ISOLATION AND CHARACTERIZATION OF *PHOTOBACTERIUM PHOSPHOREUM* FROM MIGRATING ALASKAN SALMON

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A

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Abstract

We isolated luminous bacteria from drying chum salmon, *Oncorhynchus keta*, reported by Alaska native fishermen to be "glowing in the dark." The salmon were harvested for subsistence use from the Yukon River, Alaska. We identified our luminous bacterial isolates as *Photobacterium phosphoreum* based on nutritional versatility, and 16S rDNA and *luxA* gene sequences. *P. phosphoreum* has previously only been isolated from the marine environment. We tested whether our strains, isolated from fish harvested in freshwater, represent cold-adapted, freshwater-tolerant strains of *P. phosphoreum*. We also analyzed *lux* operon composition and organization, and examined the 5' promoter region of the *lux* operon for shared genes and regulatory elements from strains of *P. phosphoreum* from Alaska, the Black Sea, Oregon, and from near the Canary Islands. Our results indicate our *P. phosphoreum* strains have a lower optimal growth temperature than other strains but rapidly lose viability after inoculation into river water. Analyses of the *P. phosphoreum lux* operon reveal a striking pattern of conservation of composition and organization, and suggest there is conservation in the location of the transcriptional start among geographically separated strains of the same species.

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I performed the research and analyses described in chapters 1 and 2. Drs. Braddock and Wimpee served as advisors for the research that was conducted as well as editors for the manuscripts.

General Introductions

Background. Since in the 1970's, salmon harvested for subsistence use from the Yukon River, Alaska, have periodically been observed to be "glowing in the dark." Salmon reported as glowing have always been in the process of drying or smoking. The phenomenon of glowing salmon has been reported in Holy Cross, Alaska, 449 km from the mouth of the Yukon River; Rampart, Alaska, located 1,228 km from the mouth; and in Eagle, Alaska, which is located 1,952 km from the mouth. Our first luminous bacterial strain was isolated in 1997 from a chum salmon harvested near Rampart, Alaska by Randy Brown of the US Fish & Wildlife Service. Mr. Brown observed glowing spots on a fish which was in the process of drying and transported it to our laboratory in Fairbanks, Alaska, where a luminous bacterium was isolated. Alaska native subsistence fishers in Holy Cross, Alaska observed this phenomenon in 2001, and expressed concern over the health and consumption of the fish. Until the residents of Holy Cross raised concerns over the safety of the "glowing" salmon, the occurrence of luminescent bacteria on salmon from the Yukon River was microbiologically interesting, but not considered to be a health concern or a potential threat to the salmon of Alaska. More recently, in 2001 and 2002, I isolated several luminous strains from chum salmon caught near Rampart, Alaska

Using tests of nutritional versatility, and sequence analysis of 16S rDNA and *luxA* genes, I identified all our luminous bacterial isolates as *Photobacterium phosphoreum*. *P. phosphoreum* is reported as an exclusively marine bacterium with a specific requirement for sodium in its growth medium (17). While it is likely the salmon are

acquiring *P. phosphoreum* in the marine environment and transporting them to the Yukon River, several questions have been raised by the isolation of an exclusively marine bacterium from freshwater: (1) are our Alaskan isolates taxonomically distinct from other strains of *P. phosphoreum*, (2) do our isolates possess physiological adaptations that allow them to remain viable in freshwater, (3) how are the bacteria transported in freshwater, (4) is the *lux* operon composition and organization of our isolates consistent with other strains of *P. phosphoreum*, and (5) what similarities exist between the 5'-promoter region of the *lux* operon in our *P. phosphoreum* isolates and other *P. phosphoreum* strains?

Bioluminescence and the *lux* operon. Bioluminescent bacteria are functionally grouped by the ability to emit visible light. Tests of nutritional versatility place luminous isolates into one of four primary groups: *Vibrio fischeri*, *Vibrio harveyi*, *Photobacterium phosphoreum*, or *Photobacterium leiognathi* (14). All four species are believed to exclusively occupy marine niches (14). Two bioluminescent bacterial species have been isolated from non-marine environments: *Vibrio cholerae* isolated from brackish waters near Kent, England (22, 23); and *Photorhabdus luminescens* isolated from the terrestrial nematode *Heterorhabditis bacteriophora* (16).

Bacterial bioluminescence is accomplished by the gene products of the *lux* operon, *luxCDABE* (Fig. 1). *luxAB* code for the α- and β- subunits of bacterial luciferase. *luxCDE* code for a fatty acid reductase, transferase, and synthetase. Some species possess an additional gene downstream of *luxE*, *luxG* (Fig. 1) (10). Most strains of *Photobacterium phosphoreum* possess a *lux* gene between *luxB* and *luxE*, *luxF* (Fig. 1)

(1, 9). Both *luxG* and *luxF* code for gene products of unknown function and are not necessary for bioluminescence (10). Upstream of *luxC* in *P. phosphoreum* is *lumP*, a gene whose product is predicted to be a secondary emitter to bacterial luciferase, resulting in the wavelength of light emitted being shifted to a higher energy level (15). *lumP* belongs to a separate transcriptional unit than the *lux* operon and the direction of transcription is divergent relative to the *lux* operon. Also, unique to *Photobacterium* species, riboflavin synthesis (*rib*) genes are located immediately downstream of *luxG* (8). This is of interest because bacterial luciferase involves the oxidation of flavin and a long-chain aldehyde, resulting in the emission of light (14). Surprisingly, LumP and RSα (riboflavin synthetase α subunit) share significant amino acid sequence similarity, which might indicate a shared evolutionary history (15).

An intergenic spacer, approximately 616 bp in length, separates *lumP* and *luxC* in *P. phosphoreum*. This spacer is the site at which RNA polymerase binds and begins transcription, where LuxR activates transcription in *V. fischeri*, and where LuxO represses transcription in *V. harveyi*. Thus, this site, immediately upstream of *luxC* is of interest for study of transcriptional control of the *lux* operon in *P. phosphoreum*.

Regulation of the *lux* operon is accomplished by quorum sensing. Quorum sensing describes regulation based on direct cell-to-cell communication mediated by autoinducer. At low cell density, autoinducer is at a low concentration and gene expression is depressed. At high cell density, autoinducer concentration increases and activation of gene expression is dramatic and coordinated. Quorum sensing has emerged as an ecologically important gene regulation paradigm for the global expression of

diverse bacterial cellular processes (11). Interestingly, two distinct quorum sensing circuits have been described, and both are reported to regulate the *lux* operon of different species of bioluminescent bacteria.

The first quorum sensing mechanism was elucidated in *V. fischeri*. The *V. fischeri* quorum sensing circuit involves two homoserine lactone (HSL) autoinducers. Each of them binds the activator protein, LuxR, under different growth conditions (5, 6). The LuxR-HSL complex then binds at the *lux* operon promoter and activates transcription of the *lux* genes (20) (Fig.2). The simplicity of the *V. fischeri* quorum sensing circuit has been complicated by identification of two additional regulatory proteins, LuxO and LitR. Both *V. fischeri luxO* and *litR* show sequence similarity to *V. harveyi luxO* and *luxR*, respectively (4, 12). Deactivation of *V. fischeri luxO* results in decreased, but not eliminated, luminescence (12). *V. fischeri* LitR is believed to bind at the 5'-end of *V. fischeri luxR*, and function as an activator for the expression of *luxR* (4).

The second quorum sensing circuit described in *V. harveyi* uses a more complex hybrid autoinduer/two-component phosphate-signaling circuit (11). Briefly, in an uninduced state, *V. harveyi lux* operon transcription is repressed by LuxO. When induced, two separate autoinducers bind to their respective sensor proteins, LuxN and LuxQ. Signals from LuxN and LuxQ are integrated through LuxU by draining phosphate. LuxU then signals LuxO (by draining phosphate), which liberates LuxO from its repressor role, allowing binding of the activator, LuxR, and transcription of the *lux* genes (Fig. 3) (11).

Identification. Identification methods have traditionally relied on numerical taxonomy. The specific taxonomic tests which apply to the luminous bacteria focus on simple characterizations like determination of growth-medium requirements, and nutritional versatility (13). The resolution of these tests is poor when compared to molecular techniques. However, these tests provide a method to quickly and reliably distinguish isolates based on more broad criteria than sequence analysis of a limited number of genes. This can be especially useful in cases where molecular analyses provide ambiguous results or horizontal gene transfer is suspected. Tests of nutritional versatility provide the ability to distinguish isolates into four primary bacterial groups: *V. harveyi* (includes *Vibrio orientalis* and *Vibrio splendidus*), *V. fischeri* (includes *Vibrio logei*), *P. phosphoreum*, and *Photobacterium leiognathi* (14).

To supplement the limited resolution of numerical taxonomy, molecular analyses based on analysis of DNA sequences have been applied to bacteria (21). Refinement of DNA sequence analysis techniques has dramatically increased the resolution of taxonomic placement of bacterial isolates. Analysis of 16S rDNA sequences from the Proteobacteria, the group to which all luminous bacteria belong, offers a well-accepted, high-resolution phylogeny for that group (2, 19). A similar high-resolution phylogeny of the bioluminescent bacteria has been established following the development of highly specific hybridization probes for *luxA* (24).

Biology of *P. phosphoreum*. *P. phosphoreum* distribution in seawater is best described as planktonic, being found at greatest abundance in deep (>200 m) and cold (<15 °C) water (18). This distribution pattern is likely the result of the thermal tolerances

of *P. phosphoreum* and specific symbioses formed with mid-water fish (7, 18). *P. phosphoreum* is known to form facultative gut-derived light-organ symbioses with several orders of fish inhabiting water >200 m in depth (7). Considerable homogeneity has been observed in *P. phosphoreum* light-organ symbionts (7, 25), leading to the hypothesis that eggs of fish with *P. phosphoreum* residing in their light organs are directly inoculated upon spawning from the light organ of the female parent (7).

Bioluminescent bacteria are generally considered to be marine organisms; however, two luminous species, *V. cholerae* and *Photorhabdus luminescens*, have been isolated from nonmarine environments. Specifically, *P. phosphoreum* is considered an exclusively marine bacterium with a specific requirement for the sodium ion its growth medium (17). Some strains have an additional requirement for L-methionine in minimal medium (17). In experiments to determine the effect of salt (NaCl) on growth and luminescence of *P. phosphoreum*, luminescence was determined to be greatest with a NaCl concentration approximately 125% seawater, while growth was greatest with a NaCl concentration approximately half that of seawater (3).

I also performed an experiment to determine whether our Alaskan *P. phosphoreum* strains have acquired freshwater tolerance, by inoculating exponentially growing *P. phosphoreum* strains AK-6 from Alaska and NZ-11-D from the Atlantic Ocean into preferred growth medium (0.38 M NaCl, 0.02 M $MgCl_2 \cdot 6H_2O$, 0.25 M $MgSO_4 \cdot 7H_2O$, 8 mM KCl, 0.5% peptone, 0.3% yeast extract, 0.3% glycerol) prepared without NaCl to assess the ability of *P. phosphoreum* to remain viable in the absence of NaCl, and river water to assess the ability of *P. phosphoreum* to survive in freshwater.

Results of this experiment suggest both *P. phosphoreum* strains are rendered nonviable in freshwater within 1 day, however, viability was preserved for up to 5 days in medium without NaCl (Fig. 4). These data indicate our Alaskan *P. phosphoreum* isolate is not freshwater tolerant. Because both *P. phosphoreum* strains were able to maintain viability in medium without NaCl, I propose *P. phosphoreum* does not have a specific requirement for sodium in its growth medium, but is able to osmotically buffer itself in complex substrates.

Research Focus

Unexpected isolation of a bioluminescent marine bacterium from salmon harvested from the Yukon River, Alaska, migrating salmon up to 1,228 km from the marine environment invoked several questions which were the subject of this thesis. An essential question to our understanding of the phenomenon of glowing salmon from the Yukon River, is specifically which bacterium is responsible for causing Alaskan salmon to be visibly luminescent? With the identity of the bacteria established as *P. phosphoreum*, questions specifically related to *P. phosphoreum* on migrating salmon include: (1) are our Alaskan *P. phosphoreum* isolates taxonomically distinct from other strains of *P. phosphoreum*, (2) do our *P. phosphoreum* isolates possess physiological adaptations that allow them to remain viable in freshwater, and (3) how are the bacteria transported in freshwater? These questions are addressed in chapter one of this thesis.

The *lux* operon of bioluminescent bacteria provides an opportunity to analyze the evolution, distribution, and maintenance of the *lux* genes, as well as enhance our understanding of quorum sensing by probing the genome of *P. phosphoreum* for

regulatory regions and genes known to be involved in the quorum sensing pathway of two close sister taxa, *V. fischeri* and *V. harveyi*. Specific questions addressed in this study are: (1) is *lux* operon composition and organization of our Alaskan isolates consistent with other strains of *P. phosphoreum*, and (2) what similarities exist between the *lux* operon promoter region in our *P. phosphoreum* isolates and other described strains? These questions are addressed in chapter 2 of this thesis.

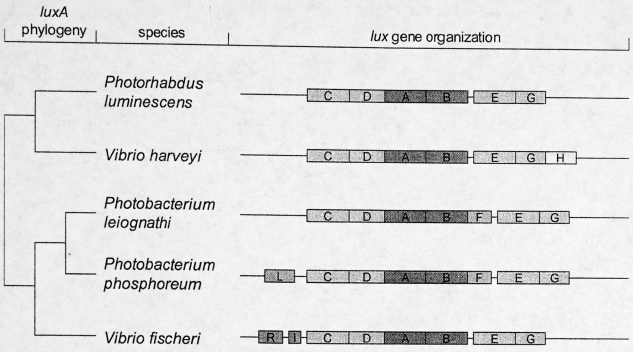
Figure Legend

Fig. 1. *lux* gene organization for common bioluminescent bacterial species and generalized phylogeny based on *luxA*.

Fig. 2. Simplified mechanism for *V. fischeri* quorum sensing circuit. LuxI generates a HSL autoinducer which is freely diffusible across the cell membrane. At a threshold concentration, HSL binds to LuxR which acts as a transcriptional activator for *luxICDABE* and a repressor for *luxR*. Modified from Miller, 2001.

Fig. 3. Generalized mechanism for *V. harveyi* quorum sensing circuit. Two autoinducers (AI-1 and AI-2) are generated by LuxLM and LuxS. LuxN senses the AI-1 and LuxQ senses AI-2. Signal from LuxN and LuxQ are integrated through LuxU, and then to LuxO. Under low density conditions, LuxO represses expression of the *lux* operon. When high density conditions occur, LuxO is deactivated by LuxU, which inactivates role of LuxO, allowing transcription of *lux* operon. Modified from Miller, 2001.

Fig. 4. Viability *P. phosphoreum* strains AK-6 and NZ-11-D in river water and SWC medium without NaCl.



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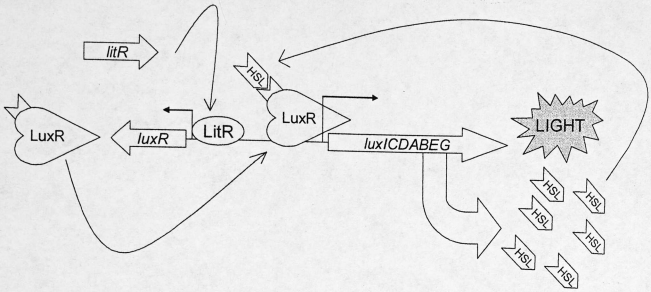

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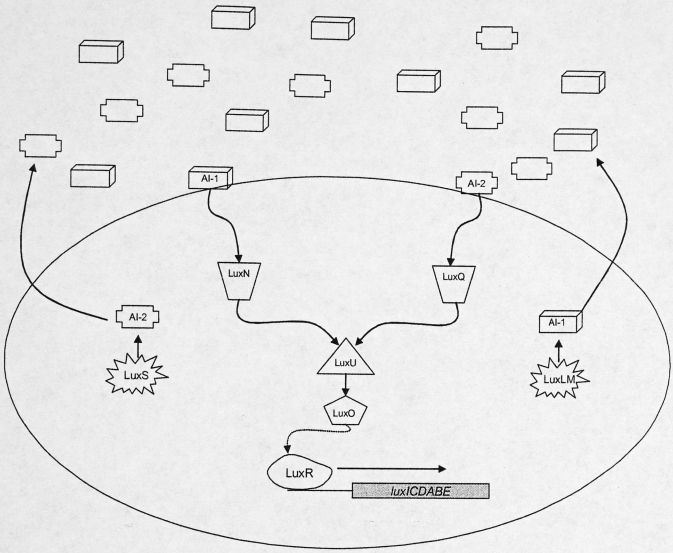

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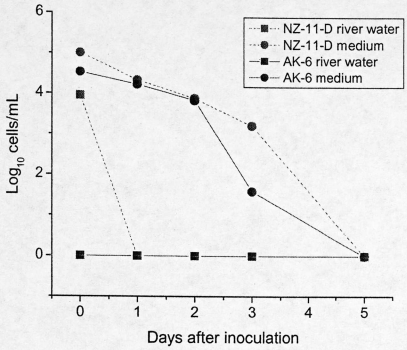

Figure 4. Viability *P. phosphoreum* strains AK-6 and NZ-11-D in river water and SWC medium without NaCl.

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Chapter 1

Isolation and identification of *Photobacterium phosphoreum* from an unexpected niche: migrating salmon

Abstract

Six luminous bacteria were isolated from migrating salmon in the Yukon River, Alaska. All isolates were identified as *Photobacterium phosphoreum*. Previous studies suggest *P. phosphoreum* is an exclusively marine bacterium, while our Alaskan isolates are from salmon which migrated up to 1,228 km from the marine environment.

Introduction

Luminous bacteria have been extensively studied and are well described phylogenetically and ecologically. Compared to the broad distribution and high abundance in the marine environment (17), only one luminous species has been isolated from fresh water (17, 30, 31) and another from soil (19). Luminous bacteria have been observed living in many ecological niches including planktonic (23, 25, 26, 31, 33), saprophytic (16), symbiotic (6, 7, 12, 13, 22-24), and parasitic (16). Some species inhabit more than one niche (10). Despite several studies describing distribution and abundance of luminous bacteria, details regarding population dynamics, ecological function, and especially niche relationships remain poorly understood.

Photobacterium phosphoreum has been well described relative to their light-organ symbioses with several families of marine fish inhabiting cold and deep ocean waters

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(11). Free-living *P. phosphoreum* also have been isolated by direct plating of seawater (20). Aside from the free-living forms and symbioses formed with marine fish, *P. phosphoreum* has been described as living saprophytically and parasitically (16). Recent reports implicate *P. phosphoreum* as an important factor in spoilage of cold-cured salmon and cod from the north Atlantic Ocean (2, 3). *P. phosphoreum* is considered an exclusively marine bacterium because of its specific requirement for sodium in the growth medium (20).

Identification of luminous environmental isolates traditionally has relied on a set of nutritional versatility tests to quickly and reliably distinguish between luminous bacterial groups (20). More recently, PCR primers have been used which are suitable for the amplification and sequencing of *luxA*. The gene product of *luxA*, α-luciferase, is necessary for the light-emitting reaction of all known luminous bacteria (14, 32).

We tested whether *P. phosphoreum* was responsible for bioluminescence from migrating salmon harvested up to 1,228 km from the marine environment. The identification of the luminous isolates as *P. phosphoreum* was accomplished by the use of three complementary methods: tests to assess nutritional versatility, and DNA sequence analysis of *luxA* and of the 16S rRNA gene.

Materials and Methods

Sampling sites and collection. Luminous bacterial strains were isolated from whole chum salmon, *Oncorhynchus kisutch*, harvested from the Yukon River near the village of Rampart (Figure 1, Table 1). Whole chum salmon were transported to Fairbanks, Alaska, within 6 h of harvest, and partially submerged in artificial seawater

(0.4 M NaCl, 0.1 M $MgSO_4 \cdot 7H_2O$, 0.02 M KCl, 0.02 M $CaCl_2 \cdot 2H_2O$) as previously described (15). The partially submerged salmon were stored at 10° C for 10 days and inspected visually for the presence of luminous areas daily. Luminous areas were swabbed and transferred to seawater complete broth (SWC), and later purified into pure culture. One additional isolate (AK-8) was received in pure culture from the Pathology Laboratory of the Alaska Department of Fish and Game from a partially smoked chum salmon caught near Holy Cross (Figure 1, Table 1).

