cDNA synthesis

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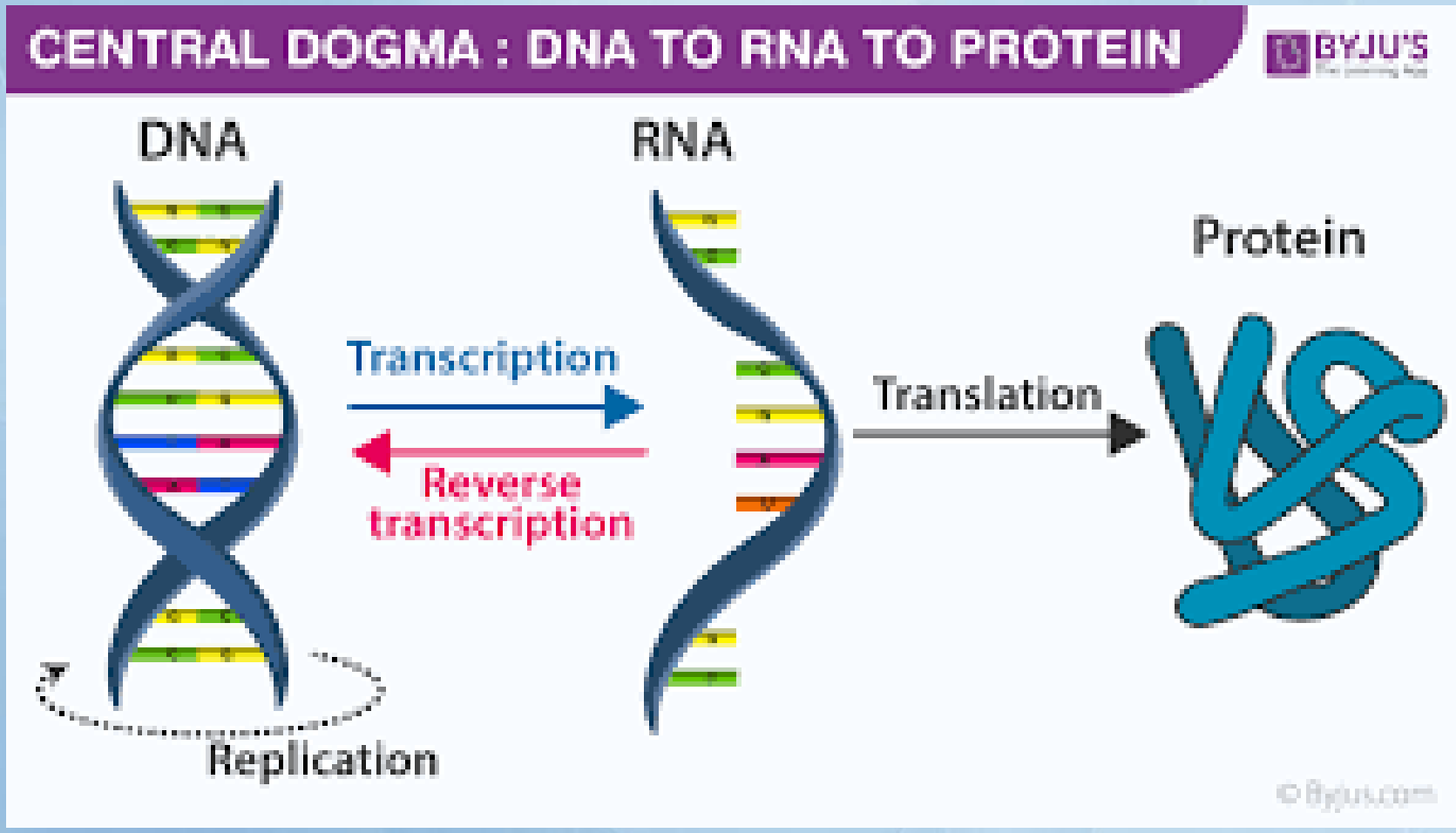
Workshop On
RNA Technologies
15-16/3/2022
Time Table

Date	Time	Activity	
Tuesday 15/3/2022	8.30- 9.00	Registration	
	9. 00- 9.20	Welcome Talk: Molecular Biology Research & Studies Institute	
	9.20 - 10.00	Lecture 1	Principles of RNA isolation.
	10.00- 10.40	Lecture 2	cDNA synthesis
	10.40 - 11.00	Break	
	11.00 - 2:00	Lab. work	RNA isolation
Wednesday 16/3/2022	9.00 - 9.30	Lecture 3	Principles of Real time PCR.
	9.30 - 10.30	Lecture 4	RNA technologies (Microarray, RNA sequencing, RNAi)
	10.30 - 11.00	Break	
	11.00- 2.00	Lab. work	qRT-PCR.

The central dogma

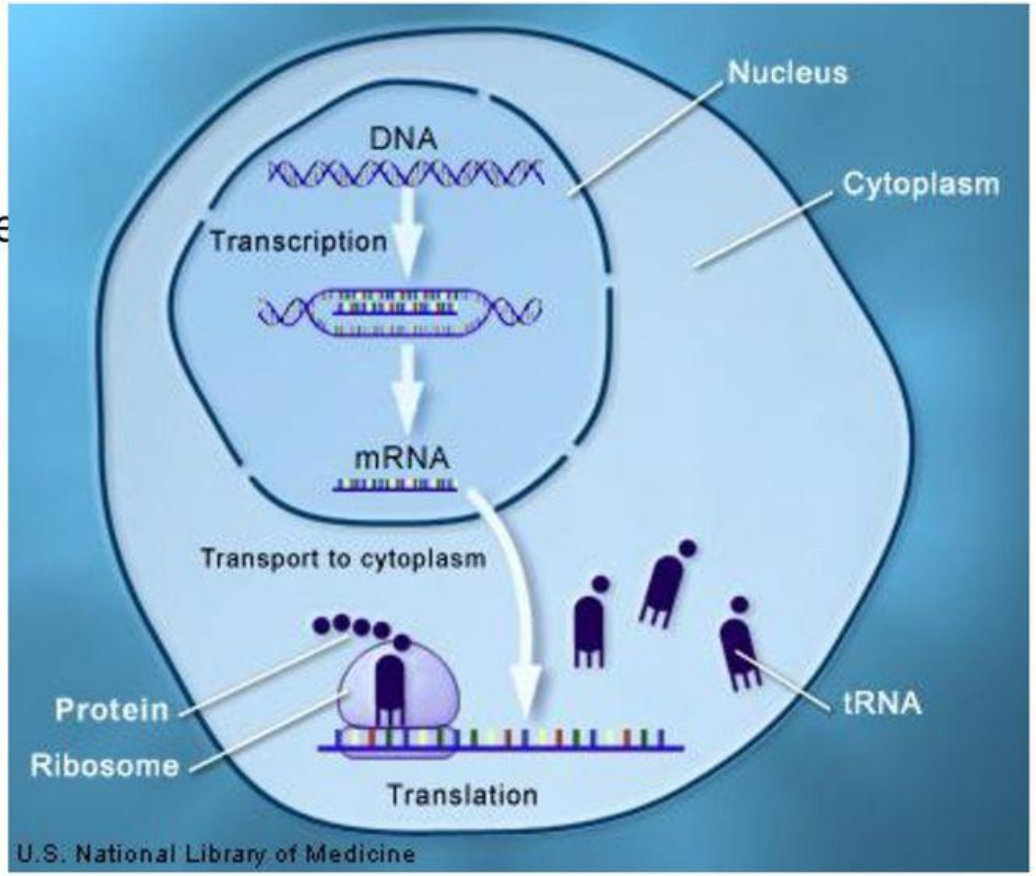


Q: Why can't DNA make proteins directly from itself?

A: DNA can't make anything- it's only information.

