Poster P20.035.B

Performance of a Combined SNP and Copy Number Pharmacogenomic Research Panel to Determine Phenotypes of Drug Metabolism Genes

Tibor Fule[1], Kathleen Hayashibara[2], Pius Brzoska[2]

[1]Thermo Fisher Scientific – Budapest, Hungary, [2]Thermo Fisher Scientific – South San Francisco, CA, USA.

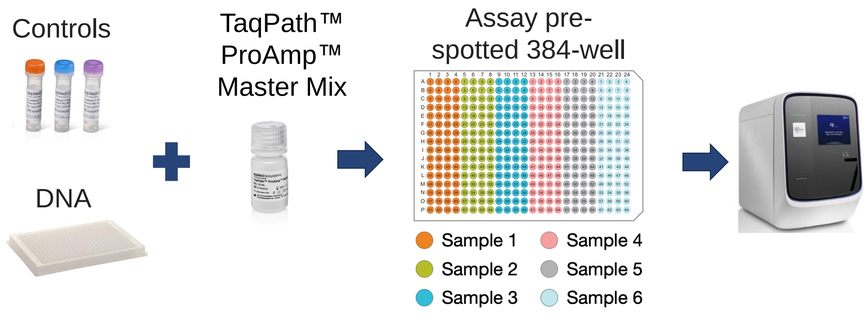
Introduction

Understanding sequence and copy number variations in genes coding for metabolizing enzymes are important when studying drug metabolism. We created a 384-well qPCR research panel containing both TaqMan® SNP Genotyping and Copy Number assays targeting CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP4F2, DPYD, NUDT15, SLCO1B1, TPMT, and VKORC1. Accuracy of phenotypes is presented using Genetic Testing Reference Material (GeT-RM) DNAs containing targeted variants.

Methods

DNAs were normalized to 4 ng/ul, mixed with TaqPath™ ProAmp™ Master Mix, loaded onto pre-spotted assay plates, and run on a QuantStudio™ 7 Flex Real-Time PCR System. CNV and genotyping results were determined by Diomni™ Design and Analysis v3.0 software. Diplotype calls were made using AlleleTyper™ Software v1.0 with translations based on information from PharmGKB.

Product Concept and Workflow



Applied Biosystems QuantStudio™ Real-Time PCR System

Instrument

QuantStudio™ 7 Flex Real-Time PCR System

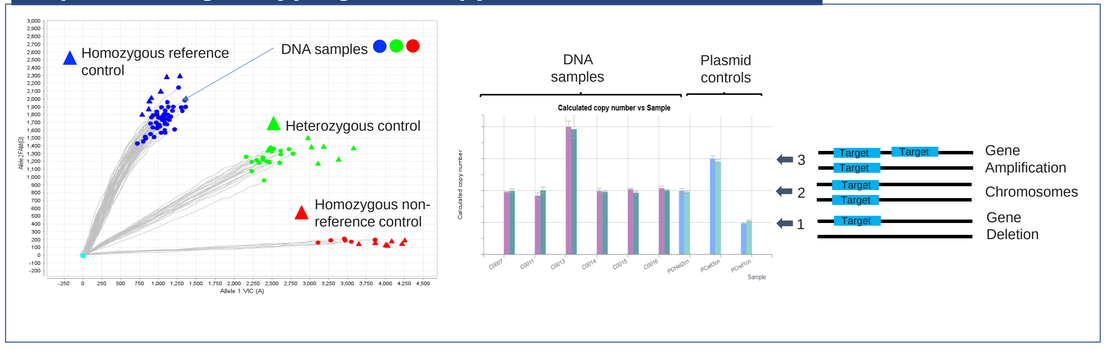
Genotyping and Copy Number data analyzed with integrated software

Diplotypes identified by Translation Table + AlleleTyper™

Results

CYP2D6 5'UTR and Exon 9 assays produced 100% call rate and 100% of calls matched consensus results from several platforms. The 56 panel genotyping assays produced results consistent with consensus star alleles in > 95% of sample-target combinations. Sample-target combinations with single diplotype calls matched consensus phenotypes in 100% of cases. Samples with multiple diplotypes produced at least one combination matching consensus phenotypes in all cases.