The Yukon River flows at a rate of approximately 6-12 km/h (9). Due to the glacial origin of some of its tributaries, the Yukon River is silty in summer and clear in winter. The climate of the Yukon River watershed is characterized by long, cold winters and brief, warm summers. Air temperatures below freezing are common in September and the Yukon River is generally frozen from late October until May (9).

Holy Cross and Rampart are located 449, and 1,228 km from the mouth of the Yukon River, respectively (Figure 1). Migration of chum salmon in the Yukon River average 35 – 40 km/day (R. Brown, personal communication); consequently, the migration time is approximately 11 days to Holy Cross, and 30 days to Rampart.

Growth and maintenance of bacterial strains. All isolates of luminous bacteria were grown in seawater complete medium (SWC) (0.38 M NaCl, 0.02 M $MgCl_2 \cdot 6H_2O$, 0.25 M $MgSO_4 \cdot 7H_2O$, 8 mM KCl, 0.5% peptone, 0.3% yeast extract, 0.3% glycerol). All Alaskan luminous isolates were grown at 15° C. The reference strain, *P. phosphoreum* strain NZ-11-D (obtained from C. W.) (24) was grown and maintained under the same conditions as Alaskan isolates. Long-term storage of strains is at -80° C in SWC medium

containing 15% glycerol. Luminous isolates of Alaskan origin are designated as "AK-" strains.

DNA isolation. We isolated DNA from 100 mL of exponentially growing cultures with a standard genomic DNA isolation protocol (1). RNA was degraded with 1-h incubation at 37° C with 10 µg/mL RNaseA (Promega, Madison, WI). We determined yield, quality and concentration of DNA isolations by gel electrophoresis.

PCR amplification and cloning. All PCR reactions were performed with PCR Core System II (Promega, Madison, WI) in a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT). PCR reactions, generally 50 µl, were conducted with final concentrations as follows: 1 X PCR buffer; 1.5 mM $MgCl_2$; 200 µM each dNTP; 1 µM each primer; 1.25 U *Taq* Polymerase; and 50–500 ng genomic DNA template. PCR conditions for 16S rRNA reactions were one cycle of denaturation of 5 min at 94° C; 25 amplification cycles consisting of denaturation (94° C for 30 sec), primer annealing (49° C for 30 sec) and primer extension (72° C for 90 sec); followed by a final extension of 7 min at 72° C. PCR conditions for *luxA* were the same except for 30 amplification cycles and a primer annealing temperature of 45° C.

Primers used to amplify the 16S rRNA gene from genomic DNA were 16S-11f (5' GTTTGATCCTGGCTCAG 3') and 16S-1512r (5' ACGGYTACCTTGTTACACTT 3') (29). All oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA). 16S rRNA amplicons were gel purified in QIAquick Gel Cleanup Kit (Qiagen, Valencia, CA) and directly sequenced. We PCR-amplified *luxA* with the primers luxA127f (5' GAICAICAITTIACIGAGTTTGG 3') and luxA1007r (5'

ATTTCITCTTCAGIICCATTIGCTTCAAAICC 3') (28) with genomic DNA as the template. *LuxA* amplicons were gel purified using Freeze 'N Squeeze DNA Gel Extraction Spin Columns (Bio-Rad, Hercules, CA). Following gel purification, *luxA* PCR products were ligated into pCR®II-TOPO vector and TOP10 cells were transformed (Invitrogen, Carlsbad, CA). Ligations and transformations were done following the manufacturer's instructions. Clones were screened with PCR for the presence of the *luxA* insert. The plasmids from one positive clone were isolated on a DNA-Pure™ Plasmid Mini-Prep Kit (CPG, Lincoln Park, NJ) following the manufacturer's instructions and used as the template in cycle sequencing reactions.

Cycle sequencing. Each 16S rRNA amplicon and *luxA* plasmid insert was bidirectionally sequenced on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). Cycle sequencing conditions for all reactions involved 40-60 ng template DNA, 3.2 pmol primer, 4 µl Big Dye (Applied Biosystems, Foster City, CA), and water to a final volume of 20 µl. 16S rRNA reactions were primed with primers 16S-11f and 16S-1512r. Internal primers were used to ensure overlapping sequences for analysis of 16S rRNA sequences: 16S-515f (5' GTGCCAGCMGCCGCGGTAA 3'), 16S-1100f (5' CAACGAGCGCAACCCT 3'), 16S-519r (5' GWATTACCGCGGCKGCTG 3'), and 16S-907r (5' CCGTCAAATCCTTTRAGTTT 3') (29). Cycle-sequencing reactions for *luxA* plasmid inserts were primed with SP6 and T7 promotor primers (Promega, Madison, WI). Cycle sequencing reactions consisted of 25 amplification cycles that included denaturation (96° C for 30 sec), primer annealing (49° C for 15 sec) and primer extension (60° C for 4 min). Cycle-sequencing conditions

for *luxA* were the same, except for a primer-annealing temperature of 45° C. Extension products were submitted for sequencing at the at the University of Alaska's Core Facility for Nucleic Acid Analysis.

Assessment of nutritional versatility. We used a set of tests to assess the ability of Alaskan isolates and the reference strain, *P. phosphoreum* NZ-11-D, to utilize each of 12 compounds as a sole carbon source in minimal media. As an additional component of this analysis, we assayed for the presence of three extracellular enzymes (15). Our *P. phosphoreum* isolates required addition of 40 µg L-methionine per mL minimal medium for growth (19, 20). Optimal growth temperature was determined by inoculating log-phase cells into SWC and observing growth at 4, 10, 15, and 20 °C.

Sequence analysis and GenBank accession numbers. Bidirectional contigs of 16S rRNA and *luxA* sequences were assembled with Sequencher ver. 4.0.5 (Gene Codes, Ann Arbor, MI). We imported contigs into ClustalX and aligned them with representative 16S rRNA and *luxA* sequences obtained from GenBank. Aligned sequences were imported into PAUP* 4.0b10 (27) where Maximum Likelihood analysis was performed and phylograms were generated. Maximum Likelihood analysis included 100 bootstrap replicates. Only bootstrap support values of > 50 are displayed.

GenBank accession numbers for sequences used in 16S rRNA sequence analyses are AE000474 for *Escherichia coli* K12 strain MG1655, X82248 for *Photorhabdus luminescens* DSM 3368, X82132 for *Shewanella hanedai* CIP 103207T, X74706 for *Vibrio harveyi* ATCC 14126, Z21729 for *Vibrio fischeri* MJ-1, X74686 for *Photobacterium leiognathi* ATCC 22551T, and X74687 for *P. phosphoreum* ATCC

11004T. Accession numbers chosen as representative for *luxA* sequence analyses are X58791 for *V. harveyi* CTP5 *luxB* (used as the outgroup), M57416 for *P. luminescens* ATCC 29999, X58791 for *V. harveyi* CTP5, X08036 for *P. leiognathi* 554, X55458 for *P. phosphoreum* NCMB 844, AB058949 for *S. hanedai* ATCC 33224, and AF170104 for *V. fischeri* MJ-1. Genbank accession numbers for *luxA* sequences derived in this study are AY345883-AY345888, 16S rRNA sequences derived for use in this study are AY345889-AY345894.

Results

Isolates from Alaskan salmon used in this study were short rods, oxidase negative, Gram-negative (8), and required L-methionine for growth in minimal media. Additionally, all isolates from Alaska grew at 4° C; however, optimal growth occurred at 15° C and growth diminished at >20° C. As compared to other species of luminous bacteria, we can assign all AK strains to the *P. phosphoreum* group based on published data on nutritional versatility (Table 2). We verified our results by including a reference strain, *P. phosphoreum* NZ-11-D (18), in our test (Table 2).

Gene sequences of *luxA* of the seven AK isolates were aligned with six representative sequences from other luminous bacteria. The alignment produced a consensus sequence 554 bp in length shared by all 13 taxa. Maximum-likelihood analysis of the alignment by PAUP* 4.0b10 revealed all AK isolates clustered closely with *P. phosphoreum* (Figure 2).

16S rRNA gene sequences of the seven AK isolates were aligned with six representative sequences from other luminous bacteria. The alignment produced a

concensus sequence 1,159 bp in length shared by all 13 taxa. Maximum-likelihood analysis of the alignment by PAUP* 4.0b10 revealed all AK isolates clustered closely with *P. phosphoreum* (Figure 3).

Discussion

We positively identified *P. phosphoreum* isolated from migrating salmon, collected up to 1,228 km from the mouth of Yukon River, Alaska. Our data on nutritional versatility allow us to confidently place our Alaskan isolates into the *P. phosphoreum* group. Molecular data, both 16S rRNA and *luxA* sequence analysis, reinforce our identification by showing our isolates cluster closely with other published accounts of *P. phosphoreum* 16S rRNA and *luxA* sequences. The significance of our results is in the scarcity of bioluminescent bacteria isolated outside of the marine environment, and that all previous studies indicate *P. phosphoreum* is an exclusively marine bacterium.

Our data indicate *P. phosphoreum* is capable of remaining viable on the external surfaces of anadromous migrating salmon. Although the possibility exists *P. phosphoreum* is colonizing salmon while in the Yukon River, we believe it is much more likely *P. phosphoreum* we isolated have their origin in the marine environment. Preliminary attempts to cultivate *P. phosphoreum* from Yukon River water have been unsuccessful (unpublished data). Despite the absence of data on the distribution of luminous bacteria in the northern Pacific Ocean, we predict *P. phosphoreum* is the primary species present because of increased abundance of *P. phosphoreum* in cold temperatures (24) and deep water (below 200 m) (21, 25).

Previous results (4, 5) suggest *P. phosphoreum* is adapted for survival in low-salt environments, showing optimal growth in media with a salt (NaCl) concentration approximately 50% that of seawater. Preliminary studies in our lab suggest Alaskan *P. phosphoreum* isolate AK-6 and the reference strain NZ-11-D are rendered non-viable after <1 day in river water, however, viability of both strains is maintained in SWC prepared without NaCl for up to 5 days. We therefore hypothesize *P. phosphoreum* remains viable in the freshwater environment of the Yukon River because the complex matrix of fish slime is of sufficient osmotic strength to protect bacterial cells from very low osmotic conditions of freshwater. We believe our *P. phosphoreum* isolates are of marine origin, forming a saprophytic association with migrating salmon while still in the ocean environment. When salmon migrate into freshwater, luminous bacteria on the salmon are protected by the slime of salmon until the fish are caught.

Our Alaskan strains of *P. phosphoreum* are nearly identical to other descriptions with respect to nutritional versatility, *luxA* and 16S rRNA sequences; however, our isolates appear to have a lower optimal growth temperature as compared to the reference strain, *P. phosphoreum* NZ-11-D. Future investigations of the osmotic requirements and temperature tolerances of Alaskan *P. phosphoreum* may reveal adaptations specific to this unique niche.

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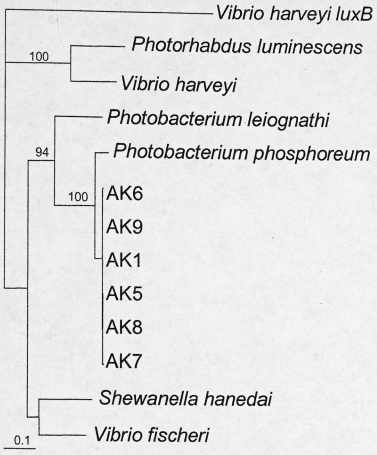
Figure Legend

Fig. 1. Sample locations along the Yukon River in Alaska where salmon with bioluminescent bacteria were caught. Distances indicate kilometers from the mouth of the Yukon River.

Fig. 2. Phylogeny of Alaskan luminous bacteria based on Maximum Liklihood analysis using PAUP* 4.0b10 with *luxA* sequences. All strains with "AK" are from salmon harvested from the Yukon River, Alaska. *V. harveyi luxB* was used as the outgroup in the Maximum Likelihood analysis of *luxA* genes. Bar represents substitutions per site

Fig. 3. Phylogeny of Alaskan luminous bacteria based on Maximum Likelihood analysis using PAUP* 4.0b10 with 16S rRNA sequences from Alaskan isolates and representative sequences from GenBank. All strains with "AK" are from salmon harvested from the Yukon River, Alaska. *E. coli* was used as the outgroup in this analysis. Bar represents substitutions per sites.

Figure 1. Sample locations along the Yukon River in Alaska where salmon with bioluminescent bacteria were caught. Distances indicate kilometers from the mouth of the Yukon River.



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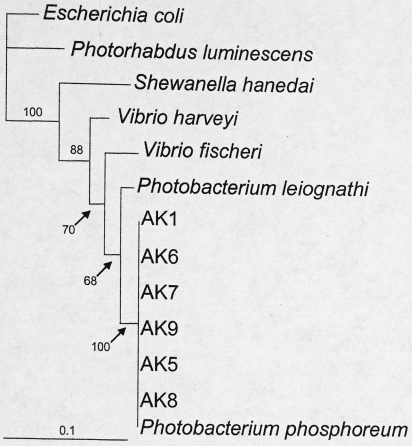

Figure 3. Phylogeny of Alaskan luminous bacteria based on Maximum Likelihood analysis using PAUP* 4.0b10 with 16S rRNA sequences from Alaskan isolates and representative sequences from GenBank. All strains with "AK" are from salmon harvested from the Yukon River, Alaska. *E. coli* was used as the outgroup in this analysis. Bar represents substitutions per sites

TABLE 1. *P. phosphoreum* isolates used in this study.

Strain	Host Fish	Location on fish	Location	Date of Isolation
AK-1	Chum salmon	Head	Rampart	August 1997
AK-5	Chum salmon (female)	Gut content	Rampart	September 2001
AK-6	Chum salmon (male)	Slime	Rampart	September 2001
AK-7	Chum salmon (male)	Liquid around fish	Rampart	September 2001
AK-8	Chum salmon	Flesh	Holy Cross	August 2001
AK-9	Chum salmon	Liquid around fish	Rampart	September 2002

[1]Brownish discharge from fish after partially submerged in artificial seawater for several days

TABLE 2. Phenotypic characteristics of Alaskan Isolates

	Published Reference Data					Tested Strains						
	V. harveyi[a]	*V. fischeri*[a]	*P. leiognathi*[a]	*P. phosphoreum*[a,b]	*P. phosphoreum* NZ-11-D[c]	NZ-11-D	AK-1	AK-5	AK-6	AK-7	AK-8	AK-9
Growth on:												
Maltose (0.2%)	+	+	-	+	+	+	+	+	+	+	+	+
Cellobiose (0.2%)	+	+	-	-	-	-	-	-	-	-	-	-
Glucuronate (0.1%)	+	-	-	(+)	(+)	+	-	-	-	-	-	-
Mannitol (0.1%)	+	+	-	-	(-)	-	-	-	-	-	-	-
Proline (0.1%)	+	+	+	(-)	(-)	-	-	-	-	-	-	-
Lactate (0.2%)	+	-	+	(-)	-	-	-	-	-	-	-	-
Pyruvate (0.1%)	+	-	+	-	-	-	-	-	-	-	-	-
Acetate (0.05%)	+	-	+	-	-	-	-	-	-	-	-	-
Propionate (0.05%)	+	-	-	-	-	-	-	-	-	-	-	-
Heptanoate (0.05%)	+	-	-	-	-	-	-	-	-	-	-	-
D-α-Alanine(0.05%)	+	(-)	-	-	-	-	-	-	-	-	-	-
L-tyrosine (0.4%)	+	-	-	-	-	-	-	-	-	-	-	-
Production of:												
Lipase	+	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	+	-	-	-	-	-	-	-	-	-	-	-
Amylase	+	-	-	-	-	-	-	-	-	-	-	-
Optimal growth temperature:					20° C	22° C	15° C	15° C	15° C	15° C	15° C	15° C

Parantheses in the published reference data indicates strain variability. Tested strains are isolates from Alaskan salmon. [a]Taxonomic information from Nealson (1978), [b]Bergey's Manual of Diagnostic Bacteriology (1994), and [c]Nealson (1993).

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Chapter 2

Organization and promoter analysis of the *Photobacterium phosphoreum lux* operon

Abstract

Bacterial bioluminescence is carried out by the gene products of the *lux* operon. Interest in the distribution and regulation of the *lux* genes has been of increasing importance due to the understanding of quorum sensing as a pervasive and ecologically important gene control paradigm. Little is known regarding *lux* operon structure, regulation, or the promoter in *P. phosphoreum*; thus, for this study, we characterized the promoter of the *lux* operon and cloned and sequenced the entire *lux* operon from strains of *P. phosphoreum* from Alaska, the Atlantic Ocean, the Black Sea, and Oregon. Our results indicate conservation in the genes present in the *lux* operon in these geographically isolated strains, as well as conservation in the transcript start site. We were unable to determine the quorum sensing circuit used by *P. phosphoreum*.

Introduction

Bacterial bioluminescence is accomplished by the gene products of the *lux* operon. DNA sequences and organization of the *lux* genes of several bacterial species have been reported revealing conservation in gene organization and requisite genes for light production (5, 6, 9, 15, 19, 21, 23, 35). Genes required for bioluminescence are *luxAB*, which code for the α- and β- subunits of bacterial luciferase, a heterodimeric protein; and *luxCDE*, which code for a fatty acid reductase complex that produces a long-chain aldehyde (9, 10, 22) (Fig.1). Light production by bacterial luciferase requires the

long chain aldehyde synthesized by the fatty acid reductase complex, and reduced flavin and molecular oxygen which are acquired as metabolic derivatives of the cell (22). In all species where *lux* operon organization has been determined, the gene order is *luxCDABE*. Unique to *Photobacterium* is *luxF*, a gene located between *luxB* and *luxE*, that codes for a gene product of unknown function (17, 21). The deduced *luxF* amino acid sequence has strong homology to *V. fischeri* and *V. harvey* LuxB (32).

Several bioluminescent bacterial species regulate the *lux* operon by quorum sensing. This gene control paradigm describes a cell density dependent regulation system in which transcription of an operon is depressed under low cell density conditions. As cell density increases and a threshold density is reached, induction of transcription among cells is coordinated and dramatic (37). Superficially, all *lux* operons under the control of quorum sensing appear the same. However, two distinct quorum sensing circuits have been described, both regulating the *lux* operon in a cell-density dependent manner. In *Vibrio fischeri*, the quorum sensing circuit resembles transcriptional control involving an activator like the *mal* operon in *Escherichia coli*. In contrast, the *Vibrio harveyi* quorum sensing circuit resembles transcriptional control with a repressor like the *lac* operon in *E. coli*. The quorum sensing circuit of *V. fischeri* involves two homoserine lactone (HSL) autoinducers, one a C8-HSL that is generated by AinS (14, 16) and is most active for the functioning of bioluminescence at intermediate cell densities, like that found in culture (20). The second HSL autoinducer, a C6-HSL, is generated by LuxI (8) and is primarily generated under high cell densities like that found in the well-studied symbiosis formed between *V. fischeri* and the squid, *Euprymna scolopes* (28). Recent work (20) suggests

the C8-HSL autoinducer is more versatile than the C6-HSL generated by LuxI. HSL binds the transcriptional activator, LuxR. Once bound, the LuxR-HSL complex binds at the *lux* operon promoter and facilitates transcription of the *lux* genes (33) (Fig. 2). The simplicity of the *V. fischeri* quorum sensing circuit has been complicated by identification of two additional regulatory proteins, LuxO (26) and LitR (11).

The quorum sensing pathway described in *V. harveyi* uses a more complex hybrid autoinducer/two-component phosphate-signaling circuit (24) (Fig. 3). Briefly, in an uninduced state, *V. harveyi lux* operon transcription is repressed by LuxO (12). When induced, two separate autoinducers bind to their respective sensor proteins, LuxN and LuxQ (2, 3). Signal from LuxN and LuxQ are integrated through LuxU (13). LuxU then signals LuxO (13, Freeman, 1999 #181, Bassler, 1994 #184), which liberates LuxO from its repressor role, allowing binding of the activator protein, LuxR (which shares no apparent sequence similarity with *V. fischeri* LuxR) (31), and transcription of the *lux* genes.

