Characterization of the IntelliCycler™ for End-point PCR Amplification in Array Tape®

ABSTRACT

Two experiments were completed to demonstrate the IntelliCycler is a viable solution for PCR amplification in Array Tape. The first experiment included SNP genotyping analysis of individual corn seeds for two polymorphic SNPs using KASP™ and probe-based assays. Results were generated using both the Soellex® and IntelliCyler for all conditions. Overall cluster quality and consistency of genotype calls were compared. The second experiment demonstrated amplification uniformity across a 384-well array using identical replicates of a GMO-positive corn sample with a duplex assay targeting the corn lipid transfer protein (LTP) gene and the 35S promoter sequence. The results of these experiments establish that the IntelliCycler is an appropriate solution for PCR thermal cycling using the Array Tape platform, and gives users an option to attain high quality results regardless of throughput needs.

INTRODUCTION

A set of end-point PCR experiments were conducted to compare the thermal cycling capabilities of the IntelliCycler and the Soellex for applications using the Array Tape Platform. Post-harvest yellow field corn seeds were genotyped using commercially available PCR master mixes with custom KASP and probe-based SNP genotyping assays. The two 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. Genotyping calls and cluster quality for all assays were used as criteria for comparison of the thermal cycling instruments.
A second experiment was performed to determine end-point fluorescent signal uniformity across a 384-well array in each water bath thermal cycling instrument. For this test, 382 identical replicates of a GMO-positive corn sample were amplified in each array using a duplex assay targeting the corn lipid transfer protein (LTP) gene and the 35S promoter sequence. Two wells served as negative controls. The combination of these experiments demonstrated that the Soellex and IntelliCycler produce similar high quality end-point PCR results in Array Tape.

MATERIALS AND METHODS

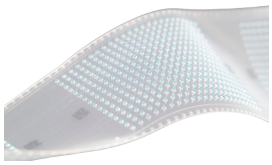
Corn Samples: Post-harvest field corn seeds for SNP genotyping analysis were donated from 10 different sources in central Minnesota, including local farmers and grain elevators. Samples were collected at random with a focus on geographic (not genetic) diversity. For the SNP genotyping analysis, two or three seeds from each source were analyzed, totaling 28 samples. The corn sample for amplification uniformity analysis was purchased from a local farm supply retailer.

DNA Extraction: Individual corn seeds were pulverized using a miniature bead beater and a dilute solution of sodium hydroxide was added to lyse the cells at 50 ^{0}C for 10 minutes. The samples were then cooled and neutralized with Tris-HCl buffer, pH 7.8. After centrifugation, the supernatant was collected and diluted 1:50 in water for the SNP analysis or 1:10 in water for the 35S/LTP end-point uniformity test.

Assays and Reagents: The two corn SNPs analyzed were SNP ID DZm2490176, Genbank Accession Number AC196688.4 and SNP ID DZm2575115, Genbank Accession Number AC185516.4. The primers and probes targeting the corn LTP gene and the 35S promoter sequence were described by Alary, 2002. The LTP probe was labeled with FAM™ and the 35S probe was labeled with HEX™. TaqMan® GTXpress™ Master Mix (TaqMan MM) from Applied BioSystems™ and KASP 1536 V4.0 2X Master Mix (KASP MM) from LGC Genomics were used in this study. Each master mix was provided at 2X concentration and 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, Inc. All primers and probes were obtained from LGC Biosearch Technologies, Inc. BHQplus probes and primers for the SNP genotyping assays and the duplex LTP/35S assay were added at 2X concentration to the 2X master mix (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. KASP primers were diluted according to the manufacturer's instructions and were added at 2X concentration to the 2X KASP MM before use.

Dispensing, Thermal Cycling, and Analysis: Douglas Scientific® instruments including the Nexar®, Soellex®, and Araya®, described in Figure 1A, were used for all sample processing and PCR reactions. The IntelliCycler is shown in Figure 1B. All DNA samples (800 nL) were dispensed into Array Tape with the multi-channel, 384-tip pipette head from CyBi® Product Line according to the 384-well array layout shown in Figure 2. For the SNP genotyping experiment, KASP MM 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 and IntelliCycler using a 65-57 ^{0}C touchdown PCR protocol with a total of 40 cycles, according to the recommended protocol for KASP genotyping chemistry. Alternatively, TaqMan MM containing 2X BHQplus probe-based assay (800 nL) was dispensed with the non-contact Dispense Jet and PCR amplification was performed in the Soellex or IntelliCycler using the recommended two-step thermal cycling protocol with 45 cycles according to the manufacturer's instructions. For the PCR uniformity experiment, TaqMan MM containing the duplex LTP/35S assay was dispensed across identical replicates of a GMO-positive corn sample and thermal cycled as described above for a total of 40 cycles. End-point fluorescence values were determined by scanning the Array Tape in the Araya for both experiments. Cluster plot analysis was completed using Douglas Scientific's Intellics® Software Suite.

