Glass based Polymerase Chain Reaction Device for DNA Amplification

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***Abstract*—Polymerase Chain Reaction (PCR) is a biomedical technique for forensic laboratory sciences in which small amount of DNA is amplified through repeated thermal cycles. It has become a powerful technique for clinical, biological, medical, forensic and genetic analysis and other areas of life science. This process actively increases the quantity of DNA by repetition of three-step procedure i.e. denaturation, annealing and extension which are performed at 95ºC, 55ºC and 72ºC respectively. In this research, two different designs of PCR device are presented. The heat transfer and flow simulation studies are performed for both designs to ensure the proper functionality of the PCR device. The pressure drop computations and heater calculations are also performed and it is observed that pressure drop and heater power are lesser for the design with larger cross-sectional area. Further, ANSYS CFX 15 is used to perform Computational Fluid Dynamics (CFD) simulations by varying the inlet velocity to calculate the residence time of fluid in PCR channel in order to attain efficient DNA amplification. The study provides a useful knowledge on the effect of variation in crossectional area on pressure drop, heater power requirement calculations and fluid residence time of PCR device.**

Keywords—Polymerase Chain Reaction; heat transfer; Computational Fluid Dynamics; residence time.

I. INTRODUCTION

Polymerase Chain Reaction (PCR) is a highly effective and well-established technique to amplify specific segments of DNA (deoxyribonucleic acid). It is largely used as a tool for diagnostics in the medical industry but can also be used for paternity testing [1] and detection of airborne pathogens [2-3].

PCR is a common method used for creating copies of specific fragments of DNA by the following three steps, denaturation, hybridization (annealing), and enzymatic polymerization (extension) [4]. PCR can amplify a single DNA molecule into many billions of molecules. The three steps of the polymerase chain reaction are typically carried out in the same vial, but at different temperatures. Denaturation occurs when the double-stranded DNA (dsDNA) helix of nucleotides, which carries the genetic information of a cell is heated to a temperature where the double helix is no longer able to keep its shape, usually between 90°C and 96°C [5], causing it to unravel into two single-stranded DNA (ssDNA) segments. The second reaction, annealing, occurs between 58°C and 65°C [4], allowing a single-stranded primer to attach to the ssDNA and identify the location to start replication. Third, extension occurs when a DNA polymerase enzyme, such as *Taq*polymerase, moves along the DNA segment and attaches nucleotides to create a complimentary DNA strand. Each polymerase has a temperature range at which it is most effective, so it can be difficult to determine a generalized ideal temperature range for this reaction. A common enzyme, *Taq*polymerase, is effective between 70°C and 74°C and is generally conducted at 72°C [6]. These three reactions are components of one PCR cycle as shown in Fig. 1. In order to obtain useful results, 25-40 cycles are generally required. In addition to achieving these temperatures, each reaction requires a residence time at the desired temperature to ensure the entire reaction has been completed. Generally the residence times for denaturation, annealing and extension have ratios of 1:1:3 or 1:1:2, resulting in a total cycle time of 4-5 minutes [7].

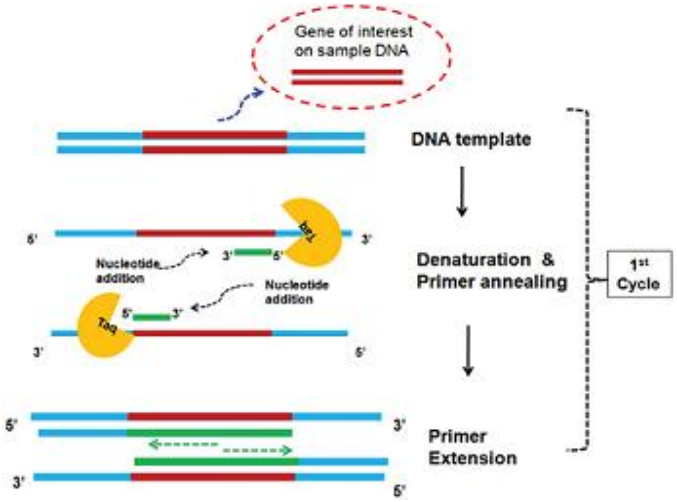


Fig. 1. DNA amplification process [8].

