Location and Timing of T-DNA Integration Events Identified Using a Next Generation Sequencing Strategy

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The standard approach to genetically modify plants relies on *Agrobacterium tumefaciens* to transfer foreign DNA (T-DNA)into plant cells where it can become a permanent part of the plant cell's genome and express engineered traits. While *A. tumefaciens* transformation of plants has been used extensively, there are aspects of the process that are incompletely understood. To study the timing and factors influencing the location of T-DNA insertions, we used a modified adapter ligation-mediated PCR strategy, coupled with next generation sequencing, to identify T-DNA integration sites into the genome of Arabidopsis (O'Malley, 2007). Previous reports examining T-DNA integration have relied on selective conditions, floral dip transformation, artificial virulence induction or use of cultured suspension plant cells. Our approach attempts to closely match natural infection conditions by using cut *Arabidopsis* root segments infected with uninduced *A. tumefaciens* and no selection for TDNA integration events. Using this approach we have identified over 1,000 integrationsites, and detected events as early as six hours post-infection. We observe integration events occurring throughout the Arabidopsis genome. Interestingly, protocols relying on the expression of selectable markers report infrequent T-DNA integration into pericentric regions, while we detect integrations in these regions at a similar frequency to nonpericentric regions, suggesting that many T-DNAs are silenced by the host. We also see differences in T-DNA integration in relationship to DNA methylation and nucleosome position, relative to insertions identified using selection.A more thorough understanding of T-DNA integration will guide future experiments to develop the techniques to engineer plants more efficiently than is currently possible.

O'Malley R.C., et al. An adapter ligation-mediated PCR method for high-throughput mapping of T-DNA inserts in the *Arabidopsis* genome. *Nature Protocols*. 2007; 2(11): 2910-7.