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Review Article

A critical review on PCR, its types and applications

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Abstract

The invention of polymerase chain reaction (PCR) has been a milestone in the history of biological and medical sciences. The applications of PCR have not only completely revolutionised the research in the field of molecular genetics as well as animal and plant biotechnology, but the technique has also proved its relevance and ingenious utility in other fields of forensic sciences, molecular systematics, molecular epidemiology, archaeology, anthropology, evolutionary genetics, *etc.* as well. The conventional PCR led to the emergence of RT-PCR, qPCR and combined RT-PCR/q-PCR. PCR has also enabled the successful completion of the 'human genome project' by enabling the amplification and sequencing of the human genes, which has further laid the foundation of genetic engineering and has now even made it possible to make useful changes in the genome of an organism. The variants of PCR are now being successfully used in most of the recent advances made in the sciences of modern era.

Keywords: Standard PCR- Variants, RT-PCR, qPCR, RT-PCR/qPCR combined.

Introduction

Polymerase Chain Reaction (PCR)

A stepping stone in the field of molecular genetics was laid by James D. Watson and Francis Crick in the year 1953 by proposing a double helix structural model of DNA (Watson and Crick, 1953). In the early 1960's, significant advances were made in elucidation of the genetic code and synthetic oligonucleotides were used as primer templates for DNA polymerase by Dr. H. Gobind Khorana, for which he was also awarded the Nobel prize in the year 1968 (Khorana *et al.*, 1976). In 1971, Kjell Kleppe, a researcher in Khorana's laboratory, described the replication of a segment of DNA by a two-primer system (Kleppe *et al.*, 1971).
Polymerase chain reaction is an *in vitro* technique that enables replication and amplification of a DNA sequence to billions fold amplitude (Mullis and Faloona, 1987; Saiki *et al.*, 1988). The technique of polymerase chain reaction (PCR) was invented by Kary Banks Mullis in the year 1983, while he was working as a biotechnologist in Cetus Corporation, Emeryville, California, USA. In 1985, a joint venture was established between Cetus Corporation and Perkin-Elmer, another US based Biotech Company to design thermal cycler instruments and reagents for PCR and in 1987, a press release announced the availability of the "PCR-1000 Thermal Cycler" and "AmpliTaq DNA Polymerase" commercially. The invention won him laurels of the Nobel Prize in Chemistry as well as the Japan Prize in the year 1993 (Shampo and Kyle, 2002).

Essential Components of Standard PCR

Component of reaction mixture:

Taq/other thermostable polymerases

Template DNA
Primers
dNTPs
$MgCl_2$
Autopipettes/Plasticwares/Gloves
Thermal cycler

Component of reaction mixture

Taq/other thermostable polymerases

In 1969, Thomas D. Brock isolated *Thermus aquaticus*, a new species of thermophilic bacterium found in the Lower Geyser Basin of Yellowstone National Park (Brock and Freeze, 1969). In 1976, thermostable enzyme 'Taq polymerase' was isolated from *Thermus aquaticus* (Scott, 2008). In 1986, Henry Erlich announced the use of Taq polymerase in PCR, since it could sustain its activity at a wide range of high temperatures. Its addition to the PCR mixture shortened the entire PCR process by removing the need to manually add fresh DNA polymerase from *E coli*, at every cycle of the reaction because of its inability to tolerate rapid heating and cooling (Saiki *et al.,* 1988). The kinetic pathway of Taq polymerase in the synthesis of template strand by incorporating nucleotides was described by Rothwell and Waksman, 2005.

Many other thermostable DNA polymerases are also discovered that can be used according to their applications **(Table1)**. Usually 1-1.5U of Taq DNA Polymerase is required in 50µl of reaction mix. However, if inhibitors (*e.g.* low purity of template DNA) are present in the reaction mix, higher amounts of Taq DNA Polymerase (2-3U) may be required to obtain a better yield of amplified products.

Template DNA

Usually 0.01-1ng of template DNA is required for plasmid or phage DNA and 0.1-1µg for genomic DNA, in a total reaction mixture of 50µl. Template DNA higher than this amount results in nonspecific PCR products. Furthermore, DNA should be pure as even a trace amount of phenol, EDTA, Proteinase K, *etc.* used during DNA isolation strongly inhibit Taq DNA Polymerase action. However, ethanol precipitation of DNA and washing with 70% ethanol to DNA pellet is usually effective in removing these contaminants from the DNA sample. (web.stanford.edu).

Primers

The most important consideration for amplification of a target site within a region of the genome is the primer designing. A successful primer is mostly designed for achieving two goals *i.e.* specificity and efficiency. Both of these goals can be achieved if the primers are designed carefully enough so as to avoid false positive results. Following are the considerations that should be kept in mind while designing the primers.

Primer Length: The length of a primer is directly proportional to the specificity of a PCR reaction. The length also determines the temperature at which the primers will anneal to the template (Chuang *et al.,* 2013). Usually primers are designed between 18 to 24 bases long though the minimum length of a primer is determined by the size of the genome (Marshall, 2007).

GC content: GC content is important as it also determines the T_m value of a sequence. 20 base pair long oligos having 50% GC content generally have a T_m value between 56-62˚C (Dieffenbach *et al.,* 1993). Most desirable GC content should be between 40 to 60˚C. More than three G or C nucleotides at the 3' end of the primer should be avoided as this may lead to nonspecific priming.

Melting temperature (T_m): Since in a PCR reaction a set of primers is used so effort should be made to select the pairs that have a T_m in the range of 5˚C within each other, therefore the GC content and length must be chosen accordingly. Usually, the T_m lies in the range of 62-70˚C (Li, 2007).

Estimation of the melting and annealing temperatures of primer: For the primer less than 25 nucleotides, the approximately T_m is calculated using the following formula:

$$T_m = 4 (G + C) + 2 (A + T)$$

G, C, A, T - number of respective nucleotides in the primer.

