

## LENTIVIRAL VECTORS

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The information in this SOP, other than introductory information, represents a summary of guidance issued by the National Institutes of Health (NIH), Office of Biotechnology Activities (OBA), Recombinant DNA Advisory Committee (RAC). The full NIH publication is available on the NIH website and is titled "[Biosafety Considerations for Research with Lentiviral Vectors.](#)" The NIH/OBA RAC produced this guidance because the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* do not explicitly address containment for research with lentiviral vectors.

### 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.

### NIH Guidance

#### Risks of Lentivirus Vectors

The major risks to be considered for research with HIV-1 based lentivirus vectors are potential for generation of replication-competent lentivirus (RCL), and potential for 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.

#### General Containment Considerations

Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include, in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the 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, of

course, 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
- 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 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
Transgene	Oncogene	Non-oncogene
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:** The 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. Safer vector systems have the following features:

- They use a heterologous coat protein in place of the native HIV-1 envelope protein (However, the use of certain coat proteins, such as VSV-G, may 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, the 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.

**Lentivirus vectors (Other than derived from HIV-1)**

Some non-human lentivirus vectors (e.g., FIV, SIV, EIAV, etc.) are also in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. In the Appendix B-V of the *NIH Guidelines*, a containment level appropriate for Risk Group 1 agents is recommended for use of certain animal viral etiologic agents not associated with disease in healthy human adults. However, replication-defective vectors in which a heterologous envelope (such as VSV-G) is used for vector packaging may require BL2 containment in the laboratory setting, since these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis.