ECONOMIC BOTANY AND PLANT BIOTECHNOLOGY

Assignment

On

PCR and RT-PCR

Tanishq

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B.Sc.(H) Zoology

iV Semester

POLYMERASE CHAIN REACTION

- PCR is an effective procedure for generating large quantities of a specific DNA sequence in vitro, without its cloning.
- Invented by American biochemist Kary Mullis in 1983.
- Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs in length, although some techniques allow for amplification of fragments upto 40 Kbp.

- The essential components for PCR amplification are:

a) DNA template containing the region to be amplified,

b) a thermostable DNA polymerase that can withstand being heated to 95°C or higher and that copies DNA with high fidelity,

c) two synthetic oligonucleotide (~20 nucleotides each) primers that are complementary to 3' ends of each of the sense and anti-sense strands of DNA target

d) deoxynucleoside triphosphates, or dNTPs, the building blocks from which the DNA polymerase synthesizes a new DNA strand.

e) a buffer solution

f) bivalent cations (Mg^{2+} or Mn^{2+}).

- The reaction is commonly carried out in a volume of 10–200 µL in small reaction tubes (0.2–0.5 mL) in a thermal cycler, which heats and cools the reaction tubes to achieve the temperatures required at each step.
- Thin-walled reaction tubes permit favourable thermal conductivity to allow for rapid thermal equilibrium.

Procedure of the Polymerase Chain Reaction:

- PCR typically consists of a series of 20-40 repeated temperature changes with each cycle of changes commonly consisting two or three discrete temperature steps.

- Temperatures used and the duration to which they are applied in each cycle depend on a variety of parameters.

- Individual steps common to most PCR methods are Denaturation, Annealing / Renaturation, and Primer Extension / Synthesis.

