**PHAGE BINTII – CLUSTER EE – ANNOTATION NOTES**

**GENE 1** **hypothetical protein**

Original Glimmer call @bp 41 has strength 10.72

SSC: 41 to 379 (Forward)

CP: yes

SCS: both

ST: SS, Start 3 [41], Found in 125 of 125 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_1, function unknown, query 1-112 subject 1-112, E value = 6e-59, Identity = 100%, Similarity = 100%

GAP: 41bp

LO: NA

RBS: Kibler7, spacer 2.050, Z score 13, Final -5.036, yes

F: NKF

SIF-blast: aligns with hypothetical protein from Microbacterium Nobel, ID YP\_009996687.1, query 1-112 subject 1-112, E value = 4e-73, Identity = 100%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

**GENE 2 hypothetical protein**

Original Glimmer call @bp 376 has strength 7.17

SSC: 376 to 1101 (Forward)

CP: yes

SCS: both

ST: SS, Start 2 [376], Found in 122 of 122 ( 100.0% ) of genes in pham, Called 95.1% of time when present

BLAST-start: aligns with Microbacterium phage PuffyCat\_2, function unknown, query 1-241 subject 1-241, E value = e-143, Identity = 100%, Similarity = 100%

GAP: ATGA overlap

LO: NA

RBS: Kibler7, spacer 13, Z score 2.400, Final -4.334, yes

F: NKF

SIF-blast: aligns with hypothetical protein from Microbacterium PuffyCat, ID WNN94210.1, query 1-241 subject 1-241, E value = 5e-169, Identity = 100%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

**GENE 3 terminase**

Original Glimmer call @bp 1104 has strength 12.04

SSC: 1104 to 2564 (Forward)

CP: yes

SCS: both

ST: NI, Start 101 [1104], Found in 125 of 1252 ( 10.0% ) of genes in pham, Called 93.6% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_3, terminase, query 1-486 subject 1-486, E value = 0.0, Identity = 100%, Similarity = 100%

GAP: 3bp

LO: NA

RBS: Kibler7, spacer 17, Z score 2.136, Final -5.818, yes

F: terminase

SIF-blast: aligns with terminase from Microbacterium phage Nobel, ID YP\_009996689.1, query 1-486 subject 1-486, E value = 0.0, Identity = 100%, Similarity = 100%

SIF-HHPred: 6Z6D\_A, Terminase large subunit, genome packaging, bacteriophage, ATPase, nuclease, VIRAL PROTEIN, HET: BR; 2.2A, Enterobacteria phage HK97, Probability= 100%, E-value= 2.6e-42

SF-Syn: This gene is pham 130373 [terminase], upstream is pham 85210, downstream is pham 131864 [portal protein], as seen in Microbacterium phage Upsilon.

**GENE 4 portal protein**

Original Glimmer call @bp 2689 has strength 12.75; GeneMark calls start at 2794

SSC: 2689 to 3816 (Forward) – all data [starterator, RBS, etc show 2689 as the start site]

CP: yes

SCS: both - GL

ST: SS, Start 16 [2689], Found in 125 of 179 ( 69.8% ) of genes in pham, Called 83.2% of time when present

Start 25 [2794], Found in 60 of 179 ( 33.5% ) of genes in pham, Called 10.0% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_4, portal protein, query 1-375 subject 1-375, E value = 0.0, Identity = 100%, Similarity = 100%

GAP: 125bp

LO: yes

RBS: Kibler7, spacer 15, Z score 1.969, Final -5.756, yes

F: portal protein

SIF-blast: aligns with portal protein from Microbacterium phage Nobel, ID YP\_009996690.1, query 1-375 subject 1-375, E value = 0.0, Identity = 100%, Similarity = 100%

SIF-HHPred: 8FQL\_L, Portal protein, Prohead I, icosahedral symmetry, HK97, phage, capsid, 3.6A, Escherichia phage HK97, Probability= 100%, E-value= 5.2e-38

SF-Syn: This gene is pham 131864 [portal protein], upstream is pham 130373 [terminase], downstream is pham 1062 [major capsid and protease fusion protein], as seen in Microbacterium phage Upsilon.

