Lecture-02

PCR and Primer Design

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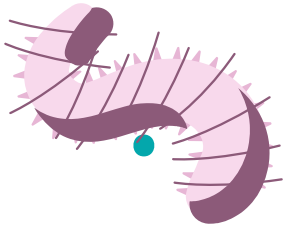
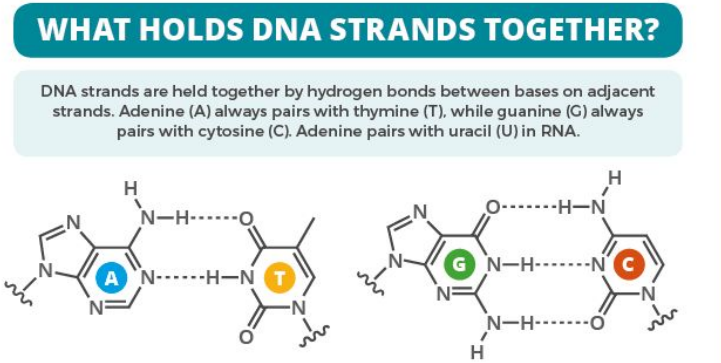


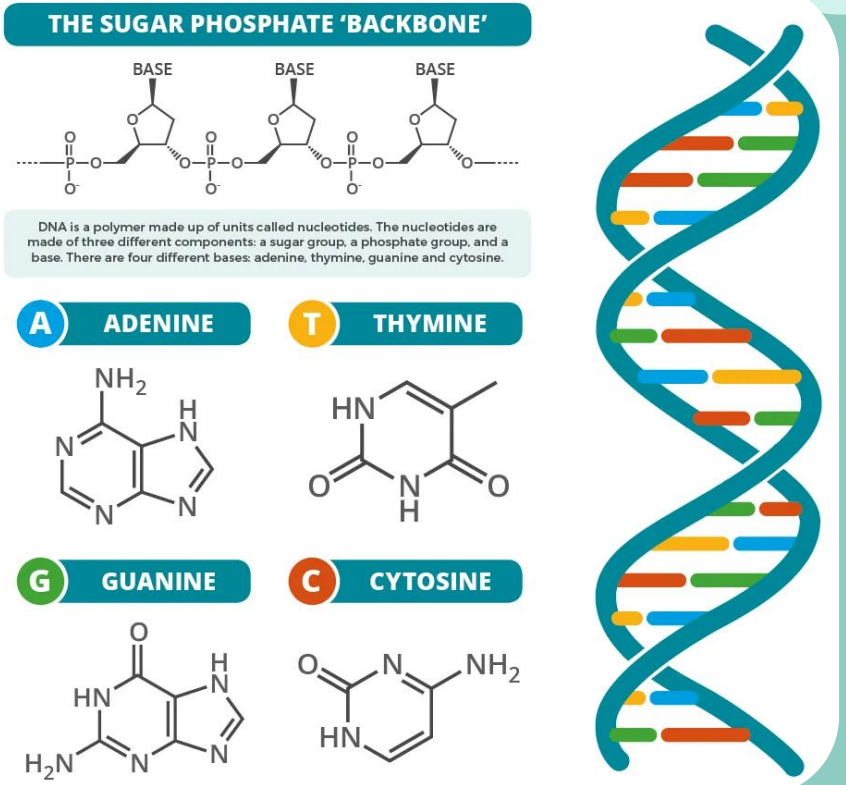
The code of life

01: DNA Recap

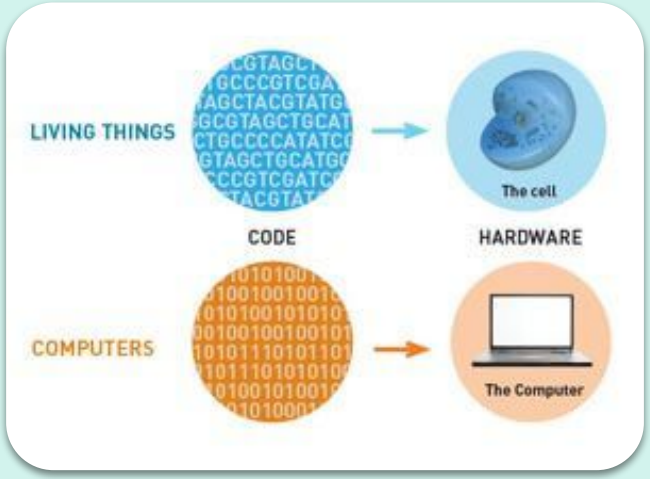
DNA

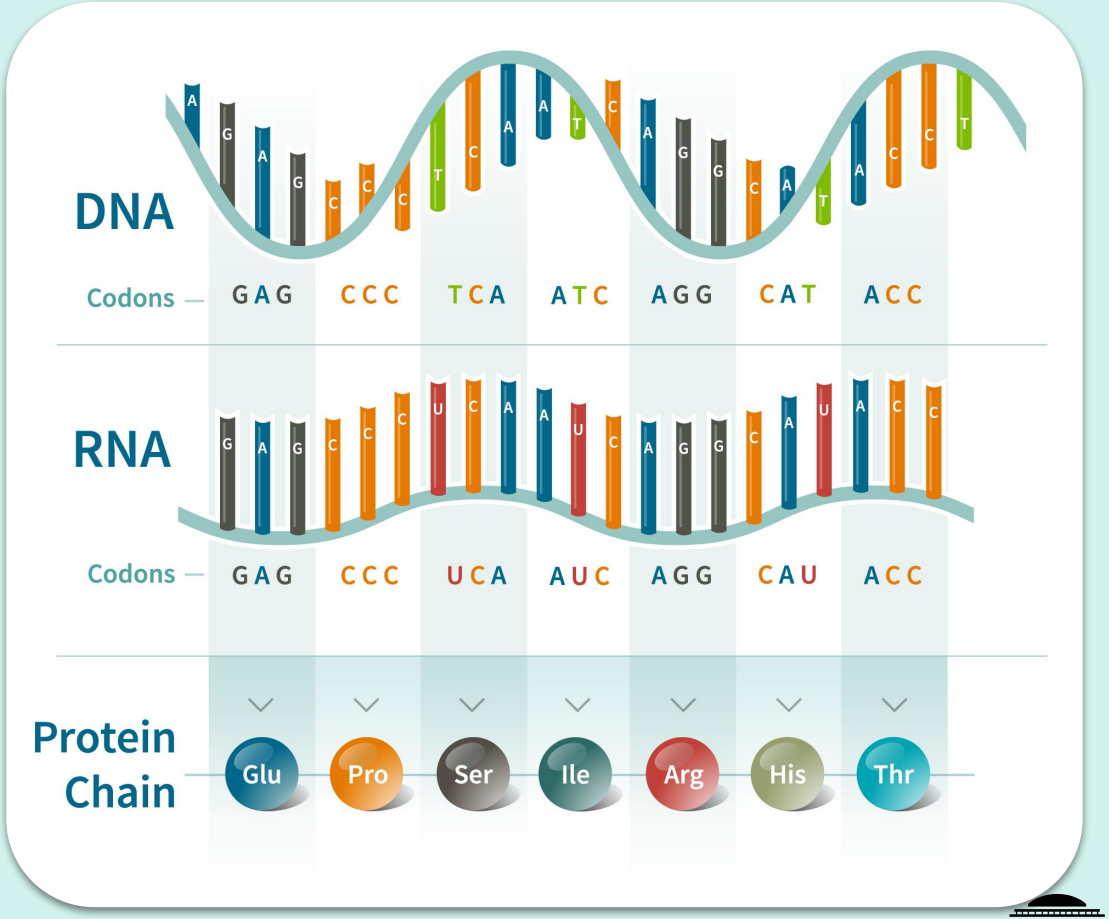
- DNA is a **nucleic acid** that is composed of two complementary nucleotide building block chains.
- The nucleotides are made up of **a phosphate group, a five carbon sugar, and a nitrogen base**.



