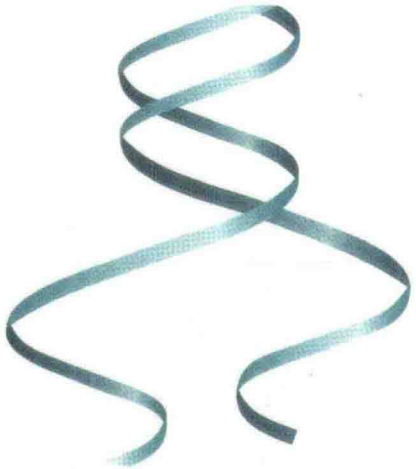
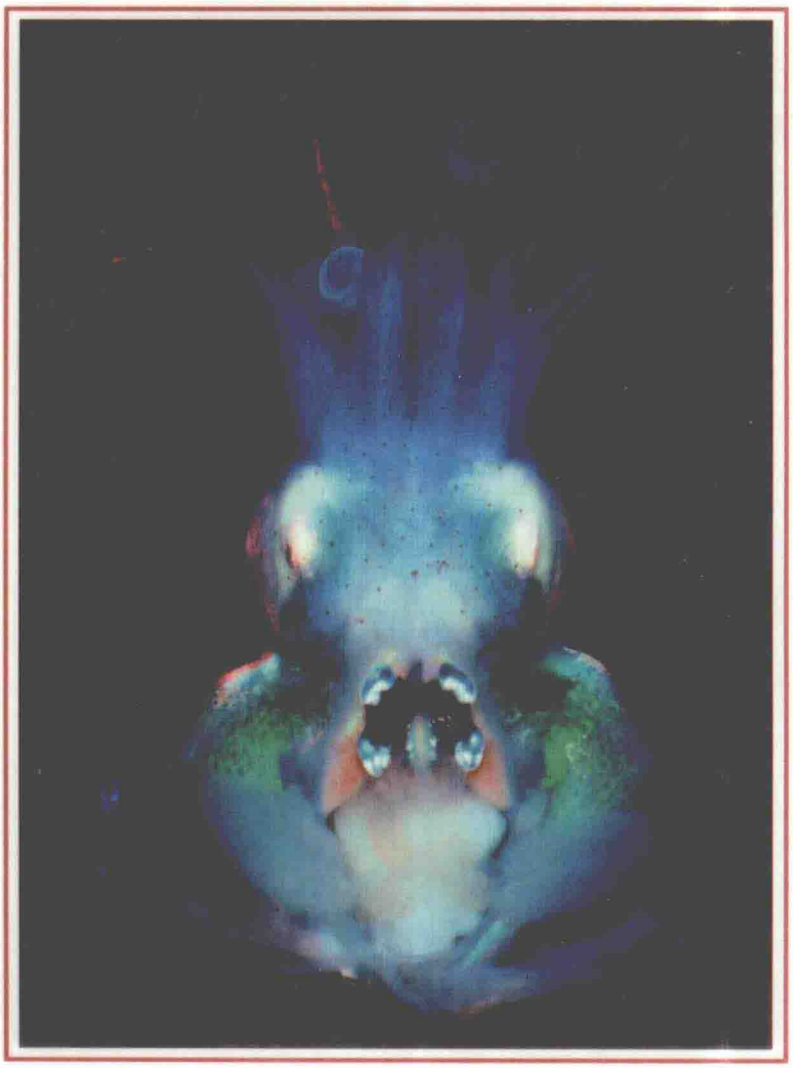
# UNRAVELING DNA

## MOLECULAR BIOLOGY FOR THE LABORATORY





WINFREY ROTT WORTMAN

# Unraveling DNA

## Molecular Biology for the Laboratory

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To Jane for showing us how to teach with high standards and
to Chuck for showing us how to keep our sanity while maintaining them
Mike and Mark

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# Unraveling DNA

## Molecular Biology for the Laboratory

# FOREWORD

Some of us have been fortunate to have worked with bioluminescent systems for part or all of our careers. For some, it was a matter of fate: being handed a project by a professor. For others, it was the attraction of studying something so totally fascinating that it was almost impossible not to study. I confess to being in the latter group—drawn to the study of bacterial bioluminescence like an insect to a flame after hearing a lecture presented by Professor J. W. Hastings at the University of Chicago in the early 1960s. This led to a thesis project on the subject of bacterial bioluminescence, and I have continued this work for more than 30 years!

