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The Good Fortune of Annotating LittleFortune: Big Functionalities and Big Opportunities for LittleFortune

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A bacteriophage is a virus that enters into bacterial cells and reproduces inside the bacteria. Bacteriophages are becoming vital for human development as they can be used to cure infections that are resistant to antibiotics. Viruses require a host to infect in order to grow and replicate. For our project, it was determined that Microbacterium foliorum is a good host due to its availability in soil and its short doubling time. Our phage, LittleFortune, was discovered in a soil sample taken from outside the VCU cafeteria under a hedge in Richmond, VA. The sample was shaken, filtered via a vacuum, isolated in the fridge, and M. foliorum host cells were added to allow for sample growth at 30 degrees C. The sample was tested for presence of phage and the positive sample went through 5 rounds of purification by plaque assay. DNA was then isolated from phage particles and submitted for sequencing. The genome length of LittleFortune is 53161, with a GC content of 69.0%, and the end characteristics are circularly permuted. We annotated the positions and functions of 91 genes of bacteriophage LittleFortune genome. This process included identifying and interpreting alignment scores, query coverage, e-value, and percent identity of all proteins. Although the number of protein coding genes is 91, only 27 (30%) have a predicted functional annotation, and there are zero tRNAs.
For annotating LittleFortune gene functions, we used Bioinformatics tools BlastP, TMHMM, Deep TMHMM, HHPred, and Phamerator. Three types of functional annotations will be described. Membrane protein: TMHMM is used to predict the amount of transmembrane helices in each gene product (gp): for example, in gp47 there were 3 predicted helices, and the sequence and structural predictions will be shared. Synteny annotation: In order for a functional annotation as tail assembly chaperone, a series of two proteins must be next to the tape measure protein as well as contain a ‘slippery sequence’ in the second protein. This slippery sequence is not present in LittleFortune gp 35 and 36. The differences in tail assembly chaperone gene features will be shared. Enzyme function: Functional annotation of glycosyltransferase was determined by the HHpred Bioinformatics tool, and previous literature exists that supports this function. The information gathered from annotating the phage, LittleFortune, adds to the library of knowledge which can be used by researchers conducting work in structural programming of proteins and protein threading. Although auto-annotation programs are helpful, the manual annotation work we have conducted increases the accuracy of our phage library of knowledge and allows us to more deeply investigate interesting genome features.