Application note
AN-161102.01

Multiplex SNP Analysis of Human Reference DNA Samples Using the IntelliQube® and BHQplus® Assays

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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® 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® according to the standard thermal cycling protocol and end-point fluorescence was captured on the IntelliQube. IntelliScore® 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

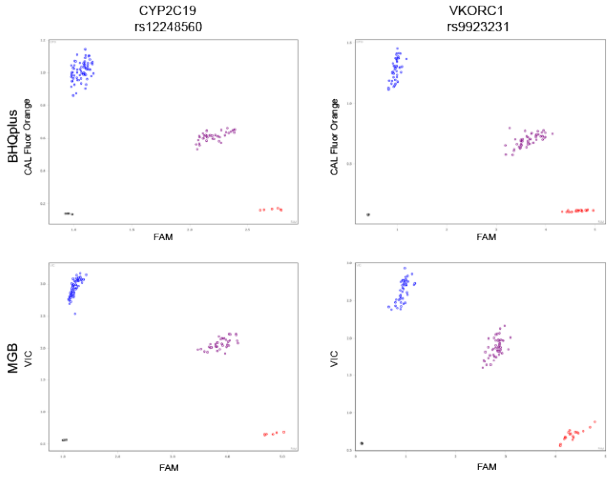

Figure 1. Comparison of BHQplus and MGB probes. BHQplus assays (top row) and commercially available MGB assays (bottom row) targeting the same SNPs were tested on the same array. CYP2C19 (rs12248560, left) and VKORC1 (rs9923231, right) were examined. For each cluster plot, FAM signal is plotted on the 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.

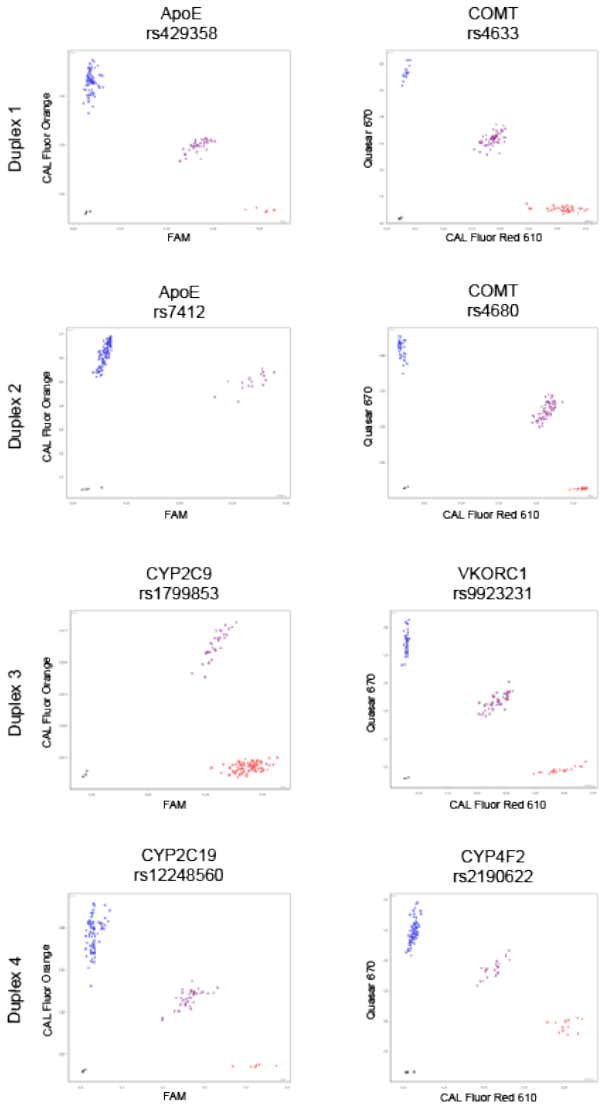

Figure 2. Duplex SNP Genotyping with BHQplus assays on the IntelliQube. Cluster plots are shown from each duplex. In each duplex, Assay 1 has FAM signal plotted on the x-axis and CAL Fluor Orange 560 plotted on the y-axis. Assay 2 has CAL Fluor Red 610 signal plotted on the x-axis and Quasar® 670 signal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

