nucleic acid segments in genomic DNA by the technique of polymerase chain reaction (PCR) (Fig. 1). This amplification technique has been incorporated into virtually all mutation detection strategies. The PCR is an *in vitro* method for producing large amounts of a specific DNA fragment of defined length and sequence from small amounts of a complex template (Fig. 1). This procedure enables one to generate millions of copies of a specific genomic fragment of DNA by exponential amplification without cloning in host cells. In fact, most cloning methods can be complemented or entirely circumvented by using PCR. The range of applications of PCR include the molecular diagnosis of inherited disorders (prenatal and carrier screening), screening for susceptibility to disease, identification of viral and bacterial pathogens, and single cell diagnosis.[1] Almost all of the diagnostic techniques discussed in this syllabus start by first amplifying the DNA target and then subjecting the PCR-amplified product to differential analysis. Alternatively, the amplified product itself can be labeled and used as a probe for the analysis of other DNAs. 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.

Genes relevant to the reproductive sciences such as prolactin, β-follicle-stimulating hormone, *etc.*, are usually present as one copy (single copy DNA). The analysis of single copy genes using standard techniques of Southern blotting and hybridization with radioactive probes require 5–10 μg of DNA. If the target sequences to be detected are repeated many times in the genome (repetitive copy DNA) then smaller amounts of extracted DNA (0.5–3 μg) are adequate. Prior to the development of PCR, the need for large amounts of starting material limited the potential applications of DNA diagnostics. A solution to the sample size problem was developed in 1985, when Saiki, Mullis and colleagues reported this brilliantly conceived method of DNA amplification called PCR.[2] In the PCR procedure, a specific gene region of interest is amplified enzymatically *in vitro* by DNA polymerase. A DNA segment of up to approximately 6000 bp in length can be amplified exponentially by this method. This approach requires sufficient knowledge of the gene or segment of DNA to develop short DNA primers flanking the region of interest. The primers are short synthetic, single stranded, oligonucleotides that are designed to be complementary to the ends of opposite DNA strands and frame the gene or region of interest. Each primer is complementary to one of the original DNA strands, to either the left (5′) or right (3′) side of the region that they have defined for amplification. These short primers will, under appropriate conditions, direct the DNA polymerase to synthesize new complementary DNA strands corresponding to the intervening region (Fig. 1). With this technique, the DNA of even a single oocyte, blastomere or sperm can be denatured and incubated with the two synthetic oligonucleotide primers corresponding to the opposite ends of the gene of interest. The primers are present in such vast molar excess that they are more likely to anneal to the dissociated strands than the strands are to reanneal with each other. The annealing of the primers is followed by the synthesis of new strands of DNA which are extensions of the primer nucleotides. The synthesis of new complimentary strands of DNA is catalyzed by a DNA polymerase that adds nucleotides complementary to those in the dissociated unpaired strand, onto the annealed primer. The enzyme used in this reaction is the DNA polymerase of *Thermus aquaticus* (TAQ polymerase). The use of this remarkable heat-stable enzyme has permitted automation of the PCR reaction, because all reaction components, nucleic acid from specimens of interest, primers, buffers, nucleotides, and enzyme can be combined at the beginning of the reaction. The number of target DNA strands doubles upon completion of each reaction cycle. After 30 cycles, a single copy of DNA can be increased up to 1,000,000 (10^6) copies. Each replication cycle is controlled by simply varying the temperature to permit denaturation

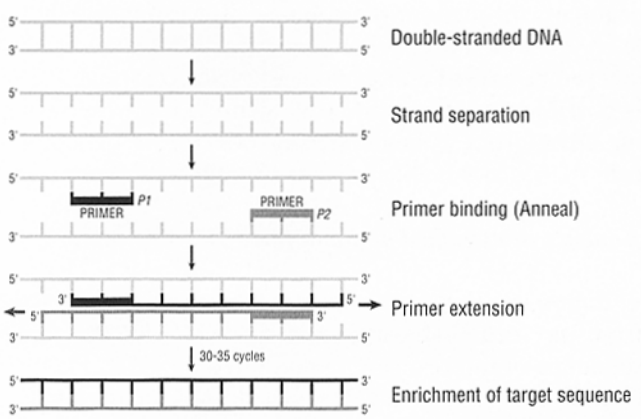

Fig. 1 Cartoon depicting a cycle of polymerase chain reaction (PCR). The double-stranded DNA is first denatured by heating (94 °C) in order to separate the strands. The temperature is then lowered (55 °C) and the primers (P1, P2) are added. The primers are complementary to the 3′ ends of each piece of the double-stranded DNA to be amplified. In the fourth step the temperature is again raised (72 °C) and the primers with the help of the "copying enzyme" (Tag – polymerase) copy their respective templates in a 3′ to 5′ direction. After 30–35 cycles of PCR the gene of interest may be amplified over 1 million-fold, such that it can be easily visualized on an agarose gel as a discrete band without the need for DNA probes.