ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 4/24)

LENTIVIRAL VECTORS

Scope

The information in this SOP, other than introductory information, represents a summary of guidance issued by the National Institutes of Health (NIH) Office of Science Policy (OSP). The full NIH publication is available on the NIH-OSP FAQ web page and is titled "Biosafety Considerations for Research with Lentiviral Vectors" https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf .

The OSP produced this guidance because the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) do not explicitly address containment for research with lentiviral vectors. Other sources used for information in this document include:

- Wooley, Dawn P. and Byers, Karen B.(ed). 2017. Biological Safety: Principles and Practices, Fifth Edition, pp 229-230, 235.
- Pauwels, K et al. "State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations", Current Gene Therapy (2009) 9: 459.

Introduction

Lentiviruses

Lentiviruses comprise a genus of the Retroviridae family and include bovine lentiviruses (e.g., Bovine immunodeficiency virus, Jembrana disease virus); equine lentiviruses (e.g., Equine infectious anemia virus); feline lentiviruses (e.g., Feline immunodeficiency virus); Ovine/caprine lentivirus (e.g., Caprine arthritis-encephalitis virus, Ovine lentivirus, Visna virus); and Primate lentiviruses (e.g., Human immunodeficiency virus (HIV) types 1 – 3, Simian AIDS retrovirus SRV-1, Human T-cell lymphotropic virus type 4, and Simian immunodeficiency virus).

Lentiviral Vectors

Most of the lentiviral vectors presently in use are HIV-derived vectors. Lentiviral vectors can transfect both dividing and non-dividing cells. Lentiviral vectors are comprised of separate transfer and packaging plasmids. Lentiviral vector systems have undergone several iterations

to engineer safety into the system. This is done by limiting the viral genes in the packaging mix to gag, pol, rev, and tat as well as separating these genes onto several plasmids. These two actions serve to prevent replication of virions (viral particles) produced after initial viral packaging and virtually eliminate the possibility of recombination events with retroviruses that may be present in cells or organisms.

NIH Guidance

Risks of Lentivirus Vectors

Major risks to be considered for research with HIV-1-based lentivirus vectors are potential for generation of replication-competent lentivirus (RCL) or oncogenesis. These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector.

A comprehensive risk assessment and determination of containment for research with lentiviral vectors should consider the nature of the vector system, transgene insert, and type of manipulations involved.

General Containment Considerations

Either BSL-2 containment or enhanced BSL-2 containment is often appropriate in the laboratory setting for research involving use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids.

Enhanced BSL-2 containment may include, in addition to attention to sharps (and use of safety needles where feasible), use of personal protective equipment intended to reduce potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L). The appropriate containment level for specific lentivirus vector research is determined following a complete risk assessment (as described below) and local IBC review.

General Criteria for Risk Assessment of Lentiviral Vectors

Decisions about containment should take into account a range of parameters/considerations including:

- Nature of the vector system and potential for regeneration of replication competent virus from the vector components
- Tropism provided by the envelope protein (e.g. what types of cells can be infected by the virus?)
- Nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential)
- Vector titer and total amount of vector

- Inherent biological containment of the animal host, if relevant
- Negative Replication Competent Lentivirus (RCL) testing

Biosafety Considerations and Risk Levels		
Biosafety Considerations	**Higher Risk**	**Lower Risk**
Vector Design	Vector packaging functions on two plasmids Expression of viral genes	Vector and packaging functions separated onto multiple plasmids Deletion of viral genes
Pseudotyping *(changing the envelope proteins of the viral particles produced)*	Replacing the envelope protein of HIV with one that allows for entry into multiple cell types	Envelope proteins that only infect a specific cell type
Transgene	Oncogene, Cell cycle/cell division, regulatory	Non-oncogene, enzymes, housekeeping, membrane proteins, etc.
Vector Generation	Large scale	Laboratory scale
Animal Hosts	Permissive host Animals engrafted with human cells	Non-permissive host
Animal Manipulations	Vector administration (e.g., use of sharps during injection)	Housing and husbandry (no use of sharps)

Potential for Generation of Replication Competent Lentivirus (RCL) from HIV-1 based lentivirus vectors:

Potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are: the number of recombination events necessary to reassemble a replication competent virus genome, and; the number of essential genes that have been deleted from the vector/packaging system. Early lentiviral systems that only used two plasmids may have a higher potential for generation of RCL. Safer vector systems have the following features:

- They use a heterologous coat protein in place of the native HIV-1 envelope protein (However, use of certain coat proteins, such as VSV-G, broaden the host cell and tissue tropism of lentivirus vectors, which should also be considered in the overall safety assessment).
- They separate vector and packing functions onto **four or more** plasmids

- They include additional safety features (e.g., they do not encode Tat, which is essential for replication of wild type HIV-1).

Animal studies

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum). IBCs may consider the biosafety issues associated with animal husbandry and housing *after* the initial injection separately from the initial inoculation itself.

In general, initial delivery of the vector should be performed under Biosafety Level 2 for Animals (ABSL-2) or enhanced ABSL-2 containment (see "*General Containment Considerations*" above) to minimize risk of autoinoculation by the investigator. However, if approved by the IBC, it may be possible to reduce the containment level at some point following vector delivery. For example, if there is no expectation of infection, the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from ABSL-2 to ABSL-1 within a few days (the specific time period will be specified by the UNL IBC). Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1.

Any reduction in containment level following injection will be assessed and approved by the IBC prior to initiation. This procedure will be described in the IBC protocol.

Lentivirus vectors (Other than derived from HIV-1)

Use of lentiviral vectors derived from animal lentiviruses that do not normally infect humans (e.g. FIV, SIV, EIAV, etc.) may require BSL-2 containment if they have modified envelope proteins allowing for the transduction of human cells. Modification of the envelope protein increases the risk of insertional mutagenesis should a human be exposed to these viral vectors.

Lenti-CRISPR systems

The popularity of the CRISPR/Cas system for gene editing has spurred the development of lentiviral vectors expressing the Cas9 or other Cas proteins and a single guide RNA (sgRNA) of choice, to improve expression efficiency. Use of a lentiviral vector with this gene-editing system increases the likelihood for insertional mutagenesis of the host genome and “off-target” effects. Additional safety and biocontainment procedures may be warranted when using this type of system. The IBC may specify additional precautions when handling these materials. Please contact the EHS Biosafety Officer for additional information and guidance.