**Application of Genotyping-By-Sequencing in Crosses of Heterozygous Grapevines: Tools for Map Construction and Marker-Trait Association Testing**

Next generation sequencing in heterozygous mapping families brings both opportunities and challenges: larger and cheaper SNP sets are easier to produce, but prone to genotyping error and harder to handle as current software is not designed to process thousands of pseudo-testcross markers.

We used Genotyping-by-Sequencing (GBS) and the *Vitis vinifera* ‘PN40024’ 12X reference genome to generate more than 40,000 SNPs from 85 F1 segregant of the cross of *Vitis rupestris* B38 and *V. vinifera* ‘Chardonnay’. Using simple filters such as minor allele frequency, linkage disequilibrium, and physical distance we selected a subset of molecular markers suitable for creating genetic maps in JoinMap 4.1. We obtained 19 linkage groups for each parental map with good coverage and correlation with the reference genome. Moreover, we were able to determine positions for SNPs tagged to unassembled portions of the 12X reference genome.

In conjunction with this genotyping effort, several large trait datasets are being generated within the VitisGen project, a USDA-NIFA SCRI funded research collaboration aimed to accelerate grapevine cultivar improvement.  We employed GBS-derived SNPs and TASSEL 4 to estimate heritability using kinship matrices and to identify single-marker associations for qualitative and quantitative traits phenotyped within F1 families of the VitisGen project.  The results obtained using TASSEL 4 were compared with segregation analysis and QTL mapping. Taken together, these analyses illustrate the successful application of the GBS method to identify marker-trait associations in heterozygous mapping families.