

Portland State University

PDXScholar

Student Research Symposium

Student Research Symposium 2024

May 8th, 11:00 AM - 1:00 PM

Construction and Mutagenesis of SSV1 Mutants in Extreme Viruses

Lou Ann F. O'Connor
Portland State University

Jono Abshier
Portland State University

Kenneth M. Stedman
Portland State University

Follow this and additional works at: <https://pdxscholar.library.pdx.edu/studentsymposium>



Part of the [Biology Commons](#), and the [Public Health Commons](#)

Let us know how access to this document benefits you.

O'Connor, Lou Ann F.; Abshier, Jono; and Stedman, Kenneth M., "Construction and Mutagenesis of SSV1 Mutants in Extreme Viruses" (2024). *Student Research Symposium*. 72.
<https://pdxscholar.library.pdx.edu/studentsymposium/2024/posters/72>

This Oral Presentation is brought to you for free and open access. It has been accepted for inclusion in Student Research Symposium by an authorized administrator of PDXScholar. Please contact us if we can make this document more accessible: pdxscholar@pdx.edu.

A wide-angle photograph of a geothermal landscape. In the foreground, there is a large, flat, light-colored rock formation with some cracks. In the middle ground, several smaller rock formations are visible, some with wisps of white steam rising from them. The background shows a dense forest of evergreen trees under a heavy, grey, overcast sky. A bright light source, possibly the sun, is visible behind a break in the clouds on the left side of the frame, creating a lens flare effect.

CONSTRUCTION AND MUTAGENESIS OF SSV1 MUTANTS IN VIRUSES FROM HELL

Lou Ann O'Connor

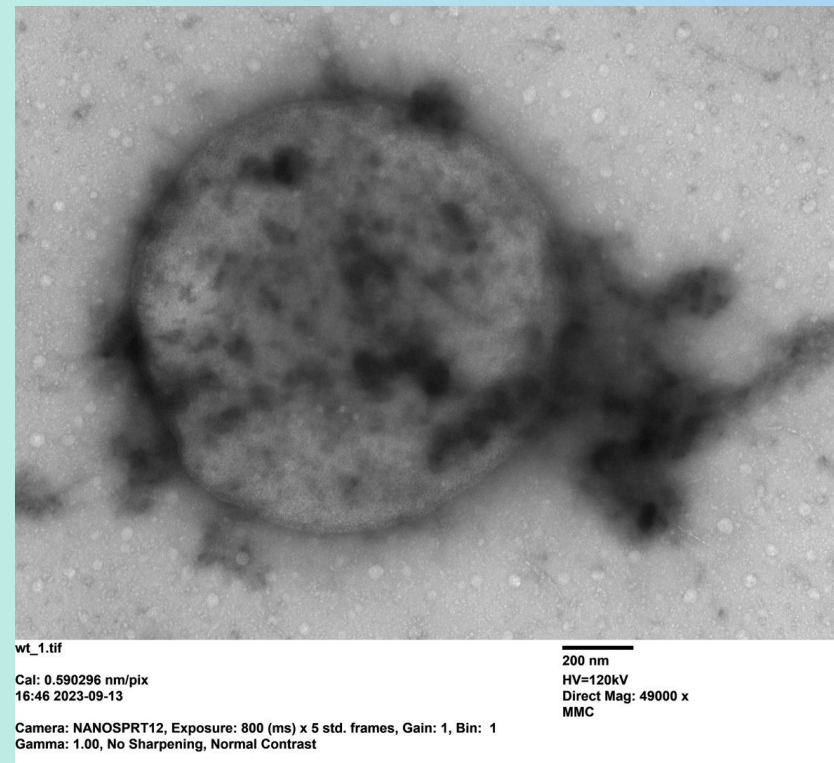
Jono Abshier

Dr. Stedman

SSV1 Overview

SSV1(Sulfolobus Spindle-Shaped Virus 1)

- Archaeal Virus with circular double-stranded DNA genome
- Habitat: Extreme Environments- Hotsprings
pH level: 4 and below and temperatures above 80
- Natural host: *Saccharalobus solfataricus*



