Targeted Next Generation Sequencing Approaches in Corn, Cucumber and Soy for High Throughput Genotyping

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ABSTRACT

With advances in plant phenotyping approaches for quantitative trait analysis and increasing complexity of gene pyramiding schemes, the number of markers required for successful molecular breeding programs in agriculture is increasing. Historically, technology has been polarized between high marker, high cost microarrays or low cost singleplex approaches that are not easily scalable. Targeted genotyping by sequencing (GBS) offers an attractive alternative for high-density genotyping of tens to thousands of markers in a high throughput and cost-effective manner.

We have applied AgriSeq targeted GBS, a high throughput amplicon based sequencing workflow to three important crops, Maize, Cucumber and Soybean. Here we demonstrate the utility of the AgriSeq GBS technology offers flexibility between sample throughput and marker density depending on the application requirements. Custom GBS panel designs allow the user to find the right balance between cost and quality of results, which when combined with the AgriSeq library prep offers a robust and efficient workflow for SNP genotyping.

CONCLUSIONS

Next generation sequencing offers great potential to fundamentally change the way plant genotyping is delivered. With the availability of 768 barcodes, the AgriSeq GBS workflow can generate genotypes on up to 1536 samples per day. The technology is flexible to scale to high markers with fewer samples and has demonstrated performance across several agriculturally important plant species.

INTRODUCTION

Single nucleotide polymorphisms (SNPs), which are faithful and generally have a low mutation rate, have emerged as the most widely used genotyping markers in agricultural applications such as trait monitoring, marker-assisted breeding selection or germplasm identification. With advances in next generation sequencing technologies and targeted resequencing approaches, genotyping by sequencing (GBS) provides an attractive alternative to traditionally more costly arrays for high-density SNP genotyping.

The AgriSeq GBS workflow is a targeted resequencing high-throughput workflow, designed to amplify and sequence up to 5000 genetic markers in a single multiplexed reaction. It offers a low-cost, reproducible and robust solution to deliver up to 1.6M genotypes per day.

Here we demonstrate the utility of the AgriSeq GBS workflow to genotype three different economically important crops, Maize, Cucumber and Soybean.

MATERIALS AND METHODS

Four crop genotyping primer panels were designed using a reference-based GBS automated pipeline, which selects primers based on optimized parameters such as amplicon length, melting temperature and GC content, for multiplexing 100s to 1000s of single nucleotide polymorphisms in a single PCR reaction. Primers were also designed to avoid overlapping nearby SNPs and prevent the formation of spurious PCR products, characteristics which were assessed in silico (Figure 1).

RESULTS

These panels were tested using the AgriSeq GBS workflow using either the 96 or 384-well AgriSeq™ HTS Library prep protocol with 10 ng of genomic DNA input. Barcoded amplicon libraries were pooled 1:1 and loaded onto an Ion Chef™ System for template prep and ship loading onto an Ion 540 Chip, and sequenced on an Ion S5™ XL System. Data were analyzed using the Torrent Variant Caller plugin available as part of the Torrent Suite software package, to determine the genotypes calls for each marker and sample tested with each panel (Figure 2).

CONCLUSIONS


TRADEMARKS/LICENSEING

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