

**Construction of an HIV-1 subtype C vector system
for
phenotypic drug resistance studies**

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Abstract

Acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) is one of the world's most deadly infectious disease. HIV-1 infections accounts for over 60% of global infections, yet majority of researchers are focused on subtype B viruses which only accounts for <12% of the global pandemic. UNAIDS has estimated 33.3 million HIV-1 infections globally in 2011, and South Africa accounts for 5.6 million of these infections. HIV-1 subtype C viruses predominate in Southern Africa and are rapidly spreading across the world accounting for more than 50% of infections worldwide. The focus on reverse transcriptase (RT) and protease (PR) gene as targets of antiviral therapy has led to the discovery of various major, minor and polymorphic mutations harbored thereof. There are mutations in HIV-1 subtype B strains that develop only under drug pressure leading to ARV drug resistance. These mutations have been observed in subtype C strains as polymorphisms. The aim of this study was to construct an HIV-1 subtype C vector system which could be used to evaluate the phenotypic significance of putative resistant mutations observed in HIV-1 subtype C polymerase gene sequences.

A mammalian derived plasmid pCMVGagPol(IndieC)RRE comprising unique Apal and Hpal restriction sites was previously modified. Viral RNA from 14 drug naïve patient samples was reverse transcribed to cDNA using transcriptor reverse transcriptase thereafter amplified using nested PCR. The amplified 1650bp gene was cloned into pGEMT easy cloning vector and expanded in DH5 α competent cells. Viral DNA was subsequently extracted from the pGEMT vector by double digestion using Apal and Hpal restriction enzymes. A 1650 bp fragment was excised from pCMVGagPol(IndieC)RRE and replaced by a 1650 bp purified viral DNA to construct pCMVGagPol(Patient)RRE. Chimeric virus was expanded in DH5 α cells followed by successive restriction digestion and colony PCR to confirm the cloned HIV-1 C *GagPol* gene. Orientation of the cloned viral DNA was confirmed by sequencing the vector and identifying the restriction enzyme positions.

Of the analyzed sample sequences, the identified significant polymorphisms observed were K20R, M36L, I93L and L89M, these mutations are known to be associated with susceptibility to PI's in subtype B viruses.

The constructed pCMVGagPol(patient)RRE chimeric virus can be used for subsequent evaluation of mutations that are present in naïve and treatment exposed viruses but whose importance is not clear.