POLYMERASE CHAIN REACTION (PCR)

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Definition

It is a method of making multiple copies of a DNA sequence, involving repeated reactions with a polymerase.

(Oxford Languages)

It is a laboratory technique used to amplify DNA sequences.

(NIH – National Human Genome Research Institute)

It is a method widely used to rapidly make millions to billions of copies (complete copies or partial copies) of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it (or a part of it) to a large enough amount to study in detail.

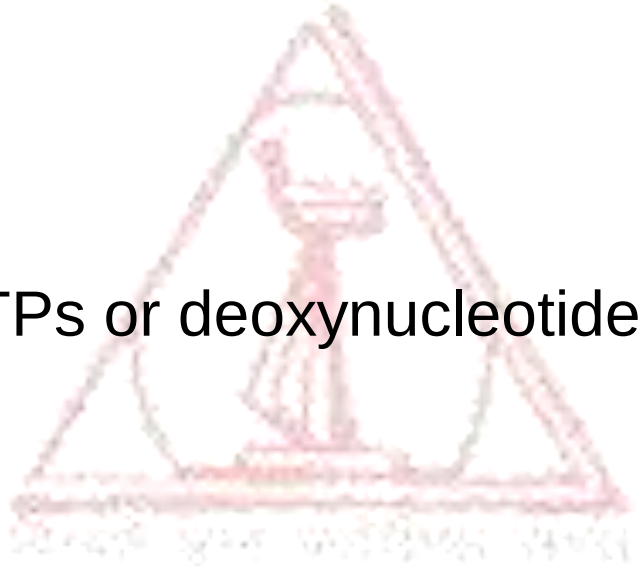
(Wikipedia)

Introduction

- By definition, it is a molecular biological technique used to amplify a single copy or a few copies of a specific DNA segment across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- This revolutionary method was developed by Kary Mullis in the 1980s for which he was awarded with Nobel Prize in Chemistry in 1993.
- It is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand.
- It involves using short DNA sequences called primers to select the portion of the genome to be amplified.
- The temperature of the sample is repeatedly raised and lowered to help a DNA replication enzyme copy the target DNA sequence.
- The technique can produce a billion copies of the target sequence in just a few hours.

Component of PCR

- Thermal cycler machine
- DNA template
- Primers
- DNA polymerase
- Nucleotides (dNTPs or deoxynucleotide triphosphate)
- Buffer solution
- Divalent cations



Component of PCR

Thermal cycler machine

- Thermal cycler or thermocyclers, or PCR machine, are instruments used to amplify DNA and RNA samples by the polymerase chain reaction.
- It can be programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.
- It has a *thermal block* with holes where tubes holding the PCR reaction mixtures can be inserted.
- The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps.

Component of PCR

Thermal cycler.

An older, three-temperature thermal cycler for PCR

A 96-well plate modern thermal cycler for PCR

Component of PCR

The Kary Mullis' Thermal Cycler

http://hpc.ilri.cgiar.org/beca/training/IMBB/lectures

Component of PCR

Thermal cycler machine today

- Standard PCR
- Nested PCR
- Touch down PCR
- Sequencing PCR
- Intersequence-specific PCR (ISSR)
- Many others
  - Real-time PCR machine
  - Digital PCR systme (dPCR)
  - Multi-block PCR thermal cyclers
  - 384-Well PCR thermal cyclers

Component of PCR

DNA template

- It is the DNA molecule that contains the segment of DNA to be amplified; it is basically a target sequence.
- Amount of DNA template is determined empirically; if too little template is used, primers may not be able to find their complementary sequences and if too much template is used, it may lead to increased mispriming events.

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- Generally, used concentration is 100-250ng for mammalian genomic DNA and 20ng for linearized plasmid DNA (circular plasmid DNA is slightly less efficiently amplified) per 50µl reaction.

Component of PCR

DNA Primers

- Primers are short synthetic DNA strands of about 18 to 25 nucleotides complementary to 3' end of the template strand.
- DNA polymerase starts synthesizing new DNA from the 3' end of the primer.

Two types:

Forward primer: It is complimentary to the 3' end of antisense strand (3' – 5').

