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Chapter 26

Synthesis of complementary DNA (C-DNA): Principles, methodology, and applications in gene expression studies

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1. Introduction:

Complementary DNA (cDNA) synthesis from RNA is a pivotal technique in molecular biology that enables the conversion of RNA molecules into DNA copies (Gubler, and Hoffman, 1983). This process holds immense significance in various research fields, including gene expression analysis, functional genomics, and molecular cloning. By synthesizing cDNA from RNA templates, researchers can access a stable DNA form of RNA sequences, allowing for easier manipulation, amplification, and analysis (Green, and Sambrook, 2012). The synthesis of cDNA from RNA involves the reverse transcription (RT) of RNA molecules using a specialized enzyme called reverse transcriptase (Okayama, and Berg, 1982). This enzyme catalyzes the synthesis of DNA strands complementary to the RNA template. Through this process, the genetic information contained within RNA molecules can be preserved and further analyzed using various molecular techniques commonly applied to DNA (Srivastava et al., 2022). cDNA synthesis plays a crucial role in numerous experimental procedures, such as gene expression profiling using techniques like quantitative reverse transcription PCR (qRT-PCR) and RNA sequencing (RNA-seq) (Brown, 2010; Tripathi et al 2013a,b). Additionally, cDNA libraries constructed from specific tissues, cells, or organisms serve as valuable resources for studying gene expression patterns, identifying novel transcripts, and understanding cellular processes at the molecular level.

2. Materials required:

1. Total RNA sample
2. Oligo(dT) primer
3. RNase-free water
4. RNase inhibitor
5. Reverse transcriptase enzyme
6. Nucleotide mix (dNTPs)
7. Reaction buffer

8. RNase inhibitor
9. Thermal cycler
10. PCR tubes or plates
11. Microcentrifuge tubes
12. Pipettes and tips
13. Ice bucket

3. Procedure:

1. Prepare a master mix for cDNA synthesis by combining the following components in a microcentrifuge tube on ice:

Table 1: Master mix

Component	Volume (µL)
Oligo(dT) primer	1
Total RNA sample	X (as per instructions)
RNase inhibitor	1
RNase-free water	Adjust to 10

2. Thoroughly blend the master mix by gently vortexing and briefly centrifuging.
3. Subject the master mix to a thermal cycler, incubating it at 65°C for 5 minutes to initiate RNA and primer denaturation.
4. Promptly transfer the tube to an ice bath for 2 minutes to induce cooling.
5. Create a reverse transcription reaction mix by incorporating the following components into the master mix:

Table 2: Add on components in master mix

Component	Volume (µL)
5X Reaction Buffer	4
RNase Inhibitor	1
Reverse Transcriptase	1
Nucleotide Mix (dNTPs)	2
RNase-Free Water	Adjust to 20

6. Thoroughly blend the reaction mix by pipetting up and down gently.
7. Place the reaction mix in a thermal cycler and incubate it at 42°C for 60-90 minutes to facilitate reverse transcription.
8. Inactivate the reverse transcriptase enzyme by heating the reaction mix at 70°C for 10 minutes.
9. Utilize the cDNA promptly for PCR amplification or store it at -20°C for future applications.

4. PCR Amplification of cDNA (Optional):

1. Prepare a PCR reaction mixture by combining the following components in a PCR tube or plate (10 µL) :

Table 3: PCR reaction mixture

Component	Volume (µL)
cDNA template	1
Forward and Reverse Primers	2
Taq DNA Polymerase	1
PCR Buffer	4
dNTPs	0.5
RNase-Free Water	1.5

2. Modify the volume to achieve the desired reaction volume.
3. Thoroughly blend the PCR reaction mixture by gently pipetting up and down.
4. Position the PCR tubes or plate in a thermal cycler and execute the PCR program as per the assay's specifications.
5. Assess the PCR products through agarose gel electrophoresis or an alternative suitable method for analysis.

5. Precautions:

1. Ensure all equipment and surfaces are clean and RNase-free to prevent RNA degradation.
2. Use RNase inhibitors and wear gloves to minimize RNA degradation during handling.
3. Work quickly and efficiently to minimize RNA exposure to RNases.
4. Use a suitable reverse transcriptase enzyme and buffer according to manufacturer's instructions.
5. Ensure RNA samples are of high quality and integrity before starting cDNA synthesis.
6. Perform a genomic DNA elimination step if necessary to prevent contamination with genomic DNA.
7. Maintain consistent and appropriate incubation temperatures during the cDNA synthesis reaction.
8. Perform a negative control reaction without reverse transcriptase to detect genomic DNA contamination.

9. Use random primers or gene-specific primers based on the application and experimental design.
10. Verify the quality and quantity of synthesized cDNA using gel electrophoresis or qPCR.
11. Store synthesized cDNA at -20°C or -80°C to maintain stability and integrity.
12. Dispose of unused reagents and contaminated materials properly according to laboratory waste disposal guidelines.

6. References:

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