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Microarrays

NIMBUS Target Preparation Instrument and SwiftArrayStudio solution for pharmacogenomic research

In this application note, we:

- Describe a script available for performing the Axiom SwiftArray assay on the NIMBUS Target Preparation Instrument
- Demonstrate how this method can be used for pharmacogenomic research using the Axiom PharmacoPro microarray

Introduction

Advances in genomics have made accurate genotyping an increasingly important tool for informing lifestyle and clinical decision-making. As the value of genomic data continues to grow, so does the need for precise and reliable sample handling and processing methods. Even small errors during target preparation can significantly impact downstream results, prompting many translational and clinical researchers to adopt automated workflows to reduce variability and minimize the risk of human error.

The benefits of using automation for liquid handling steps during sample processing are many. First, it reduces potential errors caused by mispipetting or inadvertently omitting important steps. Second, it helps minimize run-to-run variability associated with repeated and complex manual pipetting steps. Finally, while the robot operates, it frees up laboratory personnel for other tasks. Together, these benefits help improve test reproducibility and laboratory efficiency.

We have previously developed an automated target preparation protocol for the Applied Biosystems™ Axiom™ 2.0 assay on the Applied Biosystems™ NIMBUS® Target Preparation Instrument. Developed by Hamilton Robotics, the NIMBUS instrument is a compact, high-speed automated multichannel pipetting robot that is widely recognized for its reliable pipetting performance and durability. It features a 96-channel 1 mL multi-pipetting head for plate-based pipetting using a combination of capacitance-based and pressure-based liquid level detection for accuracy. It also features a heat block for temperature-sensitive incubation steps, and a cooling block for storing heat-labile reagents.

To help meet growing demands for speed in high-throughput microarray analyses, we recently introduced the Applied Biosystems™ SwiftArrayStudio™ solution, which combines the Applied Biosystems™ SwiftArrayStudio™ Microarray Analyzer, robust Axiom™ SwiftArray™ reagents, and high-performance Axiom™ arrays. This integrated, next-generation microarray solution reduces the time needed to collect data from 5 days to approximately 30 hours. To assist researchers who also need automated target preparation, we adapted the NIMBUS instrument for processing 96 samples using the SwiftArrayStudio solution.

Rapid, high-throughput workflows support timely decision-making and operational efficiency in pharmacogenomic studies. By determining how genes affect responses to medications, pharmacogenomic analyses can provide faster paths to effective drug and dose decisions, reduce undesirable side effects, and lower overall healthcare costs [1-4]. To support this area of research, we launched the Applied Biosystems™ Axiom™ PharmacoPro™ Array Kit for the SwiftArrayStudio solution. The kit includes arrays, reagents, processing consumables, and integrated analysis software. Each 96-format array plate provides information on 94 samples plus two controls, enabling high-throughput efficiency without compromising data quality.

The Axiom PharmacoPro assay targets functionally significant variants across major ADME genes, including relevant SNPs, indels, CNVs, and HLA types, providing robust coverage of clinically relevant pharmacogenomic content. For more information, see the **Axiom PharmacoPro Array data sheet**.

Here we demonstrate that high-quality data can be obtained using the Axiom PharmacoPro Array, Axiom SwiftArray reagents for target preparation, and the SwiftArrayStudio Microarray Analyzer. Although this application note focuses on the Axiom PharmacoPro assay, the script we developed can in principle be used with other 96-format arrays and the SwiftArrayStudio solution. Please note that this script was only designed to prepare 96 samples to fill an entire Axiom array plate.

Overview of the method

The workflow described here breaks the target preparation method into seven different sub-methods. The names of the sub-methods align with those shown on the NIMBUS instrument during the automated workflow. Briefly, samples and consumables are loaded on the NIMBUS instrument, and DNA Amplification (sub-method 1) is run (Figure 1). When this sub-method is completed, the samples are either moved to the 48°C heat block on-instrument, or placed in a 48°C convection oven for 150 min for amplification. Next, the amplification reactions are placed back on the instrument to run Fragmentation (sub-method 2). When the run is completed, the samples are centrifuged at 4°C and dried off-instrument.

Reagents for resuspending the pellets are added to the deck to execute Resuspension (sub-method 3) followed by shaking off-instrument. Next, a plate with samples ready for hybridization (HybReady plate) is prepared using Hybridization Preparation (sub-method 4). The resulting HybReady plate can be used as a source for preloading quality control (Sample QC, sub-method 5) or stored. Finally, the array-processing plates that will be loaded into the SwiftArrayStudio MicroArray Analyzer are either prepared manually (off-instrument) or using Prepare Hybridization Tray and Prepare SwiftArray Reagent Plates (sub-methods 6 and 7). The entire process to prepare for loading takes approximately 6 hours. Minimal hands-on time is needed to move samples after certain steps and to load and unload the NIMBUS instrument after each sub-method.

