Hyperfine Resolution of Fluorescent DNA Melting

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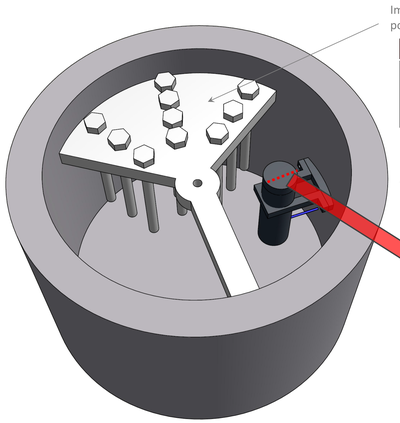


Figure 1

Mineral oil bath containing immersion heaters, melting chamber, and fiber optics.
[not shown: magnetic stir bar, oil bath lid, and mineral oil temperature probe]

Introduction and Background: Melting analysis in the presence of fluorescent dsDNA has become a standard genetic tool for rapid DNA genotyping and mutation scanning. The HR-1 (Idaho Technology) is a melting instrument that currently has the best temperature resolution of available melting instruments. Researchers typically use the HR-1 to melt DNA samples at a rate of 0.1-0.3 degrees/second. Using Lambda DNA and a low mass DNA ladder (see Table 1 for fragment sizes) as our test samples, we compared the derivative plots of a new prototype instrument with the derivative plots of the same samples using the HR-1. Our goal was to further improve melting resolution by applying our prototype's very fine temperature control and extremely uniform and constant heating rates.

Materials and Methods: A diagram of our custom prototype melting instrument is shown in Figure 1, and a cross-section of the optical configuration is shown in Figure 2. This instrument was constructed to obtain extremely uniform heating rates of 0.001-0.05 deg/sec. The 10-12 µl DNA sample was contained in a standard glass LightCycler capillary tube and overlaid with 40-45 µL of mineral oil. The sample was positioned inside a custom-built black Delrin Melting Chamber that keeps fiber optics in alignment and acts as a low-pass filter (T = ~35-40 seconds) for heat fluctuations. The Melting Chamber resides inside a large mineral oil bath. The oil bath was mixed vigorously using a magnetic stir bar and heated using immersion heaters. Fiber optics pierce the oil bath and melting chamber to provide excitation light to the bottom of the capillary and collect emitted fluorescence from the side. Excitation was provided by a 449 nm diode laser, and the emission was filtered through a 484-504 nm band-pass filter (see Figure 3). A thermocouple was placed inside the capillary to measure temperature of the sample, and a thermistor in the oil bath controlled heating rates via a custom LabVIEW software. The analog voltage output of the PMT collecting the LCGreen emission passed through a 1st order RC Low-pass filter (f_c = 2300 Hz) before entering the computer's A/D conversion card. PMT measurements were collected as fast as possible and every 520 measurements were averaged as one fluorescence intensity measurement yielding a final fluorescence samping rate of 10Hz. The fluorescence signal was then filtered through a 5th order Butterworth low-pass filter with a cutoff frequency of 0.4Hz at 0.01 deg/sec, and 2Hz at 0.05 deg/sec. Data analysis and derivative plots were done by custom MATLAB software.

Figure 2

Melting Chamber, made of delrin, keeps capillary and fiber optics in alignment, and acts as a low-pass heating filter (T = ~34-40 secs) that provides a uniformly increasing heating environment for the DNA solution.