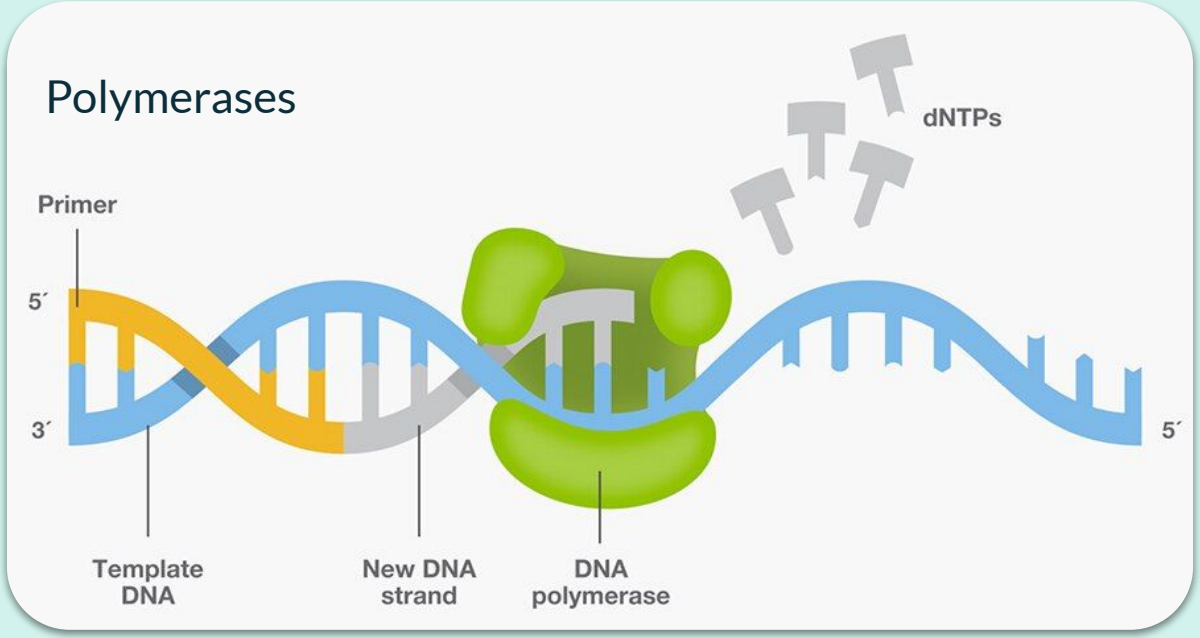


DNA Codes


LIVING THINGS
The cell
CODE
HARDWARE
COMPUTERS
The Computer


DNA
Codons
GAG CCC TCA ATC AGG CAT ACC
RNA
Codons
GAG CCC UCA AUC AGG CAU ACC
Protein Chain
Glu Pro Ser Ile Arg His Thr

DNA Replication



How fast is DNA Replication?

A typical human cell takes around **1 hour** to replicate its entire genome while *E. coli* takes about **40 minutes**.

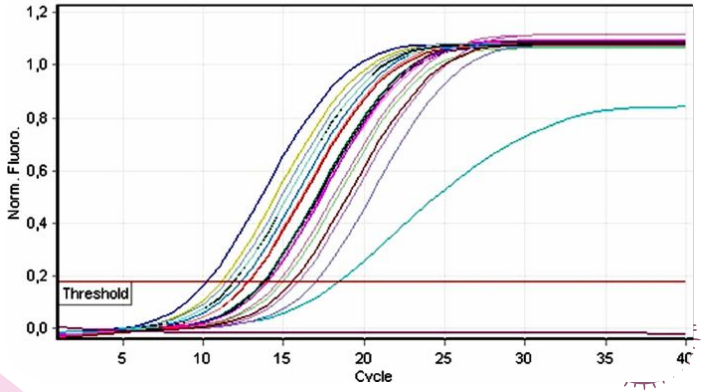
In SynBio, you use **a lot** of DNA. Hence, you need a way to quickly make a lot of it and make sure that it is accurately.

