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John G. Webster

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POLYMERASE CHAIN REACTION

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INTRODUCTION

Few techniques rival the impact that the polymerase chain reaction (PCR) has made in the age of molecular biology. Cloning and deoxyribonucleic acid (DNA) sequencing are other such techniques that have become embedded into everyday life on the molecular biologist's bench. Over 60 books alone (not to mention the tens of thousands of research articles) have been devoted to the strategies, methods and applications of PCR for the identification, detection and diagnosis of genetic and infectious diseases. Rightfully so, the inventor of PCR, Kary B. Mullis, was awarded the Nobel Prize in Chemistry for his discovery of the technique in 1993. However, PCR has not been without controversy. In 1989, DuPont challenged the validity of the Cetus PCR patents in federal court and with the Office of Patents and Trade Marks, and by 1991 the Cetus patents were unanimously upheld and later sold to Hoffman La Roche for $300 million. More recently, in 1993, Promega has challenged the validity of the Hoffmann La Roche *Taq* DNA polymerase patent that is currently pending. In this article, we attempt to provide a comprehensive overview for the molecular biologist when applying PCR to his/her application of interest.

DNA POLYMERASE REACTION

The DNA replication is an inherent process for the generation and evolution of future progeny in all living organisms. At the heart of this process is the DNA polymerase that primarily synthesizes new strands of DNA in a $5' \rightarrow 3'$ direction from a single-stranded template. Most native DNA polymerases, however, are polyfunctional and show 5′-exonuclease and/or 3′-exonuclease activities that are important for cellular DNA repair and proofreading functions. Numerous molecular biology applications have harnessed these activities, such as labelling DNA by nick translation and TaqMan assays (see below), and endrepair of sheared DNA fragments and improving DNA synthesis fidelities, respectively. The PCR is an elegant, but simple, technique for the *In vitro* amplification of target DNA utilizing DNA polymerase and two specific oligonucleotide or primer sequences flanking the region of interest. PCR is a cyclic process of double-strand separation of DNA by heat denaturation, specific hybridization or annealing of short oligonucleotide primers to singlestranded DNA, and synthesis by DNA polymerase (1,2). Each cycle doubles the region marked by the primer sequences. By sequential iteration of the process, PCR exponentially generates up to a billion of copies of the target within just a few hours (Fig. 1).

The specificity of PCR is highly dependent on the careful design of unique primers with respect to the genome under investigation and the nucleotide composition of the primer sequences. Theoretically, a 16-mer (4^{16}) is of sufficient length to represent all unique primer sequences from a completely random genome size of 3 billion base pairs. In the real world, however, all genomes are not random and contain varying degrees of repetitive elements. For the human genome, Alus, LINEs (long interspersed DNA elements) and low complexity repeats are frequently observed and should be avoided in primer design when possible. There are a few simple rules for designing primer sequences that work well in PCR. In practice, PCR primers should be between 18 and 25 nucleotides long, have roughly an equal number of the four nucleotides, and show a G + C composition of 50–60%. Commercially available oligonucleotide synthesizers that show phosphamidite coupling efficiencies $> 98\%$ mean that primers of this size can usually be used in PCR without purification. A variety of computer programs are available for selecting primer sequences from a target region. Many of these programs will reveal internal hairpin structures and self-annealing primer sequences, but manual inspection of the oligonucleotide is still necessary to maximize successful PCR amplifications.

The concentrations of the PCR cocktail ingredients are also important for product specificity, fidelity and yield. In addition to *Taq* DNA polymerase and primers, the PCR mixture contains the cofactor magnesium ion (Mg^{2+}), the four 2′-deoxyribonucleoside-5′-triphosphates (dNTPs) and the buffer. In general, PCR reagent concentrations that are too high from standard conditions result in nonspecific products with high misincorporation errors, and those that are too low result in insufficient product. A typical 50 μL PCR cocktail that contains 0.4 $\mu mol{\cdot}L^{-1}$ of each primer, 200 $\mu mol{\cdot}L^{-1}$ of each dNTP, 1.5 $mmol{\cdot}L^{-1}$ $MgCl_2$, and 1.25 units *Taq* DNA polymerase in 10 $mmol{\cdot}L^{-1}$ tris-HCl, pH 8.3, 50 $mmol{\cdot}L^{-1}$ KCl buffer works well for most PCR applications. The optimal Mg^{2+} concentration, however, may need to be determined empirically for difficult target templates. The performance and fidelity of *Taq* DNA polymerase are sensitive to the free Mg^{2+} concentration (3), which ionically interacts with not only the dNTPs but also with the primers, the template DNA, ethylenediaminetetraacetic acid (EDTA), and other chelating agents. In most cases, the Mg^{2+} concentration will range from 1.0 to 4.0 $mmol{\cdot}L^{-1}$.

