

Lecture-02

PCR and Primer Design



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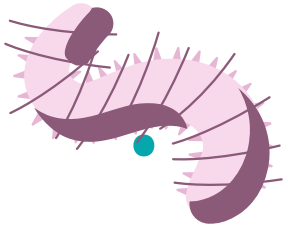
PCR

Overview of the Polymerase Chain Reaction

04

Optimisation

Ways to optimise the PCR mechanism with primer design and more





The code of life

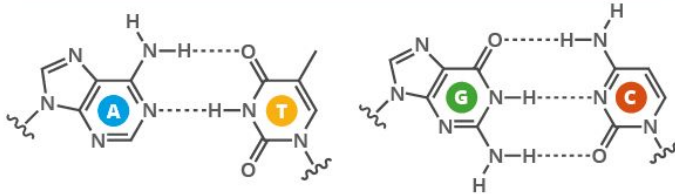
01: DNA Recap

DNA

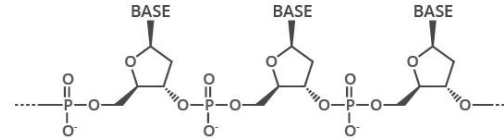
- DNA is a **nucleic acid** that is composed of two complementary nucleotide building block chains.
- The nucleotides are made up of a **phosphate group**, a **five carbon sugar**, and a **nitrogen base**.

WHAT HOLDS DNA STRANDS TOGETHER?

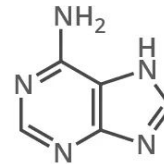
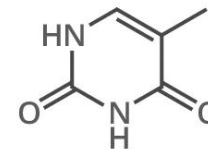
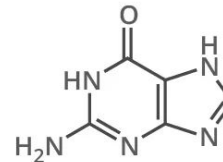
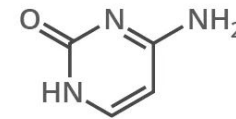
DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



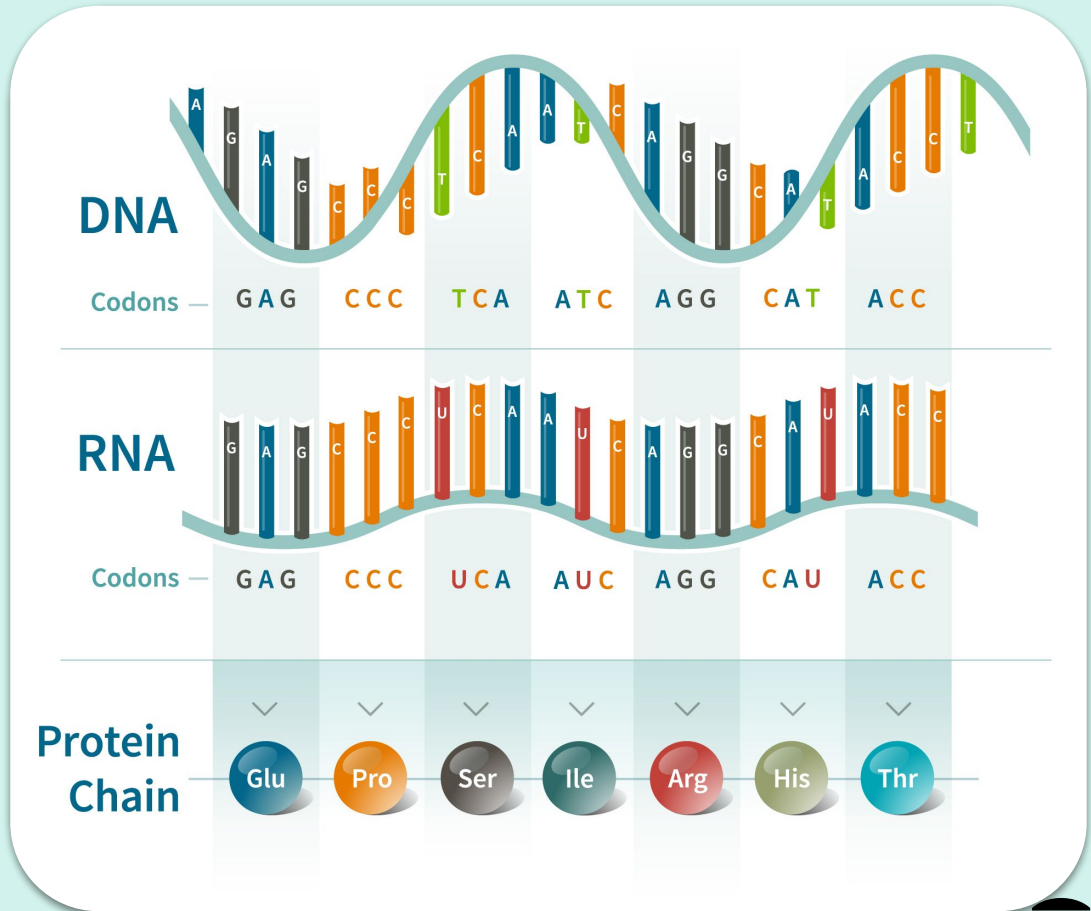
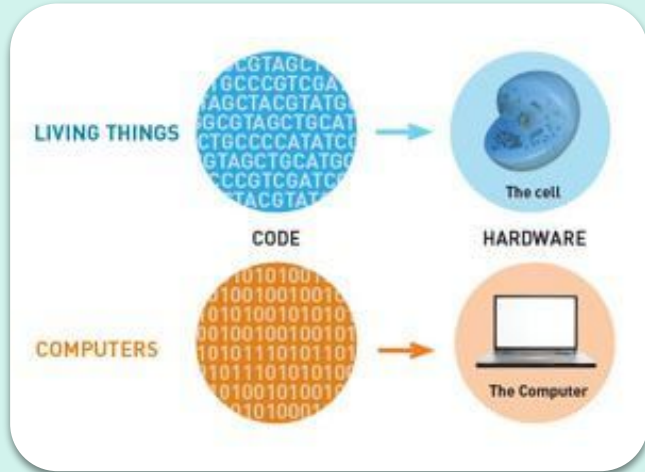
THE SUGAR PHOSPHATE 'BACKBONE'



