

Cost-Effective Genotyping by Resequencing Using Tecan Allegro Targeted Genotyping V2

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Genotyping has been used broadly for years in multiple scientific programs to provide DNA information by detecting sequence variations. These genetic variations can be designed into molecular markers and are useful for linkage mapping and genome-wide association studies. Traditional ways of genotyping use molecular markers by running gel electrophoresis, which is now considered low-throughput due to its limitation of sample numbers per run, the need of larger amount of DNA, extra time, and effort. Recent years, it has been popular to use high-throughput approaches such as next-generation sequencing (NGS) and single nucleotide polymorphism (SNP) chip DNA microarray for generating thousands of SNP markers. However, it could be expensive for labs that require frequent use as a routine tool, such as breeding programs that need to perform genotyping on large populations with hundreds of individuals. We aim to develop a cost-effective genotyping system by using Tecan Allegro Targeted Resequencing V2 kit. It provides customized probes design, which indicates that all the DNA fragments synthesized are known targets. In one of our peanut projects, polymorphic fragments that are distinguishable from possible homologs were designed into 5,154 probes for 2,770 SNP targets. This can help increase the rate of true SNPs with reduced chance of misalignment due to short reads, poor quality SNP calls, or low read depth. One of the projects in NCSU were done by using this kit with around 15K probes, and the results showed that 79 percent of targets were recovered. The kit is compatible with Illumina HiSeq, MiSeq, NextSeq, and NovaSeq, and can help lower the price from \$28 (SNP chip and GBS) to \$15. This kit has been used for many different species; besides peanut, TAMU has used in for rat, turkey, and cotton projects with customized probes. The information of validated target primers from our project will be public for the peanut community to use and become useful tools for peanut breeding programs.