Both quorum sensing circuits shown to regulate the *lux* operon in bioluminescent bacteria have signature recognition sequences in the promoter region upstream of *luxC*. In *V. fischeri*, the lux box, a 20 bp palindrome upstream of *luxC*, has been identified and has been shown to be critical for binding of the *V. fischeri* LuxR-HSL complex, and, induction of the *lux* operon (7). In the *V. harveyi* quorum sensing circuit, two regions upstream of *luxC* have been shown to be critical for binding of *V. harveyi* LuxR (34).

To date, two closely related bacteria have been shown to regulate bioluminescence with two distinct quorum sensing circuits. One objective of this study

was to probe the genome of *P. phosphoreum* for genes involved in the *V. fischeri* quorum sensing circuit. Because of the close phylogenetic relationship between *V. fischeri* and *P. phosphoreum* (4), we hypothesize both bacterial species share some of the genes involved in the regulation of bioluminescence, specifically *luxR* and *luxI*. Over the last several years, the ecological importance of quorum sensing has been realized, and we believe identification of the quorum sensing pathway used by *P. phosphoreum* may provide insight into the distribution and diversity of quorum sensing components used to regulate bioluminescence in a closely related group of bacteria.

Currently, little is known regarding regulation of bioluminescence in *P. phosphoreum* other than bioluminescence appears to follow the same general pattern of autoinduction found in *V. fischeri* and *V. harveyi*, and *luxR* is not immediately upstream of *luxC* as in *V. fischeri*. Another objective of this study is to determine the complete DNA sequence of the *lux* operon from three geographically isolated strains of *P. phosphoreum*: AK-6 isolated from Alaska, BS-2 isolated from the Black Sea, and NZ-11-D isolated from the Atlantic Ocean. Finally, we analyzed the 5' promoter region from *P. phosphoreum* AK-6, BS-2, and NZ-11-D, as well as *P. phosphoreum* BS-1 isolated from the Black Sea and OIMB-1 isolated from Oregon to determine sequence similarities and to look for regulatory regions reported upstream of *luxC* in *V. fischeri* or *V. harveyi* and to identify regions in the promoter that are conserved among five geographically separated strains of the same species.

Materials and Methods

Bacterial strains. *P. phosphoreum* AK-6 was isolated from a salmon migrating in the Yukon River 1,228 km from the mouth (4), *P. phosphoreum* NZ-11-D was isolated from the light organ of a marine fish off the west coast of Africa near the Canary Islands (29), *P. phosphoreum* OIMB-1 was isolated by one of us (CFW) from the gut of a marine fish from Coos Bay, Oregon, and *P. phosphoreum* BS-1 and BS-2 were isolated from the Black Sea (27). All *P. phosphoreum* strains were grown at 15 °C in seawater complete medium as previously described (4). Long-term storage of the strains is at -80 °C in SWC medium containing 15% glycerol. Growth of *Escherichia coli* was in LB medium at 37 °C. Ampicillin at 100mg/ml and Kanamycin at 50 μg /ml were added as needed.

Nucleic acid isolation. Genomic DNA was isolated from 100 ml exponentially growing cells following a standard protocol for DNA isolation (1). RNA was degraded by 1-h incubation at 37 °C with 10 μg of RNaseA/ml. DNA quality and yield were determined by agarose gel electrophoresis. RNA was isolated from 50 ml brightly luminous, exponentially growing cells. Cells were pelleted by centrifugation, and snap-frozen on dry ice. We extracted RNA from cell pellets with TRI Reagent (Sigma, St. Louis, MO) following the manufacturer's protocol. Isolated RNA was suspended in nuclease-free water and stored at -80 °C. RNA quality and yield were checked with gel electrophoresis and UV spectrophotometery.

Restriction enzyme digestion of genomic DNA. Twenty μg AK-6 genomic DNA was completely digested, separately, with 10 u Nsi I (Promega, Madison, WI) and 10 u Sal I (Promega) for 2-h at 37 °C. Fifty μg BS-2 and NZ-11-D genomic DNA were

partially digested with Sau 3A I (Promega) according to the method of Sambrook (30). Following digestions, active enzyme was denatured by 15-min incubation at 65 °C.

Southern blotting and hybridization. Enzymatically-digested genomic DNA was electrophoresed in a 1% agarose gel. The resulting gel was photographed, and DNA contained in the gel was transferred to a Magna nylon membrane (Osmonics, Westborough, MA) by capillary transfer (30). DNA was fixed to the membrane by UV crosslinking. Blots were prehybridized for 6-h at 20 °C in approximately 10 ml prehybridization solution containing 50% formamide, 5X SSC (0.75 M NaCl, 0.075 M sodium citrate), 10X Denhardt's solution (1% BSA, 1% Ficoll, 1% PVP-40), and 200 µg yeast RNA/ml. A *P. phosphoreum luxA*-[α-^{32}P]dCTP probe was generated (36), and approximately 10^6 cpm of the probe were added per ml prehybridization solution. Blots were hybridized for 36-h at 20 °C. Following hybridization, blots were washed in a solution containing 0.5X SSC and 0.1% SDS for 1-h at 50 °C. Blots were then exposed to x-ray film (Fuji, Elmsford, NY) at -80 °C for 18 to 48-h. Probes for *P. phosphoreum luxC* and *luxE* were generated with a protocol similar to that of *luxA*.

Genomic cloning and screening. To clone the *lux* operon of AK-6 we constructed genomic libraries with DNA that we completely digested with Sal I and Nsi I. Genomic libraries for BS-2 and NZ-11-D were generated with DNA that we partially-digested with Sau3A I. We size-selected the partially-digested genomic DNA for fragments approximately 8-10 kb by gel purification and extraction using Qiaex II gel extraction kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Recovery and yield of the DNA size selection was determined by agarose gel

electrophoresis. pGEM-3Zf+ vector (Promega) was linearized by digestion with Sal I or HindIII for AK-6, and Bam HI (Promega) for BS-2 and NZ-11-D. The linearized plasmid was then dephosphorylated with shrimp alkaline phosphatase (USB, Cleveland, OH). Vector concentration was adjusted to 100 ng/µl and mixed with 500 ng size-selected, enzyme-digested genomic DNA and 2 u T4 DNA ligase (Promega). Ligations were preformed at 20 °C for 1-h. XL10-Gold ultracompetent cells (Stratagene, La Jolla, CA) were transformed following the manufacturer's instructions. All clone libraries were screened with colony hybridization (30). Libraries for AK-6 were screened with the same *luxA* probe generated for probing the *P. phosphoreum* Southern blots described above. Plasmids from the AK-6 Sal I and HindIII clone libraries that were positive for *luxA* were harvested and purified with CsCl-gradient centrifugation (30). To ensure the entire *lux* operon was successfully cloned from strains BS-2 and NZ-11-D, we screened our genomic libraries with two *P. phosphoreum* probes: one for *luxC* and another for *luxE*. We isolated plasmids from the BS-2 and NZ-11-D libraries that were positive for both *P. phosphoreum luxC* and *luxE* on a QIAfilter plasmid maxi kit (Qiagen).

DNA sequencing. To facilitate sequencing of the AK-6 genomic clones, we utilized the EZ::TN <KAN-2> transposon insertion kit (Epicentre, Madison, WI) following the manufacturer's instructions. Gaps in the sequence were closed by primer walking. After determining the AK-6 *lux* operon DNA sequence, we compared the DNA sequence of AK-6 to *lux* gene sequences for other strains of *P. phosphoreum* deposited in GenBank, and chose conserved regions as priming sites. A complete list of primers and their sequences are listed in Table 3. For all sequencing reactions, we used 200 to 500 ng

plasmid DNA template, 3.2 pmol primer, 4 µl Big Dye (Applied Biosystems, Foster City, CA) and water to a final volume of 20 µl. We programmed our thermal cycler (GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT)) for 25 amplification cycles consisting of denaturation (96 °C for 30 s), primer annealing (primer-appropriate temperature for 15 s), and primer extension (60 °C for 4 min). Extension products were submitted to the University of Alaska's Core Facility for Nucleic Acid Analysis for sequencing on an ABI 3100 DNA sequencer.

Primer extension analysis. Ten pmol primer PphLuxC89r (Table 1) was 5' labeled with [γ-^{32}P] ATP with 5 u T4 polynucleotide kinase (Promega) at 37 °C for 10 min. Labeled primer was annealed with 15 to 30 µg AK-6, NZ-11-D, OIMB, BS-1, and BS-2 RNA for 20-min at 45 °C. Primers were then extended with AMV reverse transcriptase (Promega) following the manufacturer's instructions. Primer extension products were visualized on an 8% denaturing acrylamide gel. The gel was dried and exposed to x-ray film (Fuji) for 18 h at -80 °C. For accurate size determination of primer extension products, we generated a sequencing ladder for the DNA region corresponding to the 5' *lux* operon promoter region from AK-6. Four µg AK-6 Hind III plasmid (the genomic clone from AK-6 that we determined contains the *lux* promoter region) was sequenced using the same labeled primer used in the primer extensions with the Sequenase quick denature plasmid DNA sequencing kit (USB), following the manufacturer's instructions. Sequencing reaction products were run alongside primer extension products from AK-6, NZ-11-D, and BS-2 on an 8% acrylamide gel. Primer

extension products for BS-1 were not included because of low yield. The gel was dried and exposed to x-ray film (Fuji) for 48-h at -80 °C.

Computer based sequence analysis. A bidirectional contig for each *lux* operon was assembled with Sequencher, version 4.0.5 (Gene Codes, Ann Arbor, MI). Individual gene sequences for the *lux* operon, *luxCDABFE*, were imported into ClustalX and aligned with representative gene sequences in GenBank. Alignments were imported into PAUP* (D. Swofford, Phylogentic analysis using parsimony and other methods, ed. 4.0b10, Sinauer Associates, Sutherland, Mass., 2000) where phylogenetic analyses were performed. We utilized maximum-likelihood inference methods for *luxCDABE*. Our *luxA* gene tree is rooted in *V. harveyi luxB*, and our *luxB* tree is rooted in *V. harveyi luxA*. For *luxF* we used maximum parsimony methods on deduced amino acid sequences. The tree generated for *luxF* is rooted with *V. harveyi luxB* amino acid sequence because of the strong amino acid similarity between *V. harveyi luxB* and *P. phosphoreum luxF* (32).

GenBank accession numbers. Accession numbers for complete *lux* operon DNA sequences for *P. leiognathi* ATCC 25521 is M63594, *V. fischeri* MJ-1 is AF170104, *Shewanella hanedai* ATCC 33224 is AB058949, *Photorhabdus luminescens* ZM1 is AF403784; the complete *lux* operon sequence for *Vibrio cholerae* ATCC 14547 was determined by Webb (35). Accession numbers used for *V. harveyi* were X07084 for *luxC*, J03950 for *luxD*, M28815 for *luxE*, and X58791 for *luxAB*. Accession numbers used to generate a partial operon sequence for *P. phosphoreum* were X54690 and M64224 for a partial *luxC* sequence, M64224 for *luxD*, X55458 for *luxAB*, M22128 for *luxF*, and X55459 for *luxE*. *V. harveyi luxAB* sequences are for strain CTP5, *luxCDE*

sequences are from an unknown strain; *P. phosphoreum luxAB* are for strain NCMB 844, *luxCDFE* are from unknown strains.

Results

***lux* operon organization**. We cloned the *lux* operon for AK-6, BS-2 and NZ-11-D. For AK-6, we generated two genomic clones which together possess the *lux* operon, partial sequence for *lumP* (the gene immediately upstream of *luxC*), and four genes downstream of the *lux* operon (Fig 4). For NZ-11-D, we generated two clones from partially Sau 3A I digested genomic DNA which contain a partial sequence for *lumP*, the entire *lux* operon, and a partial sequence for *ribA*, the gene downstream of the *lux* operon (Fig. 4). Our clones for BS-2 contain a partial sequence for *lumP*, the entire *lux* operon, and a partial sequence for *ribA* (Fig. 4). The sequences for the *lux* operon from AK-6, BS-2 and NZ-11-D do possess *luxG* downstream of *luxE*, consistent with reports of its presence in *P. phosphoreum* (18).

Regardless of which *lux* gene we analyzed, a similar phylogenetic pattern emerged in which the closest sister taxon to the *P. phosphoreum* group is *Photobacterium leiognathi* (Fig. 5). Additionally, in phylogenetic inferences that included *S. hanedai*, the phylograms suggest that the lux operons fall into two clades: one contains the *Photobacterium* species, *V. fischeri*, and *S. hanedai*; the other contains *V. harveyi*, *P. luminescens*, and *V. cholerae*. Our analysis of *luxF*, both on DNA and amino acid sequence, was ambiguous because there were too few taxa for comparison.

Transcript mapping and promoter analysis. Our analysis of the location of the transcriptional start for these geographically isolated strains of *P. phosphoreum* revealed

apparent conservation in the location of the transcriptional start site, with the start site differing by only one nucleotide position for strains AK-6, OIMB-1, NZ-11-D, and BS-1 (Fig. 6, 7). For AK-6, the transcriptional start site is 34 nucleotides upstream of the start codon for *luxC*; and for OIMB-1, BS-1, and NZ-11-D, the transcriptional start site is located 33 bases upstream of the start codon for *luxC* (Fig. 7).

Alignment of the promoter regions of the *P. phosphoreum* strains demonstrates significant sequence similarity in the untranslated region upstream of the *luxC* (Fig. 8). When we compared *P. phosphoreum* strains AK6, NZ-11-D, and BS-2, 52% (125 of 240) of the bases are identical. Conservation of the promoter region becomes even more striking when only *P. phosphoreum* strains NZ-11-D and BS-2 are compared: 93% (223 of 240) of the bases are identical. These results are consistent with our phylogenetic comparisons based on the *lux* genes where AK-6 is clearly part of the *P. phosphoreum* group, but divergent from BS-2 and NZ-11-D.

We were unable to locate regions of the *P. phosphoreum* promoter which are essential for regulatory function based on sequence similarity, specifically the identification of a *V. fischeri*-like lux box located 40 bases upstream of the transcriptional start site in *V. fischeri* (7), or the regions identified as LuxR binding sites in *V. harveyi* located -290 to -253 and -170 to -116 upstream of the transcriptional start site (34). We were, however, unable to locate either region believed to be important to regulation of the *lux* system in *P. phosphoreum*.

Because of the close phylogenetic relationship between *P. phosphoreum* and *V. fischeri* based on the *lux* genes, we hypothesized *P. phosphoreum* most likely utilize the

V. fisheri quorum sensing system. Thus, as an additional component to the search for regulatory mechanisms in *P. phosphoreum*, we probed Southern blots prepared with *P. phosphoreum* genomic DNA with *V. fischeri luxR* and *luxI* gene probes (15). Results of these hybridizations were unsuccessful for both the *luxR* and *luxI* probe (data not shown).

Discussion

Our analysis of the *lux* operon and *lux* promoter region from three geographically isolated strains of *P. phosphoreum* (AK-6, BS-2, and NZ-11-D) revealed a general pattern of conservation of the organization of the *P. phosphoreum lux* operon relative to other species of bioluminescent bacteria. The *lux* operon of all three *P. phosphoreum* strains consists of *luxCDABFEG*.

Phylogenetic analysis of the individual *lux* genes from the geographically isolated strains of *P. phosphoreum* we examined revealed a consistent pattern of relationships. We found that all three strains are closely related, but BS-2 and NZ-11-D are consistently more closely related to each other than to AK-6. This may not be surprising due to the fact that the Black Sea is connected to the Atlantic Ocean via the Mediterranean Sea, which is close in proximity to the source of strain NZ-11-D.

The deduced amino acid sequence of LuxF from *P. phosphoreum* has strong similarity with LuxB of *V. fischeri* and *V. harveyi* (32), which we also found to be true for the *P. phosphoreum* strains examined in this study. Further phylogenetic analysis of *luxF* was not performed because we had too few gene sequences to make the analysis informative. Because the function of the gene product of *luxF* has not been determined, and expression of *luxF* is apparent, elucidation of the function of this gene product may

provide insight into how the bioluminescent system is integrated into the physiology of *P. phosphoreum*.

Results of our phylogenetic analysis of the individual *lux* genes are reinforced by analysis of the *lux* promoter regions for the same strains. We propose regulatory regions can be used as phylogenetic markers. They are not under the same selective pressures as the structural genes; however, regions used as protein binding sites are certainly influenced by natural selection, and similarities in these regions should reflect common ancestry. Our results show significant conservation of the 216 bases preceding the transcriptional start site of the *lux* operon when all strains are compared, but the conservation is even more striking when strains BS-2 and NZ-11-D are compared. Our data show the *lux* promoter region of AK-6 is similar, but readily distinguishable from the promoters of strains BS-2 and NZ-11-D.

We were unable to locate a *V. fischeri*-like *luxR* or *luxI* with Southern-blot hybridization (data not shown) or a *V. fischeri*-like lux box in the promoter region of *P. phosphoreum*. We were also unable to recognize *V. harveyi*-like LuxR binding regions in the *lux* promoter. We recognize that similarity in structural motifs that comprise regulatory sites might be too subtle to detect by simple sequence comparisons.

Recent studies (25, 26) suggest *V. fischeri* uses a LuxO that is homologous to *V. harveyi* LuxO for regulation of bioluminescence by indirectly repressing *litR* transcription. Miyamoto (25) also proposes that LuxO is a shared regulatory protein for the regulation of bioluminescence between *V. harveyi* and *V. fischeri*, and we believe development of LuxO as a probe for quorum sensing systems may prove useful for

determining the quorum sensing system used by *P. phosphoreum* to regulate bioluminescence.

Finally, the genes downstream of the *lux* operon in *P. phosphoreum* have been shown to be involved in riboflavin synthesis (18). We found *ribA* immediately downstream of *luxG* in the three *P. phosphoreum* strains we examined. Furthermore, the *rib* genes are transcribed in the same direction as the *lux* operon. Riboflavin has been determined to be a necessary substrate for the emission of light by bacterial luciferase (22), but it is not clear whether the *lux* and *rib* genes are part of the same transcriptional unit. Those results may provide insight regarding the integration of the luminescence system into the biology of *P. phosphoreum* and the functional importance of light emission to this bacterium.

In this study, for the first time, we have sequenced and reported the complete DNA sequence of the *lux* operon and promoter region from three geographically isolated strains of *P. phosphoreum*. We also mapped the 5' promoter region of the *lux* mRNA from the same three strains. We were unable to locate a *V. fischeri*-like *luxR* or *luxI* or recognize promoter regions that are critical for the activation of luminescence in *V. fischeri* or *V. harveyi*.

Acknowledgements

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Figure Legend

Fig. 1. *lux* operon organization of bioluminescent bacteria from which the *lux* operon has been sequenced. Organization of *lux* genes required for bioluminescence, *luxCDABE*, is conserved. Some *Photobacterium* species possess *luxF*, between *luxB* and *luxE*. Modified from Meighen, 1994.

Figure 2. Fully induced bioluminescence pathway for *Vibrio fischeri*. Under the current model, LitR activates transcription of *luxR*. LuxR binds to HSL autoinducer, forming LuxR-HSL complex which acts as a transcriptional activator for the *lux* operon, *luxICDABEG*. Repression of this system can occur by repression of *litR* or failure of LuxR-HSL complex to bind upstream of the *lux* operon. The events required for induction of the luminescence pathway occur sequentially and are under the control of two quorum-sensing circuits: one at the level of transcription of *litR*, and the other at the level of LuxR-HSL complex formation by limitation of LuxI-generated HSL. Modified from Lupp, 2003.

