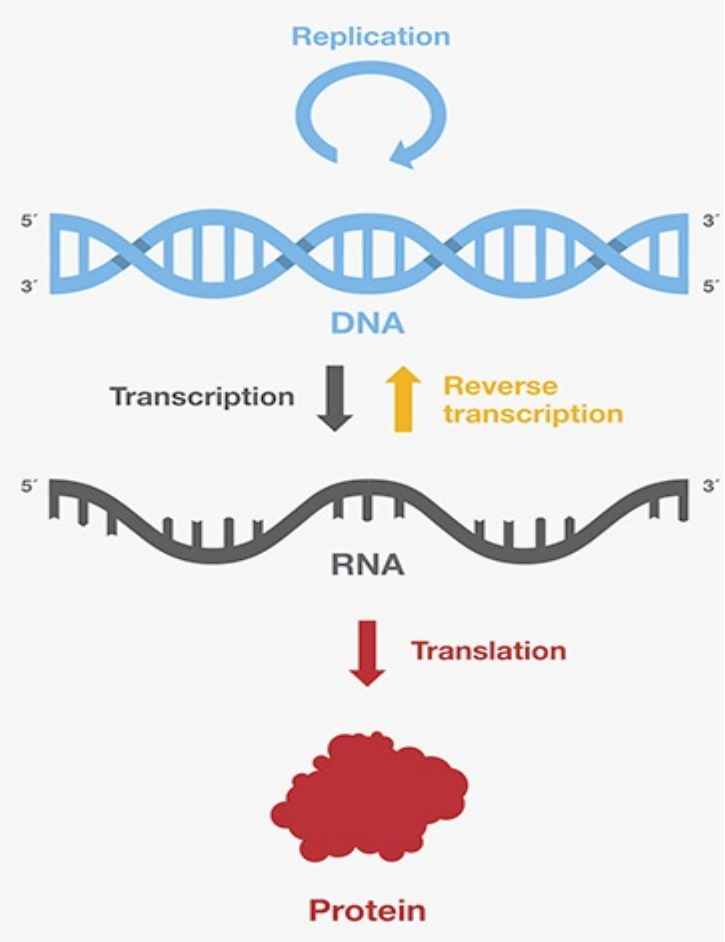
cDNA Synthesis

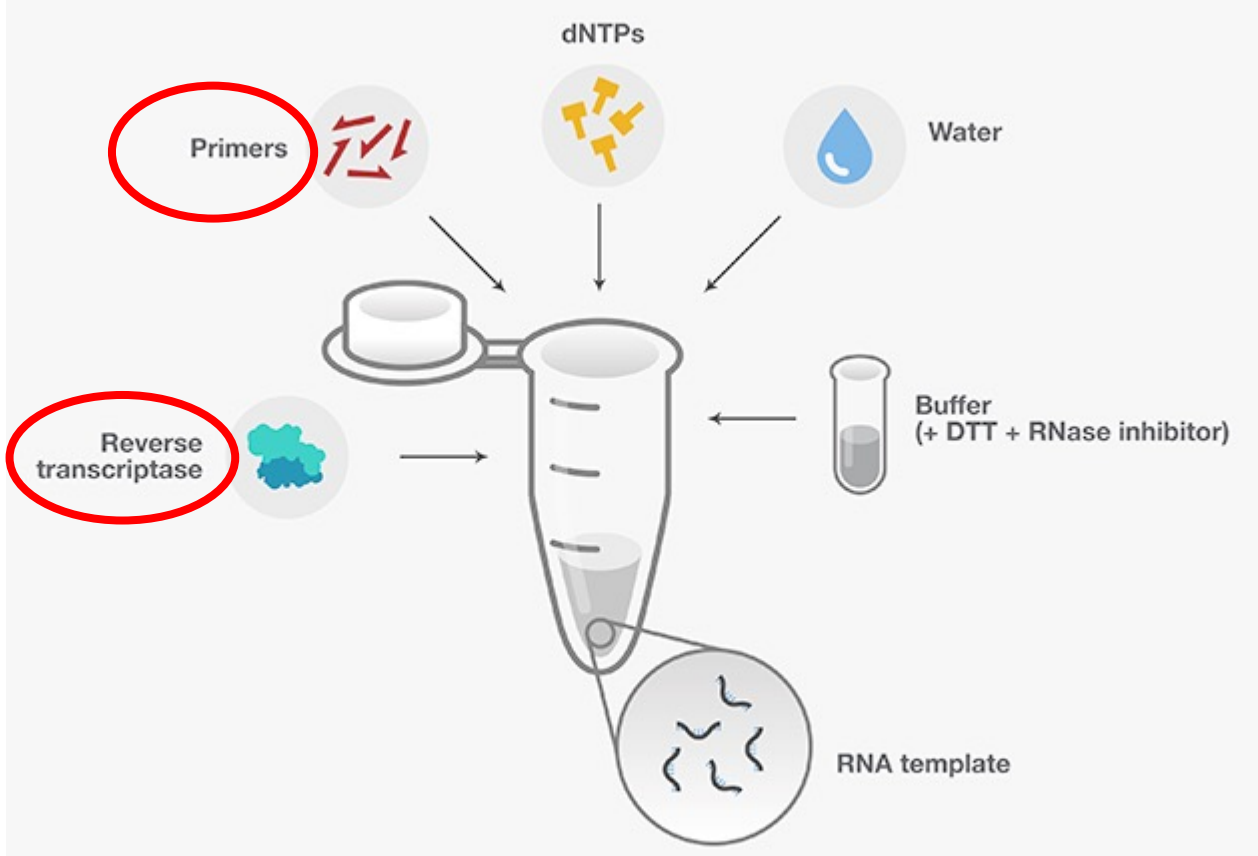
Introduction-cDNA

