

# Thermal Gradient PCR in a Continuous-Flow Microchip

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## ABSTRACT

A new continuous-flow PCR microchip has been developed that operates by cycling a prepared sample within a spatial temperature gradient. This design allows for minimal thermal residence times – a key feature of the protocols used by the fastest commercial PCR equipment. Since thermal gradients are a natural effect of heat dissipation, the appropriate temperature distribution for PCR can be generated by a minimum of one heater held at a steady state temperature. With a thermal gradient of more than 3°C/mm across the width of the chip, each complete PCR cycle requires approximately 2cm of channel length. These glass chips were manufactured using standard glass microfabrication methods as well as the Xurographic rapid prototyping technique. Targets of 110bp and 181bp were amplified from  $\Phi$ X174 plasmid DNA on these thermal gradient chips as well as on commercial PCR equipment, then subsequently analyzed by gel electrophoresis. Visual inspection of fluorescent images of the stained gels shows that the amplicon size and yield for the systems are comparable.

**Keywords:** PCR, continuous-flow, thermal gradient, DNA, amplification, microfluidics, Xurography, rapid prototyping

## 1. INTRODUCTION

A basic need for researchers and clinicians working with DNA involves gathering information on select portions of often minute biological samples. By creating a large number of identical copies of a small section of DNA, referred to as the target, the signals being monitored (fluorescence, force, absorbance, etc.) become strong enough to be clearly measured by the applicable sensors.

### 1.1 PCR

Under natural circumstances, DNA is replicated by the cell machinery. When living cells divide, biological stimuli prompt the cell to generate an exact duplicate of its entire genomic sequence. Under specific in vitro conditions (thermal, chemical, etc.), similar duplication processes can be forced to occur in rapid succession. The most popular amplification process, called the polymerase chain reaction (PCR), was invented in 1985<sup>1</sup>. For this technique, a single duplication (called an amplification cycle) is performed by causing three distinct reactions to occur in succession within the PCR mixture. First, the double-stranded DNA (dsDNA) must be separated into single-stranded DNA (ssDNA). This phenomenon is called denaturing, or melting, and occurs near a specific temperature (80°-95°C, depending on the properties of the DNA). Once the DNA is denatured, shorter oligonucleotide primers are allowed to hybridize to the ssDNA segments. This reaction, which is referred to as the annealing stage, occurs at a lower temperature (40°-65°C, depending on the properties of the primers). The PCR mixture is then brought to an intermediate temperature (70°-75°C, depending on the properties of the DNA polymerase) while DNA polymerase molecules attach to the annealed primers and construct a complementary DNA strand. In this way, each ssDNA is built into a dsDNA that is identical to the original target. Although this extension reaction occurs over a broad temperature range (~60°-78°C), the temperature which maximizes the activity of the polymerase is typically established as the extension temperature. After the extension, the temperature is increased again to the denaturing temperature to begin another amplification cycle. The total number of cycles needed to achieve a certain increase in concentration depends on the quality and completeness of the three reactions: denaturing, annealing, and extension.

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Since common PCR protocols can require many tens of amplification cycles, the temperature cycling time is very important. In traditional PCR, the surface-to-volume ratio of the sample is relatively low, which impedes the heat transfer between the sample and the sample container. This increases the time needed to reach thermal equilibrium. This, among other factors, has led to the establishment of an “Equilibrium Paradigm”, in which it was understood that the PCR mixture should be heated to each of the three temperatures and held for a specific length of time. Such a procedure is necessary to allow *large* volumes to reach thermal equilibrium sufficient for the corresponding reactions to occur. However, both denaturation and annealing occur very quickly (<1 sec) once the entire sample has reached the required temperatures, and typical extension rates are on the order of 100 bases per second, under ideal conditions<sup>2</sup>. In addition, when relatively small DNA targets are being amplified, full extension can occur during the transition between temperatures, and no hold time is required. Such understanding has led to the development of the “Kinetic Paradigm”, which allows for faster amplification cycles, no loss in amplification efficiency, and superior amplification selectivity<sup>3</sup>. Figure 1 compares the theoretical temperature cycling protocols associated with the two PCR paradigms. Rapid cycling systems founded upon the Kinetic Paradigm are currently used by the fastest of the commercially available PCR systems.

