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Fabrication of a 3D Microreactor Utilizing a Screw and Its Application in a Continuous Polymerase Chain Reaction

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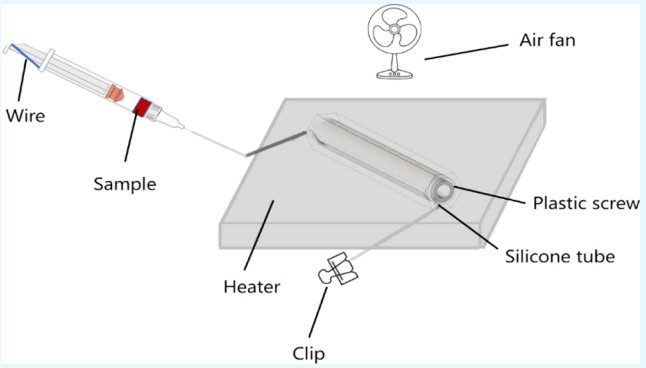
ABSTRACT: A simple and low-cost strategy for a three-dimensional (3D) helical microreactor was introduced by wrapping a thin silicone or poly(vinyl chloride) tube around a plastic screw mold. Through this method, the helical shape of the 3D microreactor was fabricated and complicated traditional processes were eliminated. After the investigation of the continuous polymerase chain reaction (PCR), a simple microreactor with a novel component was successfully fabricated; such a method could be convenient for designing a miniaturized PCR device.



INTRODUCTION

With the advent of "micro total analysis systems", microfluidics has been regarded as a promising technology applied in biotechnology and biochemistry, depending on its advantages of low cost, high sensitivity, and portability.[1−3] The flexible patterns obtained from traditional methods (e.g., photolithography and replica molding) and widely used materials (e.g., poly(dimethylsiloxane) (PDMS) and plastic) are the two contributions for the development of microfluidics.[4−7] To date, the underlying disadvantages of traditional methods are slowly being discovered, since more microreactors with flexible shapes and sizes are expected to increase efficiency and superb three-dimensional (3D) systems are expected to be designed as an alternative.[8−10] Numerous microreactors, such as 3D helical microchannels, have been fabricated using the new 3D method.[11,12] Nevertheless, these 3D fabrication systems are time consuming and fail to meet the requirements for designing special forms of microreactors.[13,14]

To eliminate the obstacles hindering the development of microfluidics, novel methods and materials have been developed. Maltezos et al. constructed a new 3D microfluidic device by molding perfluoropolyether onto printed wax molds, which exhibited strong resistance to the most organic solvents.[15] Lee et al. constructed a portable 3D microreactor by wrapping a poly(tetrafluoroethylene) (PTFE) tube around PDMS, exhibiting excellent stability of sample transport inside a microchannel over 2 m long.[16] Moreover, in the previous study conducted by the investigators, a size-controlled seamless 3D microreactor was fabricated using a silicone tube and paraffin mold and the single commercial hot plate largely decreased these bulky heating accessories.[17−19] Taking full advantage of the vertical direction and artificial shapes, a simple approach could be explored for the fabrication of the miniaturized polymerase chain reaction (PCR) device. Thus, compared with the traditional system, the advantages of the 3D microreactor were highlighted, particularly for informing the design of the microreactor in fulfilling different requirements.

PDMS, which has emerged as an inexpensive polymer, is largely used in manufacturing microfluidic devices.[20−24] It has been well-accepted that it has the highest flexibility, porosity, and resistance to corrosion under most organic solvents.[25] These advantages are useful for increasing the efficiency and economics of microreactors, allowing various types of PDMS-based PCR devices to be successively designed.[26,27] However, these satisfying factors are also responsible for its diffusion properties of vapor and liquid, which result in the unexpected loss of reagents during the PCR process.[28,29]

On the basis of the above surveys, the key factors of cost-effective devices are a simple operating system with minimized accessories and appropriate materials and these are important for the temperature control and stable operation of PCR platforms.[30] Thus, a plastic screw was introduced in the present study, which exhibited superb properties of a stable spiral shape and was inexpensive and resistant to corrosion (Table 1).

