DNA Amplification: A Comparison of Different Methods

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Abstract:

The development of the Polymerase Chain Reaction by Kary Mullis has led to the establishment and refinement of many new DNA amplification techniques that exponentially increase miniscule amounts of DNA. Each method has both its advantages and disadvantages; however, no one method has yet been identified as superior to all others. Therefore, all relevant data on the most prominent techniques, mainly PCR, LCR, RCA and HDA, was compiled for this review in order to weigh their advantages and drawbacks and determine which technique is the most effective. This review found that because HDA can thermally unwind double stranded DNA without extreme heating using a simple, inexpensive, and effective method, it is preferable to all other current methods.

Introduction

Initially invented in the 1980s by Kary B. Mullis[8], Polymerase Chain Reaction (PCR) was the first DNA amplification method developed. Originally designed as a way to study DNA that could only be obtained in small quantities, today numerous other applications for PCR have been found when combined with other technologies. For instance, PCR is now commonly used to detect and isolate different DNA sequences of interest for research; in particular, it is used as a means to detect mutations or polymorphisms in different cell lines. Forensic scientists trying to match DNA between different samples taken from crime scenes also require PCR; hospitals utilize it in order to determine the paternity of a child[1]. In fact, without PCR the human genome project would not have been possible[7].

Due in part to the overwhelming success and popularity of PCR, many new methods have been devolved which take the basic ideas of PCR and build upon them to create novel ways to amplify DNA. Today, numerous different methods are now in wide use, including Strand-Displacement Amplification, Rolling Circle Amplification, Ligase Chain Reaction, and Helicase-Dependent DNA amplification. Many of these newer methods for DNA amplification attempt to fix some of the fundamental problems associated with PCR, such as its dependence on thermo cycling. These recent advancements have been critical to the advancements of fields such as molecular biology and genomics.

However, with so many different methods that all preform the same task, knowing which method is best suited for the research at hand can be hard to determine as each method presents its own set of disadvantages. For instance, PCR is one of the easier reactions to perform; however, it can cause the chromosomal structural information of the sample DNA to be lost. Furthermore, certain techniques require expensive machinery to perform the amplification with ease, which may not be feasible for smaller laboratories. If instead a method is used that offers better applicability than PCR because it eliminates the need for these thermal cyclers, it can often be very complex and difficult to perform. Therefore, this paper attempts to review the most

popular DNA amplification techniques and determine which one provides the most advantages with the least amount of drawbacks. Of all the many methods of DNA amplification, this review has found that Helicase-Dependent DNA amplification (HDA) is superior to all others because it has the capability to exponentially increase the initial amount of DNA present without the need to repeatedly heat the sample and risk denaturation while still being relatively cheap and simple to perform.

Mechanisms of Different Methods

Polymerase Chain Reaction (PCR)

PCR is the classical technique used to amplify DNA. This method mimics the cellular process of DNA replication normally found in cells. There are three major components involved with PCR: a DNA polymerase (typically Taq DNA polymerase) which is stable at high temperatures, a mixture of four different deoxyribonucleotide triphosphates (dNTP), and two small synthesized oligonucleotide primer designed to attach to the DNA sequence of interest[10].

The first step of PCR begins with the denaturation of the double stranded DNA. In order to do this, the mixture must be raised to a high temperature, usually around 95°C[10]. After the DNA has been denatured, the oligonucleotide primers hybridize to the denatured DNA at the specific targets that they were synthesized to bind with. This step is known as the Annealing step and takes place between 50°C and 70°C[11]. In the final step, the DNA polymerase sequentially adds the dNTPs to the template DNA strands. This effectively synthesizes two new identical double stranded DNA molecules. This step occurs around 70°C[10]. These three steps make up one cycle of the PCR reaction. Many subsequent cycles are then preformed which amplifies the original amount of DNA present up to about 10^9-10^{12} molecules per every original single molecule[10].

