

SNP Genotyping of Field Corn Samples with LGC Genomics Chemistry on the Nexar[®] System

ABSTRACT

Single Nucleotide Polymorphism (SNP) genotyping must be accurate, reliable, and cost-effective for agricultural marker-assisted selection and genomic selection programs to fully benefit from SNP analysis during the breeding process. To address these needs, Douglas Scientific[®] developed the Nexar System, a flexible and automated high-capacity laboratory system for rapid processing of PCR-based SNP genotype analysis in miniaturized reaction volumes. The study described here demonstrates the efficacy and economic advantages of the Nexar System for agricultural breeding programs. Eighty-eight field corn seeds were genotyped for four high-quality, polymorphic SNPs using crude DNA extracts and two PCR-based SNP genotyping methods—KASP[™] chemistry and probe-based assays with KlearKall[™] Master Mix. Both methods produced 100% call rates and matching genotypes for all replicates of each sample.

INTRODUCTION

Plant and animal breeding programs rely heavily on marker-assisted selection (MAS) and genomic selection (GS) techniques. Single Nucleotide Polymorphisms, or SNPs, are genetic markers found in plant and animal genomes. They are stable from generation to generation and prevalent throughout the genome, making them ideal candidates for use in MAS and GS programs. The development of PCR-based SNP genotyping protocols has enabled researchers to analyze SNPs quickly and accurately. Additionally, improvements in laboratory automation have increased the capacity of MAS and GS laboratories, enabling high-throughput sample screening, and thus a more rapid development of new agricultural products.

Field corn (*Zea mays*) is an economically important crop grown around the world. According to the United States Department of Agriculture National Agricultural Statistics Service, 13.9 billion bushels of corn were produced in the United States alone in 2013, and 90 percent of the corn acres planted were biotech varieties (Young and Honig). Corn is an important source of food globally and is used for many industrial applications. These uses, combined with the prevalence of biotech varieties, make corn one of the most widely tested crops in MAS and GS programs worldwide.

Here we describe an experiment in which individual field corn seeds were genotyped using commercially-available PCR-based reagents in miniaturized reactions on the Nexar System from Douglas Scientific. The four SNPs analyzed in this study were selected from a subset of 120 high-quality SNP markers identified through a stringent statistical method described by Mammadov, et al. The SNPs were chosen for this study not because of an association with specific traits but for their quality and polymorphic nature. The post-harvest corn seeds were donated from local farmers and grain elevators in central Minnesota and were provided without any genetic information. PCR-based SNP genotyping was completed with custom probe-based SNP genotyping assays and KASP SNP genotyping assays.

MATERIALS AND METHODS

Corn Samples

Post-harvest field corn seeds were donated by 11 different sources in central Minnesota, including local farmers and grain elevators. Samples were collected at random with a focus on local geographic (not genetic) diversity. All farmers and elevators were asked to not provide any breeder, variety, or genetic information about their samples. Eight individual seeds from each source were analyzed, totaling 88 samples.

DNA Extraction

A sodium hydroxide method was used to prepare crude corn DNA samples. Individual corn seeds were pulverized using a mini bead beater and a dilute solution of sodium hydroxide was added to lyse the cells at 50 °C for 10 minutes. The samples were cooled and neutralized with Tris-HCL buffer, pH 7.8. After centrifugation, the supernatant was collected and diluted 1:9 in water before use.

Assays and Reagents

Genomic information about the SNPs analyzed in this study can be found in Table 1. KASP 1536 V4.0 2X Master Mix or KlearKall Master Mix (LGC Genomics) were used for all SNP genotyping reactions. Both master mixes were provided at 2X concentration, and were used according to the manufacturer's instructions. KASP SNP genotyping assays were designed by LGC Genomics; BHQplus[®] probe-based SNP genotyping assays were designed using RealTimeDesign[™] Software from LGC Biosearch Technologies. All primers and probes were obtained from LGC Biosearch

SNP ID	Genbank Accession Number	Chromosome
DZm2490176	AC196688.4	3
DZm2575115	AC185516.4	1
DZm2571611	AC219032.4	4
DZm2521843	EU972344.1	10

Table 1: SNP information

Reagent	Source
KlearKall Master Mix	LGC Genomics
KASP 1536 v4.0	LGC Genomics
Primers and BHQplus probes for probe-based SNP assays	Biosearch Technologies
Unlabeled primers for KASP SNP assays	Biosearch Technologies

Table 2: Reagents and assays

Technologies, as shown in Table 2. KASP SNP genotyping assays differ from traditional probe-based assays, as described in Figure 1. KASP primers were diluted according to the manufacturer's instructions and were added at 2X concentration to the 2X KASP 1536 V4.0 Master Mix before use. BHQplus probes and primers were added at 2X concentration to the 2X KlearKall Master Mix (400 nM and 1.8 μM, respectively).

