Rapid multi sample DNA amplification using rotary-linear polymerase chain reaction device (PCRDisc)

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Multiple sample DNA amplification was done by using a novel rotary-linear motion polymerase chain reaction (PCR) device. A simple compact disc was used to create the stationary sample chambers which are individually temperature controlled. The PCR was performed by shuttling the samples to different temperature zones by using a combined rotary-linear movement of the disc. The device was successfully used to amplify up to 12 samples in less than 30 min with a sample volume of 5 μ l. A simple spring loaded heater mechanism was introduced to enable good thermal contact between the samples and the heaters. Each of the heater temperatures are controlled by using a simple proportional–integral–derivative pulse width modulation control system. The results show a good improvement in the amplification rate and duration of the samples. The reagent volume used was reduced to nearly 25% of that used in conventional method. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.3690469>]

I. INTRODUCTION

Polymerase chain reaction (PCR) has become one of the most popular and widely used diagnostics tool in molecular medicine due to its specificity and sensitivity since its inception in mid 1980s.¹ Conventional method of performing PCR has its limitation. Conventional thermocyclers are sizable devices that accommodate many PCR reaction tubes but require invariably longer duration of cycling time. Furthermore, the current method of performing PCR has its limitation. Even though it is sensitive and specific, it would have limited use in underdeveloped countries because of the need for skilled personnel, the tedious nature of the application, longer time to results, and multiple pipetting steps.²

Most of the time, the amplification time of conventional PCR devices are spent on heating and cooling the thermal blocks to acquire the desired temperatures. By miniaturising the PCR devices and the sample volume, it is expected that the heat transfer rate between the sample and the heaters can be greatly reduced. The advantages of miniaturizing PCR devices were first mooted by the pioneering work of Wittwer.³ Although the original work of Wittwer does not mention anything about PCR chips, the underlying idea presented inspired Northrup⁴ to develop silicon based PCR chip. In the past 15 years, different types of miniaturised PCR formats have been introduced. The two most favoured formats are the stationary chamber^{4–7} and continuous flow PCR devices.^{5,8–10} Other formats include hybrid methods combining stationary-continuous flow¹¹ and integrated electrorheological-fluid actuated micromixer and micropump with micro-heater arrays.¹² These formats make use of highly heat conductive substrates such as silicon and low sample volume to accelerate the amplification rate. However, some of the major setbacks in using these types of designs are high cost of substrate and its associated manufacturing cost and the usage of expensive external devices such as syringe pump.

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The objective of the current research is to develop a temperature cycling reaction microchip that combines the benefits of the two main chip PCR formats mentioned earlier. That is, the efficient temperature cycling can be performed, as is in the flow-through microchannel PCR chip, while the flexibility of varying the cycle number and varying the number of temperature zones is maintained, as is in the stationary chamber PCR chip. For this, a novel rotary-linear motion device was designed and fabricated to perform multi-sample PCR. The design is aptly named PCRDisc. Inhibition and adsorption problems associated with miniaturized PCR devices were also addressed.¹³ In the following discussion, the PCRDisc design concept is thoroughly explained.

II. PCRDisc CONCEPT

In this concept, instead of using an external pump to move the sample to different temperature zones as in continuous flow device, the samples are shifted from one zone to another by using a rotary-linear motion system. Another feature of the design is the temperature of each individual sample chamber is controlled separately which is capable to amplify samples with different annealing temperatures simultaneously.

In this design, the disc has 12 sample amplification chambers, compared to a single sample amplification capability of the continuous flow PCR format. The corresponding number of individually controlled heaters is also 12 units. Figure 1 shows the schematic illustration of the design.

The discs are made of low cost polymer material to reduce the cost of the PCR chip. A special housing is designed and fabricated to accommodate the heaters and also the rotary-linear motion system. A separate motor control unit is developed to control the rotational and linear movement of the disc. There are 3 heaters each for both the denaturing and annealing temperature zones or rows. For the extension temperature zone, there are 2 rows of 3 heaters each. The reason for the additional row of heaters in the extension temperature zone is to reduce the total cycle time as longer duration is needed to complete extension cycle.

The sample chambers are rotated in a clock wise direction using the rotary system to shift it from one temperature zone to next zone. Once the sample chambers are positioned directly on the top of the heaters, the whole disc is retracted downward on to the heaters using the linear motion system. In order for all the sample chambers to come in perfect contact with the heaters, each of the heaters are loaded with a spring to retract a few millimetres from its original position when pressed down with minimal force. Once the disc is at lower position (pressed against the heaters), the disc will be allowed to remain in this position for the samples to complete the temperature cycling for a pre-determined duration. The disc is then automatically pushed upward using the linear motion system. After the disc has ample clearance from the heaters, the sample chambers or the disc are rotated 90° to the next row of heaters to start a new temperature cycling. Thereafter, the same linear and rotary movements are executed in a loop. The sample will

FIG. 1. Schematic illustration of the PCRDisc and heaters.

be deemed to complete one PCR cycle after the sample chambers are rotated 360° from its initial position at the denaturing row heaters. The number of PCR cycles is controlled by the number of rotary motion of the disc.

III. EXPERIMENTAL SET-UP

A. Rotary-Linear motion system configuration and assembly

The PCRDisc consists of 2 separate sub-systems: a rotary system to move the sample chambers from one temperature zone to another (or heater location) in a rotary movement and a linear movement to lift the disc upward before the rotary movement begins as shown in Figure 2. Once the disc is rotated to a specific location, the linear movement will then retract the disc downward in order for the sample chambers to come in contact with the heaters. In order for all the sample chambers to be in contact with heaters, the heaters are specially designed to move downward when the disc is pressed downward. This is achieved by having a spring loading mechanism for the heaters.

The rotary sub-system consists of a stepper motor, a motor driver and a controller. The linear motion to control the up and down movement of the PCRDisc is accomplished by utilizing Oriental Motors DRL series, including a linear motor and associated driver unit. The DRL model used in this system is the LIMO DRL42PA2G-O4N motor and the driver model is the CRD5107P. VEXTA EMP402 dual axis programmable motion controller from oriental motors was acquired to control the two motors. Once the rotary and linear motion system was successfully set-up, the supporting mechanical system was designed and fabricated. The mechanical system is required to mount both the rotary and linear motion system. The mechanical parts for the moving parts were fabricated from aluminium alloy to provide good support and rigidity. A plate was mounted on the top of the linear motor's rail to lift the disc for upward and downward motion as well as to support the rotary motion motor. A rigid coupler was attached to the rotary motion motor to support the spindle that is used to mount the disc. The disc was attached to the spindle with a bolt so that the disc does not slip during the fast rotary motion. All the metal parts were fabricated using a conventional computer numerical control (CNC) machine.

The rotary-linear motion of the system was programmed offline using a conventional text editor. The program is then saved in text format and downloaded to the controller via HyperTerminal's transmission function. The downloaded program is saved in the EMP402 controller memory and may also be uploaded to a personal computer via HyperTerminal's transmission function and saved in text format. The program contains the method of motor operation as well as speed

FIG. 2. Rotary-linear motion system.

settings and other parameters. Once the program is started, the motors will execute the commands contained in the program according to a specified order. The program is stored in the controller memory during operation.

The system was configured to provide maximum motion speed. Since there are two types of motion involved, the rotary and the linear motors were programmed to their maximum speed. The rotary motion was configured to rotate at 90° for each step. Therefore, each temperature cycling is completed in four steps. The linear motor is configured to minimize the up and down motion of the disc. Minimal clearance was set-up between the heaters and the disc to minimize the linear traverse of the disc when pulled downwards to have contact with the heaters. It is also necessary for the clearance between the heaters and the disc to be maintained large enough to avoid collision when the disc moves in rotational motion.

Time delay method is used in the program when the disc is at the lower position (in contact with the heaters). The time delay will provide ample duration for the PCR samples to achieve the desired cycling temperature. Loop control was used to repeat the motion and complete the desired number of temperature cycling. Since each temperature cycling completes in 4 steps of 90° rotation of the disc, totally 120 steps are needed to complete 30 cycles. The 120 steps are programmed as a loop in the program. Once all cycles are completed, the program terminates and the system becomes stationary (the disc will not be in contact with the heaters when the program terminates). The time incurred for moving the disc in each complete cycle i.e., non-working time is about 8 s. Therefore, for a 30 cycles, the total time incurred to move the disc is 240 s. As there is a 15 s time delay for each temperature zone, a total duration of 34 min is needed to complete 30 cycles.

B. PCR sample chamber design and fabrication

In the initial PCR disc design, it was decided to source readily available material for the disc. To reduce the cost of the design, it was decided to use commercially available polymer material. Some of the common polymer materials used include polycarbonates (PCs)^{14,15} and polydimethylsiloxane.^{12,16,17} For this research, a polycarbonate compact disc base material was selected. Studies have shown that polycarbonate is PCR friendly.^{14,15} It can also be easily machined using conventional machining technology. The discs are in average about 1.18 mm in thickness and have a diameter of 120 mm. The centre of the discs has a 30 mm hole to be mounted on a spindle. In order to create the sample chambers, a hole which corresponds to each of the heater alignment and positioning is drilled through the disc. Each of the holes is 3 mm in diameter. The holes or the chambers are fabricated using the conventional CNC machine. In order to create the chambers, each of the machined holes is then sealed on one side of the disc with a PCR friendly aluminium foil from *Eppendorf*. The foils are self adhesive and have a thickness of $70\ \mu\text{m}$. The self adhesive nature of the foil facilitates the adhesion of the foils onto the disc. However, the bare surface of the foil which forms the chamber base was covered with adhesives. Even though the adhesives are considered as PCR friendly, they might influence the heat transfer efficiency. Therefore, the adhesives were removed after forming the chamber with cotton swabs dampened with alcohol. Thereafter, the disc was thoroughly rinsed with distilled water to remove any traces of alcohol. Each of the open chambers can accommodate up to $10\ \mu\text{l}$ volume. Figure 3 shows the disc with sealed chambers.

C. PCR sample preparation

Samples for conventional PCR were prepared in a $20\ \mu\text{l}$ reaction mixture volume. Each reaction mixture contained 1X PCR reaction buffer containing 75 mM Tris-HCl, 20 mM $(\text{NH}_4)_2\text{SO}_4$ and 0.01% Tween 20 (Fermentas, Lithuania), 2.5 mM MgCl_2 (Fermentas, Lithuania), 0.16 mM of each dNTP (dATP, dTTP, dCTP, dGTP; Fermentas, Lithuania), 1 pmol/ μl of forward primer SSP-S_2a (5'-TCTTG TAGGTTGTCATCCATC-3'), 1 pmol/ μl of reverse primer SSP2-AS_4a (5'-GACCATTCGTCCCAAACACCA-3'), 1.125 Units of *Taq* DNA polymerase (Fermentas, Lithuania), and $2\ \mu\text{l}$ of SSP150 DNA template. An inert orange coloured dye (Orange G, Sigma) was added to the reaction mixture at a final concentration of 0.2% (w/v). Thermal cycling was performed using the following parameters for 30 cycles: denaturation at

FIG. 3. Disc with sealed chamber.

95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The anticipated product length was 150bp, calculated from the primer alignment to the template sequence. The control reaction mixture was subjected to several cycles of amplification in a thermal cycler (MJ Research, USA). The amplified PCR fragments of 150bp were electrophoresed through a 1.5% agarose gel (Promega, USA) stained with ethidium bromide (final concentration of 0.5 $\mu\text{g/ml}$) in Tris-borate-EDTA (TBE) buffer and then visualized under UV light (UVP, Upland, USA). Similar product was also reported in other research.¹¹

D. Temperature control system set-up

Figure 4 shows the schematic diagram of the temperature control system. The configuration is similar to the system used for the oscillating flow design. Instead of using thermistors, this system uses *Pt100* for temperature sensing. Due to the increase in the number of temperature sensors used in this system, the National Instruments SCXI system was used to perform the temperature sensing.

The system still uses the PXI-6602 counter/timer device to control the heater loading using the pulse width modulation (PWM) method but due to the increase in the number of heaters, two PXI-6602 devices were employed. The heaters were assembled into the heater housing by inserting the spring loaded copper heater shafts from the top of the heater housing. Thereafter, the cartridge heater is inserted from the bottom of the heater housing. A rigid coupler was fixed to the rotary motion motor shaft to enable the attachment of the disc spindle. The coupler was sourced from *Farnell components*. The disc spindle is used to fix the disc to the system to perform the rotary-linear PCR process. Figure 5 shows the complete system set-up of the PCRDisc device which is ready for experimental validation.

