Supporting Information

Fast detection of genetic information by an optimized PCR in an interchangeable chip

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Cycling Temperature, Time, and Cycle Number Considerations

Given our plan to amplify short DNA fragments, we determined to keep the amplification time to the minimum. In fact, high-speed polymerases can amplify a 50 bp fragment in less than one second. Since DNA polymerase is active over a wide temperature range, we are able to merge the extension step (usually performed at 72 °C) with the annealing step to realize two-temperature PCR for microfluidic applications.

Usually in microfluidics, it is desirable to minimize the difference between the T_a and the Td in order to shorten the heating and cooling times. Moreover, a very low T_a might lead to undesirable amplification products. Therefore, the T_a should be kept no lower than usual ~55 °C. Besides, the annealing time can be reduced to a very short span. For the current experiment, we were interested in amplifying short DNA fragments and high-speed polymerases performed extreme high productivity, 20 seconds for the combined annealing/extension step. In addition, it is preferable also to keep the Td as low as possible which allows for a wider choice of material for chip production while the denaturing time is needed to be extended. It is known that a longer denaturing time, certainly, is helpful for amplification of specific DNA from complex human bodily fluids such as saliva as well as the activation of a hot-start DNA polymerase. Based on these considerations, a 20 seconds denaturation step should be sufficient. By minimizing the denaturing time, it is possible to shorten the total assay time even further.

Desiring PCR conditions suitable both for conventional and real-time PCR, we decided to allow the PCR reaction to amplify the DNA, providing most of the expected product without any side products. We chose 35 cycles, as this is enough to amplify even from thousands of molecules for a signal detectable on agarose gel. In

the course of 35 cycles, the DNA polymerase is exposed to 700 seconds (<12 minutes) of high T_d: that is, 3-fold less time than the half-life of ExTaq at 95 °C (35 minutes). The cycle number can be further reduced to shorten the total PCR time, especially for high-template-number PCR or real-time PCR detection.

The final PCR was set up for a T_d of 20 seconds, a T_a of 20 seconds, 35 repetitive cycles, and individual T_d and T_a optimizations for each primer pair. The temperature optimization was done on bench thermocycler using 96 well PCR plates.

Reaction components considerations

Magnesium ions, as enzymatic co-factors, form soluble complexes with dNTPs and promote their hybridization. Magnesium facilitates the annealing of the oligo primer to the template DNA by stabilizing the oligo-template interaction. It also stabilizes the replication complex, which consists of polymerase with a template primer. Overall, Mg^{2+}, included in the PCR mixture, stabilizes dsDNA and raises the melting temperature (T_m). However, excessive amounts of Mg^{2+} lower specificity and can indirectly promote the formation of non-specific products; this effect is roughly comparable to the result of lowering the T_a. Insufficient Mg^{2+}, in contrast, will lead to low productivity. There is also the potential to lose Mg^{2+} ions because of the adsorption by negatively charged walls (such as SiO_2). Therefore, we decided to incorporate into the PCR reaction a higher limit of $MgCl_2$, 3.5 mM, in order to ensure that free Mg^{2+} ions remain available for DNA polymerase.

The dNTP concentration should be balanced in such a way that still-free Mg^{2+} ions remain in the solution. The usual dNTP concentration is between 40 μM and 200 μM. For longer PCR fragments a higher dNTP concentration is preferred. Excessive dNTP concentrations can inhibit the PCR, preventing the formation of product. However, concentrations up to 400 μM for each dNTP have been reported to work adequately.[1]

Therefore, we set the dNTP concentration for amplifying short PCR fragments to 200 μM.

