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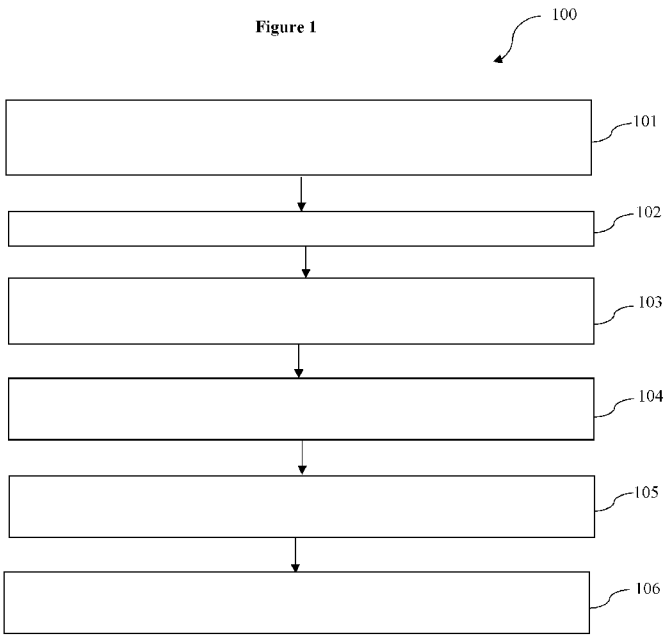
(54) Title: METHOD OF DEVELOPING HEAT CONTROL PLATFORM FOR ISOTHERMAL NUCLEIC ACID AMPLIFICATION TECHNIQUES

Figure 1



(57) Abstract: The present invention relates to a method (100) of developing heat control platform required for isothermal nucleic acid amplification techniques.

[Continued on next page]

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METHOD OF DEVELOPING HEAT CONTROL PLATFORM FOR ISOTHERMAL NUCLEIC ACID AMPLIFICATION TECHNIQUES

Technical Field

The present invention relates to a method of developing heat control platform required for isothermal nucleic acid amplification techniques.

Background of the Invention

Nucleic Acid Amplification methods are one of the most important tools used in pathogen and disease detection. In this method, Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) molecules –which are aimed to be detected– are amplified with the help of different polymerase and transcriptase enzymes by being targeted with binding oligomers (primers) designed specifically for the target sequence and thus, the signal to be created by the amplified product by interacting with the relevant nucleic acid dyes are made traceable. Quantitative Polymerase Chain Reaction (K-PCR) method is the method which is specified as the gold standard for nucleic acid amplification methods in the literature. This method can amplify target nucleic acids by means of heat cycles provided with the help of an electronic device. However, both due to the need for expensive equipment and specialized personnel, this method is not suitable for field application and widespread use at low-cost. Isothermal Nucleic Acid Amplification Methods are nucleic acid amplification methods developed as an alternative to this method and they can be performed at a constant temperature. Due to these properties thereof, it has been extensively studied in the literature and it is thought that it may be an alternative to the K-PCR method. Although this method is much more suitable for application in the field, it is necessary to develop the cheap and portable heat stabilization systems it requires.

It is very critical to ensure the correct temperature conditions required according to the nucleic acid amplification technique used. These conditions may consist of a single constant temperature value in reactions such as LAMP and RPA (for example, 65°C for LAMP, 37°C for RPA) and they may require circulation between a plurality of different temperature values in more complex reactions such as PCR as well.

In this process, for the reactions to run as efficiently as possible, it is necessary to reach the target temperature quickly and then to maintain that temperature throughout the required process. The systems responsible for controlling and maintaining the temperature also need to maintain the target temperature against unwanted fluctuations.

