

## Response to Reviewer Comments – JoVE61283R1

We would like to thank the reviewers for their helpful suggestions, which have improved the manuscript. Below are our detailed responses to their comments and suggestions.

### Editorial comments:

Changes to be made by the Author(s) regarding the written manuscript:

1. Figure 1: Please provide scale bars.

**Response:** Figure 1 has been updated with scale bars.

Changes to be made by the Author(s) regarding the video:

1. Video Delivery:

- The duration of the video should be 15 minutes or less.

2. Editing & Narration:

- General note: This is a long protocol. There are sections that are well covered with dissection or pipetting footage and the rest are lacking in visual content. Additionally, for this video to pass our video production quality control and ultimately be published, the video must be less than fifteen minutes. Filming and editing in more video is not going to shorten the video, so I would suggest this video be examined in a broad sense by the authors to determine the real "heart of the protocol" and to address the essential questions of: "What is the most important thing to show the viewers, and what can be compressed, omitted, or referred to in the manuscript?" Some notable areas lacking in visual content and places that may benefit from omission from the video are given below:
- 00:08-01:25 This first protocol section has verbal instructions for preparation but no visuals other than a reagent list. This section would benefit greatly from two changes: 1. It's probably not necessary to read all of the reagents out loud, just make sure you emphasize any important or novel ones and leave the text on-screen long enough for it to be read. 2. Add shots of the specific instructions you're issuing here. It would help greatly in us visualizing what is being said.
- 01:31-02:57 This time range consists of two shots of the talent placing a substance into the wells, but there are many more instructions that follow that no longer correspond to what we're seeing on screen.. All of these steps that are mentioned in the verbal protocol need visuals to correspond to them- there's too much information being given here without enough visual correspondence, and the shot of coating the plates becomes irrelevant a few moments after the narration moves on to the next instructions. We strongly suggest additional shooting and editing in this section to better illustrate what is being said.
- 02:57-04:10 In this time range, the reagents and tools for dissection are introduced on a text card and on still images of the hood setup. Consider cutting down or eliminating this section since it will be somewhat redundant when following along in the manuscript and also since the tools and materials will be mentioned when they are used in the protocol. You can compress sections like this down to narrations along the lines of: "Refer to the manuscript for instructions on setting up the hood workspace and preparing the dissection media." (Something like that.) This narration would then go at the beginning of the next section, which will show the use of the materials and media.
- 13:26-18:03 This time range consists of written on-screen instructions accompanied by an audible narration of the text. Having these sections in the video without any real visuals is not ideal

and questionable in terms of necessity. Consider filming and editing these steps into the video if they are important or even eliminating these sections by pointing the viewers to the manuscript.

3. Please ensure that a protocol is presented in the protocol section of the video at all times. A powerpoint

**Response:**

Thank you for your suggestions regarding video edits.

We have edited the video to ensure that the focus of the video is on the isolation and culturing of sympathetic neurons. Therefore, we have eliminated the details pertaining to media preparation (00:08-01:25) and pertaining to methods for immunocytochemistry and proteomics (13:26 – 18:03) and included a statement referring to the manuscript for detailed protocols. In addition, we have added visuals to the plate treatment (1:31 – 2:57). We have also included additional arrows during the dissection to identify the different tissues being removed during the process. The updated video has been uploaded onto Dropbox.