Analysis of defective viral genomes during influenza replication

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Background

RNA virus populations exhibit substantial genetic heterogeneity. Defective viral genomes (DVGs) are defined as incomplete or altered versions of virus genomes with insertions or deletions (indels). DVGs cannot replicate independently but may interact cooperatively or competitively during viral replication cycles. This project aims to develop a platform to detect and analyze influenza DVGs using long-read sequencing.

Methods

A(H1N1)pdm09 was serial passaged 7 times in MDCK cells under high and low multiplicity of infection (MOI). Viral RNA was extracted, reverse transcribed, amplified with uni12 and uni 13 primers. The PCR amplicons were sequenced using Nanopore MinION. Reads without the conserved 12 and 13 nucleotides of the 3' and 5' ends of viral genome were filtered using Samtools (v1.20), and indels were parsed from CIGAR strings using GenomicAlignments (v1.38.2).

Changes in MOI lead to fluctuations in infectious viral titre during serial passage of A/California/04/2009 *in vitro*

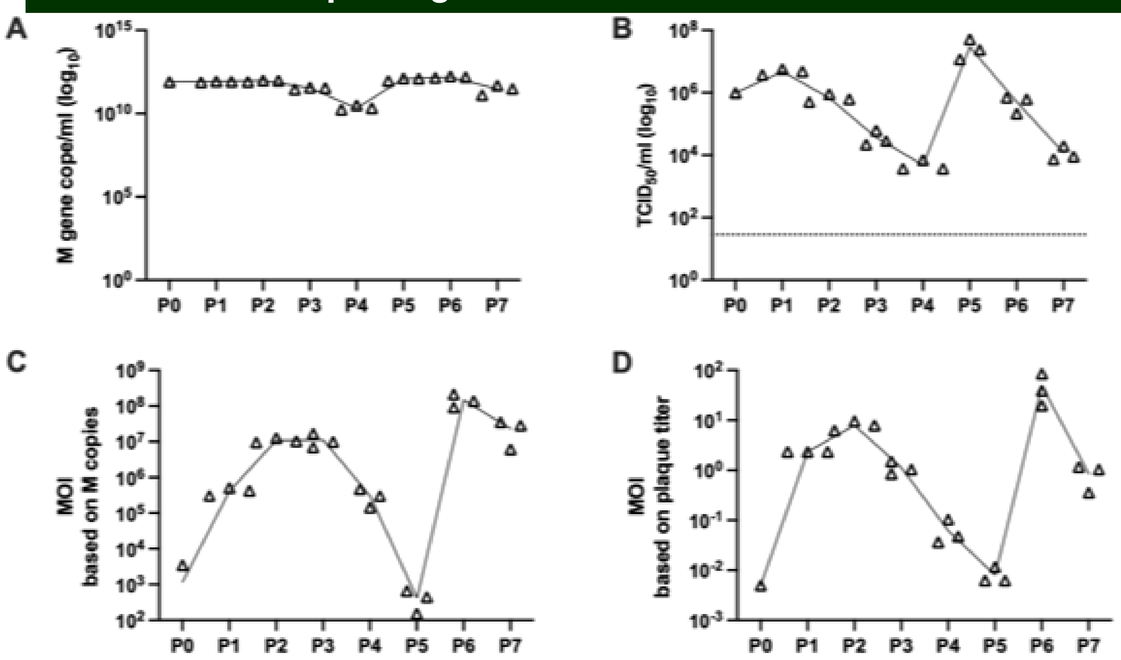

Fig 1. Serial passage of A/California/04/2009(H1N1) viruses in MDCK cells. The serial passaged were conducted with three biological replicates under MOI of 2 at P1. To avoid titre loss during freeze and thawing, serial passages from P1 to P7 were conducted continuously in MDCK cells by aiming at transferring 2×10^{12} M gene copies to the next passage. The (A) viral M gene copies and (B) infectious viral load in the culture supernatant during serial passages were determined by qRT-PCR and $TCID_{50}$ assay, respectively. The MOI at each passages was calculated based on M gene copies (C) and infectious viral load (D). P0: stock virus, P1-P7: passage 1 to passage 7.

