Hi-Res Melting™ For Mutation Scanning Using LCGreen® Plus Dye

TECHNICAL ::: NOTE

Introduction

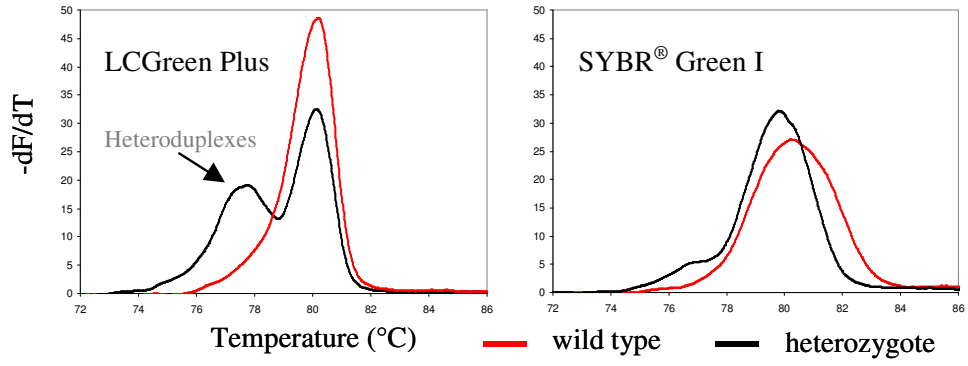
Screening amplified DNA for sequence variation, also known as "mutation scanning", is an important tool for genetic research and clinical applications. Mutation scanning techniques detect the presence of sequence variation in a fragment of amplified DNA. Conventional scanning techniques are not homogenous and require a separation step to identify heteroduplexes. The LightScanner® system is unique in that it allows homogenous mutation scanning in standard micro-titer format using a dsDNA binding dye, LCGreen Plus and High Resolution Melting.

Hi-Res Melting Overview

High Resolution Melting (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. Mutations in PCR products are detected by changes in the shape of the melting curve compared to a reference sample.

LCGreen Dyes

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.

LCGreen Plus Dye	
Optimum excitation	440-470 nm
Optimum emission	470-520 nm
Melting Temperature (Tm) Adjustment	1-3° C*

*Increases Tm by 1-3° C, may require adjustment of cycling parameters.

LCGreen Plus is added to the reaction prior to PCR. Supplied at a 10X concentration, simply add 1 volume of the dye solution for every 9 volumes of the PCR mixture during PCR set up. For enhanced ease of use, a complete PCR master mix is available from Idaho Technology Inc. The LightScanner Master Mix is an optimized PCR master mix, which includes LCGreen Plus, developed specifically for Hi-Res Melting applications.

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.

Related Products

Description	Part Number
LightScanner Instrument (96 well)	LCN-ASY-0011
LightScanner Instrument (384 well)	LCN-ASY-0001
LCGreen Plus Dye Kit (1,000 reactions)	BCHM-ASY-0005
LCGreen Plus Dye Kit (10,000 reactions)	BCHM-ASY-0006
LightScanner Master Mix (100 reactions)	HRLS-ASY-0002
LightScanner Master Mix (500 reactions)	HRLS-ASY-0003

Additional Information and References

Website : www.idahotech.com
Phone: 1-800-735-6544

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