Third Generation Recombinant Lentiviral Vectors: Lowering Biocontainment for Oncogenic Inserts

Recently, the UW Institutional Biosafety Committee (IBC) voted to lower the biocontainment level for third generation lentiviral vectors with oncogenic inserts. The Viral Vector Subcommittee determined that third generation lentiviral vectors are essentially incapable of generating replication-competent virus. Therefore, testing for replication competent virus (RCV testing) will no longer be used to lower biocontainment for research with third generation lentiviral vectors that express certain hazardous genes (e.g. oncogenes and tumor suppressor gene knockouts).

For this specific class of lentiviral vector with oncogenic inserts, work can now be approved at biosafety level 2 (BSL-2 for laboratory research and ABSL-2 for animal research) provided the researcher can verify the lentiviral vector is a third generation vector system.

What is a Third Generation Lentiviral Vector? For the purpose of lowering containment, third generation lentiviral vectors must meet all of the following conditions:

1) Vectors containing less than 2/3 of a lentiviral genome

2) Pseudo typed with the vesicular stomatitis virus G (VSV-G) protein or similar non-HIV envelope protein

3) Packaging system composed of a gene delivery vector plasmid and 3 or more additional packaging plasmids **

4) A vector genome with self-inactivating (internally deleting) long terminal repeats (aka SIN LTR's) or similar means of deleting essential portions of the viral LTRs **

**See the diagram on page 2.

Verification: The Principal Investigator must provide documentation that describes how the proposed vector system meets the definition of a third generation lentiviral vector described above. This may include:

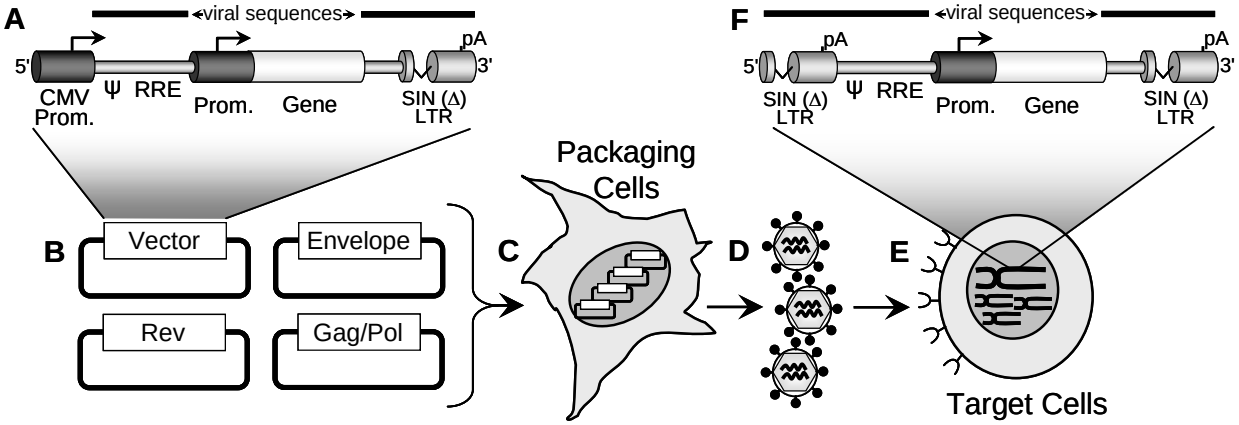
- Description from a vendor catalog
- Restriction mapping or sequencing data as annotated plasmid map
- Reference from the scientific literature

How Do I Request Lowering Containment? To request lowering containment of 3rd generation recombinant lentiviral vectors with oncogenic inserts from BSL-2 with 3 practices to BSL-2:

1) For an existing Biological Use Authorization (BUA), submit a Request for Change to BUA Application with documentation that shows the viral vector meets all four required conditions. For new and renewal applications, submit supporting documentation with the full application and state request to lower containment.
2) Obtain IBC approval. The request to lower containment will be reviewed and approved at the monthly IBC meeting. If approved, an updated BUA letter will be issued.

Questions? Contact EH&S Research & Occupational Safety at ehsbio@uw.edu or 206.221.7770.

Basic components of lentiviral vectors: The basic components of a lentiviral vector system are outlined in the following figure. (Figure courtesy of David Emery, PhD, former UW IBC Chair)



Panel A – Structure of a typical third generation lentiviral vector with self-inactivating long terminal repeats (aka SIN LTR's).

Panel B – The plasmid encoding the vector genome is combined with 3 additional packaging plasmids expressing the viral genes deleted from the vector backbone.

Panel C – Vector plasmid and packaging plasmids are introduced into the packaging cell line.

Panel D – Viral particles are collected from the culture media in which the plasmid-transfected packaging cells are grown (often referred to as viral supernatant).

Panel E – Shows that viral particles can be used to transfer the viral vector genome to target cells.

Panel F – Structure of the vector provirus following chromosomal integration.

Safety features of third generation lentiviral vector packaging systems:

1) The packaging system is completely devoid of the HIV envelope gene (*env*), making it impossible to regenerate wild type HIV from the packaging system due to the critical role of this gene in the virus life cycle.

2) The vector genome is completely devoid of intact viral long-terminal repeat (LTR) sequences through the use of self-inactivating (SIN) LTRs, making it impossible to regenerate a functional lentiviral genome from the packaging system due to the critical role of these LTR sequences in the virus life cycle.

3) The sequences encoding the genes for the packaging system are split into three or more independent plasmids with very little homology overlap, so that the chances of multiple independent recombination events leading to rejoining of all sequences is vanishingly small.

4) The vector system has been studied extensively for the appearance of wild-type and atypical replication-competent virus, with no evidence of such virus arising (for example, see Cornetta K *et al.* Mol Ther. 19:557-566, PMID: 2011). Further, the literature has no reports of such vectors giving rise to replication-competent virus, despite the very strong incentive to publish such a finding.