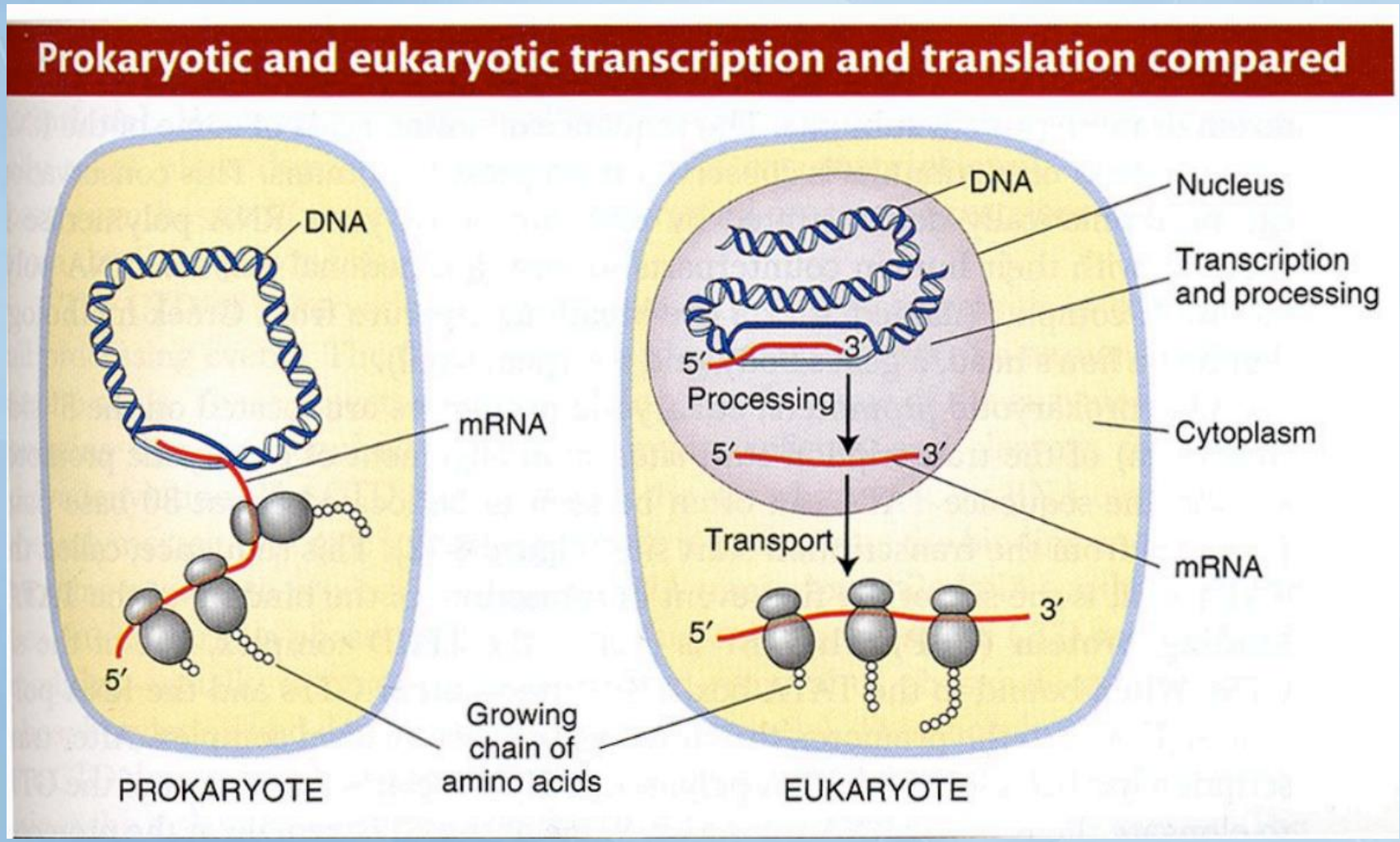
DNA can't leave the nucleus, only copies of the message in mRNA form can!

When mRNA copies a DNA molecules message, the process is called ***transcription***.

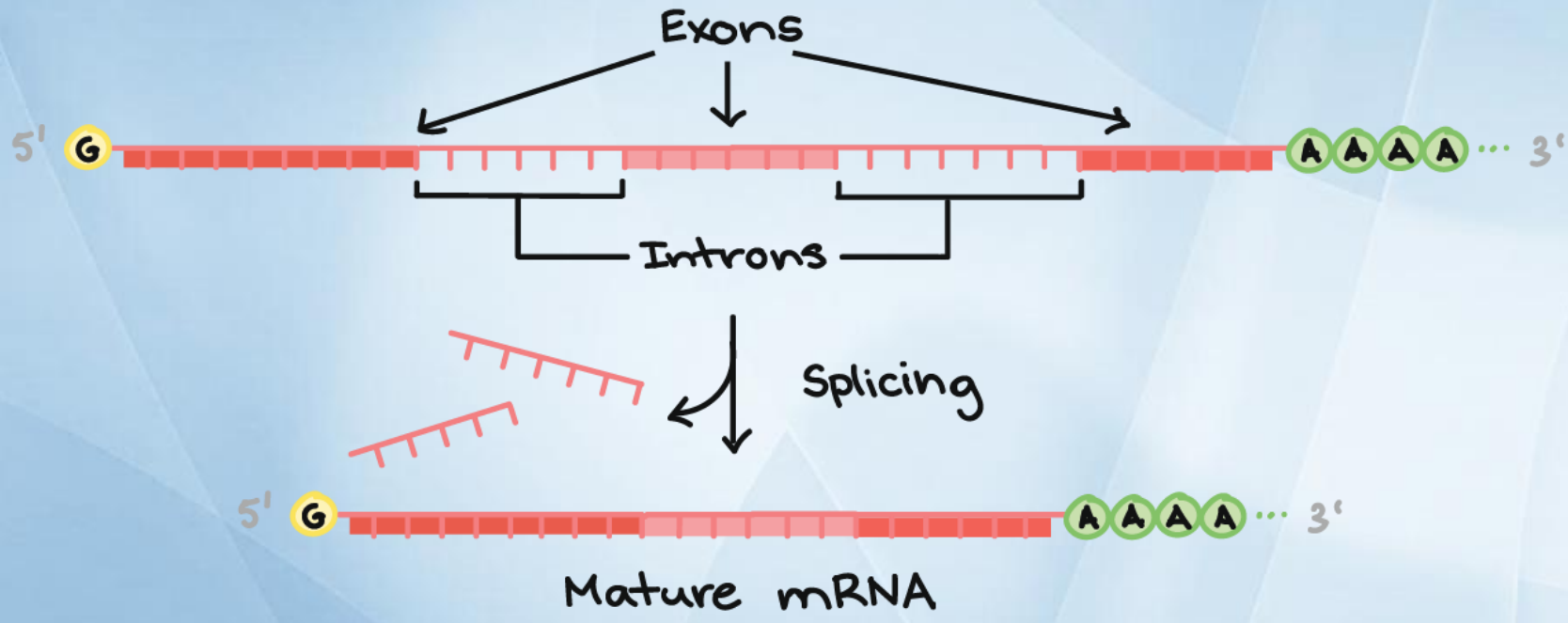


The Role of RNA

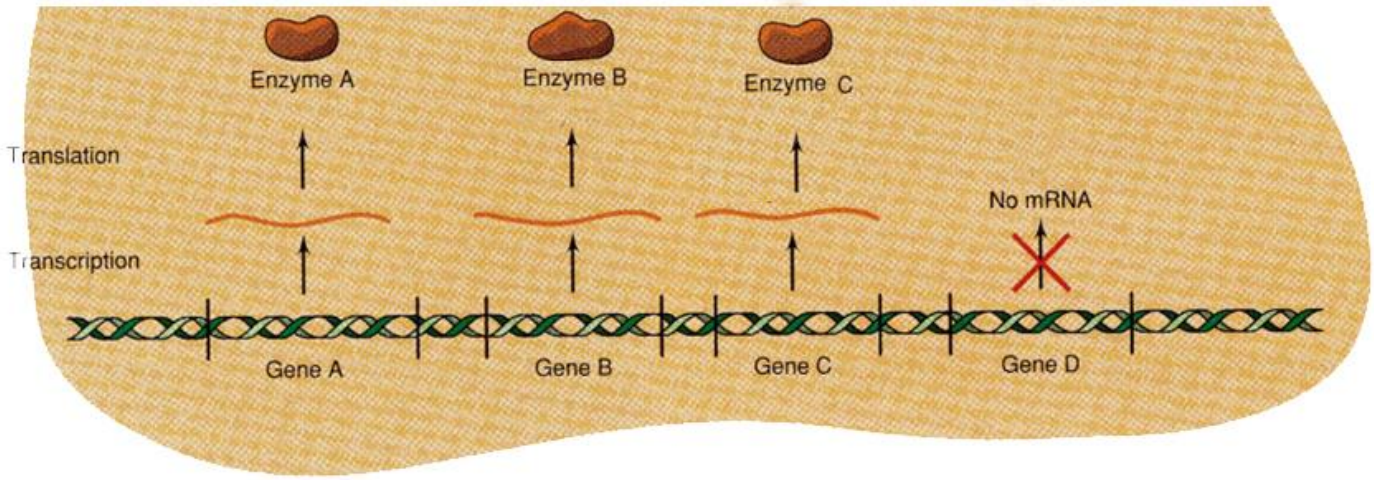
- Genes contain coded DNA instructions that tell cells how to build proteins.
- The first step in decoding these genetic instructions is to copy part of the base sequence from DNA into RNA.
- **RNA**, like DNA, is a nucleic acid that consists of a long chain of nucleotides.
- RNA then uses the base sequence copied from DNA to direct the production of proteins.


