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412 BRYAN RESEARCH BUILDING

Testing lentiviruses for appearance of replication-competent retroviruses (RCRs)- safety tests

This service is mandatory before the concentrated vector prep is released to investigators. The concentrated HIV-vector preps are evaluated for the appearance of replication-competent retrovirus (RCR) by the three independent assays described below.

1. Tat-transfer assay. The assay described here is based on a reporter HeLa-CD4-LTR-βgal cells containing a copy of the HIV-1's LTR which is linked to the β-galactosidase gene. The reporter cell line is highly susceptible to the infection. In the case of viral genome recombination that results in the reconstitution of replication-competent HIV-1, the recombined vector will be capable to generate functional tat protein. The tat-expression will lead to activation of the viral LTR-promoter driven the expression of β-galactosidase gene of the reporter cell line. To execute the assay, lentiviral vectors-contained supernatant is collected and serially-applied to fresh HEK293T cells four times (about two weeks in culture). Then, the supernatant is transferred to a reporter HeLa-CD4-LTR-βgal cells. The HIV-1-tat activity is determined by X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) staining. By this method, vector preparation is considered helper negative when no expression of β-gal is detected.

2. Gag-transfer assay. This assay is based on the detection of p^{24}gag-protein of the virus in conditioned media obtained from vector-transduced cells. The cells are serially passaged three-four times (about 2 weeks), after which the supernatant of the cells is collected for assessing the level of p24gag by ELISA (p24 ELISA kit, NIH). The detection limit of this method is ≥100 pg/mL of p^{24}, which is about 10^3 copies of viral genome per mL. By this method, vector preparation is considered helper negative when p24 is not detected.

3. Marker-rescue assay. This assay is based on the direct detection of GFP or other reporter following the transfer. Viral vector stocks are assessed as follows. The cells transduced with lentiviral vector harboring a reporter (GFP) are serially passaged four times, after which the supernatant of the cells is collected and transferred to HEK293T cells cultured in a 10-cm plate. Seventy-two hours' post-transduction, the cells are scored for a reporter expression. Vector stock was considered helper free when no reporter is detected.

References

1. Charneau, P., G. Mirambeau, P. Roux, S. Paulous, H. Buc, and F. Clavel. 1994. HIV-1 reverse transcription. A termination step at the center of the genome. J. Mol. Biol. 241:651–662.
2. Kimpton J, Emerman M. Detection of replication competent and pseudotyped HIV with a sensitive cell line based on activation of an integrated beta-galactasidase gene. J Virol 66:2232-2239, 1992.
3. Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono. 1996. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272:263–267.