Table 1: Thermostable polymerases and their applications.

Polymerases (Source)	Habitat	Applications	References
Tfl *(Thermus Flavus)*	nd	Tolerate higher concentrations of blood, high temperature DNA sequencing	Kaledin *et al.,* 1980
T4 (Bacteriophage T4 of *E. coli)*	nd	3’ overhang and 5’ fill-in to form blunt ends, probe labelling, single strand deletion subcloning, second strand synthesis in site-directed mutagenesis	Dale *et al.,* 1985; Kunkel *et al.,* 1987; Sambrook *et al.,* 1989
T7 (Bacteriophage T7, and *trxA* gene of *E. coli)*	nd	Strand extensions in site-directed mutagenesis, *in situ* detection of apoptotic DNA fragments	Bebenek *et al.,* 1989; Wood *et al.,* 1993
Vent/*Tli* *(Thermococcus litoralis)*	Deep sea hydrothermal vents	5-15 fold higher activity than Taq DNA polymerase, 3'→5' exonuclease activity	Mattila *et al.,* 1991
r*Tth* *(Thermus thermophilus)*	Hot spring in Izu, Japan	Tolerate high concentration of blood, reverse transcriptase activity in addition to a 5’→3’ polymerase activity, 5’→ 3’ exonuclease activity	Myers and Gelfand, 1991
Ultma *(Thermotoga maritime)*	Marine geothermal area near Vulcano, Italy	3′-5′ exonuclease activity	Diaz and Sabino, 1998
KOD *(Thermococcus kodakaraensis)*	Solfatara on Kodakara Island, Kagoshima, Japan	High processivity, fidelity, and extension rate without the complexity introduced by terminal transferase activity	Bensona *et al.,* 2003
Pwo *(Pyrococcus woesei)*	Marine sediments, beach of Porto Levante, Vulcano Island, Italy	3′-to-5′ exonuclease activity, tolerate high concentration of blood	Cahill, *et al.,* 2003; Kanoksilapatham *et al.,* 2004
Pfu *(Pyrococcus furiosus)*	Marine sediments, beach of Porto Levante, Vulcano Island, Italy	3′-to-5′ exonuclease activity, lowest error rate	Cahill, *et al.,* 2003; Kanoksilapatham *et al.,* 2004
Hot*Tub* *(Thermus ubiquitous)*	nd	Tolerate high concentration of blood, cDNA synthesis, second strand synthesis in site- directed mutagenesis, production of ssDNA probes by primer extension.	Kermekchiev *et al.,* 2009

[nd-] not defined

***Restriction site integration*:** 3-6 nucleotides are added at the 5' end of the primers so as to successfully provide a site for restriction cutting of the amplified sequence (Chuang *et al.*, 2008).

Primer complementarity: The primer should not be self-complementary or complementary to any other primer in the reaction mixture, so as to avoid the intra or inter primer homology as it will lead to primer dimers (Vallone and Butler, 2004).

***Redundancy*:** Repeat of a single base or two bases for 4 or more times should be avoided (www.lifetechnologies.com).

Terminal nucleotide in PCR primer: The terminal position in a primer is essential for eliminating mispriming. To avoid it, care should be taken to avoid primers that are complementary at their 3' ends as this may lead to unnecessary primer dimer formation (Kwok *et al.*, 1990; Liu *et al.*, 2007).

Secondary structures: The secondary structures should be avoided to the maximum wherever possible. Secondary structures arise as a result of intermolecular or intramolecular interactions. Hairpins, self dimers or cross dimers, all arise as a result of secondary structures (Mergny and Lecroix, 2003).

dNTPs

The concentration of each dNTP in the final reaction mixture is usually 200µM and the concentrations of each dNTPs (dATP, dCTP, dGTP, dTTP), should be equal. The inaccuracy in the concentration of even a single dNTP may lead to increase in the misincorporation of nucleotides in the new strand.

$MgCl_2$

During replication, a lone pair of electron appears in the 3'-OH region of the growing chain which is used for the formation of phosphodiester bond by the Taq Polymerase. This lone pair of electrons is used to convert dNTP to dNMP by nucleophilic attack on the phosphate atom of α-phosphate, releasing the pyrophosphate (β and γ). But the incoming dNTP has four negative charges and due to the presence of these negative charges, the nucleophilic attack is retarded. Therefore, Mg^{2+} comes to rescue by chelating extra negative charges of the incoming dNTP, facilitating the nucleophilic attack and bond formation and hence polymerization. Also, every enzyme needs cofactor for their activation and Mg^{2+} metal ions act as essential cofactor for the DNA polymerase in PCR. Mg^{2+} ion enter into the protein for joining and creating forces making the polymerase stronger and capable to join dNTPs but at the expense of specificity. So, increased concentration of Mg^{2+} in PCR, results in strong band but chances of non-specific amplification also rises. For this, its concentration must be optimized for every primer:template system. Also, other components of the PCR reaction bind to Mg^{2+} ion, including primers, template, PCR products and dNTPs. Out of these, dNTPs have more affinity to bind to the Mg^{2+} ion and as free Mg^{2+} ion are needed to serve as an enzyme cofactor in PCR, the total Mg^{2+} ion concentration must exceed the total dNTP concentration (http://www.promega.com/paguide/chap1.htm). The recommended range of $MgCl_2$ concentration is 1-4 mM in the standard reaction mixture (web.stanford.edu).

Autopipettes/Plasticwares/Gloves

Autopipettes (1-10µl, 10-100µl & 100-1000µl), 0.2ml-1.5ml microcentrifuge tubes, tips, PCR stands *etc.* are required during PCR and for loading the amplified product in the agarose gels.