A)



B)

ARRAY TAPE	NEXAR	SOELLEX	ARAYA
• Flexible microplate replacement	• Liquid handler optimized for Array Tape	• High capacity water bath PCR	• 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

INTELLICYCLER
• Medium capacity PCR water bath
• Optimized for Array Tape
• Three tanks for PCR optimization
• Touchdown or Traditional PCR

Figure 1: (A) Array Tape Platform Overview. (B) IntelliCycler PCR thermal cycling water bath. The temperature of each of the three tanks is individually controlled for PCR protocol optimization. The instrument is adapted for use with Array Tape and can hold up to 50 arrays at one time.

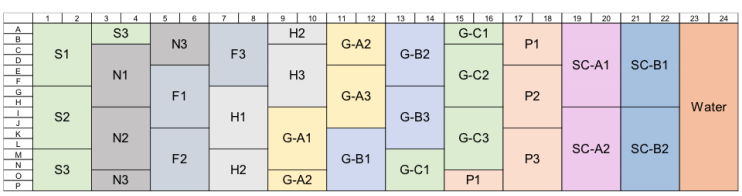


Figure 2: Corn sample layout in the 384-well array. There were 12 replicates of each seed with the exception of SC-A1, SC-A2, SC-B1, and SC-B2, which had 16 replicates. The last two columns of the array contained water controls.

RESULTS AND CONCLUSIONS

All SNP genotyping reactions in this experiment produced clusters that were easily scored using the Douglas Scientific IntelliScore software. Cluster plot images with all assays and master mixes are shown in Figure 3. PCR-based SNP genotyping reactions thermal cycled in both the Soellex and IntelliCycler produced identical consensus genotype calls for each corn seed with both KASP and probe-based assay types. A summary of the calls is provided in Table 1. The LTP/35S duplex assay successfully demonstrated thermal cycling uniformity across a 384-well array. The end-point clusters produced by the Soellex and IntelliCycler were comparable in shape and overall fluorescence intensity, as shown in Figure 4. In conclusion, these experiments demonstrate that the IntelliCycler is a viable solution for medium throughput end-point genotyping applications with the Array Tape platform.

REFERENCES

Alary R., Serin, A., Maury D., Jouira H.B., Sirven, J.P., Gautier M.F., Joudrier, P. Comparison of simplex and duplex real-time PCR for the quantification of GMO in maize and soybean. Food Control 2002; 13:235-244.

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.

	SNP Dzm2575115				SNP Dzm2490176			
	KASP		GTXpress		KASP		GTXpress	
Sample	Soellex	IntelliCycler	Soellex	IntelliCycler	Soellex	IntelliCycler	Soellex	IntelliCycler
S1	TT	TT	TT	TT	CC	CC	CC	CC
S2	AA	AA	AA	AA	TT	TT	TT	TT
S3	AT	AT	AT	AT	CT	CT	CT	CT
N1	TT	TT	TT	TT	TT	TT	TT	TT
N2	AA	AA	AA	AA	TT	TT	TT	TT
N3	AT	AT	AT	AT	TT	TT	TT	TT
F1	TT	TT	TT	TT	CT	CT	CT	CT
F2	AT	AT	AT	AT	TT	TT	TT	TT
F3	TT	TT	TT	TT	CT	CT	CT	CT
H1	TT	TT	TT	TT	CC	CC	CC	CC
H2	AA	AA	AA	AA	CC	CC	CC	CC
H3	AT	AT	AT	AT	CT	CT	CT	CT
G-A1	TT	TT	TT	TT	TT	TT	TT	TT
G-A2	AT	AT	AT	AT	TT	TT	TT	TT
G-A3	AA	AA	AA	AA	TT	TT	TT	TT
G-B1	TT	TT	TT	TT	TT	TT	TT	TT
G-B2	TT	TT	TT	TT	TT	TT	TT	TT
G-B3	TT	TT	TT	TT	TT	TT	TT	TT
G-C1	AT	AT	AT	AT	TT	TT	TT	TT
G-C2	AA	AA	AA	AA	TT	TT	TT	TT
G-C3	TT	TT	TT	TT	CT	CT	CT	CT
P1	AA	AA	AA	AA	CT	CT	CT	CT
P2	AT	AT	AT	AT	CC	CC	CC	CC
P3	AA	AA	AA	AA	CT	CT	CT	CT
SC-A1	TT	TT	TT	TT	TT	TT	TT	TT
SC-A2	AA	AA	AA	AA	TT	TT	TT	TT
SC-B1	AA	AA	AA	AA	CC	CC	CC	CC
SC-B2	AA	AA	AA	AA	TT	TT	TT	TT

