Genotyping of Human Reference DNA Samples with Pharmacologically Important Single Nucleotide Polymorphisms using KASP™ Genotyping Chemistry on the Nexar® System

ABSTRACT

Genetic analysis of Single Nucleotide Polymorphisms (SNPs) must be accurate, reliable, and economical to be a viable part of research for human genetics, drug discovery, and diagnostics. To address these needs, Douglas Scientific® has developed a highly accurate and automated platform for SNP genotyping in miniaturized reaction volumes. The study described here demonstrates the efficacy and economic advantages of the Nexar System and KASP™ chemistry for the analysis of human reference DNA samples using five pharmacologically significant SNPs. Results produced in Array Tape® matched those previously published for all SNPs and samples included in the study.

INTRODUCTION

Single Nucleotide Polymorphisms (SNPs) are powerful tools for genetic analysis. They are used in many fields, ranging from plant breeding to human diagnostics. Certain SNPs in human genomic DNA are associated with significant variation in drug metabolism or response to drug treatment between individuals. Therefore, development of accurate and economical methods for SNP genotype analysis is of utmost importance to customizing healthcare research. While there are several methods and instruments for this purpose on the market, there remains an unmet need for the development of an economical and automated method of SNP analysis to ensure that the benefits of personalized medicine reach those who need it most.

Metabolism of the commonly prescribed anticoagulant drug warfarin and the associated dosage requirements can vary up to 20-fold in Caucasian populations. Takeuchi, et al. demonstrated the link between warfarin dosage and SNP alleles within VKORC1, CYP2C9 (two SNPs) and CYP4F2. Likewise, Ference, et al. analyzed the genetic link between the SNP alleles in the KIF6 gene and response to statin drug therapy for reduction of cardiovascular disease. These SNPs have significant impacts on patient health, and the diseases with which they are associated are widespread.

In this study, we analyzed five pharmacologically important SNPs (VKORC1, CYP2C9*2, CYP2C9*3, CYP4F2, and KIF6) with a miniaturized PCR-based SNP genotyping method using Kompetitive Allele Specific PCR genotyping reagents (KASP™) from LGC Genomics and the Nexar System from Douglas Scientific. This study included 34 highly characterized human genomic DNA reference samples purchased from the Coriell Institute for Medical Research. By using reference samples from Coriell and KASP SNP assays designed by LGC Genomics, we were able to directly compare the genotyping results generated with the Nexar System to those previously published by Pratt, et al.

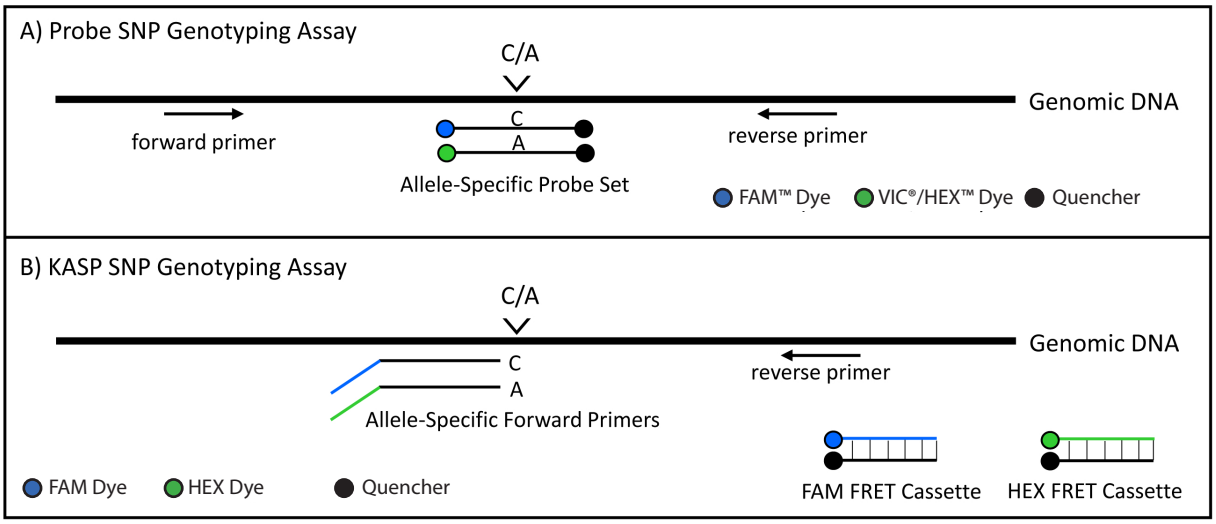

Figure 1: Assay design for probe and KASP SNP genotyping reactions. A) Probe-based SNP genotyping assays utilize forward and reverse primers to amplify a segment of genomic DNA surrounding a SNP. Two fluorogenic probes differentially bind to their allele-specific complement. Fluorescent signal is produced 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.