**GENE 5 major capsid and protease fusion protein**

Original Glimmer call @bp 3813 has strength 14.83

SSC: 3813 to 5399 (Forward)

CP: yes

SCS: both

ST: SS, Start 2 [3813], Found in 125 of 125 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_5, major capsid and protease fusion protein, query 1-528 subject 1-528, E value = 0.0, Identity = 99%, Similarity = 99%

GAP: ATGA overlap

LO: NA

RBS: Kibler7, spacer 15, Z score 3.089, Final -3.509, yes

F: major capsid and protease fusion protein

SIF-blast: aligns with major capsid and protease fusion protein from Microbacterium phage Miaurora, ID AYD84501.1, query 1-528 subject 1-528, E value = 0.0, Identity = 99%, Similarity = 99%

SIF-HHPred: 6TSU\_A4, Major capsid protein Rcc01687, "capsid", "jelly roll", "spike", "HK97", 3.42A, Rhodobacter capsulatus DE442, Probability= 99.92%, E-value: 2.3e-23

SF-Syn: This gene is pham 1062 [major capsid and protease fusion protein], upstream is pham 131864 [portal protein], downstream is pham 1065 [head-to-tail adaptor], as seen in Microbacterium phage Upsilon.

**GENE 6 head-to-tail adaptor**

Original Glimmer call @bp 5403 has strength 14.88

SSC: 5403 to 5753 (Forward)

CP: yes

SCS: both

ST: SS, Start 2 [5403], Found in 129 of 129 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Kaijohn\_6, head-to-tail adaptor, query 1-116 subject 1-116, E value = 6e-59, Identity = 99%, Similarity = 99%

GAP: 3bp

LO: NA

RBS: Kibler7, spacer 16, Z score 2.665, Final -4.553, yes

F: head-to-tail adaptor

SIF-blast: aligns with head-tail connector protein from Microbacterium phage Noelani, ID YP\_009996717.1, query 1-116 subject 1-116, E value = 3e-76, Identity = 98%, Similarity = 98%

SIF-HHPred: 7Z4W\_l, Head completion protein gp15, Bacteriophage, SPP1, Portal Protein, Head completion proteins, Connector Complex, DNA Channel, 2.7A, Bacillus subtilis, Probability: 99.48%, E-value: 8.8e-13

SF-Syn: This gene is pham 1065 [head-to-tail adaptor], upstream is pham 1062 [major capsid and protease fusion protein], downstream is pham 1057 [tail terminator], as seen in Microbacterium phage Upsilon.

**GENE 7 tail terminator**

Original Glimmer call @bp 5750 has strength 17.55

SSC: 5750 to 6130 (Forward)

CP: yes

SCS: both

ST: SS, Start 2[5750], Found in 129 of 129 ( 100.0% ) of genes in pham, Called 99.2% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_7, tail terminator, query 1-126 subject 1-126, E value = 2e-65, Identity = 100%, Similarity = 100%

GAP: ATGA overlap

LO: NA

RBS: Kibler7, spacer 7, Z score 1.513, Final -6.591, yes

F: tail terminator

SIF-blast: aligns with tail terminator from Microbacterium phage TinyTruffula, ID QKY79162.1, query 1-126 subject 1-126, E value = 2e-83, Identity = 99%, Similarity = 99%

SIF-HHPred: 8HQO\_b, Tail terminator protein; Neck, Portal, T5, VIRUS, VIRAL PROTEIN; 3.2A, Escherichia phage DT57C, Probability: 97.99%, E-value: 0.0013,

SF-Syn: This gene is pham 1057 [tail terminator], upstream is pham 1065 [head-to-tail adaptor], downstream is pham 139535 [major tail protein], as seen in Microbacterium phage Upsilon.