The number of cycles and the cycle temperature–length of time for template denaturation and primer annealing and extension are important parameters for high quality PCR results. The optimal number of cycles is dependent on the starting concentration or copy number of the target DNA and typically ranges from 25 to 35 cycles. Too many cycles will significantly increase the amount of nonspecific PCR products. For low copy number targets, such as the integrated provirus of *Human immunodeficiency virus type 1* (HIV-1) from human genomic DNA, two rounds of PCR are employed first using an outer primer pair set followed by an internal (nested) primer pair set flanking the region of interest to yield positive and specific PCR products. Brief, but effective denaturation conditions, that

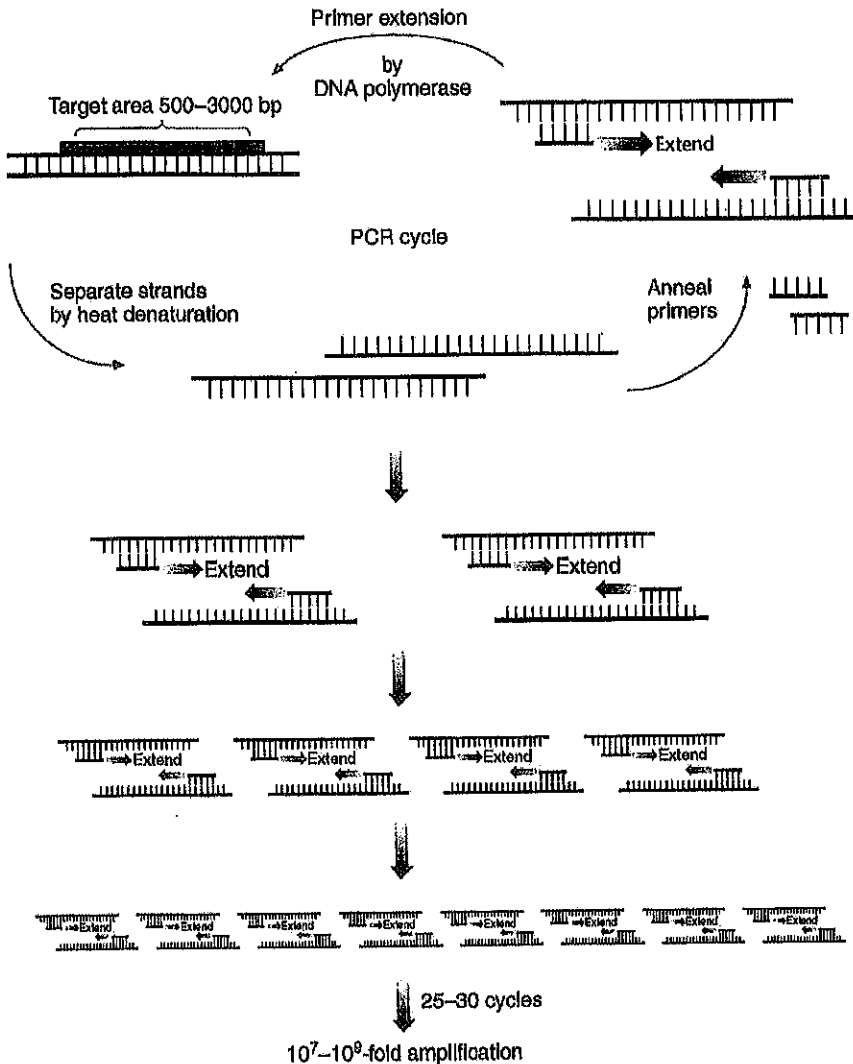

Figure 1. The PCR amplification cycle.

is 94–97 °C for 15–30 s, are necessary as *Taq* DNA polymerase has a half-life of only 40 min at 95 °C. Annealing conditions, on the other hand, are dependent on the concentration, base composition and the length of the oligonucleotide and typically range between 55 and 68 °C for 30–60 s. The length of the amplified target is directly proportional to the primer extension length of time. Primer extension is performed between 68 and 72 °C and, as a rule of thumb, is ~ 60 s for every 1 kb.

Crude extracts from blood, cerebral spinal fluid, urine, buccal smears, bacterial colonies, yeast spores, and so on are routinely used as sources of DNA for PCR templates. Due to the high sensitivity of PCR, rapid isolation protocols, such as heat and detergent disruptions, and enzymatic digestion of biological samples have been frequently used. Caution should be invoked when using crude extracts as starting materials for PCR amplifications because a number of impurities are known to inhibit *Taq* DNA polymerase. These include red blood cell components, sodium dodecyl sulfate (SDS), high salts, EDTA and too much DNA. Since only a few hundred target molecules are needed for successful PCRs, in most cases, these impurities can be effectively removed by simply diluting the starting material. Each sample should then be tested with control primers that specifically amplify a known target to determine the integrity of the crude extract. Alternatively, the isolation of the desired organism, such as HIV-1, human *Hepatitis A virus*, influenza virus, cytomegalovirus, and so on, or the isolation of specific cell fractions, such as peripheral blood mononuclear cells, can significantly increase the sensitivity and specificity of the PCR amplifications.

