Reviewer’s report

Title: A streamlined method for analysing genome-wide DNA methylation patterns from low amounts of FFPE DNA

Version: 0 Date: 20 Mar 2017

Reviewer: Mourad Assidi

Reviewer's report:

This is a very interesting study to optimize a genome-wide approach of methylation profiling using FFPE sections/rolls as a starting material. In fact, in the OMICs era and the international trend toward precision medicine, it is crucial to enhance the current protocols to benefit from the billions of FFPE blocs with full clinico-pathological data so far stored in hospitals and/or pathological departments. I believe that these type of technical and protocol refinement studies should be promoted. The study was well designed with clear objectives and steps. Except minor English revision required for the text, the manuscript was well written. However some comments needs to be addressed and answered properly in order to enhance the quality of manuscript and make it as a useful guide for future studies.

Major comments:

1. Please add a section about FFPE, the patients and patient consent? which disease? IRB? This is crucial for any study.

2. Please include a figure that summarize all your experimental design to make it easy for the reader to follow the sequence of experiments and their associated results.

3. In the introduction, please discuss in details the impact of formalin fixation on DNA quality either double or single strand and the possible ways to deparaffinize tissues. Make sure to use good references available so far in the literature.

4. Using the answer and details provided in 2), please justify the addition of xylene, ethanol and heating steps in your protocol prior DNA extraction.
5. Why only nanodrop was used to quantify the DNA yield after extraction and purification? Nanodrop is less sensitive compared to Qubit and mainly Bioanalyzer you used later on.

6. How to assess incomplete bisulfute conversion? Why authors did not used fresh versus FFPE tissues from the same patients?

7. I am not sure that 120 bp band is due to primers homodimers/heterodimers? please elaborate more on this.

8. Methylation profile may vary between genes and chromosomes due to several reasons. Why the MLH1 gene was used for your PCR-based test of bisulfite conversion efficiency? why not you assessed the methylation profile of repetitive elements? why not using a panel of genes with different positions on the chromosome and across the whole genome?

9. For RRBS library preparation and since this is a technical or protocol-based study, I recommend to put more details on the procedure of library preparation section although it is published elsewhere. This will make this manuscript more comprehensive and a reference document for subsequent studies.

Minor comments:

- Please revise the English of manuscript for very few mistakes or missing words (e.g. lines 17-18, ...)

- Include full name of abbreviations when first mentioned in the text since not everybody is familiar with RRBS for example.
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.

Not relevant to this manuscript

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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