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Tilling A Reverse Genetic Approach For Identification And Mapping Of Genes

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Introduction

Genome sequencing projects have identified a large number of genes whose function remains unknown and many techniques have been developed to enable researchers to identify gene function using knock out mutations in the known sequence. Many gene functions have been defined through the use of forward genetics, where a phenotype is identified and used to clone the gene responsible. However, in most instances, genes of known sequence are not associated with a phenotype. This is particularly true in non-model species where forward genetics can be more challenging due to genetic redundancy. Reverse genetics is a powerful tool to identify the phenotype that results from disruption of a specific sequence, even with no prior knowledge of its function. TILLING (Targeted Induced Local Lesions in Genomes) is a non-transgenic reverse genetic technique that identifies primarily random point mutations of missense and truncation mutations created by chemical mutagens. TILLING is a powerful technology that employed heteroduplex analysis to detect which organism in a population carry a single nucleotide mutation in specific genes.

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 the population.
- A 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

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Thus with only a small population, multiple alleles may be obtained regardless of the size of the gene. Molecular screening for point mutations was performed by TILLING PCR aided CEL1 mismatch cleavage. Gene regions are targeted for mutation discovery using PCR and standard SNP discovery methods (Greene *et al*., 2003).

Chemical mutagenesis

Chemical mutagenesis was used to generate populations of mutants for forward genetics long before the advent of DNA sequencing and reverse genetics. Point mutations are, generally, less deleterious than large rearrangements and so a high degree of saturation can be achieved in a mutant population using chemicals that generate single base pair changes or small insertions and deletions. This approach is, therefore, useful for the examination of gene function using genome-wide approaches. Two chemicals, in particular, are known to cause primarily single base-pair mutations in DNA in all organisms in which they have been tested: Ethyl methane sulphonate (EMS) and Ethyl nitrosourea (ENU). EMS is a highly mutagenic substance that alkylates guanine bases favouring the placement of a thymine residue instead of a cytosine residue by the DNA-polymerase opposite to the O-6-ethyl guanine in the subsequent DNA-replication step, resulting in a random point mutation wherein G-C base-pairs (bps) are switched to A-T pairs (*Comai and Henikoff*, 2006).

While many reverse genetics techniques provide only loss-of-function alleles, chemical mutagenesis can result in either loss-of-function, reduction-of-function, or gain-of-function phenotypes. The frequency of induced missense alleles is, on average, three times higher than that of nonsense alleles. Many missense alleles will not affect gene function since they may not alter the gene product(s) significantly, but examples of dominant point mutations caused by missense alleles have been well documented, including ones that affect plant hormone responses, leaf polarity and host-pathogen defense. The difficulty with using point mutations for reverse genetics screens is that there are few cost-effective ways of screening the mutagenized population for individuals that carry mutations in specific genes.

TILLING Methodology

Generally, in this procedure, the mutagenized generation (M_1) is grown up and then the progeny of these plants (the M_2 generation) were used for screening. This ensures that the mutations that are identified in this process are heritable and eliminates the background somatic mutations that may be present in the M_1 generation.

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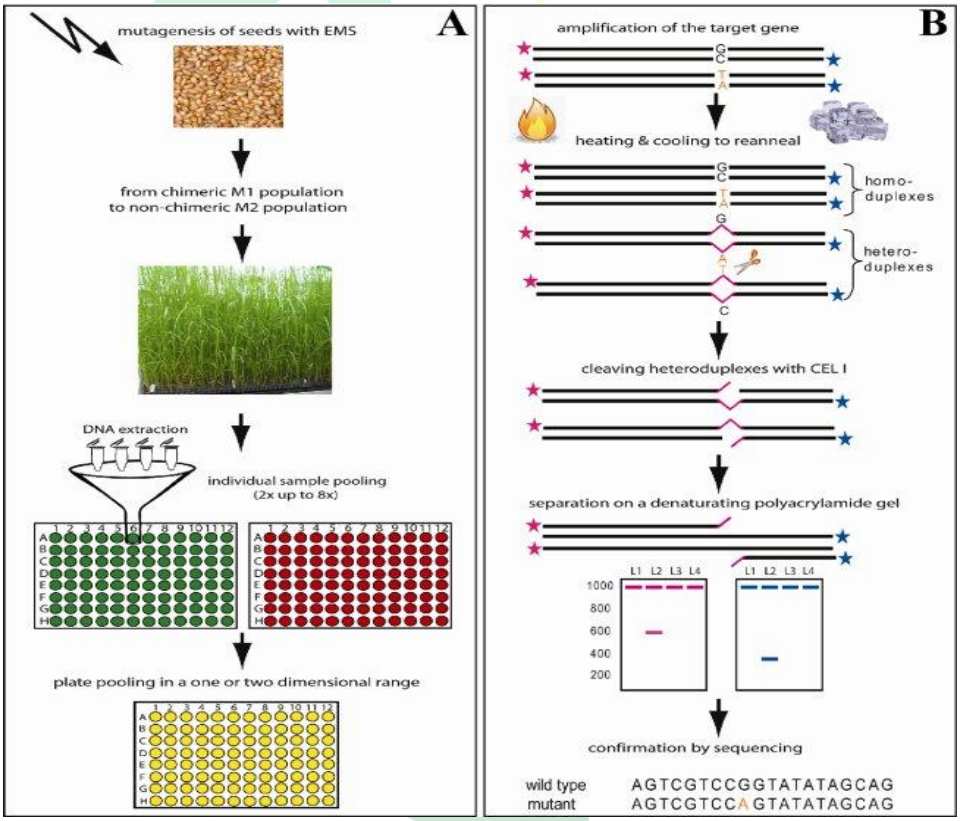
After collecting seeds and DNA from the M_2 plants, the DNA from several mutagenized individuals is pooled, and then the polymerase chain reaction (PCR) is used to amplify a target gene of interest. In conventional TILLING, the PCR products (amplicons) are denatured and allowed to randomly re-anneal before being digested with a celery juice extract (CJE). Mismatches in the amplicons occur when mutant and wild-type strands of DNA are re-annealed together to form a heteroduplex. This heteroduplex then becomes a target for the mismatch-specific enzyme. Only the samples carrying a mismatch are cleaved, and these novel fragments can be detected using DNA separation technology such as the LI-COR DNA Analyzer, or AdvanCE F96 (Advanced Analytical Technologies). Identification of the mutant individual and Sequencing of the Mutant PCR product (*Slade and Knauf*, 2005).



Advantages:

- Its applicability to virtually any organism.
- Its facility for high-throughput and its independence of genome size, reproductive system or generation time.

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- Since it uses Chemical mutagenesis virtually all genes can be targeted by screening a few individuals.
- A high degree of mutational saturation can be achieved without excessive collateral DNA damage.

Conclusion:

The advent of TILLING, New Generation Sequencing (NGS), and High-Resolution Melting (HRM) analysis, however, have made possible the screening of large populations, at a reasonable cost, within an acceptable time frame. TILLING operations use a variety of techniques for creating mutant populations and screening them, which employs a mismatch-specific endonuclease for identifying point mutations in the target gene of interest. Chemical mutagenesis is an effective approach to generate mutants with altered agronomic traits for genetic studies and to predict the gene function through the identification of an allelic series by TILLING.

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