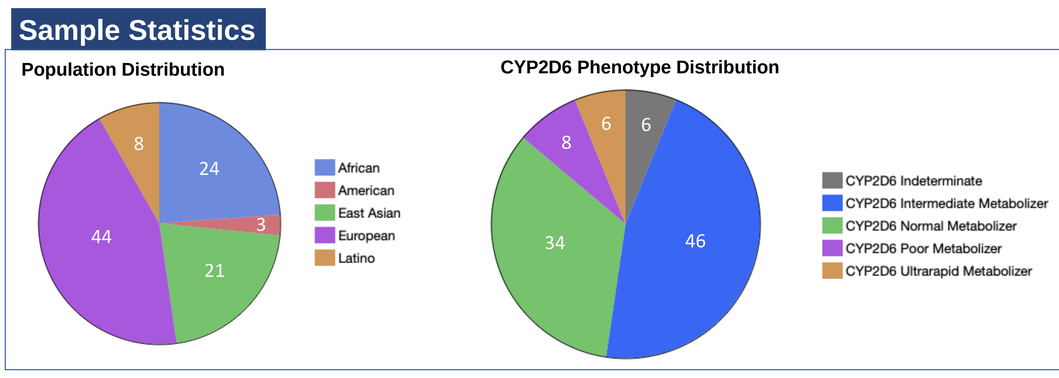
TaqMan SNP genotyping and Copy Number Variation Data



Variant Coverage

Gene	Haplotypes
CYP2B6	*4, *6, *9, *18, *36
CYP2C19	*2, *3 ,*6, *8, *9, * 10, *17
CYP2C9	*2, *3, *5, *6, *8, *11, *13
CYP2D6	*2, *3, *4, *5, *6, *7, *9, *10, *11, *12, *17, *21, *29, *40, *41, *49, *53, *69, *1x2, *2x2, *4x2, *10x2, *17x2, *29x2, *41x2, *4+*68, *10+*36, *10+*36x2, *1>=3, *2>=3
CYP3A4	*22
CYP3A5	*3, *6, *7
CYP4F2	c.1297G>A (*3/*4)
DPYD	c.557A>G, c.868A>G, c.1129-5923C>G (HapB3), c.1129-5923C>G (HapB3)+c.1236G>A (HapB3), c.1679T>G (*13), c.1905+1G>A (*2A), c.2279C>T, c.2846A>T
NUDT15	*2/*3, *4
SLCO1B1	*5, *9, *14/*37, *15
TPMT	*2, *3A, *3B, *3C
VKORC1	c.-1639G>A
2C Cluster	rs12777823

Sample Statistics



Diplotype Results

Gene	Samples with Known Diplotypes	Samples with Diplotype Calls	Call Rate	Total Known Diplotypes Containing Target Alleles	Total Calls from Diplotypes with Target Alleles	Target Allele Diplotype Call Rate	Target Allele Diplotypes w/ Correct Phenotypes	Phenotype Accuracy of Target Allele Diplotypes*	Samples with Non-target Alleles	Known Incorrect Phenotypes from Non-target Allele Samples**
CYP2B6	120	118	98.3%	115	115	100%	115	100%	5	2
CYP2C19	120	120	100.0%	113	113	100%	113	100%	7	1
CYP2C9	120	120	100.0%	112	112	100%	112	100%	8	0
CYP2D6	120	118	98.3%	103	103	100%	103	100%	17	1
CYP3A4	120	120	100.0%	114	114	100%	114	100%	6	0
CYP3A5	120	120	100.0%	120	120	100%	120	100%	0	0
CYP4F2	120	120	100.0%	97	97	100%	97	100%	23	0
DPYD	131	131	100.0%	131	131	100%	131	100%	0	0
NUDT15	12	12	100.0%	6	6	100%	6	100%	6	0
SLCO1B1	120	118	98.3%	111	111	100%	111	100%	9	0
TPMT	136	136	100.0%	122	122	100%	122	100%	14	0
VKORC1	120	120	100.0%	120	120	100%	120	100%	0	0
2C Cluster	84	84	100.0%	84	84	100%	84	100%	0	0

*Based on diplotypes with targeted variants only
** Non-target alleles with unknown or uncertain function are not counted.

CYP2D6 Copy Number Variation Results

Target	Sample CNV	Number of Samples	Percentage	Call Rate	Accuracy
CYP2D6 5UTR	1	12	10.0%	100%	100%
	2	77	64.2%	100%	100%
	3	23	19.2%	100%	100%
	4	8	6.7%	100%	100%
CYP2D6 Exon 9	1	14	11.7%	100%	100%
	2	91	75.8%	100%	100%
	3	13	10.8%	100%	100%
	4	2	1.7%	100%	100%

Conclusion

A 384-well qPCR research panel incorporating both TaqMan® SNP and Copy Number assays in a single workflow suggests possibilities for the development of a robust and accurate tool for determining phenotypes of key pharmacogenetic markers. The panel demonstrated high accuracy and reliability. These results underscore the potential of this panel to enable accurate phenotypic predictions in a streamlined workflow.

All authors are employees of Thermo Fisher Scientific.