Genetic profiling of ADME markers using PharmacoScan Solution

D. Black, C. Bruckner, R. Varma, J. Law, M. Mittmann, K. Scott, M. Shapero, J. Gollub

Affymetrix, Inc., Santa Clara, CA 95051

affymetrix
part of Thermo Fisher Scientific

ABSTRACT

Background

Genetic variation in drug absorption, distribution, metabolism, and excretion (ADME) genes forms the basis for each individual's clinical response to xenobiotics. This variation includes bi-allelic and multi-allelic polymorphisms as well as more complex structural variations, including insertions, duplications, and deletions.

PharmacoScan™ Solution is designed to enable comprehensive and accurate genotyping of specific polymorphisms involved in drug-metabolizing enzymes and transporters. 4,627 markers in 1,191 genes sourced from Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines, Pharmacogenomics Knowledgebase (PharmGKB), and other databases are analyzed on the Axiom™ genotyping platform. A novel incorporation of multiplex PCR into the Axiom™ workflow solves the genotyping challenges of key variants that are part of highly homologous, multi-gene families such as CYP2D6. Copy number (CN) changes in pharmacogenomically relevant genes, such as CYP2D6, CYP2A6, GSTM1, GSTT1, and UGT2B17, are accurately called using new algorithms for probe design and analysis. Lastly, Axiom™ Analysis Suite software enables data visualization and conversion of genotype calls to clinically recognized star nomenclature via the Allele Translation Tool.

Experimental design

Validation studies tested approximately 1,000 DNA samples from multiple sources including blood, saliva, and buccal swabs. Assay performance was evaluated based on overall call rate (>99%) and concordance to independent genotypes (1000 Genomes Project and DMET™ Plus Solution; ≥99.5%); inter-run reproducibility (≥99.8%); and the ability to discriminate between homozygous copy loss (CN = 0), hemizygous loss (CN = 1), CN = 2, and CN ≥ 3, where applicable for select copy number variable regions.

Conclusions

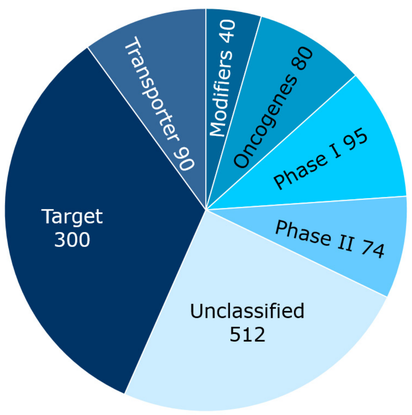
PharmacoScan Solution offers a streamlined workflow consistent with the needs of studies aimed at pharmacogenetic profiling. Integration of both SNP genotypes and CNV calls coupled with the conversion to clinically recognized star nomenclature could significantly impact future drug development processes.

