Reviewer’s report

Title: Comprehensive routine diagnostic screening to identify predictive mutations, gene amplifications, and microsatellite instability in FFPE tumor material

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Reviewer: Bart M. G. Smits

Reviewer's report:

This manuscript describes implementation of a smMIP-based NGS approach for simultaneous detection of low frequency variants, gene amplifications, and microsatellite markers in clinical cancer samples. After establishing thresholds in a small sample set, over 700 routine diagnostic samples were analyzed. While the smMIP technology has been available since 2013, the novelty of this study is its utility to reliably detect 3 types of genetic variation in routine cancer diagnostics.

The manuscript is well-written and data presentation is clear and complete. A few comments need to be addressed before publication:

Major comments:
1. At several places in the manuscript, failure of the technique was attributed to poor DNA quality. However, the manuscript does not contain data suggesting the authors have examined DNA degradation (e.g. by Bio-analyzer analysis) or other measures of poor sample quality (e.g. contaminants in DNA solution). Can the authors establish a DNA/sample quality threshold at which it would no longer be worth running the smMIP analysis?

2. The authors classify their current panel as 'small' and mention the availability of large commercial cancer panels that could be used in diagnostics. The versatility of this approach is mentioned in the manuscript as an advantage in that additional genes, MSI or amplifications can easily be added to the panel. Can the authors discuss how scalable their current setup with custom probes really is? Would doubling the number of target genes affect sensitivity? Is there a maximum number of probes that can be reliably used in 1 reaction? What is the limiting factor in terms of scalability?

Minor comments:
1. 'NGS analysis' is sometimes used interchangeably with 'smMIP', while NGS is a broader term encompassing different NGS-based variant detection technologies, of which smMIP is one. I recommend using consistent terminology to mention smMIP-based NGS.

2. Line170: The GC content is suggested as a contributing factor to the underperformance of a few targets. While these targets indeed have somewhat higher GC% than the average of the entire set, they are not among the absolute highest regarding GC%. Could the authors discuss other root causes of underperformance of certain probes?
3. Line 175: (miscellaneous)? What is meant by adding this word here?

4. Line 178: What was the average age of the stored specimens? Are the dropout samples outliers in terms of age?

5. Line 227: For detection of amplifications, the authors propose to use a threshold of 3 relative coverage and a threshold of 2-3 to be marked as potentially amplified. However, a bonafide amplification of 1 of the genes, namely PDGFRA, even fell below the 2.0 threshold. Did the authors consider an even lower threshold? Would that lead to a significant increase in false positivity for amplifications to follow up on?

6. Discussion: Please discuss the effectiveness of the smMIP NGS technology as compared with OncoScan array (similar to the comparison to FISH) to detect moderately amplified genes in low% tumor cell samples or heterogeneous samples.

7. Discussion: Do the authors think the number of microsatellite markers included in the panel is appropriate to detect MSI? Please discuss the need or advantage of surveying MSI at many microsatellite loci, as compared to the pentaplex PCR assay.

**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.

I am able to assess the statistics

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