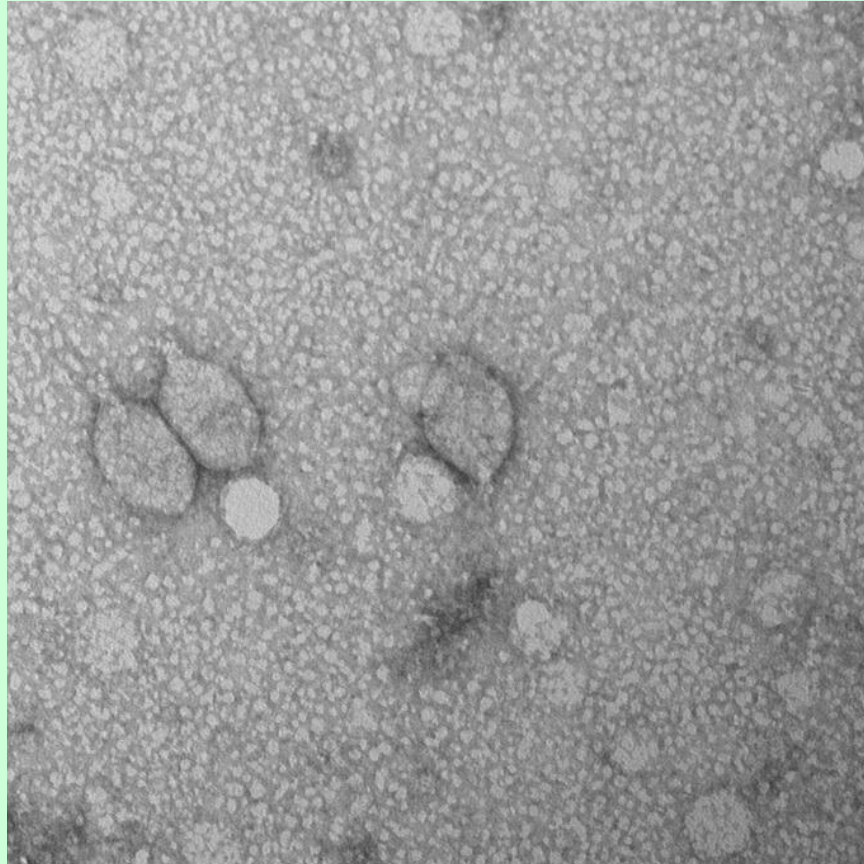
What composes SSV1?

- VP1: Major Capsid Protein
- VP3: Minor Capsid Protein
- VP2: DNA binding
- VP4 & Cysteine: Tail formation

