**INTRODUCTION**

High resolution melting applications include gene scanning, unlabeled probe (LunaProbes™) and Small Amplicon genotyping. Several instruments currently being marketed as capable of high resolution melting (HRM). These instruments generate sufficient data density to detect subtle temperature and fluorescence differences caused by a range of sequence variants. The LightScanner® (Idaho Technology, Inc.) and the LightCycler®480 (Roche) are two such plate-based instruments. There are several commercial HRM mastermix products that use different dyes, such as LightScanner Mastermix with LCGreen® PLUS dye (Idaho Technology, Inc.), and LightCycler®480 High Resolution Melting Master with Resolight dye (Roche). The purpose of this study was to compare overall system performance (instrument, analysis software, and HRM mastermix) for scanning, LunaProbes genotyping, and Small Amplicon genotyping HRM applications.

**METHODS**

To evaluate total system performance, genomic targets with known SNPs in the LIPC (scanning), CPS1 (small amplicon genotyping), OTC (LunaProbes), ADH4 (LunaProbes), human tyrosine hydroxylase (scanning), and HFE (multiplexed LunaProbes) genes were used. The CPS1 and OTC SNPs were base-neutral A:T changes with nearest neighbor base symmetry. The homozygous forms of these SNPs represent the greatest genotyping challenge due to extremely small delta Tm’s between homozygous genotypes. Assays were independently optimized for each instrument using both HRM mastermix products. Samples were loaded on both instruments and results analyzed using both software packages.

**RESULTS**

Both mastermix products generated robust and specific PCR product appropriate for HRM analysis. Data collected on the LightScanner using both mastermix products produced greater sensitivity and specificity, particularly for the LunaProbes and Small Amplicon genotyping applications. LunaProbes and Small Amplicon genotyping on the LC480 resulted in decreased sensitivity and specificity when analyzed with the LC480 software presumably due to the lack of application specific (i.e. LunaProbes or Small Amplicon genotyping) analysis modules. Analysis of the same data imported into the LightScanner software improved sensitivity and specificity for both LunaProbes and Small Amplicon genotyping in all assays tested. This was particularly evident for the HFE multiplexed LunaProbes assay, where results were unable to be analyzed with the Resolight software. Post analysis of the same data using the LightScanner software yielded 100% correct genotypes when LCGreen mastermix was used on the LC480 instrument (Figure 6, panels D and E).

**CONCLUSION**

Both the LightScanner and LC480 are capable high resolution melting instruments. The data produced by the LightScanner is higher density, allowing for increased resolution and greater sensitivity when confronted by base-neutral SNPs. However, data from the LC480 was analyzed with LightScanner software with improvements in sensitivity. Small Amplicon genotyping with internal temperature calibration data was generated using the High Sensitivity Master Mix (containing temperature calibration probes). This data was able to be analyzed in the LightScanner software with the Amplicon Genotyping module using the calibration feature (Figure 2). LunaProbes genotyping data was consistently more sensitive and specific when the LightScanner mastermix with LCGreen Plus dye was used across both instruments (Figures 3 and 6). Accurate LunaProbes genotyping can be obtained using the LC480 instrument, but primer asymmetry had to be increased and LCGreen dye was needed in order to generate a sufficient probe target signal ratio. Overall, large PCR product scanning applications are similar across both instruments and mastermix products. Smaller PCR product applications (i.e. LunaProbes and Small Amplicon genotyping) were more accurately performed using the LightScanner or the LCGreen PLUS mastermix product on the LC480 instrument.