PCR Primer: from Design to Order

By

A/Prof Dr Layla Hashim

A/Prof Dr Anwar Ibrahim

19th-20th March 2024

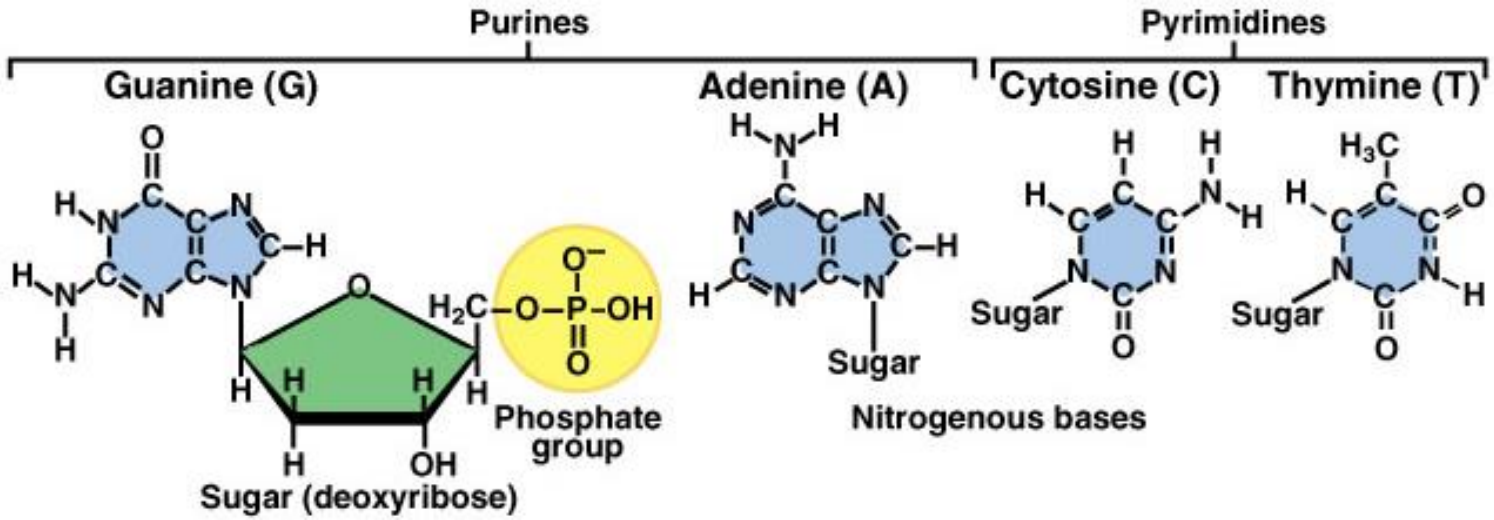
Workshop goals and Objectives

In this workshop, participants will be able:

- **To learn how to design primer.**
- **To become familiar with primer criteria .**
- **To be sure from the efficiency and the accuracy of the PCR technique.**
- **To be familiar with Primer order.**

DNA
Deoxyribonucleic Acid

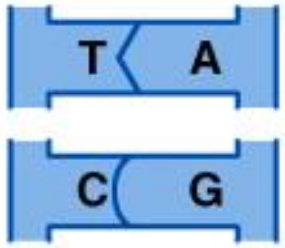
- **DNA is a chain of nucleotides**
- **Nucleotides are composed of:**
  - **Phosphate**
  - **Sugar - deoxyribose**
  - **Base - one of four types: adenine (A), thymine (T)**
    guanine (G), cytosine (C)



DNA has directionality

DNA is double stranded

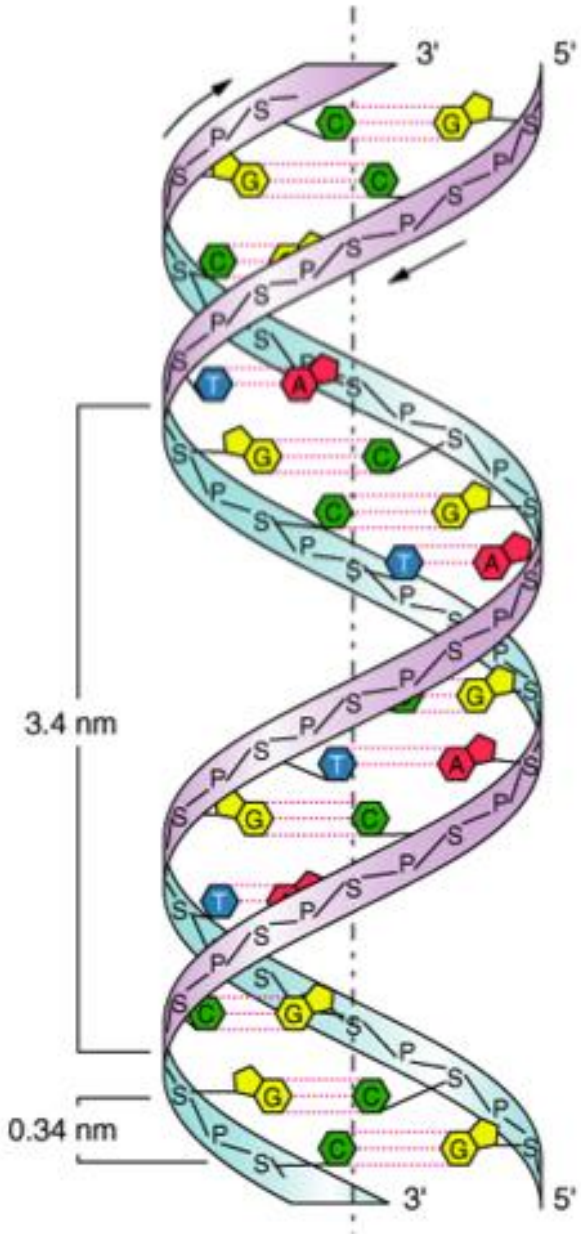
- New bases are always added to the 3’ end
  - DNA is synthesised in the 5’ to 3’ direction
- DNA bases pairs with bases on opposite strand via hydrogen bonds
  - thymine can only pair with adenine
  - cytosine can only pair with guanine



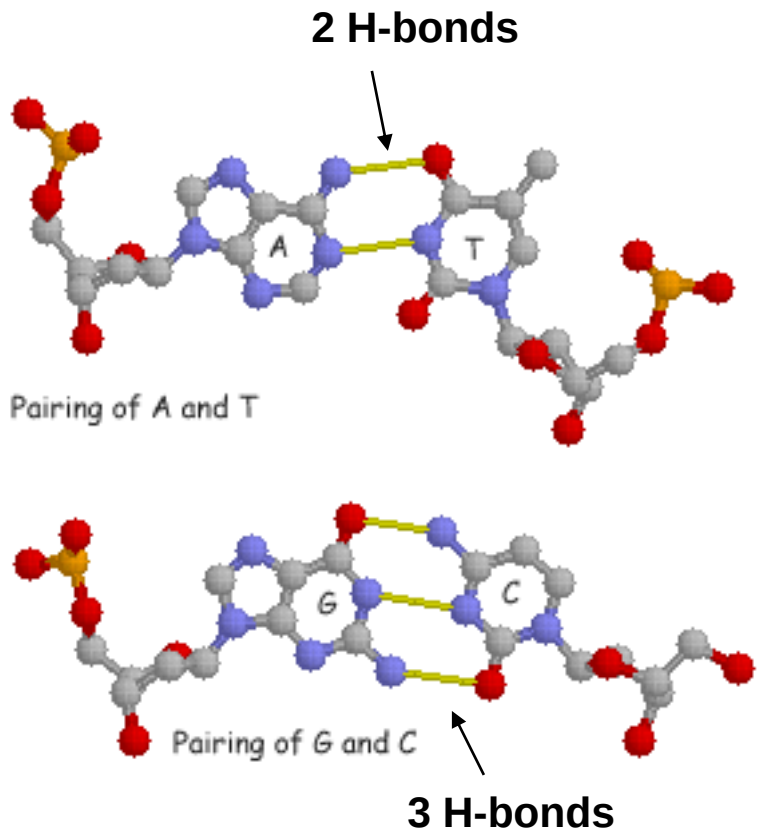
Because of this pairing, each strand is complementary to the other

5’ ACGTC 3’

3’ TGCAG 5’

