Genotyping of Human Reference DNA Samples with Pharmacologically Important Single Nucleotide Polymorphisms using BHQplus® Probe-Based Assays 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, flexibility, and economic advantages of the Nexar System for the analysis of human reference DNA samples using five pharmacologically significant SNPs. Custom BHQplus probe-based SNP genotyping assays were used with six different commercially available master mixes. 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 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 are associated with a number of human diseases and have a significant impact on patient health.

In this study we analyzed five pharmacologically important SNPs (VKORC1, CYP2C9*2, CYP2C9*3, CYP4F2, and KIF6) using BHQplus SNP assays from LGC Biosearch Technologies™ and the Nexar System from Douglas Scientific. Six different commercially available PCR master mixes were compared. 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, we were able to directly compare the genotyping results generated with the Nexar System to those previously 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.) were used in this study. DNA samples were diluted to 5 ng/µL in molecular grade water before use. The PCR master mixes used in this study can be found in Table 1. 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. Genomic information about the SNPs analyzed in this study can be found in Table 2. All primers and probes were obtained from LGC Biosearch Technologies. Oligos were added at 2X concentration to the 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.

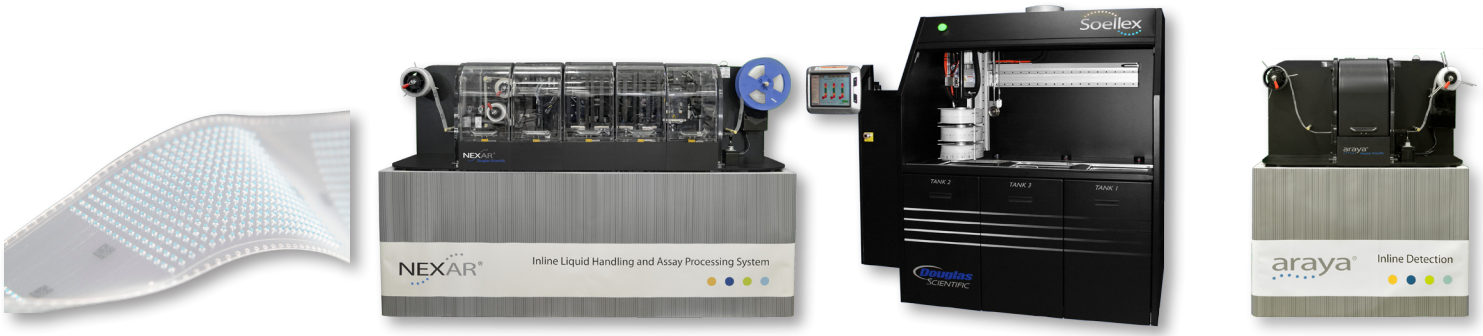
Reagent	Source
TaqMan® GTXpress™ Master Mix	Applied BioSystems
TaqMan Genotyping Master Mix	Life Technologies
PerfeCTa® qPCR ToughMix®	Quanta BioSciences
SensiMix™ II Probe Lo-ROX Kit	Bioline Reagents Ltd.
SsoAdvanced™ Universal Probes Supermix	Bio-Rad® Laboratories, Inc.
KlearKall Master Mix	LGC Genomics
Unlabeled primers and BHQplus probes	LGC Biosearch Technologies

Table 1: Reagents and assays.

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 and are described in Figure 1. DNA samples (800 nL) were dispensed into Array Tape with the multi-channel, 384 tip pipette dispense head from CyBi® Product Line. 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 according to the standard protocol for each master mix, respectively. 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 performed in duplicate.

