African Journal of Biotechnology Vol. 7 (13), pp. 2124-2127, 4 July, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.152
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Evaluation of a novel luciferase reporter construct: a positive control plasmid for reporter gene assay

Tewin Tencomnao[1]*, Varaporn Rakkhitawatthana[2] and Kanya Sukhontasing[2]

[1]Center for Excellence in Omics-Nano Medical Technology Project, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
[2]Undergraduate Program in Medical Technology, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.

Accepted 2 May, 2008

Reporter gene technology has been increasingly important in the post-genomic era to explain human complexity and diversity. The pGL3-Basic vector has been prevalently used as a tool for analyzing *cis*-acting elements critical for transcriptional mechanisms. In this work, we constructed and evaluated the pGL3-Basic plasmid containing the cytomegalovirus (CMV) enhancer/promoter aiming to establish a positive control of pGL3-Basic vector. Using a human melanoma cell line UACC-903 for transient transfection, the novel luciferase reporter construct, pGL3-CMV, showed an extremely high transcriptional activity approximately 4,260-fold greater than that of pGL3-Basic, indicating its qualification as a positive control for luciferase reporter gene assays.

Key words: Reporter gene plasmid, luciferase assay, cytomegalovirus promoter/enhancer, human melanoma cell line.

INTRODUCTION

Reporter genes, often called reporters, have become a precious tool in studies of gene expression (Alam and Cook, 1990). They are commonly employed in biomedical and scientific research since such techniques are crucial in defining the molecular events associated with gene transcription, which has implications for our understanding of the molecular basis of disorder and will influence our approach to pharmaceutical biotechnology (Naylor, 1999). In two functional parts of a gene, one is a DNA sequence for coding region giving the information about the protein produced and another is a regulatory DNA motif linked to the coding region (Boulin et al., 2006). The purpose of the reporter gene assay is to evaluate the regulatory potential of an unknown DNA sequence or gene of interest. This can be accomplished by connecting a target DNA fragment such as promoter sequence to a readily detectable reporter gene such as luciferase, green fluorescent protein (GFP), β-galactosidase, β-glucuronidase and chloramphenicol acetyltransferase (CAT). Human reporter genes have also attracted much attention in recent years for their potential clinical applications (Serganova et al., 2007). The number of genes revealed from the human genome project is not responsible for the enormous complexity of the human species. In part, the greater complexity of the human species occurs because during the process of decoding genes into proteins, human genes more than the genes of other species are transcriptionally regulated to create a greater variety of proteins. Therefore, the reporter gene technology will be growingly vital in the post-genomic era.

In analysis for transcriptional regulation, firefly luciferase has become increasingly popular due to its unique advantages such as high sensitivity, absence for intrinsic luciferase activity for most of the cell types, wide dynamic range and functionally active luciferase activity without requirement for post-translational modification (Gould and Subramani, 1988; Bronstein et al., 1994; Sherf and Wood, 1994; Naylor, 1999). According to our experience, we successfully performed reporter gene assays by transient transfection of luciferase reporter constructs, promoterless vector with or without our gene of interest,

*Corresponding author. E-mail: tewin.t@chula.ac.th. Tel: (662) 218-1081 ext. 313. Fax: (662) 218-1082.

Abbreviations: bp, base pair; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; FBS, fetal bovine serum; GFP, green fluorescent protein; PCR, polymerase chain reaction; RPMI, Rosewell Park Memorial Institute; and SV40, simian virus 40.

into mammalian cells (Tencomnao et al., 2001, 2004; Prasansuklab, 2006). Although a second reporter gene was utilized as an internal experimental standard to normalize for the differences in transfection efficiencies and other experimental variations, it would be of great advantage if a luciferase reporter construct linked to a strong promoter as a positive control reporter plasmid was readily accessible in our laboratory. In this report, we therefore generated the new reporter construct containing a strong promoter/enhancer and evaluated its capability to function as a positive control.

