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Preparation and Characterization of an ORF54 Transposon Library for Mutagenesis Studies of the Varicella-Zoster Virus Portal Protein

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Varicella zoster virus (VZV) is a double-stranded DNA virus that causes a self-limiting primary infection known as chickenpox and can manifest into a painful debilitating disease known as shingles in its latent form. Current therapies focus on inhibiting the DNA replication process, but novel studies have proposed shifting the focus onto the encapsidation process of VZV. The encapsidation process of VZV is based on indirect information from its homologous counterpart, Herpes Simplex 1 (HSV-1). Seven genes or ORFs are shown to be essential for HSV-1 DNA encapsidation. The portal protein homolog pORF54 could prove to be a novel target for antiviral therapy. Little is known about the role of specific functional regions within the portal polypeptides. The purpose of this study is to prepare and characterize an ORF54 transposon library for use in functional studies. It is expected that specific regions of pORF54 will be essential for DNA encapsidation. Briefly, ORF54 with flanking regions was isolated from the VZV genome of Ellen strain by PCR, inserted fragment into Invitrogen’s Zero Blunt TOPO PCR cloning vector, and transformed into E. coli. The Epicentre EZ-Tn5 In-Frame Linker Insertion Kit was used to prepare in frame insertions of the 19 amino acid transposon throughout the ORF54 plasmid for mutagenesis. Characterization of wild type ORF54 and mutated ORF54 will be accomplished by complementation with a null ORF54 bacterial artificial chromosome (BAC) via transfection (Roche’s FuGene6) into human retina cells (ARPE-19). The BAC ORF54 null virus was a kind gift from Dr. Hua Zhu’s (Rutgers University). The importance of ORF54 regions will be determined by complementing the ORF54 null virus with the mutated ORF54 gene. It is hypothesized that specific regions of pORF54 will be essential or detrimental since it is known that deletion of the HSV-1 portal protein counterpart is lethal. This will be the first comprehensive study to characterize potential important function regions within any large DNA virus portal protein.