GeneCopoeia™
Expressway to Discovery

Testing for Replication Competent Lentivirus (RCL) in Lentiviral Vector Based Products

Genecopoeia's HIV-based lentiviral packaging system includes the following key safety features:

- The transfer vectors contain a deletion in the 3' LTR that results in self-inactivation (SIN) of the lentivirus after the transduction of target cells.
- A four plasmid third generation system with enhanced safety designs is adopted. By splitting the vector system into 4 plasmids (3 helper plasmids and 1 transfer vector), the number of recombination events required to form a complete RCL increases, subsequently the chance of producing RCLs is significantly reduced. To date there are no known cases where this type of construct has generated RCLs.
- The HIV-1 envelope gene *env* has been completely removed from the vector. The *env* gene is replaced with the *VSV-G* gene from Vesicular Stomatitis Virus, which is placed on a helper plasmid.
- The genes for accessory proteins include tat, vrf, vpr and nef have been deleted from the plasmid. None of the HIV-1 structural genes are present in the packaged viral genome, and the reverse transcriptase and integrase proteins are provided in trans, therefore no replication-competent virus can be produced.

Test

Random samples of lentiviral particles produced with GeneCopoeia's packaging system were used to infect cultured H1299 cells and HT-1080 cells. The virus-containing culture medium was removed the next day. The cells were washed 3 times with PBS, and fresh complete medium was added. On the 4th day (3 days after transduction) the culture supernatants were collected, concentrated by ultrafiltration with a 50 kD MWCO PES filter, and used for qRT-PCR analysis (1), or used to infect new H1299 cells and HT-1080 cells (2).

Result:

(1) The qRT-PCR assay with HIV-1 specific primers did not detect any viral RNA.

(2) After growing H1299 cells and HT-1080 cells treated with concentrated supernatants in selection medium that contained selection drug, no viable colonies formed.

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