

Engineering A Chimeric ICP27 Protein Containing a Domain From VZV ORF4

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Study Aims

Test the functionality of a HSV-1 ICP27 chimeric protein containing a domain from Varicella-Zoster virus ORF4, a homolog to ICP27.
Is the dimerization function of VZV ORF4 C-terminal domain sufficient to contribute to replication of virus when used as a replacement for the ICP27 C-terminal domain

Background

- Herpesvirus DNA localizes to nucleus, where mRNA is produced, but most mRNA is unspliced and not exported by the host cell¹.

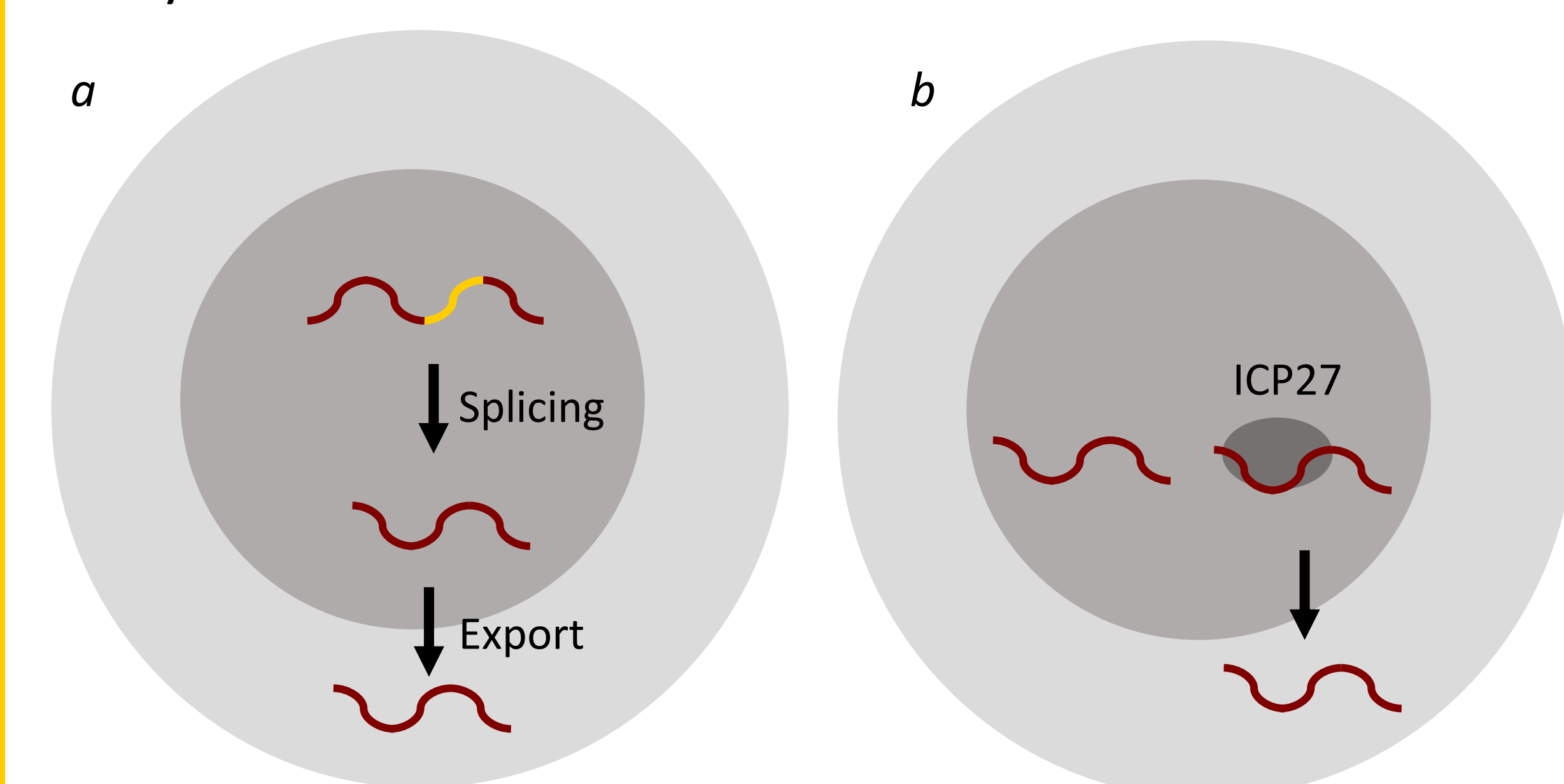


Figure 1: a) Splicing of pre-mRNA in eukaryotic cells is a prerequisite to export of mRNA to the cytoplasm. b). Viral pre-mRNA is not spliced, and is not exported on its own, but ICP27 is able to mediate export.

