Author’s response to reviews

Title: Accurate Detection of KRAS, NRAS and BRAF Mutations in Metastatic Colorectal Cancers by Bridged Nucleic Acid-Clamp Real-Time PCR

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Author’s response to reviews:

Dr. Matteo Pasini
Editor
BMC Medical Genomics

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Dear Dr. Pasini,

Please find attached a revised version of our manuscript “Accurate Detection of KRAS, NRAS and BRAF Mutations in Metastatic Colorectal Cancers by Bridged Nucleic Acid-Clamp Real-Time PCR,” (Manuscript Number: MGNM-D-19-00188R2), which we would like to submit for publication as a Research Article in BMC Medical Genomics.

We would like to thank the editors and reviewers for their helpful comments. Following the reviewers’ advice, we revised the manuscript and corrected the mistakes. We hope that this revision makes our manuscript suitable for publication in BMC Medical Genomics. The modified sections are highlighted in red in the revised manuscript.

Yours sincerely,

Yosuke Hirotsu, PhD.
Responses to the comments of Reviewer #1
The authors have now provided data on a sensitivity determination of the assay. I would suggest that "Sensitivity determination" is used in the Methods section instead of "Serial dilution assay". Page p.16 line 286 should be "constructed" not "construct".

<Response>
Thank you for your comments. I revised manuscript according to the reviewer’s suggestions as follow:

- I rewrote in Method section (page 10, line 164).
- I rewrote in Discussion section (page 17, line 297).

Responses to the comments of Reviewer #3

The authors did not delete "designed with TaqMan probe" in the METHODS of revised manuscript. The revision should be confirmed before it is submitted.

<Response>
Thank you for your comments. I deleted "designed with TaqMan probe" in the sentence.

According to the authors' responses to the comments of Reviewer #3, to detect of mutations in KRAS, NRAS and BRAF genes accurately, both the one-step real-time PCR method using BNA-probe and subsequent Sanger sequencing were needed in the manuscript. But there are concerns based on the above in the manuscript.

1. In the sections of ABSTRACT, BACKGROUND and METHODS, BNA-clamp PCR was mentioned as bridged nucleic acid-clamp real-time PCR method by the BNA Real-time PCR Mutation Detection Kit Extended RAS. On the other hand, in the section of RESULTS, it was described as combination of the real-time PCR method and subsequent Sanger sequencing. Furthermore, the former and the latter coexist in the DISCUSSION and the CONCLUSIONS. It is inconsistent in the manuscript.

<Response>
Thank you for your comment. I used the “BNA Real-time PCR with Sanger sequencing” when both assays were needed for results in entire manuscript. Contrary, I used only “BNA Real-time PCR” when single assay needed for results. I also described the results more detail (page 10-11, line 180-182).

2. In the page 13, 14 and 17, turnaround time and running cost of the BNA Real-time PCR Mutation Detection Kit Extended RAS only were mentioned. The authors need to describe these of the method combined the real-time PCR and subsequent Sanger sequencing.

<Response>
In revised manuscript, I provided the additional information of turnaround time and cost for Sanger sequencing (page 13, line 232-234 ; page 15, line 256-260).