Generating a Deep-Mutationally Scanned (DMS) CHIKV E3/E2 Virus Library to Map Virus-Antibody Interactions

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Abstract

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that can cause fever and severe, debilitating acute and chronic joint pain. There is currently no approved vaccine or specific treatment available for CHIKV. Using deep mutational-scanning (DMS) to individually substitute all amino acids at each position in the ectodomain of the E3/E2 glycoproteins of CHIKV, we can evaluate the effects of individual mutations on CHIKV cell entry, egress, and antibody-mediated neutralization. Using a novel CHIKV plasmid encoding a CMV promoter and mKate fluorescent reporter (pCHIKV-CMV-mKate), the E3/E2 ectodomain was mutagenized using NNK forward and reverse primer pools. Fragments were joined in a single joining PCR reaction and reintroduced back into a pCHIKV-CMV-mKate recipient plasmid encoding stop codons in E3 to prevent selection for WT virus. The ligation reaction was electroporated into DH10B Electromax cells and plated on LB-Amp agar plates to obtain 22,000 clones. 40 clones were miniprepped and sequenced by Sanger sequencing to evaluate mutation efficiency. We found that 36/40 (90%) clones contained a new, mutated E3/E2 insert and 32/36 (89%) contained no stop codons. The average number of mutations per productive clone (no stop codons) was 2.0 amino acid mutations/clone. The viral plasmid library was transfected into HEK293 cells to generate the initial virus library passage (p1) and then characterized for titer and mKate expression. P1 was then used to infect HEK293 cells at an MOI of 0.01 FFU/cell and the second passage (p2) harvested and characterized. Percent mKate positive cells for p1 and p2 were 39.6% and 89.7%, respectively. The plasmid library was submitted for deep sequencing using the NovaSeq 6000 platform. The average mutational frequency per codon is 8.0 x 10-3 for the plasmid library and the average number of amino acids represented at each codon is 16.4 [range = 5-21]. These results demonstrate a new method for characterizing viral mutations in an alphavirus, such as CHIKV. This comprehensive virus library can be used to characterize viral escape mutants in response to a variety of E3/E2 targeted antibody, vaccine, or drug challenges and guide decisions for therapies in clinical settings.

Background

Mosquito-borne alphavirus

Acute and chronic joint pain

No vaccine or cure available

- Since 2004, CHIKV has infected millions of people globally
- Alphavirus particles contain an ~11.5 kb plus-sense RNA genome
- CHIKV genome encodes non-structural proteins (nsP1, nsP2, nsP3, and nsP4) and the C, E3/E2 (p62), 6K/TF, and E1 structural proteins
- The function of virion-associated E3 remains unknown but the E2 ectodomain is the primary target of neutralizing antibodies (nAbs)

