

# Chapter 25

## Polymerase Chain Reaction (PCR)



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**Abstract** Polymerase chain reaction (PCR) is one of the most important techniques for prenatal diagnosis. It can amplify a specific DNA sequence from tiny fetal samples with high efficiency within a few hours. PCR is a cyclic DNA synthesis reaction by DNA polymerase in an automated system that amplifies the target sequence to over 100 million copies in a test tube. This technology is useful not only for invasive prenatal genetic diagnostic testing but also for noninvasive prenatal genetic testing.

**Keywords** Polymerase chain reaction · PCR · Taq polymerase · DNA · Direct sequencing · Sanger sequencing · Electrophoresis

### 25.1 The Technical Advantages of Polymerase Chain Reaction in Prenatal Diagnosis

One of the most important molecular technologies for prenatal diagnosis of single gene disorders is polymerase chain reaction (PCR). PCR amplifies a specific DNA sequence against a background of the entire genome. The target DNA sequence of interest is only a small part of the whole genome. When we try to read the sequence of one exon of a single target gene, we need to amplify the genomic region flanking the exon. Assuming that the average size of exons in the human genome is ~300 base pairs, the ratio to the size of whole genome (~3000 Mb) is 1:10,000,000. Moreover, in prenatal diagnosis, we can obtain only a small amount of sample from chorionic villi or amniotic fluid. PCR is able to amplify DNA fragments from such tiny amounts of tissue sample.

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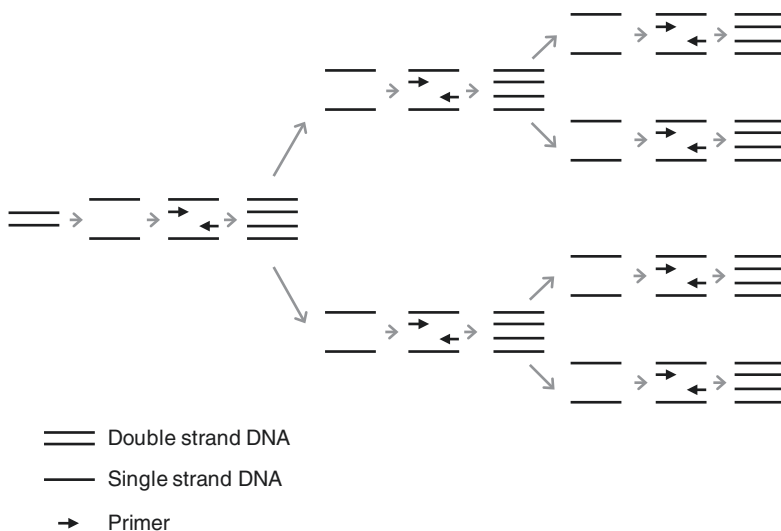
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Another indispensable condition of the technique to be useful for prenatal diagnosis is speed to obtain a short turnaround time. PCR is able to complete the amplification of target DNA within a few hours in a test tube. Although clients with affected fetuses may choose the termination of the pregnancy based on the result of prenatal diagnosis, elective abortion is allowed only when the gestational age is less than 22 weeks in Japan. Most countries also have a set limit on gestational weeks for elective abortion. From the point of view, this is important advantage of PCR.

## 25.2 The PCR Procedure (Fig. 25.1)

First, for prenatal diagnosis using fetal samples from amniocentesis or chorionic villous sampling, DNA must be extracted. The extracted genomic DNA is used as a template for PCR. DNA can be extracted from amniotic fetal cells after cell culture for 2 weeks, whereas DNA can be extracted from chorionic villi just after sampling. Next, we designed and synthesized a pair of unique primers to the sequences upstream and downstream of a region of interest. The primers were oligonucleotides of 20–25 base pairs that are homologous to sequences that flank the DNA segment to be amplified. The length between the primer binding sites is limited by the type of DNA polymerase (e.g., Taq polymerase) and is usually to around 1000 bases or fewer. The primers are designed to bind to opposite strands of the target DNA, which is denatured into single strands by heating to  $\sim 95^\circ\text{C}$ . The primers are allowed to anneal to the opposite genomic strands by cooling to the annealing temperature specific for the primer pairs being used (around  $60^\circ\text{C}$ ). Following