In order to maintain the homogeneity of the temperature measurement for both the PCRDisc and the conventional PCR device, the *Pt100* temperature sensors were calibrated using the PTC200 thermal cycler as a reference temperature. The application of two wire configurations for the *Pt100* in the PCRDisc device will give some variance from the reference temperature. This is due to the fact that the resistance of the connecting wires for the two wire configurations of *Pt100* sensors adds up the resistance reading. From the calibration procedures conducted, it is discovered that the temperature reading errors are almost linear. The temperature error is then computed in the *LabVIEW* program so that the output temperature reading is calibrated to the reference temperature.

E. PCR sample inhibition and adsorption analysis

Even though the PC compact disc material and the aluminium foil used to create the chamber is considered PCR friendly, it is nevertheless important to verify that the materials used are

FIG. 4. Schematic diagram of the 12 heater temperature control system.

indeed PCR friendly. In this experiment, a single row of chamber from the disc was cut into small strips to be placed in a conventional thermal cycler. Each of the 3 chambers was loaded with 5 μ l of SSP150 PCR mix and coated with 2 μ l light mineral oil. The strip is then placed on a 2 mm thickness copper plate inside the thermal cycler chamber. The samples in the CD strip are then amplified using the standard temperature cycling parameters using a conventional thermal cycler.

After the amplification process, the samples were then unloaded from the strip. A total of about 4 μ l of PCR product were successfully recovered from each of the 3 chambers. The samples are then loaded into 1.5% agarose gel to perform the gel electrophoresis. Figure 6 shows the gel electrophoresis results. Lane M is the 100bp ladder, lane C is the control using the conventional PCR tube, and lane 1, 2, and 3 is the CD PCR samples.

The result shows that the SSP150 gene fragment was indeed amplified even though the resolution is quite poor. The poor resolution is expected as the PCR samples in the strip were not adequately heated. This is due to the heating block of conventional thermal cycler was designed to accommodate PCR tubes. The use of copper plate to improve the thermal conduction to the strip was not adequate. However, the amplification results show that the compact disc material and the aluminium foil do not inhibit PCR process, and adsorption of PCR sample components does not take place.

FIG. 5. The completed PCRDisc system set-up ready for experimental procedures.

FIG. 6. Slab gel electrophoresis results for the inhibition and absorption experiment for the compact disc material. Lane M represents the 100bp ladder; lane 1, 2, and 3 represents the SPPS Internal control product with 150bp for the 3 PCR chambers.

IV. RESULTS AND DISCUSSION

The optimum duration for each of the temperature cycle is very important and must be determined before reliable results can be secured. Due to the way the system is configured, it is impossible to vary the temperature cycling duration for each the individual temperature zone. Therefore, it is critical to balance the duration required for the denaturing, annealing, and extension process to perform efficiently. In the first set of experiment, only 4 inner chambers of the disc were loaded with PCR samples.

The motion control system was configured to have a time delay of 10 s, 15 s, and 20 s when the disc is in contact with the heaters. This is performed to determine the optimal temperature cycling time. For a 10 s delay for each temperature zone, the samples will go through denaturing and annealing for 10 s each and 20 s for the extension. Due to the configuration of the rotary device, only one row of sample chambers will achieve a complete 30 cycle amplification when the rotary system is configured to perform 30 cycles. Three other rows of sample chambers will have 29, 28, and 27 cycles in total. This is due to the fact that the other 3 rows of sample chambers start off with either annealing or extension temperature when the device is activated. In order to maximize the amplification efficiency, the minimum number of cycles the samples must go through is set at 30 cycles. Therefore, one row of samples will have a maximum of 33 cycles and one row has a minimum of 30 cycles. The standard PCR temperature protocol was used for the SSP150. For the first set of experiment, the heating duration for each temperature zone was fixed at 10 s. Therefore, one complete cycle takes 40 s to complete with an additional 8 s for the rotary-linear motion. In total, it takes about 26.4 min to complete the maximum 33 cycles.