Fig. 3. Induced luminescence pathway of *Vibrio harveyi*. This regulatory system uses two AI, AI-1 and AI-2 generated by LuxLM and LuxS, respectively. Both autoinducers accumulate in growth medium. Upon reaching threshold concentration, each autoinducer binds to its respective sensor protein, LuxN for AI-1 and LuxQ for AI-2. Signal is integrated through LuxU and passed to LuxO. When the luminescence system of *V. harveyi* is induced, LuxU is hypothesized to liberate LuxO from its repressor role, allowing binding of luxR, and transcription of the *lux* operon. Modified from Miller, 2001.

Fig. 4. Locations of inserts of genomic clones used in this study.

Fig. 5. Phylogenetic analysis of the individual *lux* genes. A) ML tree based on *luxA* gene sequence, rooted in *V. harveyi luxB*; B) ML tree based on *luxB* gene sequence; rooted in *V. harveyi luxA*; C) ML tree based on *luxC* gene sequence, rooted in *V. harveyi luxC*; D) ML tree based on *luxD* gene sequence, rooted in *V. harveyi luxD*; E) ML tree based on *luxE* gene sequence, rooted in *V. harveyi luxE.*

Fig. 6. Primer extension products for AK6, NZ11D, OIMB, BS1, and BS2. Marker in first lane is ΦX174 DNA/*Hinf* I. Volumes loaded were 5 µl 1:50 dilution of ΦX174 DNA/*Hinf* I marker, 5 µl AK6, 5 µl NZ11D, 5 µl 1:10 dilution OIMB, 15 µl BS1, and 5 µl BS2.

Fig. 7. Autoradiogram of primer extension products for the lux operon of Photobacterium phosphoreum strains AK6, OIMB, NZ-11D, and BS1. Lanes 1-4 are a sequencing ladder generated for AK6.

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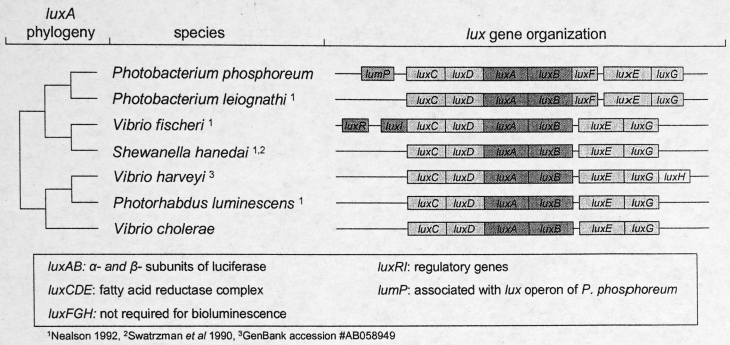

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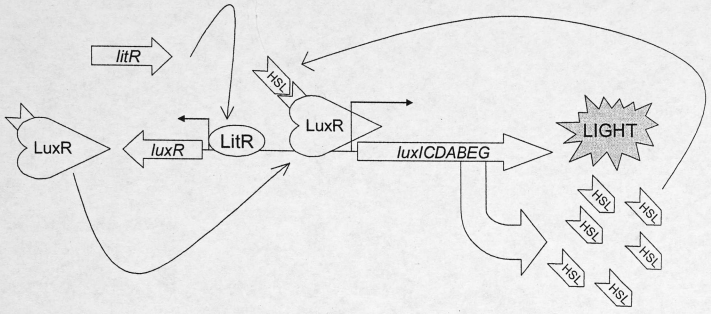

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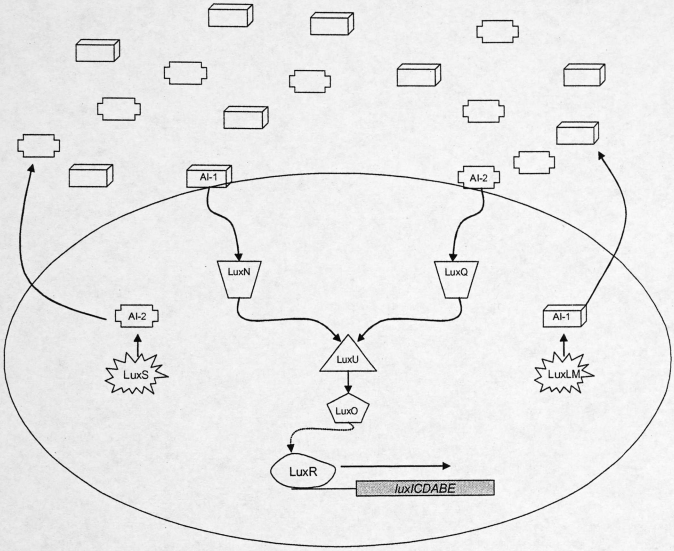

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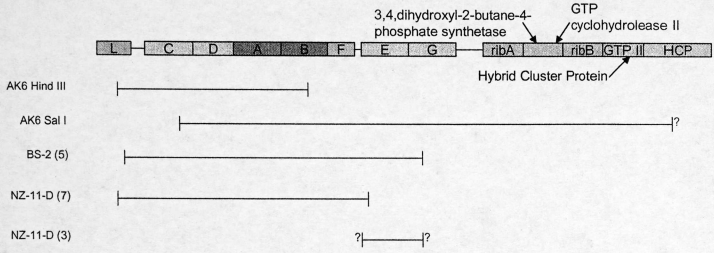
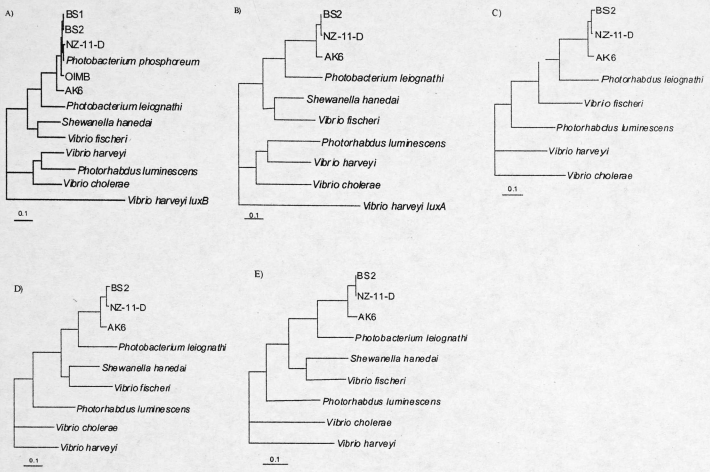


Figure 4. Locations of inserts of genomic clones used in this study.



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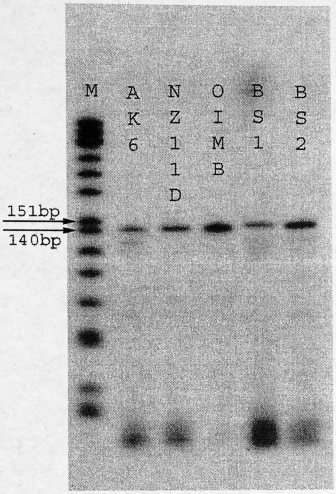

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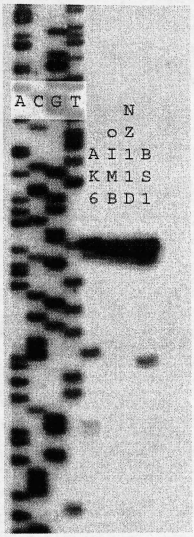

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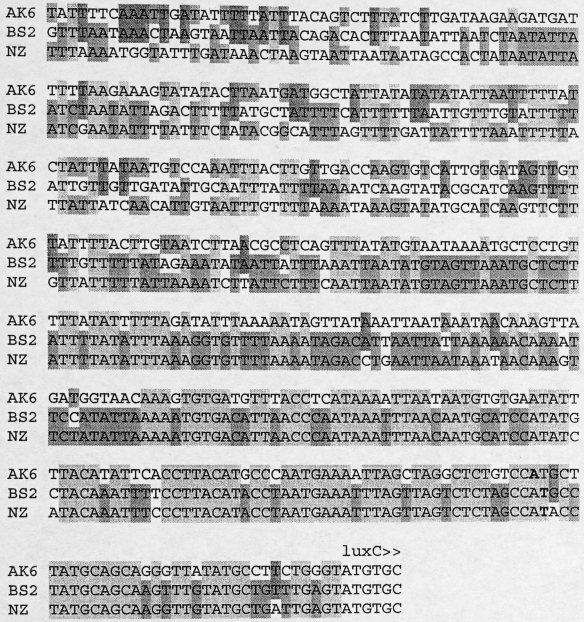

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TABLE 3. Oligonucleotide primers used in this study

Primer designation	Sequence (5'-3')	Tm (°C)
luxA127f	GAI CAI CAI TTI ACI GAG TTT GG	61.8
luxA275f	TIY TIG ATC AAI TGY CIA AAG GIC G	65.3
luxA1007r	ATT TCI TCT TCA GII CCA TTI GCT TCA AAI CC	68.1
PphLuxC89r	AGT GTG AGY TCA CGA TAT T	50.6
PphLuxC506f	TCT ATT ATT AGA GCA A	36.7
PphLuxD772f	TCA TCA CAT GAT TTA GGT GA	49.8
PphLuxB944r	TAA AAT CTT TTG AAT CTT CT	43.4
PphLuxE366f	CGA CCT CAA TGT TTA AGN AT	50.1
SalIT7-walk#1	TAC GGG TGC AAA TAA TCA T	50.2
HindIII T7 719r	CCG TTG GGA GAG GTG	51.3
H21FP 610f	CTT GAT AAG AAG ATG	35.5
Pph luxC993r	NGA TCG CCT TTT GGC	50.1
Pph luxD783r	TCA TGT GAT GAA CCT	43.1
Pph luxA646r	ATT GTA GCT GCT GTG A	47.9
Pph luxA631f	TCA CAG CAG CTA CAA T	47.9
Pph luxB943f	ACC CAG CTC GTA AGC A	53.5
Pph luxE455f	TAC NSC ACT AGT ACC AC	48.8
Pph luxE1062r	CYT CCT GAC NAT CTT C	45.3
S7FP 357r	CTT GAG GGC CGC AAA CA	56.4
T7 RP#1	ACC TTG CTC GAT CAC ACG T	57.6
T7 walk#2f	AGT TAA TGC TAT GTG GCG AT	53.1
S7FP Walk#1f	GCT GAT GCT TTT TGC A	48.7
SalI SP6-515f	GTT ACT CCT TGG GAA	43.6
HindIII SP6-581f	CAG TAT GAC CCT CAT	42.9
S44:RP-527f	GAC CAA ATG TCA GAT	41.6
B943f-516f	CCG CTT ATA ATG GCA	45.3
E366f-615f	GCC AGA TCC ACT CCA	50.2
C993r-38r	ATG ATC AGC AGG CCT	49.9
C89r-50r	TTG CTG GAG ATG TCG	48.0
T7 Walk#3f	TTA CCT GCT CAC AGC	47.9
S7:FP-401f	GCG AAT GCC GAT AAA	46.5
PphLuxB77r	ATT GAT CAT ATT ATC TAA AA	39.8
Pph luxA230r	GGT ACA AGC CAC GTA CA	52.0
1156r	TGA TGA GAC CCA AAG G	48.0
2949r	TAT CCG AGA CGT GAT G	47.4
8883f	TAT GAC GTA TAG TGT	38.2
fp407f-150r	AAC CTG AAA TTA ACG	39.5
fp407f-760f	AAT GAA TGA TGA CGG	42.2
S31:FP-478f	TCA ATC ACG TCA GGT	46.6
Walk#3-109f	GCA CAA TCA ATC ACT	42.9
Walk#3-713r	TGG CTA TTA TTG CGG	45.3
13092f	CAG CAA TAA CCG CTT G	48.4
10827f	AGT GTA CTT GAG ATG	40.3
10122r	TTT CAG GAT CTT CTG	40.7
9457r	TTC ACC ATC AAA GGT	43.9
Pph1754r	AGC ATG GAC AGA GCC	51.0
7628r	AAC TCA ACA AGA CTC	41.4
7916f	GTC AGA TTC GAG ATA C	42.4
8547r	NCC ATG AGG TGC ATC	48.6
1758r	GCA GCA RGK TTR TAT GC	50.3
2917r	GCT AAT GGT TGA TTA CC	44.8
4298r	GCA ACA AAG CGA TCC ATG AC	55.2
3747f	GAT GAA ATA CCA GAA G	40.2
5518f	GCR AGC TTC CTA TTA GG	48.2
5935r	GGC TAT CGC TCC AAC	49.0
2432r	CAT TAC CGA CAT TGA ACG	49.1
3507r	CAC CGC TAC TTA ATC	42.4
3283f	CTG TRC CAA TTG ATC ATG	47.6
4471r	CAG CAA GCC AAG TCG	50.3
6215r	CCT GTA CGC TCT AAC GC	52.8
6199f	GCG TTA GAG CGT ACA GG	52.8
6813r	GTA CAT GCT ACA TAG	38.2
8026r	CAG GAT CAA GTG CAC G	50.3
8528f	CAA ATA GAT GCA CCT C	43.8
KAN-2 FP-1	ACC TAC AAC AAA GCT CTC ATC AAC C	63.0
KAN-2 RP-1	GCA ATG TAA CAT CAG AGA TTT TGA G	60.0

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General Conclusions

I identified all our luminous bacterial isolates from Alaskan salmon to be *P. phosphoreum* based on tests of nutritional versatility and phylogenetic inferences based on 16S rRNA and *luxA* gene sequences. The identification of *P. phosphoreum* from a freshwater niche is unexpected because *P. phosphoreum* is believed to be an exclusively marine bacterium with a requirement for sodium in its growth medium (1). While the distribution of the luminous bacteria in several marine environments has been reported, a survey of the northern Pacific Ocean has not been undertaken, so there is no information on the abundance or distribution of luminous bacteria in the Bering Sea. However, based on optimal growth temperature one would expect *P. phosphoreum* to be the dominant bioluminescent bacterial species present (2). Thus, it is my hypothesis that salmon are acquiring *P. phosphoreum* while maturing in the northern Pacific Ocean in a saprophytic (i.e., non-pathogenic) association. The bacteria are likely protected from the very low osmotic environment of freshwater by the salmon until the fish are caught at least 1,228 km from the mouth of the Yukon River.

It is not clear where *P. phosphoreum* is located on or in the salmon. All of our isolates, except AK-5 which was isolated from the gut, are presumed to be from external surfaces on the fish. However, the possibility that luminous bacteria are present in the gut and are discharged through the mouth or anus after being caught cannot be dismissed. But, I propose our isolates, with the exception of AK-5, have their origin on the external surfaces of the fish because the digestive tract of the fish from which I isolated luminous

bacteria from were empty, except for the gut of the fish that was the source of AK-5.

Other possible hypotheses to explain the isolation of a marine bacterium from freshwater are (1) *P. phosphoreum* has acquired freshwater tolerance, and (2) the *lux* operon has been transferred by horizontal gene transfer to a bacterium that is capable of surviving in freshwater.

The second hypothesis, that the *P. phosphoreum lux* operon has been horizontally transferred, was eliminated by comparing data on nutritional versatility and 16S rRNA phylogeny to *luxA* phylogeny for our Alaskan *P. phosphoreum* isolates. Phylogeny of *luxA* based on DNA sequence similarity reflects the history and source species of the *lux* operon. By comparing the species from which the *lux* operon has originated to information specific to the organism like physiology (assessed by tests of nutritional versatility) or 16S rRNA gene sequence, one can determine whether horizontal gene transfer has occurred. Data that would suggest a horizontal gene transfer event occurred would be incongruence between organismal history based on physiology or 16S rRNA gene sequences and *luxA*. In the experiments described in chapter 1, I estimated the phylogeny of our luminous isolates based on *luxA* and 16S rRNA, and performed tests of nutritional versatility that are diagnostic for the bioluminescent bacteria. All three tests concurred that our luminous Alaskan isolates are *P. phosphoreum* based on physiology, and 16S rRNA and *luxA* gene sequences. These data taken together eliminate the possibility that *P. phosphoreum lux* operon has been horizontally transferred.

Specific analyses of the *lux* operon from three geographically isolated strains of *P. phosphoreum*, including one from Alaska, one from the Black Sea, and one from the Atlantic Ocean revealed several interesting results. First, all strains tested had the same gene organization of the *lux* operon, *luxCDABFEG*; and second, flanking genes for all strains are the same as those previously reported. Analysis of the individual *lux* genes consistently showed a clustering pattern reflective of the gene tree for *luxA* in which AK-6 is part of the *P. phosphoreum* group, but is slightly divergent from other strains. It is possible that the minor differences in DNA sequences would be reduced if amino acid sequences were analyzed. My analysis of the *lux* promoter region resulted in a similar conclusion: Alaskan strain AK-6 is slightly divergent from the two other strains of *P. phosphoreum*.

My attempts to determine the quorum sensing circuit utilized by *P. phosphoreum* were unsuccessful. The methods used were mapping the *lux* transcript, looking for sequences in the promoter reported as critical for density-dependent regulation in closely related bioluminescent bacteria, and probing the genome of *P. phosphoreum* with probes for *V. fischeri luxR* and *luxI*. I recognize that similarity in structural motifs that comprise regulatory sites might be too subtle to detect by simple sequence comparisons.

Taken together, results of these studies report, for the first time, isolation of the luminous bacterium *P. phosphoreum* from a freshwater environment. Additionally, I report the complete DNA sequence of the *lux* operon from three geographically isolated

P. phosphoreum strains. Finally, I report the transcriptional start site and lux promoter sequence of the same three strains of *P. phosphoreum*.

