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Designing CRISPR/Cas9 gene knockout system for merozoite surface protein 1 in *Plasmodium knowlesi*.

Muhammad Aliff Aiman Rosman[a], Tengku Idzzan Nadzirah Tengku Idris[a*]

Structured Abstract

Background: Malaria cases have been steadily increased over the years and merozoite surface protein 1 of *Plasmodium knowlesi* allows free parasite infection on human and causes malaria. There are also estimated 249 million malaria cases in 2022. Therefore, it is important to create a design of CRISPR/ Cas9 gene knockout system to merozoite surface protein 1 of *Plasmodium knowlesi.* This system functions to inhibit malaria infection that occurs through the merozoite surface protein 1 of *Plasmodium knowlesi* to human red blood cells.

Methods: The methodology of this study include firstly identification of guide RNA (gRNA) from Protospacer Adjacent Motif (PAM) sequence of *Plasmodium knowlesi* on GenBank. Secondly, analysis and design of guide RNA sequence and score by using EuPaGDT software. Thirdly, primer design using OligoCalc software and fourth, annotation of sgRNA and Cas9 complexes in recombinant plasmids by using SnapGene viewer software.

Results: The gene sequence of merozoite surface protein 1 (MSP1) of *Plasmodium knowlesi* of accession number ON926546.1 was selected from GenBank based on the multiple sequence alignment (MSA). Secondly, top three gRNAs were selected which were gRNA ON926546.1 - Plasmodium - knowlesi - isolate - PRK30008 - merozoite - surfa _ 42, 84 and 222. Fourth, the selected gRNAs were analyzed using BLAST showing no similarity with *Homo sapien.* Fifth, three pairs of primers were designed with melting temperature of 56.7°C, 62.7°C and 58°C based on the gRNA in plasmid pPbU6_hdhfr/yfcu_Cas9. Sixth, the recombinant plasmids were annotated by using SnapGene viewer software.

Conclusion: In conclusion, the step before selecting the best potential gRNAs to carry out the intended genetic alterations was the evaluation of a number of the gRNAs. The ability of gRNAs, which were small RNA molecules that attach to the Cas9 nuclease and dictate its sequence specificity, to induce DNA cleavage varies in a significant way. For the majority of applications, choosing the right target sites and, consequently, the spacer sequence, is crucial. Thus, the study's results imply that the intended gRNA targets may be employed as viable options to produce total CRISPR cas9 gene knockout system.

Keywords: Malaria, Parasite, gRNA, CRISPR/Cas9

*Correspondence: tengkuidzzan@uitm.edu.my

[a] School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia