The polymerase chain reaction (PCR)

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Lab 7

- Definition
- PCR principle
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PCR

- The polymerase chain reaction(PCR): is a scientific technique in molecular biology to amplify single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- Developed in 1983 by Kary Mullis.

PCR Principle

- PCR is used to amplify a specific region of a DNA strand (the DNA target).
- A basic PCR set up requires several components.
- <u>These components include:</u>

- DNA template:

DNA template is DNA target sequence.

- DNA polymerase:

DNA polymerase sequentially adds nucleotides complimentary to template strand at 3'-OH of the bound primers and synthesizes new strands of DNA complementary to the target sequence. The most commonly used DNA polymerase is Taq DNA polymerase (from Thermos aquatics, a thermophiles bacterium)because of high temperature stability.

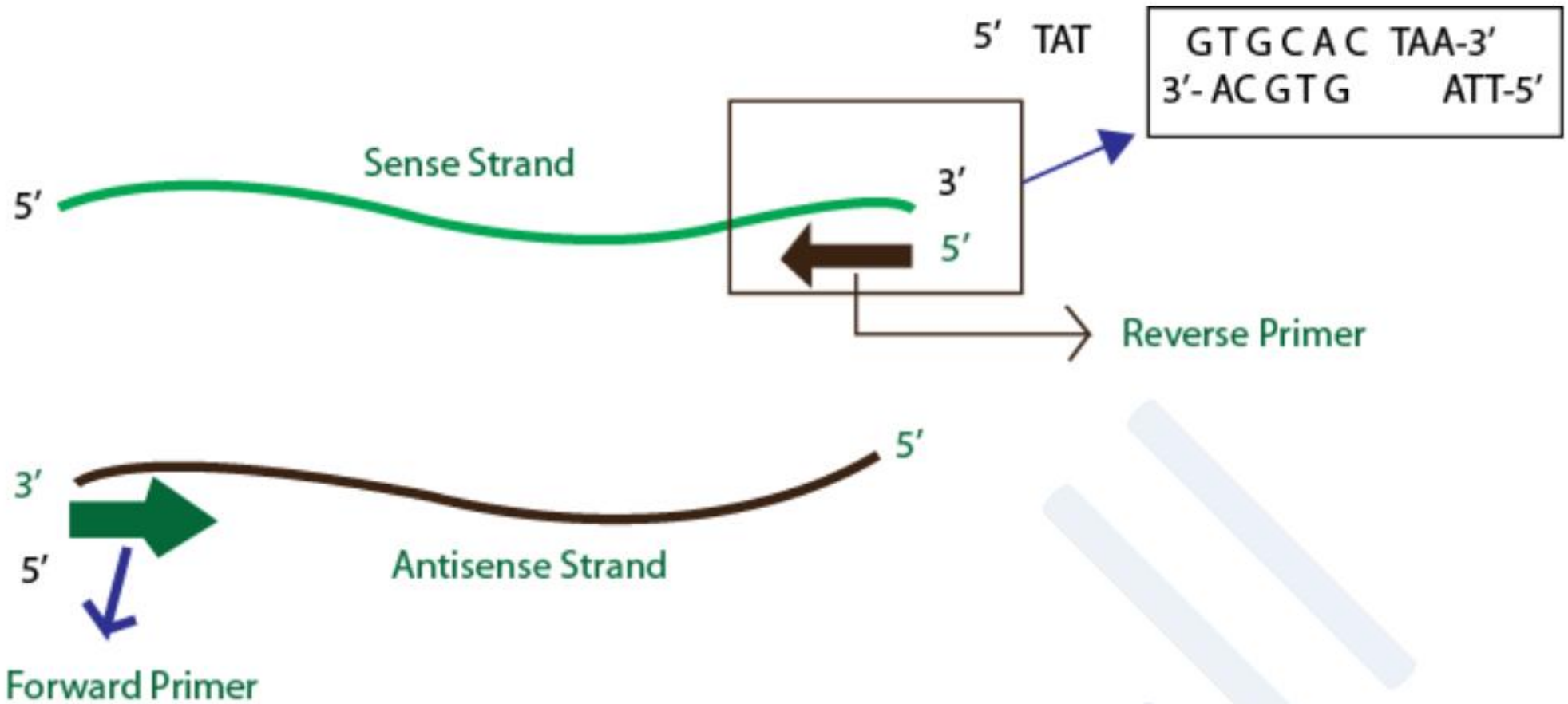
- Nucleotides (dNTPs or deoxynucleotide triphosphates):

