# High-Throughput Detection of T-DNA Insertion Sites for Multiple Transgenes in Complex Genomes

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**Project Goals:** Integrating multiple transgenes into elite lines can be a time consuming and labor-intensive process. Generation of stable homozygous lines often requires multiple generations and screening of large numbers of plants especially in polyploid species. Our goal was to develop a fast, high-throughput method to identify transgene insertion sites in the T1 generation that accelerates identification of stable lines with the desired numbers of transgene copies.

**Abstract:** Genetic engineering of crop plants has been successful in transferring traits into elite lines that could not be achieved with breeding techniques. Introduction of transgenes originating from other species have conferred resistance to biotic and abiotic stresses, increased efficiencies and modified developmental programs. Many of these traits focus on herbicide and insecticide resistance and are widely used as single gene traits while genetic engineering of new pathways often require multiple genes and regulators. In recent years, the development of resistance to pesticides in wild species has driven companies to combine transgenes for different pesticide resistances into crops via gene stacking. However, generating stable homozygous lines with multiple transgenes requires selection over several segregating generations and therefore is time consuming and labor intensive, especially if the crop is polyploid. Insertion site effects and transgene copy numbers are important metrics for commercialization and trait efficiency.

We have developed a method based on a genome-walking PCR approach and demonstrate how it can be easily adapted for high-throughput screening of multiple lines and transgenes at a time using a short-read sequencing platform. Sites identified via HT-sequencing could then be used to design screening primers to test for zygosity of subsequent generations at every locus and also enable segregation of irregular or unidentified insertions. The overall process of identification of transgene insertion sites is accessible, as it involves commonly used laboratory techniques.

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