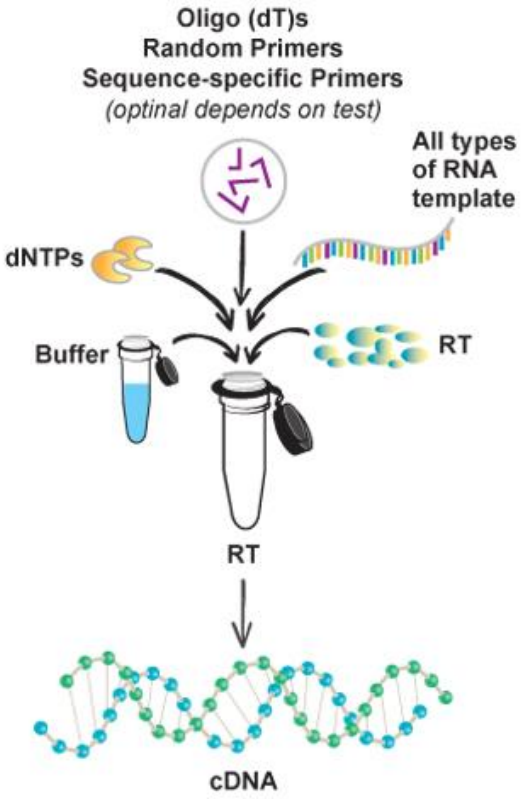
cDNA Synthesis



BCH361- Practical

Introduction

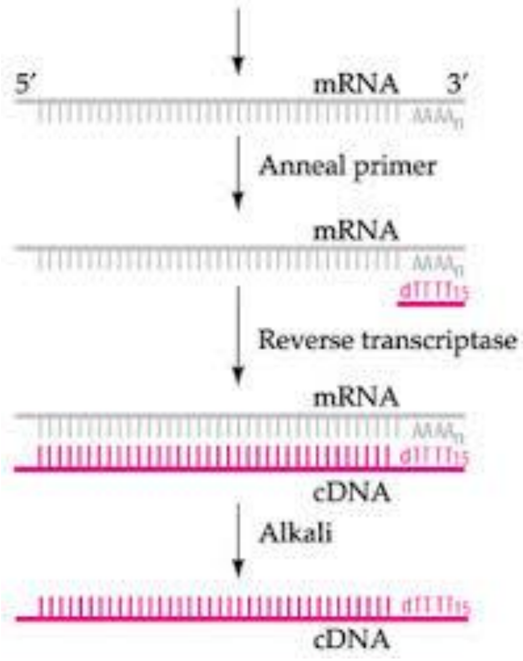
- The synthesis of DNA from an **RNA template**, via reverse transcription, produces **complementary DNA (cDNA).**

- In case of gene expression study, cDNA synthesised from mRNA.

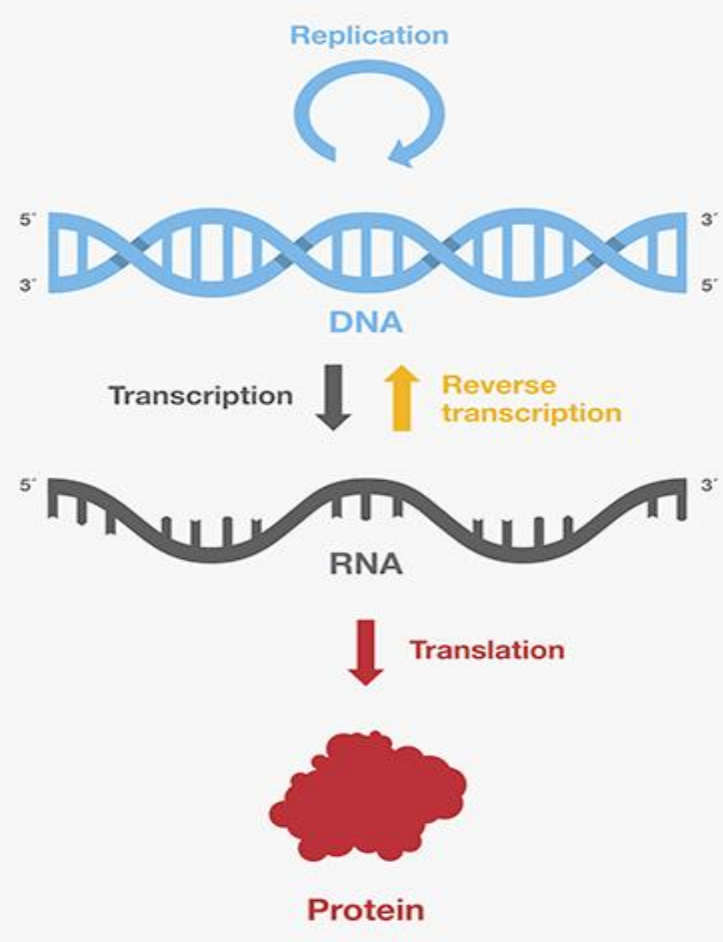
- cDNA is more stable than RNA.