**GENE 8 major tail protein**

Original Glimmer call @bp 6169 has strength 18.08

SSC: 6169 to 6609 (Forward)

CP: yes

SCS: both

ST: NI, Start 5 [6169], Found in 129 of 193 ( 66.8% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_8, major tail protein, query 1-146 subject 1-146, E value = 3e-83, Identity = 100%, Similarity = 100%

GAP: 39bp

LO: yes

RBS: Kibler7, spacer 10, Z score 1.975, Final -4.836, yes

F: major tail protein

SIF-blast: aligns with major tail protein [Microbacterium phage PoRanda, ID YP\_009996744.1, query 1-146 subject 1-146, E value = 2e-101, Identity = 100%, Similarity = 100%

SIF-HHPred: 8GTC\_F, Major tail protein; Marine bacteriophage, Cryo-EM, Siphophage, Baseplate, Megatron protein, Tail fibre protein, Distal tail protein, Hub protein, VIRAL; 4.5A, Dinoroseobacter phage vB\_DshS-R4C, Probability: 99.56%, E-value: 1.1e-13,

SF-Syn: This gene is pham 139535 [major tail protein], upstream is pham 1057 [tail terminator], downstream is pham 143854, as seen in Microbacterium phage Upsilon

**GENE 9 hypothetical protein**

Original Glimmer call @bp 6622 has strength 10.60

SSC: 6622 to 7005 (Forward)

CP: yes

SCS: both

ST: SS, Start 7 [6622], Found in 136 of 190 ( 71.6% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_9, function unknown, query 1-127 subject 1-127, E value = 1e-66, Identity = 100%, Similarity = 100%

GAP: 13bp

LO: yes

RBS: Kibler7, spacer 10, Z score 2.245, Final -4.294, yes

F: NKF

SIF-blast: aligns with hypothetical protein from Microbacterium phage PoRanda, ID YP\_009996745.1, query 1-127 subject 1-127, E value = 3e-85, Identity = 100%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

**GENE 10 tail assembly chaperone**

Original Glimmer call @bp 7019 has strength 18.73

SSC: 7019 to 7339 (Forward)

CP: yes

SCS: both

ST: SS, Start 6 [7019], Found in 130 of 130 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_10, tail assembly chaperone, query 1-106 subject 1-106, E value = 3e-54, Identity = 100%, Similarity = 100 %

GAP: 14bp

LO: yes

RBS: Kibler7, spacer 10, Z score 2.478, Final -3.826, yes

F: tail assembly chaperone

SIF-blast: aligns with tail assembly chaperone from Microbacterium phage Nobel, ID YP\_009996697.1, query 1-106 subject 1-106, E value = 2e-68, Identity = 100%, Similarity= 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: This gene is pham 85197 [tail assembly chaperone], upstream is pham 131854, downstream is pham 85363 [tail assembly chaperone]; as seen in Microbacterium phage Upsilon

this is the first of the tail assembly chaperone proteins the next gene [11] will use gene 10 and a frameshift to form the larger tail assembly chaperone protein

**GENE 11 tail assembly chaperone**

to be changed due to being part of a frameshift – see below

>Upsilon gp10

MDTTTKPKKFDLEQLTLGEVAAIEDLSGVAIGGVTTETPQGKFLAALYMVAKRRNGEPTFTFNAALQASMGEAQSFLGLDASDDEADEAE**SSAEG**NGDGSPESAPA

>Upsilon gp11

MDTTTKPKKFDLEQLTLGEVAAIEDLSGVAIGGVTTETPQGKFLAALYMVAKRRNGEPTFTFNAALQASMGEAQSFLGLDASDDEADEAE**SSAEG**KRRRLARERARLKAQFIVQLGMDPAAYERLTIAERDAIVRELNKRSRRRR

>Bintii\_Draft gp10

MDTTTKPKKFDLEQLTLGEVAAIEDLSGVAIGGVTTETPQGKFLAALYMVAKRRNGEPTFTFNAALQASMGEAQSFLGLDASDDEADEAE**SSAEG**NGDGSPESAPA

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Description automatically generated A colorful lines and dots on a white background

Description automatically generated

gene 10 = in frame 2 - ends at amino acid G [gga]

frameshift to frame 1 [-1 frameshift] – final a of gga becomes first a in aaa sequence of amino acid K

section 1 = 7019 - 7303

section 2 = 7303 to 7455

**GENE 12 tape measure protein**

Original Glimmer call @bp 7572 has strength 15.46

SSC: 7572 to 9677 (Forward)

