

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

Background

The use of lentivirus vector systems based on HIV-1 is becoming commonplace with the advent of commercially-available later-generation 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 systems are most common. These separate packaging and gene transfer functions into three distinct plasmids 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 go further by using 3 helper plasmids: a packaging construct, a VSV-G construct and a Rev construct, along with a Tat-independent gene transfer vector, providing 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 becoming 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 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 separated vector and packaging functions onto multiple plasmids, were produced at laboratory scale quantities, and lacked expression of oncogenic transgenes. They 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 individual university committees.

IBC recommendation

The University of Pittsburgh IBC aims to adopt 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. 4-plasmid systems provided by other investigators from within the University of Pittsburgh or from outside can be used if absolutely necessary. 3-plasmid systems from commercial vendors or provided by other investigators can be used, but certain restrictions apply.

Specific requirements of lentivirus vector use - see Table 1 below

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.

Scale of production

Lentivirus vectors made at a level of production > 100 ml volume must be generated and used at BSL-2+ containment regardless of whether second or third generation systems are used.

4-plasmid (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+**

May downgrade to BSL-2/ABSL-2 with negative RCV testing

3-plasmid lentivirus systems should be generated and used at BSL-2+ (laboratory research) and at ABSL-2+ (animal research). The investigator may request a downgrade in biosafety level to BSL-2/ABSL-2 following demonstration that virus preparations have no detectable RCV based on results of an accepted RCV assay as described below. A protocol modification requesting reduction in biosafety level and including data from the RCV test must be submitted to and approved by the IBC before any BSL-2 or ABSL-2 work can be performed. Since the modification involves changes to the originally approved biosafety level, the modification request must be submitted as either a new application or a full renewal according to current IBC policy.

Approval for BSL-2/ABSL-2 use with 3-plasmid systems will be specific to each virus preparation made. If separate lentivirus preparations containing different transgenes are generated each must be shown to be free of RCV before a downgrade can be approved.

RCV assay – 3-plasmid system

This assay can be performed by the investigator using a standard p24 ELISA kit (i.e., from Cell Biolabs or Perkin Elmer) providing the assay has a sensitivity of ≤ 12.5 pg/ml. If the investigator chooses, the Magee-Women's Research Institute Transgenic and Molecular Core (Director: Kyle Orwig, Manager: Yi Sheng, Administrator: Jennifer Shuttleworth; ph. 412 641-2415) will run RCV assays for individual investigators on a fee-for-service basis.

A positive control for virus infection is not required; the IBC does not want the investigator to work with infectious HIV-1 for this assay. However, the assay must contain a positive control for the ELISA itself in the form of p24 antigen.

Virus should be tested for RCV by serial passage of tissue culture supernatant on 293T cells for 3 passages with subsequent testing of supernatant from each passage for p24 antigen by ELISA. Optical density readings from each passage along with positive controls and/or standards should be submitted to the rDNA office.

If the MWRI runs the assay, the p24 ELISA assay report from the Core must be submitted to the rDNA office.

Exceptions to the requirement for RCV testing of 3-plasmid lentivirus stock

Investigators who are not generating their own viruses from the 3-plasmid system but are acquiring already constructed virus stocks from a commercial source that has documentation filed with the rDNA office of acceptable RCV testing (i.e., Mission siRNA products from Sigma Aldrich) will not be required to test for RCV.

Investigators who are not generating their own viruses from a 3-plasmid system but are acquiring already constructed viruses from a University of Pittsburgh or other investigator should contact the rDNA office regarding requirements for RCV testing, which will be reviewed on a case-by-case basis.

Table 1: Summary of biosafety level requirements for lentivirus vector production and use

Oncogenic transgene or >100 ml production	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	4 or more	Not required	BSL-2	BSL-2	ABSL-2	ABSL-2
	3	Elect to test for RCV	BSL-2+	BSL-2 if negative RCV test	ABSL-2 if negative RCV test	ABSL-2 if negative RCV test
		No RCV test	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+

Lentivirus Vector Systems

4-plasmid lentiviral system

The 3rd generation lentiviral system comprises four plasmids (the expression plasmid plus three packaging vectors: pMD2.g(VSVG), pRSV-REV and pMDLg/pRRE). This generation 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.

If you wish to use this system, you need to have a lentiviral vector with a chimeric 5' LTR in which the HIV promoter is replaced with CMV or RSV, thus making it TAT-independent. Examples of these vectors include pLKO.1, pLL3.7, pLB, pLenti6, pSico, pCL, and pCS. A lentiviral vector carrying a chimeric 5' LTR can be packaged with either the 2nd or 3rd generation packaging system.

This system is commonly available from InVitrogen (ViraPower; <http://www.invitrogen.com>), using the expression vector such as pLenti6 (InVitrogen) and the packaging vectors pMDLg/pRRE (gag/pol elements), pRSV-REV, and pMD.G (env elements) (sold as the ViraPower™ Lentiviral Packaging Mix). A related system is also available from Clontech (Lenti-X; <http://www.clontech.com>). Many of these vectors are also available from Addgene (<http://www.addgene.org>).

3-plasmid lentiviral system (2nd generation systems)

The 2nd generation lentiviral system comprises an expression plasmid and 2 packaging vectors. [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.

Below are two 2nd generation systems.

2nd generation system (Developed by the Trono lab)

Plasmid	Description
psPAX2	2 nd generation packaging plasmid for producing viral particles. psPAX2 contains a robust CAG promoter for efficient expression of packaging proteins.
pMD2.G	Envelope plasmid for producing viral particles

2nd generation system (Developed by the Weinberg lab)

Plasmid	Description
pCMV-dR8.2 dvpr	2 nd generation packaging plasmid for producing viral particles
pCMV-VSVG	Envelope plasmid for producing viral particles

A second generation system for expression of shRNA is available from Sigma (Mission RNAi). Many of these vectors are also available from Addgene (<http://www.addgene.org>).