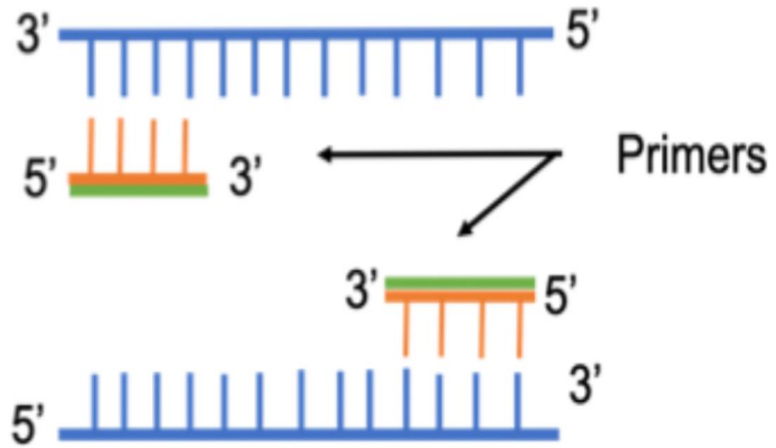


G/C base pair stronger than an A/T base pair



Purine/Pyrimidine base pairing

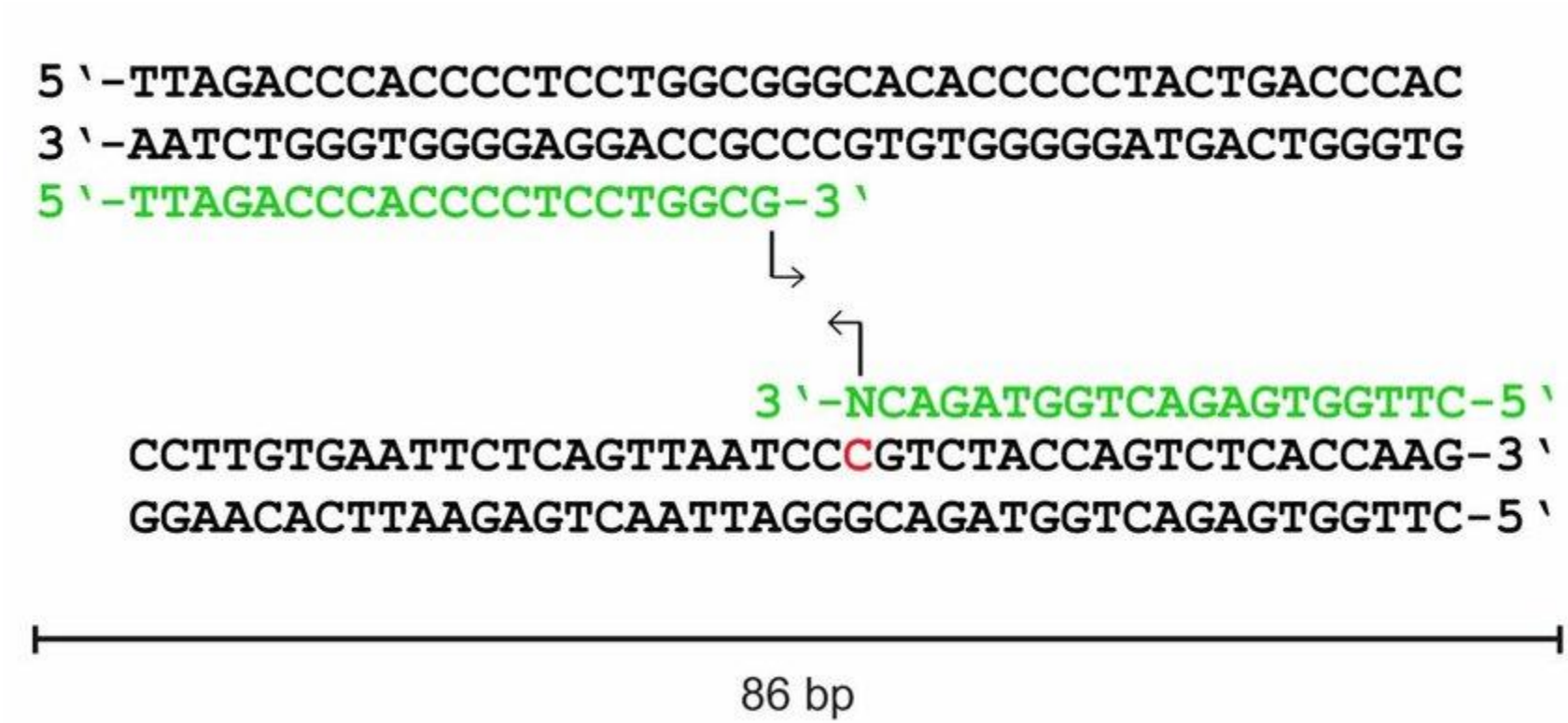
What is the Primer?