Prokaryotic and eukaryotic transcription and translation compared
DNA
mRNA
5′
PROKARYOTE
Growing chain of amino acids
DNA
Nucleus
Transcription and processing
5′
3′
Processing
5′
3′
Cytoplasm
Transport
mRNA
5′
3′
EUKARYOTE

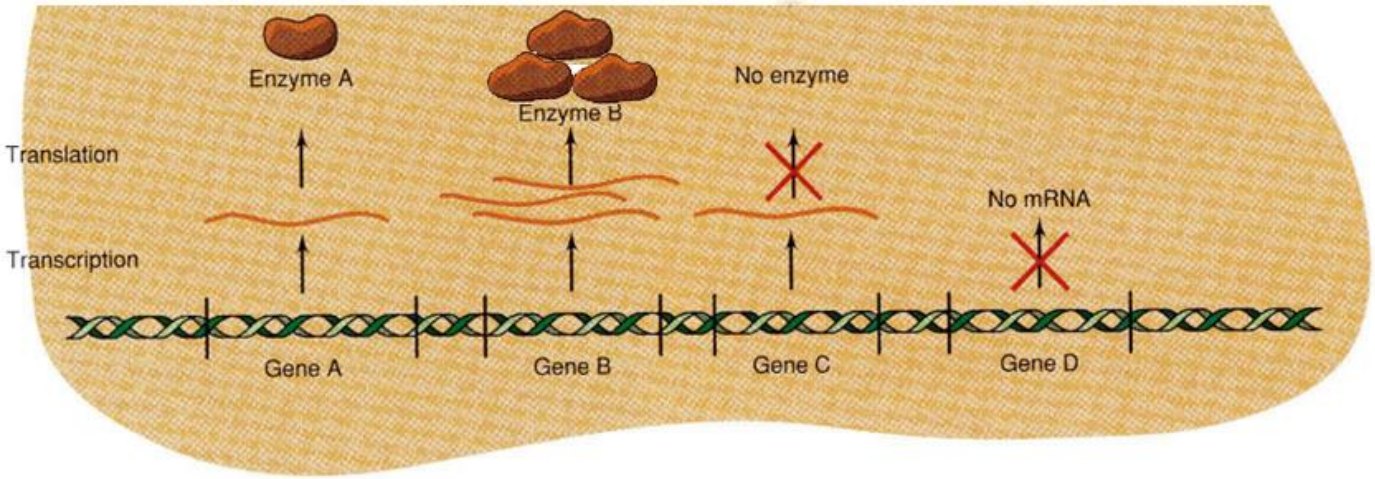
RNA processing in eukaryotes



Condition 1



Condition 2



Expression analysis

Many issues about gene function are best addressed by examining the product that they encode.

Protein

UV absorption

Colorimetric methods

- Biuret
- Lowry
- Bradford

SDS PAGE

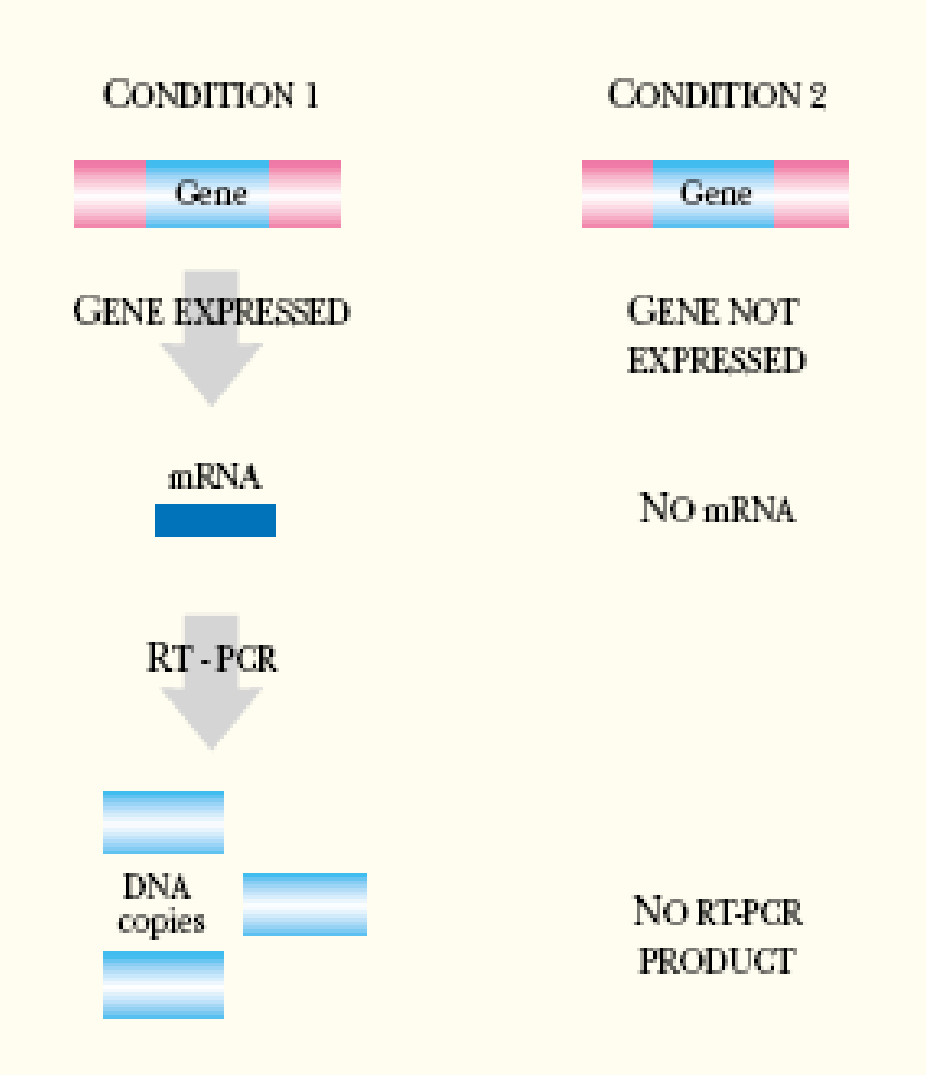
mRNA

Reverse transcriptase PCR

Microarray

RNA Seq

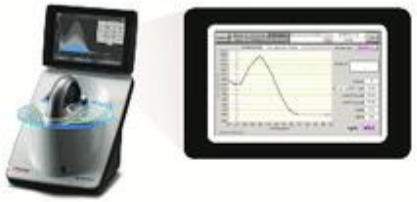
Expression analysis
By Reverse transcriptase PCR



