Axiom PharmacoFocus Array

For population-scale preemptive pharmacogenomic testing with targeted, high-evidence content

Understanding common variations in genes coding for drug metabolism and transport proteins can inform decisions in medication management research that prevent adverse drug reactions (ADRs) and lead to better health outcomes and cost savings in the future [1,2]. Evidence also indicates that use of pharmacogenomic information earlier in clinical research enables better understanding of participant drug response, leading to optimal research outcomes [3].

The Applied Biosystems™ Axiom™ PharmacoFocus™ Array was developed to accelerate pharmacogenomic research in labs, academic hospitals, health care centers, and the pharmaceutical industry. It offers comprehensive coverage of high-evidence functional variants (Pharmacogenomics Knowledge Base (PharmGKB) clinical annotation levels of evidence 1A–2B) that influence absorption, distribution, metabolism, and excretion (ADME) of commonly prescribed medications [4] (Table 1).

Table 1. Content of the Axiom PharmacoFocus Array (categories may overlap).

Category	Number of variants
Pharmacogenomics (PGx) variants	>2,100
Number of PGx genes covered	150
PGx variants by PharmGKB levels of evidence	
1	114
2	155
3	300
4	198
Highly predictive markers in regions of high homology within *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP1A2*, *CYP2A6*, *CYP2B6*, *GSTM1*, and *SULT1A1*	>110
Variants for calling over 970 unique haplotypes across 66 gene regions	>850
HLA—variants enabling HLA typing for 11 major histocompatibility complex loci (3 MHC Class I and 8 Class II)	>1,560
Applied Biosystems™ TaqMan® OpenArray™ catalog panel variants	>119
Dutch Working Group Pharmacogenomic passport markers (2019) [7]	65
Medicaid (CMS) reimbursement policy, including psychiatry panel [8]	>19 variants in CYP genes and HLA types

Key features

- 2,000 markers in 150 ADME genes covering population diversity
- 88% coverage of targeted, high-evidence pharmacogenomic research markers (PharmGKB clinical annotation levels 1A–2B)
- A single array developed and optimized for high-accuracy genotyping, copy number variation (CNV) detection, and HLA typing
  - Accurate genotyping of highly predictive markers in regions of high homology within *CYP2C19*, *CYP2C8*, *CYP2D6*, *CYP2A6*, *CYP2B6*, *GSTM1*, and *SULT1A1*
  - CNV analysis with high-resolution copy number calls (0 to 3+) for key ADME genes: *CYP2D6*, *CYP2A6*, *GSTM1*, *GSTT1*, *UGB2B17*, and *SULT1A1*
  - HLA analysis with high resolution of typing (2-digit and 4-digit) of the major histocompatibility complex (3 MHC Class I and 8 Class II) loci, including HLA-A* and HLA-B* types with known relevance in drug metabolism [5,6]
- Star allele reporting with predicted gene phenotype

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With over 35% of high-evidence functional variants implicated in toxicity and ADRs (Figure 1), the Axiom PharmacoFocus Array is ideal for research on population-scale preemptive pharmacogenomic strategies or early-phase clinical research to support better research outcomes.

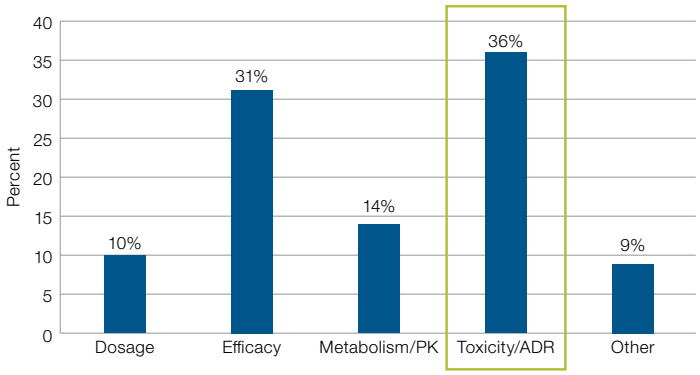

Figure 1. ADME status of Axiom PharmacoFocus Array markers with PharmGKB levels 1A, 1B, 2A, and 2B (Nov 2019).

The Axiom PharmacoFocus Array has been designed to enable accurate genotyping of even technically challenging ADME variants in complex genes.

