Dear Dr. Casanueva,

Your manuscript, JoVE61132R1 "A protocol for high-throughput quantitative RT-qPCR in single and bulk C. elegans samples using nanofluidic technology," has been editorially reviewed and the following comments need to be addressed. Please track the changes to identify all of the manuscript edits. After revising the submission, please also upload a separate document that addresses each of the editorial comments individually with the revised manuscript.

Your revision is due by **Mar 03, 2020**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

Xiaoyan Cao, Ph.D.
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We thank the editor for all the comments on the manuscript. Below is how we have addressed them in the version of the manuscript that was sent by the Editor.

Editorial comments:

1. Please note that the editor has formatted the manuscript and made changes to the protocol (updated numbering, combined shorter steps, highlighted additional steps for continuity) according to JoVE guidelines. The updated manuscript is attached and please review for accuracy and use this version to incorporate the changes that are requested.

We have checked the accuracy of the edits (step 2.2, 2.3 and 2.4) lines 173 to 193

2. Please address specific comments marked in the attached manuscript.

We have addressed these comments through the manuscript.

- *We have updated TRIZOL for guanidium thiocyanate-phenol-chloroform throughout the protocol and we have updated the Table of Materials.*
- *We have updates references 16 to 20 to ensure they are in the correct order (see lines 128 to 156)*
- *We have changed TE buffer for Tris-EDTA buffer in the protocol (see line 307-8)*
- We have changed Eva green supermix into fluorescent probe supermix with low ROX, lines 344 and 356
- *We have updated the volumes of the total loading assay, line 326 and 336*
- *We have replace the word "overage" by surplus throughout the protocol (lines 326 and 336)* and in figure 1/2
- *Unfortunately, we could not find the spelling out of the passive reference dye ROX. It seems that it always simply refer to ROX in the manuals and protocols (see* https://www.thermofisher.com/order/catalog/product/12223012#/12223012)
- *Unfortunately, we could not indicate the speed of the centrifugation in step 5.4 and 6.2, as it deals with a tabletop plate centrifuge with only one possible speed. We have updated the Table of materials with its reference and we have added the precision: centrifuge with the tabletop plate spinner in steps 5.4 and 6.2, lines 285-6 and 301-2.*
- *We have precised RNAse free water in step 3.2.3, line 255*
- *We have precised 5.4 uL per well in step 7.2.1, line 332*
- *We have modified 4uL to 6uL in step 7.2.2, line 336*
- *We have verified the step number reference in step 8.1.2 (line 350) and in step 8.2.2 (line 360)*
- *We kept the sentence "with the barcode facing outward" in step 9.3 (line 381), which is the step where the chip is loaded into the controller. We could not include it to section 10, as this section deals with the loading of the reagents (samples and assay mixes) into the wells of the nanofluidic chip.*

3. Please cross check Figure 1/2 and protocols to ensure that experimental details mentioned there match each other.

We have updated figure 1 to ensure its description matches the protocol. In particular:

- we have changed 10 min for 5 min in figure 1, in order to match the protocol (line 165)

- we have also modified figure 1 so that the stop solution is added at the end of the lysis of the worm, and not during RT. It now matches the protocol

- we have changed 1.24 uL to 1.25 uL of RT mix in figure 1 so that it now matches the protocol, line 223.

- we have changed 90C for 95C in figure 1 for the RT program, so that it matches the protocol

4. Figures: Please replace commercial language with generic terms (SYBR, Biomark, Trizol, EvaGreen Supermix, etc.).

-we have replace Evagreen, Biomark, HX controller, SYBR RT buffer by fluorescent probe supermix, nanofluidics thermocycler and nanofluidics PCR priming machine and RT buffer respectively

-in figure 3, we have replaced TRIZOL by phenol chloroform in the figure and by guanidium thiocyanate-phenol-chloroform in the legend of figure 3, lines 535 to 543.

5. Table of Materials: Please remove any ™/®/© symbols.

We have removed all commercial symbols from the Table of Materials