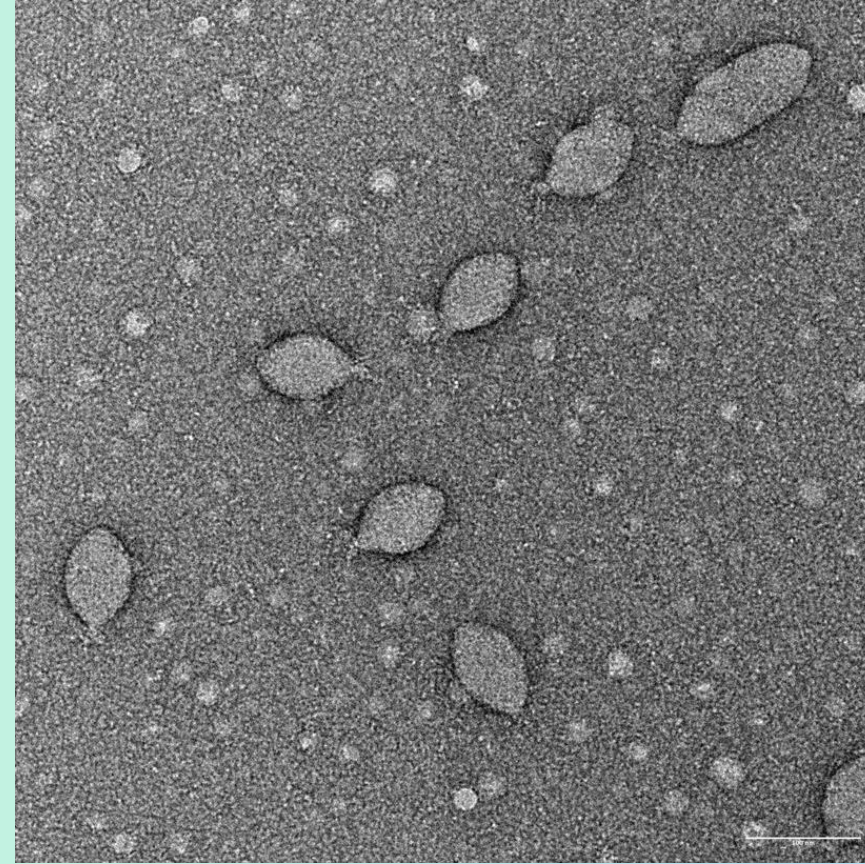


Major Capsid Protein: VP1 Mutants

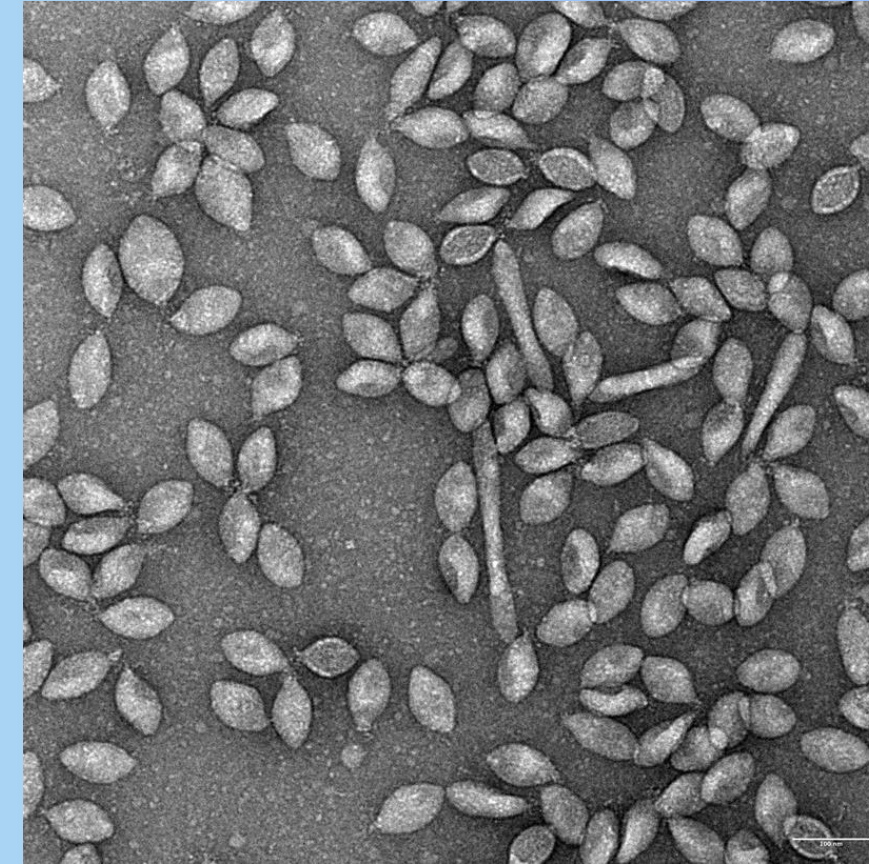
- Cleavage site at position 66 of of the residue Glutamate (E)
 - Mutation: Amino acid substitution of the residue -Glutamate
Glutamine mutant (E66Q), Methionine mutant (E66M), Serine mutant (E66S), Phenylalanine mutant (E66F), **Isoleucine mutant (E66I)**.
- Aim:** Introduce hexahistidine (6x) to VP1 mutants for purification.



Wild-type Glutamate
(E66)