During the ensuing years, I have used the bacterial bioluminescence system as a teaching tool, using its beauty and simplicity to capture the interest and imagination of many a student, and have often mused about the possibility of designing a whole course in bacteriology (or some part of it) using a bioluminescent bacterium and bioluminescence as the major organism and system of study. However, as often happens, due to the limitations of time and energy, such endeavors languish on the shelf as "great ideas" but fail to mature into working systems.

It was thus with some excitement that I have observed Mike Winfrey, Marc Rott, and Al Wortman design, implement, and now publish, in manual format, a series of exercises in molecular biology using the bacterial luminescence (*lux*) genes. Since the original cloning of these genes in 1982 by Dan Cohn, and the resulting excitement of seeing *Escherichia coli* glowing in the darkroom, it has seemed obvious that this could and should be a powerful teaching tool. Yet until now there was no procedural manual that allowed this to be done in the classroom format.

It has been my great pleasure to follow the development of this manual, to see summer students struggle with and complete the experiments, and to watch it mature into a usable manual for teaching at many levels. The feedback from students has been uniformly positive—and the exercises are enjoyable and intellectually challenging. Winfrey, Rott, and Wortman have produced an excellent manual with a selection of experiments that goes far beyond just molecular biology.

They step beyond molecular biology in experiments that examine the ecology and distribution of the luminous bacteria using molecular probes. The ecology and distribution of the luminous bacteria remains one of the unexplored horizons of marine (and soil) microbiology, and when such discussions are added to the molecular biology of bioluminescence, the usefulness of these techniques and approaches for studying organisms, populations, and ecology becomes apparent. I believe that this manual has tremendous potential for educating, exciting, and challenging minds at all levels—from beginning students to jaded veterans—and I am pleased to see its publication.

Kenneth H. Nealson
*Distinguished Professor of Biological Sciences*
*University of Wisconsin-Milwaukee*
*Center for Great Lakes Studies*

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# PREFACE

In slightly less than two decades, the biological sciences have been revolutionized by new procedures used to manipulate and study genetic material. At the heart of this "biological revolution" are procedures known as **recombinant DNA techniques**—the ability to splice pieces of foreign DNA into a vector and transfer these recombinant DNA molecules into a living organism. In the early 1980s, like many university faculty, I realized that this revolution in how biological systems are studied also required changes in how the biological sciences are taught. The applications of recombinant DNA techniques were so widespread and were having such a universal impact that it was important for young scientists to have training in the principles, methods, and applications of these procedures. Although it was relatively easy to incorporate this novel information into lectures, performing recombinant DNA techniques in a teaching lab seemed almost overwhelming.

However, I had always been inspired by the Chinese proverb that states:

I hear and I forget,
I see and I remember,
I do and I understand

and felt that for students to truly comprehend the elegance and power of these techniques, they should actually perform gene cloning experiments in a laboratory setting. Thus, in the mid-1980s, I began developing a curriculum to bring the relatively new procedures in recombinant DNA techniques into undergraduate teaching laboratories. My first attempts in 1985 involved the use of homemade gel boxes put together with scraps of plexiglass and nicrome wire, a single micropipet capable of measuring 2–10 µl (for a class of 20 students), and a handheld UV light.

During these early attempts at teaching recombinant DNA techniques, I was intrigued by the possibility of having students clone a gene from an entire genome, even though this seemed far beyond the scope of undergraduate teaching laboratories. In 1988, while pondering how to develop teaching labs that would allow such a cloning, I heard a talk by Ken Nealson on bacterial bioluminescence. I had been fascinated with these organisms since graduate school, where I had isolated them from fresh shrimp in a dark (and rather smelly) room. Ken described, amid beautiful slides of bacteria producing a soothing blue luminescence, how they had cloned the genes. The usually extremely time-consuming process of screening the thousands of clones from a genomic library for the clone of interest was done in mere minutes in a dark room! This launched the idea of developing a laboratory teaching curriculum centered around the cloning of the bioluminescence genes.

In 1990, Al Wortman, a new molecular biologist at University of Wisconsin–La Crosse, and I wrote an Undergraduate Faculty Enhancement Program grant through the National Science Foundation to offer a two-week summer laboratory workshop in molecular biology for college faculty. We were funded for three years, and these workshops, along with a new undergraduate course in microbial genetics, resulted in the birth of this manual. We wanted to create a curriculum that not only allowed the molecular biology research techniques to be done within the limitations of college teaching laboratories, but also provided students with a sense of completing a major

research project rather than just presenting a collection of seemingly unrelated techniques.