SENSITIVITY AND CONTAMINATION OF POLYMERASE CHAIN REACTION

Contamination is the dark side of the PCR force. The exquisite sensitivity of PCR can result in contamination from even a single molecule of foreign or exogenous DNA (4,5). To minimize false positives, standard operating procedures have been described, including the physical isolation of PCR reagents from the preparation of DNA templates and PCR products, using autoclaved solutions, premixing and aliquoting reagents, the use of disposable

gloves, avoiding splashes, the use of positive displacement pipettes, adding DNA last, and carefully choosing positive and negative controls (6). Contamination is likely to surface for DNA samples that are difficult to amplify because of sequence content, or due to poor primer design and chemical impurities in DNA extractions. This is especially true for low copy number targets or degraded samples, as greater numbers of amplification cycles are generally required to achieve the desired product. In these cases, residual amounts of exogenous DNAs can compete and override the amplification process, resulting in spurious data. The best approach to challenge dubious results is to repeat the experiment with scrupulous care to details and controls. Biological samples collected at a single time point should be divided into multiple aliquots such that independent DNA extractions and PCR experiments can be performed to verify and validate initial results. Data should be discarded if inconsistent positive and negative PCR results occur upon repetition of the experiment. While negative controls can rule out reagent contamination, sporadic contamination can go unchecked. The probability of repeating spurious contamination in a consistent manner is extremely low.

There are three sources of contaminating DNA: (*1*) carryover contamination from previously amplified PCR products; (*2*) cross-contamination between multiple source materials; and (*3*) plasmid contamination from a recombinant clone that contains the target sequence. Of the three, carryover contamination is considered to be the major source of contamination because of the relative abundance of amplified target sequences. The substitution of dUTP for dTTP in the PCR cocktail has been routinely used as a method of preventing carryover contamination. Pretreatment of subsequent PCR mixtures prior to thermal cycling with uracil DNA glycosylase results in the removal of dU from any carryover PCR product, but does not affect the template DNA or dUTP. The dU removal creates an abasic site that is heat labile and degrades during thermal cycling, thus preventing carryover amplification. Moreover, ultraviolet (UV) light can reduce work surface and reagent contamination. Cross-contamination between samples is more difficult to diagnose, and suspicious results should be repeated from independent DNA extracts and PCR experiments for samples in question. Plasmid contamination, on the other hand, can be identified by sequence analysis and comparison to all laboratory plasmid sequences.

POLYMERASE CHAIN REACTION INTRODUCES MUTATIONS

The power and ease of PCR, however, were not fully appreciated until the introduction of the thermostable DNA polymerase isolated from *Thermus aquaticus* (*Taq*) (7) and automated instrumentation in 1988. It was here that PCR could be run in fully closed and automated systems. Fresh Klenow DNA polymerase did not have to be added at each cycle and PCR could be performed at higher annealing and extension temperatures, which increased the specificity and yields of the reactions while minimizing the risks of contamination. A hot start PCR further enhances specificity by preventing the formation of nonspecific products that arise during the initial steps of thermal cycling in PCR.

Taq DNA polymerase has been shown to incorporate nucleotides incorrectly at a frequency of 1 in 9000 bases by a mutation reversion assay (8). From sequence analysis of cloned PCR products, a slightly higher error frequency was determined (1 in 4000–5000 bp) for *Taq* DNA polymerase (9). The fidelity of DNA synthesis for *Taq* DNA polymerase, however, can vary significantly with changes in free Mg^{2+} concentration, changes in the pH of the buffer, or an imbalance in the four dNTP concentrations. Polymerase misincorporation errors are minimized when the four dNTPs are equimolar and between 50 and 200 $\mu mol \cdot L^{-1}$ (9). Since *Taq* DNA polymerase lacks a 3′-exonuclease activity, misincorporated bases typically cause chain termination of DNA synthesis that are not propagated in subsequent PCR cycles. In a worst-case scenario, a mutation occurring during the first round of PCR from a single target molecule and propagated thereafter would exist at a frequency of 25% in the final PCR product. Since hundreds of target copies are routinely used as starting DNA in PCR and most misincorporations terminate DNA synthesis, the observed error frequency is $\ll$ 25%.

