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High throughput and flexible genotyping in soybean by Ion AmpliSeq Custom Panel

*Eri Ogiso-Tanaka**, National Agriculture and Food Research Organization, Ibaraki, Japan
Fumio Taguchi, National Agriculture and Food Research Organization, Ibaraki, Japan
Akito Kaga, National Agriculture and Food Research Organization, Ibaraki, Japan
Makita Hajika, National Agriculture and Food Research Organization, Ibaraki, Japan
Masao Ishimoto, National Agriculture and Food Research Organization, Ibaraki, Japan

The development of Next generation sequencing (NGS) technology enable us to generate the amount of genome information to be applicable to genomic selection and marker support selection (MAS). However, while whole genome resequencing by NGS can comprehensively detect polymorphisms at the entire genome level, it is costly and time consuming. As alternate approaches, AmpliSeq was developed. This is one of the genotyping-by-sequencing (GBS) method and highly flexible targeted sequencing technology. AmpliSeq enable us to highly multiplex PCR that a single PCR reaction can be used to generate greater than 3000 different amplicons by designing primers using the reference genome. Therefore, in this study, we applied AmpliSeq to Soybean and evaluated the results from the following view points; genome wide genotyping and target resequencing for the MAS analysis. First, we have detected polymorphisms from whole genome sequence data of 300 varieties/lines of soybean, and designed 2,688 amplicon primers based on effects of polymorphisms, allele frequency and linkage disequilibrium for genome wide genotyping. Second, we designed 384 markers on breeding target QTL regions and known genes for MAS. For known genes, AmpliSeq markers were designed directly on functional mutations including large InDels and copy number variation. NGS libraries were constructed with one PCR program that used a total of 3,072 oligo pairs and could be barcoded to run up to 768 samples on the Ion S5 system. With this grouping of samples and oligo pairs a read depth of more than 50 per sample per amplicon could be achieved. We will discuss the efficiency of this technique to the soybean genotyping and the application towards MAS and genomic selection.