- cDNA used for expression study, cloning and creation of cDNA library.

- Regarding template preparation, it is critical to maintain RNA integrity, **HOW?**

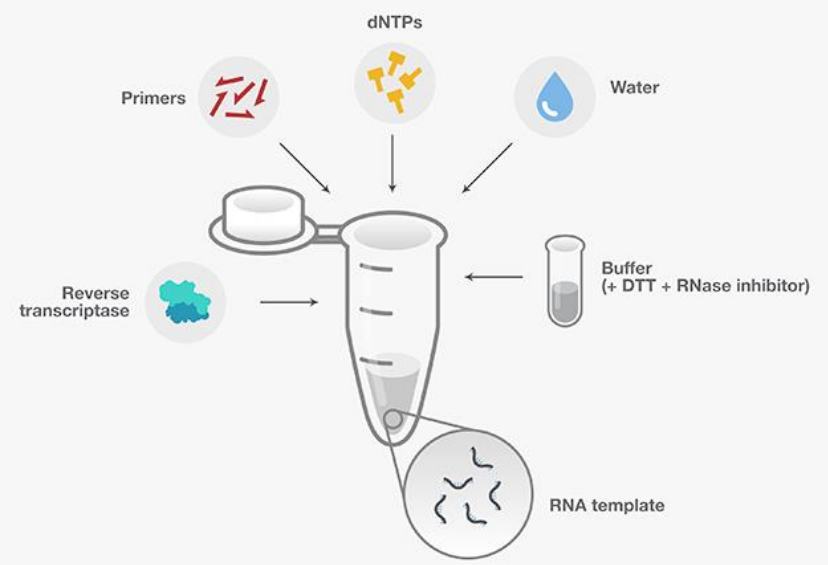


Reverse Transcription:



Reaction components:

➔ For the cDNA to be synthesized the following components are needed:



