*Science Advances*

29 June 2019

Dear Dr. Jeremy Berg and Dr. Ali Shilatifard:

I am submitting the research article entitled “Circulating cell-free DNA based low-pass genome-wide bisulfite sequencing aids non-invasive surveillance to hepatocellular carcinoma” on behalf of all authors for consideration of publication in Science Advances.

Circulating cell-free DNA has been demonstrated to provide a promising opportunity for non-invasive cancer diagnosis, especially DNA methylation in circulating cell-free DNA which have been demonstrated in cancer diagnosis and tissue-of-origin mapping. We know that genome-wide DNA hypo-methylation is the hallmark phenotypes of human cancer genome and therefore can be applied in cell-free based cancer diagnosis. However, the amount of cell-free DNA is too limited for conventional high-depth/coverage genome-wide bisulfite sequencing (WGBS). In this original manuscript, we proposed a novel strategy to apply low-pass WGBS to monitor DNA methylation levels in cell-free DNA fragments. We developed a novel measurement of Long-region hypo-methylation to be the biomarker for cancer surveillance ranging from hepatitis, cirrhosis, early stage HCC and advanced HCC. We find low-pass based WGBS could provide stable and powerful diagnosis for HCC. Furthermore, the method provided a stable approach for surgery quality evaluation. We also found over-represented differential methylation CpGs based on low-pass WGBS data enriched in HBV integration regions which is the most important risk factors of liver cancer, indicating our method is suitable for HCC diagnosis and clinical decision-making with low-cost characteristic. HBV integration based DNA methylation regions (MethyHBV) showed better prediction performance. Finally, we also introduced cell-free DNA fragment size into the prediction model and we found methylHBV and cfDNAsize could provide powerful discriminating ability. Since the novel strategy and interesting findings, we believe the readers of Science Advances will find this manuscript highly interesting.

This manuscript has not been submitted elsewhere.

Thank you for your consideration.

Sincerely,

Steven J. Schrodi, Ph.D.

Center for Precision Medicine Research

Marshfield Clinic Research Institute

Marshfield, WI  54449

Tel: (715) 221-6443

Email: [schrodi.steven@mcrf.mfldclin.edu](mailto:schrodi.steven@mcrf.mfldclin.edu)

Computation and Informatics in Biology and Medicine

University of Wisconsin-Madison

Madison, WI, 53706

Email: [schrodi@wisc.edu](mailto:schrodi@wisc.edu)

Dake Zhang, Ph.D.

Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics

Chinese Academy of Sciences, Beijing, 100101,

Tel: (xxx) xxx-xxxx

Email: [dakezhang@gmail.com](mailto:dakezhang@gmail.com)

Changqing Zeng, Ph.D.

Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics

Chinese Academy of Sciences, Beijing, 100101,

Tel: (xxx)-xxxx-xxxx

Email: [czeng@big.ac.cn](mailto:czeng@big.ac.cn)