Produksi dCas9 Rekombinan dari Bakteri Geobacillus kaustophilus Strain TBUI01 pada Escherichia coli dengan Purifikasi Immobilized Metal Affinity Chromatography = Production of Recombinant dCas9 from Geobacillus kaustophilus Strain TBUI01 in Escherichia coli by Immobilized Metal Affinity Chromatography Purification

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Abstrak

Perkembangan teknologi penyuntingan genom clustered regularly interspaced short palindromic repeat (CRISPR) memberikan kemampuan pada ilmuwan untuk memodifikasi sekuens genom pada sebagian besar sel eukariot. Selain untuk insersi dan delesi gen, penelitian sistem CRISPR-Cas9 juga telah menuju ke arah perkembangan represor transkripsional artifisial CRISPR interference (CRISPRi) dengan sistem dCas9 untuk rekayasa metabolisme melalui penyuntingan metabolic pathways. dCas9 yang merupakan turunan dari Cas9 saat ini umumnya diproduksi oleh bakteri Streptococcus pyogenes yang merupakan bakteri mesofilik dan menyebabkan Cas9 dan dCas9 yang berasal dari Sterptococcus pyogenes memiliki limitasi terhadap suhu tinggi. Saat ini eksplorasi terhadap bakteri termofilik sebagai sumber gen Cas9-dCas9 sedang berkembang. Salah satunya adalah bakteri Geobacillus kaustophilus yang tumbuh pada suhu optimal 60C dan dapat hidup hingga suhu 74C. Dalam penelitian ini, produksi enzim dCas9 dilakukan menggunakan Escherichia coli BL21 sebagai host dan dipurifikasi Immobilized metal affinity chromatography (IMAC). Variasi pemanasan supernatant dilakukan untuk suhu 50C, 60C, dan 70C sebelum purifikasi. Terdapat penurunan konsentrasi protein total dengan semakin tinggi suhu pemanasan, dengan konsentrasi protein total tertinggi pada suhu 50C. Purifikasi dilakukan menggunakan 3 buffer elusi dengan konsentrasi imidazole berbeda (250 mM, 350 mM, dan 450 mM). Konsentrasi imidazole 350 mM pada buffer elusi menghasilkan protein dengan konsentrasi total paling tinggi. SDS PAGE silver staining dilakukan untuk melihat berat molekul protein rekombinan yang telah dipurifikasi, dan protein terpurifikasi muncul pada pita ~50 kDa. .....The development of clustered regularly interspaced short palindromic repeat (CRISPR) genome editing technology has given scientists the ability to modify the genome sequences of most eukaryotic cells. In addition to gene insertion and deletion, research on the CRISPR-Cas9 system has also led to the development of artificial transcriptional CRISPR interference repressors (CRISPRi) with the dCas9 system for metabolic engineering through editing of metabolic pathways. dCas9 which is a derivative of Cas9 is currently generally produced by Streptococcus pyogenes which is a mesophilic bacterium and causes Cas9 and dCas9 derived from Streptococcus pyogenes to have limitations against high temperatures. Currently, exploration of thermophilic bacteria as a source of Cas9-dCas9 genes is rising. One of them is the bacterium Geobacillus kaustophilus which grows at an optimal temperature of 60C and can live up to 74C. In this study dCas9 recombinant production was carried out using Escherichia coli BL21 as the host and Immobilized Metal Affinity Chromatography (IMAC) purification. Variation of supernatant heating was carried out for temperatures of 50C, 60 C, and 70 C before purification. There was a decrease in total protein concentration with higher heating temperature, with the highest total protein concentration at 50 C. Purification was carried out using 3 elution buffers with varying imidazole concentrations (250 mM, 350 mM, and 450 mM). The imidazole concentration of 350 mM in the elution buffer produced fractions with

the highest total protein concentration. SDS PAGE silver staining was performed to determine the molecular weight the purified fraction, and bands appeared in the ~50 kDa band.