MATERIALS AND METHODS

Making a reporter construct containing CMV regulatory elements

The pRL-CMV vector and pGL3-Basic vector (generous gifts from Dr. Robert K. Yu, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Georgia's Health Sciences University, USA) were used in this study. A 1,075-base pairs (bp) polymerase chain reaction (PCR) fragment containing the CMV enhancer and early promoter elements was generated using PRL-CMV vector as a template with specific primers encompassing sequence reference points between 2 and 1,058 of the vector, and the two primers designed with a *Kpn*I-containing tail were forward primer (5'-AAT GGT ACC GAT CTT CAA TAT TGG CCA TTA GCC-3') and reverse primer (5'-AAT GGT ACC CCT ATA GTG AGT CGT ATT AAG TAC TC-3'). PCR amplification was performed following a procedure described elsewhere (Tencomnao et al., 2001, 2004; Prasansuklab, 2006). The PCR product of expected size was purified and cloned into pGEM-T Easy vector (Promega, Madison, WI) to produce pGEM-CMV plasmid. The target DNA fragment was digested from pGEM-CMV using *Kpn*I, purified and subsequently ligated into the *Kpn*I site upstream from the luciferase coding region in the pGL3-Basic, the promoterless luciferase expression vector. The integrity and orientation of the new reporter construct referred to as pGL3-CMV plasmid were verified by restriction digestion.

Cell culture

The human melanoma cell line UACC-903 was kindly provided by Dr. Nattiya Hirankarn (Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand). The cells were cultured in Rosewell Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal bovine serum (FBS), and they were maintained at 37°C in a humidified atmosphere at 5% CO_2.

Transient transfection and luciferase reporter gene assay

Transient transfections were performed using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) following a procedure described by the manufacturer with minor modifications. Briefly, cultured UACC-903 cells at about 90% confluence in a ninety six-well plate were co-transfected with 0.2 µg of either pGL3-Basic vector or pGL3-CMV reporter construct and 0.02 µg of the pRL-CMV vector which expresses *Renilla* luciferase under the control of the CMV promoter, to monitor the transfection efficiency. The cultured medium was changed in the following day, and cells were analyzed for the firefly and *Renilla* luciferase activities after an additional 24 h in culture using Dual-Glo™ luciferase assay system (Promega, Madison, WI) following a procedure given by the manufacturer. Chemiluminescence was read using a VICTOR^3 luminometer (PerkinElmer, Waltham, MA). In each transfection experiment, untransfected cells were included as controls for a background level of firefly and *Renilla* luciferase.

Statistical analysis

The firefly luciferase activities were normalized to *Renilla* luciferase activities. Triplicate transfections were performed in each experiment. Luciferase levels were reported as fold elevation in activity over that seen in transfections with the promoterless pGL3-Basic vector. The data represented the mean ± S.D. of three independent experiments and were analyzed by the Student's *t*-test. Differences at $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

As illustrated in Figure 1, transient transfections of pGL3-CMV reporter construct into the UACC-903 cell line demonstrated that this newly produced plasmid, which contained the CMV enhancer and early promoter elements, possessed an extremely high transcriptional potency, which was about 4,260-fold higher than that of the parent pGL3-Basic. The difference in transcriptional potential between the two plasmids was regarded as statistically significant ($P < 0.05$). This result indicated that the pGL3-CMV reporter construct might be utilized as a positive control for reporter gene assay.

Although pGL3-Control luciferase vector, which contains simian virus 40 (SV40) promoter and enhancer sequences, has been commercially available as a positive control plasmid for luciferase reporter gene assays, a novel positive control plasmid with higher transcriptional capability and more versatility should be established. CMV positive regulatory elements were chosen in this work because the promiscuous nature of the CMV enhancer/promoter has been demonstrated in transgenic mice, where its transcriptional activity was found in 24 of the 28 murine tissues tested (Schmidt et al., 1990). In fact, the strength of different promoters, both in cell culture and *in vivo*, has been compared in numerous studies. The human CMV promoter has been shown to be at least one order of magnitude stronger than the SV40 promoter and others (Doll et al., 1996). The CMV enhancer, which has little cell-type or species preference, is several-fold more active than the SV40 enhancer (Boshart et al., 1985). Using either CAT or luciferase as a reporter gene, the CMV regulatory elements were found to have greater transcriptional activity than any of the other viral positive regulatory elements examined (Lee et al., 1997; Tucker et al., 2000; Xu et al., 2001), suggesting their potential applications in DNA vaccine development (Garmory et al., 2003). In particular, despite taking four major different experimental approaches, our current study is in agreement with the previous report with the use of luciferase reporter gene (Xu et al., 2001). Firstly, the Xu group utilized the firefly luciferase gene derived from pGL3-Control, while deriving from pGL3-Basic in our study. Secondly, the CMV promoter and enhancer ele-

