applied sciences

MDPI

Article

Low-Cost Temperature Logger for a Polymerase Chain Reaction Thermal Cycler

Chan-Young Park [1,2], Jae-Hyeon Cho [1,2], Yu-Seop Kim [1,2], Hye-Jeong Song [1,2] and Jong-Dae Kim [1,2,*]

[1] Department of Convergence Software, Hallym University, Chuncheon 24252, Korea; cypark@hallym.ac.kr (C.-Y.P.); jhcho1028@hallym.ac.kr (J.-H.C.); yskim01@hallym.ac.kr (Y.-S.K.); hjsong@hallym.ac.kr (H.-J.S.)

[2] Bio-IT Research Center, Hallym University, Chuncheon 24252, Korea

[*] Correspondence: kimjd@hallym.ac.kr; Tel.: +82-10-8705-5567

Academic Editor: Wen-Hsiang Hsieh

Received: 13 September 2016; Accepted: 27 October 2016; Published: 31 October 2016

Abstract: Polymerase chain reaction (PCR) is a method of amplifying DNA which is normally carried out with a thermal cycler. To obtain more accurate and reliable PCR results, the temperature change within the chamber of the thermal cycler needs to be verified and calibrated regularly. Commercially available temperature loggers commonly used for temperature verification tests usually require a graphical user interface (GUI) attached to the logger for convenience and straightforward understanding of the device. In this study, a host-local architecture for the temperature logger that significantly reduces the development time and cost is proposed. Employing standard computing devices as the host gives better development environment and user-friendly GUI. This paper presents the hardware and software design of the host-local temperature logger, and demonstrates the use of the local temperature logger connected to a personal computer with a Windows operating system. The probe design, thermistor resistance measurement, temperature filtering, and temperature calibration is described in detail. The thermistor self-heating problem was investigated in particular to determine the reference resistor that was serially connected to the thermistor. The temperature accuracy and temporal precision of the proposed system was 0.1 K.

Keywords: PCR; the negative temperature coefficient (NTC) thermistor; GUI; calibration; filtering; log; self-heating

1. Introduction

Polymerase chain reaction (PCR), a method of amplifying segments or parts of DNA with a thermal cycler, is the most potent technology in molecular biology which is employed in various biochemical analysis, clinical laboratories, and basic research areas [1–5]. The efficiency or amplification results are very sensitive to the reaction conditions due to the exponential DNA amplification during the reaction [6–8]. Therefore, the reagent used or the performance of the thermal cycler to control and maintain the chamber temperature greatly influences the reproducibility or inter-laboratory variability. To obtain globally reliable PCR results, the PCR thermal cycler must be stable in temperature control and maintenance, and the inter-laboratory temperature variation should be reduced by temperature calibration [6–9].

To measure the temperature of the well within a thermal cycler, a temperature probe and data logger are required [6,8–11]. The probe with a temperature sensor should be designed to provide tight thermal-coupling with the well of the aluminum block, and the logger should read and store the temperature of the sensor. In a common research laboratory, the temperature is read through a high-cost data acquisition system and a probe composed of a reagent container with a thermocouple is inserted [6,8–10]. A PCR tube made of polypropylene is adapted as the reagent container which is susceptible to wear out over the course of time and therefore is not permanently reusable, and should be built anew for every test. However, employing a high-cost acquisition system to read the probe sensor has disadvantages regarding portability and cost. The temperature sensing probe is usually designed for repetitive use in a commercial thermal cycler temperature verification system [12–16].