Considering the dominating role of the pump in the microreactor, the silicon and poly(vinyl chloride) (PVC) tube with good gas permeability were chosen as the main assembly

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Table 1. Different Types of PCR Microsystems

references	materials	process	advantages	disadvantages
the present study	screw, silicone tube and PVC tube	tube wrapped around the mold	low cost simple fabrication single heater	the diameter of the screw needs to be controlled
17	silicone tube and paraffin	tube wrapped around the mold	simple fabrication single heater fast reaction (30 min)	the height of the microdevice needs to be controlled
22	PTFE−PDMS	tube wrapped around the mold	simple fabrication single heater stable flow rate	the height of the microdevice needs to be controlled
35	PDMS−glass	photolithography	single heater multiplex PCR fast reaction (<25 min)	complicated fabrication the curvature of the microdevice needs to be calculated
19	PMMA−PC	CO_2-laser ablation	improved heat tolerance rapid fabrication low cost	complicated temperature control system a high temperature needed for the bonding of PMMA and PC (165 °C, 30 min)
34	PDMS−glass	photolithography and soft lithography	amplification of large size (580 and 1450 bp) DNA fragments	complicated temperature control system (six pairs of heaters and sensors)

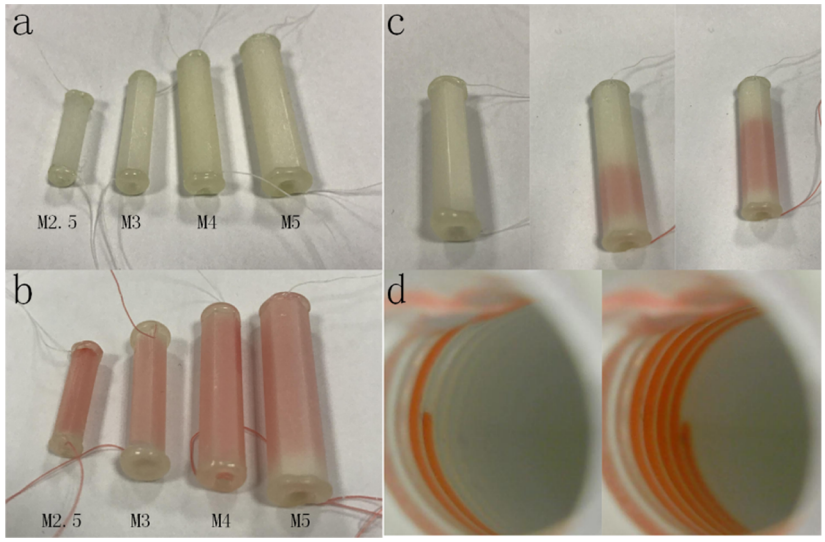

Figure 1. Various 3D helical microreactors were fabricated by utilizing silicone tubes wrapped inside the plastic screws. (a) Plastic screws with different diameters (2.5, 3.0, 4.0, and 5.0 mm) and the corresponding silicone tubes (1.5 mm ID/2.5 mm OD, 2 mm ID/3 mm OD, 2 mm ID/4 mm OD, and 4 mm ID/5 mm OD). (b) Self-actuation of red ink solution in the microreactors. (c) The red ink solution flowed through the silicone tubes. (d) Cross section of the microreactor.

part.[31,32] As shown in the previous study of the investigators, the air diffusion rate was changed between the posterior position near the sample plug and the anterior end of the position. It was the faster diffusion rate at the anterior end that generated the pressure gradient to propel the samples to the outlet. In the present study, this mechanism was used and both tubes were wrapped around the screws, exhibiting the helical route of the sample. The inside air molecules could diffuse through the tube to the environment and result in the driving force for the sample fluxion. In addition, the speed of the fluid would be reduced as the pressure gradient gradually decreased. Since the circular interface of the tube could attain enough temperature of nucleic acid denaturation and the long circulation path around the screw, this pumping was perfect enough to propel the sample through the continuous PCR process. Using this system, the problem of the redundant hot plates needed in the planar surface of the traditional system was resolved,[33,34] minimizing complicated accessories.