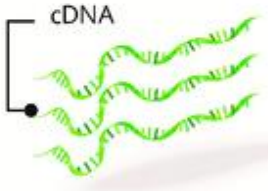
① Sample Acquisition & Handling

② RNA Extraction

③ RNA Assessment

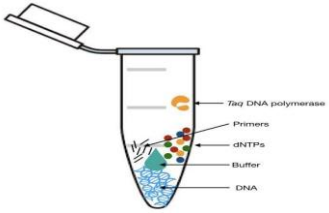


④ Reverse Transcription

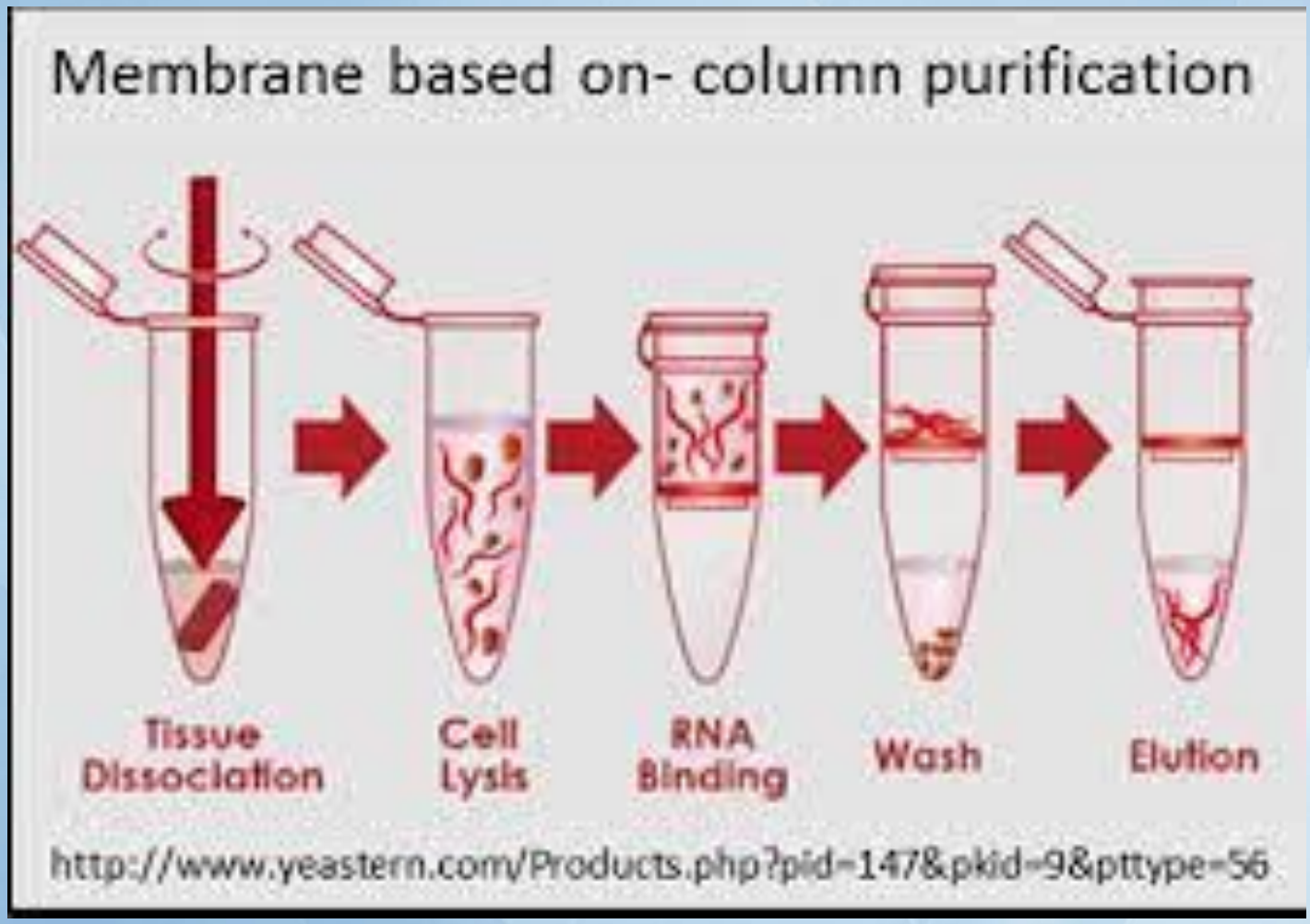


⑤ qPCR Optimisation

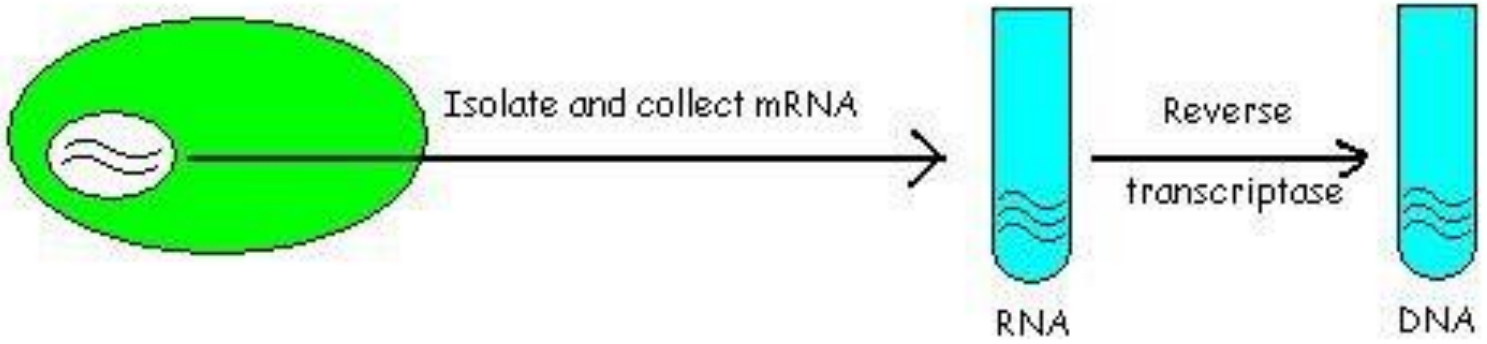
⑥ qPCR Assay



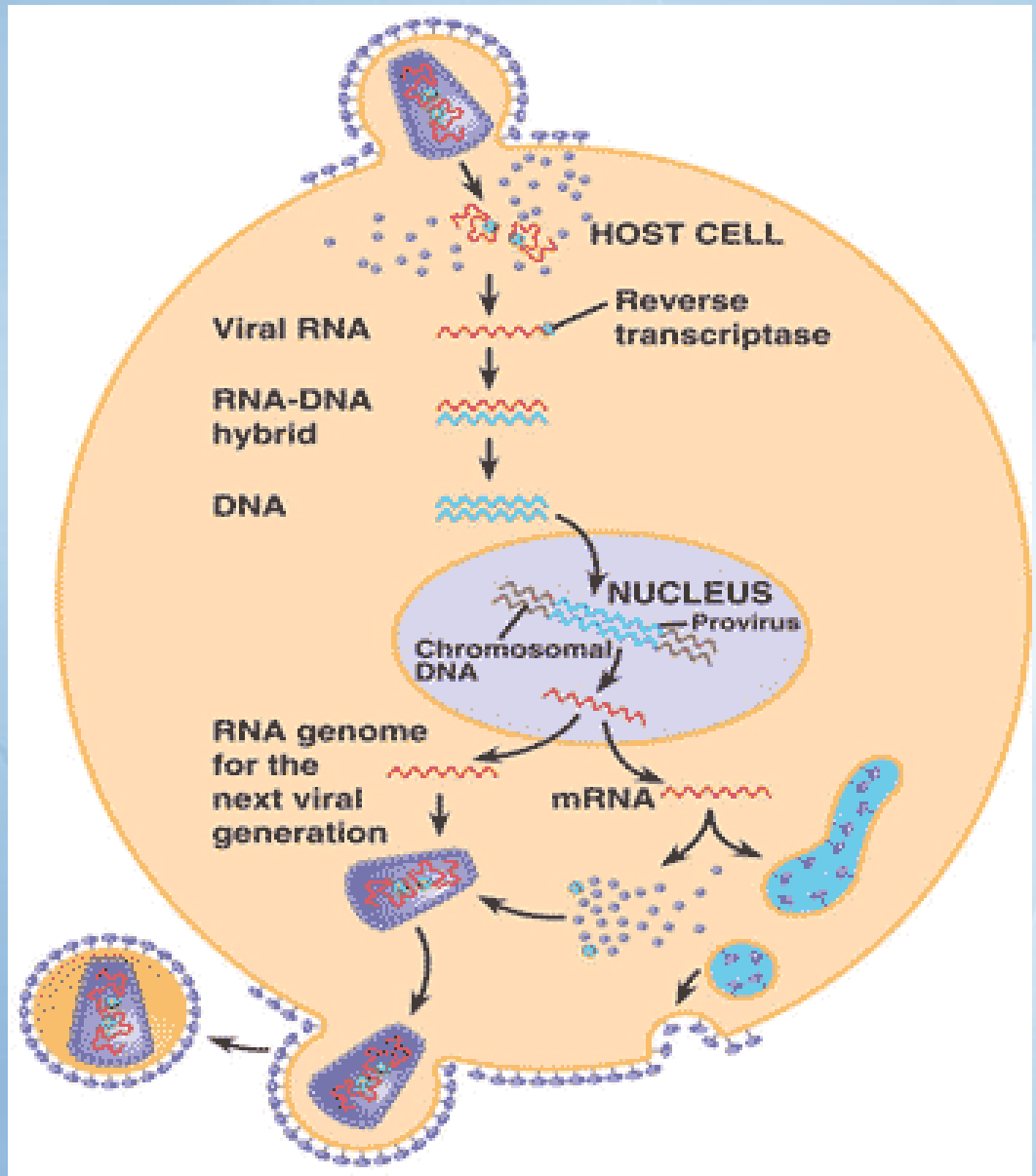
⑦ Data Analysis


Membrane based on- column purification
Tissue Dissociation
Cell Lysis
RNA Binding
Wash
Elution
http://www.yeastern.com/Products.php?pid=147&pkid=9&pttype=56

