

We would like to thank the Editorial and Production team for carefully reading our manuscript and the opportunity to address all concerns raised by Reviewers, improving greatly our manuscript.

Editorial and production comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please use American English throughout.

We thank you for your comment, we revised our document and we corrected any spelling/grammar mistakes and changed the text to American English.

2. Please provide an email address for each author.

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3. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep, and note in the protocol section. Please use Calibri 12 points.

The manuscript is now formatted as requested.

4. Please include a Summary section to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here we present a protocol ..."

In the end of abstract as well as in the introduction we included a paragraph with the protocol summary.

5. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

We included a paragraph with the ethics statement for animal handling and welfare guidelines followed by our institution.

6. Furthermore, please revise the protocol text to be more homogenous with the video narration. Ideally, the narration is a word-for-word reading of the written protocol.

However, there can be parts that are not present in the video but are there in the text.

We thank you for raising this concern. We tried to homogenize both the video and written protocol.

7. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes. Please ensure that all subheadings, steps and substeps are numbered.

We have now adjusted the numbering list and removed dashes and almost all bullets – there are some that we think they should be maintained to facilitate reading- these are not steps/actions but possibilities.

8. Please revise the protocol text to avoid the use of any personal pronouns in the protocol (e.g., "we", "you", "our" etc.).

We removed any personal pronouns used in the protocol section.

9. The Protocol should contain only action items that direct the reader to do something. We changed our protocol section so that now there are only action items.

10. Please use complete sentences to describe all the actions. We changed the sentences to be as complete as possible.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. 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."
We transformed the text and added as a Note our recommendations.

12. Some of the shorter steps can be combined to have 2-3 actions per step. We combined small sentences and reduced the number of steps.

13. Tips: Please either convert this to note below the steps which is directly related to it, as and where applicable or move to the discussion section.
We converted some of the "Tips" paragraphs into notes and moved others to the discussion section.

14. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?
We added more details in the sentence and some of the steps are now numbered as a subsection.

15. Line 100: How do you prepare the agarose plate?
We included a paragraph describing the steps for the preparation of an agarose plate.

16. Line 102: Please include how this is done?
We included a paragraph and added a new figure with a step-by-step description on how to assemble a hairpin loop.

17. Line 115: Please convert to a note.
We converted as a note as requested.

18. Lines 120-139: Please move this as notes below the steps which are directly related to it.
We move the paragraphs to the discussion section.

19. For decimals, please use period and not comma. e.g., 1.5 mL and not 1,5 ml.
We changed all commas to periods in the decimals of our manuscript.

20. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example, Falcon, Eppendorf, TrypLE, MilliQ, VectaShield, Mowiol, Vaseline, etc.

We thank you for clarifying this point, we removed any commercial language from our protocol.

21. Line 215: What is the volume of the medium used?

We apologize for not specifying this. We included the information in Table 4, properly highlighted in the protocol for the reader to understand.

22. Please define all abbreviations during the first time use e.g., CIRC and NO CIRC.

We defined all abbreviations.

23. Please include the dilution of primary and secondary antibodies used for immunostaining.

We included the dilution of primary and secondary antibodies used for immunostaining in the accompanying Materials excel sheet.

24. Cell Quantification: Please include how each step is performed. Please include button clicks in the software, command lines, etc.

We expanded the description of the cell quantification procedure and included a more detailed explanation of software use.

25. Please include at least one paragraph of text to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.

We included a section of Representative Results in which we described all the points raised by the editors.

26. Please include a figure or a table in the Representative Results showing the effectiveness of your technique backed up with data. Please ensure all figures are cited in the text.

We do not understand this point – please clarify – see if now Figure 9 is in accordance.

27. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

All the figures used in this manuscript have not been published before and are from our own authorship.

28. Please include all the Figure/Table Legends together at the end of the Representative Results in the manuscript text. Please remove it from the uploaded figures/table.

We included all the Tables and Figures Legends at the end of the Representative Results.

29. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods
- e) Any future applications of the technique

We have taken into consideration the suggestions and expanded our Discussion section.

30. Please upload all table individually as .xlsx file to your editorial manager account

We have done as requested

31. Please upload all figures individually to your editorial manager account and ensure all figures are cited in the text.

OK

32. Please sort the materials table in alphabetical order and remove any trademark (™) and registered (®) symbols.

ok