- How does HSV-1 get around this problem?
 - ICP27 is a HSV-1 protein that binds proteins involved in the NXF1/TAP pathway, a host pathway typically involved in mRNA export².
 - Homologs exist throughout herpesvirus family³
- Structure of ICP27
 - The N-terminal domain is unstructured and contains a binding site for cellular protein Aly-Ref and possibly NXF1⁴.
 - The C-terminal domain is required for interaction with TAP/NXF1 and is also responsible for dimerization³.

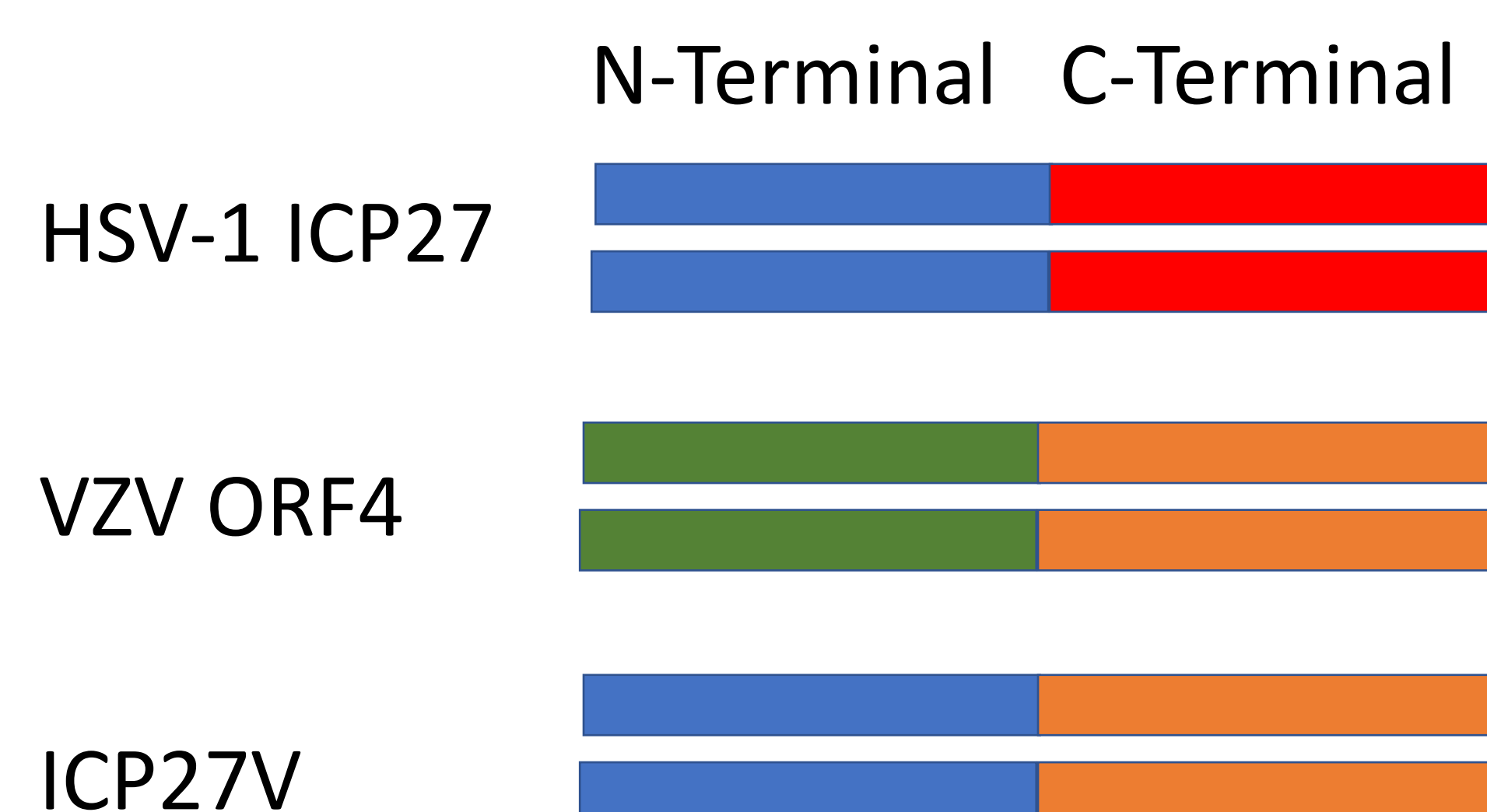


Figure 2: Domains of ICP27, ORF4, and the chimeric protein to be produced in this project