RESULTS AND DISCUSSION

Microreactor Fabrication and Continuous-Flow Demonstration. Figure 1a shows the different diameters (2.5, 3.0, 4.0, and 5.0 mm) of plastic screws studied in devices and the corresponding sizes of the silicone tubes (1.5 mm ID/2.5 mm OD, 2 mm ID/3 mm OD, 2 mm ID/4 mm OD, and 4 mm ID/5 mm OD). The simple 3D helical microreactor with different sizes and shapes could easily be fabricated using the screw, solving the disadvantages of complicated procedures that exist in the traditional method. Finally, the Teflon tubes were connected into the inlet and outlet position and the completed device was examined with red ink solution (Figure 1b−d). As for the other type of microreactor, the PVC tubes

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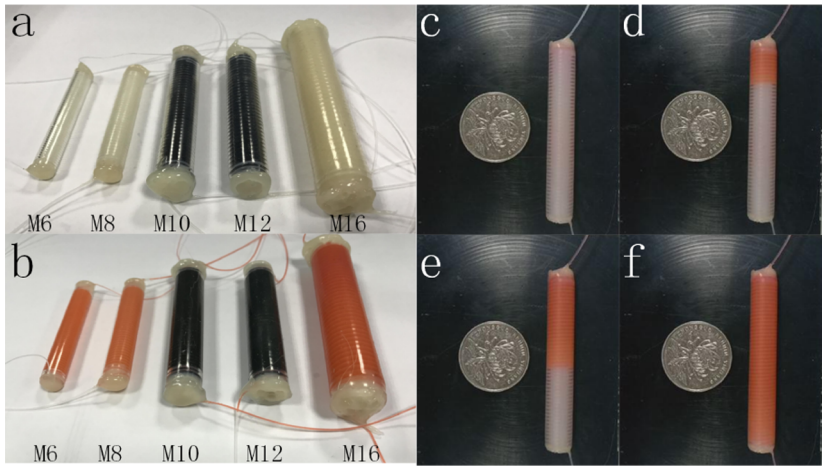

Figure 2. Various 3D helical microreactors were fabricated by utilizing PVC tubes wrapped outside the plastic screws. (a) Plastic screws with different diameters (6, 8, 10, 12, and 16 mm) and the corresponding PVC tubes (5 mm ID/8 mm OD, 6 mm ID/8 mm OD, 9 mm ID/12 mm OD, 10 mm ID/12 mm OD, and 14 mm ID/16 mm OD). (b) Self-actuation of red and black ink solution in the microreactors. (c–f) The red ink solution flowed through the PVC tubes.

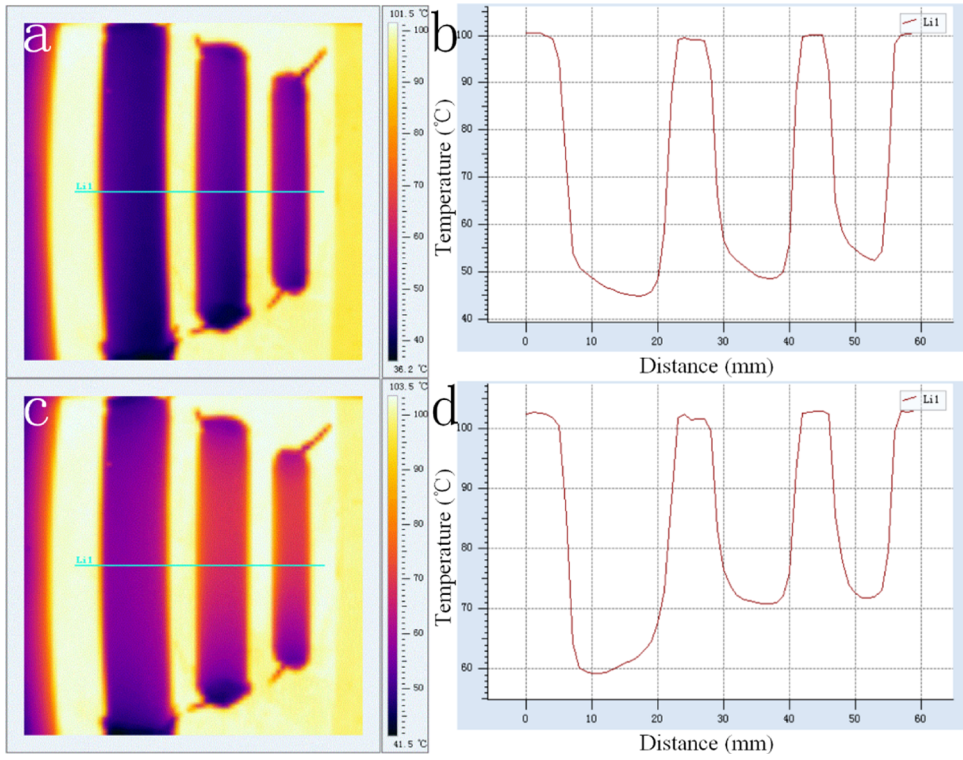

Figure 3. Results of temperature measurements under the actual heating condition: (a) the infrared (IR) camera photo of a PVC tube wrapped outside the plastic screw with different diameters (8, 10, and 16 mm) and (b) the temperature tendency of the microreactor under the heating condition with an air fan. (c) The IR camera photo of a PVC tube wrapped outside the screw with different diameters (8, 10, and 16 mm), and (d) the temperature tendency of the microreactor under the heating condition without an air fan.

