PHARMACOGENOMICS

CNV Detection for Pharmacogenetic and Mental Health Research Applications

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Abstract

Purpose: This research aims to demonstrate the development and performance of the Applied Biosystems™ Axiom™ PharmacoPro™ array to support accurate CNV detection in genes of pharmacogenomic interest for research applications including mental health.

Methods: The study involved amplifying and precipitating gDNA from various sample types using the Axiom™ SwiftArray™ Assay, followed by hybridization and measurement of PharmacoPro™ targets using the SwiftArrayStudio™ Microarray Analyzer.

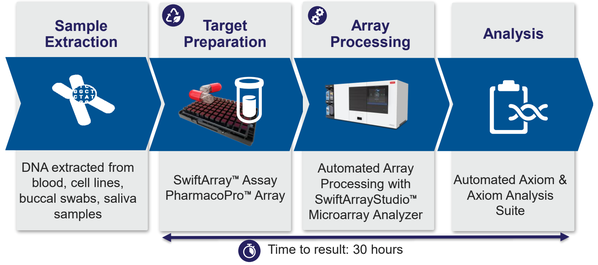
Results: Copy number states from 0 to 3 were called for most regions with some regions up to 5. For the combined SULT1A3_1A4 region, copy number states from 2 to 5 were called, with 4 being the normal copy number in the general population. Many rare events were detected in *DPYD*, *BCHE*, *PTGIS* and *TBXAS1*. Median analytical reproducibility was greater than 99% for all regions and all copy number states. For the challenging SULT1A3_1A4 region, reproducibility was about 90%.

Introduction

Applied Biosystems™ Axiom™ SwiftArray™ Assay and SwiftArrayStudio™ platform enables sample-to-report time within 30 hours. The workflow utilizes novel chemistry and optimized enzymes to improve turn around time and uses fewer pipette tips with a pour-and-go compatible consumables. In addition, the intuitive user interface on SwiftArrayStudio™ Microarray Analyzer facilitates automated processing with guided loading/unloading on the instrument. Its ergonomic design simplifies lab operations while enabling consistent, high-quality results across multiple batches.

Copy number variations (CNVs) in genes responsible for drug metabolism can significantly alter enzyme activity and affect response. Cytochrome P450 genes for pharmacokinetics, sulfotransferases (SULTs) for mental health, and other genes of pharmacogenomics importance belong to families with highly similar sequences that make CNV detection challenging. For the almost identical SULT1A3 and SULT1A4 genes, probes were designed with sequences that were identical to both genes to find a combined copy number. Additionally, probes for all regions were selected based on signal response to copy number.

Materials and Methods



The full workflow from gDNA to report generation is shown above;

- Genomic DNA (gDNA) from human cell line, blood, buccal and saliva samples is isothermally amplified using Axiom™ SwiftArray™ Assay
- Key markers in pseudogenes like CYP2D6 are amplified with the mPCR module in the SwiftArray™ Assay Reagent Kit.
- Fragments are precipitated, resuspended, & hybridized to novel PharmacoPro™ Microarray.
- Bound target is washed automatically under stringent conditions to remove non-specific background on the SwiftArrayStudio™ Microarray Analyzer.
- After ligation, the arrays are stained and imaged and the data is transferred to AutoAx for data analysis and generation of PGx relevant reports

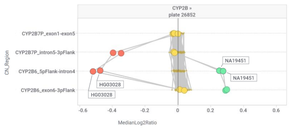
Gain or Loss of ADME Genes

ADME Genes with Copy Number Changes: 36 genes that measures CNV are shown below along with wider drug-disease substrates that they are associated with. Deletion or amplification of these genes impact the rate of drug metabolism, with significant implications for treatment.

