# Polymerase Chain Reaction

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The advent of the polymerase chain reaction (PCR) radically transformed biological science from the time it was discovered (Mullis, 1990). For the first time, PCR allowed for specific detection and production of large amounts of DNA. PCR-based strategies have propelled vast scientific endeavors such as the Human Genome Project. The technique is widely used by clinicians and researchers to diagnose diseases, clone and sequence genes, and carry out sophisticated quantitative and genomic studies in a rapid and very sensitive manner. One of the most important medical applications of the classic PCR method is detection of pathogens. In addition, the PCR assay is used in forensic medicine to identify criminals. Because of its widespread use, it is important to understand the basic principles of PCR and how its use can be modified to provide for sophisticated analysis of genes and the genome.

## WHAT PCR DOES

- PCR is a very sensitive technique that allows rapid amplification of a specific segment of DNA.
- PCR makes billions of copies of a specific DNA fragment or gene, which allows detection and identification of gene sequences using visual techniques based on size and charge.
- Modified versions of PCR have allowed quantitative measurements of gene expression with techniques called real-time PCR.

## LIMITATIONS

- The DNA polymerase used in the PCR reaction is prone to errors and can lead to mutations in the fragment generated.
- The specificity of the generated PCR product may be altered by nonspecific binding of the primers to other similar sequences on the template DNA.
- To design primers to generate a PCR product, some prior sequence information is usually necessary.

## THE PCR PROCESS

PCR is a simple, yet elegant, enzymatic assay that enables amplification of a specific DNA fragment from a complex pool of DNA. Kary Mullis, who conceptualized the PCR assay, explained that it "lets you pick the piece of DNA you're interested in and have as much of it as you want" (Mullis, 1990). PCR can be performed using source DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes. Only trace amounts of DNA are needed for PCR to generate enough copies to be analyzed using conventional laboratory methods. For this reason, PCR is a sensitive assay.

Each PCR assay requires the presence of template DNA, primers, nucleotides, and DNA polymerase. The DNA polymerase is the key enzyme that links individual nucleotides together to form the PCR product. The nucleotides include the four bases—adenine, thymine, cytosine, and guanine (A, T, C, G)—that are found in DNA. These act as the building blocks that are used by the DNA polymerase to create the PCR product. The primers in the reaction specify the exact DNA product to be amplified. The primers are short DNA fragments with a defined sequence complementary to the target DNA that is to be detected and amplified. These serve as an extension point for the DNA polymerase to build on.

The above-mentioned components are mixed in a test tube or 96-well plate and then placed in a machine that allows repeated cycles of DNA amplification to occur in three basic steps. The machine—essentially a thermal cycler—has a thermal block with holes into which the test tubes or plates holding the PCR reaction mixture are inserted. The machine raises and lowers the temperature of the block in discrete, precise, and preprogrammed steps (Weier and Gray, 1988). The reaction solution is first heated above the melting point of the two complementary DNA strands of the target DNA, which allows the strands to separate, a process called denaturation. The temperature is then lowered to allow the specific primers to bind to the target DNA segments, a process known as hybridization or annealing. Annealing between primers and the target DNA occurs only if they are complementary in sequence (e.g., A binding to G). The temperature is raised again, at which time the DNA polymerase is able to extend the primers by adding nucleotides to the developing DNA strand (Figure 1). With each repetition of these three steps, the number of copied DNA molecules doubles.

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## ANALYSIS OF THE PCR PRODUCT

There are two main methods of visualizing the PCR products: (i) staining of the amplified DNA product with a chemical dye such as ethidium bromide, which intercalates between the two strands of the duplex, and (ii) labeling the PCR primers or nucleotides with fluorescent dyes (fluorophores) prior to PCR amplification. The latter method allows the labels to be directly incorporated in the PCR product. The most widely used method for analyzing the PCR product is agarose gel electrophoresis, which separates DNA products on the basis of size and charge. Agarose gel electrophoresis is the easiest method for visualizing and analyzing the PCR product. It enables determination of the presence and size of the PCR product (Figure 2). A predetermined set of DNA products with known sizes is run simultaneously on the gel as standardized molecular markers to help determine the size of the product.