(5 mm ID/8 mm OD, 6 mm ID/8 mm OD, 9 mm ID/12 mm OD, 10 mm ID/12 mm OD, and 14 mm ID/16 mm OD) were wrapped outside the screws (6, 8, 10, 12, and 16 mm) (Figure 2a) and the consistency of the microchannels was also examined with red ink solution (Figure 2b–f). Both types of the microdevices revealed the perfect seamlessness of fluid flow and the novel shapes of the 3D microchannel.

Temperature Control of 3D Microreactors. From the two types of fabricated 3D microreactors, the microreactor of the PVC tube wrapping outside the plastic screw was chosen for the continuous PCR process. In the present study, the two-temperature PCR for deoxyribonucleic acid (DNA) amplifications was introduced, which was directly connected with temperature control.[35] The single hot plate maintained at 99 °C was sufficient enough for the heating supply. The PVC tube wrapped at the bottom of the device was directly connected with the hot plate, and the temperature of the denaturation was controlled at 94.2 ± 0.5 °C (CV = 0.2%, n = 10). Moreover, the tube at the top of the device would decrease the temperature to continue the annealing/extension process,

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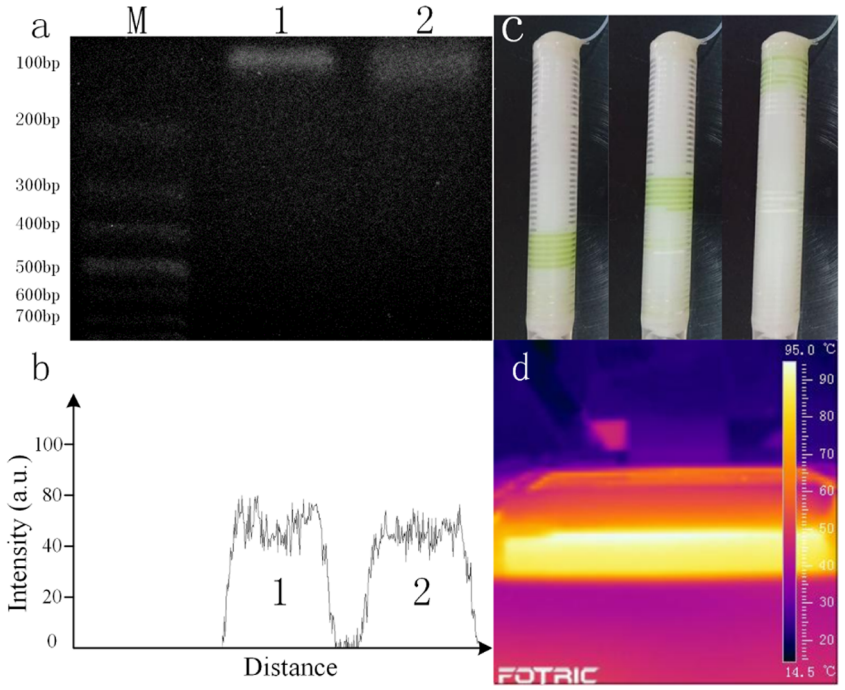

Figure 4. Results of the continuous PCR process conducted in the microdevice. (a) The DNA amplification result. Lane M was the DNA marker with a 99 bp size. Lanes 1 and 2 are the results obtained from the thermal cycler and microreactor, respectively. (b) The image shows the relative intensity of targeted genes amplified by the thermal cycler and microreactor and analyzed using ImageJ software. (c) The plastic screw with a diameter of 8 mm was chosen as the suitable component, and the corresponding PVC tube (6 mm ID/8 mm OD) was wrapped outside the plastic screw. (d) The side view of the temperature distribution obtained from the IR camera.