Deep Mutational Scanning Overview

Methodology

Figure 1. Project Overview

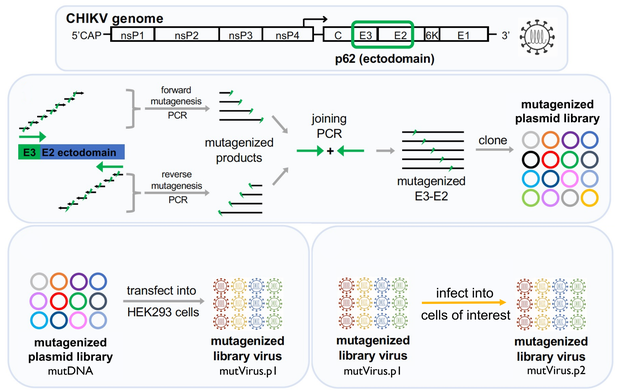


Figure 2. pCHIKV-CMV-mKate Design, Modifications, and Validation

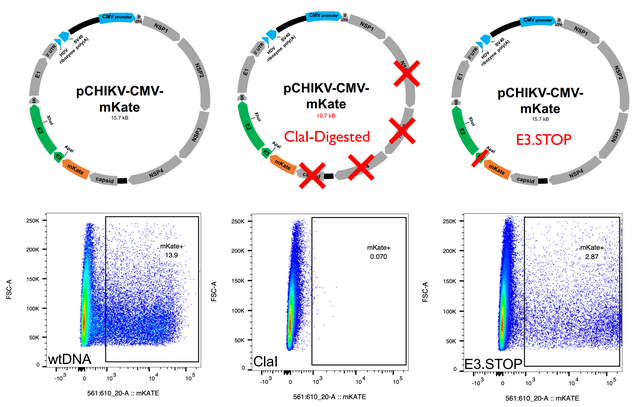
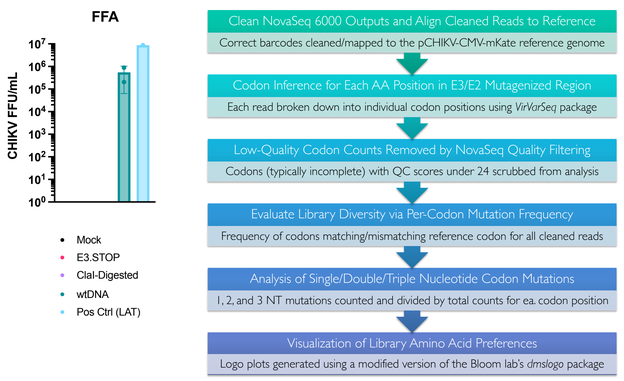
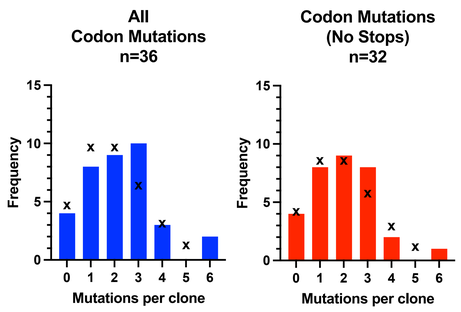


Figure 3. Analysis Plan of Mutagenized Plasmid and Virus Libraries



Results

Figure 4 (right). Sanger sequencing analysis of mutagenized inserts isolated from individual *E. coli* colonies reveals an average of 2.0 mutations/clone among clones without stop codons. A total of 40 bacterial colonies were miniprepped and analyzed for nonsynonymous mutations by Geneious. X's represent ideal Poisson distribution.



Results (cont.)

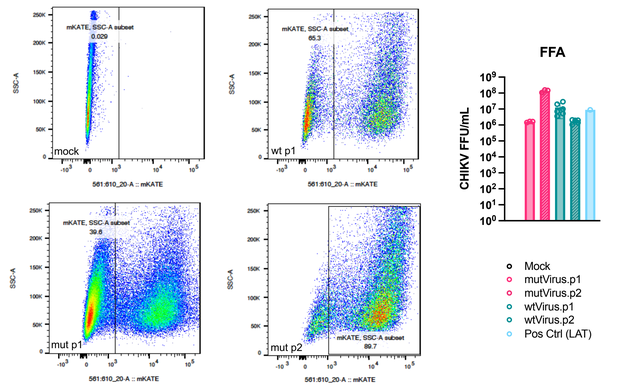


Figure 5 (above). HEK-293 cells were transfected with either mutDNA (plasmid library) or wtDNA (pCHIKV-CMV-mKate) using Lipofectamine 3000, or infected with 0.01 MOI of mutVirus.p1, and assayed for mKate expression 48 hours later by flow cytometry. Aliquots of virus were subsequently assayed for viral titer using a focus-formation assay (FFA). Positive controls include wtVirus.p1/p2 and left-ankle tissue (LAT) from CHIKV-infected mice at 24 hpi.

