# DEVELOPMENT OF THE POCT-ORIENTED PCR DEVICE DRIVEN BY CENTRIFUGATION ASSISTED THERMAL CONVECTION

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## ABSTRACT

We have developed the Point-of-Care Testing (POCT)-oriented polymerase chain reaction (PCR) device which is driven by centrifugation assisted thermal convection. The PCR solution could be driven by thermal convection and continuously exchang high/low temperatures in a ring structured micro-channel without the use of typical syringe pump. The flow rate was controlled by the relative gravitational acceleration of the centrifugation device attached to the bottom of the micro-channel. We have successfully achieved the on-chip DNA amplification of β–ACT from 120 pg human genomic DNA within only 10 min at 5G. Moreover, we have succeeded the on-chip PCR by direct injection of the buccal cell solution to the device which was pre-loaded with PCR solution without template DNA. This result indicated that the easy and rapid PCR could be done without sample pre-treatment and regardless of the technical level of the users.

**KEYWORDS:** PCR, POCT, Thermal convection, Centrifugation

## INTRODUCTION

Rapid and easy POCT devices have been playing an increasingly prominent role in the infectious diseases diagnosis as well as in the immune chromatography kits. On the other hand, PCR-based genetic testing has found its applications in many fields such as detection of cancers, infectious diseases, prenatal identification of fetal alleles and genetically modified foods. Variety of PCR device technologies have previously been developed, not only conventional PCR using sampling tube and thermal cycler, but also the continuous micro fluidic PCR [1], the thermal convection PCR [2], etc. However, these techniques require complicated sample preparations and trained users for using on-site. Herein, to overcome such strict requirements, we utilized Bénard convection which includes gravity-dependent term in our proposed device. It means that the thermal convection speed can be controlled by changing the gravitational force field to the centrifugal field i.e. relative gravitational acceleration (rpm) (Figure 1). As a result, it was expected to accelerate PCR time by the improvement of thermal exchange time. Moreover, the injection of sample and the mixing with PCR solution which was pre-loaded into the micro-channel could be possible.

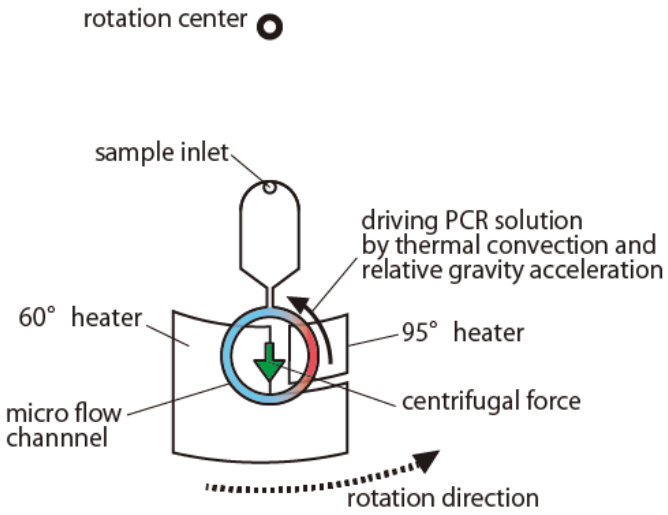


*Figure 1. Schematic illustration of the micro flow chip PCR driven by thermal convection under controlling relative gravity acceleration.*

## EXPERIMENTAL and RESULT

PDMS/glass micro-ring structured channel chip is 500 μm width, 400 μm depth channel and 6 mm diameter (Figure 2a). We fabricated the centrifugal and thermal device in which the ring shaped heaters (95 degrees C and 60 degrees C) are attached to the heater stage and connected to DC rotation motor. Each ring heater has 4 projections to contact chip's micro-channels. The distribution of these portions was shown in figure 1.