Methodology

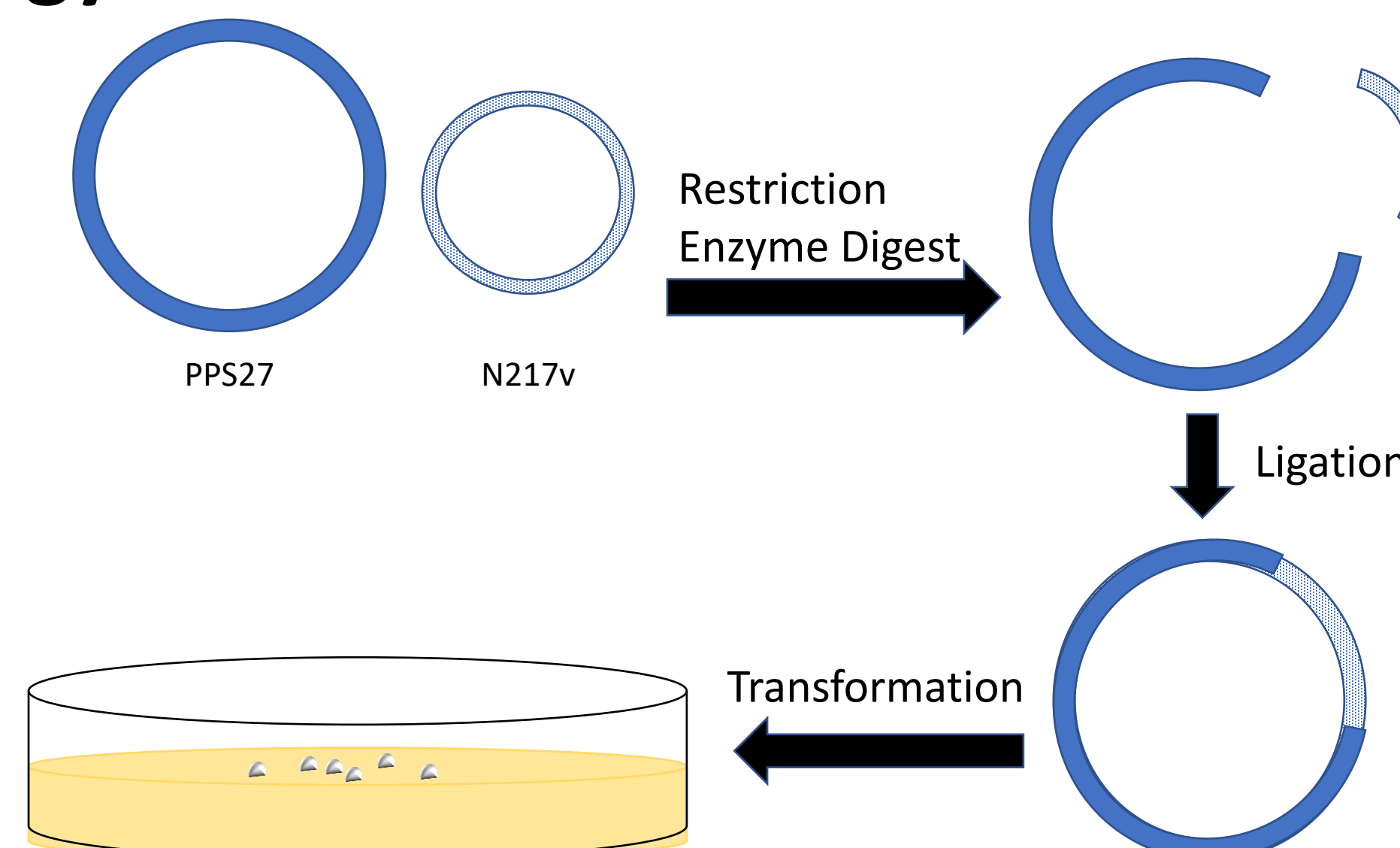


Figure 3: Cloning strategy to produce ICP27v plasmid by inserting N217v gene into PPS27 expression plasmid.

