POLYMERASE CHAIN REACTION

- **Use to amplify the number of DNA molecules.**
- **Amplify a single piece of DNA**
- **Over many cycles = millions of copies of original DNA.**

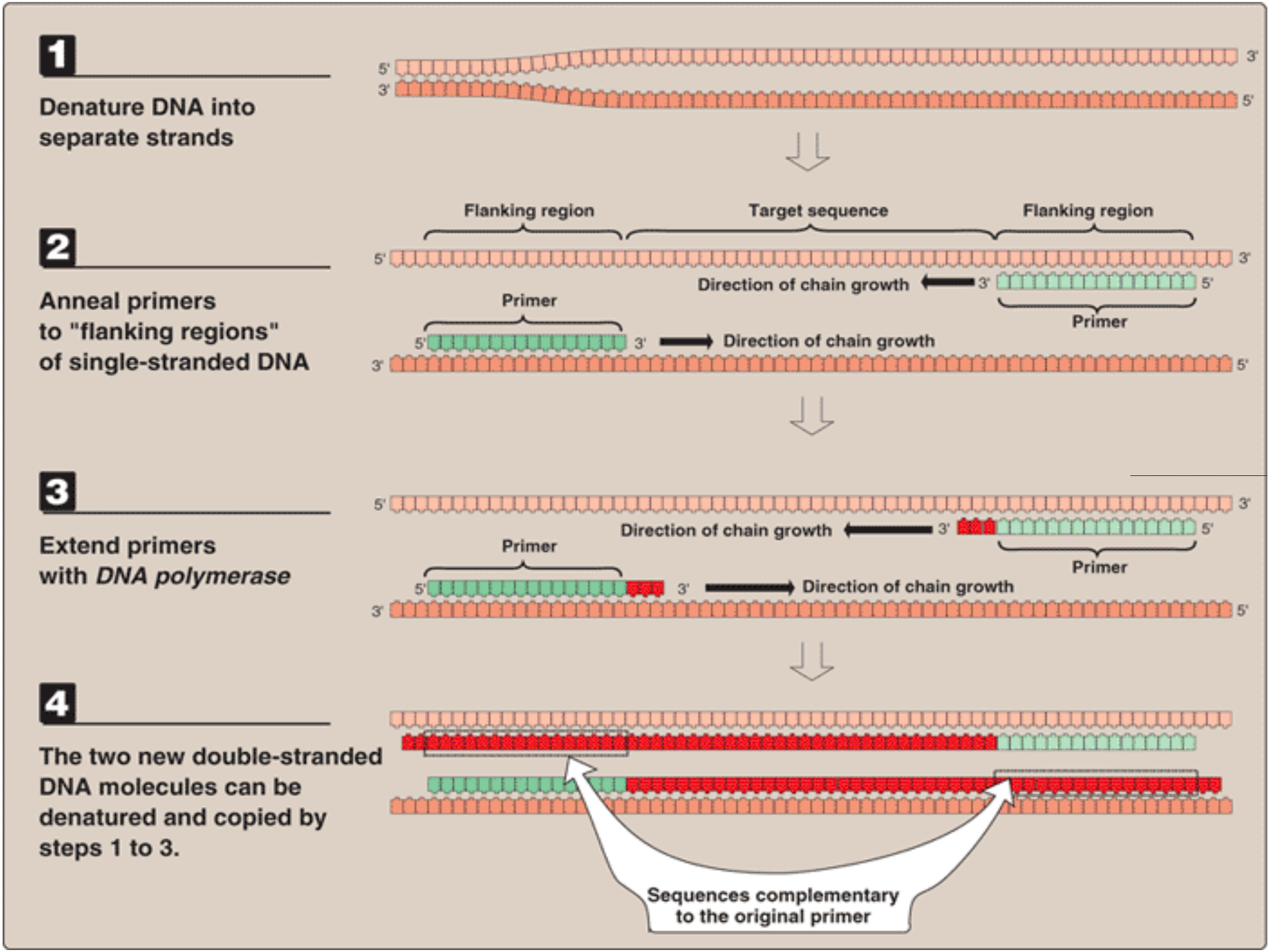
- **Technique widely used in**
  - **Molecular biology**
  - **Microbiology**
  - **Genetics**
  - **Diagnostic clinical laboratories**
- The name comes from the ***DNA polymerase*** used to amplify
- DNA replication with a piece of DNA by *in vitro* enzymatic replication.

