

Fast, accurate SNP detection using high resolution thermal melt analysis on a microfluidic chip. Boles DJ¹, Rulison A², Dong W², Knight IT¹. ¹Canon U.S. Life Sciences, Inc. Rockville, MD and ²Caliper Life Sciences Inc. Mountain View, CA.

Single nucleotide polymorphisms (SNPs) in the human genome can be responsible for coding changes that result in disease or other clinically relevant phenotypes and represent a significant challenge for accurate detection. High resolution thermal melt (HRTm) analysis is a novel method that can distinguish SNP variants in PCR amplicons by using a saturating DNA binding dye, such as LC Green I, to visualize changes in thermal melt profiles. We have developed a microfluidic lab-on-a-chip system for conducting HRTm analysis and demonstrated fast, accurate SNP detection using a heterozygote scanning application.

An 110bp PCR product was generated from a homozygous wild type and heterozygous sickle cell, human DNA sample in the presence of LC Green I. The PCR products were loaded onto a microfluidic chip mounted in a Caliper LabChip LC3000S instrument and continuously flowed through the chip while fluorescence was monitored during thermal denaturation. The melting temperature and thermal profile was determined for both genotypes at three different thermal ramp rates (0.1 °C/s, 0.5 °C/s, 1 °C/s).

Both genotypes were accurately and reproducibly identified on the LC3000S under all conditions. Results show no effect of flow rate on melting temperature and standard deviation values of the melting temperature were almost identical at the different thermal ramp rates (0.18 °C, 0.08 °C, 0.12 °C at 0.1 °C/s, 0.5 °C/s, 1 °C/s respectively). Figure 1 shows comparable thermal melt resolution between the LC3000S and the gold standard HR1 (Idaho Technology). Signal to noise values on the LC3000S were equivalent to conventional plate-based thermal cyclers (SNR = 470 and 170 for ramp rates of 0.1 °C/s and 1 °C/s respectively).

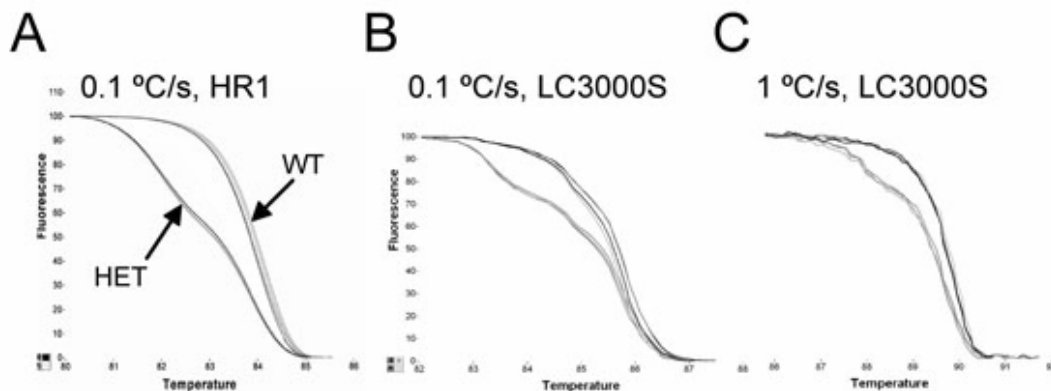


Figure 1: Thermal melt profiles for 110 bp amplicons generated from a homozygous wild type (WT) genomic DNA sample and a heterozygous sickle (HET) sample with SNPs at two locations. Data were generated on a HR1 at 0.1 °C/s thermal ramp (A) and a LC3000S at 0.1 °C/s (B) and 1 °C/s thermal ramp (C). Melts profiles for 4 replicate microfluidic channels for each genotype are shown in B and C.