The United States patent document no. **US2021069709**, an application included in the state of the art, discloses a thermal cycling apparatus used for isothermal nucleic acid amplification. The said thermal cycling apparatus comprises a sample holder for heating and cooling of a sample rapidly, a heating source, a cooler, a temperature control system and a signal monitoring system. The temperature control system and the signal monitoring system are used for controlling the output of the heating source. A signal sensor is included to detect the optical signals received from the samples in the sample holder. The heating layer included in the system is obtained by using materials with high thermal conductivity such as aluminum, silver, copper or gold. Temperatures of the samples in the sample holder are maintained at 65°C for isothermal nucleic acid amplification. In addition, the temperature control system may be any of fiber optic thermometer, infrared thermometer, fluid crystal thermometer, pyrometer, quartz thermometer, silicon bandgap temperature sensor, temperature strip, thermistor and thermocouple. With the inventive system, specific DNA or RNA is detected from biological samples and pathogen detection is performed.

Summary of the Invention

An objective of the present invention is to realize a method of developing heat control platform required for isothermal nucleic acid amplification techniques.

Another objective of the present invention is to realize a method of developing heat control platform required for isothermal nucleic acid amplification techniques by combining parts (ready for direct sale) which are inexpensive and easily accessible and available without modification.

Detailed Description of the Invention

A "Method (100) of Developing Heat Control Platform for Isothermal Nucleic Acid Amplification Techniques" realized to fulfil the objectives of the present invention is shown in the figure attached, in which:

Figure 1 is a flow chart of the inventive method.

The components illustrated in the figures are individually numbered, where the numbers refer to the following:

100. Method

The inventive method (100) of developing heat control platform required for isothermal nucleic acid amplification techniques, comprises steps of

- preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101);
- adding at least one heating/cooling element under the mold (102);
- adding a UV source and UV detection sensor on opposite side surfaces of the mold (103);
- connecting a temperature detection sensor to the chamber (104);
- connecting a control unit to the reservoir (105); and

- obtaining a heat control platform for isothermal nucleic acid amplification techniques by attaching a tracking screen to the device (106).

In the step of preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101) of the inventive method (100); heat blocks being included in 3D printer extruders and having suitable dimensions for placing PCR tubes – which are reaction tubes– are used as chambers. It is ensured that the chamber is selected to be made of a material such as copper, or aluminum –which is a good conductive material– in order that the reaction tubes placed in the chamber can reach the desired temperature quickly. Then, a mold is added to the outer part of the chamber with polystyrene polymer –which is a thermally conductive material– so as to spread the heat evenly to the reaction tube inside the chamber. The mold surrounds the chamber from the outside, excluding the upper part where the sample is placed.

In the step of adding at least one heating/cooling element under the mold (102) of the inventive method (100); a peltier, 3D printer heating elements or cooling fans are placed under the thermal mold surrounding the chamber, in order to provide heating and/or cooling. The heating elements are connected to any power supply which is capable of providing the current and the power required for operation and is electronically controllable. The peltier –which is used in one embodiment of the invention– is a peltier plate which is TEC1-12706 branded, operates with a power of 12 V - 51.4 W in the range of -30°C to ~70°C at 40 x 40 mm sizes.

In the step of adding a UV source and UV detection sensor on opposite side surfaces of the mold (103) of the inventive method (100); a UV source which is a 265-275 nm UV-C cooled LED lamp emitting UV light for detecting DNAs of the target pathogen inside the reaction tubes wherein reactions of isothermal nucleic acid amplification method are performed, is placed on one surface of the mold. A UV detection sensor, which can detect light at wavelengths of 240-370 nm, is placed on the opposite surface of the mold where the V source is placed. Thus, the absorbance

changes –which occur in the reaction liquid inside the reaction tube included in the light path reaching from the UV source to the UV detection sensor– and the changes in the nucleic acid concentration in the sample can be monitored. The values of absorbance change and the changes in the nucleic acid concentration in the sample obtained are transmitted to the control unit and displayed on the monitoring screen.

In the step of connecting a temperature detection sensor to the chamber (104) of the inventive method (100); sensors, electrical properties of which vary by temperature, such as thermocouple or thermistor are used for tracking the chamber temperature instantly. The data received from the temperature detection sensor is transmitted to the control unit and it can be tracked on the tracking screen.

