



# Multiplex SNP Analysis of Human Reference DNA Samples Using the IntelliQube<sup>®</sup> and BHQplus<sup>®</sup> Assays

Alexander Kolb, Ph.D., Luke Linz, Ph.D., James Flynn, Ph.D.;  
LGC Douglas Scientific, 3600 Minnesota Street, Alexandria, MN 56308

## Abstract

The genetic information associated with nucleic acid samples can be used in research to understand how genetic variation plays a role in human health, which could ultimately lead to improvements in personalized medicine. For example, certain SNPs are associated with significant variations in drug metabolism between individuals or can serve as biomarkers for various diseases. In this study, we assess the accuracy and reproducibility of the IntelliQube PCR instrument\* for routine SNP analysis using purified gDNA samples from cell lines obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. Custom BHQplus genotyping assays were designed to target representative SNP markers from selected panels. The results demonstrate that the IntelliQube, when used in conjunction with BHQplus assays, provides an accurate and streamlined PCR-based method for genotype analysis of human DNA to maximize human research.

## Introduction

Single Nucleotide Polymorphisms (SNPs) have become powerful tools for genetic analysis of biological specimens and important to human health research by ultimately leading to improvements in personalized medicine. As genetic research continues to expand, there is a need for an accurate, automated, and low-cost method for performing genetic analysis such as SNP genotyping. The IntelliQube end-point and real-time qPCR instrument in conjunction with BHQplus probe-based SNP genotyping assays provides an effective solution to address this need. BHQplus probes incorporate duplex-stabilizers allowing enhanced binding stability, enabling compact probe sequences with excellent mismatch discrimination. Utilizing Array Tape technology, the IntelliQube integrates liquid handling and thermal cycling with qPCR analysis in miniaturized reaction volumes. In this study, custom BHQplus genotyping assays were designed to target representative SNP markers from selected panels including drug metabolism, pain management, oncology,

and neurological disorders (Table 1). Assay performance was assessed using purified gDNA samples from cell lines obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. The results demonstrate the accuracy, flexibility, and efficiency of the IntelliQube and associated BHQplus chemistry for SNP genotyping of human DNA samples.

## Methods

Purified gDNA samples from 56 cell lines was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. Custom BHQplus probe-based genotyping assays were designed and synthesized by LGC Biosearch Technologies™.

Commercially available Minor Groove Binder (MGB) probe-based assays were purchased for comparison. BHQplus SNP genotyping assays were tested in singleplex or duplex formats using a commercially available PCR master mix. Oligos were used at a final concentration of 200 nM probes and 900 nM primers in the final PCR reaction. The IntelliQube from LGC Douglas Scientific<sup>®</sup> was used for the automated assembly of 1.6 µL reactions in Array Tape consisting of 800 nL of gDNA (6.25 ng/µL) and 800 nL of master mix containing the PCR assay. Reactions were performed in duplicate. For real-time workflows, thermal cycling and fluorescence detection were performed on the IntelliQube. The thermal cycling conditions are described in Table 2. For water bath workflows, thermal cycling was performed on the IntelliCycler<sup>®</sup> according to the standard thermal cycling protocol and end-point fluorescence was captured on the IntelliQube. IntelliScore<sup>®</sup> Software was used for SNP data analysis.

Table 1: SNP Target Information

| SNP rs #   | Gene    | Significance of Minor Allele                               |
|------------|---------|--|
| rs1799853  | CYP2C9  | Linked to poor warfarin metabolism                         |
| rs12248560 | CYP2C19 | Ultra fast metabolizer phenotype, drug metabolism          |
| rs2108622  | CYP4F2  | Linked to poor warfarin metabolism                         |
| rs9923231  | VKORC1  | Linked to warfarin sensitivity                             |
| rs429358   | ApoE    | Influences the risk of Alzheimer's disease                 |
| rs7412     | ApoE    | Influences the risk of Alzheimer's disease                 |
| rs1801131  | MTHFR   | Linked to increased risk for several types of brain cancer |
| rs1801133  | MTHFR   | Linked to increased risk for several types of brain cancer |
| rs4633     | COMT    | Schizophrenia susceptibility, pain response/tolerance      |
| rs4680     | COMT    | Schizophrenia susceptibility, pain response/tolerance      |

Table 2: Thermal Cycling Protocols

| Thermal Cycling Protocol | Activation | Annealing/Extension |      |
|--------------------------|------------|---------------------|------|
|                          | 1 cycle    | 45 cycles           |      |
|                          | 95°C       | 95°C                | 60°C |
| Standard                 | 3 min      | 15 s                | 60 s |
| Fast                     | 3 min      | 1 s                 | 15 s |