Currently, the provider of a thermal cycler offers periodic validation services with a large scale thermal cycler temperature logger [17,18]. However, this service is not readily available to users who want to immediately measure and calibrate the temperature when the PCR amplification results seem to be off. Therefore, the users commonly employ a general temperature logger and a hand-made probe to serve that purpose. Many companies also provide hand-held type thermal cycler checkers for this purpose [14,15]. They commonly embed both of the temperature reading logic in a portable system and graphical user interface (GUI) with an LCD screen [12–16]. However, providing GUI with a LCD screen increases the system size and its production and maintenance cost. Owing to the recent portability enhancement of standard computing devices, including PCs and smart devices, it is more beneficial to perform the user interaction such as saving the log information with the standard computing devices [19–22]. If a user adopts a standard computing device as a host system, it is possible to reduce the system cost drastically by using a low cost microcontroller only to interface the probe sensor. The maintenance cost can also be reduced by leaving complex tasks to the host PC and simplifying the embedded firmware. Although a thermocouple is mainly adopted in temperature sensing and calibration, the negative temperature coefficient (NTC) thermistor is more advantageous in cases where the temperature range is limited such as in the PCR thermal cycler. In addition, an NTC thermistor which is a lower cost temperature sensor providing highest sensitivity at a narrow temperature range, can also contribute to decreasing the system cost.

The existing portable thermal cycler validation systems provide an accuracy of 0.1 °C and the drift of 0.1 °C/year which are acceptable in the PCR application [14,15,23]. The calibration of the systems is required for them to be regularly authenticated by an official certification authority for traceability. The calibration process normally requires two iteration steps: the system is sent for the first measurement and it is calibrated based on the measurement report and then sent again after calibration to obtain the final measurement. We created a convenient calibration tool so that the calibration could be easily performed at the certified institution of accredited authority, which enables the elimination of the iteration process. The measurement drift, which is related to the precision of the system, should be controlled during the calibration interval. We also present the intermediate report on the ongoing long term measurements for the precision of the introduced system.

This paper proposes a permanent probe with an NTC thermistor and a temperature logger that are composed of a structurally low-cost temperature reader and a host PC responsible for the user interaction and saving and calibrating the measured temperature. In addition, the various design factors are investigated and analyzed for reducing the cost of the system, and for securing its accuracy and precision. First, this paper presents a temperature probe that enables tight thermal coupling with the chamber of the thermal cycler. Second, possible solutions to resolve issues related to the precise measurement of the temperature are introduced with regards to the temperature filtering, calibration of measured temperature, temperature conversion methods, and resistance measurement of the thermistor. Lastly, the thermistor self-heating issue is investigated. The low resistance thermistor increases in self-heating while it delivers the decreased thermal noise [24]. Therefore, the thermistor resistance should be set as low as possible so that the self-heating can be negligible. The materials and methods of the aforementioned design variables and factors are presented in Section 2. The experimental results of long-term precision and self-heating tests are presented in Section 3, followed by the conclusion and discussions in Section 4.

2. Materials and Methods

2.1. Probe and Local System Implementation

Figure 1 illustrates the cross-sectional view of the probe presented in this paper. An aluminum bolt was used to attach the NTC thermistor (standard precision interchangeable thermistor, US Sensors, Orange, CA, USA) to the aluminum head. The empty space between the aluminum head and the NTC thermistor was filled with thermal grease, which was also used when assembling bolts to the aluminum head to allow proper thermal conduction. The exterior of the aluminum head was designed to achieve satisfactory thermal-coupling with the well by matching the slope of the cone to that of the thermal cycler well. Components other than the aluminum head which are completely inserted into the well, are made with a plastic material (acrylonitrile butadiene styrene, ABS) to minimize heat radiation of the material. The heat radiation will result in creating a temperature gradient between the well and the sensor, hence effecting the efficiency of the measurement and calibration. The height from the probe cap to the aluminum head was equal to that of a 25 mL PCR tube to perform the measurement under the same environment as the actual run of a PCR reaction, where the thermal cycler's lid heater was closed. The leg of the NTC thermistor was fixed using a Teflon tube. A high-end earphone cable (Nobunaga Labs MMCX TR-SE3, WiseTech Inc., Taito, Japan) which was well known for low frequency noises, was used to connect the thermistor and reader.