Higher DVGs abundance in Polymerase genes and NA genes

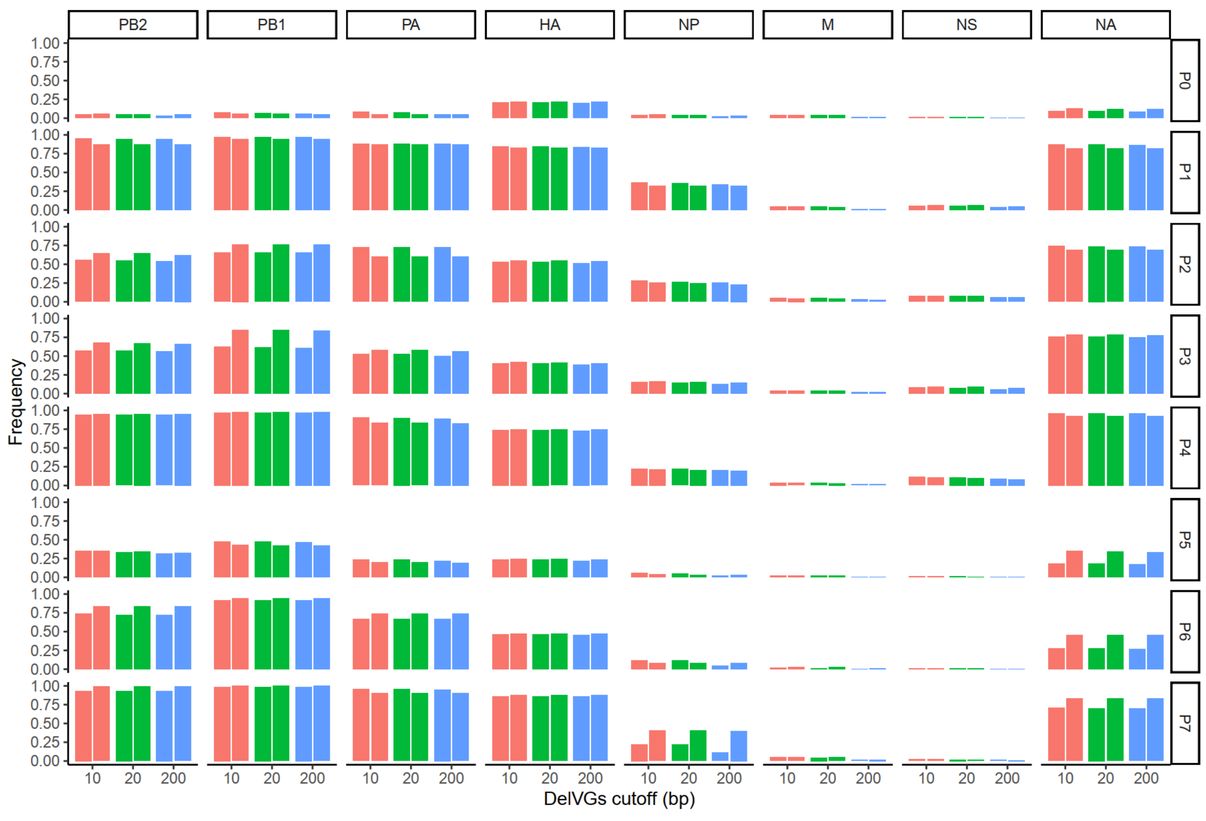

Fig 2. The impact of deletion cutoff on the detection frequency of DVGs. Nanopore reads containing deletions larger than the specified cutoff values (10 bp, 20 bp, or 200 bp) were used as proxies for large internally deleted viral genomes. The detection frequency of these deleted genomes was stratified by gene segments and passages. Two independent biological replicates were presented.

Objectives

This project aims to develop a platform to detect and analyze influenza DVGs using long-read sequencing.

Results

During the serial passages, the frequency of deleted genomes varied depending on the MOI and the gene segment length. A single passage at MOI of 2 increased the mean frequency of deleted genomes in long segments (PB2, PB1, PA, HA, NA) from 10% to 90%, while shorter segments (NP, M and NS) maintained at 4% to 34%. With two consecutive passage at MOI below 1 (P4 to P5), the frequency of deleted genomes in long segments dropped to 31%. Notably, in PB2 and NA segments, deleted genomes with unique break points can be detected above 5% from P2 to P7.