## Results

Genomic DNA from human reference samples was successfully genotyped using ten BHQplus SNP genotyping assays in 1.6 µL reactions in Array Tape. Initially, performance of a few BHQplus assays was compared to commercially available MGB assays targeting the same SNPs (Figure 1). The results demonstrate equivalent performance of the two chemistries on the IntelliQube system. Subsequently, we demonstrated effective duplexing of genotyping assays in a single well utilizing the multiple fluorescence channels on the IntelliQube and the variety of available BHQplus probe fluorophores. Cluster plots from four of the duplexed assays are shown in Figure 2. Duplexing the SNP genotyping assays did not impact performance, retaining well separated, scorable clusters with sufficient signal-to-noise ratios. Each sample was tested in duplicate and identical allele calls among replicates were observed across all assays tested. The genotype results are summarized in Table 3. Concordance of genotype calls to published data was observed for CYP2C9 (rs1799853), CYP2C19 (rs12248560), and VKORC1 (rs9923231) based on available information from

## BHQplus vs MGB Probes

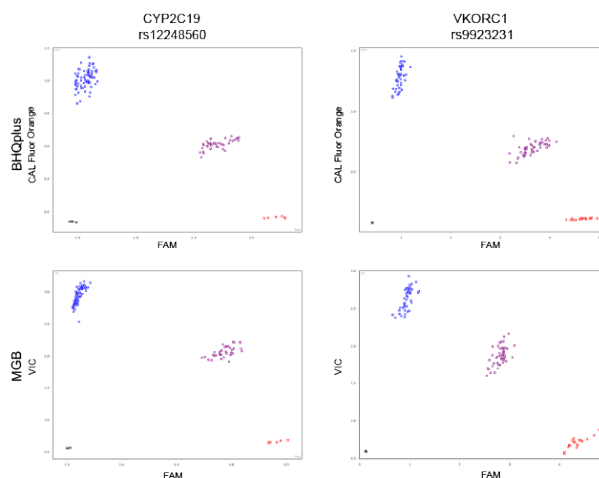


Figure 1. Comparison of BHQplus and MGB probes. BHQplus assays (top row) and commercially available MGB assays (bottom row) targeting the same SNPs were tested on the same array. CYP2C19 (rs12248560, left) and VKORC1 (rs9923231, right) were examined. For each cluster plot, FAM signal is plotted on the x-axis and CAL Fluor® Orange 560 (BHQplus) or VIC® (MGB) signal is plotted on the y-axis

Pratt et al., 2010. Prior genotype information was not available for all assays and samples.

## Workflow Comparisons

Testing using a duplexed SNP assay targeting two mutations in the MTHFR gene successfully demonstrated the flexibility of the IntelliQube to handle multiple workflows based on throughput needs. Results obtained using a fast thermal cycling protocol produced high-quality clusters comparable to data obtained using the standard thermal cycling protocol. A comparison of the thermal protocols can be found in Table 2. Data quality was also maintained using the IntelliCycler for thermal cycling. Figure 3 shows the comparison of workflows and achievable data points per day. A comparison of the cluster plots generated using all three methods is shown in Figure 4 and genotype results obtained from all three workflows were identical.

