



Polymerase Chain Reaction in African Research Setting: Oppurtunities and Limitations

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TOO and OJN conceptualized, designed and prepared the manuscript. Final editing and approval of the version to be submitted were done by authors TOO, OJN, BOE, OAK and OJA. All authors read and approved the final manuscript.

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ABSTRACT

The polymerase chain reaction (PCR) technique was first discovered in 1985 and has since then shown to be an important tool in the world of research and medical diagnostics. Previous techniques of DNA replication, which relied on microorganisms and may take weeks to complete, have been supplanted by PCR due to its timeliness, as well as its high degree of sensitivity and specificity. Since its invention, the PCR has played many roles in specimen analysis, mutation analysis, forensic science and in the human genome project, among others. While the developed nations of the world have seized the several opportunities that lie in the utilization of the polymerase chain reaction technique to advance the field of medicine and research, many Africa countries just seem to be scratching the surface in its use. The advent of COVID-19 in the last two years has brought about an increase in the awareness of the technique and brought to light the fact that many African countries are ill-equipped with the adequate tools to meet the challenges of the world of medical sciences and research. This review aims to give an insight into the opportunities that exist with the use of PCR and the limitations that may hinder these opportunities especially among developing countries in Africa.

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1. INTRODUCTION

We live in an era where through research, ground breaking discoveries are being made. Through the course of time, these ground breaking discoveries have been able to help in not just the diagnosis of diseases but also in their detection. In medical sciences, innovative discoveries have also been astronomical. From the work of Louis Pasteur on vaccines, to the life changing researches on DNA in the 20th century, the medical field has been filled with high level and remarkable scientific breakthroughs.

One of such ground breaking discovery in research is the invention of the PCR technique. The polymerase chain reaction (PCR) is a laboratory method that generates millions of copies of a single strand of DNA. It's essentially an amplification technique in which the tiniest bits of DNA found in blood, hair, or tissues are replicated to make enough copies for analysis [1].

While the developed nations of the world have seized the several opportunities that lie in the utilization of this technique to advance the field of medicine and research, many Africa countries just seem to be scratching the surface in the use of the PCR technique.

The advent of COVID-19 in the last two years has brought about an increase in the awareness of the technique and brought to light the fact that many African countries are ill-equipped with the adequate tools to meet the challenges of the world of medical sciences and research. The PCR technique which is particularly important for early detection of the virus, was discovered to be in short supply in many African countries at the wake of the pandemic [2]. Africa is poised with certain challenges that if left unattended to, could mar the growth of research in the continent.

This review aims to give an insight into the opportunities that exist with the use of PCR and the limitations that may hinder these opportunities especially among developing countries in Africa.

2. PCR

The name PCR comes from the vital component of the DNA polymerase activity, which essentially performs DNA replication. PCR makes use of a

naturally occurring enzyme, Taq polymerase, derived from the bacteria *Thermus aquaticus*. This enzyme performs best at temperatures about 70^o c. It can make a new DNA strand from scratch, using the old DNA as a template as well as the use of DNA oligonucleotides (also known as primers). In PCR, primers are short DNA sequences that are produced to match the ends of the DNA area to be replicated perfectly [1].

Previous techniques of DNA replication, which relied on microorganisms and may take weeks to complete, have been supplanted by PCR. PCR can be completed in a matter of hours, making it a very quick experiment. When urgent results are required in a diagnostic context, speed is often required. As a diagnostic and research technique, PCR has been widely employed. It has numerous uses in molecular biology, microbiology, genetics, clinical diagnostics, forensic research, environmental science, hereditary studies, and paternity testing, to name a few. Research applications of PCR technology are numerous. A partial listing would include direct genomic cloning of DNA or cDNA, genetic fingerprinting of forensic samples, the analysis of allelic sequence variations, and direct nucleotide sequencing. The PCR has the potential to replace many conventional diagnostic techniques for infectious and genetic diseases in clinical medicine.

