Mutant Viruses from Hell

Mutagenizing the cleavage site in VP1

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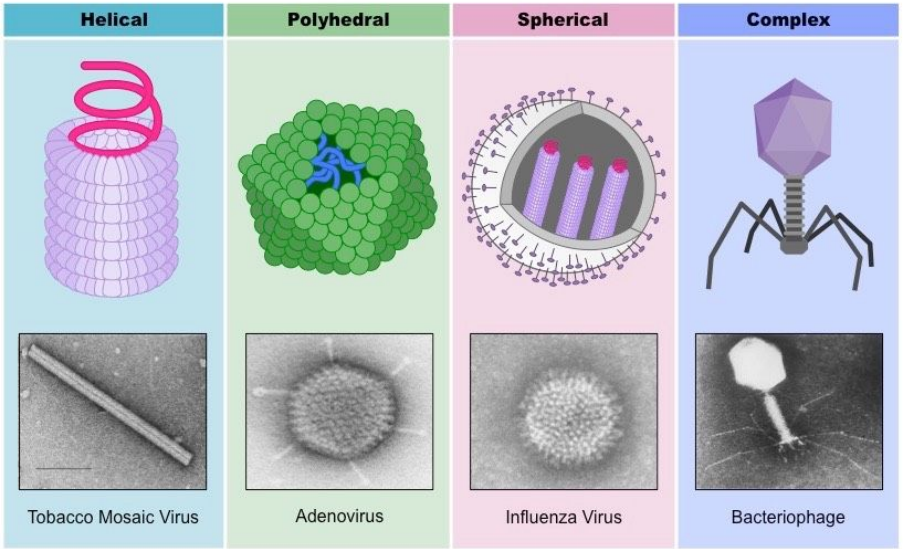
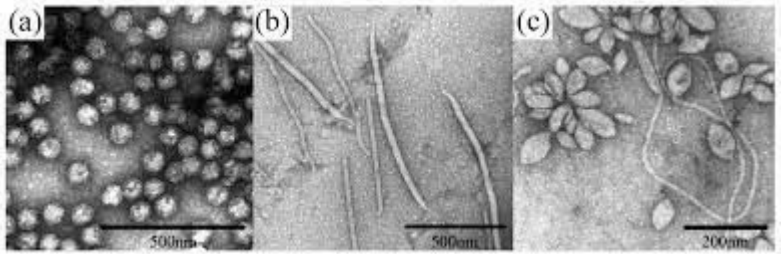
Viruses in general

… Nucleic acids wrapped up in proteins (CAPSID)



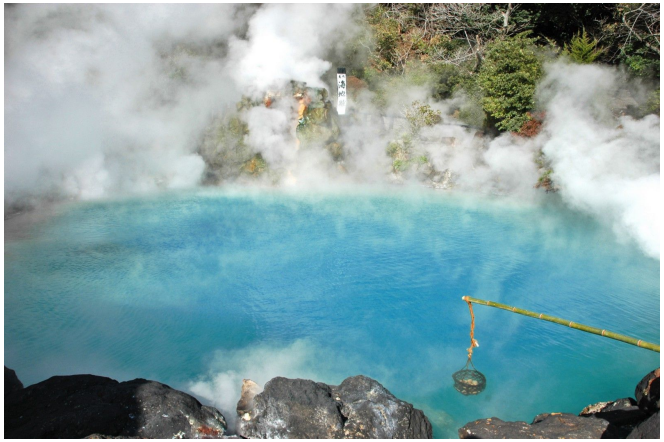


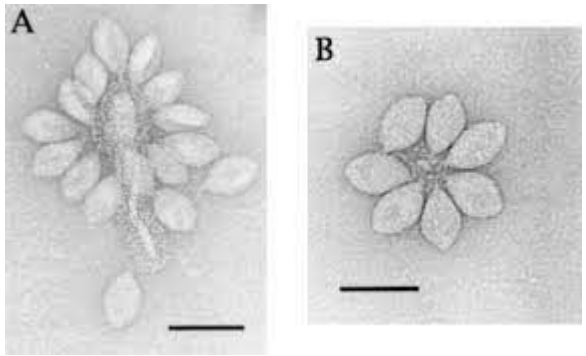
Background on SSV's

- Thermophilic, acidophilic viruses
- Sulfolobus Spindle shaped viruses
- Hosts: *saccharolobus solfataricus*
- To date there are 40 known SSV's worldwide