II. MOTIVATION AND CONTRIBUTION

In current world due to flaws in judicial system and country's law, criminal activity cases often rely on witnesses which are easily bribed or intimidated. Further, recent terrorism crisis in Pakistan increases the unidentified cases in criminal investigation. Long hours in investigations based on the current system causes delay in critical judgments and often results in wrong judicial decisions. So, there is a need to improve the existing strategies which should be done through forensic studies based on DNA identification. DNA testing

can play a key role in solving real cases of paternity disputes, murder, robbery and dead body identification etc. This work involves serological testing of human body fluids, extraction of minute quantity of DNA from evidentiary material, separation of male and female DNAs by differential extraction, real-time PCR DNA quantification, multiplex PCR of identifier kit, genotyping and report writing for courts and law enforcing agencies. For performing the said studies, the available quantity of DNA is usually very low. Therefore, there is a need for the design and development of a PCR device which can perform DNA amplification. A glass based PCR devices are commonly used in the world for the amplication of DNA and improvements in the functionality and performance of these devices is the recent area of research all over the world. PCR based device helps the sensitive institutes and speeds up the long hours of old & manual forensic analysis. The current study, motivated by the above mentioned factors propose a better, cheaper, and faster glass based PCR device for DNA amplification.

The contributions for this research include modeling, simulation and investigation of various parameters of PCR for two different designs. Pressure drop calculations are performed for both designs. It is observed that the pressure drop across the channel of larger cross-sectional area is lesser than narrow channel for same velocity. Further, optimized heater power calculations are performed and compared with already published studies. Finally, we computed optimized residence time for DNA amplification. The objective of this study is to conduct a 3Dimensional heat transfer and fluid flow simulations on PCR device to improve DNA amplification process.

III. Channel Design

PCR device presented in the current study consists of three NiCr heaters, saline filled PCR channel, glass substrate on which the serpentine PCR channel is etched and a glass cover which is applied on top of it. The PCR channel consists of 18 cycles with each cycle containing denaturation, annealing and extension zones at temperatures 95ºC, 55ºC and 72ºC, respectively. The length of the whole channel is approximately 1.9m. The NiCr thin film heaters of 9mm lateral dimensions are attached to the bottom of the one cycle model. Air gaps of 1mm are created to maintain the uniform temperatures in the process zones. The channel width is 120µm and height is 30µm. The exploded view of PCR device assembly is presented in Fig. 2. The channel dimensions are presented in Fig. 3 which shows that the longitudinal length of the channel is 30mm and has 25mm width. The gap between the channel tubes is 110 micron. An initial long preheat section is also shown.

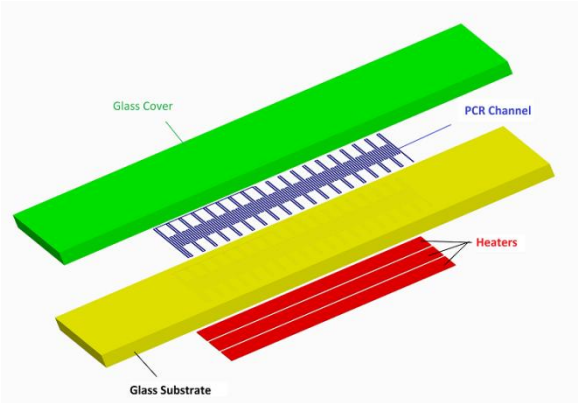


Fig. 2. Exploded view of PCR

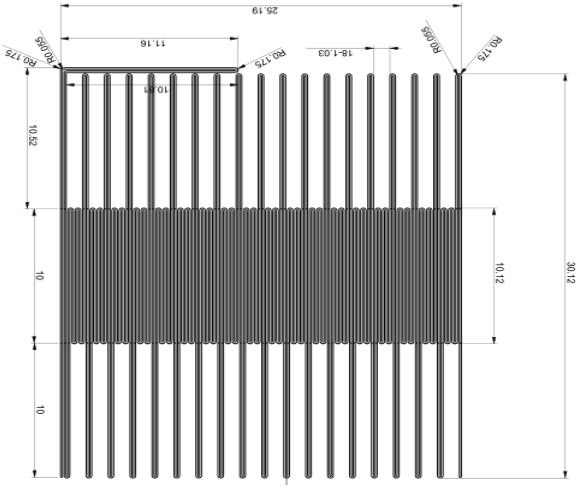


Fig. 3. Full Channel Dimensions

IV. Numerical Mehtodology

The velocities of the fluid in continuous flow PCR chips are usually of the order of mm/sec. Because of low velocity, the viscous effects are usually dominant over inertial effects and the Reynolds number determines the flow pattern and is defined as.

$$Re = \frac{\rho V L}{\mu} \tag{1}$$

Where ρ is density of fluid, v is the velocity of fluid, L is the characteristics length of the channel (channel width or depth) and μ is the dynamic viscosity.

