Components of a PCR Reaction

- **Buffer**

  Tris-HCl is the best buffer for the performance of thermostable DNA polymerase in the PCR reaction. Tris buffers are temperature sensitive, pH decreases by 0.1 for every 5°C increase in temperature, so the pH of the manufacturer supplied PCR buffer is usually in the range of 8.3-8.5.

  K^{+} – (KCl ~ 50 mM), stimulatory for DNA polymerases in PCR reactions. Shields negatively charged phosphate backbone of the DNA molecule, decreasing the electro-repulsive forces between the primers and target DNA molecule.

  Mg^{++} – ($MgCl_2$0.5-5 mM), co-factor for thermostable DNA polymerases.

- **Primers (oligonuclotides)**
  - **18-30 nucleotides** in length for best results
  - **0.1- 0.5 μM** concentration (1-2 pmol) in PCR rxn
  - **10,000,000:1 ratio** of primers to target DNA sequence at the beginning of the reaction, this is necessary because of the exponential increase of target DNA during the PCR reaction.
  - **Tm** = annealing temperature – temperature that results in complete and accurate annealing of the primers to the target DNA. This value can be calculated based on primer length, G/C , A/T content, and the thermodynamics of the DNA molecules helical structure. The Tm value is supplied by the oligo manufacturer and is included in the information provided by them upon delivery of the primers.
  - Primer set's Tm should not vary more than **+/- 5°C** between the 2 primers.
  - **PCR rxn. annealing temperature should be ~5°C below the Tm of the primer set.**
  - **Primer-dimers** can form if the primers contain complementary nucleotide sequence. This will generate ~50-100 bp PCR artifacts that are favored over the production of the desired, larger DNA fragment. The production of primer-dimers also reduces the pool of nucleotides and primers that are needed for later rounds of PCR.

- **dNTPs (deoxynucleotide triphosphates)**
  - 20-200 mM dNTPs
  - 10% are destroyed by heat after 25 PCR cycles, 50% loss after 40 cycles

- **Thermostable DNA dependent DNA polymerase**

  - Taq –Thermus aquaticus- from hot thermal springs (1976)
  - 1st PCR publication: Saiki et. al., (1986) Nature
  - **Hot-Start Pol-** prohibits mis-hybridization between primers and non-target DNA sequence, and subsequent elongation that can occur before PCR cycles begin. There are several mechanisms for prohibiting the production of these nonspecific products: 1) Genetically modified themostable DNA polymerase is folded into a non-active confirmation, but upon heating above 48°C the structure changes to an active conformation. 2) A monoclonal antibody binds the active site of the thermostable DNA polymerase, blocking activity. Heating denatures the antibody, exposing the active site of the polymerase.

- **PCR Targets**

  - **nucleic acids** – RNA and DNA
  - **10fg – 10 μg DNA**
  - **Sources** –eukaryotic single cells and tissue, eubacteria, virus, archaea, protozoa

- **H_2O**

  Deionized, Sterile, RNase, DNase free.