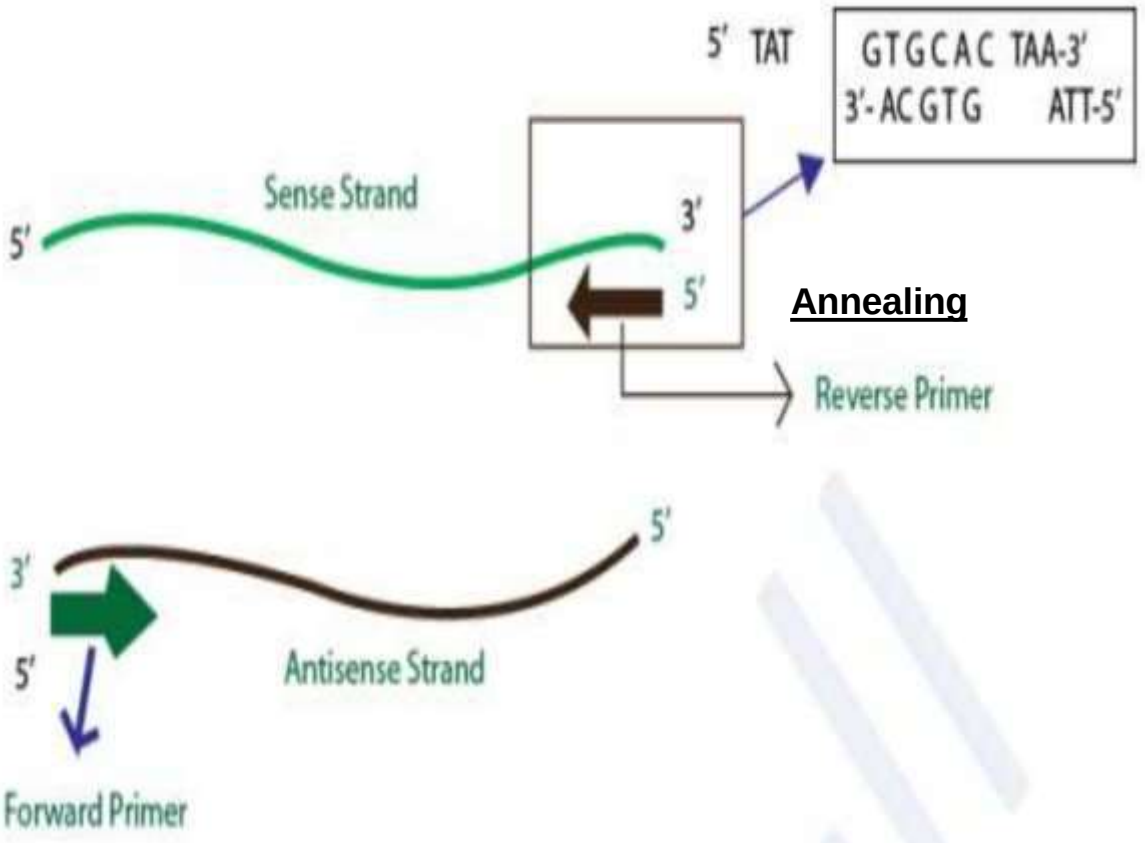
Reverse primer: It is complimentary to the 3' end of the sense strand (5' – 3').

- *The concentration of each primer should be between 0.1 and 0.5 µm (0.2 µm is also said to produce satisfactory results.*

Component of PCR

DNA Primers

- It we consider the sense strand (5’ – 3’) of a gene, for designing primers, then forward primer is the beginning of the gene and the reverse primer is the reverse-compliment of the 3’ end of the gene.



Source: slideshare/pcr-76618045

Component of PCR

DNA Primers – Designing

Consideration

- Typical primers are 18-28 nucleotides in length
- 50-60% GC compositon
- Have a balanced distributon of G/C and A/T domains
- No long strings of a single

Good

5’ ATGCACTCAGACGTACAACGTGAC 3’

24 bases; AT: 12 GC: 12 (50% GC); Balanced distribution; Ta = 65ºC

Bad

5’ AAAACAAACGATTTTTT 3’

17 bases; AT: 14; GC: 3 (18 % GC); Unbalance distribution; Ta = 38ºC

Component of PCR

DNA Primers – Designing

Characteristics

Resources for primer design – *Primer design tool*

- CLC Workbench ◄ our focus
- Prime-BLAST
- PrimerQuest
- Primer3
- Primer3 plus
- PrimerZ
- PerlPrimer
- OligoPerfect
- OLIGO
- GenScript Real-time PCR (TaqMan) Primer Design
- AutoPrime
- RExPrimer
- BatchPrimer3
- Eurofins Genomics' Primer Design Tools

Component of PCR

DNA polymerase

It sequentially adds nucleotides complimentary to template strand at 3'-OH of the bound primers and synthesizes new strnds of DNA complementary to the target sequence.