The incompressible Navier-Stokes equations are the governing equations for the problem and are represented as

$$\nabla . v = 0 \tag{2}$$

$$\rho \left(\frac{\partial v}{\partial t} + (v . \nabla) v \right) = \mu \nabla^2 v - \nabla P \tag{3}$$

$$\rho C_p \left(\frac{\partial T}{\partial t} + v . \nabla T \right) = k \nabla^2 T + \tau . \nabla v \tag{4}$$

where v is velocity vector, g is the gravitational acceleration, Cp is the heat specific capacity, T is temperature and k is thermal conductivity.

The three dimensional energy transfer in the PCR chip for steady state analysis is governed by the steady state heat equation.

$$\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} = 0 \quad (5)$$

For transient analysis, the three dimensional heat transfer is governed by the following equation.

$$\frac{\rho_f Cp_f}{\kappa_f}\left(\frac{\partial T}{\partial t} + u\frac{\partial T}{\partial x}\right) = \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial x^2} \quad (6)$$

V. Pressure drop Calculations

In the present study, the velocity magnitudes considered are in the range of 0.5 mm/sec to 10 mm/sec. The corresponding Reynolds number, therefore, is in the range of 0.027-0.54 and lie in the laminar regime as presented in Table below. As the flow is laminar, the pressure drop calculations are performed using the friction coefficient in the laminar regime and presented in table 1. The formulas for friction coefficient and pressure drop are presented below.

$$\text{Friction Coefficient (Fc)} = 16/\text{Re} \quad (7)$$

$$\text{Hydraulic Diameter (Dh)} = \frac{2ab}{(a+b)} \quad (8)$$

$$\text{Pressure Drop} = 2Fc\frac{L}{Dh}\rho v^2 \quad (9)$$

Table I. Reynolds's no calculations

Velocity (mm)	Diameter (mm)	Reonolds No	Friction Coefficient	Pressure drop (psi)
0.1	0.048	0.005	2965.2	0.346
0.5	0.048	0.027	593.0	1.74
1	0.048	0.054	296.5	3.47
4	0.048	0.216	74.1	13.87
8	0.048	0.432	37.1	27.75
10	0.048	0.540	29.7	34.67

In order to compute the velocity and pressure drop across the channel, we performed the flow simulations in ANSYS CFX. The inlet condition is set as mass flow rate of 1.447e-8 kg/sec and the outlet condition is set as Pgauge = 0 pa. The pressure drop computed from flow simulation is comparable with the analytical pressure drop. The desired velocity of 4mm/sec is successfully maintained across the PCR Channel. The contours are presented in the figures 4 and 5 below. The results indicate that for mass flow rate inlet condition corresponding to the velocity of 4mm/sec, the pressure drop computed from the simulations is 12.36 psi which is lower than the calculated pressure drop of 13.87 psi. The error in the analytical and CFD computed pressure drop is around 10 percent.