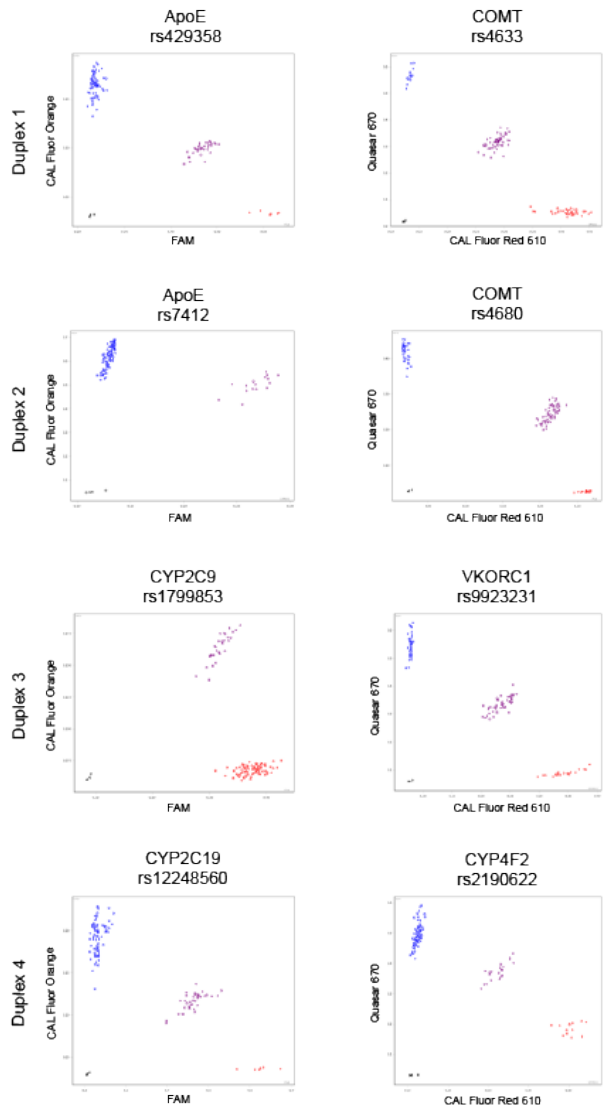


Figure 2. Duplex SNP Genotyping with BHQplus assays on the IntelliQube. Cluster plots are shown from each duplex. In each duplex, Assay 1 has FAM signal plotted on the x-axis and CAL Fluor Orange 560 plotted on the y-axis. Assay 2 has CAL Fluor Red 610 signal plotted on the x-axis and Quasar® 670 signal plotted on the y-axis.

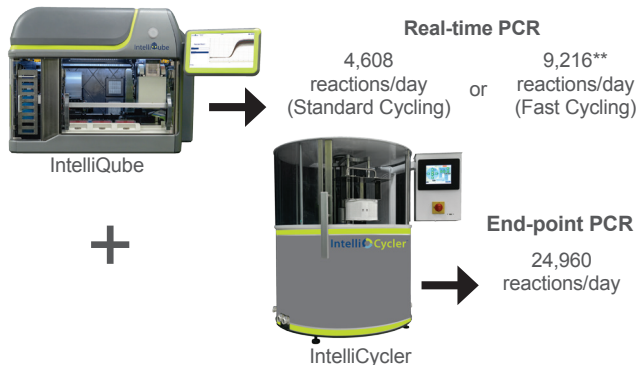


Figure 3. The number of achievable reactions that can be performed in a standard 8 hour work day is indicated for each workflow. The real-time workflows assume six 768-well arrays using standard cycling or 12 768-well arrays using fast cycling conditions. The end-point workflow assumes 65 384-well arrays. \*\*This throughput is based on the 45-cycle protocol in Table 2. Actual throughput may vary based on the thermal cycling protocol selected.