The most commonly used DNA polymerase is Taq DNA polymerase.

Taq DNA polymerase

- This enzyme is extremely heat resistant with a half-life of 40 minutes at 95°C (Optimal temp range is 75 – 80ºC).
- It was originally isolated from thermophilic bacterium of the Deinococcus-Thermus group located near the Lower Geyser Basin of Yellowstone National Park by Thomas D. Brock and Hudson Freeze, in **1969**.
- *Pfu* DNA polymerase (isolated from *Pyrococcus furiosus*) is also used widely because of its higher fidelity (accuracy of adding complimentary nucleotide).

Component of PCR

dNTPs or deoxynucleotide triphosphate (nucleotides)

All types of nucleotides are “building blocks” for new DNA strands and essential for reaction. It includes:

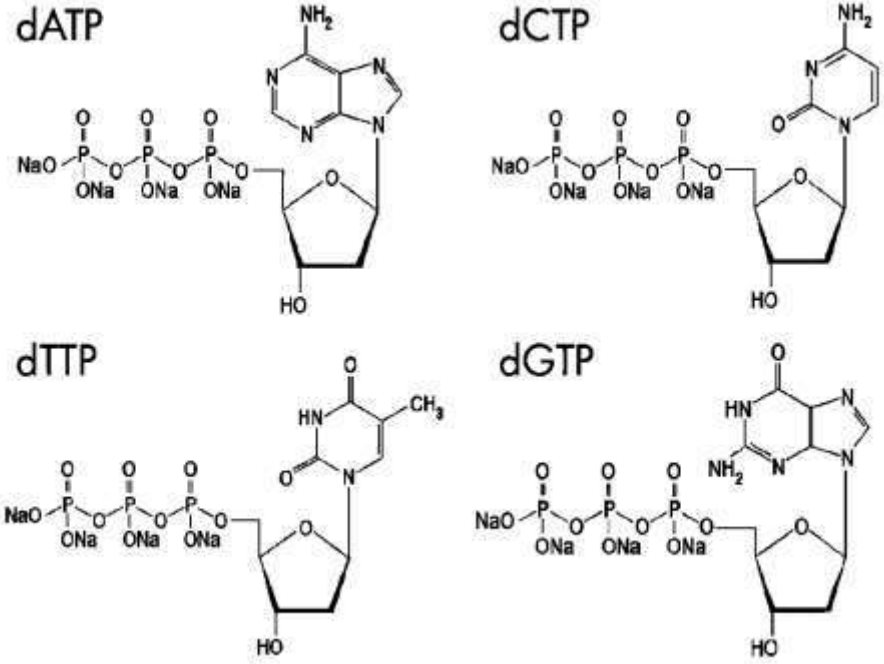
Deoxyadenosine triphosphate (dATP)

Deoxyguonosine triphosphate (dGTP

Deoxycytidine triphosphate (dCTP)

Deoxythymidine triphosphate (dTTP)

Deoxyuridine triphosphate (dUTP)



Component of PCR

Divalent cation

- Typically magnesium (Mg) or manganese (Mn) ions are used.
- Mg^{2+} is the most commonly used divalent cation – Mn^{2+} is used for PCR-mediated DNA mutagenesis (higher Mn^{2+} con. Increases the error rate during DNA synthesis).

Magnesium chloride ($MgCl_2$)

- ✓ It affects primer annealing and template denaturation, as well as enzyme activity.
- ✓ An excess of Mg^{2+} gives non-specific amplification products, while low Mg^{2+} yield lesser amount of desired product.
- ✓ It is also an essential cofactor for the synthesis and salvage of purine and pyrimidine nucleotides.
- ✓ It also affect their interaction with proteins and other ligands.

Component of PCR

Polymerase buffer

- All DNA polymerases are supplied with their own optimal polymerase buffer.
- However, buffer can also be prepared in lab.

Constituent of standard Polymerase buffer is:

- ✓ 10 mM Tris-HCl pH 8.3 (at room temperature)
- ✓ 50 mM KCl
- ✓ 1.5 mM $MgCl_2$

Procedure of PCR

It is a multistep process which consists of following steps:

Initialization: It consists of heating the reaction chamber to a temperature of 94–96 °C (94 °C) for 1 to 5 min. It causes separation of DNA double helix – *Initial denaturation.*

Denaturation: It is the first regular cycling event which consists of heating the reaction chamber to a temperature of 94–98 °C for 20 – 30 seconds. It causes melting, or denaturation of DNA by breaking the hydrogen bonds between complimentary bases, yielding two single-stranded DNA molecules. Each strand acts as a template for synthesis of complimentary strand.