WHAT IS PCR USED FOR?

- PCR is used in research laboratories in
  - ***DNA cloning procedures,***
  - ***Southern blotting,***
  - ***DNA sequencing,***
  - ***recombinant DNA technology***.
- Clinical microbiology
  - Diagnosis of microbial infections
- Forensics laboratories

These components require in PCR:

- *DNA template* that contains the DNA region (target) to be amplified.
- *Primers* which are complementary to 5' ends DNA regions
  - *Flanking sequences* *Short* nucleotide sequence on each side of target DNA = Which used to synthesis primer
- DNA polymerase such as *Taq polymerase* or another DNA polymerase with a temperature optimum at around 70°C.
  - From Thermus Aquaticus
- *Deoxynucleotide triphosphates* (dNTPs), the building blocks from which the DNA polymerases synthesizes a new DNA strand.
- *Buffer solution* for optimum activity and stability of the DNA polymerase.
- Thermal cycler allows heating and cooling of the reaction tubes to control the temperature required at each reaction step


1
Denature DNA into separate strands
5'
3'
3'
5'
2
Anneal primers to "flanking regions" of single-stranded DNA
Flanking region
Target sequence
Flanking region
5'
3'
Direction of chain growth
3'
5'
Primer
Primer
5'
3'
Direction of chain growth
3'
5'
3
Extend primers with DNA polymerase
5'
3'
Direction of chain growth
3'
5'
Primer
Primer
5'
3'
Direction of chain growth
3'
5'
4
The two new double-stranded DNA molecules can be denatured and copied by steps 1 to 3.
Sequences complementary to the original primer

PROCEDURE

- Consists of 20 to 35 repeated cycles
- Each cycle consists of 2-3 discrete temperature steps.

Denaturation step

- Consist of heating to 94-98°C for 20-30 secconds.
- Causes melting of DNA template and primers
- Disrupting the hydrogen bonds
- Yielding single strands of DNA.