The second set of experiments extends the heating duration to 15 s for each zone and it takes 37.4 min to complete the 33 cycles. The third set of experiments further extends the heating duration to 20 s. For this, the total time required for 33 cycles is 48.4 min. The heating duration was limited to a maximum of 20 s because since the volume of samples used was only 5 μ l, it is assumed that the thermal efficiency will be considerably improved compared to a 20 μ l sample volume used in a conventional thermal cycler. The typical heating duration used for SSP150 gene in a conventional thermal cycler is 30 s for each temperature cycle.

Figure 7 shows the slab gel electrophoresis results for the three different heating duration using PCRDisk. Lanes M, C, 1, 2, and 3 represents the 100bp ladder, control, 10 s heating, 15 s

FIG. 7. Slab gel electrophoresis results for different heating duration at each temperature zone. Lane M, C, 1, 2, and 3 represent the 100bp ladder, control, 10 s heating, 15 s heating, and 20 s heating, respectively.

heating, and 20 s heating, respectively. From the figure, it is observed that lane 2 which represents the 15 s heating duration yields a better amplification compared to 10 s and 20 s heating durations. Shorter duration of 10 s seems to retard the amplification efficiency, probably due to incomplete denaturation or extension of DNA template. The longer duration of 20 s compared to the 15 s shows a slight degradation. This is probably due to the *Taq* polymerase being exposed for too long in the denaturing process. For the 150bp gene fragment used in this study, the optimal duration of heating is found to be 15 s or 37.4 min for 33 cycles. Using this optimal setting, the multiple sample amplification with the PCRDisc is performed for the SSP150 DNA templates.

Once the optimal heating duration is established for the SSP150 DNA template, multi sample experiment is performed. In the first multi sample test, the device was set to amplify only four chambers closest to the centre of the disc. Sample volume of 5 μ l together with the same volume of oil was loaded into the chambers and the disc was mounted on the device. In this experiment, the total number of temperature cycle was set at 33. As mentioned earlier, this will give a minimum of 30 cycles for each sample chamber. Once the amplification is completed, the samples were unloaded from the chambers with a standard micropipette. The samples were then electrophoresis on a 1.5% agarose gel. Figure 8 shows the results for the amplification of four samples. Satisfactory amplification with PCRDisc (lane 1-4) was achieved compared to the conventional thermal cycler (lane C).

As simultaneous amplification of four samples has produced convincing results, the number of samples is increased to eight. Similar procedures explained earlier were applied to the eight sample amplification. Figure 9 shows the agarose gel electrophoresis for the eight sample amplification. The amplification results are consistent with the results of four samples shown in Figure 8. It is interesting to note that every amplification produces quite consistent result with no failed amplification. This suggests that the system is very reliable wherever the sample is amplified.

In order to determine the maximum sample amplification capability of the PCRDisc, all twelve sample chambers available on the disc were used to perform the PCR simultaneously. As per previous procedures, each chamber was filled with 5 μ l SSP150 PCR mix and coated with 5 μ l light mineral oil to prevent evaporation. Once the 33 cycles were completed, the samples were unloaded and electrophoresis with 1.5% agarose gel. Figure 10 shows the agarose gel

FIG. 8. Amplification results of four samples using the PCRDisc. Lane M and C correspond to the 100bp ladder and control, respectively. Lane 1, 2, 3, and 4 represents the amplified samples from the disc.

electrophoresis for the twelve samples amplification. The results obtained are also consistent with the four and eight sample amplification.

The simple technology using the conventional compact disc shows a promising result in successfully amplifying the 150bp DNA samples. This low cost method proves that it can perform multiple sample analysis within a short duration and uses fraction of the samples that is required for a conventional PCR device.

A. Thermal simulation analysis

Direct measurement of sample temperature in a microfluidic PCR device has always been an issue. Due to the small volume of samples and geometry of the PCR chip, it is difficult to

FIG. 9. Amplification of eight samples using the PCRDisc. Lane M and C correspond to the 100bp ladder and control, respectively. Lane 1-8 represents the amplified samples from the disc.

FIG. 10. Twelve sample amplification using PCRDisc. Lane M and C correspond to the 100bp ladder and control, respectively. Lane 1-12 represents the amplified samples from the disc.

attach a temperature sensor to the sample. Furthermore, the mass of the temperature sensor will distort the actual temperature to be measured. In order to solve these issues, non contact method has been applied by researchers. Some of the methods that have been employed include numerical simulation, thermo chromic liquid (TLC), and infra red thermometry. In the following discussion, the thermal behaviour of the PCR chip is investigated using numerical simulation.