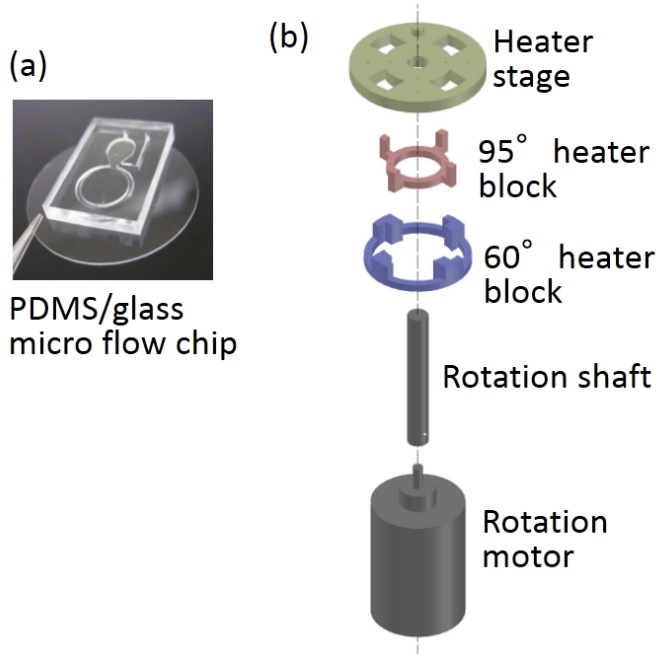


*Figure 2. (a) Fabricated PDMS/glass micro flow chip. (b) Device configuration.*

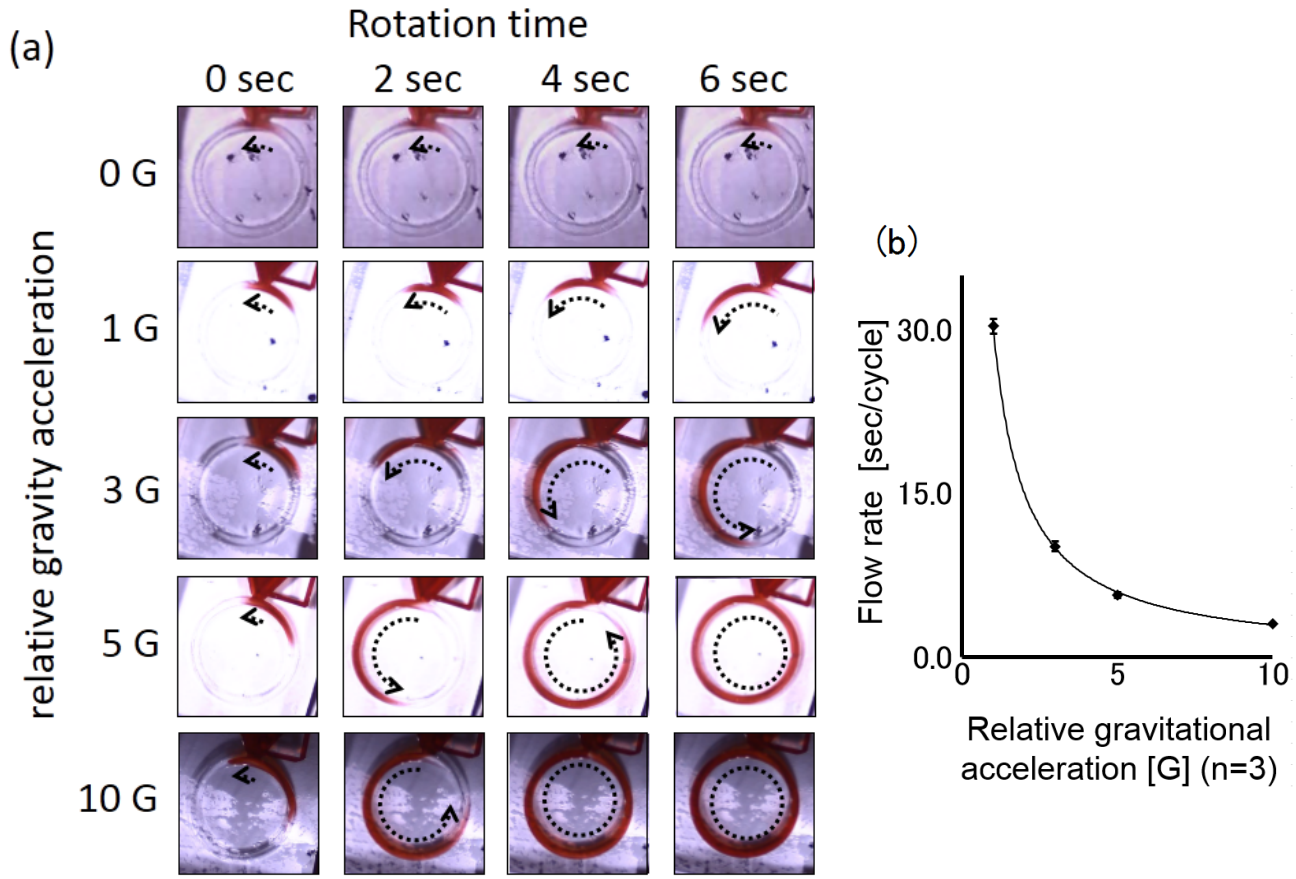


*Figure 3. Relation between thermal convection speed and relative gravitational acceleration. The fabricated chip which was filled with water (clear) and New Coccine solution (red), was set onto the heater stage, then rotated at various relative gravitational acceleration (0-10 G) with heating. (a) The motions of thermal convection in the chip's micro-channel were observed by high speed camera (nac imaging, HX-3). (b) Flow rate was calculated by measuring the time of the leading New coccine solution for one cycle.*

