FAQs on Working with Lentiviral Vector Systems

Q1. What are lentiviral vector systems?

Lentiviral vectors are derived from the human immunodeficiency virus (HIV-1) and are efficient tools for gene delivery into cells, by harnessing their ability to integrate transgenes into dividing and non-dividing cells both *in vitro* and *in vivo*.

To increase the safety of lentivirus, the components necessary for virus production are split across multiple plasmids. Lentiviral vector systems comprise components of multiple plasmids as listed follows:

1. Lentiviral transfer plasmid encoding your insert of interest
2. Packaging plasmid(s)
3. Envelope plasmid

Q2. What are generations of lentivirus vectors and how do I determine the generations of the lentivirus vectors?

Lentivirus vectors have progressed over time with features to achieve higher efficiency and biosafety:

- ***1st Generation*** is composed of all HIV genes minus the envelope protein.
- ***2nd Generation*** has 5 of the 9 genes deleted leaving only gag/pol which encode structural and enzymatic components and the tat/rev genes for transcriptional and post-transcriptional functions.
- ***3rd Generation*** packaging systems contain only gag, pol, and rev genes. It utilizes a chimeric 5' LTR to ensure transcription in the absence of Tat.

In Singapore, the use of lentivirus vector systems is governed by Ministry of Health (MOH) under the Biological Agents and Toxin Act (BATA). Under the BATA list, HIV lentivirus vectors are classified as "advanced" generation under Fourth Schedule biological agents with risk equivalent to a Risk Group 2 biological agent, or "non-advanced" generation lentivirus vectors under First Schedule Part I biological agents with risk higher than Risk Group 2.

Q3. What is the definition of advanced generation lentiviral vectors under BATA?

Under the Fourth Schedule of BATA list, advanced generation refers to any HIV lentiviral vector system which has at least 2 of the following features:

I. the U3 region of the 3'LTR in the transfer vector is absent or altered, which results in a stable self-inactivating (SIN) configuration;

II. the HIV genes for packaging function are split to a minimum of 2 packaging plasmids (excluding the env plasmid);
III. the vpr, vpu, vif and nef genes are either absent or altered to be non-functional
IV. the vector system requires minimally 4- recombination to achieve Replication Competent Lentivirus (RCL)

If the HIV lentiviral vector does not satisfy the above given definition, it could possibly be a "non-advanced" generation lentiviral vector. The possession and use of all lentiviral vectors shall comply with the BATA requirements corresponding to their respective Schedules.

Q4. What approvals do I need for non-advanced and advanced generation lentiviral vector system?

The approvals required are as follows:

1. ***Non-advanced generation lentiviral vector system***
   Principal Investigators shall obtain prior approvals from NUS Institutional Biosafety Committee (IBC), Genetic Modification Advisory Committee (GMAC), and Ministry of Ministry of Health (MOH) before possession and use of the non-advanced generation lentivirus vector systems.
2. ***Advanced generation lentiviral vector system***
   Principal Investigators shall obtain prior approvals from IBC for first-time users of advanced generation lentiviral vectors system, and GMAC prior to import and/or if the experiments are not exempt from the guidelines according to The Singapore Biosafety Guidelines for Research on Genetically Modified Organisms.

Refer to NUS Laboratory Biorisk Management Manual, Section 9.1.4 on Lentiviral Vectors, for detailed description and steps to obtain approvals required for working with lentiviral vectors.

Q5. What are the risks associated with the use of lentiviral vectors?

The two main risks are:

1. Potential generation of replication-competent lentivirus (dependent on nature of lentiviral vector system)
2. Potential for oncogenesis through insertional mutagenesis (dependent on nature of transgene)

The Principal Investigator shall conduct risk assessment on the use of lentiviral vectors, which shall take into consideration the following:

1. The nature of the vector system and the potential for generation of replication-competent virus from the vector components
2. The nature of the transgene insert (e.g. known or potential oncogenes, etc.)
3. The nature of the recipient / host (e.g. susceptibility, pathogenicity, modification of host range)
4. The vector titer and the total amount of vector
5. The exposure potential for HIV positive individuals whose native virus may recombine with or complement the vector (risk associated with earlier generation lentiviral vectors)
6. The inherent biological containment of the animal host, if relevant (e.g. can the native lentivirus replicate in the animal)
7. The potential risk of insertional mutagenesis as a result of an exposure incident

Principal Investigators and researchers shall refer to Section 9.1.4 on Lentiviral Vectors in the NUS Laboratory Biorisk Management Manual for detailed requirements and precautions when working with lentiviral vectors.

Q6. How are replication-defective lentiviral vectors safer than replication-competent lentiviral vectors?
Replication-competent lentiviral vectors contain the necessary genes for virion synthesis and are capable of propagating themselves after the initial transduction. To improve safety, higher generation systems comprise transfer vectors that are all replication incompetent and may additionally contain a deletion in the 3'LTR, rendering the virus "self-inactivating" (SIN) after integration.

Q7. What are the potential routes of lentivirus exposure?
Potential routes of lentivirus exposure are:

1. Percutaneous (skin) exposure from needlestick injury or scratch
2. Mucous membrane exposure (eyes, nose, mouth) from splash or aerosols

Q8. Are there any pre-exposure prophylaxis/vaccination requirements?
Currently there are no pre-exposure requirements for lentiviral work. If you have any health concerns, contact NUS Occupational Health Clinic (oh.nurse@nus.edu.sg / 65167333) to arrange for a consultation.

Q9. What should I do if I am exposed to lentivirus particles (post-exposure response)?
Immediately wash the area of exposure thoroughly with soap and water. For splashes onto mucous membrane, rinse gently and thoroughly wash with water. Inform your Academic Supervisor and seek treatment at the University Health Centre (weekdays from 8.30am to 5.30pm, Tel: 6516 7333). After office hours, proceed to the National University Hospital's Accident & Emergency.

Refer to Chapter 10 on Emergency Response due to Exposure to Potentially Infectious Materials in the NUS Laboratory Biorisk Management Manual for further details.

References:

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4. About Lentivirus. (n.d.). In *UCSF Viracore*. Retrieved 12 Oct 2016, from http://viracore.ucsf.edu/tidbits-of-lenti
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