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# A cryptic ribosome binding site, false signals in reporter systems and avoidance of protein translation chaos

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ABSTRACT

The expression of reporter gene may be induced by activation of cryptic signalling sequences, as we found while constructing the *mutS-lacZ* fusion gene. We cloned the *Escherichia coli lacZ* gene encoding β-galactosidase into a plasmid vector carrying the *Thermus thermophilus mutS* gene. The clones expected to produce β-galactosidase as the C-terminal fusion were selected for the complementation of β-galactosidase activity in a *lacZ* deficient *E. coli* strain. Surprisingly, one of the clones, though displaying β-galactosidase activity, did not produce the fusion protein. As shown by DNA sequencing a 92 bp fragment in the 3′ part of *mutS* gene was substituted by a 19 bp sequence. As the consequence of the resultant frameshift, a truncated MutS peptide was translated instead of β-galactosidase fusion. The cloned *lacZ* gene lacked its ribosome binding site, so *lacZ* expression could be explained by activation of a cryptic ribosome binding site in the 3′ end of *mutS* gene. This observation shows that fusion domains in reporter systems are possible to produce accidentally misleading signals. This observation also suggests that some triplets like AGG and AGA, present in the canonical ribosome binding sequence, are rarely used codons to prevent chaotic protein translation.



## 1. Introduction

DNA cloning techniques allow efficient construction of fusion genes, thus enabling the production of chimeric proteins, also known as fusion proteins. The fusion domains may originate from different organisms and also could be artificial constructs. Spontaneous formation of fusion proteins, the result of chromosomal translocations were shown to cause leukaemia (Alcalay et al., 2003).

The fusions with reporter domains, which facilitate imaging/tracking/detection of target proteins, including various variants of fluorescent proteins, derived from *Aquourea victoria* (Chudakov et al., 2005) and *Escherichia coli* β-galactosidase (Tung et al., 2004) became invaluable research tools. β-Galactosidase as a fusion domain is also applied in mutagen testing (Quillardet et al., 1982). *E. coli* β-galactosidase fusions to the N-terminus of target protein are reported to be unstable due to the activation of proteolytic cleavage at the β-galactosidase C-terminus (Viaplana et al., 1997; Corchero and Villaverde, 1999). In the C-terminal fusions, the β-galactosidase reporter domain is expected to confirm the expression of N-terminal target protein.

We engineered a fusion gene consisting of *mutS* and *lacZ* genes so as to produce a chimeric protein including the N-terminal MutS domain and the C-terminal β-galactosidase domain. Despite the reporter signal of β-galactosidase, the fusion MutS-β-galactosidase protein was not expressed. We present a single case of accidental reporter activation but such misleading signals are possible to occur in other reporter systems. We showed that the unexpected expression of the reporter domain resulted from activation of a cryptic ribosome binding site. This observation suggested us that nucleotide triplets which may form a ribosome binding sequence should be avoided as codons, thus preventing from the translation of incomplete and nonsense peptides.

## 2. Materials and methods

The MutS-β-galactosidase fusion was obtained by in-frame cloning of the *E. coli lacZ* gene into a plasmid vector carrying the *Thermus thermophilus mutS* gene (Sachadyn et al., 2007). The clones of interest were identified using blue/white selection on LB agar plates containing X-Gal (10 g/l Bacto-Tryptone, 5 g/l Bacto-Yeast extract, 10 g/l NaCl, pH 7; agar 1.5% ampicillin 0.1 mg/ml, IPTG 80 μg/ml, X-Gal 32 μg/ml). The *lacZ* deficient *E. coli* ER2566 (New England BioLabs) and *E. coli* cells (RosettaBlue, Novagen) cells transformed with the plasmid carrying

*Abbreviations:* IPTG, isopropyl-beta-D-thiogalactopyranoside; rbs, ribosome binding site; X-Gal, 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside.

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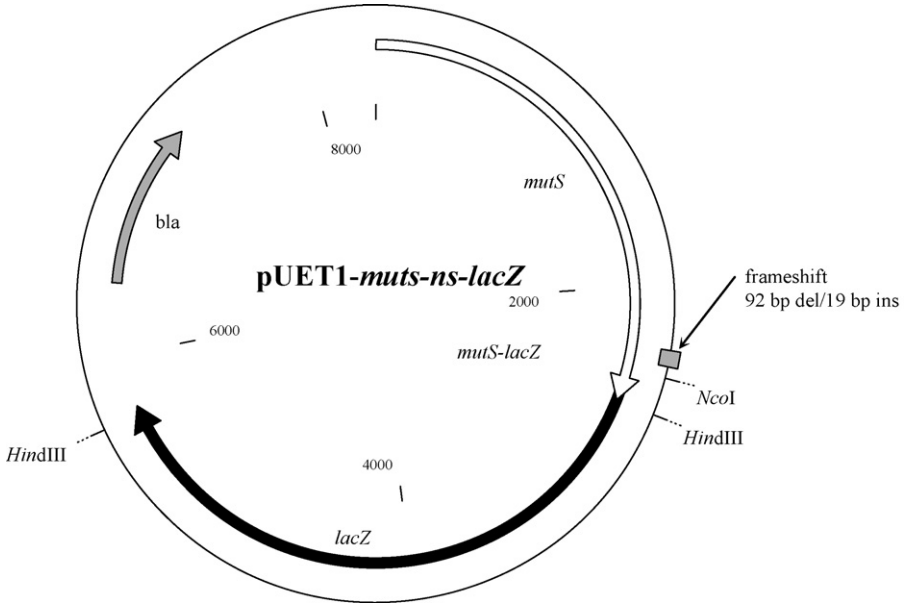


**Fig. 1.** The construction of *mutS-lacZ* gene. The fusion *mutS-lacZ* gene was constructed by in-frame cloning the *lacZ* gene into the plasmid carrying the *T. thermophilus mutS* gene. One of the obtained β-galactosidase positive clones has a spontaneous frameshift mutation: a deletion of 92 bp and insertion of 19 bp in the 3′ end of *mutS* gene. As the result, two separate ORFs instead of a single fusion ORF were formed.