CP: yes

SCS: both

ST: SS, Start 6 [7572], Found in 130 of 139 ( 93.5% ) of genes in pham, Called 97.7% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_12, tape measure protein, query 1-701 subject 1-701, E value = 0.0, Identity = 99%, Similarity = 100%

GAP: 117bp

LO: no

RBS: Kibler7, spacer 16, Z score 2.478, Final -4.386, yes

F: tape measure protein

SIF-blast: aligns with tape measure protein from Microbacterium phage Miaurora, ID AYD84507.1, query 1-701 subject 1-701, E value = 0.0, Identity = 99%, Similarity = 100%

SIF-HHPred: 6V8I\_BF, Tape Measure Protein, gp57; phage tail, tail tip, tape measure protein, VIRAL PROTEIN; 3.7A, Staphylococcus virus 80alpha, Probability: 99.7%, E-value: 2.8e-9

SF-Syn: this gene is pham 146929 [tape measure protein], upstream is pham 146929 [tail assembly chaperone], and downstream is pham 76903 [minor tail protein], as seen in Microbacterium phage Upsilon

**GENE 13 minor tail protein**

Original Glimmer call @bp 9674 has strength 15.92

SSC: 9674 to 10699 (Forward)

CP: yes

SCS: both

ST: SS, Start 1 [9674], Found in 107 of 107 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_13, minor tail protein, query 1-341 subject 1-341, E value = 0.0, Identity = 100%, Similarity = 100%

GAP: GTGA overlap

LO: NA

RBS: Kibler7, spacer 12, Z score 2.001, Final -4.924, yes

F: minor tail protein

SIF-blast: aligns with minor tail protein from Microbacterium phage PoRanda, ID YP\_009996749.1, query 1-341 subject 1-341, E value = 0.0, Identity 99= %, Similarity = 99%

SIF-HHPred: 6ZPV\_AAA, MgGH51; arabinofuranosidse, glycosidase, GH51, HYDROLASE; HET: ACT, SO4, NAG, GOL, MAN, BMA; 1.2A, Meripilus giganteus, Probability: 98.81%, E-value: 2e-7

SF-Syn: This gene is pham 76903 [minor tail protein], upstream is pham 146929 [tape measure protein], downstream is pham 1068 [minor tail protein], as seen in Microbacterium phage Upsilon

**GENE 14 minor tail protein**

Original Glimmer call @bp 10699 has strength 16.06

SSC: 10699 to 12756 (Forward)

CP: yes

SCS: both

ST: SS, Start 1 [10699], Found in 130 of 130 ( 100.0% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_14, minor tail protein, ID YP\_009996750.1, query 1-685 subject 1-685, E value = 0.0, Identity = 99%, Similarity = 99%

GAP: TAATG overlap

LO: NA

RBS: Kibler7, spacer 15, Z score 2.486, Final -4.717, yes

F: minor tail protein

SIF-blast: aligns with Microbacterium phage PoRanda\_14, minor tail protein, query 1-685 subject 1-685, E value = 0.0, Identity = 99%, Similarity = 99%

SIF-HHPred: 6TPW\_A, Receptor-type tyrosine-protein phosphatase F; Fibronectin type-III, adhesion protein, CELL ADHESION; HET: SO4; 2.9A, Homo sapiens, Probability: 99.76%, E-value: 1.1e-14,

SF-Syn: This gene is pham 1068 [minor tail protein], upstream is pham 76903 [minor tail protein], downstream is pham 85094 [minor tail protein], as seen in Microbacterium phage Upsilon

**GENE 15 minor tail protein**

Original Glimmer call @bp 12758 has strength 18.99

SSC: 12758 to 13306 (Forward)

CP: yes

SCS: both

ST: SS, Start 3 [12758], Found in 127 of 159 ( 79.9% ) of genes in pham, Called 98.4% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_15, minor tail protein, query 1-182 subject 1-182, E value = e-102, Identity = 100%, Similarity = 100%