Thermal cycler

It is an instrument which changes temperature very rapidly during each cycles for denaturation, annealing, extension and hold process.

Principle, Procedure and Post amplification detection of PCR

Principle

The principle of PCR is based on the fact that at high denaturing temperatures nearing 95°C, the two strands of the target DNA molecule separate due to breaking of A-T and G-C bonds. At the annealing temperatures in the range of 50-65°C, the complimentary forward and reverse primers bind at the 3' end of the flanking regions of the separated single stranded target DNA molecule. The Taq polymerase then extends the new DNA strand by adding dNTPs and the double stranded molecule restructures itself at the extension temperature of 72°C. This process is repeated several times, generating multiple copies of the target DNA molecule **(Fig. 1)**. For best results, The European Molecular Genetics Quality Network (EMQN) good practice guidelines should be followed (Muller, 2001).

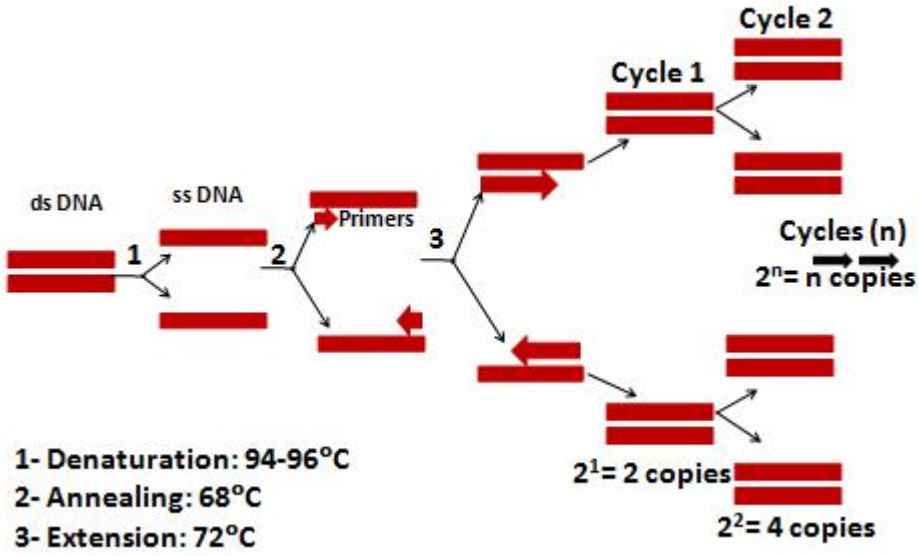

Fig. 1: Principle of PCR.

Procedure

Initial denaturation occurs at 90-95^{o}C for 3-5 minutes, where the two strands of the double stranded target DNA molecule separate.

Initial denaturation is followed by 30-35 cycles of denaturation, annealing and extension. The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. Denaturation involves heating the double stranded target DNA molecule at 90-95^{o}C for 30-55 seconds.

Annealing step allows binding of the complimentary forward and reverse primers to the 3' flanking regions at 50-65^{o}C for 30-55 seconds.

Extension step occurs at 72^{o}C for 30-55 seconds by adding complimentary dNTPs to the new strands.
After the last cycle, the samples are usually incubated at 72°C for 5-15 minutes to fill-in the protruding ends of newly synthesized PCR products.
Holding or storage of the PCR products at 4^{o}C for infinity.

Post amplification detection

The amplified PCR product is observed as a fluorescent pink band by agarose gel electrophoresis following ultraviolet transillumination of the agarose gel stained in Ethidium bromide (EtBr) solution. EtBr has been used since many years for the visualization of nucleic acids in agarose gel. EtBr is also a potent mutagen, causing mutations in the living cell. Therefore, gels should be disposed in an appropriately labelled hazardous waste container with date and handed over to the Environmental Health & Safety (EHS) department (www.ehs.harvard.edu).

Types of PCR

Standard PCR- Variants
Reverse Transcription-PCR (RT- PCR)
Real time-PCR or quantitative PCR (qPCR)
RT-PCR/qPCR combined

Standard PCR- Variants

The modifications in the basic technique of PCR led to the development of variants in PCR that are described below:

Allele specific PCR (Tetra-primer ARMS PCR)

Allele specific PCR allows direct detection of point mutation in DNA. This technique requires prior knowledge of the target DNA sequence such as differences between alleles and utilises the primer with 3' mismatch ends encompassing the single nucleotide variations (Newton *et al.,* 1989; Ugozzoli and Wallace, 1991).

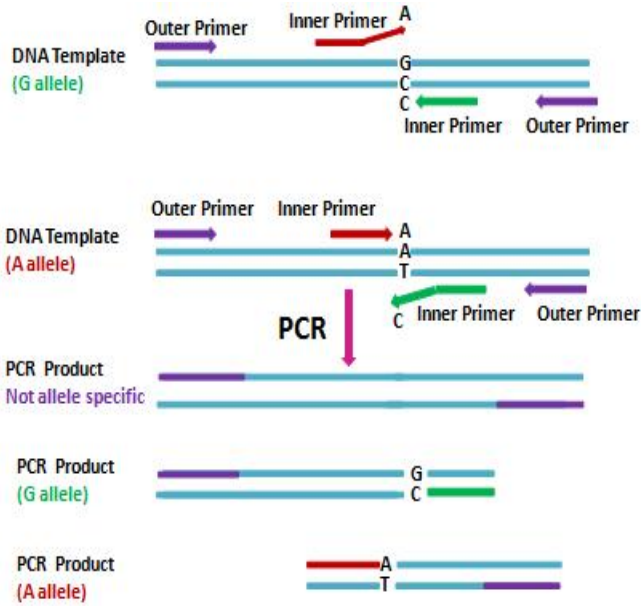

Fig. 2: Allele specific PCR.

