# Integrated DNA preparation for pyrosquencer onto a polymer microfluidic device

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## ABSTRACT

We present a microfluidic system capable of performing various DNA sample preparation processes like enzymatic digestion and purification. The system consisted of a disposable polymeric chip and a controlling system. Mixing chambers, incubation chambers and DNA purification modules were integrated on the polymeric chip. Optimization tests were carried out using the genome of HCT116 cells to study the effect of mixing time and velocity, temperature, pH, and concentration of solution. Using the optimized parameters, we managed to demonstrate the feasibility of the chip to perform enzymatic digestion and purification processes on pUC19 DNA. This microfluidic system can be further developed into a comprehensive system to automate the protocols required by molecular biologists.

***Keywords***: Lab on Chip, microfluidic chip, DNA sample preparation, enzymatic, DNA digestion

## 1 INTRODUCTION

The advancement in microfluidic technologies has resulted in adoption of different novel approaches in handling conventional molecular biology protocols. Notably, capillary electrophoresis is used to quantify DNA sample in commercial product such as Agilent Bioanalyzer, and the attempt to build faster DNA sequencing chip. However, the upstream work of DNA sample preparation has yet to receive significant attention [1]. Such DNA sample preparation for sequencing is labor intensive and time consuming, and requires well-trained personnel to carry out the protocols. Here, we discus and demonstrate an approach to automate the DNA sample preparation steps, where enzymatic and purification processes are the two primary steps.

## 2 METHODOLOGY

### 2.1 System and chip design

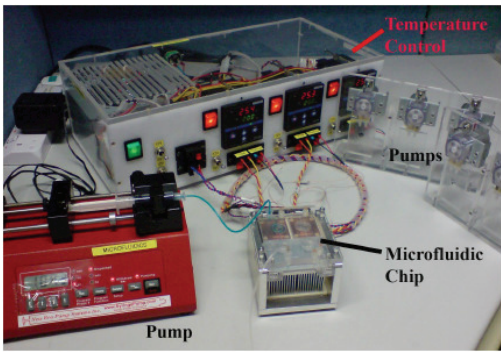
The DNA sample preparation system consisted of a disposable polymeric chip and a controlling system, as shown in Figure 1(a). The polymeric chip was made from a thermoplastic. Mixer, DNA digestion and DNA purification modules were integrated onto the polymeric chip, as shown in Figure 1(b). The spiral channel was designed to enhance mixing of liquid. A programmable pump of liquids controls the oscillation of liquid back and forward between 1st and 2nd spiral channel.

The disposable polymeric chip was fabricated from two pieces of polymethyl methacrylate (PMMA) of 4.0 mm and 2.0 mm thickness, respectively. The channel width and height were 1.0 mm and 0.5 mm, respectively, which provided a total capacity of 250 µl in each spiral-compartment. Conventional machining technique was used to make channels and drill holes. The channels were treated with a monolayer coating of perfluorinated monomers by pulsed plasma to create hydrophobic surfaces. The treatment helped to minimize liquid residual in t he channel. The commercial machine from P2i, UK was used in this purpose. The top plate was used to enclose the channel using thermal bonding technique. Oxygen plasma surface treatment was used to enhance adhesion prior to the thermal bonding. A thin polymer sheet was used to protect the channel areas coated with hydrophobic surfaces before oxygen plasma treatment. At conditions of 98°C, 4 minutes and 3 MPa, the top and bottom plates were bonded to form the final chip.