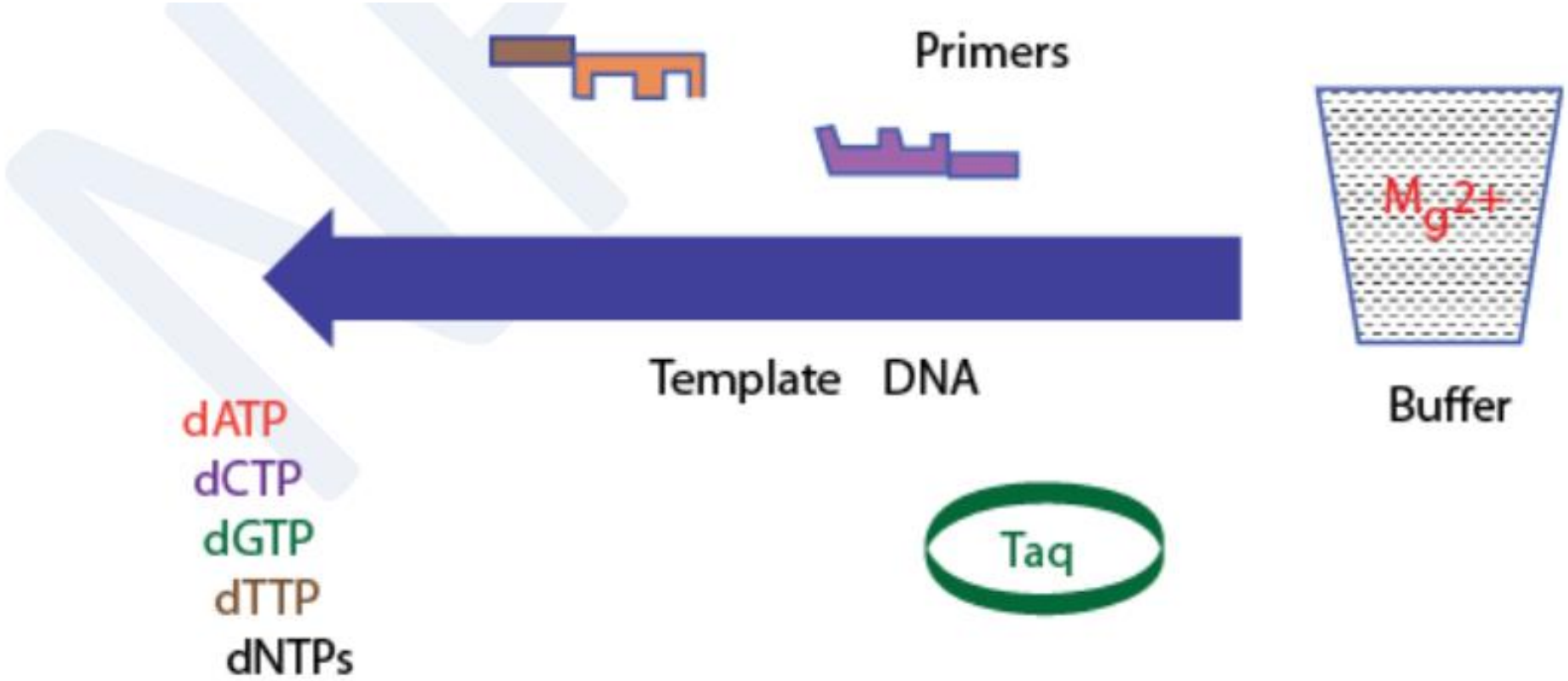
All types of nucleotides are "building blocks" for new DNA strands and essential for reaction. It includes: Adenine(A), Guanine(G),Cytosine(C), Thymine(T).

