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System Validation of a New Real Time PCR and Hi-Res Melting® instrument: the LightScanner® 32 (LS32)

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ABSTRACT

The LightScanner32 (LS32), capable of both rapid real-time PCR and high-resolution melting, has been developed by Idaho Technology and launched in March, 2009. This instrument is compatible with all major chemistry types such as SYBR® Green, Taqman® or hydrolysis probes, HybProbe® or FRET hybridization probes, and the benchmark high resolution melting dye, LCGreen® Plus.

The LS32 combines features of the LightCycler® and HR-1™ instruments (see Table 1). During the GMP-compliant system validation we tested instrument and software functionality independently and as integrated components. Electronic circuit design, hardware and optical validation tests were performed using standard engineering methods and measurement tools. CE mark testing for electrical safety was also performed. Instrument control and analysis modules were tested with independent 16-rotor experiments as part of the formal system validation plan. Integrated system testing included: multiplex qPCR with color-compensation, qPCR dynamic range, qPCR precision and accuracy, and multiple applications related to high-resolution melting. These tests were performed with multiple users and different database access levels, which is important in regulated environments. Over 100 wet qPCR and high resolution melting experiments were performed and specifically tested the design inputs and product specifications. All design inputs and product specifications related to system performance and experimental applications were successfully verified with the planned test cases. System validation resulted in final modifications to software, firmware, and the operator manual. Software and firmware modifications were validated by re-running the appropriate test cases. LS32 system performance was documented via Idaho Technology's quality system and approved for launch in March, 2009.

Rapid air-thermocycling was introduced by Idaho Technology in 1991, and led to the development of the LightCycler in 1996. Licensed to Roche (1998), the capillary-based LightCycler instruments are now commonplace throughout the world with an install base >7000. High resolution melting was introduced by Idaho Technology in 2003 with the HR-1 instrument and is now a validated technology for mutation scanning and genotyping.

Table 1: qPCR and Hi-Res Melting Instrument Comparisons

This figure shows the assay and matrix combinations that have been validated.

	LightCycler (Roche)	HR-1 (Idaho Technology)	LS32 (Idaho Technology)
Amplification	Yes	No	Yes
Analysis Mode	qPCR, Melting Curves	Hi-Res Melting	qPCR, Melting Curves, Hi-Res Melting
Acquisition Mode	Single/Step/Continuous	Continuous	Single/Step/Continuous
Data Resolution	4.1 pts/C°	400 pts/C°	400 pts/C°
Sample Capacity	32	1	32

Validation Study

The purpose of this system validation was to evaluate the performance of the LightScanner 32 system using a range of assays and applications that have been determined to represent the applications for which the customers will use the system. This validation included testing by engineering, production, and R&D biochemistry department personnel. Idaho Technology is a GMP-compliant company and as such requires all the appropriate documentation such as design inputs, design history reviews, complete device history records, and documentation of any process deviations with appropriate review and approval.

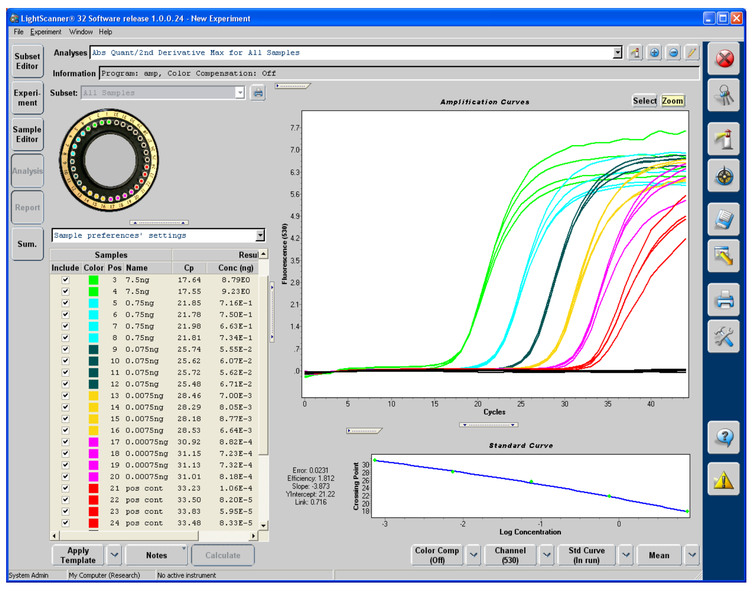
PHASE 1

The engineering and production departments performed system validation and verification tests to confirm that the pre-production instruments were calibrated, subjected to quality control specifications, and released according to standard practices. Each mechanical and electronic system that controlled various functions of the instrument such as cycling parameters, temperature control, fluorescence acquisition, and carousel movement was validated to perform within the defined specifications. Instruments were continuously cycled through intense heating and cooling for long periods of time to ensure that components would not fail in extreme conditions.

