of the DNA strands, annealing of the primers and synthesizing new DNA strands. PCR reaction allows the replication of a discrete segment of DNA to be manipulated in a tube under controlled conditions. After 30 or 60 cycles of replication, the reaction product is electrophoresed on a polyacrylamide gel, stained with ethidium bromide, and inspected under ultraviolet light. The elapsed time from the initiation of the PCR reaction to direct visualization of the amplified DNA is usually 4–6 hours (Fig. 1).[3]

For overall purposes of gene analysis, PCR offers the advantage of increased signal intensity for any subsequent detection system, including some of the newer techniques to scan the amplified product for single base substitutions (denaturing gradient gels, heteroduplex analysis, *etc.*). Another major advantage of PCR is that sufficient quantities of the segment of gene of interest are usually available for direct nucleotide sequencing. An RNA sequence can be similarly amplified, but a DNA copy of it (cDNA) must be initially synthesized by using reverse transcriptase before the PCR is begun.[4]

Multiplex genomic analysis: Innovative PCR has made possible many new and different approaches to problems in molecular genetics, evolutionary biology, forensic medicine, and development. For example, it is possible with PCR to amplify and screen multiple segments of a gene for deleted regions. This rapid and simple technique known as multiplex genomic DNA amplification permits the detection of deletions (X-linked locus) over megabase regions in a hemizygous gene. This technique does requires sequence data over the gene or region of interest in order to synthesize oligonucleotide primers for PCR. Multiple primers homologous to sequences flanking a number of exons over a large region of interest are used to amplify five or six different segments of the region with PCR. Lack of signal for any of these segments flanked by the primers would indicate a deletion. This technique can be performed in less than 5 hours and is amenable to automation. It is especially practical for X-localized genes.[5]

Reverse transcription-PCR (RT-PCR): It is important to point out that PCR can be carried out with either an RNA (total RNA or mRNA) or a DNA template. DNA can be amplified from RNA by synthesizing the first strand of DNA by reverse transcription with the enzyme reverse transcriptase and either a downstream primer, oligo (dT), or random hexamers. In this way, PCR in conjunction with reverse transcriptase provides a convenient and highly sensitive method for examining and even quantitating gene expression. One approach to quantitate RNA is the use of a known number of competitive internal standard molecules that are reverse-transcribed and subsequently coamplified under the same reaction conditions as the target sequence. This is important in reproductive endocrinology because many occasions arise in which accurate quantitative information concerning the level of gene expression is desirable. Reverse transcription PCR is often the only way to analyze RNA when it is produced in exceedingly low numbers. Various modifications of the RT-PCR method to accomplish this goal have been introduced. Starting with the mRNA they all have in common the first step with reverse transcriptase to convert the mRNA into a DNA:RNA double-strand hybrid and then a step to convert that hybrid into double-stranded cDNA. A standard PCR reaction is then performed on the synthesized cDNA. Quantitation of mRNA may have important implications for treatment strategies and clinical outcome when the target sequence is a viral or bacterial organism that is present in a patient's serum.

Primer extension preamplification: One of the important byproducts of PCR technology that has important implications for preimplantation diagnosis is Primer-Extension Preamplification or PEP. PEP is an *in vitro* procedure developed to duplicate a large fraction or entire genome from limited amounts of DNA, such as that derived from a single haploid cell. PEP involves repeated primer extensions using a mixture of 15-base random oligonucleotides (Fig. 2). The diversity of oligonucleotide sequences helps to ensure amplification of segments throughout the genome. It is estimated that at least 78% of the genome sequence in a single haploid cell can be copied no less than 30 cycles. Through PEP it is possible to perform multiple genotyping experiments on DNA from a single sperm, oocyte, or blastomere cell. PEP is a valuable adjunct to single-cell diagnosis.[6]

Analysis of extracted and amplified DNA

Southern Blot: For analysis, DNA can be extracted from any cell that contains a nucleus, and the target sequence of interest can be amplified by PCR. Platelets contain mitochondrial DNA but do not have a nuclear genome for study. Apart from this exception, DNA can be extracted from peripheral blood leukocytes, tissue, amniocytes, sperm, oocytes, and even mummified ancient tissue samples. The most common source is a peripheral blood sample collected in a heparinized or EDTA tube. Following extraction, DNA (with or without prior PCR) is restricted or cut into pieces by specific enzymes (restriction enzymes). These restriction endonucleases which cut double stranded DNA at specific sequences are the most important tools for handling DNA in a reproducible manner. Each of the restriction