Comprehensive pharmacogenomics (PGx) coverage

Phase III/Transporter genes
Example genes:
- ATP7A
- ABCG2
- SLCO1A2

Drug target genes
Example genes:
- MEFV
- VKORC1
- HMGCR

Phase II enzymes
Example genes:
- NAT1
- GSTM1
- GSTT1
- UGT1A1

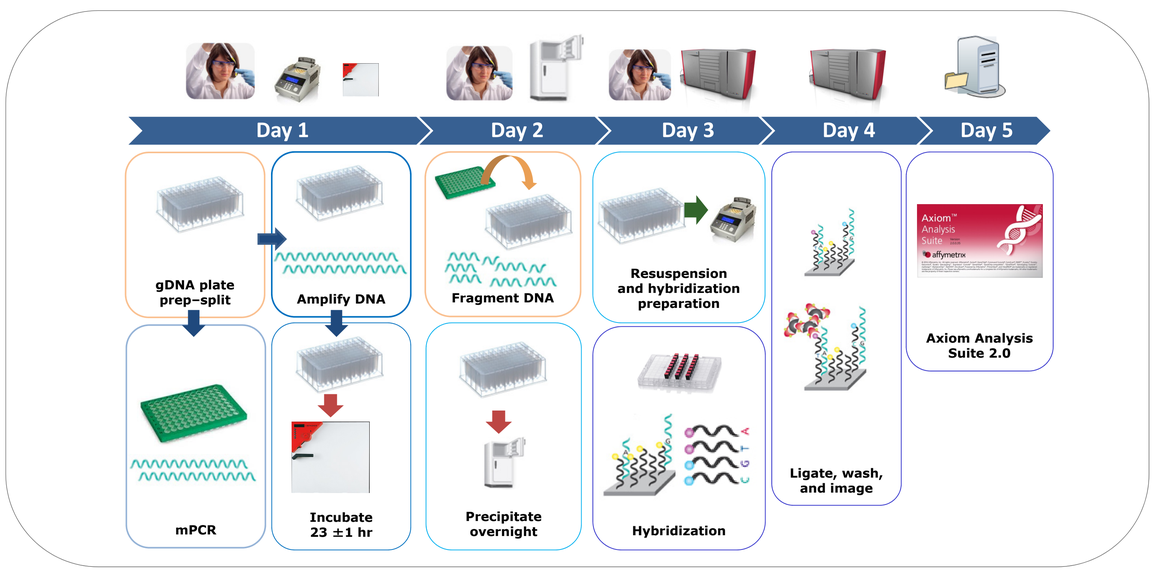


Regulatory/modifier genes
Example genes:
- NOS1
- HNF4A
- PPARD
- PPARG

Phase I enzymes
Example genes:
- CYP2D6
- CYP2C9
- CYP1A1
- CYP1B1
- CYP2C19
- CYP4F2

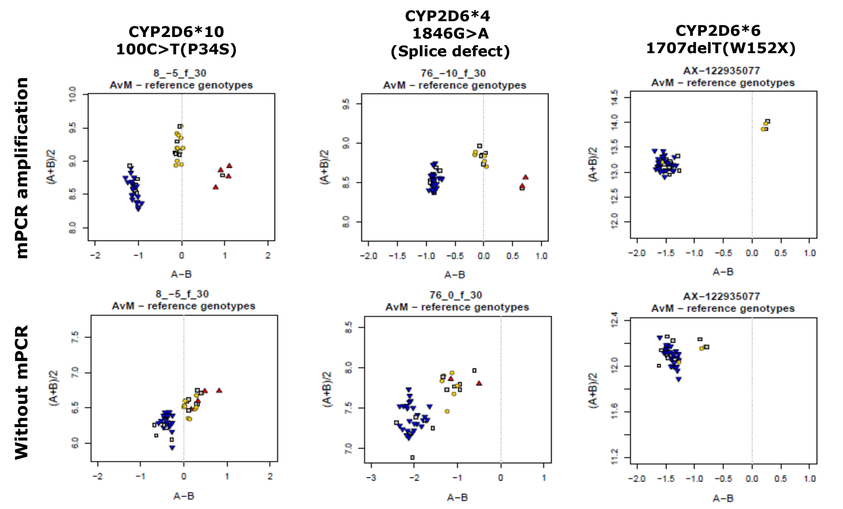
Axiom platform workflow

- Genomic DNA (gDNA) is isothermally amplified, mPCR amplicons added, and target is randomly fragmented into roughly 25–125 base pair fragments.
- Fragments are precipitated, resuspended, and hybridized to PharmacoScan™ array plates.
- Bound target is washed under stringent conditions to remove non-specific background. Each polymorphic nucleotide is queried via a multicolor ligation event carried out on the array surface.
- After ligation, the arrays are stained and imaged on GeneTitan™ MC Instrument.



Rescue of key ADME markers with mPCR addition

Cluster plots in contrast versus size space for CYP2D6*10, CYP2D6*4, and CYP2D6*6 alleles. Each symbol represents a sample. BB homozygous calls are shown in blue, yellow dots represent heterozygous calls, and AA homozygous calls are shown in red. Samples colored according to 1000 Genomes Project calls.