- 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.

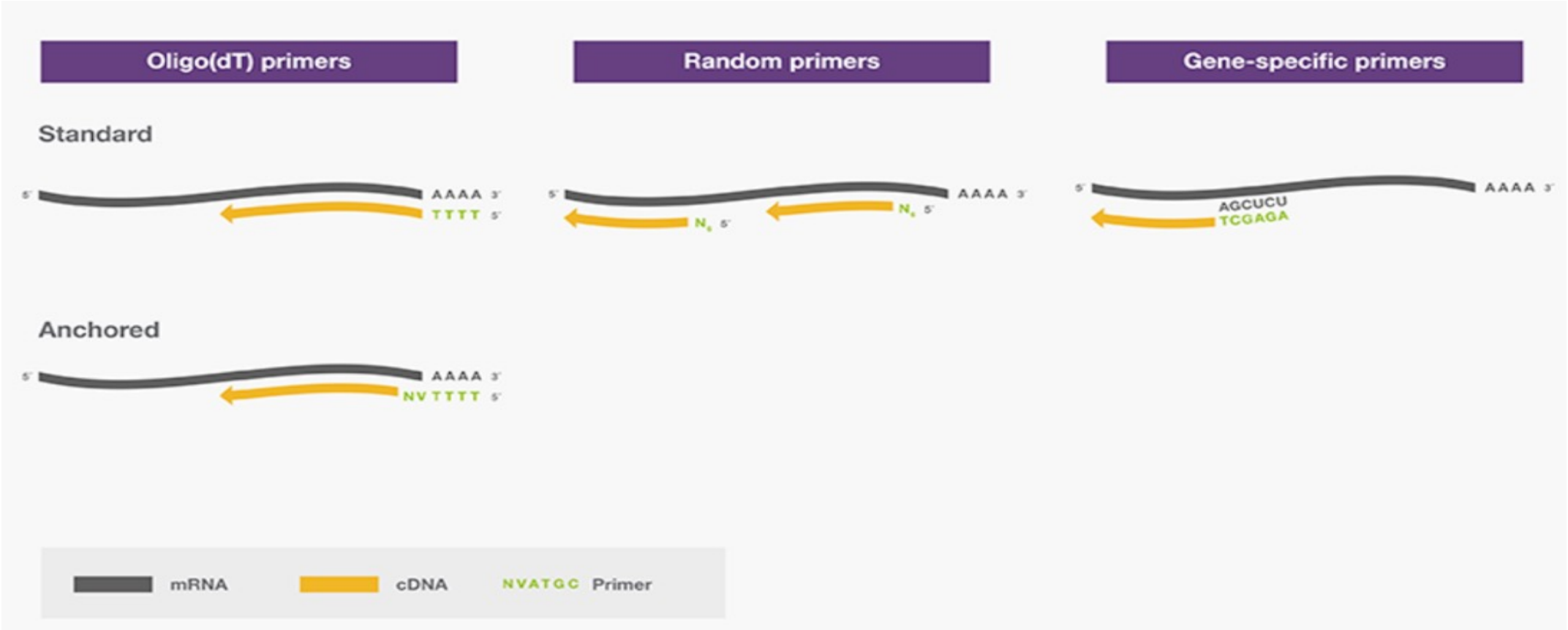
Reverse Transcription:



