INTISARI

MUTASI TERARAH *OPEN READING FRAME LUCIFERASE-LIKE MONOOXYGENASE* 2

Pada penelitian sebelumnya, *Open Reading Frame* (ORF) *Luciferase-like monooxygenase* 1 (*llm*1) dan *Luciferase-like monooxygenase* 2 (*llm*2) telah berhasil dikloning dan diekspresikan pada *Escherichia coli* BL21 (DE3). Analisis solubilitas dari kedua rekombinan protein menyatakan bahwa LLM1 diekspresikan dalam bentuk terlarut, tetapi LLM2 diekspresikan dalam bentuk badan inklusi. Pada penelitian ini, sistem ko-ekspresi dilakukan untuk meningkatkan kelarutan LLM2. Untuk pembuatan sistem tersebut, situs pengenalan *Eco*RI yang ada pada sekuens *llm*2 perlu dihilangkan. Dengan melakukan metode mutasi terarah melalui *overlap* PCR, situs *Eco*RI telah berhasil dimutasi. Basa kedua timin (T) pada sekuens GAATTC diubah menjadi adenin (A) untuk menghasilkan sekuens GAATAC. Melalui mutasi tersebut, *llm*2 tidak dapat dipotong oleh *Eco*RI. Pada penelitian ini, desain sistem ko-ekspresi belum berhasil dilakukan.

Kata kunci: Mutasi terarah, *overlapping* PCR, *Luciferase-like monooxygenase* 2, kloning, ko-ekspresi.

ABSTRACT

SITE-DIRECTED MUTAGENESIS OF LUCIFERASE-LIKE MONOOXYGENASE 2 OPEN READING FRAME

In previous research, open reading frame (ORF) Luciferase-like monooxygenase1 (*llm*1) and Luciferase-like monooxygenase2 (*llm*2) were successfully cloned and expressed in *Escherichia coli* BL21 (DE3). Solubility check of both recombinant proteins showed that LLM1 was expressed in soluble form while LLM2 was in inclusion body form. In this work, the co-expression system was employed to enhance the solubility of the LLM2. To implement such system, however, the *Eco*RI recognition site that presence in *llm*2 sequence must be removed. By implementing of site directed mutation method by overlapping PCR, the *Eco*RI site was successfully mutated. The second thymine base of the GAATTC sequence was replaced by adenine (A), to produce GAATAC sequence. By such mutation, the *llm*2 was resistant to *Eco*RI digestion. In this work, however the co-expression construction has not succeeded.

Keywords: Site-directed mutagenesis, overlapping PCR, Luciferase-like monooxygenase 2, cloning, co-expression.