Evaluation and Optimization of Genomic DNA Extraction from Food Sample for Microfluidic Purpose

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Abstract. Contamination of various food samples became one of the critical issues in food pathogen infection. Food pathogen can be detected by using digital polymerase chain reaction (PCR) and sequencing. These methods were reliable but consuming and take a longer time for detection. The present work describes the innovation to develop a technology to extract double-stranded deoxyribonucleic acid (dsDNA) from food samples and then denatured dsDNA into and single-strand DNA (ssDNA) for further use on the chip using microfluidic device. Microfluidic device is a lab-on-chip device that consist of microfluidic channels that provide paths for biomolecules to flow to individual point of care. DNA extraction is the process by which DNA is separated from proteins, membranes, and other cellular material contained in the cell from which it is recovered. Lysis solution is used in the process of extraction the DNA to break up the cells containing DNA from protein and other cellular materials. This extraction firstly be done in the most labour-intensive in obtaining the DNA biomolecules. Extraction methods may require an overnight incubation, may be a protocol that can be completed in minutes or a couple of hours by using a commercial kit. The disadvantages of the laboratory and commercial kit is due to time-consuming, poor cost-effectiveness, the need to use big laboratory and a complicated process which need an expertise to conduct the experiment and interpret the data. This research is proposed to design and fabricate a microfluidic device that has DNA extraction capabilities. In this research DNA extraction using a commercial kit will be used as a comparison for the quality of the result. The microfluidic device can be used in health care delivery system and will help the doctors in diagnostic process to identify disease of a patient rapidly. Other than that, the output extracted from microfluidic device will be used for DNA probe target interaction for diagnostic kit. The major advantage of microfluidic device is that it consumes less time compared to the conventional chemical methods.

1. Introduction

Nowadays, there are many pressing need in science fields especially in the medical fields and forensics community that seek for a sensitive, robust, and reliable integrated method of DNA extraction that is rapid, cost effective, and neither labour nor space intensive [1]. DNA extraction is a process of purification of DNA from sample through chemical and physical methods [2-6]. The DNA extraction purposes is to separate it from proteins, membrane and other cellular materials. The purification of DNA
is to make sure that a pure DNA biomolecule is obtained during this process. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labour-intensive and limited in terms of overall throughput [7]. The first DNA extraction process was done in the chemical laboratory where they needed to use a lot of equipment and go through complex procedures to get DNA biomolecule. This method takes three days for the result to be obtained including the culturing process. Then, the DNA extraction kit was manufactured and takes one hour for the doctor to obtain the result. DNA extraction method was improved to modify approach that takes less those 20 minutes [8]–[10]. Due to time-consuming problem in obtaining the result, this optimization was used to accelerate the time of result attainment by creating a lab-on-chip device [11]. The lab-on-chip device is an idea of a microfluidic chip with a pattern of micro channel which is moulded or engraved which is shown in figure 1. On the chip, there are pathways that allows liquids to flow through. This microfluidic chip provide paths for biomolecules to flow to the point of care that will detect the result of the fluids [12]. Microfluidic device is only taking a few minutes for the users to attain the result.

Figure 1. Microfluidic chip that consists of microfluidic channels.

In this study, developing a PDMS microfluidic device with DNA extraction capabilities to extract a single-stranded DNA to obtain rapid and quality result of DNA biomolecules is the main existential of this research. In order to attain the single-stranded DNA, process of mixing, homogenising, incubation, centrifuge and precipitation is being repeated until the outcome was obtained. Single-stranded DNA is obtained from double-stranded DNA. The process is done when using the commercial kit DNA extraction to obtain the single-stranded DNA. However, as micro and nanotechnology have been developed, integration of a single micro device such as the lab-on-chip device are suitable approach to be used for sampling and monitoring as well as mixing, reaction, incubation and analysis of small volumes of fluids which will give a rapid result [4]. The design of the microfluidic is done by AutoCAD software and fabrication of microfluidic by using PDMS.