**Fig. 25.1** Diagram of polymerase chain reaction

annealing, DNA polymerase (Taq polymerase) directs DNA synthesis during incubation at  $\sim 72^\circ\text{C}$  using four kinds of nucleotides. This produces a pair of hybrid molecules, which are once again separated into single strands by heating. Again, the primers bind and DNA synthesis reactions are allowed to begin. DNA polymerases are derived from bacteria that thrive at high temperatures, allowing the same polymerase to be used in spite of multiple cycles of heating and cooling of the reaction mixture. The process is repeated multiple times, usually 30 or more, using an automated system, which leads to an exponential increase in the target DNA sequence. This results in over 100 million copies of the target sequence in a matter of 2 or 3 h in a test tube.

## 25.3 Clinical Use of PCR in Prenatal Diagnosis

### 25.3.1 PCR in Invasive Prenatal Genetic Testing

Prenatal diagnosis of genetic disorders by DNA analysis can be performed either by direct detection of the mutation or by means of closely linked markers. For direct detection of mutations, there are a variety of methods including Southern blotting, PCR, DNA sequencing, and others. Detection of relatively small sized mutation begins with the amplification of the DNA region of interest.

Separation and accurate size estimation of PCR products is the final step for the prenatal diagnosis of single-gene disorder caused by mutation that alters the length of the target sequence (e.g., a triplet repeat). Amplified DNA molecules migrate toward the positive electrode at a different rate depending on its length in a non-denaturing gel. After electrophoresis, DNA is usually visualized by staining the gel with a fluorescent dye, such as ethidium bromide, which binds to DNA.

Detection of each nucleotide change, deletion, or insertion is performed with the use of the Sanger sequencing method. In principle, after denaturing the double stranded amplified target DNA, DNA polymerase is used to synthesize a complementary strand. During the reaction, different kinds of fluorescently labeled 2',3'-dideoxynucleotides of adenine, cytosine, guanine, and thymidine (fluorescent dye terminator) are added. When one of the dideoxynucleotides is incorporated, the 3'-end of the reaction is no longer a substrate for chain elongation and the growing DNA chain is terminated. Thus, in the reaction, there are DNA molecules that are fluorescent with different colors according to the type of nucleotide with a common 5'-end, but of varying length because of the incorporation of a specific 3'-end. Next, the reaction product is subjected to electrophoresis in an automated sequencer.

An indirect linkage method by means of closely linked markers also needs PCR. After amplification of the DNA sequence flanking a polymorphic marker, the mutated allele is detected using a restriction fragment length polymorphism, variable number tandem repeat, or other diagnostic feature.

### 25.3.2 *PCR in Noninvasive Prenatal Genetic Testing*

PCR plays important roles in noninvasive prenatal genetic testing (NIPT) using cell-free fetal DNA (cfDNA). NIPT for the detection of a fetal chromosomal disease was first applied clinically by massive parallel sequencing using a next-generation sequencer in October 2011 [1]. However, in the early days of NIPT research, the analysis of single-gene disorders by PCR preceded. Lo et al. started with the use of the most obvious difference between maternally and paternally derived genetic material, the Y chromosome [2]. With the use of PCR technology, amplification of a single-gene copy sequence of *DYS14* from the Y chromosome was performed. The detection of this means that the fetus is male. After this research for fetal sex determination, PCR analysis of cfDNA in maternal plasma started to be used for RhD genotyping in RhD-negative pregnant mothers [3] and diagnosis of single-gene disorders in fetuses [4].

### References

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