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Designing CRISPR/Cas9 gene knockout system for *Plasmodium falciparum* erythrocyte membrane protein 1 (*Pf*emp1)

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Structured Abstract

Background: Malaria remains a significant global health challenge, with *Plasmodium falciparum* responsible for the most severe cases. The erythrocyte membrane protein 1 (*Pf*EMP1) plays a critical role in the parasite's ability to invade human red blood cells, contributing to malaria's pathogenesis. This study aims to design a CRISPR/Cas9 gene knockout strategy targeting *Pf*EMP1 to inhibit its role in red blood cell invasion and reduce malaria transmission.

Methods: The methodology of this study involves four key steps. First, guide RNA (gRNA) sequences were identified from Protospacer Adjacent Motif (PAM) sequences of *Plasmodium falciparum* obtained from GenBank. Second, the gRNA sequences were analyzed and scored for on-target efficiency and off-target risks using the EuPaGDT software. Third, primers for CRISPR/Cas9 plasmid construction were designed using OligoCalc software. Finally, the sgRNA and Cas9 complexes in recombinant plasmids were annotated using SnapGene Viewer software.

Results: The gene sequence encoding *Pf*EMP1 of accession number EF158078.1 was retrieved from GenBank based on the multiple sequence alignment (MSA). Secondly, three optimal gRNAs were selected which were EF158078.1 - *Plasmodium* - *falciparum* - erythrocyte - membrane - protein - 1_61, 77 and 131. Thirdly, these gRNAs were evaluated through BLAST to confirm their specificity and showed no similarity with *Homo sapien*. Fourth, three pairs of primers were successfully designed with melting temperatures of 59.7°C, 61.3°C and 58.0°C based on the gRNA in plasmid pPbU6_hdhfr/yfcu_Cas9. Fifth, the recombinant plasmids were annotated using SnapGene Viewer software.

Conclusion: In conclusion, the study successfully identified potential gRNA targets and validated their suitability for CRISPR/Cas9-based gene editing. The selected gRNAs demonstrated the ability to induce specific DNA cleavage, which is crucial for achieving an effective *Pf*EMP1 gene knockout. These findings suggest that the proposed CRISPR/Cas9 gene knockout system holds promise as a novel tool for malaria control by disrupting the *Pf*EMP1-mediated invasion of human red blood cells and reducing the disease's transmission.

Keywords: Malaria, parasite, gRNA, CRISPR/Cas9

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