In the first year of the NSF-funded workshop, we compiled an integrated series of exercises involving the cloning, mapping, subcloning, and sequencing of the bioluminescence (*lux*) genes from the marine bacterium *Vibrio fischeri*. Following the first year of the course, Al Wortman resigned and was replaced by Marc Rott. Marc had extensive experience in bacterial molecular biology, assisted in part of the 1992 NSF workshop, and was a co-instructor in the 1993 course. Marc and I received an additional two years of funding from NSF to offer an expanded two-and-a-half-week workshop for college faculty in the summers of 1994 and 1995. Through the NSF-sponsored summer courses (attended by 100 college faculty) and our undergraduate microbiology courses, the exercises were thoroughly and repeatedly tested. We also extensively revised the manual each year based on the experiences and valuable comments of college faculty and our students.

So, more than a decade after the inception of an idea, and after over seven years of development, we are pleased to publish this manual—an integrated series of laboratory exercises based on the cloning and analysis of genes encoding bacterial bioluminescence. In *Unraveling DNA: Molecular Biology for the Laboratory,* we guide students and instructors alike through the tangled maze of modern protocols in molecular biology and make them feasible in the time constraints of undergraduate laboratories. Although in many cases we have pushed the envelope in determining how short one can make incubations, these exercises still yield excellent results in the hands of students. We have not varied conceptually from **how** these fundamental procedures in molecular biology are done, and the protocols in this manual are suitable for use in research labs as well.

At first glance, cloning a set of genes from an entire genome in an undergraduate course seems overly ambitious. We received some criticism from reviewers in our NSF proposals that it was not possible to clone a gene in a two-week course. What they failed to realize was that we cloned the genes in the first week and spent the second week analyzing the clones! Conducting a series of integrated exercises where subsequent exercises depend on the successful completion of previous labs may also seem problematic. Students, like all scientists, will make mistakes and not all will be successful in completing each exercise. However, the techniques provided here have been exhaustively tested by thousands of undergraduate students and more than a hundred faculty, and have proven to work exceptionally well. In addition, we have designed the exercises such that each group generates a large excess of the material needed for subsequent labs. Thus, student groups that encounter problems in any exercise will be able to borrow materials, DNA, or strains from other groups. This approach allows everyone to complete the entire cloning project successfully.

Besides providing basic experience and understanding in the principles and practice of modern molecular techniques, it is also our intention to present this material in a format that is exciting and fun for students. With this objective in mind, we have based the entire series of exercises on one of the world's most fascinating biological systems: the biological production of light. We have found that this adds an additional level of interest and biological relevance to the already intriguing study of molecular biology.

One limitation of laboratory manuals in rapidly advancing areas such as molecular biology is that they often become out of date even before they are published. This

is particularly true with the use of computers in molecular biology. To circumvent this, we have established a Molecular Biology Home Page on the World Wide Web:

**http://www.uwlax.edu/MoBio**

The computer analysis of DNA (Exercise 26) is linked to our home page to allow students to use the most recent and relevant Internet sites to conduct the exercise. Our home page also provides links to numerous other sites on the World Wide Web of relevance to many of the exercises in the manual.

In addition to the 28 exercises in the manual, 19 appendices provide a wealth of information on basic procedures, principles and precautions in molecular biology, recipes for media and reagents, lists of suppliers of equipment and materials used in the course, and current references. These provide a valuable resource for student and instructor alike and are used in many of the exercises. An instructor's manual is also available with detailed instructions on how to prepare the materials for each exercise, tips on interpreting results, troubleshooting potential errors, and answers to the questions following each exercise. Instructors are also referred to our home page for updated preparation tips, suppliers, and so on.

Finally, it is easy to get lost in the details of molecular biology and forget the big picture. Molecular biology is an elegant science in its own right, but today it is most frequently used as a powerful set of tools to study a myriad of biological processes. In this manual, we have attempted not to lose the connection with the organisms we are examining and the environment from which they came. Thus, we hope you come away from using this manual with not only an appreciation of the power of molecular biology but also an interest in a unique group of luminous bacteria and the often bizarre deep-sea creatures that provide these organisms a home.

