

## การทำงานของโปรโมเตอร์จากไวรัสในเซลล์ Sf9 และเซลล์ปฐมภูมิของกุ้ง

### Activity of viral promoters in Sf9 and primary shrimp cells

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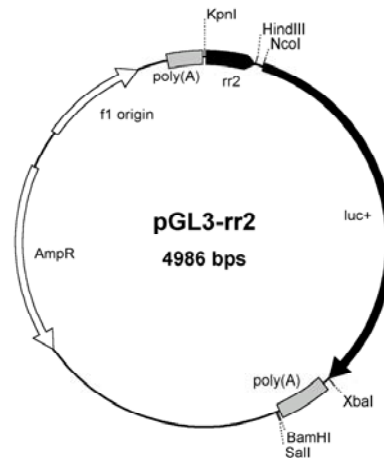
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**บทคัดย่อ:** ปัจจุบันกุ้งเป็นสัตว์เศรษฐกิจที่สำคัญในหลายประเทศรวมทั้งประเทศไทย อย่างไรก็ตาม การศึกษาเกี่ยวกับหน้าที่และการแสดงออกของยีน โดยเฉพาะอย่างยิ่งเกี่ยวกับโปรโมเตอร์ที่ทำงานได้ในเซลล์กุ้ง ยังมีอยู่จำกัด การทดลองในครั้งนี้ เพื่อศึกษาถึงการทำงานของโปรโมเตอร์จากไวรัสต่างชนิดในเซลล์กุ้ง ซึ่งโปรโมเตอร์ที่ใช้ ได้แก่ โปรโมเตอร์ p1 และ p50 จาก hepatopancreatic parvovirus (HPV), โปรโมเตอร์ rr1 และ rr2 จาก white spot syndrome virus (WSSV) รวมทั้งโปรโมเตอร์ CMV จาก human cytomegalovirus โดยมี luciferase เป็นยีนรายงานผล แต่เนื่องจากปัจจุบันยังไม่สามารถผลิต cell line ของกุ้งได้ อีกทั้งข้อจำกัดในการเลี้ยงเซลล์ปฐมภูมิของกุ้ง โปรโมเตอร์เหล่านี้จึงถูกนำไปศึกษาในเซลล์แมลง Sf9 เพื่อเป็นการศึกษาเบื้องต้นเกี่ยวกับการทำงานของโปรโมเตอร์ก่อนที่จะนำไปทดลองในเซลล์กุ้ง ผลการทดลองซึ่งวัดจากการแสดงออกของยีนรายงานผล พบว่า ทุกโปรโมเตอร์สามารถก่อให้เกิดการแสดงออกของยีนรายงานผลได้ ยกเว้น p50 โดยที่ rr2 มีระดับการทำงานสูงสุดเมื่อเปรียบเทียบกับโปรโมเตอร์อื่น ๆ อย่างไรก็ตาม โปรโมเตอร์เหล่านี้จะถูกนำไปศึกษาการทำงานในเซลล์กุ้งต่อไป

**Abstract:** The shrimp aquaculture is the economically important industry in several countries including Thailand. However, there are few reports about gene expression and gene function in shrimp, especially for the functional promoter in shrimp cells. This study is aimed to characterize the promoter activity from different viruses in shrimp cells. Putative p1 and p50 promoters controlling expression of non-structural and structural proteins, respectively, from hepatopancreatic parvovirus (HPV), putative promoters of ribonucleotide reductase large subunit (rr1) and small subunit (rr2) from white spot syndrome virus (WSSV), as well as human cytomegalovirus promoter (CMV) were tested. Firefly luciferase was used as reporter gene. Because of the unavailability of shrimp cell line and the difficulty to culture primary shrimp cells, functions of these promoters were investigated in Sf9 insect cells in order to provide the preliminary information of promoter activities. After transfection for 48 hours, the luciferase expression could be detected from all promoters, except for putative p50 promoter, in which rr2 promoter showed the highest activity. However, these promoters will be further characterized their activities in primary shrimp cells.