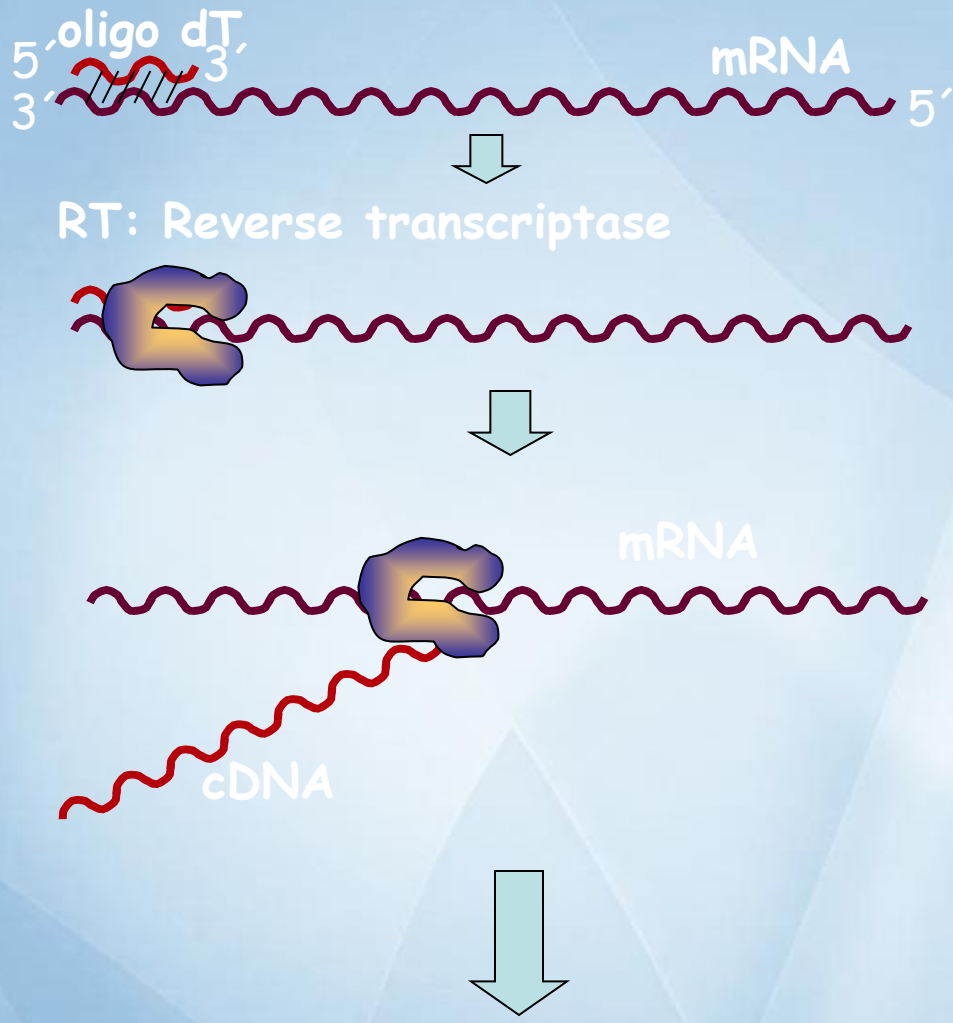
Formation of a cDNA Library



Reverse Transcription



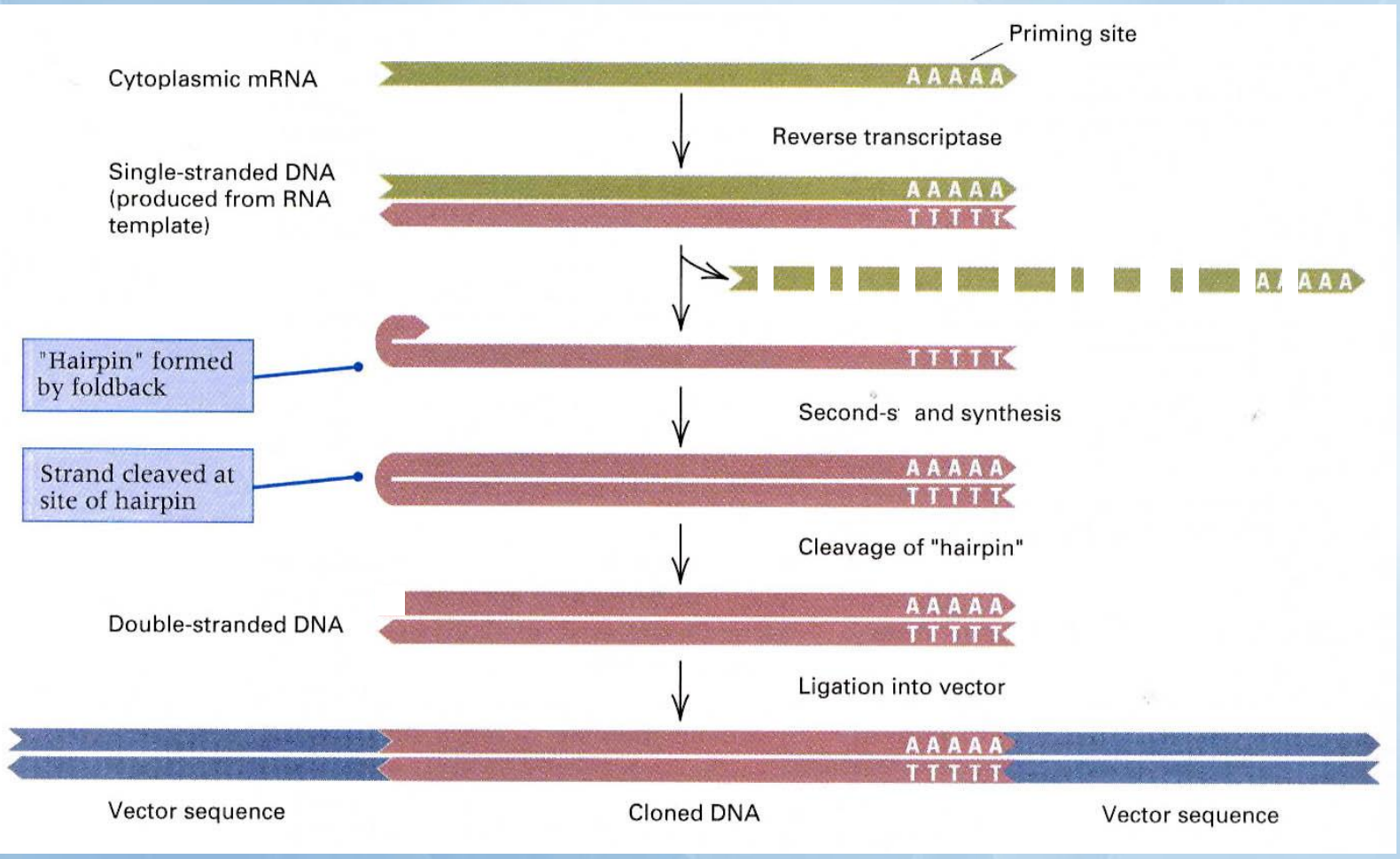
RT-PCR



Templat for RT- PCR

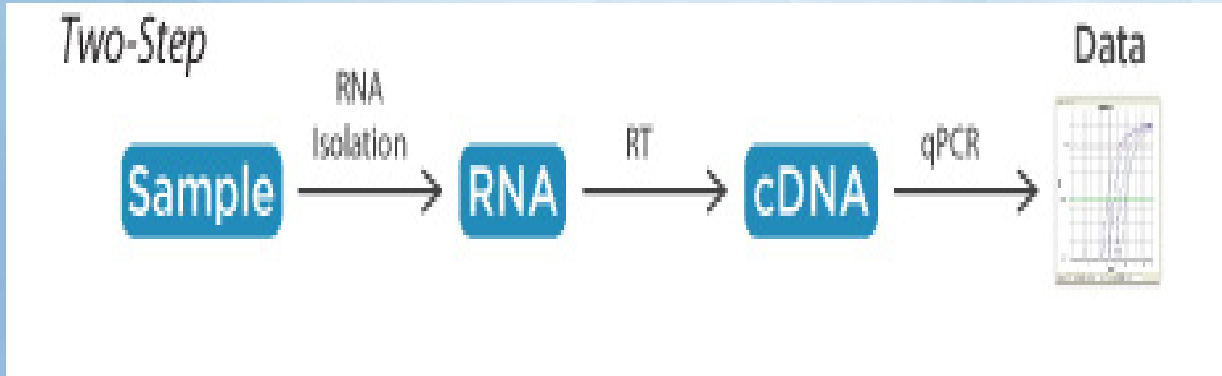
cDNA Construction

in vitro



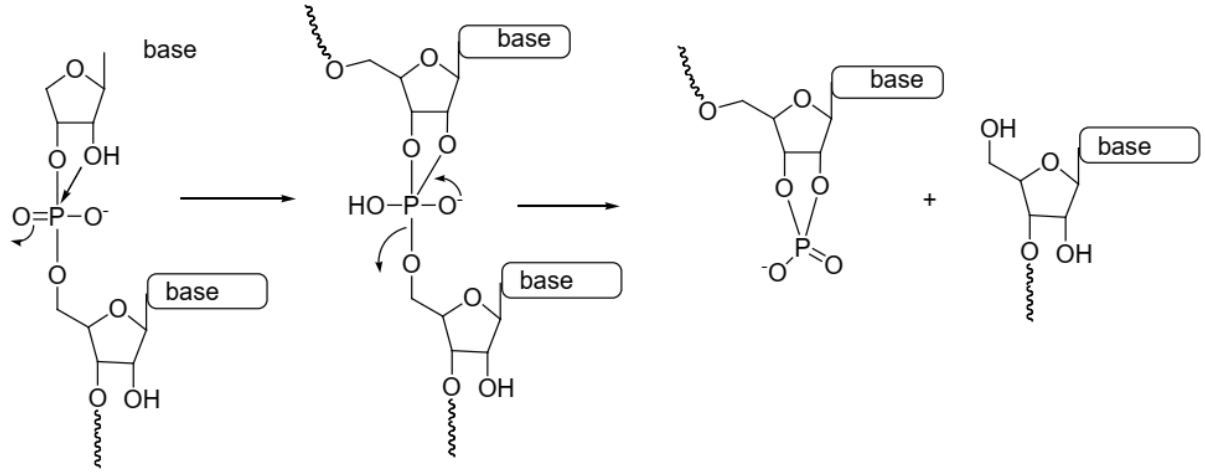
In PCR, why don't we just put RNA?

