**Manuscript JoVE52410 'Differentiation of a human neural stem cell line on three dimensional cultures, analysis of microRNA and putative target genes' Authors’ responses to editorial comments**

Dear Eric Veien, Ph.D and Jaydev Upponi, Ph.D

Thank you for the evaluation of our manuscript entitled “Differentiation of a human neural stem cell line on three dimensional cultures, analysis of microRNA and putative target genes” for publication in Journal of Visualized Experiments

We found the editorial comments both insightful and helpful. Based on these comments we have made all of the appropriate changes/edits to our manuscript. A detailed response to each question follows below. Modifications to text are shown in track changes in the Related Manuscript File.

Thank you for the opportunity to reconsider our manuscript for publication in Journal of Visualized Experiments

We look forward to hearing your responce.

Warm regards,

Lara Stevanato, PhD  
  
Your manuscript JoVE52410 'Differentiation of a human neural stem cell line on three dimensional cultures, analysis of microRNA and putative target genes' has been editorially reviewed and the following comments need to be addressed.  
  
  
**Editorial comments:**  
**1**.Your current short abstract is above our 50 word limit; please make sure the short abstract is between 10 to 50 words.  
**Modified as request, current word count is 49.**

**2.**Please state the goal of your protocol in the Short abstract.  
A goal was stated in the Short abstract: ***With the intent of characterizing changes in miRNAs on differentiated human neural stem cells (hNSCs).***

**3.**Your current long abstract is under our 150 - 300 word limit; please modify your long abstract in both the manuscript document and your editorial manager account.

**Modified as request, current word count is 158.**  
  
**4.**Please add a one line space between each step and sub-steps of your protocol section.

One line space has been added between each step and sub-steps of your protocol section.

**5.**Please adjust the numbering of your protocol section to follow JoVE instructions for authors, 1. should be followed by 1.1) and then 1.1.1) if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one line space between each protocol step.  
**Modified as request, please directly refer to manuscript document.**

**6.**Please include the paragraph mentioned below step 1 in the step wise protocol section. Please be sure the write the protocol in imperative tense. For instance, “Isolate HNSCs from …” etc. Please perform similar action in section 2.  
**The short protocol introduction was removed and replaced by a shorter statement:**

**The isolation and derivation of hNSCs, from an ethical approved tissue, are described in a previous publication2 .**

7.Please include a ethics statement below the protocol section and before the beginning of the numbered protocol steps.  
**The ethical statement has been included: The isolation and derivation of hNSCs, from an ethical approved tissue**

8.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 and replace it with a more generic term as much as possible throughout the entire manuscript. All commercial products should be sufficiently referenced in the table of materials/reagents. Examples of commercial sounding language in your manuscript are “Alvetex® (Reinnervate, UK)”, “miScript II RT kit (Qiagen)”, “LightCycler 480 system (Roche)”, “Qiagen miRNeasy® “, “GloMax™ 96 microplate luminometer (Promega)”, etc.  
**All the commercial sounded language has been removed from the manuscript, and figure.**

9.Please write step 3.1 in imperative tense.  
**Modified as request, please directly refer to manuscript document.**

10.Please use numbering and correct format and write the paragraph below step 3.2 stepwise in imperative tense.  
**Modified as request, please directly refer to manuscript document.**

11.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 no page limit for the protocol text, but there is a 3 pages limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages (or less) of text to identify which portions of the protocol are most important to include in the video; i.e. which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVEs 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.  
**The most important portions of the protocol for the video inclusion were highlighted in yellow.**

12.Please make sure that the “Discussion” section covers the following pints running between 3 – 6 paragraphs.   
a.Critical steps within the protocol.  
b.Modifications and troubleshooting.  
c.Limitations of the technique.  
d.Significance of the technique with respect to existing/alternative methods.  
e.Future applications or directions after mastering this technique.  
**We rephrased, expanded, and modified the discussion to address the above listed requirements. Please refer directly to the manuscript document.**

13.Please remove the embedded Materials Table from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends. Please remove the TM/R marketing symbols.  
**The TM/R marketing symbols were removed from the document and table.**

14.In references, issue numbers and DOI’s are missing in some references. Also the source/journal should be abbreviated and please follow correct punctuation marks. Please make sure that your references comply with JoVE instructions for authors. In-text formatting: corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text of the manuscript. Citation formatting should appear as follows: (For 6 authors or less list all authors. For more than 6 authors, list only the first author then *et al.*): [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. *Source*. **Volume** (Issue), FirstPage – LastPage, doi:DOI, (YEAR).]   
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