There are a few commercial polymerases with high extension speed. To see if there was any difference in amplification using different DNA polymerases, we tested CMV primer pairs CMV368F & CMV409R (71 bp) and CMV74F & CMV409R (364 bp) with four different polymerases and their corresponding buffers (Supplemental Figure S2) including the normal-speed polymerase ExTaq (Takara Bio Inc.), and the other three higher-speed and hot-start polymerases: Phire polymerase (Finnzymes), SpeedStar HS (Takara Bio Inc.) and KAPA2G (KapaBiosystems). Kapa2G Fast HotStart polymerase is a hot-start polymerase with a recommended one-second total extension time for amplicons <1 kb (speed ~1000 bp/second). SpeedStar HS DNA polymerase is a hot-start PCR enzyme with a speed of 100 bp/second. Phire is a hot-start polymerase with a speed of 50 bp/second. ExTaq is a non-hot-start polymerase with a speed of ~20 bp/second. The T_d was set to 91 °C and the T_a, to 71 °C. As expected, Kapa2G Fast HotStart polymerase produced most of the product. Although the PCR-product amount for the amplified 71 bp DNA fragment was similar between the standard ExTaq and the three high-speed polymerases, the difference for the amplified 364 bp fragment was obvious. Here, more PCR product was produced by the higher-speed polymerases than by ExTaq, which could be explained by the fact that ExTaq is a non-hot-start DNA polymerase.

The optimal amount of polymerase is dependent on the template size and type. We decided to use the recommended by manufacturer 0.025 U/μl amount of polymerase in our experiments. This concentration ensured a clean product with no background.

N, N, N-tri-methyl-glycine (betaine) lowers the Tm of DNA by eliminating the base pair composition (A+T and G+C) dependence.[2] Betaine also acts as an

osmoprotectant for polymerase, thereby increasing its resistance to denaturation. NMR studies have shown that betaine is a common additive in most commercial PCR optimization kits.[3]As a small N-trimethylated amino acid, betaine carries a total net charge of zero and exists in zwitterionic form at neutral pH. For these reasons we decided to include betaine as an addition to the PCR buffer in order to provide a dynamic coating for the channel walls in the chip.[4]

For visualization purposes we also included cresol red in our PCR mix. Cresol red was used in amounts up to 0.2 mM (0.008%) in the final concentration, without any PCR inhibition.

Figure S1. Oligo priming at CMV sequence. The selected primer pair (CMV368F and CMV409R) generates PCR product of the size of 71 bp.

Figure S2. DNA polymerase comparison. E stands for ExTaq, P - Phire, S - SpeedStar HS, K - Kapa2G Fast HotStart. While there is no obvious advantage of high-speed polymerases for the amplification of short (71 bp) PCR fragment (with the extension time of 20 seconds), the advantage of high-speed polymerases is seen over amplification of longer (primer pairs CMV74F & CMV409R for 364 bp product) PCR fragment.

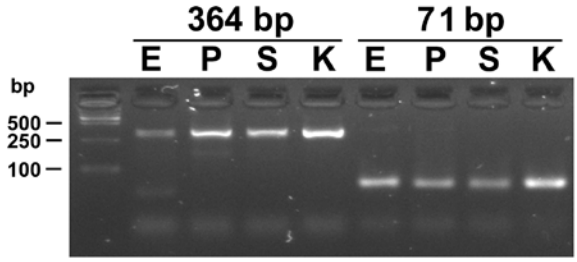


Figure S3. Amplification strategy for the SRY gene. The selected primer pair (SRY93F and SRY93R) generates PCR product of the size of 93 bp.

Figure S4

(A) The partial view of Pt thermal sensor was enlarged from black dash rectangle of top view;

(B) The chip holder was constructed by PMMA (Poly (methyl methacrylate)) planes with different thicknesses. The heater chip was mounted on upper layer of the holder. A syringe needle was fixed underneath the heater chip and used for compressed air inlet. When performing PCR, the PCR chip was attached tightly on the top-side of heater chip which can be realized by screwing them each other with two press plates.