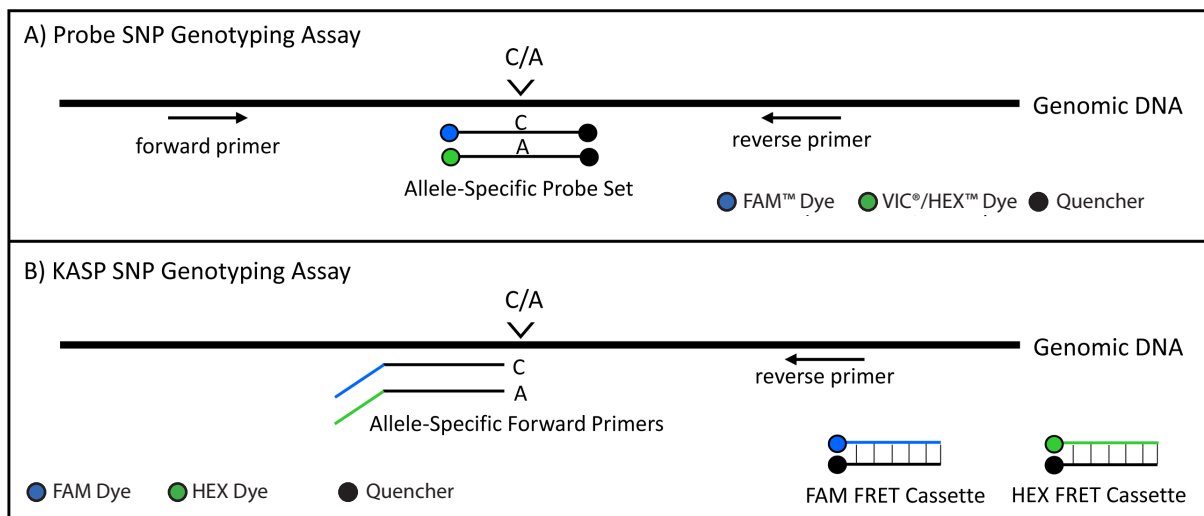
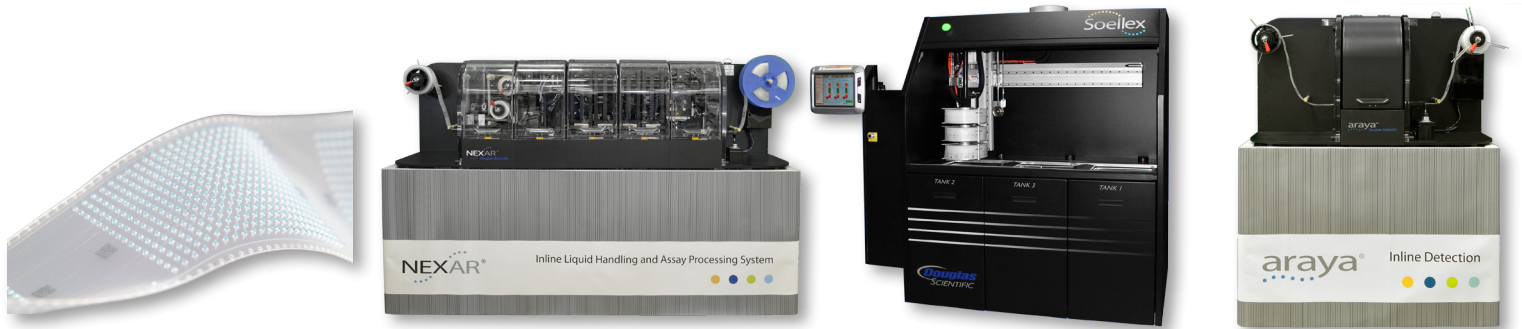