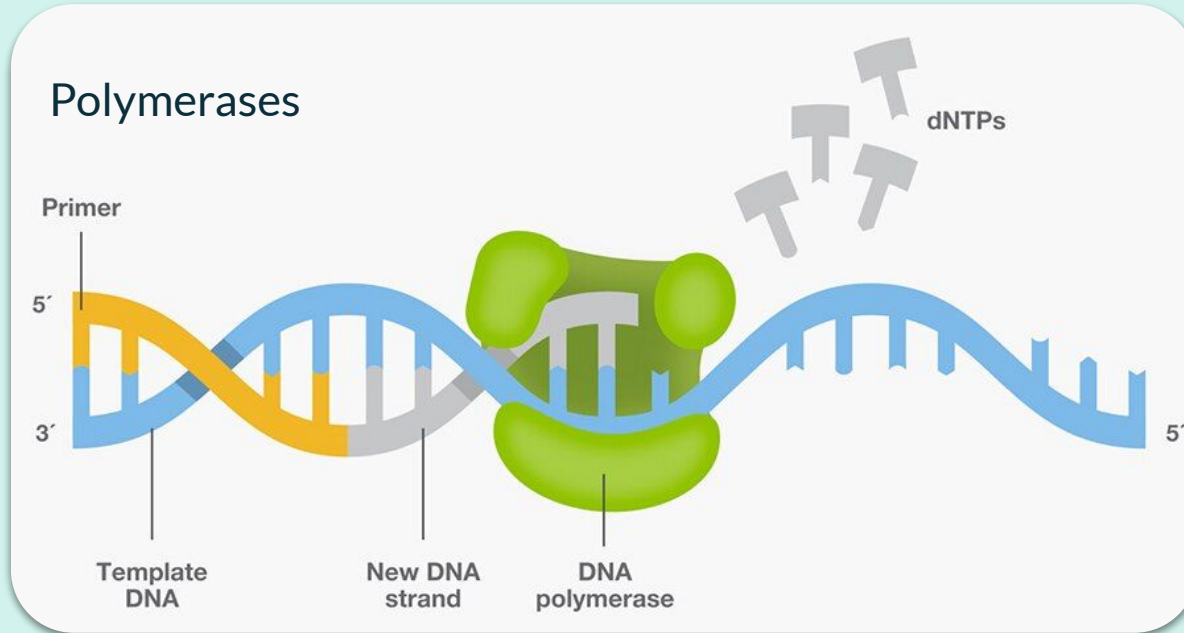
DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

A**ADENINE****T****THYMINE****G****GUANINE****C****CYTOSINE**

DNA Codes



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.