Two allele specific primers, one for each allele of the SNP are required, which contain one of two polymorphic nucleotides at the 3' end **(Fig. 2)**. A common forward or reverse primer may be used. Generally, two PCR reactions are needed for detection of both alleles of a SNP (You *et al.,* 2008).

Asymmetric PCR

This variation of PCR is used to preferentially amplify only one strand of the target DNA molecule by using unequal primer concentrations **(Fig. 3)**, as such replication occurs arithmetically by using the excess primer (Innis *et al.,* 1988).

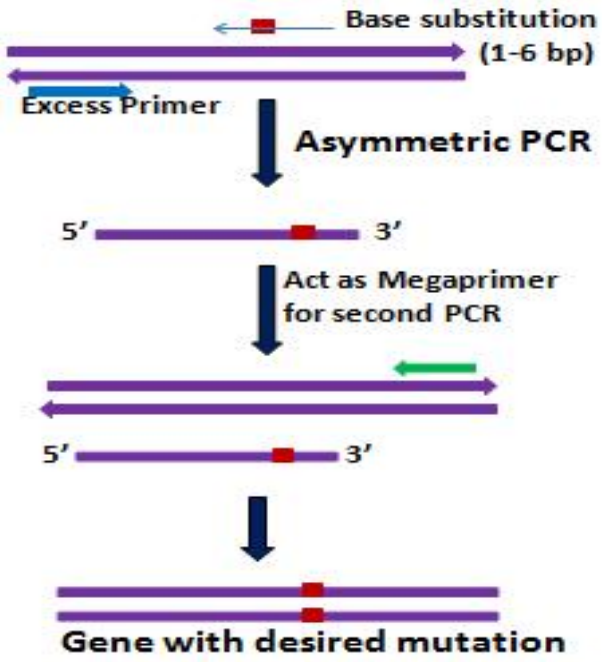

Fig. 3: Asymmetric PCR.

Colony PCR

It is a type of PCR routinely used in bacterial genomic studies. Insertion of high copy number plasmids such as pUC 18, pUC 19 or pBluescript in bacteria is routinely performed for a variety of purposes (Kilger *et al.*, 1997) and colony PCR quickly screen these plasmid inserts. It has several advantages over the traditional methods of blue/white screening as it can determine both the insert size and orientation in the vector (Plourde-Owobi *et al.,* 2005). In fact, the white colonies screened by the traditional blue white screening method has to be screened additionally by the colony PCR method so as to avoid sequencing of the false positive clones. In addition to this colony PCR also helps in generating sufficient amount of desired PCR product for sequencing (Carracedo, 2005).

Degenerate PCR

It is a variant of PCR which employs degenerate primers to amplify unknown sequences of DNA, related to a known DNA sequence. Degenerate primers are designed on the basis of known and sequenced gene homologs. This technique allows identification of new members of a gene family or orthologous genes from different organisms (Lang and Orgogozo, 2011).

Hotstart PCR

This technique involves steps of the conventional PCR, except that the Taq polymerase is added after the rest of the PCR components are heated to the DNA melting temperature, so as to avoid non-specific amplification at lower temperatures. Alternatively, covalently bound inhibitors that dissociate from Taq polymerase only after reaching the T_m can also be added (Chou *et al.,* 1992).

Inverse PCR

Whereas conventional PCR requires complimentary primer pair for both the 3' ends of the target DNA, Inverse PCR allows amplification of DNA with only one known sequence **(Fig. 4)**. This technique requires a sequence of restriction digestions and ligations which result in the formation of a looped DNA fragment which can further be primed from a section of known DNA sequence for PCR (Ochman *et al.*, 1988).

Miniprimer PCR

The standard PCR methods require Taq polymerase whose efficiency of DNA synthesis is less than other replicative enzymes due to their longer primers (20-30 nucleotides) requirement (Wang *et al*., 2004). Therefore, new PCR method has been developed called miniprimer PCR. In this PCR, engineered Taq polymerase and 10 nucleotides long 'miniprimers' are used. Miniprimer PCR is useful in understanding microbial biology for identification of conserved DNA sequences such as 16S rRNA (eukaryotic 18S rRNA) that is not possible with standard primers (Isenbarger *et al.,* 2008). Xu *et al.* evaluated the role of miniprimer PCR using Titanium Taq polymerase and short primers, for genotyping the *Pantoea stewartii* subsp. *stewartii*, the causal agent of Stewart's bacterial wilt on maize (Xu *et al.,* 2010).

Multiplex PCR

Multiplex PCR is a modification of PCR in order to rapidly detect deletions or duplications in a large gene. In 1988, deletions in the dystrophin gene were first detected by multiplex-PCR method (Chamberlain *et al.,* 1988). Multiplex- PCR mix makes use of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes which are specific for different sequences of DNA. This variant of PCR, targets multiple genes at once in a single test run which would otherwise require several times the reagents and more time to perform **(Fig. 5)**. The base pair length of the amplicons, should be different enough to segregate well and form distinct bands when visualized by gel electrophoresis. Multiplex-PCR has been successfully applied in many areas of DNA testing such as analysis of deletions, mutations and polymorphisms, microsatellites and SNPs (Hayden *et al.,* 2008).