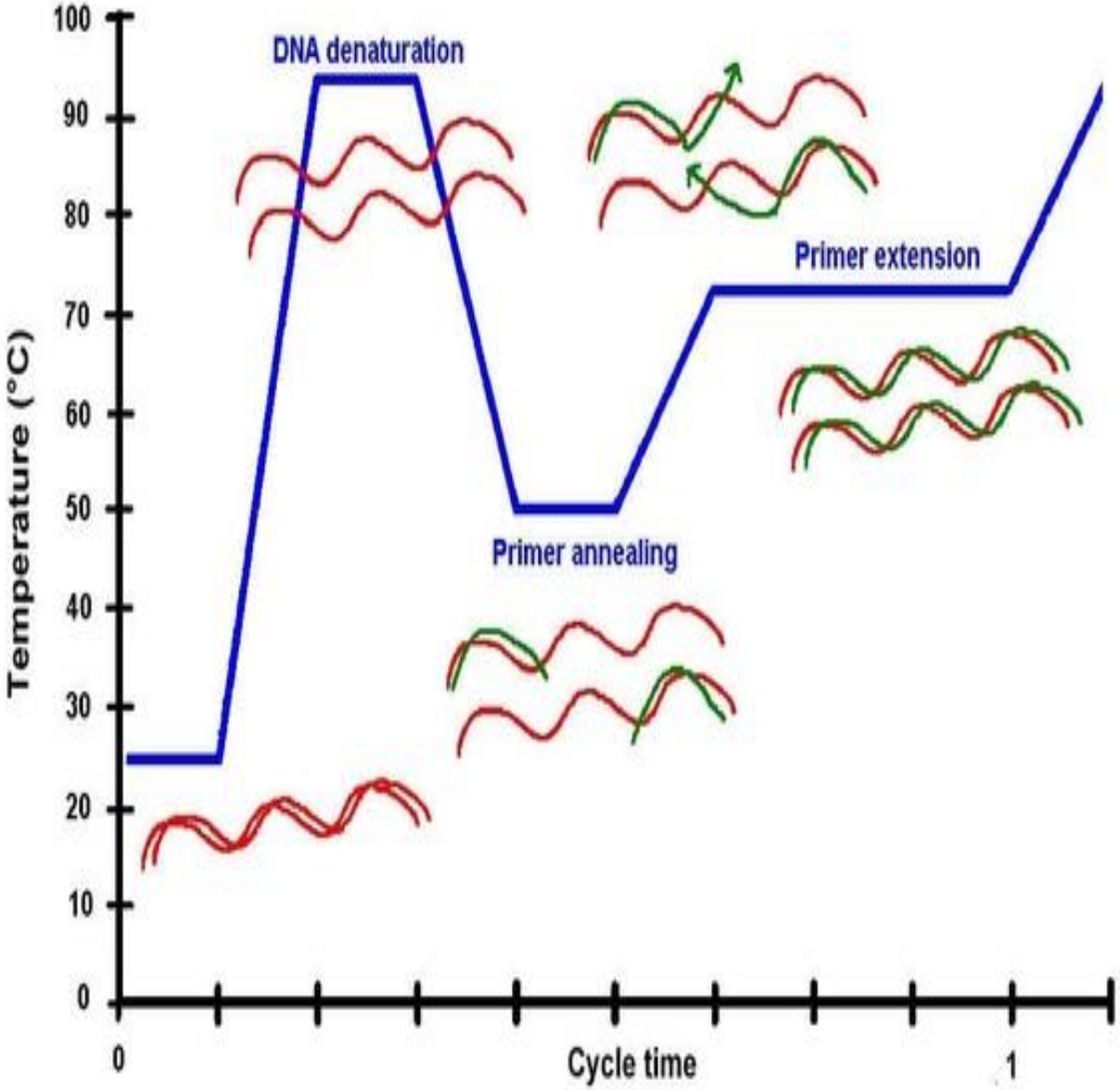


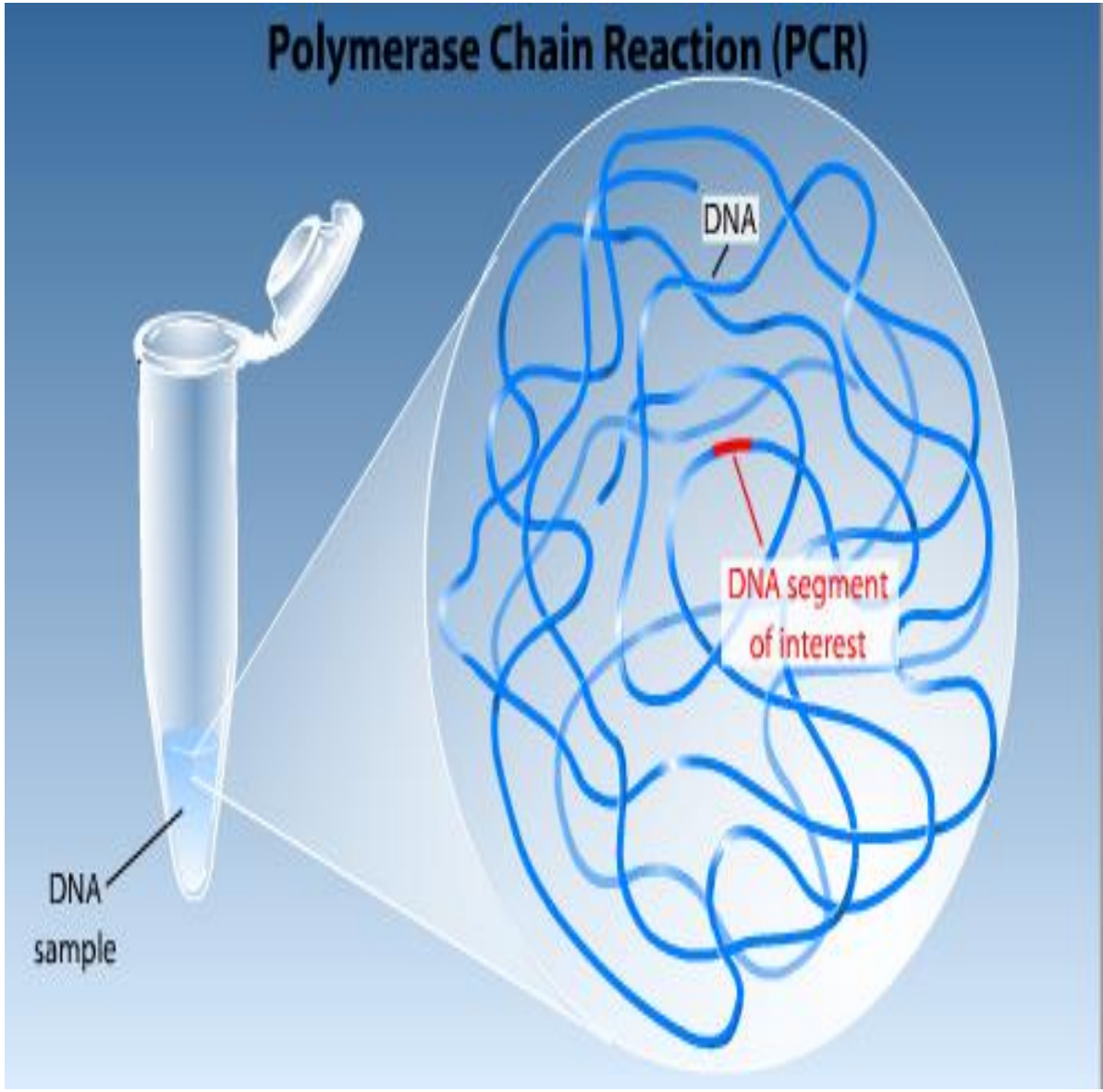
A primer, as related to genomics, is a short single-stranded DNA fragment used in certain laboratory techniques, such as the polymerase chain reaction (PCR).

