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Building reporter virus with recombinant viral proteins for analysis of resistance to antiviral compounds

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In the United States, the Centers for Disease Control and Prevention recently reported that the estimated cumulative number of diagnoses of AIDS by Human immunodeficiency virus (HIV) was about one million, and half million people were died until 2005. This HIV as a retrovirus is composed of two copies of positive single-stranded (ss)-RNA which codes for the virus's nine genes enclosed by a conical capsid protein (p24). The ss-RNA is tightly bound to nucleocapsid proteins (p7) and several enzymes needed for the development of the virion including reverse transcriptase (RT), proteases, ribonuclease and integrase. By removing and/or replacing essential coding regions, the efficient curative means on HIV infected human beings are highly required. Moreover, the creation of the new reporter virus with recombinant RT is needed to allow direct comparison of resistance to antiviral compounds. Thus, this study used the modified plasmid, pHIV-*gpt* encoding the HIV-1 provirus HXB2, with the bacterial *gpt* gene for mycophenolic acid resistance substituted for the viral *env* gene (Page et al., 1990), which was replaced by a drug selection marker. In our previous study, we removed the DNA segment of RT region in pHIV-*gpt*, called pHIV-*gpt*-ΔRT. Then, a part of pHIV-*gpt*-ΔRT was amplified by using PCR with specific primers. The amplicons were digested in the restriction sites, and then this digested fragment will be cloned into one of three target plasmids, each of which contains a mutated variant of HIV-1 strain NL4-3. The pNL4-3 carries puro-cherry, tomato or GFP in the region of viral *nef* gene. Therefore, we can make a number of reporter viruses through the mechanism of recombination, and building the reporter viruses is still on-going work in this project. This study should be to help in understanding a gene therapy approach throughout direct comparison of resistance to antiviral compounds.