Reverse transcription

Reverse transcription is a process in molecular biology where RNA is used as a template to synthesize complementary DNA (cDNA). This process is catalyzed by the enzyme reverse transcriptase. The resulting cDNA can then be used for various applications, such as polymerase chain reaction (PCR), cloning, or gene expression analysis.

Here is a step-by-step overview of reverse transcription:

RNA Template: Reverse transcription begins with an RNA template. This RNA can be messenger RNA (mRNA) or other types of RNA.

Primer Annealing: A short piece of DNA called a primer is annealed (binds) to the RNA template. This primer provides a starting point for reverse transcription.

Reverse Transcription: The enzyme reverse transcriptase synthesizes a complementary DNA strand using the RNA template. This enzyme is capable of “reading” an RNA template and synthesizing a complementary DNA strand. The RNA is used as a template to produce a single-stranded cDNA molecule.

RNA Degradation: The RNA template is usually degraded or removed, leaving behind a single-stranded cDNA molecule.

Second Strand Synthesis (optional): In some cases, a second DNA strand can be synthesized to generate a double-stranded cDNA molecule. This step is often carried out using DNA polymerase.

The resulting cDNA can be used in various downstream applications. One common application is reverse transcription polymerase chain reaction (RT-PCR), where the cDNA is amplified by PCR to study gene expression levels. Reverse transcription is also a crucial step in the generation of complementary DNA libraries for various genomic studies.

Reverse transcription is particularly important in studying gene expression in eukaryotic cells since many genes are transcribed into RNA before being translated into proteins. This process allows researchers to analyze the RNA content and infer information about gene expression patterns and regulation.

The synthesis of DNA from an RNA template, via reverse transcription, produces complementary DNA (cDNA). Reverse transcriptases (RTs) use an RNA template and a short primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for the Polymerase Chain Reaction (PCR). This combination of reverse transcription and PCR (RT-PCR) allows the detection of low abundance RNAs in a sample, and production of the corresponding cDNA, thereby facilitating the cloning of low copy genes. Alternatively, the first-strand cDNA can be made double-stranded using DNA Polymerase I and DNA Ligase. These reaction products can be used for direct cloning without amplification. In this case, RNase H activity, from either the RT or supplied exogenously, is required.