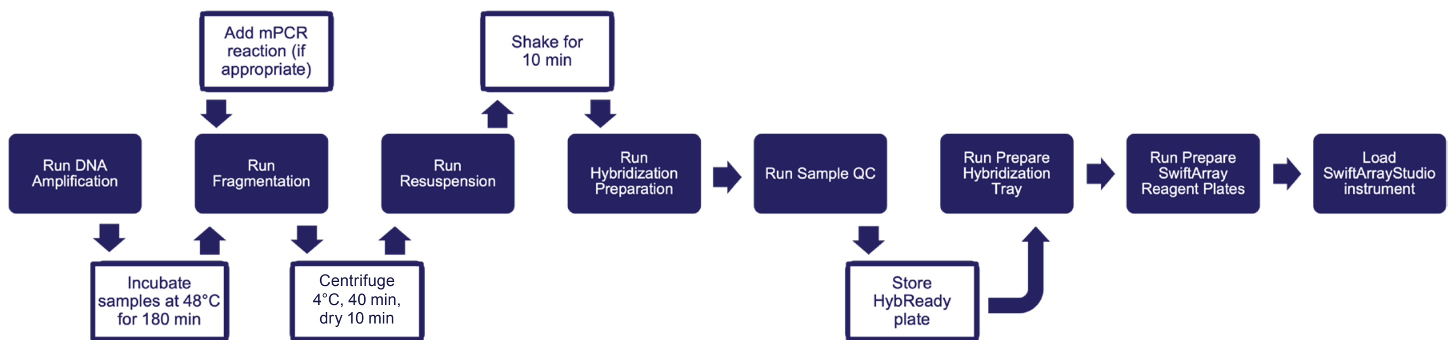


Figure 1. Workflow for running an Axiom SwiftArray assay on the NIMBUS instrument. Each filled box represents a separate sub-method of the protocol on the NIMBUS instrument. Unfilled boxes are steps run off-instrument. Overall, the process takes about 6 hours. Note: We recommend that Prepare Hybridization Tray (sub-method 6) and Prepare SwiftArray Reagent Plates (sub-method 7) be performed off-instrument.
mPCR: multiplex PCR.

Running the samples

To illustrate how the NIMBUS instrument could be used for pharmacogenomic research, we obtained 96 well-characterized human gDNA reference samples from the Coriell Institute for Medical Research (part of the Applied Biosystems™ PharmacoScan™ Training Kit, 96-format, Cat. No. 913027). To obtain the best pharmacogenomic coverage of these samples, we characterized them with the Axiom PharmacoPro assay. This assay includes a separate multiplex PCR (mPCR) reaction that amplifies difficult-to-analyze regions. Samples (50 ng input DNA) were amplified in an Applied Biosystems™ ProFlex™ PCR System using the conditions specified in the user guide. To verify amplification, representative aliquots of the sample were analyzed by agarose gel electrophoresis on 4% agarose gels with ethidium bromide (Figure 2).

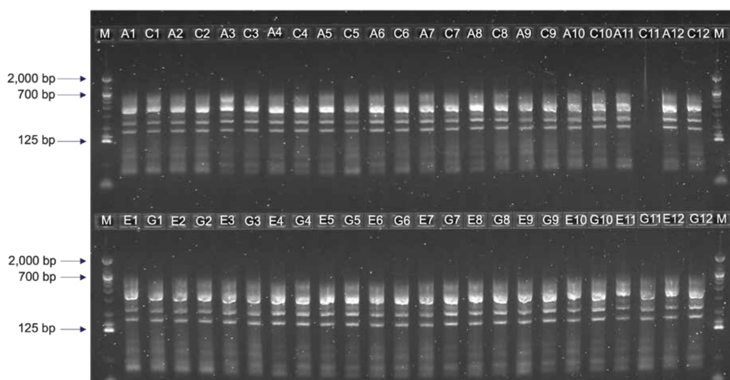


Figure 2. Agarose gel analysis of representative mPCR reactions. Multiplex PCR is expected to produce a variety of fragments of sizes between 50 and 500 bp, as shown here. Note that sample C11 failed to load into the gel; subsequent gels confirmed that the expected fragments were generated.

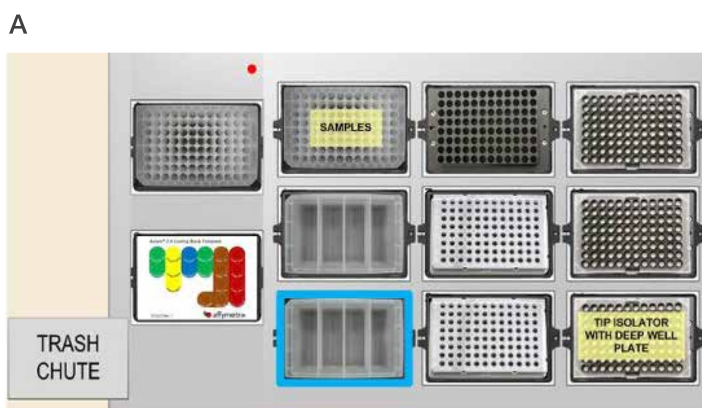
While the separate mPCR reaction was running, we initiated the DNA Amplification sub-method on the NIMBUS instrument using reagents in the Applied Biosystems™ Axiom™ SwiftArray Reagent Kit 1x (for 96 samples). A plate containing 100 ng of each gDNA sample was loaded on the instrument, along with the reagents and consumables as guided by the easy-to-follow interface (Figure 3), and the protocol was started. Sub-method 1 includes denaturing the gDNA samples, neutralizing after denaturation, and adding the whole-genome amplification (WGA) reagents to the samples. For these experiments, we sealed the sample plate, placed it in a 48°C convection oven (100% convection), and incubated for 150 min.