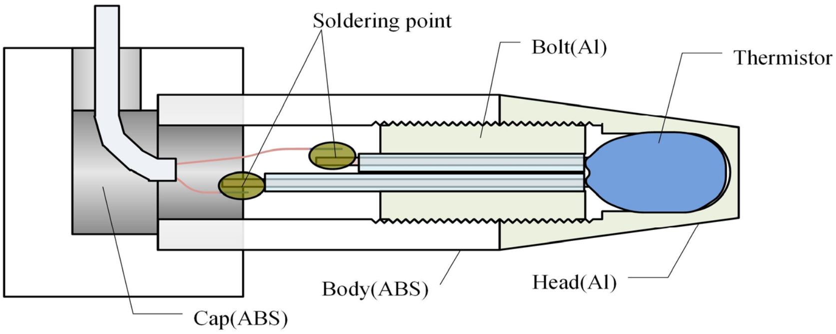
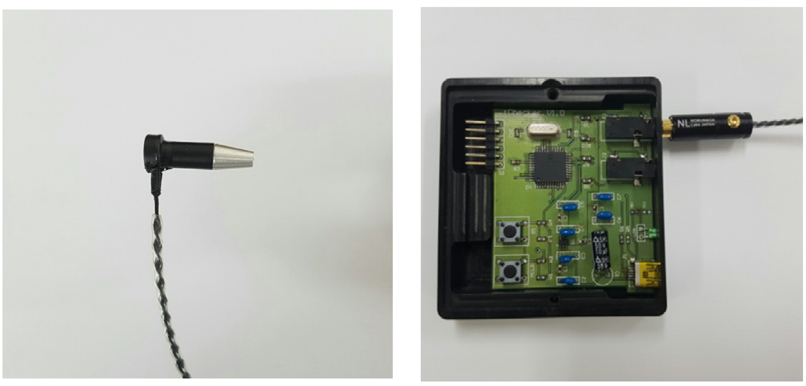


Figure 1. Cross-sectional view of probe (Al: aluminum, ABS: Acrylonitrile butadiene styrene).

The assembled probe and the temperature reader is shown in Figure 2. The earphone cable is connected to the proposed probe (Figure 2a), and the probe is connected to the temperature reader through the stereo earphone jack at the other end (Figure 2b). The temperature reader was configured to connect two earphone cables where each cable carries two probes, enabling simultaneous measurement of four probes in total.



(**a**) (**b**)

Figure 2. Assembled local system with probe and temperature reader. (**a**) probe; (**b**) temperature reader.

The reader in Figure 2b was implemented using an 8 bit microcontroller (PIC18F4553, Microchip Chip Technology Inc., Chandler, AZ, USA) with an imbedded analog–digital converter (ADC) and universal serial bus interface (USB). The thermistor resistance was calculated (Figure 3) by measuring the voltages divided from the precision reference resistor without a buffer amplifier, and connected to the imbedded 12 bit ADC inside the microcontroller. By calibrating the input impedance of the ADC in the microcontroller, long term precision and accuracy were ensured using a simple circuit [25].

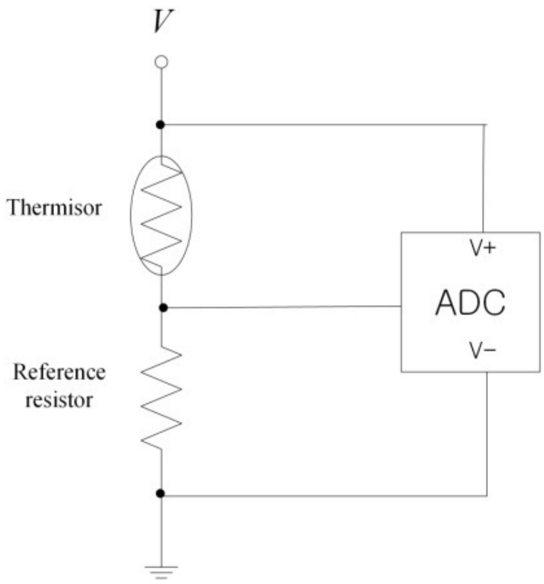


Figure 3. Thermistor resistance measuring circuit.