Results

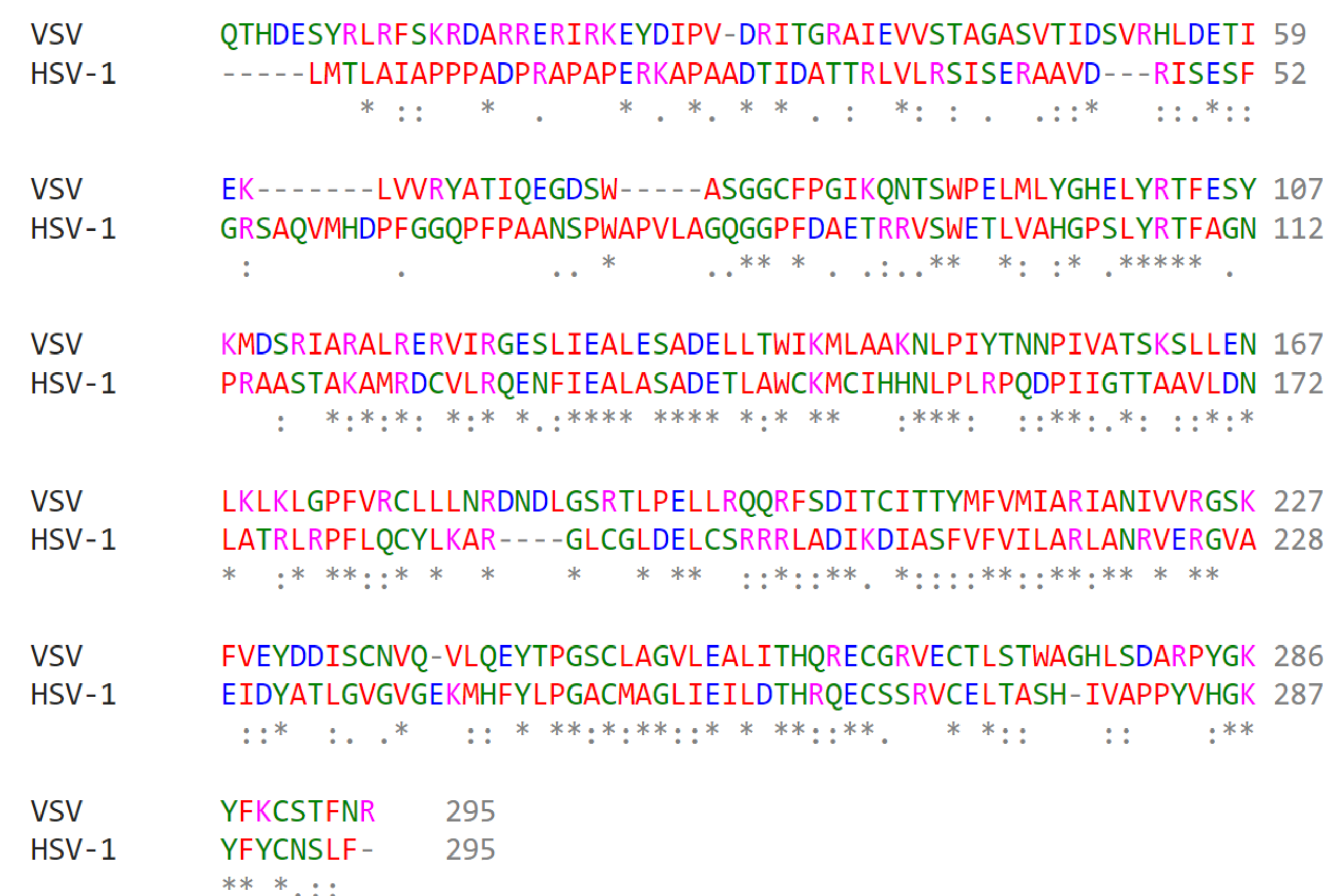


Figure 4: Sequence alignment of VZV ORF4 and HSV-1 ICP27 C-terminal domains, created using ClustalW^{5,6,7}. Red=Hydrophobic. Blue=Acidic. Magenta= Basic. Green= Hydroxyl, Sulphydral, Amine

Is the C-terminal Domain of ORF4 and the N-terminal Domain of ICP27 Sufficient for HSV-1 Replication in Vero Cells?