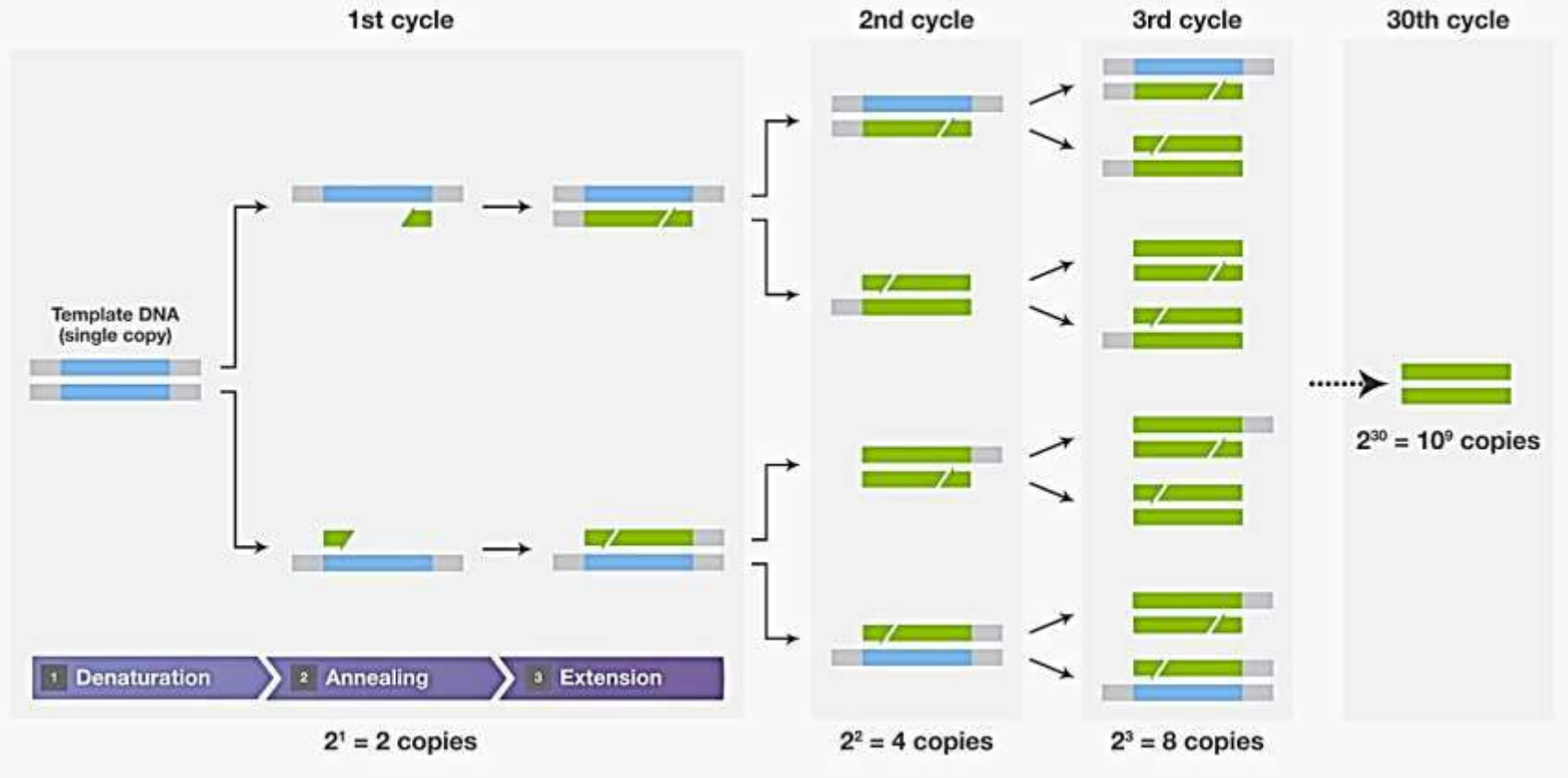
Annealing: Temp is lowered to 50 – 65 °C and kept the reaction mixture at this temp for 20 – 30 sec. This allows hybridization of primers to the primary strand.

Extension/Elongation: Reaction chamber is heated to 72 °C for 1 min. In this step, Taq DNA polymerase synthesize a new DNA stand complimentary to the DNA template strand by adding free dNTPs from the reaction mixture.