The PCR has an advantage over the competing technology of DNA hybridization in that the sensitivity is sufficient to allow the direct detection of microbial DNA in a high percentage of known positive pathological specimens, a quality not always found in DNA hybridization methods [3]. This genotypic technique however detects gene harboring strains, independent -of gene expression. Hence, a positive result in the PCR is only indicative of the presence of the targeted gene sequence and does not reflect the viability or pathogenic toxic activities of the organism in the specimen [4]. The PCR may supplement growth amplification protocols which can often fail to detect virulent strains present at low levels in pathological or food samples.

3. HISTORY OF PCR

In 1985, while working as a chemist at the Cetus Corporation in Emeryville, California, Kary Mullis devised the PCR process. Mullis' PCR process was slow and labor-intensive when done

manually [5]. As a result, Cetus researchers began exploring for ways to automate the process. Scientists had to add fresh enzyme to each cycle before discovering the thermostable Taq enzyme.

Cetus engineers created the first thermocycling machine, Mr. Cycle, to address the necessity of adding fresh enzyme to each test tube after the heating and cooling process. The purification of the Taq polymerase necessitated the use of a machine that could cycle between different temperatures more quickly. Cetus and the Perkin-Elmer Corporation in Norwalk, Connecticut launched a joint venture in 1985. The DNA Thermal Cycler was also introduced. Cetus was inundated with requests for licenses to perform PCR for commercial diagnostic reasons by 1988 [5]

4. PRINCIPLE OF PCR

On a single-stranded DNA template, the enzyme DNA polymerase guides the synthesis of DNA from deoxynucleotide substrates. When a custom-designed oligonucleotide is annealed to a longer template DNA, DNA polymerase inserts nucleotides to the 3' end. When a synthetic oligonucleotide is annealed to a single-stranded template with a complementary region, DNA polymerase can use the oligonucleotide as a primer and elongate its 3' end to form an extended area of double-stranded DNA [6].

5. STEPS INVOLVED IN PCR

5.1 Denaturation

Heat of more than 90⁰ c splits double-stranded DNA into two single strands. "Denaturation" is the term for this process. Because the hydrogen connections that connect the bases are weak, denaturation is conceivable. At high temperatures, hydrogen bonds break, while the bonds between deoxyribose and phosphates stay intact [7].

5.2 Annealing

The reaction is cooled to 50-65 degrees Celsius at this point. By hydrogen bonding, the primers can attach to a precise spot on the single-stranded template DNA (the exact temperature depends on the melting temperature of the primers being used). The primers are designed to complement short sections of DNA on both ends of the sequence to be copied in sequence.

Primers are the building blocks of DNA synthesis. Only a double strand of DNA can be added to by the polymerase enzyme. The polymerase enzyme can only bind and begin producing new complementary strands of DNA from loose DNA bases once the primer has attached [7].

The two strands of DNA that have been split are complementary and run in opposite directions (from one end of the 5' to the other which is the 3' end. As a result, there are two primers This procedure normally takes 10 to 30 seconds [7].

5.3 Extension

The reaction is then heated to 72⁰ c, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template. The DNA polymerase of bacteria is particularly stable at high temperatures, which means it can endure the temperatures required to separate DNA strands during the denaturing stage of PCR. Most other organisms' DNA polymerase would be unable to endure these high temperatures; for example, human polymerase operates best at 37°C (body temperature) [8].

With one cycle, a single segment of double-stranded DNA template is amplified into two separate pieces of double-stranded DNA. These two pieces are then available for amplification in the next cycle. As the cycles are repeated, more and more copies are generated and the number of copies of the template is increased exponentially. As the cycles are repeated, additional copies of the template are created, and the number of copies of the template grows exponentially [9].

6. TYPES OF PCR

There are over 30 types of PCR techniques [10], this review will however dwell on the common ones:

Real-time PCR: Quantitative PCR (qPCR), also known as real-time PCR or quantitative real-time PCR, is a PCR-based technique that combines the amplification of a target DNA sequence with the determination of that DNA species' concentration in a reaction. Traditional PCR is a time-consuming procedure that involves analyzing PCR results using gel electrophoresis. qPCR simplifies the analysis by detecting

products in real time during the exponential phase. The use of fluorescent dye is required for real-time PCR to work. The fluorescent dye or fluorescent labelled oligonucleotides are used to determine the concentration of nucleic acid present in the sample. Pathogen genotyping and quantification, microRNA analysis, cancer detection, microbial load assessment, and GMO detection all use q-PCR[10].