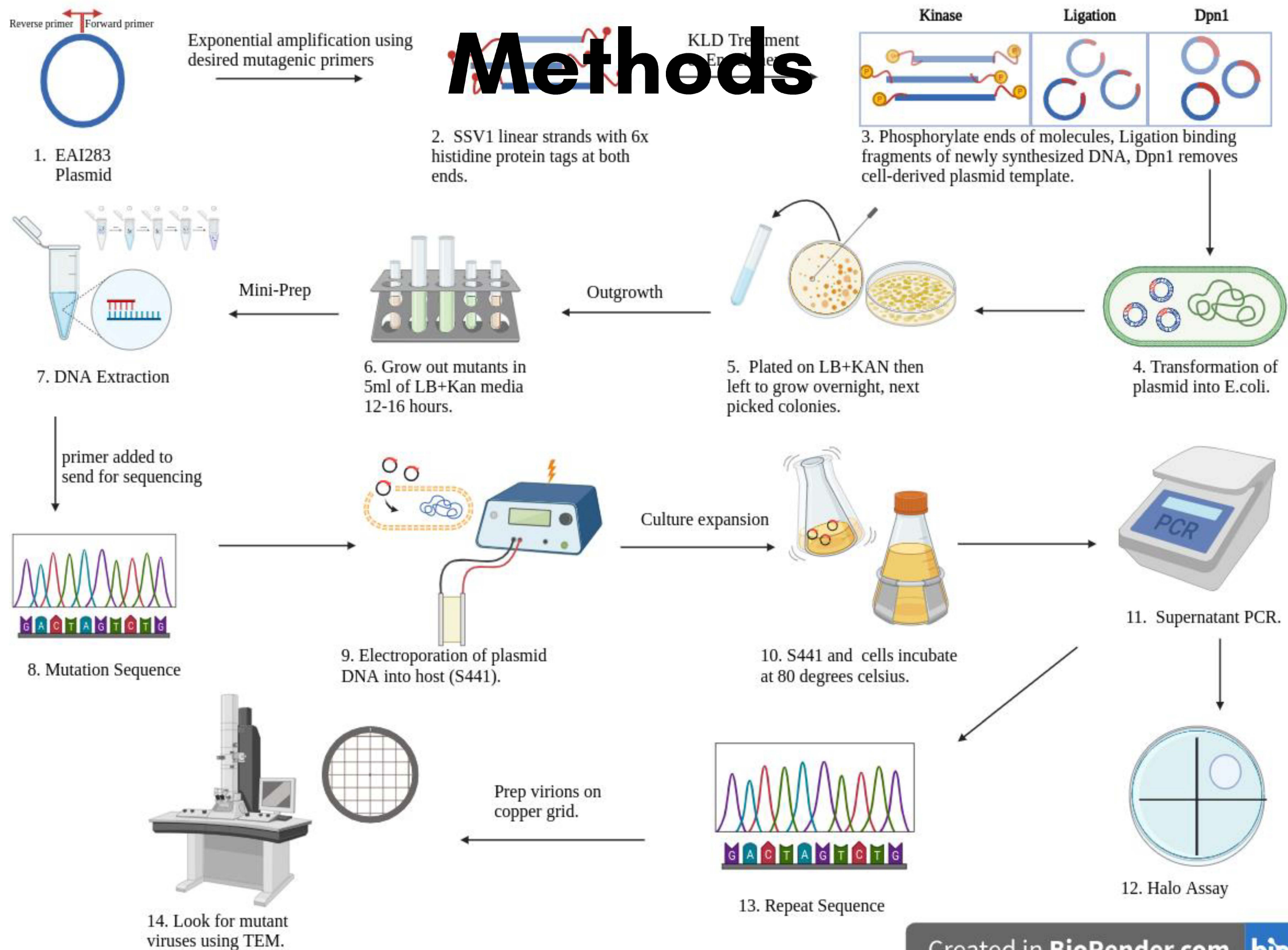


Isoleucine Mutant (E66I)



Glutamine Mutant
(E66Q)

