**Student Gene Annotation Worksheet**

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| **Basic Phage Information** | |
| **Phage Name** | **Wardwill** |
| **Gene #** | **43** |
| **Stop Coordinate** | **29527** |
| **Direction (For/Rev)** | **Rev** |
| **Gap (Overlap) with Previous Gene** | **1 bp** |
| **Selected Start Coordinate** | **29751** |
| **Selected Function** | **Membrane 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* |
| Is there evidence for coding potential? | *GeneMarkS shows coding potential* |
| Is this gene present in other annotated genomes? | *PhagesDB Blast hit: LilTerminator, gene 42, function unknown, 74 Score 154 Evalue 6e-38*  *Phamerator: phage: LilTerminator, gene: 42, pham: 219944* |
| 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: 29751*  *GeneMark Start Coordinate: 29751* |
| Does the start site have an associated Ribosome Binding Site with a high score? | *Start site 29751.*  *RBS final score: -3.854*  *Zvalue: 2.369* |
| Is the predicted start codon the longest ORF? If not, does the longest ORF result in excessive gene overlap (>30bp)? | *The predicted start codon is the longest ORF (225bp)*  *There is an overlap of 1bp with the downstream gene (gene 44)* |
| Is this start site conserved in other phage genomes as indicated by Starterator? | *This start site was found in 17 of 34 ( 50.0% ) of genes in pham.*  *Called 100.0% of time when present*  *(29751)* |
| Is this start site conserved in other phage genomes as indicated by BlastP? | *the best BlastP match from NCBI:*  *QOP65238.1 [Zayuliv]*  *e-value: 4e-42*  *Q#: 1 MDECKGYACPPST*  *S#: 1 MDECKGYACPPSK* |
| **DECISION:** | *Mantain DNA master predicted start site, 29751* |

**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: LilTerminator, function unknown*    *evalue: 6e-38*  *NCBI: membrane protein [Zayuliv]*    *evalue: 4e-42*  *DNA Master: membrane protein, Zepp*    *evalue: 1.1E-17* |
| 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? | *HHPred best match: 4Z1P\_A (Cell wall surface anchor protein; Adhesins, cell adhesion; 1.89A {Streptococcus agalactiae})*  *Probability: 92,76*  *Database: PDB* |
| 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 31, 2025 Phamerator database version, this gene is flanked upstream by a gene from Pham 7632 and downstream by a MazG-like nucleotide pyrophosphohydrolase, forming a conserved syntenic block among related phages.* |
| Is this gene a possible transmembrane protein? | *According to DeepTMHMM, this protein is an outside protein*    *The program SOSUI shows that this protein has a transmembrane region:* |
| Is the proposed function found on the SEA-PHAGES approved function list? | *YES* |
| **DECISION:** | *This is a membrane protein.* |