DESIGN AND FABRICATION OF MINI PCR MACHINE

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Introduction:
Polymerase chain reaction (PCR) machine is a device used to multiply small sequences of DNA/RNA molecules. PCR machines are also called as thermo-cyclers as PCR regulates and maintain different set temperature modes. The first mode in PCR operation is the denaturation of the DNA/RNA strands, where the two strands are broken to form two separate strands. Denaturation occurs at above 90 degree Celsius the hydrogen bonds linking the bases to one another are weak, therefore denaturation occur. The hydrogen bonds are broken at high temperatures, whereas the bonds between deoxyribose and phosphates remain intact as these are stronger. In the beginning, the DNA target sequence of interest is marked by the primers that anneal (bind) to the complementary sequence. Annealing usually takes place between 40 degrees Celsius and 65 degrees Celsius. The temperature is then raised to approximately 72 degrees Celsius after the primers anneal to the complementary DNA sequences, the enzyme Taq DNA polymerase is used to replicate the DNA strands. Taq DNA polymerase is a recombinant thermo stable DNA polymerase from the organism Thermus aquatics and unlike normal polymerase enzymes it is active at high temperatures. Extension of the strand begins at the 3’ end of the primer making a double strand out of each of the two single strands that was denatured at the first stage. This project was taken up from instructables with the reference URL:https://www.instructables.com and changes have been made in fabrication to get a reliable and portable PCR machine.

Objectives:
- To design a compact, portable PCR machine.
- To fabricate a reliable, compact, portable PCR machine.
- To fabricate a working prototype with low cost and high efficiency.
Methodology:

The project started with an aluminium block, we processed it by making it into a heat sink, three sides the block was processed the circular saw to increase the surface area for heat transfer (heat removal). We drilled holes on top of the block using a special type of drill bit that was tapered by grinding using angle grinder, on top of the block where the samples will be placed that resembles the shape of the Eppendorf tube. It was seen that all the heating blocks are placed at 120 degrees apart around the sample holder such that three heaters will heat one sample evenly. Another hole was made equidistant to the heater and the sample holder and threaded such that it holds a thermocouple where it provides the temperature data that will be processed by a microcontroller. Two Peltier units were attached to remove heat from the block. Fans were attached at all the sides of aluminium block except on the top to extract the heat from the block. All the fans, heaters and Peltier units were parallel connected to two 12 volt power supplies are connected the power up the whole system. Arduino Uno was used to regulate the temperature for denaturation, annealing and extension maintaining the temperature at different points for specific time intervals. It was programmed to maintain specific temperature points for specific time interval. The programme sends information to switch on heater and cooler through a relay. The output pins from the Arduino are 3and 9. Heat in the block was taken up by lm35 that has an accuracy of +/- 0.5 degree centigrade. The temperature sensor is fixed by a bolt placed equidistant to heater and sample port.

Results and Conclusions:

The project was switched on and monitored for temperature regulation using a thermometer the results were as expected. As it heated up to 94 degree centigrade the heaters is switched off and the coolers was switched on, as the temperature dropped to 58 degree centigrade it maintained it 30 seconds and after that it heated to 72 degree centigrade and then to 94 degree centigrade and the cycle started for about 32 cycles. The initial denaturation for 94 degrees centigrade had a longer time then during the cycle started. The final extension was about five minutes where the temperature was 72 degree centigrade. We used a thermometer to measure the temperature inside the PCR tube port. We started the sample DNA amplification with a amplification kit by aristo gene. The Regents were added and placed inside the PCR port and was powered up the process of heating and cooling started completing it two
hours the final amplified DNA was made to run on gel electrophoresis at 50 volt. After the time interval of 45 minutes the gel was placed in a gel doc and bands where observed.

**Scope For Future Work :**

- The control system can be integrated with PID controller which makes it robust.
- More sample ports can be made such that many samples can be amplified at the same time.
- Power consumption and size can be reduced if it has a compact cooling system.
- Programming can be improved with specific delay to improve the life of the power supply.
- LCD display indicating current temperature and current cycle can be installed to monitor the cycle.