SNP Name	dbSNP rs#	Chromosomal Location	Pharmacological Significance
CYP2C9*2	rs1799853	Chr10 (q23.33)	Genetic variability impacts metabolism and dosage of warfarin
CYP2C9*3	rs1057910	Chr10 (q23.33)	Genetic variability impacts metabolism and dosage of warfarin
VKORC1	rs9923231	Chr16 (p11.2)	Genetic variability impacts metabolism and dosage of warfarin
CYP4F2	rs2108622	Chr19 (p13.12)	Genetic variability impacts metabolism and dosage of warfarin
KIF6	rs20455	Chr6 (p21.2)	Genetic variability impacts risk reduction of coronary heart disease by statin therapy

Table 1: SNP information

	CYP2C9*2		CYP2C9*3		CYP4F2		VKORC1		KIF6	
CELL LINE	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
7439	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/C	C/C
10005	C/C	C/C	A/A	A/A	A/G	A/G	G/G	G/G	C/T	C/T
12244	C/T	C/T	A/C	A/C	G/G	G/G	G/G	G/G	T/T	T/T
12273	C/T	C/T	A/A	A/A	A/G	A/G	G/G	G/G	C/T	C/T
17039	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/C	C/C
17052	C/C	C/C	A/A	A/A	G/G	G/G	A/A	A/A	T/T	T/T
17057	C/C	C/C	A/A	A/A	A/A	A/A	A/G	A/G	C/C	C/C
17058	C/C	C/C	A/A	A/A	G/G	G/G	A/A	A/A	C/T	C/T
17084	C/T	C/T	A/A	A/A	A/A	A/A	A/G	A/G	C/T	C/T
17114	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/T	C/T
17115	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/C	C/C
17119	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/C	C/C
17129	C/T	C/T	A/A	A/A	G/G	G/G	G/G	G/G	C/C	C/C
17130	C/C	C/C	A/C	A/C	G/G	G/G	G/G	G/G	C/C	C/C
17203	C/C	C/C	A/A	A/A	G/G	G/G	A/G	A/G	T/T	T/T
17204	C/C	C/C	A/C	A/C	G/G	G/G	A/A	A/A	T/T	T/T
17210	C/T	C/T	A/A	A/A	A/A	A/A	A/A	A/A	C/T	C/T
17221	C/T	C/T	A/C	A/C	A/G	A/G	A/G	A/G	T/T	T/T
17227	C/T	C/T	A/A	A/A	A/G	A/G	A/G	A/G	T/T	T/T
17235	C/C	C/C	A/A	A/A	G/G	G/G	G/G	G/G	C/T	C/T
17240	C/C	C/C	A/A	A/A	G/G	G/G	A/A	A/A	C/T	C/T
17246	C/T	C/T	A/A	A/A	G/G	G/G	A/G	A/G	C/T	C/T
17247	C/C	C/C	C/C	C/C	A/A	A/A	A/G	A/G	T/T	T/T
17248	C/C	C/C	A/A	A/A	A/G	A/G	A/A	A/A	T/T	T/T
17252	C/T	C/T	A/C	A/C	G/G	G/G	A/G	A/G	C/C	C/C
17272	C/C	C/C	A/A	A/A	A/A	A/A	A/A	A/A	T/T	T/T
17280	C/T	C/T	A/A	A/A	G/G	G/G	G/G	G/G	T/T	T/T
17281	C/C	C/C	A/A	A/A	G/G	G/G	A/G	A/G	C/C	C/C
17289	C/C	C/C	A/A	A/A	A/G	A/G	A/A	A/A	T/T	T/T
17293	C/T	C/T	A/A	A/A	G/G	G/G	A/G	A/G	C/T	C/T
17296	C/C	C/C	A/A	A/A	A/A	A/A	A/G	A/G	T/T	T/T
17298	C/C	C/C	A/A	A/A	A/G	A/G	A/G	A/G	C/T	C/T
17300	C/C	C/C	A/A	A/A	A/G	A/G	A/G	A/G	C/C	C/C
2016	C/C	C/C	A/A	A/A	G/G	G/G	A/G	A/G	C/C	C/C

Table 2: Expected and observed SNP genotype alleles for genomic DNA from each cell line in this study. Expected alleles are the consensus allele calls published by Pratt, et al.

MATERIALS AND METHODS

Samples and Supplies

Purified genomic DNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. DNA from 34 individual cell lines (Set 1 described by Pratt, et al.) was used in this study. DNA samples were diluted to 5 ng/µL in molecular grade water before use. KASP 1536 V4.0 2X Master Mix (LGC Genomics) was used for all SNP genotyping reactions. Master mix was provided at 2X concentration and was used according to the manufacturer's instructions. KASP SNP genotyping assays were designed by LGC Genomics and unlabeled primers were obtained from LGC Biosearch Technologies. KASP SNP genotyping assays differ from traditional probe-based assays which consist of two labeled allele-specific probe sequences and forward and reverse primers (Figure 1A). KASP assays consist of two different allele specific forward primers with unique tag sequences and one common reverse primer (Figure 1B), and the KASP Master Mix contains two FRET cassettes specific to the unique tag sequences. All KASP assays were used according to the manufacturer's instructions and were added at 2X concentration to the 2X KASP 1536 V4.0 Master Mix before use. Information on the SNPs described in this study can be found in Table 1.