owing to the long distance from the hot plate. However, the temperature was too high to sustain the normal denaturation process (Figure 3c,d). Thus, an air fan (12 V, 2.4 W) was added and located 15 cm from the microreactor to keep the temperature below approximately 55 °C (Figure 3a,b). Moreover, the appropriate temperature could be confirmed by adjusting the diameter of the plastic screw (i.e., 8, 10, and 16 mm), since the temperature decreases in a diameter-dependent manner (Figure 3). Therefore, the portable microreactor that consisted of a PVC tube and plastic screw could be easily employed in the PCR process, with a single hot plate, air fan, and inexpensive materials. In this method, a plastic screw with a diameter of 8 mm was chosen as the most suitable component and the temperature of the annealing/extension could be controlled at approximately 56.4 ± 0.8 °C (CV = 0.8%, n = 10).

Continuous-Flow PCR. On the basis of the thorough flow examination of the ink solution, the continuous PCR was conducted using the fabricated microreactor. Then, the mixture of 20 μL of the sample plug was introduced in the system using a disposable plastic syringe. As shown in Figure 4, the DNA amplification results conducted in the microreactor were compared with those in a thermal cycler. A total of 42 cycles was counted after running through the whole fabricated microdevice, and the total consumed running time was 64 min. The obtained results are presented in Figure 4a, in which lanes 1 and 2 exhibit the 99 bp gene amplified from the thermal cycle and microdevice, respectively. Furthermore, the relative intensity of the targeted gene obtained from the microdevice was 88.6% of that obtained from the thermal cycle. Figure 4c shows the real-time position of the sample according to the green-colored solution, revealing the stable fluid flow in the microdevice. Furthermore, the side view of the temperature in the microdevice, which was heated under the actual condition, revealed the successful temperature control with a single hot plate and air fan. In this method, a completed microdevice with simple component and novel materials was readily established and this largely reduced the complicated accessories introduced in the traditional method. Furthermore, the improvement of the process could easily be adjusted by changing the size of the plastic screw.

CONCLUSIONS

In summary, a simple approach for designing a 3D microreactor was introduced by using a silicone or PVC tube wrapped around a plastic screw. Compared with the traditional microfluidic system, this novel technique constructed a 3D microreactor with only a single hot plate and the temperature of denaturation and annealing/extension could be adjusted by changing the size of the plastic screw. The air, which diffused from the gas-permeable tubes to the atmosphere, could drive the fluid flow through the microchannel, highlighting the advantage of self-actuation. The flexible shape of the microdevice could be altered by changing the size of the screws and tubes, overcoming the laborious fabrication that exists in the traditional method. Notably, the components used in this system, including the microreactor and operation accessories, were manufactured using commercially available laboratory commodities. Moreover, the external apparatuses, which consisted of an air fan, a syringe pump, and hot plate, were inevitably needed in the continuous PCR process. Thus, it was considered that this method could develop a new direction for fabricating portable and miniaturized microdevices.

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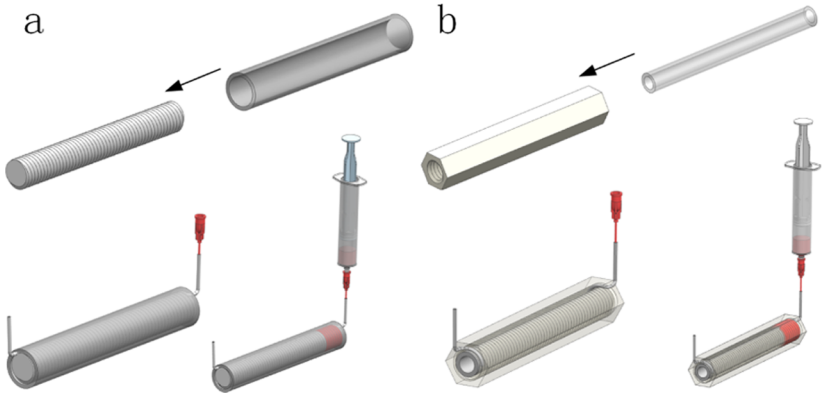

Figure 5. Schematic illustration of fabricating the 3D microreactor with a tube and plastic screw. (a) The PVC tube was wrapped outside the plastic screw. (b) The silicone tube was wrapped inside the plastic screw.

