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Strategies for enhancing bioluminescent bacterial sensor performance by promoter region manipulation

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Genetically engineered microbial reporter strains are based upon the fusion of an inducible sensing element upstream of a reporting element, so that the construct emits a dose-dependent signal when exposed to the inducing compound(s) or stress factor(s). In this communication[1] we described several general approaches undertaken in order to enhance the sensing performance of such promoter::reporter fusions. Significant improvements in detection sensitivity, response kinetics and signal intensity were achieved by modi fication of the length of the promoter-containing DNA fragment, by random or site-directed mutagenesis and by promoter duplication. The general nature of these genetics manipulations makes them applicable to other types of promoter::reporter fusions.

Key words: biosensor, bioreporter, bio luminescence, environmental stress, gene expression/regulation, promoter manipulation, error-prone PCR

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Genetically engineered bioluminescent microbial reporter strains were first reported almost 20 years ago for the detection of naphthalene.[2] Since then, numerous reports have described the construction of other bacterial sensors capable of detecting either the presence of specific compounds or global stress factors, such as toxicity or genotoxicity.[3,4] These genetically engineered reporters are mostly based on the fusion of two elements: (a) a gene regulatory element, a promoter, that is induced by the target compound(s) or stress factor(s); (b) a reporter gene(s) the expression of which is quantitatively monitored, often in real time. Once the basic promoter::reporter fusion has been constructed, a variety of molecular approaches may be used to enhance its sensing performance. Most published reports focus on promoter-specific molecular manipulations of the sensing element, such as adding a second repressor binding site for background reduction,[5,6] or by altering the ribosome binding site.[7] Our aim was to investigate universal, rather then gene-specific, approaches for enhancing the engineered bacterial sensor output, to provide a stronger and faster signal and a lower detection threshold of the target compounds. We used the bioluminescence genes *luxCDABE* of *Photorhabdus luminescens* as the reporting element, and tested four independent strategies for molecular manipulation of the sensing element in the promoter::*lux* fusion. For each strategy, an example was presented that displayed its potential.

A dilemma that often accompanies the selection of the desired gene promoter serving as the sensing element concerns the optimal boundaries of the DNA fragment to be cloned. Transcription initiation requires the interaction of RNA polymerase (RNAP) with promoter DNA and the formation of an open complex, in which the duplex DNA at the transcription start-point is unwound.[8] Four different DNA sequences elements in the promoter region have previously been indentified to be responsible for RNAP recognition, all of them located upstream of the ORF of the gene: The -10 and the -35 hexamers, a TGn motif located immediately upstream of the -10 element, and the UP element, a ~20 bp sequence upstream of the -35 element. We have posed the question of whether a DNA segment harboring all of these