SSV1

- Prototypical virus
- Important: many of the essential are conserved

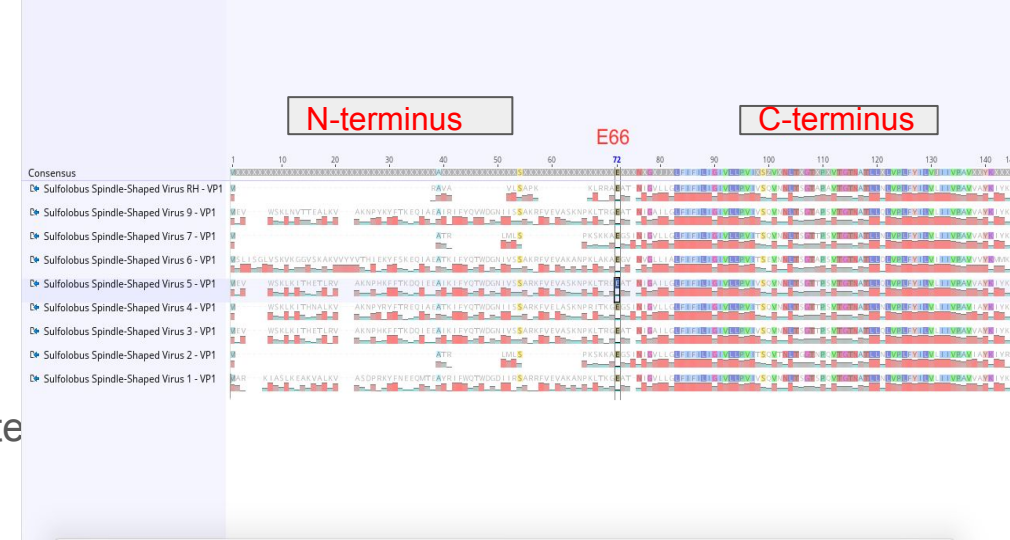




Capsid structure

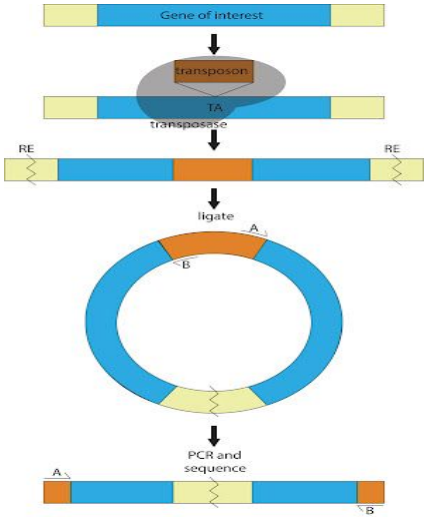
Capsid: VP1 and VP3 results in lemon shape

- Thermostability and acidophilic of virion
- VP1 has a E66 → proteolytic cleavage site
  - Maturation
- Essential
- E66A and E66Q mutants were created, infectious but abnormally shaped