Nested PCR

It is a modification of PCR designed to minimize the amplification of non-specific and spurious PCR products, which may result due to primer binding at unexpected or unwanted sites similar to the target DNA. Nested PCR involves 2 sets of primers which are utilized in two successive runs of the PCR reaction **(Fig. 6)**. The second set of primers functions to bind to

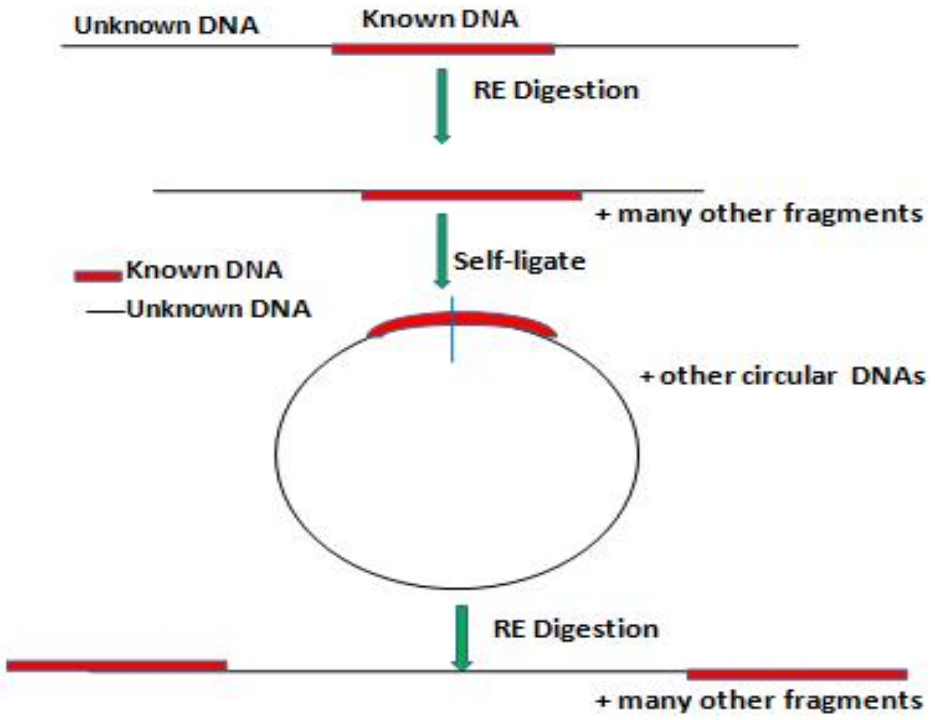

Fig. 4: Inverse PCR.

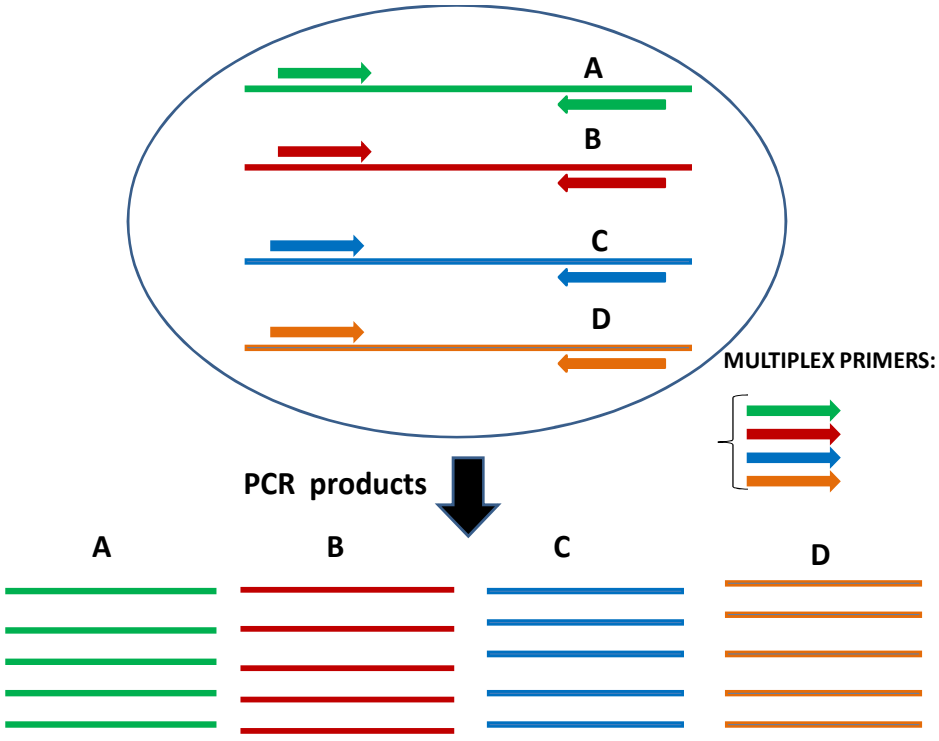

Fig. 5: Multiplex PCR.

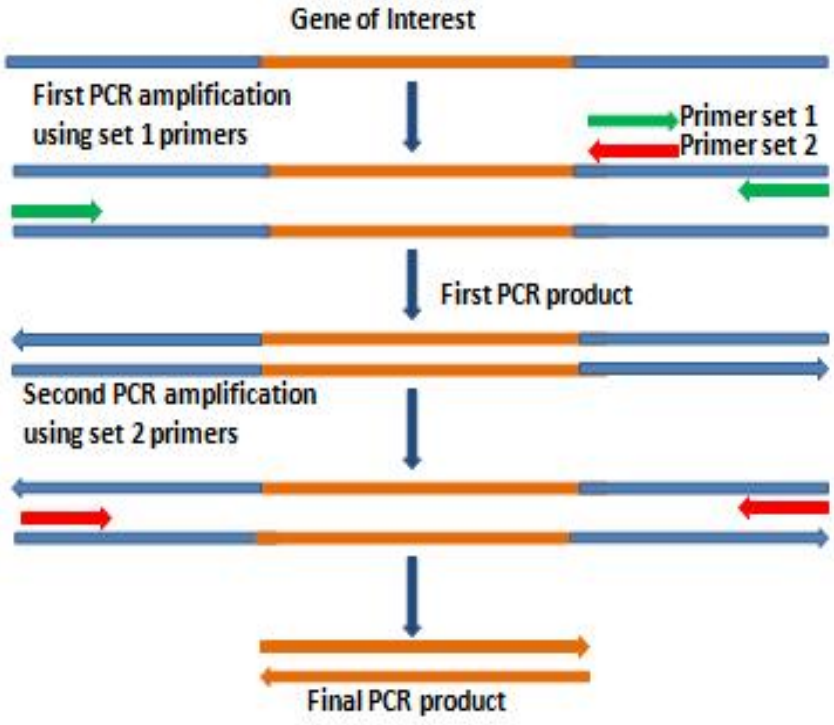

Fig. 6: Nested PCR using two set of primers.

a secondary target within the sequence amplified by the first set of primers, as it is highly unlikely that the spurious or unwanted sequence will have binding site for both the sets of primers (Haff, 1994).

