Last revised 9/7/2018

COMS Lentiviral Vector Policy

I. Purpose

To provide guidance for investigators and reviewers for biosafety requirements and best practices for laboratory work with lentiviral vectors at institutions affiliated with the Harvard Committee on Microbiological Safety (COMS).

II. Applicability

This policy applies to all new and current projects that include work with lentiviral vectors at institutions affiliated with COMS. Use of lentiviral vectors in laboratory animals is addressed in a separate COMS policy (Policy on Recommended Containment Levels for use of Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits).

III. Definitions

A. Lentivirus: Lentiviruses are a subset of retroviruses. Retroviruses are RNA viruses that use reverse transcriptase to produce DNA from their RNA template. The DNA then becomes integrated into the DNA of the host cell. Lentiviruses are characterized by slowly progressive infections, complex genomes and the ability to infect non-replicating cells. Lentiviruses include human immunodeficiency viruses (HIV-1 and HIV-2), simian immunodeficiency virus (SIV) and feline immunodeficiency virus.

B. Lentiviral vector: Lentiviral vectors are composed of recombinant or synthetic gene sequences derived from retroviruses, including genes for viral packaging and regulatory elements. Lentiviral vectors retain the ability to integrate DNA into the host genome, however, they are unable to replicate the viral genome.

C. Tropism: Tropism is the ability of a virus to infect or replicate in specific cells (e.g., from a host species) or tissue. An ecotropic virus has a host range limited to the original host. An amphotropic virus can infect cells of multiple hosts.

IV. Implementation Procedures

A. General Information

Biosafety recommendations for use of lentiviral vectors will be made on a case-by-case basis by COMS in consultation with institutional biosafety

officers. The general guidelines provided here do not limit the ability of COMS to require additional biosafety practices for specific projects.

B. Assessment of Biosafety of Lentiviral Vectors

There are several factors that affect the biosafety of lentiviral vectors and these should be considered in determining what practices must be followed in using these agents.

1. Potential for Generation of Replication Competent Lentivirus (RCL). Reducing the potential for generation of RCL increases the safety of lentiviral vectors. Modifications to lentiviral vectors have been made which reduce the chance that RCL will be generated. Specific modifications that reduce the chance that RCL will be generated include the following:
    a. limited homology between vector and helper sequences
    b. separation of genes encoding the actual vector and packaging functions on multiple plasmids (e.g. 4 or more plasmids in third and greater generation lentiviral vectors)
    c. elimination of accessory genes (e.g. *tat*) from packaging plasmid
    d. use of self-inactivating vectors.
2. Generations: Lentiviral vectors are separated into several "generations" based on some of these characteristics. The characteristics that define each generation of lentiviral vector are:
    a. First generation: A LV packaging system that includes all HIV-1 genes except *env*.
    b. Second generation: A LV packaging system that lacks *env* and all auxiliary HIV-1 genes, i.e. *vpr*, *vif*, *vpu* and *nef*. Examples: pCMV-dR8.91, pCMV-dR8.74, psPAX2
    c. Third generation: A LV packaging system that includes only *gag*, *pol*, *rev* and a chimeric 5' LTR from HIV-1. A cDNA encoding *rev* is provided on a separate plasmid. A third generation packaging system offers maximal biosafety and involves the transfection of four different plasmids into the

producer cells. Removing part of the LTR creates a self-inactivating vector unable to drive unwanted expression of host genes. Example: pMDL g/p RRE + pRSV-Rev. Researchers are encouraged to work with third generation (i.e., four-plasmid) systems or higher.

3. Nature of Inserted Genes: Use of potentially hazardous genetic inserts (e.g. oncogenes) increases the risk associated with use of lentiviral vectors. A list of high-risk gene activities can be found in Section D, entitled "High Risk Gene Activity Examples."
4. Tropism: Tropism is not usually considered as part of the risk assessment because most commonly used lentiviral vectors are capable of infecting human cells.

C. Approach to Laboratory Biosafety with Lentiviral Vectors

The determination of appropriate biosafety practices for use of lentiviral vectors will require careful consideration of the factors above by the investigator, the biosafety officer and the COMS. Minimal biosafety levels will be assigned to lentiviral work as follows. However, specific projects may require additional containment or practices:

1. BL2 for all lentiviral vectors carrying low-risk transgenes.
2. BL2+ for all lentiviral vectors either carrying high-risk transgenes or affecting the expression of a high-risk transgene. High-risk genes are described in Section D.

D. High-Risk Gene Activity Examples (note: this list is not exhaustive, and other gene activity may be high-risk based on risk assessment)

1. Gene activities that may directly or indirectly result in oncogenesis. The most notable examples include expression of oncogenes and reduction of tumor suppressor function. Other examples include modification or alternative expression of genes associated with: cell cycle maintenance and DNA repair, apoptosis, transcription factors, epithelial to mesenchymal transition (EMT), angiogenesis, and cell-cell adhesion.

2. Genes encoding functional biological toxins. Please note: library screens may contain genes that fall into a high-risk category. If so, COMS may require the entire library to be handled at BL2+.

V. Policy Authority

The Office of Biological Safety of the Harvard Medical School is responsible for supporting the COMS. This includes preparation and revising of the COMS Policy Manual for committee review and approval. COMS authorizes this policy.

VI. Related Policies

A. COMS Policy on Recommended Containment Levels for Adenoviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits

B. COMS Policy on Recommended Containment Levels for Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits

C. Sharps Policy

VII. References

- Biosafety Considerations for Research with Lentiviral Vectors, Recombinant DNA Advisory Committee (RAC) Guidance Document. Accessed from the Office of Science Policy website on 6/14/18.
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)
- Lentiviral Vector Guidelines on the web:
  - University of Kentucky guidelines for research involving viral vectors