The precision reference resistance value was set to receive maximum resolution in the temperature range mainly used during PCR. A long-term median filter and a short-term average filter were placed to manage noise filtering. To control short-term average filtering, all divided voltages measured in each channel were summed over the number of the filter tap, and thereafter sent to the host. The host divided the delivered sum by the number of filter taps to complete the average filter of the measured voltages. The filtered value of the divided voltage was converted to the thermistor resistance, and the resistance was converted to temperature based on the Steinhart–Hart equation:

$$\frac{1}{T} = A + B + \log R + C(\log R)^3, \tag{1}$$

where R and T are the thermistor resistance and absolute temperature, respectively. The calibration constants are shown in the equation as A, B, and C. Among the various calibration methods developed, the most widely used Steinhart-Hart equation was adopted in this study which provides sufficient accuracy with simplicity in the temperature range of the thermal cycler application (50~98 °C). The thermistor employed in this paper was calibrated at 4 points (50, 60, 72, 95 °C) and the maximum error from the resistance-temperature table provided from the vendor was 1.7 mK [26].

The calculated temperatures were filtered again using a median filter. The required specification of the response time was 1 s, and the temperatures could be obtained every 100 ms, enabling the application of a 10-tap median filter.

The temperature logger was calibrated and certified by an official certification authority (Korea Research Center for Measuring Instruments Co., Ltd., Anyang, Korea). The norm to receive the proper certification was to send the fully completed system to the accredited authority to obtain the measurement report, followed by the iteration process for receiving the final report after calibration. However, we created a convenient calibration tool so that the calibration could be easily performed at the certified institution of accredited authority, and the iteration process was not necessary.

2.2. Selection of the Thermistor Resistance

The thermal noise of an NTC thermistor decreases as the resistance decrease. However, to obtain the highest resolution with the divided voltage method (Figure 3), the resistance of the reference resistor should be reduced at the same rate of that of the thermistor [21]. Consequently, the self-heating effect increases due to the increase in resistive heat power when using the low resistance thermistor. Note that the current is inversely proportional to the thermistor resistance. Therefore, the lowest resistance should be selected within the range where the self-heating is negligible. Given that the ADC input impedance calibration data for the 10 kΩ thermistor at 25 °C were shown to be precise and accurate from the data we gathered, we investigated the self-heating effects of thermistors that were over 10 kΩ. The maximum value of the thermistor with the same performance as in the 10 kΩ was 100 kΩ; thus, we first studied these two types of thermistors. To determine the maximum resolution, reference resistors with the resistance of 1.8 and 18 kΩ were used for the 10 and 100 kΩ thermistors, respectively. To investigate the self-heating effect, a circuit independent of the reader could be constructed to measure the resistance/temperature while switching the current on/off through the thermistor. However, it was also possible to determine whether the self-heating could be ignored by applying a step temperature to the sensor, and observing the step response through the temperature logger. In cases where the self-heating has a significant effect on the sensor, the temperature measured within the constant temperature water bath will gradually reach the equilibrium temperature after the power is applied. However, if the self-heating is negligible, the temperature will reach the equilibrium temperature without delay as the power is applied.

When the sensor was mounted on the probe, the self-heating might have insignificant effect because of the wider heating surface of the aluminum head. Thus, in the present study, the experiments were performed to evaluate the aforementioned step responses before and after mounting the 10 and 100 kΩ NTC thermistors on the probe. The described experiments were conducted at 95, 72, 60, and 50 °C that were the critical temperatures in the PCR cycle. We waited until the measured temperature remained constant in the water bath prior to starting the experiment. After reaching the steady state temperature, the reader was switched off for a sufficient time, and switched on again to obtain the step response. This response was compared with the temperature without self-heating, which could be obtained by measuring the thermistor resistance with a digital multi-meter.

