Oxford, 30th January 2021

**Re: Letter submission to Nature Biotechnology**

Dear Editors,

We wish to submit a research manuscript entitled "Benchmarking small-variant genotyping in polyploids" for consideration by Nature Biotechnology. This work is original and has not been published elsewhere, nor is it currently under consideration for publication.

In this manuscript, we provide the first substantial evaluation of polyploid genotyping from sequencing. In particular, we establish a comprehensive and easily reproducible benchmarking pipeline using synthetic data derived from gold-standard Genome-in-a-Bottle human reference data. We evaluate several prominent variant calling tools, including our own method – Octopus – that was recently accepted for publication in this journal (NBT-A46432C). Finally, we show our results give probable upper-bounds on genotyping accuracy in plant polyploids using concordance analysis from real triploid banana whole-genome sequencing data.

We believe that our manuscript is appropriate for publication by Nature Biotechnology because of the relevance of our work to the emerging technology of sequencing-based plant molecular breeding, a field that the journal has previously published works on - notably a 2019 review article that highlighted the importance of DNA sequencing to marker-assisted selection in crops[[1]](#footnote-1). Since genotyping is a fundamental step in these technologies, our analysis highlights current limitations, guides best practice on experimental design, and provides a foundation for improvement and validation. Furthermore, our work has impact outside of the field of plant genetics because polyploidy is known to occur in animals, including as a normal part of development in some human tissues[[2]](#footnote-2).

We have made our analysis fully open-source, and our benchmarking pipeline is easily reproducible due to being implemented entirely in Snakemake.

We look forward to your reply.

Sincerely,

Daniel Cooke, David Wedge, and Gerton Lunter

1. <https://www.nature.com/articles/s41587-019-0152-9> [↑](#footnote-ref-1)
2. <https://www.nature.com/articles/s41575-020-0284-x> [↑](#footnote-ref-2)