Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 23 (2) : 2021 : 119-125

ISSN-0972-3005

INNOVATIONS IN PCR DEVICES IN TERMS OF HARDWARE PROPERTIES

IRMAK CALIK AND IREM GAZEZOGLU

[1]Department of Molecular Biology and Genetics, Faculty of Science and Letters, Baskent University, 06570, Ankara, Turkey

(Received 10 January, 2021; Accepted 9 February, 2021)

Key words : *RT-PCR, Lab on a Chip, thermal Cyclers, TECs, Microfluidics, fluorescent dyes*

Abstract – Biotechnology co-progresses along the continuous development within other technologies and science fields such as nanophysics, thermodynamics etc. Their developments had an equal contribution to PCR devices, one way or another new innovations end up being integrated within them such as different thermal cyclings or sensors used to identify fluorescent dyes. With the global 2020 pandemic, biotechnological advances quickly became a hot-topic globally and PCR marketing skyrocketed due to an increase of demands. With a rise in positive SARS-CoV-2 cases, laboratories sought more lightweight and portable devices that are less expensive but high-sensitive props and kits to work their tests with. Microchips' involvement within the PCR markets achieved just that, and has proven to be revolutionary. What these chips and their accompanying hardware properties within PCR devices provide, their types and in what areas some specific chips such as photodiodes suit best are all explained in this review along with crediting the first origins of PCR devices.

INTRODUCTION

PCR, the short for *Polymerase Chain Reaction*, dates back to 1985's when its origins are traced back in history. The main purpose of PCR is to, as its name implies, amplify the amount of nucleic acid sequences in multiple numbers via heating and cooling procedures to follow (Mullis, 1997). This method was first detailed by Saiki *et al.* (1985). In these reactions, the DNA region to be synthesized is extensively specific fragments. With correct properties and kits, PCR amplifications of nucleic acids became a key point in recombinant technologies without a doubt. They partake in important roles when it comes to: forensics, cloning, phylogenetic studies, fossil DNA amplifications, DNA sequence analysis and last but not the least molecular diagnosis. With the start of ongoing SARS-CoV-2 in late 2019s and Jan 2020, PCR kits and tests gained even more value. Swab tests being enough to diagnose those that are contaminated with the virus, specifically RT-PCR have been playing an important role for a while now, as they perform a reverse transcription, synthesizing DNA from mRNA. Real-time PCR is composed of a heater, temperature sensor, optical detection unit induced by fluorescence excitation (Ahrberg *et al.,* 2016). However RT-PCR kits are also costly. Thus people began seeking alternatives in microchips integrated within PCR devices (Zhu *et al.,* 2020). Involvement of microtechnology with PCR has made it possible to manufacture various types of devices with co-accompanying hardwares that each have their own purpose.

Microfluidic Chip: Lab-on-Chip PCR

Lab on a Chip PCR is the miniaturised and evolved version of the PCR within the microfluidics which serves for Point-of-Care (POC) diagnostics and provides several advantages over conventional PCR methods (Ahrberg *et al.,* 2016; Mauk *et al.,* 2015; Gorgannezhad *et al.,* 2019). The advantages are indicated as the decrease in the amount of sample and reagents regarding the small size and volume of the microchannels, laminar and stable flow, short diffusion length leading to reduction in the assay time, high mass and heat transfer rates due to the high surface to volume ratio of the small device (Gorgannezhad *et al.,* 2019). Majority of those devices are non-quantitative from the start and

solely capable of offering either positive or negative answers. The costly prices for sensors, electronics and read-out systems all are a negative effect on budgets, making it quite challenging to acquire any result that is quantitative (Nguyen *et al.*, 2018). In addition, the quality of PCR taking place on chip is preserved due to the superior thermal conductivity because of the silicon-based microfabrication (Zhang and Xing, 2007; Zhu *et al.*, 2020).

