CONSTRUCTION OF *Escherichia coli* MG1655 red(Km^{R})Δarnab:scar STRAIN VIA Flp-FRT RECOMBINATION AND P1 PHAGE TRANSDUCTION FOR GENES INTEGRATION

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ABSTRAK

ARINA ROSYADA AZKA. Konstruksi Strain *Escherichia coli* MG1655 red(Km^R)Δarnab:scar melalui Rekombinasi Flp-FRT dan Transduksi Faga P1 untuk Integrasi Gen. Dibimbing oleh HASIM, I MADE ARTIKA, dan MASAYUKI HASHIMOTO.

Peningkatan krisis resistensi antibiotik mendorong perlunya pengembangan strategi biokontrol seperti terapi faga. Strategi ini memerlukan pemahaman mengenai mekanisme imun bakteri, salah satunya adalah kompleks Hachiman. Penelitian ini berfokus pada konstruksi galur khusus *Escherichia coli* MG1655 red(Km^R)ΔarnAB:scar sebagai model genetik untuk mempelajari ekspresi kromosom dari sistem pertahanan ArnAB yang merupakan varian dari kompleks Hachiman. Proses konstruksi dilakukan menggunakan kombinasi rekombinasi situs spesifik dan transduksi general untuk mengatasi masalah duplikasi marka antibiotik. Pertama, sistem rekombinasi Flp-FRT yang diekspresikan dari plasmid pCP20 digunakan untuk memotong marka resistensi kanamisin sehingga menyisakan *FRT scar* yang non-fungsional. Selanjutnya, dilakukan transduksi faga P1 untuk menggantikan marka resistensi kloramfenikol pada kromosom dengan marka kanamisin. Langkah ini bertujuan untuk memastikan kompatibilitas strain inang dengan fragmen DNA sisipan pBAD-arnAB(Cm).

Kata kunci: Kompleks Hachiman, konstruksi strain, rekombinasi Flp-FRT, sistem pertahanan antifaga, transduksi faga P1

ABSTRACT

ARINA ROSYADA AZKA. Construction of *Escherichia coli* MG1655 red(Km^R)Δarnab:scar Strain via Flp-FRT Recombination and P1 Phage Transduction for Genes Integration. Supervised by HASIM, I MADE ARTIKA, and MASAYUKI HASHIMOTO.

The escalating crisis of antibiotic resistance requires the development of biocontrol strategies, such as phage therapy, which needs a deep understanding of bacterial immune mechanisms like the Hachiman complex. This study focused on the construction of a specialized strain *Escherichia coli* MG1655 red(Km^R)ΔarnAB:scar strain to serve as a genetic model for investigating the chromosomal expression of the ArnAB defense system as a variant of the Hachiman complex. The construction process utilized site-specific recombination and generalized transduction to resolve antibiotic marker duplication. First, the Flp-FRT recombination system expressed from the temperature sensitive pCP20 plasmid was used to excise the kanamycin resistance marker resulting in a non-functional FRT scar. Second, P1 phage transduction was performed to replace the chromosomal chloramphenicol resistance marker with a kanamycin marker, ensuring the host strain's compatibility with the pBAD-arnAB(Cm) insert DNA fragment. notypic screening confirmed the loss and gain of specific antibiotic resistance s, while genotypic verification via PCR amplification validated the correct sizes of the modified locus, specifically the *ΔarnAB* scar site and the *red(Km^R)* locus.

Keywords: Antiphage defense system, Flp-FRT recombination, Hachiman complex, Strain construction, P1 phage transduction

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