Detection of deletions induced by Fe-ion irradiation in *Arabidopsis thaliana* using array comparative genomic hybridization†

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Heavy-ion irradiation mainly induces deletions. The size of deletions increases with increasing linear energy transfers (LETs)[1]. Because large deletions are useful for producing null mutations as well as disrupting multiple genes arrayed in tandem, high-LET ion beams are considered powerful mutagens in the field of genome science. However, the determination of deleted chromosomal region(s) in a mutant by using classical PCR-based methods is difficult because designing primers at both sides of a large unidentified deletion is quite difficult. Thus, a method for rapidly detecting deletions at the whole-genome level is desired.

Array comparative genomic hybridization (array CGH) is a powerful tool for detecting deletions at the whole-genome level[2]. In the present study, we herein optimized the array CGH experiment to develop a method for investigating large heavy-ion induced deletions at the whole-genome level. For the array CGH, we used an *Arabidopsis* mutant having downward-pointing flowers (Fe-148-pg1) as an example of a mutant harboring large deletions, which was induced by Fe-ion beam irradiation (90 MeV/nucleon, 640 keV/μm) at a dose of 50 Gy. Through the phenotypic characterization and PCR confirmation, Fe-148-pg1 was found to have a large deletion around the *BREVIPEDICELLUS* (*BP*) gene.

Arabidopsis thaliana ecotype Columbia (Col-0) was used as a wild-type plant. The whole-genome sequence of Col-0 was tiled with oligonucleotides that started every 50 bp. Probe lengths were constrained to a minimum of 50 and a maximum of 75 bp. Considering this design, our array was expected to detect deletions of more than 200 bp at the whole-genome level.

The DNA of the wild-type and the mutant plant (Fe-148-pg1) were labelled with Cy5 and Cy3, respectively. Hybridization, washing, and scanning were conducted by Roche NimbleGen Inc (Madison, WI, USA). Raw fluorescence intensity data were obtained from scanned images of the oligonucleotide tiling arrays by using NimbleScan 2.4 extraction software (Roche NimbleGen Inc.). For each spot on the array, log2 ratios of the Cy3-labelled sample to the Cy5 reference sample were calculated.

Candidate deletions were identified as follows. First, regions supported by more than 4 consecutive probes with log2 ratios of over 1.0 were listed as candidate deletions. Then, the candidate deletions were confirmed by performing PCR. For the PCR test, 7 individual M_3 plants were tested; when some of the plants showed amplification in the candidate deletion region, the candidate deletion was determined as being heterozygous in the M_2 generation. Finally, we detected 7 deletions (Table 1). Candidate deletions supported by the higher log2 ratio tended to be homozygous. However, candidate deletions supported by log2 ratios lower than 1.209 were false positives. As expected, the Fe-148-pg1 had a large deletion covering the *BP* gene; the size of deletion was found to be 90,307 bp by conducting PCR-based confirmation and sequencing. The results suggest that our array platform can detect both homozygous and heterozygous deletions at the whole genome level, although the estimated deletion size is not completely consistent with the actual one.

Table 1 List of deletion peaks estimated by array CGH in Fe-148-pg1.

Start site of Peak area		Estimated		Detected	Genetic
Chr.	Position	deletion size	Signal*	deletion	homogeneity in M_3
4	5,129,711	88069	1.500–6.097	○	homogeneous
4	7,875,508	81108	3.375–6.047	○	homogeneous
1	17,030,769	467	2.289	-	-
3	17,831,368	450	2.076	○	homogeneous
4	8,550,408	264	1.992	○	homogeneous
5	8,060,900	84816	1.744–1.930	○	heterogeneous
4	2,179,908	850	1.777	-	-
4	2,177,558	814	1.767	-	-
4	2,185,508	723	1.743	○	heterogeneous
4	2,181,208	469	1.209	○	heterogeneous

*When several peaks were detected in a naborhood, the peaks were combined and the range of signal values were considered.

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References

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