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 2: SNP genotyping assay information



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

Figure 1: Nexar System Overview

RESULTS

Genomic DNA from 34 individual cell lines was successfully genotyped with five BHQplus SNP genotyping assays using the Nexar System with miniaturized (1.6 µL) reactions. Identical calls were obtained using six different master mixes with each assay as shown in Table 3. All reactions were run in duplicate and the results generated in Array Tape matched the consensus alleles published by Pratt, et al. Also, a matching set of reactions were completed in a ViiA™ 7 Real-Time PCR System (Life Technologies) with 5 µL reactions and these results confirmed the results generated in Array Tape (data not shown). Observed and expected SNP allele calls for each cell line and SNP assay are given in Table 4. Expected calls listed in Table 4 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 2. The cluster plot of each assay contains all 34 cell lines in duplicate, for a total of 72 data points consisting of 68 samples and four no template controls.

SNP	TaqMan GTXpress	TaqMan Genotyping	PerfeCTa ToughMix	SensiMix II Probe	Bio-Rad SsoAdvanced	KlearKall
CYP2C9*2 (CC/CT/TT)	23/11/0	23/11/0	23/11/0	23/11/0	23/11/0	23/11/0
CYP2C9*3 (AA/AC/CC)	28/5/1	28/5/1	28/5/1	28/5/1	28/5/1	28/5/1
CYP4F2 (AA/AG/GG)	6/8/20	6/8/20	6/8/20	6/8/20	6/8/20	6/8/20
VKORC1 (AA/AG/GG)	8/14/12	8/14/12	8/14/12	8/14/12	8/14/12	8/14/12
KIF6 (CC/CT/TT)	11/11/12	11/11/12	11/11/12	11/11/12	11/11/12	11/11/12

Table 3: Summary of SNP genotyping calls for the 34 cell line samples.

	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 4: 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.

