October 14, 2016
Dr. Mala Mani
Science Editor
Journal of Visualized Experiments

Dear Dr. Mani,

We appreciate the encouraging peer reviews and comments. Please, see our response to the Editorial and Reviewers' comments following this letter. All edits have been tracked in the new versions of the article files uploaded with this resubmission. Also, the invoice for publication has been paid. We hope that the current state of the paper is sufficient to begin working with the JoVE Production Team. Again, we would like to include a video of Dr. Shen introducing the CRISPR section, a video he will have to shoot as he lives in China. Additionally, we would like to include a couple animations in the video, one describing crosses/QTL and the other describing CRISPR. Once the Production Team is included, we can work with them to figure out how to accomplish these goals.

Thanks again for your Editorial oversight of this article.

Sincerely,

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Response to Editorial and Reviewers' Comments

Editorial Comments:

• Figure 1: Please modify the figure to remove the reference to Protocol section 5.

"5" has been removed from Figure 1.

•Scattered grammar issues should be corrected:
-1.3. "Scrape the monolayer with a cell scrapper" scraper

Fixed

-4.1.4.1.1. "Use the following cycling conditions for PCR: 98 °C for 1 min, followed by 25 cycles of 98 °C for 10 s, 55 °C for 30 s and 72 °C for 5 min, followed by final extension for 2 min at 72 °C." – this has no real associated filmable action, and should be converted to a note. "Refer to the mutagenesis kit manual to finish the rest of the mutagenesis reactions." can then be removed.

Changed to a Note and highlighting has been removed.

-SI units should be used throughout (mL, µL, L).

Units have been modified to reflect SI standards.

-In general, where primer sequences appear, or thermocycler settings, these do not need to be included in full within a step and can instead be placed as a note immediately following the action (and not highlighted). They can also just be included in the supplemental materials and referenced there.

Two such sections have been converted to Notes and de-highlighted.

-4.4.8 should be converted to a note.

Converted to a Note.

•Additional detail is required:
-1.3: "…pass the parasite solution through a 10 ml syringe/22 gauge blunt needle 2 - 3 times." What is the purpose this step? You are sucking up the scraped solution and passing it back out of the syringe?

"to lyse the host cells releasing parasites. Solution can be pulled back up into the syringe for multiple needle passages." Has been added to Protocol 1.3

-4.1.5 “Grow the transformants on lysogeny broth (LB) plates containing ampicillin.” What temperature condition? 37 °C?

“at 37 °C” has been indicated

•Branding – 4.4.7: We don’t need to reference Clustal W, “sequence alignment tool” is fine.

Reference to ClustalW has been removed

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
The manuscript describes QTL mapping in T. gondii using progeny from a cross to map a drug resistance gene. A detailed protocol of the bioinformatic analysis is included. The authors then describe the use of the CRISPR/Cas9 system to create disruptions of the candidate gene to validate its role in drug resistance. The paper is dense but that is likely necessary for the detailed bioinformatic analysis

Major Concerns:
None

Minor Concerns:
1. Include the type of input data that you are going to analyze for the QTL scan. Consider including any requirements in terms of estimating required depth of sequencing and replicates.

The input data format is already described in protocol 2.3, either gary’s format or csv. We have added additional description to Protocol 2.3 in the main text to alert the reader to the supplement where this information can be found.

The read depth has already been addressed in Protocol 3.2.2 with the VarScan options, i.e. minimum read depth of 5 to call SNPs.

2. Consider including a more detailed map of the Cas9GFP plasmid and targeting plasmids with guide RNAs. Otherwise the figures are very good.

The Addgene plasmid page for the CRISPR plasmids have detailed maps. We have pointed the reader to Addgene in Protocol 4.1.

3. Consider including a recipe for cytomix.

The working concentrations for cytomix were provided in Protocol 4.2.2.

4. Check spelling on Caution using pyrimethamine. Explain the risk and IBC justification for its use as a selectable marker.

Additional language as to the risks and IBC guidelines have been added to the Note after Protocol 5.3.2.

Additional Comments to Authors:
N/A

Reviewer #2:
Manuscript Summary:
This manuscript provides a detailed description on the identification of candidate gene(s) potentially responsible for drug resistance to sinefungin (SNF) based on whole genome-based QTL and the verification of the candidate gene by CRISPR/Cas9-based genetic tool. This protocol will be a great resource not only for the Toxoplasma research community, but the approaches are also applicable to the identification and verification of desired phenotypes in other organisms such as other protozoan parasites.

Major Concerns:
No major concerns. The protocols are well and clearly presented.

Minor Concerns:
I only have three minor concerns that can be easily clarified by the authors without being further reviewed:
1) In the beginning of the protocol, it may be necessary to briefly describe the progenies that are used in this protocol, or have a short paragraph to describe materials and methods.

Much of this was provided in Protocol 2 in Supp File 1. We have included specific reference to details about the progeny in this section, and have indicated that there are 24 progeny in Protocols 1.8 & 2 and in the Introduction.

2) At the beginning of individual sections of the protocol, one or two sentences to tell the purpose of the subsequent procedures may be helpful.

In keeping with the JoVE formatting guidelines for the Protocol section, we feel the titles sufficiently translate the intent of each section. Also, many of the Notes which contain further detailed description of the Protocols were moved to Supp File 1 due to length restrictions. Reference to each of these Supp Notes has been provided in the main Protocol text and we hope the reader will make the effort to access the Supp files to obtain additional details.

3) Somewhere in the section 1 (such as in section 1.5), clarify that the "parasites" are merozoites.

We have added “and the tachyzoite stage readily grows in tissue culture.” to the first Note in Protocol 1.