Time Domain PCR

Time-domain PCR implies the change in the temperature according to the phases of the PCR while the samples contact with the heater directly and only one heater is present. (Ahrberg *et al.*, 2016). Within this model, thermal and fluidic crosstalk would be reduced and their controls are performed precisely. In addition, thermal optimization of the reaction sites is required to ensure the chambers are exposed to heat equally. When the closed systems microfabricated on the chip are considered, it is also indicated that the speed of the PCR lacks flexibility which is an obstacle eliminated with spatial domain systems (Zhang and Xing, 2007).

Spatial Domain PCR

The spatial-domain PCR presents a model in which the samples move between several heaters at different temperatures each (Ahrberg *et al.*, 2016). In the cycles of the PCR, the amplification of the sample is facilitated by pumping the solution through the channels of the microfluidic chip as illustrated in Figure 1. Thus, the flow rate of the solution including the sample and the period required for reaching a thermal equilibrium affect the temperature transition times. The gas bubbles that can be formed in the channels are also eliminated by adding a highly viscous fluorinated oil cap before the sample (Zhang and Xing, 2007). The temperatures of the zones required for the model are determined as 95 °C, 77 °C, and 60 °C in

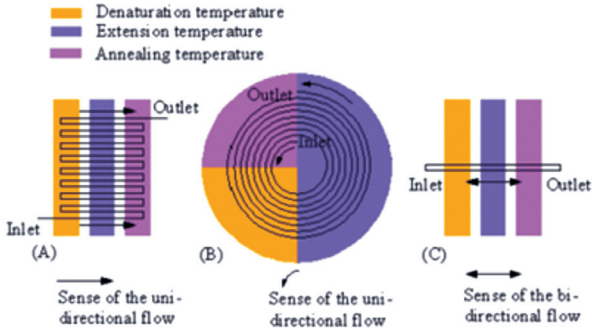

Fig. 1. Retrieved from Zhang and Xing, 2007

which the zones are generally copper blocks for the nucleic acids to pass through each of the stages of the PCR. The cross section and the length of the channels are also designed according to the residence time of a particular temperature which can be exemplified as the length of the channel in which the sample is expected to be in extension phase is more than the channels the samples would be in denaturation and annealing stages. It is also suggested that the driving force of the sample to move through the channels of the microfluidic chip is the capillary force of the liquid and air interface in a design in which the microchannels are optimized for the capillary pressure and the viscosity as a statement discarding the need of an active micropump (Gorgannezhad *et al.*, 2019).

Oscillating PCR is also introduced within the spatial domain PCR systems as a set-up the sample moves back and forth in a channel localized on the related temperature zones and the footprint of the device is reduced. It allows simple system configuration, number/dwell time flexibility, simple application in real-time (Gorgannezhad *et al.*, 2019).

Thermal Cycling System

Dating back to the early 1980's this device was an important PCR machine, invented by Kary Mullis and titled as one of the most important scientific inventions with multiple awards. It would direct the signals back to samples or measure plurality of PCR samples in real time. It is also capable of performing amplification on a target nucleotide sequence under necessary conditions. Such as the reaction mixture being a fundamental necessity (Maltezos *et al.*, 2012).

Peltier Device: Thermoelectric Cooler (TEC)

The Thermoelectric Cooler (TEC) utilizes the Peltier effect by performing the role of a steady heat pump by dissipating heat by flowing to cooler zones and ridding of the hotspots in electronic devices (Gong *et al.*, 2019). That is due to TECs being integrated onto chips in direct contact with the chip itself that they are the most viable options (Nimmagadda, 2020). As temperature control is important in performing a PCR reaction, it is not surprising to see TECs being used in PCR machines as well. In the heating blocks of thermal cycling systems, it is not uncommon to see TECs integrated. The aim is to control the thermal cycles and amplify the speed of the PCR process. Approximately making it between 20% and 30% faster (Wu *et al.*, 2019).

Microheater

Microfluidics play an important role in advancing future and current findings in the field of molecular biology and bioassays as mentioned in the previous titles. Originally, the idea for microfluidics was conceived for ink-jet printers and then adapted into scientific fields as well. Basic working principle for microfluidics involves gradients flooding into microchannels by way of external pumps and pressure. This permits precise handling of small volumes, making it an important assay when it comes to preparation and synthesis of biomolecules (Stroock *et al.,* 2008). When it comes to microfluidics roles in PCR, they are conventional for manufacturing low-cost, low-power and continuous devices as integrated devices. In the past years, this type of PCR machines had an increasing interest to them. Amplifications that are performed with these devices take minutes rather than hours and help save a lot of time when compared with other thermocyclers (Mouschou *et al.,* 2014).