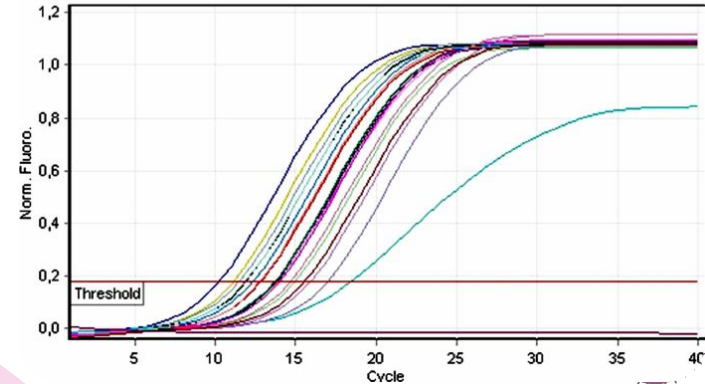
Polymerase Chain Reaction

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

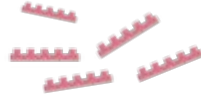


03: PCR Steps Involved

PCR Components



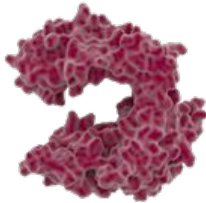
Gene of Interest



Primers



**Nucleotides
(dNTPs)**



Taq Polymerase

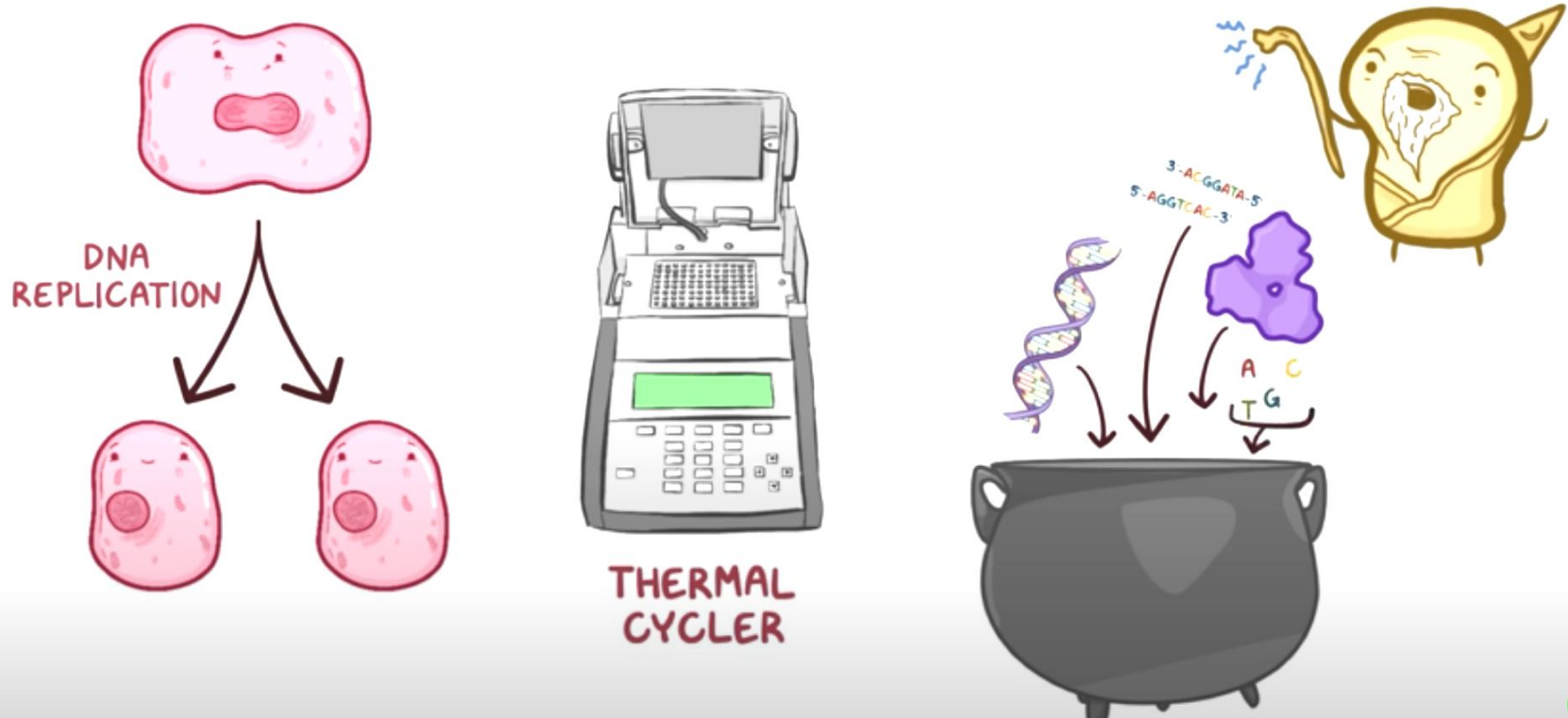


Mix Buffer



PCR Tube

PCR Components



Steps of the PCR



Steps of the PCR

5'-TTCAGGTCACAGTCCCTGTATGCCCTATGTCC-3'
3'-AAGTCCAGTGTCAAGGACATACGGATACAGG-5'

1. DENATURATION ↓ HEAT to 96 DEGREES CELSIUS

5'-TTCAGGTCACAGTCCCTGTATGCCCTATGTCC-3'
3'-AAGTCCAGTGTCAAGGACATACGGATACAGG-5'



Steps of the PCR

5'-TTCAGGTCACAGTCCCTGTATGCCCTATGTCC-3'
3'-AAGTCCAGTGTCAGGACATACGGATACAGG-5'

1. DENATURATION ↓ HEAT to 96 DEGREES CELSIUS

5'-TTCAGGTCACAGTCCCTGTATGCCCTATGTCC-3'

3'-AAGTCCAGTGTCAGGACATACGGATACAGG-5'

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS

5'-TTCAGGTCACAGTCCCTGTATGCCCTATGTCC-3'
3'-CGGATAC-5'

5'-AGGTCAC-3'

3'-AAGTCCAGTGTCAGGACATACGGATACAGG-5'