Mike Winfrey
(with Marc Rott and Al Wortman)

# ACKNOWLEDGMENTS

We would like to thank the Japanese pinecone fish, *Monocentris japonicus*, for harboring and caring for its symbiont *Vibrio fischeri* since before the dawn of humanity. We would also like to thank *Vibrio fischeri* for providing illumination for its caretaker through all those millennia. We also express a deep appreciation for the process of evolution for providing all the genes required for bioluminescence on a single 9 kb *Sal* I restriction fragment. This manual would not be possible without this extraordinary bond between these two species and the fortuitous location of the *lux* genes.

We would also like to thank Ken Nealson for his continued assistance throughout the development of this manual, providing the MJ1 strain of *Vibrio fischeri* and numerous ideas, and presenting the opening seminars at each of our NSF workshops. Ken has an infectious enthusiasm and love of science that have been an inspiration to us and all who have used this manual.

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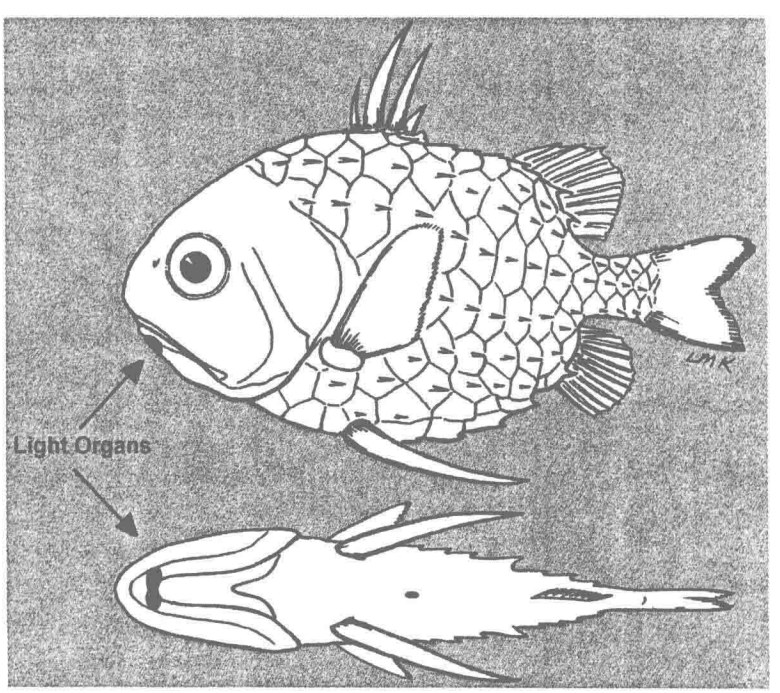


The Japanese pinecone fish (*Monocentris japonicus*). This fish, commonly referred to as the "port and starboard" fish, has two light organs under its lower jaw. The bioluminescent bacterium used in this manual, *Vibrio fischeri* MJ1, was isolated from this fish by Ken Nealson. Bar = 1.0 cm (from Hastings and Nealson, 1981, with permission).

Many people contributed in many ways to the development of this manual. We thank Chuck Whimpee and Lisa Van Ert for providing the PCR primers used in the PCR exercise. Dave Mead, Dave Essar, Ford *Lux*, Pat Singer, and Paul Barney assisted in several of the NSF-sponsored workshops and contributed valuable suggestions to the manual. We thank Jerry Davis for the photography in the manual, and Jill Rouselle, Zac Triemert, and Lindsay Dunnum for editing and assisting with the photography.

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# INTRODUCTION TO BACTERIAL BIOLUMINESCENCE

The biological production of light, or bioluminescence, has intrigued and fascinated humans for thousands of years, and scientific studies on bioluminescence date back more than 300 years (Meighen 1988). Numerous organisms have the ability to emit biologically produced light, including fireflies, fish, clams, worms, algae, and bacteria (Harvey 1952). Perhaps some of the most bizarre and fascinating of these are marine fish and squid, which have a variety of unique light organs (Goode and Bean 1895, McCosker 1977, Robison 1995, Ruby and McFall-Ngai 1992). However, most of the luminescent marine animals do not produce bioluminescence themselves, but harbor bioluminescent bacteria in specialized light organs. Due to their ease of study, extensive research has been done on bioluminescent bacteria, which has allowed a detailed understanding of the biochemistry and genetics of this process.

