A novel targeted NGS-based assay for simultaneous detection of SNVs and structural variation in pharmacogenes

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Introduction

Pharmacogenomics (PGx) can help optimizing drug therapy, yet current methods have limitations in terms of genomic coverage, costs, incidental findings and privacy concerns. Furthermore, several technologies struggle with accurate genotyping of complex but important genes like *CYP2D6* due to frequent copy number variations (CNVs) and hybrid gene formations.

To address the need for a simple, single-assay solution for detecting both single nucleotide variants (SNVs) and complex structural variations in pharmacogenes, we are developing two new digitalMLPA assays:

- Z015 Pharmacogenomics Panel 1: targets 29 actionable PGx genes, containing 205 probes for copy number determination and 217 SNV-specific probes, including all AMP Tier 1 and most Tier 2 variants.
- Z016 Pharmacogenomics Panel 2: contains all Z015 probes, plus additional probes for 52 PGx related genes (adding to a total of 436 probes for CN determination and 315 SNV-specific probes).

In this study, we evaluate the performance of these assays using reference materials to assess their ability to detect SNVs and structural variants in prominent pharmacogenes.

digitalMLPA: a high-throughput method for reliable SNV and CNV detection

digitalMLPA is a multiplex PCR-based method for detecting up to 1600 selected SNVs and structural variants in a single assay. It combines the sensitivity of CNV and SNV detection of the widely used MLPA technology with the broad scale of NGS for amplicon detection.

The Z015/Z016 pharmacogenomics assays are compatible with:

- purified genomic DNA
- crude DNA extracts from whole blood without any purification
- crude DNA extracts from 3 mm punch of a DBS without any purification
- buccal swab samples without any purification

The workflow of digitalMLPA is illustrated in ***Figure 1***.

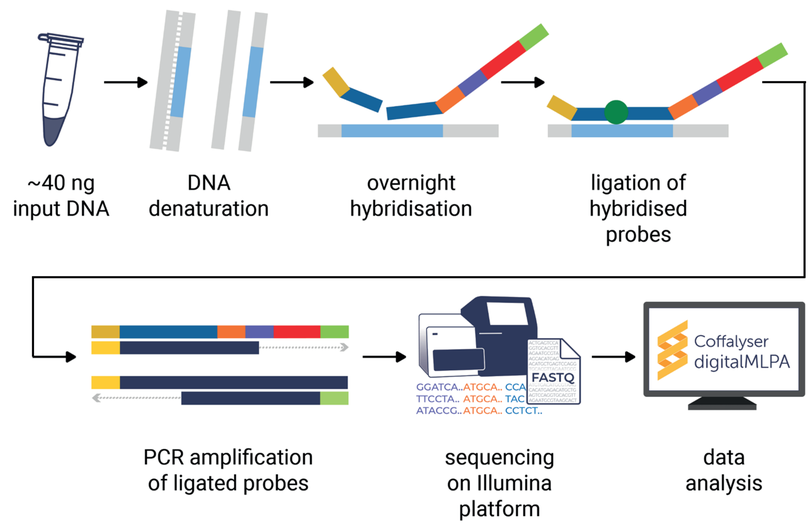

Figure 1. *Samples (containing 20-40 ng DNA) are first denatured, after which the left and right probe oligo bind to their target DNA during overnight hybridisation. This is followed by ligation of all hybridised probes. Successfully ligated probes are amplified by multiplex PCR. Amplicons of all reactions are combined and loaded on an Illumina sequencing platform (no library quantification is required). Data analysis is performed using the free analysis software Coffalyser digitalMLPA.*

Performance evaluation of digitalMLPA pharmacogenomics assays

The digitalMLPA pharmacogenomics assays were tested on ~350 DNA samples with known genotype information based on GeT-RM publications, and Ensembl for variants without published reference materials.

The assays showed high concordance with known data (see Table 1)(see ***Table 1***). Note that only a subset of tested genes is displayed. In various samples, the results did not match the GeT-RM result but did match the genotype information in Ensembl.
The number of probes that did not show concordance in all samples was limited and these are currently investigated to improve specificity.
For *CYP2D6*, discordance in 3 samples was due to involvement of hybrid alleles that were not mentioned in GeT-RM call.

	Number of probes in Z016 Pharmacogenomics Panel 2		GeT-RM		Ensembl *(n=152)*
Gene	# CNV probes	# SNV probes	# Samples	% Concordance	% Concordance
CYP2B6	8	8	95	99	100
CYP2C9	5	12	115	99	100
CYP2C19	6	9	116	100	98
CYP2D6	25	22	120	97	99
CYP3A4	3	9	110	100	100
CYP3A5	3	3	95	100	100
CYP4F2	5	3	68	97	99
DPYD	28	18	59	100	100
G6PD	4	22	-	-	100
*HLA-A *31:01*	0	1	76	100	-
*HLA-B *15:02*	0	1	76	100	-
*HLA-B *57:01*	0	1	76	100	-
*HLA-B *58:01*	0	1	73	100	-
NUDT15	2	5	13	96	96
RYR1	0	23	-	-	100
SLCO1B1	10	8	95	92	100
TPMT	9	12	102	100	100
UGT1A1	6	6	78	100	100
VKORC1	3	4	22	100	100
Other genes	316	142	-	-	>99

