392 Hi-Res Melting® on the LightScanner®-96 and LightCycler® 480: A Complete System Comparison

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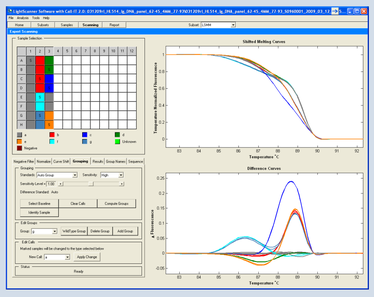
INTRODUCTION

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.

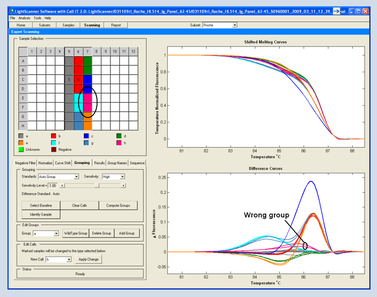
METHODS

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 melted on both instruments and results analyzed using both software packages.

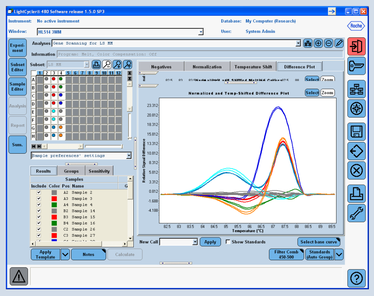
Figure 1: LIPC gene scanning - 301 bp fragment, 4 known SNPs. The use of the LCGreen mastermix allows 100% sensitivity across both platforms (panels A, C, and E). Sensitivity is improved when data from the LC480 is analyzed using LightScanner software (panels D vs. F).



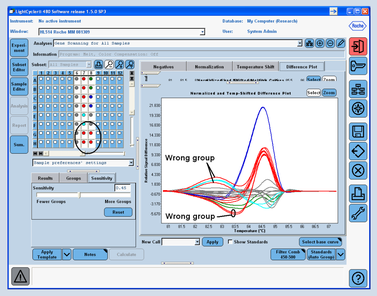
A. LightScanner – LCGreen – LS software



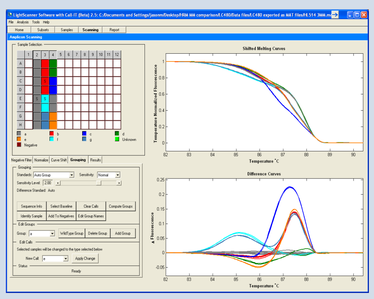
B. LightScanner – Resolight – LS software



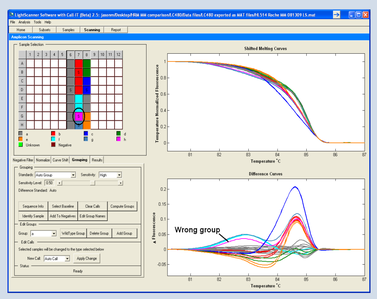
C. LC480 – LCGreen – Roche software



D. LC480 – Resolight – Roche software

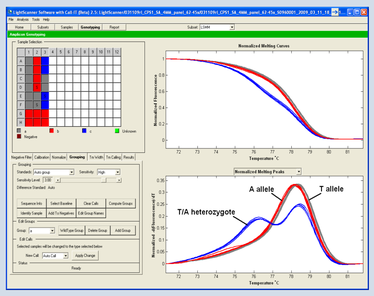


E. LC480 – LCGreen – LS software

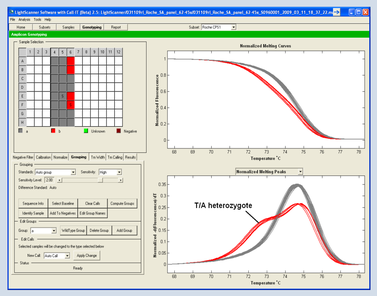


F. LC480 – Resolight – LS software

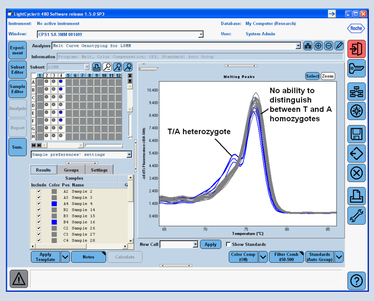
Figure 2: CPS1 gene, Small Amplicon Genotyping assay, A>T SNP with nearest neighbor base symmetry – LCGreen mastermix using internal temperature calibration and the LightScanner software Amplicon Genotyping module correctly genotyped this difficult SNP (panels A and E), regardless of which instrument was used to generate the data. There was a trend for decreased heteroduplex percentage detection with the Resolight dye relative to the LCGreen dye across all comparisons (Note the diminished "het" peak distinction in heterozygous T/A samples in panels A vs. B).