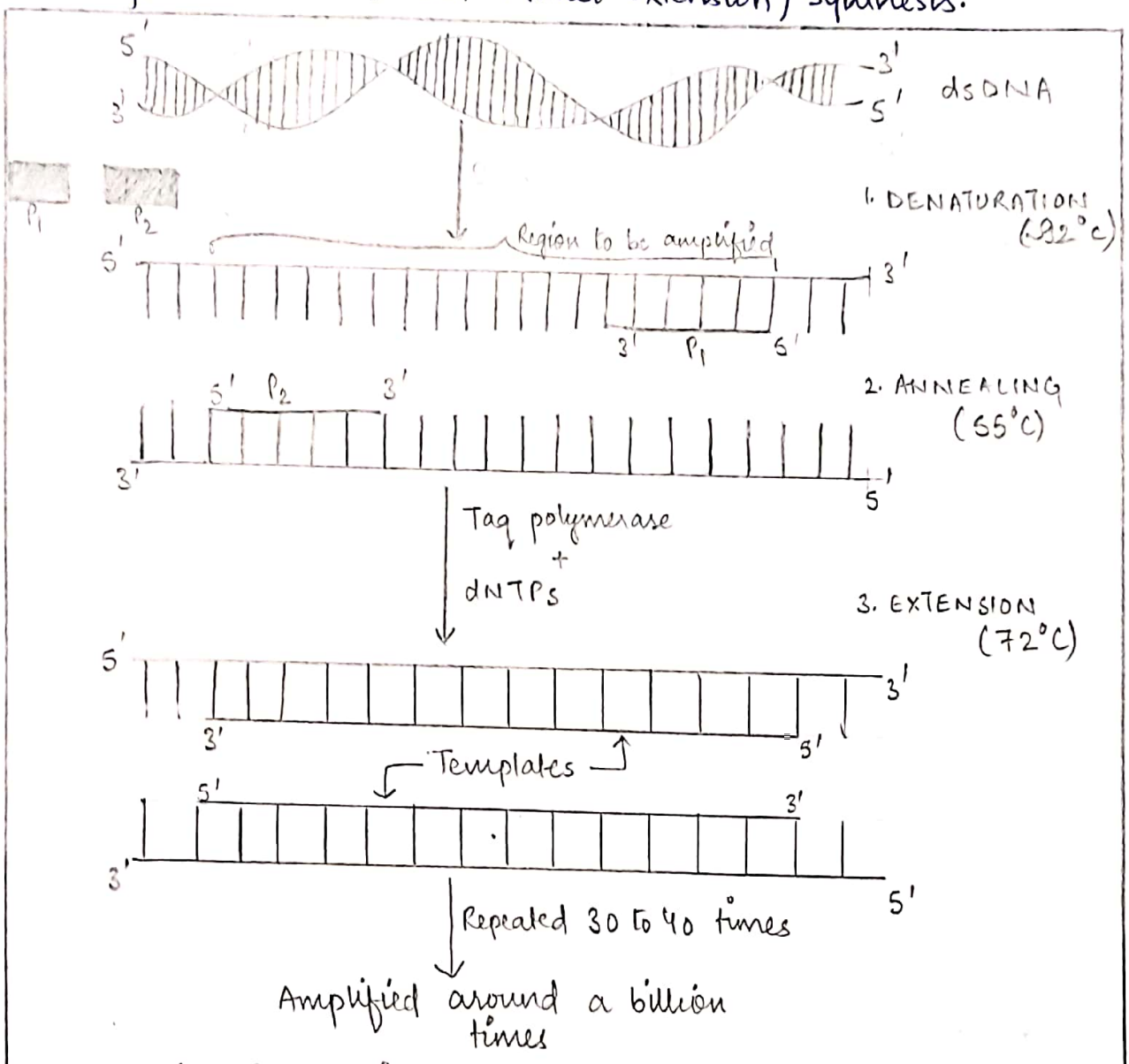


Fig: 3 successive steps in each thermal cycle of PCR.

- Each step is very temperature - sensitive.

1. Denaturation: It consists of heating the temperature within the reaction tube to 92 °C for about 1 minute.

This causes DNA melting or denaturation of the ds DNA template by breaking the hydrogen bonds between the complementary bases, yielding two ss DNA molecules.

2. Annealing: In the next step, the reaction temperature is lowered to 55 °C (50 °C to 65 °C) for 20 to 40 seconds, allowing annealing of the primers to each of the ss DNA templates. Two different primers are typically included in the reaction mixture (one for each).

A typically optimum annealing temperature is 3 °C - 5 °C below the melting temperature of the primers used.

During this step, the polymerase binds to the primer - template hybrid and begins DNA formation.

3. Extension: The thermostable Taq (Thermus aquaticus) polymerase is used, for which the optimum temperature is 72 °C.

In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture, that are complementary to the template in the 5′ to 3′ direction, condensing the 5′-Phosphate group of the dNTPs with the 3′-OH group at the end of the nascent DNA.

Applications of PCR:

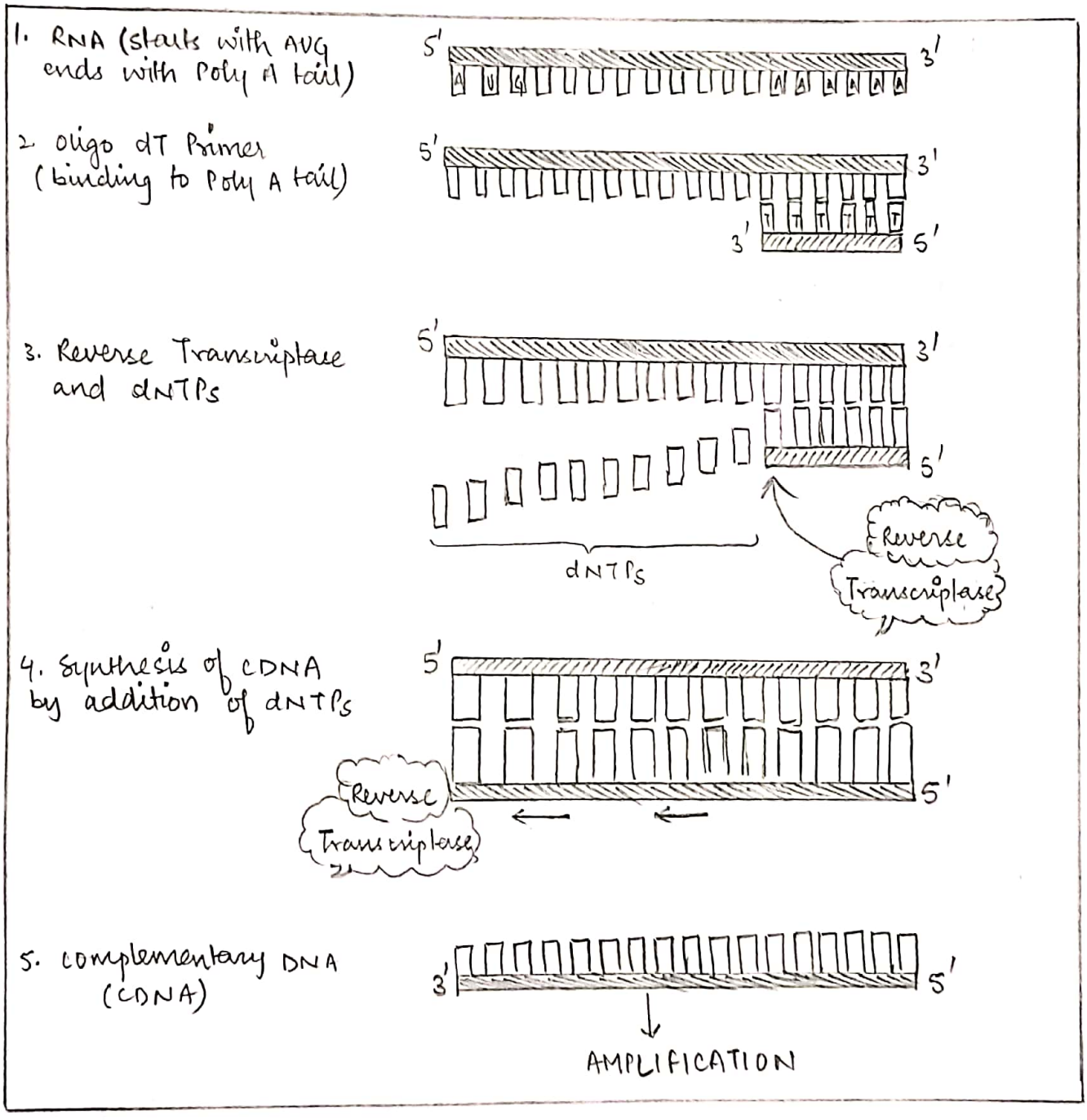
1. Diagnosis of specific mutation
2. Prenatal diagnosis
3. DNA fingerprinting
4. Diagnosis of plant pathogen
5. In molecular archaeology
6. In research.