It can be a set of primers (forward and reverse) with a sequence complementary to the template DNA- a point of initiation synthesis .

The **forward primer** runs in **3'-5'** while the **reverse primer** runs in **5'-3'.** However process of extension results in two new strands of ds DNA.

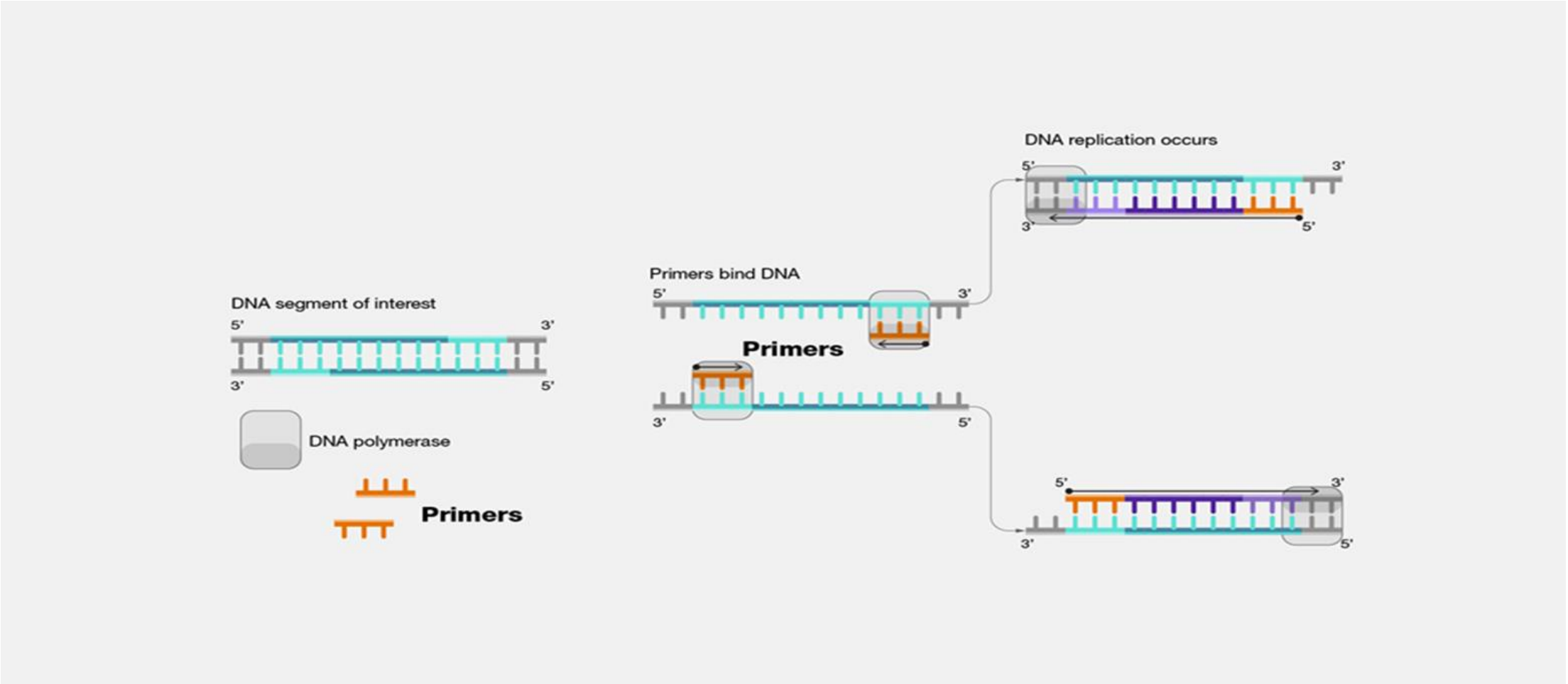

5'-TTAGACCCACCCCTCCTGGCGGGCACACCCCCTACTGACCCAC
3'-AATCTGGGTGGGGAGGACCGCCCGTGTGGGGGATGACTGGGTG
5'-TTAGACCCACCCCTCCTGGCG-3'
3'-NCAGATGGTCAGAGTGGTTC-5'
CCTTGTGAATTCTCAGTTAATCCCGTCTACCAGTCTCACCAAG-3'
GGAACACTTAAGAGTCAATTAGGGCAGATGGTCAGAGTGGTTC-5'
86 bp


DNA denaturation
Primer extension
Primer annealing
