxins must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

- *Scale of production*

Lentiviral Packaging cell line supernatant made at a level of production of more than 100 ml volume at one time must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

- *Titer greater than 10^9* which is a 50% *tissue culture infectious dose ($TCID_{50}$)*

Lentivirus vectors that are expected to have a titer higher than 10^9 $TCID_{50}$ must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

EXAMPLES

4- or more plasmids (third generation) systems – no oncogenes, laboratory scale production

IBC recommendations: **BSL-2/ABSL-2**

4-plasmid system vectors may be generated and used at BSL-2 (laboratory research) and at ABSL-2 (animal research). The IBC does not require testing for RCV when 4-plasmid (third generation) systems are used.

3-plasmid (second generation) systems – no oncogenes, laboratory scale production

IBC recommendations: **BSL-2/ABSL-2**

Investigators obtaining cells or cell lines that have been stably transfected using a second generation lentiviral vector system, and are not working or handling the lentiviral vectors, may conduct research with the cell lines under BSL-2/ABSL-2 containment conditions.

Lentivirus Vector Systems

4-or more plasmid lentiviral system

Third (3rd) generation lentiviral systems are comprised of a total of at least four plasmids (the expression plasmid (transgene) plus three packaging vectors: pMD2.g(VSVG), pRSV-REV and pMDLg/pRRE).

This split packaging system offers maximal biosafety, as described in: Dull et al "*A third generation lentivirus vector with a conditional packaging system*" (1998) J. Virol. 72, 8463-8471 and in Klages et al. "*A stable cell line for the high-titer production of third generation lentiviral vectors*". Mol. Ther. (2000) 2, 170-6.

3-plasmid lentiviral system (2nd generation systems)

Older (second generation) lentiviral systems use an expression plasmid (transgene) and 2 packaging vectors; a total of 3-plasmid. [Stewart, S.A., et al., "*Lentivirus-delivered stable gene silencing by RNAi in primary cells*", RNA, 9, 493-501 (2003)].

In general, lentiviral vectors with a wildtype 5' LTR need the 2nd generation packaging system because these vectors require TAT for activation.

Table 1:

Summary of biosafety level requirements for lentivirus vector production and use

Meets any ONE of the Specific Criteria?	Number of plasmids	RCV testing	Vector production	Use of viral vectors in vitro	Use of viral vectors in animals	Use of virus-transfected cells in animals
Yes	Any number	With or without testing	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+
No	3 or more	Not required	BSL-2	BSL-2	ABSL-2	ABSL-2

Reference: NIH OBA Guidance from 2006 RAC meeting: http://osp.od.nih.gov/sites/default/files/resources/Lenti_Containment_Guidance_0_0.pdf last visited on May 18, 2015