Why do we use cDNA instead of RNA directly when we perform RT-PCR ?



1.RNA is quite unstable in nature

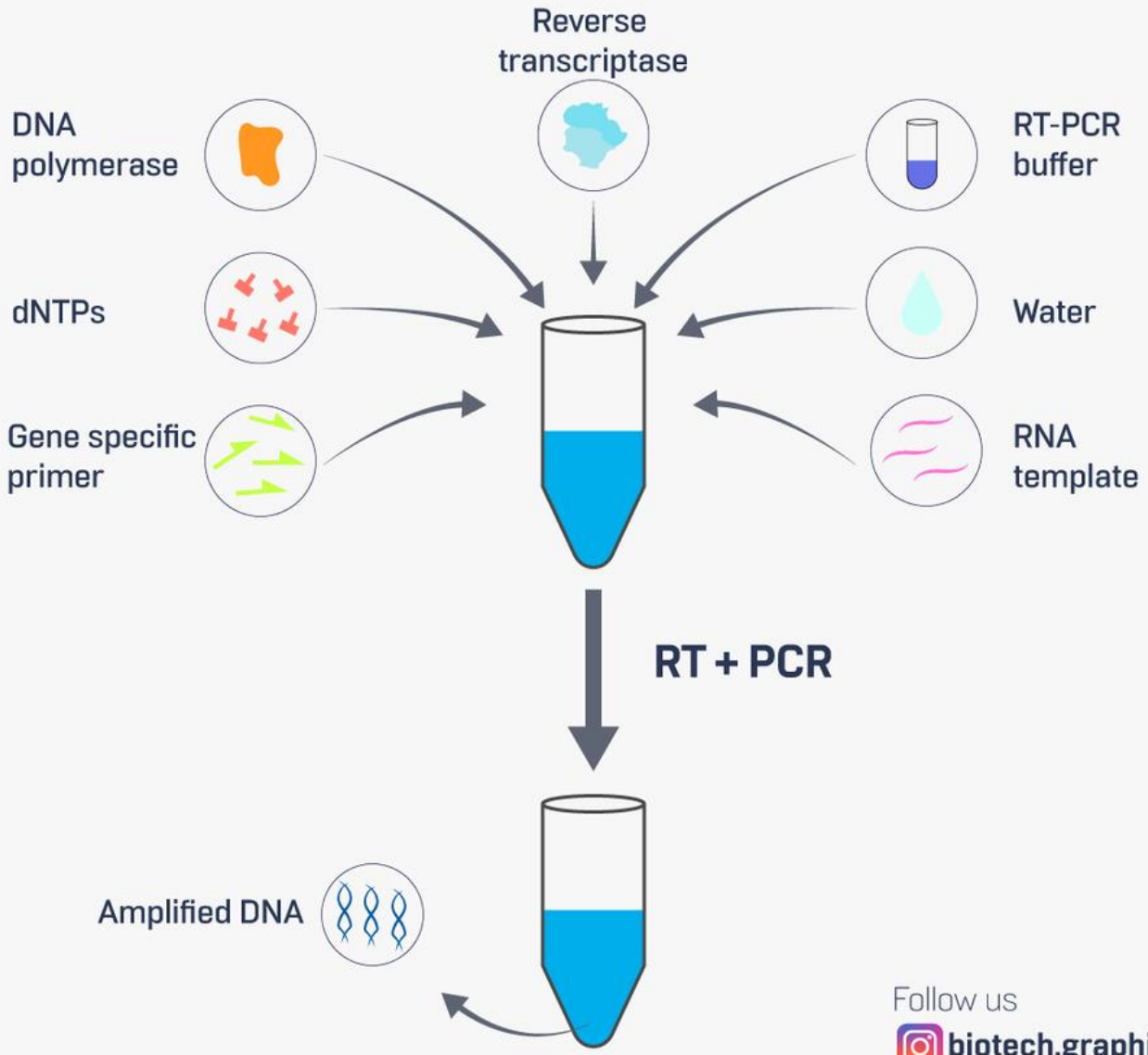
In RNA, the 2'-OH of ribose attacks the adjacent phosphodiester group that leads to the cleavage of the strand (Figure 7). This reaction does not take place in DNA, because it does not have the 2'-OH group. Thus, DNA remain intact throughout the life span of cells



2.Mostly because the thermostable DNA polymerases used in PCR will not recognize/amplify RNA

3.Using RNA dependent RNA polymerase can give high error rate.

One Step RT-PCR



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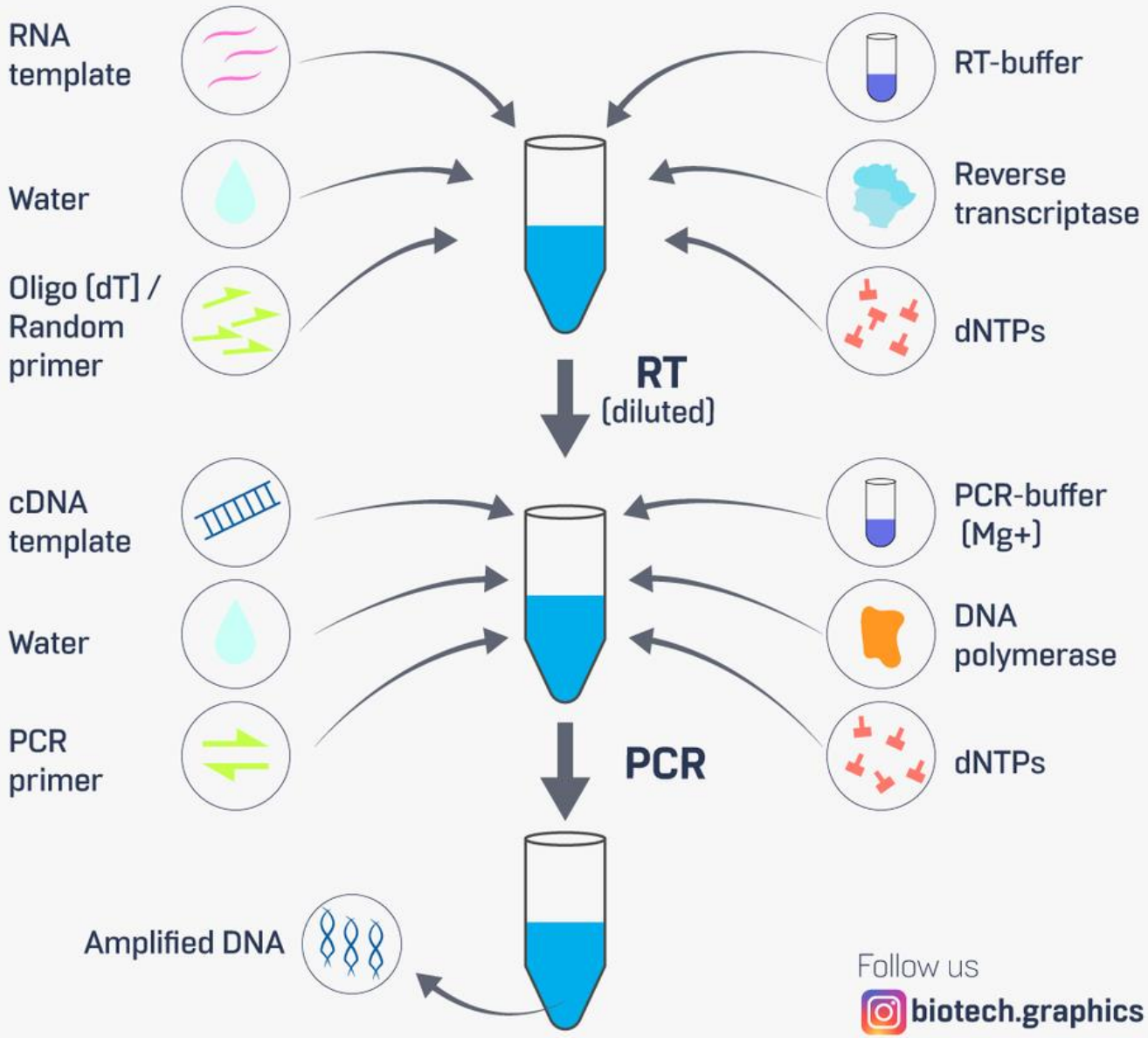
Two Step RT-PCR