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Appendices

DNA sequence of the *P. phosphoreum* AK-6 *lux* operon

lumP – partial (SEQ: 736-1228):

AATTAATGCAAATAATATCACCTGAAATTTCTTCAATATAACCAGATATCCCATTAA

TTCCAATGTCATCTCTTTCTGAAATGTTTTCTGTTAAGGTTTTTGGTATATTAATAT

AAACTTTAAGAAAATTTCCTGCTTCAATAATATCTTCAACAATAGCAACACCTTTTA

TTTTTCCAGTTAAGCCACCTCTCCCAACGGTTACGCCAAATTTTGGATGTATTTCTA

AGTTTACATGGTTACCAACGTCTAATGAATCAAATGTTGTTGTATTAAGTGCTTGAT

CTATATCAAAGTAAACGACATCTCCAGCAATGCGAACAACAGTTACGGAGCATCCAT

TTAAGAGCATTACAGTGTTTTTTTCAACTAAATCCAAGACGTTTTTGGAAAGATAAT

ACCGTGCCTTTGGGTCTCATCATTTTTTGATATTTTTTTAATAATTCCAGTTCCCTG

AACTATACCTTTGAACATAATAATCTCCTTCTGTAGG

lumP-luxC spacer (SEQ:1229-1783):

TAATATTATTTAAATGAAGTTATTAATTTTTTAATTAGTCTAGTGGTAAAAAAATAA

AATTCAACAGTAAATAATGATTTTTAATTTTAAATCTGTTTTTTAATACATAAATTAT

TACTTTTTACGTTCTATTTTTGTTTGTTTTATTTAAATATCTATTTTCAAATTGATA

TTTTTATTTACAGTCTTTATCTTGATAAGAAGATGATTTTTAAGAAAGTATATACTT

AATGATGGCTATTATATATATATTAATTTTTATCTATTTATAATGTCCAAATTTACT

TGTTGACCAAGTGTCATTGTGATAGTTGTTATTTTACTTGTAATCTTAACGCCTCAG

TTTATATGTAATAAAATGCTCCTGTTTTATATTTTTAGATATTTAAAAATAGTTATA

AATTAATAAATAACAAAGTTAGATGGTAACAAAGTGTGATGTTTACCTCATAAAATT

AATAATGTGTGAATATTTTACATATTCACCTTACATGCCCAATGAAAATTAGCTAGG

CTCTGTCCATGCTTATGCAGCAGGGTTATATGCCTTCTGGGT

***luxC* (SEQ:1784-3251):**

ATGTGCAGCAAGGTAATTTAAAGGAGATTGTATGATAAAGAAAATCCCAATGATTAT

TGGTGGCGCAGAGAGGGATACTTCAGAACATGAATATCGTGAGCTCACACTCAATAG

CTATAAAGTTAATATACCTATCATAAATCAAGATGATGTTGAGGCGATTAAATTACA

AAACGTTGAAAATAACTTAAATATCAATCAGATAGTAAATTTCTTATACACTGTTGG

CCAAAAATGGAAAAGTGAGAATTATTCTCGTCGACTCACCTATATTCGAGATTTGAT

AAGATTTCTCGGATATTCTTCTGAAATGGCCAAACTAGAAGCCAACTGGATCTCAAT

GATCTTGAGTTCAAAAAGTGCCTTATACGATATTGTTGAAACAGATTTAGGTTCTCG

TCATATTGTAGATGAATGGTTACCTCAGGGGGATTGTTATGTCAAGGCTATGCCAAA

AGGAAAATCCGTTCATTTGCTAGCAGGTAATGTGCCTCTATCTGGTGTTACTTCTAT

TATTAGAGCAATTTTGACTAAAAATGAATGTATCATTAAAACATCATCGGCTGATCC

ATTTACGGCAATAGCATTAGCTTCAAGTTTTATTGATACAGATGAGCACCATCCAAT

TAGTCGTTCAATGTCGGTAATGTATTGGTCTCATAACGAAGATATTGTAATCCCACA

ACAAATTATGAATTGTGCTGATGTTGTTGTTAGTTGGGGTGGGCATGATGCCATTAA

ATGGGCAACAGAACATACACCAGTAAACGTCGACATATTAAAATTTGGGCCGAAGAA

AAGTATTGCGATTGTTGATGATCCTGTAGATATTACAGCTTCTGCTATTGGCGTCGC

TCATGATATTTGTTTTTATGATCAGCAGGCCTGTTTTTCAACCCAAGATATCTATTA

TATAGGCGATAACATTGATGCGTTTTTTGATGAGCTTGTAGAACAATTAGATATATA

TATGGAGATATTGCCAAAAGGCGATCAAACATTTGATGAAAAGGCATCATTTTCATT

AATTGAAAAAGAGTGTCAATTCGCAAAATATAACGTTGAGAAAGGTGATAATCAATC

TTGGTTATTAGTTAAATCACCGCTAGGATCTTTTGGTAATCAACCATTAGCTAGATC

TGCATATATTCATCACGTCTCGGATATATCAGAAATAACACCTTATATAGAAAATAG

AATTACTCAAACTGTAACTGTTACTCCTTGGGAATCATCATTTAAATATAGAGATAT

TCTAGCCTCTCATGGTGCAGAGCGTATTGTTGAATCTGGGATGAATAATATCTTCCG

TGTCGGCGGTGCGCATGATGGTATGAGGCCTCTTCAACGCTTAGTTAAATATATTTC

ACATGAAAGACCTTCTACATATACAACCAAAGATGTGGCAGTAAAAATCGAACAAAC

ACGTTACCTAGAAGAAGATAAGTTTTTAGTCTTTGTACCATAA

***luxC-luxD* spacer (SEQ:3252-3263):**

AAAGGAATTAAT

***luxD* (SEQ:3264-4184):**

ATGAAAAGTGAAAACAATTCTGTGCCAATTGATCATGTTATAAAAATTGATAATGAC

CAATATATACGTGTTTGGGAAACAATCCCTAAAAATCAAGGTGATAAAAGAAATAAT

ACTATTGTTATTGCTTCTGGTTTTGCTCGAAGAATGGACCATTTTGCAGGTTTAGCT

GAATATTTATCAACCAATGGATTTCATGTTATTCGGTATGATTCACTTAATCATGTT

GGATTAAGTAGCGGTGAAATTGATCAGTTCTCAATGTCAGTCGGTAAGAAAAGTTTA

TTAACCGTTATTGATTGGTTGAAATCAGAGCATGGTATTGATCAAATTGGTTTAATT

GCATCAAGCCTTTCTGCTCGAATTGCTTATGATATTGTTGCTGATGTTAATTTGTCT

TTTTTAATTACCGCCGTTGGTGTGGTTAATTTACGAAACACTCTTGAACAAGCACTT

AAATATGATTACTTGAAGATGGAAATTGATGAAATACCAGAAGATCTAAATTTTGAT

GGATATAATTTAGGTTCAAAAGTATTTGTTACAGATTGCTTTGAAAATAACTGGGAT

ACATTAGATTCAACTATAAATAAAACGAAAAATTTAAATTTCCCTTTTATAGCTTTT

GTCGCCAATGATGACAGTTGGGTACAACAGCACGAAGTTGAAGAATTAATGAATAAT

ATTAATTCAGATAAAACGAAAATTTACTCTTTAATAGGT*TCATCACATGATTTAGGT*

*G*AAAATCTAATAGTGCTAAGAAATTTCTATCAATCAATTACGAAAGCTGCGATTGCA

TTAGATAGTAATTTATTAGGGTTAGCGAGTGAGATTGTTGAGCCACAATTTGAAGCT

CTTACAATTGCTACAGTAAATGAACGTCGCTTGAAAAACACAATAAAAAGTAAGTCA

TTAGTTTAA

***luxD-luxA* spacer (SEQ:4185-4221):**

TTACAACTGATACATAAACCAACAAAAGGAATATATT

***luxA* (SEQ:4222-5295):**

ATGAAATTTGGAAATATTTGTTTCTCATATCAGCCCCCAGGTGACTCACATAAAGAA

GTCATGGATCGCTTTGTTCGTTTAGGTGTTGCATCAGAAGAGCTAAATTTTAATACT

TACTGGGCTCTAGAGCATCATTTTACTGAATTTGGTCTAACAGGTAACCTTTTTGTT

GCTTGTGCTAACTTACTTGGTCGAACCACAAAATTACATGTTGGCACTATGGGAATT

GTACTTCCTACTGCTCACCCTGCGCGTCAAATGGAAGACTTATTACTTTTA*GATCAA*

*ATGTCAAAAGGTCG*TTTTAATTTTGGTGTTGTACGTGGCTTGTACCATAAAGATTTT

CGCGTCTTTGGTGTGACAATGGAAGATTCTCGTGCCATTACTGAAGATTTTCATACT

ATGATTATGGATGGCACAAAAACGGGTACACTTCATACTGATGGGAAAAACATCGAG

TTCCCAGATGTTAACGTTTATCCAGAGGCGTATTTAGATAAAATTCCAACATGTATG

ACAGCGGAGTCAGCAGTAACAACGACTTGGCTTGCTGAGCGTGGTTTACCGATGGTG

CTTAGCTGGATTATTACAACCAGTGAAAAGAAAGCTCAAATGGAACTCTATAATGCT

GTTGCTAGAGATAGTGGTTACAGTGAAGAGTACATTAAAAACGTTGATCA*CAGTATG*

*ACCCTCAT*CTGTTCTGTAGATGAAGATGCTAAAAAAGCTGAAGATGTATGCCGTGAG

TTTTTGGGAAATTGGTATGACTCATACGTAAATGCGACCAATATCTTTAGTGAAAGT

AACCAGACTCGTGGTTATGATTATCATAAAGGTCAATGGAAAGATTTCGTTCTTCAG

GGACATACTAATACTAAACGTCGAGTTGACTATAGCCACGATTTAAACCCTGTAGGT

ACACCTGAAAAATGTATTGAAATTATTCAGCGTGATATTGATGCAACAGGTATTACT

AATATTACCCTTGGTTTTGAAGCGAATGGTTCAGAGGAAGAAATCATTGCCTCTATG

AAACGTTTCATGACGCAAGTTGCACCATTCTTAAAAGATCCAAAATAA

***luxA-luxB* spacer (SEQ:5296-5337):**

ATCACTTAGATTAACTTTAATAAATAATATAAGGAATATAAC

***luxB* (SEQ:5338-6224):**

ATGAATTTTGGATTATTCTTCCTCAACTTTCAGCTTGAAAAGACATCGTCAGAAACT

GTTTTAGATAATATGATCAATATGGTGTCTCTGGTTGATAAAGATTATAAAAACTTT

ACAACTGTTTTAGTCAATGAGCACCATTTTTCTAAAAATGGTATTGTCGGTGCCCCG

ATCACTGCTGCGAGCTTCCTATTAGGGTTAACTGAACGTTTACACATTGGTTCTTTA

AATCAAGTTATTACAACTCATCACCCTGTTCGTATTGCAGAAGAAGCAAGTTTACTT

GACCAAATGTCAGATAGTCGTTTTATTCTAGGTCTAAGTGATTGTATTAATGATTTT

GAGATGGATTTCTTTAAACGTCAGCGTGATTCACAGCAGCTACAATTTGAAGCTTGT

TATGAGATCATTAATGAAGCAATCACAACCAATTATTGCCAAGCGAATAATGATTTT

TATAACTTCCCTCGCATCTCAATTAATCCTCATTGCCTCAGTAAAGAGAATATGAAG

CAATATATTTTAGCTTCTAGTGTGAGTGTTGTTGAGTGGGCGGCTAAAAAAGCGCTG

CCACTGACATATCGTTGGAGCGATAGCCTTGAAGATAAAGAGATTCTTTATAAGCGT

TATTTAGACGTTGCAGCAAATCATAATATTGACGTTTCTAATGTCGAGCATCAGTTC

CCACTGCTTGTAAATTTAAATCATGATCGTGATGTTGCTCATCAAGAAGCAACAACC

TATTTAGCAAGTTATATTGCCGAGGTATATCCGCATCTAAATCAGCAACAAAAAATA

GCTGAACTTATTAGCCAACATGCGATTGGTACTGATAATGATTACTATGAATCAACA

TTAAATGCGTTAGAGCGTACAGGTTCAAAAAA

***luxB-luxF* spacer (SEQ:6225-6346):**

TGTATTACTTTCTTTTGAATCAATGAAAAATCATGATGATGTTGTAAACGTGATTAA

TATGGTCAATGAGAAGATTCAAAAGAATTTA*CCAAGCTCGTAAGC*ATAAAGATGGCG

GTGTTATT

***luxF* (SEQ:6347-7042):**

ATGAATAAATGGAATTACGGAATTTTCTTCGTTAACTTTTATAGTAAAGACGAACAA

GAGTCATCAAAAATGATGAATAATGCGTTAGAAACATTACGCATTATTAATGAAGAT

ACATCTATTTATGATGTGGTTAATATTAATGATCACTATCTTGTAAAGAAAGATAGT

GAAGATAATAAGTTAGCGCCTTTTATTGCACTAGGGTCTAAATTATACGTGCTTGCT

ACCAGTGAAAACACAGTTGATAGCGCAGCAAAATATGCATTACCGCTAGTTTTTAAA

TGGGATGATACAAACGAGGAACGACTTAAATTATTGAGTTCCTATAATGCATCAGCA

AGTAAATATAAACAGAATATAGATTTGGTTCGACACCAACTTATGTTACATGTCAAT

GTCAATGAGGCAGACACTGTCGCAAAAGAAGAGCTAAAAGAATATTTTGAAAACTAT

GTAGCATGTACACAACCTAGTAATTTTAATGGCTCGATTGATAGTATTATTCAGAGT

AATGTGACAGGGTGTTATAACGACTGTTTGTCATATGTAGCGAATCTTGCTAGTGAA

TTTAATAATACTGTGGACTTCTTACTTTGTTTTGAGTCAATGCAAGATCAAAATAAG

AAAAAATCAGTAATGATAGAACTTAATAATCAAGTTATTAAGTTCCGCCAAGATAAC

AATCTAATCTAA

***luxF-luxE* spacer (SEQ:7043-7125):**

TCTACAATCATTG*CCGCTTATAATGGC*AGTGCTAATTTAAAGTTCTGCCATTATATT

TAATTATATCTTAAATAGGATTAAAC

***luxE* (SEQ:7126-8247):**

ATGACTATTATATTAGATACTTTCGAAAAAGATATTATTGTAAGTACAGAGATCGAC

GATATTATTTTTACATCCTCACCTCTTGATATTACTTATGATGAACAAGAAAGAATA

AAGCATAAATTAATATTAGAATCATTTCGTTATCATTATAACAATAATGAAGATTAT

AAGTTTTTCTGTAATACTCAGGGGATTGACGAAAATATTTCATCACTTGACGATATC

CCTGTTTTTC*CGACCTCAATGTTTAAGTAT*GCAAAAATATGTACAGCTGATGAGTCT

GACATTGAAGACTGGTTTACAAGTAGTGGTACTAGTGGTGTAAAAAGTCATATTGCT

CGTGATCGTGTAAGTATTGAACGTTTACTGGGTTCTGTAAATTATGGAATGAAATAT

CTTGGCTCATTTCATGAAAATCAGTTAGAGCTTGTTAATATGGGACCCGATCGTTTT

AATGCTAAAAATGTTTGGTTTAAGTATGTAATGAGTCTTGTTGAGTTATTATACCCA

ACCACATTTACTGTGAATAATGATGAAATAGATTTTGAACTTACCATTAAAAGTTTA

AAAGAAATCTATAATAAAGGTAAAGGCATTTGTCTCATTGGTCCTCCATATTTCATT

TACTTATTATGTCAGTACATGAAAGATAATGATATTGAGTTTAATGCTGGTAATCGA

ATCTTTATTATTACTGGTGGTGGTTGGAAAACTAAGCAAAAACAAGCGCTAAACCGT

CAAGATTTTAATCAACTATTGATGGATACCTTCCACTTAGCACATGAAAGTCAGATT

CGAGATACATTTAATCAAGTTGAATTAAATACCTGTTTCTTTGAAGATAATCGTCAG

CGTAAGCATGTTCCGCCATGGGTTTATGCGCGTGCACTTGATCCTGTGACACTAAAA

CCTGTTGAAGATGGTCAAGAGGGTCTTATTAGTTATATGGATGCATCATCAACGAGT

TACCCAACATTTATCGTTACTGATGATATTGGTATTATTCATACTATTAAAG*CGCCA*

*GATCCACTCCA*AGGTACTACGATTGATATCGTTCGCCGTTTGAATACTAGAGAACAA

AAAGGGTGTTCATTATCAATGTCATCAGGTTTAAAATAG

***luxE-luxG* spacer**

ATCATAAGGAAGATAT

luxG

ATGATTTTAAATTGTAAAATAATTAAAATTGAAGCTTCTGAATGTAATATTTTTAAA

GTATTTATTAAGCCTGATAAGTGTCTCAATTTCAAAGCTGGGCAATATGTTTTAGCG

TATTTAGATGGTAAAAAATTACCTTTTTCAATTGCTAATTGTCCAACATGTAATGAG

CTTATAGAGTTACATGTTGGAAGTTCGGTAAAAGAAACAGCAGTTAAATCTATTTCT

TACTTTGTAGATGCTTTTGTGAATAGCGGTGACATACAAATAGATGCACCTCATGGT

AATGCTTGGTTACGTGAGGGCAGTAATTCTCCATTATTACTTATTGCTGGAGGTACA

GGACTATCATATATCAATAGTATTCTTAGTAATTGTGTAAATAGGAATTTACCTCGT

TCTATTTATGTTTACTGGGGAGTTAATAATATTGACTTATTATATGCAGACACTCAA

TTGAAAGCACTTTCTAGCGACTTTAGTAATGTTAAATACGTGCCTGTTTTAGAAAAC

TTTGATAATAGTTGGTATGGGAAAAAAGGTAATGTTATTGATGCAATAATAGAAGAT

TTTTGTGATTTGTCAGATTTTGATATCTATGTTTGCGGCCCTCAAGGTATGACGTAT

AGTGTTCGAGAAAAGTTAACATCACTTAAAAAAGCGAATGCCGATAAAATGTTTGCT

GATGCTTTTGCATA

Sequence downstream of *luxG* (SEQ: to-15239)