Typically, when PCR is used to detect the presence or absence of a specific DNA product, it is called qualitative PCR. Qualitative PCR is a good technique to use when PCR is performed for cloning purposes or to identify a pathogen. For example, in a study by Dworkin *et al.* (2009), qualitative PCR was used to detect the presence of Merkel cell polyomavirus in cutaneous squamous cell carcinoma (SCC) in immunocompetent individuals. Using genomic DNA isolated from SCCs excised from immunocompetent individuals and primers specific to virus genes, the investigators were able to demonstrate the presence of a 351-base-pair (bp) viral gene in 6 of 16 samples tested by the presence of a PCR product band about 351 bp long, as seen on a 2% agarose gel with ethidium bromide (Figure 3). The experiment also included template DNA from a polyomavirus containing plasmid as a positive control (P) and a negative water control (W). The first lane (M) is the molecular marker, which is used to identify the size of the detected PCR product. The presence of a viral-specific gene detected by PCR is indicated by a plus sign; the absence of a viral gene is indicated by a minus sign.

## QUANTITATIVE PCR

Quantitative real-time PCR (qPCR) provides information beyond the mere detection of DNA. It indicates how much of a specific DNA or gene is present in the sample. qPCR allows for both detection and quantification of the PCR product in real time, while it is being synthesized (VanGuilder *et al.*, 2008). The two methods commonly used to detect and quantify the product are (i) fluorescent dyes that nonspecifically intercalate with double-stranded DNA and (ii) sequence-specific DNA

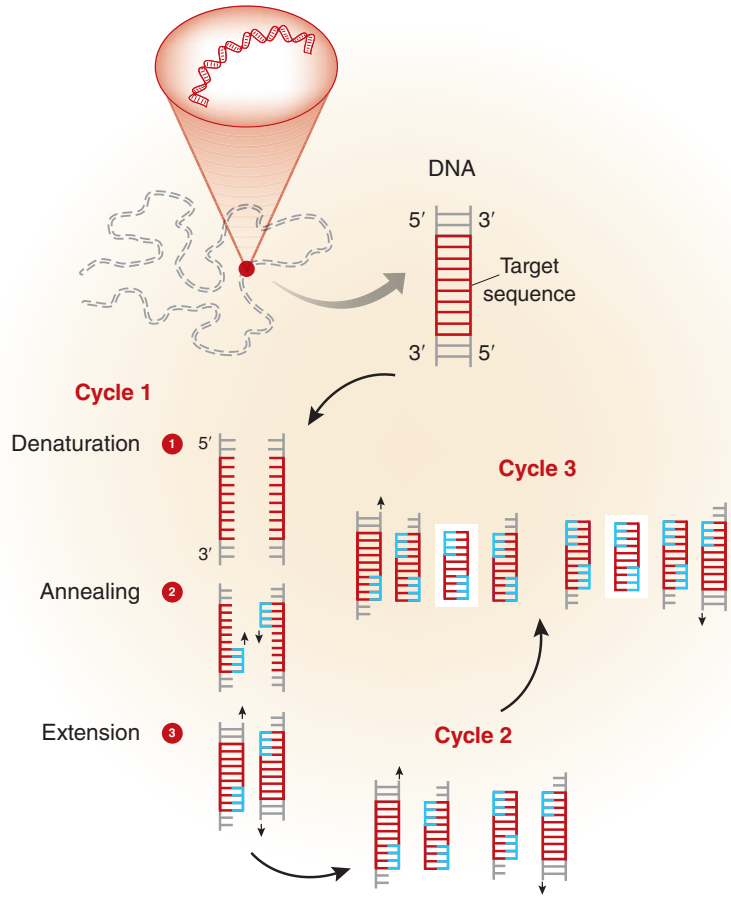


**Figure 1. Schematic presentation of the polymerase chain reaction principle.** Modified from "PCR: Uses, Steps, Purpose," SchoolWorkHelper, St. Rosemary Educational Institution (http://schoolworkhelper.net/pcr-uses-steps-purpose).

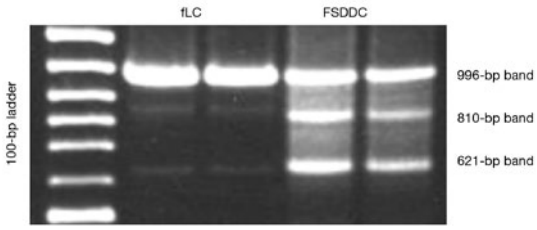


**Figure 2. Visualization of the PCR products on an agarose gel.** Identification of an alternatively spliced mouse Langerin transcript. Ethidium bromide–stained agarose gel showing PCR products from full-length mouse Langerin obtained from C57BL/6 fresh Langerhans cells (fLC) and from fetal skin–derived dendritic cells (FSDDC). From Riedl *et al*. (2004).

