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Investigating Nucleotide-binding Proteins in Bacteriophage JohnDoe

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*Siphoviridae* bacteriophage JohnDoe is a temperate bacteriophage in the AZ cluster that infects *Arthrobacter globiformis* bacteria. Here, we discuss nucleotide-binding proteins observed in the genome annotation of bacteriophage JohnDoe. In particular, we focus on unique DNA and RNA-binding proteins, as well as a DNA methyltransferase.   
   
Gp52 has a high probability of sharing structural features with several Sm- and lsm- RNA binding proteins, as well as an Hfq- RNA binding protein. This annotation is missing in many AZ cluster bacteriophages. We compare this RNA-binding protein with similarly classified proteins in other phages using the R programming language, and our preliminary analysis suggests that the JohnDoe RNA-binding protein is remarkably different from other phage RNA-binding proteins.  
   
We also analyze the DNA-binding proteins found in JohnDoe, and investigate potential immunity repressors, given that all AZ phages analyzed at the University of Ottawa are capable of forming lysogens, with several displaying superinfection immunity.  
   
Finally, we compare JohnDoe to three other AZ cluster bacteriophages: Cassia, Crewmate, and ObiToo. JohnDoe and Obitoo share a DNA methyltransferase (gp37 and gp42 in the submitted annotations), while Crewmate has a distinct methyltransferase (gp52). In contrast, Cassia does not contain either methyltransferase.  
   
To investigate whether these methyltransferases are used as a mechanism to evade host immunity, we examine the genome sensitivities of phages Cassia, Crewmate, JohnDoe and ObiToo to restriction enzymes whose activity is altered by the presence or absence of adenine (A) or cytosine (C) methylation (DpnI, DpnII, and Sau3A). More specifically, DpnI only cuts GATC motifs when A is methylated, while DpnII exhibits the opposite activity, and can only cut unmethylated DNA. Sau3A, on the other hand, will cleave GATC motifs regardless of A methylation, but its activity is blocked by C methylation. We anticipate that Cassia’s DNA will be digested by DpnII and Sau3A, and will examine whether the digestion patterns of JohnDoe, ObiToo and Crewmate reveal if GATC motifs are the target of these two DNA methyltransferases, as well as determine whether either of these two methyltransferases specifically modifies A or C nucleotides.