INTISARI

KLONING DAN EKSPRESI GEN PENYANDI *MEROZOITE SURFACE PROTEIN 1* (MSP-1) DARI *Plasmodium falciparum* (PfMSP-1 19kDa) PADA SISTEM BEBAS SEL

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Pendahuluan: PfMSP-1 19 kDa merupakan suatu gen penyandi protein pada permukaan merozoit dari *P. falciparum*. Protein ini berperan dalam patogenesis malaria dan dapat menginduksi respon imun protektif serta dapat membentuk antibodi saat terjadi infeksi malaria. Antibodi PfMSP-1 19 kDa dapat digunakan untuk mengetahui perbedaan seroprevalensi, tingkat antibodi pada setiap lokasi.

Tujuan: Penelitian ini bertujuan untuk mempelajari apakah gen PfMSP-1 19 kDa dapat dikloning dan diekspresikan pada sistem bebas sel.

Metode Penelitian: Isolat *P. falciparum* digunakan sebagai *template* untuk amplifikasi gen penyandi PfMSP-1 19 kDa dengan PCR. *Fresh* produk PCR diligasi ke dalam vektor pET SUMO kemudian ditransformasikan ke sel kompeten bakteri *E. coli* One Shot® Mach1™-T1^{R}. Hasil dari transformasi dikultur pada media LB agar yang mengandung kanamisin dan diinkubasi suhu 37^{o}C selama *overnight*. Koloni tunggal hasil kultur selanjutnya disubkultur pada media LB cair pada *incubator shaker* suhu 37^{o}C selama *overnight*. Analisis DNA *insert* dilakukan dengan PCR koloni dengan primer spesifik PfMSP-1 19 kDa dan primer T7-SUMO. Hasil PCR koloni yang mengandung plasmid rekombinan di-*sequencing* menggunakan primer T7-SUMO. Hasil *sequencing* di-*blast* dan di-*alignment*, hasil yang memiliki urutan dan orientasi yang benar diekspresikan pada sistem bebas sel. Analisis hasil ekspresi dilakukan dengan metode western blot, SDS-PAGE, dan dot blot.

Hasil Penelitian: Hasil amplifikasi menunjukkan *single band* berukuran 279 bp. Kloning berhasil dilakukan dengan terbentuknya koloni yang berwarna putih sebanyak 19 klon. Hasil analisis DNA insert terdapat 10 klon yang mengandung plasmid rekombinan. Hasil *sequencing* didapatkan 2 klon yang memiliki urutan dan orientasi yang sesuai dengan *Genebank*. Hasil western blot, SDS-PAGE, dan dot blot menunjukkan bahwa protein belum terekspresi secara maksimal.

Kesimpulan: Kloning gen penyandi PfMSP-1 19 kDa berhasil dilakukan di vektor pET SUMO. Ekspresi protein berhasil dilakukan di sistem bebas sel, namun belum didapatkan hasil yang sesuai dengan target protein yang diharapkan.

***Keywords*:** kloning, ekspresi protein, gen penyandi PfMSP-1 19 kDa.

ABSTRACT

CLONING AND EXPESSION GENE ENCODING *MEROZOITE SURFACE PROTEIN 1* (MSP-1) FROM *Plasmodium falciparum* (PfMSP-1 19kDa) IN CELL-FREE SYSTEM

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Introduction: PfMSP-1 19 kDa is a protein-encoding gene on the merozoite surface of *P. falciparum*. These proteins play a role in the pathogenesis of malaria and can induce protective immune responses and can form antibodies when malaria infections occur. Antibodies PfMSP-1 19 kDa can be used to determine differences in seroprevalence, antibody levels at each site.

Aim: The aims in research is to examine whether the PfMSP-1 19 kDa gene can be cloned and expressed in a cell-free system.

Methods: Isolates *P. falciparum* ware used as a template for the amplification of PfMSP-1 19 kDa encoding genes with PCR. Fresh product PCR are ligated into pET SUMO vectors then transformed to a competent cell of the *E. coli* One Shot® Mach1™-T1^{R}. The results of the transformation were cultured on agar plate LB medium containing kanamycin and incubated at 37° C overnight. A single colonies were selected and subculture in liquid LB medium at incubator shaker temperature 37^{o}C overnight. DNA insert analysis was performed with PCR colonies with PfMSP-1 primer 19 kDa primers and T7-SUMO primer. The results of PCR colonies containing recombinant DNA in sequencing using T7-SUMO primers. The sequencing result then in blast and alignment, results that have the correct sequence and orientation then expressed on the cell-free system. The result expression analysis was done by western blot, SDS-PAGE, and dot blot.

Result: The amplification results show the single band sized 279 bp. Cloning was successful with the formation of white colonies of 19 clones. DNA insert analysis results in 10 clones containing DNA recombinant. The results of sequencing obtained 2 clones that have the sequence and orientation in accordance with Genebank. The western blot, SDS-PAGE, and dot blot results show that the protein has not been fully expressed.

Conclusion: The cloning of encoding genes PfMSP-1 19 kDa was successfully performed in the SUMO pET vector. Protein expression was successfully carried out in a cell-free system, but no results were found that matched the expected protein target.

***Keywords*:** cloning, protein expression, gene encoding PfMSP-1 19 kDa.