KONSTRUKSI PLASMID REKOMBINAN pET-*REP* DAN EKSPRESI GEN *REP* (C1) GEMINIVIRUS KE DALAM *Escherichia coli* STARIN BL21

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Konstruksi Plasmid Rekombinan pET-*Rep* dan Ekspresi Gen *Rep* (C1) Geminivirus ke dalam *Escherichia coli* Strain Bl21

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ABSTRAK

Gen *Rep* (C1) Geminivirus menghasilkan protein replikasi (*Rep*) yang berinteraksi dengan *Retinoblastoma-related protein* (RBR) pada tanaman saat terjadi infeksi Geminivirus. Interaksi tersebut mengganggu fungsi RBR dan faktor transkripsi. Sehingga mekanisme petahanan tanaman cabai terhadap gejala penyakit kuning keriting (PepYLCVD) terblokir. Gen NPR1 merupakan koaktivator transkripsi yang terlibat dalam regulasi mekanisme pertahanan *Systemic Acquired Resistance* (SAR) tanaman cabai. Secara *in silico* protein *Rep* memblokir mekanisme SAR yang diregulasi oleh gen NPR1. Penelitian ini bertujuan untuk mendapatkan konstruksi plasmid ekspresi pET-28a(+) rekombinan gen *Rep* (C1) dan protein *Rep* untuk diekspresikan di dalam *E.coli* BL21. Penelitian ini bermanfaat untuk studi interaksi Geminivirus dengan koaktivator transkripsi tanaman cabai. Konstruksi dilakukan dengan cara meligasikan plasmid serta Gen *Rep* (C1) yang telah direstriksi menggunakan enzim restriksi *Bam*HI dan *Sac*I. Konstruksi plasmid rekombinan pET-*Rep* dikonfirmasi dengan amplifikasi dan verifikasi urutan nukleotida melalui teknik sekuensing. Hasil verifikasi urutan nukleotida membuktikan bahwa gen *Rep* (C1) berhasil dikonstruksi ke dalam plasmid pET-28a(+) dengan posisi dan orientasi yang tepat. Plasmid rekombinan pET-*Rep* dilanjutkan ke tahap uji ekspresi menggunakan *host E.coli* BL21. Ekspresi dilakukan menggunakan metode induksi dengan *IPTG*. Protein *Rep* hasil ekspresi dipurifikasi menggunakan *Magnehis™ Protein Purification System*. Protein *Rep* divisualisasi dengan SDS-PAGE. Visualisasi menunjukkan bahwa protein *Rep* berhasil diekspresikan di dalam *E.coli* BL21 dimana ukuran protein sesuai estimasi, yaitu 41,03 kDa.

Kata kunci : *Ekspresi, gen Rep (C1), konstruksi, PepYLCVD, plasmid pET-Rep dan protein Rep*

Skripsi ini telah dipertahankan di depan sidang penguji dan dinyatakan lulus tanggal 23 Januari 2018
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Construction pET-*Rep* Recombinant Plasmid and Expression Rep (C1) Gene of Geminivirus in *Escherichia coli* Bl21 Strain

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ABSTRACT

Rep (C1) gene Geminivirus produces a replication protein (*Rep*) that interacts with *Retinoblastoma-related protein* (RBR) in plants when Geminivirus infection occurs. That interactions interfere RBR and transcription factor function. So the mechanism of chili plant defense against symptoms of *Pepper Yellow Leaf Curl Desease* is blocked. The NPR1 gene is a transcriptional co-activator involved in the regulation defense mechanism of Systemic Acquired Resistance (SAR) in chili plants. In silico *Rep* protein blocks the SAR mechanism that is regulated by the NPR1 gene. The aim of this study was to obtain construction of pET-28a(+) plasmid expression recombinant *Rep* (C1) gene and *Rep* protein that can be expressed in *E.coli* BL21. This research is useful to study the interaction of Geminivirus with transcription co-activator in chili plants. The construction was done by ligating plasmid and *Rep* gene which have been cut using restriction enzymes *Bam*HI and *Sac*I. The construction of a recombinant pET-Rep plasmid is confirmed by amplification and verification of the nucleotide sequence through sequencing techniques. Nucleotide sequence verification results prove that Rep (C1) gene is successfully constructed into pET-28a(+) plasmid with proper position and orientation. The pET-Rep recombinant proceed to the expression test using the *E.coli* BL21 host. Expression was performed using induction method with IPTG. Proteins *Rep* are purified using the *Magnehis™ Protein Purification System*. Protein Rep is visualized with SDS-PAGE. Visualization showed that *Rep* protein was successfully expressed in *E.coli* BL21 where protein size according to estimation is 41,03 kDa.

Keywords: *Expression, Rep (C1) gene, construction, PepYLCVD, pET-Rep plasmid and Rep protein.*

This thesis has been defended and was passed on January,23rd 2018

Abstract Editor

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