Figure 1: Assay design for probe and KASP SNP genotyping reactions. A) Probe-based SNP genotyping assays utilize forward and reverse primers to amplify a segment of genomic DNA surrounding a SNP. Two fluorogenic probes differentially bind to their allele-specific complement. Fluorescent signal is produced during each PCR cycle by separation of the dye and quencher, as probes are hydrolyzed through Taq exonuclease activity. B) KASP SNP genotyping assays utilize a common reverse primer and two allele-specific forward primers, ending at the SNP site. Each forward primer contains an allelic discrimination nucleotide at the 3' end and a tag that is specific to one of the FRET cassettes at the 5' end. Tag sequences are identical to dye-labeled strands of the FRET cassettes, which are supplied in the master mix. The tag sequence is incorporated into the PCR product along with the forward primer as PCR amplification occurs. During subsequent PCR cycles, the dye-labeled strand of the FRET cassette acts as a forward primer. Fluorescent signal is produced by irreversible separation of the FRET cassette dye and quencher.

Dispensing, Thermal Cycling, and Analysis

Douglas Scientific instruments including the Nexar, Soellex®, and Araya® were used for all sample processing and SNP genotyping reactions in Array Tape®. The Nexar System is described in Figure 2. DNA samples (800 nL) were dispensed into Array Tape with the multi-channel, 384-tip pipette head from CyBi® Product Line. KASP 1536 V4.0 Master Mix containing 2X SNP genotyping assay (800 nL) was dispensed with the non-contact Dispense Jet to create 1.6 µL total volume reactions in Array Tape. PCR amplification

and thermal cycling were performed in the Soellex using a touchdown PCR protocol with a total of 38 cycles according to the standard protocol for KASP genotyping chemistry. Alternatively, KlearKall Master Mix containing 2X BHQplus probe-based assay (800 nL) was dispensed with the non-contact Dispense Jet and PCR amplification was performed in the Soellex using the manufacturer's instructions. End-point fluorescence values were determined by scanning the Array Tape in the Araya. Cluster plot analysis was completed in Douglas Scientific's Intellics® Software Suite. All SNP genotyping reactions were performed in duplicate.



ARRAY TAPE	NEXAR	SOELLEX	ARAYA
• Flexible microplate replacement	• Liquid handler optimized for Array Tape	• High capacity PCR waterbath	• End-point fluorescence scanner
• Reduced reaction volumes	• 800 nL DNA, 384-channel dispense	• Optimized for Array Tape	• Optimized for Array Tape
• Total well volume of 2 µL	• 800 nL master mix, 384-well dispense in 38 seconds	• Three tanks for PCR optimization	• Scan 384 wells in 28 seconds
• Optically clear cover seal	• Seal Array Tape for thermal cycling	• Touchdown or traditional PCR	• Data ready for analysis in Intellics

Figure 2: Nexar System Overview

RESULTS

All SNP genotyping reactions in this experiment produced clusters that were easily scored in Intellics data analysis software. Cluster plot images for both KASP and probe-based assays are shown in Figure 3. Duplicate reactions produced the same call for each corn seed with both assay types, and all calls matched between the KASP and probe-based assay designs. A summary of the calls is provided in Table 3. The SNPs studied in this experiment produced expected polymorphic results with three clusters for each assay whether analyzed with KASP or probe-based assays with KlearKall Master Mix.

CONCLUSION

This study demonstrates that the Nexar System can be successfully used to produce repeatable and easily scored SNP genotyping results with crude preps from field corn using either KASP or probe-based SNP genotyping assays in miniaturized reaction volumes. In addition to automating the SNP genotyping process for MAS and GS programs, this platform provides significant cost savings in the form of reduced PCR reaction volumes. The results shown in this study were generated with 1.6 μ L total volume reactions in Array Tape, a 68% volume reduction compared to standard plate-based systems, such as real-time PCR instruments, which generally require at least 5 μ L total reaction volumes. Taken together, the results of this experiment demonstrate that the Nexar System is an industry-leading solution for agricultural genotyping applications.