Nested PCR: This PCR employs two sets of primers for amplification. One pair of primers (dubbed "inner primers") has a target DNA sequence that overlaps with the target sequence of the second set of primers (dubbed "outer primers"). To amplify a target sequence, this PCR uses two distinct primer pairs [11]. Nested PCR is an effective approach for phylogenetic analysis and pathogen identification. Because the technology is more sensitive, even if the sample contains less DNA, it can be amplified, which is not possible with traditional PCR [12].

Multiplex PCR: Multiplex PCR keeps track of the amplification of many DNA sequences at the same time. Using numerous primers and a temperature-mediated DNA polymerase in a thermal cycler, this technique amplifies DNA in a sample. This technology has been used in a variety of applications, including genotyping, mutation and polymorphism analysis, microsatellite STR analysis, pathogen identification, and the detection of genetically engineered organisms, among others. Multiplex PCR is used in diagnostic laboratories to detect diverse bacteria that cause the same diseases [13].

Reverse transcription polymerase chain reaction (RT-PCR): In this type of PCR, an RNA strand is first reverse-transcribed into its DNA complement (complementary DNA, or cDNA) using the enzyme reverse transcriptase, and the cDNA is then amplified using traditional or real-time PCR. RT-PCR can be done in a single tube or in two separate tubes in two phases. With less chances of contamination and variation assimilation, the one-step technique is more effective. Research methodologies, gene insertion, genetic illness diagnostics, and cancer detection all use RT-PCR [14].

SYBR Green dye PCR: SYBR Green is a double-stranded DNA-binding dye. The intensity of fluorescent emissions increases when SYBR Green dye attaches to double-stranded DNA. The SYBR Green dye signal will rise as more double stranded amplicons are created [15].

In situ PCR: In situ PCR is a powerful method that detects minute quantities of rare or single-copy number nucleic acid sequences in a frozen or paraffin- embedded cells or tissues sections for the localization of those sequences within the cells. This type of reaction is a histological technique that exploits the advantages of PCR for detection of DNA or mRNA directly in tissue sections. In-situ PCR is widely utilized in the study of organogenesis and embryogenesis and can be used to diagnose infectious illnesses, quantify DNA, and detect even minute amounts of DNA [10].

Assembly PCR: Assembly PCR can be used to assemble two gene-sized pieces of DNA into one piece for easier cloning of fusion genes. It involves the PCR'ing the two pieces separately with primers that have a 20bp overlap and then doing an extra PCR step using the two products as the template. Assembly PCR can be used to increase the output of a desired protein or to generate vast amounts of RNA for structural or biochemical studies [10,16].

Allele- specific PCR: Allele- specific polymerase chain reaction is a technique based on allele-specific primers, which can be used to analyze single nucleotide polymorphism. It is also called the amplification refractory mutation system corresponding to the use of two different primers for two different alleles [17].

Alu PCR: This is a rapid and easy DNA fingerprinting technique based on the simultaneous analysis of many genomic loci surrounded by Alu repetitive elements. Alu elements are short stretches of DNA initially characterized by the action of the *Arthrobacter luteus* (Alu) restriction endonucleases [18].

Asymmetric PCR: This is a form of PCR that preferentially amplifies one strand of the original DNA over the other [9]. The excessive amount of primers for a single strand distinguishes this type of PCR from ordinary PCR. When only one of the two complementary strands is required, the approach can be used in particular types of sequencing and hybridization probing [19].

7. ADVANTAGES OF PCR

Decision making: The Polymerase Chain Reaction allows for quick and accurate decision-making. The results gotten using a RT-PCR machine are fast as it does not require a long-time frame for the result to be gotten. This can

make one make informed decision making in any activity that would be carried out by such individual.

