

# A destabilized bacterial luciferase for dynamic gene expression studies

Michael S. Allen · John R. Wilgus · Christopher S. Chewning · Gary S. Saylor · Michael L. Simpson

Received: 25 October 2005 / Revised: 15 May 2006 / Accepted: 20 June 2006 / Published online: 31 October 2006  
© Springer Science+Business Media B.V. 2006

**Abstract** Fusions of genetic regulatory elements with reporter genes have long been used as tools for monitoring gene expression and have become a major component in synthetic gene circuit implementation. A major limitation of many of these systems is the relatively long half-life of the reporter protein(s), which prevents monitoring both the initiation and the termination of transcription in real-time. Furthermore, when used as components in synthetic gene circuits, the long time constants associated with reporter protein decay may significantly degrade circuit performance. In this study, short half-life variants of LuxA and LuxB from *Photobacterium luminescens* were constructed in *Escherichia coli* by inclusion of an 11-amino acid carboxy-terminal tag that is recognized by endogenous tail-specific proteases. Results indicated that the addition of the C-terminal tag affected the functional half-life of the holoenzyme when the tag was added to *luxA* or to both *luxA* and *luxB*, but modification of *luxB* alone did not have a significant effect. In addition, it was also found that alteration of the terminal three

amino acid residues of the carboxy-terminal tag fused to LuxA generated variants with half-lives of intermediate length in a manner similar to that reported for GFP. This report is the first instance of the C-terminal tagging approach for the regulation of protein half-life to be applied to an enzyme or monomer of a multi-subunit enzyme complex and will extend the utility of the bacterial luciferase reporter genes for the monitoring of dynamic changes in gene expression.

**Keywords** Luciferase · Reporter genes · Gene expression · Lux · Destabilized protein · *ssrA* · *Photobacterium luminescens*

## Introduction

Reporter genes are transcriptional fusions of genetic regulatory elements to reporter proteins that provide readily detectable signal outputs. Such reporter proteins include LacZ, chloramphenicol acetyltransferase,  $\beta$ -lactamase (Zlokarnik et al. 1998), firefly luciferase (*luc*), bacterial luciferase (*lux*), and fluorescent proteins such as those derived from the green fluorescent protein of *Aequoria victoria* (e.g. GFP, EGFP, YFP) (Chalfie et al. 1994; Cormack et al. 1996; Andersen et al. 1998; Miyawaki et al. 1997; also reviewed in van der Meer et al. 2004). These gene fusions have been used in detecting xenobiotic compounds (King et al. 1990; DiGrazia et al. 1991), monitoring performance of synthetic gene circuits (Elowitz and Leibler 2000; Hasty et al. 2002; Gardner et al. 2000), in genome-wide expression arrays (Van Dyk et al. 2001), as well as in vivo imaging studies (Xiong et al. 2005; Kadurugamuwa et al. 2003; Kuklin et al. 2003; Doyle et al. 2004).

---

M. S. Allen · J. R. Wilgus · M. L. Simpson  
Molecular-Scale Engineering and Nanoscale Technologies  
(MENT) Research Group, Oak Ridge National Laboratory,  
P.O. Box 2008, Building 3500, MS 6006, Oak Ridge, TN  
37931-6006, USA

M. S. Allen · M. L. Simpson  
Department of Materials Science and Engineering,  
University of Tennessee, Knoxville, TN 37996, USA

C. S. Chewning · G. S. Saylor · M. S. Allen ·  
J. R. Wilgus · M. L. Simpson (✉)  
The Center for Environmental Biotechnology, University of  
Tennessee, Knoxville, TN 37996, USA  
e-mail: simpsonml1@ornl.gov

While these reporter proteins have numerous applications, all except the bacterial luciferase system require the additional input of reactants or UV light. The bioluminescent gene cassette comprising the *lux* system was originally isolated from *Vibrio fischeri* (Engbrecht et al. 1983). Later, more thermally stable versions of *lux* were isolated from *Photobacterium luminescens* (*Xenorhabdus luminescens*) and other species (Schmidt et al. 1989; Szittner and Meighen 1990). In these systems, the heterodimeric bacterial luciferase comprised of LuxAB oxidizes an aliphatic aldehyde substrate and FMNH<sub>2</sub> in the presence of diatomic oxygen to release a photon of light at a wavelength of ~490 nm (reviewed in Meighen (1991)). The remainder of the cassette consisting of the *luxC*, *D* and *E* genes, encode proteins that are responsible for generating and recycling the aldehyde substrate for the reaction.