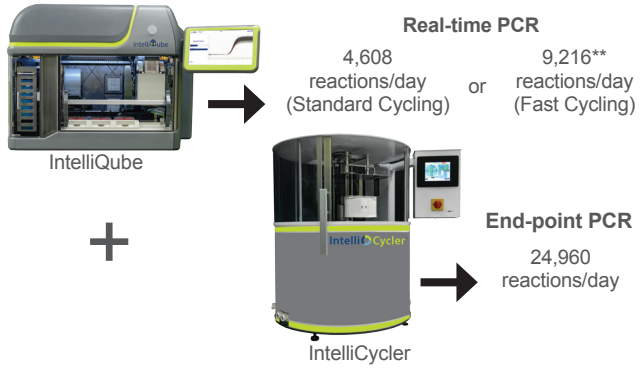

Figure 3. The number of achievable reactions that can be performed in a standard 8 hour work day is indicated for each workflow. The real-time workflows assume six 768-well arrays using standard cycling or 12 768-well arrays using fast cycling conditions. The end-point workflow assumes 65 384-well arrays.
**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 \ SNP	CYP2C9 rs1799853	CYP2C19 rs12248560	CYP4F2 rs2180622	VKORC1 rs9923231	ApoE rs429358	ApoE rs7412	MTHFR rs1801131	MTHFR rs1801133	COMT rs4633	COMT rs4680
NA01251	WT	WT	WT	WT	WT	WT	HET	WT	HET	HET
NA02016	WT	WT	WT	HET	WT	WT	HET	WT	HET	HET
NA07439	WT	WT	WT	WT	WT	WT	WT	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	WT	HET	WT	WT
NA12244	HET	WT	WT	WT	WT	WT	HET	WT	HET	HET
NA12273	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	HET	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	WT	MUT	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	HET	WT	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
NA17194	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	MUT	HET	WT	WT	MUT	HET	HET
NA17246	HET	HET	WT	HET	HET	WT	WT	WT	MUT	MUT
NA17247	WT	WT	MUT	HET	WT	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	MUT	HET	HET	WT	WT	WT
NA17276	WT	HET	WT	WT	HET	WT	WT	WT	WT	WT
NA17280	HET	WT	WT	WT	WT	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	HET	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

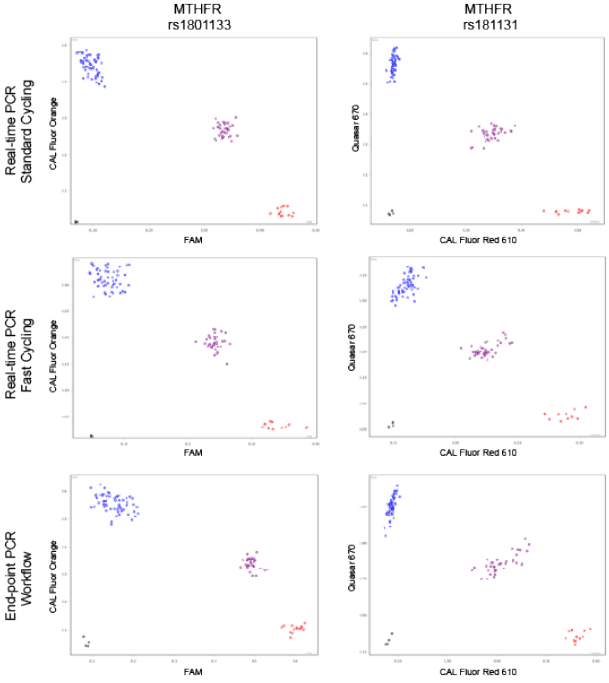

Figure 4. Comparison of cluster plot data from real-time and end-point PCR workflows on the IntelliQube. Two SNP assays were duplexed and the results were analyzed following standard (top) or fast (middle) thermal cycling conditions using real-time fluorescence detection. End-point PCR results (bottom) 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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