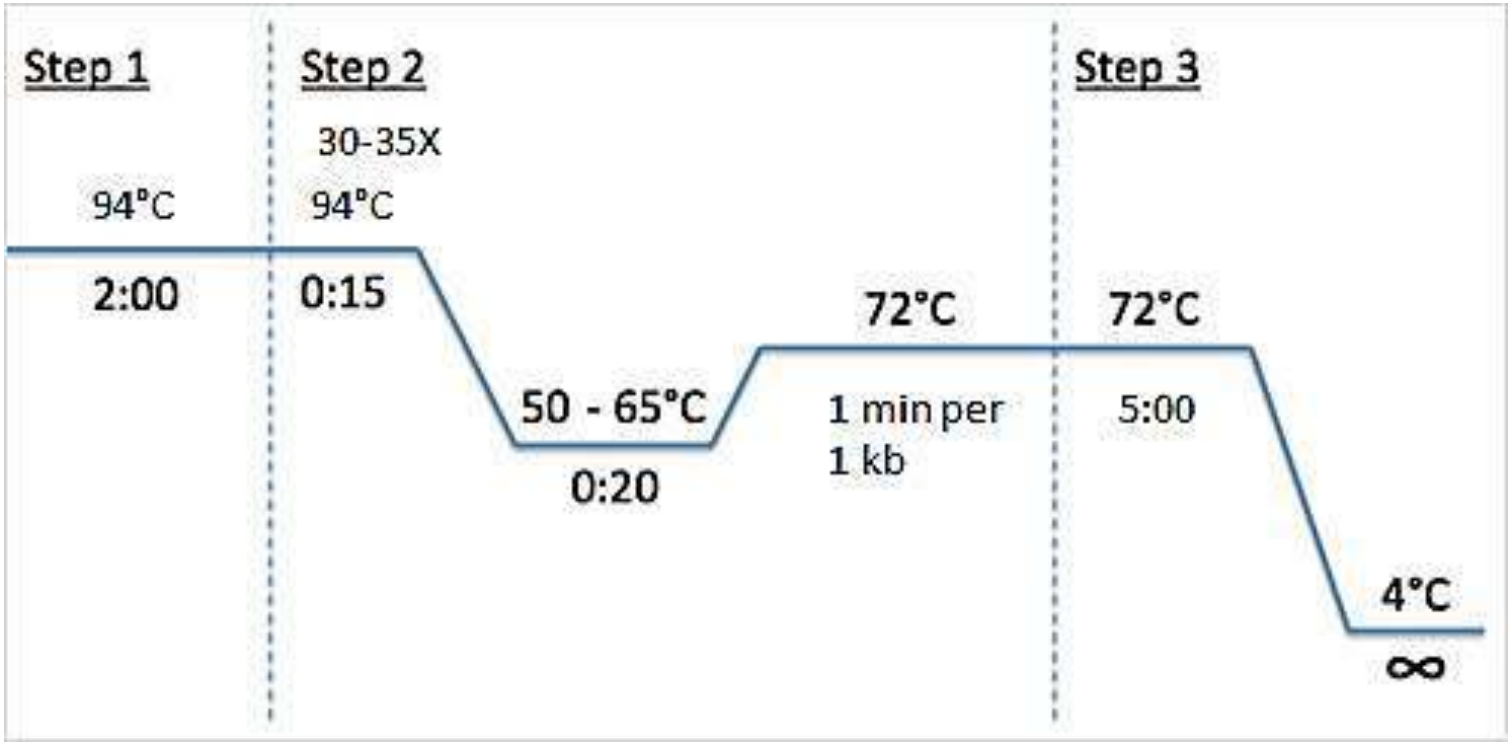
Final extension: This step is optional. It is performed at a temperature of 70 – 74 °C (72 °C) for 7 -10 min. It ensures that any remaining single-stranded DNA is fully elongated.

