

High Resolution Melting Detection of Mutations Causing Cystic Fibrosis on the FilmArray® Instrument

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INTRODUCTION

Idaho Technology has developed the FilmArray system that performs all of the steps necessary to identify pathogens from human and environmental samples. The FilmArray performs sample preparation, nucleic acid isolation, PCR and data analysis within one hour. Our goal was to enhance FilmArray pathogen detection by developing a method for genotyping using high resolution melting on this platform. Presented are genotyping of known CFTR mutations and scanning for novel variants using high resolution melting and LCGreen® Plus dye on the FilmArray.

METHODS

Nine prevalent mutations and a single common SNP within the CFTR gene were analyzed on the FilmArray. Known mutations in the CFTR gene included: delta I507, delta F508, G542X, G551D, R553X, 2184 delA, W1282X, N1303K, IVS11-1 G>A (c.1585 -1 G>A). The common M470V polymorphism was also evaluated by exon scanning assays. LunaProbe assays were developed using a nested multiplexed format to genotype all variants in a single sample.

A set of eight coding amplicons and associated splice junctions of the CFTR gene were also analyzed. The following exons were scanned as large amplicons: 10, 11, 13, 20, and 21. These amplicons survey a variety of exon sizes, GC content, and genetic variants (i.e. SNPs and In/Dels) commonly observed in the CFTR gene. Nested multiplexed assays were developed to scan these eight amplicons using LCGreen Plus. Samples bearing known heterozygous variants were used to demonstrate heteroduplex detection sensitivity on the FilmArray.

Nested multiplexed assays were freeze-dried into the FilmArray pouch (Figure 1) using Idaho Technology's standard procedure. Genomic DNA from whole blood samples was used as starting template and processed within the standard workflow of the FilmArray instrument. All data were analyzed on pre-existing high resolution melting software packages designed for Idaho Technology LightScanner instruments.

RESULTS

Data shown (Figures 2 and 3) represent final genotyping or heteroduplex scanning assay results generated on FilmArray instrument. Both the LunaProbe genotyping and exon-scanning applications yielded 100% correct calls for test samples and controls analyzed in duplicate. Where there was overlap, samples with known mutations detected with a LunaProbe genotyping assay were also correctly identified in the larger exon-scanning assay.

Mutant and normal alleles were easily differentiated using LunaProbes, with an average apparent melt temperature shift of >5°C. Correct genotyping calls were made regardless of whether the probe was designed to match the mutant or normal allele. The differential plots for the exon scanning assays clearly demonstrate successful genotyping. However, a control sample which was homozygous for the M470V minor allele was not distinguished from a homozygous wildtype sample. This is not entirely unexpected, as even the highest resolution melting instruments are often unable to distinguish homozygous polymorphisms from wildtype based on whole amplicon variant scanning techniques.

Based on these results, additional FilmArray software development efforts to implement high resolution melting data analysis capabilities, similar to those available in the LightScanner software, are being considered.

Figure 2.

