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The Luc2 gene enhances reliability of bicistronic assays

Communication

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Abstract: Luciferases are prominent reporters in molecular and cellular biology investigations including miRNA target studies and the determination of Internal Ribosome Entry Site (IRES) activities in bicistronic assays. A majority of the current bicistronic vectors contain a firefly luciferase reporter as the second cistron. One reason for this is the presence of cryptic transcription start sites inside the luciferase gene. We present here an experimental evaluation of the cryptic transcription within the latest version of the firefly luciferase gene, *luc2*. Using flow cytometric analysis, we observed a negligible amount of cryptic transcriptional activity that was only slightly above the background of untransfected cells. Nevertheless, quantitative reverse transcription PCR experiments revealed a six-to-nine-fold gradual increase of transcription along the coding region of the gene. The level of cryptic transcription from the coding region of the improved *luc2* firefly luciferase gene is significantly lower when compared to the *luc+* gene. In summary, the *luc2* better fulfills the requirements of bicistronic assays than the previous *luc+* version. The observed low cryptic transcription activity in *luc2* could be limiting only in cases where weak IRESs are studied.

Keywords: *Luciferase • Cryptic transcription • Reporter gene • Bicistronic vector • IRES*

Abbreviations:

Ct	- threshold cycle;
IRES	- Internal ribosome entry site;
NMD	- nonsense-mediated decay;
qRT-PCR	- quantitative reverse transcription PCR;
sORF	- short ORF.

1. Introduction

Firefly luciferase is the prime reporter for assaying various aspects of cell and molecular biology. It has been used in thousands of experiments since its discovery [1] due to its sensitivity, versatility and reasonable cost (compared with more recently introduced luciferases that utilize coelenterazine as a substrate). Typical research areas where firefly luciferase reporters are advantageous and often used are those focused on miRNA/siRNA, translation initiation, mRNA polyadenylation, nonsense-mediated mRNA decay (NMD), 3'UTR-mediated control of gene expression and mRNA stability, and promoter strength. The most popular firefly luciferase gene, the *luc+* variant, was introduced into a pGL3 vector series (Promega Corporation). Among other improvements over its predecessor, Promega's variant inactivated a peroxisomal targeting signal, optimized expression, and broadened the applicability of the reporter by removing common restriction sites [2].

We reported, and characterized in detail, a significant cryptic promoter activity in the *luc+* coding region that is detectable in mammalian as well as yeast cells [3]. More importantly, cryptic transcription from the *luc+* gene was only ten to sixteen times weaker than the strong, immediate-early cytomegalovirus promoter expression in human CCL13 and Huh7 cells.

The latest version of firefly luciferase released by Promega, *luc2*, is claimed by the manufacturer to display a 4.1 to 11.8-fold increased sensitivity in mammalian cells when compared to *luc+* [4]. According

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