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What is PCR?

- PCR is a technique that takes specific sequence of DNA of small amount and amplifies it to be used for further testing.
- *In vitro* technique

Short History of PCR

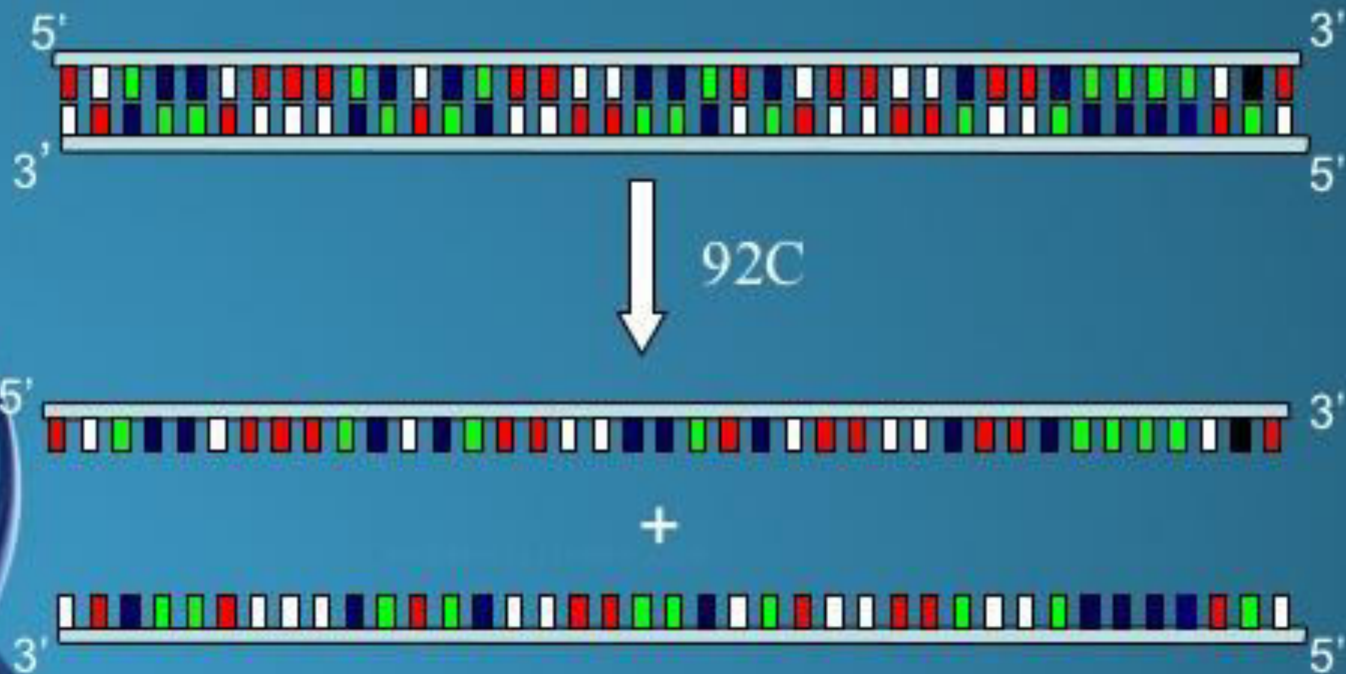
- 1983: Dr. Kary Mullis developed PCR
- 1985: First publication of PCR by Cetus Corporation appears in Science.
- 1986: Purified Taq polymerase is first used in PCR
- 1988: PerkinElmer introduces the automated thermal cycler.
- 1989: Science declares Taq polymerase "molecule of the year.

Purpose

- To amplify a lot of double-stranded DNA molecules (fragments) with same (identical) size and sequence by enzymatic method and cycling condition.