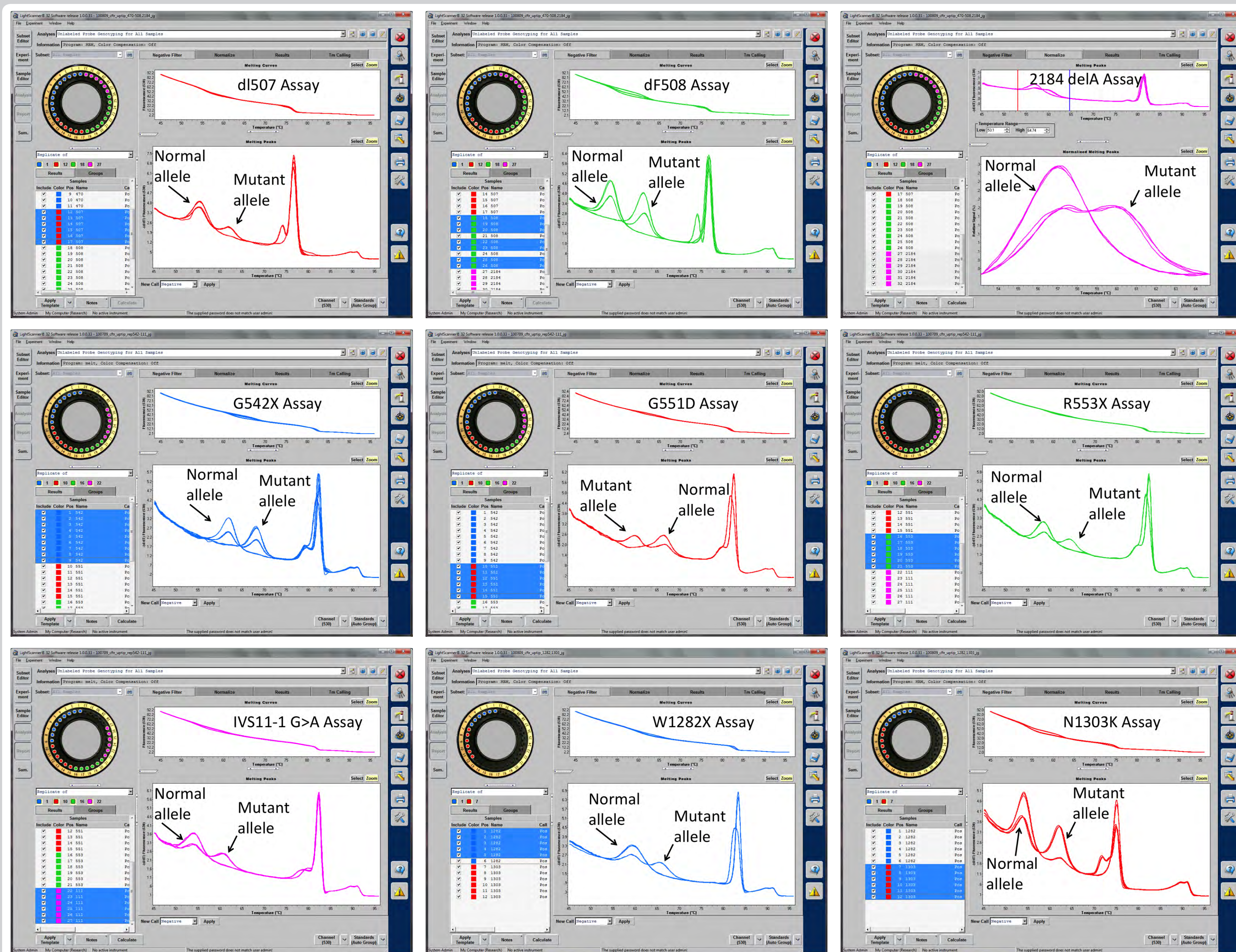


Figure 2. LunaProbe assays run on the FilmArray and analyzed on the LightScanner 32 software. All assays except the G551D use a probe that is matched to the mutant allele, eliminating the possibility of false-positive genotypes on FilmArray. All genotypes match known control samples.

Figure 1. The FilmArray Instrument and Pouch

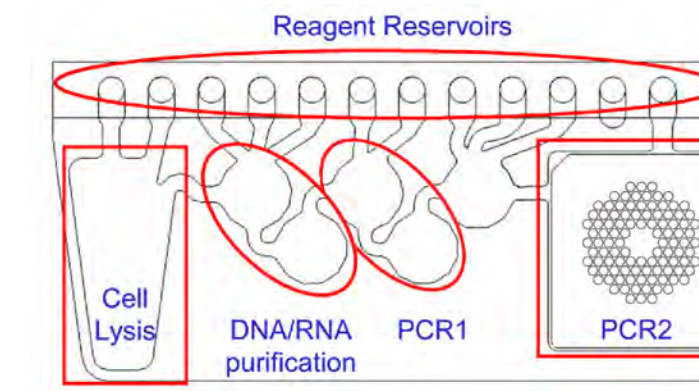


ITI has developed a lab-in-a-pouch system called "FilmArray". It is a medium-scale fluid manipulation system performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion. These system characteristics are ideal for the multiplex testing of pathogens in standard diagnostic sample matrices.

The FilmArray Test System is initiated by injecting rehydration solution and a patient blood culture sample into the FilmArray pouch and placing it in the FilmArray instrument. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run. Results are provided in ~ 1 hour.

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second stage PCR product is detected in real-time using a fluorescent-double-stranded DNA binding dye, LCGreen®.

- Fitment with freeze-dried reagents
- Plungers - deliver reagents to blisters
- Sample lysis and bead collection
- Wash station
- Magnetic bead collection blister
- Elution Station
- Multiplex Outer PCR blister
- Dilution blister
- Inner Nested PCR array



The FilmArray pouch has a fitment (see label A) containing all needed freeze-dried reagents. The film portion of the pouch has stations for:

1. Cell lysis (Blister C)
2. Magnetic-bead based nucleic acid purification (D & E)
3. First-stage multiplex PCR (F & G)
4. Array of 102, second-stage nested PCRs (I)

Figure 3.

