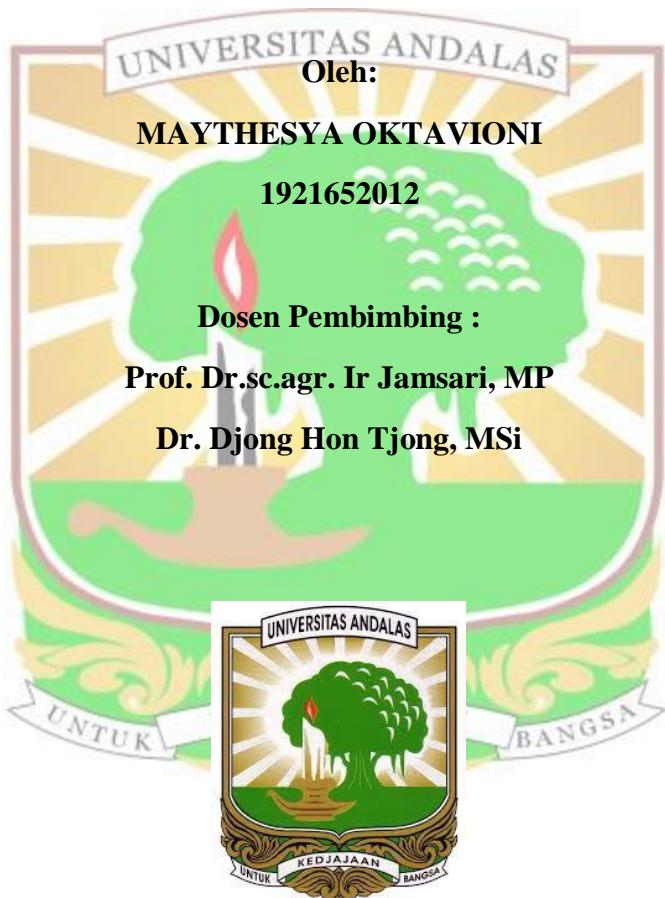


KONSTRUKSI HYBRID PROMOTER UNTUK VALIDASI FUNGSI ELEMEN CIS-ACTING DARI PD_CbNPRI

Tesis



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KONSTRUKSI HYBRID PROMOTER UNTUK VALIDASI FUNGSI ELEMENT CIS-ACTING DARI PD_CbNPRI

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Abstrak

Hybrid promoter merupakan alat validasi elemen *cis-acting*. *Hybrid promoter* direkayasa melalui penggabungan *minimal promoter* dengan fragmen yang mengandung elemen *cis-acting*. *Hybrid promoter* berperan dalam peningkatan level ekspresi gen-gen target dalam dunia bioteknologi dan berpotensi dalam pengembangan biosintesis dan bioindustri. Pada penelitian ini lima elemen *cis-acting* (*W-Box 1*, *W-Box 2*, *W-Box 3*, *RAVIAAT*, dan *WLE1*) dari *PD_CbNPRI* disintesis menjadi fragmen *g-block*. Fragmen tersebut difusikan di daerah *upstream* T7 Promotor pada plasmid *pET28a(+)*. Tujuan penelitian ini adalah untuk mengkonstruksi *hybrid promoter* yang mengandung lima elemen *cis-acting* dari *PD_CbNPRI* pada plasmid *pET28a(+)*. Konstruksi tersebut sebagai langkah awal dalam proses validasi fungsi masing-masing elemen *cis-acting* dalam regulasi ekspresi gen secara *in-vitro*. Konstruksi *hybrid promoter* dari fragmen sintetik yang mengandung lima elemen *cis-acting* dari *PD_CbNPRI* dan T7 Promotor dinamakan dengan *pET28a(+)_HP*. Konstruk *pET28a(+)_HP* yang berhasil dikonstruksi secara *in-vitro* memiliki ukuran 5.834 bp. Selanjutnya plasmid *pET28a(+)_HP* berhasil diligasi dengan gen *Rep* membentuk plasmid rekombinan *pET28a(+)_HP-Rep*. Agar fungsi dari lima elemen *cis-acting* dari *PD_CbNPRI* bisa tervalidasi secara *in-vitro*, plasmid rekombinan *pET28a(+)_HP-Rep* perlu diekspresikan pada sistem ekspresi sel bakteri.

Kata kunci: ekspresi gen, promotor sintetik, RAVIAAT, W-Box, WLE1.

HYBRID PROMOTER CONSTRUCTION FOR VALIDATION THE FUNCTION OF PD_CbNPR1 CIS-ACTING ELEMENTS

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Abstract

Hybrid promoter is a tool for validation the function of cis-acting elements. Hybrid promoters are engineered by fusing the minimal promoters with a fragment containing cis-acting elements. Hybrid promoter plays a role in increasing the expression level of target genes in biotechnology and has the potential in biosynthesis and bioindustry development. In this study, five cis-acting elements (W-Box 1, W-Box 2, W-Box 3, RAVIAAT, and WLE1) of PD_CbNPR1 were synthesized into a g-block fragment. The fragment was fused in the upstream region of the T7 Promoter on the pET28a(+) plasmid. This study aimed to construct a hybrid promoter containing five cis-acting elements of PD_CbNPR1 on the pET28a(+) plasmid. This construction is the first step in the process of validating the function of each cis-acting element in the regulation of in-vitro gene expression. The hybrid promoter construct of a synthetic fragment containing the five cis-acting elements of the PD_CbNPR1 and T7 promoter was named pET28a(+)_HP. The in-vitro construct of pET28a(+)_HP has a size of 5,834 bp. Furthermore, the pET28a(+)_HP plasmid was successfully ligated with the Rep gene to form the pET28a(+)_HP-Rep recombinant plasmid. In a further study, the recombinant plasmid pET28a(+)_HP-Rep needs to be expressed in the bacterial cell expression system so the function of the five cis-acting elements of PD_CbNPR1 can be validated in-vitro.

Keywords: gene expression, RAVIAAT, synthetic promoter, W-Box, WLE1.