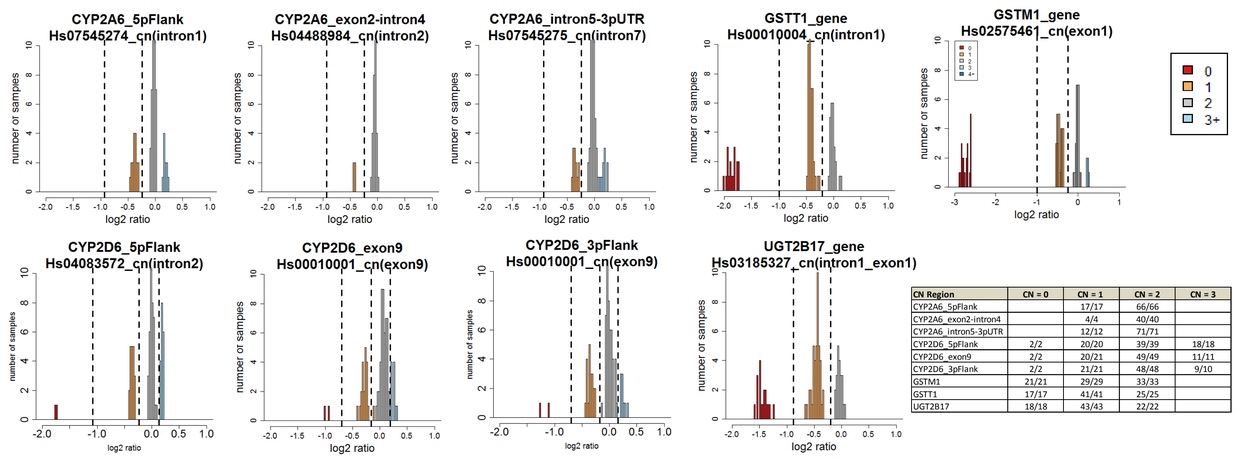
Validation

	Performance metric	Sample pass rate	QC CR	ADME CR	mPCR CR	ADME CC	ADME Het CC	mPCR CC	Reprod.* (inter-plate)
	Specification	22/24	≥98.5%	≥99.0%	≥99.0%	≥99.6%	≥98.0%	≥99.5%	≥99.8%
Test Site	Affymetrix, Santa Clara	72/72 (3 plates)	99.7%	99.9%	100.0%	99.9%	99.8%	100.0%	99.9%
	Affymetrix, Ohio	72/72 (3 plates)	99.9%	99.9%	99.4%	99.9%	99.9%	99.7%	100.0%
	EA1 Plt 1	24/24	99.9%	100.0%	100.0%	100.0%	99.9%	100.0%	100.0%
	EA1 Plt 2	24/24	99.9%	99.9%	99.9%	100.0%	100.0%	100.0%	100.0%
	EA2 Plt 1	23/24	99.7%	99.8%	98.7%	99.8%	99.7%	98.5%	99.9%
	EA2 Plt 2	24/24	99.6%	99.7%	98.3%	99.6%	99.7%	99.6%	99.9%
	EA3 Plt 1	24/24	99.8%	99.9%	100.0%	99.9%	99.9%	100.0%	99.9%
	EA3 Plt 2	24/24	99.7%	99.8%	99.9%	99.7%	99.7%	99.9%	99.9%
	EA3 Blood samples**	235/240 (10 plates)	99.7%	99.8%	99.7%	99.7%	99.6%	100.0%	99.9%
	EA4 Plt 1	24/24	99.9%	99.9%	99.8%	99.9%	100.0%	99.9%	100.0%
	EA4 Plt 2	24/24	99.9%	99.9%	100.0%	99.9%	100.0%	100.0%	100.0%
	EA5 Plt 1	22/24	99.5%	99.9%	99.7%	99.8%	99.9%	99.5%	99.9%
	EA5 Plt 2	23/24	99.6%	100.0%	99.9%	100.0%	99.9%	99.9%	99.9%

*Inter-plate reproducibility was calculated for each site; haploid markers were excluded.
**Concordance was to 1000 Genomes Project reference, except for EA3 blood samples where concordance was to DMET Plus reference data.

PharmacoScan Solution performed well during testing at five external sites (EA1–EA5). Two plates (Plt1, Plt2) of cell line samples (data shown above) as well as two plates of customer blood samples were tested. One site, EA3, ran 10 additional plates of blood samples with reference data from DMET Plus Solution. Call rate (CR) and concordance (CC) were evaluated for mPCR and ADME markers. With a few exceptions, all assay performance metrics were met.

Copy number calling in important genes



CN Region	CN = 0	CN = 1	CN = 2	CN = 3
CYP2A6_5pFlank		17/17	66/66	
CYP2A6_exon2-intron4		4/4	40/40	
CYP2A6_intron5-3pUTR		12/12	71/71	
CYP2D6_5pFlank	2/2	20/20	39/39	18/18
CYP2D6_exon9	2/2	20/21	49/49	11/11
CYP2D6_3pFlank	2/2	21/21	48/48	9/10
GSTM1	21/21	29/29	33/33	
GSTT1	17/17	41/41	25/25	
UGT2B17	18/18	43/43	22/22	

Seventy-seven samples with TaqMan® Assays copy number data were assayed with PharmacoScan Solution, six in duplicate. Figures show histograms of median log2 ratios of all samples colored by TaqMan calls. The table shows the number of concordant calls and total number of samples in each cell.

Translation to star allele nomenclature

- CYPD2D6*5 is defined as deletion of an entire gene copy.
- CN information enables reporting of single and double copy deletions.
- CN gains are captured by the Interpretation Code field.

