

A new method for detection of induced mutations in wheat

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As more and more plant genome sequence become available, reverse genetics is proven to be useful and feasible in plants to explore gene function and identify desired mutations for breeding. TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics tool that combines traditional chemical mutagenesis methods and high-throughput PCR based mutation detection techniques to yield an allelic series of point mutations in a gene of interest. Wheat is a hexaploid crop with a large and complex genome. It has much less genome sequence information than *Arabidopsis* and rice. It is sometimes difficult to design allele-specific PCR primers in a much conserved region (high homology between three homoeologous genes) or in a gene lacking intron information. Here we describe a new mutation detection method which combines High Resolution Melting (HRM) and sequencing analyses using Mutation Surveyor software. The method is sensitive enough to detect a heterozygous SNP in a PCR amplicon containing 3 homoeologous gene fragments. Therefore it can be used for screening three homoeologous genes simultaneously, especially in a conserved functional domain or some EST sequences. This method can also be used for SNP marker development and eco-TILLING.