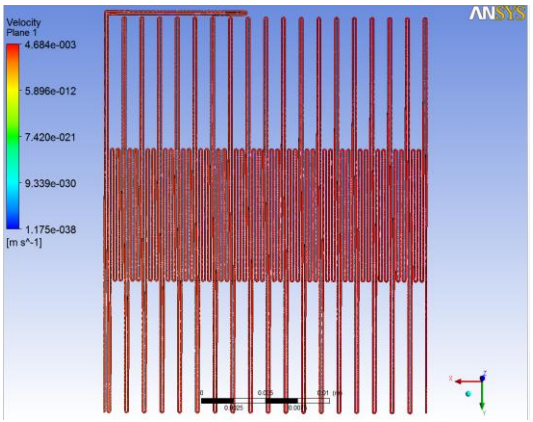


Fig. 4. Velocity Contours in PCR channel

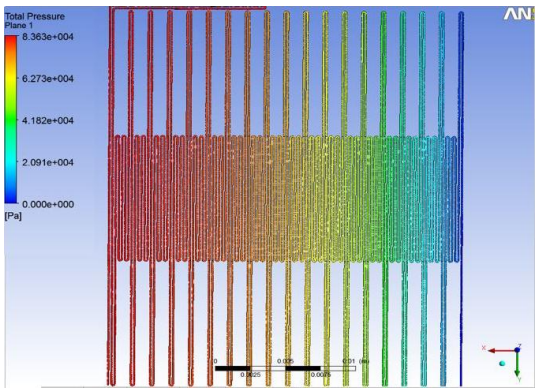
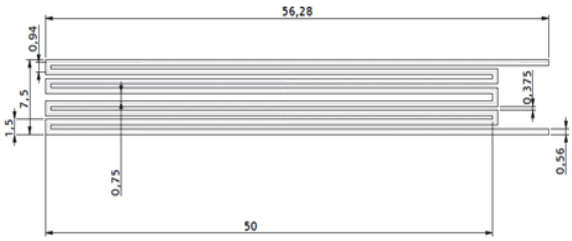


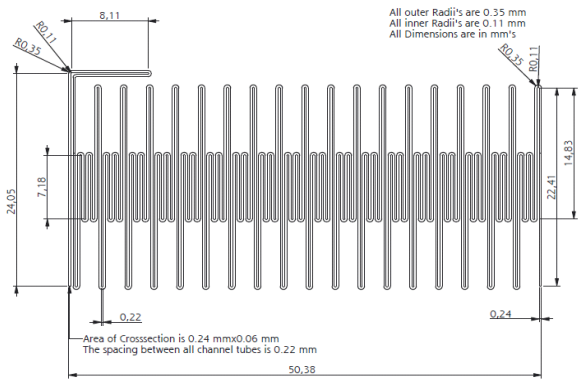
Fig. 5. Validation of Analytical calculations via Numerical Experiments.

VI. Alternate Design

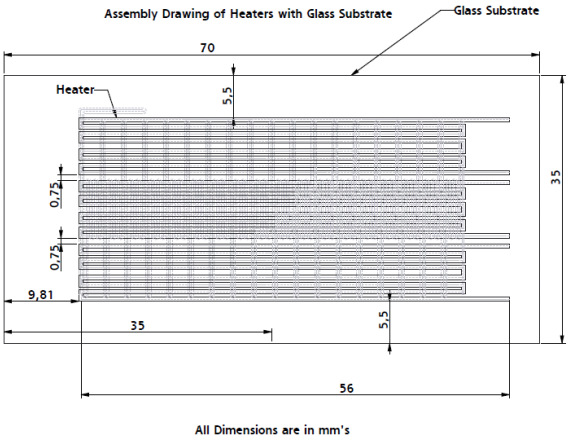
In order to avoid fabrication complexity an alternate design is suggested. The design cross-sectional area is taken as 240 mm x 60 mm and total of 18 cycles are made. The length in the vertical direction is reduced by 25 percent in order to compensate the increase in cross-sectional area. The assembly of the design is performed in Creo 2.0 assembly module. The detailed drawings of the heater, channel and assembly are presented in the Fig. 6a, 6b and 6c below. The length and width of the glass is taken as 70mm and 35mm respectively. Fig. 6a shows the heater design which is adjusted based on the new channel design. Fig. 7c shows the complete assembly of the PCR device and shows that the gap between each heater is 0.75mm. The gap between the heaters is made smaller because of decrease in longitudinal length of the PCR channel which is reduced inorder to compensate the reaction time delay due to increase in cross-sectional area in current design.



(a) Heater Design



(b) Channel Dimensions



(c) Assembly Drawing

Fig. 6. Alternate design with larger cross sectional area (a) Heater (b) Channel (c) PCR Assembly

VII. Pressure Drop Calculations

The pressure drop calculations are performed to calculate the minimum pressure required at the inlet to maintain the flow velocities in the range of 0.5mm/sec to 10 mm/sec. The same formulas as in section V are used for the analytical calculations which are presented in Table 2.