2. Material and methods

2.1 Chemicals, Reagents and Equipment

DNA extraction process using DNA extraction kit is done by using micropipette, mixer, shaker, centrifuge, nanodrop spectrophotometer and UV-Vis Spectrophotometer. The chemicals that been used are lysis buffer, buffer AW1 and AW2 and Elution Buffer AE. Buffer AW1 and AW2 serve as a washing agent for the DNA which will make sure that it is clean from other bimolecular materials. Meanwhile lysis buffer separate protein from the sample DNA. Elution Buffer AE act as a binding agent to make sure that the DNA is bind together. For fabrication process of microfluidic, there are synthetic materials that been used which are acetone, SU-8 photoresist, Isopropanol (IPA) and glass. Acetone and IPA serve in substrate cleaning to eliminate contaminants and particles from the surface of the glass. SU-8 is an
average utilized epoxy-based negative photoresist which is used to make design with high aspect ratio.
It is utilized as a high-resolution mask in fabrication process where it used most in fabrication of microfluidic and MEMS parts.

2.2 DNA Extraction Methods
DNA extraction process can be done using multiple types of kits. The kits that are used during extracting the DNA is commercial extraction kit. This kit takes about one to two hours to obtain the single-stranded DNA. The main function of DNA extraction is to obtain the single-stranded DNA. The samples that been used such as milk, yogurt drink, yakult and other liquid food samples. The DNA that have been extracted is then been tested the purity by using NanoDrop spectrophotometer and UV-Vis spectrophotometer. DNA was extracted by preparing the sample be. If the sample is in liquid form, it would be easy to extract DNA from the sample. However, if it is solid forms such as rice or chicken, the sample needs to be ground until the liquid comes out from the food. Next, is the mixing process between lysis and sample. The lysis buffer is used to isolate protein from the molecular cells. The lysis buffer that is used in the mixing process with the sample is NaOH. Then quickly incubate the sample at 65°C with 500 rpm. The main purpose of this process is to break up connective tissue holding the cell together. This will make them to be easier to lyse. It will also inactivate DNAs and enzymes that would degrade and inhibit PCR. After that, the sample will undergo cleaning process which use washing buffer that helps in purification of DNA and elimination of cellular debris. Then, the extraction process continues with elution process. Elution is a process of extracting material for other material by washing it with a solvent. The washing agent that is used in the extraction process is AE Buffer solution. The solution will help in removing the DNA from the matrix. This process is done by using the spin column tube which the tube will be spun in the centrifuge that will eventually pull the elution buffer through the matrix. After elution, the double-stranded is converted to single-stranded by adding a ratio of 1:1 of the double-stranded DNA of the sample and 0.01M of NaOH. The single-stranded DNA of the sample is then stored inside a fridge at -20°C.

2.3 Microfluidic Device Fabrication
A few steps are included in the production of the microfluidic gadgets. Sample preparation is the first step in the production of microfluidic [8-10]. The result and technique used in this production process will be influenced by this development. The manufacturing process is carried out with a microscopic glass in this project. The 75 mm x 25 mm x 1 mm microscopic glasses are used as shown in Table 1.
Glass was used as the foundation of the microfluidic device. The glass must be microscopically cleaned. The glass is cleaned from the glass surface with Isopropanol (IPA) and distilled waters (DI water).

| Properties                  | Specification                      |
|-----------------------------|------------------------------------|
| Microfluidic glass          | 23.00 mm x 31.67 mm                |
| Channel 1 width             | 0.5 mm                             |
| Channel 2 width             | 0.8 mm                             |
| Inlet radius                | 1 mm                               |
| Outlet width                | 1.6234 mm                          |
| Point of care width         | 5.3202 mm                          |