Cloning of full-length genes from PCR products, however, has been problematic because PCR-induced mutations can cause amino acid substitutions in the wildtype sequence. Thus, significant effort must be employed in the complete sequencing of multiple PCR clones to identify mutation-free clones or ones that contain synonymous substitutions that do not change the protein coding sequence. Accordingly, thermostable DNA polymerases that contain a 3′-exonuclease (3′-exo) activity for proofreading of misincorporated bases have been recently introduced and include DNA polymerases isolated from *Pyrococcus furiosus (Pfu)*, *Thermococcus litoralis* (Vent), *Pyrococcus* species GB-D (Deep Vent) and *Pyrococcus woesei* (*Pwo*). The error frequencies of these DNA polymerases are two- and sixfold < *Taq* DNA polymerase (10), but these polymerases are difficult for routine use, as the 3′-exonuclease activity can easily degrade the single-stranded PCR primers. 3′-Exo DNA polymerases, however, have been successfully used in long PCR in combination with *Taq* DNA polymerase and show an approximately twofold lower error frequency than *Taq* DNA polymerase alone (10).

POLYMERASE CHAIN REACTION LENGTH LIMITATIONS

For most applications, standard PCR conditions can reliably amplify target sizes up to 3–4 kb from a variety of source materials. Target sizes > 5 kb, however, have been described in the literature using standard PCR conditions, but generally yield low quantities of PCR product. The PCR size limitation can be attributed to the misincorporation of nucleotides that occurred 1 in 4000–5000 bp that ultimately reduced the efficiency of amplifying longer target regions. A breakthrough in long PCR came through the combined use of two thermostable DNA

polymerases, one of which contains a 3′-exonuclease activity (11,12). The principle for long PCR is that the *Taq* DNA polymerase performs the high fidelity DNA synthesis part of PCR, coupled with the proofreading activity of *Pfu*, Vent or *Pwo* DNA polymerases. Once the nucleotide error is corrected, *Taq* DNA polymerase can then complete the synthesis of long PCR templates. From empirical studies, only a trace amount of the 3′-exo DNA polymerase, roughly 1% to that of *Taq* DNA polymerase or another DNA polymerase isolated from *Thermus thermophilis (Tth)*, is needed to perform long PCRs > 20 kb. Other important factors for long PCR are the isolation of high quality, high molecular weight DNA and protection against template damage, such as depurination during thermal cycling. The use of the cosolvents glycerol and dimethyl sulfoxide (DMSO) have been shown to protect against DNA damage by efficiently lowering the denaturation temperature by several degrees centigrade. The rule of thumb for primer extensions still applies for long PCRs (60 $s{\cdot}kb^{-1}$), although for targets > 20 kb, times extension should not exceed 22 min $cycle^{-1}$. The complexity and size of the genome under investigation can also affect the size of long PCR products. For example, PCR product lengths of 42 kb have been described for the amplification of λ bacteriophage DNA (11,12), compared with a 22 kb PCR product obtained from the human β-globin gene cluster (12).

CREATION OF NOVEL RECOMBINANT MOLECULES BY POLYMERASE CHAIN REACTION

Polymerase chain reaction can amplify both single- and double-stranded DNA templates as well as complementary DNA (cDNA) from the reverse transcription of messenger ribonucleic acid (mRNA) templates. Because of the flexibility of automated DNA synthesis, *In vitro* mutagenesis experiments can easily be performed by PCR. Recombinant PCR products can be created via the primer sequences by tolerated mismatches between the primer and the template DNA or by 5′-add-on sequences. Primer mediated mutagenesis can accommodate any nucleotide substitutions and small insertions or deletions of genetic material. The desired genetic alteration can be moved to any position within the target region by use of two overlapping PCR products with similar mutagenized ends (Fig. 2, left). This is accomplished by denaturing and reannealing the two overlapping PCR products to form heteroduplexes that have 3′-recessed ends. Following the extension of the 3′-recessed ends by *Taq* DNA polymerase, the full-length recombinant product is reamplified with the outer primers only to enrich selectively the full-length recombinant PCR product. 5′-Add-on adapters can also be used to join two unrelated DNA sequences, such as the splicing of an exogenous promoter sequence with a gene of interest (Fig. 2, right). The promoter–gene sequences are joined at the

