HOMOGENEOUS DNA MELTING ANALYSIS FOR MUTATION SCANNING USING NANOLITER VOLUMES

Scott O. Sundberg[1], Jenny Greer[2], Carl T. Wittwer[3], Robert J. Pryor[3], Oluwole Elenitoba-Johnson[3], and Bruce K. Gale[2]
[1]Department of Bioengineering, University of Utah
[2]Department of Mechanical Engineering, University of Utah
[3]Department of Pathology, University of Utah

Abstract
Solution-phase, homogeneous DNA melting analysis for heterozygote scanning was performed in 10 nL and 1 nL reaction volumes on a custom microchip and melt curves were in agreement with the gold-standard 10 μL HR-1™ melting instrument (Idaho Technology, Salt Lake City, UT). The microchips were characterized for no surface coating and a bovine serum albumin (BSA) coating applied to the microchannels. It was found that BSA improved the reproducibility and S/N for melting analysis.

Keywords: DNA melting, mutation scanning, SNP genotyping, xurography

1. Introduction

In 1997 DNA melting analysis was introduced [1], using a double-stranded DNA fluorescent dye to detect single nucleotide polymorphisms (SNPs) and to perform mutation scanning following the polymerase chain reaction (PCR) for DNA amplification. This analysis method is advantageous over other DNA analysis systems because it is less complicated, fast, and prevents contamination of the sample and environment due to its closed-tube technique. Solution-phase or "homogeneous" melting analysis on a microchip is advantageous over microscale systems using hybridized oligonucleotides [2] due to the minimization of chip fabrication complexity and reduction in analysis time.

2. Materials and methods

Xurography [3] was used to create the microchips, using a knife plotter to cut out channel structures from double coated tape (9019, 3M, St. Paul, MN) and sandwiching patterned tape between glass slides, Figure 1(A&B). A Peltier heater and J-type thermocouple, with thermal grease at interfaces, were used for temperature control, Figure 1(C). Detection used a modified inverted microscope with optics designed for the double-stranded dye LCGreen® Plus (Idaho Technology), a photomultiplier tube and module (Hamamatsu, Bridgewater, NJ), and all hardware was operated using LabView 7.1 (NI, Austin, TX). PCR was performed on the DNA sample and then transferred to an HR-1™ instrument for a high-resolution reference melting curve. The sample was then injected into a microchip for melting within 10 nL and 1 nL volumes.

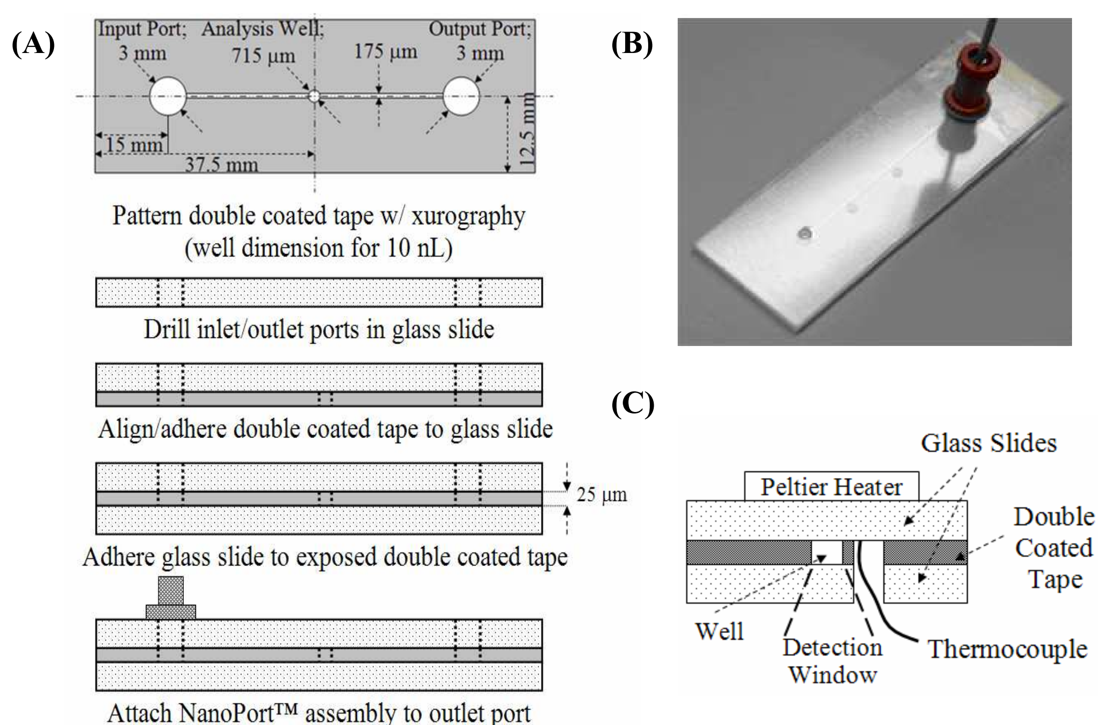

Figure 1. Fabrication process for microchip (A); photograph of final microchip assembly (B); and schematic of microchip well detection and temperature control (C).

