Rapid screening of heavy-ion-induced large deletion mutants by using quantitative real-time PCR in *Arabidopsis thaliana*

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In heavy-ion irradiation, the size of deletions can be varied according to different values of linear energy transfer (LET). In irradiation experiments on *Arabidopsis thaliana*, we previously found that beams with LET of 30 keV/μm induced small deletions (1 to 51 bp) with high frequency, and this size is appropriate for disrupting a single gene.[1] Thus, 30-keV/μm beams are suitable for use in mutation breeding as well as in reverse genetics to find single gene disruption lines in conjunction with SNP detection systems. On the other hand, beams with LET of 290 keV/μm caused larger deletions (1 kbp to several hundred kbp).[2] Such high-LET beams can be used for generating mutants lacking tandemly arrayed genes (TAGs) that have functions similar to one another, allowing us to investigate the function of TAGs. Thus, we are now developing a deletion-mutant correction by screening them from M_3 generations after being irradiated with 100-, 200-, or 290-keV/μm beams. The screening is performed using array-CGH designed for all TAGs of *A. thaliana*. Researchers will be able to screen deletion mutants of interest from our stock of over 10,000 of 290-keV/μm irradiated M_2 lines.

However, screening deletion mutants is time-consuming. Conventionally, it was performed by PCR in the M_2 generation, in which only homozygous deletions (HomDels) could be detected unless primers were designed to have deletions placed between them (Table 1). In case that the HomDel mutant of interest is lethal, no candidate would be obtained. By contrast, heterozygous deletions (HetDels) can be inherited over generations, and are found in the M_2 generation with about two times frequency compared with HomDels (Fig.1). If a HetDel is easily detected in the M_2 generation (See Fig. 1), the screening pool size can be reduced. Herein, we tested the quantitative real time PCR (qPCR) for detection of a HetDel.

For our test, we selected an *Arabidopsis* line (C200-84-N2) from our stock induced by carbon-ion beam irradiation (135 MeV/nucleon, 200 keV/μm) at a dose of 75 Gy. This line has a 3.5-kbp deletion at the 2762232-2765771 position on chromosome 4. The 3.5-kbp deletion is harbored as a HetDel in the M_2 generation. Thus, four individual progenies of the M_3 plants of C200-84-N2 were used for qPCR, since some of them would have a 3.5-kbp deletion as the HetDel.

qPCR was performed by using LightCycler and the Universal Probe Library detection format (Roche Diagnostics, Penzberg, Germany). Relative amplification ratios between the 3.5-kbp deleted and non-deleted regions were calculated by using the ΔΔCp method. Sample A showed the same relative amplification ratio as wild-type (Fig. 2). Samples B and C showed about half-level of the ratio as compared to wild-type, indicating that they contain HetDels. Sample D showed no amplification. These results indicate that large deletions can be detected even if they are heterozygous. Our irradiated M_2 seed stocks in conjunction with this method will prove a powerful tool for analyzing the functions of TAGs.

Table1. Features of Hom and Het deletions

	Detection by normal PCR	Incidence in M_2 generation
HomDel	Possible	Low
HetDel	Impossible	High

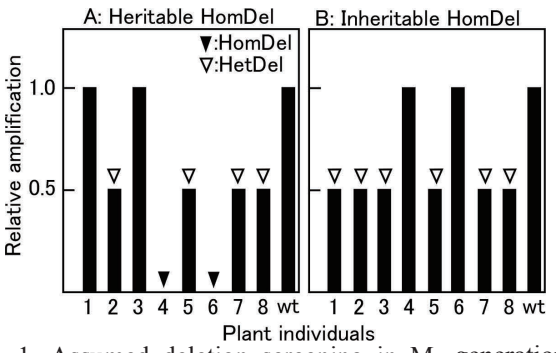

Fig. 1. Assumed deletion screening in M_2 generation by qPCR. HetDels are detected more frequently than HomDels for heritable (A) and inheritable (B) cases. No symbol indicates individuals that have no deletion.

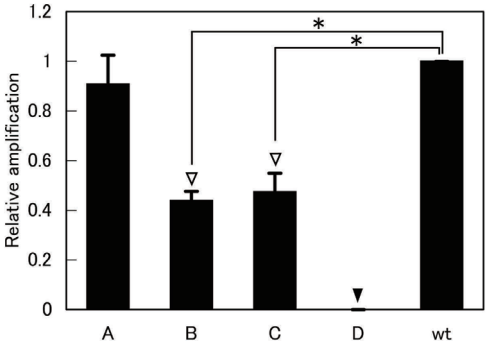

Fig. 2. qPCR in four M_3 plants (A to D). Data are expressed as fold changes compared with values in wild-type (wt). Error bars indicate ±SD. N=3 in each plant. White arrowheads and a black arrowhead indicate HetDel and HomDel, respectively. *, $p < 0.01$ compared to wild-type.

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References

1) Y. Kazama et al.: BMC Plant Biol. **11**, 161 (2011).
2) T. Hirano et al.: Mutat. Res. **735**, 19 (2012).