TATGTGATCTTAATTTAAGTTAATAGAATTTAAAAAATACCTTAAACTCTCTATGAG

GTTGTATTTATATAAATTAATTTTTTAACTCTATTTTTTGGCTTTTAATATTATTAT

TCTCTCAATAAATAGAGTTATTACTAAATTTGTAATTAACGTTAATTTCAGGTTTTT

GATATATTTGTTAGGGGTGTTGGAAATAGTAATAATATATTACCTGTAACTTTGGTA

ATTTTTCTTTAATAGAAGATAATAAAGGAATAATTATGACTTTAAGTACATCTCAAG

AAATCATTGAGGATATTCGTCAAGGGAAAATGGTAATATTAATGGATGATGAAGATC

GTGAAAATGAAGGCGATCTTATCATTGCATCAGATAAAGTGACGCCTGAAGCTATAA

ATTTTATGGCAACTTACGGTCGTGGTTTAATTTGTCTGACATTAAACAAAGCCCGTT

GCCTGCAATTAAAATTACCTTTGATGGTGAAGAACAATACCGATAAATTTGCAACCC

CGTTTACTCTTTCTATAGAAGCGGCTTCTGGGGTTACAACCGGTATTTCAGTAAAAG

ATAGAGCGCGCACTGTTCAAGCGGCTGTAGCGGCAATGGCGACGCCGGAAGATATTG

TTATGCCTGGACACATTTTTCCATTAATGGCTCAAGATGGCGGTGTATTAACTCGCG

CAGGCCATACTGAAGCTGGTTGTGATGTCGCACGGTTAGCAGGATTAGAGCCTTCCA

GTGTTATTGTTGAAATATTGAATGATGACGGTACGATGGCGAGACGGCCGCAGTTAG

AAGTCTTTGCTAATAAGCATGGCTTAAGGTTAGGCACTGTCGCTAACCTTATTGAAT

ATCGAAATAAATATGAAACCATGATTGAACGTATCTCTGAGTGTAAATTGAAGACTG

AATATGGTGAATTTAATATGATCACTTATCGAGATAAAATTAATCATCAAATTCATT

ATGCGCTACAAAAAGGTAATATTGAGCCTAATTGTCAAACCTTAGTGCGAGTGCATT

TACAAGATACATTTAAAGATATTCTGCAAACAGGATCGAATCGATGGACATTACCCG

CCGCGATGAGTCGTATTAGTTCTGAAAATGGCGTTCTTGTTATAGTAACTAAACCAG

AAGATCCTGAAATTGTAATCAGTAAAATTCAGAATCTAGCTTTGGGTAATCAAGAAA

CAGCTGTGGTTAATAGTCAATCACGTCAGGTTGGATTAGGTTCGCAAATATTATCAG

ATCTTGGCGTTAGAAAAATGCGTTTATTATCATCCAGTAGTCAGCTTTATCATTCAT

TATCTGGTTTCGGTCTTGAAATAGTTGAGTATGTGTGTGATTAAGTTTCGATACAGT

AATAAGACTAGCCGTTATTTATACTAAAATTAATTATAAATATTATAGGAGTACCCA

TGAAGCTAATTGAAGGTGCCACCGTAGCACCCAATGCTAAAGTTGCTATTGTAATTG

CACGTTTTAATAGTTTTATTAATGACAGTTTATTATCTGGCGCGCTTGATGCGTTGC

AACGTCAACGTCAAGGTCAAGTTAGCGATGATAATATTACTATTATTCGTTGCCCTG

GAGCTTATGAGCTACCTCTTGTTGCCCAGTTTACGGCCAAAACTGATCGTTATGATG

CAATTATAGCTTTAGGTGCTGTTATTCGAGGTGGTACACCGCATTTTGAATATGTGG

CTGGTGAATGTAATAAAGGTCTTGCGCAAGTCGCATTAGATTATAATATTCCAGTTG

CTTTTGGTGTGTTAACTGTTGATTCAATTGAACAAGCGATTGAACGTGCTGGCACTA

AAGCGGGAAATAAAGGTGCAGAGGCTGCATTAAGTGTACTTGAGATGGTTAATGTTT

TGGCTCAAGTTGAATCTTAACTATATAACGGTTTATTAAAATTAAGTTACGAGTGTT

TAATTACACTCGTTTATAAATACAATACCGGATAGTTATTAAATAATGAATATTAGT

CATATTAGTTGATTAAGTTTGTTGTTATCGAAAGAGAATCAATACTTCTTTATTTTA

CGTAGAAATATTTAGGAATATTATGGTCAATGTTAGGGAAAGAGTACCTTTAAACGT

GGGTATTAATAGTGATATTCCTGCTGAGTTGCTTTCGTTTAATGGTCTTGAATCGGG

AAAAGAACATATAGCACTTATTTTTAAAGAAGCAGATAAAATATTGGTTCCTTTAGT

TCGTATGCATTCTGAGTGTTTAACGGGCGATGTTTTTCATTCATCACGCTGTGATTG

TGGAGAGCAGTTAGTTGAAACCATGGAAAAAATGACTGAGCAAGGTGGTATTATTTT

ATATCTGCGTCAGGAAGGTCGCGGTATTGGGCTCTATAATAAGATCGATGCTTATAA

GCTACAAAGTCAAGGGATGAATACTTATGAAGCGAATAATTATTTAGGTTTTGATGA

CGACTTACGAGAGTTTTCTGAAGCAGCTCAAATGCTTACTGCTCTTGGTATTCAGAA

TATACATTTAGTAACGAACAATCCTAAGAAAATTTTCGATTTACAGCAAAACGGTAT

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TTTAAAAACCAAAGCCTCTTATGGTCATCATCGTTTTAATTTTGACGAATGAATAGC

GAATAATAAATATTGATTGTTGCAATGAATAAAATAAGCCTCTCTGTTGGCGAGGCT

TATTTATTTATAAAGAATACGAATAAGGTTAAAGTTAGGCGCCTAAGATAGTTTTAA

GATCGGCCTCAGGGGTTGAGATCGAACGCATATCGAATTTTTCGGCAATAATAGCCA

ATAGATTATCAGTTAAAAATGCAGGAGCCGTTGGACCGGTGTAAATACCTTTAACAC

CCAATGCAAACAAGGTCAGTAGGATCACAATCGCTTTTTGTTCAAACCAAGATAGCA

CTAACGTCAGCGGTAATTCGTTAATACCACAATCGAACTCTTTCGCTAATGCGAGTG

CCAATTGAATCGCGGAATAAGCATCATTACATTGACCCACATCGAGTAGTCGTGGAA

TACCATTAATGTCGCCAAAGTTATTTTTATTAAAGCGATATTTGCCACAAGCTAAGG

TTAAGATCAGTGAGTCTTGAGGTACTTGTGCCGCAAAATCCGTAAAGTAACTACGTT

CTGCTTTATCGCCGTCACAACCGCCAACAAGGAAGAAGTGGCTGATATTGCCTTGTT

TAACTTGATCGATAACCGCGGGTGCTGCATTCATTAATGCATTACGCCCAAAACCGA

TAGTGATCATATGTTCGATTTCAGTGTGCTTAAAGCCGTCTAATGCTAATGCACAAT

CAATCACTTGAGTAAAATCATCGTCTTCAATGTGTACTACATCTGGCCATGCCACAA

TGCTGCGAGTAAAGATACGATCGGCATAGTGGCCCACATTAGGGTTAATTAGGCAGT

TTGATGTCATTACGATTGCGCCAGGGAAATTAGCAAATTCAAGCTGTTGGTTCTGCC

AAGCACTACCGTAGTTACCGACTAGGTGAGGGTATTTCTTCAGTTCAGGGTAGCTGT

GAGCAGGTAACATTTCACCATTAGTGTAAACATTAATGCCTTTACCTTCAGTTTGTT

GCAATATTTTTTCAAGATCGTGTAAGTCATGACCTGAAACTAAAATACATTTGCCTT

GAACCGGTTTAACGTTAACTTGAGTGGGGACTGGGTGACCAAAGGTGTTGGTTTCGC

CTAAATCAAGCATCTCCATCACTTTGTAGTTCATTAGACCAATTTCCATTGAGCATT

CAAGTAAGGCATTAAGATCGGTTGGATCGGTGCCTAGCCAGCCCATGATGTGATGGT

ATTGGGCGTAAATAGCATTGTCTGTTTGTTCTAAGACACGCGCATGCTCCATGTAAG

CCGCTGCGCCTTTGAGGCCATATAGACACAATAAACGTAAACCAATCACATCTTCAT

TAATGGTTTCATAACCGCGGTTAACGGCCACTTGTGGCGCAAACGCTAAGATAGCAT

CAATATTTTGTGGTAATTCAAATTCAGCCACGGCTGGTAGGGGCGGTAATGACTGTT

GTGTGAGTGTCATCGCAGCAATAACCGCTTGTTTTAGACGTGCCTTATAACTCGCCG

CAAGATTTGTCAGCGCTAAAATACGGTCGCGATCAAAGTTCACATTGGTTAAGGTGG

CGAAGAATGCACGCGGTGCCCAATGATTGATTTCATCATCGATAATATGGTATTGAT

GAGCAAGATCTGCCCAATATGATACCCCTTGTAGCGTGTAAACTAATACATCTTGTA

GATCTGAAATTTCAGCGGTTTTACCACACATACCTGCGGCATAAGCACAGCCTTTTT

TCTTTGGCGCTTCAAGGGTTTGTTCACATTGAATACAGAACATTAAAAATCTCCAGC

GTTTGATTATCTAATTATAAATATTATCTAATCGCCACATAGCATTAACTATGCCAC

TTTTTATACTGTTGTTTTTAATCGGTTTATTTTAATTCTCGTTTATTGATGATGTTA

TAAACACATCGTAATTGTTGTGAATATGACATCATTAGTTCGATGGTTCTGTTATTT

GATACTTAATTGATACCTAATTTCTTTCCCATTCGATAGAGGTTGCCGCGATCCATT

TGTAAAAATTCAGCCGATTTTGACCAGTTTTTGTTTGTTTTTTCAAGAGTGTGTTCA

ATTAACTGTTTTTGATAATCTTCGACTAATTGCCGCATCGGTTGCGATTGTTCAGGG

AAAAGTGGTGATGTGTTTTTAACATCATTAGTTGGTGATAAATCAAAATAGTGATGA

GTAATGGTCTTTTGTTCATCTTGTATAGCACGTAATGCTGCGCGTGTAAGTGTATGT

TCTAATTCGCGAATATTACCCGGCCATTTGCTTTGCTTCAAGCATGTTCAAGACTTT

TGGATGAATATGTAGATTAGGCAAATTAAATTGATGGCGTACTTTTTCTAGTAAATA

ACCGGCGAGAACGGGAATATCATCACAACGCTCACGTAATGATGGCACAAAGATAGG

GAAGACATTAAGGCGATGGTATAAATCAGCTCGAAAATGACCGAGTTCGACTTCTGT

TTCTAGCTGACGGTTGGTGGCGGCGATAATACGTACATTAACGTGTAAATGTTGATC

ACTGCCAACCCGTTGCAGTTCACCTTGCTGGATCACACGTAATAATTCGTGCTTGTA

AAAGTAGCGGTAATTCGCCAATTTCATCTAAAAATAAGGTGCCGCCATCAGCGAGTT

CAAATTTACCCGCTCGATGATTATTTGCACCCGTAAATGCCCCTTTTACATGACCAA

ACAGTTCGCTTTCAGCTAACCCTTCAGGCAGTGCCGCACAGTTAATATAAATCATTG

GCGTATCTGCGCGCGTAGATTGAGCGTGAAGTTCATGTGCAACCAGCTCTTTACCTG

TGCCTGTTTCACCACTGATTAGTACTGCATAATCAGATTTTGCGACAGTAGCAATAT

TGGTACGCAGTCGTTGCATTGCTTGGCTAATACCAACCATTTCACCTTGTTGTGAAC

GCGCCTGTTGGATCAACGTATTTGTGAGTGACTTTTGCTTTTTGTTTTGATCTTTAA

GGGCATTAAATTGGGCAATATTACGTAATGTTGCCGCAGCTAGAGCGGCAAAGGTTT

CAATAGCAACGGTTTCTATATGATCAAAGGCACCTACTGTTAATGAATCTAGTGTCA

GTACACCAACAAGTTGTCCTTCAACATAGAGACTACAGCCAAGACAATCATGAATAT

CAATCGCTTGATCTTCATTTAATAAGATCCCGTCAAAGGGATCGGGCAGGGCGCAAT

CAGCATCAAATCTTACTGGGTGTTTACTGGCCATTATGGCTTGTAAACGGGGATGCG

CTTGAGGGAAAAAACGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGCC

CTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGA

AAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTG

GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAA

TGGCGAATGGAATTGTAAGCGTAATATTTGGTAAAATTCGCGTTAATTTTGGTAATC

AGCTCATTTTTTACCATAGGCCGAATCGCAAATCCTTATAATCAAAGATAACCGAAT

AGGGTGAG

Deduced amino acid sequence of the *P. phosphoreum* AK-6 *lux* operon

LuxC

MIKKIPMIIGGAERDTSEHEYRELTLNSYKVNIPIINQDDVEAIKLQNVENNLNINQ

IVNFLYTVGQKWKSENYSRRLTYIRDLIRFLGYSSEMAKLEANWISMILSSKSALYD

IVETDLGSRHIVDEWLPQGDCYVKAMPKGKSVHLLAGNVPLSGVTSIIRAILTKNEC

IIKTSSADPFTAIALASSFIDTDEHHPISRSMSVMYWSHNEDIVIPQQIMNCADVVV

SWGGHDAIKWATEHTPVNVDILKFGPKKSIAIVDDPVDITASAIGVAHDICFYDQQA

CFSTQDIYYIGDNIDAFFDELVEQLDIYMEILPKGDQTFDEKASFSLIEKECQFAKY

NVEKGDNQSWLLVKSPLGSFGNQPLARSAYIHHVSDISEITPYIENRITQTVTVTPW

ESSFKYRDILASHGAERIVESGMNNIFRVGGAHDGMRPLQRLVKYISHERPSTYTTK

DVAVKIEQTRYLEEDKFLVFVP

LuxD

MKSENNSVPIDHVIKIDNDQYIRVWETIPKNQGDKRNNTIVIASGFARRMDHFAGLA

EYLSTNGFHVIRYDSLNHVGLSSGEIDQFSMSVGKKSLLTVIDWLKSEHGIDQIGLI

ASSLSARIAYDIVADVNLSFLITAVGVVNLRNTLEQALKYDYLKMEIDEIPEDLNFD

GYNLGSKVFVTDCFENNWDTLDSTINKTKNLNFPFIAFVANDDSWVQQHEVEELMNN

INSDKTKIYSLIGSSHDLGENLIVLRNFYQSITKAAIALDSNLLGLASEIVEPQFEA

LTIATVNERRLKNTIKSKSL

LuxA

MKFGNICFSYQPPGDSHKEVMDRFVRLGVASEELNFNTYWALEHHFTEFGLTGNLFV
ACANLLGRTTKLHVGTMGIVLPTAHPARQMEDLLLLDQMSKGRFNFGVVRGLYHKDF
RVFGVTMEDSRAITEDFHTMIMDGTKTGTLHTDGKNIEFPDVNVYPEAYLDKIPTCM
TAESAVTTTWLAERGLPMVLSWIITTSEKKAQMELYNAVARDSGYSEEYIKNVDHSM
TLICSVDEDAKKAEDVCREFLGNWYDSYVNATNIFSESNQTRGYDYHKGQWKDFVLQ
GHTNTKRRVDYSHDLNPVGTPEKCIEIIQRDIDATGITNITLGFEANGSEEEIIASM
KRFMTQVAPFLKDPK

LuxB

MNFGLFFLNFQLEKTSSETVLDNMINMVSLVDKDYKNFTTVLVNEHHFSKNGIVGAP
ITAASFLLGLTERLHIGSLNQVITTHHPVRIAEEASLLDQMSDSRFILGLSDCINDF
EMDFFKRQRDSQQLQFEACYEIINEAITTNYCQANNDFYNFPRISINPHCLSKENMK
QYILASSVSVVEWAAKKALPLTYRWSDSLEDKEILYKRYLDVAANHNIDVSNVEHQF
PLLVNLNHDRDVAHQEATTYLASYIAEVYPHLNQQQKIAELISQHAIGTDNDYYEST
LNALERTGSK

LuxF

MNKWNYGIFFVNFYSKDEQESSKMMNNALETLRIINEDTSIYDVVNINDHYLVKKDS
EDNKLAPFIALGSKLYVLATSENTVDSAAKYALPLVFKWDDTNEERLKLLSSYNASA
SKYKQNIDLVRHQLMLHVNVNEADTVAKEELKEYFENYVACTQPSNFNGSIDSIIQS
NVTGCYNDCLSYVANLASEFNNTVDFLLCFESMQDQNKKKSVMIELNNQVIKFRQDN
NLI