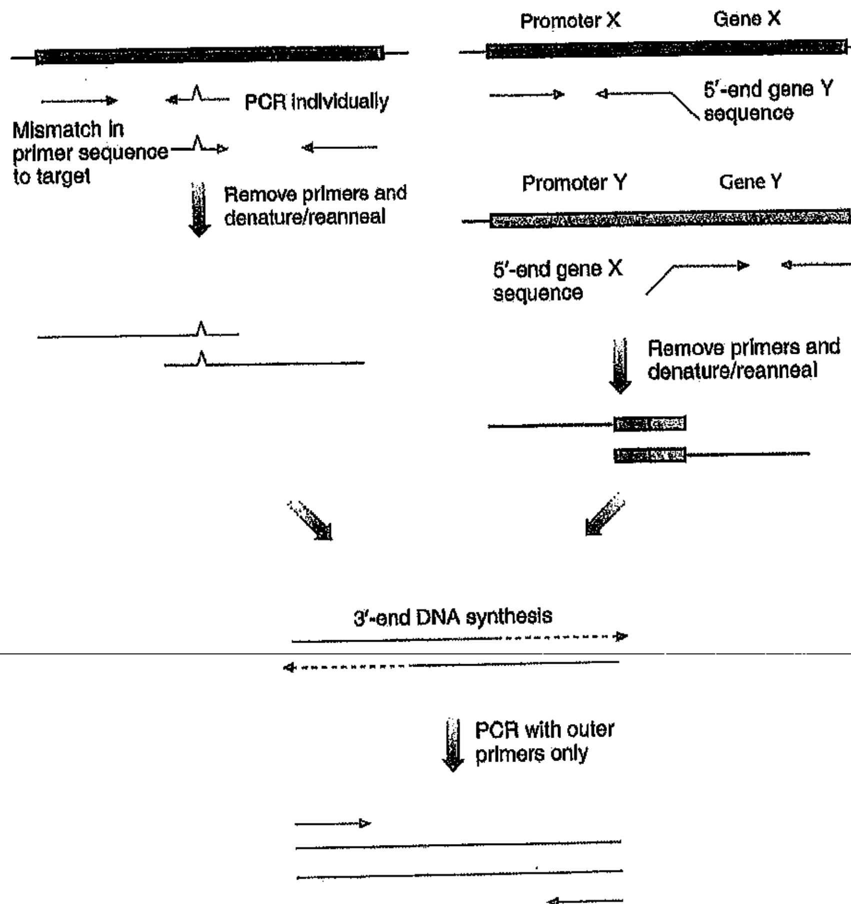

Figure 2. Creation of mutagenized or recombinant PCR products via primer mismatches (left) or 5′-add-on sequences (right).

desired junction by 5′-add-on gene specific and 5′-add-on promoter-specific adapters that can PCR amplify the promoter and the gene targets, respectively. Heteroduplexes can then be formed, as described above, from the two overlapping PCR products, which are then selectively amplified with outer primers to generate the desired full-length recombinant PCR product.

POLYMERASE CHAIN REACTION AS A DETECTION SYSTEM

Polymerase chain reaction is a powerful tool for the detection of human polymorphic variation that has been associated with hereditary diseases. Many PCR techniques have been described that can discriminate between wild-type and mutant alleles, but in this section only a few of the most frequently used techniques are discussed. Of these, DNA sequencing of PCR products is the most widely used and most sensitive method for the detection of both novel and known polymorphic differences between individuals. Complementing scanning technologies, however, have been developed for the rapid detection of allelic differences because of the high costs associated with DNA sequencing and the ability to process large numbers of samples. Singlestrand conformation polymorphism (SSCP) has been commonly used as a technique for the identification of genetic polymorphisms. Following PCR, the product is heat denatured and subjected to native or nondenaturing gel electrophoresis. Allelic differences between samples are detected as mobility band shifts by radioactive and non-radioactive labeling procedures. PCR–SSCP, however, is limited in fragment size to ~ 200 bp because the accuracy in discriminating between different alleles diminishes significantly with an increase in the fragment length.

Multiplex PCR allows for the simultaneous amplification of multiple target regions and has been particularly useful for the detection of exon deletion(s) in X-linked disorders, such as Duchenne muscular dystrophy (13) and Lesch–Nyhan syndrome (14). The multiplex PCR products are resolved by gel electrophoresis and are visualized by ethidium bromide staining. The absence of specific PCR product(s) is diagnostic of exon deletion(s) in affected males, and half-dosage PCR products are diagnostic of carrier mothers (15). Moreover, up to 46 primer pairs have been simultaneously amplified by multiplex PCR with excellent success (90%) for the large-scale identification of human single nucleotide polymorphisms (SNPs) by hybridization to high density DNA chip arrays.

Genetic polymorphisms can also be identified by immobilizing the PCR product on to a nylon membrane in a dot blot format and probing by hybridization with an allele-specific oligonucleotide (ASO) that contains a 5′-biotin group. The ASO hybridization is detected by adding streptavidin–horseradish peroxidase, which binds to the biotinylated probe, followed by a colorimeter assay. The colorimeter ASO assay has been applied to the genotyping of human leucocyte antigen (HLA)–DQA alleles and the detection of β-thalassaemia mutations. More recently, multiplex PCR and colorimeter ASO methodologies have been combined in a reverse fashion, in which ASOs are immobilized on to nylon membrane strips and probed against biotinylated gene-specific multiplex PCR reactions. Allele-specific PCR products are detected by hybridization and conversion of a colourless substrate to a blue precipitate for the simultaneous genotyping of HLA–DQA 1, low density lipoprotein receptor, glycophorin A, hemoglobin G gammaglobin, D7S8, and groupspecific component.