The microheaters are designed with a special shape in order to provide a consistent temperature during the whole process through the PCR chamber (Barman *et al.,* 2018). Other than time, due to their low-cost nature and the fact that cell cultures have to be incubated at a uniform temperature, and require a thermal control *in vitro* microheaters are once again sought upon as a much less expensive alternative. The same reasons that apply to cultures again apply to PCR mechanisms, thermal control. Microheaters' ability to preserve a consistent temperature paves the way for them taking an important part as a core part of PCR mechanisms. The pulsing due to pressure mechanism in microheater chips quicken the thermal cycling procedure about 6 times (Nieto *et al.,* 2017).

The Rotary Zone Thermal Cycler

Deriving from the standard cycle denaturation, annealing and elongation temperatures a method to without any sacrifices from performance was developed. The aim was to benefit the speed of cycles by performing logistical factors and how suitable power required for dispersed PCR. Not only necessary for replacements and supplements, but also portability measurements such as its weight and height are included alongside environmental adaptation, reagent stabilization is all considered. Given sensitivity in regard to mono-molecular PCR and how careful technicians are expected to be in laboratories, a level of rigor must be replicated (Bartsch *et al.,* 2015).

To aid all the extra laboratory PCR elements listed above without losing any, and modular integration with other functional units, the rotary zone thermal cycler was introduced, shortened to RTZC. This system further contributes to the power efficiency of spatial cycling whilst preserving the low surface-volume ratios found in temporary cyclers used as samples. Working procedure involves rotary valves closing and blocking the bounding waters in order to lock samples in one place. Then, wheel rotation to these stationary samples brings preheated blocks in contact with the reactor in pre-set sequences (Fig. 2). Finally, at the end sample gets taken out and the tube is cleaned for reuse in the next sample (Bartsch *et al.,* 2015).

Optical Detection System

Optical detection systems in biotechnologies come with economical advantages due to their low costs compared to their equivalents that were being previously used. Their high sensitivity alongside economical advantages has turned these systems into quite advantageous and favorable ones (Storhoff *et al.,* 2004).

Photomultiplier Tubes (PMT)

Photomultiplier tube (PMT) is utilized as a tool of fluorescence detection system which occupies a smaller area serving for portability and compactness of the LoC PCR devices (Lee, 2010; Zhang *et al.,* 2006). The advantages of using PMT can be listed as gain, increase in sensitivity and linear output in a wide range of optical densities. The gain stands for the detection of sufficient amounts of photons and the maximum signal obtained from the fluorescence. The sensitivity and high Signal-to-Noise Ratio (SNR) of the PMT is preferred to be high in order to increase the quality of the fluorescence images for the detection of the wavelength of the fluorescent (Zhang *et al.,* 2006).

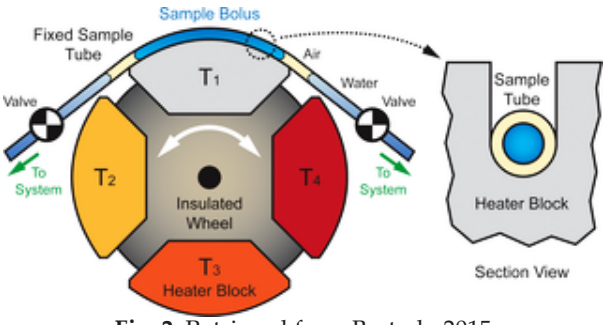

Fig. 2. Retrieved from Bartsch, 2015

Silicon Photomultipliers (SiPM) / Multi-Pixel Photon Counters (MPPC)

Silicon Photomultipliers (SiPM) also go by the name Multi-Pixel Photon Counters (MPPC) and are a result of the advances in photomultipliers. This system had become available during 2015's. SiPMs are from highly compatible solid photodetectors that are suited for high-energy physics fields. Due to their relations with photons they were also quickly favored in astrophysics (Bonanno *et al.*, 2016).