2.3. Software Implementation

The software from the host system is responsible for two functions: measuring the temperatures from the four probes and providing the user interface. The temperature measurement function covers the analog-to-digital conversion (ADC) and filtering of the sampled data. The user interface function is categorized into two subsets, each specifically aimed for the two groups of users. One group of users is the thermal cycler users who will use the proposed system to calibrate the thermal cycler. For the thermal cycler users, the user interface displays and records the current temperature, as well as the statistical comparison between the probe temperatures. The calibration status of the probes is also displayed in this subset. The other group of users is the people in charge of the calibration authorization office. The person who will authenticate the calibration needs another tool that will calculate the Steinhart-Hart constants based on the measured temperature. We reported these user interface tools in our previous work [27].

The average and median filter are employed to filter the temperature measurement. These filters are functionally partitioned into the local system and the host to minimize the complexity of the local system, as described in the above section. Therefore, the local system firmware is very simple, where the firmware is on standby after system initialization until it receives orders from the host USB. Then the local firmware will send the averaged divided voltages from the four probes back to the host for analysis.

2.4. Long Term Precision Test

After calibrating the temperature logger presented in this research, periodical tests were performed for more than seven months. A probe was placed in a water bath at a constant temperature. Thereafter, its temperature was measured to observe its deviation from the temperature of the initial calibration. The temperature was measured at 85 and 55 °C corresponding to the temperature points used in the verification of the commercial PCR thermal cycler.

3. Results

3.1. Step Response Test Results for the Self-Heating Effect

Figure 4 shows the step response at 95 °C in constant temperature water bath. The interval where the temperature measured is less than 94.5 °C shown in the middle of the figures (denoted as 'B' interval) represents when the reader power was shut off. The dashed and solid line depicts the temperature changes of the assembled probe and the thermistor directly put in the water bath, respectively. When placing the bare thermistor in the water bath without mounting it on the probe, the initial temperature when the power was turned back on gradually increased and reached equilibrium as shown by the solid line. We measured the resistance of the thermistors with a digital multi-meter (Fluke 189, Fluke Corp., Everett, WA, USA) to obtain the resistance without self-heating. Note that the multi-meter delivered negligible current when measuring the resistance. The measured resistance was converted to the temperature using the same conversion method as that in the proposed system. The converted temperatures for the bare thermistor and the probe were 95 and 95.2 °C, respectively. The steady state temperature difference between the thermistor (95.5 °C) and water bath (95 °C) was caused by self-heating of the thermistor as mentioned above. In contrast, the dashed line showed that the equilibrium and initial temperatures were the same, implying that sufficient release of heat occurred through the wide surface of the probe, maintaining the temperature as it was, without the effect of self-heating.