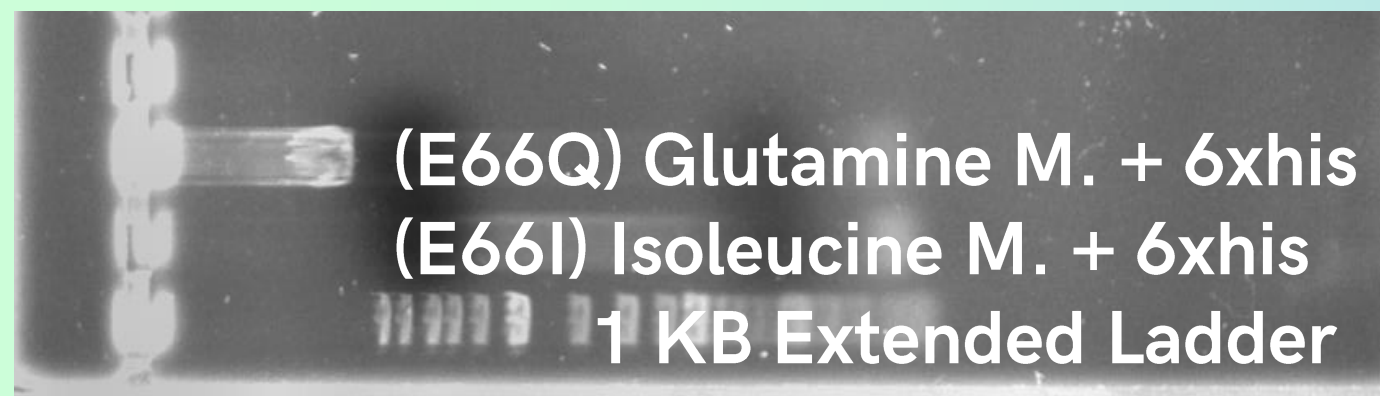
Materials and Methods



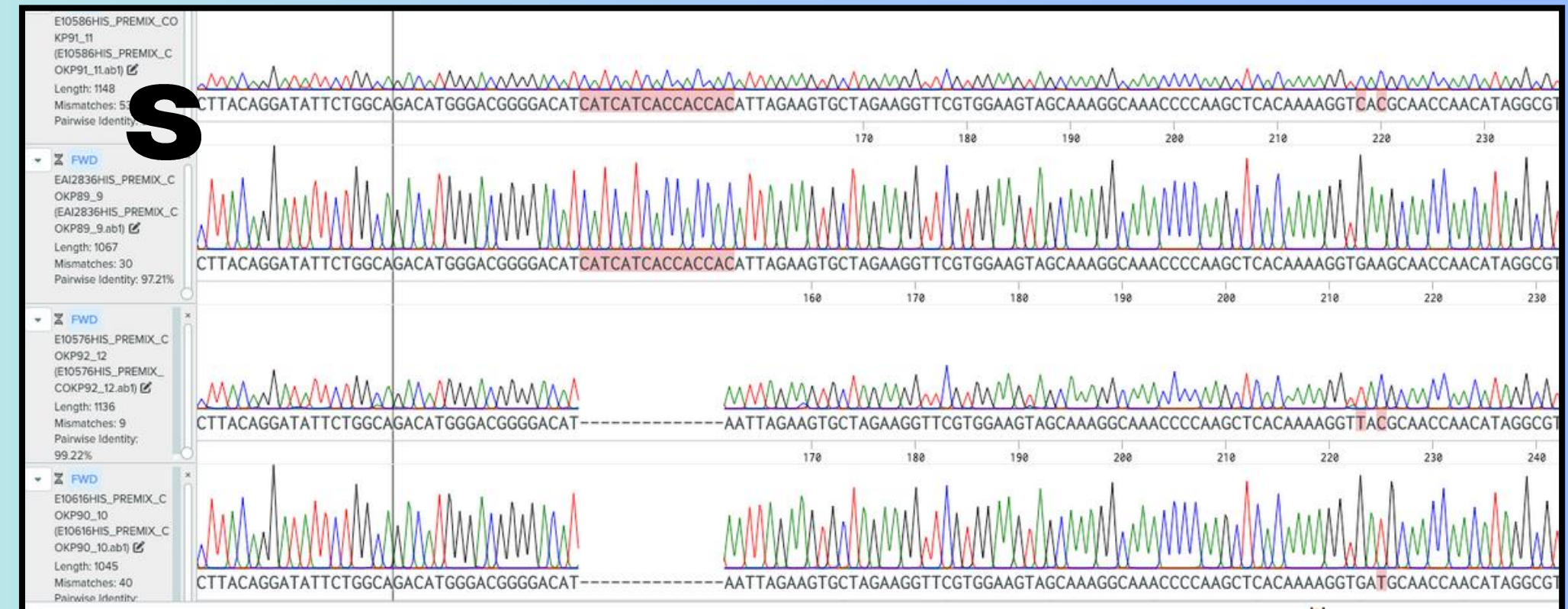
Result



Mutagenic PCR introducing 6xhis tag primers to additional VP1 mutants.



