Research Article | Molecular Diagnostics and Genetics

High-Resolution Genotyping by Amplicon Melting Analysis Using LCGreen

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Abstract

Background: High-resolution amplicon melting analysis was recently introduced as a closed-tube method for genotyping and mutation scanning (Gundry et al. *Clin Chem* 2003;49:396–406). The technique required a fluorescently labeled primer and was limited to the detection of mutations residing in the melting domain of the labeled primer. Our aim was to develop a closed-tube system for genotyping and mutation scanning that did not require labeled oligonucleotides.

Methods: We studied polymorphisms in the hydroxytryptamine receptor 2A (HTR2A) gene (T102C), β-globin (hemoglobins S and C) gene, and cystic fibrosis (F508del, F508C, I507del) gene. PCR was performed in the presence of the double-stranded DNA dye LCGreen, and high-resolution amplicon melting curves were obtained. After fluorescence normalization, temperature adjustment, and/or difference analysis, sequence alterations were distinguished by curve shape and/or position. Heterozygous DNA was identified by the low-temperature melting of heteroduplexes not observed with other dyes commonly used in real-time PCR.

Results: The six common β-globin genotypes (AA, AS, AC, SS, CC, and SC) were all distinguished in a 110-bp amplicon. The HTR2A single-nucleotide polymorphism was genotyped in a 544-bp fragment that split into two melting domains. Because melting curve acquisition required only 1–2 min, amplification and analysis were achieved in 10–20 min with rapid cycling conditions.

Conclusions: High-resolution melting analysis of PCR products amplified in the presence of LCGreen can identify both heterozygous and homozygous sequence variants. The technique requires only the usual unlabeled primers and a generic double-stranded DNA dye added before PCR for amplicon genotyping, and is a promising method for mutation screening.

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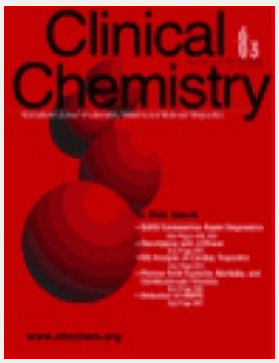

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Here we show that High Resolution Melt Analysis meets these criteria, it is suitable for closed-tube genotyping of all allele types and current genotyping assays can be converted to this technology with little or no effort. 17. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ (2003) High-resolution genotyping by amplicon melting analysis using LCGreen. Clin Chem 49: 853–860. View Article. High resolution melting was performed after the temperature cycling by slowly increasing the temperature from 65–95 °C in increments of 0.1 °C. Fluorescent signal was recorded for 5 s after each 0.1 °C increment. Primers used for the real-time PCR and HRM were selected by the Variant Melting Profile tool, checked for specificity by ePCR ordered from IDTDNA, with the following base composition: “Reverse” primer 5′CAACACGTTTCACCAGTGCA3′ and “forward” primer 5′TGCAAGATTGTACCTTCCTTGGT3′. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin Chem. 2003;49:853–60.View ArticlePubMedGoogle Scholar. High-resolution genotyping by amplicon melting analysis using LCGreen. Carl T. Wittwer, Gudrun H Reed, Cameron N. Gundry, Joshua G Vandersteen, Robert J. Pryor. Clinical chemistry. 2003. BACKGROUND High-resolution amplicon melting analysis was recently introduced as a closed-tube method for genotyping and mutation scanning (Gundry et al. Clin Chem 2003;49:396-406). The technique… BACKGROUND High-resolution melting of PCR amplicons with the DNA dye LCGreen I was recently introduced as a homogeneous, closed-tube method of genotyping that does not require probes or real-time… (More). 21. View Paper. Cite. Save. Continuous fluorescence monitoring of rapid cycle DNA amplification.