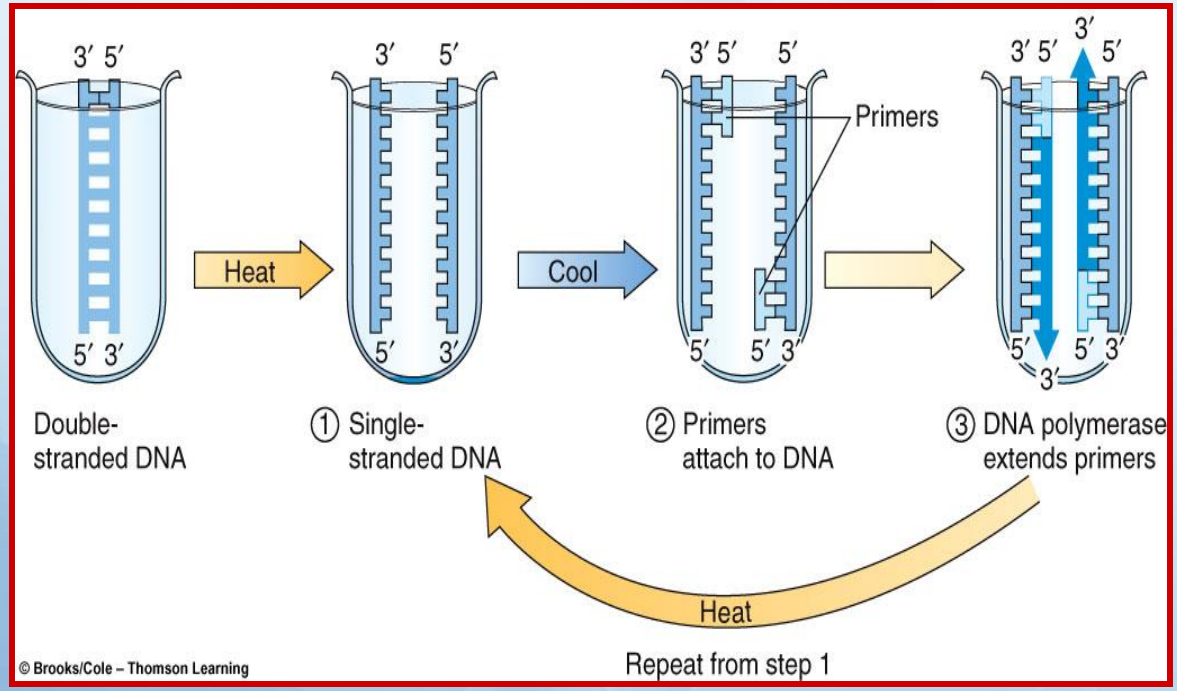


Primers for "first strand" cDNA synthesis

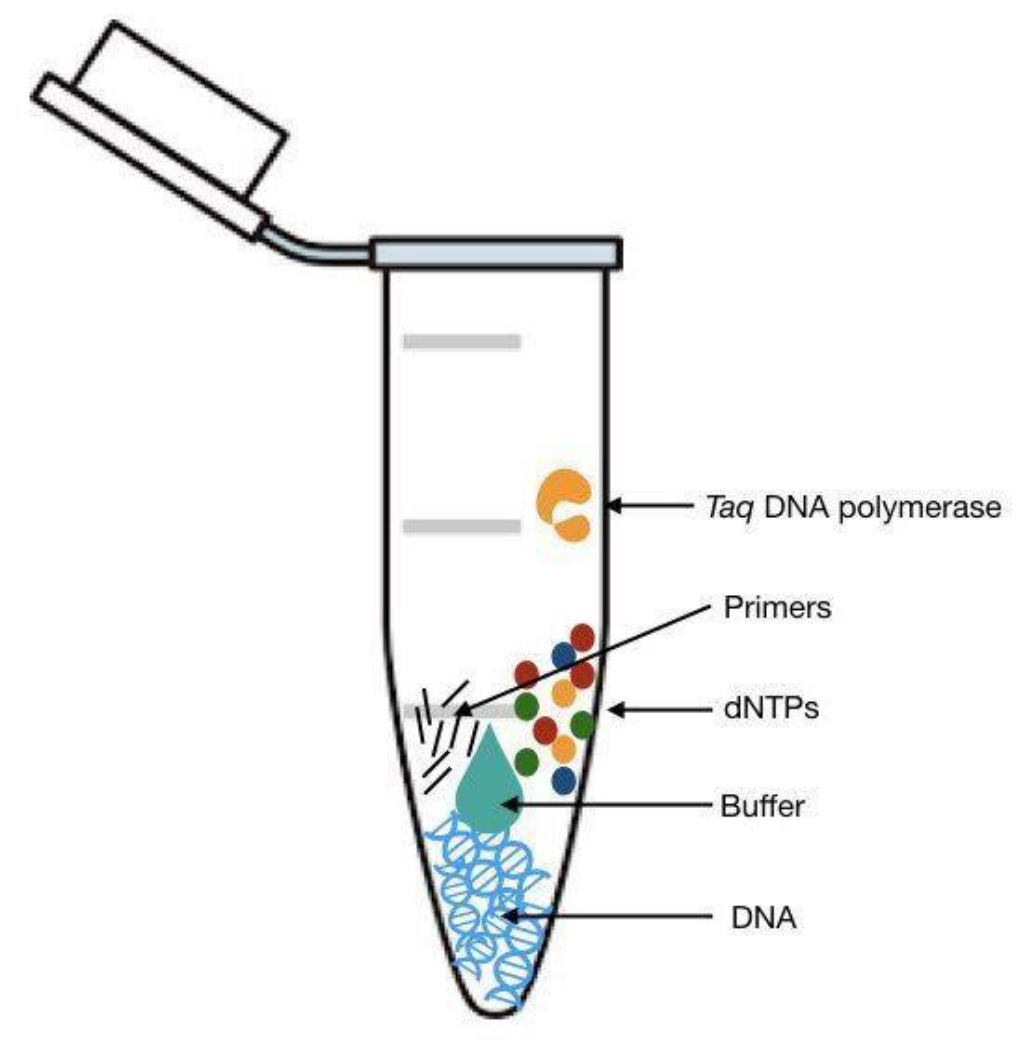
1) Oligo dT (binds polyA tails)
2) Random primers

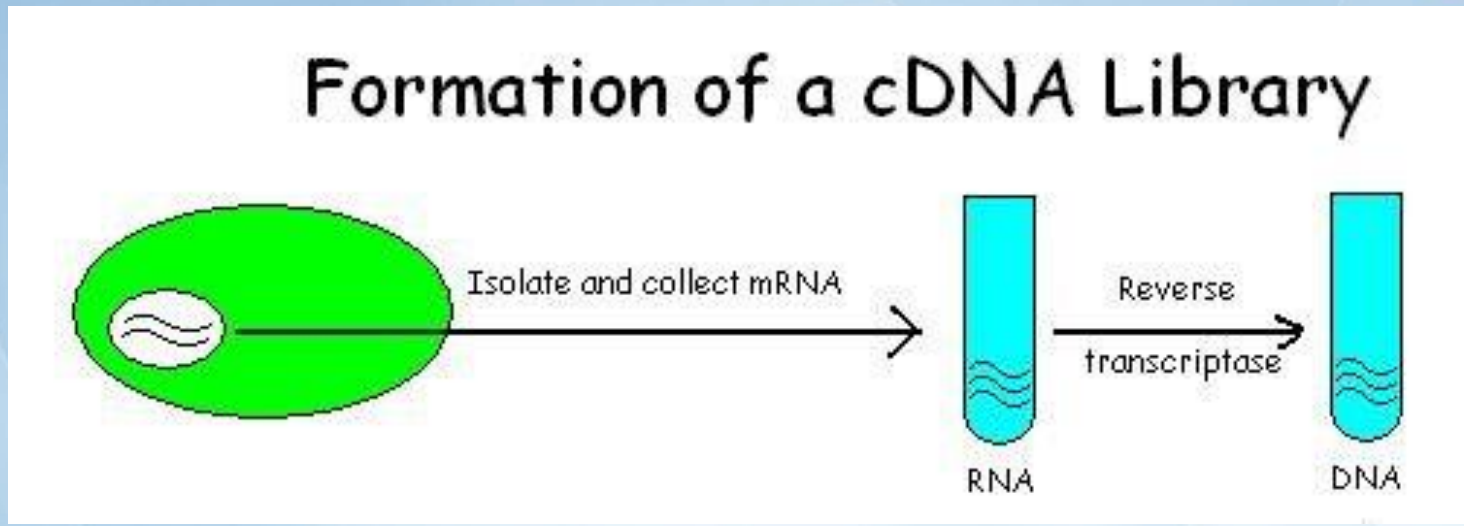
PCR Cycle

- **Each cycle (Round) of PCR contains 3 steps:**
  - **1- Denaturation**
  - **2- Primer annealing**
  - **3- Primer extension**
- **The cycle usually repeated for 25 – 40 times.**