Next, we initiated the Fragmentation sub-method. Since we were using the Axiom PharmacoPro Array, we added 10 µL of a 1:10 dilution of the completed mPCR reaction to the completed WGA plate before starting the Fragmentation sub-method. The sample plate was loaded on the instrument with reagents and consumables as guided by the user interface.

The Fragmentation sub-method performs the 20 min fragmentation reaction, stops the fragmentation, and adds reagents for isopropanol precipitation to the samples. When this sub-method was completed, the sample plate was spun at 3,200 x *g* at 4°C for 40 minutes. The resulting supernatant was discarded, the plate was blotted to drain, and the plate was dried for 10 minutes in the 48°C convection oven.

The samples were resuspended by initiating sub-method 3 (Resuspension). Here, the plate containing the DNA pellets was loaded on the NIMBUS instrument along with reagents and consumables for the Resuspension sub-method. This brief protocol added buffer to resuspend the pellet; the plate was then sealed and shaken for 10 minutes off-instrument to dissolve the DNA pellet. The plate was spun briefly before running the Hybridization Preparation sub-method for getting samples ready for hybridization (sub-method 4). When this sub-method was completed, the plate was sealed and stored at –20°C.

A



B

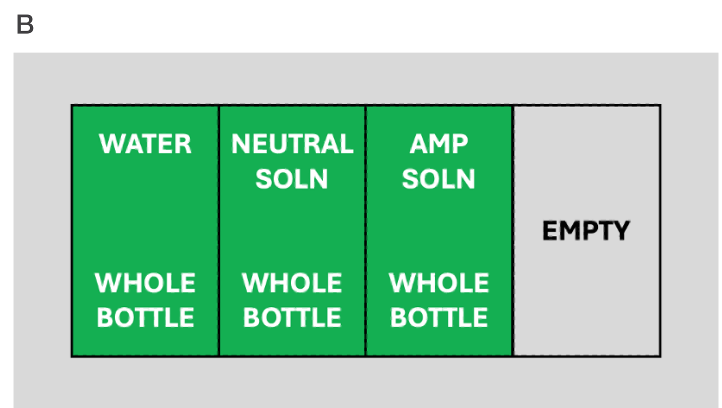


Figure 3. The SwiftArrayStudio Microarray Analyzer is controlled by an intuitive GUI. Users are guided to **(A)** placement of consumables on the deck and **(B)** reagent filling in the reservoirs.

Sample QC following the liquid handler runs

The Sample QC sub-method follows the recommendations in the user guide of the SwiftArrayStudio Microarray Analyzer for brief QC analysis of the samples, before proceeding with the hybridization. This sub-method makes dilutions of the hybridization-ready samples so they can be quantified by UV absorption, and so they can be analyzed by size using agarose gel electrophoresis.

We used this sub-method to perform QC on the DNA obtained using the NIMBUS instrument. Quantification of the recovery from one representative plate ranged from 832 µg to 1,397 µg (average = 1,076 µg, SD = 104, Figure 4A). A replicate plate of the same samples gave similar results (average = 1,126 µg, SD = 207). We analyzed the size distribution of some representative samples, and determined that the fragments were in the desired range between about 25 and 125 bp (Figure 4B).

Preparing trays for hybridization, staining, washing, and scanning is trivial with the Axiom SwiftArray assay. For the hybridization tray, we simply denatured the samples in the HybReady plate at 95°C for 10 minutes, held the plate at 48°C, and manually pipetted them into the 96-well hybridization tray before loading into the SwiftArrayStudio Microarray Analyzer. We manually prepared the ligation and staining solutions in 15 mL conical tubes according to the user guide for the SwiftArrayStudio Microarray Analyzer, and simply poured them into the universal trays. Similarly, the hold solution and stabilization solution were poured into the scan tray and universal tray, respectively. Although scripts were written such that these steps could be run on the NIMBUS instrument, they are easier and faster to perform manually. Once all the trays were loaded into the SwiftArrayStudio Microarray Analyzer, it was started with a 16 hr hybridization, followed by automated washing and scanning.

QC of resulting scans

We analyzed the resulting scans using the Axiom Analysis Suite Best Practices workflow in the user guide. The output of this analysis includes standardized QC metrics for scan quality. One of these metrics, DishQC, measures signal-to-noise ratios in nonpolymorphic regions of the genome. One of the samples did not meet the DishQC metric threshold; further analysis showed this was due to a bubble or some other type of blemish during imaging. Rescanning the plate can sometimes recover data from such failed samples; however, this was not done with this plate. Another metric is the average passing QC call rate (average percentage of autosomal SNPs with a call other than NoCall, as measured at the sample QC step of passing samples within a plate). One sample fell below the default threshold set using the best practices workflow. When the two samples that failed default QC were excluded, the average call rate for passing samples was 99.908%. Because the genotype QC metric was so high, these results demonstrate that the workflow with the NIMBUS instrument produces high-quality data.