After the cleaning process is completed, the next step is resistant coating. In this process, the thin SU-8 layer is spread across the glass surface and the grip of the entire SU-8 laying onto the glass surface is enhanced. On the spinning coater, the glass plate is attached. In the center of the glass plate, about 1 ml SU-8 is then dropped. Spin velocity is set for 30 seconds to 1000rpm and for 10 seconds to 50rpm. The sample is then softly baked at temperature of 95°C for 45 minutes using a hot plate after spinning coating with SU-8 3050. During the soft baking process, the solvent level of SU-8 layer is reduced, and thus diminishes the risk of exposed resist loss, swelling and the adhesion defects. The sample remains on the cold plate for 20 minutes. Then, the designation mask was exposed to UV light. The exposed region remained after the development since negative photo resistance is employed in our cases. The exposure time is 55 sec. The glass slide is then moved to the warm plate for 45 minutes to bake hard at 95°C. The glass diaphragm is then developed. The sample is developed using the developer solution that is SU-8 developer. The purpose of the process is to remove the undesirable resistance and shape the pattern. The rinsing and drying process is followed by SU-8 developers, Isopropanol (IPA) and Distilled Water so that the process is not developed after removal from the glass surface. The rest of the pattern on the glass slide is referred to as the microfluidic mold used in the following process. The form SU-8 received from the last step is used as a form in soft lithography following the development procedure.

2.4 DNA Extraction using Fabricated Microfluidic Device

DNA extraction process by using microfluidic device does have a mixing process that is same with the DNA extraction process using the DNA extraction commercial kit. The difference is the time taken to obtain the double-stranded DNA and reducing the equipment that needed to be used during the DNA extraction process. Mixing the lysis buffer to samples of food is the first mixing process. This procedure is performed for cell disruption and cellular content extraction. The second process to obtain a single beam DNA is to mix NaOH solution into a double beamed DNA. This mixing process is done by simultaneously drop the sample and lysis solution into the first and the second inlet of the Microfluidic device. The double-stranded DNA is obtained at the first output and been tested using UV-Vis and NanoDrop. Then, the double-stranded DNA is converted to single-stranded DNA by dropping a 0.01M of NaOH at the third inlet of the Microfluidic device.
3. Result and Discussion

3.1 DNA Extraction using Commercial Kit

DNA quantification by using NanoDrop spectrophotometer was used to quantify the concentration the specimen of the nucleic acid since the efficiency of downstream reactions rely on the concentration of these nucleic acids. DNA can be quantified from several types of specimens such as genomic DNA, PCR product, plasmid DNA. There are two main methods which the extracted DNA amount can be determined. DNA yield can be assessed using four different methods which are absorbance (optical density), agarose gel electrophoresis, fluorescent DNA binding dyes and a luciferase-pyro phosphorylation-coupled quantitation system. In this research, absorbance method was used to obtain the results.

After the entire extraction process was completed, double-stranded DNA was obtained. It took between one and a half hours to obtain the double stranded DNA. However, the ssDNA only took five minutes processing where the dsDNA was mixed with a 0.01µM of NaOH solution with 1µL:1µL ratio to denature the dsDNA. There are 24 samples of dsDNA and ssDNA that been obtained from DNA extraction process using commercial Kit. The 24 samples is obtained from 6 fluid samples which one fluid sample have 4 output which is a pure sample double-stranded DNA, a sample mix with synthetic E.coli double-stranded DNA, a sample mix with synthetic Listeria double-stranded DNA and a sample mix with synthetic Salmonella double-stranded DNA. The sample is tested using NanoDrop Spectrophotometer. The result was plotted in a graph as shown in Figure 3.