Most bioluminescent bacteria are marine in origin and include both free living forms and species that form symbiotic relationships with fish or squid. The light organs are often highly specialized and specifically adapted to harbor essentially pure cultures of the luminescent bacteria (see Figure 1). Bioluminescence in fish is particularly common in the deep sea, where up to 96% of all deep-sea fish are reported to be bioluminescent (Harvey 1952). The exact role of bioluminescence is not clearly known, although numerous advantages to the fish have been proposed, such as warding off predators, attracting prey, or communicating. In return for producing light for the fish, the bacteria are provided with a protected environment and a rich supply of nutrients. Recently, some understanding of the relationship between bioluminescent

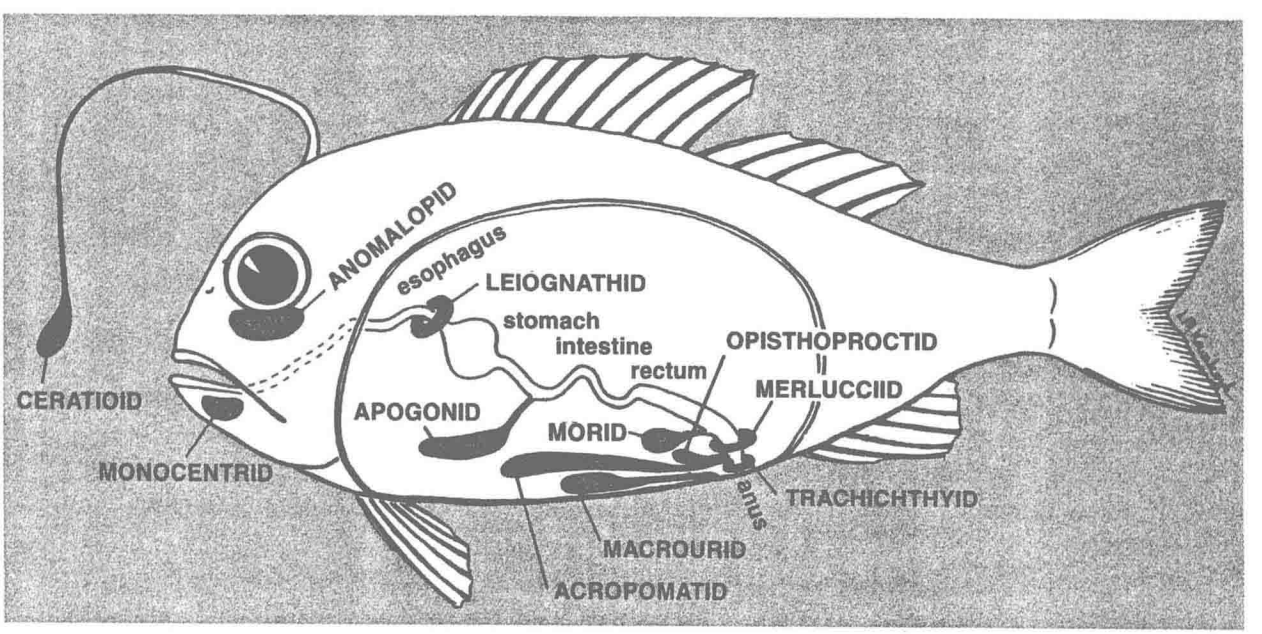


Figure 1
A hypothetical fish illustrating the approximate location and size of light organs (indicated in blue) of the various types of luminescent fishes (modified from Hastings and Nealson 1981, with permission)

marine species and their bacterial symbionts has evolved from the study of the squid *Euprymna scolopes* (see front cover) and its symbiont *Vibrio fischeri* (Boettcher, et. al 1996, McFall-Ngai and Ruby 1991, Ruby and McFall-Ngai 1992), and this system has become an excellent model for studying animal-bacterial symbioses.

Besides the mutualistic relationships, bioluminescent bacteria are common in a variety of other associations with marine animals. Many are saprophytic, found on living or dead fish or shellfish. In fact, one of the easiest ways to isolate bioluminescent bacteria is to allow bacterial growth on the surface of fresh fish and then to examine the fish for bioluminescent patches in a dark room. Luminescent bacteria have also been isolated from stored meat and even open human wounds (Hastings and Nealson 1981). Numerous species are parasites on marine crustaceans, such as sand fleas (Harvey 1952), and other species are commensal in the intestinal tracts of marine animals (Hastings and Nealson 1981).