A

	1	2	3	4	5	6	7	8	9	10	11	12
A	1,005	978.1	1,013	977.2	846.8	1,010	1,026	992.2	1,020	1,088	1,098	1,309
B	876.8	1,085	1,025	1,067	1,112	1,048	1,048	1,022	1,014	1,036	1,062	1,363
C	923.6	1,127	1,034	1,010	1,166	1,156	1,024	1,076	1,050	966.2	1,070	1,397
D	832.0	1,131	1,080	1,082	1,025	1,047	1,022	960.1	1,007	1,036	1,062	1,367
E	851.6	1,045	1,081	1,086	1,130	1,030	1,126	1,028	1,148	1,063	1,106	1,350
F	1,017	1,084	1,131	1,080	1,079	1,128	1,139	1,092	1,145	1,124	1,057	1,340
G	1,112	1,077	1,101	1,179	1,055	1,008	1,004	1,091	1,006	1,116	1,105	1,274
H	1,082	1,078	1,077	1,085	1,067	932.0	1,035	1,027	1,026	1,083	1,114	1,334

B

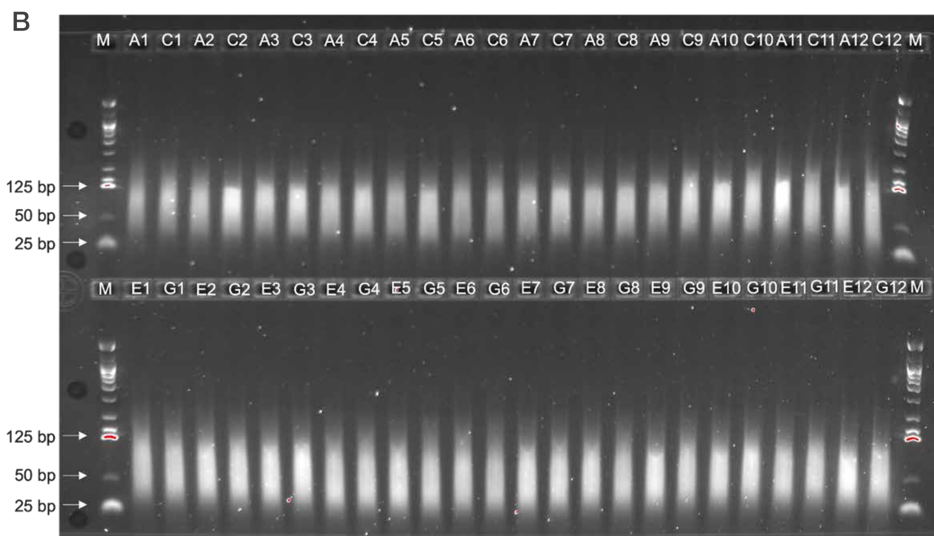


Figure 4. Quality control analysis of samples produced on the NIMBUS instrument. (A) Amount of DNA recovered (in µg) after the resuspension step (median = 1,065.5 µg). **(B)** Agarose gel analysis of samples after running the Hybridization Preparation sub-method. Marker was a 25 bp ladder. Note that a majority of the fragments were around 50 bp, with a good distribution between 25 and 125 bp.

Concordance of data with known genotypes

To characterize the accuracy of this workflow, we compared the results obtained in the two replicates to the known genotypes of these samples, as determined by sequencing (Figure 5). For all the samples in plate 1, the average concordance was 99.85%; for plate 2 the average was 99.83% (median for both was 99.86%).

We also determined reproducibility and accuracy of copy number calls compared to known truth of these samples. For accuracy, we computed the sensitivity and specificity of the calls for each CNV region queried on the array. The average sensitivity was 98.8% on both plates, and average specificity was 99.6% for both. To compare reproducibility, we calculated the correlation of number of samples that were called for each copy number variant at each locus, as measured by the two different plates. Here, the correlation between the two sets of data was 0.9963, indicating that the copy numbers that fell into each class were similarly determined.

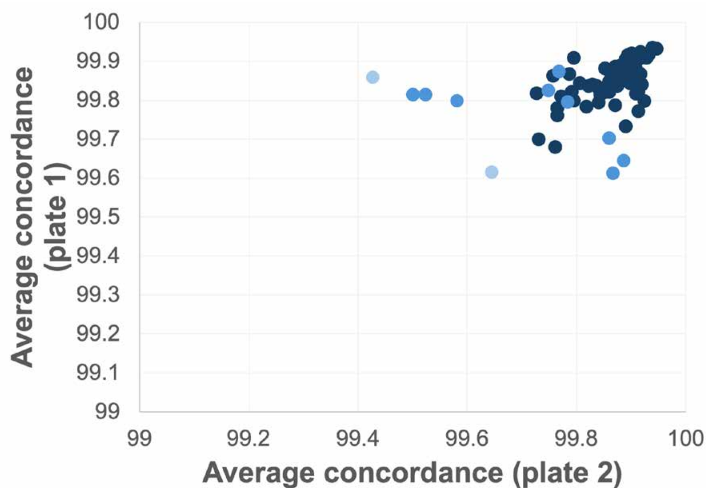


Figure 5. Concordance with known truth of genotyping calls made with the Axiom SwiftArray assay workflow with target preparation on the NIMBUS instrument. All loci, including those queried by mPCR, were compared against known truth. The concordance of each sample correlates extremely well across the two replicates (R^2 >0.999). Data points are colored according to total call rate (CR): dark blue = CR >99.9%, medium blue = CR >99.75%, light blue = CR >99.5%.

Considerations

In this application note, we describe a workflow that takes advantage of the automated liquid handling capabilities of the NIMBUS Target Preparation Instrument. A script with a method and user interface to execute the target preparation steps was developed, where all steps of the Axiom SwiftArray assay can be set up on and incubated on the instrument. However, in most cases it can be more efficient to perform some steps manually. For example, the sample incubation step at 48°C (DNA Amplification sub-method) can be performed off-instrument, in a convection oven. This frees up the instrument for processing more samples during the incubation step.