There are many advantages associated with PCR. To begin, the entire PCR reaction occurs rapidly and only takes a few hours to perform[11]. This method is also extremely sensitive. It can amplify sequences from miniscule amounts of original DNA, even from that of a single cell[12]. Finally, PCR can amplify material coming from DNA which has been degraded or which is in a medium that in not typically conducive to DNA isolation[12].

However, there are many disadvantages associated with PCR. The main drawback is that it relies on temperature cycling in order to separate the double stranded DNA. Therefore, this requires special machines that have an automated thermal cycler and these high temperatures can potentially destroy the original morphology of the chromosome structures[6]. Furthermore, before the PCR reaction can take place, the primers need to be synthesized. This requires the researcher to know at least some of the DNA sequence prior to amplifying it[11]. This is not always the case when studying a new or unknown DNA sequence.

Ligase Chain Reaction (LCR)

The LCR reaction is similar to PCR in that both use synthesized primers to amplify the DNA. However, while PCR primers are normally relatively short, LCR oligonucleotide primers

are much longer as they are designed to cover the entire sequence to be amplified[2]. Also like PCR, LCR relies on temperature cycling in order to break the double stranded DNA.

The first step in LCR is the denaturation of the double stranded DNA by heating the reaction mixture. After denaturation, the two complimentary pairs of primers bind to their respective DNA strands. One pair is complimentary to one strand of the DNA while the other is complimentary to the other template DNA strand. These primers cover the entire length of the sequence that will be amplified by the reaction and nothing else. There is no space between these primers and the space between the two is then sealed by a thermo stable DNA ligase. This generates a fragment that is as long as the total length of each pair of primers. These products of one cycle then serve as the templates for subsequent cycles[2].

The main advantage of this type of amplification is that a single point mutation in the original template DNA can prevent the reaction. Therefore, an absence of product from this type of amplification can be an indicator of mutations[2].

There are several drawbacks to the LCR reaction. Like PCR, it relies on extreme heat to denature the DNA, and therefore information about that sequence may be lost. Also, because this reaction is very specific and only the sequences encoded by the primers are replicated, any mutation outside of this amplified sequence is not detected[2].

Rolling Circle Amplification (RCA)

With RCA, first two ends of the DNA of interest are joined together using a DNA ligase to form a circular single stranded DNA template. Next, the primer is attached to this template during an annealed step similar to PCR[4]. The amplification is then carried out by a suitable DNA polymerase, which extends the primer until the circle is complete at which point the synthesized strand is displaced due to the intrinsic property of the polymerase[6].

The main advantage that this method has is that the synthesis can occur at room temperature and does not require heating. This solves the problem encountered by PCR and LCR[6]. Also, this reaction occurs relatively quickly, only taking a few hours[4].

However, the main disadvantage to RCA is that the protocol is extremely complicated and it is not capable of amplifying a satisfactory length of nucleic acids[6]. Therefore, the reactions are not always successful. Also, while the bulk of the reaction takes place at room temperature, the initial step in which the single stranded cyclic DNA is formed does require an initial heat denaturation step[6].

Helicase-Dependent DNA Amplification (HDA)

HDA mimics DNA replication found in cells in that it relies on a DNA helicase to separate the double stranded DNA to generate the single stranded DNA templates[5]. After the helicase has unwound the DNA, single-stranded binding proteins bind to the unwound DNA, which prevent these unwound pieces from re-annealing to one another or degrading[6]. Next, like PCR, two-sequence specific primers are annealed to the single stranded DNA, and a DNA polymerase extends these primers until a new double stranded DNA molecule is formed. These newly

synthesized double stranded DNA molecules are then used as templates for the next round of amplification[6].

The major advantage to HDA is that the DNA helicase can operate at room temperature and therefore the thermal cycling found with both PCR and LCR is unnecessary. This both preserves the structure of the chromosomes being replicated as well as illuminating the expense of buying an expensive thermo-cycler. Furthermore, unlike RCA the procedure for HDA is very simple and straightforward[6]. Studies have also been preformed that show that this method is extremely specific for the target DNA[5].

The only real disadvantage to HDA is that because it is a relatively new method, it is not as efficient as PCR[5]. Therefore, it takes a little longer to amplify the same amount of DNA to the target concentration using HDA than it would by using PCR.