Sensitivity: The smallest absolute amount of an organism that can be detected by a measurement is referred to as Sensitivity. The Polymerase Chain Reaction can detect the tiniest amount of an organism present in a sample once it has been amplified. Reports have shown that the PCR is better than traditional staining and culturing. While gram staining can correctly identify bacteria and fungi 60-75%, the sensitivity of the PCR has a positivity rate of about 83% [20]. From this report we can hence see that the PCR has a better sensitivity than traditional staining methods.

Specificity: This is a metric that measures a test's ability to correctly designate a person as healthy or disease-free. The choice of the primer and probe sequences is critical for accurate diagnosis of a disease in the laboratory as each primer differs from one another. It is through the primer that the PCR is said to be specific because each primer are different based on the organism causing diseases that they are used for.

Reduced Time Duration: The PCR procedure takes approximately 4-8 hours, which is about three times faster than those of cultures. A particular type, known as multiplex PCR, can amplify multiple sequences of DNA in one reaction [21].

8. RELEVANCE OF PCR TO RESEARCH

The human Genome Project: The Human Genome Project is the operating manual that contains all of the instructions that have assisted man's development from a single cell to the person he is today. Understanding how the genome works might help you better understand your own health and make informed decisions about it. This is where PCR comes in, as it can aid in the development of disease prediction and diagnostic tests. A predictive test is used for people who have a family member who has a genetic disorder, while a diagnostic test is performed to confirm or rule out a genetic disorder [22].

Analysis of Specimen: In the examination of clinical specimens for the presence of infectious agents, PCR has become the gold standard. PCR has drastically shortened the time required

to reach diagnosis while also reducing the false positives.

Prognosis of the Patient: PCR can be used to determine a patient's prognosis and predict treatment response or resistance. Small alterations in specific genes characterize many malignancies, and PCR is used to detect these mutations.

8.1 Mutation Analysis

PCR is used to examine mutations that occur in a variety of genetic illnesses (e.g., cystic fibrosis, sickle cell anemia, phenylketonuria, muscular dystrophy). The PCR can help in the examination of mutated gene in order to make early diagnosis. A person who has a family history of cancer can through the help of the PCR determine if the presence of such mutated gene is present in such individual.

In Forensics Science: In forensics laboratories, PCR is also utilized, and it is particularly effective because just a small amount of original DNA is required that is gotten from the crime scene. The PCR purpose here is to amplify the samples that have been gotten from the crime scene so that it can be compared with that from the data base for criminals [23].

9. OPPORTUNITIES OF PCR TO RESEARCH IN AFRICA

9.1 Forensics Science

According to a data published by Statista Research Department September 14, 2021, two African nations, which were Congo and Nigeria, ranked 1st and 2nd respectively and were said to be the nations with the highest crime rate in the continent. The Democratic Republic of Congo had the highest organized crime rate in Africa as of 2021, while Nigerians were reported to be the most worried about muggers and robbers. The level of concern about it stood at 66.7 points on a scale from zero to 100, where 100 represents the highest concern. Some other crimes causing high levels of worry in the country were burglary, theft, and attacks. The source identified human and arms trafficking as well as wildlife crimes as particularly widespread on the continent.

In forensic science, forensic scientists examine and analyze evidence from crime scenes and elsewhere to develop findings that can help in prosecuting the perpetrators of the crime and

also absolve an innocent person from suspicion. PCR serves as a means to an end in forensics science as samples that are being collected from the crime scene are first of all visualized for staining in order to check for the presence of blood, saliva, semen or hairs. Then the samples undergo DNA extraction in order to purify the sample after which PCR is carried out to amplify the DNA sequence. Once amplification is done by the PCR, scientists compare the biological evidence from the crime scene to a known DNA profile of a suspect.

Adopting this method in African countries would ensure that criminals won't get away with crimes that they commit as they would always be found and brought to justice and also decrease the amount of unsolved crimes which has become a regional burden.

9.2 Paternity Dispute

According to a report published on Vanguard newspaper on January 10, 2021, Nigeria has the 2nd highest rate of paternity fraud in the world after Jamaica. "Three out of ten Nigerian men are not biological fathers of their children" [24]. This report shows that in many homes in Nigeria, children are raised by their non-biological fathers. There could be many reasons why this could occur ranging from either the husband is impotent making the woman to have extra marital affair or a wife who actually engages in extra marital affairs. Such reasons vary from house to house and would only be revealed once a paternity test has been carried out.

