Reviewer’s report

Title: The application of Methylation specific electrophoresis (MSE) to DNA methylation analysis of the 5’ CpG island of mucin in cancer cells

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Reviewer: Long-Cheng C Li

Reviewer’s report:

In the revised manuscript entitled “The application of methylation specific electrophoresis to DNA methylation analysis to the 5’ CpG island of mucin in cancer cells”, Yokoyama et al. used both unmethylated and methylated DNA as controls and also revised some text. However the way the paper is presented and the method is described are still problematic to me and may make readers hard to follow their method.

Major comments:

1. I previously commented the following: “The authors should have given description about the principle behind the method, and how band patterns on the gel should be interpreted: What size of the PCR products are expected, what patterns of the bands are expected for methylated, unmethylated and partially methylated samples? In the revised paper, I still did not much improvement to address my comments. Because this is a method paper, those questions should be clearly spelled out to allow others to follow your method.

2. It appears to me that the only difference between MSE and bisulfite-DGGE is the use of nested primers. If that is the case, the new method does not really deserve a new term because it is only a very minor improvement. And it is also intuitive to do nested PCR to obtain enough products for electrophoresis.

3. The authors did not mention at all how the PCR primers should be designed, at least specify whether the primers should be bisulfite genomic sequencing (BSP) or MSP type of primers.

Minor comments

1. Full name for MSP should be used in line 21 of page 4
2. The paper should be further grammatically edited.

Level of interest: An article of limited interest

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.