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PCR is a **cycle** of three steps:

1. DENATURATION - the strands of the DNA are melted apart by heating to 95°C

2. ANNEALING - the temperature is reduced to ~ 55°C to allow the primers to anneal to
the target DNA

3. POLYMERISATION / EXTENSION - the temperature is changed to the optimum temperature for the DNA polymerase to catalyse extension of the primers, i.e. to copy the DNA between the primers.

The **cycle** is repeated over and over again - as many times as needed to produce a detectable amount of product.

Discovery of a thermostable DNA polymerase

The breakthrough came with the discovery of the thermostable DNA polymerase Taq polymerase, from the thermophilic bacterium, Thermus aquaticus, which lives in hot springs.

Taq polymerase enzyme can resist high temperatures required to melt the template DNA apart without denaturation (loss of activity) and works best at high temperatures (72°C). This led to improved specificity & sensitivity. Annealing of primers to sites other than the target sequence is significantly reduced at the higher temperatures used for Taq polymerase.

Applications of PCR

1) Cloning a gene encoding a known protein

2) Amplifying 'old DNA'

3) Amplifying cloned DNA from vectors

4) Creating mutations in cloned genes

5) Rapid amplification of cDNA ends - RACE

6) Detecting bacterial or viral infection

* AIDs infection
* Tuberculosis (Mycobacterium tuberculosis)

7) Cancer Detecting mutations that occur in cancer and monitoring cancer

therapy. Determining if a patient is free of malignant cells

8) Genetic diagnosis

a. Diagnosing inherited disorders :-

* Cystic fibrosis
* Muscular dystrophy
* Haemophilia A and B
* Sickle cell anemia