Enabling preemptive pharmacogenomics—a comprehensive, accurate, flexible, efficient, and scalable solution

Comprehensive

Pharmacogenomics implementation promises to reduce unnecessary costs associated with ineffective medication management and adverse drug reactions [9]. To realize this promise, it will be important to utilize cost-efficient multigene test panels that capture a more complete pharmacogenomic profile. The Axiom PharmacoFocus Array offers comprehensive coverage of high-evidence content (88% of variants in PharmGKB highest levels of evidence) with 2,000 markers in 150 genes across diverse populations.

Accurate and robust

Currently, ~25% of prescription drugs in the United States have pharmacogenomic labeling, and over ~25% of all referenced alleles are in the *CYP2D6* gene [10]. Often, *CYP2D6* alleles are found in regions that have high homology with pseudogenes, and many technologies cannot accurately genotype them [10,11].

The Axiom PharmacoFocus Array includes a gene-specific amplification step that overcomes this problem and enables accurate genotyping of high-evidence variants within the *CYP2D6*, *GSTM1*, *CYP2B6*, *CYP2A6*, *CYP2C19*, *CYP2C8*, and *SULT1A1* genes.

The array leverages the proven Axiom platform and the automated Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument for an efficient workflow used worldwide by genetics researchers to provide accurate and highly reproducible results.

Axiom arrays are manufactured using photolithography technology, known for 100% fidelity, ensuring that every marker is present in every manufacturing batch, which is critical for pharmacogenomic profiling. For details on array performance see, Table 2.

Flexible panel content

The array offers options for a configurable gene test panel aligned with your pharmacogenomics interests and goals. The array content can be selected by the end user to focus on genes of interest from one of the three configurations, and choice of marker density:

- Premium configuration
  - Predesigned multigene test panel covering 2,000 variants in 150 genes
- Advanced configuration
  - Select up to 50 genes and 1,000 markers
- Lite configuration
  - Select up to 15 genes and 200 markers

Each configuration is offered with optional star allele reporting.

- Down-selecting the content does not require reverification of the array.
- Tailor the gene test panel for any phase of your pharmacogenomics implementation [12], and extend access to broader content with an easy-to-upgrade process.

Efficient—achieving the tasks of three different technologies in a single workflow

Pharmacogenomics clinical research often requires more than one molecular assay to address complexities in key ADME genes, assess copy number states, and obtain HLA types. This increases costs and delays results. The Axiom PharmacoFocus Array helps overcome these challenges with one accurate array for:

- Genotyping of high-evidence markers (SNPs and insertions and deletions) in regions of high homology
- Copy number analysis in regions of key pharmacogenes (*CYP2A6*, *CYP2D6*, *GSTM1*, *GSTT1*, *UGT2B17*, and *SULT1A1*)
- HLA analysis at 11 HLA MHC loci (*HLA-A*, *HLA-B*, *HLA-C*, *DPA1*, *DPB1*, *DQA1*, *DQB1*, *DRB1*, *DRB3*, *DRB4*, and *DRB5*)

Easily scalable

The Axiom PharmacoFocus Array offers a batching option, of 96 samples. Each mini 96-array plate can process 94 samples and 2 controls, enabling a throughput of 768 samples per week per instrument.

Analysis software and results

Data generated using the Axiom PharmacoFocus Array are analyzed with Applied Biosystems™ Axiom™ Analysis Suite software [13]. Standard analysis includes quality control checks and genotypes. Copy number (CN) results are also reported and include CN 0/1/2+ for *UGT2B17*, and CN 0/1/2/3+ for *CYP2A6* (3 regions), *CYP2D6* (3 regions), *GSTT1*, and *GSTM1*. With an optional analysis workflow, *SULT1A1* CN states 0/1/2/3/4+ are also reported.

Star allele reports

The software can also translate genotype calls to functional allele calls (star alleles), enabling the following functions:

- Identification of haplotype and SNP-level sequence variation
- Annotation of reported genotypes across translated SNPs to indicate genomic, mRNA, or amino acid changes
- Prediction of general gene activity based on detected diplotypes

The databases used to curate the allele translation tables include: PharmGKB—Stanford University pharmacogenomics reference database, Pharmacogene Variation Consortium, Database of NAT genes (Democritus University of Thrace), UGT SNPs and haplotypes (Laval University), TPMT nomenclature (Linkoping University), and PubMed—online National Library of Medicine publication database.

