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Contact: COMS@hms.harvard.edu

Lentiviral Vector Policy

Purpose

The purpose of this policy is 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).

Scope

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).

Policy

1. General Information
 - a. 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.
2. Assessment of Biosafety of Lentiviral Vectors
 - a. There are several factors that affect the biosafety of lentiviral vectors, and these should be considered in determining which practices must be followed in using these agents.
 - i. *Potential for Generation of Replication Competent Lentivirus (RCL)*. Reducing the potential for the generation of RCL increases the safety of lentiviral vectors. Modifications to the design and construction of 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:
 - Limited homology between vector and helper sequences.
 - 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).

- Elimination of accessory genes (e.g., *tat*) from the packaging plasmid.
 - Use of self-inactivating (SIN) vectors that may contain an additional deletion in the 3'LTR.
- ii. *Generations.* Lentiviral vectors are separated into several “generations” based on some of these characteristics. The characteristics that define each generation of lentiviral vector are:
- First generation: A LV packaging system that includes all HIV-1 genes except *env*.
 - 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.
 - 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 (SIN) vector unable to drive unwanted expression of host genes. Example: pMDL g/p RRE + pRSV-Rev.
 - Fourth generation: This system divides the viral genome into five plasmids, increasing the number of recombination events required to generate RCL. High expression of essential viral components is driven by the Tet-Off and Tat transactivators. The *pol* gene is fused to *vpr* to ensure transport of the reverse transcriptase/integrase protein into the recombinant viral particle. Third-generation systems do not contain separate *gag* and *pol* sequences.

Researchers are strongly encouraged to work with third or fourth generation (i.e., four or five-plasmid) systems or higher.

- iii. *Nature of Inserted Genes:* Expression of potentially hazardous genetic inserts (e.g., oncogenes or shRNAs targeting tumor suppressors) increases the risk associated with use of lentiviral vectors. A list of high-risk gene activities can be found in Section 4, 'High Risk Gene Activity Examples.'
- iv. *Tropism.* Tropism (infection of specific cell types) may be determined by pseudotyping.
- VSV-G lentiviral packaging—produces VSV-G pseudotyped lentivirus, which readily infects virtually all types of cells.
 - Ecotropic lentiviral packaging—produces lentivirus pseudotyped with the MLV ecotropic envelope glycoprotein, which limits transduction to mouse and rat cells.

- v. *Scale of Vector Generation.* Manipulation of higher-titer virus preparations in larger volumes is a higher risk activity from a biosafety standpoint. The implementation of BL2 measures is adequate for the production or handling of most replication –defective lentiviral vectors, unless large volumes are exceeded.
- 3. Approach to Laboratory Biosafety with Lentiviral Vectors
 - a. 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:
 - i. BL2 for all lentiviral vectors carrying low-risk transgenes.
 - ii. BL2+ for certain lentiviral vectors encoding for high-risk gene function or affecting the expression of a high-risk gene, as described in Section 4.
- 4. High-Risk Gene Activity Examples (note: this list is not exhaustive—the assessment of whether transgene activity may be identified as high risk should consider the functional outcome of transgene expression within the biological constraints of the research described. This means that viral vector tropism, transgene promoter function and tropism, scale of vector use, and expected routes of entry for exposures based on research activities should be considered).
 - a. Transgene activities that may directly or indirectly result in oncogenesis are considered high risk. The most notable examples include expression of oncogenes or reduction of tumor suppressor function. If the oncogenic activity is specific to a certain cell type, risk assessments should consider the potential hazard for that cell type upon human exposure in the laboratory. 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, or cell-cell adhesion. In the case of library screens with lentiviral vectors encoding transgenes or guide RNAs of unknown function or targets, only a small subset of the transgenes or guide RNAs encoded by the library, and carried by individual lentivirus particles, will involve overexpression of oncogenes or the targeting of genes encoding known or potential tumor suppressors. These library screens may be handled at BL2.
 - b. Genes encoding functional biological toxins.

Definitions

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.

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.

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. Tropisms, in which particular tissues may be expressed, may lead to the committee deciding that BL2+ containment is not necessary.

Related Materials/References

- COMS Policy on Recommended Containment Levels for Adenoviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits
- COMS Policy on Recommended Containment Levels for Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits
- [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 \(NIH Guidelines\)](#)
- [Viral Vector Biosafety in Laboratory Animal Research](#)
- Lentiviral Vector Guidelines on the web:
 - [University of Kentucky guidelines for research involving viral vectors](#)
 - [Stanford University guidelines](#) for viral vectors
 - [ABSA Lentivirus Fact Sheet](#)