Lastly, *In situ* PCR enables the amplification of target sequences from sections of formalin-fixed, paraffin-embedded tissue specimens to determine the levels of gene expression in specific cell types that otherwise could not be detected by conventional *In situ* hybridization. The PCR is performed directly on glass slides by overlaying the PCR mixture on to the specimen, sealing the slides to prevent evaporation, and temperature cycling using a thermal sensor or modified thermal cycler that holds glass slides.

DEGENERATE POLYMERASE CHAIN REACTION

Degenerate PCR is a powerful strategy for obtaining novel full-length cDNA sequences from limited amino acid sequence information (16). The PCR primer sequences are derived from the reverse translation of 6–9 amino acid codons, which will result in varying levels of degeneracy except for methionine and tryptophan residues. Careful attention should be exercised in the design of degenerate primers because increasing the primer complexity (i.e., using codons that show more than twofold degeneracy) will typically result in an increase in nonspecific PCR products. One approach in reducing the complexity of the degenerate primer is the use of codon bias for the particular organism from which the gene will be cloned. Alternatively, the alignment of orthologous gene sequences from other species can greatly improve the specificity of cloning the gene of interest by revealing evolutionarily conserved domains. Once the optimal primer sequence is determined, the mixture of oligonucleotides can be simultaneously synthesized and will represent all possible amino acid combinations of the degenerate sequence. The specificity of PCR should then selectively amplify the correct primer sequences to generate a gene or gene family-specific probe from which the full-length cDNA can be obtained. Degenerate PCR has been successfully used in the screening of novel gene family members such as G-protein-coupled receptors, nuclear steroid receptors and protein tyrosine kinases.

ANCIENT DNA

Phylogenetics is the study of evolutionary relationships between specimens that are inferred from contemporaneous sequences. The ability to obtain DNA sequences from specimens or even fossils that are millions of years old could equip the phylogeneticist with a powerful means of directly testing an a priori hypothesis. Following death of the tissue or organism, however, DNA is rapidly degraded by, presumably, nuclease activities and hydrolytic processes, resulting in short fragment sizes that are generally no longer than 100–150 bp. Moreover, this old DNA is largely modified by oxidative processes and by intermolecular crosslinks that render it unsuitable for cloning by standard molecular biology procedures. Short PCRs,

however, have been successfully performed from DNA samples isolated from archival and ancient specimens (17).

Museums hold vast collections of archived hospital files of patient specimens and of different species that have been collected over the last century. In a recent study, phylogenetic analyses of DNA sequences were performed from reverse transcriptase PCR (RT-PCR) of formalin-fixed, paraffin-embedded tissue specimens obtained from U.S. servicemen killed in the 1918 Spanish influenza pandemic. Viral sequences from three different gene regions were consistent with a novel H1N1 *Influenza A virus* that was most closely related to influenza strains that infect humans and swine, but not wild waterfowl, considered to be the natural reservoir for the influenza virus (18). Moreover, PCR and DNA sequencing have been performed on DNA extractions of archaeological findings, such as amplifying mitochondrial DNA sequences from a 7000 year old human brain, amplifying both mitochondrial and nuclear DNA sequences from bone specimens from a 14,000 year old saber-toothed cat, and amplifying chloroplast DNA sequences from fossil leaf samples from a 17 million-year-old Miocene *Magnolia* species.

QUANTITATIVE POLYMERASE CHAIN REACTION

Quantitative PCR (QPCR) has been widely used for detecting and diagnosing genetic deletions, for studying gene expression and for estimating the viral load of HIV-1. While DNA quantitation by multiplex PCR has been previously described (15), the quantitation of RNA has been wide reaching for the latter two areas. For many applications, estimating the relative amount of PCR product is sufficient to describe a biological observation. The absolute quantitation of RNA molecules, however, has been more difficult than for DNA because of the difficulty of generating accurate controls. Internal standards derived from synthetic RNA or cRNA have been designed to contain the same primer sequences as the target but yield a different-sized PCR product that can be easily separated by gel electrophoresis. cRNAs are not only coamplified with target sequences, but also coreverse transcribed to account for the variable efficiencies of cDNA syntheses. Moreover, QPCR is typically performed in the exponential or log phase of the amplification process (typically 14–22 cycles) to obtain accurate quantitative results. The absolute amount of target mRNA can be quantitated by serial dilutions of the target/internal control mixture and by extrapolating against the standard curve.

