**Student Gene Annotation Worksheet**

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| **Basic Phage Information** | |
| **Phage Name** | **Wardwill** |
| **Gene #** | **14** |
| **Stop Coordinate** | **8203** |
| **Direction (For/Rev)** | **For** |
| **Gap (Overlap) with Previous Gene** | **Overlap 3pb** |
| **Selected Start Coordinate** | **7853** |
| **Selected Function** | ***tail terminator*** |

**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* |
| Is there evidence for coding potential? | *GeneMarkS and GeneMark-M. folio show coding potential.* |
| Is this gene present in other annotated genomes? | *PhagesDB Blast hit: QuadZero\_13 with e-value=8e-63*  *Phamerator: phage: QuadZero; gene: 13; pham: 640 (05/05/2025)* |
| 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 Coordinate: 7853*  *GeneMark Start Coordinate: 7853* |
| Does the start site have an associated Ribosome Binding Site with a high score? | *Final RBS score: -3.593*  *Z-score: 2.521*  *This is the best score.* |
| Is the predicted start codon the longest ORF? If not, does the longest ORF result in excessive gene overlap (>30bp)? | *YES.* |
| Is this start site conserved in other phage genomes as indicated by Starterator? | *The start number called is the most often in the published annotations.*  *This start number was called in 143 of 182 non-draft genes in the pham.*  *Called 97.5% of time when present* |
| Is this start site conserved in other phage genomes as indicated by BlastP? | *DNA Master:*  *Best hit: QWY84459 (QuadZero)*  *E-value: 0*  *Q#1 MSIWDDIHAL*  *S#1 MSIWDDIHAL* |
| **DECISION:** | *Keep DNA Master predicted start site* ***7853*** |

**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? | *PhagesDB: QuadZero\_13, tail terminator E value 8e-63*  *NCBI: QuadZero\_13, tail terminator E value 4e-79* |
| 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? | *Tail terminator protein Rcc01690; "neck", "portal", "capsid", "tail tube", VIRUS; 3.58A {Rhodobacter capsulatus}*  *Probability:97.55*  *E value :0.0011* |
| 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 Phamerator, this gene is flanked upstream by a scaffolding protein and downstream by a hypothetical protein belonging to a pham family conserved among related phages.* |
| Is this gene a possible transmembrane protein? | *NO* |
| Is the proposed function found on the SEA-PHAGES approved function list? | *YES* |
| **DECISION:** | *tail terminator* |