Paternity test is the use of DNA profiles to determine whether an individual is the biological father to a child [25]. In carrying out paternity test, a PCR machine serves an essential role in DNA amplification after which other techniques like Electrophoresis are used to compare the DNA structure of the suspected father to that of the child.

In achieving this, samples (blood, saliva) are gotten first both the father and the child. While in the laboratory, they undergo DNA extraction before they are amplified by the use of a PCR. After amplification, electrophoresis is carried out so as to compare the result of both the father and that of the child.

Through the advent of PCR in Africa, paternity disputes are gradually becoming settled and unraveling a lot of buried family secrets.

9.3 Detection of Disease

The PCR machine can be used in the detection of diseases. In the wake of COVID 19, the PCR machine has become a vital tool in the detection of the virus. A swab gotten from the nasopharyngeal tract of an individual is taken to the laboratory where DNA extraction is carried out before amplification is done making use of the PCR machine. The PCR machine then detects the presence of the virus after the amplification has been done. The PCR machine can also be used to detect other micro-organisms causing diseases such as fungi, bacteria, etc.

With the advent of so many diseases in Africa, the PCR machine serves as an indispensable tool not just in the diagnosis of these diseases but also in the futuristic detection of diseases that can affect man.

9.4 Human Genome Project

The Human Genome is the instruction manual that has guided man's development from a single cell to the person he is today.

Understanding how the genome works might help one better understand one's own health and make informed decisions about it. Africa holds one of the largest ethnic and cultural diversities in the world, which may infer greater genetic diversity than on any other continent [26]. This diversity could aid in the understanding of human evolution and common diseases, as well as the development of medications tailored to these genetic variants. Despite this, African genomes account for less than 2% of all genomes studied [26].

10. LIMITATIONS IN THE USE OF PCR FOR RESEARCH IN AFRICA

Cost: Most research laboratories in developing countries in Africa cannot afford a PCR machine without fundings from private investment or funding from outside Africa. In Nigeria for example, although regarded as Africa's largest economy, its research budget languishes at 0.2% of gross domestic product (GDP) [26]. These could serve as a major problem as it limits the growth of Africa.

Electricity: For a PCR machine to work, it needs to be powered by electricity. Over 640 million Africans have no access to electricity,

corresponding to an electricity access rate for African countries at just over 40 percent, the lowest in the world [27]. Although organizations that can afford, either have their own generators or power plants to help them carry out their day-to-day activity, these usually does not meet up with the demand of a standard research activity.

Without a stable source of electricity, the usefulness of the PCR machine cannot be met as the effectiveness of the machine would be reduced.

Knowledge: Not many scientists are trained in the use of PCR and with this, there are few scientists who are skilled to make use of the PCR machine. Furthermore, some institutions do not include in their curriculum the need to train their students on the use of the PCR machine. This would make such students not have an adept understanding on the usefulness of the PCR machine. Lastly, the PCR machine has undergone some modernization through the years and scientists who aren't aware of such modernization would not be able to use the PCR machine adequately.

Research Funding: For many developing countries in Africa, government does not fund much research in science. According to an article published by Friday Okonofua in August 15, 2021 on "The Conversation" [28]. Nigeria is currently one of the countries with the lowest health rsearch fundings in the world. It contributes 0.22% of its GDP to research. It has always been that the government pays little or no attention to science as the money allocated to science is allocated to other areas. These could really hinder research as scientists would have to rely on their own personal fundings in order to carry out the research.

11. CONCLUSION

The future of PCR appears promising. New versions of the classic PCR have drastically shortened the time required to reach a diagnosis particularly in a COVID era while also reducing the number of false positives. Even in its present state, PCR has shown to match and even exceed the gold standard. Its uses have also been shown to be diverse, cutting across many areas of life and science. African countries have a lot to benefit from this diagnostic tool but would have to be intentional in its approach of tapping into its world of opportunities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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