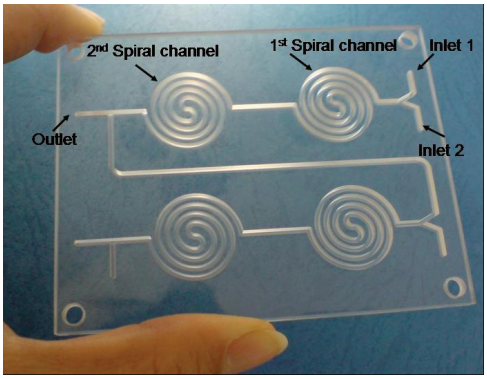
### 2.2 Preliminary tests to find optimum operating conditions on Eppendorf tube

To understand the effect of DNA purification solutions, mixing techniques and processing time, the DNA sample protocols were first run on Eppendorf tube. The basic protocol for the DNA sample preparation was based on the Paired-End diTag (PET) library construction technique developed by Genome Institute of Singapore [2, 3]. First, the genomic DNA of HCT116 cell was incubated in 1 x TE buffer at pH 8.0 for 30 min at 37°C. Mixing was performed at every 10 min interval during incubation using vortex machine. DNA was sheared to fragment size of 10 kb using Hydroshear machine. The sheared DNA was treated with blunt-end process using commercial end-repair kit. A 336 µl of sheared DNA were mixed with 50 µl 10x Epicentre Endit Buffer, 50 µl Epicentre Endit ATP, 50 µl Endit dNTPs, and 14 µl Endit Enzyme. The mixed solution was incubated at room temperature for 40 min.

Subsequently, DNA purification was performed using silica beads separation technique. This technique was based on the electrostatic interactions between the silica surface and the phosphate groups of DNA backbone, which resulted in binding and de-binding of DNA onto the silica surface under proper ionic conditions [4,5]. The silica beads, Dynabeads® MyOne™ SILANE, from Invitrogen were used. The binding solutions of 5M GuHCL, 10 mM Tris-HCL, and 1 mM EDTA, were used to bind 175 µl of DNA onto 25 µl of silica beads buffer. The solution was measured to have a pH of 6.1. Mixing was applied during binding state.




(a)




(b)

Figure 1. (a) Controlling System which consisted of thermal, fluid flow and magnetic unit, (b) Disposable polymeric chip for automated DNA processing. The PMMA chip consisted of four spiral shape channels

Next, a permanent magnet was used to immobilize the silica beads, which had magnetic cores. The solution was removed by using a pipette. The DNA and silica beads were washed by 500 µl 10mM Tris-HCl, 1mM EDTA, 80% ethanol, pH 7.5. The washing buffer was removed using the same immobilization technique. The silica beads were dried at room temperature for 10~15 min. DNA were eluted from the silica beads using 375 µl elution solution of 10mM Tris-HCl, 1mM EDTA, pH7.5-8.0. Again, the silica beads were immobilized using a permanent magnet so that the solution containing purified DNA can be extracted. The solutions in binding and elution steps were collected and analyzed using spectrophotometer (NanoDrop) to quantify the concentration of DNA in the solution. The effect on binding efficiency due to change in mixing time, mixing method, and temperature were analyzed.

### 2.3 Enzymatic digestion and purification processes on polymeric chip

Next, we implemented the DNA sample preparation steps, namely enzymatic digestion and DNA purification processes, on a polymeric chip. New polymeric chips were washed with elution buffer (TE) for 15 min, followed by 5M GuHCL in 10 mM Tris without DNA for a further 15 min. The chip were loaded with 10 µl of pUC19 DNA at 100 ng/µl, 2µl of 10 x Fastdigest buffer, 1µl of Fastdigest BamHI, and 16µl of Nuclease free water. The solution was incubated at room temperature for 30 minutes. During incubation, a bi-directional syringe pump was used to move the solution to flow between the first and the second spiral-channels at a speed of 20µl/min. This step helped to ensure proper mixing in the solution.

The digested DNA was separated from the solutions by silica purification process, which consisted of loading, washing, and elution steps. In the loading step, 100 µl of binding solution and 10 µl of silica beads were flowed into the chip and mixed with chip solutions. The mixing was performed for 15 min at a flow speed of 15 ml/hr using the same bi-directional flow technique. DNA was now bound to silica beads. Magnet was inserted beneath the second spiral-channel compartment. The silica beads were immobilized by magnetic field. The enzymatic solutions were removed by flowing 200µl, 3ml/hr of washing buffer through the fixed silica beads at the channel bottom. The spiral-channels were dried with atmospheric air flow for 15 min. Finally, the DNA was eluted in the elution solution. Again the mixing was performed to elute DNA from the silica beads for 15 min at a flow speed of 15 ml/hr.

