PCR

polymerase chain reaction (pcr) is a laboratory technique for rapidly producing (amplifying) millions to billions of copies of a specific segment of DNA, which can then be studied in greater detail. PCR involves using short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment.

PCR requirements needed

Four components (reagents or chemicals) are needed for the PCR process:

1. A DNA or RNA sample (from saliva, blood, hair, skin scraping, etc(.
2. DNA primers: short single-stranded DNA that promotes synthesis of a complementary strand of nucleotides
3. DNA polymerase: an enzyme that aids in the synthesis of a complementary strand of DNA
4. Nucleotide solution mix containing adenine (A), thymidine (T), cytosine (C), and guanine (G) used to build duplicate DNA strands

PCR machine steps

Step 1 – Denaturation The solution contained in the tube is heated to at least 94°C (201.2°F) using a thermal cycler. The heat breaks the hydrogen bonds of the original DNA sample and separates the DNA into single strands (this is termed denaturation of double-stranded DNA.(

Step 2 – Annealing The sample mixture is then cooled to between 50 to 60°C (122 to 140°F) allowing the DNA primers and the DNA polymerase enzyme to bind to the individual strands of DNA that were separated by the heat (this is termed annealing of the primers). At this point, the nucleotides (A, T, C, G) from the added mixture solution will pair with the individual separated strands of DNA that resulted from the heating process.

Step 3 – Extension Once joined together, they form a new complementary strand of DNA (termed extension of the DNA). Thus, a new duplicate double-stranded DNA molecule has been formed from each of the single strands of the original sample molecule. The temperature cycles from 95°C to 50 to 60°C. The cycle is then repeated about 35 to 40 times using the thermal cycler which automatically repeats

the heating and cooling cycles of the process. Resulting DNA sequence is doubled each time the heating/cooling cycle is conducted by the cycler. Thus, what started out as a single short segment of DNA from one sample can be amplified to form millions of copies after 35 doubling cycles.

Step 4 - Analysis with Electrophoresis Once the PCR process is completed, the resulting amplified (replicated) segments generated can then be compared to other nucleotide segments from a known source. The PCR-generated nucleotide sequences are then placed next to known nucleotide sequences from humans, pathogens, or other sources in a separating gel. Electrical current is then run through the gel, and the various nucleotide sequences within the gel form bands that resemble a ladder, according to their electrical charge and molecular size. This is termed gel electrophoresis. Bands or ladder-like steps that migrate to the same levels in the gel show identity of nucleotide sequences. This method is one of the most popular ways PCR tests are completed.

Types of PCR

1. Multiplex PCR
2. Long-range PCR
3. Single-cell PCR
4. Fast-cycling PCR
5. Methylation-specific PCR (MSP)
6. Hot start PCR
7. High-fidelity PCR
8. In situ PCR
9. Differential display PCR
10. Real-Time PCR (quantitative PCR or qPCR