Both the variable range of initial target amounts and the presence of various inhibitors can, however, adversely affect the kinetics and efficiencies of PCR. Alternatively, a strategy based on a quantitative competitive (QC) approach has been used to minimize the effects of these variables. Known quantities of the competitor template, which contains the same primer sequences as the target but differs in size, are introduced into replicate PCRs containing identical quantities of the target. The point at which the intensities of the PCR products derived from the target sequence and the competitor template are equivalent is used to estimate the amount of target sequence in the original sample (19).

Recently, real-time QPCR and QCPCR (20) using a 5′-nuclease fluorogenic or TaqMan assay (21) has been developed to measure accurately the starting amounts of target sequences. Unlike gel electrophoresis, real-time QPCR has the unique advantage of being a closed-tube system, which can significantly reduce carryover contamination. Using this technique, one can easily monitor and quantitate the accumulation of PCR products during log phase amplification. The TaqMan assay utilizes dual reporter and quencher fluorescent dyes that are attached to a nonextendible probe sequence. During the extension phase of PCR, the 5′-nuclease activity of *Taq* DNA polymerase cleaves the hybridized fluorogenic probe, which releases the reporter signal and is measured during each cycle. In addition to real-time QPCR, TaqMan assays have broad utility for the identification of SNPs.

RELATED NUCLEIC ACID AMPLIFICATION PROCEDURES

Other *in vitro* systems can amplify nucleic acid targets such as the transcription-based amplification system (TAS) (6), its more recent version called the self-sustained sequence replication (3SR) (22) and the ligation-dependent Qβ-replication assay (23). These methods are best suited for the detection and semiquantitation of RNA target sequences. The strategy for TAS and 3SR is a continuous series of reverse transcription and transcription reactions that mimic retroviral replication by amplifying specific RNA sequences via cDNA intermediates. The primers contain 5′- add-on sequences for T7, T3, or SP6 promoters that are incorporated into the cDNA intermediates. The rapid kinetics of transcription-based amplifications is an attractive feature of these systems, which can amplify up to 10^7 molecules in 60 min. Short amplify products, however, which are due to incomplete transcription of the target region and incomplete RNase H digestion of the RNA–DNA hybrids, can be problematic in the TAS and 3SR assays.

Unlike PCR, TAS, or 3SR assays, the ligation-dependent Qβ-replication assay results in the amplification of probe, not target, sequences. This assay utilizes a single hybridization to the target sequence, which is embedded within, and divided between, a pair of adjacently positioned midvariant (MDV-1) RNA probes. MDV-1 RNA is the naturally occurring template for the bacteriophage Qβ RNA replicase. Following the isolation of the probe–target hybrids, ligation of the binary probes creates a full-length amplifiable MDV-1 RNA reporter. When Qβ replicase is added, newly synthesized MDV-1 RNA molecules are amplified from ligated binary probes that originally hybridized to the target sequence (23). Similar to TAS and 3SR, the Qβ-replication assay shows rapid kinetics, generating up to 10^9 molecules in 30 min, and all three methods have been successfully used for the detection and quantitation of HIV-1 RNA molecules.

LIGATION CHAIN REACTION

The ligase chain reaction (LCR) can also amplify short DNA regions of interest by iterative cycles of denaturation

and annealing/ligation steps (24). The LCR utilizes four primers: two adjacent ones that specifically hybridize to one strand of target DNA and a complementary set of adjacent primers that hybridize to the opposite strand. LCR primers must contain a 5′-end phosphate group, such that thermostable ligase (24) can join the 3′-end hydroxyl group of the upstream primer to the 5′-end phosphate group of the downstream primer. Successful ligations of adjacent primers can subsequently act as the LCR template, resulting in an exponential amplification of the target region. The LCR is well suited for the detection of SNPs because a single-nucleotide mismatch at the 3′ end of the upstream primer will not ligate and amplify, thus discriminating it from the correct base. Although LCR is generally not quantitative, linear amplifications using one set of adjacent primers, called the ligase detection reaction, can be quantitative. Coupled to PCR, linear ligation assays can also be used as a mutation detection system for the identification of SNPs using both wild-type-specific and mutant-specific primers in separate reactions. The oligonucleotide ligase assay was first reported to detect SNPs from both cloned and clinical materials using a 5′-end biotin group attached to the upstream primer and a nonisotopic label attached to the downstream primer (25). Allele-specific hybridizations and ligations can be separated by immobilization to a streptavidin-coated solid support and directly imaged under appropriate conditions without the need for gel electrophoretic analysis.