With how often fluorescence is used in PCR technologies, SiPMs were quick to become a part of these devices as well. Using silicon photomultipliers along fluorescence dyes offer a high performance yet low cost RT-PCR performances. The semiconductor SiPM serves as a high gain passive quencher and is used as a detector whenever photon rates are high. The SYBR Green I as a fluorescent dye has been proven to be efficient in these systems without the need of optical filters (Siminfar *et al.*, 2019).

Photodiode

With the development of previously described PCR techniques and devices, along the heavy influence microchips have over them an increase over these biomedical and technological assays' demands is seen. To meet aforesaid interest, the investment over these devices also increases correspondingly. One of them includes introducing photodiodes to PCR. During past pandemics, they have been a great help due to their high sensitivities, i.e EBOLA (Soares *et al.*, 2019). Meaning PCR techniques with photodiodes may as well be useful in SARS-CoV-2 pandemic and in identifying the cases.

The aim in this hybrid is to develop a combination of RCA-based nucleic acid amplifications with selective silica-bead amplicon chips. These chips are not only micro but they also carry a photodiode film that covers 200/x/200/µm area for fluorescence readings. Normally, fluorescence readings come in costy without any mobility but with the introduction of photodiodes, this problem is solved. And as microfabricated

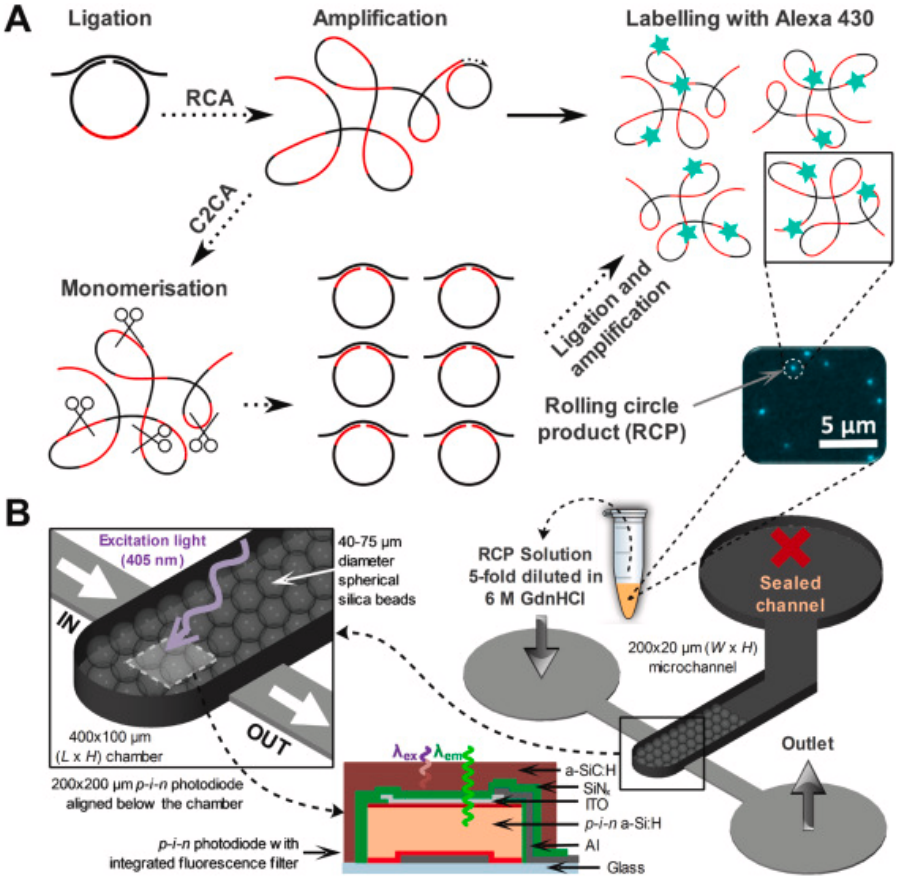

Fig. 3. Retrieved from Soares, 2019

photodiodes are reusable that makes them an affordable and one of most ideal choices. The silica microbeads practically perform the isolation and concentration of nucleic acids. The optimal environmental values include neutral pH. When the environment is at a neutral pH level a hydrophobic and ionic phosphate-silanol interaction occurs. By thus, it leads to a large overcome over two molecules mentioned. These interactions require guanidinium chloride (GdnHCl) or another chaotropic salt's presence in the environment in order for them to occur. An example of off-chip PCR is shown (Fig. 3) as a sum of how these PCR devices work. Excitation light (λex) = 405 nm and emission light(λem) = 540 nm (Soares *et al.,* 2019).

CMOS Sensors