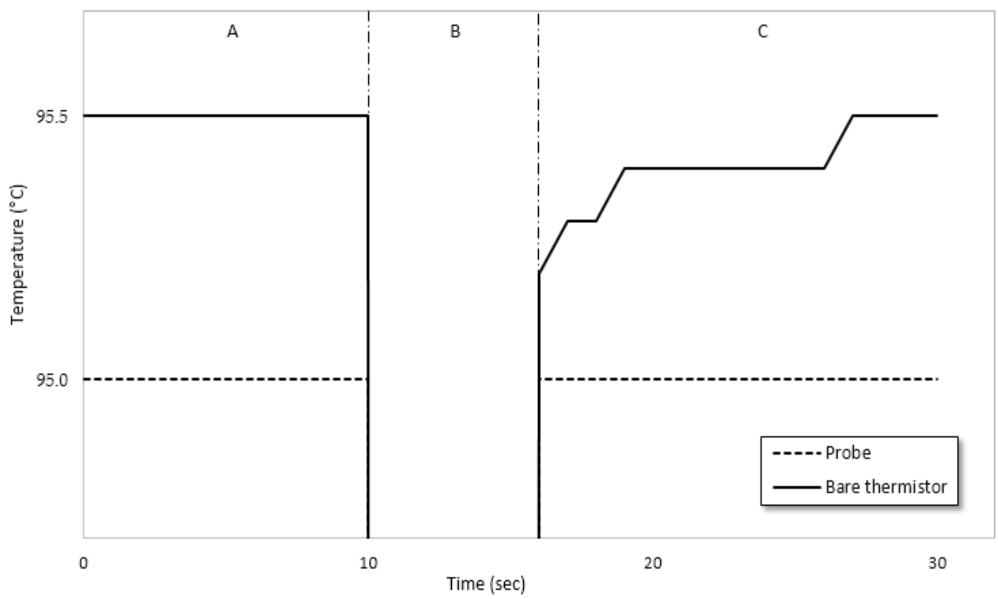


Figure 4. Self-heating test at 95 °C.

Table 1 shows the self-heating effects of the 10 and 100 kΩ thermistors. As expected, self-heating was not observed in the 100 kΩ thermistor. However, the 10 kΩ thermistor was 0.2–0.3 °C higher than the water bath temperature. Table 2 shows that the self-heating effect of the 10 kΩ thermistor was cancelled out by the thermal spread through the probe head. The row labeled as "Bare thermistor," which shows the results from the "10 kΩ" row in Table 1, shows higher temperatures than those of the water bath due to self-heating. In contrast, the row labeled as "probe" showed no self-heating effect. This implied that the thermistor was thermally coupled well with the aluminum head and the surface area of aluminum head fully released the heat caused by self-heating.

Table 1. Self-heating effect of 10 and 100 kΩ thermistor.

Thermistor Resistance	50 (°C)	60 (°C)	72 (°C)	95 (°C)
10 kΩ	0.2	0.2	0.2	0.3
100 kΩ	0.0	0.0	0.0	0.0

Table 2. Self-heating cancellation for the probe aluminum head radiation (10 kΩ).

Bare or Probe	50 (°C)	60 (°C)	72 (°C)	95 (°C)
Bare thermistor	0.2	0.2	0.2	0.3
Probe	0.0	0.0	0.0	0.0

3.2. Long Term Stability Test Results

Table 3 shows the results tracked for two months after calibrating the presented temperature logger. The first test included the temperature measured immediately after calibration. The four probes of the temperature logger were measured at 85 and 55 °C by simultaneously placing them in the water bath. The results of repeated measurements conducted five times over the course of two months showed a variation within 0.1 °C, demonstrating the stability of temporal precision.

Table 3. Temporal precision test.

True Temperature	Probe ID	55 (°C)	85 (°C)
1st test (14 March 2016)	Probe 1	55	85.1
	Probe 2	55	85
	Probe 3	55	85
	Probe 4	55	85
2nd test (17 March 2016)	Probe 1	55	85.1
	Probe 2	55.1	85
	Probe 3	55	85
	Probe 4	55	85
3nd test (22 March 2016)	Probe 1	55	85
	Probe 2	55.1	85
	Probe 3	55.1	85
	Probe 4	55	85
4th test (28 March 2016)	Probe 1	55	85
	Probe 2	55.1	85
	Probe 3	55	84.9
	Probe 4	55	85
5th test (30 March 2016)	Probe 1	55	85
	Probe 2	55.1	85
	Probe 3	55	85
	Probe 4	55	85
6th test (10 May 2016)	Probe 1	54.9	85
	Probe 2	55	85
	Probe 3	55	85
	Probe 4	55	85.1
7th test (20 October 2016)	Probe 1	55	85
	Probe 2	55.1	84.9
	Probe 3	55.1	85
	Probe 4	55	85

4. Discussion and Conclusions

This paper presents a host-local architecture for a temperature logger used to verify the temperature of the well within the PCR thermal cycler. This system is comprised of the host PC responsible for the user interface, the reader system to measure the temperature of the sensor, and the probe that was designed to enable sufficient thermal coupling between the temperature sensor and the chamber of the thermal cycler. The ADC input impedance calibration and sensor interface for reducing the system cost were also introduced. In addition, tools for temperature certification that eliminated the certification iteration were also described. A method for selecting the thermistor resistance value to solve the thermistor self-heating issue was established, and the cancellation capability of the self-heating effect by the presented probe was experimentally demonstrated.