Polymerase Chain Reaction

02: PCR

PCR Overview

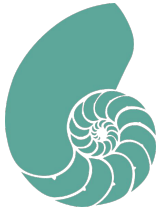
- Invented by Kary Mullis and his colleagues in the 1983 (Nobel Prize 1993).
- PCR or Polymerase Chain Reaction works like the *photocopier* in SynBio.



Uses of PCR

Research

PCR is used in SynBio extensively as a reliable and fast way to generate DNA for cloning.



Ancient DNA Detection

Fossilised DNA can be detected and amplified using PCR

Viral Detection

PCR can be used to detect viral sequences in other cell genomes (for diagnosis).

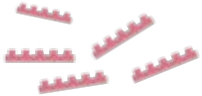
Forensic DNA Fingerprinting

PCR is used to amplify genetic sequences extracted from the accused to establish heredity

03: PCR Steps Involved

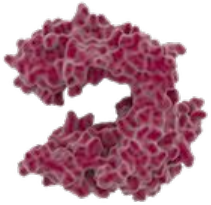
PCR Components

Gene of Interest



Primers

Nucleotides (dNTPs)

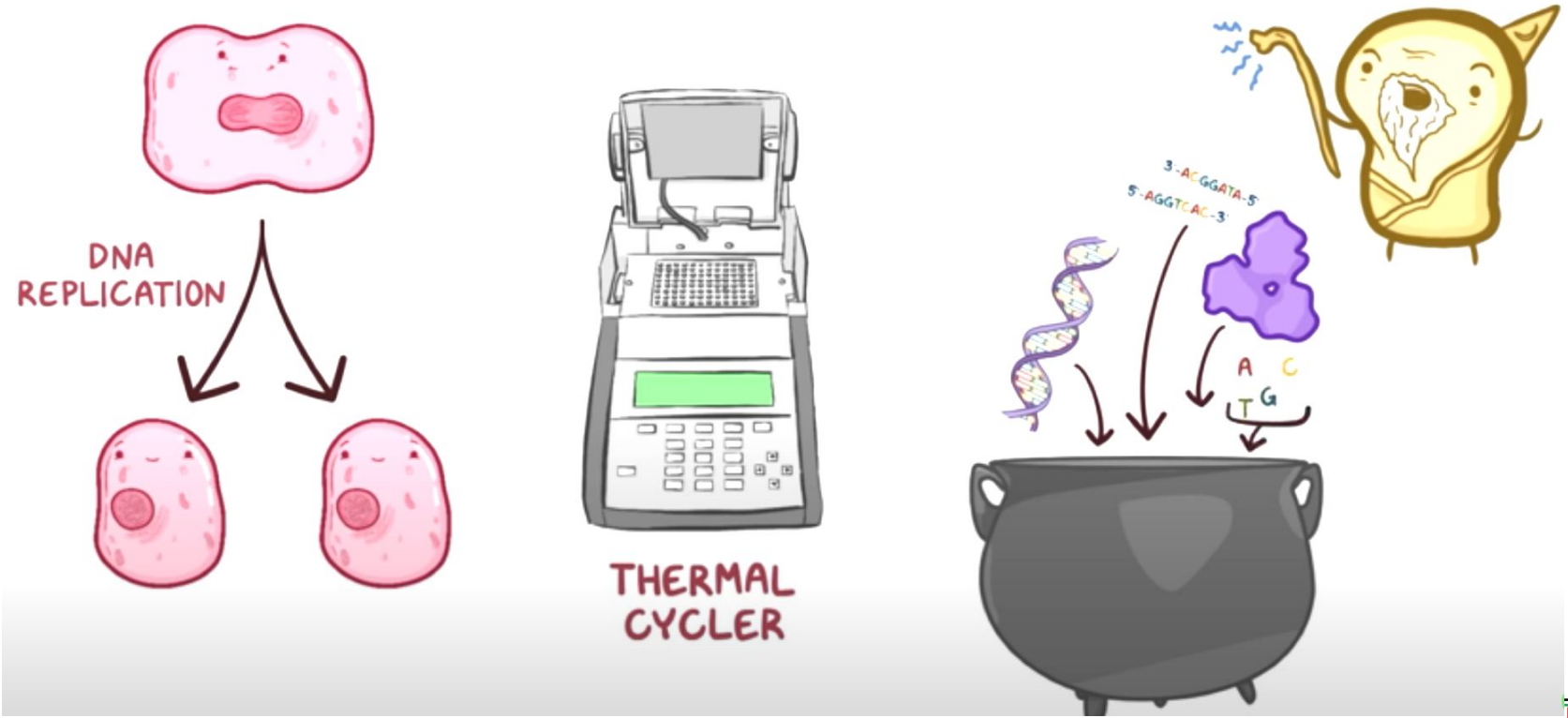


Taq Polymerase

Mix Buffer

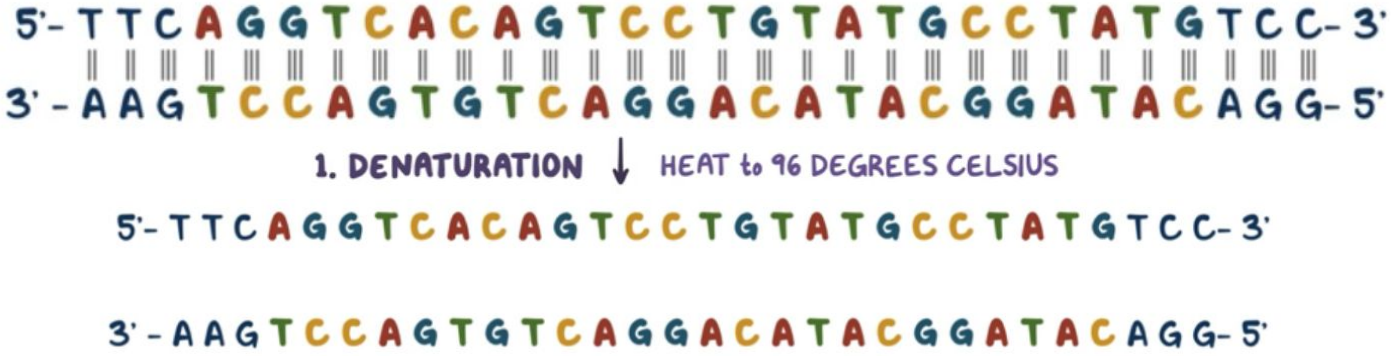
PCR Tube

PCR Components

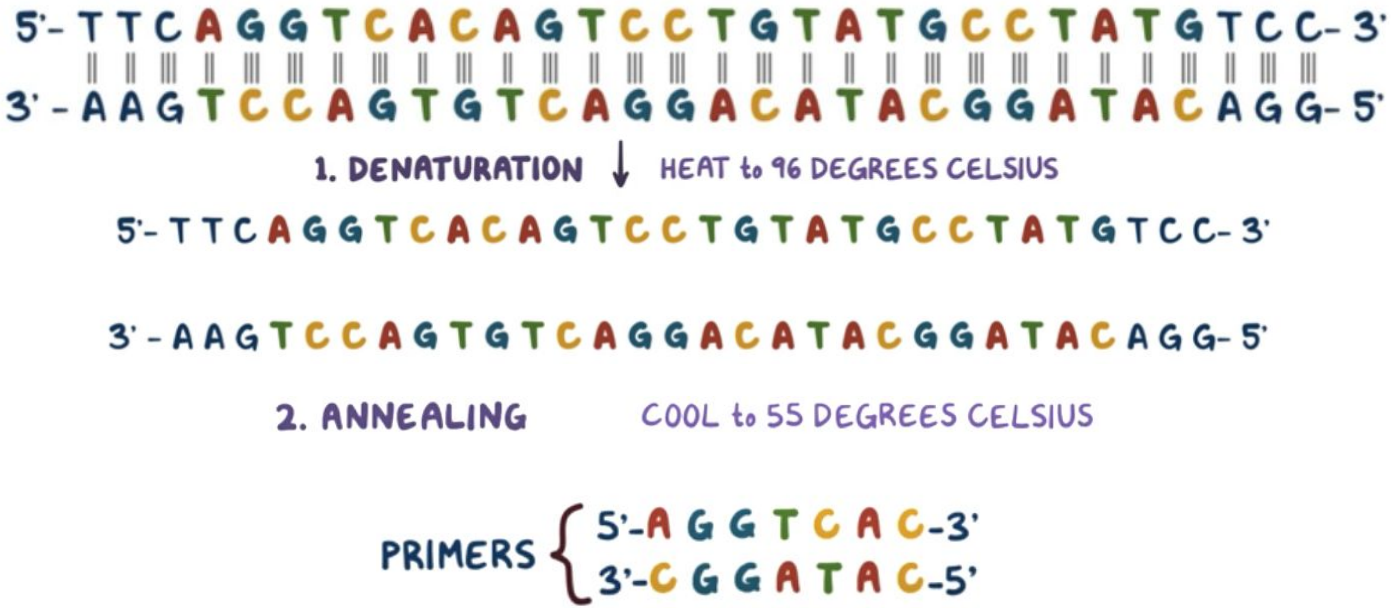


Steps of the PCR

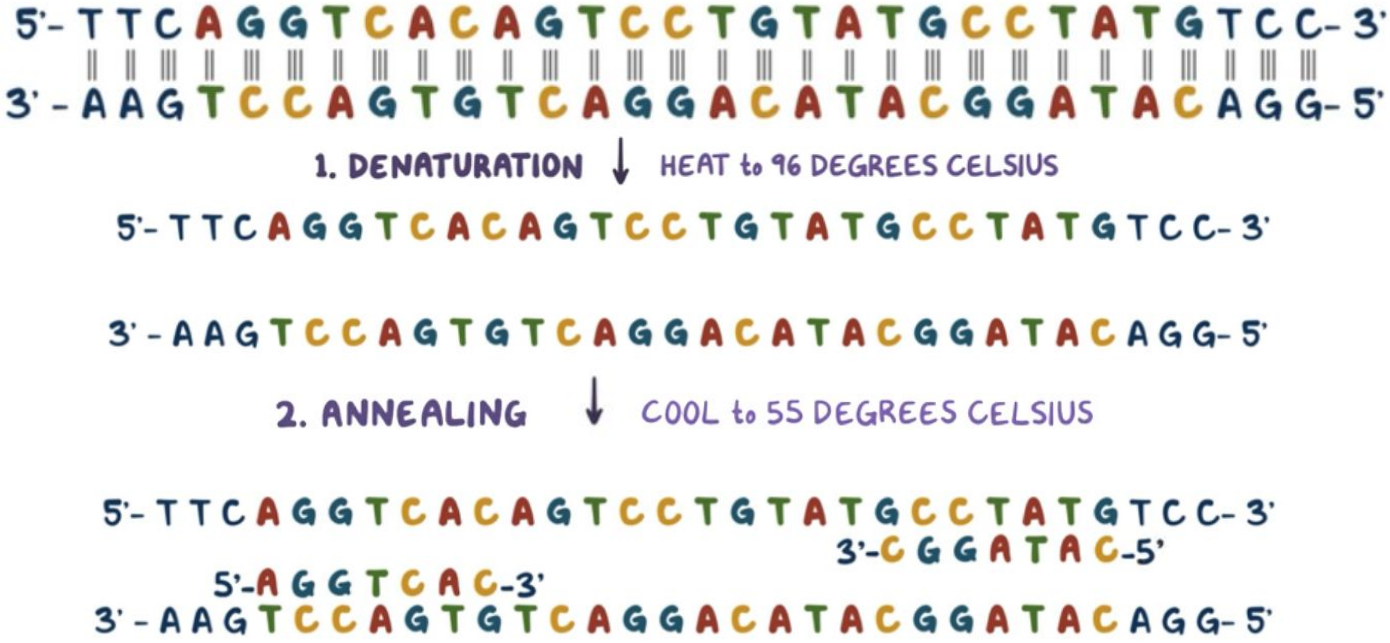
Steps of the PCR



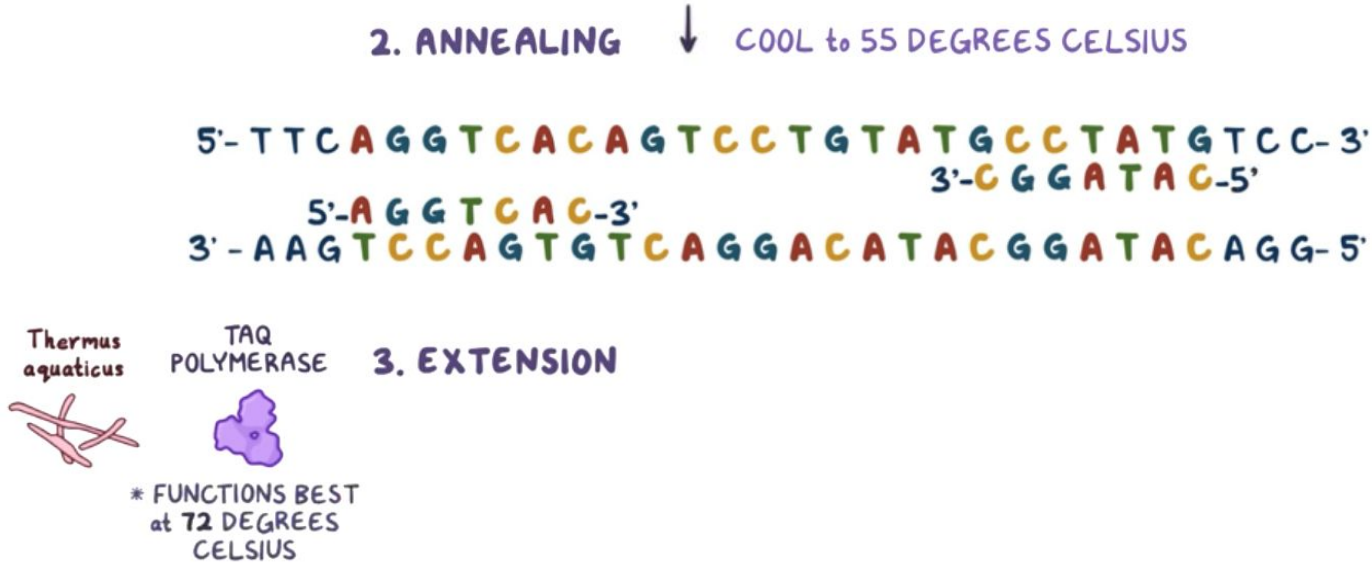
Steps of the PCR



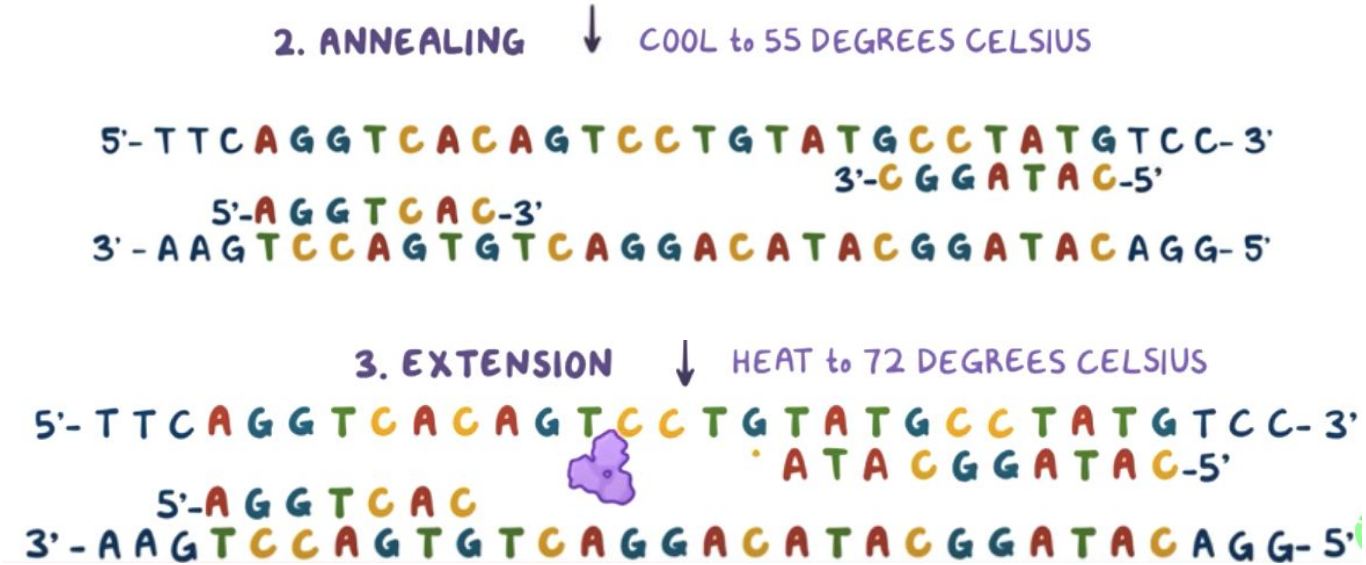
Steps of the PCR



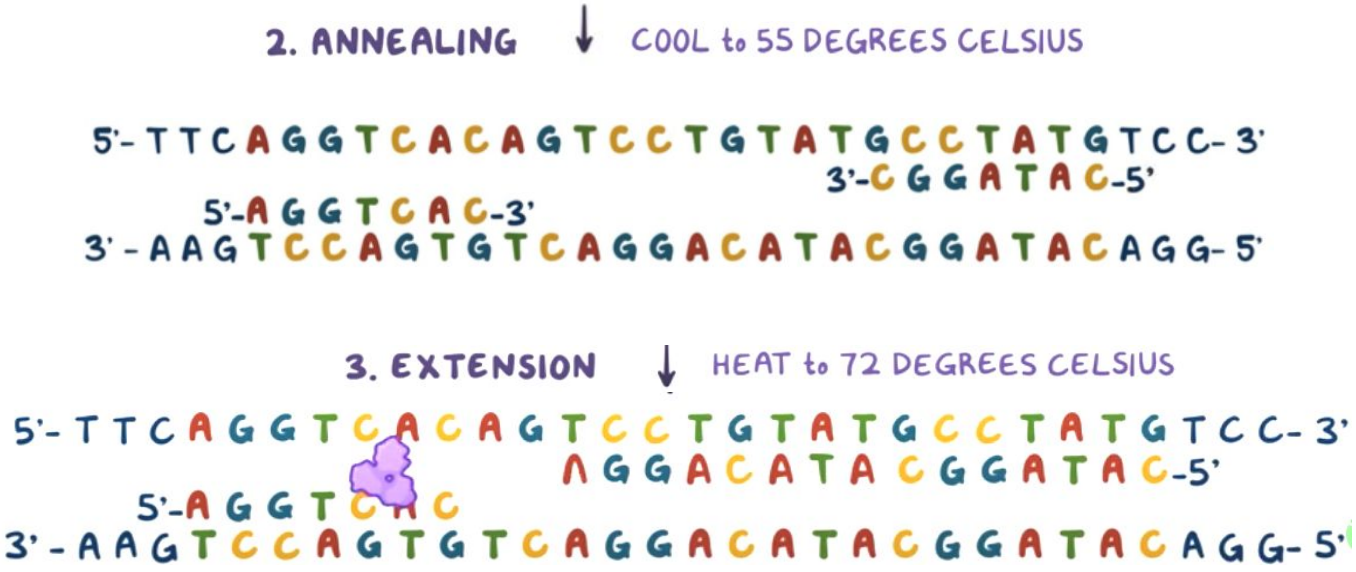
Steps of the PCR