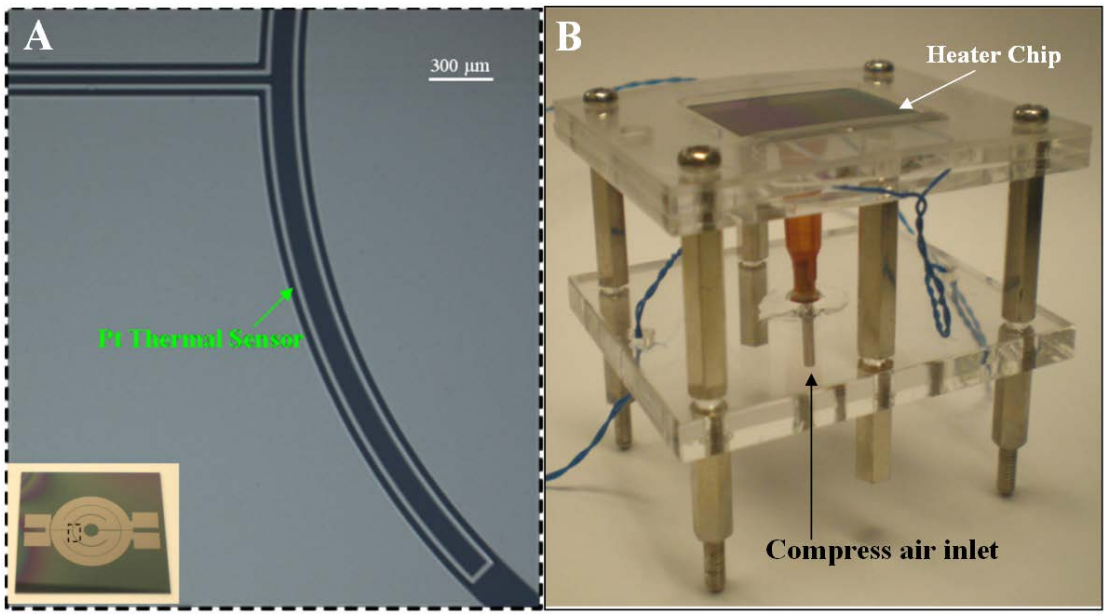


Figure S5

(A) The state machine composes of two states (I and II) as illustrated in supplemental figure S5A. The state I is for 20 second denaturation step (86 ºC); the state II is for 20 second annealing step (67 ºC). When we start the program, the state machine sets

target temperature to 86 ºC, the PID controller automatically heats up the temperature to 86 ºC. Once $T_{ms} = T_s$, it begins 20-second countdown and keeps T_s=86 ºC. Then it goes to state II until finishing countdown. The state machine repeats the process except that T_s=86 ºC and when it finishes countdown, it adds one to total cycle and then compare the total cycle to 35. If the total cycle = 35, the state machine stops. Otherwise it goes to state I.

(B) Pt thermal sensor and heater are integrated together on the topside of heater chip for real-time temperature monitoring and heating generation, respectively. When an electrical current is applied to the heater, the temperature rise affects the electrical resistance of the nearby thermal sensor. This electrical resistance variation can be precisely calibrated to determine the corresponding temperature, which can be seen in Figure S5B. From Figure S5B, the resistance of Pt thermal sensor shows a very good linearity to temperature in the whole range (25-100 °C, correlation coefficient r = 0.9998). The linear property of the Pt thermal sensor can be fitted to the following equation:

$$R = R_o[1 + \alpha(T - T_o)]$$

Where R (Ω) is the resistance of the thermal sensor at temperature T (°C), R0 (Ω) is the resistance of the thermal sensor at initial temperature T0 (°C) and α is the temperature coefficient resistance (TRC) of the Pt thermal sensor. By linear fit of the data in Figure S5B, the TRC of the Pt thermal sensor is found to be 3.09×10-3 °C-1. Once calibrated, the Pt thermal sensor can used to measure the temperature of PCR chamber *in-situ*, based on its own resistance In order to know resistance-temperature relationship, we calibrate our Pt thermal sensor with a commercial 450 μm K-type junction thermocouple. The Pt heater's temperature is different from PCR chamber's since there are two layers of Si between them. Therefore, instead of the Pt heater, we

should measure and calibrate the temperature in the PCR chamber. To do so, the junction of thermocouple was inserted into PCR chamber thought a hole drilled in the centre of glass layer in a dummy PCR chip. Then we applied different currents to the heater and recorded the temperature of thermal couple and resistance of Pt thermal sensor. The data was plotted in Figure S5B.

