Feedback on tGBS with Allegro : Does it hit the target?

SNP genotyping is widely used as a 'fairy dust', both for research and breeding for diversity and genetic association studies, building genetic maps, confirming varieties identification, detect QTL and genomic selection. However, species diversity in term of genomic features (such as genome size, ploidy, heterozygosity) challenges the emergence of new and useful approaches. Chip arrays are considered as a "gold standard" but show limits in flexibility and associated costs. In addition, they return information on target SNP only, masking potential useful diversity around the target. We recently implemented a Targeting-By-sequencing (tGBS) approach, namely the Allegro Targeted Genotyping v2 (TECAN).

IGBS Workflow : from the SNP panel design up to genotyping.

- Three parts construction of targeted SNP panel between geneticians, TECAN and EPGV.
- TECAN protocols with EPGV adaptations (optimized normalisation and PCR amplification).
- STAMPS developed at EPGV with integration of ProbeFilter tool of Interval Bio (Carey et al. knox@interval.bio https://interval.bio/allegro.html)
- Genotyping matrix and experimental report (SNP coverage, missing data, reproducibility).
- Specific analysis (Frequencies analysis, haplotypes construction...)

Target and de novo SNP discovery.

Conclusions

SPET is a powerful flexible genotyping solution (from 1000 SNP up to 100 000, and 192 to 3 072 samples in one experiment) for organisms with reference genome or not.

The collaboration between geneticians, TECAN and EPGV leads to specific probes design illustrated by the high percent of bases alignment on the targeted regions. The P. viticola exception is potentially due to the lower specificity of SE mapping regarding to the PE mapping.

The targeted SNP coverage variability obtained, had no impact on the genotyping success rate. And a good sample repeatability was observed within the same experiment. According to our comparison results between genotypes from IGSB and available SNP array considered as golden standard, we can inferred that IGSB could be an effective alternative to SNP array.

Moreover, the SNP de novo discovery allows haplotype reconstruction that could be useful to limit ascertainment bias. Additionally, the tGBS workflow can be adapted to genotype structural variations.