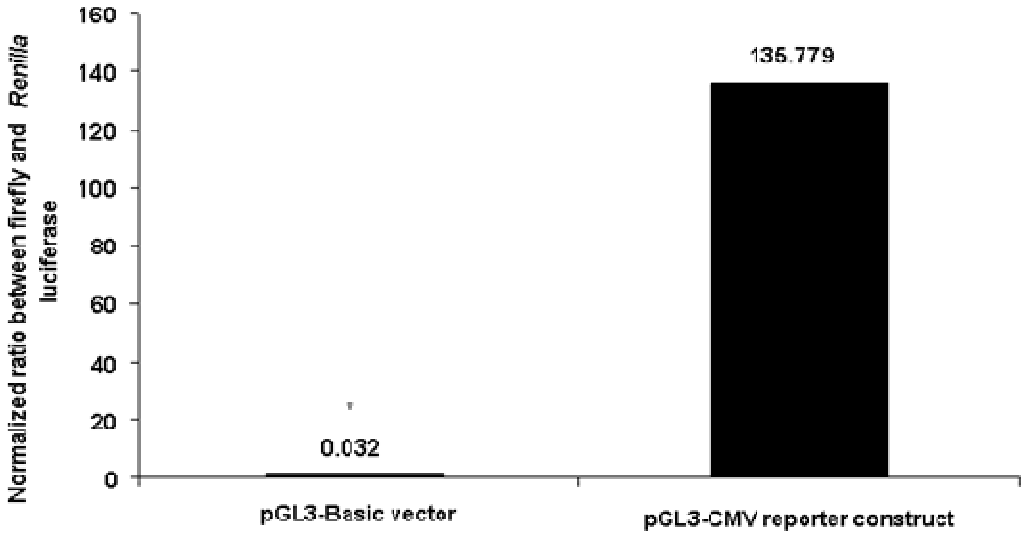

Figure 1. Transcriptional activity of the luciferase reporter gene plasmids, pGL3-Basic and pGL3-CMV, in UACC-903 cells. Data are mean ± S.D. for triplicate determinations, * $P < 0.05$ by student's *t*-test.

ments were derived from pHMCMV6 in the study of Xu and colleagues, while deriving from pRL-CMV in our work. Thirdly, concerning the reagent for transfection, Lipofectamine™ 2000 was used for our experiments, whereas SuperFect for the Xu group. Fourthly, UACC-903 (human melanoma cell line) was employed as a cell culture model in our report, but HeLa cells (human epitheloid carcinoma of the cervix), HepG2 cells (human hepatoma) and ECV304 cells (endothelial cell line derived from human umbilical cord vein) were employed by the Xu group.

Based on the physiological expression of the distinct promoters, the CMV promoter has been demonstrated to drive gene expression in all cell types tested and more skin layers, while other promoters studied are more restricted (Lin et al., 2001). The UACC-903 cell line derived from human skin was chosen as an *in vitro* cell culture model because its availability during the time of study. In terms of versatility for reporter gene assays, the CMV promoter has been shown to exhibit high activity in both attached and suspended cells, but the SV40 promoter is decreased in the latter ones (Feng et al., 2003).

Therefore, we have established the novel positive control plasmid with greater transcriptional activity and additional versatility for luciferase reporter gene assays.