Table 3: SNP Genotyping Results

| Sample 1 SNP | CYP2C9 rs1728853 | CYP2C19 rs1248560 | CYP4F2 rs2190622 | VKORC1 rs9923231 | ApoE rs429358 | ApoE rs7412 | MTHFR rs1801131 | MTHFR rs181133 | COMT rs4633 | COMT rs4680 |
|--------------|------------------|-------------------|------------------|------------------|---------------|-------------|-----------------|----------------|-------------|-------------|
| NA01251      | WT               | WT                | WT               | WT               | WT            | WT          | HET             | HET            | HET         | HET         |
| NA02016      | WT               | WT                | WT               | HET              | WT            | WT          | HET             | WT             | HET         | HET         |
| NA07439      | WT               | WT                | WT               | WT               | WT            | WT          | HET             | WT             | MUT         | MUT         |
| NA08873      | WT               | WT                | WT               | HET              | WT            | HET         | HET             | HET            | HET         | HET         |
| NA09912      | WT               | WT                | WT               | MUT              | WT            | HET         | MUT             | WT             | HET         | HET         |
| NA10005      | WT               | HET               | HET              | WT               | HET           | WT          | HET             | HET            | WT          | WT          |
| NA12244      | HET              | WT                | WT               | WT               | WT            | WT          | HET             | WT             | HET         | HET         |
| NA12276      | HET              | WT                | HET              | WT               | WT            | WT          | HET             | HET            | HET         | HET         |
| NA12877      | WT               | WT                | WT               | HET              | WT            | WT          | WT              | MUT            | HET         | HET         |
| NA12878      | HET              | WT                | WT               | HET              | WT            | WT          | HET             | HET            | HET         | HET         |
| NA12891      | WT               | WT                | WT               | WT               | WT            | WT          | HET             | WT             | HET         | HET         |
| NA12892      | HET              | WT                | WT               | HET              | WT            | WT          | MUT             | WT             | HET         | HET         |
| NA17039      | WT               | WT                | WT               | WT               | WT            | WT          | WT              | WT             | HET         | HET         |
| NA17052      | WT               | WT                | WT               | MUT              | WT            | WT          | WT              | MUT            | HET         | HET         |
| NA17057      | WT               | WT                | MUT              | HET              | WT            | WT          | HET             | HET            | HET         | MUT         |
| NA17058      | WT               | WT                | WT               | MUT              | WT            | WT          | HET             | HET            | WT          | WT          |
| NA17084      | HET              | WT                | MUT              | HET              | WT            | WT          | MUT             | WT             | HET         | HET         |
| NA17104      | WT               | HET               | WT               | WT               | HET           | HET         | WT              | WT             | MUT         | MUT         |
| NA17105      | WT               | WT                | WT               | WT               | WT            | WT          | WT              | WT             | HET         | WT          |
| NA17107      | WT               | HET               | WT               | HET              | HET           | WT          | WT              | HET            | WT          | WT          |
| NA17109      | WT               | HET               | WT               | HET              | HET           | WT          | WT              | HET            | WT          | WT          |
| NA17113      | WT               | HET               | WT               | HET              | HET           | WT          | HET             | WT             | HET         | HET         |
| NA17114      | WT               | WT                | WT               | WT               | WT            | WT          | HET             | WT             | HET         | HET         |
| NA17115      | WT               | WT                | WT               | WT               | WT            | WT          | WT              | WT             | HET         | HET         |
| NA17117      | WT               | WT                | WT               | WT               | WT            | WT          | WT              | WT             | WT          | WT          |
| NA17119      | WT               | HET               | WT               | WT               | HET           | HET         | WT              | WT             | WT          | WT          |
| NA17123      | WT               | WT                | WT               | WT               | WT            | WT          | WT              | WT             | WT          | WT          |
| NA17129      | HET              | WT                | WT               | WT               | WT            | WT          | HET             | WT             | HET         | HET         |
| NA17130      | WT               | HET               | WT               | WT               | HET           | HET         | WT              | WT             | WT          | WT          |
| NA17131      | WT               | WT                | WT               | HET              | WT            | WT          | WT              | WT             | HET         | HET         |
| NA17155      | WT               | HET               | WT               | HET              | HET           | WT          | WT              | WT             | WT          | WT          |
| NA17184      | WT               | HET               | WT               | WT               | HET           | WT          | WT              | WT             | MUT         | MUT         |
| NA17203      | WT               | HET               | WT               | HET              | HET           | WT          | MUT             | WT             | WT          | WT          |
| NA17204      | WT               | WT                | WT               | MUT              | WT            | WT          | HET             | HET            | WT          | WT          |
| NA17209      | HET              | WT                | HET              | WT               | WT            | WT          | HET             | HET            | WT          | WT          |
| NA17210      | HET              | WT                | MUT              | MUT              | WT            | WT          | MUT             | WT             | HET         | WT          |
| NA17221      | HET              | WT                | HET              | HET              | WT            | WT          | WT              | HET            | HET         | HET         |
| NA17226      | HET              | WT                | HET              | HET              | WT            | HET         | WT              | WT             | HET         | HET         |
| NA17227      | HET              | WT                | HET              | HET              | WT            | HET         | WT              | HET            | MUT         | MUT         |
| NA17232      | WT               | HET               | HET              | HET              | HET           | WT          | HET             | HET            | HET         | HET         |
| NA17235      | WT               | WT                | WT               | WT               | WT            | WT          | HET             | WT             | WT          | WT          |
| NA17240      | WT               | HET               | WT               | WT               | HET           | WT          | WT              | MUT            | HET         | HET         |
| NA17246      | HET              | HET               | WT               | HET              | HET           | WT          | WT              | WT             | MUT         | MUT         |
| NA17247      | WT               | WT                | MUT              | HET              | HET           | WT          | WT              | MUT            | WT          | WT          |
| NA17248      | WT               | MUT               | HET              | MUT              | MUT           | WT          | WT              | HET            | HET         | HET         |
| NA17252      | HET              | WT                | WT               | HET              | WT            | WT          | WT              | MUT            | HET         | HET         |
| NA17272      | WT               | MUT               | MUT              | MUT              | HET           | HET         | WT              | WT             | WT          | WT          |
| NA17276      | WT               | HET               | WT               | WT               | HET           | WT          | WT              | WT             | WT          | WT          |
| NA17280      | HET              | WT                | WT               | WT               | HET           | WT          | MUT             | WT             | HET         | HET         |
| NA17281      | WT               | HET               | WT               | HET              | HET           | WT          | HET             | HET            | HET         | HET         |
| NA17289      | WT               | WT                | HET              | MUT              | WT            | WT          | HET             | WT             | MUT         | MUT         |
| NA17293      | HET              | HET               | WT               | HET              | HET           | WT          | WT              | MUT            | HET         | HET         |
| NA17296      | WT               | MUT               | MUT              | MUT              | MUT           | WT          | HET             | HET            | HET         | HET         |
| NA17298      | WT               | WT                | HET              | HET              | WT            | WT          | WT              | HET            | HET         | HET         |
| NA17300      | WT               | HET               | HET              | HET              | HET           | WT          | HET             | HET            | MUT         | MUT         |
| NA19240      | WT               | HET               | WT               | WT               | HET           | WT          | WT              | WT             | WT          | HET         |

