Polymerase Chain Reaction (PCR)

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PCR:

PCR is a technique for amplifying DNA sequences in vitro.

PCR properties:

- Rapid & easy.
- Sensitive.
- Robust.
- Widespread applications.

Basic requirements for PCR reaction:

- ❑ DNA sequence of target region
- ❑ Primers
- ❑ Taq polymerase (Thermo-stable DNA polymerase)
- ❑ dNTPs
- ❑ DNA thermal cycler

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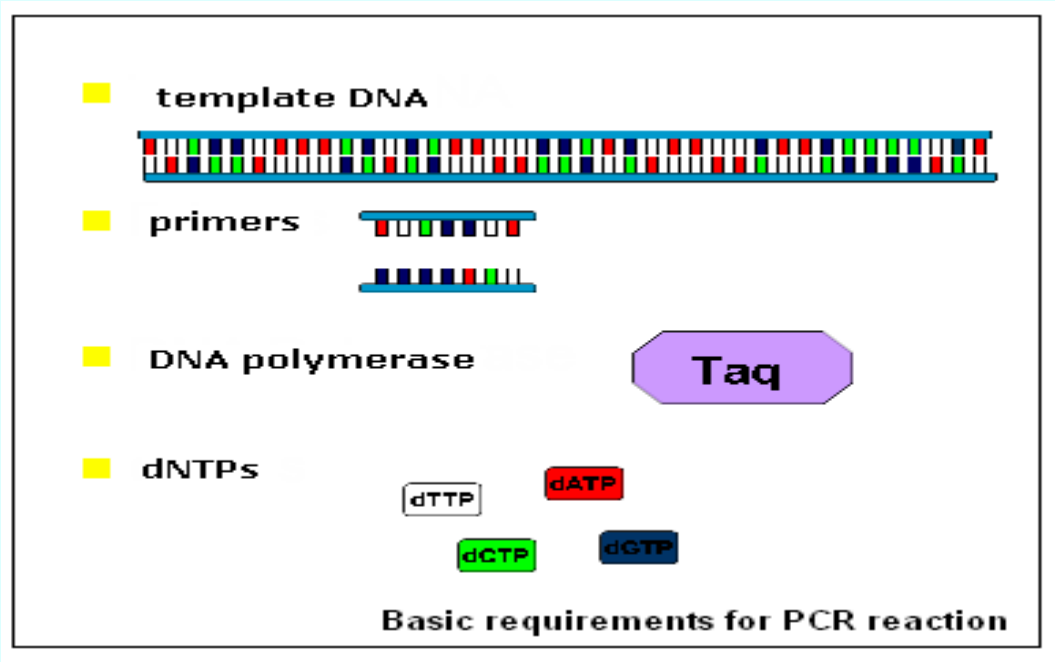


Standard PCR reaction:

- DNA template (target) to be amplified. 1-5uL.
- Two primers (forward and reverse).
- Master mix.

Master mix consist of :

- 1-2ul Taq polymerase. (optimum temperature at around 70C.)
- 1-2.5ul dNTPs.
- 4-7ul Buffer solution
- 1-2ul Divalent cations (Mg2+)
- 1-2ul Monovalent cation (k+1)

PCR cycling steps:

- Denaturation
- Annealing
- Extension

Denaturation:

Double stranded DNA are separated to form single stranded DNA.

Temperature: 93-95C°.

Annealing:

Primers bind to their complementary sequences.