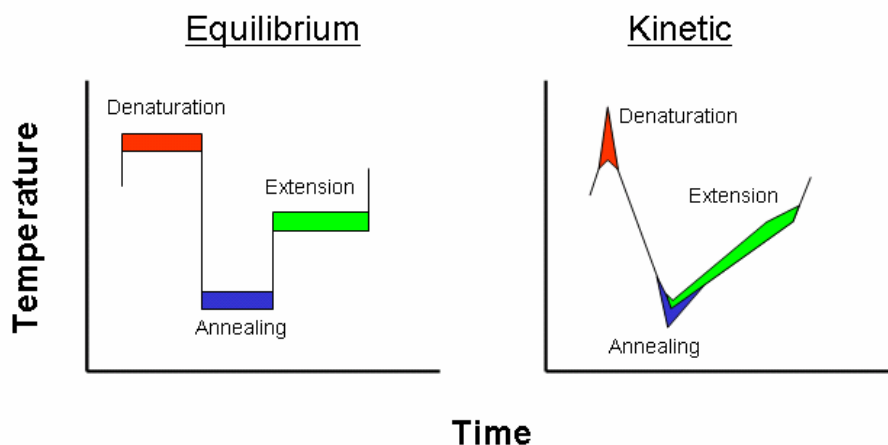


Figure 1: The temperature cycling protocols associated with the Equilibrium and Kinetic PCR paradigms<sup>3</sup>.

## 1.2 Microchip PCR platforms

Due to the PCR’s wide expanse of biological applications and extensive use in so many research and clinical areas, much work is ongoing in both commercial and academic environments to improve the speed and precision of the process. A principal direction in which this research is moving is toward performing PCR in microfluidic chips. Doing so allows for a reduction in the thermal mass of the system and sample, lower reagent consumption, and possible integration with sample preparation processes, heating and cooling elements, and detection systems. The design and operation of microchip PCR can be divided into three principal categories: well-based PCR, shuttle PCR, and continuous-flow PCR.

**Well-based PCR** is performed in a sample-containing chamber that is cyclically heated and cooled to the appropriate temperatures<sup>4,5,6,7,8</sup>. This approach can produce PCR systems with the smallest footprint, but complex closed-loop control instrumentation is required to cycle the PCR sample through the required temperatures<sup>9</sup>. Also, the thermal mass associated with the heater and PCR well limits the achievable thermal ramp rates of the solution<sup>10</sup>.

**Shuttle PCR** refers to techniques in which the thermal cycling is performed by shuttling small plugs of the PCR mixture back and forth between isolated temperature zones<sup>11,12</sup>. The limitations set by the thermal mass of the system are eliminated by designing the temperature variations to occur spatially, instead of over time. The different thermal regions are established through local heating and/or cooling systems that maintain a specific section of the device at a unique steady state temperature. Although shuttle PCR removes the challenges associated with the time-dependent temperature cycling protocols of well-based systems, shuttle PCR introduces technical challenges with respect to the fluid handling<sup>10</sup>, since the transport of the PCR mixture becomes the time-dependent feature of the device.

**Continuous-flow PCR (CF-PCR)** is performed by pumping the PCR mixture at a steady volume flow rate through a microfluidic channel that passes through different temperature regions<sup>13,14,15,16,17,18</sup>. Similar to shuttle PCR, amplification can be performed with a steady-state temperature distribution. The sample transport for CF-PCR becomes trivial, since only a constant positive flow rate is required. The temperature cycling is achieved by fabricating a single serpentine channel that passes repeatedly through the distinct temperature regions of the microfluidic chip. Significant effort has been applied to improve the functionality and feasibility of the original design<sup>13</sup>. Sun et al.<sup>14</sup> have integrated transparent heating elements onto the microfluidic chip to improve the optical exposure of the channel. Obeid et al.<sup>18</sup> have researched various port/interface arrangements on the chip, thus allowing for some cycle number selection. A number of research groups<sup>16,17,19</sup> have included specific features that provide greater thermal isolation between the separate regions and/or to reduce the time the sample spends *between* the established PCR temperatures.

### 1.3 Thermal gradient platforms

Thermal gradients occur naturally as heat dissipates through material. With localized heating, regions in the vicinity of heating elements are hotter, while cooler temperatures exist further from the heat source. When net heat gains are eliminated, a steady state thermal gradient is established. The spatial variance in temperature (linear or higher-order) is a characteristic of the thermal conductivity of the material as well as the arrangement of the several heat sources and drains. Mao et al.<sup>20</sup> have shown that linear thermal gradients can be generated for use with microfluidic chips. Thermal gradients of up to 25°C/mm were shown to be achievable<sup>21</sup>. Cheng et al.<sup>12</sup> used a radial temperature gradient to perform PCR. However, the device was designed such that the PCR sample was only to be shuttled between isothermal regions.

## 2. METHODOLOGY

A microfluidic channel running through a spatial temperature gradient was used to perform PCR, using a novel heating platform and a compatible microfluidic chip. The critical characteristics of the heater include the spatial rate of temperature change (°C/mm) and the overall range of temperatures. Features of the microfluidic chip must allow for an adequate number of thermal cycles, fast cooling rates, and moderate heating rates.