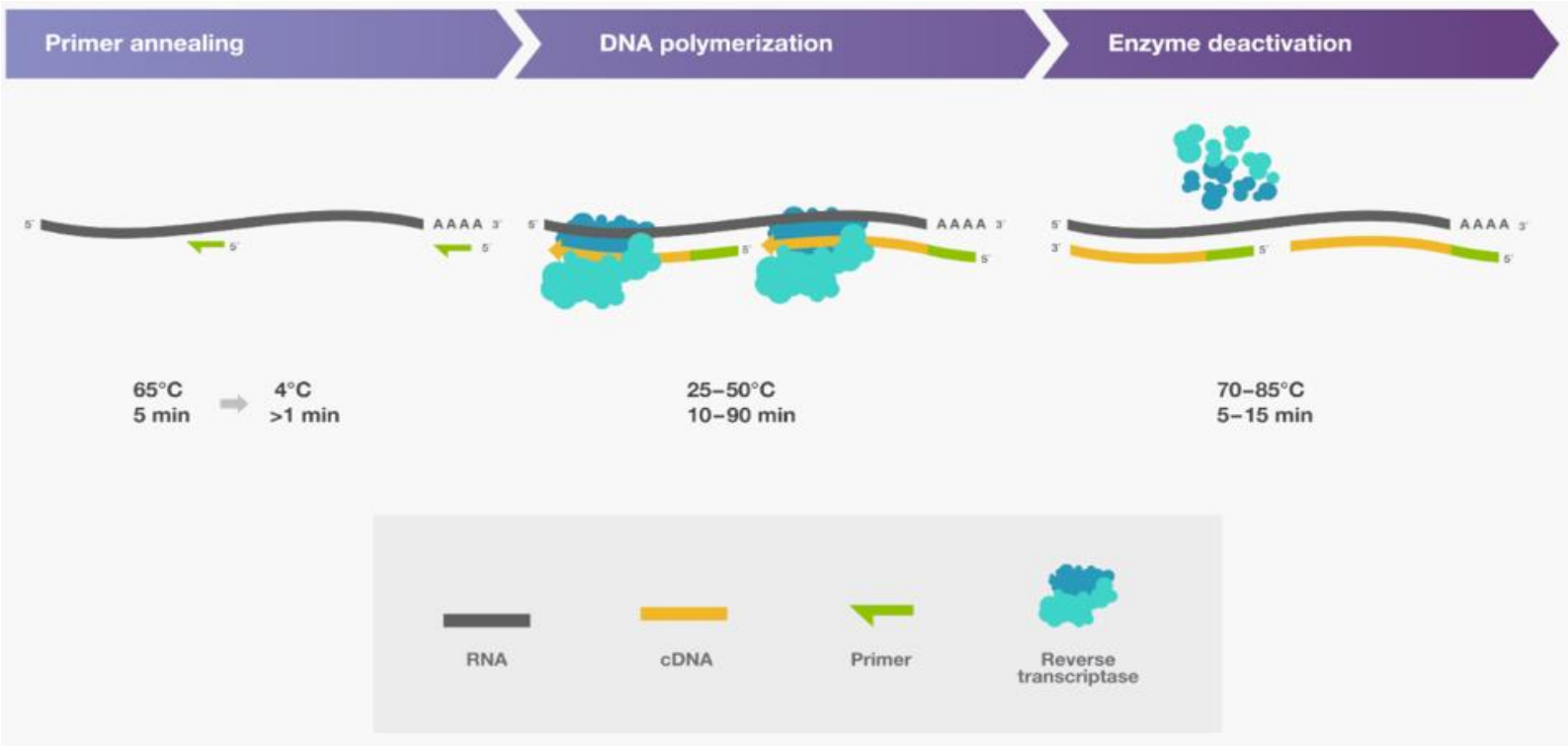
➔ **The reverse transcriptase** which synthesizes cDNA using RNA as template .

➔ Reverse transcriptase require a short DNA oligonucleotide called a **primer** to bind to its **complementary sequences** on the RNA template and serve as a **starting point** for synthesis of a new strand.

Type of primers

Reaction conditions:

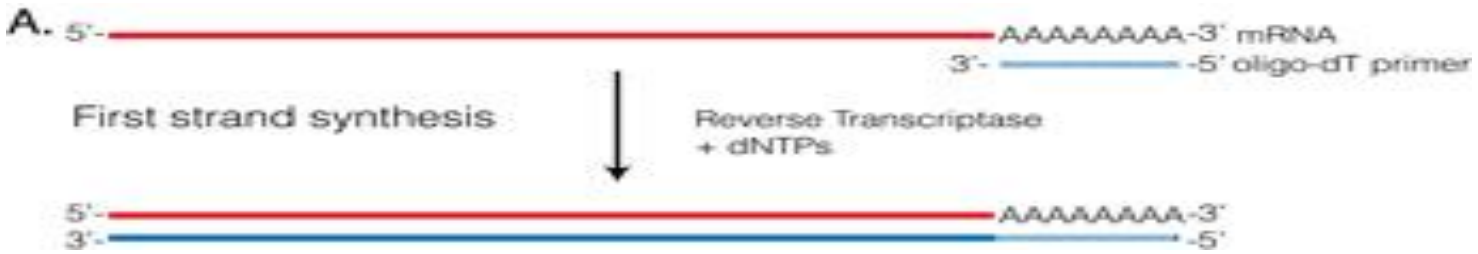
Reverse transcription reactions involve three main steps:



