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Potential of Sriwijaya Thermal Cycler Smart Controlling-Based as a Tool for DNA Sequence Polymerase Chain Reaction

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ABSTRACT

Background. Along with the COVID-19 pandemic, the need for a thermal cycler device for examining COVID-19 is getting bigger. Many laboratories have overused the use of thermal cyclers due to the limited availability of this tool. This study aims to test the effectiveness of the Sriwijaya thermal cycler based on a smart controller in conducting PCR DNA sequences. **Methods**. The research design was an experimental study with a posttest control group approach, in order to see an overview of the results of the polymerase chain reaction (PCR) in the form of intermediate DNA bands using Sriwijaya Thermal Cycler compared to groups using factory-made Thermal cyclers. **Results.** The PCR results showed precise results from the image and band separation, and there was a clear separation of the bands on the marker. The PCR results from the ACE I / D gene showed quite good and optimal results in the separation of the PCR results band. **Conclusion**. Sriwijaya thermal cycler is effective in DNA polymerase chain reaction (PCR) sequences comparable to that of the manufacturer's Thermal Cycler.

1. Introduction

Polymerase chain reaction (PCR) technique is a technology that is increasingly being used in various biomolecular and genetic examinations.[1] PCR is a technique used to apply or multiply the DNA sequence so that the nitrogen base sequence in the DNA strand can be studied and assessed.[2,3] Without carrying out the DNA sequence amplification process, it will not be easy to study the nitrogen base strands in DNA. The PCR technique, which was initially only performed for advanced research and not routine examinations, is now becoming recognized along with the use of this technique for the gold-standard examination for the diagnosis of COVID-19. Along with the COVID-19 pandemic, the need for a thermal cycler device for examining COVID-19 is getting bigger. Many laboratories have overused the use of thermal cyclers due to the limited availability of this tool. The need for a thermal cycler is increasing while the price of this tool in the market is relatively high. This encourages ideas and creativity to develop a thermal cycler device called the Sriwijaya thermal cycler based on a smart controller. This domestically produced thermal cycler device is much more economical and easy to use, so it is hoped that it can answer the need for domestic thermal cycler equipment.

Thermal cycler has a working principle of regulating the temperature regulation of annealing, denaturation and elongation. The ability of this tool to regulate

temperature regulation and the length of exposure to temperature are essential points in the development of a smart controlling-based thermal cycler device. Arduino is used as a microcontroller that regulates the length of temperature exposure to the DNA sample. The use of the Arduino microcontroller is a critical point and the novelty of the thermal cycler technology being developed. This study aims to test the effectiveness of the Sriwijaya Thermal Cycler based on a smart controller in conducting PCR DNA sequences.

2. Research Methods

Study design

The research design was an experimental study with a posttest control group approach, in order to see an overview of the results of the polymerase chain reaction (PCR) in the form of intermediate DNA bands using Sriwijaya Thermal Cycler compared to groups using factory-made Thermal cyclers. The sample tested in this study was the DNA of healthy respondents, where this study has received approval from the Ethics Committee for Medical and Health Research, Faculty of Medicine, Universitas Sriwijaya (No.154 / KEPKK-FKUNSRI / X / 2020).

Preparation of DNA sequences for PCR

A sample of 3 mL of blood was taken from a healthy individual and then inserted into an EDTA tube, then the blood sample was centrifuged at a speed of 5000 rpm, for 10 minutes, 25oC. The centrifuged supernatant was separated from the blood portion of the sample. The part that contains blood cells is used for the isolation of DNA from white blood cells. The next stage, the blood sample was isolated by DNA, starting with the sample mixed with 0.5% saponins in PBS 1x, then incubated for 4 hours and centrifuged at a speed of 12,000 rpm for 10 minutes, resulting in precipitates. The precipitate was separated and added back with ddH2O, then again centrifuged. The precipitate was then added with 100ul ddH2O and 50 ul Chelex-100 20%, then boiled for 10 minutes, then returned to centrifuge at 12,000 rpm for 10 minutes. The supernatant was collected and stored at -20oC for PCR.