PHASE 2

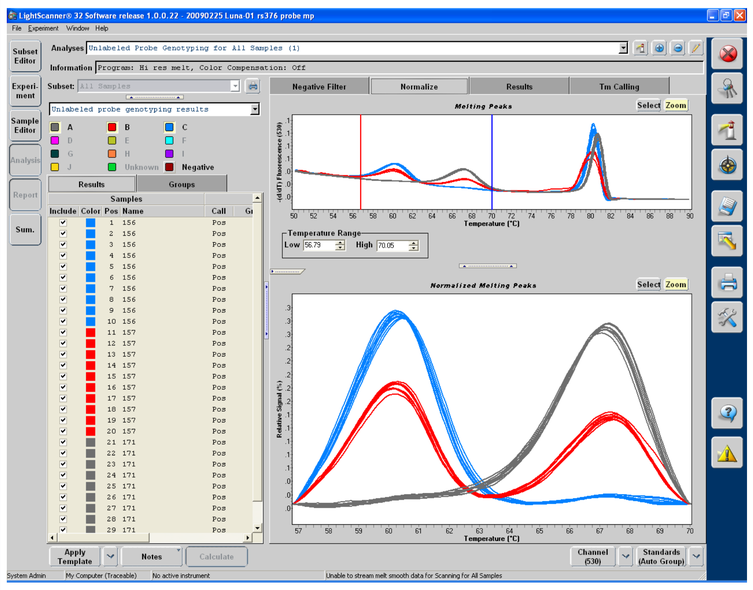
The R&D biochemistry department ran a series of 16-rotor test plans using a panel of assays representing each of the 5 analysis modules supported on the LS32 instrument: real-time qPCR quantification, scanning using Hi-Res Melting, LunaProbes genotyping, Small Amplicon genotyping, and Labeled probe genotyping using SimpleProbes® as well as HybProbe probe chemistry. These 16-rotor test plans were designed to verify that all possible workflows supported by the software (importing/exporting templates, macros, sample editor templates, internal/external standards, etc.) were functional. Five of these 16-rotor test plans were executed with each type of data analysis module represented. Multiple assays were used within each 16-rotor test plan to provide a reasonable variety of independent assay targets to adequately test the software analysis algorithms. In addition to this 16-rotor test plan over 100 additional rotors of various types were run using all of the common chemistry types as mentioned in the abstract.

Figure 1.



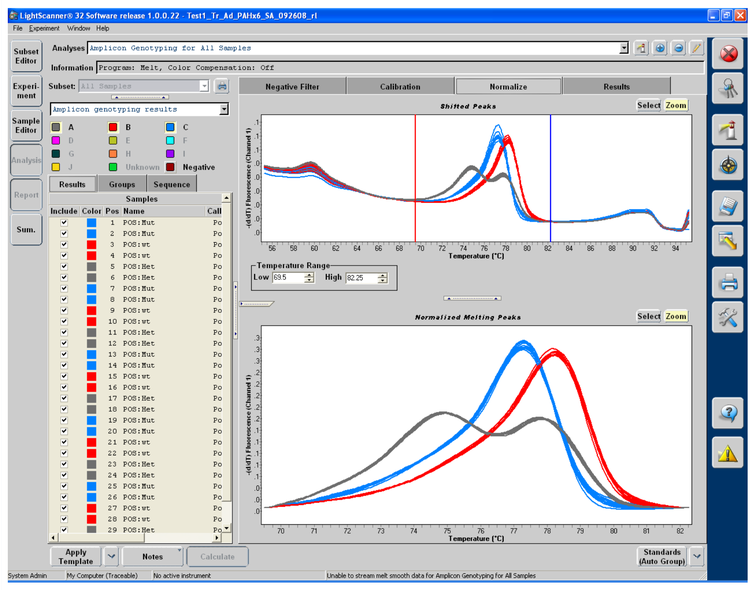
Hydrolysis probe experiment showing five 10-fold dilutions of Plasmodium falciparum genomic DNA beginning at 7.5 ng (330,000 genomic equivalents, light green) down to 750fg (33 genomic equivalents, pink). Also, freeze-dried positive controls are seen (red) as well as no template controls (black flat lines). One sample from each dilution is selected as standard and software calculates the PCR efficiency as well as Cp statistics within each dilution and concentration estimates based on entered in values.

Figure 2.