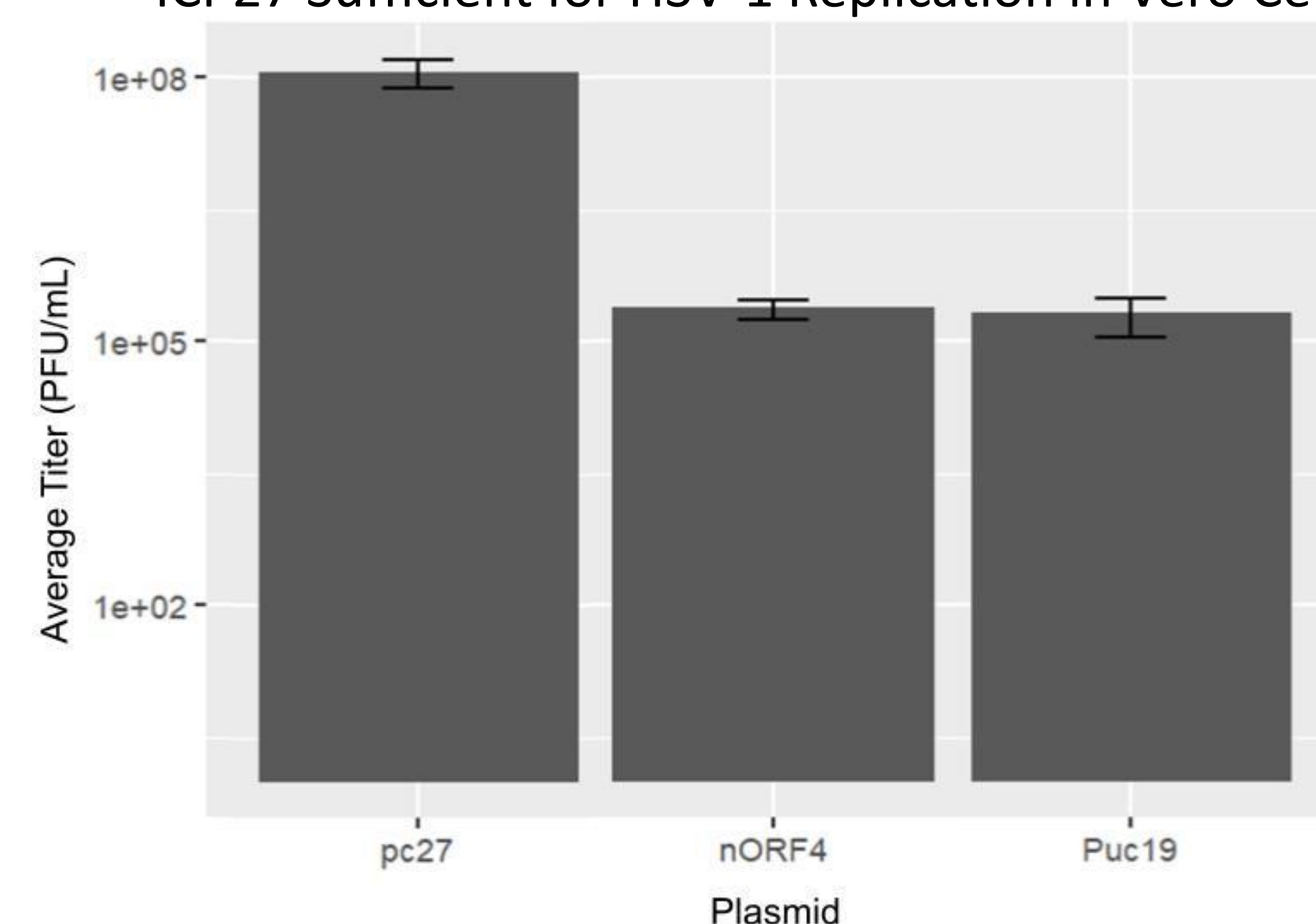


Figure 5: Complementation assay in which Vero cells were first transfected with one of three plasmids; pc27 (which produces WT ICP27, pORF4, and puc19). Following incubation, the cells were infected with n504r virus. Such an assay tests if plasmids are capable of rescuing a defective virus. Viral titers were determined using a plaque assay.