Bioluminescence is catalyzed by an enzyme known as **luciferase** (Meighen 1988). The bacterial enzyme is a heterodimer with a molecular weight of approximately 80,000 daltons and consists of an $\alpha$ and $\beta$ subunit with molecular weights of approximately 42,000 and 38,000 daltons, respectively. The active site is on the $\alpha$ subunit, although the $\beta$ subunit is required for activity. Luciferase is a mixed function oxidase that produces a blue-green light via the simultaneous oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long chain aldehyde (tetradecanal) by $O_2$:

$$FMNH_2 + O_2 + R\text{-}CHO \longrightarrow FMN + R\text{-}COOH + H_2O + \text{light}$$

The energy for light production is supplied by the oxidation of the aldehyde and $FMNH_2$. The actual mechanism of light emission is not clearly understood but is thought to result from the formation of a hydroperoxy flavin via the reaction of $FMNH_2$ and $O_2$. These molecules have been shown to emit light in the presence of aldehydes (Meighen 1988).

Three additional enzymes are necessary to generate the aldehyde required in the reaction. The fatty acids for this fatty acid reductase enzyme complex are removed from the fatty acid biosynthesis pathway via the enzyme **acyl-transferase**. This enzyme reacts with acyl-ACP (*A*cyl *C*arrier *P*rotein) to release free fatty acids (R-COOH). The fatty acids are then reduced to an aldehyde by a two-enzyme system via the following reaction:

$$R\text{-}COOH + ATP + NADPH \longrightarrow R\text{-}CHO + AMP + PP + NADP^+$$

One enzyme, **acyl-protein synthetase,** activates the fatty acid via the cleavage of ATP to form R-CO-AMP. This serves as the substrate for the final enzyme, **acyl-reductase**, that catalyzes the NADPH-dependent reduction of the activated fatty acid to an aldehyde. The role of each enzyme involved in light production is summarized in Figure 2.

Bacterial bioluminescence is observed only at very high cell densities because of a unique type of regulation known as autoinduction (Nealson and Hastings 1979). The bacteria produce a diffusible compound (an N-acyl homoserine lactone) known as an autoinducer, which induces transcription of the genes encoding the enzymes required for light production. However, light production occurs only when a threshold concen-

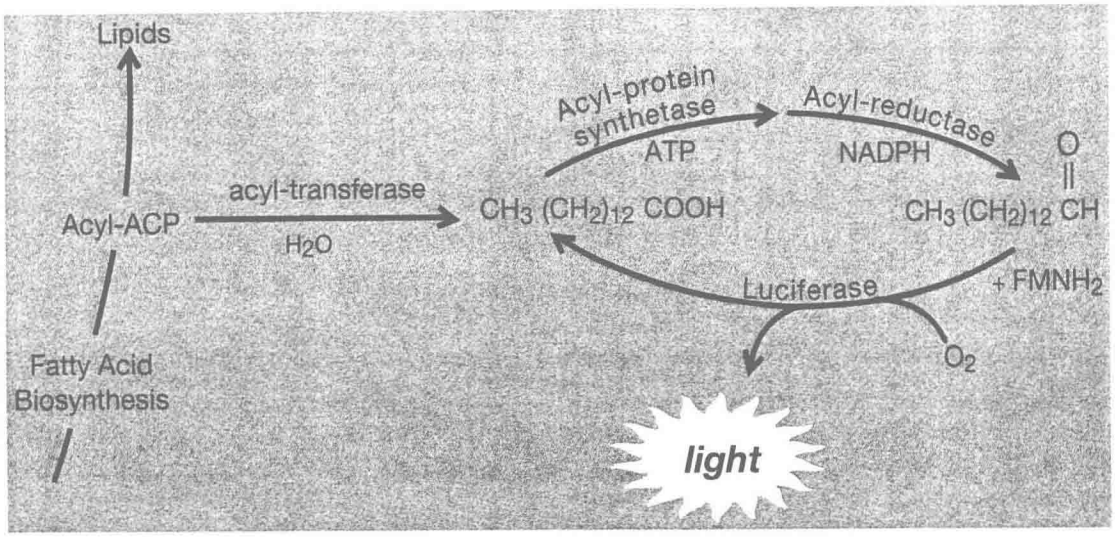


Figure 2
Pathway of aldehyde formation and light production in the bacterial bioluminescence system (modified from Meighen 1988, with permission).

tration of the autoinducer accumulates. Thus, bacteria at high cell density in the light organ of a fish accumulate sufficient autoinducer to bioluminesce, while free living bacteria do not. The requirement for an autoinducer represents a significant ecological adaptation. Because light production requires a tremendous amount of energy and cellular reducing power, free living bacteria in the ocean—which are nutrient limited—will not waste this energy expenditure that probably offers them no benefit.

