Reviewer’s report

Title: Targeted High Throughput Sequencing in Clinical Cancer Settings: Formaldehyde fixed-paraffin embedded (FFPE) tumor tissues, input amount and tumor heterogeneity.

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Reviewer: Kurt Zatloukal

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The authors present interesting data on application of next generation sequencing on formalin-fixed, paraffin-embedded (FFPE) human tissues, which is of potential great importance for future use in molecular cancer diagnosis. They demonstrated that enrichment of DNA sequences could be readily applied on DNA isolated from FFPE prostate tissues, and how the amount of DNA used influenced the sequencing results. Furthermore, it was demonstrated that tumor heterogeneity may affect analysis of copy number variations (CNVs) but had no obvious effect on single nucleotide variations (at least in those cases of prostate cancer investigated).

Major compulsory revisions:

The previous findings published by the same group (Schweiger et. al., 2009) on next generation sequencing of FFPE tissues should be better described in the introduction. The rationale and novelty of the present study has to be elaborated more clearly.

The criteria used for selecting different loci should be described in detail. How has it been verified that the loci selected are not linked at another level of the specimen. Has 3D reconstruction of the tumor been performed on consecutive sections? Images of the loci analyzed should be provided as supplementary material. A detailed description of these features is important since a key finding of the study refers to possible implications of tumor heterogeneity.

How do the authors exclude that the criterion for concordant CNVs (see page 13) that a CNV has to be found in at least one read of locus B is not a random effect related to sequencing errors?

How does the finding of one somatic CNV per tumor analyzed correspond to results reported in the literature?

Minor essential revisions:
Page 3: A reference should be provided on global cancer deaths.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I have no competing interests