ACE I / D polymorphisms were examined using the polymerase chain reaction (PCR) method. In brief, ACE is obtained by a separate reaction, using an oligonucleotide. PCR primers were 5'GCCCTGCAGGTGTCTGCAGCATGT3' (forward primary) and 5'GGATGGCTCCCCGCCTTGTCTC3' (reverse primary). The final volume of the PCR reaction mixture was 20 µL containing one mM of each primer (Invitrogen®), 1.5 mM MgCl2, 0.2 mM of each deoxynucleoside triphosphate - dNTp, 2.5 µL of 10X PCR Buffer and 0.5 U of Taq polymerase (Invitrogen ®). Amplification was performed in a thermocycler (Biorad®) with an initial denaturation step at 94 ° C for 2 minutes followed by 40 cycles consisting of denaturation at 94 ° C for 10 seconds, annealing at 60 ° C for 30 seconds and extension at 72 ° C for 30 seconds. PCR products were separated on 8% polyacrylamide gel and DNA visualized with silver nitrate. This gel is prepared with 15 mL of polyacrylamide solution, 129 µL ammonium persulfate and 15 µL TEMED (N, N, N', N'-Tetramethylethylenediamine). The DNA fragment measures 190 bp for the D allele and 490 bp for the I allele.

Sriwijaya thermal cycler developing

The manufacture of the thermal cycler device is carried out with a rectangular aluminium container. Furthermore, the construction of a thermal regulator with Wiremound resistors, 150 ohms / 50 Watts, Arctic Silver Thermal Epoxy, Solid-state relay 25A AC / DC SSR, MAX31855 breakout Thermocouple wire 60mm fan 12 V 12 DC transistor, 0.5A power supply, Regular power cable was carried out. Furthermore, developing the Arduino microcontroller regulator coding and the final stage of construction will be carried out.

Data analysis

The data analysis in this study was carried out qualitatively by visualizing the comparison between the PCR results using a manufacturer's thermal cycler and the Sriwijaya thermal cycler. Comparison of the bands formed and the clarity of the band image are essential

points in the PCR result comparison process.

3. Results

Figure 1 shows the results of the polymerase chain reaction (PCR) of the ACE I / D gene with these primers and reverses in the method. The PCR results showed precise results from the image and band separation, and there was a clear separation of the bands on the marker. The PCR results from the ACE I / D gene showed quite good and optimal results in the separation of the PCR results band.

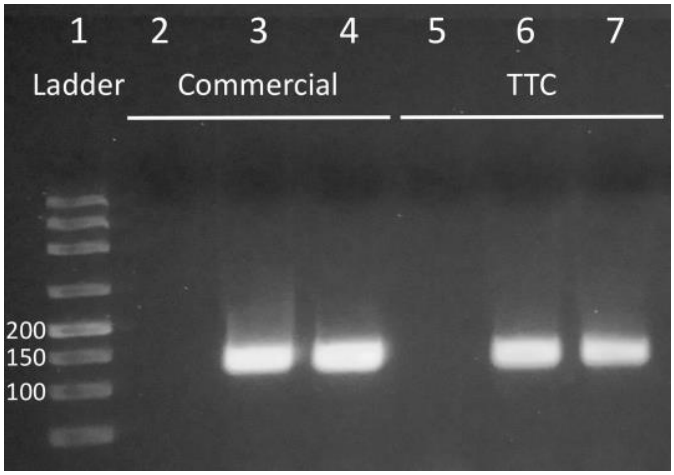

Figure 1. Comparison of PCR Results with Thermal Cycler Commercial and Thermal Cycler Creation (TTC)

4. Discussion

Sometimes called "molecular photocopying," the polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" - copy - small segments of DNA.[4-6] Because significant amounts of a sample of DNA are necessary for molecular and genetic analyzes, studies of isolated pieces of DNA are nearly impossible without PCR amplification. For amplifying a segment of DNA using PCR, the sample is first heated, so the DNA denatures or separates into two pieces of single-stranded DNA.[7,8] Next, an enzyme called "Taq polymerase" synthesizes - builds - two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment.[9-11]

The entire cycling process of PCR is automated and can be completed in just a few hours. It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.[12-15] Sriwijaya Thermal Cycler with temperature control using a sufficient microcontroller as a thermal cycler. This study shows that the use of a microcontroller is beneficial and efficient to maintain the temperature regulator and heating time of the thermal cycler. The identical results between the thermal cycler manufacturer and the Sriwijaya thermal cycler show the potential and success of Arduino as a master temperature and heating time regulator of the thermal cycler.

5. Conclusion

Sriwijaya thermal cycler is effective in DNA polymerase chain reaction (PCR) sequences comparable to that of the manufacturer's Thermal Cycler.

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