Another major problem with most reporter systems are the relatively long half-lives of the reporter proteins, which complicate the detection and monitoring of dynamic changes in gene regulation at the transcriptional level. To address these issues, modifications to the protein coding sequences of some of these reporter systems have been made to include amino acids sequences that are recognized by host protein degradation machinery resulting in more rapid turnover of the reporter protein (Andersen et al. 1998; Mateus and Avery 2000; Li et al. 1998; Triccas et al. 2002). Similar tactics have been employed in the engineering of synthetic gene constructs such as the Repressilator (Elowitz and Leibler 2000).

To build on the existing utility of the bacterial *lux* reporter system, we modified each of the individual *luxA* and *luxB* monomers of the bacterial luciferase holoenzyme and tested them both individually and in combination. The modification employed a naturally occurring protein degradation pathway in *Escherichia coli*. This system, used for removing peptides synthesized from truncated mRNA, involves the addition of an 11-amino acid carboxy-terminal peptide tag (AANDENYALAA) and requires the SsrA RNA (Tu et al. 1995; Keiler et al. 1996). Cytoplasmic proteins containing the tag are rapidly degraded by the ClpP protease in conjunction with either ClpA or ClpX (Gottesman et al. 1998; Weber-Ban et al. 1999; Kim et al. 2000). In addition, the essential protease HflB may also play a role in degradation (Herman et al. 1998). Direct modification of a DNA sequence to encode the 11-amino acid SsrA tag on the 3' end of the gene to be expressed on the carboxy-terminus of a protein has been previously shown to result in rapid degradation of proteins within the cell (Andersen et al. 1998; Elowitz and Leibler 2000; Herman et al. 1998).

Furthermore, alteration of the last three amino acids on the tag has been used to alter the recognition specificity of the proteases, thereby generating a variety of protein half-lives of intermediate lengths (Andersen et al. 1998). Addition of the SsrA tag has also been shown to result in short half-life GFP variants in *Pseudomonas* and *Mycobacterium* species (Andersen et al. 1998; Triccas et al. 2002).

We have employed a similar approach for the modification of the bacterial luciferase holoenzyme and show that modification of a single monomer of the holoenzyme, LuxA, is sufficient for altering the functional half-life of the holoenzyme. Conversely, modification of the LuxB monomer alone neither impacts the performance of the holoenzyme nor results in decay of the bioluminescent signal. Based on these results, additional variants of *luxA* were generated containing modifications of the last three amino acids of the tag, and their half-lives were found to range between that containing the native SsrA tag (abbreviated here as -laa) and the wild type holoenzyme. Finally, the carboxy-terminal tags were cloned into an existing plasmid in a manner that should facilitate retrofitting of other *P. luminescens lux*-based reporters for the monitoring of rapid changes in transcription, or inclusion into synthetic gene circuits where dynamic signal performance is critical.

## Materials and methods

### Genetic constructs

Wild type and modified variants of the *luxA* and *luxB* genes were amplified by PCR from a vector containing the *P. luminescens lux CDABE* operon (pCDABE) using the primers listed in Table 1. Abbreviations for the tag (e.g. -aav) are indicated in the name of the construct after the gene being affected. Wild type *luxAB* and *luxABaav* were amplified by PCR, gel purified, and cloned into the TOPO-TA cloning vector pCR2.1 (Invitrogen) per the manufacturer's instructions. For *luxA* variants, *luxBaav*, and wild-type *luxB*, the genes were independently amplified and cloned. The intergenic sequence upstream of the ribosomal binding site between *luxA* and *luxB* was modified to create a unique *XmaI* site and facilitate subsequent construction. The *luxA* variants were digested overnight with *XmaI* and *XbaI* and ligated upstream of the *luxB* or *luxBaav* genes in pCR2.1. The wild type *luxAB* and the newly assembled variants were then each double digested overnight from their respective TA-cloning vectors with *BamHI* and *KpnI*, gel purified, and directionally cloned in place