Table 1: Summary of SNP genotyping calls for each seed sample. There was 100% concordance between each condition tested. FAM = Red, HET = Purple, HEX = Blue

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

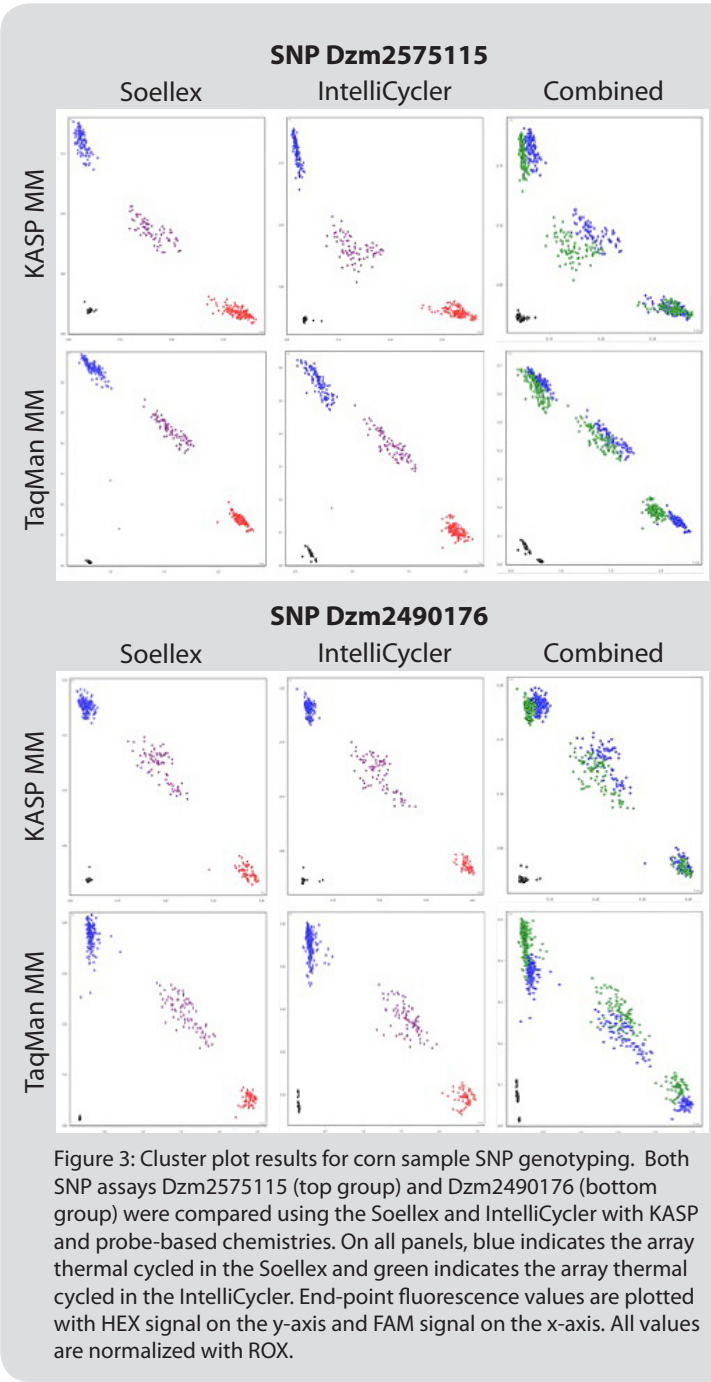


Figure 3: Cluster plot results for corn sample SNP genotyping. Both SNP assays Dzm2575115 (top group) and Dzm2490176 (bottom group) were compared using the Soellex and IntelliCycler with KASP and probe-based chemistries. On all panels, blue indicates the array thermal cycled in the Soellex and green indicates the array thermal cycled in the IntelliCycler. End-point fluorescence values are plotted with HEX signal on the y-axis and FAM signal on the x-axis. All values are normalized with ROX.

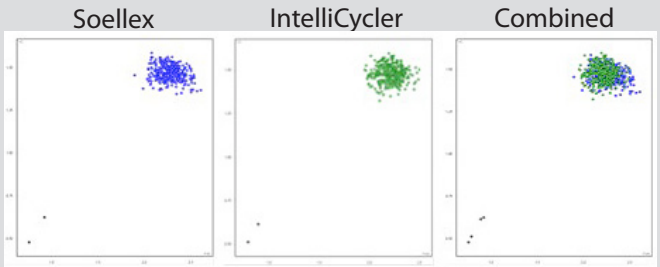


Figure 4: Cluster plot analysis of 382 replicates of crude corn DNA with the LTP/35S duplex PCR assay. Two wells served as negative controls. Cluster shape and overall fluorescence intensity were similar from data generated using the Soellex and the IntelliCycler. End-point fluorescence values are plotted with HEX signal on the y-axis and FAM signal on the x-axis. All values are normalized with ROX.