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. End-point fluorescent 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 peformed 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

Genomic DNA from 34 individual cell lines was successfully genotyped with five SNP genotyping assays using the Nexar System in miniaturized (1.6 µL) reactions. Identical allele calls were observed for duplicate reactions and all results generated in Array Tape matched the consensus alleles published by Pratt, et al. A matching set of reactions were completed with TaqMan® SNP genotyping assays and TaqMan Genotyping Master mix using both a ViiA™ 7 Real-Time PCR System (Life Technologies) with 5 µL reactions and the Nexar System with 1.6 µL reactions (data not shown), and these results confirmed those generated by KASP assays in Array Tape. Observed and expected SNP allele calls for each cell line and SNP assay are given in Table 2. Expected calls listed in Table 2 are from previously published results by Pratt, et al. Cluster plot analysis of each SNP assay was performed to determine allele calls, as shown in Figure 3. The cluster plot of each assay contains all 34 cell lines in duplicate for a total of 72 data points—68 samples and four no template controls.

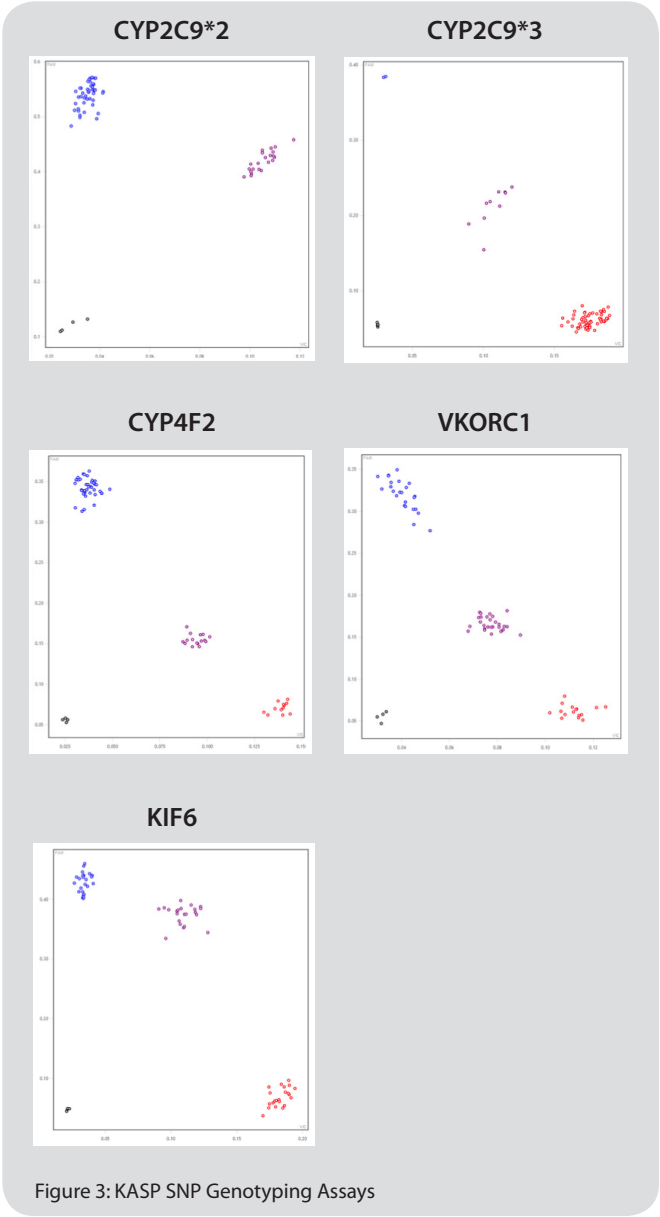

Figure 3: KASP SNP Genotyping Assays

CONCLUSIONS

This experiment demonstrates that expected SNP alleles can accurately be determined for human DNA samples by using miniaturized PCR reactions with KASP genotyping chemistry in the Nexar System. Genomic DNA samples from previously analyzed cell lines were genotyped for pharmacologically significant markers using 1.6 µL reactions in Array Tape, and the results matched the previously published alleles with 100% concordance. Array Tape and the accompanying automation significantly reduces reaction volumes, hands-on effort and over-all cost of SNP genotyping, leading to a reliable and economical alternative to traditional PCR-based SNP genotyping techniques.

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