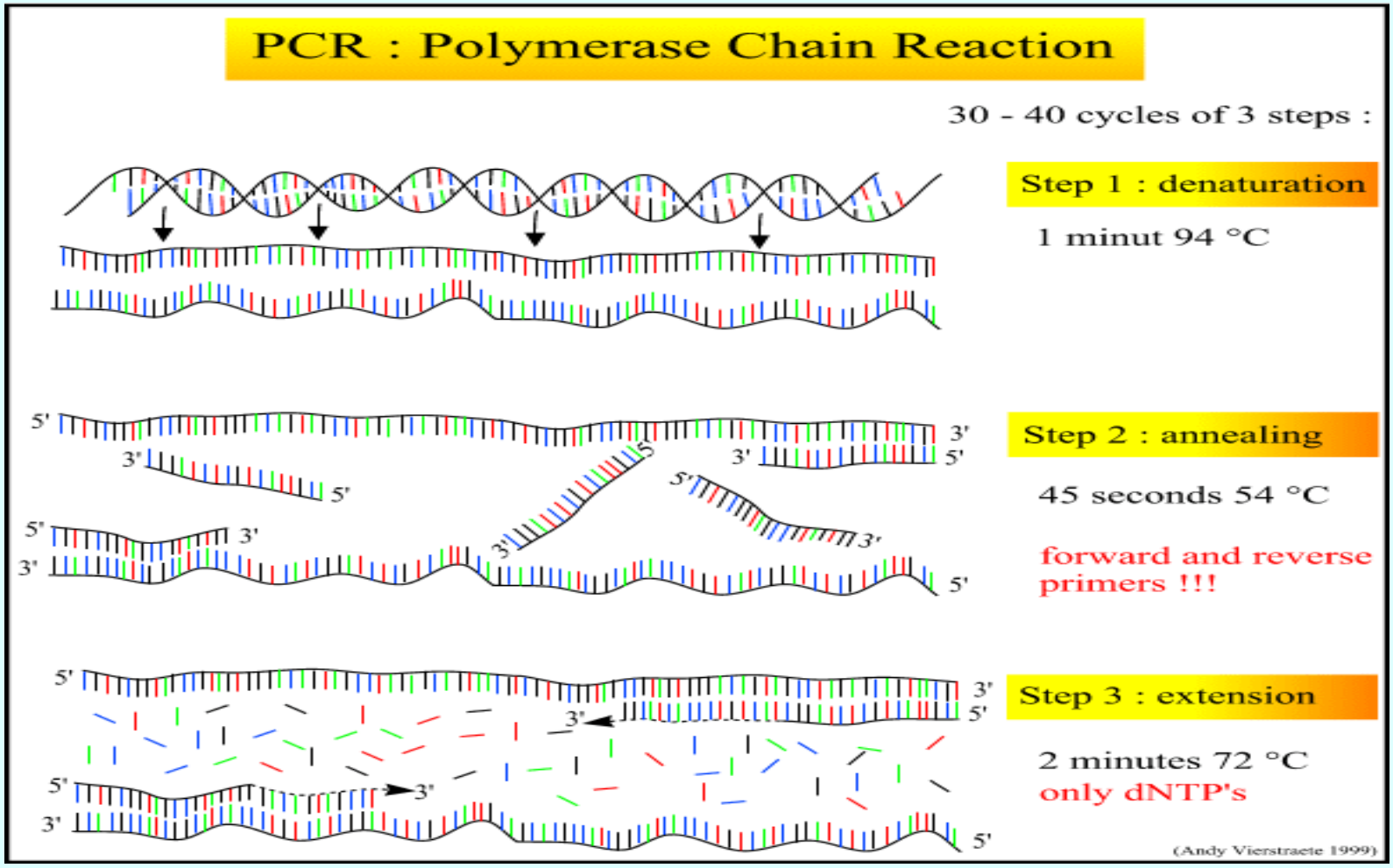
Temperature: 50-70C° (depending on the melting temperature of the expected duplex).

Extension:

DNA polymerase binds to the annealed primers and extends DNA at the 3’ end of the chain.

Temperature: 72C°

PCR cycling steps:



Standard PCR method:

- Prepare mix containing primers, dNTP's, buffer, Taq polymerase and water sufficient for all reaction tubes.
- Aliquot appropriate volume to each tube.
- Add DNA to each tube using a new tip for each sample.

Standard PCR method:

- Load tubes on PCR machine.
- Cover the plate.

N.B If PCR machine does not have heated lid, 1 drop of mineral oil should be added to each tube before loading then start the program.

Properties of Polymerase:

- Taq polymerase originally isolated from Thermus aquaticus.
- Heat stable (half life of about 30 min at 95C°).
- Taq DNA polymerase has no proof-reading function in 3’ to 5’ direction.
- Primer extension occurs at up to 100 bases/sec.
- Plateau is eventually reached.

Trouble Shooting:

Problem	Possible reasons
No product	Primers annealing?
Product of incorrect size	Primers annealing elsewhere in genome?
Several Products formed	Contamination? Several annealing sites?

PCR Rxn inhibitors:

- protinase K: digest polymerase
- phenol: denature the polymerase.
- EDTA: chelates Mg+2

Advantages of PCR:

- Uses less patient DNA.
- Result obtained more quickly about 3hr for PCR.
- No need to use radioactive material for PCR.
- Precise in determining sizes of alleles.
- Can be used to detect point mutations.

Disadvantages of PCR:

- Target DNA sequence must be known.
- Errors made by Taq polymerase.
- Size limitations particularly in GC rich triplet repeats.

PCR Based Technologies:

- Multiplex PCR
- QF-PCR
- RT-PCR