### 2.1 Heating Assembly

The heating of the microfluidic chips is achieved by placing single or multiple heaters (#HR5200, Minco, MN, USA) against the underside of the microchip. Both “centerline” and “edge” heating have been examined. Centerline heating involves placing a single heater down the middle of the chip. In this configuration, the middle of the chip is at the highest temperature (the denaturing temperature) while the temperature decreases to its coolest point at the outer edges of the chip. Heat dissipates to the atmosphere from all exposed surfaces of the chip. For the edge heating scheme, a single edge is held at a high temperature, while the opposite edge is in contact with a heat sink, through which the majority of the heat is drawn from the chip, thus creating the gradient from one side of the chip to the other. Both types of heating platforms were fabricated out of high temperature polymers (acrylic and/or Teflon), to which the heaters and the microchips were attached. Figure 2 shows exploded CAD images of the assembled heater platforms.

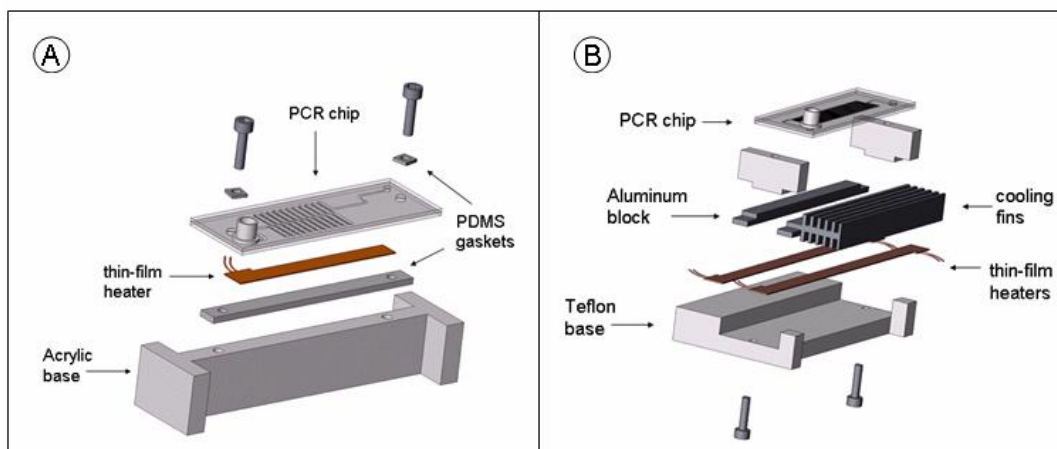


Figure 2: Heating platforms for the A) centerline heating assembly, and the B) edge heating assembly

## 2.2 Chip Design

During PCR cycling, as the sample cools from the denaturation temperature to the annealing temperature, single stranded product begins to form double stranded product, preventing further primer annealing. Therefore, this cooling should be as fast as possible. Since both annealing and denaturing occur nearly instantaneously, the PCR sample should not be held at these temperatures. The heating rate, specifically in the vicinity of the extension temperature, should be moderated according to the size of the product being amplified. Therefore, adapting a linear temperature gradient to an optimum PCR temperature cycle would require either: a) placing the microchannel at different angles in relation to the direction of the gradient, or b) flowing a sample at different velocities within each amplification cycle. This latter result can be achieved by changing the cross-sectional area of the channel within each cycle, as shown in Figure 3. Li et al.<sup>16</sup> have obtained substantial numerical and experimental data to show the effect of cross-sectional area on fluid velocity.

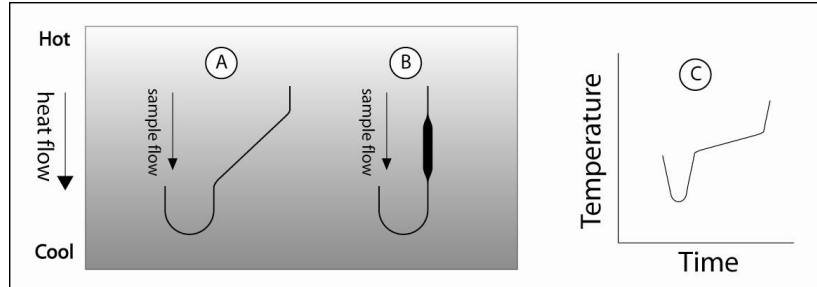


Figure 3: Two different methods to generate variable heating and cooling rates from a constant fluid volume flow rate. Microchannels A and B are placed within a spatial temperature gradient. Channel A is laid at varying angles to the direction of the gradient, while channel B has varying cross-sectional area. Fluid passing through both channels exhibit the same temperature profile, which is represented by graph C.

