Reviewer's report

Title: Evaluation of an integrated clinical workflow for targeted next-generation sequencing of low-quality tumor DNA using a 51-gene enrichment panel

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Reviewer: Zhifu Sun

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The manuscript “Evaluation of an integrated clinical workflow for targeted next generation sequencing of low-quality tumor DNA using a 51-gene enrichment panel” describes a series of strategies in variant/mutation detection from targeted amplicon sequence in FFPE samples, a challenging yet very important application in clinical settings. The authors demonstrated the importance and consideration of FFPE sample quality (QFI), input DNA amount, systematic variants (background noise), platform specific noise..., which can be very useful to the field. However, the work is presented in a way not easy for others to understand and the key messages are not clear. I have following questions and suggestions:

Major Compulsory Revisions

1) The abstract is too general and does not contain any specific messages for readers to appreciate the value of the manuscript. The same is for the conclusion section.

2) It is not clear what the authors considered as “variants”. GATK was used but were all variants made from GATK (i.e., raw calls without any filtering) used for comparison? GATK is designed for WGS or WES of genomic DNA and does not work well for TAS, particularly for tumor samples. What were the parameters used in the study? Did realignment work at the region with super coverage? Were sub-sampling used in variant calls? Were duplicates removed?

3) Did the author try using VQSR (or not applicable) to filter low quality variants and what would be the impact to the results and conclusions?

4) Up to 2 base indels are included in the panel but there is no mentioning of the performance for indel variant calling.

5) For the sentence “According to mixed-effect modeling with SV filtering, variant callers and sequencing-depth filtering as random effects and cell mixtures as fixed effects, 10% of the variation in average precision can be attributed to SV filtering, while 17% is attributed to sequencing depth filtering and 57% to the variant caller. The remaining 16% variation can be attributed to error from the mixed-effect”, what is the interpretation or implications in more understandable language? If I understood correctly, variant callers contributed dramatically to SVs. If this is true, what are the recommendations here?

6) Low DNA quality and amount may be reflected in some bioinformatics QC measures such as alignment rates, duplicate rate, on target read percentage....
The authors did not present these results. Can these be used in the pipeline?

Minor Essential Revisions

1) Are there any data or programs made available to public for others to replicate the results or benefit from the study? I did not see any.
2) The limitations of the study appear not discussed.
3) There are some minor typos in the text and Table 1 (incorrect entry in the body with column headers)

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests