

Small Amplicon Genotyping Using the LightScanner® System

Method Background

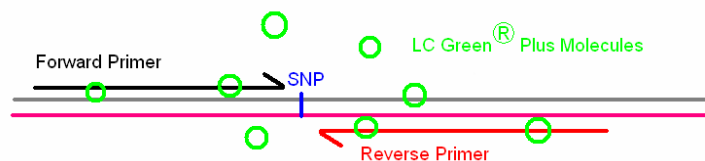
Small amplicon genotyping is a simple yet powerful genotyping technique. No probes are required, making this technique rapid and inexpensive. Until recently, without probes, accurate closed-tube genotyping has been difficult. Now, with the high-resolution LightScanner instrument and a specialized genotyping master mix containing LCGreen® Plus dye and internal calibrators, small amplicon genotyping can produce the same high accuracy results associated with probe-based techniques.

High Sensitivity Genotyping

Most SNPs can be genotyped easily by monitoring the melting profiles of small amplicons (50-100 bp) in the presence of LCGreen dye. In some cases where the polymorphism consists of a base pair neutral change (A>T, G>C) the homozygous species have such subtle differences in melting temperature that the noise in the system can mask the difference between the two alleles. This limitation can now be overcome by using the LightScanner High Sensitive Genotyping Master Mix along with the LightScanner System. The signal provided by temperature calibrators included in the mix is used to correct for small mechanical and chemical variations resulting in highly precise genotyping calls generated automatically by the software. In general, this Master Mix should be used for small amplicon genotyping of polymorphisms where the melting temperature of homozygous alleles is expected to differ by less than 0.5° C.

Assay Design

Small amplicon genotyping can be successfully performed for SNPs, and small deletions and insertions that are located between the 3' -ends of the forward and reverse primer, as illustrated below.



For best results, choose appropriate primers using LightScanner Primer design software and perform our simple optimization protocol, using an annealing gradient. The specific annealing gradient for each primer set is automatically suggested by the software.

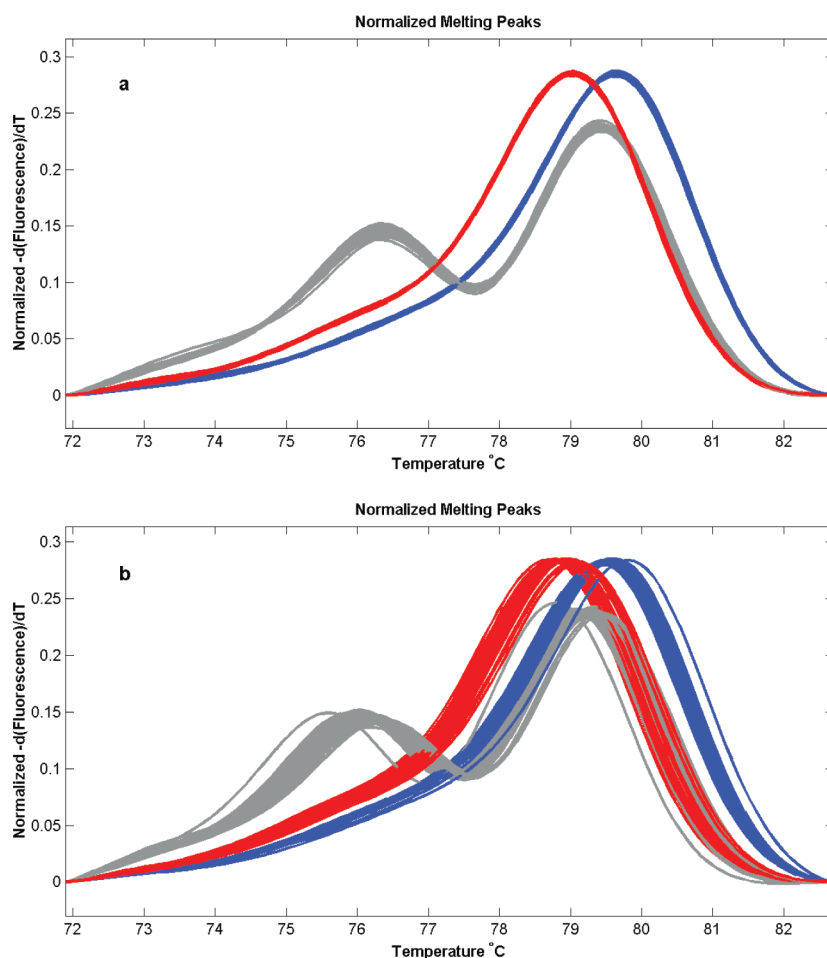
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Genotyping Results - Example

The panels below illustrate the genotyping results obtained with (Panel a) and without (Panel b) the LightScanner High Sensitivity Genotyping Master Mix for a G>C SNP in a 42 bp product. Homozygotes display single melting peaks. Heterozygous samples (in grey) show broad melting profiles indicating the presence of both heteroduplexes and homoduplexes.



These results demonstrate the benefits of using the specialized master mix for small amplicon genotyping.

References

Liew, M., et al. (2004) *Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons*. Clin Chem. Jul;50(7):1156-64.

Liew, M., et al. (2007) *Closed-tube SNP genotyping without labeled probes/a comparison between unlabeled probe and amplicon melting*. Am. J. Clin. Pathol. 127, 341-348

Seipp, M. T. et al. (2007) *Unlabeled Oligonucleotides as Internal Temperature Controls for Genotyping by Amplicon Melting*. J Mol Diagn., 9, 284-9

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