SUMMARY

Some of the general concepts and practices of PCR have been reviewed here. Not only has PCR made a major and significant impact on basic and clinical research, but it has also been well accepted and utilized in forensic science. For any scientific methodology to be accepted in the courts as evidence, it must satisfy four criteria: that the method (*1*) be subject to empirical testing, (*2*) be subject to peer review and publication, (*3*) has a known error rate, and (*4*) is generally accepted in the scientific community. The application of PCR has been admitted in the U.S. courts as evidence in criminal cases for the analysis of human DNA sequences, and in January 1997 as evidence for the phylogenetic analysis of HIV DNA sequences (26). Clearly, the scope of applications for PCR seems endless and it is truly a remarkable technique that has been widely used in molecular biology.

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See also ANALYTICAL METHODS, AUTOMATED; DNA SEQUENCE; MICROARRAYS.

POLYMERIC MATERIALS

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INTRODUCTION

This article aims to provide basic and contemporary information on polymeric materials used in medical devices and instrumentation. The fundamental concepts and features of polymeric materials are introduced in the first section. In the second section, the major commodity polymers used in medicine are reviewed in terms of their basic chemical and physical properties. The main part of this article, however, is devoted to polymers in biomedical engineering applications, including tissue engineering and drug delivery systems.

Polymers are a very important class of materials. A polymer can be defined as a long-chain molecule that is composed of a large number of repeating units of identical structure. Some polymers, (e.g., proteins, cellulose, and starch) are found in Nature, while many others, including polyethylene, polystyrene, and polycarbonate, are produced only by synthetic routes. Hundreds of thousands of polymers have been synthesized since the birth of polymer science. Today, polymeric materials are used in nearly all areas of daily life.

Polymers can simply be divided into two distinct groups based on their thermal processing behavior: thermoplastics and thermosets. Thermoplastics are linear or branched polymers, and they soften or melt when heated, so that they can be molded and remolded by heating. This property allows for easy processing and recycling. In comparison, thermosets are three-dimensional (3D) network polymers, and cannot be remelted. Once these polymers are formed, reheating will cause the material to scorch.

In addition to classification based on processing characteristics, polymers may also be grouped based on the chemical structure of their backbone. Polymers with one identical repeating unit in their chains are called homopolymers. The term copolymer is often used to describe a polymer with two or more repeating units. The sequence of repeating units along the polymer chain can form different structures, and copolymers can be further classified as random copolymers, alternating copolymers, block copolymers, and graft copolymers. In random copolymers, the sequence distribution of the repeating units is random, while in alternating copolymers the repeating unit are arranged alternately along the polymer chain. A block copolymer is one in which identical repeating units are clustered in blocks along the chain. In graft copolymers, the blocks of one type of repeating unit are attached as side chains to the backbone chains.

Unlike simple pure compounds, most polymers are not composed of identical molecules. A typical synthetic polymer sample contains chains with a wide distribution of chain lengths. Therefore, polymer molecular weights are usually given as averages. The number average molecular weight (M_n), which is calculated from the mole fraction distribution of different sized molecules in a sample, and the weight average molecular weight (M_w), which is calculated from the weight fraction distribution of different sized molecules, are two commonly used values. The statistical nature of polymerization reaction makes it impossible to characterize a polymer by a single molecular weight. A measure of the breadth of the molecular weight distribution is given by the ratios of molecular weight averages. The most commonly used ratio is M_w/M_n. As the weight dispersion of molecules in a sample narrows, M_w approaches M_n, and in the unlikely case that all the polymer molecules have identical weights, the ratio M_w/M_n becomes unity. Most commercial polymers have the molecular weight distribution of 1.5–10. In general, increasing molecular weight corresponds to increasing physical properties and decreasing polymer processability.

In many cases, individual polymer chains are randomly coiled and interviewed with no molecular order or structure. Such a physical state is termed amorphous. Amorphous polymers exhibit two distinctly different types of mechanical behavior. Some, like poly(methyl methacrylate, PMMA) and polystyrene are hard, rigid, glassy plastics at room temperature, while others, like polybutadiene and poly(ethyl acrylate), are soft, flexible, rubbery materials at room temperature. There is a temperature, or range of temperatures, below which an amorphous polymer is in a glassy state, and above which it is rubbery. This temperature is called the glass transition temperature (T_g). The value of T_g for a specific polymer will depend on the structure of the polymer. Side groups attached to the polymer chain will generally hinder rotation in the polymer backbone, necessitating higher temperatures to give enough energy to enable rotation to occur.

For most polymers, the T_g constitutes their most important mechanical properties. At low temperatures ($< T_g$), an amorphous polymer is glass-like, with a value of Young's modulus in the range of 10^9–10^{10} Pa, and it will break or yield at strains greater than a few percent. When the temperature is $> T_g$, the polymer becomes rubber-like, with a modulus in the range of 10^5–10^6 Pa, and it may withstand large extensions with no permanent deformation. At even