Steps of the PCR

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS



*Thermus
aquaticus*



TAQ
POLYMERASE



3. EXTENSION

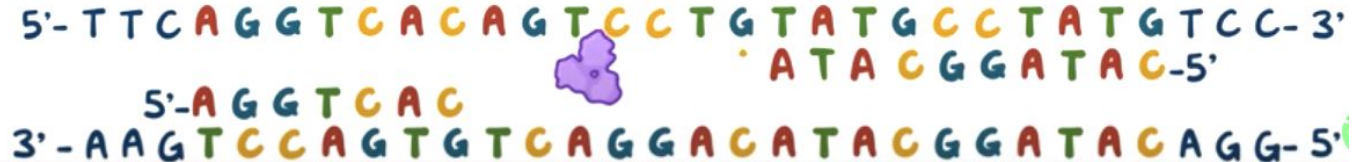
* FUNCTIONS BEST
at 72 DEGREES
CELSIUS

Steps of the PCR

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS



3. EXTENSION ↓ HEAT to 72 DEGREES CELSIUS



Steps of the PCR

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS



3. EXTENSION ↓ HEAT to 72 DEGREES CELSIUS



Steps of the PCR

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS



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Steps of the PCR

2. ANNEALING ↓ COOL to 55 DEGREES CELSIUS



3. EXTENSION ↓ HEAT to 72 DEGREES CELSIUS



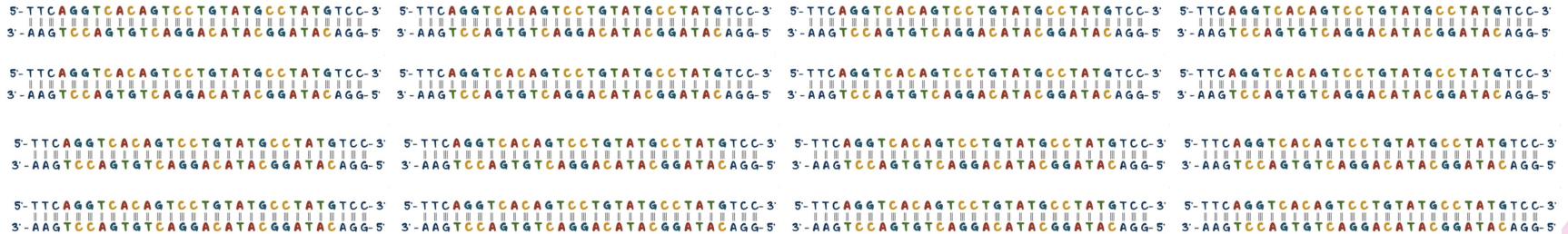
Steps of the PCR



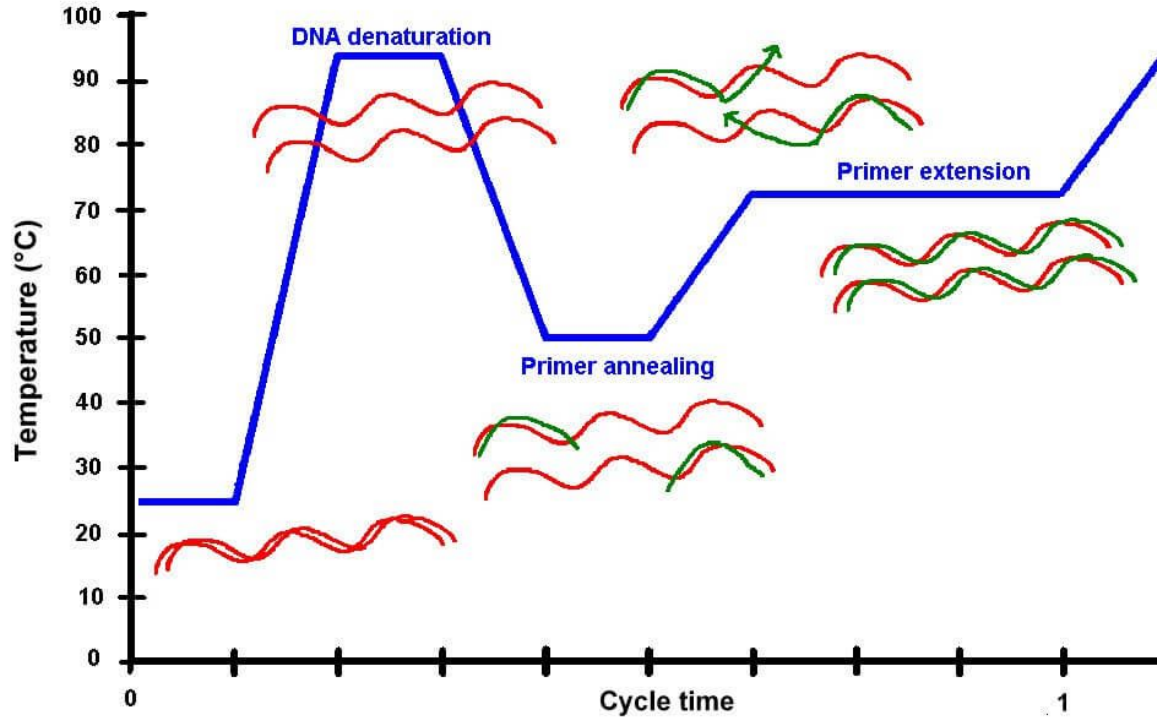
