

Lentivirus vector stocks generated with 2<sup>nd</sup> generation packaging systems AND transfer plasmids with wild type LTRs (i.e., not self-inactivating transfer plasmid constructs) must be tested for replication competent virus (RCV) by serial transfer in a cell line documented to be capable of propagating wild type HIV if:

- 1) The PI proposes to reduce biocontainment after target cell transduction,
- 2) The PI proposes to transduce mammalian cells capable of supporting lentiviral replication, introducing the cells into animals (e.g., xenografts) and maintain the animals at ABSL1, or
- 3) The PI proposes to introduce lentiviral vectors or transduced mammalian cells capable of supporting lentiviral replication into animals that could support viral replication.

Testing should be conducted on the initial preparations of viral stocks.

Lentivirus vector stocks generated with 3<sup>rd</sup> generation (4 plasmid, including self-inactivating transfer vectors) or with 2<sup>nd</sup> generation packaging systems and 3<sup>rd</sup> generation transfer vectors (SIN) are exempt from these requirements. However, if the PI proposes to introduce lentiviral vectors, or transduced mammalian cells capable of supporting lentiviral replication, into animals that could support viral replication, then the PI should provide a complete risk assessment to the IBC.

If none of these criteria apply RCV testing will not typically be required. **However, the Institutional Biosafety Committee (IBC) may determine that, following the risk assessment, RCV testing is still required.**

The vector stock should be tested for RCV at a limit of sensitivity of 1 infectious unit per milliliter (mL). Several sensitive RCV assays have been described, including an ELISA-based p24 Gag antigen assay, a product enhanced reverse transcriptase (PERT) assay that involves the vector's reverse transcriptase, a PCR-based assay that detects Psi-Gag sequences from a recombination event between vector and packaging constructs, and PCR-based assays to detect the VSV-G Env used for pseudotyping. All represent acceptable methods for RCV testing. The protocol must be provided to the IBC for approval.