An artifact in studies of gene regulation using β-galactosidase reporter gene assays

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Abstract

Reporter gene assays are important tools for evaluating gene expression. A frequently used assay measures the activity of β-galactosidase (β-gal) expressed from lacZ in plasmid or genomic constructions. Such constructions are often used to interrogate the ability of DNA (query DNA), potentially encoding a transcription factor, to regulate in trans the expression of a promoter fused to the reporter lacZ. Query DNA is frequently inserted into a second plasmid within the α-subunit of β-gal, interrupting its function. However, this plasmid can induce up-expression of β-gal even when void of query DNA, leading to confusion between artifact and authentic regulation.

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Notes & Tips

The reporter gene assay is an important tool for biological and biomedical research, permitting the detection of in vivo gene expression and the identification and analysis of cis and trans regulators that affect such expression. Several commonly used assays include the use of reporter genes encoding β-galactosidase (β-gal),1 chloramphenicol acetyltransferase (CAT), alkaline phosphatase (AP), luciferases, and green fluorescent protein (GFP) (reviewed in Ref. [1]). However, artifacts have been described for the β-gal system and suggest that it can be most readily circumvented by substituting one of the other common reporter genes in the offending part of the protocol.

An artifact was discovered during experiments designed to detect regulators of gene expression. The artifact was first detected in a screen for potential regulators of a candidate promoter for fur (ferric uptake regulator) from the extreme acidophile Acidithiobacillus ferrooxidans. The experimental setup used in the screen is shown in Fig. 1. Briefly, the candidate promoter for fur (Pfurα) was cloned upstream of lacZ encoding β-gal, and the resulting construction was inserted into the chromosome of a β-gal negative strain of Escherichia coli H2331 (ΔlacU169) [4]. The modified strain, termed E. coli H2331 Pfurα::lacZ, weakly produced β-gal as determined by colony color (white vs. blue) on agar plates containing X-gal [5] and by the Miller assay (70 Miller units) [6], suggesting that Pfurα exhibited weak transcriptional activity. Subsequently, E. coli H2331 Pfurα::lacZ was transformed with pUC18 containing a library of A. ferrooxidans genomic DNA cloned into the BamHI restriction enzyme site within the multiple cloning site (MCS) of pUC18. The library was constructed by partial digest of A. ferrooxidans DNA with Sau3AI, followed by size selection (~1–4 kb) by Q2 agarose gel electrophoresis [7]. The objective was to detect colony transformers that potentially encoded up- or down-regulators of Pfurα by evaluating β-gal production. Colonies with up-regulated expression of β-gal were detected, but on isolation and examination approximately 50% (14 of 27) were found to be recircularized restriction enzyme sites (3–24) that do not inactivate α-peptide function. To explain this up-regulation, it is hypothesized that the α-peptide of β-gal encoded by pUC18 is able to increase the activity of the wild-type β-gal expressed from the intact chromosomal copy of lacZ of E. coli H2331 Pfurα::lacZ. To test this hypothesis, several different plasmids encoding the α-peptide of β-gal were transformed into the host E. coli BL21(DE3) that contains lacZ expressed from its native promoter (Plac) [8]. The plasmids tested were pUC18 [9], pGEM-T (Promega), and pMCL200 [10]. Each transformant gave increased β-gal enzymatic activity even when void of query DNA, leading to confusion between artifact and authentic regulation.

Abbreviations used:
β-gal, β-galactosidase; CAT, chloramphenicol acetyltransferase; AP, alkaline phosphatase; fur, ferric uptake regulator; MCS, multiple cloning site; RT-PCR, reverse transcription polymerase chain reaction.

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activity compared with the untransformed host (Fig. 2). To evaluate whether increased β-gal activity required an intact α-peptide, the corresponding encoding region was interrupted by the introduction of a foreign DNA fragment to give rise to recombinant plasmid PUC18-X. This construction did not induce an increase in β-gal activity in E. coli BL21(DE3), showing that an intact α-peptide was required (Fig. 2). Finally, E. coli BL21(DE3) was transformed with the plasmid PET15B (Novagen) that does not encode the α-peptide of β-gal, and no increase in β-gal activity was detected (Fig. 2), consistent with the idea that the α-peptide is responsible.

A necessary component of this hypothesis is the need for the expression of the α-peptide from each of the plasmids under evaluation. To test this requirement, plasmids PUC18, pGEM-T, and pMCL200 were transformed into E. coli JM109 (containing alpha acceptor of β-gal to analyze alpha complementation). Each transformant expressed functional β-gal, indicating that the respective plasmid encoded α-peptide.

An alternate hypothesis is that PUC18, pGEM-T, and pMCL200 are able to up-regulate the expression of lacZ at the transcriptional level. To test this hypothesis, semiquantitative reverse transcription polymerase chain reaction (RT-PCR) experiments were performed as described previously [11] using template RNA prepared from E. coli BL21(DE3) either with or without these plasmids. Results show that the expression of lacZ RNA is the same in all cases, demonstrating that these plasmids do not up-regulate transcriptional expression of lacZ (Fig. 2B). Additional evidence for this conjecture comes from experiments with two different strains in which the native promoter of lacZ was replaced by other promoters such as Fiu, a promoter for a gene encoding an outer membrane ferric iron uptake protein [12], and Phuf, a promoter for a gene encoding a ferric reductase [12]. Increased β-gal activity was observed in both cases following transformation with pUC18 (data not shown), indicating that increased β-gal activity is promoter independent.

All results are consistent with the hypothesis that the α-peptide of β-gal is able to increase the activity of wild-type β-gal. It is not known how increased activity is accomplished, but we speculate that the presence of excess α-peptide may help to stabilize β-gal, which is a tetramer of identical subunits [13]. This phenomenon is most likely different from alpha complementation, where α-peptides reconstitute the dimer–dimer interface to recover active tetramers [14]. The β-gal activity in this case is lower than that obtained in the artifact. Alternately, the α-peptide may serve as a decoy for proteases, or other cellular mechanisms that might inactivate β-gal, helping to extend the life of β-gal.

The artifact reported here can be diagnosed at the end of the experiment by sequencing candidate plasmids. However, the frequency of occurrence of the artifact reduces the efficiency of detecting bona fide transcription factors and increases DNA sequencing costs. These problems can be avoided by choosing plasmids or other vectors that do not encode the α-peptide of β-gal as vehicles for the query DNA. The suggested artifact might have been the cause of the observation that a plasmid construction in which DNA had been inserted at a site that did not disrupt the α-peptide could subsequently give rise to an increase in β-gal activity when it was used in experiments with E. coli strains carrying intact lacZ [15].

In conclusion, based on our data, an artifact can arise in gene regulation assays in which an empty plasmid encoding the α-peptide of β-gal, but void of query DNA, can induce up-expression.
of β-gal from a reporter gene, leading to confusion between artifact and authentic regulation from query DNA. It is suggested that this problem can be circumvented by using reporter plasmids or other vectors that do not encode the α-peptide of β-gal for interrogating regulatory activity of query DNA.

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