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TECHNICAL
::: NOTE

DNA Melting Analysis: Benefits of High Resolution Systems for Mutation Scanning and Genotyping

Introduction

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® Green I, to characterize primer-related non-specific amplification (primer dimer) in order to optimize assays for specific target detection.
Introduction of instruments that offer high-resolution amplicon melting capabilities, and new fluorescent dyes, have advanced this method into a powerful tool for mutation scanning and genotyping.

Hi-Res Melting™ Platforms

Idaho Technology Inc, enables DNA melting analysis with instruments and chemistries designed specifically for Hi-Res Melting. The HR-1® instrument detects subtle differences in fluorescent signals over temperature change in a single sample tube, and recently introduced, the LightScanner® instrument combines the same detection capabilities in a higher throughput format, 96- or 384-well plates. Both instruments are designed to be used in combination with LCGreen® dye. LCGreen dye is manufactured exclusively by Idaho Technology Inc. and is unique in its ability to detect the presence of heteroduplexes formed during PCR.
Other commercially available real-time PCR instruments lack the precise temperature control, the data density acquisition and the analysis software required for successful implementation of the method.

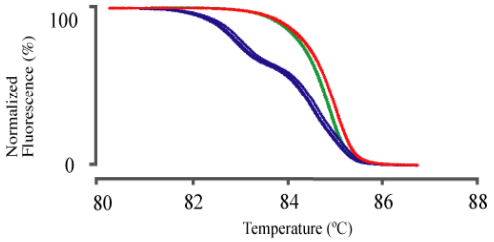
Hi-Res Melting Platform Comparison

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

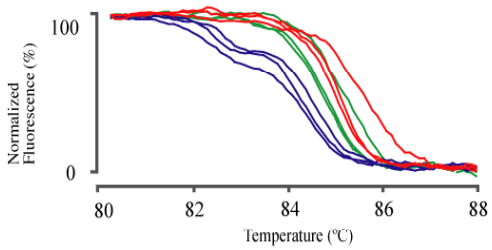
*not all instruments are compatible with LCGreen dye

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

Conclusion

Although there are multiple real-time PCR instruments on the market, 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.

References

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