# SNP Genotyping of Field Corn Crude Samples with Probe-Based Assays and a Variety of Master Mixes 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 probe-based SNP genotyping assays and three different master mixes. All conditions produced call rates above 98%, with 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 Stated 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 essential 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 master mixes and custom assays 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 because of their quality and polymorphic nature. The post-harvest corn seeds were donated by 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 three different master mixes.

# 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 not to 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. TaqMan® GTXpress™ Master Mix (Applied BioSystems), PerfeCTa® qPCR ToughMix® (Quanta Biosciences), and SsoAdvanced™ Universal Probes Supermix (Bio-Rad) were used to genotype all samples with four SNP assays. Each master mix was provided at 2X concentration, and used according to the manufacturer's instructions. BHQplus® probe-based SNP genotyping assays were designed using RealTimeDesign™ Software from LGC Biosearch Technologies. All primers and probes were obtained from LGC Biosearch Technologies, as shown in Table 2. Probe-based SNP genotyping assays are made up of two primers and two allele-specific probes with different fluorogenic dye labels, as described in Figure 1. BHQplus probes and primers were added at 2X concentration to the 2X master mixes (400 nM and 1.8 µM, respectively) to achieve a final concentration in the PCR reaction of 200 nM probes, 900 nM primers, and 1X master mix.

| 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 |
|---|---|
| TaqMan GTXpress Master Mix | Applied BioSystems |
| PerfeCTa qPCR ToughMix | Quanta BioSciences |
| SsoAdvanced™ Universal Probes Supermix | Bio-Rad® Laboratories |
| Unlabeled primers and BHQplus probes | LGC Biosearch Technologies |

Table 2: Reagents and assays

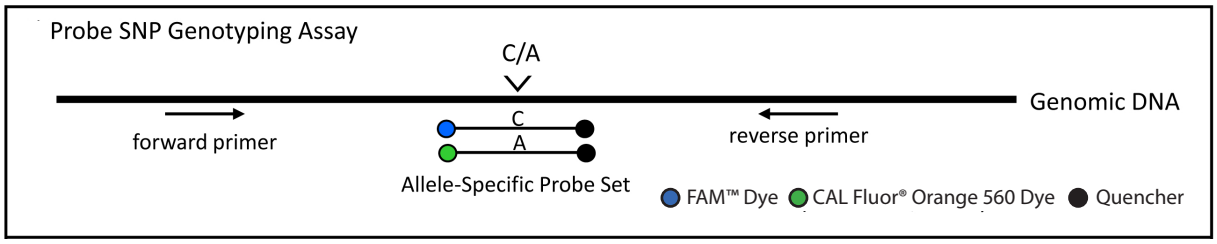
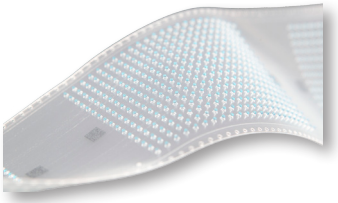
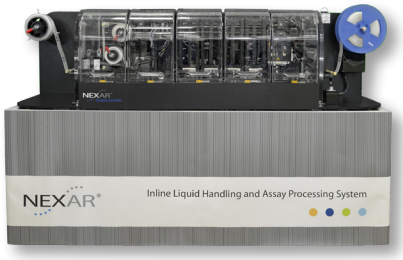



Figure 1: Assay design for probe-based SNP genotyping reactions. Probe-based SNP genotyping assays utilize forward and reverse primers to amplify a segment of genomic DNA surrounding a SNP. Two fluorogenic probes differentially bind to their allele-specific complement. Fluorescent signal is produced during each PCR cycle by separation of the dye and quencher, as probes are hydrolyzed through Taq exonuclease activity.

## Dispensing, Thermal Cycling, and Analysis

Douglas Scientific instruments including the Nexar, Soellex®, and Araya® make up the Nexar System and were used for all sample processing and SNP genotyping reactions. 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. Master mix containing 2X BHQplus probe-based assay (800 nL) was dispensed with the non-contact Dispense Jet to create 1.6 µL total volume reactions. PCR amplification and thermal cycling were performed in the Soellex using the manufacturer's instructions for each mix. Briefly, an initial activation step at 95 °C (1 min for GTXpress, 3 min for PerfeCTa and SsoAdvanced) was followed by 45 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds. 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 duplicate reactions with all assays and master mixes 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 mixes, with one individual exception. One corn sample was not scored for SNP DZm2521843 with SsoAdvanced Supermix because of poor amplification. 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 using GTXpress, PerfeCTa, and SsoAdvanced master mixes.

