Reviewers report

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

Version: 0 Date: 22 Jun 2019

Reviewer: Sung-Kun Kim

Reviewers report:

This manuscript describes mutation detections in the KRAS, NRAS, and BRAF genes. The methods employed were PCR-rSSO and BNA-clamp PCR techniques. To confirm the detection accuracy, the authors used a next generation sequencing technique (NGS). They basically analyzed 50 patient samples to identify the mutations using the two techniques. This manuscript touched an interesting method with the help of BNA materials in order to look at single point nucleotide mutations, but there are considerable changes needed. The concerns are as follows:

1. The title of this manuscript is "Rapid Detection ....", but the content didn't sufficiently show the details of rate. The title or content should be modified.

2. In the page 3, the authors mentioned, "high sensitivity and accuracy" in the conclusion section of the abstract; however, any sensitivity in the text cannot be found.

3. In the page 5, the second line describes PCR-rSSO. The authors should justify why the method is legitimate.

4. In the page 5, BNA-clamp PCR is mentioned. An example of BNA-clamp PCR should be added, or if Figure 1 is the example, the details of the conditions and Ct values should be enumerated.

5. In the page 6, the meaning of "94% positive and 95% negative agreement" is not clear.

6. In the page 6, it would be clearer if the authors details the meaning of "98% on target sites and an average of 11,768-fold coverage depth".

7. In the page 7, BRAF mutation detections were described, but there are no data shown in the manuscript. A table or a figure can be added with adequate description.

8. In the page 8, the authors mentioned "PCR-rSSO takes 4.5 hours per run" and "BNA-clamp PCR takes only 2 hours per run" in the Discussion section. These observations could belong to the Results section and describe more.
9. In the page 9, for the further visualization, an Ion Reporter Genomic Viewer was used. The authors should show the data in the Supplement data section.

10. In the page 11, the conclusion is insufficient.

11. In the page 13, the method real time RCR was used, but it is not clear whether SYBR green or TaqMan was used. More description would be needed.

12. In Table 1, at least one NGS results obtained from both PCR-rSSO and BNA-clamp PCR should be shown. For example, #4 or a higher number of the samples should be checked.

13. Table 2 should include the meaning of Mapped reads, on target, Mean depth, and Uniformity.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable
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