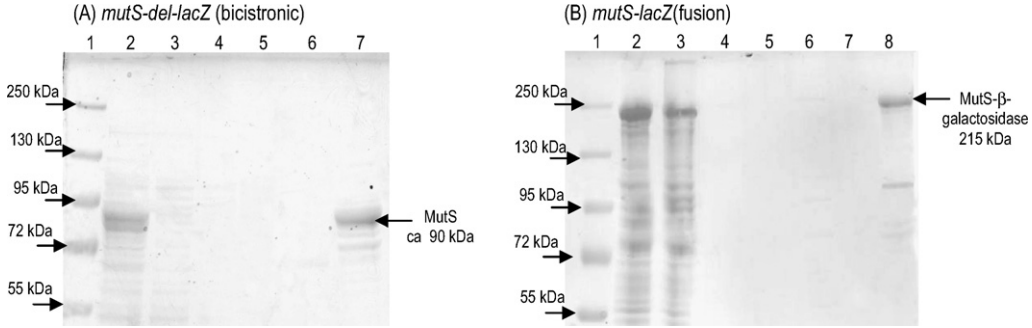


**Fig. 2.** The expression of fusion *mutS-lacZ* and bicistronic *mutS-lacZ* genes in β-galactosidase deficient *E. coli* strain and purification of his-tagged proteins by metal-chelate affinity. SDS-PAGE electrophoresis in 6% polyacrylamide gels stained with Coomassie Brilliant Blue. (A) *mutSdel-lacZ*—bicistronic clone. (1) Protein MW marker (Fermentas SM1811: 250, 130, 95, 72, 55 kDa); (2) cell free extract from *E. coli* expressing *mutS-lacZ* bicistronic gene. The determined β-galactosidase activity was 1.5 units/mg of total protein; (3–6) washing fractions; (7) his-tagged MutS peptide truncated as the result of frame-shift mutation purified by metal-chelate affinity chromatography. (B) *mutS-lacZ*—fusion clone. (1) Protein MW marker (Fermentas SM1811: 250, 130, 95, 72, 55 kDa); (2) cell free extract from *E. coli* over-expressing MutS-β-galactosidase fusion protein. The determined β-galactosidase activity was 10.2 units/mg of total protein; (3–7) washing fractions; (8) his-tagged MutS-β-galactosidase purified by metal-chelate affinity chromatography.

the fusion *mutS-lacZ* gene acquired β-galactosidase activity. The nucleotide sequences of the cloned genes were confirmed by DNA sequencing using PerkinElmer ABI Prism 377 automatic sequencing system. The potential translation initiation sites were predicted using Glimmer (Delcher et al., 1999) (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/glimmer_3.cgi).

Proteins were expressed in β-galactosidase deficient *E. coli* cells (RosettaBlue, Novagen), transformed with the plasmids carrying the *mutS-lacZ* fusion genes. The cells were cultured in the flasks containing 500 ml LB broth (10 g/l Bacto-Tryptone, 5 g/l Bacto-Yeast extract, 10 g/l NaCl, pH 7) supplemented with 0.1 mg/ml ampicillin and 0.034 mg/ml chloramphenicol, at 30 °C. Upon reaching an OD (600 nm) of 0.5 the cell culture was induced with IPTG added to the final concentration of 1 mM, then grown for an additional 6 h and harvested by centrifugation. The bacterial pellets were suspended in buffer M (100 mM Tris–Cl; pH 7.5; 100 mM NaCl; 0.1% Triton-X-100; 1 mM DTT). The cells were disrupted by sonication on ice, and then the total lysate was centrifuged at 16,000 × *g* for 20 min.

The his-tagged MutS and MutS-β-galactosidase were purified by metal-chelate affinity chromatography on a $Ni^{2+}$-IDA agarose resin (his·Bind Resin, Novagen) according to the manufacturer's protocol.

β-Galactosidase activity of cell free extracts was determined by hydrolysis of orthonitrophenyl-β-D-galactopyranoside (ONPG) at 28 °C for 10 min in reaction buffer (100 mM sodium phosphate, pH 7.0; 10 mM KCl, 1 mM $MgSO_4$; 50 mM β-mercaptoethanol) supplemented with 4 mg/ml ONPG. The reaction was stopped by adding $Na_2CO_3$ to 0.3 M concentration. The released o-nitrophenol (ONP) was measured spectrophotometrically at 420 nm. The calibration curve for ONP was constructed in the concentration range of 0.03–0.6 mM. One enzyme unit is defined as the quantity of enzyme that catalyzes the release of 1 μmol of ONP/min. Total protein concentrations were determined according to the procedure of Bradford.

## 3. Results and discussion

We previously reported the construction of a plasmid vector carrying *mutS-lacZ*, a fusion gene designed to obtain a bifunctional chimeric protein consisting of MutS (mismatch binding protein) and β-galactosidase (Sachadyn et al., 2007). The fusion gene was