HLA analysis

Applied Biosystems™ Axiom™ HLA Analysis Software can be used for HLA typing [14].

Integration of genotype results into reporting systems

Axiom PharmacoFocus Array data can easily be integrated into third-party reporting systems, such as the one from Coriell Life Sciences [15]. By incorporating a participant's genetics, along with lifestyle information and health history, a researcher can be informed of potential drug–drug interactions and options under the appropriate Institutional Review Board (IRB) approval process followed by their institution.

Axiom PharmacoFocus Assay workflow

The Axiom PharmacoFocus Assay workflow consists of the following steps (Figure 2):

- Genomic DNA (gDNA) is isothermally amplified, mPCR amplicons added, and target is randomly fragmented into roughly 25–125 bp fragments.
- Fragments are precipitated, resuspended, and hybridized to Axiom PharmacoFocus Array plates.
- Bound target is washed under stringent conditions to remove nonspecific 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.

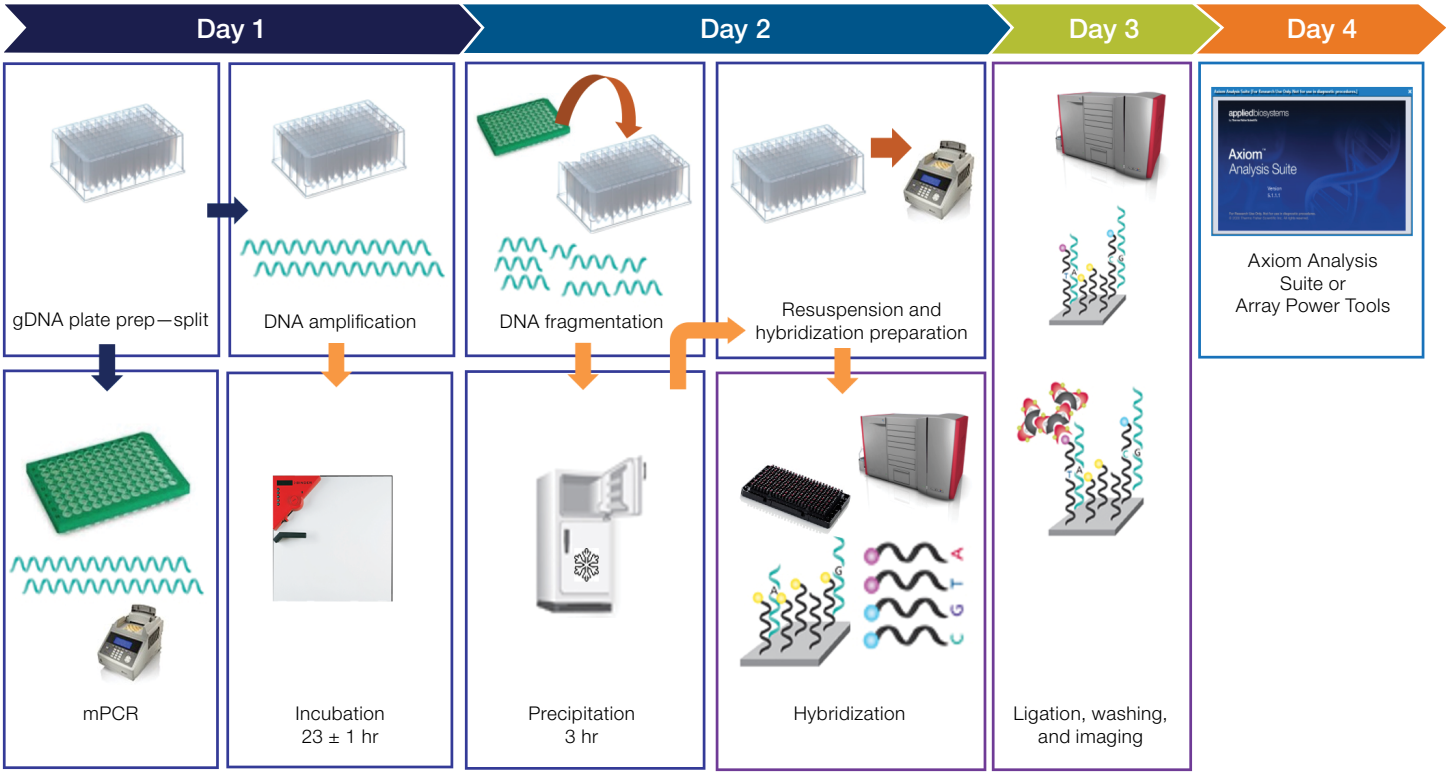

Figure 2. Axiom PharmacoFocus Assay workflow.

