

Author's Response To Reviewer Comments

Reviewer reports:

Reviewer #1: Re-review on manuscript

BS-virus-finder: virus integration calling using bisulfite-sequencing data

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by Lada A Koneva, PhD

Introduction

The reviewed manuscript (BS-virus-finder: virus integration calling using bisulfite-sequencing data) describes developed software to detect viral integration breakpoints in whole-genome bisulfite-sequencing data (WGBS).

The manuscript was improved according to my suggested concerns and questions, but there are still some questions.

Questions

As a consequence of choosing HBV for simulation and PLC/PRF/5 hepatocellular carcinoma cell lines (which harboring HBV) as a real data, there is still unanswered my question about how this algorithm will work in case of HPV integration. There are about 170 types of HPV, with high commonality between different types, and about 15 types are carcinogenic. How authors suggest creating a "hybrid reference that contains both human genome and virus sequences" in case of HPV? Which viral types could be chosen for alignment of assembled clipping regions in case of HPV contamination? Probably this algorithm could not accurately predict which type(s) of HPV contaminates the sample. It would be useful if authors could provide their thoughts if this algorithm also will work in case of virus like HPV or this should be restricted to analysis of HBV integration only.

Although most programs input only one virus sequence, BSVF users are allowed to set their own multiple virus sequences. For each custom virus genome, BSVF constructs a hybrid reference by combining the virus sequences and human reference. For viruses with large number of strains, such as HPV, to get more meaningful results, we suggest users select some representative sequences from highly similar (such as >90% similarity) sequences.

How many reads were simulated in each simulation scenario? Did the authors vary the sequencing depth in the simulations?

We simulated all possible reads base by base with selected insert size in each scenario, and the number of reads in each scenario are not equal. The varied depth and the number of reads were listed for each scenario in updated Supplementary Table 6.

Minor

1. Section "Description in silico and real data": authors mentioned that simulated reads were selected from chr 18 of GRCh38 and then next sentence "Input fragments were selected from chr

18 in the GRCh37 assembly (hg19) of the human genome". Please, correct this if it's your typo, or clarify why different versions of reference genome were used for simulation.
All data used in the manuscript were based on simulation of chr1, although we have also simulated chr18. Thanks. We have revised the text.

2. Also they are still using the word "stimulated" instead of "simulated". And in the section "Availability of supporting data" the chromosome 1 was mentioned as chosen for simulation. Please correct it. Also typo on page 14-15: "We stimulated three kinds of reads, PE50, 90, and 50" should be 150.

As the answer to the above question, all descriptions in the manuscript were revised to chr1. Thank you, we have also revised the typo.

3. Legend to Figure 1: The sentence "Methylation sites were showed as read bases" do you mean "red" bases?

Thank you for your comments, we revised the typo.

4. The information for which virus was used for the simulation of the viral integration is mentioned only at the end of the manuscript (Availability of supporting data). Please provide this information in section "Description in silico and real data".

Thank you for your comments, we revised the text based on your suggestion.