The manuscript from Dr. Kang provided an interesting multiplex bisulfite-sequencing technique which can be applied for hundreds of CpG sites methylation detection simultaneously. This technique provided a bridge between WGBS, RRBS and BSP as well as MSP. What’s more, Dr. Kang applied this technique detected the methylation status of ~250 CpGs in 46 cancer cell lines and identified large number of cancer specific DNA methylation biomarkers which would be valuable for relevant clinical biomarker research. I only have several comments to the manuscript as the following:

1, In the background, the authors are encouraged to introduce the latest progress on methylation biomarker research in cancers. Especially, there are some paper shown 5-10 DNA methylation biomarker could provide higher diagnosis. In your opinion or practice, how many biomarker combinations could provide enough sensitivity and specificity for cancer diagnosis?

2, In page 7, between 9 and 12, the most interesting questions is how to design the multiplex PCR primers and group them within in each subgroup. However, this information was exactly missed in the manuscript.

3, In page 7, between 21 and 25, I don’t understand why the methylation level of CpGs after SssI-treatment was 60%+, not 100%? It’s batch effect, specific region effect, enzyme effect or what reasons?

4, In page 8, between 9 and 19, it is quite hard to make conclusion that methylation status of MHLH1 and LIFR was correlated with the gene expression level since only two cell lines were selected. Now that, there are 46 cell lines in the study, all the samples should be detected and the more robust analysis should be provided.

5, In page 9, between 21 and 27, why only cancer samples were shown in Figure 6. What about the adjacent normal tissues from TCGA?

6, In page 10, between 10 and 23, it is a great discussion. However, the authors seem didn’t give further answers, even didn’t provide possible relevant reference in this field.

7, In page 11, between 8 and 20, it is quite interesting example, especially MLH1 promoter methylation. Did the author check any TFBS or potential TF interacted with DNA methylation? Why the methylation will influence the expression of MLH1 in this special case?

8, Figure 1, please add the basic information for these covered regions, CpGI or not? Meanwhile, Mean count can be replaced with average methylation levels (Beta)

9, Figure 2b, please change to density plot with more detail distributions of coverage for each depth.

10, Figure 3a, color key should be provided.

11, The ‘e’ among all the P-values should be changed to 10

12, The data should be uploaded to GEO.

13, Could the authors compare the present technique with these similar techniques, such as bisulfite padlock probes (BSPPs) sequencing, CpG tandems amplification and sequencing (MCTA-Seq)?