Similarly, we found it is faster to prepare the staining and ligation solutions and load the trays for the SwiftArrayStudio analyzer manually than utilize the NIMBUS instrument for these steps. The Axiom SwiftArray assay features new reagent trays with an open-tray design, meaning it only requires pouring the reagent into the tray and tilting to distribute across the entire tray. It is no longer necessary to pipet reagents into confined, specific wells.

Nevertheless, the script does allow for automated loading of the trays (sub-methods 6 and 7). To do this, it is necessary to transfer Applied Biosystems™ SwiftArray™ Ligation Solution 1 and 2, and SwiftArray™ Ligation Enzyme from the tubes supplied with the kit into tubes that are compatible with these steps on the robot (see Appendix for details).

Labs needing to process large numbers of samples might want to perform numerous runs on the NIMBUS instrument in a day. To facilitate these labs, we outlined 3 scenarios where the use of the NIMBUS instrument is optimized for multiple plates (Figure 6). In these scenarios, the NIMBUS instrument is used to process more samples when it would otherwise be idle. For example, three hybridization-ready plates (288 samples) can be prepared in a single day with normal work shifts. One of these can be loaded into the SwiftArrayStudio analyzer; a second plate can be loaded in a longer day. Plates that are made to be hybridization-ready using the workflow on the NIMBUS instrument can be stored at –20°C for up to 10 days before analysis on the SwiftArrayStudio analyzer.

To assist users of the automated workflow for the Axiom 2.0 assay to transition to the Axiom SwiftArray assay, some modifications to the script for automated target preparation on the NIMBUS instrument were incorporated. These modifications were verified using more than 20 dry runs, water runs, and generic DNA runs. Furthermore, the script was tested on two different NIMBUS instruments, producing results that were accurate and reproducible each time. The data presented here provides a proof of concept of how the NIMBUS instrument could be adapted for workflows using the Axiom SwiftArray assay.

Scenario 1: trays prepared manually

	Run 1	Run 2	Run 3
9:00	DNA Amplification		
9:15			
9:30			
9:45	Sample incubation at 48°C	DNA Amplification	
10:00			
10:15			
10:30		Sample incubation at 48°C	DNA Amplification
10:45			
11:00			
11:15			Sample incubation at 48°C
11:30			
11:45			
12:00			
12:15			
12:30	Fragmentation		
12:45			
13:00			
13:15	Centrifugation at 4°C and drying	Fragmentation	
13:30			
13:45			
14:00		Centrifugation at 4°C and drying	Fragmentation
14:15			
14:30			
14:45	Resuspension		Centrifugation at 4°C and drying
15:00	Hybridization Preparation		
15:15		Resuspension	
15:30	Sample QC		
15:45			Resuspension
16:00	Tray preparation	Hybridization Preparation	
16:15	Load analyzer	Sample QC	
16:30			Hybridization Preparation
16:45			Sample QC
17:00			Run later
17:15			
17:30			
17:45			
18:00			
18:15			
18:30		Tray preparation	
18:45		Load analyzer	
19:00			
19:15			
19:30			
19:45			
20:00			
20:15			

Scenario 2: trays prepared on-instrument

	Run 1	Run 2	Run 3
9:00	DNA Amplification		
9:15			
9:30			
9:45	Sample incubation at 48°C	DNA Amplification	
10:00			
10:15			
10:30		Sample incubation at 48°C	DNA Amplification
10:45			
11:00			
11:15			Sample incubation at 48°C
11:30			
11:45			
12:00			
12:15			
12:30	Fragmentation		
12:45			
13:00			
13:15	Centrifugation at 4°C and drying	Fragmentation	
13:30			
13:45			
14:00		Centrifugation at 4°C and drying	Fragmentation
14:15			
14:30			
14:45	Resuspension		Centrifugation at 4°C and drying
15:00	Hybridization Preparation		
15:15		Resuspension	
15:30	Sample QC		
15:45			Resuspension
16:00	Prepare Hybridization Tray		
16:15	Prepare SwiftArray Reagent Plates		
16:30			
16:45	Load analyzer	Hybridization Preparation	
17:00		Sample QC	
17:15			Hybridization Preparation
17:30			Sample QC
17:45			Run later
18:00			
18:15			
18:30		Prepare Hybridization Tray	
18:45		Prepare SwiftArray Reagent Plates	
19:00			
19:15		Load analyzer	
19:30			
19:45			
20:00			
20:15			

Scenario 3: full run on-instrument

	Run 1	Run 2
9:00	DNA Amplification	
9:15		
9:30		
9:45	Sample incubation at 48°C	
10:00		
10:15		
10:30		
10:45		
11:00		
11:15		
11:30		
11:45		
12:00		
12:15	Fragmentation	
12:30		
12:45		
13:00	Centrifugation at 4°C and drying	
13:15		
13:30		
13:45		
14:00	Resuspension	
14:15		DNA Amplification
14:30		
14:45		
15:00		Sample incubation at 48°C
15:15		
15:30		
15:45		
16:00		
16:15		
16:30		
16:45		
17:00		
17:15		
17:30		Fragmentation
17:45		
18:00		
18:15	Hybridization Preparation	Centrifugation at 4°C and drying
18:30	Sample QC	
18:45	Prepare Hybridization Tray	
19:00	Prepare SwiftArray Reagent Plates	
19:15		
19:30		Resuspension
19:45	Load analyzer	Hybridization Preparation
20:00		Sample QC
20:15		Run later