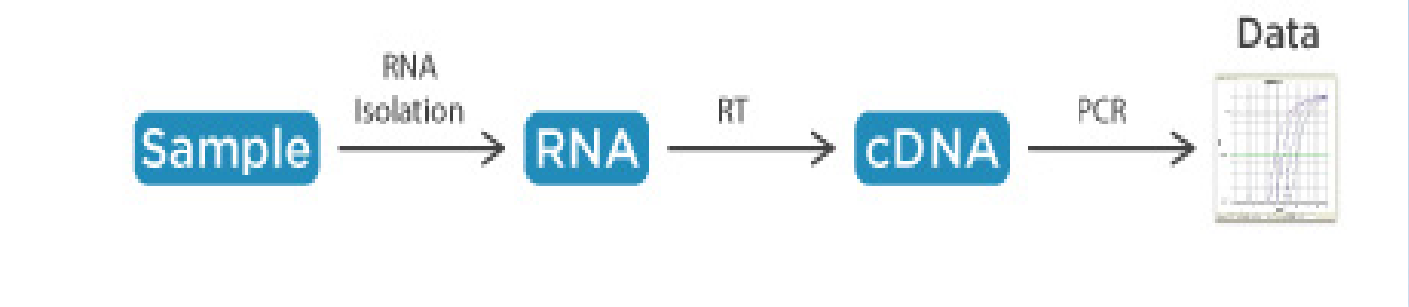
PCR Procedure



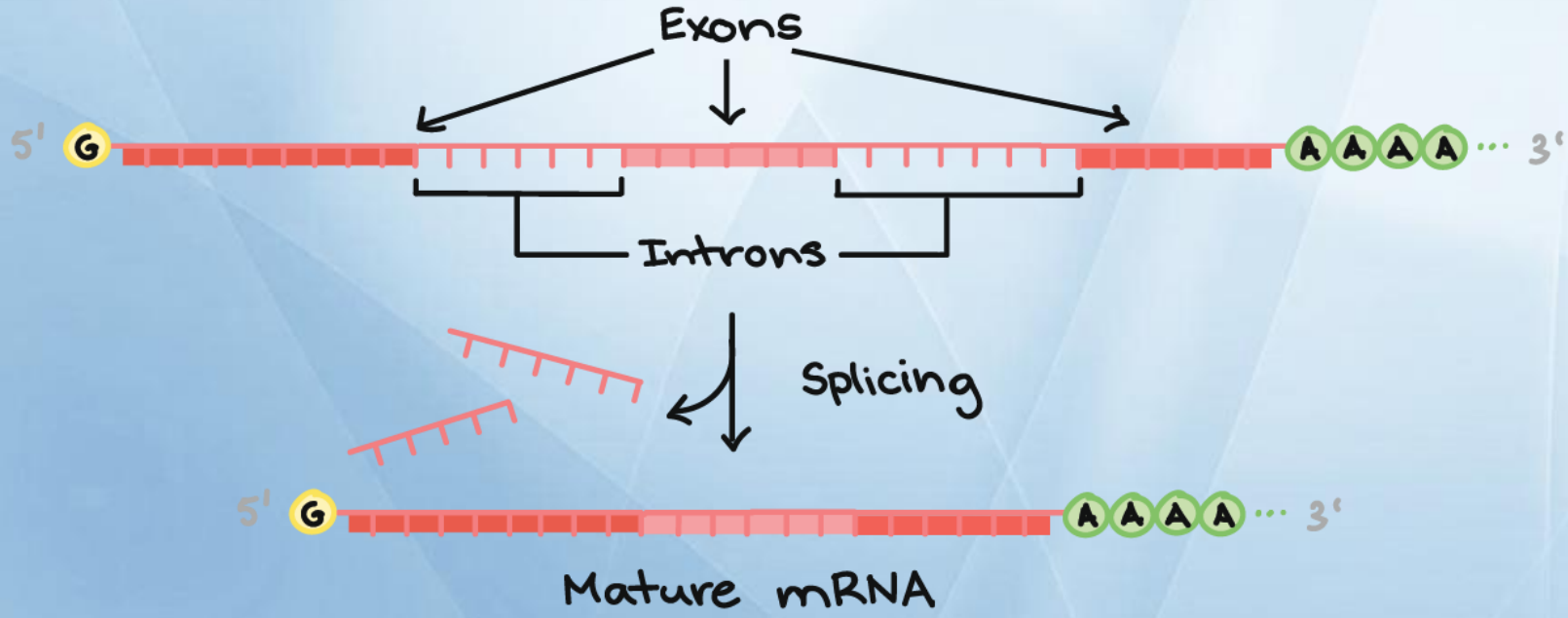


- cDNA clones are useful for many studies:
  - **Gene Expression quantification**
  - **Cloning of cDNA**

Gene Expression quantification



Cloning of cDNA



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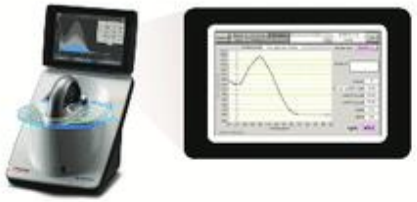

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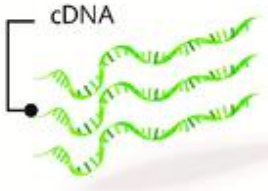

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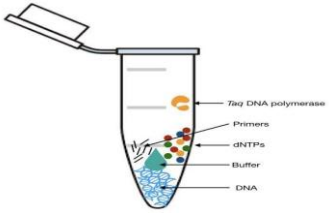

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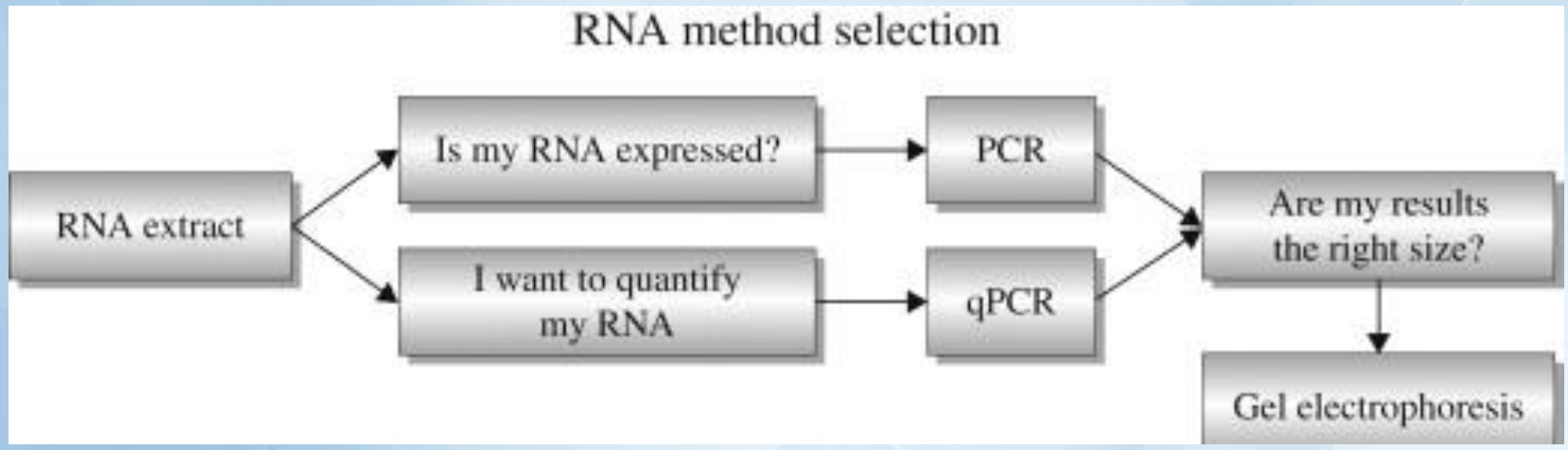
RNA method selection
RNA extract
Is my RNA expressed?
I want to quantify my RNA
PCR
qPCR
Are my results the right size?
Gel electrophoresis