INTEGRATED PCR AND THERMAL MELT ANALYSIS ON A MICROFLUIDIC CHIP

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Differentiation of PCR products by fluorescent melting analysis was first introduced in 1997 (1), and has since been demonstrated as a reliable genotyping technique for diagnostic applications (2). Conventional instrumentation for thermal melt analysis utilizes slow thermal ramp rates (0.1 – 0.2 ºC/s) to allow for heat transfer to reaction volumes in the microliter range (3). We have modified and tested the microfluidic LabChip® system (4) to integrate thermal melt analysis and PCR in a continuous flow format utilizing reaction volumes of several nanoliters. Results demonstrate high resolution thermal melt analysis at ramp rates up to 1ºC/s. To our knowledge, this is the first demonstration of a continuous flow system integrating PCR and thermal melt analysis.

The microfluidic chip, Figure 1, consisted of two bonded quartz plates; one piece with etched channels, the other piece with nine metal traces and through holes to serve as reagent reservoirs. A capillary inserted perpendicular to the plates allowed samples to be introduced into the channels directly from a microtiter plate by pressure driven flow. DNA samples were mixed with PCR reagents stored on the chip and then split into 8 parallel channels each of which was supplied with a different set of primers. Samples were introduced serially onto the chip with buffer spacers between adjacent samples. Varying power levels were applied to the metal traces to rapidly cycle the temperature of the samples flowing through the amplification region. After amplification, samples in the detection region were thermally melted by ramping the temperature of a heater block beneath the detection region. Fluorescence was measured and converted to plots of the negative derivative of fluorescence as a function of temperature.

A study of melting temperature of two PCR products, 85bp and 606bp, was performed in the apparatus using the double-stranded DNA binding dye SYBR Green I. Figure 2 shows the typical melting profile for the two products, when PCR and thermal melt are integrated. Figures 2A and B show the raw melt curves where fluorescence drops off most steeply as the DNA melts. In Figure 2C the melting transitions can easily be seen as peaks and are reported as Tm values. Note that the 606bp product has a complex melting curve with two transitions that are easily resolved in this device.

A comprehensive study of the effect of flow rate on Tm values at two thermal ramp rates was performed using the same two PCR products. In these experiments PCR was performed off-chip and melt analysis was performed in the chip. Figure 3 shows that at each ramp rate, Tm values were consistent across all flow rates. Table 1 shows that ΔTm, the parameter that differentiates the PCR products, was consistent across all tests, ($\Delta Tm = 3.7 \pm 0.05$ ºC).

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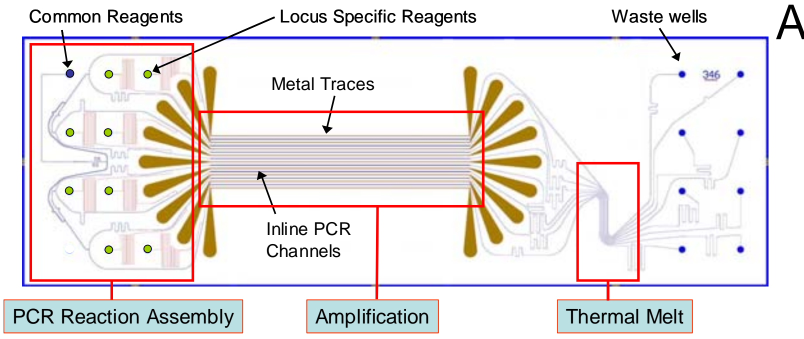