**Table 1** Primers used in this study

LuxA forward:	<i>KpnI</i>	Start
	5'- <u>GGTACC</u> GC ATG AAA TTT GGA AAC TTT TTG -3'	
LuxB forward:	<i>XmaI</i>	Start
	5'- <u>CCCGGG</u> CT AAG GAG AAA GAA ATG AAA T -3'	
LuxB reverse:	<i>BamHI</i>	Stop
	5'- TTA <u>GGATCC</u> TA TTA G GTA TAT TCC ATG TGG TAC TTC -3'	
LuxAaav reverse:	<i>XmaI</i>	Stop V A A
	5'-TTA <u>CCCGGG</u> A CTA AAC TGC TGC AGC GTA GTT TTC GTC GTT TGC TGC AGG CCT ATA TAA TAG CGA ACG TTG TTT -3'	
LuxAlaa reverse:	<i>XmaI</i>	Stop A A L
	5'-TTA <u>CCCGGG</u> TA TTA AAC TGC TGC TAA AGC GTA GTT TTC GTC GTT TGC TGC AGG CCT ATA TAA TAG CGA ACG TTG TTT -3'	
LuxAasv reverse:	<i>XmaI</i>	Stop V S A
	5'-TTA <u>CCCGGG</u> A CTA AAC TGA TGC AGC GTA GTT TTC GTC GTT TGC TGC AGG CCT ATA TAA TAG CGA ACG TTG TTT -3'	
LuxBaav reverse:	<i>BamHI</i>	Stop V A A
	5'-TTA <u>GGATCC</u> TA TTA AAC TGC TGC AGC GTA GTT TTC GTC GTT TGC TGC AGG ACT GGT ATA TTC C ATG TGG TAC TTC -3'	

Unique restriction sites used in the final assembly are underlined. Start and stop codons and pertinent amino acids are indicated above the respective sequences

of the *GFP<sub>asv</sub>* gene of the repressilator reporter plasmid (Elowitz and Leibler 2000). For insertion of the tagged *luxA* into an existing full *lux* cassette, the appropriate primary construct was subsequently digested internal to both *luxA* (*AgeI*) and *luxB* (*BfuAI*) and the tag-bearing fragment was directionally cloned into the similarly digested pCDABE. Cloning reactions were conducted at room temperature for 10 min and included: 100 ng vector DNA, a 2:1 molar ratio of insert to vector DNA, 4 U of T4 DNA ligase (Fisher Scientific, Pittsburgh, PA) and 2 × LigaFast rapid ligation buffer (Promega, Madison, WI) in 25–35 μl reactions. Restriction enzymes were purchased from New England Biolabs (Beverly, MA), and all digestions were conducted using 5 U of each enzyme and incubated overnight at 37°C except in the case of *BfuAI*, in which digestion was incubated for 4 h at 50°C, followed by transfer to 37°C and the addition of *AgeI*.

#### Strains and growth conditions

Strains used in this study are listed in Table 2. Luria-Bertani media (Sambrook et al. 1989) was used for all experiments with 50 μg/ml kanamycin as necessary, and incubated at 37°C with shaking at 100 r.p.m. when appropriate.

#### Bioluminescence assays

Overnight cultures of the four variants of *luxAB* and a negative control carrying the parent vector with the gene for destabilized green fluorescent protein, *Gfp<sub>asv</sub>*, were transferred into triplicate tubes of fresh LB medium containing 50 μg/ml kanamycin. Cultures were then grown to an optical density at 546 nm ( $OD_{546}$ ) of ~0.6. Initial bioluminescence measurements were made at  $t = 0$  as described below. Subsequently, rifampicin (150 μg/ml) and tetracycline (30 μg/ml) were added to all cultures to block transcription and translation, respectively. For bioluminescence measurements, 1-ml samples were taken immediately ( $t = 0$ ) and at 30 min intervals thereafter and assayed for optical density at 546 nm ( $OD_{546}$ ) and bioluminescence.  $OD_{546}$  was measured using a Beckman DU-640B spectrophotometer. Bioluminescence was assayed by first adding 10 μl of *N*-decanal (Sigma, St. Louis, MO) to the 1-ml samples, inverting 10 times, and measuring light with a SDI Deltatox handheld luminometer. Three light measurements were taken in rapid succession and these values were averaged for each sample. Error bars represent the standard deviations between the averages of three independent samples.