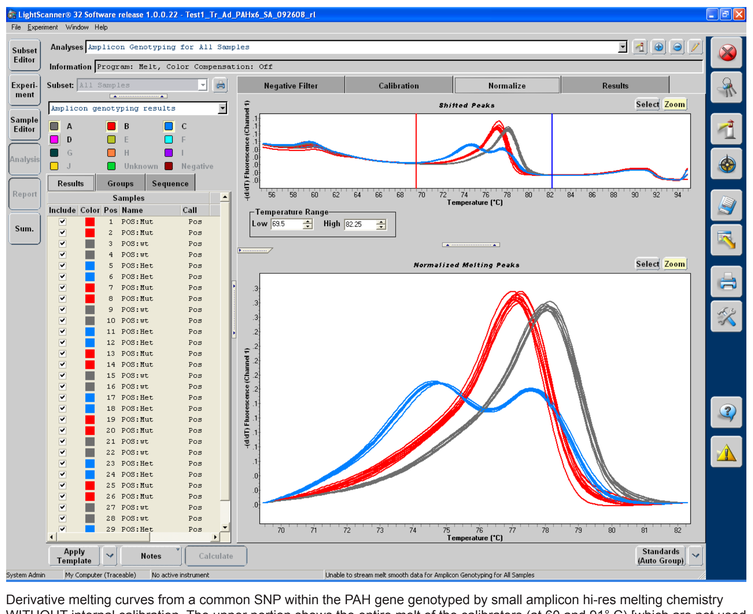
Derivative melting curves from a common SNP genotyped by LunaProbes chemistry. The upper portion shows probe peaks at approximately 60 to 68° C bounded by a red and a blue cursor as well as the amplicon melts at approximately 80° C. The lower portion shows the normalized peak curves representing the three genotypes. Software automatically assigns groups based on curve shape and correct genotyping calls are easily and rapidly made.

Figure 3.



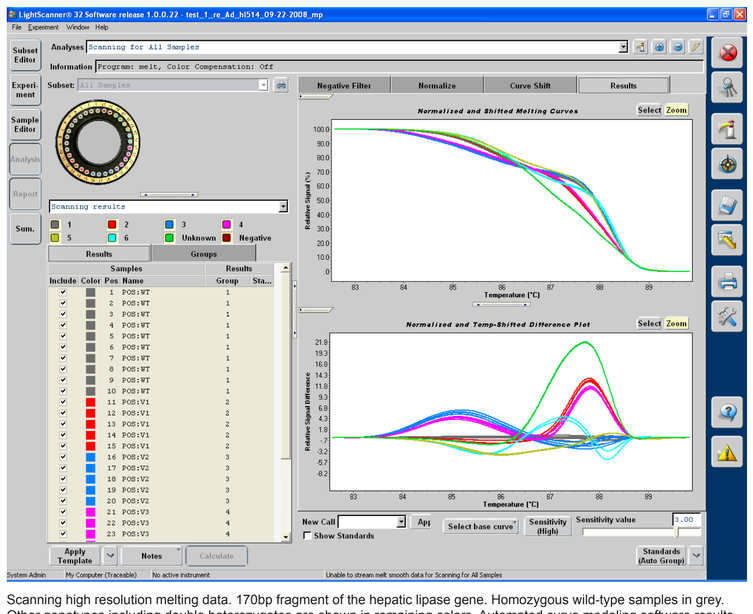
Derivative melting curves from a common SNP within the PAH gene genotyped by small amplicon hi-res melting chemistry WITH internal calibration. The upper portion shows the entire melt of the calibrators (at 60 and 91° C) as well as the amplicon melts bounded by a red and a blue cursors at approximately 70 and 82° C. The lower portion shows the normalized and temperature corrected small amplicon derivative melting peaks representing the three genotypes in grey (G homozygote), blue (A homozygote), and red (G/A heterozygote). Software automatically assigns groups based on curve shape and correct genotyping calls are easily and rapidly made.

Figure 4.



Derivative melting curves from a common SNP within the PAH gene genotyped by small amplicon hi-res melting chemistry WITHOUT internal calibration. The upper portion shows the entire melt of the calibrators (at 60 and 91° C) [which are not used to adjust each sample's temperature] as well as the amplicon melts bounded by a red and a blue cursor at approximately 70 and 82° C. The lower portion shows the normalized and temperature corrected small amplicon derivative melting peaks representing the three genotypes in grey (G homozygote), blue (A homozygote), and red (G/A heterozygote). The data is looser than the data in Figure 3--which is the same experiment but WITH internal calibration algorithms automatically applied. This demonstrates the utility of internal melting calibrators. However, due to the exquisite temperature uniformity inherent in this hi-res melting system, homozygotes are easily differentiable and all correct genotypes are easily and automatically called.

Figure 5.



Scanning high resolution melting data. 170bp fragment of the hepatic lipase gene. Homozygous wild-type samples in grey. Other genotypes including double heterozygotes are shown in remaining colors. Automated curve modeling software results in unique groups corresponding to all genotypes at several loci.

RESULTS

All test phases were completed prior to launching the machine for commercial sale. Software and firmware bugs were found during this testing and resolved. In addition, electronic and mechanical instrumentation fixes were implemented and re-validated.
Expert as well as novice users were involved in this validation to maximize the likelihood of finding software and machine design problems, which were resolved, resulting in an excellent multi-function instrument for organism detection, gene expression studies and mutation scanning and genotyping. Representative data from this system validation is shown throughout the remainder of this poster.

CONCLUSION

These data are a sampling of actual validation data run on the LS32. Precision and linearity measurements met expectations and this instrument is being sold to medium throughput laboratories that require the most flexible qPCR and HRM instrument without sacrificing quality of either system.