Main reaction components

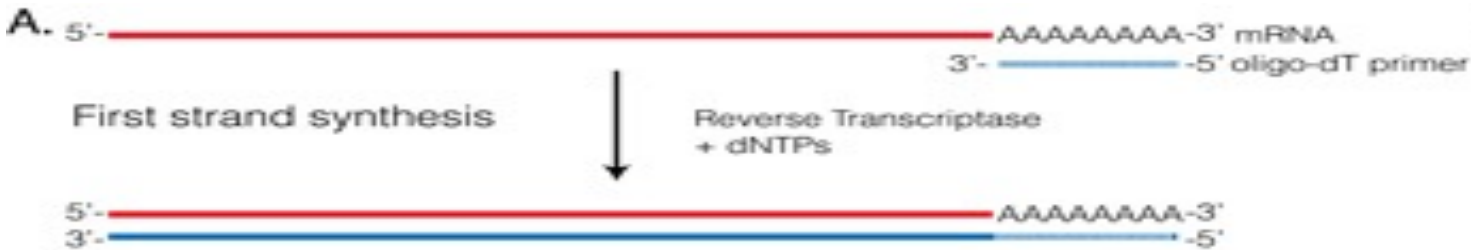


Type of primers

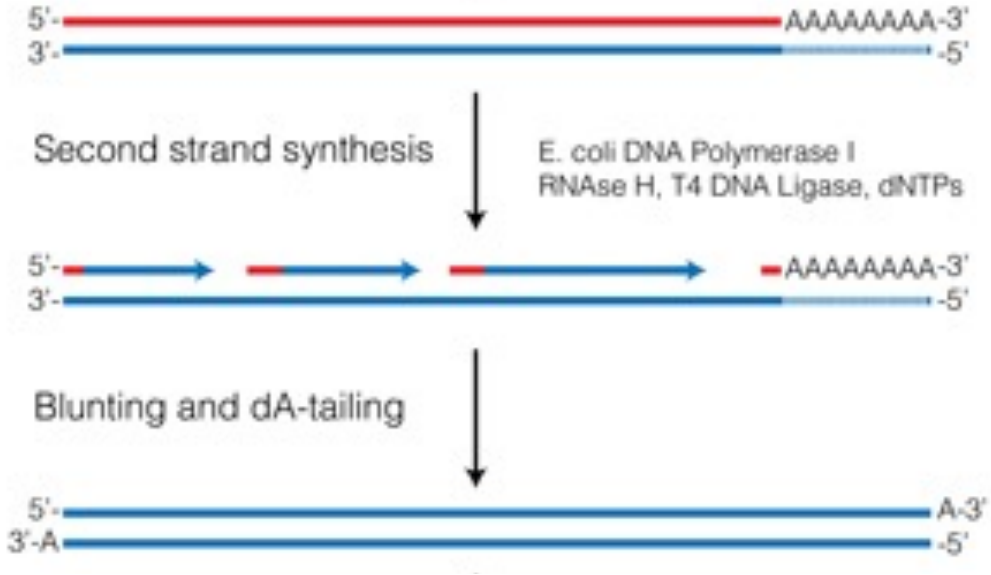


cDNA synthesis

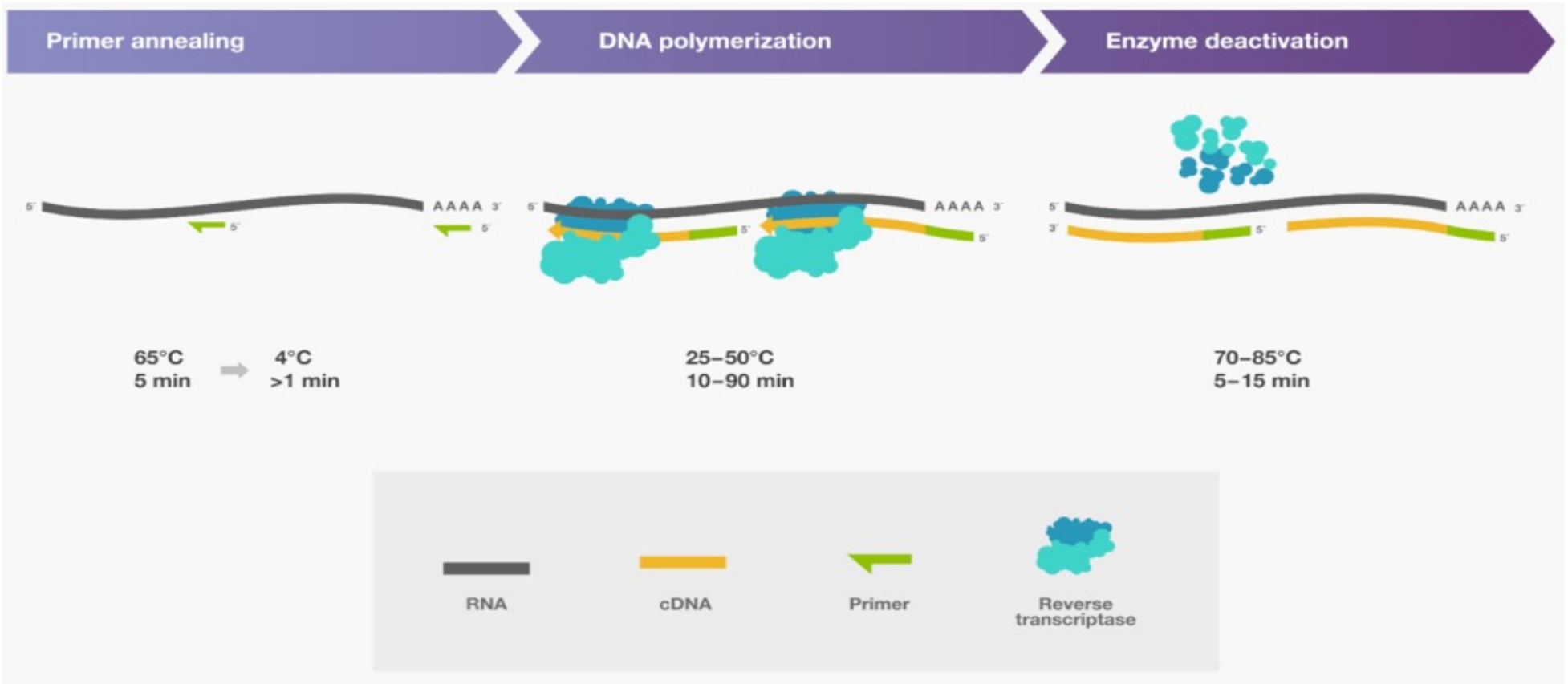
First-strand cDNA synthesis.



Second-strand cDNA synthesis.



Reaction conditions:



Practical Part

Prepare the 2X RT master mix

1. Allow the kit components to thaw on ice.
2. Calculate the volume of components needed to prepare the required number of reactions.

   Note: Prepare the RT master mix on ice.

Component	Volume	
	With RNase Inhibitor	Without RNase Inhibitor
10X RT Buffer	2.0 µL	2.0 µL
25X dNTP Mix (100 mM)	0.8 µL	0.8 µL
10X RT Random Primers	2.0 µL	2.0 µL
MultiScribe™ Reverse Transcriptase	1.0 µL	1.0 µL
RNase Inhibitor	1.0 µL	—
Nuclease-free H_2O	3.2 µL	4.2 µL
Total per reaction	10.0 µL	10.0 µL

IMPORTANT! Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.

3. Place the 2X RT master mix on ice and mix gently.

Prepare the reverse transcription reactions