**Table 2** Strains and plasmids used in this work

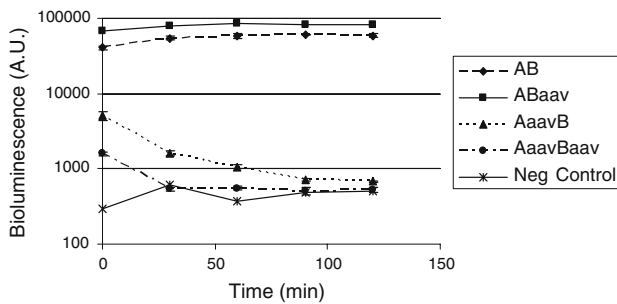
Name	Properties	Source
<i>E. coli</i> TOP10 and TOP10F'	Cloning and expression strains. Expression from the lac promoter in F' cells is IPTG inducible	Invitrogen (Carlsbad, CA)
pCR2.1	TOPO TA Cloning vector. Am <sup>R</sup> , Km <sup>R</sup>	Invitrogen
pCDABE	pCRXL (Invitrogen) containing PCR-amplified <i>luxCDABE</i> from <i>Photobacterium luminescens</i>	Gift from J. Sanseverino
pZE21-GFPasv	Km <sup>r</sup> ; Plasmid containing short half-life GFPasv constitutively expressed from the P <sub>Ltet01</sub> promoter	Elowitz and Leibler
pAB	pZE21-GFPasv with the wild-type <i>luxAB</i> of <i>P. luminescens</i> cloned in place of <i>GFPasv</i>	This work
pABaav	Similar to pAB using the alternative tag AANDENYAAAV translationally fused to <i>luxB</i> by PCR	This work
pAaavBaav	Similar to pAB using the <i>luxA</i> and <i>luxB</i> genes each modified to include the gene sequence for the 11-amino acid carboxy-terminal tag AANDENYAAAV	This work
pAaavB	pAB containing the <i>luxA</i> gene modified to include the gene sequence of the 11-amino acid carboxy-terminal tag AANDENYAAAV, and the wild type <i>luxB</i>	This work
pAlaavB	Similar to pAaavB using the alternative tag AANDENYALAA	This work
pAasvB	Similar to pAaavB using the alternative tag AANDENYAASV	This work
pCDAaavBE	pCDABE containing the 3' end of the modified <i>luxA</i> gene and the 5' end of <i>luxB</i> from pAsvB	This work
pCDAaavBE	As in pCDAaavBE but using pAaavB	This work
pCDAalaaBE	As in pCDAaavBE but using pAlaavB	This work

Variants of *luxA* containing the *-laa*, *-aav* and *-asv* tags were chosen for further analysis. Plasmids containing the constructs were digested with *AgeI* and *BfuAI*, gel purified, and cloned as described above into the similarly treated pCDABE. The latter contains the entire *P. luminescens lux* operon under the control of the *lac* promoter in the multi-copy pCR-XL-TOPO vector (Invitrogen). Resulting plasmids were then transformed into *E. coli* TOP10 F' cells (Invitrogen) for IPTG-inducible expression. For induction experiments, overnight cultures of cells grown in LB with 0.2 mM IPTG were diluted 1:5 in the same, fresh, preheated medium, and incubated at 37°C until reaching an OD<sub>600</sub> of 0.15–0.2 (approximately 4 h). Cells were subsequently collected by centrifugation, washed with fresh M9 containing 100 mM leucine medium without inducer, and incubated in M9 + leucine medium at 37°C. One milliliter samples were removed every 20 min for bioluminescent measurements using a SDI Deltatox handheld luminometer. Three measurements were taken in rapid succession for each sample and their values were averaged, and three independent replicates were performed for each variant.

## Results and discussion

Bioluminescence measurements of cells harboring plasmids containing the wild type *luxAB* genes of

*P. luminescens* behind a constitutive promoter were compared to those of *luxA* and *luxB* variants containing carboxy-terminal tags designed to accelerate degradation of the protein(s) within the cell. Assays using constructs containing only *luxA* and *luxB* (i.e. which lack *luxC*, *D*, and *E* and hence are unable to produce the substrate) were conducted by adding antibiotics to block transcription and translation (rifampicin and tetracycline, respectively), followed by monitoring bioluminescence with the addition of aldehyde over time. As shown in Fig. 1, modification of the LuxB protein did not result in a decrease of bioluminescence. Bioluminescence in this variant was found to remain relatively constant similar to the wild type over the course of the experiments. Addition of the same *-aav* tag (encoding AANDENYAAAV) to the *luxA* gene resulted in a rapid decrease in bioluminescence after time  $t_0$ . An even more dramatic decrease in bioluminescence was observed when both LuxA and LuxB included the *-aav* tag. Interestingly, the clone containing *luxA-Baav* (wild type *luxA* with modified *luxB*) appeared to produce more light than did the wild-type *luxAB*. Since this result was reproducible within the experimental parameters (Fig. 1) yet inconsistent with those generated in the absence of antibiotics (Table 3), it is believed that this result is an experimental artifact.



