

INTISARI

Ekspresi gen rekombinan sangat dipengaruhi oleh karakteristik genetik dari vektor ekspresi yang digunakan, seperti jenis promotor, sistem induksi, serta keberadaan tag fusi. Pemilihan vektor yang tepat menjadi faktor kunci untuk menghasilkan transkrip mRNA dan protein dalam jumlah yang optimal, terutama pada sistem ekspresi bakteri seperti *Escherichia coli*. Penelitian ini bertujuan untuk membandingkan ekspresi gen Ag85B-SpyCatcher dan ESAT6-SpyCatcher menggunakan dua vektor ekspresi berbeda, yaitu pGEX-6P-1 dan pET-21d(+), pada *E. coli* BL21(DE3). Konfirmasi gen rekombinan dilakukan melalui PCR, sekuensing, dan analisis penjajaran menggunakan BioEdit untuk memastikan tidak adanya mutasi. Analisis transkripsi gen dilakukan menggunakan RT-qPCR untuk membandingkan produksi mRNA dari masing-masing vektor dan masing-masing gen. Translasi protein dianalisis menggunakan SDS-PAGE dan Western blot dengan antibodi anti-Ag85B dan anti-ESAT6, serta kuantifikasi pita protein dilakukan dengan perangkat lunak ImageJ. Hasil penelitian menunjukkan bahwa transkrip mRNA pada vektor pET-21d(+) lebih tinggi dibandingkan pGEX-6P-1. Protein rekombinan berdasarkan hasil SDS-PAGE dan western blot juga lebih tinggi pada vektor pET-21d(+). Produksi mRNA dan protein tersebut menunjukkan kecenderungan hasil yang sejalan, dengan nilai yang lebih tinggi pada vektor pET-21d(+). Namun demikian, transkripsi mRNA maupun translasi protein Ag85B-SpyCatcher dan ESAT6-SpyCatcher menunjukkan adanya perbedaan meskipun diekspresikan dalam jenis vektor yang sama. Temuan ini membuktikan bahwa ekspresi gen rekombinan tidak hanya dipengaruhi oleh vektor ekspresi, tetapi juga oleh struktur dan karakteristik dari masing-masing gen target.

Kata kunci: Ag85B-SpyCatcher, ESAT6-SpyCatcher, Ekspresi gen, vektor pGEX-6P-1, vektor pET-21d(+).

ABSTRACT

Several factors related to the genetic characteristics of the expression vector can affect recombinant protein expression, such as the type of promoter used, the induction system, and the presence of a fusion tag. Thus, selecting an appropriate vector is a critical for achieving optimal protein yield, particularly in bacterial expression systems such as *Escherichia coli*. This study aimed to compare the expression of Ag85B-SpyCatcher and ESAT6-SpyCatcher genes using two different expression vectors, pGEX-6P-1 and pET-21d(+), in *E. coli* BL21(DE3). The recombinant genes Ag85B-SpyCatcher and ESAT6-SpyCatcher for this study were confirmed by PCR method, sequencing, and sequence alignment analysis using BioEdit to ensure there are no mutation from the original sequence. Transcriptional analysis was conducted using RT-qPCR to compare mRNA levels of each gene in both vectors. Protein translation was assessed by SDS-PAGE and Western blot using anti-Ag85B and anti-ESAT6 antibodies, and band intensity was quantified using ImageJ software. The results showed that mRNA levels were higher in the pET-21d(+) vector compared to pGEX-6P-1. The recombinant protein, as determined by SDS-PAGE and Western blot analysis, was also higher in pET-21d(+) vector. Both mRNA and protein levels followed a similar pattern, showing higher expression in the pET-21d(+) vector compared to pGEX-6P-1. Nevertheless, differences in transcription and translation levels between Ag85B-SpyCatcher and ESAT6-SpyCatcher were still observed even within the same vector. This suggests that recombinant gene expression is influenced not only by the expression vector but also by the structure and intrinsic characteristics of each recombinant gene.

Keywords: Ag85B-SpyCatcher, ESAT6-SpyCatcher, gene expression, pGEX-6P-1 vector, pET-21d(+) vector.