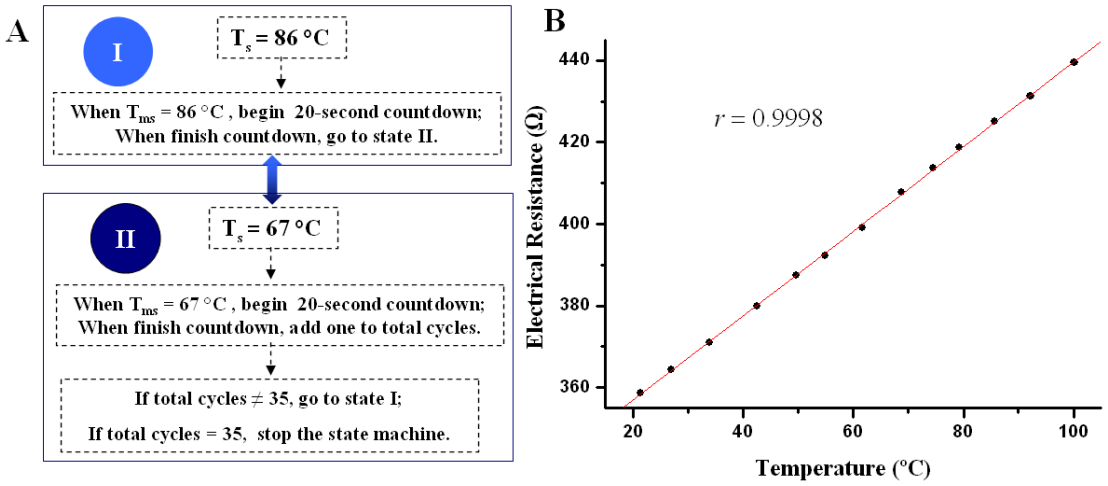
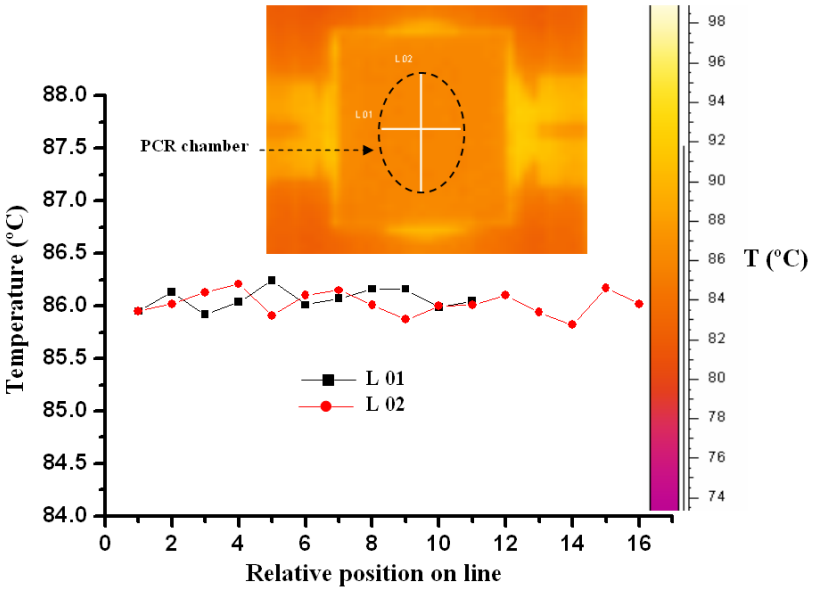


Figure S6

To observe the thermal distribution across the PCR chamber area, an infrared IR camera (FLIR Systems, Thermovision A40) was employed to detect both the heat image and the local temperatures. In figure S6, the upper panel is an infrared image showing the thermal distribution of the PCR chip at 86 °C. The lower plot is the local temperature along two lines (L o1 and L 02). The average temperature of two lines is 86°C and the temperature variation is less than 0.3 °C. The thermal distribution round the PCR chamber is uniform and no distinct temperature difference is found from center to edge.



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