

Developing and Testing a High-throughput Protocol to Measure Viral Fitness

Abstract

- Develop a sequencing-based high-throughput method of measuring viral fitness
- Study the affect of combinations of mutations in the G spike protein on viral fitness
- Phase One: Compare developed method to traditional fitness measures
- Compare data gathered to traditional fitness measures already collected for a several G protein mutants
- This comparison is important for gaining confidence in the high-throughput method
- Phase Two: Use the developed method to identify combinations of mutations in the viral spike protein that physically interact with a variety of host lipopolysaccharides (LPS) (Figure 1)

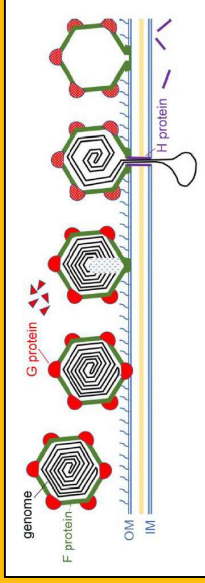


Figure 1: The major spike protein (G) and major capsid protein (F) recognize and bind to lipopolysaccharides (LPS) found on the outer membrane of host bacteria to transfer the PhiX174 genome into the host cell

Motivation

- Studying evolution of host-virus interactions
- Predicting viral mutations in nature
- Utilizing a sequencing-based competition assay of viral fitness
- Using high-throughput method of measuring viral fitness to study how combinations of mutations affect viral fitness

PhiX174 as a Model Virus

- An Icosahedral bacteriophage
 - Small, circular genome that is easy to manipulate
 - Site directed mutagenesis
 - Engineered fragments inserted using assembly platform (Figure 2)
- Ease of cultivation in laboratory conditions (non-pathogenic, large populations)
- Typically grown on *E. coli* C.

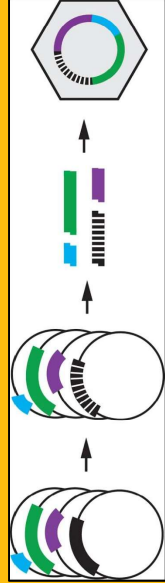


Figure 2: Depiction of phage assembly platform. Engineered fragments are ligated together to recreate viral genome with mutations incorporated.

Aims

- Phase One:** Compare developed method to traditional fitness measures
- Compare growth rate of individual viral genotypes through growth assays to already available data on G mutations
 - Gain confidence in high-throughput method
- Phase Two:** Characterize F protein viral mutations
- Use the developed method with hosts that have a different LPS variants for the media and assays.
 - Identify combinations of mutations in the viral spike protein that physically interact with host LPS

Methods

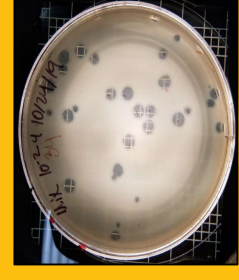


Figure 3: Plating of double G mutants after concentration.

- Mutant fragment libraries generated
 - Fragments assembled into viruses
 - Pools of mutant viruses are transformed, recovered in SOC & DNase I
 - Pellet cells, remove supernatant with phage
 - Filter to remove remaining cells, concentrate phage solution
 - Run high throughput fitness assay
- Plating occurs after steps 3, 4, 5

Results

- Phase One**
- Addition of DNase I removes extracellular DNA (Figure 5)
 - Transforming more ligation mix results in more phage (Figure 4)
 - Characterizing coverage of library in transformations is ongoing (Figure 6)
 - In process: preliminary high throughput assays with G double mutants

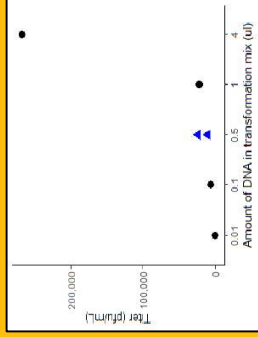


Figure 4: Titters of phage forming units per mL when various amounts of ligation mix were used in the transformation (Faber et al. 2019)

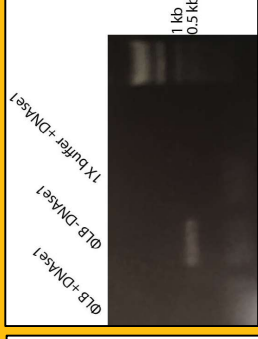


Figure 5: The use of DNase I removes fragments of viral DNA not taken up by cells during transformation before PCR, so as to not effect sequencing data (Faber et al. 2019)

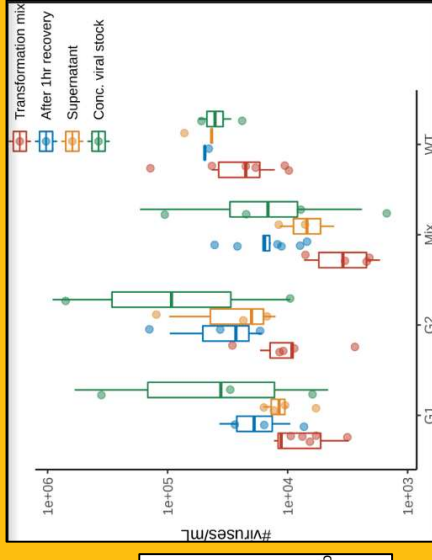


Figure 6: Viruses per mL of solution for G1 mutations, G2 mutations, G mixed mutations, and wild type virus after various stages of recovery and concentration.

Future Work

- Sequence gathered samples from G double mutants (IBEST Genomics Core)
- Analyze sequencing data and compare to pre-existing data on mutations of protein G
- Complete phase two of viral fitness assays on variety of hosts
- Analyze the generated sequencing data to characterize protein F mutations (Figure 7)

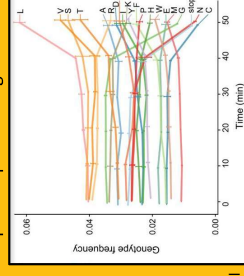


Figure 7: Changes in genotype frequency over time represents viral fitness of each genotype.

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Sources

Matthew S. Faber, James T. Van Leuven, Martina M. Ederer, Yesol Sapozhnikov, Zoë L. Wilson, Holly A. Wichman, Timothy A. Whitehead, Craig R. Miller. 2019. Saturation mutagenesis genome engineering of infective ΦX174 bacteriophage via unamplified oligo pools and golden gate assembly. *Biorxiv*.