LuxE

MTIILDTFEKDIIVSTEIDDIIFTSSPLDITYDEQERIKHKLILESFRYHYNNNEDY

KFFCNTQGIDENISSLDDIPVFPTSMFKYAKICTADESDIEDWFTSSGTSGVKSHIA

RDRVSIERLLGSVNYGMKYLGSFHENQLELVNMGPDRFNAKNVWFKYVMSLVELLYP

TTFTVNNDEIDFELTIKSLKEIYNKGKGICLIGPPYFIYLLCQYMKDNDIEFNAGNR

IFIITGGGWKTKQKQALNRQDFNQLLMDTFHLAHESQIRDTFNQVELNTCFFEDNRQ

RKHVPPWVYARALDPVTLKPVEDGQEGLISYMDASSTSYPTFIVTDDIGIIHTIKAP

DPLQGTTIDIVRRLNTREQKGCSLSMSSGLK

LuxG

MILNCKIIKIEASECNIFKVFIKPDKCLNFKAGQYVLAYLDGKKLPFSIANCPTCNE

LIELHVGSSVKETAVKSISYFVDAFVNSGDIQIDAPHGNAWLREGSNSPLLLIAGGT

GLSYINSILSNCVNRNLPRSIYVYWGVNNIDLLYADTQLKALSSDFSNVKYVPVLEN

FDNSWYGKKGNVIDAIIEDFCDLSDFDIYVCGPQGMTYSVREKLTSLKKANADKMFA

DAFAY

DNA sequence of the *P. phosphoreum* BS-2 *lux* operon

***lumP* –partial (SEQ:1-1031):**

AGGAGAAATACCGCATCAGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGT

GGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT

CGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAA

TCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAA

ACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCG

CCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAAC

AACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTC

GGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAA

AATATTAACGCTTACAATTTCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGG

CGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCA

AGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACG

GCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGA

TCTTCTTCTTCAGTAACATTATCAACAGTAGCGACCCCCTTTATATTTCCAGTTAAA

GCACCTTTCCCAAGGAGTGAGCCAAATTCTGGACGTAGTTCTAAGTTTACCTTGTCA

CCGATTTCTAATGAATCAAAGGTTGTGGTGTTAAGTGCTTGATCTATATCGAAATAA

ACGATATCACCAGTGATGCGAACAACAGTTAATGAGCATCCATTTACGAGCATGACA

GTATCTTTTGCAACTAAGTCCAATATATTTTTTTGAAAAGTAATGCCGTGTCTTTGG

GTATCATCATTTTTTGATATTTTTTTAATTATTCCAGTGCCATGAACTATACCTTTG

AACAT

***lump-luxC* spacer (SEQ:1032-1670):**

AATAATCTCCTTTTGTAGACAACCGTATTTAAAATGAAAATATTCATTTTGAAATTA

GTCTAATGGTAAAAAAATAAAATTCAATGATAAATAATATTTTTTTGACTTTTAATC

TGTTTTTGATAATAAGATTTAGATTTGTTATTTCTATTTAAGACGATTTTTTAAGGT

TTAAATTTTTTTAAATGGATATTTTTAGTTGAGATTTAGGCTGGTAAGTGGATGATT

TTAAACTGGTGTTTAATAAACTAAGTAATTAATTACAGACACTTTAATATTAATCTA

ATATTAATCTAATATTAGACTTTTTATGCTATTTTCATTTTTTAATTGTTTGTATTT

TTATTGTTGTTGATATTGCAATTTATTTTAAAATCAAGTATACGCATCAAGTTTTTT

TGTTTTTATAGAAATATAATTATTTAAATTAATATGTAGTTAAATGCTCTTATTTTA

TATTTAAAGGTGTTTTAAAATAGACATTAATTATTAAAAAACAAAATTCCATATTAA

AAATGTGACATTAACCCAATAAATTTAACAATGCATCCATATGCTACAAATTTTCCT

TACATACCTAATGAAATTTAGTTAGTCTCTAGCCATGCCTATGCAGCAAGTTTGTAT

GCTGTTTGAGT

***luxC* (SEQ:1671-3136):**

ATGTGCAATGCGGAATTTAAAGGAGATTGTATGATAAAGAAAATCCCAATGATTATT

GGTGGCGCAGAGAGGGATACTTCAGAACATGAATATCGTGAACTCACACTAAATAGC

TATAAAGTTAGTATACCTATCATTAATCAAGATGATGTTGAGGCGATTAAATCACAA

AGTGTAGAAAATAATCTAAATATCAATCAGATAGTGAATTTCTTATACACTGTTGGT

CAGAAATGGAAAAGTGAGAATTATTCTCGTCGACTAACCTATATTCGTGATTTGGTA

AGATTTCTCGGATATTCTCCTGAAATGGCAAAGCTAGAAGCTAACTGGATCTCAATG

ATCTTGAGCTCAAAAAGTGCCTTATATGATATTGTTGAGACAGAGTTAGGTTCTCGT

CATATTGTAGATGAATGGTTACCTCAGGGGGATTGTTATGTTAAGGCCATGCCAAAA

GGAAAATCTGTTCATTTGCTAGCCGGTAATGTGCCTCTATCTGGTGTTACTTCTATA

ATTAGAGCAATTCTGACTAAAAATGAATGTATCATTAAAACATCATCAGCTGATCCA

TTTACGGCAATAGCATTAGCTTCAAGTTTTATTGATACAGATGAACACCATCCAATT

AGCCGTTCAATGTCGGTAATGTATTGGTCTCATAACGAAGATATTGCAATCCCACAA

CAAATTATGAATTGTGCTGATGTTGTTGTTAGTTGGGGTGGATATGATGCAATTAAA

TGGGCAACAGAACATACACCGGTAAATGTAGACATATTAAAATTTGGGCCGAAGAAA

AGTATTGCAATTGTTGATAATCCTGTAGATATTACAGCTTCTGCTGTTGGTGTGGCT

CATGATATTTGTTTTTATGATCAGCAGGCCTGTTTTTCAACTCAAGATATTTATTAT

ATAGGCGATAACATTGATGCGTTCTTTGATGAGCTTGTAGAACAATTAAATCTATAT

ATGGATATATTGCCAAAAGGCGATCAAACATTTGATGAAAAGGCATCATTTTCATTA

ATTGAAAAAGAGTGTCAATTTGCAAAATATAAAGTTGAGAAAGGCGATAATCAATCT

TGGTTACTTGTTAAATCACCGCTAGGATCTTTTGGTAATCAACCATTAGCTCGATCT

GCATATATTCACCACGTCTTCGATATATCAGAAATAACGCCTTATATAGAAAATAGA

ATTACTCAAACTGTAACAGTTACTCCTTGGGAGTCATCATTTAAATATAGAGATGTT

CTAGCCTCTCATGGTGCTGAACGTATTGTTGAGTCCGGAATGAATAATATTTTCCGT

GTTGGTGGTGCGCATGATGGTATGAGACCTCTTCAACGTTTAGTTAAATATATTTCA

CATGAAAGACCTTATACATATTCAACCAAAGATGTAGCAGTAAAAATCGAACAAACA

CGTTATCTAGAAGAAGATAAGTTTTTAGTCTTTGTACCATAA

***luxC-luxD* spacer (SEQ:3137-3149):**

AGGGGAATTAAT

***luxD* (SEQ:3150-4069):**

ATGAAAAGTGAAAACAATTCTGTACCAATTGATCATGTTATAAAAGTTGATAACGAA

CGTCATATACGTGTTTGGGAAACTTTCCCTAAAGATCAATGTGATAAAAGAAATAAT

ACTGTTATTATTGCTTCTGGTTTTGCTCGAAGAATGGACCATTTTGCAGGTTTAGCT

GAATATTTATCAACTAATGGATTTCATGTTATTCGTTATGATTCACTTAATCATGTT

GGATTAAGTAGCGGTGAAATTGATCAGTTCTCAATGTCAGTAGGTAAGGAAAGTTTA

TTAACCGTTATTGATTGGTTGAAATCAGAGCATGGTATTGATCAGGTCGGTTTAATT

GCATCGAGTCTTTCTGCTCGAATTGCTTATGATATTGTCGCTGATGTTAATTTGTCT

TTTTTAATTACCGCCGTTGGTGTGGTTAATTTGCGAAATACTCTAGAACAAGCACTT

AAATATGATTATTTGCAGATGGAGATAGACGAAATACCAGAAGATCTAGATTTCGAT

GGATATAATTTAGGTTCGAAAGTATTTGTTACAGATTGTTTTGAAAATAATTGGGAT

ACATTAGATTCAACTATAAATAAAACAAAAAATTTAAATTTCCCATTTATAGCTTTT

GTCGCCAATGGTGATAGTTGGGTACAACAGCATGAAGTCGAAGAATTAATTAGTAAT

ATCAATTCAGATAAAACCAAAATTTACTCTTTAATAGGTTCATCACATGATTTAGGT

GAAAACCTAATTGTGTTAAGAAATTTCTATCAATCAATTACTAAAGCAGCAATTGCA

TTAGATAGTAATTTAGTCGGATTGGTGAGTGAGATTATTGAACCACAATTTGAAGCT

CTCACTATTGCTACAGTAAATGAACGTCGTTTGAAAAATAAAATACAAAGTAAGTCA

TTAGCTTAA

***luxD-luxA* spacer (SEQ:4070-4096):**

AACTGATACATAAACCAACAAAAGGAATATATT

***luxA* (SEQ:4097-5181):**

ATGAAGTTTGGAAATATTTGTTTCTCATATCAGCCCCCAGGTGAGTCACATAAAGAA

GTCATGGATCGCTTTGTTCGTTTAGGCGTTGCATCAGAAGAACTAAATTTTGATACT

TACTGGACTCTAGAGCATCATTTTACTGAATTTGGACTAACAGGTAACTTGTTTGTT

GCTTGTGCTAACTTACTTGGTCGAACCACCAAACTTAATGTTGGTACTATGGGTATT

GTTCTTCCAACAGCTCACCCTGCACGTCAGATGGAAGATTTATTACTTTTAGATCAA

ATGTCAAAAGGTCGTTTTAATTTTGGTGTTGTGCGTGGCTTATACCACAAAGATTTC

CGCGTTTTTGGTGTAACGATGGAAGATTCTCGTGCTATCACTGAAGACTTTCATACC

ATGATTATGGATGGTACAAAAACGGGTACACTTCATACTGATGGTAAAAATATCGAG

TTCCCAGATGTAAACGTTTACCCGGAAGCATATTTAGCGAAAATTCCTACATGCATG

ACTGCTGAATCAGCAGTAACAACGACTTGGCTTGCTGAGCGTGGCTTACCGATGGTT

CTTAGTTGGATTATTACAACGAGTGAAAAGAAAGCTCAAATGGAACTCTATAATGCT

GTTGCTCGAGATAGCGGTTACAGTGAAGAGTACATTAAAAACGTTGATCACAGTATG

ACCCTCATCTGTTCTGTAGATGAAGATGGCAAAAAAGCTGAAGATGTGTGCCGTGAG

TTTTTAGGTAATTGGTATGATTCATACGTAAATGCAACCAATATCTTTAGTGAAAGT

AACCAAACTCGTGGTTATGATTATCATAAAGGTCAATGGAAAGATTTTGTTCTTCAA

GGACATACTAATACTAAACGTCGTGTTGATTATAGCCACGATCTAAACCCTGTAGGT

ACACCTGAAAAATGTATTGAAATTATTCAGCGTGATATTGATGCAACAGGTATTACT

AATATTACCCTTGGTTTCGAAGCAAATGGCTCTGAGGAAGAAATCATTGCCTCTATG

AAATGCTTCATGACGCAAGTTGCACCATTCTTAAAAGATCCAAAATAAATAA

***luxA-luxB* spacer (SEQ:5182-5226):**

ATCACTCAGATTAACTTTAATAAATAATATAAGGAATATAAC

***luxB* (SEQ:5227-6213):**

ATGAATTTTGGATTATTCTTCCTCAACTTTCAGCCTGAAAATACATCGTCAGAAACA

GTTTTAGATAATATGATCAATACTGTCTCTTTAGTTGATAAAGATTATAAAAACTTT

ACAACTGCTTTAGTCAACGAGCACCATTTTTCTAAAAATGGTATTGTCGGTGCTCCG

ATGACAGCTGCAAGCTTCCTATTAGGACTAACTGAACGTTTACATATTGGTTCTTTA

AATCAAGTAATTACAACGCATCACCCGGTTCGTATTGCAGAAGAAGCAAGTTTGCTT

GATCAATGTCAGAAAGCCGCTTTATTCTAGGTCTAAGTGATTGTGTTAATGATTTTG

AGATGGATTTCTTTAAACGGCAACGTGACTCACAGCAGCTACAATTTGAAGCTTGCT

ATGACATCATTAATGAAGCTATCACAACTAATTACTGCCAAGCTAATAATGATTTTT

ATAACTTCCCTCGTATCTCAATTAATCCTCATTGCTTAAGTAAAGAGAATATGAAGC

AATATATTTTGGCTTCTAGTGTGAGTGTTGTTGAGTGGGCTGCTAAAAAAGCGCTTC

CACTAACATATCGTTGGAGCGATAGCCTTGAAGATAAAGAGATTCTTTATAAGCGTT

ATTTAGACGTTGCAGCAAAGCATAATATTGACGTTTCTAATGTCGAGCATCAGTTCC

CACTGCTTGTAAATTTAAATCATGATCGTGATGTTGCTCATCAAGAAGCAACGGCCT

ATTTAGTAAGTTATGTTGCTGAAGTATACCCACATCTAAATCAGCAACAAAAAATTG

CTGAACTTATTAGCCAACATGCGATTGGTACTGATAATGATTACTATGAATCAACAT

TAAATGCGTTAGAGCGTACAGGTTCAAAAAATGTATTACTTTCTTTTGAATCAATGA

AGAATCATGATGATGTTGTAAAAGTGATTAATATGGTTAATGAGAAGATTCAAAAGA

ATTTACCAAGCTCGTAA

***luxB-luxF* spacer (SEQ:6214-6234):**

GTGTAAAGGAAGCGGTGTTATT

***luxF* (SEQ:6235-6930):**

ATGAATAAATGGAATTACGGAGTCTTCTTCGTTAACTTTTATAATAAAGGCCAACAA

GAGCCATCAAAAATGATGAATAATACATTAGAAACATTACGTATTATTGATGAAGAT

ACATCTATTTATGATGTGATTAATATTGATGACCATTATCTTGTAAAGAAAGACAGT

GAAGATAAAAAGCTAGCGCCTTTTATTACACTAGGGGAAAAACTATATGTGCTTGCT

ACCAGTGAAAACACAGTTGATATTGCAGCGAAATATGCATTACCATTAGTTTTTAAA

TGGGATGATATAAATGAGGAACGACTTAAATTGTTGAGTTTTTATAATGCATCCGCA

AGTAAATATAACAAGAATATAGATTTGGTTCGACACCAGTTTATGTTACATGTAAAT

GTTAATGAGGCAGAAACTGTAGCAAAAGAAGAACTCAAATTATATATTGAAAACTAT

GTAGCATGTACACAGCCTAGTAATTTCAATGGCTCGATTGATAGTATTATTCAGAGT

AACGTGACAGGGAGTTATAAAGACTGATTGTCATATGTAGCGAATCTTGCTGGTAAA

TTTGATAATACTGTGGACTTCTTACTTTGTTTTGAGTCAATGCAAGATCAAAATAAG

AAAAAATCAGTAATGATAGATCTTAATAATCAAGTTATTAAGTTTCGCCAAGATAAT

AATCTAATATAA

***luxF-luxE* spacer (SEQ:6931-7013):**

TCTACAATCATTGCCTCTTATAATAGCAGTGCTAATTTAAAGTTCTGCCATTATATT

TAATTATATTTTAAATAGGATTAAAC

***luxE* (SEQ:7014-8136):**

ATGACTATTACATTAGATACTTGCGAAAAAAATATTATTGTAAGTACAGAGATCGAC

GATATTATTTTTACATCATCACCTCTTGATATTACTTACGATGAACAAGAAAGAATA

AAGCATAAATTAATATTAGAATCATTTCGTTACCACTATAATAATAATGAAGATTAT

AAGTCTTTCTGTAATACTCAGGGGGTAGACGAAAATATTTCATCACTTGATGATATC

CCTGTTTTTCCGACCTCAATGTTTAAGTATGCAAAAATATGCACAGCAGATGAGTCT

AACATTGAAAACTGGTTCACAAGTAGTGGTACGAGTGGTGTAAAAAGTCATATTGCC

CGTGATCGTGTAAGTATTGAACGTTTACTTGGTTCTGTAAATTATGGAATGAAATAT

CTTGGTTCATTTCATGAAAATCAGCTAGAACTTGTTAATATGGGACCTGATCGTTTT

AATGCTAAAAATGTTTGGTTTAAGTATGTAATGAGTCTTGTTGAGTTATTATATCCA

ACTACATTTACTGTAAATAACGATGAAATAGATTTTGAACTTACTATCAAAAGTTTA

AAAGAAATCTATAATAAAGGAAAAGGTATTTGTTTAATTGGCCCTCCGTATTTCATT

TATTTGCTATGCCAGTACATGAAAGAGAATGATATTGAATTTAATGCAGGTAATCGT

ATCTTTATTATTACTGGCGGTGGTTGGAAAACTAAGCAAAAACAAGCACTAAATCGT

CAAGATTTTAATCAACTATTGATGGAAACCTTCCATTTAGCACATGAAAGTCAGATT

CGAGATACATTTAATCAAGTTGAATTAAATACGTGTTTCTTTGAAGATAACCGTCAG

CGTAAGCATGTTCCGCCATGGGTTTATGCACGTGCACTTGATCCTGTAACTCTAAAG

CCTGTTGAAGATGGTCAAGAAGGGCTTATTAGTTACATGGATGCATCATCAACGAGT

TATCCAACATTTATCGTTACTGACGATATCGGTATAATTCATACAATTAAAGATCCA

GATCCGTACCAAGGCACTACGATTGATATTGTCCGTCGTTTGAATACGAGAGAGCAG

AAAGGGTGTTCATTATCAATGGCATCAGGCTTGAAATAG

***luxE-luxG* spacer:**

CTTACAAGGAAGATGT

***luxG* -- partial (to 8447):**

ATGATTTTAAATTGTAAAATAATTAAAATTGAAGCTTCTGAATGTAATATTTTTAAA

GTATTTATTAAGCCTGATAAGTGTCTCAATTTTAAAGCTGGGCAATATGTTTTAGCA

TATTTAGATGGTAAAAAATTACCTTTTTCAATTGCTAATTGTCCAACATGTAATGAA

CTTATAGAGTTACATGTTGGGAGTTCGGTAAAAGAAACCGCAGTTAAATCTATTTCT

CATTTTGTAGATGCTTTTGTGAATAGCTCTGAAAATACAAATAGATGCACCTCATGG

GTAATGCTTGG

Deduced amino acid sequence of the *P. phosphoreum* BS-2 *lux* operon

LuxC

MIKKIPMIIGGAERDTSEHEYRELTLNSYKVSIPIINQDDVEAIKSQSVENNLNINQ

IVNFLYTVGQKWKSENYSRRLTYIRDLVRFLGYSPEMAKLEANWISMILSSKSALYD

IVETELGSRHIVDEWLPQGDCYVKAMPKGKSVHLLAGNVPLSGVTSIIRAILTKNEC

IIKTSSADPFTAIALASSFIDTDEHHPISRSMSVMYWSHNEDIAIPQQIMNCADVVV

SWGGYDAIKWATEHTPVNVDILKFGPKKSIAIVDNPVDITASAVGVAHDICFYDQQA

CFSTQDIYYIGDNIDAFFDELVEQLNLYMDILPKGDQTFDEKASFSLIEKECQFAKY

KVEKGDNQSWLLVKSPLGSFGNQPLARSAYIHHVFDISEITPYIENRITQTVTVTPW

ESSFKYRDVLASHGAERIVESGMNNIFRVGGAHDGMRPLQRLVKYISHERPYTYSTK

DVAVKIEQTRYLEEDKFLVFVP

LuxD

MKSENNSVPIDHVIKVDNERHIRVWETFPKDQCDKRNNTVIIASGFARRMDHFAGLA

EYLSTNGFHVIRYDSLNHVGLSSGEIDQFSMSVGKESLLTVIDWLKSEHGIDQVGLI

ASSLSARIAYDIVADVNLSFLITAVGVVNLRNTLEQALKYDYLQMEIDEIPEDLDFD

GYNLGSKVFVTDCFENNWDTLDSTINKTKNLNFPFIAFVANGDSWVQQHEVEELISN

INSDKTKIYSLIGSSHDLGENLIVLRNFYQSITKAAIALDSNLVGLVSEIIEPQFEA

LTIATVNERRLKNKIQSKSLA

LuxA

MKFGNICFSYQPPGESHKEVMDRFVRLGVASEELNFDTYWTLEHHFTEFGLTGNLFV

ACANLLGRTTKLNVGTMGIVLPTAHPARQMEDLLLLDQMSKGRFNFGVVRGLYHKDF

RVFGVTMEDSRAITEDFHTMIMDGTKTGTLHTDGKNIEFPDVNVYPEAYLAKIPTCM

TAESAVTTTWLAERGLPMVLSWIITTSEKKAQMELYNAVARDSGYSEEYIKNVDHSM

TLICSVDEDGKKAEDVCREFLGNWYDSYVNATNIFSESNQTRGYDYHKGQWKDFVLQ

GHTNTKRRVDYSHDLNPVGTPEKCIEIIQRDIDATGITNITLGFEANGSEEEIIASM

KCFMTQVAPFLKDPK

LuxB

MSESRFILGLSDCVNDFEMDFFKRQRDSQQLQFEACYDIINEAITTNYCQANNDFYN

FPRISINPHCLSKENMKQYILASSVSVVEWAAKKALPLTYRWSDSLEDKEILYKRYL

DVAAKHNIDVSNVEHQFPLLVNLNHDRDVAHQEATAYLVSYVAEVYPHLNQQQKIAE

LISQHAIGTDNDYYESTLNALERTGSKNVLLSFESMKNHDDVVKVINMVNEKIQKNL

PSS

LuxF

MNKWNYGVFFVNFYNKGQQEPSKMMNNTLETLRIIDEDTSIYDVINIDDHYLVKKDS

EDKKLAPFITLGEKLYVLATSENTVDIAAKYALPLVFKWDDINEERLKLLSFYNASA

SKYNKNIDLVRHQFMLHVNVNEAETVAKEELKLYIENYVACTQPSNFNGSIDSIIQS

NVTGSYKD*LSYVANLAGKFDNTVDFLLCFESMQDQNKKKSVMIDLNNQVIKFRQDN

NLI

LuxE

MTITLDTCEKNIIVSTEIDDIIFTSSPLDITYDEQERIKHKLILESFRYHYNNNEDY

KSFCNTQGVDENISSLDDIPVFPTSMFKYAKICTADESNIENWFTSSGTSGVKSHIA

RDRVSIERLLGSVNYGMKYLGSFHENQLELVNMGPDRFNAKNVWFKYVMSLVELLYP

TTFTVNNDEIDFELTIKSLKEIYNKGKGICLIGPPYFIYLLCQYMKENDIEFNAGNR