For reasons of compactness and ease of fabrication, the design of the thermal gradient PCR chip presented here incorporates variations in the channel widths to achieve optimum heating and cooling rates. For simplicity, these designs are such that the channels are wider in the regions where the fluid flow is from the annealing to the denaturing temperatures. Thus, the average heating ramp rate is established by the volume flow rate at the inlet to the device, while the ratio of the heating and cooling rates is determined by the geometry of the channel. In this manner, a constant volume fluid flow rate will produce optimum heating rates combined with rapid cooling. Figure 4 shows the general designs for both the centerline heating device and the edge heating assembly. For centerline heating, each PCR cycle begins in the narrow channel segment at the center of the chip. The sample rapidly cools as it moves toward the outer edge of the chip. The channel's farthest distance from the center of the chip corresponds to the annealing temperature of the PCR sample. As the channel turns and widens, the sample begins its moderate ramp through the extension temperature and to the denaturing temperature, where the one cycle ends and a successive cycle begins. The PCR chip for edge heating operates the same, only with the denaturing and annealing temperatures being on opposite edges of the chip, as explained previously.

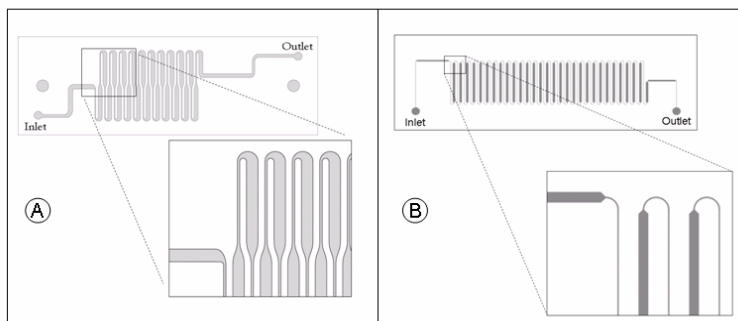


Figure 4: Microfluidic channel geometries associated with A) the centerline heating device, and B) the edge heating apparatus. The masks shown correspond to A) the rapid prototyping technique, and B) the photolithographic etching technique.

### 2.3 Rapid Prototyping

Functional prototypes of the centerline heating chips have been fabricated using the recently developed “Xurographic” process<sup>22</sup>. The “mask” for the fabrication consists of a two-dimensional outline of the channel geometry, drawn to scale in Adobe Illustrator CS (Adobe Systems, Inc., CA, USA). The design is then exported to a cutting plotter (Model #FC5100-75, Graphtec, CA, USA) where a very fine blade traces out the pattern on a double-coated adhesive tape (#9019, 3M, St. Paul, MN) which has a uniform thickness of 25 $\mu$ m (30 $\mu$ m, including adhesive). Tweezers are then used to “weed” the thin film by removing the interior of the cut channel design. The patterned film is then manually aligned and sandwiched between two pre-cleaned glass microscope slides (#12-550A, Fisher Scientific, NH, USA) that have been prepared with pre-drilled holes and Nanoport fluidic interconnects (Upchurch Scientific, WA, USA). The final step involves applying moderate pressure and curing at 65°C for 20 minutes. Using this rapid prototyping method, a single thermal gradient chip can be fabricated in less than one hour. These chips were designed to include 22 amplification cycles. The channel widths were designed to be 1mm and 200 $\mu$ m for the heating and cooling sections, respectively, thus resulting in a 5:1 ratio between the average heating and cooling rates. Including segments for a longer initial denature and final extension, the overall channel length is approximately 25cm, with a total volume on the order of 6 $\mu$ l. The mask geometry is shown to scale in Figure 4-A. The lone circles in the mask design show the location of through-holes used to fasten the chip to the centerline heating platform.

