

The LightScanner[®] System: Ultra Fast Mutation Discovery and Gene Scanning

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Introduction

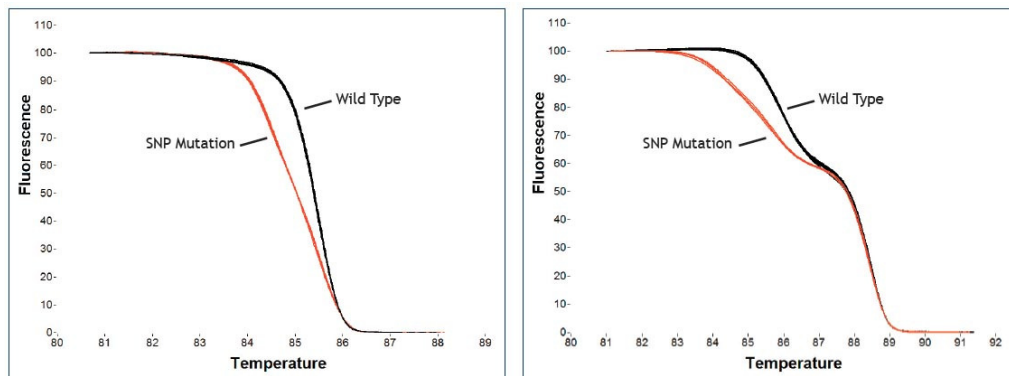
The LightScanner System enables DNA melting analysis, also referred to as Hi-Res Melting™, to perform high-throughput mutation discovery and gene scanning. Hi-Res Melting of nucleic acid depends on the ability to collect high-density information of fluorescence as a function of temperature in a mixture that contains a fluorescent double-strand DNA binding dye and PCR product. In the LightScanner Instrument images of DNA melting are captured by a CCD camera and magnified to reveal subtle details in DNA melting profiles. Sample-to-sample comparisons of these images are then used to interrogate the sequences of amplified DNA. Correct interpretation of the data depends to a large extent on the software algorithms that are used. The LightScanner System uses software specifically developed to provide the most accurate analysis of Hi-Res Melting curves.

Mutation Scanning

Screening amplified DNA for sequence variation, also known as, mutation scanning is an important tool for genetic research and clinical applications. The LightScanner is unique in that it allows homogenous mutation scanning in standard micro-titer format using a dsDNA binding dye and Hi-Res Melting. Mutation scanning techniques detect the presence of sequence variation in a fragment of amplified DNA. The DNA fragments are analyzed for completely matched hybrids called homoduplexes, and mismatched hybrids called heteroduplexes. Conventional scanning techniques are not homogenous and require a separation step to identify heteroduplexes. Mutations in PCR products are detected by changes in the shape of the melting curve compared to a reference sample. Below are examples of SNPs identified in two exons using the LightScanner Instrument and LCGreen[®] Plus dye. Superior reproducibility is demonstrated by the overlapping curves of duplicate samples for both the wild type and the mutant samples.

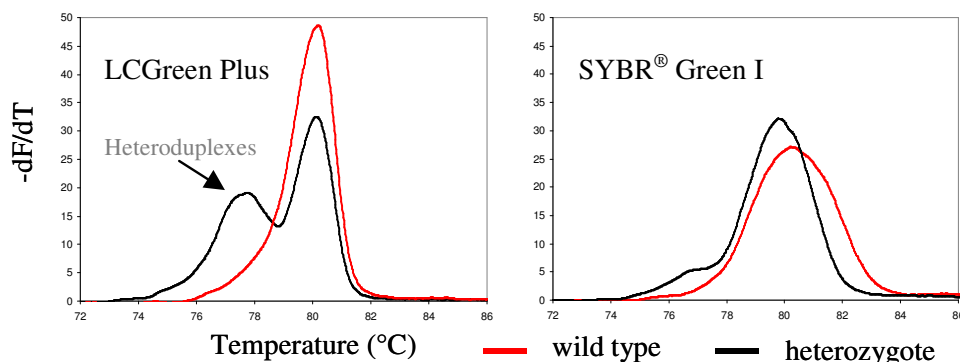
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Idaho Technology Inc.
www.idahotech.com



LCGreen Plus Dye

The LightScanner System utilizes the fluorescence of a new category of dsDNA binding dye, LCGreen Plus, to identify sequence variations without the need for dye-labeled probes. LCGreen Plus dye is specifically designed for Hi-Res Melting curve analysis for detecting DNA sequence variants. LCGreen Plus is unique in its ability to detect the presence of heteroduplexes formed during PCR.