GAP: 2bp

LO: NA

RBS: Kibler7, spacer 12, Z score 2.484, Final -3.955, yes

F: minor tail protein

SIF-blast: aligns with minor tail protein from Microbacterium phage Yasuo, ID UOW93316.1, query 1-182 subject 1-182, E value = 3e-119, Identity = 93%, Similarity = 95%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: this gene is pham 85094 [minor tail protein], upstream is pham 1068 [minor tail protein] and downstream is pham 137948, as seen in Microbacterium phage Upsilon

**GENE 16 hypothetical protein**

Original Glimmer call @bp 13343 has strength 13.83; GeneMark calls start at 13319

SSC: 13343 to 13630 (Forward) – starterator, RBS and alignment suggest 13343 as the start

CP: yes

SCS: both - GL

ST: SS, Start 5 [13343], Found in 54 of 55 ( 98.2% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_16, function unknown, query 1-95 subject 1-95, E value = 6e-46, Identity = 98%, Similarity = 100%

GAP: 37bp

LO: no

RBS: Kibler7, spacer 9, Z score 2.634, Final -3.589, yes

F: NKF

SIF-blast: aligns with hypothetical protein from Microbacterium PoRanda, ID YP\_009996752.1, query 1-95 subject 1-95, E value = 5e-55, Identity = 99%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

**GENE 17 endolysin**

Original Glimmer call @bp 13650 has strength 10.26

SSC: 13650 to 14345 (Forward)

CP: yes

SCS: both

ST: SS, Start 7 [13650], Found in 128 of 130 ( 98.5% ) of genes in pham, Called 95.3% of time when present

BLAST-start: aligns with Microbacterium phage Trireme\_17, endolysin, query 1-231 subject 1-231, E value = e-136, Identity = 99%, Similarity = 99%

GAP: 20bp

LO: yes

RBS: Kibler7, spacer 11, Z score 2.473, Final -2.473, yes

F: endolysin

SIF-blast: aligns with endolysin from Microbacterium phage Miaurora, ID AYD84512.1, query 1-231 subject 1-231, E value = 2e-165, Identity = 99%, Similarity = 99%

SIF-HHPred: 8TZL\_E, Peptidase M23; membrane protein, enzyme, TRANSPORT PROTEIN; HET: ADP, Vibrio cholerae, Probability: 99.64%, E-value: 1.1e-14,

SF-Syn: this gene is pham 141078 [endolysin], upstream is pham 137948 and downstream is pham 1063, as seen in Microbacterium phage Upsilon

**GENE 18 membrane protein**

Original Glimmer call @bp 14312 has strength 10.81

SSC: 14312 to 14575 (Forward)

CP: yes

SCS: both

ST: SS, Start 7 [14312], Found in 128 of 131 ( 97.7% ) of genes in pham, Called 85.2% of time when present

BLAST-start: aligns with Microbacterium phage PoRanda\_18, function unknown, query 1-87 subject 1-87, E value = 9e-47, Identity = 100%, Similarity = 100%

GAP: 14312 gives an overlap of 33bp, 14342 gives a GTGA overlap – starterator and alignment suggest 14312

LO: yes

RBS: Kibler7, spacer 16, Z score 1.583, Final –6.724, yes

F: membrane protein

SIF-blast: aligns with membrane protein from Microbacterium phage Miaurora, ID AYD84513.2, query 1-87 subject 1-87, E value = 2e-56, Identity = 99%, Similarity = 98%

SIF-HHPred: 8HHF\_B, Cell division protein FtsB; Bacterial cell division, divisome, FtsB, FtsL, FtsQ, FtsBLQ, FtsQLB, membrane protein complex, heterotrimer, MEMBRANE PROTEIN; 3.04A, Escherichia coli, Probability: 96%, E-value: 0.23

SF-Syn: NKF

SIF-Mem: 1 domain found by DEEPTMHMM

**GENE 19 holin**

Original Glimmer call @bp 14572 has strength 10.77

SSC: 14572 to 14796 (Forward)

