Poster G089

Use of OpenArray TaqMan SNP Genotyping and Copy Number Assay Pharmacogenomic Research Panels to Determine Phenotypes of Drug Metabolism Genes

Kathleen Hayashibara, Kevin Papenfuss, Jeff Marks, Greg Govoni, Kerri Miller, Namrata Pabbati, Kelly Li

Thermo Fisher Scientific, South San Francisco, CA 94080 USA

Introduction

Variations in sequence or copy number of drug metabolizing enzymes may result in absent or altered activity towards substrates. Determining phenotypes based on SNP and copy number variation (CNV) is challenging in terms of workflow, data analysis, and cost per sample. Results are presented from a 120 SNP and CNV assay research panel on TaqMan® OpenArray® Real-Time PCR Plates with Genetic Testing Reference Material (GeT-RM) DNAs.

TaqMan DME Genotyping Assays use two allele specific probes labeled with different dyes in combination with 5´-nuclease chemistry for amplifying and detecting specific polymorphisms in DNA.

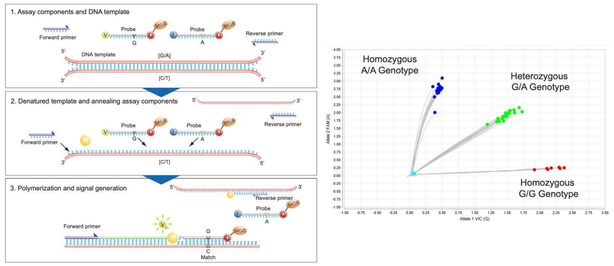

Figure 1: TaqMan SNP Genotyping Assay concept and example data

TaqMan CNV Assays determine the number of copies of a target in an unknown sample by measuring ΔCq between a reference assay and target assay using a calibrator DNA with known copy number and measuring ΔCq between the same assays for an unknown sample. The ΔΔCq between known and unknown CNV samples is then used to calculate the copy number based on the equation: Copy Number = $2^{-\Delta\Delta Cq}$ x 2.

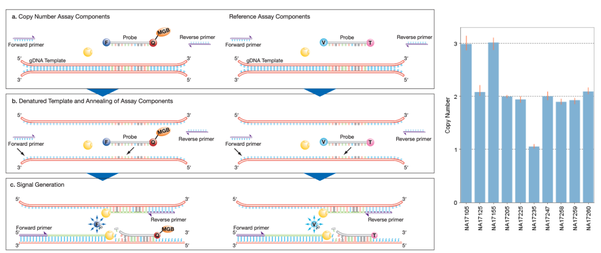

Figure 2: TaqMan Copy Number Variation Assay concept and example data

Combining results from both assays allows identification of star alleles correlated with enzyme activity.

Previously DME SNP and CNV assays were run on separate qPCR plates. Here we present data from an integrated workflow where both assay types are run on the same plate for a more streamlined workflow.

Materials and Methods

DNA was quantified by qPCR with a known standard vs. TaqMan™ Copy Number Reference Assay, human, TERT (4403316). DNA was normalized to 20 ng/ul, mixed with an equal volume of TaqMan OpenArray Genotyping Master Mix and loaded onto OpenArray plates with both SNP and CNV assays. Plates were run on a QuantStudio 12K Flex Real-Time PCR System with v1.3 software. Genotype calls were made with Diomni™ Design and Analysis v3.0 software and a reference panel of pooled synthetic plasmid controls for each genotype. CYP2D6 Exon 9 and 5'UTR CNV was determined from multiple replicates on each plate using a PCR efficiency-modified ΔΔCq between known and unknown CNV samples which was then used to calculate the copy number based on CN = $2^{-\Delta\Delta Cq}$ x 2. Diplotype calls were made using AlleleTyper Software v1.0 with translations based on information from PharmGKB.

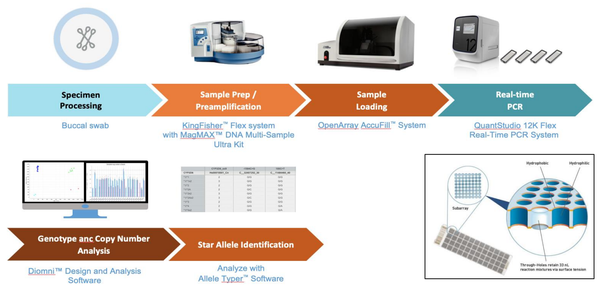

Figure 3: Integrated SNP + CNV workflow with OpenArray detail (inset)

