TECHNICAL NOTE

Manual workflow delivers high performance for Infinium™ Global Diversity Array with Enhanced PGx-8

- Fully manual workflow retains the high accuracy and reproducibility of the semiautomated workflow
- Targeted gene amplification enables accurate detection of high-impact pharmacogenomics genes
- Three-day Infinium workflow provides compatibility with low- to high-throughput applications

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Introduction

Microarrays are powerful tools for precision medicine research, enabling researchers to identify thousands of pharmacogenomics (PGx) variants in a single assay. The Infinium Global Diversity Array with Enhanced PGx-8 (Figure 1) is the most comprehensive genotyping array on the market, featuring over 44,000 genome-wide PGx markers, including more than 6000 variants from globally recognized PGx databases. These variants comprise high-impact genes, like *CYP2D6*, *CYP2B6*, and *TPMT*, that have historically been challenging to discern but are now accessible as a result of significant workflow improvements.

The Infinium Global Diversity Array with Enhanced PGx-8 uses the Infinium LCG assay with a targeted gene amplification (TGA) step for improved accuracy and pseudogene disambiguation. Currently this assay is supported by a semiautomated workflow validated for mid- to high-throughput applications. Lower throughput laboratories without automation capabilities, however, typically use the manual Infinium workflow to process BeadChips. In this technical note, we demonstrate that highly accurate and reproducible genotyping results are obtained when the Infinium Global Diversity Array with Enhanced PGx-8 is run with a fully manual workflow. Using a high-performance manual workflow with an 8-sample BeadChip and comprehensive PGx content make the Infinium Global Diversity Array with Enhanced PGx-8 a powerful pharmacogenomics research tool for lower throughput laboratories.

Figure 1: Infinium Global Diversity Array with Enhanced PGx-8 BeadChip—Built on the trusted eight-sample Infinium LCG assay platform.

Methods

Twelve BeadChips with eight samples per BeadChip were processed using the fully manual Infinium LCG Assay to demonstrate the high performance of the Infinium Global Diversity Array with Enhanced PGx-8 processed with the fully manual workflow. Test samples were obtained from the Coriell Institute. Each DNA sample contained 200 ng DNA diluted to 20 ng/µl. The 96 samples comprise 48 unique single nucleotide variant (SNV) and copy number variant (CNV) reproducibility samples, including replicates of different concentration levels. The Infinium workflow was processed using the three-day fully manual protocol in which the extension and staining (X-stain) steps were also performed manually. BeadChips were scanned using the iScan™ System with standard scan parameters for the Infinium LCG assay. Data analysis was performed on all 96 samples simultaneously using Illumina Microarray Analytics with PGx analysis on the Illumina Connected Analytics platform. An in-house Jupyter notebook Python script was used to compute genotype, PGx, and CNV concordance to control semiautomated run outputs.

Results

The Infinium Global Diversity Array with Enhanced PGx-8 run on a fully manual workflow shows a high degree of concordance with data obtained using the semiautomated workflow. Overall, the fully manual workflow performance met all quality control passing criteria. High concordance with the semiatomated workflow was observed for genotype, copy number call rate, copy number gain/loss, and PGx concordance. (Figure 2, Table 1).

The overall TGA performance was compared between the semiautomated and fully manual X-stain runs. The true positive rate (TPR), which is a measure of accuracy, and concordance (≥ 0.98) were comparable between the semiautomated and fully manual X-stain runs for all probes tested (Figure 3). TPR and concordance were compared for key PGx markers, including CYP2B6, CYP2D6, and TPMT. The accuracy was high and overall PGx performance using the fully manual X-stain workflow was comparable to the semiautomated X-stain metrics (Figure 4).