Complementary Metal Oxide Semiconductor (CMOS) image sensor is a tool used in the biological and chemical detection systems. It is designed to detect the changes in the photon number via its photodiode and to convert them into electrical energy in the presence of an analog to digital converter (ADC) (Gupta *et al.,* 2016; Wang *et al.,* 2016). The changes in the photon number is regulated with the increasing yield of magnesium pyrophosphate and color changes. It is also known that the quantity of the photon declines while the amplification process of nucleic acid takes place (Wang *et al.,* 2016).

The (CMOS) image sensor does not require any additional equipment during the detection, which is considered as a factor reducing the cost. Because it is cost-efficient and consumes lower power as a device whose sensitivity is stated within the range of CCDs, it can be an alternative to CCDs and Photomultiplier Tubes (Wang *et al.,* 2016). As it can be seen in Fig. 4; the reaction takes place on a disposable CMOS sensor array surface, indicating a promising innovation in terms of handheld, point-of-care, inexpensive PCR devices (Gupta *et al.,* 2016; Wang *et al.,* 2016).

CCD (Charge Coupled Device) Camera

The Charged Coupled Device (CCD) sensor is a photodetector used for real-time imaging within the fluorescence detection system of PCR devices (Lee, 2010; Gorgannezhad *et al.,* 2019; Zhang *et al.,* 2006). As the sensitivity of the CCD is considered to be close to the sensitivity of PMT it can be an option to be utilized in PCR devices. Peltier elements are also used to cool the CCD which is in the form of a chip in order to increase the Signal-to-Noise Ratio (SNR) and decrease the level of noise. On the other hand, CCDs are not proper to be used in real-time PCR microfluidics in terms of compactness and portability (Zhang *et al.,* 2006).

CONCLUSION

LoC and microchip including PCR devices are being developed by biotechnology companies continuously, each having different advantages to one another or different methods to operate them. Just as mentioned a few times throughout the review, the PCR and RT-PCR market is continuously developing. One of the leading biotechnology companies, QIAGEN periodically shares their future plans on their website. With the recent developments of RNA vaccines for SARS-CoV-2, PCR and RT-PCR has repetitively been a part of people's lives. With how constant these devices have become in our lives it is to be expected of them to gain even more value in the future (Zhu *et al.,* 2019). Especially with newer methods like Next Gen Sequencing (NGS) being used in SARS-CoV-2

