

The LightScanner® System: Ultra Fast Mutation Discovery and Genotyping

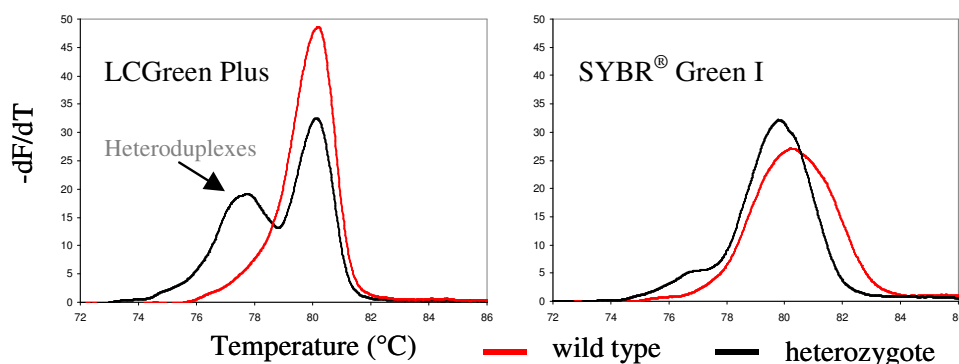
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Introduction

The LightScanner System enables DNA melting analysis, also referred to as Hi-Res Melting™, to perform high-throughput mutation discovery and genotyping. 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.

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.

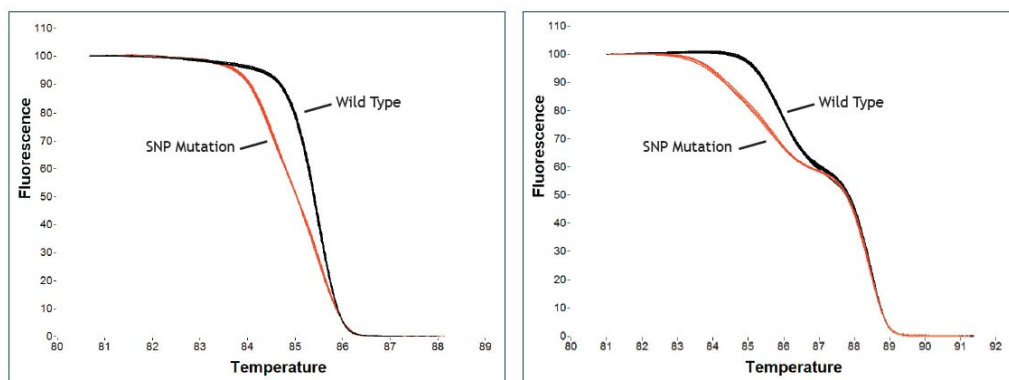
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Heteroduplex detection is a feature not shared with other dyes traditionally used in real-time PCR, such as SYBR Green I or ethidium bromide.

Mutation Discovery

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.



Genotyping

Homogenous PCR methods for genotyping require expensive fluorescently labeled oligonucleotide probes. We use a single 3'-blocked, unlabeled oligonucleotide probe and the saturating dye LCGreen Plus to generate melting curves characteristic of the genotype under the probe. Melting curves are generated and analyzed using the LightScanner Instrument System.

Genotyping with unlabeled probes is an homogeneous, end-point assay. The probe is included in the PCR mix but is not consumed during amplification. Genotyping is accomplished by monitoring the melting of probe-target duplexes post-PCR. Key to the method is

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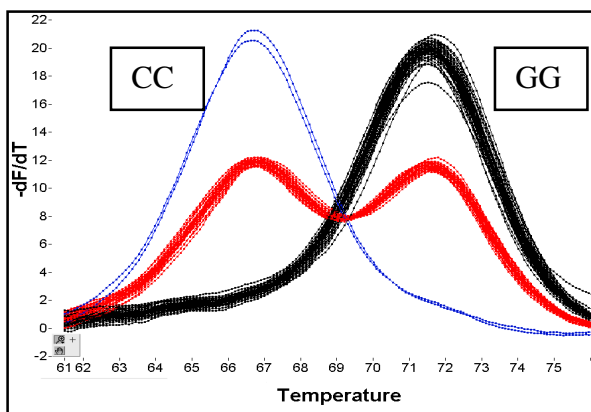
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the use of one primer in excess, which leads to the overproduction of the target strand. The method provides the same specificity as current methods without the cost.

Genotyping Results - Example

The mismatched probe-target hybrid (C variant) generates a melting curve with lower T_m , shown below in blue, while the matched hybrid (G allele) generates melting with higher T_m , shown below in black. The heterozygous samples demonstrate a melting profile with curves resembling in smaller scale the combination of homozygous samples, shown below in red.



Conclusion

High-resolution melting analysis for scanning and genotyping is enabled with 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. The best results for high throughput mutation scanning and genotyping are enabled using the LightScanner System.

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Technology Comparisons

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