In the step of connecting a control unit to the reservoir (105) of the inventive method (100); the control unit is in connection with the temperature detection sensor, heater/cooler elements and UV sensor and it enables data input related to the temperature value of the reaction tube that is desired to be kept constant. With the control unit –which can run control algorithms on itself and may be microcontroller cards such as Arduino or DeneYap– instant chamber temperature data is received from the temperature detection sensor and the need for heating or cooling is determined by controlling the compliance with the temperature value set by the control unit. If it is determined that heating is required, the control unit operates the heating element or if it is determined that cooling is required, the cooling element is operated. Thereby, the constant temperature required for isothermal amplification techniques can be provided.

In the step of obtaining a heat control platform for isothermal nucleic acid amplification techniques by attaching a tracking screen to the device (106) of the inventive method (100); waiting times of the reaction tubes in the chamber, the chamber temperature, the UV sensor and the change values of DNA amount in the reaction tube are displayed.

With the inventive method (100), a temperature control platform which will provide the constant temperature required for isothermal nucleic acid amplification techniques is obtained by combining parts that are inexpensive and easily accessible and available without modification. In one example of the invention, a constant temperature of 65°C can be provided for the LAMP method, which is an isothermal nucleic acid amplification technique.

The absorbance provided by DNA molecule to light at 260 nm wavelength has been characterized for many different studies in the literature. The aim of the inventive method (100) is to make the absorbance-based nucleic acid characterization system feasible with inexpensive and easily accessible electronic components and thus to ensure detection of DNAs to be formed by the LAMP reaction, which is a model of an isothermal nucleic acid amplification method, if it contains the target pathogen genome area. For this method (100), a UV detection sensor capable of detecting light at wavelengths of 240-370 nm and a LED lamp with 265-275 nm UV-C cooler are used. Operation of the reaction is tested by placing these two correspondingly and measuring the UV absorbance such that it will be passed through the reaction liquid inside the reaction tube to be placed in the light path reaching from the UV lamp to the sensor.

In the method (100), in summary, isothermal reaction tubes are placed in the chamber and a waiting period of half an hour is started and it is easily tracked on the tracking screen. In the meantime, while the system is heated by the peltier module, the change of the DNA amount in the reaction is periodically controlled by UV lamp and UV sensor. Periodic time intervals of 1 minute/measurement; 2 minutes/measurement; 5 minutes/measurement; 10 minutes/measurement are tested at the first step and it is determined which one is more suitable. An increase in the amount of absorbance at 260 nm, depending on the amount of DNA, is considered as a positive test and target pathogen is detected.

Within these basic concepts; it is possible to develop various embodiments of the inventive “Method (100) of Developing Heat Control Platform for Isothermal Nucleic Acid Amplification Techniques”; the invention cannot be limited to examples disclosed herein and it is essentially according to claims.

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CLAIMS

1. A method (100) of developing heat control platform required for isothermal nucleic acid amplification techniques; **characterized by** steps of
   - preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101);
   - adding at least one heating/cooling element under the mold (102);
   - adding a UV source and UV detection sensor on opposite side surfaces of the mold (103);
   - connecting a temperature detection sensor to the chamber (104);
   - connecting a control unit to the reservoir (105); and
   - obtaining a heat control platform for isothermal nucleic acid amplification techniques by attaching a tracking screen to the device (106).

2. A method (100) according to Claim 1; **characterized in that** in the step of preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101); heat blocks being included in 3D printer extruders and having suitable dimensions for placing PCR tubes –which are reaction tubes– are used as chambers.

3. A method (100) according to Claim 2; **characterized in that** in the step of preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101); the chamber is selected to be made of a material such as copper, or aluminum –which is a good conductive material– in order that the reaction tubes placed in the chamber can reach the desired temperature quickly.

4. A method (100) according to Claim 3; **characterized in that** in the step of preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101); a mold is added to the outer part of the chamber with

polystyrene polymer –which is a thermally conductive material– so as to spread the heat evenly to the reaction tube inside the chamber.