- Primers:

Primers are 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 primers must be designed for PCR; the forward primer and the reverse primer. The forward primer is complimentary to the 3' end of antisense strand (3'-5') and the reverse primer is complimentary to the 3' end of sense strand(5'-3').


5′ TAT
GTGCAC TAA-3′
3′- ACGTG ATT-5′
Sense Strand
5′
3′
5′
Reverse Primer
3′
5′
5′
Antisense Strand
Forward Primer


Primers
Mg^{2+}
Template DNA
Buffer
dATP
dCTP
dGTP
dTTP
dNTPs
Taq

PCR procedure

- There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on the Thermocycler, which can heat and cool the tubes with the reaction mixture in a very short time

- 1.Denaturation at 94°C :

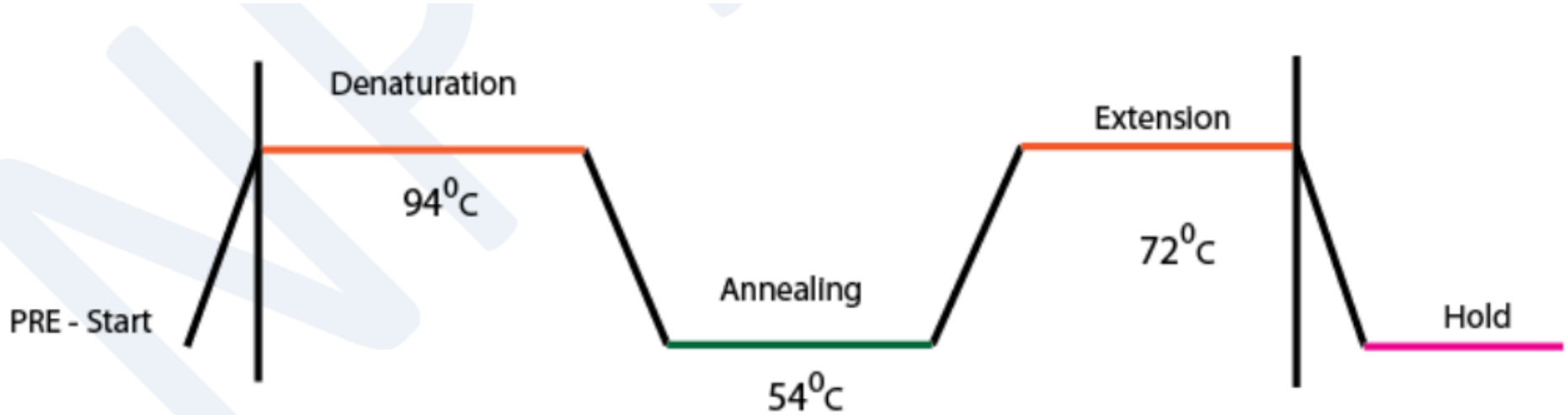
During the heating step (denaturation), the reaction mixture is heated to 94°C for 1 min, which causes separation of DNA double stranded. Now, each strand acts as template for synthesis of complimentary strand.

