

August 8, 2016
Dr. Mala Mani
Science Editor
Journal of Visualized Experiments

Dear Dr. Mani,

We appreciate the post-review Editorial comments for our manuscript entitled “QTL mapping and CRISPR/Cas9 editing to identify a drug resistance gene in *Toxoplasma gondii*”. We have made the recommended changes which make the article more consistent and clear. Please see the document “55185_R0_072516-edited” which has tracked changes for all edits. Also, we have included detailed responses (red) to the Editorial comments below. Lastly, please see the Rightslink document which has a statement from ASM, the publisher of Eukaryotic Cell, granting permission to re-publish figures.

Sincerely,



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Editorial Comments

- JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript (text and figures). Examples of commercial sounding language in your manuscript are: REDHORSE (if REDHORSE is an open source software, please ignore this), Illumina, Addgene, NEB, nanodrop, BTX ECM-830, Q5 High-Fidelity, Gibson Assembly, Excel, etc. All commercial products should be sufficiently referenced in the table of materials/reagents. Please replace all commercial sounding language in your manuscript with generic names that are not company-specific.

We have changed all commercial related language to generic terms, occasionally pointing the reader to the Materials list for further information.

- Please ensure that all items mentioned have been included in the Materials/Equipment list, and are accompanied by a catalog number.

The Materials list has been completed.

- Please define all abbreviations before use. For e.g., DMEM, LB, GSH, PBS, etc.

Abbreviated terms have been defined at their first instance in the article.

- Your Long Abstract exceeds our 300 word limit. Please revise the Long Abstract so that it clearly states the goal of the protocol within 300 words.

The Long Abstract is now 300 words.

- Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.). Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

- Please rewrite the following steps in the imperative tense: 3.1.1, 3.1.2, 3.2.1, 4.1.2 (last sentence), 4.1.3.1, 4.1.4.1.1, 4.3.2.1, 4.4.2, and 4.4.4.1.

Tense for the listed sections has been modified.

- 1.1: Please provide additional details on how to maintain and culture the parasite. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Additional details and reference have been added.

- 1.5: Is it a hemocytometer?

Instances of hemacytometer has been changed to hemocytometer.

- 1.6: Please add stepwise detail on how to pass the parasite to a HFF culture. Please mention the sinefungin concentration used in your studies.

Details have been added.

- 1.7: Please add details on how to monitor the parasite growth.

Wording has been changed to indicate growth of the parasite.

- 1.8: Please add additional details on how to score the progeny. How is growth/no growth and lysis observed?

Details have been added.

- Hyperlinks: Please move all hyperlinks to the Reference section and provide the superscript reference number in the relevant protocol steps.

Hyperlinks have been removed from the Protocols sections and added as references.

- Please note that the highlight was removed from sections 2 and 3 as these sections cannot be filmed.

We would like to work with the Editor and script writing team to determine what will be filmed.

- 3.4: The protocol section should be made up almost entirely of discrete steps, without large paragraphs of text between sections. Please break up the paragraphs into simple steps (with 2-3 actions per step) as much as possible throughout the protocol. The Protocol should contain only action items that direct the reader to do something. Alternatively, please consider moving long paragraph style text to the Discussion.

Discrete steps have been added to section 3.4.

- 4.1.1: If this step is to be filmed, please add stepwise detail on how to obtain the genomic sequence. Alternatively, remove the highlight and provide a citation.

The proper citation was added.

- 4.1.3.1: If this step is to be filmed, please add stepwise detail on how to synthesize the two primers. Alternatively, remove the highlight and provide a citation.

The proper citation was added.

- 4.1.4.1: If this step is to be filmed, please add stepwise detail on how to perform Q5 site-directed mutagenesis.

The proper citation was added.

- 4.1.5: Please add details on how to transform the mutagenesis product. Please mention the mutagenesis products used in your experiments.

Additional details have been added.

- 4.1.5.1: If this step is to be filmed, please add details on how to extract the plasmids, analyze DNA and sequence the plasmid. Please note that steps referring to manufacturer's instructions and cite a reference on how to perform a method cannot be filmed.

Highlighting has been removed.

- 4.2.1: Please add stepwise detail on how to inoculate HFF with the parasite. In addition, please mention the culture conditions.

Additional details have been added.

- 4.2.2: How are lysed cells observed?

Additional details have been added.

- 4.2.9: Please provide a reference.

Reference has been added.

- 4.3.1: Please add details on how to seed HFF cells on the coverslip. How many cell? When are the cells seeded on the coverslips? Please mention the culture conditions, medium, volume, etc.

Additional steps have been added describing these methods.

- 4.3.2.1: What volume of 4% formaldehyde? Please mention the antibody dilutions. Please add stepwise detail on how to perform IFA or provide a reference.

The appropriate changes have been made.

- 4.3.3.2: Please define "regular medium". Please mention the media volumes used.

Media volumes and details have been added.

- 4.3.3.3: Please mention the cell culture conditions. What volume of ethanol and crystal violet? Please add more details on how to stain and how to observe the plaques.

Details have been added.

- 4.4.1: Please mention the media, volume and culture conditions. Please add details on how to determine if the resistant pool becomes stable.

The appropriate details have been included.

- 4.4.2: Please add details on how to subclone the resistant pool. How many HFF cells? What medium?

Details for the media have been added. HFFs grown in 96 wells indicates how many cells.

- 4.4.3: Please mention what “contents”. How are the contents transferred?

“Contents” changed to “cells”, and pipetting details have been added.

- 4.4.5: Please mention the PCR amplification conditions. For e.g., reagents for the PCR reaction, cycles, temperature, etc.

PCR conditions have been added.

- 4.4.7: If this step is to be filmed, please add stepwise detail on how to compare the sequences. Alternatively, please provide a reference.

Reference has been added.

- Section 5 (including sub-steps): Please simplify steps of your protocol section so that individual steps contain only 2-3 related actions per step and a maximum of 4 sentences per step. We recommend that you split your longer steps into sub-steps numbered accordingly, i.e. step 1 is followed by sub-step 1.1 then 1.1.1 as necessary. The Protocol should contain only action items that direct the reader to do something. Please move any discussion about the protocol to the Representative Results or Discussion section.

Section 5 has been modified to discrete steps.

- Section 5.6 and 5.7: Please provide references for Western blotting, RT-PCR, and microscopy. Please add details on how to freeze the stocks (medium, etc.).

Appropriate references have been added.

- After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

- Please disregard the comment below if all of your figures are original. If you are re-using figures from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]." *We do not require the permission to re-use the figures until after internal and peer review. You can however, upload the permission to re-use email/file any time during the review process via your editorial manager account.*

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- Figure 1: Please provide a short description of each panel and/or a general description in the legend.

The legend has been added.

- Please expand your discussion to cover the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

The Discussion has been extended by three paragraphs providing further details about protocol limitations, critical steps, and significance and future applications.

- Please revise the protocol text to avoid the use of any pronouns (i.e. "we", "you", "your", "our" etc.).

Language using pronouns has been changed.