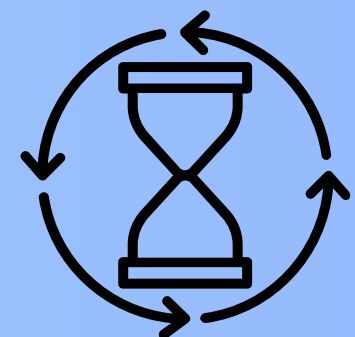
Mutagenic PCR introducing New 6xhis tag primers to additional VP1 mutants.



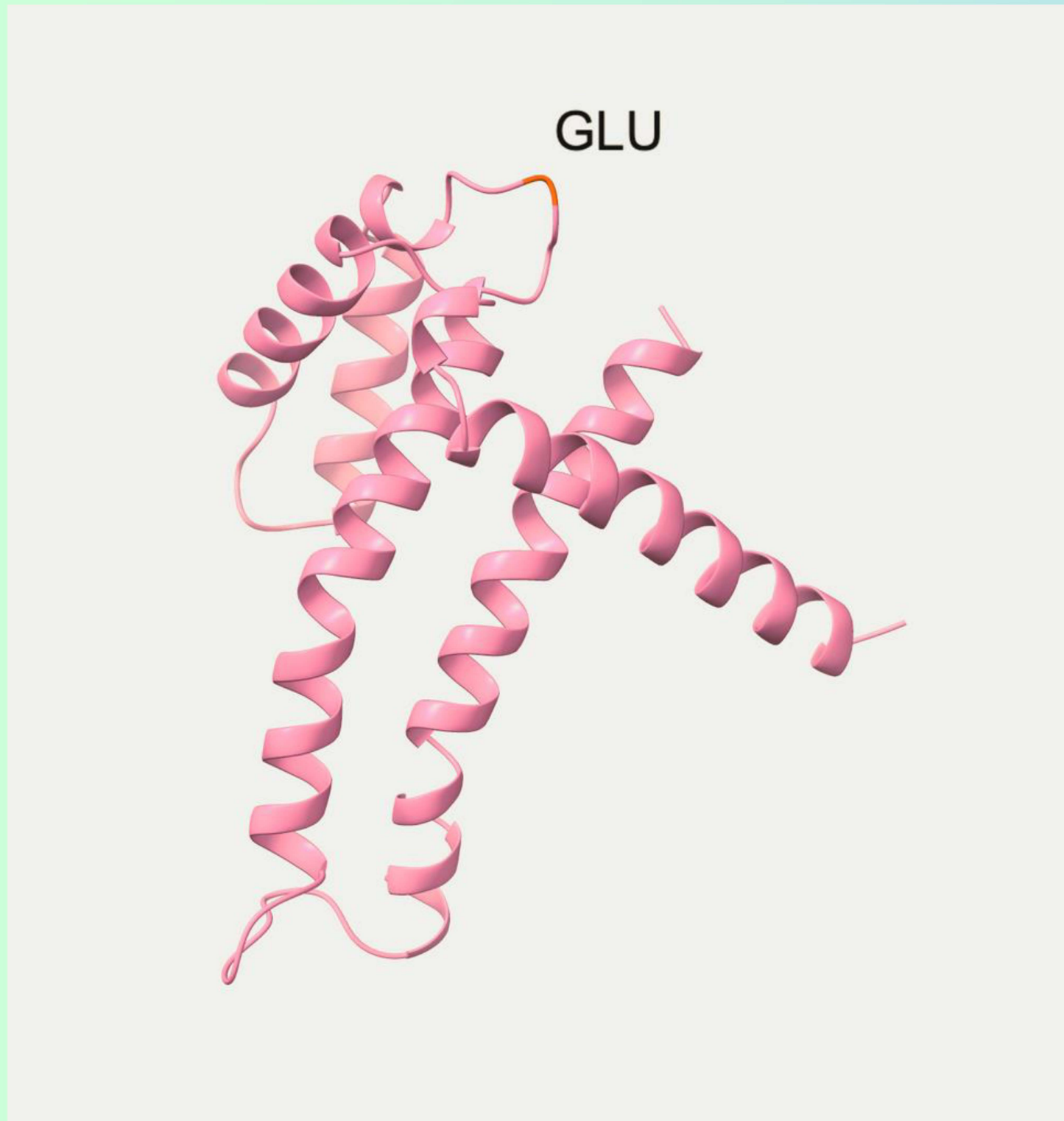
Mutation sequence:

- (E66H) Histidine M. 6xhis tag present
- (E66) Glutamate M. 6xhis tag present
- (E66Y) Tyrosine M. No 6xhis tag
- (E66D) Asparate M. No 6xhis tag

Mutation Sequence for (E66Q) Glutamine M. + 6his tag and (E66I) Isoleucine M. + 6his tag ??

