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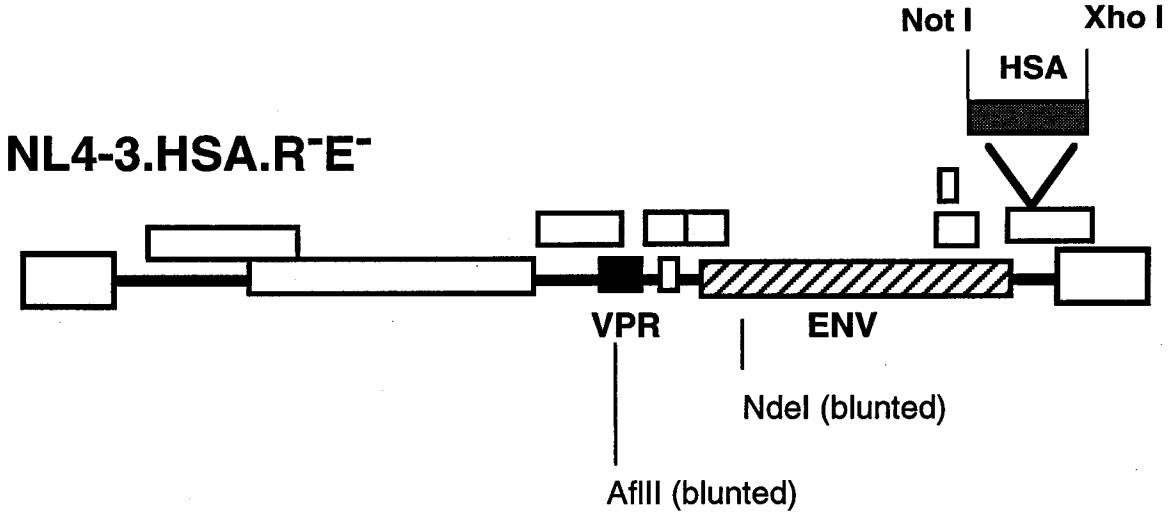

HEAT STABLE ANTIGEN REPORTER VIRUS

pNL.HSA.R⁻E⁻ is based on the HIV-1 proviral clone pNL4-3. It contains the murine heat stable antigen (CD24) cDNA in the *nef* position, is *env*- due to a frameshift near its 5'-end and has a frameshift in *vpr* that prevents its production. The resulting virus is competent for a single round of replication. The plasmid is grown in *E. coli* with ampicillin selection.

To make infectious virus, 293 or 293T cells are transfected with a mixture of the reporter virus DNA and Env expression vector DNA (generally at a ratio of reporter to Env DNA of 10ug:10ug). Virus is harvested 48 hr. after transfection, titred by p24 ELISA, aliquoted and frozen at -80°. Typically, 1 X 10^5 cells are infected with 10ng p24 in 0.5ml total volume. Infected cultures are harvested two to five days postinfection and then analysed by FACS using commercial monoclonal CD24 antibody (Pharmingen). Amphotropic pseudotypes generally have a much higher infectivity than those bearing HIV-1 Env. Construction and use of this vector is described in He, et al.(1995) *J. Virology* **69**(11):6705-6711.