Testing of Lentiviral Vector Stocks for Replication Competent Particles

Scope/Summary: This policy outlines the requirements for testing of lentiviral vector stocks for replication competent particles at MIT.

Background: The CAB/ESCRO recognizes the enhanced safety features embedded in the latest lentiviral vector systems. These safety features, the use of multiple plasmids for vector packaging, the deletion of overlapping sequences between the packaging plasmids plus the minimalist design of the vector backbone itself, are all aimed at reducing the risk of generation of replication competent virus during vector stock preparation. Publications, along with data and personal communication from various investigators developing and using these plasmid systems indicate that detection of replication competent virus is difficult to none detected[i]. Despite the improvements in lentiviral vector generations, there is the theoretical risk of generation of replication competent particles.

I. Testing requirements:

- Lentiviral vector particles generated from 2nd generation lentiviral (using 3 plasmids) systems need to be tested for replication competent particles as described below.
- Lentiviral vector particles generated from 3rd generation lentiviral systems or higher (using 4 plasmids and that are "self inactivating") do not require testing for replication competent particles.
- These requirements also apply when obtaining lentiviral vectors from Core facilities or other laboratories.

II. Testing procedures and frequency:

- Investigators should select and use one assay for replication competent viral contaminants and show that the assay works and is sensitive. This assay should be one that the PI is familiar with and able to do on a relatively routine basis. Contact the Biosafety Program for advice on available testing procedures.
- Once researchers have gained enough experience with these vectors, and these vectors work in their lab as expected (based on the results from two vector preps), researchers will not be required to test every vector preparation. Depending on the frequency that vector preps are generated within a group, vector preps should be tested on a periodic basis. Some groups will generate and use multiple preps a week, others will be making far fewer. Laboratories that generate multiple vector preps a month should test 3 representative preps every 2-3 months. Low frequency (low number of preps generated) labs should test 2 vector preps every 4-6 months. A low frequency lab generates and uses up a prep approximately every month or so. This is difficult to specify but the committee is asking that about 1 in every three preps be tested. This will provide "ongoing" testing but will not require that every prep be tested as long as the investigators are able to show that they are able to generate at least two consecutive independent preps with no detectable rep+ virus.
- The CAB/ESCRO requires that the data on detection of rep+ viral particles be made available upon request.

References:

[i] From personal communication, Prof. Inder Verma, Salk Institute

Revision History:

Revised and approved 3/15/2018