Annealing step

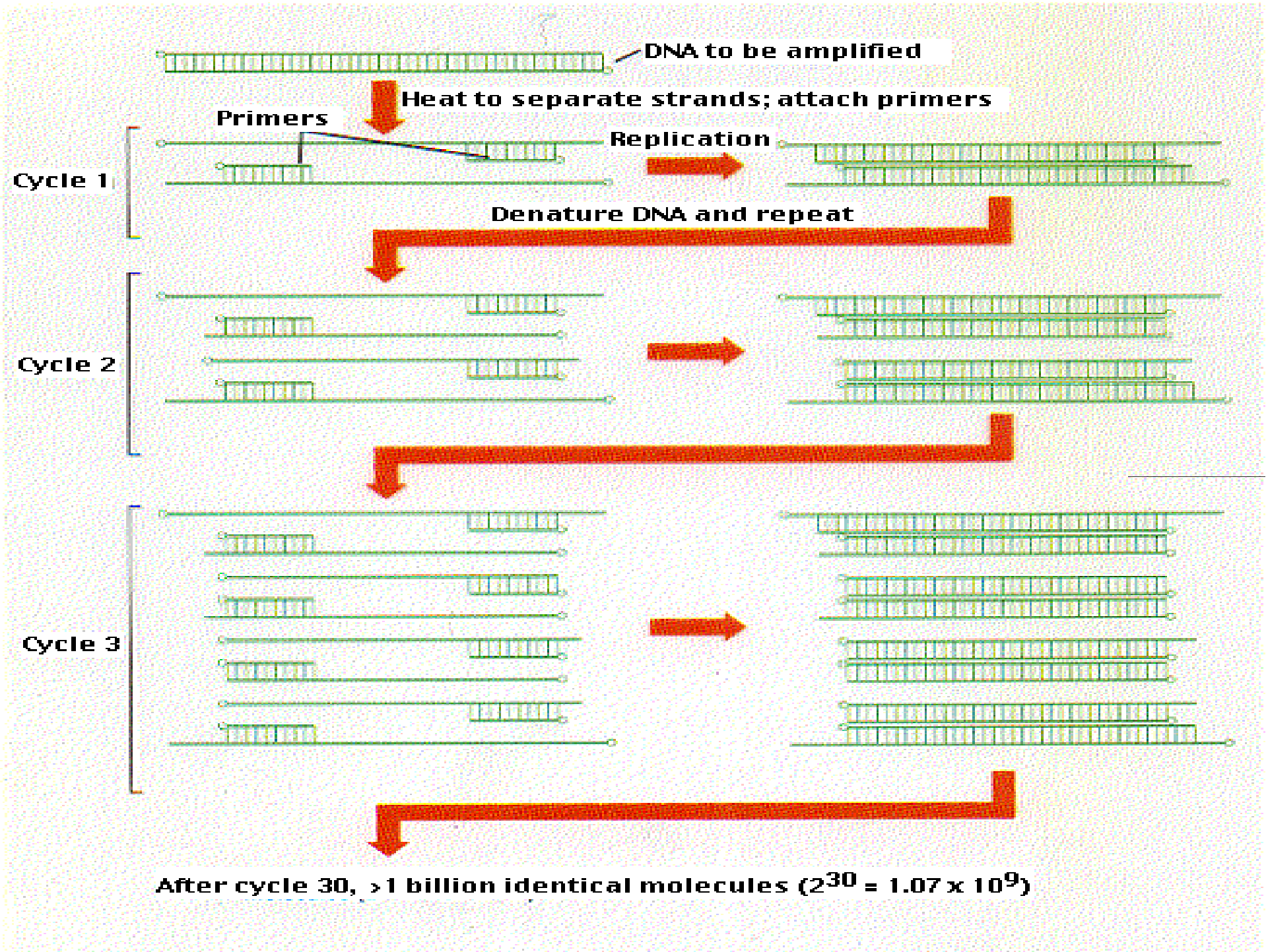
- Temperature lowered to 50-65°C for 20-40 seconds
- Allowing annealing of the primers to the single-stranded DNA template.
- Annealing temperature is about 3-5° Celsius below Tm of the primers used.
- Polymerase binds to the primer-template hybrid
- And begins DNA synthesis.