ACKNOWLEDGEMENTS

This work was financially supported by a research grant from the Asahi Glass Foundation and a research grant for development of Chulalongkorn University's new faculty staff, whereas KS received a research project for undergraduate student (RPUS) scholarship from the Thailand Research Fund. The authors gratefully acknowledge the generous gift of the UACC-903 cell line from Dr. Nattiya Hirankarn, and plasmid vectors from Dr. Robert K. Yu. We thank Anchalee Prasansuklab and Ingorn Kimkong for their technical assistance.

REFERENCES

Alam J, Cook JL (1990). Reporter genes: Application to the study of mammalian gene transcription. Anal. Biochem. 188: 245-254.

Boshart M, Weber F, Jahn G, Dorsch Häsler K, Fleckenstein B, Schaffner W (1985). A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41: 521-530.

Boulin T, Etchberger JF, Hobert O (2006). Reporter gene fusions. In WormBook (ed) The *C. elegans* Research Community, pp 1-23, www.wormbook.org.

Bronstein I, Fortin J, Stanley PE, Stewart GS, Kricka LJ (1994). Chemiluminescent and bioluminescent reporter gene assays. Anal. Biochem. 219: 169-181.

Doll RF, Crandal JE, Dyaer CA, Aucoin JM, Smith FI (1996). Comparison of promoter strength on gene delivery into mammalian brain cells using AAV vectors. Gene Ther. 3: 437-447.

Feng G, Hicks P, Chang PL (2003). Differential expression of mammalian or viral promoter-driven gene in adherent versus suspension cells. In Vitro Cell Dev. Biol. Anim., 39: 420-423.

Garmory HS, Brown KA, Titball RW (2003). DNA vaccines: improving expression of antigens. Genet. Vaccines Ther. 1: 2.

Gould SJ, Subramani S (1988). Firefly luciferase as a tool in molecular and cell biology. Anal. Biochem. 175: 5-13.

Lee AH, Suh YS, Sung JH, Yang SH, Sung YC (1997). Comparison of various expression plasmids for the induction of immune response by DNA immunization. Mol. Cells 7: 495-501.

Lin MT, Wang F, Uitto J, Yoon K (2001). Differential expression of tissue-specific promoters by gene gun. Br. J. Dermatol. 144: 34-39.

Naylor LH (1999). Reporter gene technology: the future looks bright. Biochem. Pharmacol. 58: 749-757.

Prasansuklab A (2006). Role of genetic polymorphism in regulating the expression of serotonin transporter. MSc thesis, Chulalongkorn University, Bangkok, Thailand.

Schmidt EV, Christoph G, Zeller R, Leder P (1990). The cytomegalovirus enhancer: A pan-active control element in transgenic mice. Mol. Cell. Biol. 10: 4406-11.
Serganova I, Ponomarev V, Blasberg R (2007). Human reporter genes: potential use in clinical studies. Nucl. Med. Biol. 34: 791-807.
Sherf BA, Wood KV (1994). Firefly Luciferase Engineered for Improved Genetic Reporting. Promega Notes Magazine 49: 14.
Tencomnao T, Yu RK, Kapitonov D (2001). Characterization of the human UDP-galactose:ceramide galactosyltransferase gene promoter. Biochim. Biophys. Acta. 1517: 416-23.
Tencomnao T, Kapitonov D, Bieberich E, Yu RK (2004). Transcriptional regulation of the human UDP-galactose:ceramide galactosyltransferase (hCGT) gene expression: functional role of GC-box and CRE. Glycoconj. J. 20: 339-51.
Tucker C, Endo M, Hirono I, Aoki T (2000). Assessment of DNA vaccine potential for juvenile Japanese flounder Paralichthys olivaceus, through the introduction of reporter genes by particle bombardment and histopathology. Vaccine 19: 801-9.
Xu ZL, Mizuguchi H, Ishii-Watabe A, Uchida E, Mayumi T, Hayakawa T (2001). Optimization of transcriptional regulatory elements for constructing plasmid vectors. Gene 272: 149-56.