**Fig. 1** Total bioluminescence (arbitrary units) of clones containing wild type *luxAB*, and *luxABaav* (left axis), and *luxAaavB*, *luxAaavBaav* and the negative control (right axis) over time following the addition of tetracycline and rifampicin ( $t = 0$ ). Measurements were made following the addition of *N*-decanal as described in the “Materials and methods”. Values are averages of triplicate samples with error bars representing standard deviations among the replicates. The negative control contains the parent plasmid without the luciferase genes

In the unregulated case where enzyme was allowed to accumulate to a steady state level dependent upon the relative half-lives of their luciferases, mid-log cultures of pLuxAaavB produced approximately 100-fold less bioluminescence than the wild-type (Table 3). The double modified holoenzyme produced bioluminescence nearly 800-fold less than the wild type (Table 3).

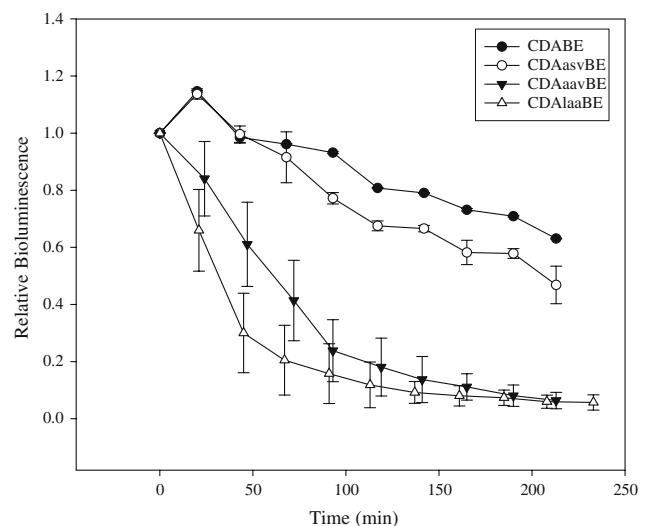
To determine if the drop in bioluminescence output could be attributed solely to interference of the carboxy-terminal tag to enzymatic activity, two additional constructs were made with predicted half-lives greater (*-asv* encoding AANDENYAASV) and lesser (*-laa* encoding AANDENYALAA) than those predicted for *-aav*. Total bioluminescent output for all of these constructs is shown in Table 3.

Variants of *luxA* were subsequently cloned into a vector containing the entire *lux* operon by restriction digestion interior to each of the *luxA* and *B* genes and replacement of the wild type fragment with fragments containing the modified *luxA* gene. Results of

experiments on luminescent decay following the removal of inducer (isopropyl- $\beta$ -D-thiogalactopyranoside; IPTG) are shown in Fig. 2.

**Conclusions**

The results for variants containing the modified *luxA* gene are consistent with those previously reported by Anderson et al. (Andersen et al. 1998) for GFP, and indicate that the single modification of this monomer is sufficient to result in decreased functional half-life of the heterodimeric holoenzyme (Fig. 1 and Table 3). This modification may impact enzymatic activity of the holoenzyme since the carboxy-terminus of LuxA is thought to be involved in substrate binding (Valkova et al. 1999). However, bioluminescence outputs for all variants were readily detectable under our test conditions. Interestingly, modification of the LuxB monomer



**Fig. 2** Relative bioluminescence over time for *luxA* variants contained within the entire *lux* cassette following removal of the IPTG inducer

**Table 3** Comparison of total (arbitrary units) and relative (% w.t.) bioluminescence output from 1-ml samples of mid-log cultures ( $OD_{546} = -0.4$ ) containing the indicated plasmids after addition of  $10 \mu l$  *N*-decanal

Plasmid	Total bioluminescence	Std. dev.	% w.t.	Est. half-life
pAaavBaav	2,510	77.56	0.13	N.D.
pAlaaB	11,998	713.5	0.61	46
pAaavB	17,057	1,524	0.87	48
pAsvB	632,260	9,424	32.21	145
pABaav	1,994,751	79,169	101.64	> 240
pAB	1,962,650	121,346	100.00	> 240
Negative control	504	42	N.D.	N.A.