Figure 6. Scenarios for multiple runs on a single NIMBUS instrument. In scenario 1, staggering the runs on-instrument can allow up to three plates of 96 samples each that can be made ready for hybridization and quality analysis. By manually preparing the hybridization trays and Axiom SwiftArray reagent trays (tray preparation), two array plates can be run 2.5 hours apart. In scenario 2, the same trays are prepared on the instrument (Prepare Hybridization Tray and Prepare SwiftArray Reagent Plates). Again, up to three sets of 96 samples can be made ready, but in a slightly longer day. In the final scenario, all steps, including genome amplification, are run on the instrument. This requires the least amount of active monitoring but only results in two sets of 96 samples, ready to load on the SwiftArrayStudio analyzer, in a long day. Other scenarios not indicated here are also possible. Filled boxes represent steps performed on-instrument, and unfilled boxes represent steps performed off-instrument. The green, yellow, blue, and red colors align with cap colors used in the Axiom SwiftArray Reagent Kit at each sub-method (see Appendix). For Sample QC and Prepare Hybridization Tray (gray), all materials are user-supplied.

Conclusion

In this application note, we illustrate how the NIMBUS instrument could be used to facilitate pharmacogenomic analyses. We developed a script specific for the Axiom SwiftArray assay and used the Axiom PharmacoPro Array to show how high-quality and reproducible data can be obtained using this workflow. Notably, although we focus on the Axiom PharmacoPro Array, in principle, the new script can be used with any other 96-format Axiom array and the SwiftArrayStudio solution. Together, this workflow can help to improve data reproducibility and laboratory efficiency in pharmacogenomic research.

References

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2. Krebs K et al. (2019) Translating pharmacogenomics into clinical decisions: do not let the perfect be the enemy of the good. *Hum Genomics* 13:39. doi.org/10.1186/s40246-019-0229-z
3. Way H et al. (2021) Genomics as a clinical decision support tool: successful proof of concept for improved ASD outcomes. *J Pers Med* 11:596. doi.org/10.3390/jpm.11070596
4. Morris SA et al. (2022) Cost effectiveness of pharmacogenetic testing for drugs with Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines: A systematic review. *Clin Pharmacol Ther* 112:1318–1328. doi.org/10.1002/cpt.2754

Ordering information

Product	Quantity	Cat. No.
SwiftArrayStudio Microarray Analyzer	1 instrument	00-0471
Axiom PharmacoPro Array Kit 1x	1 kit for 96 samples	952647
Axiom PharmacoPro Array Kit 2x	1 kit for 2 x 96 samples	952648
Axiom SwiftArray Reagent Kit 1x	1 kit for 96 samples	952634
Axiom SwiftArray Reagent Kit 2x	1 kit for 2 x 96 samples	952636
Axiom SwiftArray Reagent Kit with Wash Buffer 1x	1 kit for 96 samples	952639
Axiom SwiftArray Reagent Kit with Wash Buffer 2x	1 kit for 2 x 96 samples	952641
Axiom Consumables Kit for NIMBUS Target Preparation Instrument	Labware sufficient for 4 x 96 reactions	902366
Axiom Consumables Kit for QC	1 kit	902909

Appendix

Obtaining and installing new script

1. To obtain the script for performing the Axiom SwiftArray assay on the NIMBUS instrument, please speak with your field applications scientist (FAS).
2. Open the Hamilton Method Editor.
3. From the File menu, select “File” and then “Import”.
4. Select the “…” button under the Import Method/Library/ Workflow section of the window.
5. Select the “SwiftArray1.2.pkg” file and press the “Next” button.
6. On the next screen, choose the “Recovery” button, followed by “Import original Hamilton files” at the bottom of the window. Then press “Next”.
7. Select the “Finish” button. The import path for the files will be shown at the bottom of the window. When the process is complete, close the window.
8. Ensure that the supplied files “Axiom 2.096.xml” and “Axiom_ Logo.png” are in the same directory as the .med file. If files with these names are already in the directory, rename them (i.e., Axiom 2.096_backup.xml) before copying over the new files, otherwise they will be overwritten.
9. Unzip the “Images” folder and copy to the same directory as the .med file. If a folder with this name is already in the directory, rename it (i.e., AxiomImages_Backup) before copying over the new files; otherwise it will be overwritten.

General protocol notes

- When a sub-method finishes, it prompts the user to clean off the deck and exits to the beginning interface. To move on to the next sub-method, click the green arrow in the toolbar. The script should start the incremented sub-method after the one that was just completed.
- Cap colors of the Axiom SwiftArray reagent bottles or tubes are indicated in the tables. Rows in the tables without cap colors are not part of the kit.
- MLS: major laboratory supplier

DNA Amplification (sub-method 1)

- **Note:** Samples must be in a round-bottom deep-well plate (such as Cat. No. 278743).

