

## APPLYING GENOTYPING BY SEQUENCING TO PEACH BREEDING GERMPLASM FROM EMBRAPA

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Genotyping by sequencing (GBS) is a multiplexed, high-throughput, and low-cost approach that allows simultaneous SNP discovery and genotyping. In the present study, we explored suitability of GBS as a genome scan in a panel with 220 peach cultivars and advanced selections from Brazilian Agricultural Research Corporation (EMBRAPA) peach breeding program. This selected panel includes only accessions belonging to *Prunus persica* with contrasting phenotypes for bacterial spot, brown rot and tolerance to abiotic stresses such as heat tolerance at the flowering stage and chilling requirement. Genomic DNA was extracted and normalized to 10 ng/μl for subsequently library preparation. 96-plex libraries comprising each 95 peach DNA samples and a negative control (no DNA) were *ApeKI* restricted, ligated to barcoded adaptors, pooled and amplified for multiplex sequencing on the Illumina HiSeq 2000 platform. Default parameters of Tassel 4.0 pipeline software were used. This method produced 274 MB of sequence in total, with on average 1.3 million sequence reads per genotype, and majority of the accessions having more than 500,000 reads. We identified 18,914 single-nucleotide polymorphism (SNP) markers, with minor-allele frequency (MAF) > 0.05, present in at least ≥ 80% analyzed accessions distributed evenly across the entire peach genome. When data filtered to show only markers present in all accessions, 4,268 SNPs were identified. The high quality SNPs obtained will enable better understanding and utilization of variability and population stratification in the available germplasm, and provide basis for genome-wide association studies (GWAS) for valuable traits in peach.

Keywords: *Prunus persica*; GBS; Next-generation sequencing; SNP diversity.

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