Figure 3 shows the motion of thermal convection with heating and centrifugation. The motion of thermal convection of fluid took about 30 sec for one cycle at 1 G. The fluid speeds increased by increasing relative gravitational acceleration and took about 6 sec at 5 G for one cycle.

The on-chip DNA amplification at each relative gravitational acceleration was carried out by constructed system. Target DNA was human β-ACT (ABI, #401846, fluorescence probe: FAM) and template DNA was human genome DNA. As a result, the target gene of human β–actin successfully amplified 12 ng human genomic DNA in 10 min and was mostly amplified at 5G. Then, we have estimated the initial concentration dependency of genomic DNA as a template DNA. As shown in figure 4a, fluorescence intensity increased with increasing initial template DNA concentration and we successfully obtained calibration curve. Detection limit was 120 pg human genomic DNA.

We also successfully achieved the on-chip PCR by direct injection of the buccal cell solution which was taken from mucosa of the oral cavity, to the device which was pre-loaded with PCR solution without template DNA. This result indicated that the easy and rapid PCR could be done without sample pre-treatment and trained users.

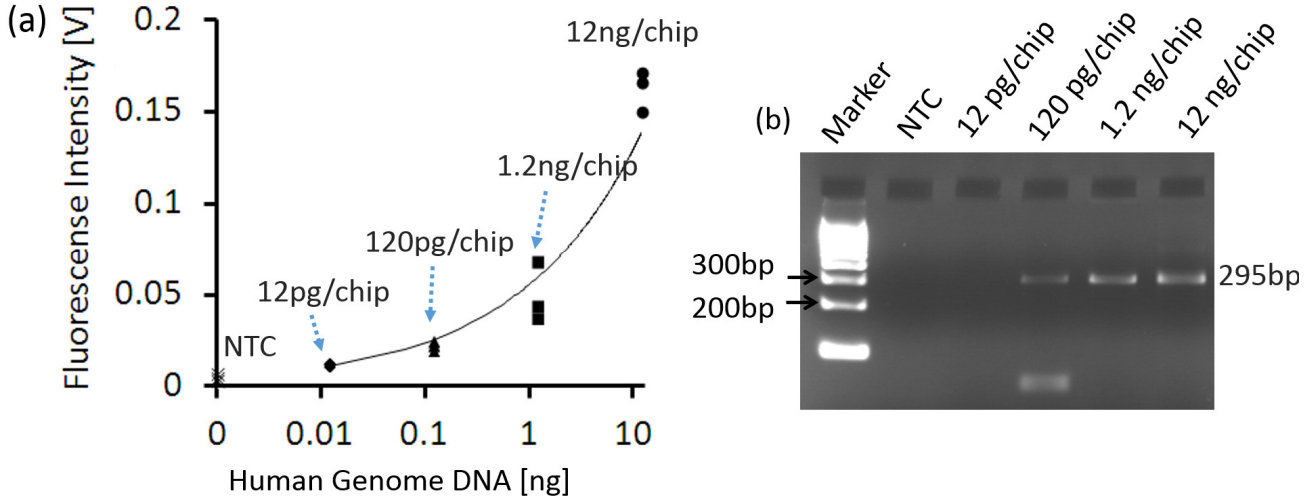


*Figure 4. On-chip DNA amplification of human beta actin by our devices. (a) Calibration curve of the fluorescence intensity after on-chip PCR from the human genomic DNA. (b) Images of gel shift assays performed with different amplicons.*

## CONCLUSION

We have designed and fabricated the POCT-oriented chip PCR device. The chip has a ring structured micro-channel and the PCR solution in the channel was flowed by thermal convection and centrifugation. The flow rate was controlled by the relative gravitational acceleration of the centrifugation. We have successfully achieved the on-chip DNA amplification of β–ACT from 120 pg human genomic DNA within only 10 min at 5G. Moreover, we have succeeded the on-chip PCR by direct injection of the buccal cell solution to the device which was pre-loaded with PCR solution without template DNA. We hope this result will be step toward the practical use of the easy and rapid PCR device for all users.

## ACKNOWLEDGEMENTS

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