36. Application of FTA Card technology for identification of transgenes in transgenic rice

M. HAKATA[1, 2)], H. NAKAMURA[1, 3)], M. KAJIKAWA[1)] and H. ICHIKAWA[1)]

1) Genome Resource Center, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602 Japan

2) Present address: Hokuriku Research Center, National Agricultural Research Center, 1-2-1 Inada, Joetsu, Niigata 943-0193 Japan

3) Present address: Department of Applied Biological Chemistry, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657 Japan

For large-scale analyses of gene structures and functions, a simple and high-throughput process is desirable to isolate genomic DNA. FTA Card technology (Whatman) allows the easy collection of genomic DNA without an extraction process. By simply pressing tissues onto the FTA Cards, DNA in the samples is protected from degradation, oxidative and UV stress, and so forth. This technology is widely applicable to various DNA sources including plants. Furthermore, the FTA Card is useful for sampling DNA from wild plants in remote areas such as the upland region of the Himalayas (Tsukaya and Ikeda, 2005). We applied the Full-length cDNA Over-eXpresser gene (FOX) hunting system for random over-expression of individual full-length (FL)-cDNAs in rice using a rice-FOX *Agrobacterium* library (Nakamura et al., 2007). In this study, we demonstrated genomic PCR analysis of FOX-rice lines with FTA Cards, and compared the performance of the FTA Card method with that of alternative DNA purification procedures.

FOX-rice lines (T_0 generation), produced using the rice-FOX *Agrobacterium* library, were grown as previously described (Nakamura et al., 2007). The FTA Classic Card, a DNeasy Plant Mini Kit (QIAGEN), and a MagAttract 96 DNA Plant Core Kit (QIAGEN) were used according to the manufacturer's instructions. Genomic DNA samples were prepared from leaf blades of individual transgenic plants grown for 2 weeks after transplanting to soil. Each young leaf blade was pressed onto the FTA Card using a pestle, and a 2-mm disk punched out of the FTA Card carrying plant material was used for PCR. Transgenes inserted into the genome of FOX-rice lines were amplified by PCR using DNA polymerases KOD Dash (Toyobo) or TaKaRa Ex Taq (Takara Bio) with a forward primer FOX5-2 (located on P*Ubi-1*; 5′-AGCCCTGCCTTCATACGCTATTTATTTGCTTGGTACTGTTTC-3′) and a reverse primer FOX3 (located on T*nos*; 5′-GAAACTTTATTGCCAAATGTTTGAACGATCGGGGAAATTCGAG-3′) (Fig. 1A). The PCR conditions for KOD Dash were as follows: 1 min at 94°C for denaturation, followed by 35 (or 40) cycles of 30 s at 94°C for denaturation, with 20 s at 62°C for annealing and 3 min at 74°C for elongation. PCR analysis with TaKaRa Ex Taq was performed as previously described (Nakamura et al., 2007). An *hpt* gene fragment was similarly amplified using the forward primer H5 (5′-TGGGAATCCCCGAACATCGCC-3′) and the reverse primer H3 (5′-CCGATTCCGGAAGTGCTTGAC-3′) (Fig. 1A). The PCR conditions for KOD Dash were as follows: 1 min at 94°C for denaturation, followed by 35 cycles of 30 s at 94°C for denaturation, with 20 s at 60°C for annealing and 30 s at 74°C for elongation. The size(s) and number of the PCR products were checked by agarose gel electrophoresis.

Transgenes in five independent FOX-rice lines were analyzed using the FTA Card or the DNeasy Plant Mini Kit, and were amplified by genomic PCR using KOD Dash. The PCR analysis using genomic DNA extracted with the DNeasy Plant Mini Kit showed that FL-cDNAs were integrated in all the lines. However, distinct cDNA fragments were only detected from two lines in the FTA Card preparations, and a cDNA fragment from line AA205 was very faint (Fig. 1B). To assess the reproducibility of this result, we analyzed transgenes in lines 2635-1, 2635-2, and 2635-3, all of which were transformed with the same FL-cDNA. When genomic DNA from these three lines was trapped in the FTA Cards and used as a template, the PCR analysis only detected 2.5 kbp FL-cDNA fragments from line 2635-2 (Fig. 1C, panel 1). Then, we repeated the same experiment using the newly prepared FTA Card specimens, and only line 2635-3, not 2635-2, was PCR-positive (Fig. 1C, panel 2). Increased PCR cycles, using the third FTA Card specimens from individual plants, did not improve the frequency of the PCR-positivity, and smear bands were observed in lines 2635-1 and 2635-3 (Fig. 1C, panel 3). The same results were obtained when other disks punched out of each FTA Card were used for the genomic PCR experiments (data not shown). No amplified fragment was detected from any lines when Ex Taq was used (Fig. 1C, panel 4). As a control experiment, genomic DNA extracted with the MagAttract Kit was used for the PCR analysis, and all the three lines were PCR-positive (Fig. 1C,