REVERSE TRANISCRIPTASE - POLYMERASE CHAIN REACTION

- Primarily used to measure the amount of a specific RNA.
- Achieved by monitoring the amplification reaction using fluorescence, a technique called real-time PCR.

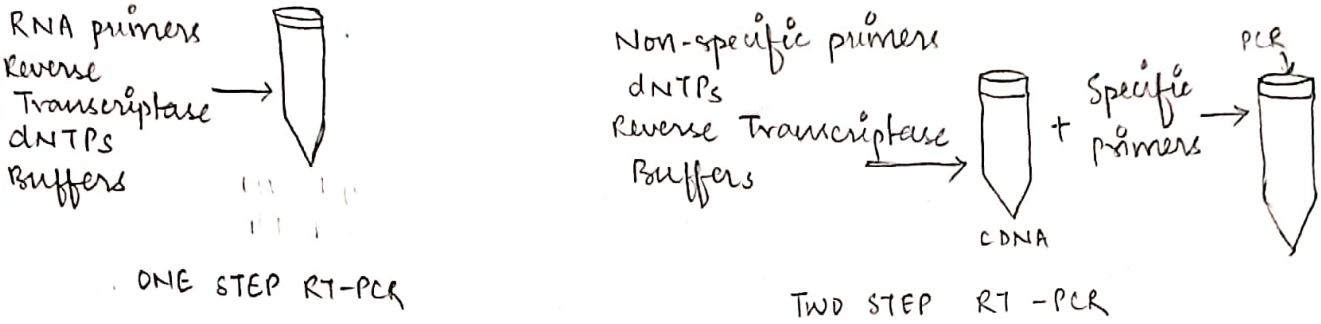
Principles of RT-PCR:

In RT-PCR, the RNA population is converted to CDNA by reverse transcription, and then the CDNA is amplified using PCR.



One-step RT-PCR v/s Two-step RT-PCR:

- Quantification of mRNA using RT-PCR can be achieved as either a one-step or a two-step reaction.
- Difference between the two approaches is that the two-step reaction requires the amplification of the synthesized cDNA by PCR to be done in a seperate tube, whereas the one-step reaction dictates that both synthesis and amplification occur in the same tube.



ONE STEP RT-PCR

TWO STEP RT-PCR

- Two-step reaction renders the cDNA vulnerable to contamination due to frequent sample handling.
- On the other hand, the RNA templates are prone to degradation in the one-step approach.

Applications of RT-PCR:

- RT-PCR is commonly used in research methods to measure gene expression.
- Can also be very useful in the insertion of eukaryotic genes into prokaryotes.
- RT-PCR plays a role in diagnosis of genetic diseases (such as the Lesch Nyhan syndrome)
- Scientists are working on ways to use RT-PCR in cancer detection.
- It is also commonly used to study genomes of Riboviruses (such as HIV and Sars-Cov2 (causing CoVID-2019)).