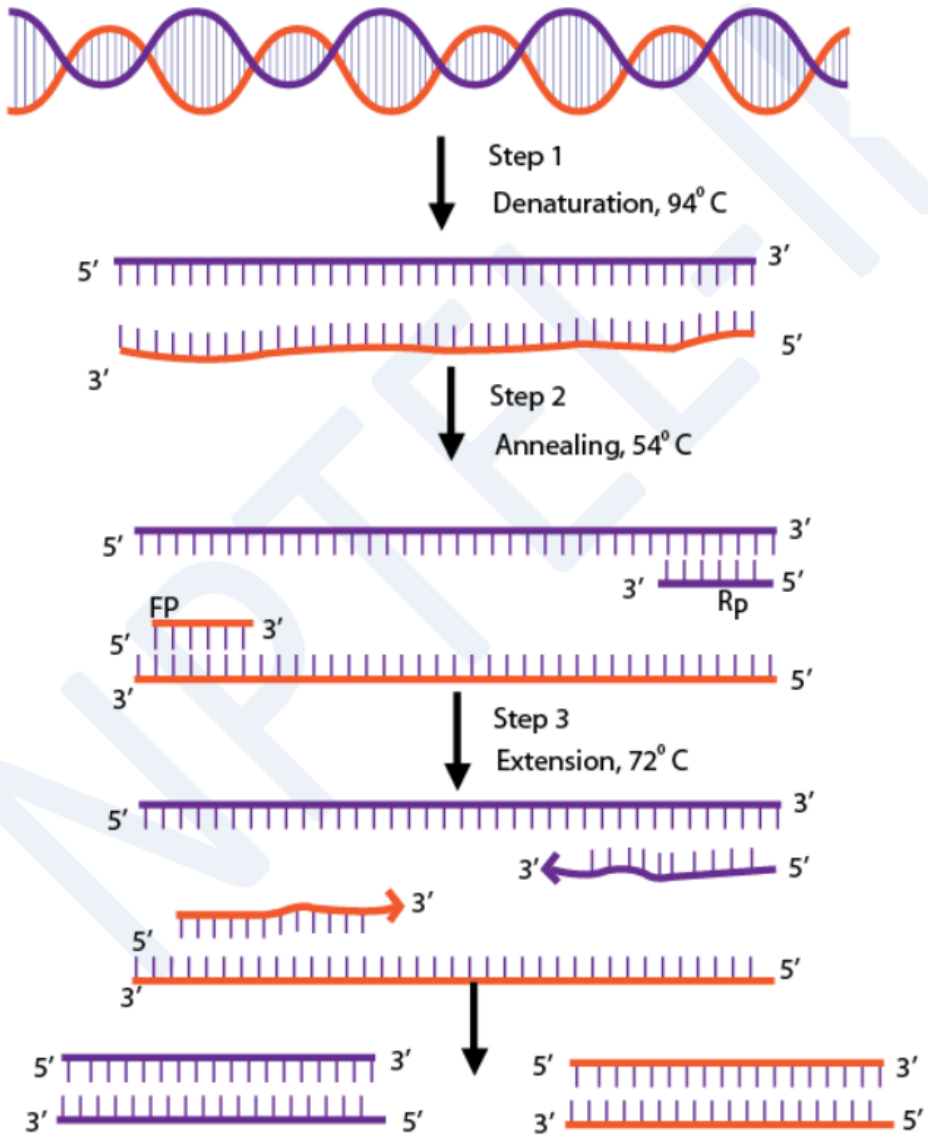
- 2.Annealing at 54°C :

This step consist of cooling of reaction mixture after denaturation step to 54°C,which causes hybridization (annealing) of primers to separated strand of DNA (template).

3.Extension at 72°C :

The reaction mixture is heated to 72°C which is the ideal working temperature for the Taq polymerase. The polymerase adds nucleotide (dNTP's) complimentary to template on 3' –OH of primers thereby extending the new strand