CP: yes

SCS: both

ST: SS, Start 5 [14572], Found in 129 of 131 ( 98.5% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_19, function unknown, query 1-74 subject 1-74, E value = 4e-36, Identity = 100%, Similarity = 100%

GAP: ATGA overlap

LO: NA

RBS: Kibler7, spacer 11, Z score 2.836, Final -3.171, yes

F: holin

SIF-blast: aligns with holin from Microbacterium phage Kaijohn, ID , query 1-74 subject 1-74, E value = 9e-43, Identity = 100%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

SIF-Mem: 2 domains found by DEEPTMHMM

**GENE 20 Lsr2-like DNA bridging protein**

Original Glimmer call @bp 15073 has strength 10.12

SSC: 15073 to 14864 (Reverse)

CP: yes

SCS: both

ST: NI, Start 12 [15073], Found in 128 of 163 ( 78.5% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_20, Lsr2-like DNA bridging protein, query 1-69 subject 1-69, E value = 1e-32, Identity = 100%, Similarity = 100%

GAP: 3bp

LO: NA

RBS: Kibler7, spacer 12, Z score 2.648, Final -3.627, yes

F: Lsr2-like DNA bridging protein

SIF-blast: aligns with Lsr2-like DNA bridging protein from Microbacterium phage PoRanda, ID YP\_009996756.1, query 1-69 subject 1-69, E value = 1e-40, Identity = 100%, Similarity = 100%

SIF-HHPred: 4E1P\_A, Protein lsr2; anti-parallel beta sheet, dimer, DNA BINDING PROTEIN; 1.728A, Mycobacterium tuberculosis, Probability: 99.87%, E-value: 4.4e-21

SF-Syn: this gene is pham 85067 [Lsr2-like DNA bridging protein], upstream is pham 1053 and downstream is pham 1059 [helix-turn-helix DNA binding domain protein], as seen in Microbacterium phage Upsilon

**GENE 21 helix-turn-helix DNA binding domain protein**

Original Glimmer call @bp 15510 has strength 10.99

SSC: 15510 to 15076 (Reverse)

CP: yes

SCS: both

ST: NI, Start 12 [15510], Found in 127 of 130 ( 97.7% ) of genes in pham, Called 22.8% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_21, helix-turn-helix DNA binding domain protein, query 1-144 subject 1-144, E value = 5e-76, Identity = 99%, Similarity = 99%

GAP: 145bp – BLAST performed – no matches

LO: yes

RBS: Kibler7, spacer 9, Z score 2.214, Final -4.437, yes

F: helix-turn-helix DNA binding domain protein

SIF-blast: aligns with DNA binding protein from Microbacterium phage PoRanda, ID YP\_009996757.1, query 1-144 subject 1-144, E value = 1e-96, Identity = 99%, Similarity = 99%

SIF-HHPred: 6G1T\_A AM32, Repressor, Protein-DNA complex, Type IV secretion system, DNA BINDING PROTEIN; HET: EPE; 1.93A, Enterococcus faecalis, Probability: 99.61%, E-value: 9.2e-15

SF-Syn: this gene is pham 1059 [helix-turn-helix DNA binding domain protein], upstream is pham 85067 [Lsr2-like DNA bridging protein] and downstream is pham 85194 [helix-turn-helix DNA binding domain], as seen in Microbacterium phage Upsilon

**GENE 22 helix-turn-helix DNA binding domain protein**

Original Glimmer call @bp 15885 has strength 5.05

SSC: 15885 to 15655 (Reverse)

CP: yes

SCS: both

ST: SS, Start 5 [15885], Found in 130 of 130 ( 100.0% ) of genes in pham, Called 95.4% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_22, helix-turn-helix DNA binding domain protein, query 1-76 subject 1-76, E value = 6e-37, Identity = 100%, Similarity = 100%

GAP: 512bp – BLAST performed – no matches

LO: no

RBS: Kibler7, spacer 8, Z score 0.897, Final -7.526, no

F: helix-turn-helix DNA binding domain protein

SIF-blast: aligns with transcriptional repressor from Microbacterium phage PoRanda, ID YP\_009996758.1, query 1-76 subject 1-76, E value = 2e-45, Identity = 100%, Similarity = 100%