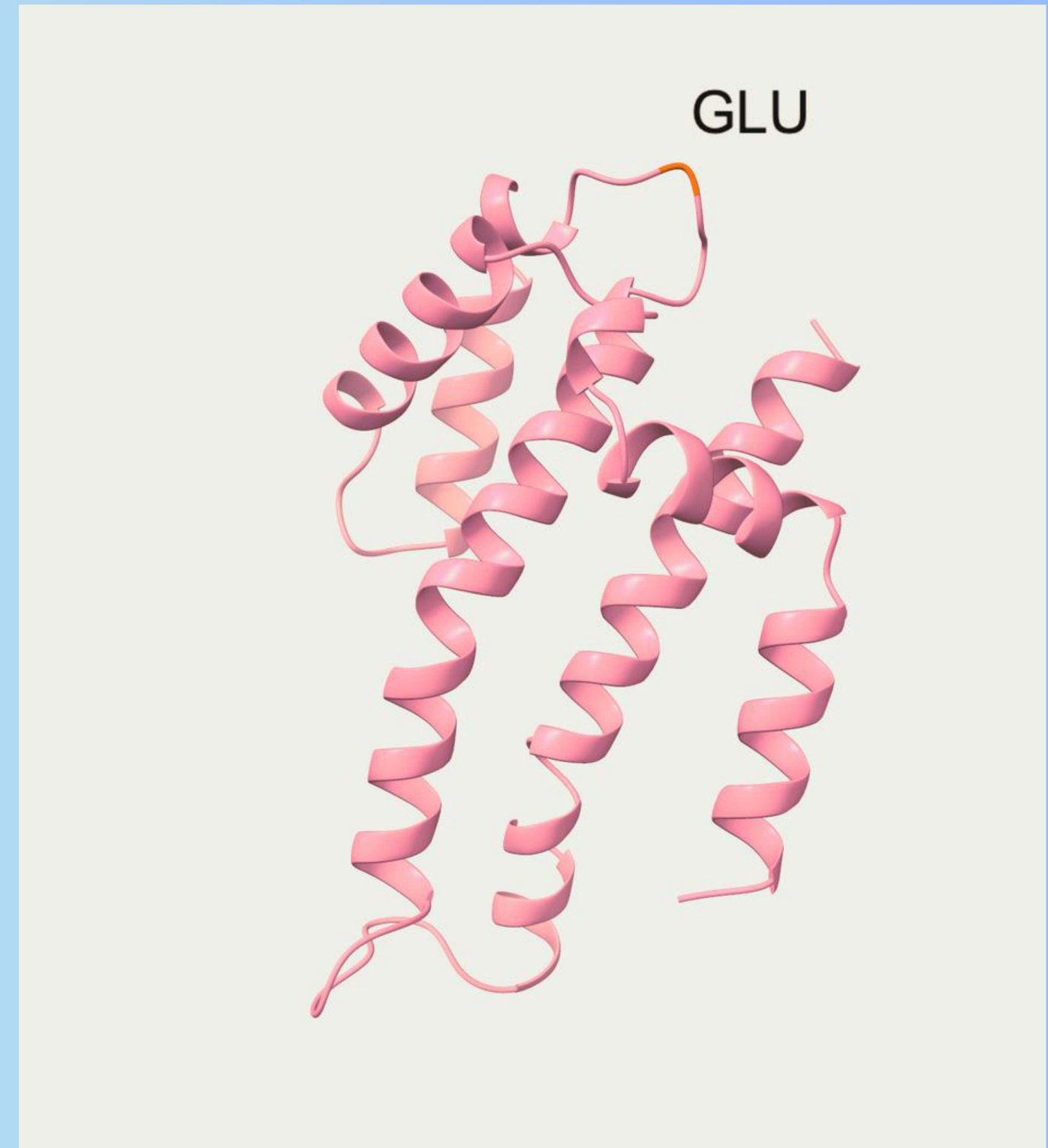


Result



VP1 Alphafold Wild-type predicted structure

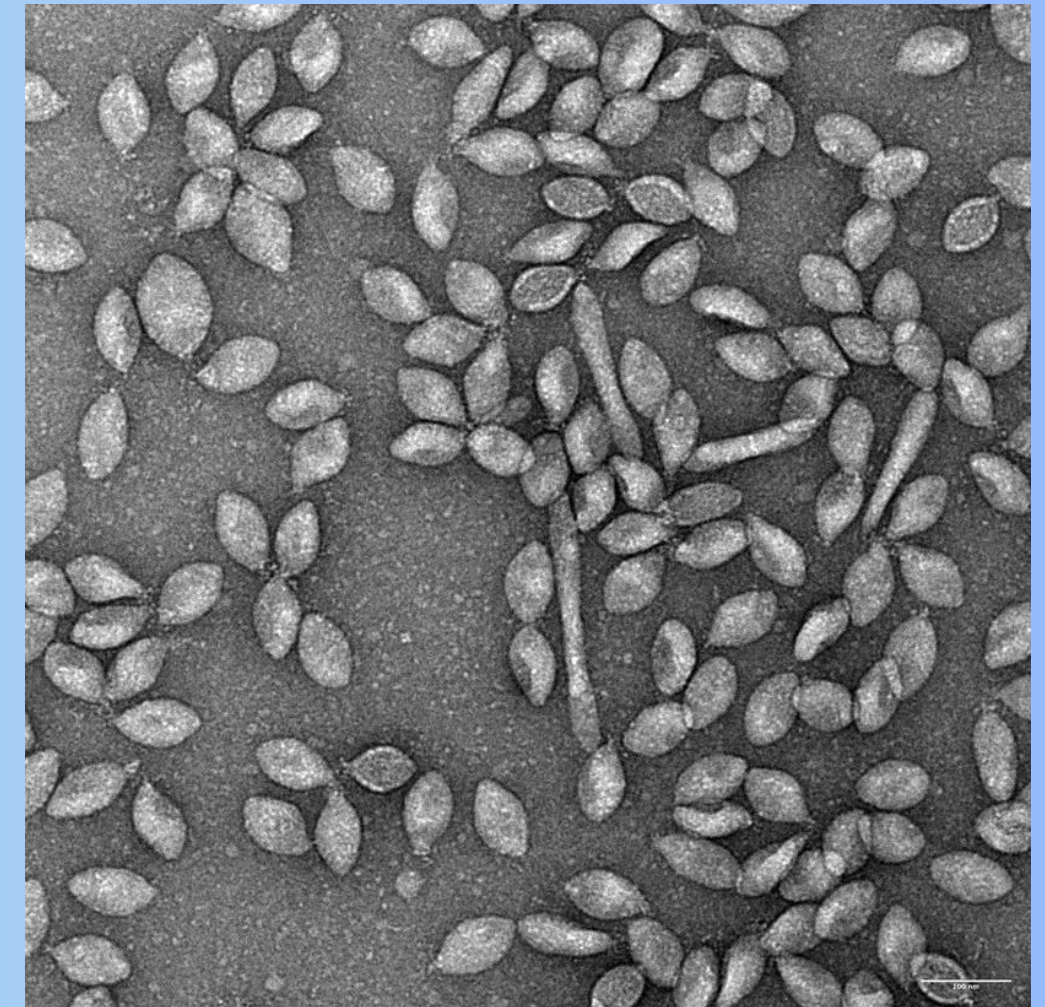
S



VP1 Alphafold 6xhis Tag Mutant predicted structure

Future Directions

- Electroporate mutants with VP1 6xhis tag mutants into S441 Host
- Sequencing for VP1 Mutants, (E66Q) Glutamine mutant.
- Look for VP1 mutant virions at TEM Microscope



Acknowledgement



Special thanks to my Principal Investigator Dr. Stedman, my mentor Jono, and the eXtreme Virus Lab for the opportunity and support to work on this research project, and great thanks to BUILD EXITO for funding and supporting this research project.

This research was supported and funded by NIH BUILD EXITO (Grant numbers: 5TL4GM118965-09, 5RL5GM118963-10, 5TL4GM118965-09).