Reviewer's report

Title: Quality assessment of DNA derived from up to 20 years old formalin fixed paraffin embedded tissue (FFPE) for PCR-based methylation analysis using SMART-MSP and MS-HRM

Version: 1 Date: 7 July 2009

Reviewer: Catherine Gratas

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Major Compulsory revisions

1) No data in fresh or frozen NSCLC tissues are presented to validate their methods in this type of tissue. This prevents comparison of their results with any other published data and of the efficiency of the techniques in paraffin versus frozen.

2) It is not precised if the histology and quality of the tissue (necrosis, % of tumoral tissue) was checked before extraction of DNA.

3) For a study in paraffin-embedded samples usually easily available, the initial number of samples is not sufficient: as all samples are not methylated, the number of tumors by groups to assess the reproducibility is then too small. Moreover many tumors have very low percentage of methylation.

4) For DNA study, DNA quality from paraffin-embedded is always a big issue. However in immunohistochemistry it is well known that the quality of the samples is good enough. A correlation between % of methylation and immunostaining of the protein in the same samples would reinforce the conclusion.

5) It is unclear to me if the triplicates are done at the level of the PCR or at the bisulfitation level. The reproducibility level should be studied on both levels.

6) The method of calculation of $2^{{\Delta\Delta CT}}$ is not well described. The formulae should be clearly presented.

7) The authors do not comment on the clinical relevance of very low percentage of methylation. Is it clinically significatif? They do not comment on samples with 0% methylation. Was the quality of DNA correct?

Minor revisions

1) The methylation CDKN2A (p16) and RARB genes is not well presented in the context of NSCLC. We have to wait until the end of the discussion to get one reference to this subject.

2) Some references are not well introduced in material and methods
3) Fig4: the non template controls do not appear clearly

4) For the SMART-PCR The authors do not explain why they used Alu primers instead of Col2A1 primers, used in the paper they refer to.

5) The e-mails of the authors are missing

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.