DARTMOUTH
ENVIRONMENTAL HEALTH & SAFETY
Biological Safety Program

Policy 111: Replication Competency Testing of Retroviral and Lentiviral Vectors

I. PURPOSE

Retroviral and lentiviral vectors are routinely used in biomedical research given their ability to infect dividing and non-dividing cells, transduce with high efficiency, and stably express genetic elements of interest in target cells. Despite their utility and ubiquitous use, many biosafety concerns should be considered during the experimental design and use of these viral vectors. In order to minimize the risk associated with these vectors, the IBC has adopted the following policy for safety and consistency.

II. BACKGROUND

According to Recombinant DNA Advisory Committee (RAC) Guidance Document, "Biosafety Considerations for Research with Lentiviral Vectors", the major risks associated with research involving lentiviral or retroviral vectors are:

i. the potential for generation of replication competent lentiviruses (RCL) or replication competent retroviruses (RCR), and

ii. potential for oncogenesis through insertional mutagenesis or containing an oncogene.

The probability of RCL or RCR formation is higher in earlier generation vector systems because fewer recombination events are needed to generate a virus that has regained sequences required for replication. These recombination events may occur in the packaging cells (i.e. HEK293), in target cells, or in lab animals. The probability of RCL or RCR is significantly increased in the presence of wild-type lentivirus or retrovirus.

The risk of RCL or RCR may be lowered by the nature of the vector system. For example, 3rd generation (and later) packaging systems have incorporated increased safety features to reduce the probability for RCL/RCR formation. However, risk of potential oncogenesis may be elevated by the nature of the inserted transgene.

III. DEFINITIONS

2nd generation packaging system: Second generation transfer vectors may contain *tat*, and the packaging vectors contain *rev*, *gag*, *pol*. Together with the envelope plasmid (i.e., pVSV-g), this results in a three plasmid system.

3rd generation packaging system: Third generation transfer vectors contain self-inactivating sequences (SIN) and do not express *tat*. *Rev* is separated from *gag*/*pol* onto different vectors for packaging, resulting in a four plasmid system.

Note: 3rd generation transfer vectors may be packaged with 2nd generation packaging systems; however, these viral vectors have the same potential for RCL as a 2nd generation system.

Oncogene: A gene that contributes to the development of a cancer through the dysregulation of cell growth, cell division, or cell death. This function must be supported by multiple studies from multiple laboratories. A list of common oncogenes can be found here: https://www.cancerquest.org/cancer-biology/cancer-genes

Proto-oncogene: The wild-type expression or regulation of an oncogene.

Tumor suppressor genes: Genes that function to suppress or block tumor formation or growth based on multiple studies from multiple laboratories. A list of common tumor suppressors can be found here: https://www.cancerquest.org/cancer-biology/cancer-genes

IV. RCL/RCR TESTING REQUIREMENTS

A. RCL/RCR testing is not generally required, if all four of the following are true:

i. Experiments are conducted entirely in tissue culture.

ii. Less than 100 mL of supernatant is produced.

iii. The transgene is not an oncogene, proto-oncogene, shRNA or CRISPR/Cas sequence to a tumor suppressor, and does not code for a potential toxin.

iv. A third generation SIN (self-inactivating) commercially available lentiviral system is used with a third generation or later packaging system (total 4 plasmid system) according to manufacturer's specifications.

NOTE: Testing may be still required at the discretion of the IBC after conducting a full risk assessment of experimental procedures.

B. RCL/RCR Testing IS required for second-generation lentiviral systems.

C. RCL/RCR Testing may be required at the discretion of the IBC, if any of the following are true:

i. A third generation system is used in the presence of wild-type lentivirus or retrovirus.

ii. Greater than 100 mL of supernatant is produced.

iii. The transgene is an oncogene, proto-oncogene, shRNA or CRISPR/Cas sequence to a tumor suppressor, or codes for a potential toxin.

iv. The viral vector will be used in animals.

D. Testing Procedures

i. Testing should be conducted with every new batch of viral production or every 6 months.

ii. Confirmation of the absence of RCL or RCR must be documented by the researcher prior to use in animals.

iii. Documentation of methodology and results must be available to the Dartmouth Biosafety Officer (BSO) upon request.

iv. Researchers will destroy all batches in which replication competent virus is detected and will notify the BSO within one business day of competent virus detection.

v. A current procedure of demonstrated sensitivity and specificity must be used for RCL/RCR testing.

vi. A positive control is required. However, working with infectious HIV-1 when testing for RCL would not be appropriate. A standard p24 ELISA kit with a sensitivity of ≤12.5 pg/ml is recommended. Acceptable kits can be obtained from Cell Biolabs or Perkin Elmer.

V. RESOURCES

- *Biosafety Considerations for Research with Lentiviral Vectors*. National Institutes of Health Recombinant DNA Advisory Committee 2006 https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf

- Sastry L and Cornetta K. *Detection of replication competent retrovirus and lentivirus*. Methods Mol Biol 2009; 506:243-63

- Sastry L, Xu Y, Johnson T, Desai K, Rissing D, Marsh J, Cornetta K. *Certification assays for HIV-1-based vectors: frequent passage of gag sequences without evidence of replication-competent viruses*. Molecular Therapy 2003; 8(5):830–9

- Skrdlant LM, Armstrong RJ, Keidaisch BM, Lorente MF, DiGiusto DL. *Detection of Replication-Competent Lentivirus Using a qPCR Assay for VSV-G*. Mol Ther Methods Clin Dev. 2017; 8:1-7

- Policy 110: Viral Vectors. Dartmouth IBC 2022 http://www.dartmouth.edu/~ehs/biological/policies_sops.html

- *Testing for Replication Competent Retrovirus (RCR)/Lentivirus (RCL) in Retroviral and Lentiviral Vector Based Gene Therapy Products — Revisiting Current FDA Recommendations*. Food and Drug Administration 2010 https://sites.duke.edu/dvvc/files/2016/05/FDA-recommendation-for-RCR-testing.pdf