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
0
Cycle time
1

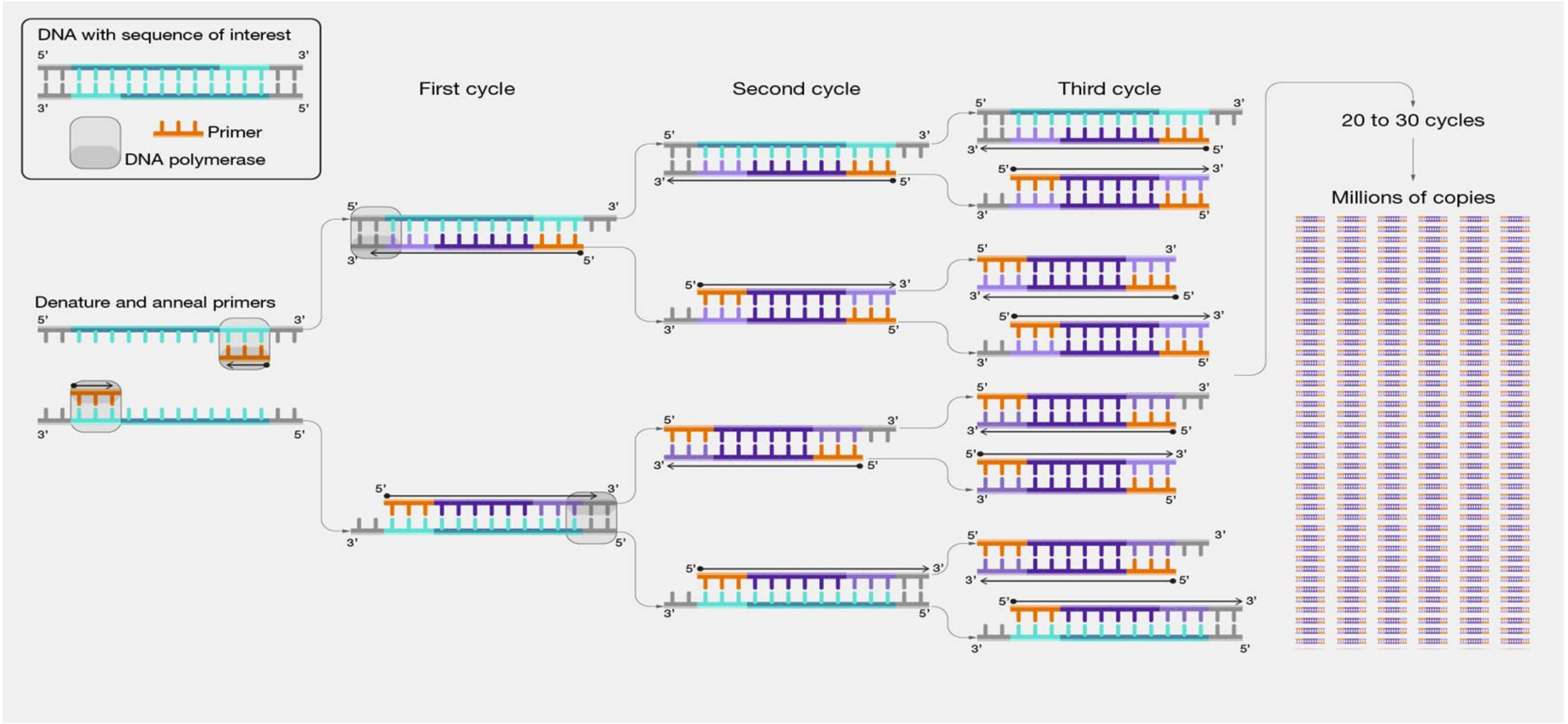

Polymerase Chain Reaction (PCR)
DNA
DNA segment
of interest
DNA
sample

In the PCR method, a pair of primers hybridizes with DNA sample and defines the region that will be amplified, resulting in billions of copies in a very short timeframe.

Primers are also used in DNA sequencing and other experimental processes.



Polymerase Chain Reaction (PCR)



Types of Primers

- DNA primers
- RNA primers

DNA Primers: *In vitro*: PCR amplification, DNA sequencing.
RNA Primers: *In vivo*: DNA replication, cloning.