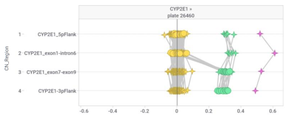
Genes (No. CN regions)	Associated substrate / disease area
ABCC1 (7)	Chemotherapy drugs, MDR
ABCC6	PXE, Calcification disorders
FCGR2A FCGR2B FCGR2C FCGR3A FCGR3B	Modulation of efficacy, toxicity or risk of Autoimmune diseases (Lupus, SLE, RA): Rituximab, margetuximab
ABCG2 (3)	Statins, Cancer MDR, Gout
BCHE (3)	Cocaine, Acetylcholine levels (Alzheimer's disease)
CYP1A1 (3) CYP1A2 (3)	Chlozapine (Antipsychotic related ADRs), Caffeine, Opiods
GSTM1	Toxicity of Platinum Drugs (cancer), Reduced Detox of carcinogens
CYP2A6 (3) CYP2A7 (3)	Nicotine, tegafur (5-FU pro-drug), Coumarin
GSTT1	Platinum drugs, Streptomycin, hydrocarbons
CYP2B6 (2) CYP2B7P (2)	Efavirenz (HIV), cyclophosphamide (Chemotherapy), bupropion
PTGIS (3)	Prostacyclin (Hypertension)
SLC22A2 (4)	Metformin, Platinum Drugs
SCN1A (3)	Carbamazepine, antiepileptics
CYP2C18 (3)	NA
SLCO1A2	Chemotherapy drug (imatinib)
CYP2C19 (4)	Diazepam (antidepressants),
CYP2C18 (3)	Clopidogrel (Thrombosis)
SLCO1B1 (4)	Atorvastatin, rosuvastatin,
SLCO1B3 (7)	Methotrexate (anticancer drug)
CYP2D6 (3)	Opioids, Antidepressants, beta-blockers, antiarrhythmics
CYP2D7 (3)	
SLCO1C1	TNF-alpha inhibitors
CYP2E1 (4)	Acetaminophen, ethanol metabolism, cisplatin
SULT1A1	Estrogens, Acetaminophen, tamoxifen (Cancer)
CYP4F2	Warfarin, Vitamin K1, Vitamin E
TBXAS1 (3)	Aspirin
DPYD (12)	Fluoropyrimidines (5-FU)
UGT2B17	Steroid hormones, osteoporosis
SULT1A3_ SULT1A4	Acetaminophen, Drugs related to neurologic disorders

Results

In an internal study, gDNA extracted from whole blood, buccal swab, saliva, and around 750 unique cell line samples were amplified and precipitated using the SwiftArray™ Assay. WGA target along with spiked mPCR amplicons was then hybridized and measured on PharmacoPro™ microarray using the SwiftArrayStudio™ Analyzer.



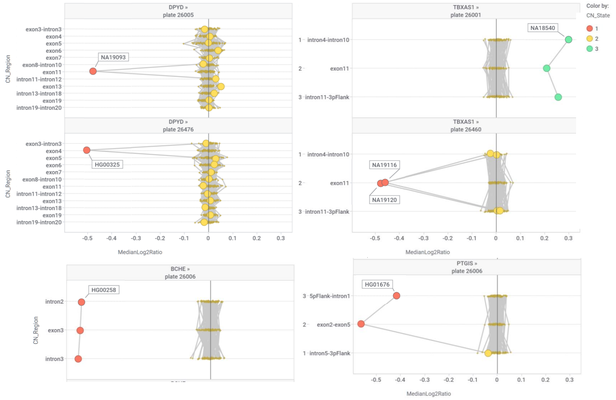
CYP2B6 and CYP2E1 deletion and duplication events are more commonly observed. HG03028 (run in replicate on the plate) reports a deletion event for only the first several exons of CYP2B6, and also for part of the adjacent CYP2B7P pseudogene, which is located 5prime of CYP2B6. NA19451 (also run in replicate) reports a whole-gene CYP2B6 duplication (total copy number = 3).



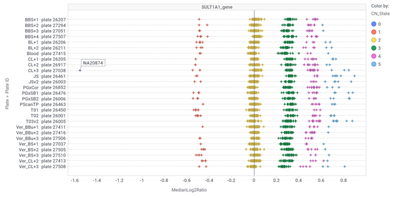
Eleven Yoruban samples in a test plate report a partial duplication of CYP2E1 (CN=2 in exon 1 to intron6, then CN=3 in exon7 to exon9). This especially signifies the benefits of PharmacoPro™ that measures CNVs in multiple regions enabling higher PPV.