Touchdown PCR

This technique enables ruling out the amplification of non-specific sequences by using early steps of PCR cycles at high temperatures and with subsequent cycles, the annealing temperatures are decreased in increments **(Fig. 7)**. This allows specific primer to anneal at the highest temperature that is least permissive for non-specific binding and generates only the sequence of interest (Don *et al.*, 1991).

Reverse Transcription-PCR (RT- PCR)

This technique enables quantitative detection of levels of RNA expression by creating complimentary DNA (cDNA) from RNA with the help of reverse transcriptase, followed by further amplification of cDNA using standard PCR. Howard Temin from the University of Wisconsin–Madison made the discovery of reverse transcriptases in RSV (Rous Sarcoma Virus) (Temin and Mizutani, 1970), which were then later independently isolated by David Baltimore in 1970 from two RNA tumour viruses: R-MLV (Rauscher- Murine Leukemia Virus) and RSV (Baltimore, 1970). Both shared the 1975 Nobel Prize in Physiology or Medicine for their aforesaid achievements. Reverse transcriptase enzyme includes an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase and ribonuclease H activity which work in sync to perform transcription. Apart from functioning in the process of transcription, retroviral reverse transcriptases have a domain belonging to the RNase H family which is vital to their replication. The idea of reverse transcription was very unpopular at first as it contradicted the central dogma of molecular biology but finally accepted in 1970 when the scientists Howard Temin and David Baltimore both independently discovered the enzyme responsible for reverse transcription, named reverse transcriptase.

The retroviral reverse transcriptases, including Avian Myeloblastosis Virus (AMV) and Moloney murine leukemia virus (MMLV) are the most characterised reverse transcriptases used in the field of molecular biology.

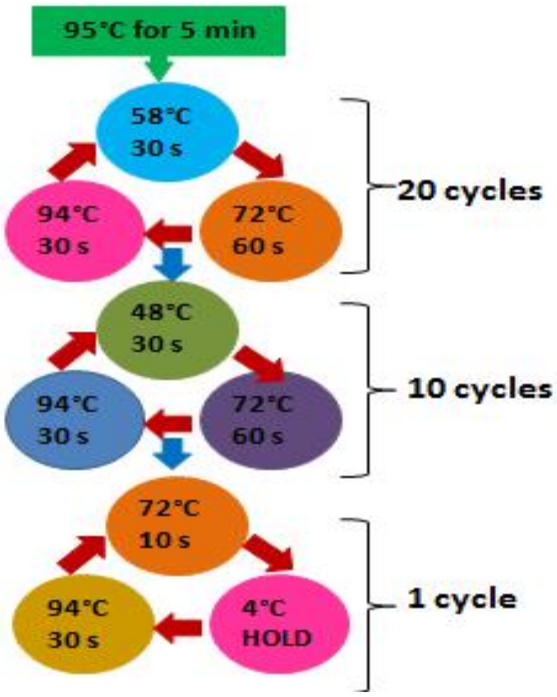

Fig. 7: Cycling method of Touchdown PCR.

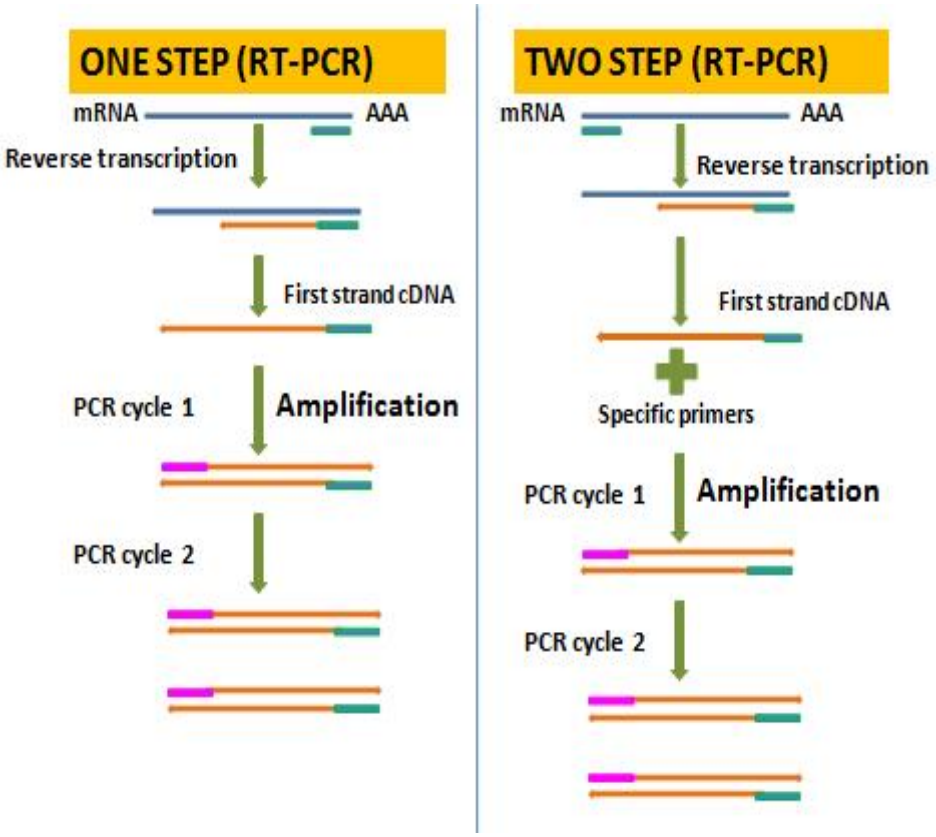

Fig. 8: One step and two step methods of RT- PCR.

