

**Detection of multiple cystic fibrosis transmembrane conductance regulator (CFTR) mutations using high resolution thermal melt analysis on a microfluidic chip.** Mason JA<sup>1</sup>, Boles DJ<sup>1</sup>, Pryor RJ<sup>2</sup>, Poulson MD<sup>2</sup>, Wittwer CT<sup>2</sup>, Inoue H<sup>1</sup>, Knight IT<sup>1</sup>. <sup>1</sup>Canon U.S. Life Sciences, Inc., Rockville, MD and <sup>2</sup>The University of Utah, Salt Lake City, UT.

Cystic Fibrosis is an autosomal recessive genetic disease that is caused by mutations in the CFTR gene. The current method of detection of this disease is via a positive sweat chloride test followed by sequencing of the 23 mutations recommended by the American College of Medical Genetics which is very costly and time consuming. We have utilized an unlabeled probe assay followed by high resolution thermal melt analysis to distinguish genotypes by differences in melting temperature using a saturating double stranded DNA binding dye (LC Green Plus). This method utilizes PCR primers and a 3' phosphate-modified probe that covers the mutation of interest. As the DNA is denatured, the intercalating dye is released from the helix and becomes non-fluorescent in a sequence dependent manner. Differences in the probe melting temperatures indicate the presence or absence of a mutation. We have developed a microfluidic lab-on-a-chip system capable of differentiating homozygous, heterozygous and compound heterozygous mutants of all 23 CFTR ACMG mutations.

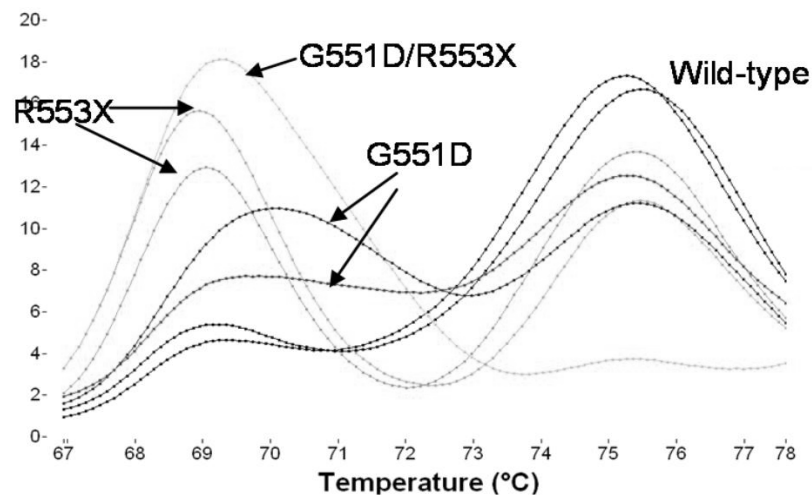


Figure 1

A 93 bp PCR product was generated in the presence of LC Green Plus from samples representing wild type, heterozygous and double heterozygous CFTR human DNA. The DNA samples were loaded on a microfluidic chip mounted in a modified Caliper LabChip 3000S instrument. A 15 nL volume of PCR reagents and DNA samples were first loaded onto the microfluidic chip where they were mixed on chip via lateral flow and amplified while continuously flowing through the microchip. Fluorescence was monitored in the microfluidic channel during thermal denaturation and the melting temperature was extrapolated using a third party software system. The results above display four melting profiles of wild type and mutations in Exon 11 that were accurately genotyped on our microfluidic chip. The thermal melt profiles for the unlabeled probe that binds to the 93bp product are shown above and represent 7 different human genomic DNA samples.