CNV Measurement in Rare CN Regions

CNV data run on PharmacoPro microarray is shown in Figure 2a for DPYD, BCHE, PTGIS and TBXAS genes. 1000 Genomes samples with rare deletion or duplication events (in 5 samples out of ~2400) are labeled in each plotted plate of samples.

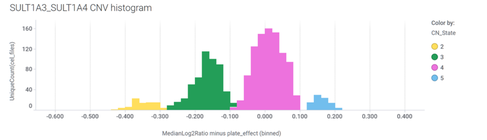


CNV in SULT Gene



SULT1A1 CNVs reported by PharmacoPro™ are shown in Figure 3. Updated controls in the SwiftArray™ Assay Reagent Kit are also CN neutral for this region, supporting reproducible CN calling up to CN=5.

SULT1A3 and 1A4 share more than 99% sequence homology and have been linked with risk of developing Alzheimer's disease and influencing Parkinsons disease therapy[1]. The Figure 4 histogram shows the PharmacoPro™ reported CN states from 2 to 5 for the 84% of 1896 samples that report an integer CN. CN4 is the neutral state of the combined gene region.



Sensitivity and Reproducibility

688 unique gDNA samples were run in 2 to 35 replicates to obtain reproducibility. It is calculated by comparing individual CN calls to the consensus CN for each unique DNA and region. As shown below, calls were >99% reproducible for most regions.

CNVs (deletions & duplications) were compared with dbVar along with external/internal data to obtain concordance, sensitivity and PPV. Homozygous deletions were detected with 100% concordance. Single copy gains and losses were detected with >99% analytical sensitivity and PPV as shown below.

# of samples for each consensus CN state

Reported CN State	0	1	2	3	4	5	PPV
0	2217	0	0	0	0	0	1
1	0	5693	16	0	0	0	0.997
2	0	0	216997	24	0	0	0.999
3	0	0	13	3487	4	0	0.995
4	0	0	0	14	390	2	0.960
5	0	0	0	0	4	98	0.960
Sensitivity	1	1	0.999	0.989	0.979	0.98	

Reproducibility	CN Regions
85%-90%	SULT1A3_SULT1A4
98.9%-99.0%	FCGR2C_exon2-intron6
99.7%-99.8%	SULT1A1_gene
99.8%-99.9%	CYP2D6_exon9 CYP2D7_exon9 CYP2D7_exon3-intron6 CYP2D7_intron2 CYP2D6-3pFlank
99.9%-100%	ABCC1_exon1 SLCO1B3_5pFlank-exon3 CYP2E1_exon7-exon9 CYP2E1-3pFlank CYP2A6_intron2-intron4 CYP2D6_5pFlank
100%	All other regions

Conclusions

The Axiom™ PharmacoPro™ Microarray represents a significant advancement in the field of pharmacogenomics, addressing the critical need for rapid and comprehensive genetic characterization in drug metabolism and transport. This innovative solution is poised to support the transition of personalized medicine into clinical research practice by offering a robust platform for the identification of functionally significant genetic variants across major drug absorption, distribution, metabolism, and excretion (ADME) genes.

- The novel PharmacoPro™ Microarray reports CN variation for 6x as many pharmacogenetic genes as the previous generation array in this product line.
- The PharmacoPro™ array together with SwiftArray™ Assay resolves CN states up to CN5, which can be useful for drug metabolism studies.
- The PharmacoPro™ Array together with SwiftArray™ Assay and SwiftArrayStudio™ generates data within 30 hours, which is especially relevant for preemptive pharmacogenetics in research settings. Overall, the streamlined two-day workflow from gDNA to data supports timely decision-making, operational efficiency and relevance of pharmacogenetic insights.

References

1. NJ Butcher et al. Pharmacogenomics Journal (2018), 209-214
2. https://cpicpgx.org/guidelines/
3. https://api.clinpgx.org/v1/download/file/data/relationships.zip

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