The most preferred reverse transcriptase for long mRNA templates is the M-MLV RT because its RNase H activity is weaker than the commonly used AMV reverse transcriptase. Progress in the field of genetic engineering and the development of RT-enhancing buffers have led to the commercial availability of newer enzymes that offer superior performance over the naturally occurring reverse transcriptases which have a higher level of thermostability and a longer shelf life at 50°C. M-MLV RT used these days is purified from *E. coli* expressing the *pol* gene of M-MLV on a plasmid (www.lifetechnologies.com) while insect cells infected with baculovirus containing the pol gene of AMV are used for purification of cloned Avian Myeloblastosis Virus (AMV) reverse transcriptase (www.lifetechnologies.com).

There are two primary ways to carried out RT-PCR *i.e.* one-step and two-step method **(Fig. 8)**. In one-step, all the components including specific primers are put into a single tube as same as the PCR reaction. In a two-step method, the first reaction involves the formation of cDNA with the help of a separate reverse transcription reaction and then addition of cDNA to the PCR reaction.

One-step method is a highly advantageous method as it takes lesser time, is cheaper and requires less handling of samples, thereby reducing pipetting errors, contamination *etc.* However, the drawback is the later analysis of other genes of interest that cannot be amplified as gene-specific primers are used in one reaction tube for cDNA formation and amplification. Therefore, aliquot of RNA from the original sample must be stored for future testing. But two-step method proves to be advantageous as in this method the RNA samples are not used in single processing and future analysis of the gene of interest can be done (Wacker and Godard, 2005).

Real time-PCR or quantitative PCR (qPCR)

qPCR introduced in 1992 by Higuchi and co-workers (Higuchi *et al.,* 1992) enables detection of fluorescent reporter dye, such as SYBR Green I to measure the amplification of DNA at each cycle of PCR. During the log linear phase of amplification, the fluorescence increases to a point which becomes measurable and is called as the Threshold cycle (C_T) or Crossing point. Therefore, by using serial dilutions of a known quantity of standard DNA, the amount of DNA or cDNA of unknown sample can be calculated as C_T value by plotting a standard curve of log concentration vs C_T.

qPCR combines the amplification and detection into a single step thereby eliminating the need for any post amplification processing of the sample (Mackay, 2004). The other advantages of qPCR are sensitivity, real time detection of reaction progress, speed of analysis and precise measurement of the examined material in the sample (Gachon *et al.,* 2004).This is due to the presence of either fluorescent dyes or fluorescently-tagged oligonucleotide probes whose intensity correlates to the amount of DNA product formed (Wong and Medrano, 2005). To facilitate different types of qPCR reactions, different types of polymerases are used including high fidelity, hot start and fast enzymes. Therefore, real-time PCR instruments are designed to carry out these reactions that comprise a thermal cycler for DNA amplification, an optical system to excite fluorophores and capture emitted fluorescence from the detection chemistry **(Fig. 9-A&B)**, and specialized software to collect and analyze the quantitative data generated (www.thermoscientificbio.com).

RT-PCR/qPCR combined

In case of qualitative detection of RNA expression, reverse transcription (RT-PCR) polymerase chain reaction technique is used through conversion of RNA template to cDNA where as for quantitative detection of RNA expression, both RT-PCR and qPCR techniques are merged and this combined technique is called qRT-PCR/ quantitative RT-PCR or RT-qPCR (Joyce, 2002; Taylor *et al.,* 2010; Varkonyi-Gasic and Hellens, 2010).

Advantages and Disadvantages of PCR Technique

Since amplification is carried out by designing the complementary primers, the technique is highly specific. It is relatively fast enough generating a billion copies of amplification in less than three hours. Based on the type of genetic material (DNA or RNA) suitable modifications can be made easily and the technique can be easily used for a wide range of

