

Division of Biosafety and Biotechnology

Construction of a chimeric vector using Human and Simian immunodeficiency viruses

1. Aim of the project

Lentiviral vectors have the ability to efficiently transduce non-dividing cells and many Human Immunodeficiency Virus (HIV)-based vectors have already been developed for gene therapy. However, concerns regarding safety in humans still exist and novel “safer” lentiviral vectors are continuously designed. One of the main safety concerns arises from the possibility of recombination during vector production (between vector plasmids), which may give rise to infectious replication-competent lentiviruses (RCLs).

To develop a “safer” viral vector system for gene therapy by curtailing potential recombination events, the goal of this project is to construct a vector system that is based on two different lentiviruses: a Human Immunodeficiency Virus-derived genome (HIV-1) expressing the Green Fluorescent Protein (GFP) is encapsidated by Simian Immunodeficiency Virus (SIV) core particles, which are pseudotyped with the Vesicular Stomatitis Virus (VSV) glycoprotein G. HIV and SIV are phylogenetically closed enough to ensure a correct encapsidation of an HIV-derived genome by SIV proteins and such SIV/HIV chimeric vectors offer greater safety since the lack of homology between the transfer vector and the packaging construct eliminates the potential for homologous recombination during vector production. The pseudotyping with VSV glycoprotein G enhances the host spectrum and allows the transfection of a large variety of cell types (stem, dendritic and T cells, neurons).

2. Vector/plasmid

The production of recombinant viruses (HIV/SIV/VSV) requires the transfection of cell culture with three distinct plasmids:

- The Human Immunodeficiency Virus (HIV)-based transfer vector: HIV belongs to the genus *Lentivirus* of the *Retroviridae* family and is responsible for the acquired immunodeficiency syndrome (AIDS) in human. The parental virus, not infectious by air, belongs to the class of risk 3 for human. For this application, some important virulence factors are deleted from the HIV genome. These viruses are therefore unable to replicate and to propagate themselves. They are classified in class of risk 2 for human.
- The Simian Immunodeficiency Virus (SIV)-based packaging construct: The packaging plasmid is the backbone of the virus system and contains all the elements required for vector packaging. SIV also belongs to the genus *Lentivirus* of the *Retroviridae* family and is responsible for AIDS-like disease in some non-African primates, such as rhesus monkeys (*Macaca mulatta*). This virus, not infectious by air, belongs to the class of risk 3 for human and animals. For this application, SIV is derived from SIVmac1A11, which corresponds to an attenuated strain of SIV adapted for growth in human cells. This virus belongs to the class of risk 2 for human and animals.
- The VSV-G-expressing construct: This construct allows replacement of the natural HIV envelope gene with the Vesicular Stomatitis Virus glycoprotein (VSV-G) gene, which greatly broadens the host range of the lentiviral vector. The parental virus VSV belongs to the class of risk 2 for human and the class of risk 3 for animals.

None of the plasmids are, by themselves, capable of functioning as autonomous viruses.



3. Insert

The HIV-based transfer vector is engineered to express the Green Fluorescent Protein (GFP). The donor organism of this fluorescent marker-protein corresponds to the jellyfish *Aequorea victoria*, which belongs to class of risk 1. The nature of this insert is unlikely to alter the class of risk of the transfer vector.

4. Receptor organism

HIV/SIV/VSV recombinant vectors were produced in COS cells (immortalized kidney cell line from Green African Monkeys) after transfection of the three plasmids described above. For more information on the risk assessment of animal cell cultures, see Pauwels *et al.*, 2007.

5. Biological hazards and/or considerations related to the manipulation of the resulting GMO

The potential safety of the produced vector particles containing GFP insert is based on:

- the existence of low nucleotide sequence homology between HIV-1 and SIVmac, diminishing the possibility of RCL production;
- the fact that the packaging construct is derived from a non-virulent SIVmac isolate;
- the fact that most necessary genes involved in HIV virulence have been removed;
- the fact that the nature of the insert confer no hazardous properties.

However, the resulting vector particles are:

- still able to transduce human cells. Within this respect, it has to be noted that the use of the heterologous coat protein VSV-G protein broadens the host cell and tissue tropism of this vector;
- still able to integrate into the chromosomes of human cells: risks of insertional mutagenesis still remain;
- theoretically still prone to mobilization upon inadvertent infection of the producer cell line with wild type virus.

6. Class of risk of the resulting GMO

The vector producing cells (COS cells) and the non-replicative recombinant lentiviral vectors produced in this activity belong to class of risk 2.

7. Class of risk of the activity

This activity involves the culture and production (small scale) of non-replicative lentiviral vectors. This activity is of class of risk 2.

8. Recommended containment measures

The manipulation and production of this non-replicative lentiviral vector expressing gene products that are not intrinsically harmful or do not induce hazardous properties in the transduced cells require a Biosafety Level 2 (BSL-2). Any manipulation involving cell cultures and recombinant viral vectors should be handled in a biosafety cabinet class II.

In the laboratory, the vector producing cell cultures, materials derived from these cultures, and all equipment and devices coming into direct contact with any of these materials are potentially contaminated with lentiviral vector particles.

- The skin (especially when scratches, cuts or other lesions are present) and mucous membranes of the eye, nose, and mouth should be considered as potential pathways for entry of these retroviruses during laboratory activities. Gloves should therefore be worn during any manipulation



of these recombinant viruses and care must be taken to avoid spilling and splashing infected cell-culture liquid and other potentially infected materials.

- The need for using sharps in the laboratory should be evaluated. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded.
- Simultaneous manipulation of replication competent viruses or other vector systems in the same class II biosafety cabinet should be prohibited
- Infection occurring via the respiratory tract has not been documented and has not been reported.

9. References

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- Pauwels K, Herman P, Van Vaerenbergh B, Do thi CD, Berghmans L, Waeterloos G, Van Bockstaele D., Dorsch-Häsler K, Sneyers M. Animal Cell Cultures: Risk Assessment and Biosafety Recommendations. *Applied Biosafety*, 2007, 12 (1): 27-39.
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