

High Resolution Melting: an efficient tool in mutational screening of *DMP1* and *MEPE* genes

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INTRODUCTION

Mutation scanning using high-resolution melting (HRM) is an effective and sensitive method that can replace gel-based methods and dHPLC to detect sequence variations. HRM increases simplicity and improves turn around time without compromising assay sensitivity and accuracy. HRM is a closed-tube assay without the requirement of any post-PCR handling. A saturating DNA binding dye is introduced during DNA amplification enabling differentiation of PCR products based on their dissociation behavior as they are subjected to increasing temperature.

The *DMP1* (MIM *600980) and *MEPE* (MIM *605912) genes play a crucial role in bone mineralization and mutations in *DMP1* have been described as a cause of hypophosphatemic rickets (HYP).

METHODS

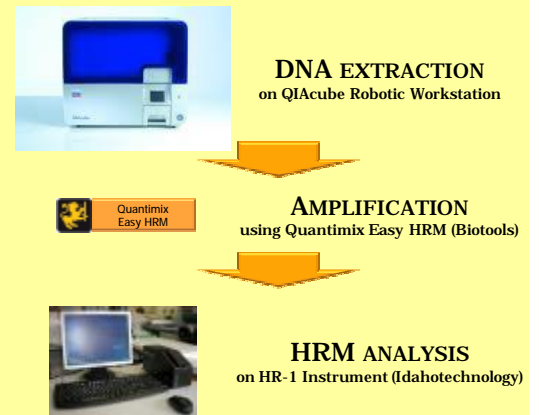
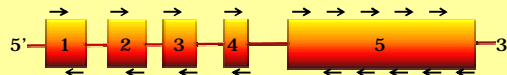


Fig. 1: Workflow of HRM analysis

DMP1 gene
5 exons
9 amplicons



MEPE gene
4 exons
9 amplicons

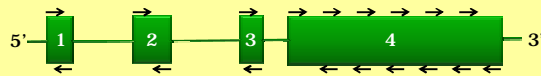


Fig. 2: *DMP1* and *MEPE* amplicons to perform HRM analysis. The amplicons span to 121 bp from 350 bp

A potential weakness of HRM is the detection of homozygous variants. Because *DMP1* and *MEPE* have a recessive pattern of inheritance, to generate heteroduplexes and ensure the detection of all homozygous variants we mixed the proband DNA with a wild-type DNA (1:1 ratio) in the PCR reaction.

AACCC **CAAAFTCTAGGCCAAATGAAAGACCC** **CAGTGA** **AGCACTCAGTCAGAGGAG** **GGCCTG**

Fig. 3: Oligonucleotide pairs used to perform SNP genotyping of c.A205T (p.S69C) in *DMP1* gene .

RESULTS

We performed mutational screening by HRM of the complete coding region and of intron/exon boundaries of *DMP1* e *MEPE* genes in a pediatric cohort of 12 HYP patients. Moreover, we carried out SNP genotyping of the non-synonymous SNP c.A205T (p.S69C) in *DMP1* gene, identified in 9 of our patients, to identify the frequency in 50 healthy subjects.

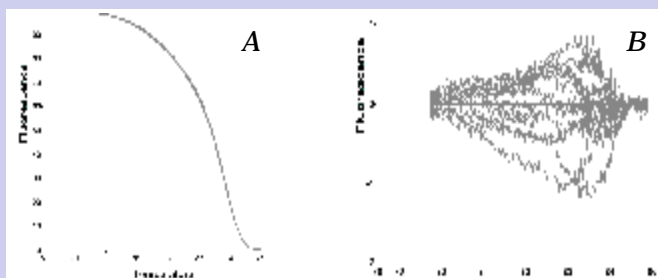


Fig. 4: Mutational screening of exon 3 of *DMP1* gene. Normalized curves (A) and difference plot (B) obtained after HRM analysis. The analysis shows exclusively wild-type subjects.

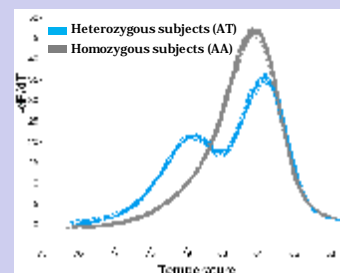


Fig. 4: SNP genotyping of c.A205T in *DMP1* gene. Derivative plot obtained after HRM analysis. The analysis shows heterozygous subjects (blu) and homozygous subjects in grey (AA genotype).

CONCLUSIONS

In our experience HRM, for its ease of use, rate of analysis, sensitivity, specificity and low cost is an optimal tool that can be used for high-throughput mutation screening and genotyping both for research and molecular diagnostic.