The VeriDose CYP2D6 CNV Panel: A One-Well Solution for Copy Number and Hybrid Allele Detection of CYP2D6

Robin E. Everts, Augusto Lois, Aleksey Nakorchevsky, Helen Tao, Keith Jackson, Kate Rhodes

Agena Bioscience, 4755 Eastgate Mall, San Diego, CA 92121, USA

INTRODUCTION:

Detection of copy number variation (CNV) in the CYP2D6 gene is an important component of pharmacogenetic testing. CYP2D6 copy number influences the metabolizer status of a person and which drug and dose are likely to be the most effective. Hybrid alleles are CYP2D6 alleles with unique rearrangements with the CYP2D7 gene, rendering the allele inactive. Several CYP2D6 hybrid alleles have been defined in the literature, of which the most common are *13, *36, *4N and *68. Prevalence of hybrid alleles varies by population, but it is estimated that up to 13% of the general population possess a CYP2D6 gene hybrid. The *36 allele is the most prevalent of these alleles, as up to 40% of people of Asian descent possess a *36 allele. The *13 and *68 alleles are present across many ethnicities and occur in about 1%-2% of individuals. Detecting the presence of these non-functional CYP2D6 alleles is critical in pharmacogenetic testing to ensure an accurate metabolizer status is determined.

METHODS

Assay Design: Agena Bioscience has created a single-well panel that uses 22 assays which simultaneously interrogate 7 regions of the CYP2D6 gene. The CYP2D6 gene region was aligned vs the genome and PCR primers were designed to only amplify CYP2D6 and CYP2D7 or CYP2D6 and CYP2D8. Each region contained several mismatches between the 2 genes, allowing us to use as an artificial SNP and thus design Single Base Extend Primers.

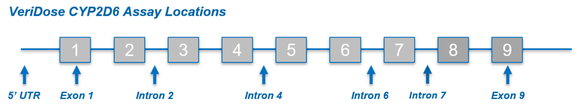
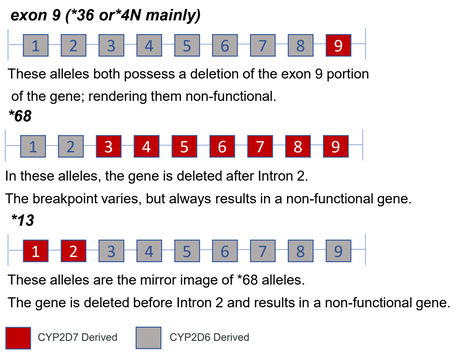


Figure 1. Overview of CYP2D6 gene structure and approximate location of the fragments amplified and tested

Software Design: The VeriDose CYP2D6 CNV Panel is accompanied by a reporting software that automatically analyzes each “SNP”. A dedicated 2N control sample has to be present on each chip to anchor and normalize the data. By detecting peak height at each SNP, the algorithm calculates the copy number for CYP2D6 and a CNV value, which represents the confidence level in the call. These values are then analyzed, and a final result is displayed in an easy to interpret report (Figure 2). The software will perform several comparisons to detect whether there is a hybrid allele or not, which hybrid allele is present, and what the overall copy number of CYP2D6 is as well as the copy number of the CYP2D6 alleles without a hybrid portion.

Summary of key software features:

- Automatic analysis of peak heights to identify copy number for each assay
- Automatic analysis for hybrid alleles
- Calculation of overall copy number as well as copy number for non-hybrid alleles
- All results displayed in easy to interpret reports



Experiment 1:

881 unique clinical samples (Buccal swabs) were run with the VeriDose CYP2D6 CNV panel to determine accuracy. All samples were tested with Thermo Fisher TaqMan Probes for intron 2, intron 6 and exon 9 for CYP2D6 CNV as an orthogonal validation of copy number. Copy numbers and hybrids were compared to determine overall concordance of the assay

Experiment 2:

Thirteen samples were repeated 20 times on 96- and 384-well plates using 10 ng of DNA input to assess the repeatability. After iPLEX SBE reaction, the plates were spotted using an RS1000 as well as the CPM module. Samples were divided over 1N, 2N, 3N+ copy numbers. Orthogonal copy number data was obtained by TaqMan CNV analysis as above.

Both experiments were performed using standard iPLEX PRO reagents and protocols.

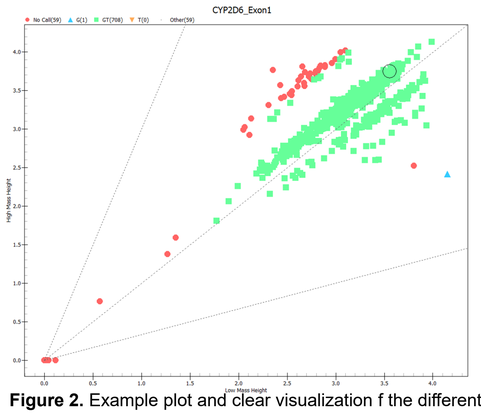


Figure 2. Example plot and clear visualization f the different groupings for 1N, 2N, 3N, 4N etc

RESULTS

This panel was shown to accurately determine CYP2D6 copy number. For the CYP2D6 copy number, the concordance with TaqMan CNV assays for intron 2, intron 6 and exon 9 exceeded 97%, based on 881 DNA samples. See Table 1.

When looking at the overall data, 68 samples identified as *68 carrying which were assigned correct if their intron 2, intron 6 and exon 9 data were similar to what was observed for the VeriDose CNV panel.