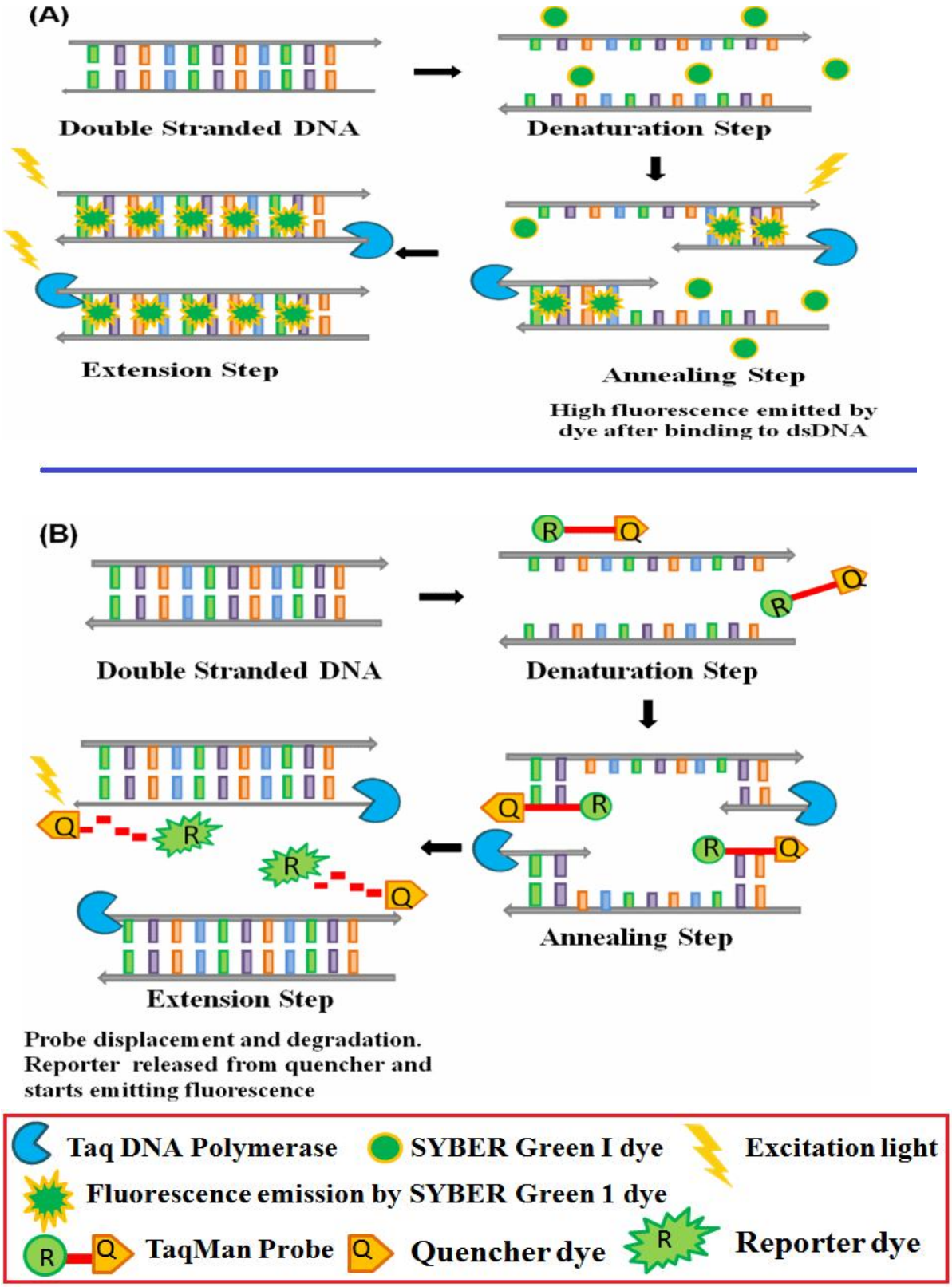

Fig. 9: qPCR: (A) SYBR Green I assay and (B) TaqMan assay.

applications in almost all sorts of organisms ranging from microorganisms to plant and animal kingdom.
Along with huge benefits and applicability, it also has potential drawbacks. The first and foremost drawback is its cost. It is an expensive technique in comparison to the conventional tests. Performing a PCR require a great degree of skill and expertise. Furthermore, to carry out the PCR one must have a sound knowledge of the bioinformatics to design primers, to incorporate restriction sites *etc.* The technique is available in only those labs that have specialized molecular biology testing and analysis techniques. Most of the times nucleic acid from non-viable organisms is also amplified alongwith the desired samples. The analysis of samples after PCR exposes a researcher to harmful chemicals like EtBr, dyes, fluorochromes and UV light which are carcinogenic (Baechtel, 1989; Butler, 2005).

Applications of PCR

Forensic science

PCR is an important tool in DNA profiling, fingerprinting, DNA typing and DNA testing. This technique enables identification of one person among millions of others. Samples of DNA extracted from crime scene can be compared with DNA of suspects or DNA database. Also DNA fingerprinting enables parental testing to identify biological parentage of a child (Saiki *et al.,* 1985; Butler, 2005).

Medicine and diagnostics

Prospective parents can be subjected to gene testing for the presence of genetic diseases and hence the probability of children being carriers of the same can be ascertained. Prenatal testing can be performed by amniocentesis, chronic villus sampling or fetal cells circulating in mother's blood to ascertain the possibility of mutations in the embryo (Saiki *et al.,* 1985).

Tissue-typing can be done prior to performing organ transplantation by using PCR for checking compatibility between donor and recipient. This method has replaced the traditional antibody based blood type test for identifying antigens on the surface of the body cells and tissues (Quill, 2008). Therapy regimens can be customised for individual patients by using PCR based tests to study mutations in oncogenes in certain forms of cancer.

Since antibodies to HIV do not appear until many weeks after infection, PCR based tests have been developed that enable detection of even a single viral genome among the host cells. Similarly, donated blood, newborns and effects of antiviral treatments can be done immediately (Kwok *et al.,* 1987). Moreover, donated blood can also be tested for bacterial contamination using real-time PCR (Dreier *et al.,* 2007).

In case of Tuberculosis, which otherwise requires sputum sample collection and culture in laboratory, PCR based tests have enabled detection of both live and dead microorganisms. Moreover, detailed gene analysis enables detection of antibiotic resistance as well as effects of therapy (Lindstrom and Korkeala, 2006).

PCR based testing has enabled detection of spread of infectious microorganisms in domestic or wild animals.

Conclusions

PCR is a highly advanced yet simple technique which has proved its versatility in most fields of biological and medical sciences due to its ability to yield not only qualitative but quantitative results also. The invention of PCR has been a boon to the modern science with its applicability in clinical diagnostics, DNA fingerprinting, DNA profiling, recombinant DNA technology apart from its role in other fields of archaeology, anthropology, forensics as well. The unravelling of the human genome as well as genomes of many other organisms including several plant species has led to PCR being applied robustly in either one form or another for detailed scientific analysis. These advancements have resulted in the modification of basic PCR technique together with RT-PCR, qPCR and combined RT-PCR/qPCR. The future will bring further novel application of PCR in biological sciences which will be accompanied by more sophisticated instrument design having high sample throughput and atomisation. Above all, the prerequisite is the curious and ingenious scientists that will upbring these inventions.

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