LENTIVIRUS VECTOR FACT SHEET

LENTIVIRUSES:

Lentiviral vector constructs are derived from HIV and are therefore highly efficient vehicles for *in vivo* gene delivery. Use of these vector systems is particularly desirable because of their ability to integrate transgenes into dividing, as well as, non-dividing cells. However, there are risks associated with working with lentiviral vectors and these must be carefully evaluated. The two major risks are: 1) the potential generation of replication competent virus; and 2) the potential for oncogenesis through insertional mutagenesis. These risks are largely based upon the vector system used and the transgene insert encoded by the vector. Therefore, it is imperative that prior to utilizing a lentiviral vector system, a risk assessment must be completed and documented. Also, because construction and/or use of lentiviral vectors falls under the definition of rDNA research as outlined in the NIH Recombinant DNA Guidelines, possession must be reported in the workplace's biological agent inventory and experiments must be submitted for review and approval by the site IBC prior to initiation. Typically, lentiviral vectors may be safely handled using either BSL-2 or BSL-2 enhanced controls depending upon the risk assessment.

LENTIVIRAL VECTORS:

Lentiviruses can infect not only dividing, but also non-dividing host cells, because their pre-integration complex (virus "shell") can get through the intact membrane of the nucleus of the target cell. Lentiviral vectors can be used to provide highly effective gene therapy as they can provide long-term expression of the vectored transgene in target cells.

First-generation lentiviral vectors were manufactured using a single vector packaging system that contained all HIV genes, except the envelope (*env*) gene, in one plasmid.

In the second-generation system, five of nine HIV-1 genes were eliminated, leaving the two genes which encode structural and enzymatic components, and the two genes for transcriptional and post-transcriptional functions.

A four plasmid vector system is used in a third generation lentiviral vector. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 containing the vector genome plus transgene), the third generation lentiviral vector system offers advantages over the previous generations because the number of recombination events required to form a complete replication-competent virus increases, thereby reducing the possibility of making a replication-competent viral particle.

CONTAINMENT LEVELS:

The Principle Investigator shall conduct a proper risk assessment when working with lentiviral vectors. This includes considering the following to determine the appropriate biosafety containment level:

- The nature of the vector system (e.g. generation and pseudotype – VSV-G or other envelope gene) and the potential for generation of replication-competent virus from the vector components
- The nature of the transgene insert (e.g., known or potential oncogenes, immunoregulatory genes, metabolic genes)
- The nature of the recipient/ host (e.g., susceptibility, pathogenicity, modification of host range)
- The vector titer and the total amount of vector
- The exposure potential for HIV positive individuals whose native virus may recombine with or complement the vector (this is more of a concern with earlier generation lentiviral vectors)
- The inherent biological containment of the animal host, if relevant (e.g., can the native lentivirus replicate in the animal)

- The potential risk of insertional mutagenesis as a result of an exposure incident

Based upon the Risk Assessment factors, a decision to use BSL-2 (Biological Safety Level 2) or BSL-2+ (enhanced BSL-2 containment including BSL-3 work practices and personal protective equipment) is generally appropriate in the laboratory setting for lentiviral vectors.

Enhanced BSL-2 containment includes:

- Discussion with Employee Health Management to determine if medical surveillance is needed.
- Specific training in the handling of lentiviral vectors and a proficiency test provided by the Principal Investigator (PI).
- Solid front, back closure or "wrap around" gown usage, preferably disposable.
- Gloves (double gloves should be considered) used at all times while handling viral samples.
- Gloves must be pulled over the cuffs of disposable clothing. Sleeves may be used to ensure wrists are covered.
- Containment recommended for all manipulations of lentiviral vectors (e.g., a BSC or centrifuge containment devices).
- Mucous membrane protection for occasional applications (e.g., FAC Sorters, column purification, and certain animal work) which is not conducive to containment
- Strict attention to surface/equipment decontamination and handwashing.
- Elimination of sharps or strict attention to the use of safety sharps and safety devices, such as sharps containers with roller top lids that prevent overfilling of containers and reduce needlestick injuries.
- All work must be conducted in a lab with limited access.
- All animal work must be carried out initially at Animal Biosafety Level 2 (ABSL-2); NIH guidance allows reduction to ABSL-1 for mice after 1-7 days and a cage changing.

BIOSAFETY NOTIFICATION:

Work involving use of lentiviral vectors, **including those purchased in cloning kits**, constitutes recombinant DNA (rDNA) experimentation. Therefore, these activities fall under the definition of rDNA research as outlined in the NIH Recombinant DNA Guidelines. Possession of lentiviral vectors must be reported in the workplace's biological agent inventory. Experiments must be submitted for review and approval by the site Institutional Biosafety Committee (IBC) **before** being conducted.

FACILITY:

The laboratory must contain a biosafety cabinet (BSC) and centrifuges must be equipped with bioaerosol containment devices. It is preferable this be an inner lab with multiple doors between the BSC and hallway. Air should flow from the hallway to this lab (negative to the hallway).