Table 1*. Concordance between output of the digitalMLPA Z015/Z016 pharmacogenomics assays and published genotype data (GeT-RM and Ensembl), per gene. The number of investigated samples is indicated.*

Tackling complexity: analysing structural rearrangements in *CYP2D6*

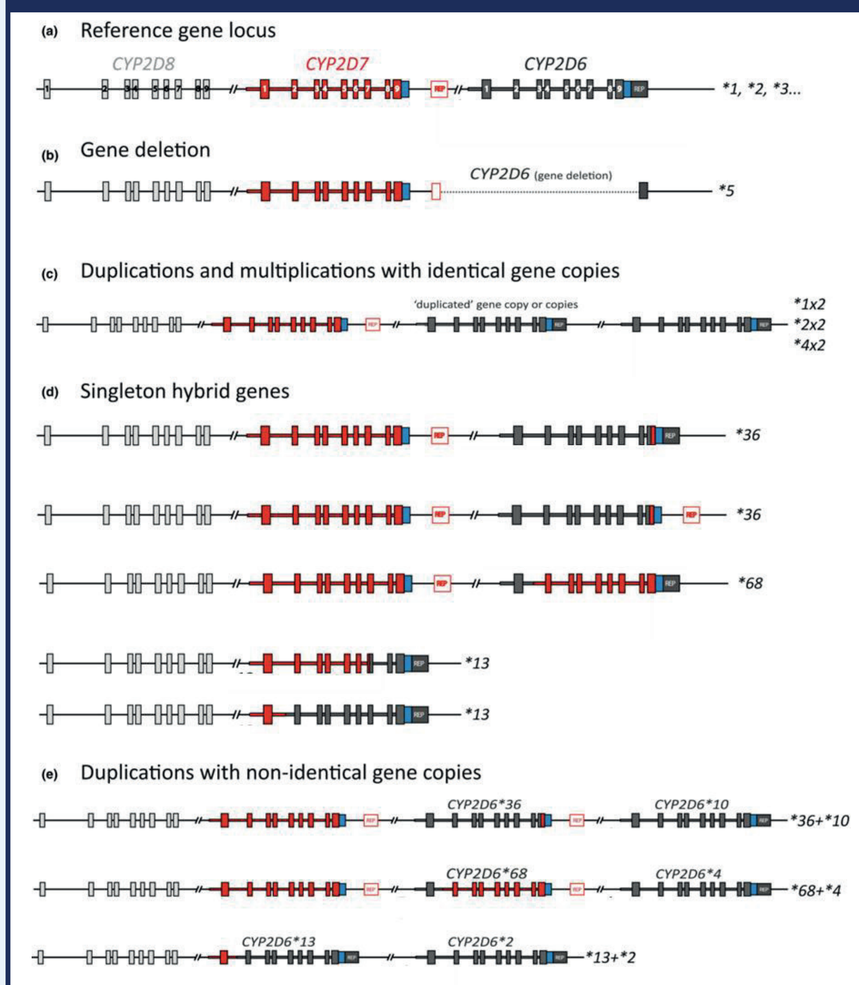

Figure 2*. Schematic representation of the* CYP2D6 *locus, its frequent structural variants and corresponding star alleles. Adapted from Turner et al., 2023. Clin Pharmacol Ther.*

