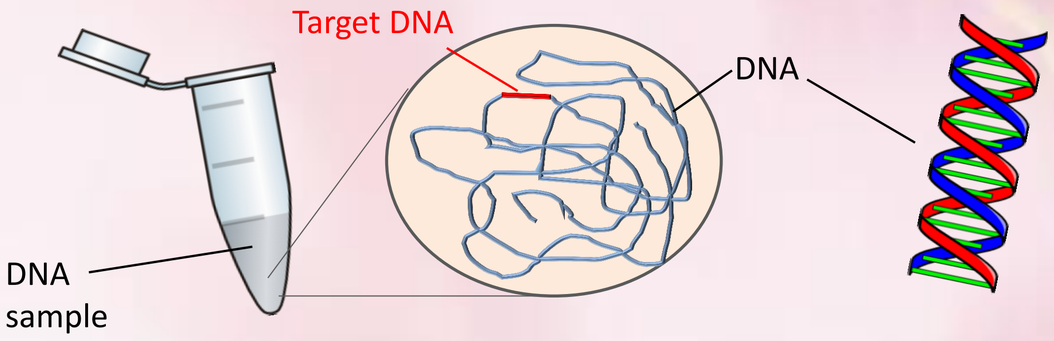
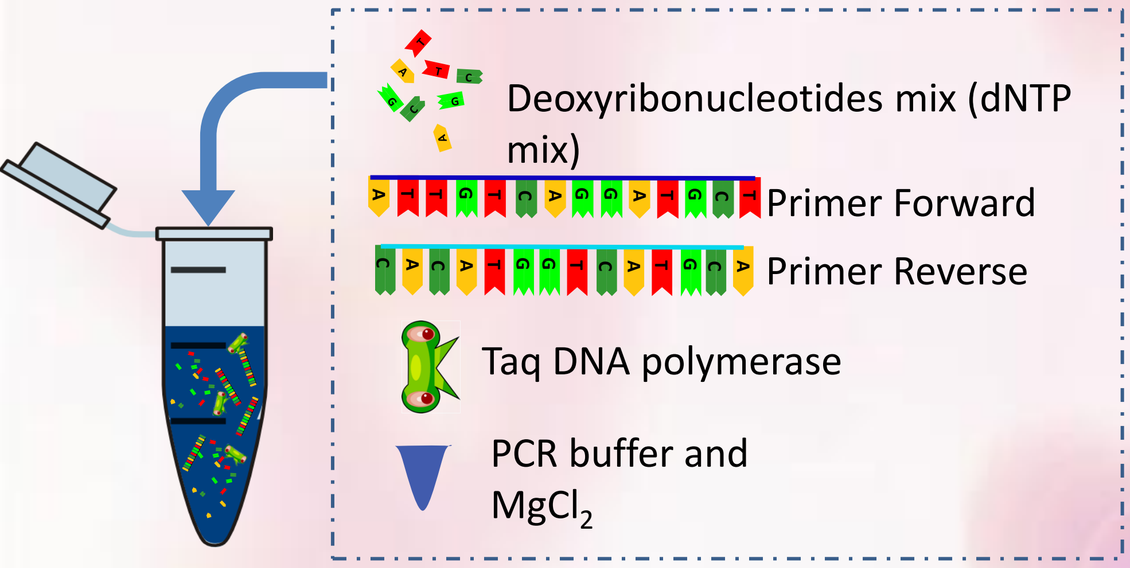
The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a technique that amplifies a small amount of deoxyribonucleic acid (DNA) into a large amount by repeating a simple three step process: denaturation, annealing and synthesis. PCR is frequently employed on this platform to detect bacteria and fungi, and is used in diversity analyses : Metagenomic, Denaturing Gradient Gel Electrophoresis (DGGE), Random Amplified Polymorphic DNA (RAPD).



DNA is composed of two long polynucleotide chains that associate to one another in an antiparallel arrangement by forming hydrogen bonds between complementary base pairs. PCR amplifies a small amount of DNA (Target DNA).