The methods and system proposed in this study can lead to the development of a low cost PCR thermal cycler temperature verification system. Additionally, the proposed host-local system will provide a relatively inexpensive solution from both the maintenance and development perspective by using a standard computing device as the host. Further applications employing a cloud interface or a smart device will also improve the cost efficiency and portability of the proposed system. In addition, the temperature calibration tool implemented in this study will also help reduce the cost and time for calibrating the temperature of the PCR thermal cycler. The target temperature accuracy of the proposed system was 0.1 K and it had high temporal precision stability.

Acknowledgments: This material is based upon work supported by the Ministry of Trade, Industry & Energy (MOTIE, Korea) under Industrial Technology Innovation Program. No. 10052106, 'Development of CMOS/MEMS hybrid biosensor array platform'.

Author Contributions: Chan-Young Park and Jong-Dae Kim conceived of and designed the system architecture. Jae-Hyeon Cho and Yu-Seop Kim and Hye-Jeong Song designed and performed the experiments. Chan-Young Park and Jong-Dae Kim analyzed the data and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Verdoy, D.; Barrenetxea, Z.; Berganzo, J.; Agirregabiria, M.; Ruano-López, J.M.; Marimón, J.M.; Olabarría, G. A novel Real Time micro PCR based Point-of-Care device for Salmonella detection in human clinical samples. *Biosens. Bioelectron.* **2012**, *32*, 259–265. [CrossRef] [PubMed]
2. Wu, J.; Kodzius, R.; Xiao, K.; Qin, J.; Wen, W. Fast detection of genetic information by an optimized PCR in an interchangeable chip. *Biomed. Microdevices* **2012**, *14*, 179–186. [CrossRef] [PubMed]
3. Srinivasan, B.; Tung, S. Development and applications of portable biosensors. *J. Lab. Autom.* **2015**, *20*, 365–389. [CrossRef] [PubMed]
4. Jiang, X.; Jing, W.; Zheng, L.; Liu, S.; Wu, W.; Sui, G. A continuous-flow high-throughput microfluidic device for airborne bacteria PCR detection. *Lab Chip* **2014**, *14*, 671–676. [CrossRef] [PubMed]
5. Volpatti, L.R.; Yetisen, A.K. Commercialization of microfluidic devices. *Trends Biotechnol.* **2014**, *32*, 347–350. [CrossRef] [PubMed]
6. Young, H.K.; Yang, I.; Bae, Y.S.; Park, S.R. Performance evaluation of thermal cyclers for PCR in a rapid cycling condition. *Biotechniques* **2008**, *44*, 495–505.
7. Saunders, G.C.; Dukes, J.; Parkes, H.C.; Cornett, J.H. Interlaboratory study on thermal cycler performance in controlled PCR and random amplified polymorphic DNA analyses. *Clin. Chem.* **2001**, *47*, 47–55. [PubMed]
8. Schoder, D.; Schmalwieser, A.; Schauberger, G.; Kuhn, M.; Hoorfar, J.; Wagner, M. Physical characteristics of six new thermocyclers. *Clin. Chem.* **2003**, *49*, 960–963. [CrossRef] [PubMed]
9. Yang, I.; Kim, Y.-H.; Byun, J.-Y.; Park, S.-R. Use of multiplex polymerase chain reactions to indicate the accuracy of the annealing temperature of thermal cycling. *Anal. Biochem.* **2005**, *338*, 192–200. [CrossRef] [PubMed]
10. Xiang, Q.; Xu, B.; Fu, R.; Li, D. Real time PCR on disposable PDMS chip with a miniaturized thermal cycler. *Biomed. Microdevices* **2005**, *7*, 273–279. [CrossRef] [PubMed]
11. Xiang, Q.; Xu, B.; Li, D. Miniature real time PCR on chip with multi-channel fiber optical fluorescence detection module. *Biomed. Microdevices* **2007**, *9*, 443–449. [CrossRef] [PubMed]