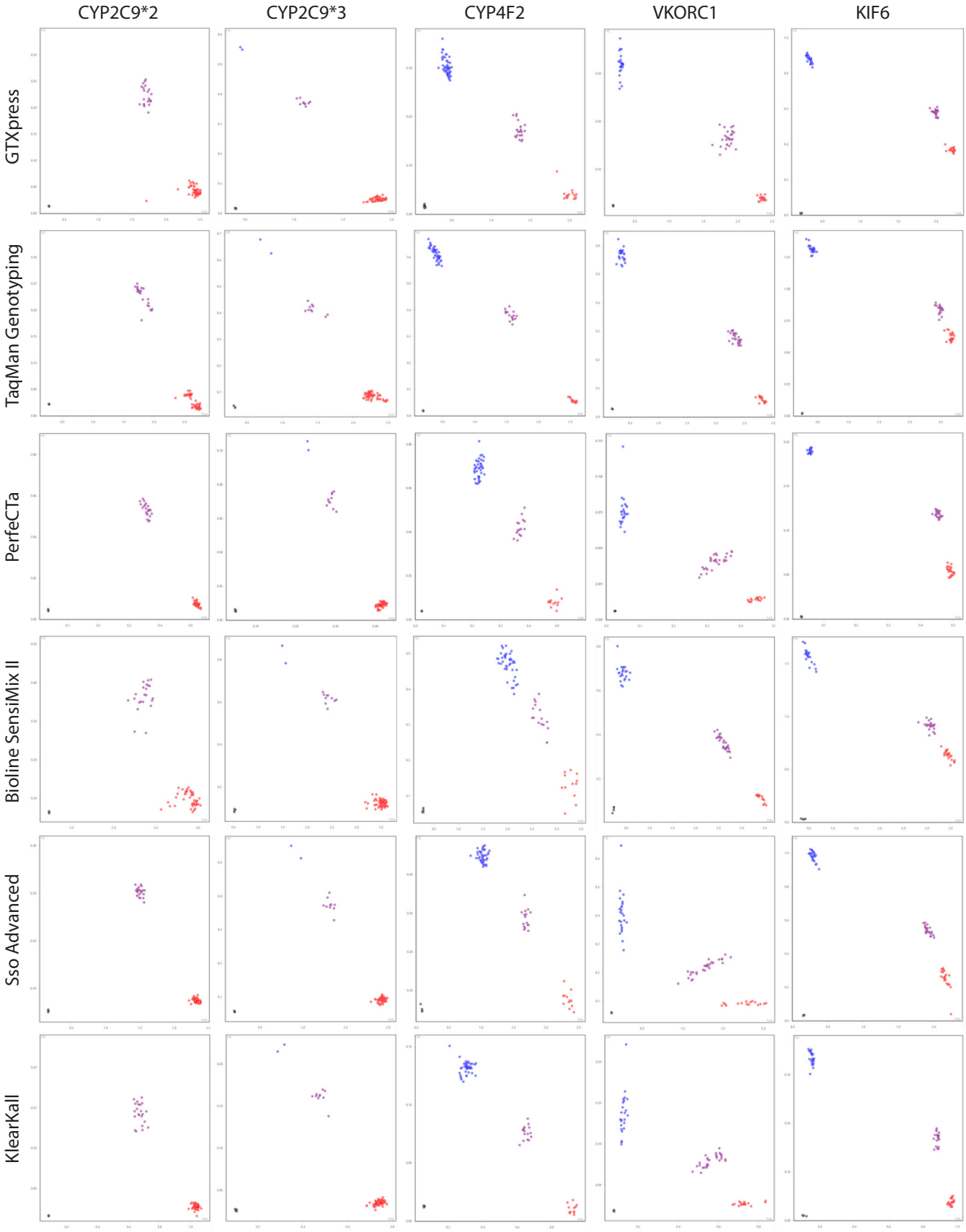


Figure 2: SNP genotyping cluster plots. Endpoint fluorescence values are plotted with CAL Fluor® Orange signal on the y-axis and FAM™ signal on the x-axis. All values are normalized with ROX™.

CONCLUSIONS

The Nexar System used in conjunction with BHQplus SNP genotyping assays provides an accurate and flexible solution for genotyping human DNA samples. Genomic DNA samples from previously analyzed cell lines were successfully genotyped for pharmacologically significant markers using 1.6 µL reactions in Array Tape, and the results matched the previously published alleles with 100% concordance. All of the master mixes used in this study produced consistent allele calls for each SNP tested. Array Tape and the accompanying automation significantly reduces reaction volumes, hands-on effort and overall cost of SNP genotyping, leading to a reliable and economical alternative to traditional PCR-based SNP genotyping techniques using microplates.

REFERENCES

Brian A. Ference, Wonsuk Yoo, John M. Flack, Michael Clarke. A Common KIF6 Polymorphism Increases Vulnerability to Low-Density Lipoprotein Cholesterol: Two Meta-Analyses and a Meta-Regression Analysis. PLoS ONE 2011 6(12):e28834

Victoria M. Pratt, Barbara Zehnbauer, Jean Amos Wilson, Ruth Baak, Nikolina Babic, Maria Bettinotti, Arlene Buller, Ken Butz, Matthew Campbell, Chris Civalier, Abdalla El-Badry, Daniel H. Farkas, Elaine Lyon, Saptarshi Mandal, Jason McKinney, Kasinathan Muralidharan, LeAnne Noll, Tara Sander, Junaid Shabbeer, Chingying Smith, Milhan Telatar, Lorraine Toji, Anand Vairavan, Carlos Vance, Karen E. Weck, Alan H.B. Wu, Kiang-Teck J. Yeo, Markus Zeller, Lisa Kalman. Characterization of 107 genomic DNA reference materials for CYP2D6, CYP2C19, CYP2C9, VKORC1 and UGT1A1: A GeT-RM and Association for Molecular Pathology collaborative project. J Mol Diag 2010 12(6):835-846

Fumihiko Takeuchi, Ralph McGinnis, Stephane Bourgeois, Chris Barnes, Niclas Eriksson, NicoleSoranzo, Pamela Whittaker, Venkatesh Ranganath, Vasudev Kumanduri, William McLaren, Lennart Holm, Jonatan Lindh, Anders Rane, Mia Wadelius, Panos Deloukas. A Genome-Wide Association Study Confirms VKORC1, CYP2C9, and CYP4F2 as Principal Genetic Determinants of Warfarin Dose. PLoS Genetics 2009 5(3):e1000433

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