### 2.4 Cleanroom Fabrication

Thermal gradient PCR chips for the edge heating PCR device were fabricated using mature microfabrication technologies. Initially, soda lime glass microscope slides (#12-550A, Fisher Scientific, NH, USA) were pre-cleaned with a piranha etch (3 H<sub>2</sub>SO<sub>4</sub> : 1 H<sub>2</sub>O<sub>2</sub>) for ten minutes. A 900nm thick film of chromium was then sputtered onto the glass blanks. Following the chromium deposition, a 2 $\mu$ m thick layer of photoresist (Shipley, #1813) was spun onto the slides. The photoresist was exposed to UV light through a darkfield photomask, then developed and baked for 1 hour. The geometry of the exposed regions of the photomask is shown in Figure 4-B. After curing the photoresist, the glass was immersed into a chromium etchant to remove the exposed metal. With the cured photoresist and the thin chromium film serving as an etch mask for the top side of the glass, the backside of the glass was protected from the glass etchant with DuPont Kapton tape. The glass slides were then immersed in an etchant bath (1 HF : 3 HNO<sub>3</sub> : 10 H<sub>2</sub>O) for 34 minutes. After stripping off the remaining photoresist and chromium, inlet and outlet holes were drilled through the patterned glass using a diamond-tipped drill bit and drill press. The glass slides were again cleaned in a piranha etch, along with an equal number of blank slides. Each patterned and drilled slide was then fused to a blank slide by baking at 620°C for four hours. Upon cooling, a Nanoport fluidic interconnect (Upchurch Scientific, WA, USA) was attached over the inlet hole of each chip. The chip was designed to have 30 amplification cycles placed within the center 1cm of the chip, as well as a longer initial denaturing time and final extension. The serpentine channel was to be 50 $\mu$ m deep, and having widths of 650 $\mu$ m and 110 $\mu$ m for the heating and cooling regions, respectively. Considering the isotropic nature of the acid etch, these widths would correspond to a heating to cooling ratio of approximately 7:1.

### 2.5 Thermometry

To quantify the temperature profile generated across the PCR chips, it was necessary to measure the temperature extremes in addition to the general gradient shape. Thermocouples (#5SC-GG-K-30-3, Omega Engineering, CT, USA) were affixed over the locations corresponding to the annealing and denaturing regions of the microchannel. In addition, the entire surface of the chip was imaged with an infrared camera (Thermacam PM390, Inframetrics Inc., MA, USA). Since borosilicate glass is partially transparent in the infrared spectrum, a number of sacrificial chips were coated with a flat black Krylon spray paint prior to assembly, thus giving these a known emissivity. Upon assembly of the device, current was applied to the heater(s) using a DC power supply (#E3642A, Agilent Technologies Inc., CA, USA). When the temperature equilibrated, the chips were characterized using both the thermocouple measurements and the IR data. Upon measuring the surface temperatures, it was assumed that the temperature of the glass at the depth of the channel was within 2° of surface temperature. Additionally, it was assumed that any PCR sample flowing through the channel would be within this same margin, as long as the fluid velocity was under 10mm/s. These two assumptions were derived from experimental results previously published by other researchers<sup>11,14</sup>.

## 2.6 PCR Protocol

To demonstrate the capability of these thermal gradient PCR chips, multiple PCR samples were prepared and amplified on the fabricated devices. In addition to the specific DNA template concentration used, each mixture consisted of 0.5  $\mu\text{M}$  of each primer (forward and reverse primers specific to the sequence to be amplified), 200  $\mu\text{M}$  of each deoxynucleotide triphosphate (dNTP), 0.4 U of KlenTaqI polymerase (AB Peptides, MO, USA), 88 ng of TaqStart antibody (ClonTech, CA, USA), 3 mM  $\text{MgCl}_2$ , and 1X LCGreen Plus (Idaho Technology, UT, USA) in 50 mM Tris (pH 8.3) with 250 ng/mL bovine serum albumin (BSA). The samples prepared for the centerline PCR device contained approximately  $10^7$  copies/ $\mu\text{l}$  of a phage DNA as the template ( $\Phi\text{X174}$  RF1, 5386-bp, New England Biolabs, MA, USA). Primers for these samples corresponded to a 110bp segment of the plasmid. The samples for the edge heating device included  $10^5$  copies/ $\mu\text{l}$  of the phage DNA. These samples contained different primer sets, one targeting a 110bp segment, and the other a 181bp region. In addition to being amplified on the gradient chips, portions of each master mix (including negative controls lacking template) were amplified on two commercial PCR systems, the MJ PTC100 Thermocycler (MJ Research, MA, USA) and the LightCycler<sup>®</sup> (Roche, IN, USA). Amplicon from the thermal gradient microchip and the commercial equipment was compared for size and relative concentration using gel electrophoresis.

A 30-cycle microfabricated glass chip was placed in the edge heating assembly, and heated so that the denaturing and annealing temperatures stabilized at approximately 87°C and 58°, respectively ( $\pm 1^\circ\text{C}$ ). Prior to running each PCR sample, the channel was washed and primed. The flow rates during these flush protocols were as fast as possible without risking the delamination of the Nanoport interconnects. The wash protocol consisted of manually injecting 40 $\mu\text{l}$  of 10% bleach through the channel. This was followed by 100 $\mu\text{l}$  injection of deionized water. When this was quickly expelled from the chip, another 50 $\mu\text{l}$  of water was passed through. The channel priming protocol consisted of passing 40 $\mu\text{l}$  of the unamplified PCR mixture rapidly through the channel, followed by 40 $\mu\text{l}$  of water. This priming was done at lower temperatures ( $\sim 75^\circ\text{C}$ ) so that the priming mix would not denature. Sample was then passed through the chip at approximately 1 $\mu\text{l}/\text{min}$  using a syringe pump (#KDS120, KD Scientific, MA, USA). The sample was collected at the outlet and stored in an aliquot for prompt analysis. In this way (repeated wash – prime – amplify), multiple positive (containing phage DNA template) PCR samples of 110bp and 181bp were obtained. To show the lack of contamination between samples, negative (no DNA template) samples were interspersed between the positive runs.