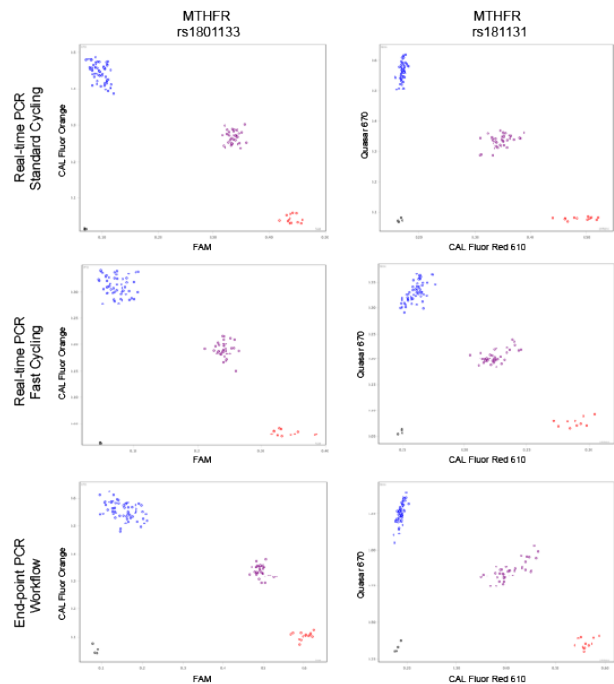


Figure 4. Comparison of cluster plot data from real-time and end-point PCR workflows on the IntelliQube. Two SNP assays were duplexed and the results were analyzed following standard (top) or fast (middle) thermal cycling conditions using real-time fluorescence detection. End-point PCR results (bottom) using the IntelliCycler for thermal cycling are also shown. The cluster plots for rs1801133 have FAM signal plotted on the x-axis and CAL Fluor Orange 560 plotted on the y-axis. The cluster plots for rs1801131 have CAL Fluor Red 610 signal plotted on the x-axis and Quasar 670 signal plotted on the y-axis.

## Conclusions

The IntelliQube, when used in conjunction with BHQplus SNP genotyping assays, provides an accurate and economical solution for genotyping human DNA samples. The genomic DNA samples used in this study were successfully genotyped using 1.6 µL reactions in Array Tape, and the results demonstrated the reproducibility of the system and concordance of the data with the published genotypes. By seamlessly integrating liquid handling, thermal cycling, and detection systems, the IntelliQube enables users to benefit from more efficient and economical end-point PCR and qPCR workflows. The IntelliQube systems offers the flexibility to meet the needs of researchers with varying throughput requirements. Using real-time workflows, fast PCR capabilities can increase the number of achievable data points per day to 9,216 based on the 45 cycle protocol used in this study. By reducing activation time and the number of total PCR cycles, throughput can be further increased, assuming the master mix and assays are compatible with the thermal protocol. When real-time data is not required, adding an IntelliCycler to the workflow can dramatically increase throughput up to 24,960 data points per day. Duplexing capabilities can increase efficiency by reducing the number of runs required to obtain the same number of data points. BHQplus probes, Array Tape, and associated automation enable a streamlined workflow from start to finish, while maintaining accurate, reliable, and economical SNP genotyping results. With the demonstrated ability to generate accurate and reproducible SNP genotyping results, the IntelliQube provides research laboratories a compelling new alternative to traditional PCR-based techniques.

## References

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