Numerical simulation was carried out to investigate the thermal behaviour of the PCR device and only the PCR sample element was considered. The heater housing temperature effect is assumed to be negligible to the PCR samples. The thermal analysis and simulation was performed by using the commercial finite element analysis (FEA) software ANSYS. Thermal analysis is performed for both the compact disc and the cartridge type PCR chip design. The geometry was created using SOLIDWORKS computer aided design software. The geometry is then imported to ANSYS to perform FEA. Table I shows the physical properties of the materials used in the FEA simulation.

Due to a large surface area of the compact disc, only a fraction of the surface covering the four sample chambers is taken into consideration for the thermal analysis. This is due to the large computational power requirement that will be needed to perform complete FEA on the whole disc. As a general rule, a natural convection boundary condition, with a coefficient of $5 \text{ W/m}^2 \text{ K}$, was assumed on all external surfaces of the chip except for the bottom surface of each heater. The ambient temperature is assumed to be 25°C . A constant heat flux is assumed at each of the heaters at the pre-determined temperature.

Furthermore, due to the low thermal conductivity of the compact disc material, the thermal response is assumed to be more concentrated on the heated region only. Figure 11(a) shows the simplified cross sectional view of the FEA model for one of the sample chamber.

Three sample chambers are heated directly below the aluminium foil seal. It is assumed that the contact between the heater and the aluminium foil is rough. This will be the worst case scenario for the thermal contact for the heated sample. The sample and the light mineral oil inside the chamber are assumed to be evenly distributed with the bottom layer consisting of PCR sample and the top layer the mineral oil. It is assumed that there is no separation in the thermal contact between the sample, mineral oil, and the disc. The thermal analysis is performed for both steady and transient state.

Initial simulation result shows that the temperature distribution at 95°C is bounded within the region of $20 \text{ mm} \times 50 \text{ mm}$ of the disc. Therefore, the following simulation results were based on the above defined surface area only.

TABLE I. Material physical properties.

Material	Thermal conductivity (W/m °C)	Specific heat capacity (J/kg °C)	Density (kg/m ³)
PC	0.179	1700	1200
Aluminium	150	875	2770
Water	0.609	4180	998
Mineral oil	0.11	1800	840

In the following thermal analysis, the reference temperatures were taken at the interface of the PCR sample and the mineral oil coating. One of the temperature probes in the analysis was placed at the centre of the sample. The remaining two probes were placed near to the interface of the sample and the compact disc wall as shown in Figure 11(b).

In the initial FEA analysis, a steady state thermal simulation was performed on the PCR chip for room temperature. The result was then used as an initial condition for the following simulations using transient method. Different set of heating duration was analysed. The ambient temperature was set at 25 °C to monitor the effect of convection related heat losses. The convection in the analysis was defined as temperature dependent and the correlation as stagnant air for simplified case. The denaturing heater temperature was set at 95 °C, annealing heaters at 60 °C, and the extension heaters at 72 °C. Analysis was performed for the first two complete

FIG. 11. (a) Simplified cross sectional view of a single chamber of the compact disc PCR chip. (b) Location of the temperature probes in the sample chamber and boundary conditions.

FIG. 12. PCRDisc temperature cycling using 15 s heating duration.

cycles. It is assumed that the analysis of the remaining cycles will be the same. Several optimisation temperature cycles were performed on the disc and the optimal condition was obtained at 15 s cycle time.

Figure 12 shows the temperature gradient for the 15 s heating duration for two complete cycles. As expected, for the first cycle, the denaturing temperature was achieved but not sustained to complete the denaturing process. The annealing and extension temperatures achieved an extended heating duration in the first cycle itself already. From the second cycle onwards, all three temperature zones had sufficient heating duration to perform optimal PCR process. Therefore, for this PCR device, the minimum heating duration required was 15s for each temperature zone.