5. A method (100) according to Claim 4; **characterized in that** in the step of preparing a chamber wherein at least one reaction tube will be placed and a mold outside (101); the mold surrounds the chamber from the outside, excluding the upper part where the sample is placed.

6. A method (100) according to Claim 1; **characterized in that** in the step of adding at least one heating/cooling element under the mold (102); a peltier, 3D printer heating elements or cooling fans are placed under the thermal mold surrounding the chamber, in order to provide heating and/or cooling.

7. A method (100) according to Claim 6; **characterized in that** in the step of adding at least one heating/cooling element under the mold (102); the heating elements are connected to any power supply which is capable of providing the current and the power required for operation and is electronically controllable.

8. A method (100) according to Claim 1; **characterized in that** in the step of adding a UV source and UV detection sensor on opposite side surfaces of the mold (103); a UV source which emits UV light for detecting DNAs of the target pathogen inside the reaction tubes wherein reactions of isothermal nucleic acid amplification method are performed, is placed on one surface of the mold.

9. A method (100) according to Claim 8; **characterized in that** in the step of adding a UV source and UV detection sensor on opposite side surfaces of the mold (103); a UV detection sensor is placed on the opposite surface of the mold where the V source is placed, thus the absorbance changes –which occur in the reaction liquid inside the reaction tube included in the light path

reaching from the UV source to the UV detection sensor– and the changes in the nucleic acid concentration in the sample can be monitored.

10. A method (100) according to Claim 9; **characterized in that** in the step of adding a UV source and UV detection sensor on opposite side surfaces of the mold (103); the values of absorbance change and the changes in the nucleic acid concentration in the sample obtained are transmitted to the control unit and displayed on the monitoring screen.

11. A method (100) according to Claim 1; **characterized in that** in the step of connecting a temperature detection sensor to the chamber (104); sensors, electrical properties of which vary by temperature, such as thermocouple or thermistor are used for tracking the chamber temperature instantly.

12. A method (100) according to Claim 11; **characterized in that** in the step of connecting a temperature detection sensor to the chamber (104); the data received from the temperature detection sensor is transmitted to the control unit and can be tracked on the tracking screen.