DVG abundance of polymerase genes was depended on MOI

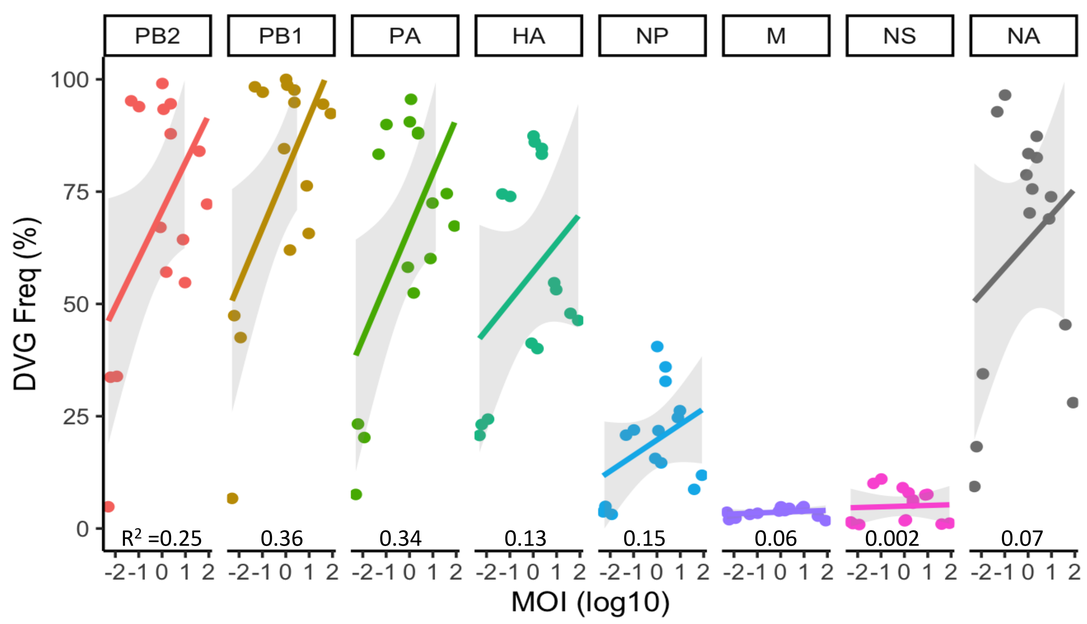

Fig 3. Relationship between MOI and detection frequency of deleted viral genomes. Deleted viral genomes were identified using a 20 bp deletion threshold. Each data point represents the detection frequency of deleted viral genomes for a specific gene segment. The linear regression analysis was performed, with a 95% confidence interval displayed around the trend line. The coefficient of determination (R^2) is provided at the bottom of the graph.

Identification of DVGs with unique breakpoints

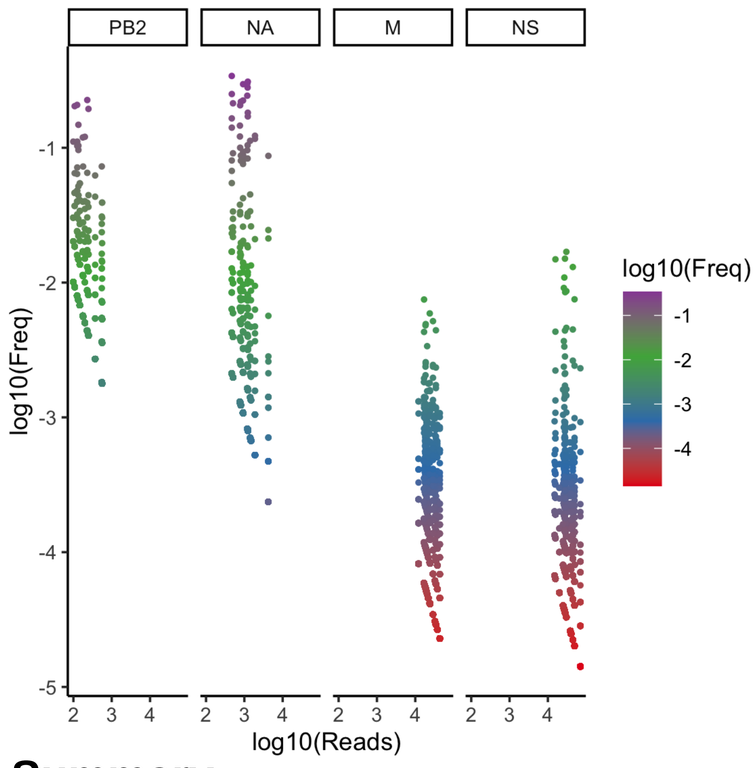

Fig 4: Frequency of individual DVGs with unique break points. Each dot represented a DVG with a unique breakpoint, color-coded according to its detection frequency. The detection frequency for individual DVGs was determined by dividing the read support by the sequencing depth of the corresponding gene segment.

Summary

1. Nanopore sequencing facilitated the comprehensive characterization of DVGs during influenza infection.
2. The established protocol and computational pipeline can be extended to investigate the role of DVGs in modulating viral infectivity within and across hosts.

References

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Acknowledgements

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