

First Strand cDNA Synthesis



General Aim

To synthesize cDNA from RNA templates using reverse transcriptase enzyme.

Method

First Strand cDNA Synthesis for RT-PCR using Avian Myeloblastosis Virus (AMV) reverse transcriptase.

Learning Objectives (ILOs)

- Demonstrate proficiency with the protocol involved in cDNA Synthesis.
- Identify the role of specific reagents and equipment in cDNA Synthesis.
- Practice basic laboratory techniques.
- Conclude downstream applications of cDNA Synthesis.

Theoretical Background/Context

cDNA Synthesis describes the generation of complementary DNA (cDNA) from an RNA template by reverse transcription. Reverse transcriptases (RTase) use an RNA template and a primer complementary to the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for several processes. Viruses synthesize cDNA so that they can be incorporated efficiently into the host chromosome.

cDNA could be used in the following applications:

- Gene cloning.
- Creation of a cDNA library.
- DNA microarray.
- qPCR.
- Studying gene expressions.

Principle of Work

cDNA Synthesis describes the generation of complementary DNA (cDNA) from an RNA template by reverse transcription. Reverse transcriptases (RTase) use an RNA template and a primer complementary to the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for several processes.