Reviewer’s report

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

Version: 1 Date: 28 Aug 2019

Reviewer: Takao Iida

Reviewer's report:

This manuscript was revised in accordance with the reviewers' comments, but still I have concerns on several issues in this paper.

1. According to Supplemental Figure 1, BNA-probe clamps wild-type allele. On the other hand, the authors mentioned "BNA-probe designed with TaqMan probe" in the page 7. The role of BNA-probe is inconsistent in the figure and the text.

2. The authors set the threshold line at 0.04 in order to decide whether mutations exist. Then results of some samples that amplification plot did not reach to threshold line by the BNA-clamp PCR should be determined mutation negative.

3. The limit of detection of the BNA-clamp PCR was 1-5%. In sample #3, although the VAF of KRAS A146T and NRAS G12D by the NGS were 28.3% and 9.1% respectively, amplification plot of these did not reach to threshold line using the BNA-clamp PCR. The authors need to describe the explanation in the manuscript.

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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