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: Robert Bristow

Reviewer's report:

The manuscript by Kerick et al addresses a key question in the prostate cancer field for the use of FFPE versus frozen material in CNV and sequencing experiments. They conclude that FFPE tissue material can supplement for fresh frozen tissues for the detection of SNVs. Their enrichment experiments show that this can be accomplished with 500 ng of DNA with only small effects on enrichment uniformity and data variance; but the latter requires greater coverage for targeted re-sequencing. In a small series of 3 patients with 2 foci per prostate, the authors show heterogeneity in CNV but not somatic variations. I found the manuscript easy to read and the results supported the authors’ conclusions. These are important and useful data for the sequencing community.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

Discretionary Revisions
None

Minor Essential Revisions
I have only minimal comments/query:

(1) What is the heterogeneity within small regions of the pathologic material at the level of what would be needed to call “hits” with diagnostic biopsies? For example-do the authors have data that the use of diagnostic biopsies can be used for the purpose of targeted sequencing, Other groups (Ishkanian et al; Prostate; 2009) have shown CNVs using BAC arrays can be detected in small amounts of frozen material similarly macrodissected to the present study. The authors might wish to address this issue. Have the author’s compared the diagnostic biopsy from the patient in the location of a dominant nodule to the final prostatectomy specimen in terms of CNVs?

(2) What were the Gleason scores of the two foci-the same?

(3) Can the authors be more clear about changes in pathologic “normal” epithelium versus pathologic “malignant epithelium”-is this true normal compared to the patient’s blood for example?

Major Compulsory Revisions
None

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.