

[TILLING (Targeting-Induced Local Lesions in Genomes)]

TILLING is a method for detecting single-nucleotide polymorphisms (SNPs) in genes within a genome, which enables high-throughput selection of target-gene mutants.

(1) Wild-type genomic DNA is mixed with genomic DNA derived from mutants containing single-nucleotide substitutions, and the mixture is used as a PCR template. Three types of amplified products, i.e., wild-type double-helix DNA (2), mutant double-helix DNA (3), and heteroduplex DNA (4), are obtained from PCR. Mismatches occur at SNP sites in the heteroduplex DNA. SNP sites are cleaved using the CEL1 enzyme, which recognizes and cuts specifically at mismatch sites.

(5) SNPs can be confirmed through agarose gel electrophoresis of the resulting DNA fragments. A figure below shows that a SNP occurred between the 200-bp and 700-bp amplified fragments.