Values for half-lives are in minutes. N.D. = not determined; N.A. = not applicable. Negative control consisted of the parent strain containing no plasmid

alone neither decreased peak bioluminescence nor resulted in decay of bioluminescence over time when compared to the wild type. Modification of both *luxA* and *luxB* genes to include the *-aav* tag (*luxAaavBaav*), however, resulted in lower total bioluminescence than modification of *luxA* alone (Fig. 1). These results suggest that the carboxy-terminus of LuxB may be protected in the heteroduplex of the holoenzyme and inaccessible to cellular proteases, but its release following the degradation of LuxA may facilitate its subsequent degradation and account for the reduced bioluminescence of the double-modified variant.

Modification of protein half-lives in eukaryotic proteins by the carboxy-terminal additions of the PEST-rich region from the G<sub>1</sub> cyclin Cln2 and the degradation domain of mouse ornithine decarboxylase have been used to generate destabilized variants of GFP in yeast and mammalian systems (Mateus and Avery 2000; Li et al. 1998), as well as firefly luciferase (Leclerc et al. 2000). Such an approach could also be applied to the *luxA* and *luxB* genes, which have been codon-optimized for expression in eukaryotic systems (Gupta et al. 2003; Patterson et al. 2005). Furthermore, the approach taken here in which the insertion of the *luxA-tag-B* fragment into an existing construct containing the entire *P. luminescens lux* cassette should be widely applicable to a number of existing *lux*-based reporter systems, and will allow relatively simple retrofitting of those constructs to generate short half-life variants for dynamic monitoring of changes in gene expression in reporter gene transcription, in vivo imaging, and synthetic biology applications.

**Statement of competing interests** MSA and GSS have applied for patents on the materials described here.

**Author's contributions** MSA, JRW, and CSC carried out the molecular biology experiments and bioluminescence assays. MSA, GSS, and MLS conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

**Acknowledgements** We would like to thank R. Gupta and C. Cox for helpful discussions, M. Elowitz for the repressilator reporter plasmid pGFP<sub>asv</sub>, and J. Sanseverino for pCDABE. This work was supported in part by an LDRD grant from Oak Ridge National Lab and the U.S. Department of Energy and by the University of Tennessee Center for Environmental Biotechnology Research Center of Excellence.

## References

- Andersen J, Sternberg C, Poulsen L, Bjørn SP, Givskov M, Molin S (1998) New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Environ Microbiol* 64:2240–2246
- Chalfie M, Tu Y, Euskirchen G, Ward W, Prasher D (1994) Green fluorescent protein as a marker for gene expression. *Science* 263:802–805
- Cormack B, Valdivia R, Falkow S (1996) FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* 173:33–38
- DiGrazia P, King J, Blackburn J, Applegate B, Bienkowski P, Hilton B, Saylor G (1991) Dynamic response of naphthalene biodegradation in a continuous flow soil slurry reactor. *Biodegradation* 2:81–91
- Doyle TC, Burns SM, Contag CH (2004) In vivo bioluminescence imaging for integrated studies of infection. *Cell Microbiol* 6:303–317
- Elowitz M, Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* 403:335–338
- Engbrecht J, Nealson K, Silverman M (1983) Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32:773–781
- Gardner T, Cantor C, Collins J (2000) Construction of a genetic toggle switch in *Escherichia coli*. *Nature* 403:339–342
- Gottesman S, Roche E, Zhou Y, Sauer R (1998) The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. *Gene Dev* 12:1338–1347
- Gupta R, Patterson S, Ripp S, Simpson M, Saylor G (2003) Expression of the *Photobacterium luminescens lux* genes (*luxA*, *B*, *C*, *D*, and *E*) in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 4:305–313
- Hasty J, McMillen D, Collins J (2002) Engineered gene circuits. *Nature* 420:224–230
- Herman C, Thévenet D, Bouloc P, Walker G, D'Ari R (1998) Degradation of carboxy-terminal-tagged cytoplasmic proteins by the *Escherichia coli* protease HflB (FtsH). *Gene Dev* 12:1348–1355
- Kadurugamuwa JL, Sin LV, Yu J, Francis KP, Kimura R, Purchio T, Contag PR (2003) Rapid direct method for monitoring antibiotics in a mouse model of bacterial biofilm infection. *Antimicrob Agents Chemother* 47:3130–3137
- Keiler K, Waller P, Sauer R (1996) Role of peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* 271:990–993
- Kim Y-I, Burton R, Burton B, Sauer R, Baker T (2000) Dynamics of substrate denaturation and translocation by the ClpXP degradation machine. *Mol Cell* 5:639–648
- King J, DiGrazia P, Applegate B, Sanseverino J, Dunbar P, Larimer F, Saylor G (1990) Rapid and sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. *Science* 249:778–781
- Kuklin NA, Pancari GD, Tobery TW, Cope L, Jackson J, Gill C, Overbye K, Francis KP, Yu J, Montgomery D et al (2003) Real-time monitoring of bacterial infection in vivo: development of bioluminescent staphylococcal foreign-body and deep-thigh-wound mouse infection models. *Antimicrob Agents Chemother* 47:2740–2748
- Leclerc G, Bookfor F, Faught W, Frawley L (2000) Development of a destabilized firefly luciferase enzyme for measurement of gene expression. *BioTechniques* 29:590–601
- Li X, Zhao X, Fang Y, Jiang X, Duong T, Fan C, Huang C-C, Kain SR (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *J Biol Chem* 273:34970–34975
- Mateus C, Avery SV (2000) Destabilized green fluorescent protein for monitoring dynamic changes in yeast gene expression with flow cytometry. *Yeast* 16:1313–1323
- van der Meer JR, Tropel D, Jaspers M (2004) Illuminating the detection chain of bacterial bioreporters. *Environ Microbiol* 6:1005–1020