In addition, a 22-cycle Xurographic prototype chip was assembled onto a centerline heating platform for amplification experiments. The voltage applied to the single heater was adjusted until the denaturing temperature (down the center of the chip) and the annealing temperature (the coolest region through which the serpentine channel passes) were established at 95°C and 53°C, respectively. Sample was passed through a newly fabricated chip at a rate of approximately 1 $\mu\text{l}/\text{min}$  and removed in the way explained above. For the Xurographic chip, multiple samples were passed through the chip prior to washing/priming. Following these samples, the chip was washed, primed, and then used for additional amplification experiments. The wash and priming protocols were the same used for the edge heating experiments.

## 3. RESULTS

The microchips were fabricated with both the Xurographic technique and the microfabrication processes. Figure 5 shows photographs of the assembled devices.

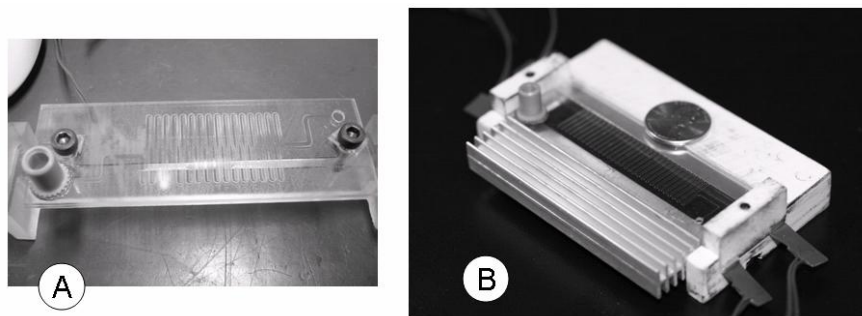


Figure 5: Assembled thermal gradient PCR devices for A) the centerline heating and, B) the edge heating technique

Voltage was applied to the heaters and the systems were allowed approximately 30 minutes to thermally stabilize before measurements were taken with the IR camera. For visualization of the nature of the gradient, whether linear or higher-order thermal decay, the data retrieved from the infrared camera was plotted using Matlab (Mathworks, MA, USA). The resulting pseudo-3D graphs clearly show the profile. Figure 6 shows this data for both the centerline heating assembly and the edge heating device.

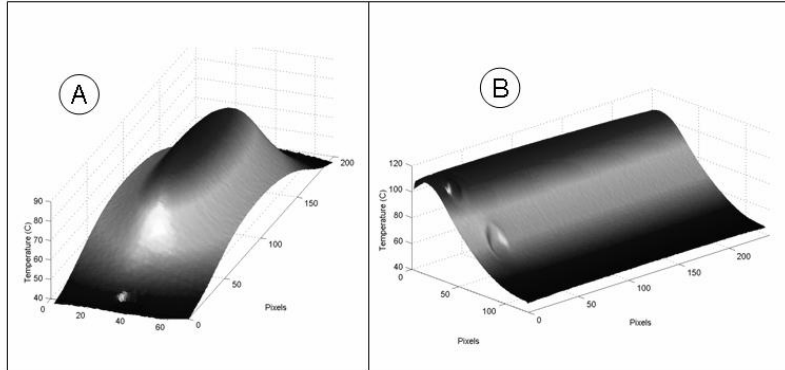


Figure 6: Pseudo 3-D images of temperature data obtained from an infrared camera. The centerline heated chip (A) is heated only partially down the center, resulting in cooler temperatures at the outer edges. The edge heated chip (B) is heated along its entire length, thus providing a more even gradient.

With the thermal gradient devices stabilized at the appropriate temperatures, the PCR experiments were run according to the wash – prime – amplify protocol explained previously. Operating under the designed flow conditions, sample began exiting the edge heated chip after approximately 9:40 (min:sec). Each of the thirty amplification cycles was performed in less than 20 seconds (average). The samples were cooled from the denaturing temperature to the annealing temperature in slightly more than 2 seconds. Heating from the annealing temperature, through the extension temperature, and back to the denaturing temperature took approximately 17 seconds. Local average velocities did not exceed 5mm/s. After removal from the chip, 10µl of the amplified samples were run on a 1.5% agarose gel. Figure 7 and Figure 8 show a comparison between amplicon from the commercial PCR systems and multiple samples amplified on the edge heated glass chip.

