Junior Science Communications
Faculty of Applied Sciences, UiTM Shah Alam
https://journal.uitm.edu.my/ojs/index.php/JSC

E-ISSN 2637-0689
Vol 63 2026

Colloquium on Applied Sciences 1 2026
7-12 January 2026, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Designing CRISPR/Cas9 Gene Knockout System for Granule Antigen 2 (*GRA2*) Gene in *Toxoplasma gondii*

Danial Asyraf Salim[a], Tengku Idzzan Nadzirah Tengku Idris[a*]

Structured Abstract

Background: *Toxoplasma gondii* is an obligate intracellular protozoan parasite and the causative agent of toxoplasmosis. Although toxoplasmosis is generally asymptomatic in healthy individuals, it can cause severe complications in immunocompromised patients and during congenital infection. Current treatment options are limited and mainly target the acute stage of infection, highlighting the need for further research into parasite biology and gene function. Dense granule proteins (GRAs) play important roles in host–parasite interactions, including the formation and maintenance of the parasitophorous vacuole. However, the functional role of several GRA proteins, including GRA2, remains insufficiently understood. Therefore, this study aimed to design potential CRISPR/Cas9 gene knockout system targeting the *GRA2* gene in *T. gondii* via in-silico analyses.

Methods: The nucleotide sequence of the *GRA2* gene from *T. gondii* strain RH (GenBank: HM014012.1) was retrieved from GenBank and used as the reference sequence for guide RNA design. Potential CRISPR target sites were identified based on the presence of Cas9-compatible protospacer adjacent motif (PAM) sequences. Single guide RNAs (sgRNAs) were designed and evaluated using EuPaGDT software according to predicted cleavage efficiency, GC content, and off-target effects. Specificity of the selected sgRNAs was further verified using BLAST analysis. Primers for validation of recombinant plasmids were designed using OligoCalc and assessed based on primer length, melting temperature, and secondary structure formation. In-silico plasmid construction and annotation were performed using SnapGene Viewer with the pSAG1::Cas9-U6 plasmid backbone.

Results: Three potential sgRNAs targeting different regions of the *GRA2* gene were successfully designed. All selected sgRNAs showed high predicted efficiency, optimal GC content, and zero predicted off-target effects within the *T. gondii* genome. BLAST analysis confirmed specific alignment to the *GRA2* gene and no similarity with any human gene. All designed primers met standard molecular biology criteria. SnapGene annotation confirmed correct insertion and orientation of all sgRNAs within the plasmid backbone.

Conclusion: In conclusion, this study successfully designed three potential CRISPR/Cas9 gene knockout system targeting the *GRA2* gene in *T. gondii* via in-silico analyses. The findings provide a reliable foundation for future experimental validation and functional analyses of the *GRA2* gene.

Keywords: CRISPR/Cas9, *Toxoplasma gondii*, *GRA2*, sgRNA, Gene Knockout

***Correspondence: tengkuidzzan@uitm.edu.my

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