12. Rpbert, L.; Grossman, J.W.F. Temperature Verification for Polymerase Chain Reaction Systems. U.S. Patent 5,224,778 A, 6 July 1993.
13. Brothers, B.; Christianson, W.; Hunter, L.; Hunter, M.; Sitler, C.; Stauder, B. Portable Electronic Thermometer and Method of Temperature Measurement. U.S. Patent 3,822,598 A, 9 July 1974.
14. Thermal Cycler Temperature Verification System. Available online: https://tools.thermofisher.com/content/sfs/manuals/cms_070449.pdf (accessed on 20 October 2016).
15. 1 Channel Temperature Verification Kit. Available online: http://www.alphatechnics.com/precision-thermometry/1-channel-temperature-verification-kit/ (accessed on 20 October 2016).
16. Knieriem, A.S.; Quinn, D.E.; Wawro, J.T.; Lane, J. Sealed Probe Chamber for Thermometry Apparatus. U.S. Patent 6,827,488 B2, 7 December 2004.
17. On-Site Temperature Verification Service. Available online: https://www.thermofisher.com/us/en/home/products-and-services/services/instrument-qualification-services/compliance-and-validation/on-site-temperature-verification.html (accessed on 20 October 2016).
18. Thermal Validation Services. Available online: http://www.bio-rad.com/en-ch/product/thermal-validation-services (accessed on 20 October 2016).
19. Nicolae, M.; Lucaci, L.; Moise, I. Embedding Android devices in automation systems. In Proceedings of the 2013 IEEE 19th International Symposium Design Technology Electronic Packaging (SIITME), Galati, Romania, 24–27 October 2013; pp. 215–218.
20. Drumea, A. Control of industrial systems using android-based devices. In Proceedings of the 36th International Spring Seminar on Electronics Technology, Alba Iulia, Romania, 8–12 May 2013; pp. 405–408.
21. Diab, M.O.; Brome, R.A.M.; Dichari, M.; Moslem, B. The smartphone accessory heart rate monitor. In Proceedings of the International Conference on Computer Medical Application (ICCMA), Sousse, Tunisia, 20–22 January 2013.
22. Kim, J.D.; Park, C.Y.; Yeon, J.; Kim, Y.S.; Song, H.J. Development of PCR controller and smart-phone application based on bluetooth communication. *Int. J. Multimed. Ubiquitous Eng.* **2013**, *8*, 223–230. [CrossRef]
23. Driftcon Operations Manual. Available online: http://cyclertest.com/documentation/driftcon/manual.aspx (accessed on 20 October 2016).
24. Sapoff, M.; Oppenheim, R.M. Theory and application of self-heated thermistors. *Proc. IEEE* **1963**, *51*, 1292–1305. [CrossRef]
25. Park, C.; Kim, J.; Kim, J.; Kim, Y.; Song, H.; Kim, J. Buffer-less system for thermistor temperature measurement. In Proceedings of the 2012 IEEE International Conference on ICT Convergence (ICTC), Jeju, Korea, 15–17 October 2012; Volume 2012, pp. 240–242.
26. Chen, C. Evaluation of resistance-temperature calibration equations for NTC thermistors. *Meas. J. Int. Meas. Confed.* **2009**, *42*, 1103–1111. [CrossRef]
27. Kim, J.-D.; Jeong, D.-H.; Song, H.-J.; Kim, Y.-S.; Park, C.-Y. Efficient calibration tool for thermistor. *Sens. Mater.* **2015**, *27*, 593–598.