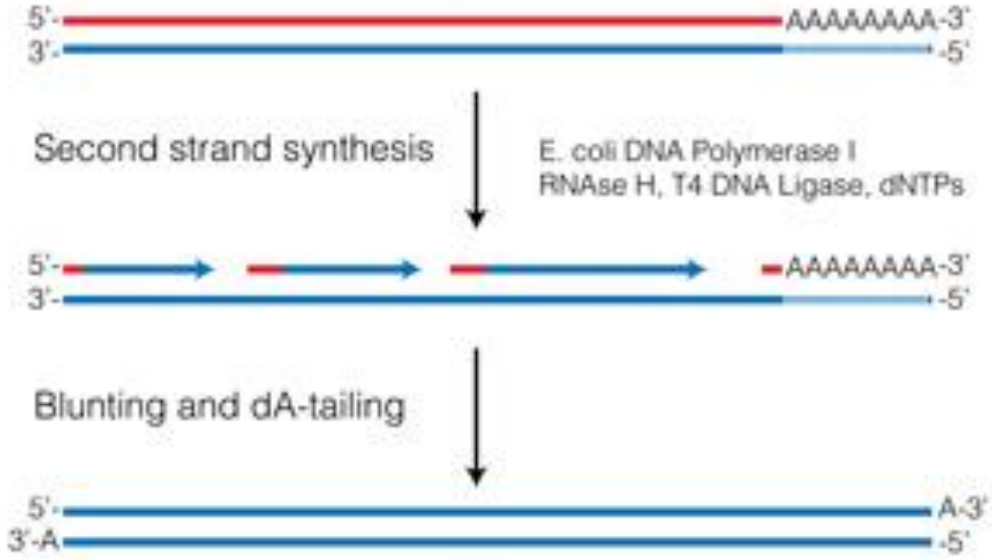
Step	Temperature	Duration
Primer annealing	65 °C	5 min
DNA polymerization*	Oligo(dT): 35–50 °C	>60 min
	Random hexamers: 10–15 °C	
	Primer mixture: 25 °C	
Enzyme deactivation	70-85 °C	5-15 min

cDNA synthesis

First-strand cDNA synthesis.



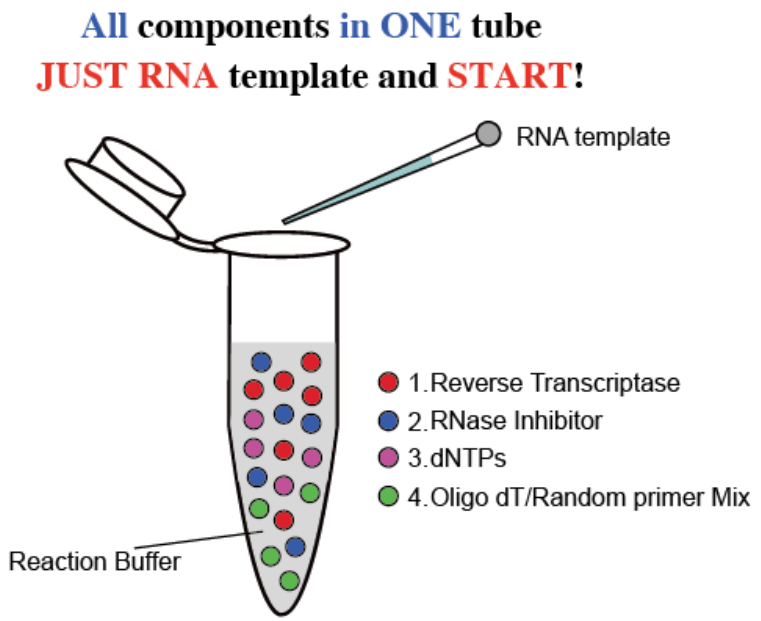
Second-strand cDNA synthesis.



Practical Part

Aim:

- Reverse transcript RNA to cDNA.



Method:

1. Label a clean microcentrifuge tube, and add the following:

component	volume
Reverse transcriptase	
dNTP	
Primers	
RNA template	
water	

2. Adjust the reaction conditions in the Thermal cycler.