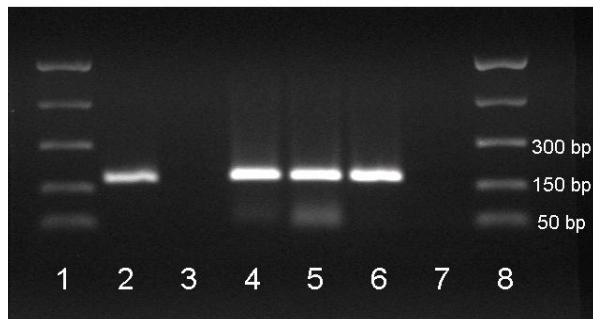


Figure 7: Gel electrophoresis comparing the 181bp product amplified on the LightCycler® and the edge heating thermal gradient chip. Lanes 1 and 7 show the bands associated with a marker DNA ladder (PCR Marker, New England Biolabs, MA, USA). Lanes 2 & 3 show a positive and negative sample amplified on the LightCycler® (30 cycles in less than 8 minutes). Lanes 4-6 show the product from successive runs on the edge heating chip (30 cycles in less than 10 minutes). Lane 7 shows a negative sample that was passed through the same amplification cycle after the samples shown in Lanes 4-6.

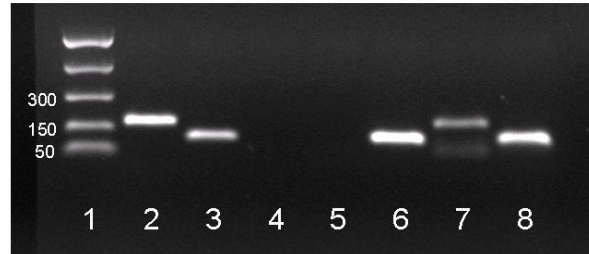


Figure 8: Gel electrophoresis comparing amplicon from the MJ Thermocycler and the edge heating microchip. Lane 1 shows the marker ladder. Lanes 2-4 show a 181bp sample, a 110bp sample, and a negative control sample that were amplified on the MJ Thermocycler (30 cycles in approximately 45 minutes). Lanes 5-8 show a negative sample, an 110bp sample, an 181bp sample, and another 110bp sample that were amplified on the edge heating chip (30 cycles in less than 10 minutes).

The samples taken from the 22-cycle Xurographic chip were amplified in approximately 6 minutes, with each amplification cycle lasting approximately 15 seconds. After removal from the chip, 5 $\mu$ l of the amplified samples were run on a 1.5% agarose gel. Figure 9 shows a comparison between amplicon from the LightCycler<sup>®</sup> and multiple samples amplified on the edge heated glass chip. These chips were found to consistently fail after several runs. Some necking of the channels was observed to occur between sample runs, when the channel was hot, moist, and empty. The glass eventual separated from the adhesive, allowing the sample to exit the sides of the chip.

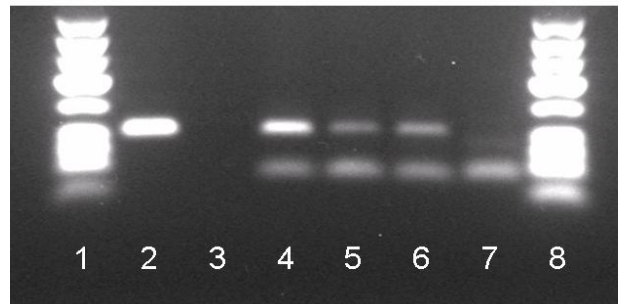


Figure 9: Gel electrophoresis comparing amplicon from the LightCycler<sup>®</sup> and the centerline heater with the Xurographic PCR chip. Lanes 1 & 8 shown the DNA marker ladder, Lane 2 & 3 show the positive and negative samples amplified on the LightCycler<sup>®</sup>. Lanes 4-6 show successive PCR samples with no wash step between. Lane 7 shows a sample that was amplified on the chip after a wash. For lanes 2-7, the primers were selected to amplify a single target having a length of 110bp.