Reaction
DNA primers: The process of amplification is temperature dependent with fewer proteins.
RNA primers: The replication process is a catalytic reaction in an enzyme-dependent manner with several proteins.

Length
DNA primers: 18- 24 base pairs.
RNA primers: 10- 20 base pairs.

Synthesis: DNA primers are synthesized chemically while RNA primers require Primase enzyme.

DNA primers are long-lived and more stable while RNA primers are short-lived and are more reactive.

General Rules for Primer Design

1. **Primer Length:** It is generally accepted that the optimal length of PCR primers is 18-22 bp. This length is long enough for adequate specificity and short enough for primers to bind easily to the template at the annealing temperature.

If primer is too short, the annealing temp becomes lower and reduce amplification. If primer too long, the annealing temp will be high and leads to non-specific binding .

* amplicon size 50-150 bp

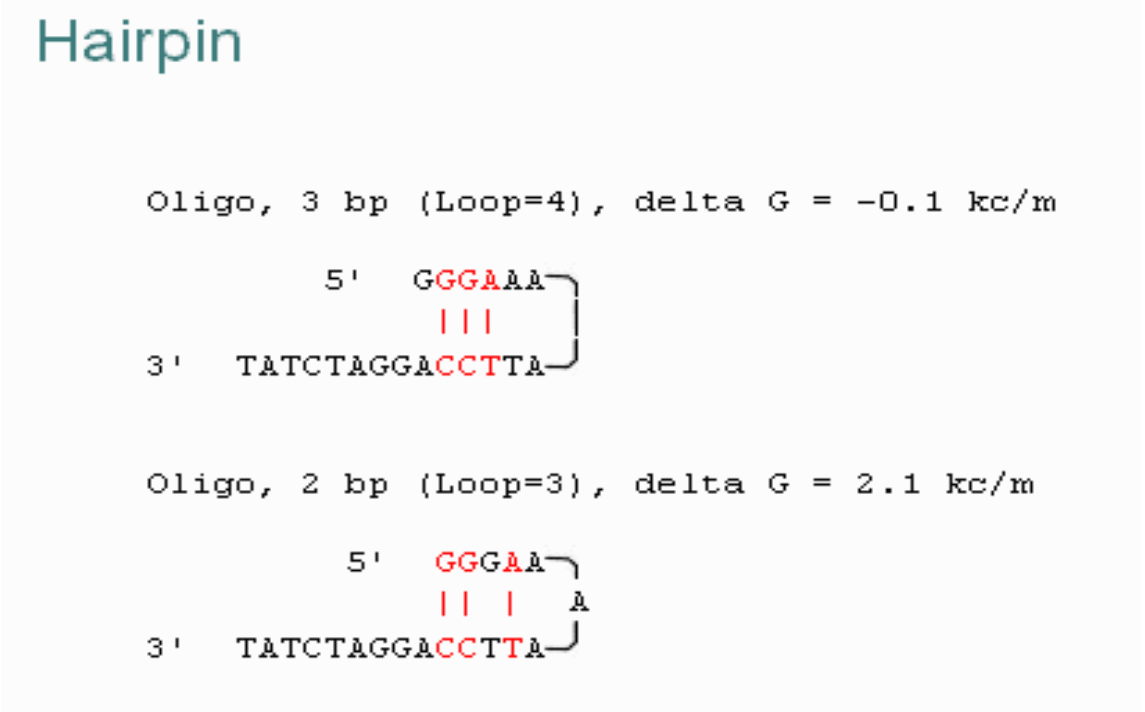
2. Primer Melting Temperature

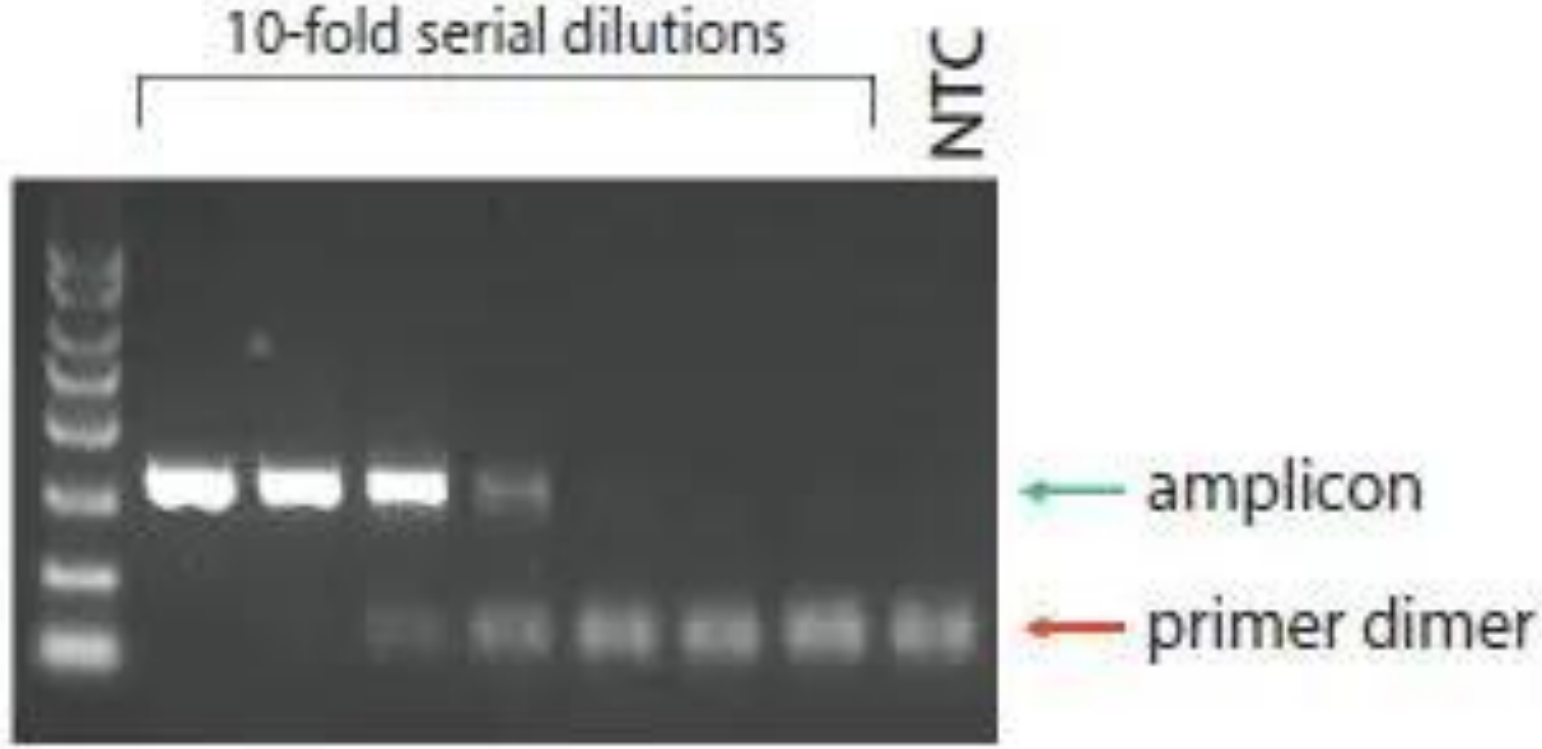
Primers with melting temperatures in the range of **52-58 °C** generally produce the best results. Primers with melting temperatures above **65°C** have a tendency for secondary annealing.

3. GC Content: The GC content (the number of G's and C's in the primer as a percentage of the total bases) of primer should be 40-60%.

4. **GC Clamp:** The presence of G or C bases within the last five bases from the 3' end of primers (GC clamp) helps promote specific binding at the 3' end due to the stronger bonding of G and C bases. More than 3 G's or C's should be avoided in the last 5 bases at the 3' end of the primer.

5 . Primer Secondary Structures: Presence of the primer secondary structures produced by intermolecular or intramolecular interactions can lead to poor or no yield of the product. They adversely affect primer template annealing and thus the amplification. They greatly reduce the availability of primers to the reaction.