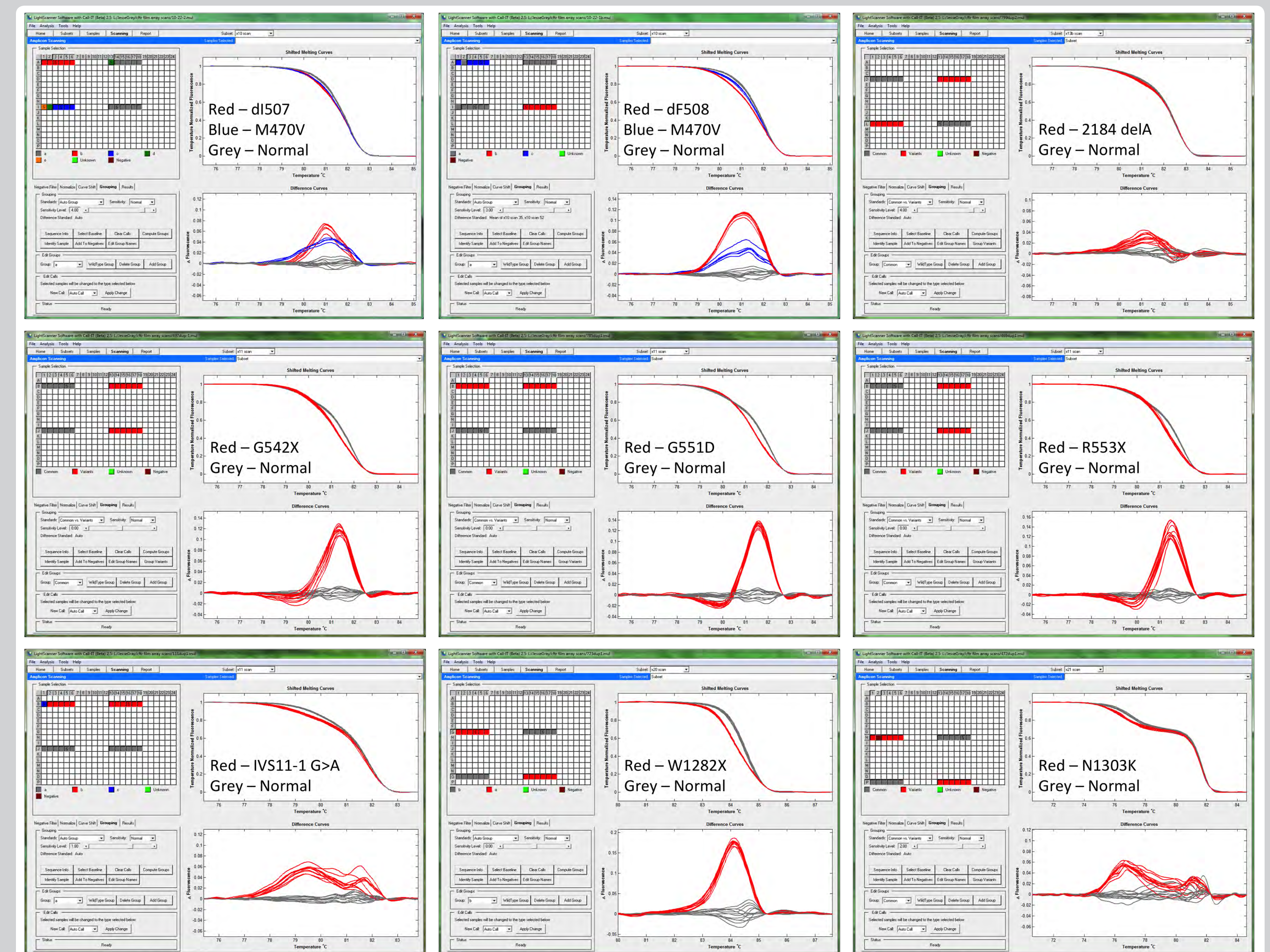


Figure 3. FilmArray data ported into LightScanner-384 software analysis. All heterozygous variants were successfully identified relative to known normal (wildtype) melt profiles. Further, the exon 10 scanning assay was able to distinguish between a sample heterozygous for either the delta I507 or delta F508 mutation and the common M470V polymorphism. However, a control sample which was homozygous for the M470V minor allele was not distinguished from a homozygous wildtype sample. This is not entirely unexpected, as even the highest resolution melting instruments are often unable to distinguish homozygous polymorphisms from wildtype based on whole amplicon variant scanning techniques. Similar adjustments in PCR reagents were required for these scanning assays, as mentioned above for the LunaProbe genotyping assays, when FilmArray freeze-dried pouches were formulated.

CONCLUSION

This study demonstrates that high resolution melting on the FilmArray instrument is a viable approach for highly multiplexed genotyping of a single disease or disorder. Both LunaProbes and exon scanning were capable of yielding reliable genotyping results on the FilmArray. Further instrument and assay development will enable a wider range of genetic targets and sample types to be assayed in a highly efficient, accurate, and rapid fashion.

ACKNOWLEDGMENTS

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