Interestingly, the autoinduction mechanism has recently been shown to be a general mode of regulation in many gram-negative bacteria. There is considerable interest in this process as a result of the discovery that some plant and animal pathogens, as well as plant symbionts, also produce homoserine lactone autoinducers and have *lux*I and *lux*R analogs to control host colonization. It appears that many bacterial behaviors involved in host colonization (such as bioluminescence) require a large population, or "**quorum**," of bacteria and use the *lux*I and *lux*R system of autoinduction. This has resulted in use of the phrase "**quorum sensing**" (Fuqua, et al. 1994) to describe cell activities that require a threshold cell density.

With the advent of recombinant DNA techniques, it has been possible to clone and determine the genetic organization of the bacterial bioluminescence genes from numerous species (see Meighen 1988, 1991, 1994 for examples). The α and β subunits of luciferase and the three enzymes required for aldehyde formation are encoded in a single operon (the *lux* operon) in all luminescent bacteria examined (see Figure 3). The first two structural genes, *lux*C and *lux*D code for the acyl-reductase and acyl-transferase, respectively. These are followed by *lux*A and *lux*B, which code for the α and β subunits of luciferase, and finally *lux*E, which codes for the acyl-protein synthetase. Two regulatory genes, *lux*I and *lux*R, have also been identified in *Vibrio fischeri* (which has recently been reclassified as *Photobacterium fischeri*). The *lux*I gene is on the same operon as the structural genes, whereas *lux*R is transcribed in the opposite direction. *Lux*R codes for a transcriptional activator that binds the autoinducer synthesized by the *lux*I gene product.

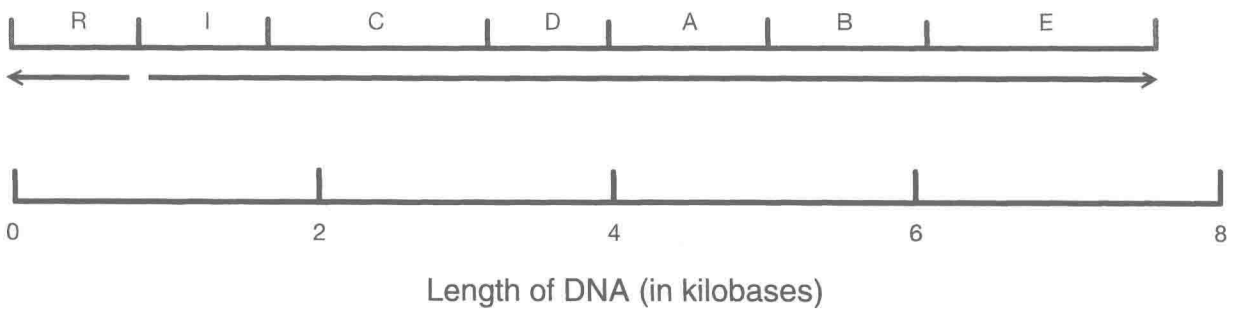


Figure 3
The *lux* operon of *Vibrio fischeri*. *lux*C codes for the acyl-reductase; *lux*D codes for the acyl-transferase; *lux*A and *lux*B code for the α and β subunits of luciferase, respectively; *lux*E codes for the acyl-protein synthetase; *lux*R and *lux*I code for regulatory proteins. Arrows under the operon indicate the directions of transcription.

Detailed study of the molecular genetics of bacterial *lux* operons has allowed use of this genetic system in applied and basic research. The *lux* system is now used in toxicity testing (Schiewe, et al. 1985), as a reporter in gene fusions to indicate the level of expression of various operons (Heitzer, et al. 1992, Nealson and Hastings 1991, Selifonova, et al. 1993), in promoter probe vectors (Sohaskey, et al. 1992), and as a method of monitoring the fate of genetically engineered microorganisms in the environment (Shaw, et al. 1992). The genes for bacterial bioluminescence have also been engineered into bacteriophage to allow sensitive testing for bacterial pathogens (Stewart, et al. 1996). Although bioluminescence will always hold its appeal and excitement due to the soothing blue light produced, this unique biological process is likely to find additional practical applications in molecular biology and biotechnology in years to come.