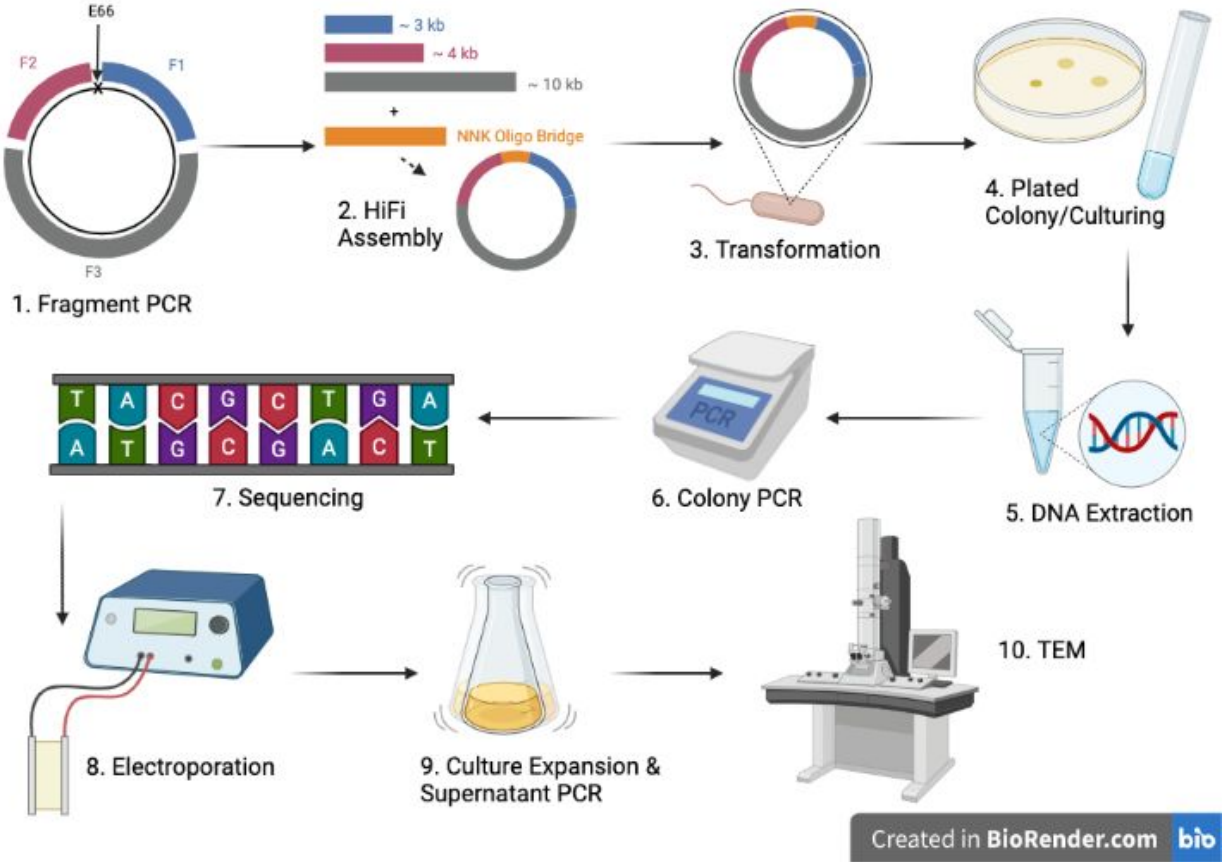


Aims

1. Mutagenizing the E66 position to the to the other 19 amino acids
    - Alanine and glutamine were infectious but with abnormal shapes



Methods


E66
F2
F1
F3
1. Fragment PCR
~ 3 kb
~ 4 kb
~ 10 kb
+
NNK Oligo Bridge
2. HiFi Assembly
3. Transformation
4. Plated Colony/Culturing
5. DNA Extraction
PCR
6. Colony PCR
7. Sequencing
8. Electroporation
9. Culture Expansion & Supernatant PCR
10. TEM
Created in BioRender.com

Results (So far…)/ Discussions

1. 14 amino acid changes, 47 mutants: Mutants have been created but infectivity and impact to capsid structure are up for debate.

Table 3: Rounds of assembly and the mutants created within them.

Round of HiFi assembly	Number of mutants
1	E66C E66G
2	E66R
3	E66N E66R (4X) E66I E66Q (3X) E66L (4X) E66K (2X) E66P (3X) E66S (3X) E66T (3X) E66F E66A

Future work

- Work of checking infectivity of these mutants
  - Halo assays are the usual methods → issue with growth on plates
  - New approach using qPCR
- Check structure of mutants
  - TEM
- Produce the remain mutants
- Electroporate all mutant into host

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Deniz

Jono

Jenni

References

1.Iverson, Eric, & Stedman, Kenneth. (2012). A genetic study of SSV1, the prototypical fusellovirus. Frontiers in Microbiology, 3, 200–200. https://doi.org/10.3389/fmicb.2012.00200

2. Iverson, Eric A, Goodman, David A, Gorchels, Madeline E, & Stedman, Kenneth M. (2017). Genetic Analysis of the Major Capsid Protein of the Archaeal Fusellovirus SSV1: Mutational Flexibility and Conformational Change. Genes, 8(12), 373. https://doi.org/10.3390/genes8120373

3. Quemin, Emmanuelle R J, Pietilä, Maija K, Oksanen, Hanna M, Forterre, Patrick, Rijpstra, W Irene C, Schouten, Stefan, Bamford, Dennis H, Prangishvili, David, & Krupovic, Mart. (2015). Sulfolobus Spindle-Shaped Virus 1 Contains Glycosylated Capsid Proteins, a Cellular Chromatin Protein, and Host-Derived Lipids. Journal of Virology, 89(22), 11681–11691. https://doi.org/10.1128/JVI.02270-15

4. Researchers at University of Texas Medical Branch Target Virology. (2018). Structural insights into the architecture of the hyperthermophilic Fusellovirus SSV1. In Life Science Weekly (p. 3664). NewsRX LLC.

5.. Iverson, Eric A, Goodman, David A, Gorchels, Madeline E, & Stedman, Kenneth M. (2017). Extreme Mutation Tolerance: Nearly Half of the Archaeal Fusellovirus Sulfolobus Spindle-Shaped Virus 1 Genes Are Not Required for Virus Function, Including the Minor Capsid Protein Gene vp3. Journal of Virology, 91(10). https://doi.org/10.1128/JVI.02406-16