Master Mix PCR

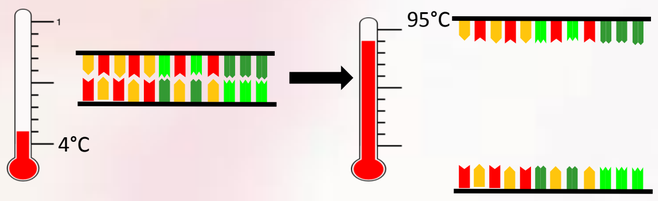


Master Mix PCR . Master mix PCR is composed of:

- Mix of nucleotides (dNTP mix) which are the building blocks of new DNA strands.
- Two primers. A primer is a single strand DNA sequence (17-24 bases) that define the boundaries of the sequence of the DNA template to be amplified.
- An enzyme (Taq DNA polymerase) that helps catalyze the polymerization of the deoxynucleotides into a DNA strand.
- PCR buffer for creates an environment for optimum activity of the Taq DNA polymerase.
- $MgCl_2$ that an essential cofactor for the Taq DNA polymerase.

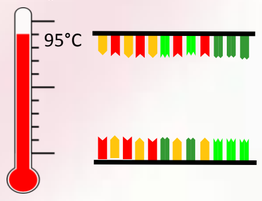
PCR reaction

Step 1 : Initial denaturation; 95°C, 5 minutes



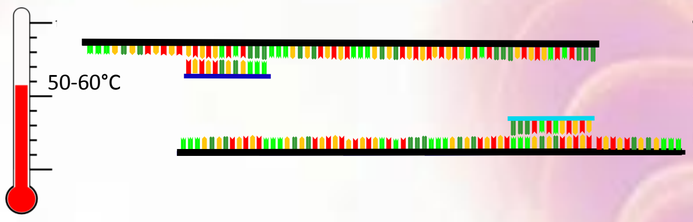
The mix PCR and the DNA template are heated to 95°C to activate Taq DNA polymerase and disrupting the hydrogen bonds between the base pairs resulting two single strands of DNA from the one double stranded DNA template.

Step 2 : Denaturation; 95°C, 30 seconds



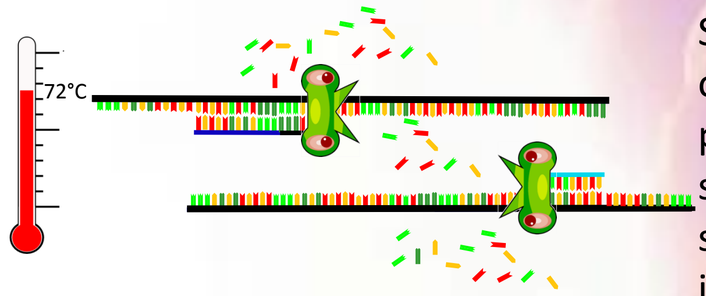
The DNA template are heated to 95°C disrupting the hydrogen bonds between the base pairs resulting two single strands of DNA.

Step 3 : Annealing of the primers; 50-60°C, 30 seconds - 1 minute



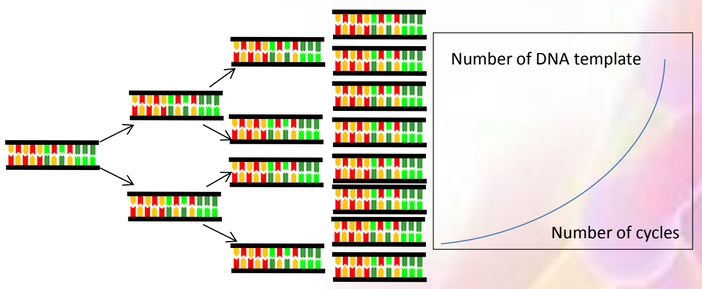
The primer are required to initiate the Taq DNA polymerase and define the boundaries of the sequence of DNA template to be amplified. After denaturation the temperature is reduced to 50-60°C to allow primers to bind with high specificity to their complementary bases on the DNA template. Annealing temperature varies depending on the nucleotide composition of the primer.

Step 4 : Extension; 72°C, 30 seconds - 1 minute



Synthesis of a new DNA. After annealing the primers, the temperature is increased to 72°C the optimal temperature for Taq DNA polymerase activity. The Taq DNA Polymerase beginning at the primer to synthesizes new DNA strand by adding complementary nucleotide bases to the single strand DNA template. The time of the reaction depend of the size of the DNA template and the speed of the Taq DNA polymerase. The result of synthesis is tow double stranded DNA template identical to the initial DNA template.

Step 5 : Repeat step 2, 3 and 4 for 25-30 cycles



The three step process of denaturation, annealing and synthesis is typically repeated for 25-30 cycles. The number of DNA template will grow exponentially (2^n at the end of each cycle). After 30 cycles, there will be 230 (over 1 billion) double stranded DNA molecules identical to the initial template fragment.

Step 6 : Final extension; 72°C, 5 minutes

This step is performed to ensure the remaining single strands are fully extended

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