![Figure 3. Synthetic samples, double-stranded DNA sample and single-stranded DNA sample.](image)

Figure 3 shows that there are huge different between the sample that is not added with synthetic samples and the samples that is added with synthetic samples. DNA concentration and purity both can be determined by measuring the absorption of ultraviolet light. DNA absorbs UV more or less strongly depending upon the wavelength. NanoDrop spectrophotometer, will takes measurements at wavelengths of 260 and 280 nm, and in addition determines an absorption spectrum from 220 – 330 nm. The graph shows that all the synthetic samples have a high peak on the 260 wavelength. This indicate that there are high reading on DNA being detected in the synthetic samples. 260/280 - ratio of absorbance at 260 nm and 280 nm. The ratio of absorbance at 260 and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.
Table 2. Double-stranded and single-stranded DNA purity and concentration.

| Samples               | Double-Stranded DNA | Single-Stranded DNA |
|-----------------------|---------------------|---------------------|
|                       | Concentration(ng/µL)| Purity              | Concentration(ng/µL)| Purity  |
| Synthetic E.coli      | -                   | -                   | 819.763             | 1.81    |
| Synthetic Listeria    | -                   | -                   | 832.265             | 1.85    |
| Synthetic Salmonella  | -                   | -                   | 847.502             | 1.77    |
| Chocolate Milk        | 3.927               | 2.07                | 1.966               | 2.28    |
| Chocolate Milk with E.coli | 3.628           | 2.12                | 75.767              | 1.83    |
| Chocolate Milk with Listeria | 5.704          | 2.02                | 75.984              | 1.81    |
| Chocolate Milk with Salmonella | 3.696       | 2.26                | 77.837              | 1.78    |

Table 2 shows the concentration and purity of double-stranded DNA and single-stranded DNA. Based on the sample, the purity of the DNA could be determined. This is to make sure that the DNA is totally free from other biomolecules such as protein. In the table, most of the samples range between 1.8-2.0 which was still in the range of a clear DNA.

3.2 DNA Purity and Concentration Calculation

The concentration of the extracted DNA was estimated spectrophotometrically by using UV/Visible spectrophotometer Ultraspec 2100 pro [16]. Spectrophotometric assay was used to determine the quantity and purity of the DNA. The quantitation of DNA was done by taking absorbance at 260 nm, 280 nm and 320 nm[17]. The ratio of absorbance at 260 nm and 280 nm were calculated using formula 2. The value of pure DNA sample should be in the range of 1.8 to 2.0. A lower ratio is an indication of protein contamination. DNA solution with A260 nm contains approximately 50 µg/mL of single-stranded DNA. Thus, the concentration of DNA was calculated according to the formula 1.

Concentration of DNA µg/mL = \((A_{260} - A_{320}) \times \text{dilution factor (df)} \times 50 \frac{\mu g}{ml}\)  

\[
\text{DNA Purity} = \frac{A_{260} - A_{320}}{A_{280} - A_{320}}
\]

4. Conclusion

This research goal has been successfully achieved by developing a double-stranded and single-stranded chip microfluidic device. The effort is made by using PDSS with a lab glass slide as the base for mouth to design and manufacture a Microfluidic Chip for a lab on chip devices. The device functional test shows that the sample can be extracted using the Microfluidic Chip according to the path designed. Therefore, as set out in Chapter 1, the research has effectively met three objectives. The first goal is achieved by successfully designing and producing the Microfluidic device using the PDMS technology in soft lithography. Evaluation of single-stranded DNA and double-stranded DNA is done using NanoDrop spectrophotometer and UV-Vis spectroscopy which lead to the achievement to the second and third objectives.

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Reference

[1] Azizah N., Hashim U., S. C. B. Gopinath, and S. Nadzirah, “A direct detection of human papillomavirus 16 genomic DNA using gold nanoprobe,” *Int. J. Biol. Macromol.*, vol. 94, pp. 571–575, 2017.

[2] A. Ayoib, U. Hashim, S. C. B. Gopinath, and M. K. Md Arshad, “DNA extraction on bio-chip: history and preeminence over conventional and solid-phase extraction methods,” *Applied Microbiology and Biotechnology*. 2017.