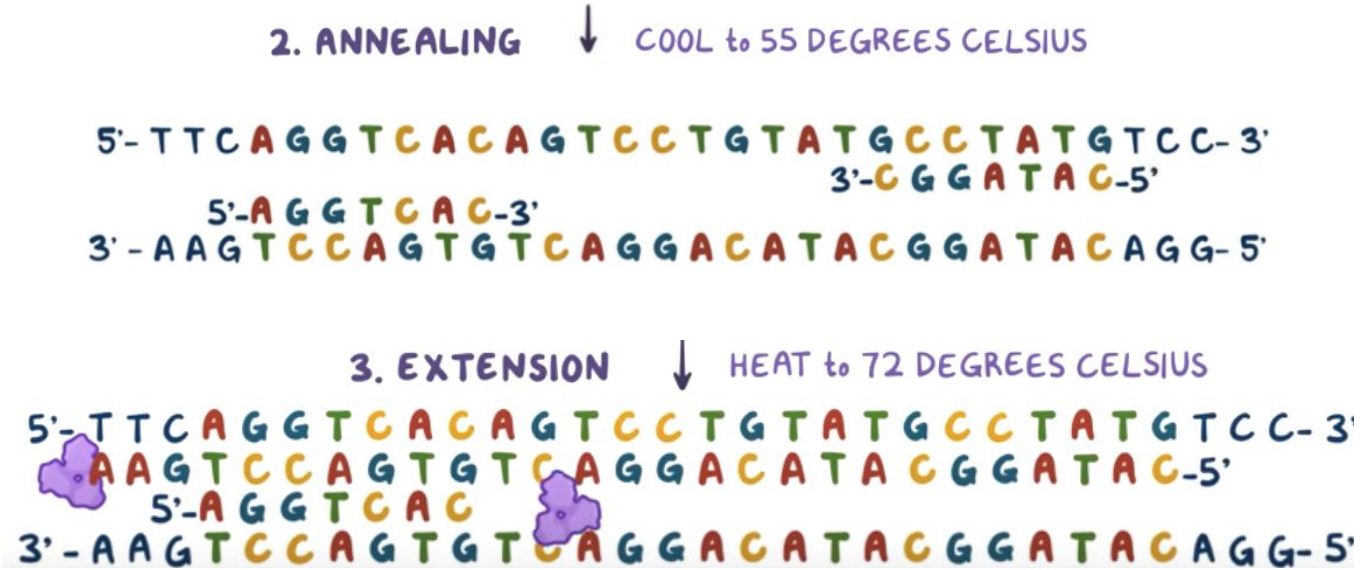
Steps of the PCR



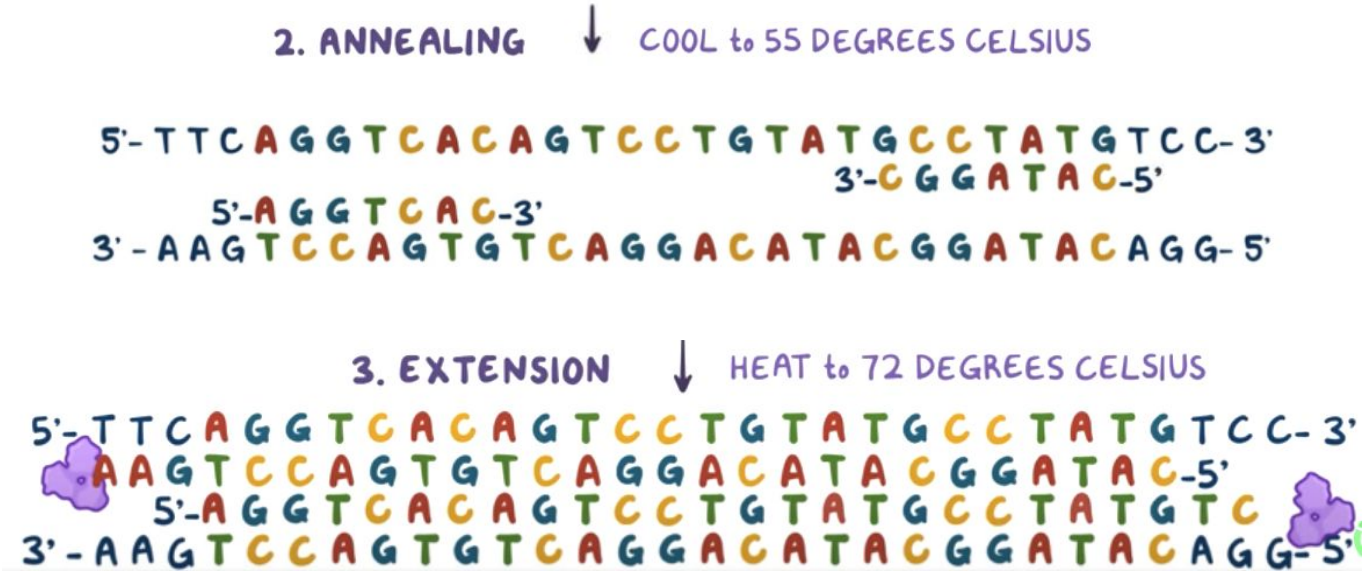
Steps of the PCR



Steps of the PCR



Steps of the PCR

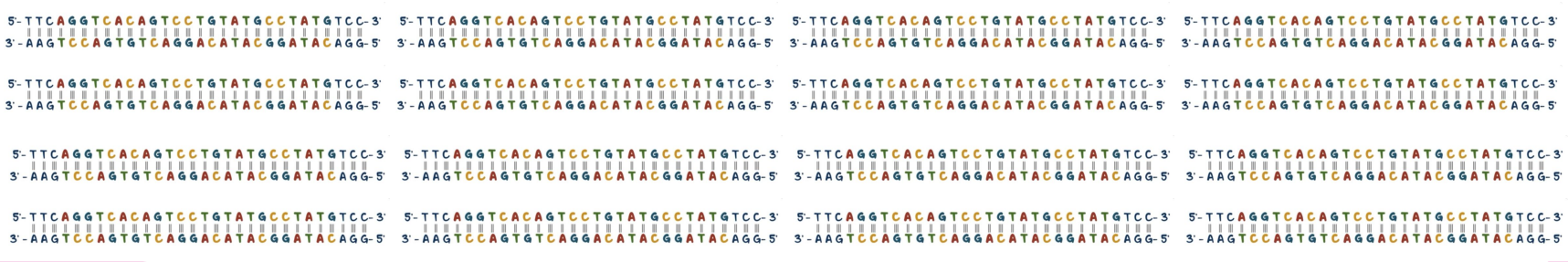


Steps of the PCR

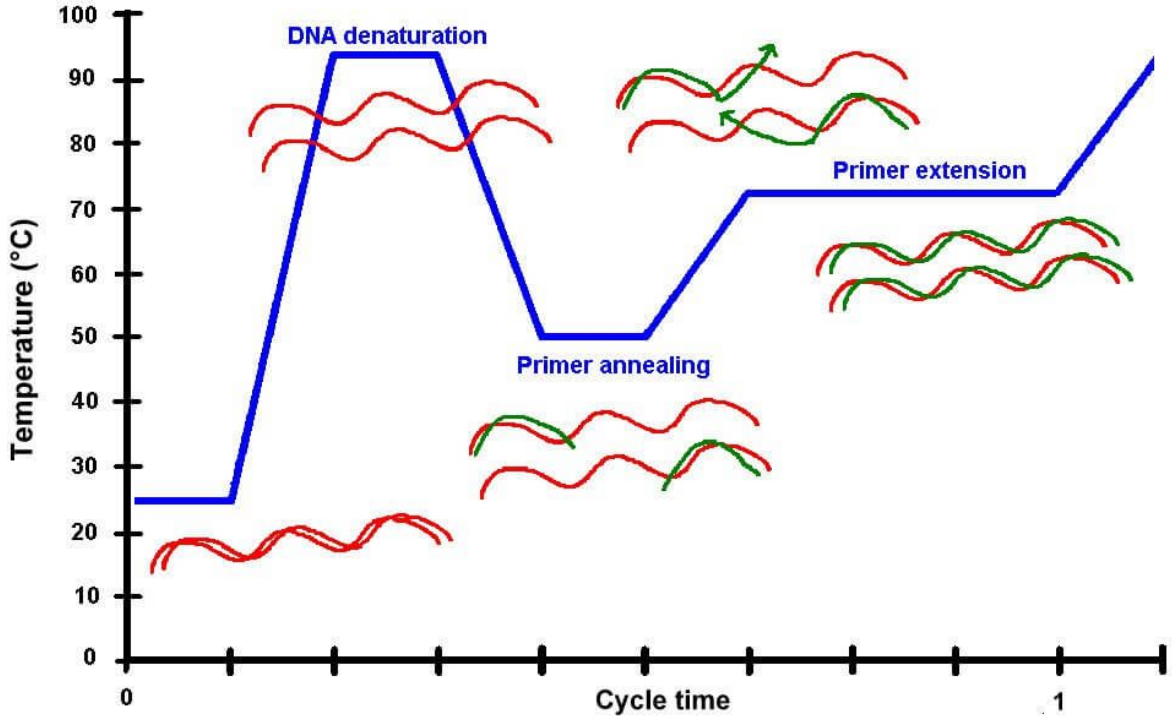
DNA DOUBLES EACH CYCLE

2 → 4 → 8 → 16 → 32 → 64 → 128 COPIES

AFTER 40 CYCLES: 2^{40} or 1,099,511,627,776 COPIES



Steps of the PCR



Types of PCR