3. Results and discussion

Table 1 compares S/N of melts with no microchannel coating and BSA (2.5 mg/mL). BSA coated channels provided a higher S/N and a more reproducible system. Figure 2 shows negative derivative melting plots of homozygous wild-type and heterozygous DNA for a SNP within an 84-bp fragment of *ATM* exon 17, where the heterozygotes were easily distinguished from the homozygotes by comparing the melting curve shape. Both 1 nL and 10 nL melts are in good agreement with the 10 µL HR-1™ melt curves. Temperature shifts between plots is due to a Peltier heater temperature gradient and varying thermocouple placement between microchips. It was found that the homozygous wild-type S/N values were 51, 69, and 2450 and the heterozygous mutant S/N values were 59, 63, and 2800, reported from smallest to largest volumes.

Table 1. S/N calculations of microchannels with no coating and with BSA coating.

Run #	S/N	
	No Coating	BSA
1	29.8	40.2
2	37.5	67.8
3	27.7	53
4	no melt	54.9
5	22.3	70.7

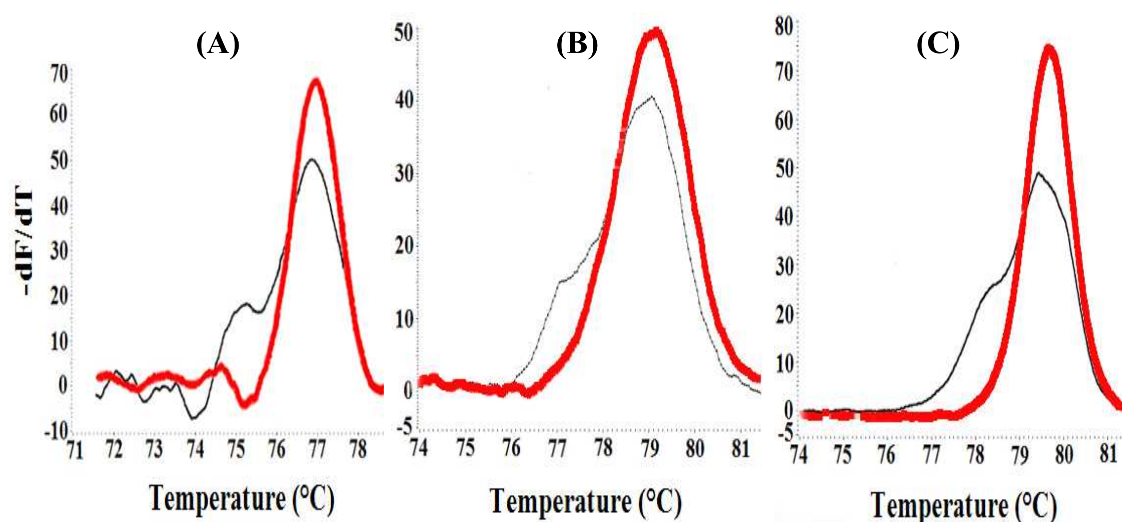

Figure 2. Negative derivative plots for the microchip (A:1 nL, B:10 nL) and the HR-1™ (C:10 μL) using the *ATM* exon 17 target. Homozygous wild-type template (thick line); heterozygous mutant template (thin line).

4. Conclusions

Mutation scanning can be performed using homogeneous DNA melting analysis within nanoliter volumes. The 1,000 and 10,000-fold reduction in sample volume from the 10 μL HR-1™ to 10 nL and 1 nL microchips does result in a lower S/N, but is still adequate for mutation scanning and would also work for SNP genotyping. It was further seen that the Xurography fabrication method provided adequate microchips for testing this chemistry. The next step for this research is to add PCR amplification with melting analysis within the microchip and move to an array format. Such a system could provide an inexpensive, high-throughput mutation scanning platform.

References

1. K. Ririe, R. Rasmussen, and C. Wittwer, *Product differentiation by analysis DNA melting curves during the polymerase chain reaction*, Anal. Biochem., *245*, pp. 154-160, (1997).
2. A. Dodge, G. Turchatti, I. Lawrence, N. Rooij, and E. Verpoorte, *A microfluidic platform using molecular beacon-based temperature calibration for thermal dehybridization of surface-bound DNA*, Anal. Chem., *76*, pp. 1778-87, (2004).
3. D. Bartholomeusz, R. Boutte, and J. Andrade, *Xurography: rapid prototyping of micro-structures using a cutting plotter*, IEEE J. MEMS, *14*, pp. 1364-74, (2005).