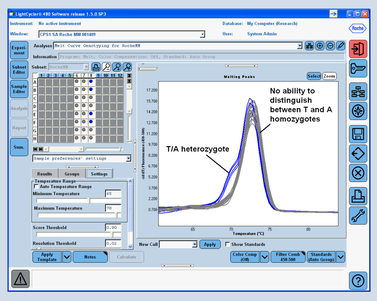
A. LightScanner – LCGreen – LS software



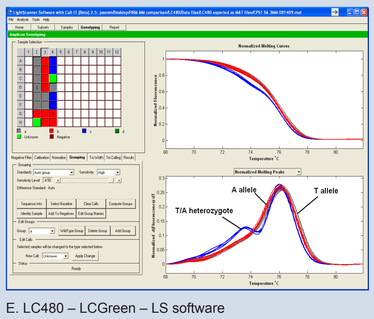
B. LightScanner – Resolight – LS software



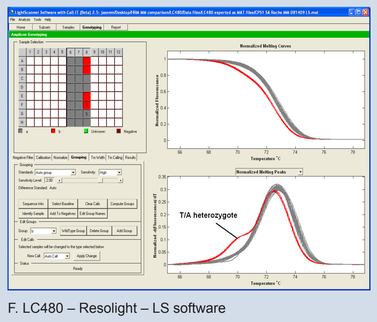
C. LC480 – LCGreen – Roche software



D. LC480 – Resolight – Roche software

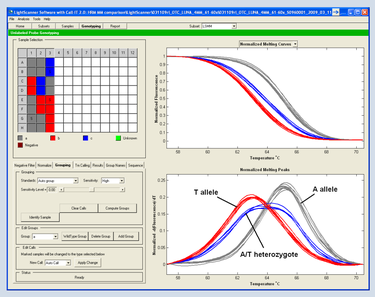


E. LC480 – LCGreen – LS software

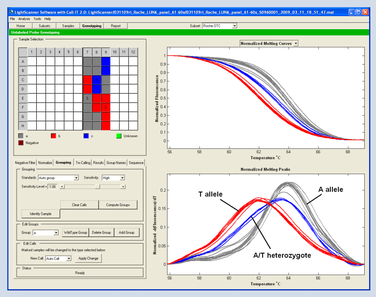


F. LC480 – Resolight – LS software

Figure 3: OTC gene – LunaProbes assay A>T SNP with nearest neighbor base symmetry. Both LCGreen and Resolight mastermix products gave accurate genotypes on the LightScanner instrument (panels A and B). Analysis of LCGreen mastermix data from the LC480 instrument (panel C) using the LightScanner software also provided correct genotype results (panel E).



A. LightScanner – LCGreen – LS software



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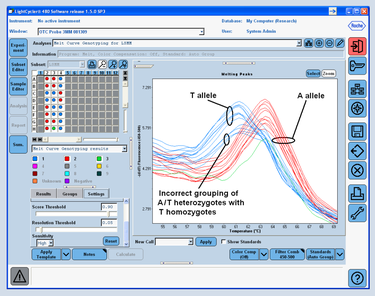
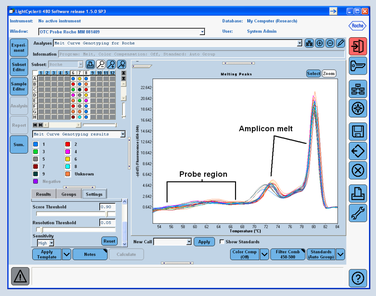
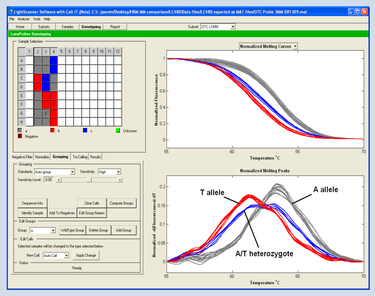