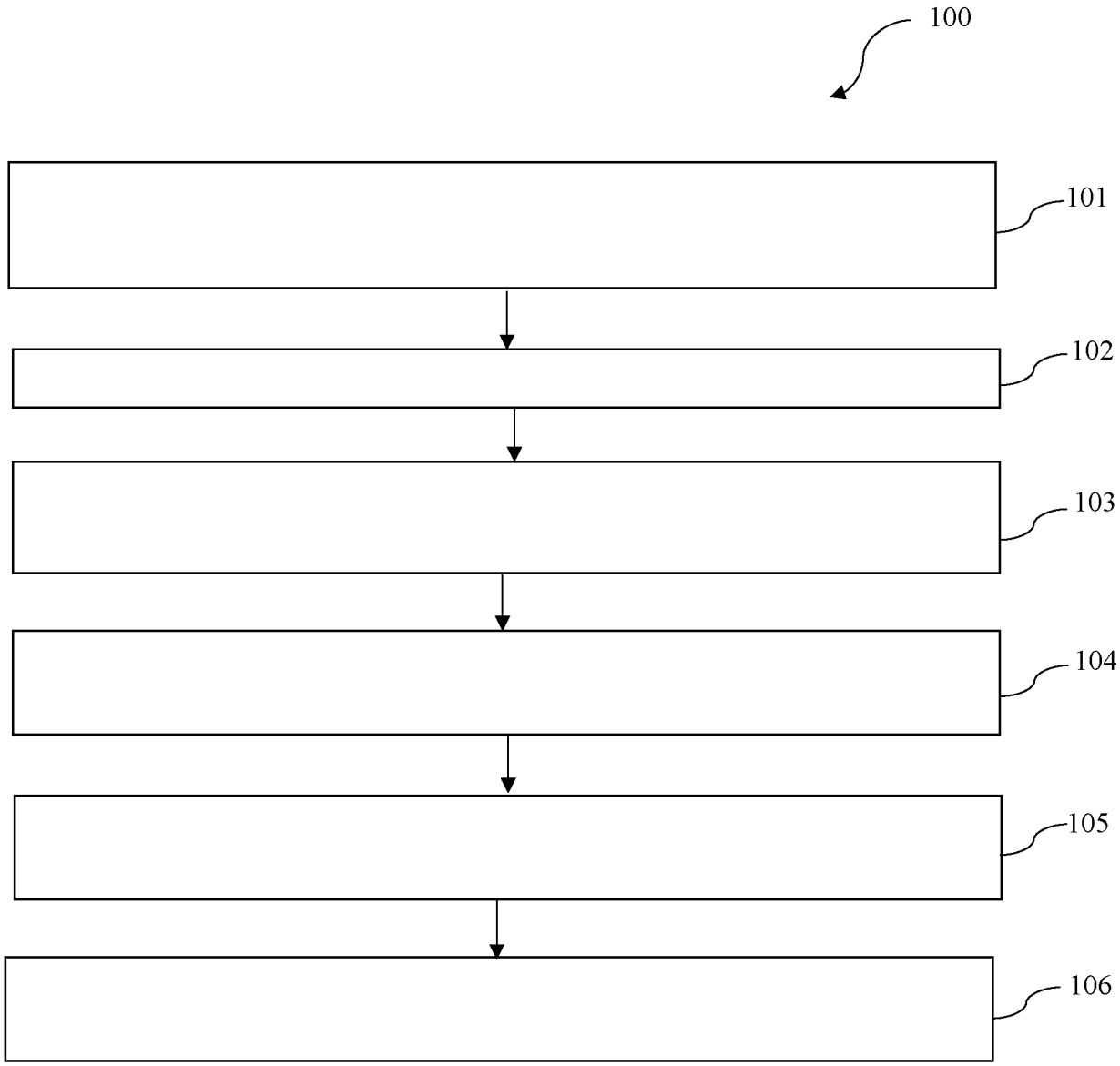
13. A method (100) according to Claim 1; **characterized in that** in the step of connecting a control unit to the reservoir (105); the control unit is in connection with the temperature detection sensor, heater/cooler elements and UV sensor and it enables data input related to the temperature value of the reaction tube that is desired to be kept constant.

14. A method (100) according to Claim 13; **characterized in that** in the step of connecting a control unit to the reservoir (105); with the control unit –which can run control algorithms on itself and may be microcontroller cards such as Arduino or DeneYap– instant chamber temperature data is received from the temperature detection sensor and the need for heating or cooling is determined by controlling the compliance with the temperature value set by the control unit.

15. A method (100) according to Claim 14; **characterized in that** in the step of connecting a control unit to the reservoir (105); if it is determined that heating is required, the control unit operates the heating element or if it is determined that cooling is required, the cooling element is operated; thereby, the constant temperature required for isothermal amplification techniques can be provided.

16. A method (100) according to Claim 1; **characterized in that** in the step of obtaining a heat control platform for isothermal nucleic acid amplification techniques by attaching a tracking screen to the device (106); waiting times of the reaction tubes in the chamber, the chamber temperature, the UV sensor and the change values of DNA amount in the reaction tube are displayed.

Figure 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/TR2023/051340

A. CLASSIFICATION OF SUBJECT MATTER

B01L 7/00(2006.01)i; ***C12Q 1/686***(2018.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

B01L; C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL & KEYWORDS: PCR, heat, control, tube, vessel, cool, fan

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017098521 A1 (INDIAN INST OF TECH GUWAHATI [IN]) 15 June 2017 (2017-06-15) page 6, page 7, lines 9-18, page 12, lines 20-28, page 13, lines 9 and 10, page 14, lines 24-28, page 17, lines 15-25, page 19, lines 1-7	1-16
X	WO 2019231339 A1 (UBIQUITOME LTD [NZ]) 05 December 2019 (2019-12-05) figure 3, page 10, paragraphs 1 and 3, page 11, paragraphs 2 and 3, page 12, paragraph 2	1-16

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
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Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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