Performance—high-accuracy results, even for complex ADME gene variants

The Axiom PharmacoFocus Array's performance was evaluated using a set of 480 samples comprising whole blood, buccal, and saliva samples. The data were analyzed using Axiom Analysis Suite Software version 5.1.1 [13] and the Axiom Best Practices Workflow.

The results of the evaluation study, which had stringent quality metrics, are outlined in Table 2. Examples of cluster plots and copy number results are shown in Figures 3 and 4, respectively.

Table 2. Axiom PharmacoFocus Array performance (mini 96-well array plate format).

Metric	Specification	Performance
Number of samples	–	480
Sample pass rate	>96.9%	98.9%
Average call rate	≥99.0%	99.92%
Reproducibility	≥99.8%	99.98%
Average concordance with 1KGP phase III	≥99.6%	99.91%
Average call rate of markers that require gene-specific amplification	≥99.0%	99.99%
Concordance of markers that require gene-specific amplification with 1KGP phase III	≥99.0%	99.91%

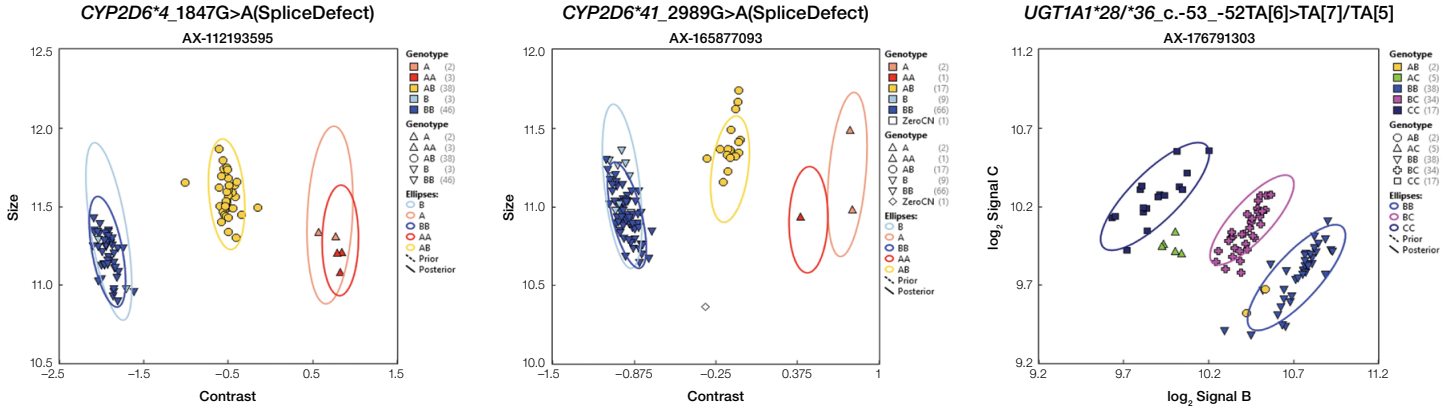

Figure 3. Cluster plots for CYP2D6*4 (rs3892097), CYP2D6*41 (rs28371725), and UGT1A1*28/*36 (rs3064744) markers.

