Reviewer 2
The authors verified the performance of the PAX gene tissue fixation reagent by parallel analysis of 19 prospective pancreatic cancer cases. Samples from each tissue wes fixed by formalin or PAXgene in parallel, and paraffin embedded. DNA and RNA was extracted and quality and quantity parameters measured. Amplifiability of the extracted DNA/RNA was tested by PCR. Eight samples were also subjected to NGS with the oncomine panel, based on AmpliSeq/Ion Torrent technology.

Comment:
The study focuses on a very precise aim and on a disease (pancreatic cancer) that may be relevant to many labs performing molecular analyses on the same type of samples. For this reason, the data herein presented, though interesting, should be expanded and better contextualized. Please find below a list of main points of concern.

1. PAXgene has been around for several years, the FP7 EU project that validated its use (SPIDIA), led by QIAGEN, started in 2008 and ended in 2013. Several papers (more than those cited by the authors) already reported similar results. Some focused more on IHC and ISH, other on NGS or PCR. This should be presented to the reader, clarifying what are the scope and the novelty of the present manuscript.

We appreciate the reviewer's valuable and constructive comments. We have expanded the introduction in the final paragraph to cite the SPIDIA project and more references and to emphasize the scope and novelties of our current studies.

2. Importantly Groelz et al, researchers from QIAGEN involved in the SPIDIA project, recently (2018) published a work (https://doi.org/10.1371/journal.pone.0203608) showing that the performance of samples treated by PAXgene fix reagent and stored at RT for several years seem to degrade more than FFPE samples. This improved when PFPE/FFPE blocks were stored at 4°C or -20°C, but storage at low temperatures poses other issues that should be discussed.

We thank the reviewer suggesting an important reference for our study and we have cited Groelz et al's study in our revised manuscript. We also acknowledge that our PET blocks in the study were stored at room temperature only, as this is the current standard of practice for storing pathology specimens; as such, exploring storage conditions for future clinical studies are beyond the scope of our study and are not entirely relevant to the question of our study which is exploring the use of PAX-gene fixative for routine pathological diagnosis of PDCA. Nevertheless, we have discussed the above shortcoming in the revised discussion.

We would like to quote the abstract from Groelz et al's study, which concluded that PAXgene fixation out-performed formalin fixation, regardless storage time and temperature. Their conclusion appears inconsistent with reviewer's comment.

"Chemically unmodified nucleic acids from PFPE tissue performed superior compared to FFPE tissue, regardless of storage time and temperature in both human and rat tissues. In

conclusion molecular analyses from PET can be greatly improved by using a non-crosslinking fixative and storage at lower temperatures such as 4˚C, which should be considered in prospective clinical studies."

3. It is true that the use of PFPE for pancreatic cancer samples was never explored, yet I believe the experiment sould be presented from a different perspective. Since pancreas is rich in nucleases, fixation with formalin is effective in removing their function because it causes the formation of covalent bonds between macromolecules. Conversely, PFPE could be less effective, because by definition is a not-crosslinking method. Indeed, its lesser stability on the long run could be partly due to this. Thus, showing that its use allows to obtain good DNA/RNA has merit. However, from the data herein presented it is impossible to know wether degradation will be superior to FFPE after some years of sample storage and this should be discussed.

We thank that the reviewer raised a very interesting question regarding long-term storage conditions of pancreatic tumors. We have discussed this matter in the revised discussion.

4. A comparison of unit costs for FFPE vs PFPE (cost/block) should also be presented and discussed. Indeed, previous publications were hazy about the cost increment that the introduction of PAXgene in the routine processes would yield and the authors could add some really useful, yet unpublished information.

We have added a paragraph about comparing cost of PEPE vs. FFPE in our revised discussion.

5. The authors show that PFPE samples yield longer amplicons, yet this is limited to rather short designs (around 100-150 nucleotides). Previous works showed the tracks of Agilent Tape station for isolated DNA/RNA and performed multiple PCR at increasing amplicon length (e.g. Groelz et al, Plos ONE 2018). The authors could provide similar information to show that indeed degradation is not an issue in PFPE pancreatic cancer samples, albeit when processed soon after fixation.

Unfortunately, our lab does not have an Agilent Tape station. However, we did run the extracted NA on agarose gels, which showed denser smears around 1000 bps of genomic DNA from PFPE tissues and denser degraded DNA at the bottom of gels from FFPE tissues, suggesting the presence of less degraded DNA from PFPE tissues. We decided not to include this result in our manuscript, as the gels exhibited high background, a common issue of running genomic DNA using agarose gel electrophoresis. They are likely to draw criticism of subjectivity and unacceptable for publication. Nevertheless, we have used two-qPCR assays for shorter fragments to infer the presence of less degraded DNA from PFPE tissues.

6. Methods: How many samples were from surgery and how many from biopsy? Were there any differences in these samples concerning amount and quality of obtained nucleic acids?

In the method section, we have clearly stated that all samples were from surgical specimens and all tissues cores were cutting into a standardized size in our study. We acknowledge that there will be variable amounts of NA within individual cores. Therefore, it requires statistical analysis (as we did) to demonstrate the improved NA quality of PFPE samples.

7. Methods/results: Was any IHC/ISH performed on PFPE samples?

The absence of histo-/immunohistochemical analyses in this study is an acknowledged limitation, which relates to resource availability for the study. We have included a paragraph acknowledging this limitation in the revised manuscript. From a practical perspective, in routine surgical pathology specimens obtained in pancreatic ductal adenocarcinoma, special stains are not typically required. Nevertheless, future study to further clarify the antigen preservation in tissues is desirable.

8. Another interesting observation regards MAPD. It is interesting that PFPE provide a stabler signal at NGS with AmpliSeq/IonTorrent technology, as high MAPD of FFPE DNA has been an issue for some time in that field. Did the authors find any difference in CNV reports from IonReporter of parallel samples extracted with PFPE vs FFPE? This would add a lot to the statement that PFPE could improve CNV detection in AmpliSeq/IonTorrent NGS.

We appreciate that the reviewer concurred that improved MAPD score of PFPE tissue is an interesting finding. We relooked into our NGS data, and unfortunately, there were no CNV detected in all 8 samples with NGS analysis. As we acknowledged in our original discussion, it would be crucial in future studies to confirm if improved MAPD metrics of PFPE would result in accurately CNV calling in tumor samples using amplicon-based targeted NGS assays.