Ongoing and Planned Experiments

- Plasmid Construction
 - Diagnostic restriction enzyme digest.
 - Transfect into cells and conduct a western blot to confirm that chimeric protein is produced.
- Conduct a complementation assay

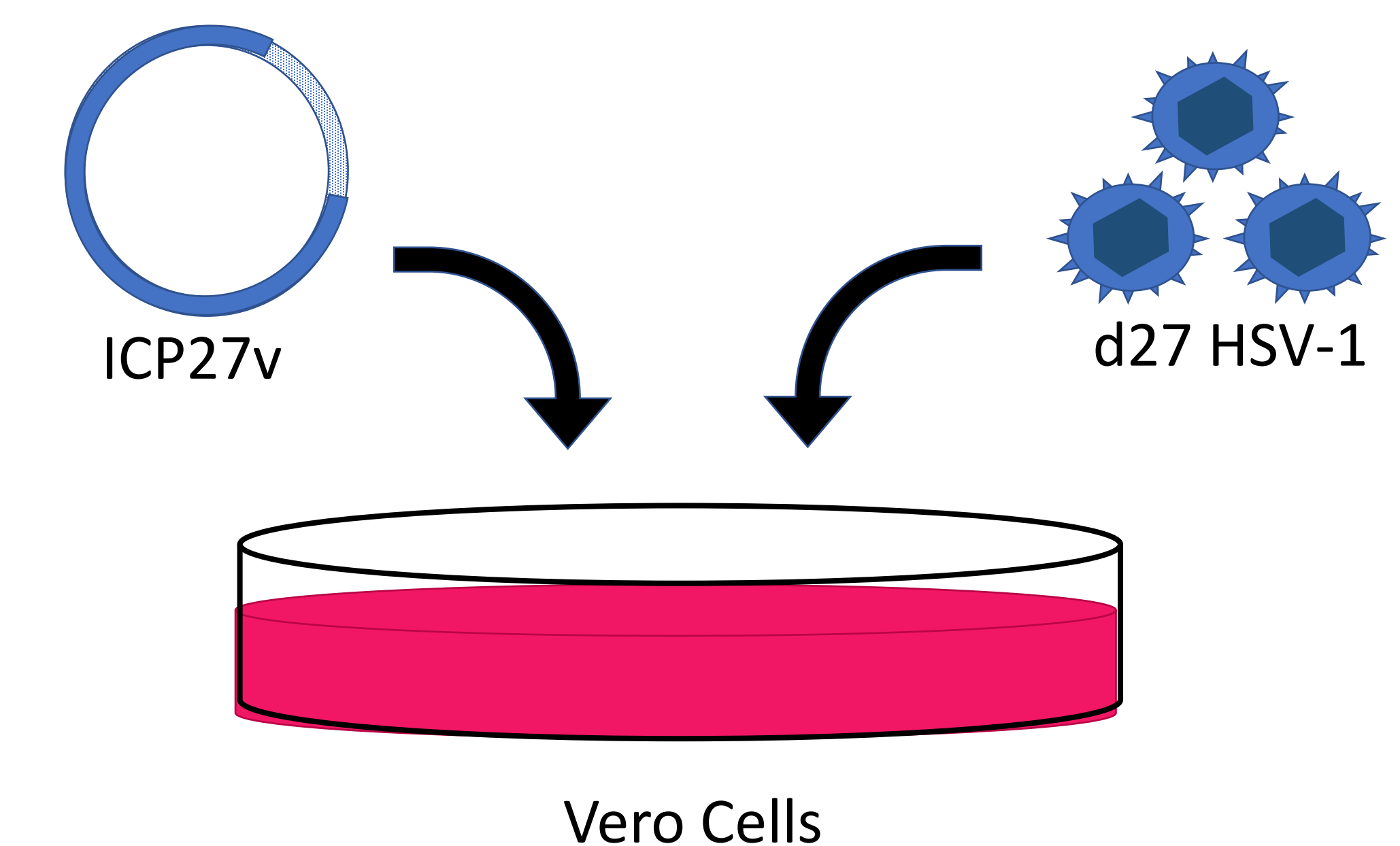


Figure 6: Set up of complementation assay. Vero cells are transfected with a plasmid (ICP27v), and these cells are then infected with HSV-1 lacking a functional ICP27

- Confirm dimerization of ICP27V protein
- Create a virus with ICP27V
 - Homologous recombination with d27-LacZ virus
- Test complementation in multiple cell lines.

Future Directions

- Expand experiment to additional members of herpesvirus family
- Identify conserved sequences and test knockouts with targeted mutagenesis
- Create a chimeric protein with VZV ORF4 N-terminal domain and ICP27 C-terminal domain

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