# 1. Basic Reporting

# 2. Experimental design

# 3. Validity of the findings

# 4. General comments

# 5. Confidential notes to the editor

# Notes

1. After some quick search, I find no existing publications on the effect of FASTQ read orders on SV calling for long-reads. I think this is a non-trivial question and an important sanity check. I'm glad someone is doing it and sharing the results. I do believe that these findings hold value for the field.
2. Personally, I have some concerns regarding the data presented in the article to support the main finding that "**the order of input data had a large impact on SV prediction**" (Line 22, Line 142).
    a. For Table 1 and Figure 1, The criteria for matching/consistent VCF entries appear to be overly stringent ("**All VCF fields, except for the variant ID, were required to be equal in both the original and permutated SV calls to be classified as intersecting**."). I don't feel comfortable drawing any major conclusions based on this criteria of intersecting.
    b. (Con.) Even with this stringent criteria, I still don't think the data sufficiently support the language enough. Figure 1 demonstrates that the effect is dependent on the SV caller and large only for pbsv.
    c. The criteria used for Figure 2 and Table 2 is already stringent enough. And I would recommend basing all the major conclusions on this intersecting criteria.
    d. (Con.) This will make the finding less shocking and more of something like "**the order of input data has some but limited impact on SV prediction**." And this will be more in-line with people's expectation and thus less "ground-breaking", but I think the results are just as valuable.
    e. I would recommend moving Table 1 and Figure 1 to supplementary, but I will leave that to your own discretion.
3. Related to the last point, I think Figure 3 should use the matching criteria used in Figure 2 instead of Figure 1.
4. It is not clear which BAM sorting methods were used in Figure 1 & 2 and Table 1 & 2. Clarifying this information would enhance the paper's clarity.
5. Regarding lines 167-168, it is not clear which two elements are being referred to as identical. The clarification of this point is crucial as it directly impacts the core findings of the paper. For example, it could be "The discrepancy between the sorted BAM files from the original and permuted FASTQ files

using SAMtools was also observed when using Picard for sorting" or "The Picard-sorted BAM file from the original and permuted FASTQ files was found to be identical."

6. (If you want to add a little more to the paper) Within the 15 samples, one of the samples is the reference. Compare it to the rest of the samples would be a good sanity-check and yield some useful insights. It is expected to have less SVs. Will it also have a very different intersecting to non-intersecting SV ratios?
7. Line 168: “aligned using Picard”. Probably a typo?
8. As a new reviewer, please consider my comments with your own discretion. Thanks a lot.