**Student Gene Annotation Worksheet**

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| **Basic Phage Information** | |
| **Phage Name** | **Wardwill** |
| **Gene #** | **58** |
| **Stop Coordinate** | **37124** |
| **Direction (For/Rev)** | **Rev** |
| **Gap (Overlap) with Previous Gene** | **No** |
| **Selected Start Coordinate** | **37372** |
| **Selected Function** | **Hypothetical Protein** |

**Annotation Decision #1: Is this a Gene?**

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| **Gathering Evidence** | **Explain Your Rationale** |
| Was the gene called by an auto-annotation program (Glimmer, GeneMark)? | *YES BOTH (different start sites)* |
| Is there evidence for coding potential? | *GeneMarkS andr GeneMark-M. folio show coding potential.* |
| Is this gene present in other annotated genomes? | *PhagesDB Blast hit: QuadZero\_57 ; e-value= 4e-41*  *Phamerator: phage: Neferthenea; gene: 58; pham: 2778* (4-18-25) |
| Does the gene violate any major guiding principles? | *No* |
| **DECISION:** | *YES* |

**Annotation Decision #2: What is the best possible start site for this gene?**

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| **Gathering Evidence** | **Explain Your Rationale** |
| What start site do Glimmer and GeneMark suggest? | *Glimmer start site: 37372*  *GenMark start site: 37375* |
| Does the start site have an associated Ribosome Binding Site with a high score? | *Start site 37372*  *Final Score: -3,598*  *Z-value: 2,452*  *This is the best score.*  *Start site 37375*  *Final score: -4,426*  *Z-value: 2,452* |
| Is the predicted start codon the longest ORF? If not, does the longest ORF result in excessive gene overlap (>30bp)? | *It’s not the longest ORF and doesn’t result in gene overlap.* |
| Is this start site conserved in other phage genomes as indicated by Starterator? | *The start site predicted by Glimmer is indicated by starterator as 13, and it’s conserved in other phage genomes.* |
| Is this start site conserved in other phage genomes as indicated by BlastP? | ***PhagesDB***  *Best Hit: QuadZero\_57, function unknown*  *E-value: 4e-41*  *Q#1: MAEAYTIVLVDESLW*  *S#1: MAEAYTIVLVDESLW*  ***BlastP match from NCBI***  *Best Hit: hypothetical protein SEA\_QUADZERO\_57 [Microbacterium phage QuadZero]*  *E-value: 1e-53*  *Q#1: MAEAYTIVLVDESLWK*  *S#1: MAEAYTIVLVDESLWK*  ***DNA Master***  *Best Hit: hypothetical protein SEA\_QUADZERO\_56 [Microbacterium phage QuadZero]*  *E-value: 0*  *Q#1: MNADRRVAGK*  *S#1: MNADRRIAGK* |
| **DECISION:** | *The gene should start at 37372* |

**Annotation Decision #3: What is the Function of the Putative Protein?**

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| **Gathering Evidence** | **Explain Your Rationale** |
| Does this protein align with a protein having a functional assignment in BlastP (phagesDB and/or GenBank) with an alignment of 10-4 or smaller with appropriate coverage? | *List the most informative BlastP match from each source PhagesDB: QuadZero\_57, function unknown*  *NCBI: hypothetical protein SEA\_QUADZERO\_57 [Microbacterium phage QuadZero]*  *DNA Master: hypothetical protein SEA\_QUADZERO\_56 [Microbacterium phage QuadZero]* |
| Does this protein align with a protein having a functional assignment in the PDB or other database in HHPred with a probability of 90% or greater with appropriate coverage? | *Best match: 2RAW\_B Borealin; Cell cycle, DasraB, chromosomal passender complex, IAP, BIR, Apoptosis, Cell division, Centromere, Chromosomal protein, Metal-binding*  *Probability: 68.03%, E-value: 18* |
| Is this gene located adjacent to genes of known function and in a region of the genome that shows high conservation of gene order? | *According to the July 30, 2025 Phamerator database version, Pham 2778 is flanked upstream by Pham 197878 and downstream by Pham 87511, forming a conserved syntenic arrangement among related phages.* |
| Is this gene a possible transmembrane protein? | *NO* |
| Is the proposed function found on the SEA-PHAGES approved function list? | *NO* |
| **DECISION:** | *Hypothetical Protein* |