10-fold serial dilutions
NTC
amplicon
primer dimer

6 . Repeats: A repeat is a di-nucleotide occurring many times consecutively and should be avoided because they can misprime.
For example: ATATATAT. A maximum number of di-nucleotide repeats acceptable in an oligo is 4 di-nucleotides.

7 . Runs: Primers with long runs of a single base should generally be avoided as they can misprime. For example, AGCGGGGGATGGGG has runs of base 'G' of value 5 and 4. A maximum number of runs accepted is 4bp.

Steps to design Primers

Go to the following web site :-
http://www.ensembl.org/index.html
You should select:-
- Animal species
- Your target gene
- The cDNA sequence
- Download the sequence

Go to this web site

http://primer3.wi.mit.edu

Here you should :-

- Paste the cDNA sequence
- Choose product size ranges (amplicon size 50-150)
- Pick primer

In this web site

https://www.ncbi.nlm.nih.gov/tools/primer-blast/

- You should paste the sequence of primer (forward and reverse)
- Tick get the primer

Another way for Primer Design

Steps to design Primers

1) Go to the web site of the National Center for Biotechnology Information (NCBI) by opening the following location: **https://www.ncbi.nlm.nih.gov/**

2) Find “Blast” (Basic Local Alignment Search Tool) in right menu bar and click on it.

Steps to design Primers

3) Click on “Primer BLAST”

4) Copy FASTA format of sequence including “>” from NCBI nucleotide database and paste it in PrimerBlast.

5) Click on “Get Primers”, then get detailed primer reports.

Make to Order

1 Customer

Places order with specifications

2 Production

3

Customized Product

Receives Delivery

Make To Order

Go to this Website

https://www.thermofisher.com/order/custom-standard-oligo/