DNA DOUBLES EACH CYCLE

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

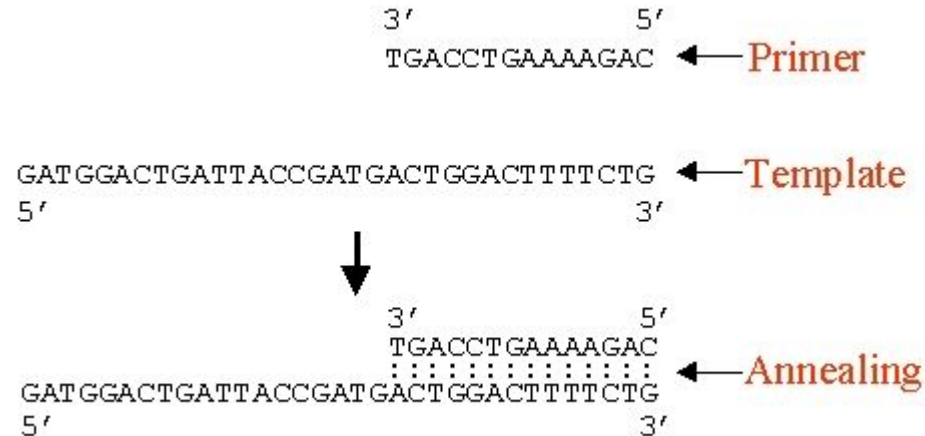
Performs PCR at a constant temperature for even quicker reaction.

The background is a dark navy blue field filled with stylized, colorful illustrations. There are three prominent DNA double helices: one in the top left with yellow and blue strands, one in the center with blue and red strands, and one in the bottom right with red and yellow strands. Scattered throughout are various geometric shapes: red hexagons, blue hexagons, green rounded rectangles, and white organic shapes. The overall style is modern and scientific.

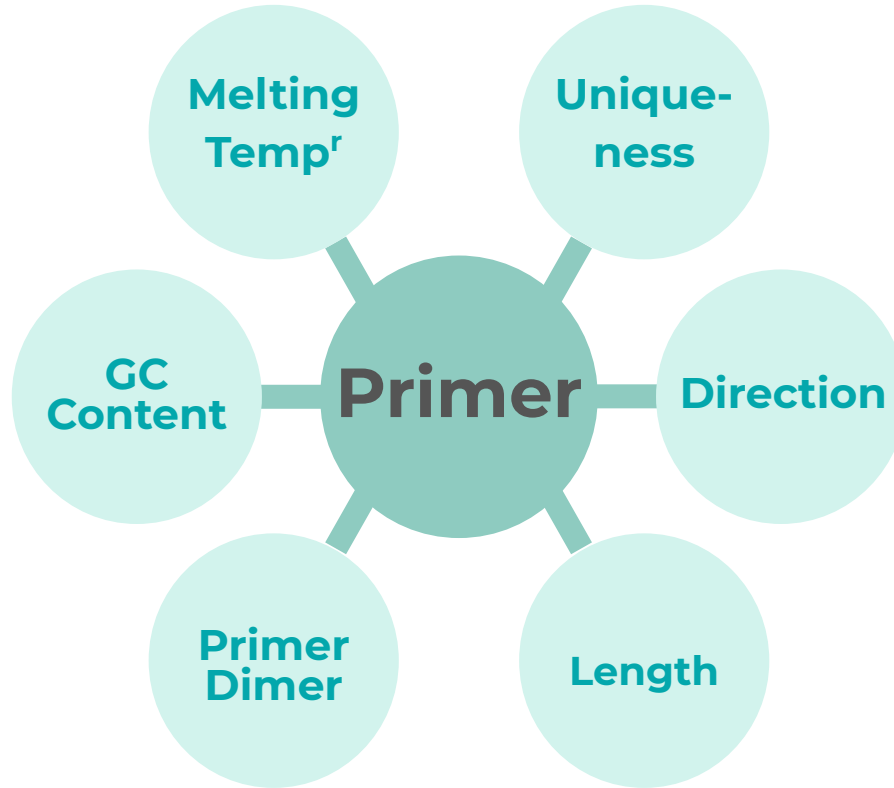
04: Primer Design and Optimisation

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!