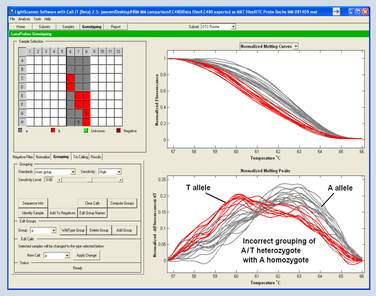
C. LC480 – LCGreen – Roche software



D. LC480 – Resolight – Roche software

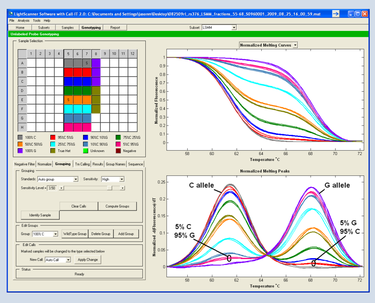


E. LC480 – LCGreen – LS software

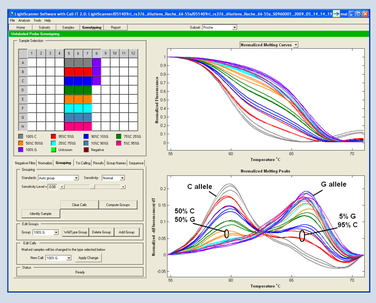


F. LC480 – Resolight – LS software

Figure 4: ADH4 gene, rs3762894 G>C SNP – LunaProbes assay for low allele fraction sensitivity – LCGreen mastermix on the LightScanner instrument provided allele sensitivity down to 5% for both alleles (panel A). Analysis of LCGreen mastermix data from the LC480 instrument (panel C) using the LightScanner analysis software improved detection sensitivity to a similar level as achieved on the LightScanner (panel E).



A. LightScanner – LCGreen – LS software



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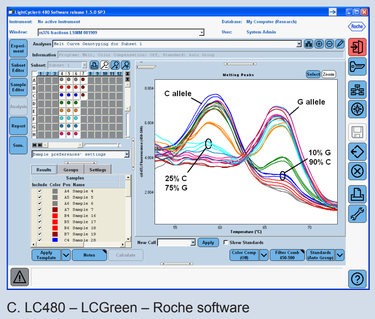

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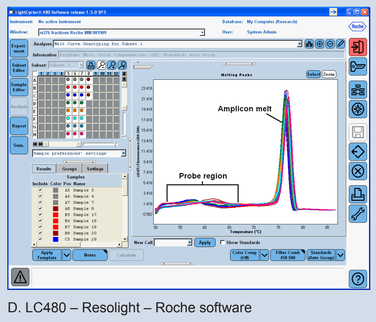

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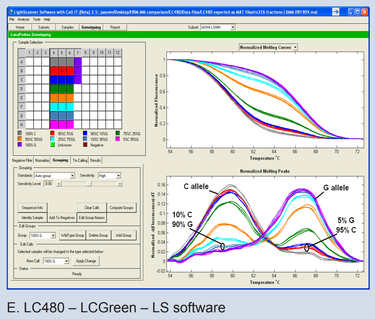
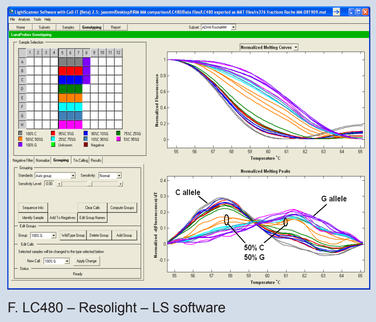
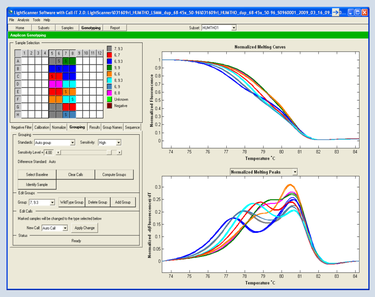


E. LC480 – LCGreen – LS software

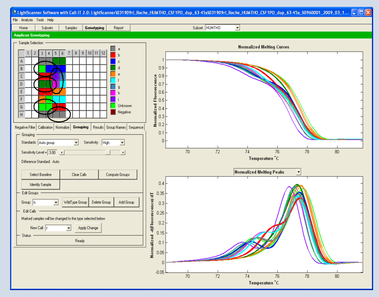


F. LC480 – Resolight – LS software

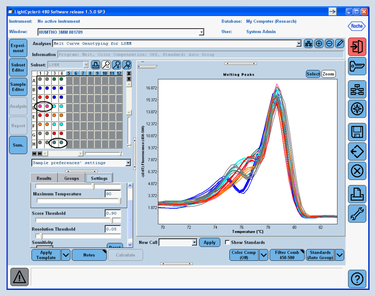
Figure 5: Human THO1 gene, common STR, tetra-nucleotide repeat – Small Amplicon Genotyping assay – LCGreen mastermix on the LightScanner produced correct genotypes and high confidence grouping for all samples (panel A). Sensitivity was significantly reduced using Resolight mastermix on both instruments (panels B and D). Detection sensitivity was improved for both LCGreen and Resolight mastermix when LC480 data was analyzed with LightScanner software (panels C vs. E, and D vs. F). Heteroduplex detection sensitivity was decreased for Resolight mastermix across both instruments (panels A vs. B, C vs. D, and E vs. F). Note the decrease in distinction of the low Tm heteroduplex peak in the BLUE samples.