- Meighen EA (1991) Molecular biology of bacterial bioluminescence. *Microbiol Rev* 55:123–142
- Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY (1997) Fluorescent indicators for  $\text{Ca}^{2+}$  based on green fluorescent proteins and calmodulin. *Nature* 388:882–887
- Patterson S, Dionisi H, Gupta R, Sayler G (2005) Codon optimization of bacterial luciferase (*lux*) for expression in mammalian cells. *J Ind Microbiol Biotechnol* 32:115–123
- Sambrook J, Fritsch E, Maniatis T (1989) *Molecular cloning – a laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Schmidt T, Kopecky K, Nealon K (1989) Bioluminescence of the insect pathogen *Xenorhabdus luminescens*. *Appl Environ Microbiol* 55:2607–2612
- Szittner R, Meighen E (1990) Nucleotide sequence, expression, and properties of luciferase coded by *lux* genes from a terrestrial bacterium. *J Biol Chem* 265:16581–16587
- Triccas J, Pinto R, Britton W (2002) Destabilized green fluorescent protein for monitoring transient changes in mycobacterial gene expression. *Res Microbiol* 153:379–383
- Tu G, Reid G, Zhang J, Moritz R, Simpson R (1995) C-terminal extension of truncated recombinant proteins in *Escherichia coli* with a 10Sa RNA decapeptide. *J Biol Chem* 270:9322–9326
- Valkova N, Szittner R, Meighen E (1999) Control of luminescence decay and flavin binding by the LuxA carboxyl-terminal regions in chimeric bacterial luciferases. *Biochemistry* 38:13820–13828
- Van Dyk T, DeRose E, Gonye G (2001) LuxArray, a high-density, genomewide transcription analysis of *Escherichia coli* using bioluminescent reporter strains. *J Bacteriol* 183:5496–5505
- Weber-Ban E, Reid B, Miranker A, Horwich A (1999) Global unfolding of a substrate protein by the Hsp100 chaperone ClpA. *Nature* 401:90–93
- Xiong YQ, Willard J, Kadurugamuwa JL, Yu J, Francis KP, Bayer AS (2005) Real-time *in vivo* bioluminescent imaging for evaluating the efficacy of antibiotics in a rat *Staphylococcus aureus* endocarditis model. *Antimicrob Agents Chemother* 49:380–387
- Zlokarnik G, Negulescu PA, Knapp TE, Mere L, Burres N, Feng L, Whitney M, Roemer K, Tsien RY (1998) Quantitation of transcription and clonal selection of single living cells with beta-lactamase as reporter. *Science* 279:84–88