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

Approved February 11, 2008
Revision Approved April 9, 2012
Revision Approved December 14, 2015
Revision Approved: August 13, 2018

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.

Older (3-plasmid) systems are still used by investigators having background and knowledge in virology for specialized manipulations. The three vectors separate packaging and gene transfer functions into three distinct plasmids:

1) a single packaging plasmid,
2) a transfer plasmid containing the sequence of interest (e.g. gene or shRNA) to be expressed lacking certain viral accessory genes, and
3) a plasmid encoding an envelope protein, often the vesicular stomatitis virus G (VSV-G) protein in place of viral Env to increase cell tropism.

Far more common and safer to use are the 4 plasmids:

1) a packaging construct,
2) a Rev construct,
3) a Tat-independent gene transfer vector, and
4) an envelope construct (e.g. VSV-G).

The elimination of the accessory gene *tat* is an important component as this protein is essential for transcription of the wild-type HIV-1 genome and is often replaced with a CMV-promotor upstream of the sequence of interest. Vector systems that use more than 4 plasmids are available with even higher levels of biosafety.

In addition to separation of the viral genome on multiple plasmids, lentiviral vectors are generally self-inactivating due to promoter disabling mutations engineered into the U3 region of the 3' long terminal repeat (LTR). These deletions provide an additional level of safety as vectors should not be able to generate full-length viral RNA after viral integration.

NIH Opinion

The Recombinant DNA Advisory Committee (RAC) of the NIH Office of Science Policy 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 NIH recommendations. As such, the IBC strongly recommends that investigators use 4-plasmid lentiviral vectors from commercial vendors when at all possible.

Specific criteria the IBC uses to determine containment of lentivirus:

- Nature of the vector and potential for RCV from vector components
- Nature of transgene (e.g. oncogenic potential)
- Vector production volume and titer
- Inherent host-range: amphotropic versus ecotropic

Use of oncogenes, toxins, large scale production, or high titer (3- or more plasmids)

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

- *Oncogenic transgenes*

Lentivirus vectors that incorporate transgenes with oncogenic potential must be generated and used at BSL-2+ containment regardless of the number of plasmids used to generate the vector.

- *Expression of toxins*

Lentivirus vectors that incorporate transgenes that express toxins must be generated and used at BSL-2+ containment regardless of the number of plasmids used to generate the vector.

- *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 the number of plasmids used to generate the vector.

- *Titer greater than 10^9* 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 the number of plasmids used to generate the vector.

Investigators proposing research with lentiviral vectors or transfected cells using a lentiviral vector system that meets any of the specific criteria identified must conduct the research with the agents under BSL-2 containment with enhanced biosafety practices and procedures (BSL-2+). Any of the agents administered to animals is appropriate under ABSL-2 (animal research) containment. Animal containment may not be downgraded.

3- or more plasmids systems – no oncogenes, used in human cells or animals engrafted with human cells or tissues permissive for HIV replication

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

Investigators obtaining cells or cell lines that have been stably transfected using a lentiviral vector system, or generating the lentiviral vectors, may conduct research with the cell lines under BSL-2 containment. Vectors in human or non-human primate cells may be used under BSL-2 (laboratory research) and administered to animals under ABSL-2 (animal research). Animal containment may not be downgraded.

3- or more plasmids systems – no oncogenes, non-human cells

IBC recommendations: **BSL-2/ABSL-2 with downgrade to ABSL-1 after 72 hours**

Investigators obtaining cells or cell lines that have been stably transfected using a lentiviral vector system, and are not working or handling the lentiviral vectors, may conduct research with the cell lines under BSL-2 containment. Vectors in non-human cells (e.g. murine) may be used under BSL-2 (laboratory research) then administered to animals directly under ABSL-2 (animal research) and after 72 hours, the animal containment may be reduced to ABSL-1. The laboratory personnel must provide the change to the clean ABSL-1 containment cages. Alternatively, the *non-human* cells may be maintained (ex vivo) under BSL-2 conditions for 72 hours after transduction and then administered under ABSL-1 containment.

Table 1:

Summary of biosafety level requirements for lentivirus vector production and use

Meets any ONE of the Specific Criteria?	**Vector production**	**Use of viral vectors *in vitro***	**Use of viral vectors or virus-transduced or -transfected cells *in vivo***
Yes	BSL-2+	BSL-2+	ABSL-2
No	BSL-2	BSL-2	ABSL-2, may be downgraded to ABSL-1 after 72 hours

Reference: NIH OSP Guidance from 2006 RAC meeting; https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf, last visited July 23, 2018