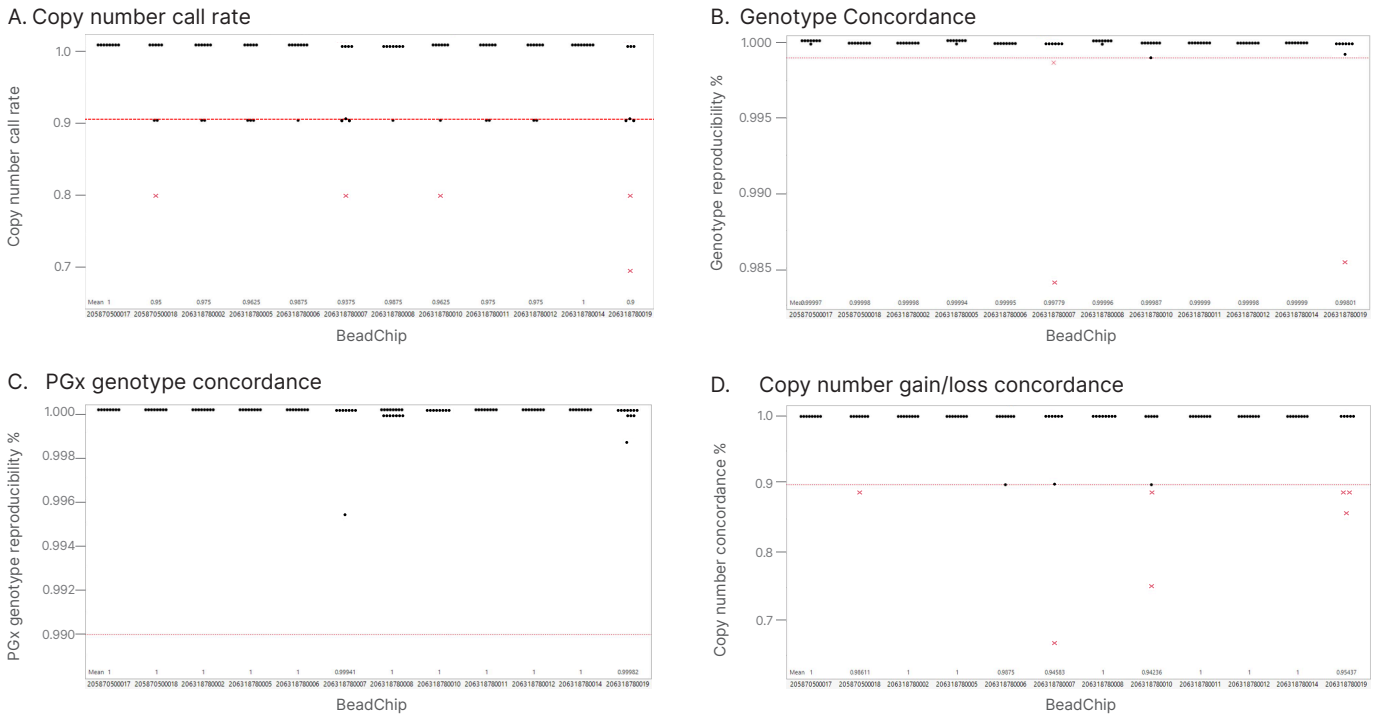

Figure 2: Concordance metrics for Infinium Global Diversity Array with Enhanced PGx-8 run on a fully manual workflow—A high degree of concordance was observed between fully manual and semiautomated workflows for (A) copy number call rate, (B) genotype, (C) PGx genotype, and (D) copy number gain/loss. Outliers (indicated by x) were observed as expected due to increased variability on a fully manual workflow. However, the overall concordance was high and met the quality control passing criteria (indicated by dotted red line). Optimal performance may be achieved by implementing a custom-generated cluster file that captures the experimental conditions of the processing laboratory.

Table 1: Summary of concordance metrics for the Infinium Global Diversity Array with Enhanced PGx-8 run on a fully manual workflow

Concordance metric	Manual vs semiautomated workflow concordance
Genotype	0.99962
PGx genotype	0.99994
Copy number call rate	0.96771
Copy number gain/loss	0.98468