## 4. DISCUSSION

### 4.1 Thermal Gradient Profile

From the comparison shown in Figure 6, the centerline heating device does not have the same temperature uniformity as does the edge heating setup. While the centerline assembly contains only a single heater (0.25" x 2.30") down its spine, the edge heating device has a pair of the same heaters (effectively, 0.25" x 4.60") along the hot edge of the chip. For this reason, the heat can be seen to dissipate from the central region of the centerline chip in all directions, while the gradient in the edge heated chip is exclusively perpendicular to the long edge of the chip. This results in the centerline chip having a limited region where the temperature range is ideal for PCR, so that the PCR cycling must be constrained to this area (see Figure 4-A, Figure 5-A). Alternately, the uniformity of the gradient generated by the edge heater allows for the thermal cycles to fill as much of the chip as the geometry will allow. Within the optimum regions of both heating designs, the temperature decay across the chip was observed to be nearly linear, with thermal gradients slightly larger than of 3°C/mm.

### 4.2 Fabrication Methods

Two fabrication methods were used to produce the microfluidic chips. Small batches of the Xurographic chips can be produced in little more than an hour, only a fraction of the time needed for the typical microfabrication processes. Likewise, the equipment/material cost is at least two orders of magnitude less for the rapid prototyping protocol. However, it has been shown that the wash/prime protocol used did not adequately restore the initial chip conditions, thus resulting in poor amplification specificity (see Figure 9, lane 7). In addition, the observed failure of the Xurographic chips has not been shown to be preventable. The consistent failure mechanism was eventual delamination between the glass and the adhesive, likely caused by the slight flowing of the adhesive between runs. This is indicative of probable gradual rehydration of the cured adhesive. While the Xurographic chips currently do have these operational weaknesses that discourage long-term use, for single-use disposable applications, the low fabrication cost of the chips would be considered advantageous. For applications where a more enduring chip is required, the microfabricated glass microchips can be cleaned sufficiently to avoid cross-contamination between PCR samples. In instances where eventual failure of these microfabricated chips was observed, the mode was that of clogging of the channels. Solutions to this clogging were provided previously by other researchers<sup>18</sup>.

### 4.3 Amplification

The PCR samples amplified by the commercial equipment are seen in the gel electrophoregrams as bright single bands, which indicates the specific amplification of a single product. In every instance, the samples from the edge heated chips contained a similar band as that of the commercial equipment, having similar location and comparable fluorescence intensity. Although a minor number of the samples amplified on the edge heated chips did show an additional band indicative of possible primer dimer formation, these undesirable products can be easily removed by slightly improved experimental precision.

Analysis of the samples removed from the centerline heated Xurographic chip similarly confirms the presence of the desired amplicon. The samples amplified on the fresh chip, prior to washing, were shown to contain significant levels of 110bp product, while samples amplified after the chip was washed and primed contain primarily undesirable product. Although not well understood, this observed "spoiling" of the channel is attributed to the gradual reactivation of the adhesive.

As is expected, the results indicate a possible sensitivity of the larger product (181bp) to perturbations in temperature and/or flow rate. Since the heating rate and cooling rate are inseparably coupled in this chip design, required reductions in heating rates for larger amplicon produce a proportional slowing of the cooling portion of the amplification cycle. Therefore, for DNA targets in excess of 400bp in size, it is anticipated that the overall amplification time would no longer be competitive. However, for the majority of DNA analyses – which involve the amplification of smaller products – the thermal gradient PCR chip would be ideal.

## 5. CONCLUSION

Thermal gradient PCR can be used to amplify DNA targets. The specific benefits associated with the miniaturization of the PCR to the micro-scale can be found in many other works, and will not be summarized here. Specific to this research is the combination of the steady state spatial temperature gradient with a variable-velocity serpentine channel. This system is less complex to build and operate than other microfluidic PCR systems in the following ways:

- The temperature profile of the thermal gradient system is steady state, whereas well-based PCR systems have time-dependant thermal protocols. Eliminating this time domain allows for reduced instrumentation and makes thermal mass concerns virtually irrelevant. The sample flow is also steady state, thus eliminating the need for extensive fluid control systems common to the shuttle PCR approach.
- The thermal gradient, as a naturally occurring condition, is easily shaped and utilized, while shuttle and other continuous flow systems struggle against this phenomenon, seeking to reduce its effect by either incorporating additional insulating features or expanding the footprint of the device. Unhindered by such an obstacle, these thermal gradient devices have characteristically reduced channel length and footprint relative to other continuous flow systems.
- Where other continuous flow and shuttle systems incorporate multiple heating zones, each managed by an independent heater, sensor, and controller, the thermal gradient PCR device can operate with a single heater, having the entire required thermal spectrum forming around this one heat source. Such simplicity makes further advancements more attainable, such as those involving disposability, portability, and parallel processing.

While these new devices overcome certain obstacles, their usefulness may be limited to the amplification of DNA targets less than 400bp in length. Below this threshold, the thermal gradient PCR device possesses an optimum combination of simplicity and versatility.

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