To:

Vineeta Bajaj, Ph.D.

Review Editor JoVE

Leuven, October, 2018

Dear Doctor Bajaj,

**Ref: Manuscript JoVE58960**

On behalf of all the authors, I would like to thank you for handling our manuscript ‘Efficient and Flexible Protocols for Production of Adeno-Associated Virus-Based Vectors, Purification and Quality Control’ that we submitted.

We have read the comments raised by the editorial committee and found their critiques to be both considered and fair. Therefore, we have applied changes in the manuscript and addressed these modifications in a point-by-point response letter for the benefit of the editors. All the comments were addressed accordingly, specific steps modified into notes.

We hope the manuscript is now suitable for publication in *JoVE* and remain available to address any additional concerns.

Sincerely,



Matthew G. Holt

Corresponding author

VIB-KU Leuven Center for Brain & Disease research.

The **editorial comments** have been addressed by the authors as follows:

The editor has formatted the manuscript to match the journal's style. Please retain the same. We preserved the formatting done by the editor and included in the new sub steps of the protocol.

Please have the title in alignment with the highlighted section of the protocol. The title was modified following the recommendation of the editor and the reviewer comment.

.

Please address all the specific comments marked in the manuscript. All the comments marked in the manuscript were addressed. Please find the detailed response to each comment as follows:

This part is not explained in the protocol section. The title should reflect the highlighted portion of the protocol. Either change the title to reflect the protocol or include a subsection/ step that the transgene is delivered to the mouse and the expression is studied. This can reference already published protocols. Perform transgene delivery to xx mice as shown previously. The title was modified accordingly to reflect the protocol.

Results for this section is missing. Show cells with GFP and without GFP AAV transfection. How does it look? We thank the editor for bringing this point to our attention. A supplementary figure has been added to the protocol to show HEK293T cells expressing GFP after transfection.

Please use imperative tense throughout. Changed here, please check. For how many hours are the cells grown?. The text has been revised to only include actions written in imperative tense. The amount of hours during which cells are grown is variable and an exact time window is difficult to define. Therefore, we suggest monitoring the cells until they reach a confluency of 70-80%, at which point they are ready to be transfected.

* This is not an action step. Please convert this to a note and renumber the steps accordingly. The step has been converted to a note (please, check note after step 1.3) and the rest of the protocol modified accordingly.
* Please remove redundancy and write exactly how you perform your experiment in a step wise manner. The protocol has been revised to remove redundancy and all unnecessary remarks (redundancy was corrected specifically in step 1.4.1).
* Dilute to what concentration? Also 10 ml of 150 mM NaCl? The text of step 1.4.1 has been corrected. The word “dilute” has been replaced by “mix” and the rest of the section modified accordingly.

Again, this is not a step but note instead. Alternatively this can be moved to the discussion section. The step has been modified accordingly and replaced for a note.

Are the cells out at this stage? Yes, the cells are out of the incubator at this stage and handle under a flow laminar cabinet. The text was modified to clarify this comment and corresponds to step 1.5.

How much per tube? The specific step has been modified to state “6 ml of DNA mix per conical tube”. Please, check step 1.4.2

We cannot have paragraph of text in the protocol section so converted to a different step. Please check. Thank you for bringing this point to our attention. The protocol has been revised to include more frequent use of short steps, such as 1.7.

Check the step number. The mistake was corrected. Please check step 1.8.

Where is the result for this part? Test for different fractions. The proper preparation of the different iodixanol fractions can be ensured by visual confirmation. Since the fractions all have different densities, they should not intermix during the layering step (please check Figure 1C). A note after step 2.9.4 was added to clarify the issue.

Again, this is not a step and can be converted to a note. Steps should contain only action items that direct the reader to do something. Also notes cannot be filmed so please remove the highlight. The section has been converted to a note and the highlight removed, as requested.

What solution do you use to make different percentages of iodixanol? The solutions used to prepare the different iodixanol fractions are described in Table 1. Moreover, a note directing the reader to Table 1 was added at the beginning of the protocol section.

Write a one liner caution here concerning iodixanol. The description of safety measures is now included after step 2.9.

Where did you add the phenol red to the gradient? Please write exactly as you perform your experiment. Also if this needs to be filmed please convert to imperative tense. A new step (number 2.8) has been added to the text, which refers to the preparation of the iodixanol solutions as described in Table 1, in which the phenol red is mentioned.

We cannot have a paragraph of text in the protocol section so converted to substeps. Please check. We thank the editor for the comment and the remodelling of the section.

We cannot have steps which are not numbered. Converted to a substep instead. The step was changed to a substep and numbered accordingly. Please check substep 2.14.3

Please refer to the table 1 where ever you are listing the solution for the first time. Thank you for the comment. To reduce the amount of text not strictly related to the protocol, we added have added a note at the beginning of the protocol directing the reader to Table 1 for details of all solutions used during the protocol.