Denaturation
94⁰C
Extension
72⁰C
Annealing
54⁰C
PRE - Start
Hold


Step 1
Denaturation, 94° C
5′
3′
3′
5′
Step 2
Annealing, 54° C
5′
3′
3′
5′
Rp
FP
5′
3′
3′
5′
Step 3
Extension, 72° C
5′
3′
3′
5′
3′
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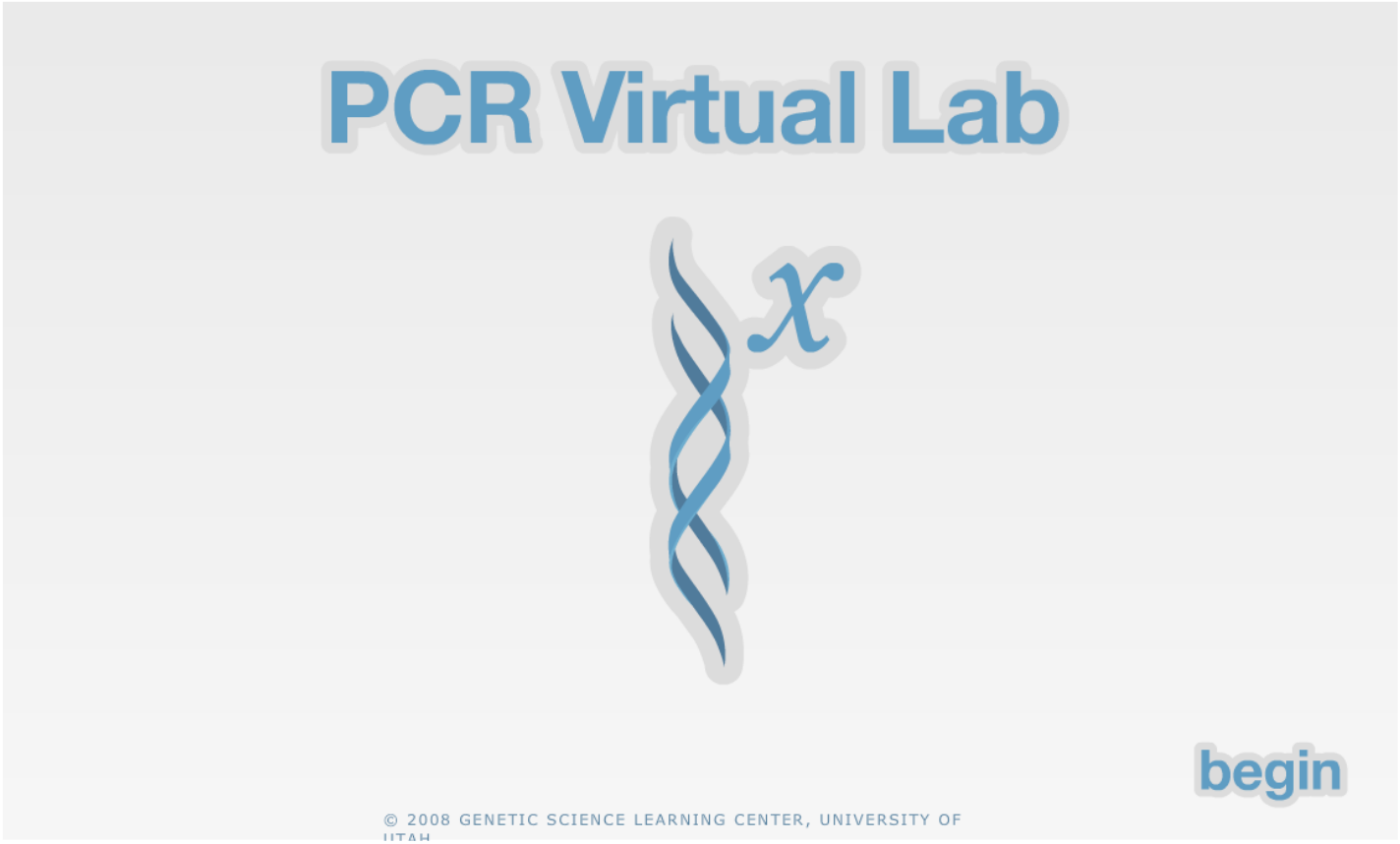
5′

PCR virtual lab



Reference

- https://nptel.ac.in/courses/102103017/pdf/lecture%2037.pdf
- http://fac.ksu.edu.sa/sites/default/files/358_bot.pdf