SIF-HHPred: 6WPZ\_A Pf4r, DNA BINDING PROTEIN; 1.993A, Pseudomonas aeruginosa, Probability: 98.76%, E-value: 3.2e-7

SF-Syn: this gene is pham 85194 [helix-turn-helix DNA binding domain protein], upstream is pham 1059 [helix-turn-helix DNA binding domain protein] and downstream is pham 1383, as seen in Microbacterium phage Upsilon

**GENE 23 hypothetical protein**

Original Glimmer call @bp 16397 has strength 5.93

SSC: 16397 to 16780 (Forward)

CP: yes

SCS: both

ST: SS, Start 2 [16397], Found in 93 of 95 ( 97.9% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_23, function unknown, query 1-127 subject 1-127, E value = 4e-65, Identity = 100%, Similarity = 100%

GAP: 512bp – no coding potential observed

LO: yes

RBS: Kibler7, spacer 8, Z score 1.957, Final -5.399, yes

F: NKF

SIF-blast: aligns with hypothetical protein from Microbacterium PoRanda, ID YP\_009996759.1, query 1-127 subject 1=127, E value = 7e-83, Identity = 100%, Similarity = 100%

SIF-HHPred: NKF, no matches with a probability above 90%

SF-Syn: NKF

**GENE 24 helix-turn-helix DNA binding domain protein**

Original Glimmer call @bp 16879 has strength 9.13

SSC: 16879 to 17097 (Forward)

CP: yes

SCS: both

ST: SS, Start 12 [16879], Found in 108 of 129 ( 83.7% ) of genes in pham, Called 98.1% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_24, helix-turn-helix DNA binding domain protein, query 1-72 subject 1-72, E value = 2e-35, Identity = 100%, Similarity = 100%

GAP: 99bp

LO: no

RBS: Kibler7, spacer 13, Z score 1.003, Final -7.139, no

F: helix-turn-helix DNA binding domain protein

SIF-blast: aligns with helix-turn-helix DNA binding domain protein from Microbacterium phage TimoTea, ID QBI97337.1, query 1-72 subject 1-72, E value = 2e-40, Identity = 93%, Similarity = 94%

SIF-HHPred: 4LHF\_A, Regulatory protein cox; helix-turn-helix, DNA binding, VIRAL PROTEIN; 2.401A, Enterobacteria phage P2, Probability: 99.09%, E-value: 2.8e-9

SF-Syn: this gene is pham 117549 [helix-turn-helix DNA binding domain protein], upstream is pham 1383 and downstream is pham 143877 [HNH endonuclease], as seen in Microbacterium phage Upsilon

**GENE 25 HNH endonuclease**

Original Glimmer call @bp 17094 has strength 10.73

SSC: 17094 to 17393 (Forward)

CP: yes

SCS: both

ST: SS, Start 23 [17094], Found in 130 of 173 ( 75.1% ) of genes in pham, Called 100.0% of time when present

BLAST-start: aligns with Microbacterium phage Upsilon\_25, HNH endonuclease, query 1-99 subject 1-99, E value = 3e-56, Identity = 100%, Similarity = 100%

GAP: ATGA overlap

LO: NA

RBS: Kibler7, spacer 5, Z score 1.896, Final -6.299, yes

F: HNH endonuclease

SIF-blast: aligns with HNH endonuclease from Microbacterium phage PoRanda, ID YP\_009996761.1, query 1-99 subject 1-99, E value = 7e-68, Identity = 100%, Similarity = 100%

SIF-HHPred: 5H0M\_A, HNH endonuclease; Thermophilic bacteriophage, HNH Endonuclease, DNA nicking, HYDROLASE; 1.52A, Geobacillus virus E2, Probability: 97.29%,E-value: 0.00027

SF-Syn: this gene is pham 143877 [HNH endonuclease] and upstream is pham 117549 [helix-turn-helix DNA binding domain protein], as seen in Microbacterium phage Upsilon