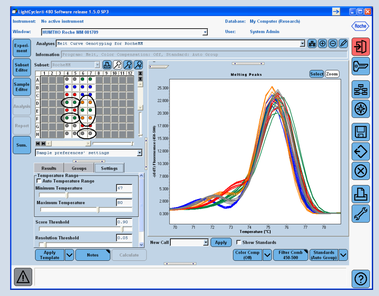
A. LightScanner – LCGreen – LS software



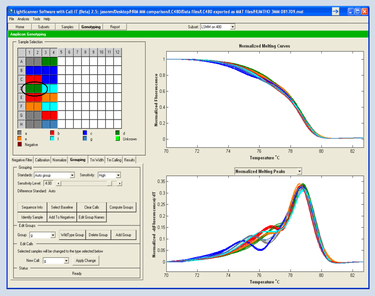
B. LightScanner – Resolight – LS software



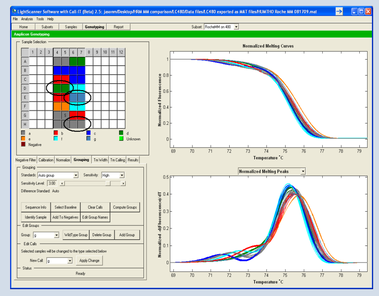
C. LC480 – LCGreen – Roche software



D. LC480 – Resolight – Roche software

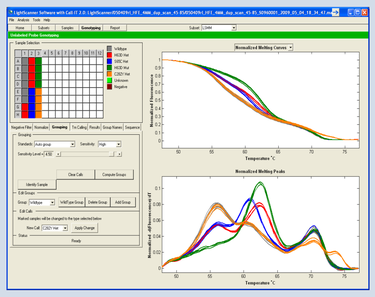


E. LC480 – LCGreen – LS software

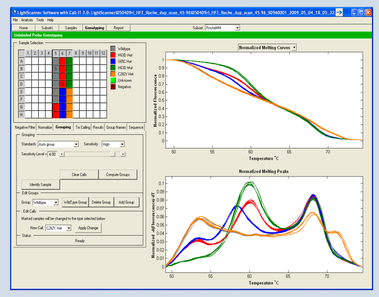


F. LC480 – Resolight – LS software

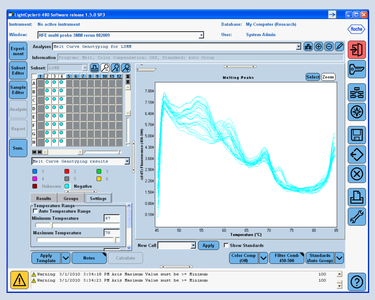
Figure 6: HFE gene, multiplexed LunaProbes assay – Correct genotypes were called using the LightScanner instrument and both LCGreen and Resolight mastermix products (panels A and B). LCGreen mastermix run on the LC480 (panel C) clearly shows that probe peaks are present, but the signal:noise ratio was too low for the samples to be analyzed. A similar issue was observed for the Resolight mastermix on the LC480 (panel D). Analysis of LC480 data using the LightScanner software resulted in correct genotype calls for LCGreen mastermix, and 3/5 genotypes correctly called with the Resolight mastermix (panels E and F).



A. LightScanner – LCGreen – LS software

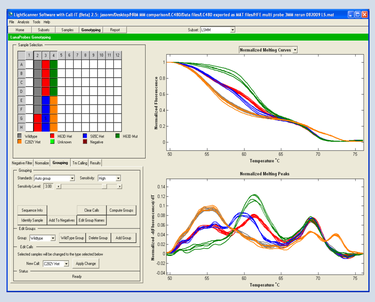


B. LightScanner – Resolight – LS software

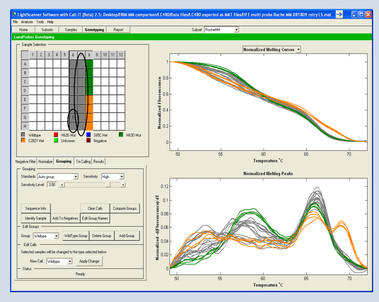


C. LC480 – LCGreen – Roche software

D. LC480 – Resolight – Roche software



E. LC480 – LCGreen – LS software



F. LC480 – Resolight – LS software

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.

Authors would like to acknowledge Cameron Gundry for development of the OTC and CPS1 assays used in Figures 2 and 3

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 Roche software yet 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 C and E).