

## **Reduced-Cost Genotyping in Peanut Breeding Programs Using Genotyping by Resequencing**

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Next-generation sequencing (NGS) has been used broadly for genomic analysis. NGS usually generates thousands of single nucleotide polymorphism (SNP) markers and can help identify useful markers that benefit some genomic projects such as genome-wide association studies (GWAS). However, the number of SNPs generated from NGS data are far more than needed for most breeding programs, instead, breeders focus on the use of hundreds of polymorphic molecular markers for analysis. One limiting factor is that the statistical power is decided more by the population size instead of the number of markers. When the breeding population is large, the cost of genotyping could become too high. Therefore, developing a more economical genotyping system of marker analysis in breeding populations can help make use of molecular markers a routine tool for breeding programs. In this research, we used previous NGS data, including SNP Chip, RAD-Seq, transcriptome, WGS, and other sources. Then we extracted the sequences flanking each SNP for BLAST against the Tifrunner reference sequence and searched for homoeologs and/or paralogs. Our goal was to select the true SNPs that are distinguishable from their homoeologs and paralogs. Selected SNPs polymorphic among different tetraploid accessions were sent for designing custom probes using the Tecan Allegro system. We have received 5,154 probes to detect 2,770 SNP targets, and are testing these on 48 selected accessions, which include some closely related sister lines as well as other accessions. An additional 144 accessions will also be tested afterwards. We expect to identify some SNP markers that are polymorphic among closely related genotypes and breeding populations. The information of markers we designed will be uploaded for use by the peanut community, where the data can be used for genotyping by resequencing for breeding programs with reduced cost.