IFIITGGGWKTKQKQALNRQDFNQLLMETFHLAHESQIRDTFNQVELNTCFFEDNRQ

RKHVPPWVYARALDPVTLKPVEDGQEGLISYMDASSTSYPTFIVTDDIGIIHTIKDP

DPYQGTTIDIVRRLNTREQKGCSLSMASGLK

DNA sequence of the *P. phosphoreum* NZ-11-D *lux* operon

***lumP* –partial (SEQ:1-172):**

TCTTTCCAATAACTACTCCCCCTATTCGACCCCAGTAAGAGCAGCCCTTACGAGCAN

GCCGNCCCTTTTCAACGATTCCATATATCTTTGGNAAAGNAATGCCANGTCTTGGGG

GTCATCATTTTTTGATATTTTTTAATTATTCCAGCGCCCTGAACTATACCTTGACCA

T

***lumP-luxC* spacer (SEQ:173-798):**

AATAATCTCCTTTNGTCGACAAACATATTTAAAATGAAAATCTTCATTTGGAAATTA

GTCTAATGATAAAAAAATAAAATTCAATACTAAATAATATTTTTTTGATATTTAATC

CGTTTTTAATAATTAACTTTTTATTTTAATTTCTATTTTAGACAAGGTGTTTAAGAT

GTGATTTTCTTTATATAGGTGTTTTTAGTTGAAATTAAAGCTAGTAAGTAGATGATT

TAAAATGGTATTTGATAAACTAAGTAATTAATAATAGCCACTATAATATTAATCGAA

TATTTTATTTCTATACGGCATTTAGTTTTGATTATTTTAAATTTTTATTATTATCAA

CATTGTAATTTGTTTTAAAATAAAGTATATGCATCAAGTTCTTGTTATTTTTATTAA

AATCTTATTCTTTCAATTAATATGTAGTTAAATGCTCTTATTTTATATTTAAAGGTG

TTTTAAAATAGACCTGAATTAATAAATAACAAAGTTCTATATTAAAAATGTGACATT

AACCCAATAAATTTAACAATGCATCCATATCATACAAATTTCCCTTACATACCTAAT

GAAATTTAGTTAGTCTCTAGCCATACCTATGCAGCAAGGTTGTATGCTGATTGAGT

***luxC* (SEQ:799-2265):**

ATGTGCAATGCGGAATTTAAAGGAGATTGTATGATAAAGAAAATCCCAATGATTATT

GGTGGCGGAGAGAGGGATACTTCAGAACATGAATATCGTGAACTCACACTAAATAGC

TATAAAGTTAGTATACCTATCATTAATCAAGATGATGTTGAGGCGATTAAATCACAA

AATGTGGAAAATAATCTAAATATCAATCAGATAGTGAATTTCTTATACACTGTTGGT

CAGAAATGGAAAAGTGAGAATTATTCTCGTCGACTAACCTATATTCGTGATTTGGTA

AGATTTCTCGGATATTCTCCTGAAATGGCAAAACTAGAAGCTAACTGGATCTCAATG

ATATTGAGCTCAAAAAGTGCCTTATATGATATTGTTGAAACAGAGTTAGGTTCTCGT

CATATTGTAGATGAATGGTTACCTCAGGGTGATTGTTATGTCAAGGCCATGCCAAAA

GGAAAATCTGTTCATTTGCTAGCCGGTAATGTGCCTCTATCTGGTGTTACTTCTATT

ATTAGAGCAATTCTGACTAAAAATGAATGTATCATTAAAACATCATCAGCTGATCCA

TTTACGGCAATAGCATTAGCTTCAAGTTTTATTGATACAGATGAACACCATCCAATT

AGCCGTTCAATGTCGGTAATGTATTGGTCTCATAACGAAGATATTGCAATCCCACAA

CAAATTATGAATTGTGCTGATGTTGTTGTTAGTTGGGGTGGATATGATGCAATTAAA

TGGGCAACAGAGCATACACCGGTAAACGTCGACATATTAAAATTTGGGCCGAAGAAA

AGTATTGCGATTGTTGATAATCCTGTAGATATTACAGCTTCTGCTATTGGTGTGGCT

CATGATATTTGTTTTTATGATCAGCAGGCCTGTTTTTCAACCCAAGATATCTATTAT

ATAGGCGATAACATTGATGCGTTTTTTGATGAGCTTGTAGAACAATTAAATCTATAT

ATGGATATATTGCCAAAAGGCGATCAAACATTTGATGAAAAGGCATCATTTTCATTA

ATTGAAAAAGAGTGTCAATTCGCAAAATATAAAGTTGAGAAAGGTGATAATCAATCT

TGGTTACTTGTTAAATCACCGCTAGGATCTTTTGGTAATCAACCATTAGCTCGATCT

GCATATATTCACCATGTCTCCGATATATCAGAAATAACGCCTTATATAGAAAATAGA

ATTACTCAAACTGTAACAGTTACTCCTTGGGAGTCATCATTTAAATATAGAGATGTT

CTAGCCTCTCATGGTGCTGAGCGTATTGTTGAGTCAGGGATGAATAATATTTTCCGT

GTTGGTGGTGCGCATGATGGTATGAGACCTCTTCAACGTTTAGTTAAATATATTTCA

CATGAAAGACCTTATACATATACAACCAAAGATGTAGCCGTAAAAATTGAACAAACA

CGTTATCTAGAAGAAGATAAGTTTTTAGTCTTTGTACCATAA

***luxC-luxD* spacer (SEQ:2266-2278):**

AAGGGAATTAAT

***luxD* (SEQ:2279-3198):**

ATGAAAAGTGAAAACAATTCTGTACCAATTGATCATGTTATAAAAGTTGATAACGAA

CGTCATATACGTGTTTGGGAAACTTTCCCTAAAAATCAATGTGATAAAAGAAATAAT

ACTATTGTTATTGCTTCTGGTTTTGCTCGAAGAATGGATCATTTTGCAGGTTTAGCT

GAATATTTATCAACCAATGGATTTCATGTTATTCGTTATGATTCACTTAATCATGTT

GGATTAAGTAGCGGTGAAATTGATCAGTTCTCAATGTCAGTAGGTAAGAAAAGTTTA

TTAACCGTTATTGACTGGTTGAAATCAGAGCATGGTATTGATCAGGTAGGTTTAATT

GCATCGAGTCTTTCTGCTCGAATTGCTTATGATATTGTCGCTGATGTTAATTTGTCT

TTTTTAATTACTGCCGTTGGTGTGGTCAATTTACGGAATACTCTTGAACAAGCGCTT

AAATATGATTACTTGCAGATGGAGATAGATGAAATACCAGAAGATTTAGATTTCGAT

GGATATAATTTAGGTTCAAAGGTATTTGTTACAGATTGTTTTGAAAATAACTGGGAT

ACATTAGATTCAACTATAAATAAAACGAAAAATTTAAATGTCCCATTTATAGCTTTT

GTCGCCAATGATGATAGTTGGGTACAACAGCACGAAGTCGAAGAATTAATGAGTAAT

ATCAATTCAGATAAAACCAAGATTTACTCTTTAATAGGTTCATCACATGATTTAGGT

GAAAACCTAATTGTGTTAAGAAATTTCTATCAATCAATTACTAAAGCAGCAATTGCA

TTAGATAGTAATTTAGTAGGGTTAGTAAGTGAGATTATTGAACCACAATTTGAAGCT

CTCACTATTGCTACAGTAAATGAACGTCGTTTGAAAAATAAAATACAAAGTAAGTCA

TTAGCTTAA

***luxD-luxA* spacer (SEQ:3199-3236):**

TTACAACTGATACATAAACCAACAAAAGGAATATATT

***luxA* (SEQ:3237-4310):**

ATGAAGTTTGGAAATATTTGTTTCTCATATCAGCCTCCAGGTGAGTCACATAAAGAA

GTCATGGATCGCTTTGTTCGTTTAGGCGTTGCATCAGAAGAACTAAATTTTGATACT

TACTGGACTCTAGAGCATCATTTTACTGAATTTGGACTAACAGGTAACCTGTTTGTT

GCTTGTGCTAACTTACTTGGTAGAACCACCAAACTGAATGTTGGTACTATGGGTATT

GTTCTTCCAACAGCTCACCCTGCACGTCAGATGGAAGATTTATTACTTTTAGATCAA

ATGTCAAAAGGCCGTTTTAATTTTGGTGTTGTGCGTGGCTTGTACCACAAAGATTTC

CGCGTTTTTGGTGTAACGATGGAAGATTCTCGTGCTATTACTGAAGATTTTCACACC

ATGATTATGGATGGTACAAAAACAGGTACACTTCATACTGATGGTAAAAACATCGAA

TTCCCAGATGTAAACGTTTACCCAGAGGCGTATTTAGAGAAAATTCCAACATGCATG

ACTGCTGAATCAGCAGTAACAACGACTTGGCTTGCTGAGCGTGGCTTACCGATGGTT

CTTAGTTGGATTATTACAACGAGTGAAAAGAAAGCTCAAATGGAACTCTATAATGCT

GTTGCTCGAGATAGCGGTTACAGTGAAGAGTACATTAAAAACGTTGATCACAGTATG

ACTCTCATCTGTTCTGTAGATGAAGATGGCAAAAAAGCTGAAGATGTGTGCCGTGAG

TTTTTAGGTAATTGGTATGATTCATACGTAAATGCAACCAATATCTTTAGTGAAAGT

AACCAAACTCGTGGTTATGATTATCATAAAGGTCAATGGAAAGATTTTGTTCTTCAA

GGACATACTAATACCAAACGTCGTGTTGATTATAGCCACGATCTAAACCCTGTAGGT

ACACCTGAAAAATGTATTGAAATTATTCAGCGTGATATTGATGCAACAGGTATTACT

AATATTACCCTTGGTTTCGAAGCAAATGGCTCTGAGGAAGAAATCATTGCCTCTATG

AAACGCTTCATGACGCAAGTTGCACCATTCTTAAAAGATCCAAAATAA

***luxA-luxB* spacer (SEQ:4311-4356):**

ATAAATCACTCAGATTAACTTTAATAAATAATATAAGGAATATAAC

***luxB* (SEQ:4357-5342):**

ATGAATTTTGGATTATTCTTCCTCAACTTTCAGCCTGAAAATACATCGTCAGAAACA

GTTTTAGATAATATGATCAATACTGTCTCTTTAGTTGATAAAGATTATAAAAACTTT

ACAACTGCTTTAGTCAACGAGCACCATTTTTCTAAAAATGGTATTGTCGGTGCTCCG

ATGACAGCTGCAAGCTTCCTATTAGGACTAACTGAACGTTTACATATTGGTTCTTTA

AATCAAGTAATTACAACGCATCACCCGGTTCGTATTGCAGAAGAAGCAAGTTTGCTT

GATCAAATGTCAGACAGCCGCTTTATTCTAGGTCTAAGTGATTGTGTTAATGATTTT

GAGATGGATTTCTTTAAACGTCAACGTGACTCACAGCAGCTACAATTTGAAGCTTGC

TATGACATCATTAATGAAGCTATCACAACTAATTACTGCCAAGCTAATAATGATTTT

TATAACTTCCCTCGTATCTCAATTAATCCTCATTGCTTAAGCAAAGAGAATATGAAG

CAATATATTTTGGCTTCTAGTGTGAGTGTTGTTGAGTGGGCTGCTAAAAAAGCGCTT

CCACTAACGTATCGTTGGAGCGATAGCCTTGAAGATAAAGAGATTCTTTATAAGCGT

TATTTAGAAGTTGCAGCAAAGCATAATATTGACGTTTCTAATGTCGAGCATCAGTTC

CCACTGCTTGTAAATTTAAATCATGATCGTGATGTTGCTCATCAAGAAGCAACGGCC

TATTTAGTAAGTTATATTGCTGAAGTATACCCACATCTAAATCAGCAACAAAAAATT

GCTGAACTTATTAGCCAACATGCGATTGGTACTGATAATGATTACTATGAATCAACA

TTAAATGCGTTAGAGCGTACAGGTTCAAAGAATGTATTACTTTCTTTTGAATCAATG

AAGAATCATGATGATGTTGTAAAAGTGATTAATATGGTTAATGAGAAGATTCAAAAG

AATTTACCAAGCTCGTAA

***luxB-luxF* spacer (SEQ:5343-5364):**

GTGTAAAGGAAGCGGTGTTATT

***luxF* (SEQ:4357-5342):**

ATGAATAAATGGAATTACGGAGTCTTCTTCGTTAACTTTTATAATAAAGGCCAACAA

GAGCCATCAAAAACGATGAATAATGCATTAGAAACATTACGTATTATTGATGAAGAT

ACATCTATTTATGATGTGATTAATATTGATGACCACTATCTTGTAAAGAAAGACAGT

GAAGATAAAAAGCTAGCGCCTTTTATTACACTAGGGGAAAAGCTATATGTGCTTGCT

ACCAGTGAAAACACAGTTGATATTGCAGCGAAATATGCATTACCGTTAGTTTTCAAA

TGGGATGATATAAATGAGGAACGACTTAAATTGTTGAGTTTTTATAATGCATCCGCA

AGTAAATATAACAAGAATATAGATTTGGTTCGACACCAGCTTATGTTACATGTCAAT

GTTAATGAGGCAGAAACTGTAGCAAAAGAAGAACTCAAATTATATATTGAAAACTAT

GTAGCATGTACACAGCCTAGTAATTTTAATGGCTCGATTGATAGTATTATTCAGAGT

AACGTGACAGGGAGTTATAAAGACTGTTTGTCATATGTAGCGAATCTTGCTGGTAAA

TTTGATAATACTGTGGACTTCTTACTTTGTTTTGAGTCAATACAAGATCAAAATAAG

AAAAAATCAGTAATGATAGATCTTAATAATCAAGTTGTTAAGTTCCGCCAAGATAAT

AATCTAATCTAA

***luxF-luxE* spacer (SEQ:6061-6148):**

TCTAATCTACAATCATTGCCTCTTATAATGGCAGTGCTAATTAAAAGTTCTGCCATT

ATATTTAATTATATTTTAAATAGGATTAAAC

***luxE* (SEQ:6149-7270):**

ATGACTATTACATTAGATACTTGCGAAAAAGATATTATTGTAAGTACAGAGATCGAC

GATATTATTTTTACATCATCACCTCTTGATATTACTTACGATGAACAAGAAAGAATA

AAGCATAAATTAATATTAGAATCATTTCGTTACCACTATAATAATAATGAAGATTAT

AAGTCTTTCTGTAATACTCAGGGGGTAGACGAAAATATTTCTTCACTTGATGATATC

CCTGTTTTTCCGACCTCAATGTTTAAGTATGCAAAAATATGTACAGCAGATGAGTCT

AACATTGAAAACTGGTTCACAAGTAGTGGTACGAGTGGTGTAAAAAGTCATATTGCC

CGTGATCGTGTAAGTATTGAACGTTTACTTGGTTCTGTAAATTATGGAATGAAATAT

CTTGGTTCATTTCATGAAAATCAGCTAGAACTTGTTAATATGGGACCTGATCGTTTT

AATGCTAAAAATGTTTGGTTTAAGTATGTAATGAGTCTTGTTGAGTTATTATATCCA

ACTACATTTACTGTAAATAACGATGAAATAGATTTTGAACTTACTATTAAAAGTTTA

AAAGAAATCTATAATAAAGGAAAAGGTATTTGTTTAATTGGCCCTCCGTATTTCATT

TATTTGCTATGCCAGTACATGAAAGAGAATGATATTGAATTTAATGCAGGTAATCGC

ATCTTTATTATTACTGGCGGTGGTTGGAAAACTAAGCAAAAACAAGCACTAAATCGT

CAAGATTTTAATCAACTATTGATGGAAACCTTCCATTTAGCACATGAAAGTCAGATT

CGAGATACATTTAATCAAGTTGAATTAAATACGTGTTTCTTTGAAGATAACCGTCAG

CGTAAGCATGTTCCGCCATGGGTTTATGCACGTGCACTTGATCCTGTAACTCTAAAG

CCTGTTGAAGATGGTCAAGAAGGGCTTATTAGTTACATGGATGCATCATCAACGAGT

TATCCAACATTTATCGTTACTGACGATATCGGTATAATTCATACAATTAAAGATCCA

GATCCGTACCAAGGCACTACGATTGATATTGTCCGTCGTTTGAATACGAGAGAGCAG

AAAGGGTGTTCATTATCAATGGCATCAGGCTTGAAATAG

***luxE-luxG* spacer**

CTTACAAGGAAGATGT

luxG

ATGATTTTAAATTGTAAAATAATTAAAATTGAAGCTTCTGAATGTAATATTTTTAAA

GTATTTATTAAGCCTGATAAGTGTCTCAATTTTAAAGCTGGTCAATATGTTTTAGCA

TATTTAGATGGTAAAAAATTACCTTTTTCAATTGCTAATTGTCCAACATGTAATGAG

CTTATAGAGTTACATGTTGGGTGTTCGGTAAAAGAAACCGCAGTTAAATCTATTTCT

CATTTTTTAGATGCTTTTGTGAATAGCTCTGAAATACAAATAGATGCACCTCATGGT

AATGCTTGGCTACGTGAAGATAGTAATTCTCCATTATTACTTATAGCTGGAGGTACT

GGGTTATCATATATCAATAGTATTCTTAGTAATTGTGTAAATAGGAATTTGCCCCGT

TCTATTTATGTTTACTGGGGAGTTAATAATATTGATTTATTATATGCAGACACTCAA

TTAAAAGCTCTTTCTAGCGATTTTAACAATGTTAAATACGTACCTGTTTTGGAAAAC

TTTGACAATAATTGGTACGGAAAAAAAGGCAATGTTATTGATGCAATAATAGAGGAC

TTTGGTGATTTATCAGAATTTGATATCTATGTTTGCGGCCCTCAAGGTATGACACGT

AGTGTTCGTGAAAAGTTAACATCACTTAAAAAAGCTGA

Sequence downstream of *luxE* (to-8448):

TACTGATAAAATGTTTGCCGATGCTTTTGCATATATGTGATACTAATTTAAATTAAT

TAAAATCTAACTGATACCTTAAAGTTTATCTGAAGGTATATTGAATTAAATTATTAA

CTCTATTTTTTGGTTTGTAATCTCACCAATAAATAGAGTTATCATCAGACTTTTAAT

TAACTTTAAACTAAGAGGTTGAATATGTTTACCGGAATTATTGAGGCTGTTGGTAAT

ATATCGGCCATCACTTCAAAAGGATCTGATTTTGAAGTCTCAGTTAATTGTGACACG

TTAGATCTAGCTGATGTGAAAATAGGTGATAGTATTGCTACCAACGGTATATGTTTA

ACGGTAGTTAAACTGACAGCCAATAGTTATGTCGCTGATCTATCTATAGAAACCATT

AGGNCGAACTGCTTTTTAATTATTATAANGGGGGCCNAGCCGTTAATTTAGAAAAAG

CGANGTTCCCTCCACTCGTTTNGGGGGCCANNTGGCCCNGG

Deduced amino acid sequence of the *P. phosphoreum* NZ-11-D *lux* operon

LuxC

RDTSEHEYRELTLNSYKVSIPIINQDDVEAIKSQNVENNLNINQIVNFLYTVGQKWK

SENYSRRLTYIRDLVRFLGYSPEMAKLEANWISMILSSKSALYDIVETELGSRHIVD

EWLPQGDCYVKAMPKGKSVHLLAGNVPLSGVTSIIRAILTKNECIIKTSSADPFTAI

ALASSFIDTDEHHPISRSMSVMYWSHNEDIAIPQQIMNCADVVVSWGGYDAIKWATE

HTPVNVDILKFGPKKSIAIVDNPVDITASAIGVAHDICFYDQQACFSTQDIYYIGDN

IDAFFDELVEQLNLYMDILPKGDQTFDEKASFSLIEKECQFAKYKVEKGDNQSWLLV

KSPLGSFGNQPLARSAYIHHVSDISEITPYIENRITQTVTVTPWESSFKYRDVLASH

GAERIVESGMNNIFRVGGAHDGMRPLQRLVKYISHERPYTYTTKDVAVKIEQTRYLE

EDKFLVFVP

LuxD

MKSENNSVPIDHVIKVDNERHIRVWETFPKNQCDKRNNTIVIASGFARRMDHFAGLA

EYLSTNGFHVIRYDSLNHVGLSSGEIDQFSMSVGKKSLLTVIDWLKSEHGIDQVGLI

ASSLSARIAYDIVADVNLSFLITAVGVVNLRNTLEQALKYDYLQMEIDEIPEDLDFD

GYNLGSKVFVTDCFENNWDTLDSTINKTKNLNVPFIAFVANDDSWVQQHEVEELMSN

INSDKTKIYSLIGSSHDLGENLIVLRNFYQSITKAAIALDSNLVGLVSEIIEPQFEA

LTIATVNERRLKNKIQSKSLA

LuxA

MKFGNICFSYQPPGESHKEVMDRFVRLGVASEELNFDTYWTLEHHFTEFGLTGNLFV

ACANLLGRTTKLNVGTMGIVLPTAHPARQMEDLLLLDQMSKGRFNFGVVRGLYHKDF

RVFGVTMEDSRAITEDFHTMIMDGTKTGTLHTDGKNIEFPDVNVYPEAYLEKIPTCM

TAESAVTTTWLAERGLPMVLSWIITTSEKKAQMELYNAVARDSGYSEEYIKNVDHSM

TLICSVDEDGKKAEDVCREFLGNWYDSYVNATNIFSESNQTRGYDYHKGQWKDFVLQ

GHTNTKRRVDYSHDLNPVGTPEKCIEIIQRDIDATGITNITLGFEANGSEEEIIASM

KRFMTQVAPFLKDPK

LuxB

MNFGLFFLNFQPENTSSETVLDNMINTVSLVDKDYKNFTTALVNEHHFSKNGIVGAP

MTAASFLLGLTERLHIGSLNQVITTHHPVRIAEEASLLDQMSDSRFILGLSDCVNDF

EMDFFKRQRDSQQLQFEACYDIINEAITTNYCQANNDFYNFPRISINPHCLSKENMK

QYILASSVSVVEWAAKKALPLTYRWSDSLEDKEILYKRYLEVAAKHNIDVSNVEHQF

PLLVNLNHDRDVAHQEATAYLVSYIAEVYPHLNQQQKIAELISQHAIGTDNDYYEST

LNALERTGSKNVLLSFESMKNHDDVVKVINMVNEKIQKNLPSS

LuxF

MNKWNYGVFFVNFYNKGQQEPSKTMNNALETLRIIDEDTSIYDVINIDDHYLVKKDS

EDKKLAPFITLGEKLYVLATSENTVDIAAKYALPLVFKWDDINEERLKLLSFYNASA

SKYNKNIDLVRHQLMLHVNVNEAETVAKEELKLYIENYVACTQPSNFNGSIDSIIQS

NVTGSYKDCLSYVANLAGKFDNTVDFLLCFESIQDQNKKKSVMIDLNNQVVKFRQDN

NLI

LuxE

MTITLDTCEKDIIVSTEIDDIIFTSSPLDITYDEQERIKHKLILESFRYHYNNNEDY

KSFCNTQGVDENISSLDDIPVFPTSMFKYAKICTADESNIENWFTSSGTSGVKSHIA

RDRVSIERLLGSVNYGMKYLGSFHENQLELVNMGPDRFNAKNVWFKYVMSLVELLYP

TTFTVNNDEIDFELTIKSLKEIYNKGKGICLIGPPYFIYLLCQYMKENDIEFNAGNR

IFIITGGGWKTKQKQALNRQDFNQLLMETFHLAHESQIRDTFNQVELNTCFFEDNRQ

RKHVPPWVYARALDPVTLKPVEDGQEGLISYMDASSTSYPTFIVTDDIGIIHTIKDP

DPYQGTTIDIVRRLNTREQKGCSLSMASGLK

luxG

MILNCKIIKIEASECNIFKVFIKPDKCLNFKAGQYVLAYLDGKKLPFSIANCPTCNE

LIELHVGCSVKETAVKSISHFLDAFVNSSEIQIDAPHGNAWLREDSNSPLLLIAGGT

GLSYINSILSNCVNRNLPRSIYVYWGVNNIDLLYADTQLKALSSDFNNVKYVPVLEN

FDNNWYGKKGNVIDAIIEDFGDLSEFDIYVCGPQGMTRSVREKLTSLKKAD