Please use imperative tense and please do not used phrases like should be, would be etc. The text in step 3.2 was modified as requested.

Imperative tense please. The text in step 3.6 was modified accordingly.

Result for this part is missing. We thank the editor for bringing this point to our attention. A note has been added which directs the readers to Table 6, where the results of an exemplary qPCR run can be found..

Some of the details can be moved to supplemental material. We believe that the calculations provided in the text are necessary for the user to accurately understand how the attached qPCR template is organised, and how to use it properly. Hence, we have left this text unaltered.

Please remove the redundancy from the protocol by removing the repetition. The protocol should only contain action steps which direct the reader to something and should be written in imperative tense. Any discussion about the protocol should be placed in the discussion section and not the protocol. The protocol has been modified accordingly. Please, check steps from 4.1.2 to 4.1.6.

Please make it substeps or convert this to a table in .xls/xlsx format and upload it separately to your editorial manager account. We cannot have bullet or dashes in the protocol section. 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 or dashes. The bullet point list has been replaced by Table 2. As requested, the numbering has been adjusted throughout the section in question.

Citation for the manual to follow. Citations of the manuals to follow to perform steps such as the gel purification have been added to the text. Please, check references number 23 and 24.

Please reword. Instead can be written as.. Now add 1 microlitre of DNase I to each tube. Step 4.2.1 has been reworded, according to the Editor’s suggestion.

Please provide step number instead. In step 4.2.3, the text has been adjusted. Reference to step 4.2.1 is now made.

Few details can be moved to the discussion. Also this is not an action, hence converted to a note instead. The text was converted into substep 4.2.3

This step is not needed as you would recommend the user to run the qPCR instead. Please remove the redundancy. The protocol was adjusted accordingly and the unnecessary step removed.

Please number it as supplemental table. Upload each table in this case individually as .xls/.xlsx file. A supplementary table (Table 6), containing the template for the qPCR-based titration of the AAV vector, has been added to the manuscript.

Details about the gel preparation are irrelevant and can be removed. We thank the editor for the comment. However, we believe that, self-made gels (made according to the details provided in our protocol) work better and offer greater flexibility for Silver Staining, compared to precast gels. Hence we have left this section in the protocol.

Either convert to a one liner note, move the discussion to the discussion section or convert to imperative tense. The text has been modified to reduce redundancy and include only direct instructions in the imperative tense. Please, check step 5.3.

Convert this to a note. Or use imperative tense. Another option is to remove the redundancy and bring out clarity by: Mix the gel components by swirling the tube by hand. Do not vortex since… The text has been modified to implement the suggestion. Please, check step 5.4.

What is caution here. Include the caution statement after the step. Thank you for bringing this point to our attention. A caution statement has been added, as requested, after step 5.6.

Please convert this to substeps and do not use dashes. Use complete sentences. We thank the editor for the feedback. The bullet point list was substituted by an additional Table (Table 7).

Please see my comment for the title of the protocol. How do you perform the transduction in the mice? What concentration of the AAV is used? Controls if any? All these steps are relevant here. For how long you leave the mouse? When do you dissect the tissues and perform IHC.. Following the recommendation of the reviewer, the title of the protocol was modified to reflect the protocol.

We cannot have commercial terms in the manuscript. Please move it to the Materials table. Any use of a commercial name has been removed from the text. Reference to the generic and commercial names, plus the relevant suppliers, is now made in the Materials Table.

How are you sure that the protein obtain is viral protein only? Western blot etc data? We are grateful to the editor for the question. However, we would like to point out that using Silver Stain (or Colloidal Coomassie) to establish vector purity is widely used amongst vector labs. AAV capsids are known to be composed of three proteins, with distinct sizes, that give a characteristic pattern when run in SDS-PAGE. Thus, any additional bands are considered contamination, irrespective of the identity of the proteins. By using a Western Blot, which is more time consuming and expensive (due to the antibody steps) gives no significant advantage over the protocol described. Hence, we have left the text largely unaltered.

We have a limit of 10 pages for the protocol section. Please consider making some of the standard details as a supplementary materials section. In the actual version the revised protocol does not exceed 10 pages (including notes)

Please ensure that the protocol is in imperative tense throughout. we have ensured that the imperative tense is used throughout the manuscript.

Please ensure that there is no redundancy and the action steps are as crisp as possible. We have ensured that any redundant text has been removed o modified into additional steps or notes if necessary.

Please ensure that the highlighted section of the protocol has an associated representative result. The highlighted section has associated a representative result as requested. Section 1 results are show in the Supplementary Figure 1, section 2 results are explained in the note after step 2.9.4. and step 2.9.14.

Once done please ensure that the protocol is no more than 10 pages and highlighted section is no more than 2.75 pages in length including heading and spacing The highlighted portion of the protocol is no longer than 2.75 pages (including heading and spaces, after excluding notes).