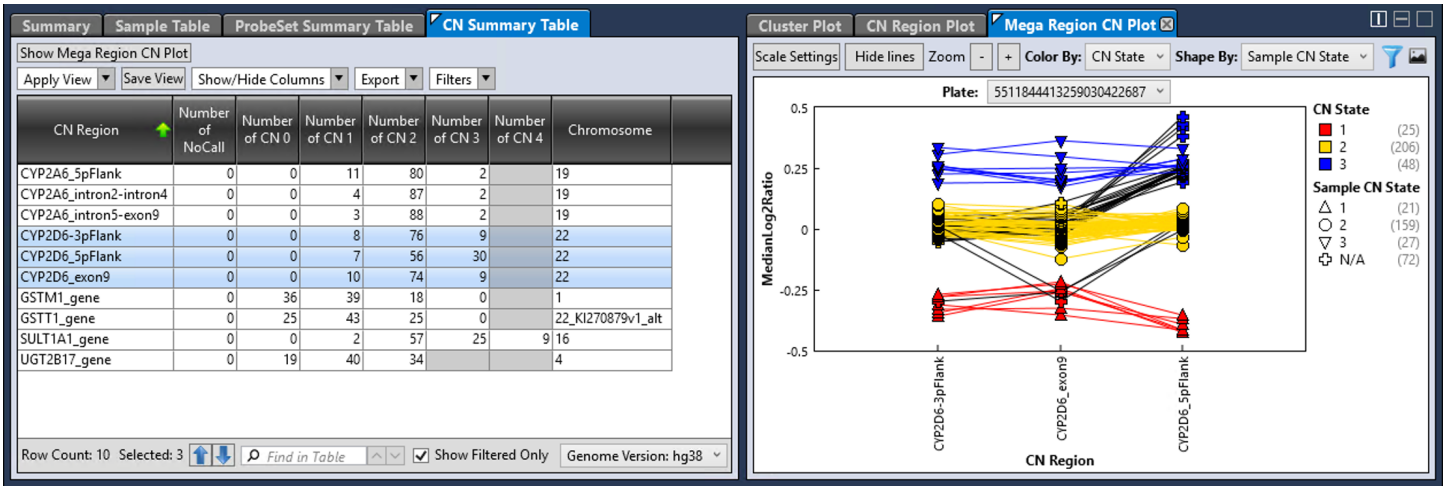

Figure 4. Copy number capabilities offered with Axiom PharmacoFocus Array. Built-in visualization (Mega Region CN Plot) shows 3 gene regions of *CYP2D6*.

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14. Axiom HLA Analysis v1.2 USER GUIDE Pub. No. 703338
15. The Power of Personalized Medicines Delivered **https://www.coriell.com/**

Ordering information

Product	Description	Quantity	Cat. No.
Premium configuration for Axiom PharmacoFocus Assay Mini 96 Kit with star allele reporting	Includes one mini 96-array plate, Axiom PharmacoFocus reagents, controls, and GeneTitan Multi-Channel Instrument consumables for running 94 samples and 2 control samples	94 samples	952396
Advanced or Lite configuration to choose content on Axiom PharmacoFocus Array that meets your pharmacogenomic goals	Advanced or Lite configuration to choose the number of genes and variants covered on Axiom PharmacoFocus array For more information contact **BioinformaticsServices@thermofisher.com**	94 samples	000870
Axiom PharmacoFocus Assay Mini 96 Reagent Kit	Includes Axiom PharmacoFocus reagents and controls required to run one mini 96-format array plate with 94 samples and 2 control samples	94 samples	952389
Axiom 384HT GeneTitan High Volume Consumables Kit	Contains all consumables required to process 5 mini 96-array plates on the GeneTitan Multi-Channel Instrument: 5 hybridization trays, 5 scan trays, and 25 stain trays with covers	470 samples	902629
Axiom PharmacoFocus Assay Core Mini 96 Kit without star allele reporting	Includes one mini 96-array plate, Axiom PharmacoFocus reagents, controls, and GeneTitan Multi-Channel Instrument consumables for running 94 samples and 2 control samples (does not include star allele reports)	94 samples	952425
Axiom PharmacoFocus Assay Mini 96 Training Kit	Includes two mini 96-format array plates and two DNA plates for training, Axiom PharmacoFocus reagents, controls, and GeneTitan Multi-Channel Instrument consumables for running 94 samples and 2 control samples on each plate (includes star alleles reports)	188 samples	952398
Axiom PharmacoFocus Assay Core Mini 96 Training Kit	Includes two mini 96-format array plates and two DNA plates for training, Axiom PharmacoFocus reagents, controls, and GeneTitan Multi-Channel Instrument consumables for running 94 samples and 2 control samples on each plate (does not include star allele reports)	188 samples	952426

Find out more at **thermofisher.com/pharmacofocus**

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