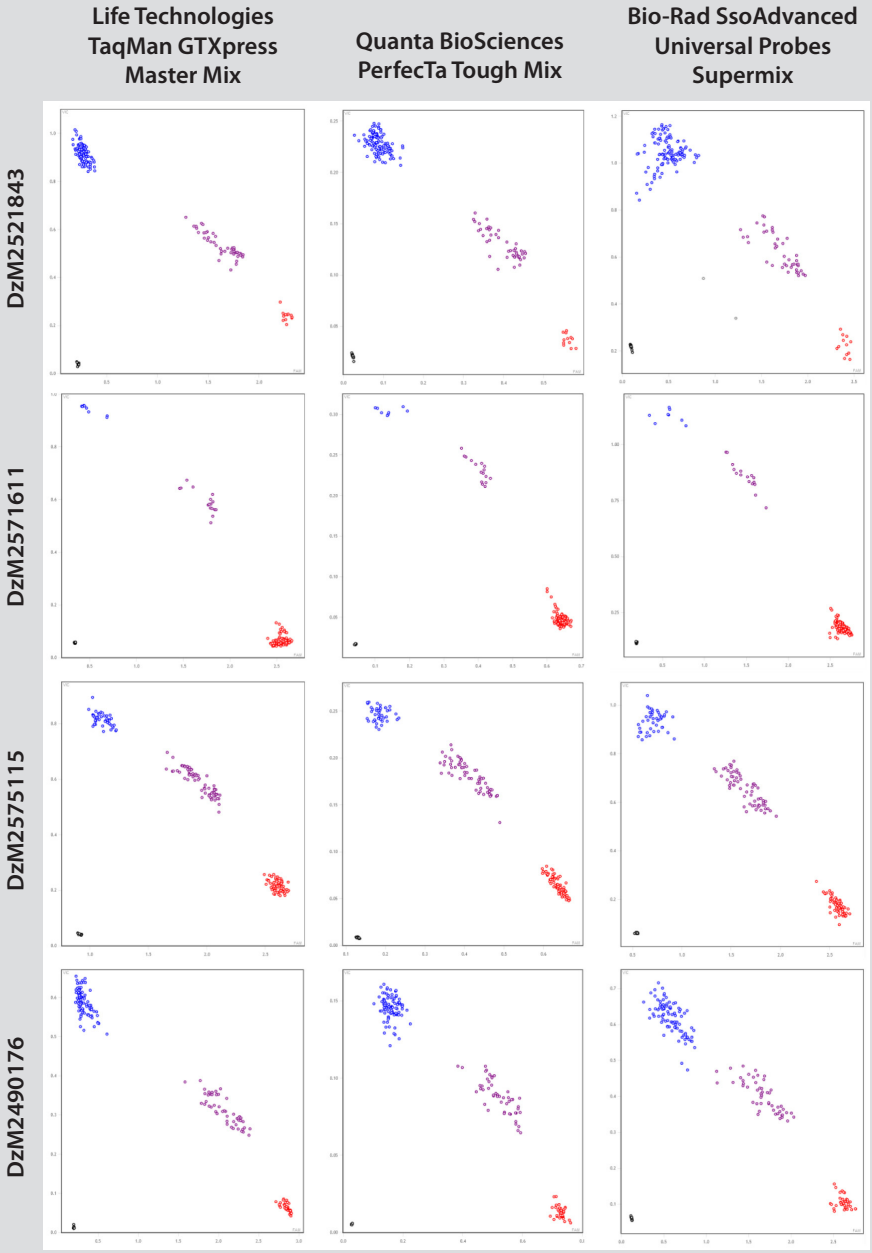



Figure 3: Cluster plot results for corn sample SNP genotyping

# 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 BHQplus probe-based SNP genotyping assays and a variety of master mixes 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 µL 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 µL total reaction volumes. Taken together, the results of this experiment demonstrate that the Nexar System is an industry-leading solution for agricultural genotyping applications.

| SNP ID | GTXpress FAM/HET/HEX | PerfeCTa FAM/HET/HEX | SsoAdvanced FAM/HET/HEX |
|---|---|---|---|
| Dzm2521843 | 6/22/60 | 6/22/60 | 6/21*/60 |
| Dzm2571611 | 76/8/4 | 76/8/4 | 76/8/4 |
| Dzm2575115 | 35/31/22 | 35/31/22 | 35/31/22 |
| Dzm2490176 | 18/25/45 | 18/25/45 | 18/25/45 |

*One sample did not fall within a cluster
Table 3: Summary of SNP genotyping calls

*For research use only. The products of Douglas Scientific, LLC are not FDA approved for use in human diagnostic procedures.

# 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.


NEXAPP-4-1