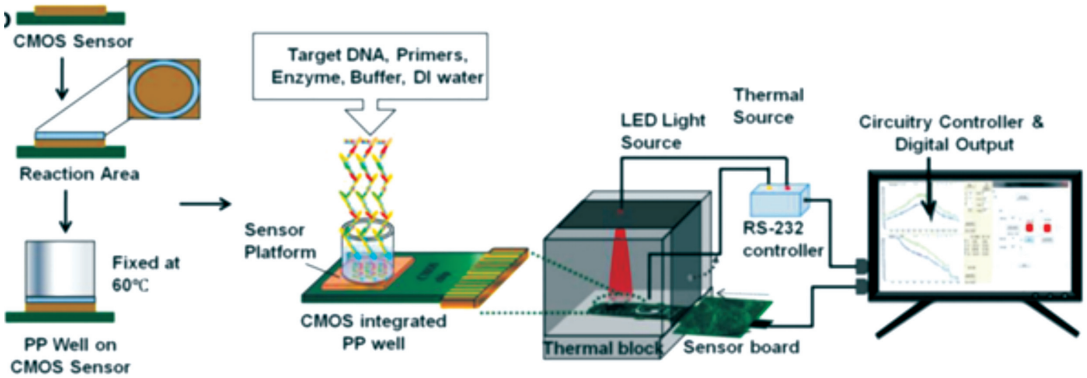

Fig. 4. Retrieved from Wang, 2016

diagnosis and PCR devices' involvement in a few NGS steps in order to perform DNA sequencing (Narayanan *et al.*, 2020; Ji *et al.*, 2020), only serves to strengthen their value.

REFERENCES

Ahrberg, C. D., Ilic, B. R., Manz, A. and Neu•il, P. 2016. Handheld real-time PCR device. *Lab on a Chip*. *16* (3): s. 586–592. doi:10.1039/c5lc01415h

Barman, U., Wiederkehr, R. S., Fiorini, P., Lagae, L. and Jones, B. 2018. A comprehensive methodology for design and development of an integrated microheater for on-chip DNA amplification. *Journal of Micromechanics and Microengineering*. *28*(8): 085014.

Bartsch, M. S., Edwards, H. S., Lee, D., Moseley, C. E., Tew, K. E., Renzi, R. F. and Patel, K. D. 2015. The rotary zone thermal cycler: a low-power system enabling automated rapid PCR. *PloS One*. 10(3), e0118182.

Bonanno, G., Marano, D., Romeo, G., Garozzo, S., Grillo, A., Timpanaro, M. C. and Sottile, G. 2016. Advances in multi-pixel photon counter technology: First characterization results. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 806: 383-394.

Gong, T., Gao, L., Wu, Y., Zhang, L., Yin, S., Li, J. and Ming, T. 2019. Numerical simulation on a compact thermoelectric cooler for the optimized design. *Applied Thermal Engineering*. 146 : 815-825.

Gorgannezhad, L., Stratton, H. and Nguyen, N. T. 2019. Microfluidic-Based Nucleic Acid Amplification Systems in Microbiology. *Micromachines*. 10 (6) : s. 408. doi:10.3390/mi10060408

Gupta, S., Ramesh, K., Ahmed, S. and Kakkar, V. 2016. Lab-on-Chip Technology: A Review on Design Trends and Future Scope in Biomedical Applications

Hung, T. Q., Chin, W. H., Sun, Y., Wolff, A. and Bang, D. D. 2017. A novel lab-on-chip platform with integrated solid phase PCR and Supercritical Angle Fluorescence (SAF) microlens array for highly sensitive and multiplexed pathogen detection. *Biosensors and Bioelectronics*. 90 : 217-223.

Ji, H., Sandstrom, P., Paredes, R., Harrigan, P. R., Brumme, C. J., Avila Rios, S. and Kantor, R. 2020. Are We Ready for NGS HIV Drug Resistance Testing? The Second "Winnipeg Consensus" Symposium. *Viruses*. 12(6): 586.

Lee, D. S. 2010. Real-time PCR machine system modeling and a systematic approach for the robust design of a real-time PCR-on-a-chip system. *Sensors (Basel, Switzerland)*. 10(1) : 697–718. https://doi.org/10.3390/s100100697

Maltezos, G., Johnston, M., Goodwin, D., Scherer, A. and Walker, C. I. 2012. *U.S. Patent No. 8 : 232,091*. Washington, DC: U.S. Patent and Trademark Office.

Mauk, M. G., Liu, C., Sadik, M. and Bau, H. H. 2015. Microfluidic devices for nucleic acid (NA) isolation, isothermal NA amplification, and real-time detection. *Methods in molecular biology (Clifton, N.J.)*, 1256: 15–40. https://doi.org/10.1007/978-1-4939-2172-0_2

Moschou, D., Vourdas, N., Kokkoris, G., Papadakis, G., Parthenios, J., Chatzandroulis, S. and Tserepi, A. 2014. All-plastic, low-power, disposable, continuous-flow PCR chip with integrated microheaters for rapid DNA amplification. *Sensors and Actuators B: Chemical*, 199 : 470-478.