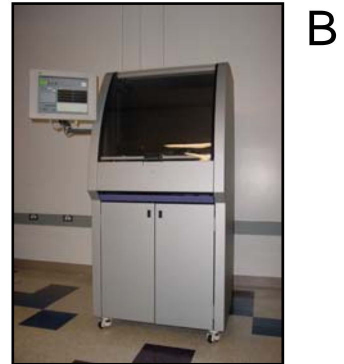
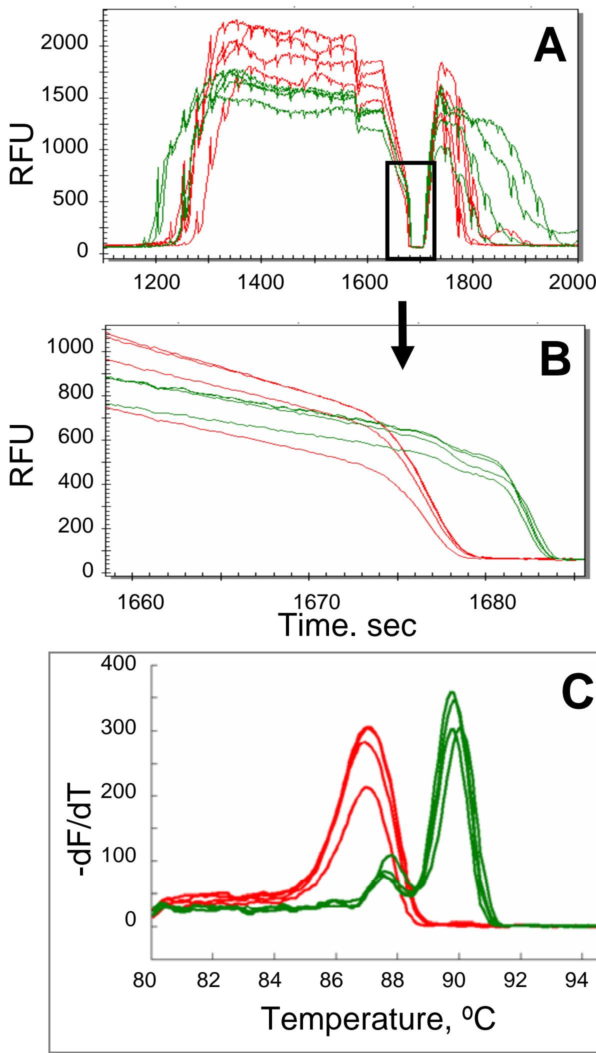


Figure 1. A schematic of the microfluidic chip design for integrated PCR and thermal melting (A) and the instrument (B).



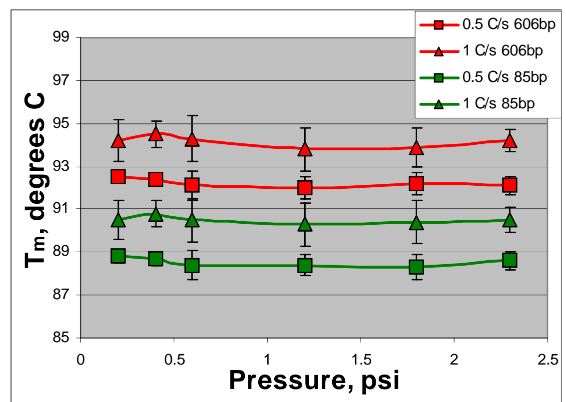


Figure 3: Effect of pressure on T_m of PCR products at different ramp rates. Each data point is a mean of 4-7 experiments. Error bars are standard deviation values.

Table 1. Comparison of T_m values for two PCR products across all flow rates and ΔT_m between the two PCR products across all flow rates for the experimental results shown in Figure 3.

Thermal Ramp Rate (ºC/s)	Mean T_m Values ± SEM (ºC)		Mean □T_m ± SEM (ºC)
	85bp	606bp	
0.5	88.5 ± 0.08	92.2 ± 0.08	3.7 ± 0.05
1	90.5 ± 0.07	94.2 ± 0.11	3.7 ± 0.05

Figure 2: Representative thermal melt data generated from four samples each of an 85 bp (red) and 606 bp (green) PCR product at 0.5 ºC/s ramp and -1.3 psi flow. Raw data showing PCR and melt signal (A) with inset detail of melt transition (B) and negative first-derivative plot (C) showing the T_m values as peaks.