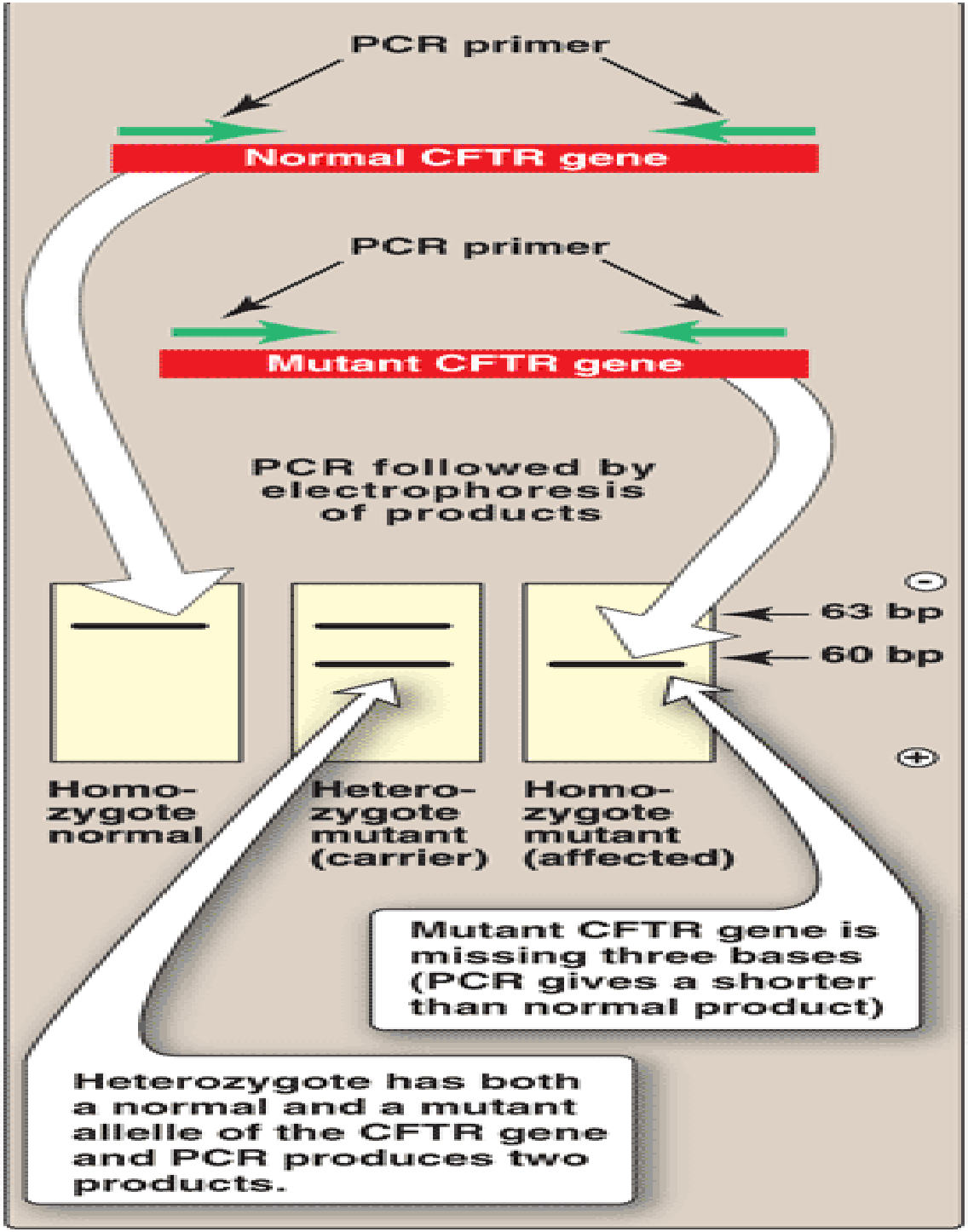
Elongation step

- Temperature in this step depends on = DNA polymerase used
- Taq polymerase = optimum activity = at 75-80°C
- DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand
- By adding dNTP's - complementary to the template
- In 5' to 3' direction
- Condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand.
- Will polymerize a thousand bases in one minute.

Final elongation

- Single step
- Performed after the last PCR cycle.
- Temperature of 70-74°C for 5-15 minutes
- To ensure that any remaining single-stranded DNA is fully extended.


DNA to be amplified
Heat to separate strands; attach primers
Primers
Replication
Cycle 1
Denature DNA and repeat
Cycle 2
Cycle 3
After cycle 30, >1 billion identical molecules ($2^{30} = 1.07 \times 10^9$)


PCR primer
Normal CFTR gene
PCR primer
Mutant CFTR gene
PCR followed by electrophoresis of products
63 bp
60 bp
Homo-zygote normal
Hetero-zygote mutant (carrier)
Homo-zygote mutant (affected)
Mutant CFTR gene is missing three bases (PCR gives a shorter than normal product)
Heterozygote has both a normal and a mutant allelle of the CFTR gene and PCR produces two products.

APPLICATION OF PCR

- PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA.
- In many Diagnostic methods, such as
  - Generating probes for Southern or northern technic
  - DNA cloning - which require larger amounts of specific DNA region.
- PCR enabling analysis of DNA samples even from very small amounts of starting material.
- Used for; a forensic technique use Genetic finger printing d to identify a person
- DNA sequencing to determine unknown PCR-amplified sequences in which one of the amplification primers may be used

REAL-TIME PCR

- Traditionally, PCR is performed in a tube and when the reaction is complete the products of the reaction (the amplified DNA fragments) are analysed and visualised by gel electrophoresis.
- Real-Time PCR permits the analysis of the products while the reaction is actually in progress.
- This is achieved by using various fluorescent dyes
- React with the amplified product
- And measured by an instrument.
- Facilitates the quantitation of the DNA.
- Not only can one tell instantly "what" DNA is present in the sample but also "how much".

- ***Quantitative PCR (Q-PCR) = Real Time PCR***
- Method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA.
- PCR is therefore often used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.
- Rapidity assay = Not necessary for electrophoresis.

REAL TIME PCR