The loading buffer and the aliquots of the elution buffers were collected and quantified using gel electrophoresis technique. A 10 µl of purified DNA was mixed with 2 µl of loading dye and run in 1% agarose gel at 100 V for 1 hr. Agarose gel electrophoresis images were used to quantify the recovery of DNA.

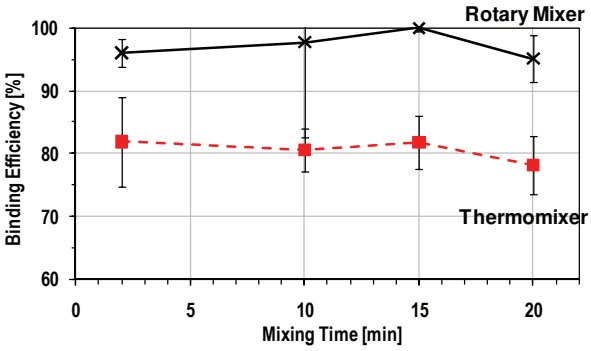
## 3 RESULT

### 3.1 Test result on Eppendorf tube

The effect of mixing technique was studied using two different types of mixers, namely rotary mixer and thermomixer. A rotary mixer can rotate left and right, and provide shaking action, while a thermomixer moves in planar motion, as shown in Figure 2(b) and 2(c). The mixing time was varied from 2 min to 20 min. The efficiency of binding process was measured using

NanoDrop, from the supernatant samples. A 100% efficiency will mean that no DNA exist in the supernatant. Figure 2(a) shows the result of the binding efficiency.

The experimental results indicated that rotary mixer provided higher binding efficiency than Thermomixer. It is believe that a lower efficiency in the Thermomixer was due to silica beads settled in the tube and resulted in reduced contact with the binding solution. Highest binding efficiency of 98% was achieved using rotary mixer at a mixing time of 15 min.




(a)

(b)

(c)

Figure 2. (a) Binding efficiency of DNA on silica beads for rotary mixer and Thermomixer; (b) Rotary Mixer; (c) Thermomixer.

Besides the mixing method, the operating temperature was known to directly affect the DNA-silica interaction, and thus, the binding efficiency [4,5]. To understand the effect of temperature on binding of DNA on silica beads, we performed further experiments at two operating temperature of 25°C and 55°C, respectively. The effect of temperature was plotted against binding time, see Figure 3. The experiment was performed using rotary mixer. The result indicated that higher operating temperature of 55 °C during binding process did not enhance the efficiency.

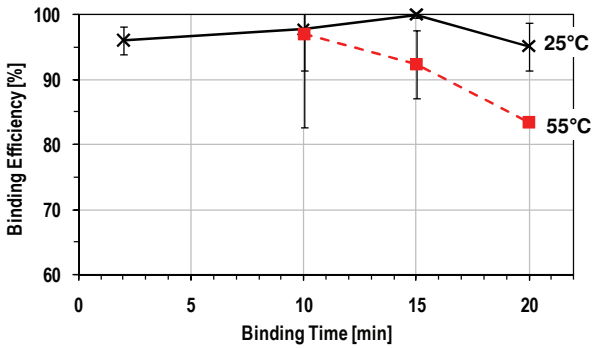



Figure 3. Binding efficiency of DNA on silica beads at 25°C and 55°C.

The elution step was performed using the optimum operating condition indicated by the previous experiments, that is, a binding step using rotary mixer for 15 minutes at room temperature. The elution time was varied from 2 min to 20 min. A total of three experiments were conducted for each setting. The experimental result is shown in Figure 4. The highest elution efficiency was recorded to be 76% at an elution time of 2 min.

