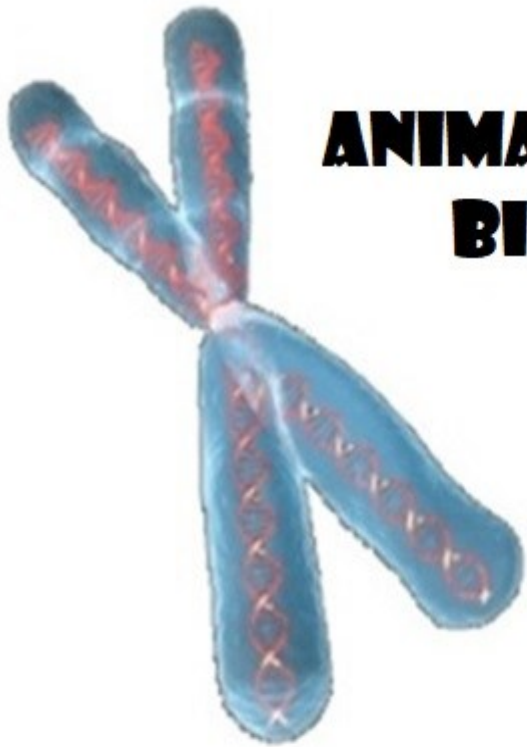


Lab: 4



زانكۆی سه‌لاحه‌دین - ههولیر  
Salahaddin University-Erbil

**PRACTICAL**



# **ANIMAL BIOTECHNOLOGY**

**Subject :**

**PRIMER DESIGN & BLAST  
PRIMER3 & NCBI**

**Department: Animal Resources**

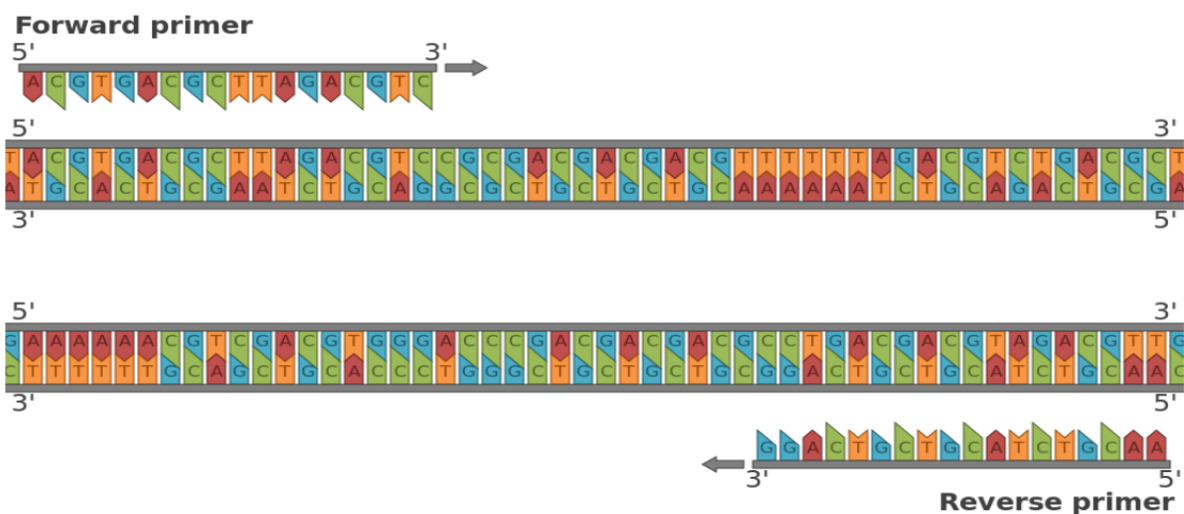
**Stage : 4**

**Done By :** *Lecturer: Kamaran M. Taha*

**Date :** */ 2 / 2023*

## Introduction:

A primer: is a strand of short nucleic acid sequences that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3'-end of the primer, and copies the opposite strand.



For a successful and reliable PCR requires efficient and specific amplification of the product, using chemically synthesized oligonucleotides – DNA primers.

## Good Primer's Characteristic:

1. Uniqueness: ensure correct priming site (There should be one and only one target site in the template DNA where the primer binds, which means the primer sequence should be unique in the template DNA, avoiding the possibility of mis-hybridization to a similar sequence nearby).

2. Length: 18-28 bases. (The longer the primer, the more chance that it is unique; and the longer the primer, the higher melting/annealing temperature – specificity)

3. Base composition: average (G+C) content around 40-60%; avoid long (A+T) and (G+C) rich region if possible (Avoid long (A+T) and (G+C) rich region if possible.

Repeats: A repeat is a dinucleotide occurring many times consecutively and should be avoided because they can mis-prime. A maximum number of dinucleotide repeats acceptable in an oligo is 4 dinucleotides). Template DNA

5'..TCGATATATATGCATG...GATGCCGGCGCGCTGTACACAA..3'

4. Optimize base pairing: The presence of G or C bases within the last five bases from the 3' end of primers (GC clamp) to enhance annealing of the end which will be extended 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 – mis-priming

5'-CCGATATGCCAGCTATCTGT-3'

5. Melting Temperature  $T_m$ : The temperature at which 50% of the primer molecules are bound to their corresponding target sequence.  $T_m$  is characteristics of the DNA/Base composition; Higher G+C content DNA, has a higher  $T_m$  due to more Hydrogen-bonds. (**3 vs. only 2 in A::T**). in general (52 -65 °C).

6. Assure that F/R primers have annealing temperature within 2 – 3 °C of each other  
Annealing Temperature, T<sub>a</sub>, anneal – the temperature at which primers anneal to the template DNA.

7. Minimize internal secondary structure: hairpins and dimers should be avoided (minimize self-complementarity and 3' end self-complementarity). If primers can anneal to themselves or anneal to each other (primer dimer) rather than anneal to the template.