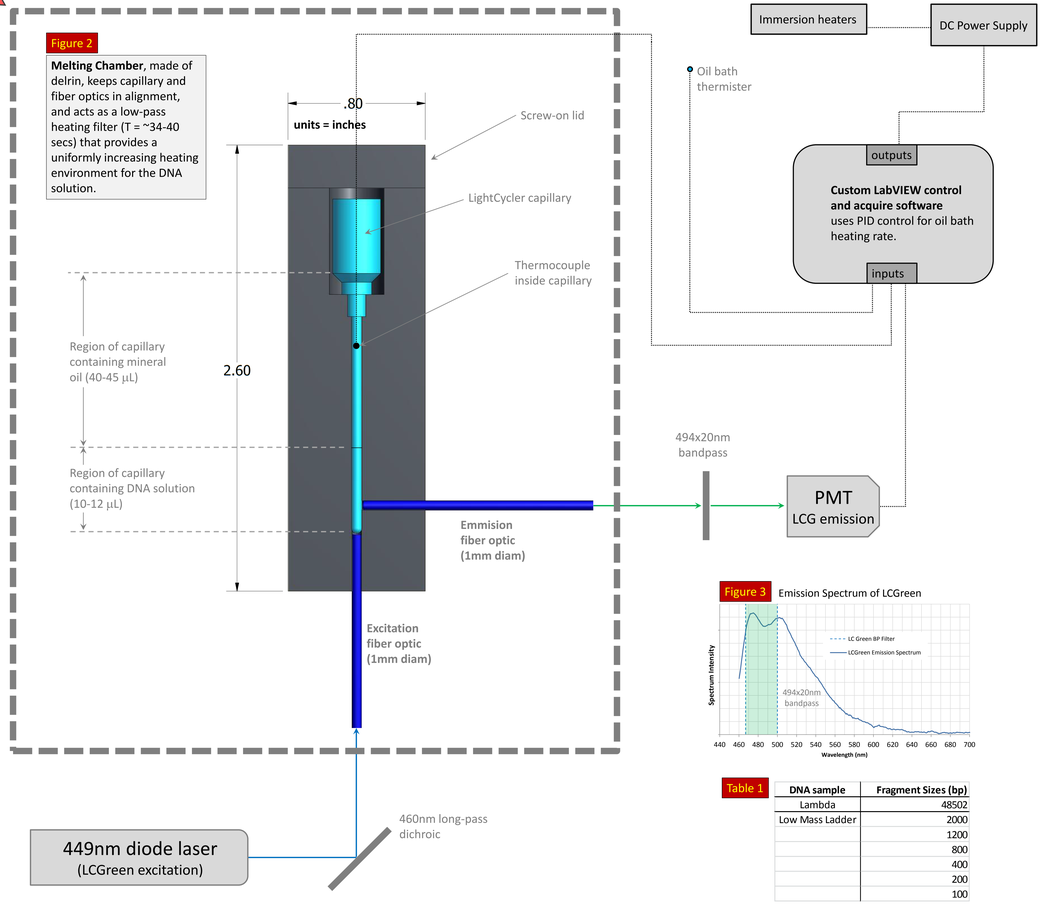


Figure 3 Emission Spectrum of LCGreen

Table 1

DNA sample	Fragment Sizes (bp)
Lambda	48502
Low Mass Ladder	2000
	1200
	800
	400
	200
	100

Results: Figures 4 and 5 show a comparison of derivative curves for the low mass ladder (Fig. 4) and Lambda (Fig. 5) DNA samples. Compared to the HR-1, the derivatives plots of our prototype instrument show additional repeatable melting transitions that are seen as new peaks. Also the reduced width of most of the prototype's peaks imply better fluorescent melting resolution. Figure 6 shows the derivative plot of the Lambda absorption melt curve measured by *Gotoh et. al.* at a rate of 0.003 deg/sec.

Conclusions: Slower heating rates and better temperature control produce derivative melting curves with more peaks and better resolution. This study represents a solid first step in pushing our prototype towards becoming a highly sensitive melting instrument that can improve on genotyping resolution and scanning sensitivity for PCR products.

Figure 4 Low Mass DNA Ladder Melt Comparison between Prototype and HR-1

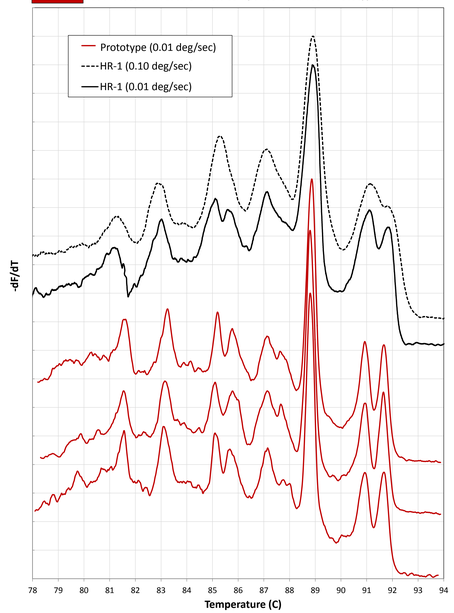


Figure 5 Lambda DNA Melt Comparison between Prototype and HR-1

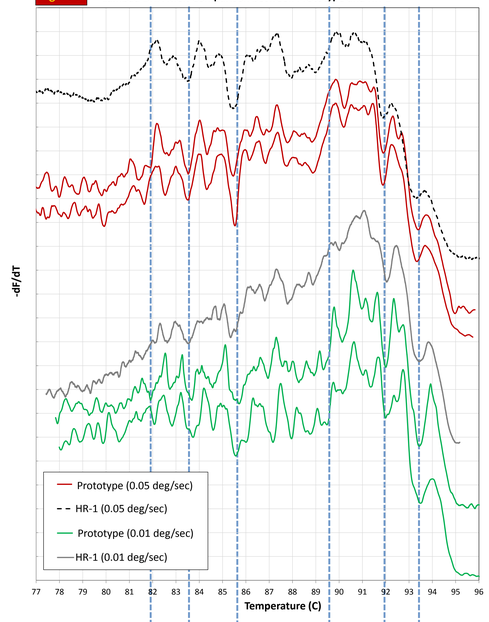
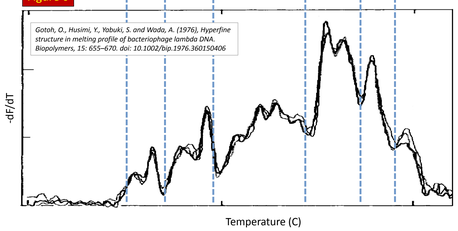


Figure 6



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Gotoh, O., Husimi, Y., Yabuki, S. and Wada, A. (1976), Hyperfine structure in melting profile of bacteriophage lambda DNA. Biopolymers, 15: 655–670. doi: 10.1002/bip.1976.360150406

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