**Methodology:** Putative p1 and p50 promoters from hepatopancreatic parvovirus (HPV), putative rr1 and rr2 promoters from white spot syndrome virus (WSSV), as well as CMV promoter from human cytomegalovirus were tested. The promoter regions were amplified by polymerase chain reaction (PCR) using specific primer pairs and the restriction sites of *HindIII* and *KpnI* were introduced into 5' and 3' ends, respectively. The amplified fragments

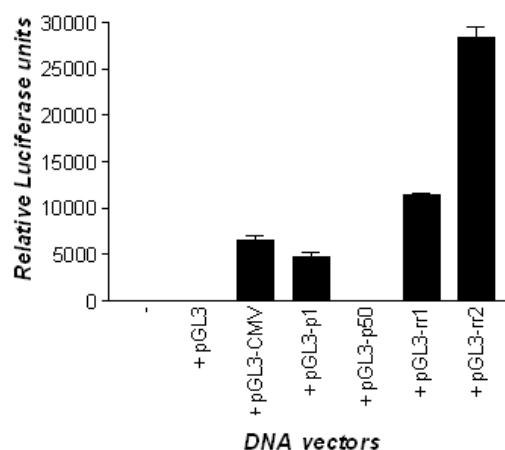
were then cloned into pGL3-Basic vector (Promega) containing firefly luciferase as reporter gene at *Hind*III and *Kpn*I sites to construct the expression cassettes. The example of DNA vector, pGL3-rr2, after plasmid construction was shown in Fig1. The recombinant plasmids were confirmed by restriction enzyme analysis and DNA sequencing.



**Fig 1.** The pGL3-rr2 vector. The amplified rr2 promoter region was cloned into pGL3-Basic vector at *Kpn*I and *Hind*III sites to construct the expression cassette containing rr2 promoter upstream of luciferase-encoding gene.

These constructs were transfected into Sf9 insect cells seeded at  $2 \times 10^5$  cells/well using Tfx-20<sup>®</sup> Reagent (Promega) with 2:1 charge ratio and 1.0  $\mu$ g plasmid DNA. The luciferase activity was measured at 48h post-transfection by GEN-PROBE<sup>®</sup> Leader 450i followed the Luciferase Assay System (Promega). The analyzed data was presented by Graphpad Software Prism 2.01.

**Results and Discussion:** The luciferase assay of tested promoters showed different levels in their activities. Figure 2 illustrates the expression level of luciferase activities driven by indicated promoters.



**Figure 2.** The expression of luciferase under the control of different viral promoters in transfected Sf9 cells. The luciferase activities were measured from 20  $\mu$ g total proteins from cell lysate at 48 hr after transfection. – represents Sf9 cells without transfection, + represents Sf9 cells transfected with indicated DNA vectors. pGL3 is a promoter-less vector containing luciferase reporter gene. pGL3-CMV, pGL3-p1, pGL3-p50, pGL3-rr1, pGL3-rr2 stand for pGL3 with CMV, p1, p50, rr1 and rr2 promoters, respectively.

The result of luciferase assay from Sf9 transfected cells showed putative rr2 promoter has the highest activity when compared with others. The activity of CMV and p1 showed the similar level, about 5 times lower than that of rr2. Whereas the activity of rr1 is about 3 times lower than that of rr2. The difference in promoter function did not effect from the promoter size since all of them are about 150-350 bp in length. The low level of promoter activities in Sf9 insect cells might due to Sf9 could not sufficiently provide the transcription factors that are essential in gene expression. Interestingly, putative p50 promoter could not show the expression of luciferase gene. The previous study of HPV genome sequences demonstrated the putative promoter region of p50 has the specific regulatory element of parvovirus family that requires trans-acting protein from its viral genome to control gene expression. However, all of these promoters will be further characterized their activities in primary shrimp cells. The level of luciferase expression from shrimp cells will provide the additional information of promoters rather than in Sf9. Moreover, the comparison of promoter activities between Sf9 insect cells and shrimp cells will demonstrate the relationship of these two cells about the regulatory elements in promoter region as well as the related transcription factors. The study of functional promoters in shrimp cells can be applied to determine gene expression either endogenous or exogenous genes in shrimp cells including to improve the shrimp production as well as to further prevent the shrimp viral infection.

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**Keywords:** Promoter, Shrimp, Sf9, CMV, HPV and WSSV