1. Pipette 10 µL of 2X RT master mix into each well of a 96-well reaction plate or individual tube.
2. Pipette 10 µL of RNA sample into each well, pipetting up and down two times to mix.
3. Seal the plates or tubes.
4. Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles.
5. Place the plate or tubes on ice until you are ready to load the thermal cycler.

Program the thermal cycling conditions

Program the thermal cycler using the conditions below.

IMPORTANT! These conditions are optimized for use with the High-Capacity cDNA Reverse Transcription Kits.

Settings	Step 1	Step 2	Step 3	Step 4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	∞

HOMEWORK

Q1: According to the figure (1): what the appearance of extracted RNA should be under AGE so you can proceed for cDNA? Write a justification of each sample.

Q2: Fill the table below with comparison (three at least)

DNA	RNA	cDNA

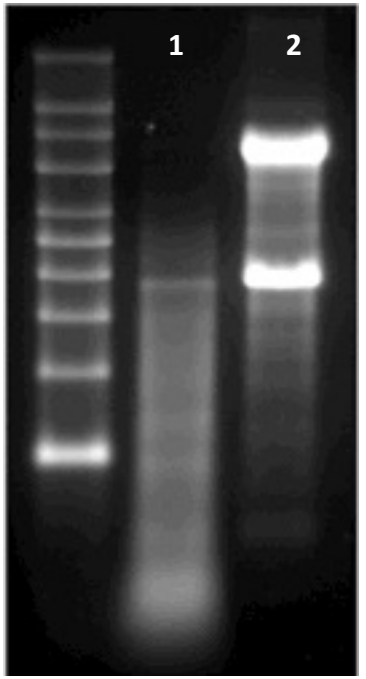


Figure (1)