Derivative melting curves illustrate detection of heteroduplexes in the heterozygous mutant using LCGreen Plus which are not detected using SYBR Green I.

Heteroduplex detection is a feature not shared with other dyes traditionally used in real-time PCR, such as SYBR Green I or ethidium bromide.

Platform Comparison

The method of melting DNA for confirmation of specific PCR products is enabled on most real-time PCR platforms. Also known as dissociation curve analysis, this technique is typically utilized in combination with a double stranded DNA binding dye like, SYBR®

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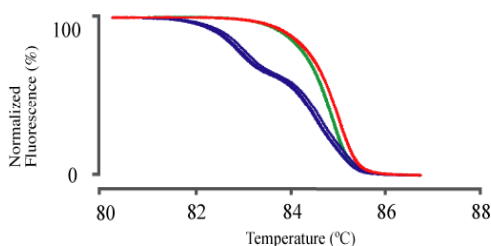
Green I, to characterize primer-related non-specific amplification (primer dimer) in order to optimize assays for specific target detection. It is important to emphasize that high resolution scanning cannot be performed on these systems. The high precision temperature control required for this technique is only available on instruments designed specifically for Hi-Res Melting, like the HR-1™ or LightScanner available from Idaho Technology Inc.

Instrument Feature	Idaho Technology	Other Platforms
Resolution	High	Low
Analysis Software specific for Hi-Res Melting	Yes	No
Fluorescent Compatibility	Yes	Some*
Time to result	5 minutes	>30 minutes

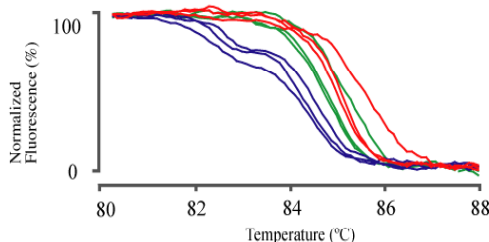
*not all instruments are compatible with LCGreen

The power of DNA melting analysis depends directly on the resolution of the melting instrument. Precision of the melt curves enables identification of different sample genotypes.

HR-1 Instrument



Commercial Real-Time PCR Instrument



Normalized melting curves of 110-bp amplicon in the presence of LCGreen Plus. Figures demonstrate the advantage of Hi-Res Melting Platform. True Hi-Res Melting profiles enable discrimination of wild type (AA) in green, heterozygotes (AT) in blue and homozygous mutants (TT) in red.

Hi-Res Melting Applications

Application	Hi-Res Melting Platforms	Other Platforms
PCR product purity confirmation	Yes	Yes
Whole amplicon mutation scanning (probe free)	Yes	No
SNP detection (unlabeled probes)	Yes	No
Genotyping (with labeled probes)	Yes	Yes
Small Insertion/Deletion detection	Yes	No

The LightScanner System

The LightScanner system requires no post-PCR addition of reagents or the need for expensive and time-consuming separation. LCGreen Plus is included in the amplification reaction. The Hi-Res Melting profile reveals heterozygous single-base changes in 2- 5 minutes with a sensitivity and specificity superior to non-homogenous techniques, such as DHPLC or TGCE. In addition to identifying anonymous heterozygous variants, the system enables identification of specific mutations, in such cases scanning and genotyping can often be combined into one simple melting analysis. The post-PCR product remains intact, thus enabling down stream analysis such as sequencing

Conclusion

High-resolution melting analysis for scanning and genotyping is enabled with the LightScanner System. Closed-tube mutation scanning using existing fluorescent chemistries (i.e SYBR Green I) is not possible and conventional real-time PCR machines perform poorly. The best results for high throughput mutation scanning are enabled using the LightScanner System.

References

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Additional Information

Address: 390 Wakara Way, Salt Lake City, Utah 84108 USA

Phone: 1-800-735-6544 or + 801-736-6354

Email: it@idahotech.com

Website: www.idahotech.com



Idaho
Technology Inc.