

### Transposon Tagged *Medicago truncatula* Lines

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Legumes constitute one of the largest crop families and stand second only to grasses in economic importance to mankind. Over the past decade, *Medicago truncatula* has been selected as one of two model legume species to study legume functional genomics. Retrotransposon tagging is preferred for plants with large genomes as their replicative transposition results in multiple stable insertions per genome. We used the tobacco retrotransposon *Tnt1* to tag the *Medicago* genome and generated over 7,600 independent lines representing an estimated 190,000 insertion events. *Tnt1* inserted on an average at 25 different locations per genome during tissue culture and insertions were stable during subsequent generations in soil. Analysis of 2,461 *Tnt1* flanking sequence tags (FSTs) revealed that *Tnt1* appears to prefer gene-rich regions. The proportion of *Tnt1* insertion in coding sequences was 34.1% compared to the expected 15.9% if random insertions were to occur. *Tnt1*, however, showed neither unique target site specificity nor strong insertion hot spots although some genes were more frequently tagged than others. Forward genetics screening of 3,237 R1 lines resulted in identification of visible mutant phenotypes in approximately 30% of the regenerated lines. Tagging efficiency appears to be high as all of the 20 mutants we examined so far were found to be tagged. Taking the properties of *Tnt1* into account and assuming 1.7 kb for the average *M. truncatula* gene size, we estimate that approximately 14,000 – 16,000 lines would be sufficient for a 90% gene tagging coverage in *M. truncatula*. This is in contrast to more than 500,000 lines that would be needed to achieve the same saturation level using T-DNA tagging. Our data demonstrate that *Tnt1* is an efficient insertional mutagen in *M. truncatula* and could be a primary choice for other plant species with large genomes. Collaborative efforts are underway to generate a sizable number of tagged mutant lines in the next four years and also to develop a flanking sequence database for reverse genetics studies.