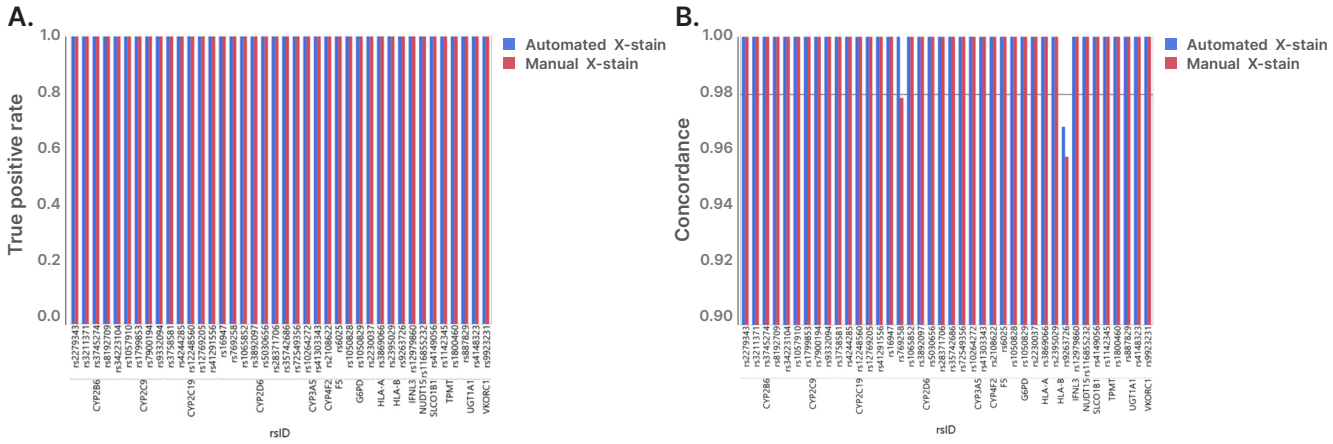

Figure 3: Targeted gene amplification (TGA) performance using the manual extension and staining (X-stain) protocol—(A) The true positive rate (TPR) and (B) concordance was compared between semiautomated and manual X-stain workflows. The overall PGx performance of the manual X-stain is comparable to the semiautomated workflow and accuracy is high for all probes tested.

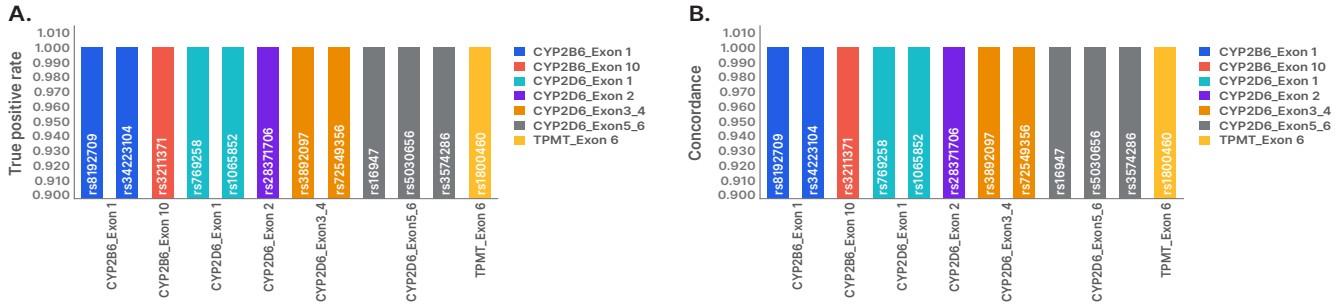

Figure 4: PGx marker performance using the manual extension and staining (X-stain) protocol—(A) The true positive rate (TPR) and (B) concordance after manual reclustering was compared between semiautomated and fully manual X-stain workflows for key PGx markers, including *CYP2B6*, *CYP2D6*, and *TPMT*. Overall PGx performance of the fully manual X-stain is comparable to the semiautomated workflow with high PGx marker accuracy for all probes tested.

Summary

The data presented in this technical note confirm that the Infinium Global Diversity Array with Enhanced PGx-8 processed using a fully manual workflow achieved the same high accuracy and overall PGx performance as the automated workflow. Compatibility with fully manual and semiautomated workflows makes this powerful tool for precision medicine research accessible to low-, mid-, and high-throughput laboratories.

Learn more

Infinium Global Diversity Array with Enhanced PGx-8, illumina.com/products/by-type/microarray-kits/infinium-global-diversity-pgx

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