Materials required

Cap color	Material	Quantity	Volume	Source	Notes
●	Water (supplied)	1 bottle	16 mL	Axiom SwiftArray Reagent Kit	–
●	SwiftArray Neutralization Solution	1 bottle	15 mL	Axiom SwiftArray Reagent Kit	–
●	SwiftArray Amplification Solution	1 bottle	30 mL	Axiom SwiftArray Reagent Kit	–
●	SwiftArray 10X Denaturation Solution	1 tube	650 µL	Axiom SwiftArray Reagent Kit	–
●	SwiftArray Amplification Enzyme	1 tube	1,100 µL	Axiom SwiftArray Reagent Kit	–
–	Round-bottom deep-well plate	1	–	Thermo Fisher Scientific	Empty
–	4-chamber reservoirs	2	–	Hamilton	Use reservoir holders
–	96-well full-skirted plates	2	–	Corning (Axygen™ brand)	–
–	1,000 µL tips	1	–	Hamilton	–
–	300 µL tips	2	–	Hamilton	–

1. Start sub-method by selecting “DNA Amplification”.
2. Follow the instructions on screen to place consumables and reagents in the right positions on deck. Time to run is approximately 26 min.
3. When the sub-method is complete:
   a. Seal the sample plate.
   b. Incubate the plate at 48°C on the NIMBUS heat block or in a convection oven for 150 min.
   c. If the plate is incubated on the NIMBUS heat block, spin down condensation at 2,000 rpm for 1 min after the 150 min incubation.
   d. Proceed to Fragmentation sub-method.

Fragmentation (sub-method 2)

- **Note:** If performing mPCR, add the diluted reactions to the sample plate just before starting the fragmentation steps.

Materials required

Cap color	Material	Quantity	Volume	Source	Notes
● (yellow)	SwiftArray Fragmentation Solution	1 bottle	10 mL	Axiom SwiftArray Reagent Kit	–
● (yellow)	SwiftArray Fragmentation Stop	1 bottle	5 mL	Axiom SwiftArray Reagent Kit	–
● (yellow)	SwiftArray Precipitation Solution	1 bottle	23 mL	Axiom SwiftArray Reagent Kit	–
● (yellow)	SwiftArray Fragmentation Enzyme	1 tube	518 µL	Axiom SwiftArray Reagent Kit	–
–	Isopropanol (not supplied)	–	75 mL	MLS	–
–	1.2 mL square-well plate	1	–	Included in the Axiom Consumables Kit for QC or available from Thomas Scientific	Thomas Scientific, Cat. No. OX1263
–	4-chamber reservoirs	2	–	Hamilton	Use reservoir holders
–	96-well full-skirted plates	2	–	Hamilton	–
–	Square deep-well plate	1	–	Thermo Fisher Scientific	–
–	1,000 µL tips	1	–	Hamilton	–
–	300 µL tips	2	–	Hamilton	–

1. Follow the prompts and run the sub-method. Time to run is approximately 37 min.
2. When the sub-method is complete:
   a. Seal the plate immediately.
   b. Spin at 3,200 x *g* for 40 min at 4°C.
   c. Carefully discard supernatant, invert and drain plate on a laboratory wipe for 5 min.
   d. Dry the plate at 48°C in a convection oven for 10 min.
   e. Proceed to Resuspension sub-method.

Resuspension (sub-method 3)

Materials required

Cap color	Material	Quantity	Volume	Source	Notes
● (blue)	SwiftArray Resuspension Solution	1 bottle	6.5 mL	Axiom SwiftArray Reagent Kit	–
–	4-chamber reservoir	1	–	Hamilton	Use reservoir holders
–	96-well full-skirted plates	1	–	Corning (Axygen brand)	–
–	300 µL tips	2	–	Hamilton	–

1. Follow the prompts and run the sub-method. Time to run is approximately 3 min.
2. When the sub-method is complete:
   a. Seal the plate and shake on shaker for 10 min. Check to see if resuspension is complete; if not, shake for an additional 10 min.
   b. Spin the plate at 2,000 rpm for 2 min.
   c. Proceed to Hybridization Preparation sub-method.

Hybridization Preparation (sub-method 4)

- **Note:** The same 4-chamber reservoir can be used from Resuspension (sub-method 3).

Materials required

Cap color	Material	Quantity	Volume	Source	Notes
●	SwiftArray Hybridization Buffer	1 bottle	11 mL	Axiom SwiftArray Reagent Kit	–
●	SwiftArray Hybridization Solution	1 tube	160 µL	Axiom SwiftArray Reagent Kit	–
–	96-well half-skirted plate	1	–	Thermo Fisher Scientific	Use white holder
–	4-chamber reservoir	1	–	Hamilton	Use reservoir holders
–	Square deep-well plate	1	–	Thermo Fisher Scientific	Resuspended samples
–	96-well full-skirted plate	1	–	Corning (Axygen brand)	–
–	300 µL tips	2	–	Hamilton	–
–	1,000 µL tips	1	–	Hamilton	–

1. Follow the prompts and run the sub-method. Time to run is approximately 10 min.
2. When the sub-method is complete:
   a. Proceed to Sample QC or to Prepare Hybridization Tray sub-method, or seal the plate and store at –20°C.
   - **Note:** This plate is the hybridization-ready plate (HybReady plate). It can be stored at –20°C for up to 10 days.

Sample QC (sub-method 5)

Materials required

Material	Quantity	Volume	Source	Notes
Water	–	20 mL	MLS	–
Diluted gel-running solution	–	20 mL	Made fresh	1:6 dilution
96-well half-skirted plate	2	–	MLS	HybReady plate
4-chamber reservoir	1	–	Hamilton	Use reservoir holders
96-well UV plate	1	–	Corning	–
96-well full-skirted plates	2	–	Corning (Axygen brand)	–
300 µL tips	1	–	Hamilton	–
50 µL tips	2	–	Hamilton	–

1. Follow the prompts and run the sub-method. Time to run is approximately 12 min.
2. When the sub-method is complete:
   a. Run agarose gel of dilutions made.
   b. Measure OD values of dilutions made in UV plate.
   c. If samples pass QC, proceed to the Prepare Hybridization Tray sub-method. For recommendations on QC metrics, see the user guide for Axiom SwiftArray assays.