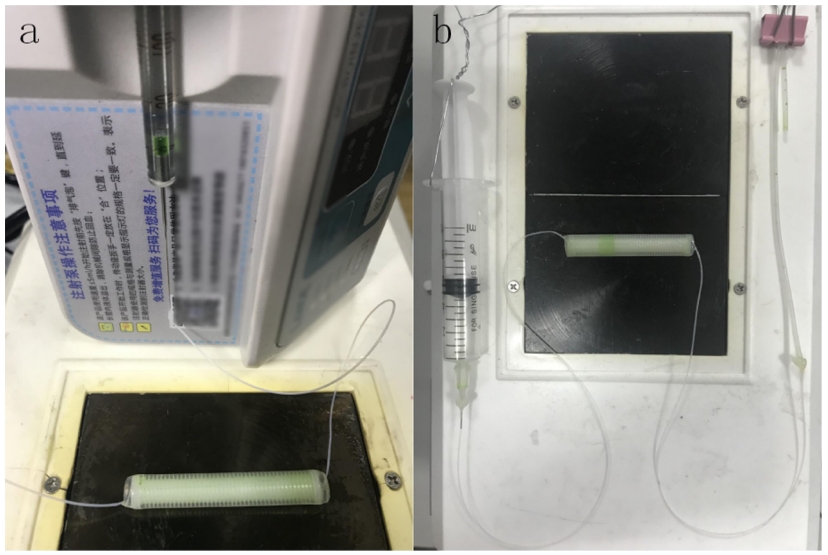

Figure 6. Photos show the two different sample injection methods.

METHODS

Fabrication of Microreactor. The process of fabricating a 3D helical microreactor was easier by using a plastic screw, when compared with traditional methods, such as photolithography. In the present study, both tubes were strictly wrapped around the screw thread, depending on their flexibility, and the surface of these tubes also provided a large potential for air diffusion. Different diameters of screws and different combinations of tubes and screws were fabricated. As shown in Figure 5, the process of fabricating the 3D microreactor by utilizing a tube and plastic screw and two different winding patterns was exhibited. The silicone tube was tightly wrapped inside and around the thread of the screw by virtue of an iron wire, and the PVC tube was tightly wrapped outside and around the thread of the screw. The final fabrication was the collection of tubes and plastic screws.

Sample Injection for a Microreactor. For continuous-flow PCRs, the sample was transported inside the screw channel that is placed on top of the single heater with a constant temperature. As shown in Figure 6a, when the sample was transported by a syringe pump, the mineral oil (M8410, Sigma-Aldrich, MO) was first introduced into the glass syringe. Then, a 20 μL sample was loaded into the glass syringe and connected to the inlet of a microreactor. The flow of a syringe pump was adjusted to form the persistent flow rate, with the samples collected from the outlet. And each cycle of the flow running through the tube was controlled to 80 s.

As shown in Figure 6b, when the sample was transported by the self-activated micropump, a 12 cm long hollow silicone tube (ID = 1 mm, OD = 2 mm) was connected to the outlet of the microreactor, which is then blunt-ended by a clip. Then, the inlet of the Teflon tube was connected to a 27G needle and combined with a disposable syringe (HD, Jiangxi, China). For flow automation, the position of the syringe (containing PCR reagent) was pushed from the initial calibration of 8−5 mL. The air in the syringe was thus compressed to guarantee a persistent flow rate inside the microreactor.

Reagents. The PCR reagent contained 1× Premix Taq (Ex Taq version 2.0 plus dye), 0.5 μM forward and reverse primers, and PCR template. The commercially available pGEM-3Zf (+) plasmid vector (Promega) was used as the PCR target and diluted to the final concentration of 10^7 copies/μL in the PCR reagent. The primer sequences of the targeted gene were as followed: 5′-CCA GTC GGG AAA CCT GTC GTG CC-3′ (forward) and 5′-GTG AGC GAG GAA GCG GAA GAG CG-3′ (reverse). Agarose powder (V900510; Sigma-Aldrich, MO), 0.5× TBE buffer (PH1755, Phygene), and Nucleic Acid Gel

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Stain (KeyGEN BioTECH, Nanjing, China) were used for agarose gel electrophoresis.

Temperature Measurement. An infrared (IR) camera (Fotric 220, ZXF Laboratory, TX) was used for measuring the heater temperatures during the continuous-flow PCRs. The diameter of the screw was used for controlling the annealing/extension temperatures on the top surface of the microreactor. Ten spots were randomly chosen, and the variable coefficients of temperatures were estimated. For guaranteeing the most proper cycling conditions, an air fan can be used to provide air cooling.

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Notes

The authors declare no competing financial interest.

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