Gene	Variants
ABCG2	c.421C>T
APOE	e2, e4
BCHE	A, F1, F2, K
CYP2B6	*4, *5, *6, *7, *8, *18, *22
CYP2C9	*2, *3, *4, *5, *6, *8, *11, *12, *13, *15, *16, *71
CYP2C19	*2, *3, *4A *5, *6, *8, *9, *10, *17
CYP2D6	*2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *17, *21, *29, *31, *32, *34, *35, *36, *39, *40, *41, *42, *45, *49, *53, *56, *59, *68, *69, *91, *109, *114, *119
CYP3A4	*20, *22
CYP3A5	*3, *6, *7
CYP4F2	c.1297G>A, *3 or *4
DPYD	c.1297G>A, *3 or *4, c.1679T>G (*13), c.1905+1G>A (*2A), c.2846A>T, c.1129-5923C>G (HapB3), c.1236G>A (HapB3), c.557A>G, c.868A>G, c.2279C>T
G6PD	c.292G>A or c.202G>A, c.466A>G or c.376A>G
NAT2	*5A, *6A, *6B,*7A,*12A, *14A, *19
NUDT15	*3, *4, *14
RNR1	chrMT:1555 A>G
SLCO1B1	*5, *9, *15
TPMT	*2, *3A, *3C, *9, *11, *29, *42
UTG1A1	*6, *27, *80
VKORC1	c.-1639G>A, c.196G>A, c.106G>T, D36Y, c. 1173T>C
2C Cluster	g.94645745G>A

Table 1: Panel genes and variants

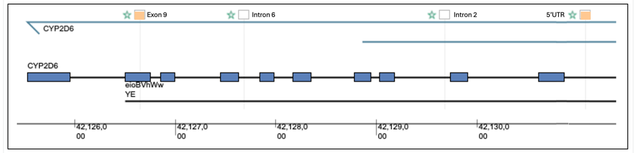

Figure 4: Location of CNV assays on CYP2D6 gene (Chr 22)

Results

Genotyping

Average genotype call rates of two replicates of 135 GeT-RM DNAs was 99.6-99.7%. At least one DNA/assay combination produced a genotype result for an overall call rate of 100%.

Table 2: OpenArray SNP genotype call rate and accuracy DNA

Metric	Replicate 1	Replicate 2
Calls (out of 14204)	14161	14159
Call Rate	99.7%	99.6%

Copy Number Variation

CYP2D6 5'UTR and Exon 9 CNV assays using TERT as a reference produced 100% call rate and 100% accuracy with copy number based on consensus GeT-RM diplotypes determined by sequencing and other platforms.

Table 3: OpenArray CNV call rate and accuracy from GeT-RM DNAs

CNV Target	DNAs	Calls	Call Rate	Correct Calls	Accuracy	Pass 2 Call Rate	Correct Calls w/ Pass 2	Accuracy
CYP2D6 5'UTR	134	131	97.8%	131	100.0%	100.0%	135	100.0%
CYP2D6 Exon 9	134	133	99.3%	133	100.0%	100.0%	135	100.0%

* CYP 2D6 5'UTR and Exon 9 copy numbers greater than 3 are treated as 3+ copies.
** DNAs that did not pass QC metrics were re-run and produced expected results.

Star Allele Analysis

Samples with CYP2D6 star alleles identified by one or more independent studies [1,2] were analyzed using the Allele Typer software. Diplotypes determined by AlleleTyper were compared to consensus results. Identical phenotypes consistent with consensus diplotypes were determined from 88.0% (118/134) of samples. DNA with > 3 copies of CYP2D6 produced multiple results from the translation, where 13 samples produced at least one call matching the consensus diplotype in addition to others with different ratios of alleles from the consensus.

Table 4: Analysis of CYP2D6 star alleles from OpenArray format 120 panel

Metric	Count	Percentage
Diplotype Match	86	64.20%
Phenotype Match	119	88.90%
Multiple diplotypes	13	9.70%
Alleles called *1 or *2 due to SNP coverage	2	1.50%

Conclusions

An OpenArray panel with both TaqMan SNP Genotyping and TaqMan Copy Number Assays was able to produce accurate SNP and CNV results using GeT-RM DNA containing a wide range of star alleles. In this non-random, diverse collection across multiple populations, 88% of samples produced diplotypes with phenotypes matching consensus results. Limitations involve a small percentage of cases where several diplotypes with different phenotypes are possible based on the SNP and CNV results. The number of samples requiring further resolution of allele ratios should be lower for a random set of samples. Buccal DNA showed SNP and CNV call rates and accuracies comparable to Coriell DNA.

References

1. Characterization of 137 Genomic DNA Reference Materials for 28 Pharmacogenetic Genes, Pratt, V.M. et al., J Mol Diagn. 2016 Jan; 18(1): 109–123.

2. Allelic decomposition and exact genotyping of highly polymorphic and structurally variant genes, Numanagić, I. et al., Nat Commun. 2018 Feb 26;9:828.

Acknowledgements

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