Conclusions:

As shown, each of these methods has their own benefits and drawbacks. Both PCR and LCR require thermocycling to occur throughout the DNA amplification reaction and RCA requires an initial heating step before the reaction can take place. HDA is the only true isothermal method of amplifying DNA because at no point in the reaction are elevated temperatures required. Consequently, HDA is advantageous in that it alone does not require expensive thermocycling equipment and can retain the chromosomal structure of original sample.

In terms of ease of performing the reaction, HDA and PCR are much more simplistic and practical than LCR and RCA. This is due to the fact that the RCA protocol is very convoluted and lengthy. Also, with LCR, no automated equipment has yet been developed for this particular reaction so it must be done by hand[13]. HDA, because it can be performed isothermally, only requires all the reagents to be added at once and the reaction takes place without any addition work, making it very practical for a laboratory setting. Similarly, because PCR has been in practice for so long, many automated machinery are available which preform the temperature cycling and only require the researcher to add all the reagents at the beginning of the reaction.

PCR seems the most efficient of all the amplification reactions. As stated, with LCR mutations to the DNA can be missed if they occur outside of the primer region being amplified. RCA is also inferior to PCR in that it can only amplify short sequences of DNA. Finally HDA has not be refined enough to match the efficiency of PCR.

While PCR is slightly more efficient than HDA, it is clear that HDA is superior to all other methods in terms of ease of performance, cost, and its ability to perform a completely isothermal reaction. Therefore, Helicase-Dependent DNA Amplification should be the chief method used whenever performing any type of DNA amplification. Although using HDA would compromise the overall efficiency of the reaction, it would cost much less and be easy to preform while still satisfactorily producing products which have not been denatured in any way. In turn, future research should focus on increasing the overall efficiency of this reaction until it rivals that of PCR.

References:

1. Moore P (2005) PCR: replicating success. Nature 435:235–238
2. Andras SC, Power JB, Cocking EC, Davey MR (2001) Strategies for signal amplification in nucleic acid detection. Mol Biotechnol 19:29-44
3. Davis JD, Riley PK, Peters CW, Rand KH. (1998) A comparison of ligase chain reaction to polymerase chain reaction in the detection of Chlamydia trachomatis endocervical infections. Infect Dis Obstet Gynecol. 6(2): 57-60.
4. Demidov VV (2002) Rolling-circle amplification in DNA diagnostics: the power of simplicity. Expert Rev Mol Diagn 2: 542-548
5. Vincent M, Xu Y, Kong H (2004) Helicase-dependent isothermal DNA amplification. EMBO Rep 5:795-800
6. Jeong YJ, Park K, Kim DE (2009) Isothermal DNA amplification in vitro: the helicase-dependent amplification system Cell Mol Life Sci DOI 10.1007/s00018-009-0094-3
7. Chial, H. (2008) DNA sequencing technologies key to the Human Genome Project. Nature Education 1(1):219
8. Mullis KB. The unusual origin of the polymerase chain reaction. Sci Am. 1990;262:56–61, 64.
9. Fakruddin M, Mannan KSB, Chowdhury A, Mazumdar RM, Hossain MN, Islam S, Chowdhury MA. (2013) Nucleic acid amplification: Alternative methods of polymerase chain reaction. J Pharm Bioallied Sci. 5(4): 245-252
10. Schadendorf D and Czarnetzki BM. (1991) Gene Amplification by Polymerase Chain Reaction in Dermatology. J Invest Dermatol 97: 751-755
11. Strachan T, Read AP. (1999) Human Molecular Genetics. 2nd ed. Wiley-Liss, New York.
12. Li H, Gyllenstein U B, Cui X, Saiki R K, Ehrlich H, Arnheim N. (1988) Amplification and analysis of DNA sequences in single human sperm and diploid cells. Nature. 335: 414–417.
13. Markoulatos P, Siafakas N, Moncany M. (2002) Multiplex Polymerase Chain Reaction: A Practical Approach. J Clin. Lab. Ana. 16:47-51