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Annotation of Cluster B3 Mycobacteriophages Obutu and Rita1961

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Mycobacteriophages Obutu and Rita1961 were isolated from enriched soil samples in Atlanta, Georgia, using *Mycobacterium smegmatis* as the host. Obutu and Rita1961 are Cluster B3 phages that are 69021 and 69026 bp long, respectively. The purpose of our research was to finalize the draft annotation of this phage by manually confirming potential genes and identifying gene functions. Utilizing the annotation site PECAAN (https://discover.kbrinsgd.org) and guided by heuristic GeneMark output we determined the most likely open reading frames to identify each gene in this genome. Starterator reports were used to help identify the most conserved starts in each pham, and BLASTp searches and HHPred searches were used to identify the function of more than half of the genes in this genome. Comparisons between the Phamerator maps showed remarkable similarity in both nucleotide sequences and protein products for all cluster B3 phage. In our completed annotation, there are a total of 103 genes in Rita1961 and 104 genes in Obutu. No tRNA or tmRNA sequences were located. The functions of 33 of the 104 genes in Rita1961 and Obutu were identified. Interestingly, there is a region of no homology between Rita1961 and Obutu at the start of the forward gene cluster at approximately 56,500 bp for about 1500 base pairs. The genes contained within this cluster do not have known functions. This is a known region of divergence in B3 genomes, with the B3 phages having either the Rita1961-like or the Obutu-like cluster. Rita1961 also contains an additional forward gene, not present in Obutu, at 67,977 bp which is a member of pham 3689 and is frequently found in B3 phages.