Prepare Hybridization Tray (sub-method 6)

- **Note:** This step can be performed without using the NIMBUS instrument, in less time and with minimal manual effort. For details on the manual method, see the user guide for Axiom SwiftArray assays.
- **Important: Before running this sub-method, denature samples by heating to 95°C for 10 min, then keep at 48°C until running this sub-method and loading onto the NIMBUS instrument.**

Materials required

Material	Quantity	Supplier	Notes
96-well half-skirted plate	1	MLS	Denatured HybReady sample plate
Hybridization tray	1	Thermo Fisher Scientific	–
300 µL tips	1	Hamilton	–

1. Follow the prompts and run the sub-method. Time to run is approximately 1 min.
2. When complete, load the hybridization tray with denatured samples into the SwiftArrayStudio instrument.

Prepare SwiftArray Reagent Plates (sub-method 7)

- **Note:** This step can be performed without using the NIMBUS instrument, in less time and with minimal manual effort. For this reason, and to avoid spillage, we recommend preparing these plates off-instrument. For details on the manual method, see the user guide for Axiom SwiftArray assays.
- **Note:** Once reagents are added to trays, if carried they can slosh about and spill onto the tray cover, interfering with performance. For best results, prepare the hold, stabilization, stain, and ligation trays close to the SwiftArrayStudio Microarray Analyzer.
- **Important: Before running the sub-method on the NIMBUS instrument, transfer the entire volume of the SwiftArray Ligation Solution 1 (1.9 mL) to a 2 mL screw cap tube such as Cat. No. 3490PK. Transfer the entire volume of SwiftArray Ligation Enzyme (600 µL) to a 500 µL skirted conical bottom microcentrifuge tube, designed for enzymes, such as Cat. No. 3422NK. Transfer the entire volume of SwiftArray Ligation Solution 2 (500 µL) to a 500 µL skirted conical bottom microcentrifuge tube, designed for enzymes, such as Cat. No. 3422NK.**
- **Important: Do not put SwiftArray Ligation Solution 2 into the CPAC block until immediately before restarting the sub-method.**

Materials required

Cap color	Material	Quantity	Volume	Source	Notes
● (green)	SwiftArray Stabilization Buffer	1 bottle	12.7 mL	Axiom SwiftArray Reagent Kit	–
–	SwiftArray Hold Buffer	1 bottle	15 mL	Axiom SwiftArray Reagent Kit	–
● (dark red)	SwiftArray Wash A Buffer	1 bottle	15 mL	Axiom SwiftArray Reagent Kit	–
● (dark red)	SwiftArray Stain Buffer	1 tube	325 µL	Axiom SwiftArray Reagent Kit	–
● (dark red)	SwiftArray Stain 1-A	1 tube	150 µL	Axiom SwiftArray Reagent Kit	–
● (dark red)	SwiftArray Stain 1-B	1 tube	150 µL	Axiom SwiftArray Reagent Kit	–
● (red)	SwiftArray Ligation Buffer	1 bottle	10 mL	Axiom SwiftArray Reagent Kit	–
● (red)	SwiftArray Ligation Solution 1	1 tube	1.9 mL	Axiom SwiftArray Reagent Kit	See important note
● (red)	SwiftArray Probe Mix 1	1 tube	1.55 mL	Axiom SwiftArray Reagent Kit	–
● (red)	SwiftArray Probe Mix 2	1 tube	1.55 mL	Axiom SwiftArray Reagent Kit	–
● (red)	SwiftArray Ligation Enzyme	1 tube	600 µL	Axiom SwiftArray Reagent Kit	See important note

Materials required (cont.)

Cap color	Material	Quantity	Volume	Source	Notes
● (red)	SwiftArray Ligation Solution 2	1 tube	500 µL	Axiom SwiftArray Reagent Kit	See important note
–	SwiftArray Scan Tray	1	–	Thermo Fisher Scientific	–
–	SwiftArray Ligation Tray	1	–	Thermo Fisher Scientific	These trays are the Universal Stain Trays
–	SwiftArray Stain Tray	1	–	Thermo Fisher Scientific	
–	SwiftArray Stabilization Tray	1	–	Thermo Fisher Scientific	
–	4-chamber reservoirs	2	–	Hamilton	Use reservoir holders
–	300 µL tips	2	–	Hamilton	–
–	1,000 µL tips	1	–	Hamilton	–
–	2 mL screw cap tube	1	–	Thermo Fisher Scientific	Cat. No. 3490PK
–	500 µL skirted conical bottom microcentrifuge tube	2	–	Thermo Fisher Scientific	Cat. No. 3422NK

1. Follow the prompts and run the sub-method. Time to run is approximately 26 min. Note that the sub-method pauses and requires plates and reagents to be changed. During this pause, place the tube of SwiftArray Ligation Solution 2 in the CPAC block and immediately continue the protocol.
2. When complete, cover the SwiftArray trays with SwiftArray tray covers and load them into the SwiftArrayStudio MicroArray Analyzer.

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