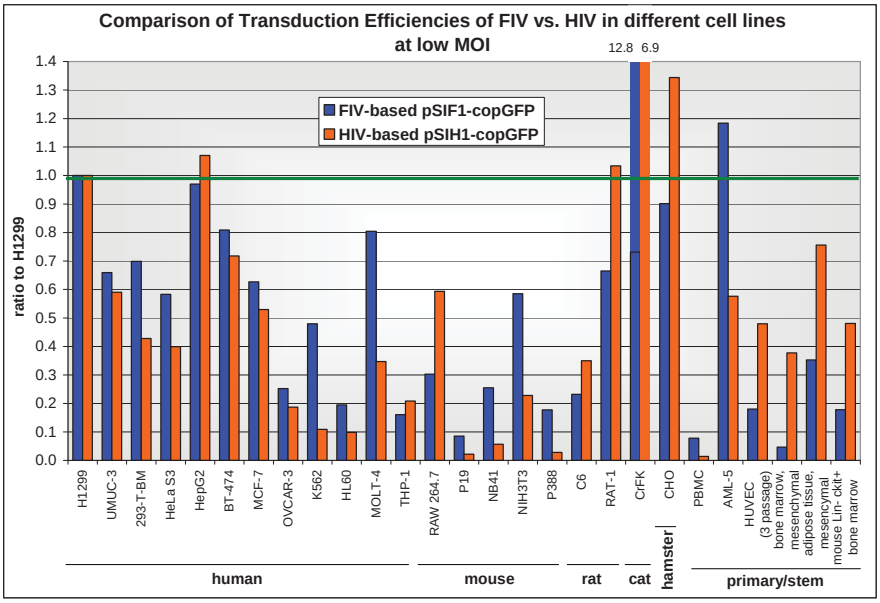
Delivery of Packaged Lentivector Constructs into Target Cells

Pantropic VSV-G pseudotyped viral particles containing the RNA copy of the lentivector expression construct can be efficiently used to deliver and stably express effector and reporter sequences in a wide range of mammalian target cells. In order to provide guidelines for the use of lentivector delivery systems, we compared transduction efficiencies of FIV-based and HIV-based vectors in different cell types. The graph below shows a comparison of transduction efficiencies of FIV-based and HIV-based lentivector systems for 27 different cell lines, including primary and stem cells.



Comparison of transduction efficiencies for FIV-based and HIV-based packaged positive transduction controls for 27 different cell lines based on FACS analysis percentage of GFP-positive cells at low Multiplicity of Infection (MOI).

These data clearly demonstrate that unlike commonly used cancer cell lines (like H1299, HeLa, HeK295, HepG2, etc.) that can be effectively transduced by lentivectors, some cell types (mouse Lin- ckit+ bone marrow, P19, PBMC, HL60, P388) are more resistant to infection. More efficient transduction of more "resistant" cell types may be possible by using a higher concentration of pseudoviral particles per cell in order to achieve the same MOI, but not in all cases. It is important to mention that FIV-based and HIVbased lentivectors have different tropism. For example, the FIV-based system is more effective at infectingseveral of the tested mouse cell lines (P1 9, NB41, NIH3T3, P38) and some of the blood cells (MOLT-4, K562, Tcells from AML patient). The HIV-based system is more effective at infecting stem and primary cells (HUVEC, bone marrow, adipose).

Pseudotyped lentiviruses have been successfully used to infect many other cell types, including neuronal, dendritic, endothelial, retinal, pancreatic, hepatic, aortic smooth muscle cells, airway epithelia, skin fibroblasts, macrophages, etc. Lentivectors have been successfully used also for direct in vivo delivery and expression of transgenes in muscle, brain, airway epithelium, liver, pancreas, retina, and skin. For a more complete list of cells or tissues, which have been successfully transduced with lentivectors, please see the Reference Section.
The pseudoviral particles can infect (or transduce) target cells and express effector or reporter molecules but cannot replicate within target cells because the viral structural genes are absent and the LTRs are designed to be self-inactivating upon transduction. Following transduction into the target cells, the expression cassette is reverse transcribed and integrated into the genome of the target cell. After integration, the expression cassette continuously and stably produces high levels of effector or reporter molecules in target cells. Target cells stably expressing the effector molecule can be isolated using the selectable marker contained in the expression vector construct (e.g., puromycin or copGFP).