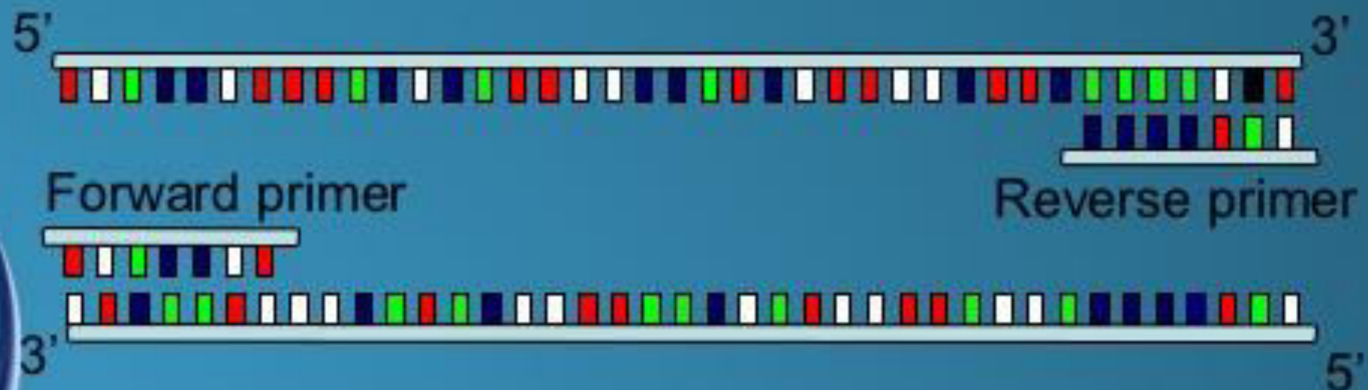
Denaturation

- Temperature: 92-94C
- Double stranded DNA melts ⟶ single stranded DNA



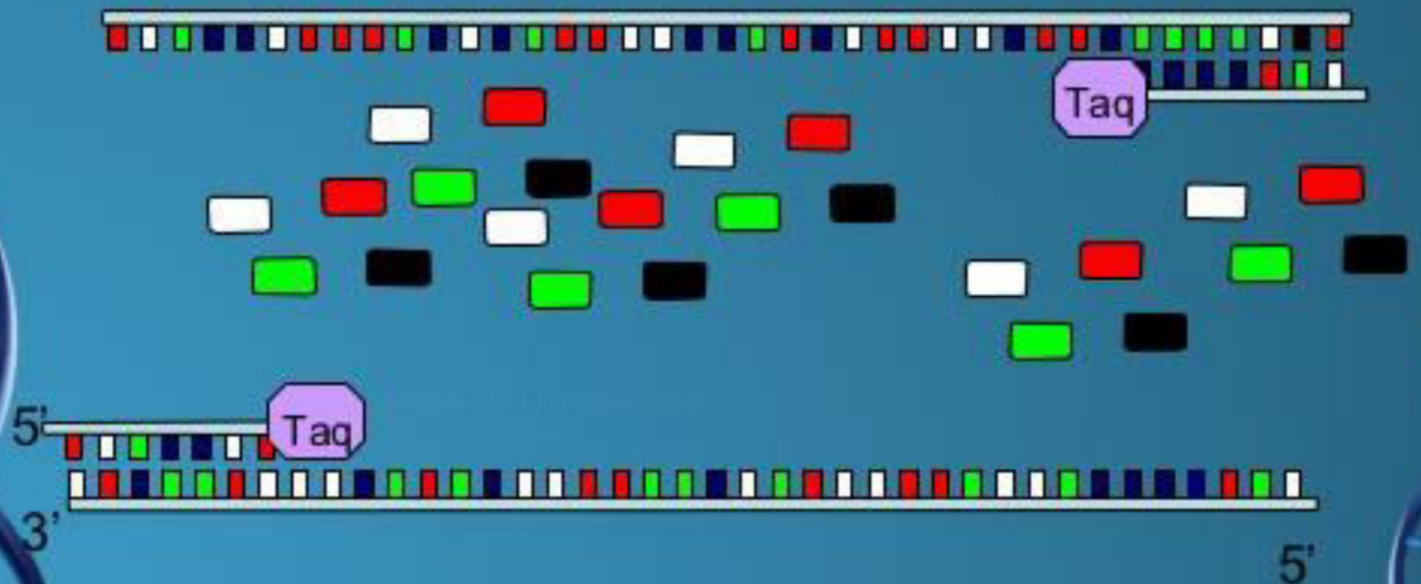
Annealing

- Temperature: ~50-70C (dependant on the melting temperature of the expected duplex)
- Primers bind to their complementary sequences

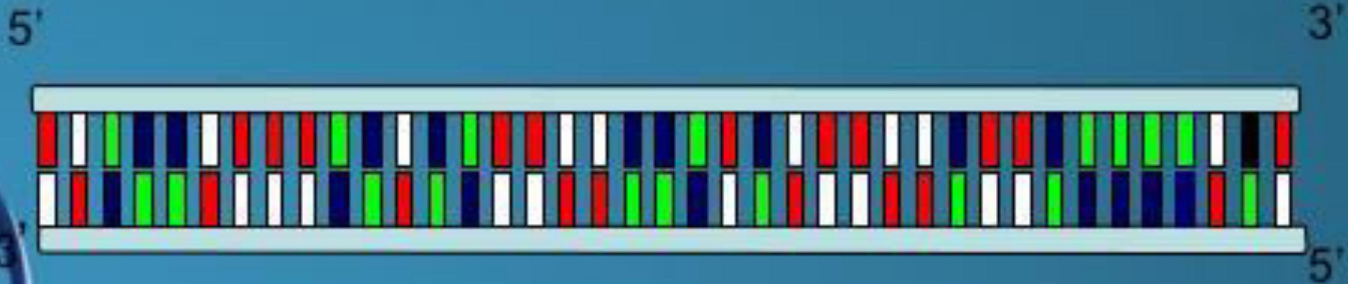


Extension

- Temperature: ~72C
- Time: 0.5-3min
- DNA polymerase binds to the annealed primers and extends DNA at the 3' end of the chain