## Literature Cited

Boettcher, K. J., E. G. Ruby, and M. J. McFall-Ngai. 1996. Bioluminescence in the symbiotic squid *Euprymna scolopes* is controlled by a daily biological rhythm. *J. Comp. Physiol.* 179:65-73.

Fuqua, W. C., S. Winans, and E. P. Greenberg. 1994. Quorum sensing in bacteria: The LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176:269-275.

Goode, G. B., and T. H. Bean. 1895. *Oceanic ichthyology: A treatise on the deep-sea and pelagic fishes of the world.* Washington D. C.: Government Printing Office.

Harvey, E. N. 1952. *Bioluminescence.* New York: Academic Press.

Hastings, J. W., and K. H. Nealson. 1981. The symbiotic luminous bacteria. M. P. Starr, H. Stolp, H. G. Truper, A. Ballows, and H. G. Schlegel, ed. 1332-1345. In *The prokaryotes: A handbook on habitats, isolation, and identification of bacteria.* New York: Springer-Verlag.

Heitzer, A., O. F. Webb, J. E. Thonnard, and G. S. Sayler. 1992. Specific and quantitative assessment of naphthalene and salicylate bioavailability by using a bioluminescent catabolic reporter bacterium. *Appl. Environ. Microbiol.* 58:1839-1846.

Meighan, E. A. 1988. Enzymes and genes from the *lux* operons of bioluminescent bacteria. *Ann. Rev. Microbiol.* 42:151-176.

Meighan, E. A. 1991. Molecular biology of bacterial bioluminescence. *Microbiol. Rev.* 55:123–142.

Meighan, E. A. 1994. Genetics of bacterial bioluminescence. *Ann. Rev. Genet.* 42:151–176.

Meighan, E. A., and P. V. Dunlap. 1993. Physiological, biochemical, and genetic control of bacterial bioluminescence. *Adv. Microbial Physiol.* 34:1–67.

McCosker, J. E. 1977. Flashlight fishes. *Scientific American.* 236:106–112.

McFall-Ngai, M. J., and E. G. Ruby. 1991. Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. *Science* 254:1491–1494.

Nealson, K. H., and J. W. Hastings. 1979. Bacterial bioluminescence: Its control and ecological significance. *Microbiol. Rev.* 43:496–518.

Nealson, K. H., and J. W. Hastings. 1991. The luminous bacteria. In *The Prokaryotes.* 2d ed., ed. A. Ballows, H. G. Truper, M. Dworkin, W. Harder, and K. Schleifer. New York: Springer-Verlag.

Robison, B. H. 1995. Light in the ocean's midwaters. *Scientific American.* 273:59–64.

Ruby, E. G., and M. J. McFall-Ngai. 1992. A squid that glows in the night: Development of an animal-bacterial mutualism. *J. Bacteriol.* 174:4865–4870.

Schiewe, M. H., E. G. Hawk, D. I. Actor, and M. M. Krahn. 1985. Use of bacterial bioluminescence assay to assess toxicity of contaminated marine sediments. *Can. J. Fish. Aquat. Sci.* 42:1244–1248.

Selifonova, O., R. Burlage, and T. Barkay. 1993. Bioluminescent sensors for detection of Hg(II) in the environment. *Appl. Environ. Microbiol.* 59:3083–3090.

Shaw, J. J., F. Dane, D. Geiger, and J. W. Kloepper. 1992. Use of bioluminescence for detection of genetically engineered microorganisms released into the environment. *Appl. Environ. Microbiol.* 58:267–273.

Sohaskey, C. D., H. Im, and A. T. Schauer. 1992. Construction and application of plasmid-based and transposon-based promoter-probe vectors for *Streptomyces spp.* that employ a *Vibrio harveyi* luciferase reporter cassette. *J. Bacteriol.* 174:367–376.

Stewart, G. S. A. B., M. J. Loessner, and S. Scherer. 1996. The bacterial *lux* gene bioluminescent biosensor revisited. *ASM News.* 62:297–301.



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