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Development of CAPS Markers Based on wide Detection SNPs using Milyang23/Gihobyeo Recombinant Inbred Lines (MGRILs) by Re-Sequencing

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[Introduction]
The development and utilization of genetic markers play important roles in genetic research for marker-assisted breeding. High-throughput sequencing has greatly advanced the discovery of single nucleotide polymorphism (SNP) markers. However, a large gap between the developed SNP markers were still exist in the narrow down of mapping. In this study, we performed re-sequencing using Milyang23 and Gihobyeo recombinant inbred lines (MGRILs) as mapping population and developed the cleaved amplified polymorphic sequences (CAPS) markers with massive SNPs for validation of their availability.

[Materials and Methods]
The genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN) and quantified NanoDrop ND-1000 spectrophotometer. Re-sequencing analysis were performed by Illumina platform and mapped on Nipponbare reference genome (IRGSP 1.0). BWA-mem was mainly used for mapping and GATK haplotyCaller for SNP detection among 162 RILs. The construction of CAPS markers were based on widely used restriction enzymes with SNPs, and also PCR primers were designed by Primer3. The validation of CAPS markers with restriction enzyme were taken by the digestion in agarose gel.

[Results and Discussion]
Re-sequencing of MGRILs including their parents was performed in this study. Sequencing yields were 35.5×10^9 bps in Milyang23, 30.0×10^9 bps in Gihobyeo and the average was 15.0×10^9 bps in RILs, respectively. The raw reads with high quality were mapped to Nipponbare genome sequences as a reference and 297,339 SNPs was detected among RILs. To identify their availability of these results, CAPS markers were developed and used for validation of SNPs as genetic markers. Furthermore, a large amount of these SNPs can be used for QTL/gene mapping to important agricultural traits.

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