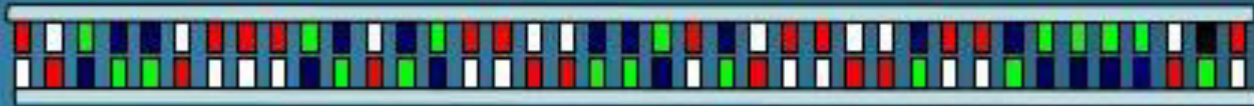


Products of Extension



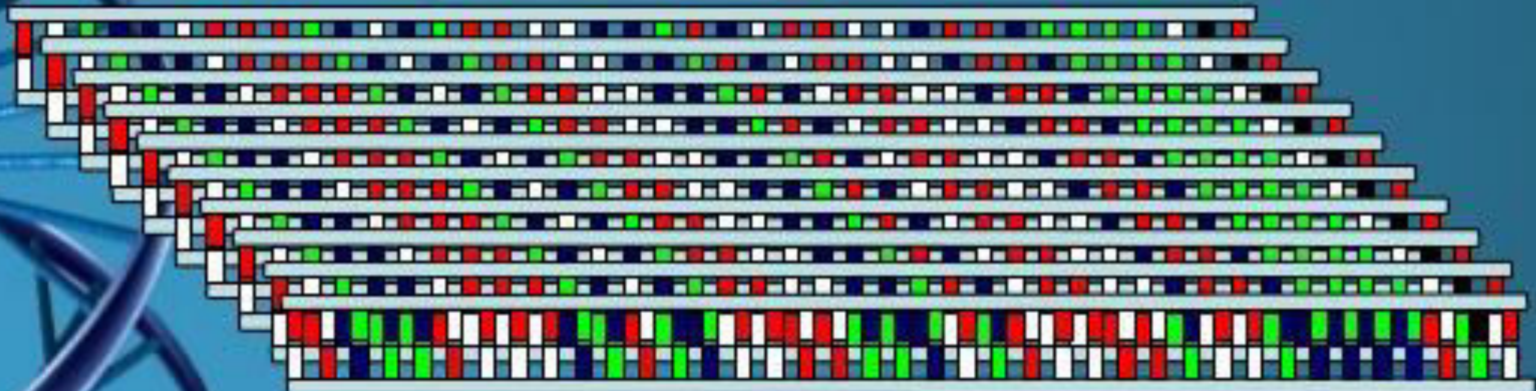
Overall Principle of PCR

- DNA – 1 copy



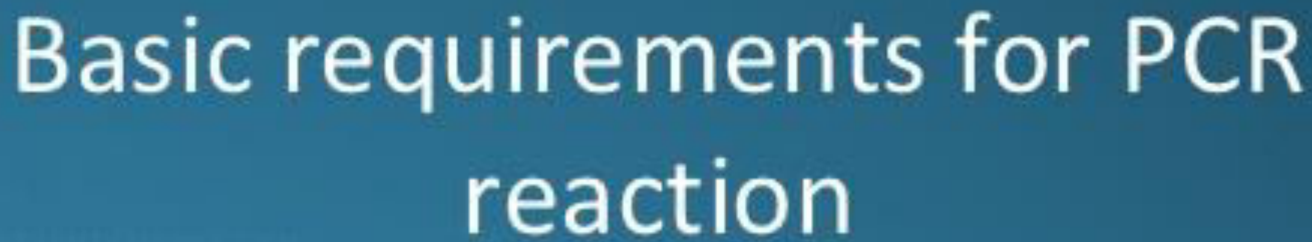
- Known sequence Sequence of interest Known sequence

- PCR



Chemical Components

- Magnesium chloride: .5-2.5mM
- Buffer: pH 8.3-8.8
- dNTPs: 20-200μM
- Primers: 0.1-0.5μM
- DNA Polymerase: 1-2.5 units
- Target DNA: $\leq$ 1 μg



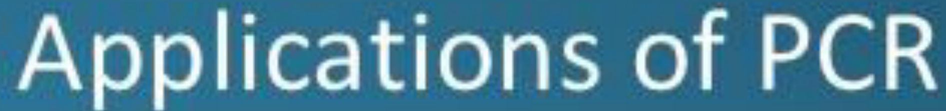
- 1) DNA sequence of target region must be known.

  2) Primers - typically 20-30 bases in size. These can be readily produced by commercial companies. Can also be prepared using a DNA synthesizer

Basic requirements for PCR reaction

- 3) Thermo-stable DNA polymerase - eg *Taq* polymerase which is not inactivated by heating to 95C

  4) DNA thermal cycler - machine which can be programmed to carry out heating and cooling of samples over a number of cycles.



Applications of PCR

Molecular Identification

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- DNA fingerprinting
- Classification of organisms
- Genotyping
- Pre-natal diagnosis
- Mutation screening
- Drug discovery
- Genetic matching
- Detection of pathogens

Sequencing

- Bioinformatics
- Genomic Cloning
- Human Genome Project

Genetic Engineering

- Site-directed mutagenesis
- Gene Expression Studies

Instrumentation