Saiki, R. Scharf, S., Faloona, F., Mallis, K., Horn, G., Erlich, H. and Arnhaim. 1985. Enzymatic amplification of beta-globulin genomic sequences and restriction site analysis of sickle cell anemia. *Science*. 230(4732) 1350-1354. doi: 10.11126/science.2999980.

Narayanan, K., Frost, I., Heidarzadeh, A., Tseng, K. K., Banerjee, S., John, J. and Laxminarayan, R. 2020. Pooling RT-PCR or NGS samples has the potential to cost-effectively generate estimates of COVID-19 prevalence in resource limited environments. *medRxiv*.

Nguyen, T., Zoëga Andreasen, S., Wolff, A. and Duong Bang, D. 2018. From lab on a chip to point of care devices: The role of open source microcontrollers. *Micromachines*. 9(8): 403.

Nieto, D., McGlynn, P., de la Fuente, M., Lopez-Lopez, R. and O'connor, G. M. 2017. Laser microfabrication of a microheater chip for cell culture outside a cell incubator. *Colloids and Surfaces B: Biointerfaces*. 154: 263-269.

Nimmagadda, L. A. and Sinha, S. 2020. Thermoelectric Property Requirements for On-Chip Cooling of Device Transients. *IEEE Transactions on Electron Devices*. 67(9): 3716-3721.

Mullis, K.B. 1994. The polymerase chain reaction (Nobel lecture) Angewandte Chemie edition in English 33(12)s.1209-1213. doi:10.1002/anie19412091.

Siminfar, A. R. and Shoaei, O. 2019. Development of a passive quenching SiPM-based detection system for quantification of real time PCR products based on fluorescence lifetime. *Journal of Instrumentation*. 14(05): P05019.

Soares, R. R., Neumann, F., Caneira, C. R., Madaboosi, N., Ciftci, S., Hernández-Neuta, I. and Nilsson, M. 2019. Silica bead-based microfluidic device with integrated photodiodes for the rapid capture and detection of rolling circle amplification products in the femtomolar range. *Biosensors and Bioelectronics*. 128: 68-75.

Storhoff, J. J., Marla, S. S., Bao, P., Hagenow, S., Mehta, H., Lucas, A. and Müller, U. R. 2004. Gold nanoparticle-based detection of genomic DNA targets on microarrays using a novel optical detection system. *Biosensors and Bioelectronics*. 19(8) : 875-883.

Stroock, A. D. 2008. Microfluidics. In *Optical Biosensors* (pp. 659-681). Elsevier.

Van Pelt-Verkuil, E., Van Belkum, A. and Hays, J. P. 2008. *Principles and technical aspects of PCR amplification*.

Springer Science and Business Media.

Wang, T., Devadhasan, J. P., Lee, D. and Kim, S. 2016. Real-time DNA Amplification and Detection System Based on a CMOS Image Sensor. *Analytical Sciences : the International Journal of the Japan Society for Analytical Chemistry*. 32(6) : 653–658. https://doi.org/10.2116/analsci.32.653

Wu, D. and Wu, W. 2019. Battery Powered Portable Thermal Cycler for Continuous-Flow Polymerase Chain Reaction Diagnosis by Single Thermostatic Thermoelectric Cooler and Open-Loop Controller. *Sensors*. 19(7) : 1609.

Zhang, C. and Xing, D. 2007. Miniaturized PCR chips for nucleic acid amplification and analysis: latest advances and future trends. *Nucleic acids research*, 35(13) : 4223–4237. https://doi.org/10.1093/nar/gkm389

Zhang, C., Xu, J., Ma, W. and Zheng, W. 2006. PCR microfluidic devices for DNA amplification. *Biotechnology Advances*. 24(3) : 243–284. https://doi.org/10.1016/j.biotechadv.2005.10.002

Zhu, H., Zhang, H., Xu, Y., Laššáková, S., Korabeèná, M., and Neu•il, P. 2020. PCR past, present and future. *BioTechniques*. 69(4): 317-325.