TABLE II. REYNOLD'S NO CALCULATIONS

Velocity (mm/sec)	Diameter (mm)	Reynolds No	Friction Coefficient	Pressure drop (psi)
0.1	0.096	0.0054	1482	0.067
0.5	0.096	0.027	296.15	0.3355
1	0.096	0.054	148.25	0.671
4	0.096	0.216	37.06	2.684
8	0.096	0.432	18.53	5.368
10	0.096	0.540	14.825	6.71

VIII. Flow Analyis

The flow simulations are performed in ANSYS CFX 15 to compute the pressure drop required to flow the desired mass flow rate in the PCR channel. For this purpose, the meshing of the full channel is performed in order to discretize the channel for flow simulations. The structured meshing is performed and total of around 1.14 million hexa elements are generated. In order to capture the velocity profile properly, total of 7 elements are generated along the thickness of the channel cross-section. The meshing snapshots are presented in Fig. 7 below.

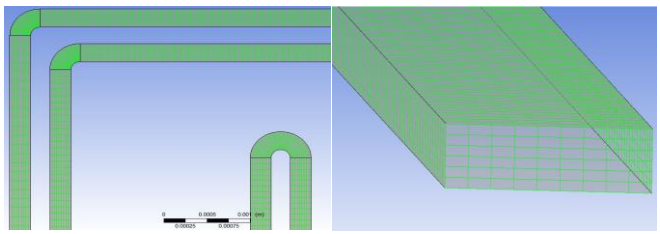


Fig. 7. Mesh of PCR Channel

For flow simulation the inlet boundary condition is mass flow rate of 5.88e-8 kg/sec and outlet boundary condition is 0 Pa.

IX. Flow Simulation Results

The results of flow simulation are presented in figures 8 and 9 below. It can be seen that for applied boundary conditions we achieved the desired velocity in the whole fluid channel i.e. 4mm/sec. The corresponding pressure drop is 2.2 psi which is lesser than the calculated flow drop. The lower pressure drop can be attributed to the use of no slip boundary condition (v=0 at the wall). The results also show that the velocity at the wall is zero.

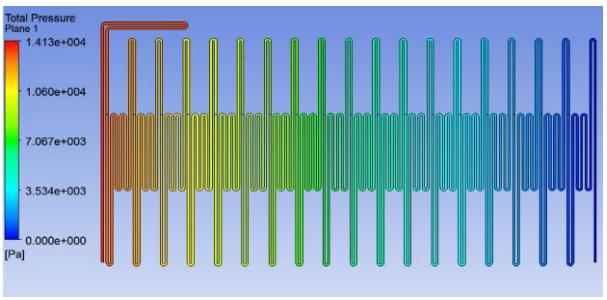


Fig. 8. Pressure drop contours

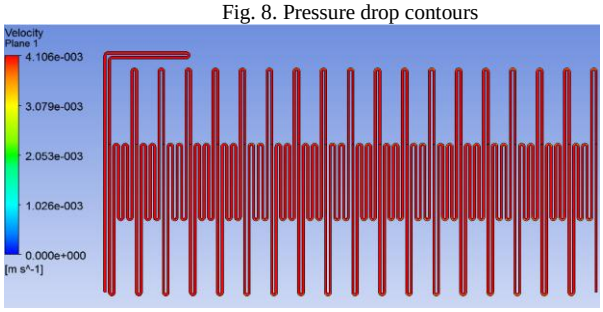


Fig. 9. Velocity contours

X. Heater Power Calculations And Comparison

Maximum heater power for old model, is found to be 1.9 W while for model B, it is 1.3 W. The power flowing through a resistor R is given by

$$P = VI = \frac{V^2}{R} \quad (10)$$

From Ohm's law,

$$I = \frac{V}{R} \tag{11}$$

The Power consumption and other parameters for the presented designs are presented in Table 3 and compared with the power consumption of the previously presented PCR devices in Table 4. It can be seen that the new design consumes lesser power because of the reduced length of heaters. Also, the comparison with previous devices has ensured that the proposed device is quite efficient and does not consume too much power.

