26. A modified TILLING system for rice mutant screening

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The TILLING (Targeting Induced Local Lesion in Genome) system is one of the most efficient methods to screen mutants in a large-scale mutant population (Till et al. 2003). The original TILLING system is composed of PCR amplification of interest genes with fluorescence-labelled primers, CELI digestion and a LI-COR gel electrophoresis. However, these steps take a fair amount of labor and time. To simplify and reduce these steps, we have modified the methods and reconstructed another efficient way for high-throughput TILLING. The new system is modified in two major steps; one is replacement of fluorescence primers with non-labelled primers in PCR amplification, and the other is a use of capillary gel electrophoresis. The electrophoresis is carried out by the HDA-GT12 (eGene corp., USA) that can separate DNA fragments below 2 kb within eight minutes.

The modified system could detect SNPs at any DNA regions examined between Indica and Japonica varieties. All SNPs could be detected clearly after amplification followed by digestion with CELI as two to four DNA fragments (Fig. 1). SNPs could also be detected using mixed genome DNAs of the Japonica and Indica varieties at 11:1 ratio (Fig. 2). This indicates that presence of one heterozygous mutant in a pool of six plants is sufficient to detect SNPs by the non-fluorescence TILLING. The modified TILLING method was then applied for mutation screening of an MNU-induced mutant population. We used over 700 M2 mutant lines in which six individuals were pooled for screening of mutations in a 600 bp coding region of a known gene. The first screening detected 10 candidate mutant lines. We examined sequences of six individual plants of all candidate lines and identified mutations in six lines. Additional screening and identification are now in progress and the results obtained so far showed that the mutation ratio per 1 kb region would be around 0.8 % for this mutant population. From these results, we can expect about eight different mutations for every 1 kb genome sequence in 1000 mutant lines. The mutant population with this high mutation ratio should serve as a promising TILLING resource and reverse genetic studies in rice.

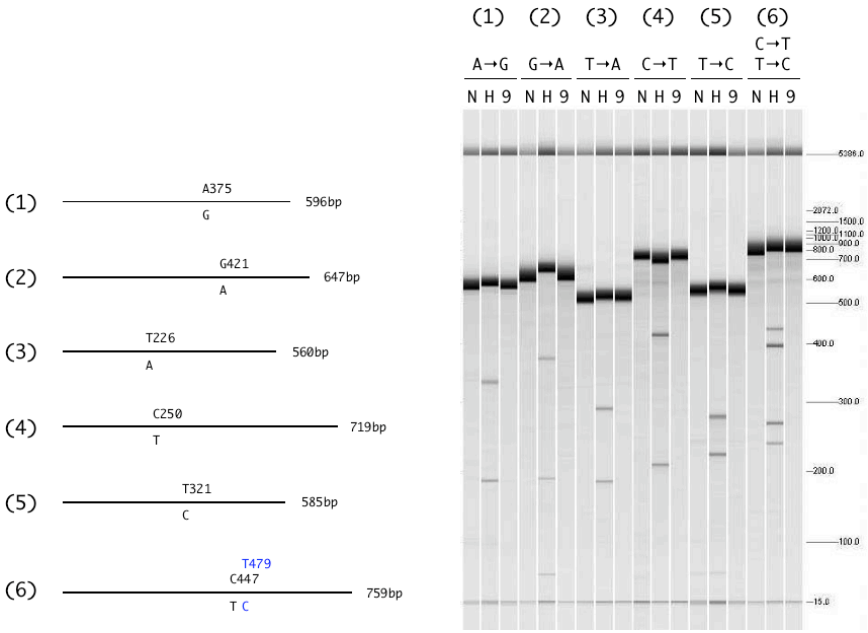

Fig. 1. Detection of six SNPs between Japonica and Indica varieties. Fragments of indicated bp length were amplified with specific primers (left panel), digested by CELI after denaturation and annealing, and electrophoresed in an HDA-GT12 capillary gel apparatus. The amplified fragments were digested at the SNP positions indicated in the left figures. Nucleotides above the DNA bars are Japonica (Nipponbare: N) and below bar are Indica (93-11; 9) at the indicated nucleotide position. Right panel shows pseudo images of electrophoresed DNA fragments. Appearance of clear fragments of expected length in the right panel revealed correct recognition and digestion of the DNA fragments at the SNP positions. N; Nipponbare, 9; 93-11, H; Mixed N and 9 by 1:1.

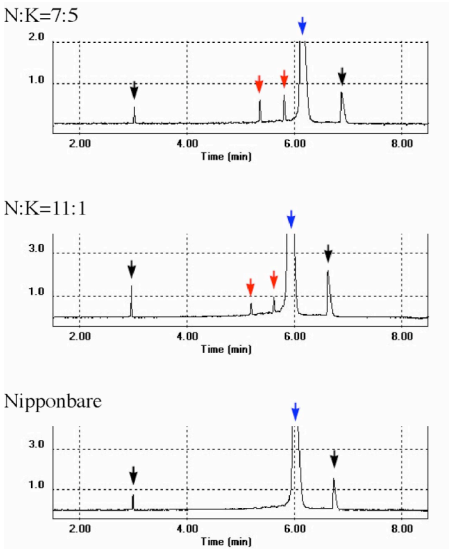

Fig. 2. SNP detection between Nipponbare (N) and Kasalath (K) genomes. A 1002 bp fragment (blue arrow) was amplified and applied for SNP detection by capillary gel electrophoresis. A SNP at 433rd base was digested and two fragments of 432 and 569 bp long (red arrows) were detected. The genome DNAs of N and K were mixed at the ratio of 7:5 (top), 11:1 (middle) and 1:0 (bottom). Black arrows are marker DNAs of 15 (left) and 5386 bp (right).

Reference

Till, B. J., S. H. Reynolds, E. A. Greene, C. A. Codomo, L. C. Enns, J. E. Johnson, C. Burtner, A. R. Odden, K. Young, N. E. Taylor, J. G. Henikoff, L. Comai and S. Henikoff, 2003. Large-scale discovery of induced point mutations with high-throughput TILLING. Genome Res. 13: 524-530.