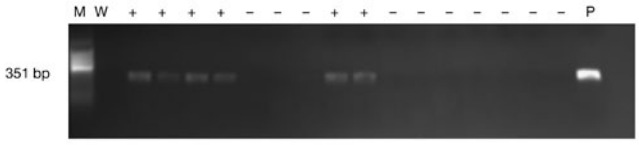


**Figure 3. Qualitative PCR detects the presence of Merkel cell polyomavirus in cutaneous squamous cell carcinoma in immunocompetent individuals.** MCPyV detection. The presence of MCPyV in squamous cell carcinomas, genomic normal DNA, and adjacent skin DNA was determined via PCR using VP1 primers. A representational result is shown, with 6 of 16 samples tested showing a PCR product at 351 bp. All experiments included DNA from an MCPyV plasmid as a positive control (P) and a negative water control (W). M, molecular weight marker; +, positive for virus; −, negative for virus. From Dworkin *et al*. (2009).

probes consisting of fluorescently labeled reports. These methods permit detection only after hybridization of the probe with its complementary DNA target. Real-time PCR can be combined with reverse transcription, which allows messenger RNA to be converted into cDNA (i.e., reverse transcription), after which quantification of the cDNA is performed with qPCR (Valasek and Repa, 2005). Quantification of the desired gene during the exponential amplification avoids problems that are associated with end-point PCR, which is analysis after completion of the final PCR cycle.

Analysis of tumors is an ideal example of the use of PCR. It can be employed to isolate and amplify DNA of tumor suppressor genes or proto-oncogenes. In turn, quantitative PCR can be used to quantify the amount of the particular gene isolated. On the other hand, quantitative PCR can be used to analyze single cells and quantify any combination of DNA, mRNAs, and proteins (Stahlberg *et al*., 2012).

## ADVANTAGES AND LIMITATIONS OF PCR

PCR has several advantages. It is a simple technique to understand and use, and it produces results rapidly (Vogel *et al*., 2012). It is highly sensitive, with the potential to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis. This is true of qPCR as well, but qPCR has the advantage of quantification of the synthesized product. Thus, it can be used to analyze alterations of gene expression levels in tumors, microbes, or other disease states.

However, PCR also has limitations. Because it is highly sensitive, any form of contamination of the sample by even trace amounts of DNA can produce misleading results (Vogel *et al*., 2012; Smith and Osborn, 2009). In addition, to design primers for PCR, some prior sequence data are needed. Therefore, PCR can be used only to identify the presence or absence of a known pathogen or gene. Another limitation is that the primers used for PCR can anneal nonspecifically to sequences that are similar, but not completely identical, to target DNA. Moreover, incorrect nucleotides can be incorporated into the PCR sequence by the DNA polymerase, albeit at a very low rate.

## QUESTIONS

**1. Qualitative PCR and quantitative PCR provide information on _____ and _____, respectively.**

A. Presence/absence of specific DNA product; how much of a specific DNA product is present.

B. How much of a specific DNA product is present; presence/absence of DNA product.

C. RNA; DNA.

D. Gene by-products; RNA.

**2. The most widely used method of analysis of the PCR product is:**

A. Agarose gel electrophoresis.

B. Western blot.

C. ELISA.

D. FISH.

**3. A major advantage of using PCR as compared with other molecular biology techniques is:**

A. Low risk of contamination.

B. Rapidity.

C. Low sensitivity.

D. Low specificity.

**4. The PCR process contains these three steps:**

A. Denaturation, extension, annealing.

B. Annealing, denaturation, extension.

C. Denaturation, annealing, extension.

D. Extension, annealing, denaturation.

Answers to the questions and an opportunity to comment on the article are available on our blog: http://blogs.nature.com/jid_jottings/.

## SUMMARY AND FUTURE DIRECTION

PCR is a simple and widely used process in which minute amounts of DNA can be amplified into multiple copies. In addition to working rapidly, it is able to quantitatively demonstrate how much of a particular sequence is present. As with all methods, the validity of the results should be compared with the specificity associated with the method. The future of PCR is promising, combining various assays and approaches to produce greater insight into various gene combinations. For example, in a study to link distinct taxa within the microbial community to specific metabolic processes, stable isotope probing was combined with qPCR (Postollec *et al.*, 2011; Smith and Osborn, 2009). Microarray experiments can be validated by qPCR approaches because of the method's rapidity.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors state no conflict of interest.

## SUPPLEMENTARY MATERIAL

Answers and a PowerPoint slide presentation appropriate for journal club or other teaching exercises are available at http://dx.doi.org/10.1038/jid.2013.1.

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