V. CONCLUSION

In this paper, a novel PCRDisc concept was tested using different disc material and method. In the first design, a commonly available low cost compact disc was used to fabricate the disc that provides up to sixteen sample chambers. A low cost aluminium foil was used to create a chamber for a volume of 10 μl . Passive PCR experiments were performed on the compact disc design to verify its PCR “friendliness.” The results were satisfactory and active PCR experiments were further performed on the disc. In order to validate experimentally the compact disc PCR chip design, 150bp DNA templates were used. The sample volume used for the PCR experiments was 5 μl . An additional 5 μl light mineral oil was used to coat the samples to prevent evaporation during high temperature cycling. In the first active PCR experiment, only the first four inner sample chambers were used. The results show a very good PCR amplification comparable to conventional PCR device. The experiments were further extended to perform eight and twelve samples simultaneously. The results were outstanding and prove that the simple and low cost compact disc design can be used to amplify multiple samples in a single experiment.

¹K. Mullis, F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich, *Cold Spring Harb Symp. Quantit Biol.* **51**, 263 (1986).

²I. Aziah, M. Ravichandran, and A. Ismail, *Diagn. Microbiol. Infect. Dis.* **59**(4), 373 (2007).

³C. T. Wittwer, G. C. Fillmore, and D. R. Hillyard, *Nucleic Acids Res.* **17**(11), 4353 (1989).

⁴M. A. Northrup, C. Gonzalez, D. Hadley, R. F. Hills, P. Landre, S. Lehew, R. Saw, J. J. Sninsky, and R. Watson, “A Mems-based miniature DNA analysis system,” in *The 8th International Conference on Solid-State Sensors and Actuators, 1995 and Eurosensors IX. Transducers '95* (IEEE, 1995), Vol. 1, pp. 764–769.

⁵M. U. Kopp, A. J. D. Mello, and A. Manz, *Science* **280**, 1046 (1998).

⁶Z. Q. Niu, W. Y. Chen, S. Y. Shao, X. Y. Jia, and W. P. Zhang, *J. Micromech. Microeng.* **16**(2), 425 (2006).

⁷Z. Q. Zou, X. Chen, Q. H. Jin, M. S. Yang, and J. L. Zhao, *J. Micromech. Microeng.* **15**(8), 1476 (2005).

- ⁸S.-R. Joung, J. Kim, Y. J. Choi, C. J. Kang, and Y.-S. Kim, "ITO-coated glass/polydimethylsiloxane continuous-flow PCR chip," in *2nd IEEE International Conference on Nano/Micro Engineered and Molecular Systems, 2007* (IEEE, 2007), Vol. 1, pp 691–694.
- ⁹S. Li, D. Y. Fozdar, M. F. Ali, H. Li, D. Shao, D. M. Vykoukal, J. Vykoukal, P. N. Floriano, M. Olsen, J. T. McDevitt, P. R. C. Gascoyne, and S. Chen, "A continuous-flow polymerase chain reaction microchip with regional velocity control," *J. Microelectromech. Syst.* **15**(1), 223 (2006).
- ¹⁰L. Yao, B. Liu, T. Chen, S. Liu, and T. Zuo, "Micro flow-through PCR in a PMMA chip fabricated by KrF excimer laser," *Biomed. Microdevices* **7**(3), 253 (2005).
- ¹¹D. Sugumar, A. Ismail, M. Ravichandran, I. Aziah, and L. X. Kong, *Biomicrofluidics* **4**(2), 024103 (2010).
- ¹²L. Liu, W. Cao, J. Wu, W. Wen, D. C. Chang, and P. Sheng, *Biomicrofluidics* **2**(3), 034103 (2008).
- ¹³L. Kricka and P. Wilding, *Anal. Bioanal. Chem.* **377**(5), 820 (2003).
- ¹⁴M. Hashimoto, M. L. Hupert, M. C. Murphy, S. A. Soper, Y. W. Cheng, and F. Barany, *Anal. Chem.* **77**(10), 3243 (2005).
- ¹⁵J. Wang, Z. Chen, M. Mauk, K.-S. Hong, M. Li, S. Yang, and H. Bau, *Biomed. Microdevices* **7**(4), 313 (2005).
- ¹⁶J. Wu, W. Cao, W. Wen, D. C. Chang, and P. Sheng, *Biomicrofluidics* **3**(1), 012005 (2009).
- ¹⁷L. Wang and P. C. H. Li, *Biomicrofluidics* **4**(3), 032209 (2010).