Reviewer's report

Title: Quantitation of DNA methylation by melt curve analysis

Version: 2 Date: 12 January 2009

Reviewer: Yaguang Xi

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In the submitted manuscript entitled “Quantitation of methylation by melt curve analysis”, the authors provided a novel method to quantitate DNA methylation using the raw fluorescence melt data obtainable following PCR amplification of bisulfite modified DNA. The development of a rapid, simple, low-cost, convenient and reliable method to quantify methylation is certainly important. However, based on the accomplishments of the current version, the manuscript is not sufficiently satisfied to be accepted for publication in BMC cancer.

Major Compulsory Revisions:

The first criticism of this work is the authors did not clearly describe the principles of primer design. Only on Page 7, under the subtitle “PCR and melt analysis”, the authors mentioned the "tricks" of primer design. However, do we also need consider the length of primers and PCR products, the possibility of secondary structure and Tm value? The informative guideline of primer design is thought to be important to perform the melt curve analysis.

Since the method in this study is newly discovered, it is critical to create a comparison to other well-established methods (e.g. bisulfite sequencing, pyrosquencing or MSP). The results would illustrate the advantage of melt curve analysis if do so.

Colorectal carcinoma FFPE tissue has been used in this study for validation purpose, whereas the authors did not state the exact number of CRC FFPE samples that were examined. Also, the authors did not clarify if the replicates had been utilized. MGMT was the marker used in the validation of the method, but no secondary evidence indicated the status of MGMT in those samples. Hence, it is difficult to convince the reviewer that the measurement generated by the new method was solid.

Minor Essential Revisions: None.

Discretionary Revisions: None.

Level of interest: An article of limited interest

Quality of written English: Acceptable
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.