



APPLICATION OF TILLING FOR ORPHAN PLANT BREEDING

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➤ **What Is Tilling?**

- **Tilling (Targeting Induced Local Lesions in Genomes)** is a method in molecular biology that allows directed identification of mutations in a specific gene or allele. TILLING was introduced in 2000 (McCallum *et al.*), using the model plant *Arabidopsis thaliana*. TILLING has since been used as a reverse genetics method in other organisms such as zebrafish, corn, wheat, rice, soybean, tomato and lettuce.
- TILLING is a general reverse genetic technique that combines chemical mutagenesis with PCR based screening to identify point mutations in regions of interest. TILLING is a powerful technology that employed heteroduplex analysis to detect which organism in a population carry single nucleotide mutation in specific genes.
- McCallum utilized reverse genetic approaches such as T-DNA lines and antisense RNA, but was unable to successfully apply these approaches to characterize CMT2. The approach that was successful turned out to be what is now known as TILLING.
- TILLING can also be used to detect naturally occurring SNP in genes among the accession, variety or cultivar. To study the gene function, or to detect genetic marker in population.

Why Tilling??

- Tool for functional genomics that can help decipher the functions of the thousands of newly identified genes.
- To identify SNPs and/or INS/DELS in a gene of interest from population.
- Genetic mutation is a powerful tool that establishes a direct link between the biochemical function of a gene product and its role *in vivo*.
- Non transgenic method for reverse genetics.
- **The basic procedure**
- TILLING is straightforward; it involves 1) the creation of a large genetically diverse population of plants; 2) the high throughput identification of individual plants whose genotype predicts a phenotype of interest; and 3) the evaluation of these individuals' phenotypes for the accelerated development of novel cultivars that exhibit traits of interest.
- It combines random mutagenesis of seeds with an alkylating agent such as ethyl methanesulfonate (EMS) or other mutagenic agent with the targeted identification of induced alterations in the genes of interest.
- **Ecotilling**
- The first publication of the EcoTILLING method in which TILLING was modified to mine for natural polymorphisms was in 2004 from work in *Arabidopsis thaliana*.

- EcoTILLING is similar to TILLING, except that its objective is to identify **natural genetic variation** as opposed to induced mutations (Comai *et al.*, 2004).
- Many species are not amenable to chemical mutagenesis; therefore, EcoTILLING can aid in the discovery of natural variants and their putative gene function
- This approach allows one to rapidly screen through many samples with a gene of interest to identify naturally occurring SNPs and / or small INs/DELS.
- **Advantages**
- Its applicability to virtually any organism.
- Its facility for high-throughput and its independence of genome size, reproductive system or generation time.
- Since it uses Chemical mutagenesis virtually all genes can be targeted by screening few individuals.
- High degree of mutational saturation can be achieved without excessive collateral DNA damage.
- Eco- TILLING is useful for association mapping study and linkage disequilibrium analysis.
- Ecotilling can be used not only to determine the extent of variation but also to assay the level of heterozygosity within a gene.

Perspective

- TILLING and EcoTILLING have been proven to be highly effective reverse genetic tools for functional genomic studies in plants and animals. Since the inception of these techniques, many researchers have gained indispensable insight on gene function and have identified natural and induced variants.
- These methods are now well established for many model plant and animal systems regardless of their mating system, genome size, or ploidy level. TILLING is one of the few reverse genetic applications that has not been proven to be applicable in a species specific manner unlike other approaches (i.e.-RNAi or homologous recombination), which potentially makes this application available for all species.
- We demonstrate that high throughput TILLING is applicable to maize, an important crop plant with a large genome but with limited reverse genetic resources currently available.
- The main limitation for TILLING is that the species is capable of being mutagenized. Therefore, for ethical reasons TILLING should not be employed for analyzing functional genomics in humans.

References

- McCallum, C. M. (2000).** Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiology* **123**, 439-442.
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