

[Mutant analysis]

It is expected that the provided mutant strains contain several thousand SNPs over the whole genome other than the target genes. Therefore, it is necessary to confirm that the SNPs within the target genes are responsible for changes in the phenotype. For the selected mutants with SNPs, it is necessary to compare and analyze the phenotypes of several individual plants from **plants with homozygous mutations in the target genes and homozygous wild-type plants** isolated from the same strain (or the same individual plant).

For TILLING, we use bulk genomic DNA of M₂ plants (6 plants) derived from the Kinmaze and Taichung-65 varieties, and M₁ plants derived from the Nipponbare variety. Therefore, for the Kinmaze- and Taichung 65-derived strains, M₃ seeds of each M₂ plant are provided to the client, whereas for the Nipponbare-derived strains, in general M₂ seeds are provided. Although the provided seeds are divided into homozygous mutants, homozygous wild types, and heterozygous mutants, they may contain no homozygous mutant plants or homozygous wild-type plants. In such cases, please **grow the heterozygous mutants**, identify homozygous mutant and homozygous wild-type plants segregated in the next generation, and analyze their phenotypes using these progeny. When no heterozygous mutants are included in the provided seeds, seeds will be provided a second time.