There were approx. 15 samples showing a very solid 2N on VeriDose CNV whereas the TaqMan data was variable between 2N and 3N and many samples had a 2.5-2.6 copy number call on TaqMan probes.

Repeat testing of these samples did not clarify the outcome and is being followed up on.

Testing the same samples multiple times showed the repeatability of the assay is high and > 98% using different equipment. See Table 2.

Table 1. Concordance analysis for the VeriDose CYP2D6 panel and data generated by TaqMan intron 2, intron 6 , and exon 9 assays.

	Samples	Concordance CN	Concordance Functional
Number	881	862*	856
Percentage		97.8	97.2

* Includes samples called *68 if their functional outcome was similar to TaqMan outcome. This as the TaqMan assays, as used, were unable to account for *68 alleles.

Table 2. Concordance analysis for the VeriDose CYP2D6 panel using 13 samples repeated 20 times each and 1) amplified using either 96 or 384 thermal cycler and 2) spotted using an RS1000 or CPM spotting device.

Repeatability	CN	Functional
Samples	1053	1053
Concordant Samples	1024	1035
Percent Concordance	97.2	98.3

CONCLUSION

The VeriDose CYP2D6 CNV panel is a single reaction and:

- ✓ Can detect copy numbers for 7 distinct regions in the gene
- ✓ Compares these regions with each other to detect hybrid allele presence of *13, exon 9 (*36, *4N, *) and *68 hybrids
- ✓ Calculates the copy number including the hybrid alleles as well as report the functional or CYP2D6 alleles without hybrid alleles
- ✓ Has greater than 95% concordance with TaqMan assays

The VeriDose CYP2D6 CNV panel can be run concurrently with any of Agena Bioscience’s SNP Pharmacogenetics panels using the same workflow. The Pharmacogenetics Report software will automatically integrate the copy number all into the CYP2D6 diplotype call. See Table 3.

Table 3. Example dataset for the VeriDose CYP2D6 panel compared to data generated by TaqMan intron 2, intron 6 , and exon 9 assays.

Sample	CYP2D6	CNV	CNV Quality	Hybrid Status	CNV Functional Outcome	qPCR Intron2	qPCR Intron6	qPCR Exon9
Sample0002	1N *1/*5	1N	(1.11-0.07-HighConf)		1N(1.11)	0.94	0.96	0.94
Sample0003	1N *9/*5	1N	(1.06-0.1-HighConf)		1N(1.06)	0.96	0.97	0.94
Sample0005	1N *1/*5	1N	(1.05-0.13-HighConf)		1N(1.05)	1	0.95	1
Sample0009	1N *4/*5	1N	(1.05-0.09-HighConf)		1N(1.05)	1.03	1.09	1.08
Sample0052	1N *13 No Call	2N	(2.01-0.14-HighConf)	*13	1N(1.01)	1.3	1.27	2.47
Sample0053	1N *13 *2/*2	2N	(2.17-0.09-HighConf)	*13	1N(1.17)	1.11	1.09	2.41
Sample0054	1N *68 *4/*5	2N	(1.93-0.09-HighConf)	*68	1N(0.96)	0.91	0.9	0.96
Sample0055	1N *68 *4/*5	2N	(1.88-0.16-HighConf)	*68	1N(1)	1	0.81	0.94
Sample0090	2N *2/*41	2N	(1.99-0.05-HighConf)		2N(1.99)	1.74	1.89	2.08
Sample0102	2N *1/*2	2N	(2.1-0.04-HighConf)		2N(2.1)	1.79	1.89	2.02
Sample0143	2N *4/*4	2N	(2-0.07-HighConf)		2N(2)	1.9	1.74	1.9
Sample0163	2N *3/*4	2N	(1.98-0.05-HighConf)		2N(1.98)	1.93	1.85	1.96
Sample0465	2N *1/*2	2N	(1.92-0.07-HighConf)		2N(1.92)	1.96	1.93	1.98
Sample0466	2N *1/*1	2N	(1.92-0.03-HighConf)		2N(1.92)	1.96	1.93	2.19
Sample0671	2N *68 *1/*4	3N+	(2.69-0.1-MedConf)	*68	2N(1.94)	1.91	2.01	1.88
Sample0672	2N *68 *4/*4	3N+	(2.61-0.08-MedConf)	*68	2N(1.91)	1.92	1.83	2
Sample0700	3N+ *68 *2/*4	3N+	(3.51-0.08-LowConf)	*68	3N+(2.97)	3.28	3.12	2.88
Sample0748	2N *Exon9 *1/*4	3N+	(2.63-0.16-MedConf)	*Exon9	2N(1.76)	3.05	3.34	2.38
Sample0750	2N *Exon9 *3/*4	3N+	(2.8-0.07-HighConf)	*Exon9	2N(1.94)	3.33	2.72	2.14
Sample0753	2N *Exon9 *1/*4	3N+	(2.62-0.1-MedConf)	*Exon9	2N(1.94)	2.63	3.02	2.09
Sample0763	3N+ *1/*10	3N+	(2.96-0.08-HighConf)		3N+(2.96)	3.43	3.27	3.18
Sample0771	3N+ *1/*2	3N+	(2.95-0.03-HighConf)		3N+(2.95)	3.48	3.2	2.91
Sample0802	3N+ *1/*2	3N+	(3.88-0.07-HighConf)		3N+(3.88)	4.37	4.03	4.38
Sample0820	3N+ *1/*2	3N+	(3.06-0.05-HighConf)		3N+(3.06)	3.22	3.06	2.98
Sample0832	3N+ *1/*2	3N+	(4.6-0.09-LowConf)		3N+(4.6)	6.44	6.69	5.24