Store: Now the final product is stored for analysis. Generally stored at 4 – 15 °C

Procedure of PCR



Procedure of PCR



Temperature changes during each cycle of PCR

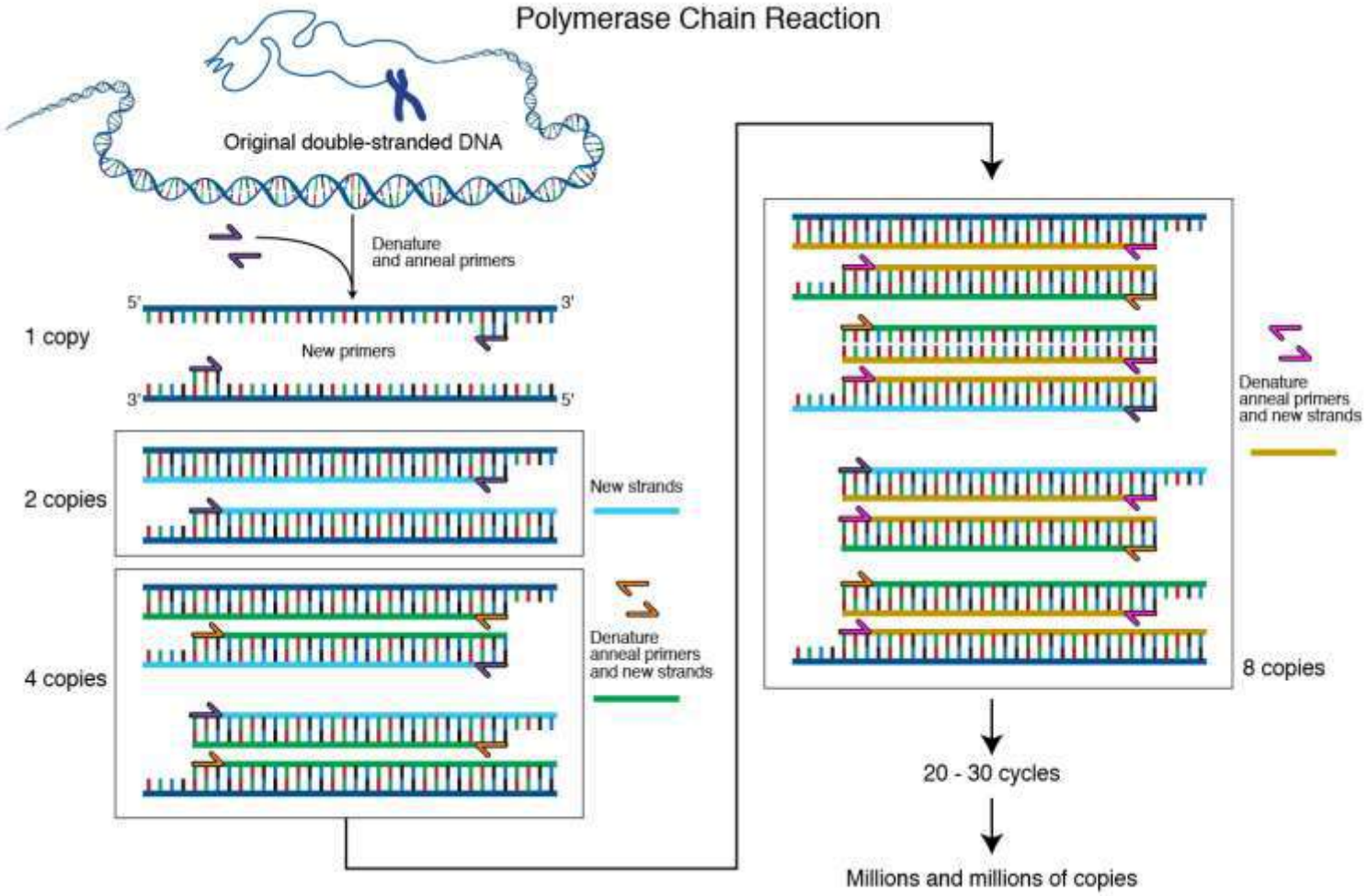
Calculation of copies in PCR

- The number of double stranded DNA pieces is doubled in each cycle, so that after n cycles you have 2^n (2 to the n^{th} power) copies of DNA.

Cycle	Calculation	Number of copies
1st	2^1	2
2^{nd}	2^2	4
3^{rd}	2^3	9
4^{th}	2^4	16
5^{th}	2^5	32
10^{th}	2^{10}	1,024
20^{th}	2^{20}	10,48,576

However it also depends on efficiency of PCR.

Overview of PCR



Further reading

- **The History of PCR (RU 9577)** http://siarchives.si.edu/research/videohistory_catalog9577.html
- **PCR: A Revolutionary Invention** https://www.nature.com/scitable/blog/bio2.0/pcr_a_revolutionary_invention/
- **How to design primers for PCR experiments – Tips and considerations for sensitive PCR assays. https://www.zymoresearch.com/blogs/blog/how-to-design-primers-for-pcr-experiments**