Table III. Power Consumption for both designs

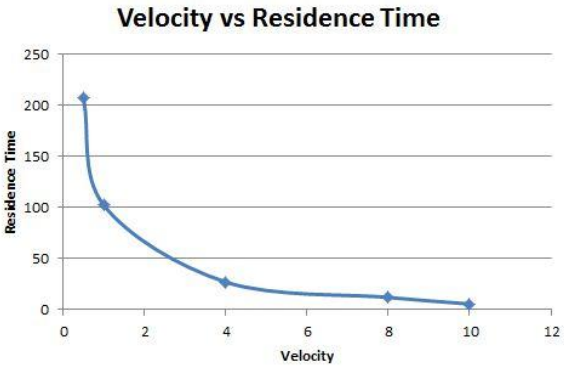
Maximum power required (W)	Narrow Design	Alternate Design
Heater resistance (Ω)	1.90	1.327
Maximum voltage (V)	52.5	75.375
Maximum current (mA)	10	10
Maximum power required (W)	0.190	0.1327

Table IV. Power Comparison with Literature Devices

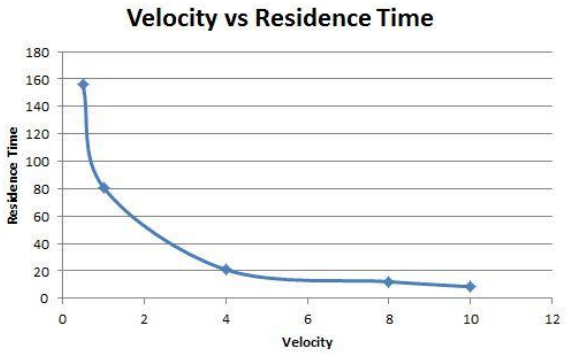
Devices	Power Consumption (W)
Traditional peltier device	264 (max)
Traditional convective devices	800 (max)
El-Ali's device	6.4 (average)
Zou's device	0.9 (average)
Current device	1.9 (average)

XI. Residance Time Comparison for Both Devices

The residence time for the narrow and larger channel designs is plotted against the velocity in Fig.10. The compasion for both designs show that the residence time for channel with larger cross-sectional area is lesser as compared to narrow design because of the reduced length. The residence time for both channels seems not to vary non linearly with velocity. The reason is because at lower velocity the viscous effects are dominat as compared to higher velocity. The particals which are near the walls have lower velocity than those away from the wall which makes average velocity in the main stream flow to vary non linearly with residence time.



(a) Narrow Design



(b) Larger Cross-section Design

Fig. 10. Residence Time Distribution vs. Velocity for Both Models

XII. CONCLUSIONS

In this study, three dimensional heat transfer simulation is performed on one pass channel configuration of the PCR device. Further, an alternate design with larger cross-sectional area is proposed. The analytical pressure drops are calculated and compared with the flow simulation results. Also, the heater power calculations are performed and two designs are compared based on the power consumption. It is observed that the channel with larger dimensions consumes less power. Finally, an important factor in residence time is compared for both designs and it is concluded that the average velocity varies non linearly with residence time.

References

[1] V. E. Ragsdale, "Three-Dimensional Heat Transfer Analysis of a Disposable, Continuous-Flow Polymerase Chain Reaction Device (Published master's thesis)." University of Utah, Salt lake City, United States, 2012.

[2] S. D. J. Pena and R. Chakraborty, "Paternity testing in the DNA era," Trends in Genetics, vol. 10, pp. 204-209, 1994.

[3] S. I. Makino, et al., "Detection of anthrax spores from the air by real-time PCR," Letters in Applied Microbiology, vol. 33, pp. 237-240, 2001.

[4] H. Cheun, et al., "Rapid and effective detection of anthrax spores in soil by PCR," Journal of Applied Microbiology, vol. 95, pp. 728-733, 2003.

[5] Stephen P. Naber. " Molecular Pathology: Diagnosis of Infectious Disease" 331: 1212- 1215, November 3, 1994, and "Molecular Pathology: detection of Neoplasia The New England Journal of Medicine." 331: 1508-1510, December 1, 1994.

[6] H. A. Erlich, "PCR Technology: Principles and Applications for DNA Amplification," 1992 ed. New York: W.H. Freeman and Company, 1992, p. 11.

[7] S. A. Bustin, "The PCR Revolution: Basic Technologies and Applications". New York: Cambridge University Press, 2010.

[8] N. Pathak, B. K. Das, "Polymerase Chain Reaction as a Diagnostic Tool in Human Viral Myocarditis". Lab Science vol. 1, Issue 2, Page 168-175, 2015.