Gene	Probe Location	Probe Target	Associated Star Allele	*1/*1	*2/*5	*5/*5	*1x2/*2x2	*1/*13+*2 intron 1 switch	*1/*13+*2 intron 4 switch	*39/*13 exon 7-8 switch	*1/*83	*36+*10/ *36+*10	*1/*36+*10	*1/*68+*4
CYP2D7	Exon 1	Copy number	*13, *36, *68, *83 (*CYP2D6*)	1,9	2,2	1,8	1,8	2,0	1,8	1,8	1,8	2,1	2,0	2,1
	Intron 4			1,8	2,1	1,8	1,9	0,9	2,0	1,9	1,6	2,3	2,0	3,1
	Exon 9			1,8	2,0	1,7	2,0	1,0	0,9	0,9	1,7	4,3	3,1	2,8
	Exon 9			2,1	2,1	1,9	1,9	1,0	1,0	1,0	1,9	3,8	3,0	3,0
	Exon 9			1,9	2,0	1,8	2,0	1,0	1,0	0,9	2,0	3,9	2,9	2,9
CYP2D6	4500 nt upstream	Copy number	*xN, *5, *13, *36, *68, *83	2,0	1,0	0,0	3,8	2,0	1,9	1,0	1,8	3,7	2,8	3,0
	2500 nt upstream			2,1	1,0	0,0	3,8	2,1	1,8	0,9	1,9	3,9	3,0	3,0
	2000 nt upstream			2,0	1,1	0,0	4,0	2,2	2,0	1,0	2,1	3,9	2,9	2,8
	1500 nt upstream			2,0	1,1	0,0	3,9	2,1	1,9	0,9	2,0	4,0	3,1	3,0
	250 nt upstream			2,1	1,1	0,1	3,8	2,0	1,9	1,0	1,9	3,9	2,9	2,9
	Exon 1			2,0	1,0	0,0	3,9	2,1	1,9	1,0	2,0	3,9	3,0	3,2
	Intron 1			2,0	1,0	0,0	4,0	2,0	2,1	0,9	2,0	3,9	3,0	2,8
	Intron 2			2,1	1,1	0,0	4,0	3,0	1,9	1,0	2,0	4,0	3,1	2,1
	Intron 2			1,9	1,1	0,0	3,8	2,9	1,9	0,9	1,9	3,7	2,9	1,9
	Intron 2			2,1	1,0	0,0	3,8	3,2	2,0	0,9	1,9	3,8	2,9	1,7
	Intron 2			2,0	1,1	0,0	3,9	3,1	1,9	0,9	2,1	3,8	3,0	2,0
	Intron 4			2,1	1,0	0,0	3,9	3,0	2,0	1,0	2,0	3,9	2,9	1,9
	Intron 4			2,0	1,0	0,0	3,9	3,1	2,9	0,9	2,0	3,8	2,9	2,0
	Intron 5			2,0	1,0	0,0	4,0	3,1	2,8	0,9	1,9	3,8	2,9	2,0
	Intron 6			2,0	1,0	0,0	4,0	3,1	2,9	0,9	2,0	4,0	3,0	2,0
	Exon 9			2,0	1,0	0,0	3,9	2,9	2,9	1,8	1,0	2,1	1,8	1,8
	Exon 9			1,8	0,9	0,0	3,7	2,9	2,9	1,9	1,0	1,8	1,8	2,0
	150 nt downstream			2,0	1,1	0,0	3,8	3,1	2,9	2,0	2,0	2,0	2,1	1,9
	450 nt downstream			2,2	1,1	0,0	4,3	3,0	3,0	2,1	2,0	2,0	2,0	2,2
	500 nt downstream			2,1	0,9	0,0	4,0	3,0	2,9	2,0	1,9	2,0	2,0	2,0
	Exon 1	rs5030862	*12 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 1	rs1065852	*10 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,8	2,0	2,0
	Intron 1	rs201377835	*11	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 2	rs28371706	*17 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 9	rs1135840	*2 (AMP Tier 1)	0,0	1,0	0,0	2,1	2,1	1,9	1,0	0,0	1,9	0,9	1,0
	Exon 3	rs1135822	*49 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 3	rs61736512	*29 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 3	rs5030655	*6 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 3	rs5030865	*14 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 3	rs5030865	*8 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Intron 3	rs3892097	*4 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0
	Exon 4	rs72549356	*40 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 5	rs35742686	*3 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 5	rs5030656	*9 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 6	rs79292917	*59 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 6	rs5030867	*7 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 6	rs16947	*2 / *17 (AMP Tier 1)	0,0	1,1	0,0	2,1	2,1	1,8	0,0	0,0	0,0	0,0	0,0
	Intron 6	rs28371725	*41 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 7	rs59421388	*29 (AMP Tier 1)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 7	rs72549347	*56 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 7	rs72549346	*42 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Exon 9	rs267608319	*31 (AMP Tier 2)	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Figure 3*. Heatmap of probes for CN determination (upper part) and SNVs (lower part) in* CYP2D6 *and* CYP2D7*, tested on various samples with known structural variations using the Z015/Z016 pharmacogenomics assays. Diplotypes of samples are displayed in upper row. Values represent the calculated number of copies.*

CYP2D6 is a key gene in drug metabolism but challenging to characterise due to frequent CNVs and hybrid alleles (see ***Figure 2***). digitalMLPA probes were designed to specifically discriminate between *CYP2D6* and *CYP2D7*. Various samples with known *CYP2D6* deletions and hybrid alleles were tested using the Z015/Z016 assays. The correct copy number status as well as the approximate breakpoint in hybrid alleles was identified (***Figure 3***), indicating the value of these digitalMLPA assays to detect structural variants in *CYP2D6*.

Conclusions

- The **digitalMLPA pharmacogenomics assays show high concordance** to known datasets when tested on ~350 DNA samples with known variants, **including all AMP Tier 1 and most Tier 2 targets**.
- These assays can be used for the detection of both selected SNVs and (complex) structural variants in pharmacogenes, **including *CYP2D6***, in a single reaction.
- As **digitalMLPA is a targeted method**, where probes are sequenced instead of DNA, this greatly reduces incidental findings and privacy concerns, making this assay a promising technique for the characterisation of pharmacogenes.

For further information please contact
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