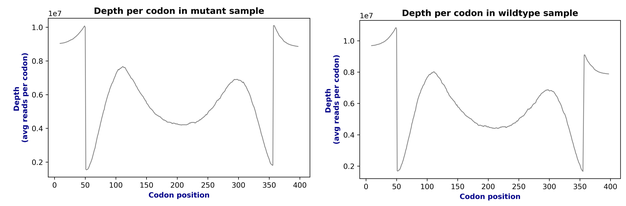
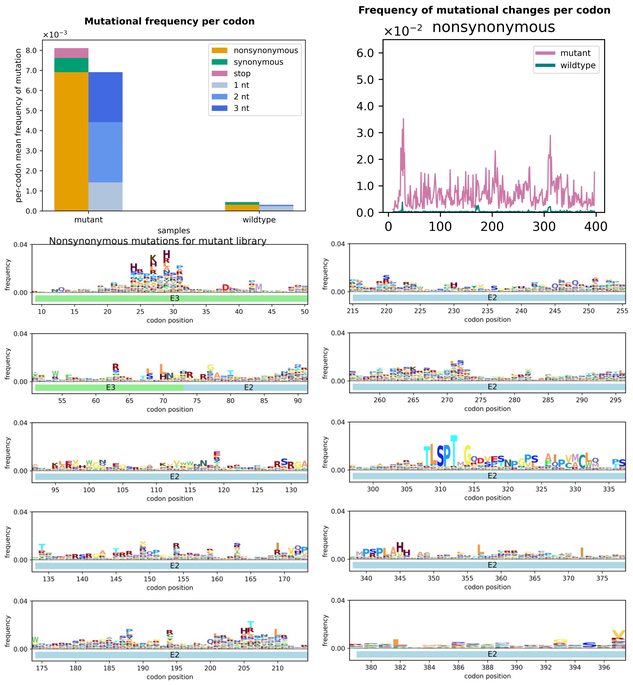


Figure 6 (above). The average read depth achieved by Illumina sequencing using the NovaSeq 6000 platform shown above. Total transformed read counts for mutDNA (left) and wtDNA (right) are shown for each amino acid position.



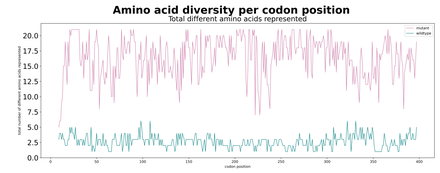


Figure 7 (above). Bar graph displays nonsynonymous, synonymous and stop codon mutation frequencies for the mutDNA and wtDNA libraries. Nonsynonymous mutations are broken into 1/2/3 nucleotide changes for both libraries. For the nonsynonymous mutations, the per codon mutation frequency is shown across the entire mutagenized region for mutDNA (pink) and wtDNA (teal) in the line plot shown above. For the mutDNA library, amino acid diversity for the mutated region is shown in the above logo plot for all nonsynonymous mutations with all WT amino acids removed for each position. The total number of codons represented for mutDNA and wtDNA are plotted across the length of the mutagenized region. The average number of codons per position is 16.4 (min: 5, max: 21) for mutDNA and 2.7 (min: 1, max: 6) for wtDNA.

Conclusions

- Deep mutational scanning (DMS) can be used to mutagenize large alphavirus proteins, like CHIKV p62.
- The pCHIKV-CMV-mKate plasmid is a useful tool in CHIKV transfection settings that removes the need for in-vitro transcription and demonstrates suitable transfection efficiency.
- The mKate reporter in the pCHIKV-CMV-mKate plasmid predicts viral titers and can be used to screen viral populations for infected cells but cannot discern populations with stop codons.
- Enzymatic-digestion and stop codon modifications to the pCHIKV-CMV-mKate plasmid successfully reduced background contamination of uncut plasmid and single-cut plasmid throughout the mutagenesis and cloning processes.
- Reduction of mutagenesis rounds resulted in sufficient product for ligation and successfully reduced the overall mutation frequency (per clone), as indicated by Sangar sequencing.
- Additional passaging of mutVirus.p1 (mutVirus.p2) increased the proportion of mKate+ cells and improved viral titers. This is likely due to additional selection of non-functional viral variants and expansion of more pathogenic mutants.
- Per-codon mutation frequency was increased ~7-fold over background with a relatively even distribution across the mutated region and representative amino acid diversity in the mutDNA library.

Future Directions

- Passage into other host cells (i.e., C6/36, fibroblasts, etc.)
- Deep sequence mutVirus.p1 and mutVirus.p2 and analyze variant diversity
- Continue to investigate optimization of mutational frequency indicated by Sangar sequencing (data not shown)
- Compare subamplicon approach for deep sequencing using *dms_tools2*
- Conduct replicate 2
- Challenge virus library with monoclonal antibody to measure selection
- Challenge virus library with naturally infected and vaccine-recipient sera

Contact Information

"Generating a Deep-Mutationally Scanned (DMS) CHIKV E3/E2 Virus Library to Map Virus-Antibody Interactions"

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