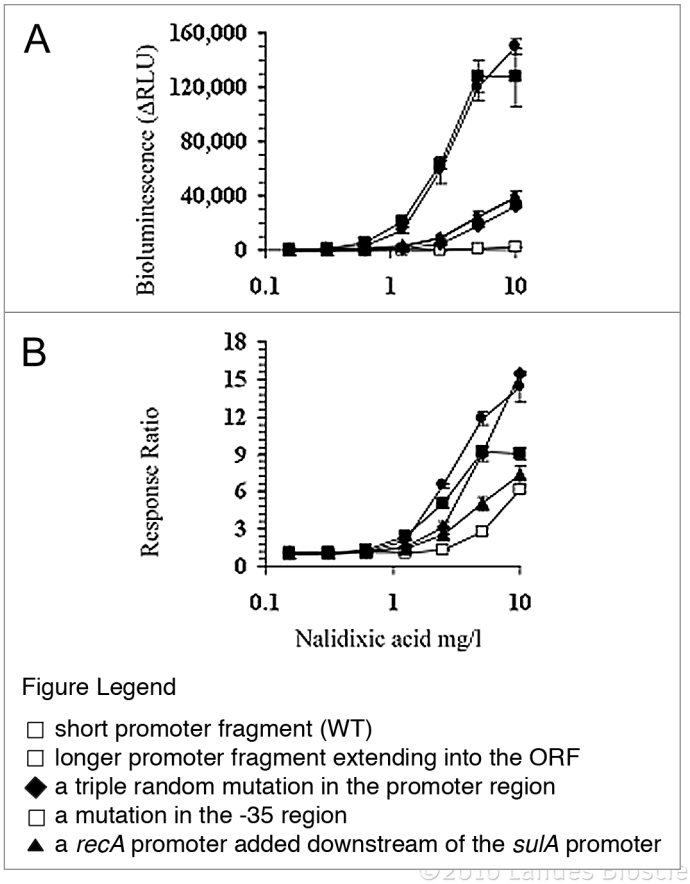

Figure 1. Bioluminescence of *E. coli* strains harboring either a wild-type *sulA* or a genetically manipulated *sulA* promoter fragment. (A) Data are presented as the difference in luminescence intensity (ΔRLU) in the presence and absence of the model inducer nalidixic acid (NA), at the designated concentration, 60 min after exposure. (B) Data are presented as the ratio of the luminescence of the induced sample to that of the non-induced control (response ratio) as a function of NA concentration after 60 min exposure.

loci is sufficient for optimal control of a reporter gene expression, or will an inclusion of an additional fragment, even containing a portion of the ORF, be beneficial. To at least partially answer this question we compared the inducibility of two promoter-harboring DNA segments, one that does not include the ORF, as constructed by Norman et al.[9] and another that extends 200 bp into the ORF of the gene, as reported by Van Dyk et al.[10] We have tested these for two gene promoters: *sulA*, that responds to SOS-inducing genotoxicants such as nalidixic acid (NA),[11,12] and *grpE* that is activated by diverse stress conditions that induce synthesis of heat shock proteins.[10,13] Comparison of the performance of the two *sulA* constructs is presented in **Figure 1A** as the difference in the intensity of the luminescence in the absence and presence of the inducer (ΔRLU), and in **Figure 1B** as the ratio of the luminescence of the induced sample to that of the non-induced control (response ratio or fold induction). Surprisingly, although no DNA sequences known to be relevant for transcription are located in the ORF of *sulA*, the longer ORF-containing fragment performed better than the short one (defined here as the wild-type): luminescence was stronger (**Fig. 1A**) and response ratios were higher (**Fig. 1B**). Furthermore, the longer fragment exhibited an enhanced detection sensitivity by responding to a three-fold lower NA concentration.[1] Interestingly, the situation was opposite for the *grpE* example; a non-ORF-containing insert displayed higher ΔRLU values upon induction by ethanol.[1] While no general conclusion can drawn from these opposite effects, it is clear that optimization of the length of the promoter-harboring insert may be a powerful tool for reporter function modulation.

Additional improvements in sensor output were obtained by the introduction of either random or site-specific mutations to the *sulA* wild-type promoter region. The most dramatic effect was obtained when the -35 element in the *sulA* promoter region was mutated to the -35 consensus sequence,[14] leading to significantly stronger bioluminescence (**Fig. 1A**), higher response ratios (**Fig. 1B**) and a lower detection threshold. In contrast to our predictions, mutating the -10 (TATA box) element in the *sulA* promoter region into the -10 consensus sequence[15] led to a complete inhibition of activity. In this context, one can appreciate the power of random mutagenesis, which introduces mutations in arbitrary locations that could not be predicted by available bioinformatics tools. Using an error-prone PCR procedure[16] we isolated a triple mutant (*sulA18*) that displayed an 22-fold stronger luminescence (**Fig. 1A**, 5 mg/l NA), a 3.2-fold higher response ratio (**Fig. 1B**, 5 mg/l NA) and an improved detection sensitivity (not shown). By separately expressing each of the three single mutations, it was shown that only one of the three base substitutions is responsible for the improved phenotype. This base substitution responsible for this effect was located neither in the RNAP recognition sites nor in the LexA-repressor binding site.

A different approach we have taken to improve *sulA* sensor performance was to add an additional SOS promoter, that of the *recA* gene, adjacent to the *sulA* promoter, thus generating a *sulA::recA::luxCDABE* fusion. As predicted, this manipulation also yielded an improved reporter strain (**Fig. 1**).

In the course of this study, we have noticed that all manipulations that improved sensor performance also led to an increase in background light emission by non-induced cells. It is thus indicated that an enhanced initiation of transcription, implying better recognition by the RNAP, allows a much stronger expression upon induction.

We believe that the general nature of the described promoter region manipulations

renders them applicable to other types of promoter::reporter fusions for whole-cell biosensor applications.

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