**Simulations**

To verify the reliability of SV calling pipeline, SVs were simulated from two reference genomes of BTx623 (Paterson et al., 2009) and Rio (Cooper et al., 2019) via R package RSVSim (version 3.10) (Bartenhagen, 2018) in R (version 3.5.1). Four types of SVs including deletions, insertions, inversions, and duplications were simulated for 2,500 SVs each with the size ranging from 1 to 10,000 bp distributed randomly across ten chromosomes. The SV-simulated FASTA of each genotype was then simulated to FASTQ sequence reads via WgSim (Li, 2011) with 125 pb read length and three coverage depths (20, 100, and 200X). These simulated FASTQ sequences were proceeded through the pipeline of SV calling via lumpy. The SVs with imprecise flags, depth less than 10X and pe < 3 were removed. The called SVs were compared and determined how many overlapped with the simulated SVs (true positive), simulated but not detected by the caller (false negative), and not simulated but detected by the caller (false positive).

**The read coverage at 20X was acceptable to call SVs from the simulation.**

To determine how reliable the SV calling pipeline performs at different depths (20X, 100X, and 200X), the simulations of SVs were conducted on two reference genomes of BTx623 and Rio. In both genotypes, 20X depth, which is the average depth used in BAP, called only 2558 and 2706 SVs but overlapped with 9471 and 9602 with 0.79 and 0.84 sensitivities, 0.78 and 0.79 accuracy, and 0.77 and 0.75 precision in BTx623 and Rio, respectively (Supplemental table 8). Expectedly, when increase the depth to 100X, the total SV calls, sensitivities, and accuracies increased from 20X, but little decreased in precision. However, 200X performed unprecedentedly that the BTx623 dropped in total SV calls, sensitivity, accuracy, and precision even lower than at 20X. Whereas, Rio at 200X did not improved from 100X but still better than 20X. This suggested the optimal depth for future study with this pipeline should have the coverage at least 100X for better sensitivity and accuracy.

The simulation suggested increasing coverage can improve both sensitivity and accuracy. The higher coverage were proven to increase sensitivity at low coverage (<10X) (Zhang et al., 2012) and at 40X (Suzuki et al., 2011; Xu et al., 2017). The other simulation also showed a sharp improvement in sensitivity 20X to 90X and reach plateau after 120X (Wilson-Sánchez et al., 2019). However, at low coverage simulation, there was no improvement in accuracy when the coverage increased (Zhang et al., 2012).

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