Title: Accurate SNP analysis using the IntelliQube® and duplex BHQplus® genotyping assays with a fast PCR protocol

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Improvements to next-generation sequencing technologies have enabled extensive discovery of single nucleotide polymorphisms (SNPs) in numerous organisms. Due to their large numbers and genome-wide distribution, SNPs are the molecular marker of choice in plant, animal, and human genetic research. As the discovery of SNP markers continues to expand, there is a need for a more efficient method for routine genetic analysis. The IntelliQube® 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 with qPCR analysis in miniaturized reaction volumes. In this study, we assess the performance of custom BHQplus genotyping assays run in a duplex fashion using fast thermal cycling protocols. Accuracy and reproducibility of this platform was assessed using purified gDNA samples from cell lines obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. Genotype results were compared to previously published literature. The results demonstrate that the IntelliQube, when used in conjunction with BHQplus assays, provides an accurate and streamlined real-time PCR-based method for genetic analysis.