We have performed exome sequencing analysis to reveal mutations induced by heavy-ion beams in rice. We used three software packages, GATK, Pindel, and BEDTools, to detect mutations from exome sequencing data. Among them, Pindel finds DNA breakpoints, decides the types of mutations such as deletion, insertion, inversion, and tandem duplication. When Pindel cannot determine types of mutations, the mutation is called an "unassigned breakpoint". We found homozygous mutations called unassigned breakpoints from the rice mutant (Mutant-1) induced by Ne-ion beam irradiation. This mutant possessed three unassigned breakpoints. Two of these were located in the same chromosome, suggesting that they were breakpoints derived from an inversion.

We confirmed the candidate inversion by polymerase chain reaction (PCR) analysis. First, we estimated the position of two breakpoints, namely, breakpoint A and breakpoint B by using Integrative Genomic viewer. Then, we designed primer F1 having the sequences in the upstream region of the breakpoint A (Fig. 1). We created primer R1 corresponding to the downstream region of this breakpoint. Similarly, primers F2 and R2 were synthesized on both sides of the breakpoint B. When PCR was carried out with primer sets F1R1 or F2R2 using wild-type genomic DNA as the template, DNA amplification was observed (Fig. 1). On the other hand, when PCR was carried out using Mutant-1 DNA as the template, no amplification of these DNA fragments should be observed (Fig. 1). When we attempted to amplify a DNA fragment with primer sets F1F2 and R1R2 using Mutant-1 DNA, DNA amplification should be observed (Fig. 1).

We investigated the existence of the inversion from DNA amplification pattern in this way.

In the PCR experiment, DNA amplification was observed when we used primer sets F1F2 or R1R2 using mutant 1 genomic DNA as the template (Fig. 2). No amplification of these DNA fragments was observed when we used wild-type DNA as the template (Fig. 2). These results demonstrated that homozygous inversion existed in the Mutant-1 plant as we expected. Sequencing analysis using PCR products of the Mutant-1 plant revealed that 2-bp deletion occurred at breakpoint A and 5-bp deletion occurred at breakpoint B (Fig. 3). The distance from breakpoint A to B was 298.6 kb, and we found a new mutant gene disrupted by breakpoint B (Fig. 3). Our findings suggest that we can identify new mutated gene from a mutation that is called an "unassigned breakpoint" by using Pindel. PCR analysis of "unassigned breakpoint" mutations generated in other mutants is in progress.

Fig. 2. Detecting an inversion generated in mutant-1. DNA amplification is detected by MCE-202 MultiNA (Shimadzu). The names of the genomic DNAs and primers are displayed above the gel image. DNA size markers are shown on the left side.

Fig. 3. Structure of inversion determined in this study. Thin arrows indicate the direction of the DNA. The gene disrupted by the inversion is shown as the yellow box.

References