[3] D. M. Prendergast *et al.*, “Application of PCR for rapid detection and serotyping of Salmonella spp. from porcine carcass swabs following enrichment in semi-solid agar,” *Food Res. Int.*, 2012.

[4] N. Azizah, U. Hashim, S. C. B. Gopinath, and S. Nadzirah, “Gold nanoparticle mediated method for spatially resolved deposition of DNA on nano-gapped interdigitated electrodes, and its application to the detection of the human Papillomavirus,” 2016.

[5] N. Z. Natasha, R. D. A. A. Rajapaksha, M. N. A. Uda, and U. Hashim, “Electrical DNA Biosensor using Aluminium Interdigitated Electrode for E. Coli O157 : H7 detection,” vol. 020235, 2017.

[6] N. A. Parmin *et al.*, “Voltammetric determination of human papillomavirus 16 DNA by using interdigitated electrodes modified with titanium dioxide nanoparticles,” *Microchim. Acta*, vol. 186, no. 6, 2019.

[7] S. C. Tan and B. C. Yiap, “DNA, RNA, and Protein Extraction: The Past and The Present,” *J. Biomed. Biotechnol.*, vol. 2009, pp. 1–10, 2009.

[8] M. N. A. Uda, R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, and A. B. Jambek, “Selective detection of E.coli O157:H7 bacteria DNA using electrical based aluminium interdigitated electrode biosensor,” *AIP Conf. Proc.*, vol. 2045, no. December, 2018.

[9] R. D. A. A. Rajapaksha, M. N. A. Uda, U. Hashim, S. C. B. Gopinath, and C. A. N. Fernando, “Impedance based Aluminium Interdigitated Electrode (Al-IDE) biosensor on silicon substrate for salmonella detection,” *IEEE Int. Conf. Semicond. Electron. Proceedings, ICSE*, vol. 2018-Augus, pp. 93–96, 2018.

[10] R. D. A. A. Rajapaksha, U. Hashim, M. N. Afnan Uda, C. A. N. Fernando, and S. N. T. De Silva, “Target ssDNA detection of E.coli O157:H7 through electrical based DNA biosensor,” *Microsyst. Technol.*, 2017.

[11] M. G. Mauk, C. Liu, M. Sadik, and H. H. Bau, “Microfluidic devices for nucleic acid (NA) isolation, isothermal NA amplification, and real-time detection,” *Methods Mol. Biol.*, 2015.

[12] G. Luka *et al.*, “Microfluidics integrated biosensors: A leading technology towards lab-on-A-chip and sensing applications,” *Sensors (Switzerland)*. 2015.

[13] A. K. Au, W. Lee, and A. Folch, “Mail-order microfluidics: Evaluation of stereolithography for the production of microfluidic devices,” *Lab Chip*, 2014.

[14] T. Adam, U. Hashim, T. S. Dhahi, and M.N.A.Uda, “Fabrication of PDMS Based Micro Fluidic Devices Fabrication of PDMS Based Micro Fluidic Devices,” in *2014 Fifth International Conference on Intelligent Systems, Modelling and Simulation*, 2014, no. January, pp. 717–721.

[15] R.D.A.A Rajapaksha, N. A. N. A. M. N. A. Uda, U.hashim, S.C.B.Gopinath, and C.A.N.Fernando, “Multichannel PDMS microfluidic based nano- biolab-on-a-chip for medical diagnostics,” in *AIP Conference Proceedings 2045*, 2018, vol. 020020, no. December, pp. 1–7.

[16] N. Azizah, U. Hashim, S. Nadzirah, M. K. M. Arshad, A. R. Ruslinda, and S. C. B. Gopinath, “Comparison of different methods for extraction and purification of human Papillomavirus (HPV) DNA from serum samples,” in *AIP Conference Proceedings*, 2017, vol. 1808.

[17] F. H. Stephenson, “Nucleic Acid Quantification,” in *Calculations for Molecular Biology and Biotechnology*, 2016.