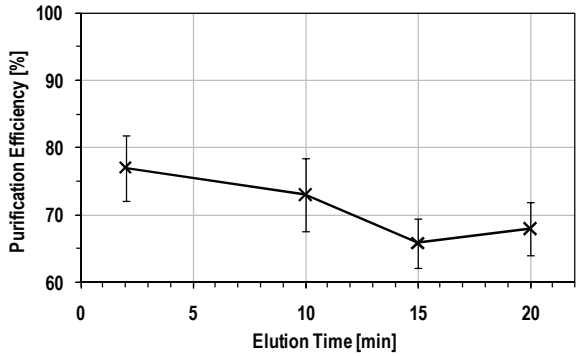



Figure 4. Purification efficiency under different elution time.

## 3.2 DNA sample preparation on polymeric chip

Integration of emzymetic and purification DNA on the polymeric chip was performed. The concentration of the DNA after enzyme digestion on the chip was quantified by running in the 1% agarose gel. Gel electrophoresis was performed on a ladder, undigested pUC19 DNA, and digested pUC19 DNA. The digested pUC19 DNA (loading 3) displayed a shorter DNA size, compared to undigested pUC19 DNA (loading 2), as shown in Figure 5. This result confirmed a successful digestion of the pUC19 DNA in our polymeric chip.

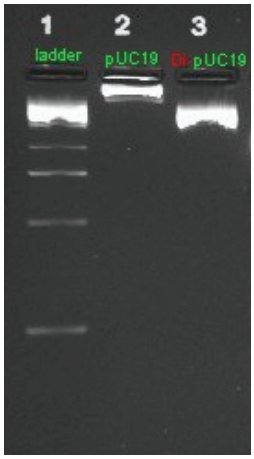



Figure 5. Agarose gel electrophoresis result for digested pUC19 DNA. Loading 1, 2, and 3 were ladder, undigested DNA, and digested DNA, respectively. Digested pUC19 DNA was shorter compared to undigested pUC19 DNA, indicating a successful digestion process on polymeric chip at room temperature.

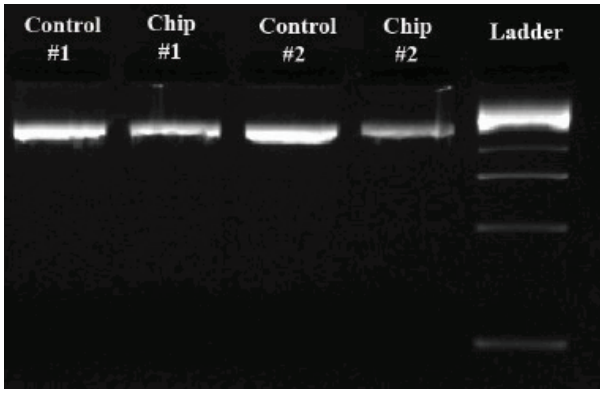



Figure 6. Comparison of purified DNA sample with control sample at 100% efficiency. Tests on two chips were conducted and the results are compared.

After DNA digestion process was completed, silica beads and binding solution were loaded to the channel to separate DNA from enzymatic solution on the same chip. 10 µl Purified DNA in elution solution was loaded in the 1% agarose gel and run with 100 V for 1 hr. Purification efficiency was compared with the control DNA solution which has same amount of DNA at 100% efficiency. Two chips were tested in enzyme digestion followed with silica purification. Figure 6 shows the result of gel electrophoresis. Image processing software was used to quantify the gel image. The purification efficiency was quantified to be at 65~68%.

## 4 DISCUSSION AND CONCLUSION

We demostrated the DNA digestion and purification processes on a disposable polymer device. The device has potential to be further developed into an automated system to handle the complex molecular biology protocols.

We are working to further increase the purification efficiency to a target of at least 80%. This maybe achieved by reducing DNA absorption in the chip and increasing mixing efficiency.

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