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Construction and Characterization of Varicella-zoster virus DNA Encapsidation Protein Expressing Cell Lines

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Mutant viruses containing deletions in DNA encapsidation genes have been characterized in detail for Herpes simplex type I, and to some extent for Pseudorabies virus, and Human cytomegalovirus. However, similar mutants for Varicella-zoster virus do not exist. It is expected that the deletion of each putative DNA encapsidation gene will yield a phenotype consistent with a defect in DNA cleavage and/or packaging. These mutant viruses will be isolated by site directed homologous recombination in complementing cell lines expressing the respective DNA encapsidation proteins. Essential to this process is the isolation of stable, DNA encapsidation protein expressing cell lines. Previously, we cloned each of the encapsidation ORFs into the mammalian expression vector, pcDNA3.1D-V5-His-Topo. Transient expression of each of the pORFs was confirmed in transiently transfected MeWo cells by performing immunofluorescence microscopy using a V5 monoclonal antibody that recognized an epitope tag at the C-terminus of each polypeptide. Each ORF (ORF25, ORF26, ORF 30, ORF34, ORF45/42, ORF43, and ORF54) will be used in the Invitrogen Flp-In two-plasmid system to isolate isogenic cell lines. Flp-In Cell Lines are designed for rapid generation of stable cell lines that express a protein of interest from a Flp-In expression vector. The cells use targeted integration of a Flp-In expression vector to ensures high-level expression of the gene of interest. Co-transfection of the Flp-In Cell Lines with a Flp-In expression vector and the Flp recombinase vector, pOG44, results in targeted integration of the expression vector ensuring homogeneous levels of gene expression. Cell lines will be tested for (i) the presence of the ORF via PCR and (ii) expression of the encapsidation gene product by western blot. Clones will be expanded and analyzed for protein expression with either the V5 monoclonal or pORF specific antisera.