CN = 1 haploid SNP calls

#Interpretation Code Description:
#UNIQ: Unique haplotype pair
#MULT: Multiple haplotype pairs possible due to phase ambiguity
#UNIQ+UNK: Unique annotated haplotype pair, with other haplotype pairs requiring unannotated haplotypes also possible
#MULT+UNK: Multiple annotated haplotype calls possible, with other haplotype pairs requiring unannotated haplotypes also possible
#UNDH: Only haplotype pairs with undefined haplotypes possible
#NC/PRA/NotAvailable: NoCall, PossibleRareAllele, or NotAvailable call for one or more markers resulting in multiple haplotype pairs
#NoHAP: No haplotypes defined at this gene
#CN_HybridLoss: Partial gene deletion detected, so haplotype pair calling not available
#CN_HybridGain: Partial gene duplication detected
#CN_Gain: Gene duplication detected, but cannot determine which haplotype(s) are duplicated
#CN_NoCall: Copy Number state not reported, so less confidence in reported haplotype pairs
#CN_Error: Genotypes from multiple Copy Number states, so Haplotype pair calling not available

Index	Filename	Gene	Known Call	Interpretation Code	Summary Flag	Common Name	Probe Set ID	Basecall	Reference Base	Variant Base	Call
0002-0029-28	HG00260_JS_CYP2D6		*2/*5	UNIQ	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	G	C	G	*5/Var
0002-0029-17	HG00260_JS_CYP2D6		*2/*5	UNIQ	R296C	CYP2D6_2850C>T(R296C)	AX-112192888	A	G	A	*5/Var
0010-0029-28	NA11829_T01	CYP2D6	*4/*5	UNIQ	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	G	C	G	*5/Var
0010-0029-09	NA11829_T01	CYP2D6	*4/*5	UNIQ	*4	CYP2D6*4_1846G>A(SpliceDefect)	AX-112193595	T	C	T	*5/Var
0010-0029-01	NA11829_T01	CYP2D6	*4/*5	UNIQ	P34S	CYP2D6_100C>T(P34S)	AX-112194311	A	G	A	*5/Var
0015-0029-28	NA18973_CS_CYP2D6		*1/*21	UNIQ+UNK	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	C/G	C	G	Ref/Var
0015-0029-17	NA18973_CS_CYP2D6		*1/*21	UNIQ+UNK	R296C	CYP2D6_2850C>T(R296C)	AX-112192888	G/A	G	A	Ref/Var
0015-0029-14	NA18973_CS_CYP2D6		*1/*21	UNIQ+UNK	*21	CYP2D6*21_2573insC	AX-112192915	-/G	-	G	Ref/Var
0019-0029-28	NA19315_T01	CYP2D6	*1/*29	UNIQ+UNK	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	C/G	C	G	Ref/Var
0019-0029-17	NA19315_T01	CYP2D6	*1/*29	UNIQ+UNK	R296C	CYP2D6_2850C>T(R296C)	AX-112192888	G/A	G	A	Ref/Var
0019-0029-20	NA19315_T01	CYP2D6	*1/*29	UNIQ+UNK	*29	CYP2D6*29_3183G>A(V338M)	AX-122945650	C/T	C	T	Ref/Var
0019-0029-05	NA19315_T01	CYP2D6	*1/*29	UNIQ+UNK	*29	CYP2D6*29_1659G>A(V136I)	AX-165876996	C/T	C	T	Ref/Var
0023-0029-01	NA19317_CS_CYP2D6		*5/*5	UNIQ	Deletion alleles have been detected for this gene, CN=0						
0025-0029-28	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	G/G	C	G	Var/Var
0025-0029-17	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	R296C	CYP2D6_2850C>T(R296C)	AX-112192888	G/A	G	A	Ref/Var
0025-0029-09	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	*4	CYP2D6*4_1846G>A(SpliceDefect)	AX-112193595	C/T	C	T	Ref/Var
0025-0029-04	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	T107I	CYP2D6_1023C>T(T107I)	AX-112193805	G/A	G	A	Ref/Var
0025-0029-01	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	P34S	CYP2D6_100C>T(P34S)	AX-112194311	G/A	G	A	Ref/Var
0025-0029-10	NA19318_CS_CYP2D6		*4/*40	UNIQ+UNK	*40	CYP2D6*40_1863ins(TTTCGCCCC)2	AX-112283720	-/GGGGCGAAAGG(	-	GGGGCGAAAGG(	Ref/Var
0031-0029-28	NA19782_JS_CYP2D6		*1/*4	UNIQ+UNK	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	C/G	C	G	Ref/Var
0031-0029-09	NA19782_JS_CYP2D6		*1/*4	UNIQ+UNK	*4	CYP2D6*4_1846G>A(SpliceDefect)	AX-112193595	C/T	C	T	Ref/Var
0031-0029-01	NA19782_JS_CYP2D6		*1/*4	UNIQ+UNK	P34S	CYP2D6_100C>T(P34S)	AX-112194311	G/A	G	A	Ref/Var
0032-0029-28	NA19785_CS_CYP2D6		*1/*2	CN_HybridGain	S486T	CYP2D6_4180G>C(S486T)	AX-11155878	C/G	C	G	Ref/Var
0032-0029-17	NA19785_CS_CYP2D6		*1/*2	CN_HybridGain	R296C	CYP2D6_2850C>T(R296C)	AX-112192888	G/A	G	A	Ref/Var

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