

**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 pertahanan 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 *MagnehisTM 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 Disease* 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*TM *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.*


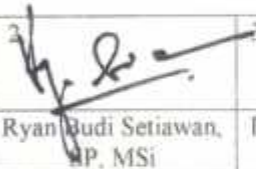

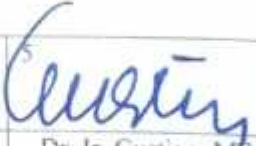
This thesis has been defended and was passed on January, 23rd 2018

Abstract Editor

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