REFERENCES

USDA National Agricultural Statistics Service (http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/cornprod.asp visited July 20, 2014)

Krissy Young and Lance Honig, USDA Newsroom (http://www.nass.usda.gov/Newsroom/2014/06_30_2014.asp posted June 30 2014; visited July 20, 2014)

Mammadov, J. A., Chen, W., Ren, R., Pai, R., Marchione, W., Yalcin, F., Witsenboer, H., Greene, T. W., Thompson, S. A., Kumpatla, S. P. Development of highly polymorphic SNP markers from the complexity reduced portion of maize [*Zea mays* L.] genome for use in marker-assisted breeding. *Theor Appl Genet* (2010) 121:577-588.

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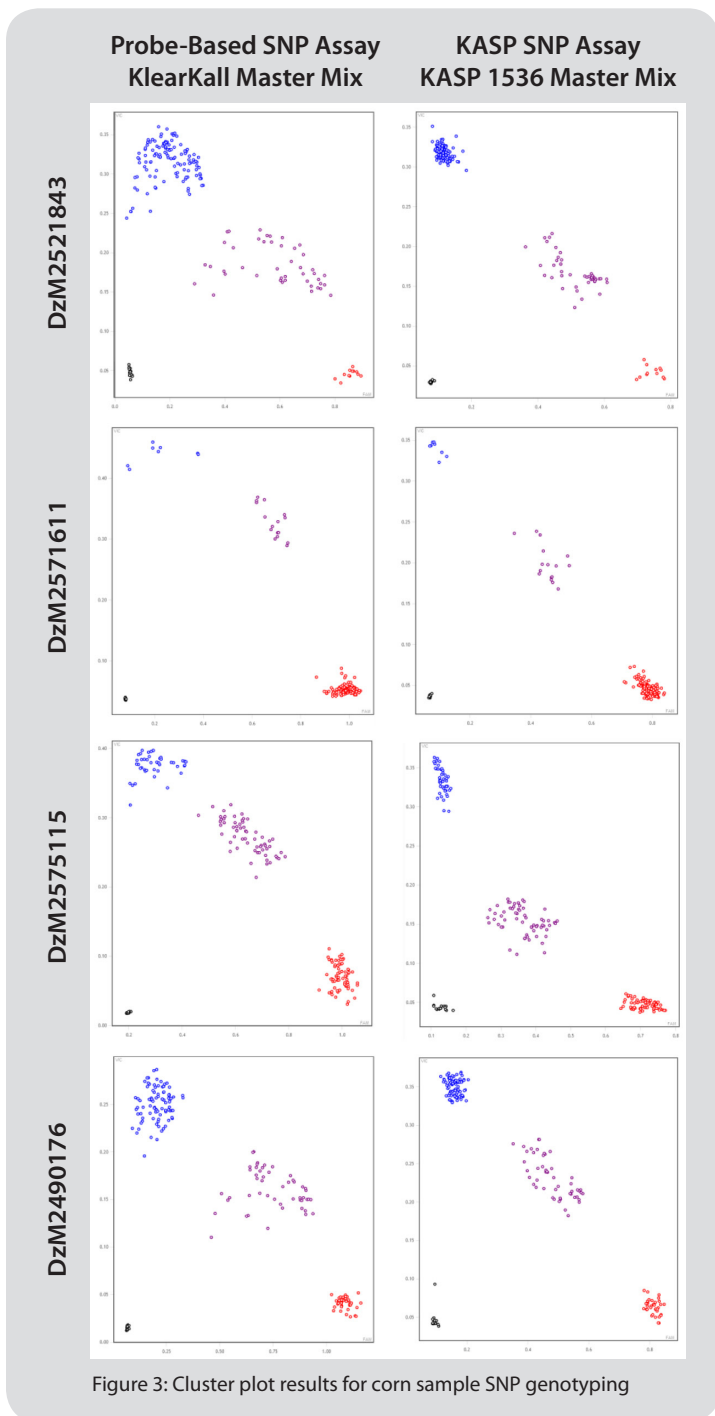


Figure 3: Cluster plot results for corn sample SNP genotyping

SNP ID	KASP FAM/HET/HEX	KlearKall FAM/HET/HEX
Dzm2521843	6/22/60	6/22/60
Dzm2571611	76/8/4	76/8/4
Dzm2575115	35/31/22	35/31/22
Dzm2490176	18/25/45	18/25/45

Table 3: Summary of SNP genotyping calls