panel 5). A 0.5 kbp fragment in the *hpt* marker gene was successfully amplified by using the FTA Card specimens from the three lines (Fig. 1C, panel 6).

Efficient amplification of FL-cDNA fragments was required for the sequencing analysis to identify introduced cDNA in individual FOX-rice lines, but we failed to amplify FL-cDNAs consistently with the FTA Card method. Additional purification steps or improvement of PCR conditions might overcome this problem. However, a short PCR fragment (0.5 kbp) from the *hpt* gene in the T-DNA was consistently amplifiable. This result suggests that the FTA Cards are applicable for large-scale detection of integration of particular DNA fragments in transgenic rice. Furthermore, since the FTA Cards are easy to handle and allow long-term storage of DNA at room temperature, the technology could be convenient for the safe storage of genomic information, especially for plants with low or no fertility which often appear in our FOX-rice plants.

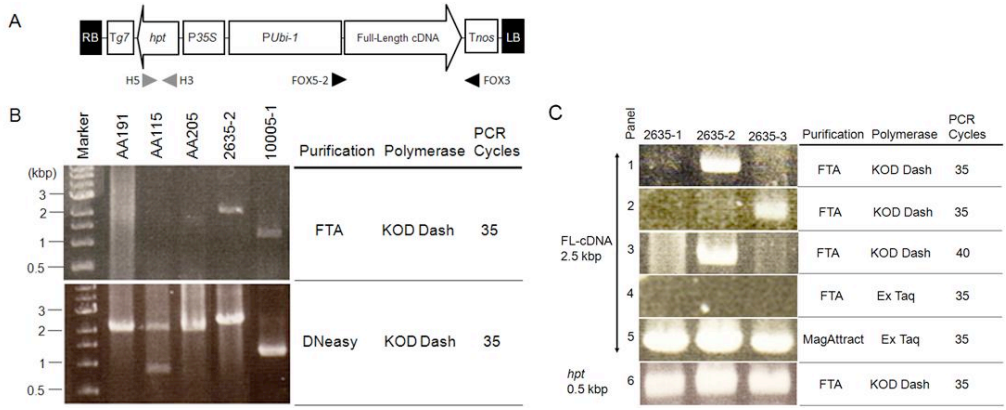

Fig. 1. Genomic PCR analysis of transgenes in the FOX-rice plants. A) A schematic structure of T-DNA in the pRiceFOX binary vector. P*35S*, CaMV *35S* promoter; P*Ubi-1*, maize *Ubiquitin-1* promoter; T*g7* and T*nos*, polyadenylation signals of *gene 7* and *nopaline synthase* gene from *Agrobacterium* T-DNA, respectively; *hpt*, *hygromycin resistance* gene; LB, left border; RB, right border. Arrowheads: positions of the PCR primers. B) Comparison of the FTA Card and the DNeasy Plant Mini Kit. Genomic DNAs were prepared from five independent FOX-rice lines, and inserted FL-cDNAs were analyzed by the methods described in the table. C) From three FOX-rice lines bearing an identical FL-cDNA, genomic DNAs were prepared, and FL-cDNA and *hpt* fragments were amplified by the methods described.

Acknowledgement

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Green Technology Project EF-1004).

References

Nakamura H., M. Hakata, K. Amano, A. Miyao, N. Toki, M. Kajikawa, J. Pang, N. Higashi, S. Ando, S. Toki, M. Fujita, A. Enju, M. Seki, M. Nakazawa, T. Ichikawa, K. Shinozaki, M. Matsui, Y. Nagamura, H. Hirochika and H. Ichikawa, 2007 A genome-wide gain-of-function analysis of rice genes using the FOX-hunting system. Plant Mol. Biol. 65: 357–371.

Tsukaya H. and H. Ikeda, 2005 Collection of plant DNA in the field by using FTA Cards. Acta Phytotaxon. Geobot. 5: 127–135.