RT-PCR

Uses RNA sequences to generate DNA.

qPCR

Quantitative PCR to quantify the amount of DNA in a sample (or detect DNA)

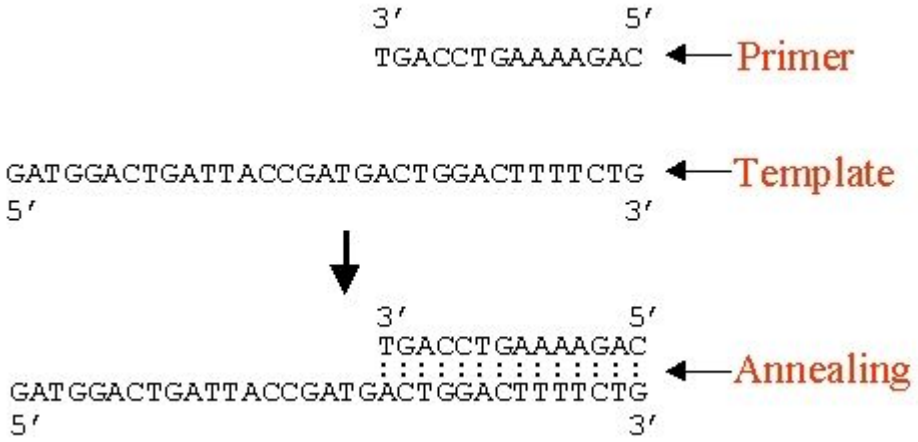
Isothermal PCR

Performs PCR at a constant temperature for even quicker reaction.

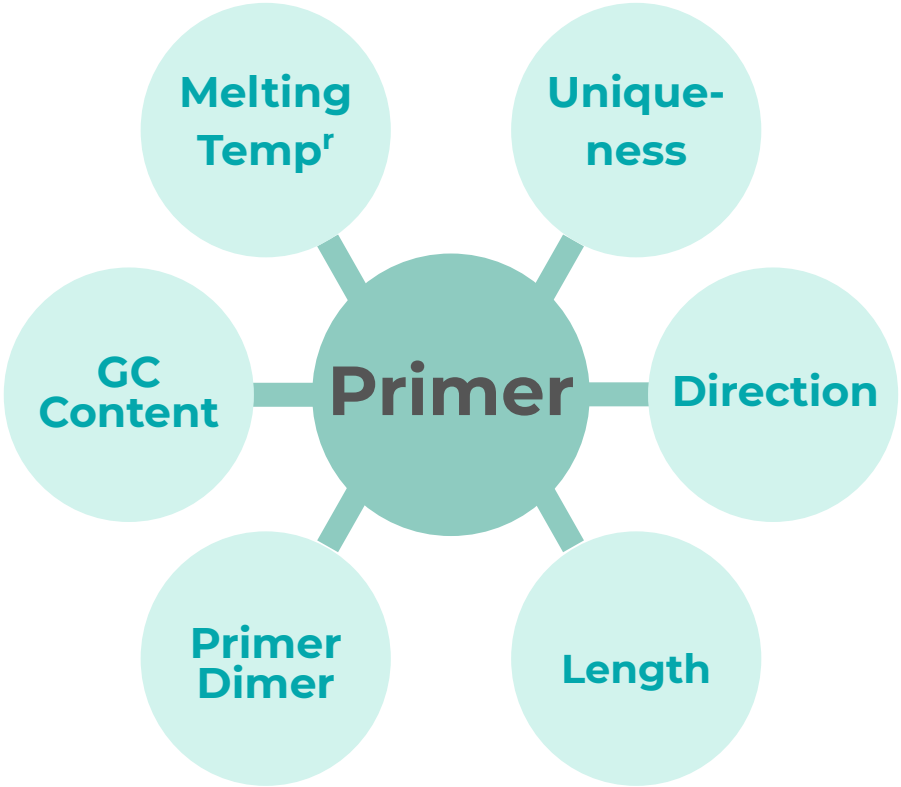
04: Primer Design and Optimisation

DNA Primers

A primer is a short synthetic oligonucleotide which is used in many molecular techniques. These primers are designed to have a sequence which is the reverse complement a region of template or target DNA to which we wish the primer to anneal.



Primer Design



In the next class:

- Further details on primer design
- Introduction to software for designing primers (Check out “Benchling” if you’re curious!)
- Following a case study of an existing project
- Designing your very own primers!

Thank you!