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2025 SEA Symposium Abstract

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A Tale of Two Tails: The Characterization and Annotation of Novel FH and FL-Cluster Bacteriophages Circuit and Vitus

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Bacteriophages are a class of viruses that can infect bacteria. In this study, we explore and compare the genomes of two related myoviridae bacteriophages Circuit and Vitus.   
Circuit was discovered in a soil sample taken from alongside the Rideau Canal in Ottawa.   
  
Circuit is an FH cluster bacteriophage with a myoviridae morphology with a genome size of 76 genes across 100 base pairs. Its genome consists of 76 genes across 100 base pairs, with a GC content of 70.3%. Infection of *Arthrobacter globiformis* NRRL B-4225 by Circuit yielded clear, medium-sized plaques, which are characteristic of lytic phages.   
  
Vitus is a myoviridae phage isolated from a flower bed in Kanata, Ottawa, that infects host bacteria *A. globiformis* NRRL B-2979. Vitus’ genome encodes 80 genes across 47597 base pairs, with a GC content of 66.1%. Infection of *A. globiformis* by Vitus yielded medium-sized turbid plaques, which are characteristic of lysogenic phages.   
  
Dot plots were used to compare Vitus and Circuit, visualizing genetic similarities between phages. Gepard Dot Plot software analyzed six FH cluster phages and five FL cluster phages, including Circuit (FH) and Vitus (FL). While phages in the same cluster are generally similar, key genetic differences were found. Circuit is the only FH phage infecting bacterial host 4225. This unique host range may explain some of the diversity seen in the dot plot, highlighting potential adaptations.  
  
Both Vitus and Circuit contain a tail assembly chaperone (TAC) gene, however, canonical slippery sequences were absent, preventing the annotation of a long form of the TAC gene in both phages. Evidence supporting the likely presence of a frameshift was present; Circuit had a potential 3' open reading frame (ORF) annotated as NKF, while Vitus showed strong coding potential despite lacking a start codon to allow prediction of a second ORF.  
  
We are testing the hypothesis that a novel slippery sequence is used in both phages allowing a long form of the TAC to be formed. We have created plasmids that inducibly express a GST-fusion of the TAC in E. coli to test if a long form is translated, and if so, to identify the site of the slippery sequence by protein sequencing using mass spectrometry. Our preliminary data suggests that long forms of both TACs are made in our E. coli expression system, and we are determining the ratio of the short to long forms.   
  
We have also been able to create a Vitus lysogen. This lysogen confers super-infection immunity to re-infection by Vitus. Despite forming a stable lysogen, our annotation has not identified an integrase or Par partitioning system, suggesting a novel mechanism of lysogeny functions in this phage. We are exploring if any NKF may have integrase activity, or whether another recombinase can function to catalyze phage insertion into the host genome.