MANUAL FOR THE USE OF LENTIVIRUS VECTORS IN IMR, NIH

IMR Institutional Biosafety and Biosecurity

1.0. Purpose of this Manual

This manual provides information about the use of the lentivirus vectors in Institute for Medical Research (IMR). The detailed standard procedure on its use will be provided by the principal investigator (PI). The lentivirus vectors will be used for production of precursor clones either as pooled or ready-to-infect, or pseudotyped lentiviral preparation. For more detailed information regarding the individual clones that comprise the library, the PI will refer to the user manual for the product line. PI is advised to read this manual before using reagent and associated materials provided in the commercial kit. PI is also advised to keep inventory on clones produced during the study.

2.0. Intended use of lentivirus vectors

2.1 The lentivirus vectors will contain a pool of precursor clones in a ready-to-infect lentivirus preparation.

2.2 PI should be aware that each virus within the pool will express an individual clone to ensure biologically relevant interactions with endogenous processing machinery and regulatory partners.

2.3 PI must prepare and take note and make inventory of the details of the clone construction and preparation of the virus pool.

2.4 As positive control virus is usually included in the kit, PI will assess transduction efficiency in the cells of interest.

2.5 PI must design the oligonucleotide primers that may be used to identify the sequence responsible for the observed phenotypes.

2.6 The lentivirus library is a tool that enables the study of phenotypic effects associated with the over-expression of individual clone.

2.7 In certain kit, the lentivirus preparation is a pseudotyped clone that allows for broad cellular tropism. Cellular tropism is possible in hard-to-transfect mammalian cell lines, primary cells, non-dividing cells and even whole animal studies.

2.8 PI should isolate by selection or sorting of the transduced cells exhibiting the phenotypes of interest.

2.9 The generated phenotypes of interest may be recovered through simple genomic PCR using lentivirus vector-specific primers followed by direct sequencing of clones.

3.0. General considerations:

3.1 The transduction efficiency of the lentivirus library varies significantly for different cells and experimental conditions.

3.2 Determine the optimal number of transducing units per cell.

3.3 Measure the expression of the clones after transduction.

3.4 Determine the transient versus stable screen.

3.5 Biosafety considerations. Although these viral particles are replication-incompetent, they can infect mammalian cells and integrate into the host cell genome. Please follow IMR Biosafety Manual, Biosafety Act 2007, WHO regulations for working with BSL-2 class viruses.

4.0 Biosafety Information

Commonly the expression of lentivectors together with the packaging plasmids comprise the third-generation lentiviral expression system. The human immunodeficiency virus (HIV)-based lentivectors are based on the vectors developed for gene therapy applications by Dr. J. G. Sodroski (US patent #5,665,577 and # 5,981,276).

Both feline immunodeficiency virus (FIV)-based and HIV-based lentivector systems were designed to maximize their biosafety features, which included:

i. A deletion in the upper region will ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.

ii. The Rous Sarcoma Virus (RSV) promoter (in HIV-based vectors) and human cytomegalovirus (CMV) promoter (in FIV-based vectors) upstream of 5'LTR in the lentivector allow efficient Tat-independent production of viral RNA, has an effect on the number of genes from HIV-1 that will be used in any system.

iii. Number of lentiviral genes necessary for packaging, replication and transduction is commonly reduced to three (*gag, pol, rev*), and the

corresponding proteins are expressed from different plasmids (for HIV-based packaging plasmids) lacking packaging signals and share no significant homology to any of the expression lentivectors, or any other vector, to prevent generation of recombinant replication-competent virus.

iv. None of the HIV-1 genes (*gag, pol, rev*) will be present in the packaged viral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.

v. Pseudoviral particles will carry only a copy of the expression construct. Despite the above safety features, use of lentivectors falls within Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self replicating virus, or the possibility of insertional mutagenesis. It is also important to check with the health and safety guidelines at IMR regarding the use of lentiviruses and always follow standard microbiological practices;

vi. Wear gloves and laboratory coat all the time when conducting the procedure.

vii. Always work with pseudoviral particles in a Class II Biosafety Cabinet.

viii. All procedures are performed carefully to minimize the creation of splashes or aerosols.

ix. Work surfaces are decontaminated at least once a day and after any spill of viable material.

x. All cultures, stocks, and other regulated wastes are decontaminated before disposal by autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leak proof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

xi. Lentivirus vectors are integrated into genomic DNA and could have a risk of insertional mutagenesis.

5.0. Training for Personnel

5.1 PI and personnel involved in the study must undergo training on usage, storage and disposal of lentivirus vectors and libraries.

5.2 PI and personnel involved in the study must be trained in standard microbiological methods.

5.3 The training record must be kept and updated annually during the duration of the study.

6.0. Medical Surveillance of Personnel

It is the responsibility of the PI and personnel to report any health events or changes in a biologic function of PI and personnel to OSH unit. (Office number 03-26162493/2509)

Reference:

Poeschla, E.M., Looney, D.J., and Wong-Staal, F. (2003) Lentiviral nucleic acids and uses thereof. US Patent NO. 6,555,107 B2