**Re: “qPCR-BASED ANALYSES OF INTESTINAL MICROBIOTA AFTER ORAL ANTIBIOTIC TREATMENT OF MICE”** by Rebeca Jimeno, Phillip M. Brailey & Patricia Barral (JoVE58481)

**Reply to reviewers**

We are very grateful for the positive and constructive comments provided by the reviewers and editors and their thoughtful questions. We have reviewed the figures and the manuscript according to their suggestions as detailed below:

**Editorial comments:**  
  
**1.** The manuscript has been carefully revised  
**2.** The protocol included in part 3 (DNA extraction) corresponds to the step-by-step protocol from a commercially available kit (Qiagen QIAamp fast DNA Stool mini kit). As this protocol is available in the manufacturer’s webpage and in response to comments from reviewer 1 (about unnecessary steps), we have deleted this part of the protocol from the current manuscript. We believe that this simplifies the manuscript making it more accessible to readers. However, if the editor considers that this part provides valuable information for the readers we would be happy to include the text back and modify it as required.

**3.** CT values don’t have a specific unit  
**4.** Figure 3B has been changed  
**5.** Tables have been revised  
**6.** Keywords have been added  
**7.** This has been done  
**8.** This has been done  
**9.** This has been done

**10.** This has been done  
**11.** Commercial language has been removed from the manuscript  
**12.** This has been included in the manuscript  
**13.** This has been included in the manuscript  
**14.** This has been changed in the manuscript  
**15.** This has been revised  
**16.** This has been revised  
**17.** This has been revised  
**18.** References have been changed  
  
**Reviewers' comments:**  
  
**Reviewer #1:**  
*Manuscript Summary:  
Authors described a protocol for analyzing intestinal micorbiota using qPCR.  
Major Concerns:  
Overall concern is that the paper lacks scientific rational for the method. Purely describe too many boring and unnecessary steps*

We appreciate the concerns from reviewer 1 and, as requested, we have simplified the protocol steps to make the manuscript more accessible to readers and the protocol easier to follow (see reply to editorial comments (2) above).

*1) No explanation how qPCR can be used to quantify microbiota.*

The method for quantification of the microbiota requires the generation of a standard curve, and unknown samples (from stool or intestinal lumen) are quantified against the standard curve. The process involved in the quantification of the bacterial samples is detailed in the results section.

However, to further clarify this part of the protocol, we have included additional steps in our protocol (steps 3.2.8-3.2.9) specifically explaining the processes used to calculate the number of 16S rDNA copy numbers in the bacterial sample.

*2) No explanation of formula used to calculate number of copies. line 271*

We have included an appropriate reference (ref 14) for a paper that explains the underlying calculations used to devise the formula included in our manuscript

We have also referred in the manuscript to online tools that use this formula for the calculation of the number of copies, such as:

<http://cels.uri.edu/gsc/cndna.html> (from the Genomics and Sequencing Center of the University of Rhode Island)

<https://www.idtdna.com/pages/education/decoded/article/calculations-converting-from-nanograms-to-copy-number> (from Integrated DNA technologies) <https://www.thermofisher.com/uk/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/dna-copy-number-calculator.html> (from Fisher)

*Minor Concerns:  
Materials: primers are incomplete*

We have revised the materials

**Reviewer #2:**  
*Manuscript Summary:  
The importance of the analysis of the microbiota in different lines of research is increasingly consolidated, but many of them do not need a deep analysis like that obtained by sequencing. The authors provide here two protocols for oral antibiotic treatment of mice and a qPCR based method to quantify antibiotic-induced changes in faecal bacteria. They may serve as a quick, cost-effective and reliable tool to manipulate the murine intestinal microbiota and to study of the effects of antibiotic treatment in intestinal homeostasis and disease.  
  
Minor Concerns:  
This is an experimental protocol, so it would be important to further detail the region for which the primers were designed. (a figure representing this region). In addition, the construction of the vector containing the 16S rRNA gene (figure)*

We thank the reviewer for his/her comments and the appreciation of our manuscript.

The primers used to amplify 16S rRNA were not designed by ourselves, but obtained from an already published paper. Consequently, we have included the appropriate reference for this in the manuscript (ref 13). Also, the plasmid used for cloning is included in a commercially available kit (TOPO TA cloning). We have included this kit in the list of materials.