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Reverse Transcriptase Polymerase Chain Reaction (RT PCR)

RT-PCR: (Reverse Transcription PCR) is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA. The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene. The 5' end of a gene (corresponding to the transcription start site) is typically identified by an RT-PCR method, named RACE-PCR, short for *Rapid Amplification of cDNA Ends*.

Procedure: Clean working area with 70% lab alcohol. Wear gloves, and thaw all the required reagents on ice, followed by vortex mix and quick spin all the reagents. Arrange reagents on ice as per sequence of addition. Add reagents in 200 ul capacity PCR tube, as per sequence given below:

1. Pre- RT step

Nuclease free water	5.0 ul
Total RNA from HeLa cell line	4.0 ul
<u>Oligo dT primer</u>	<u>1.0 ul</u>
Total reaction volume =	10.0 ul

Mix all added reagents by micropipetting, vortex it and quick spin to collect entire volume at the end of the bottom of PCR tube. Incubate at 65 °C for 10 minutes followed by incubate at RT for 2 minutes. *This is called as pre RT step where RNA gets linearized, and breaks any possible folded structure, hair pin loop structure, because of intrastrand complementarity.*

To the same tube containing RNA for RT reaction, add following reagents as given below:

2. RT step

RT mix	8.0 ul
RNAsin	1.0 ul
<u>MMLVRT</u>	<u>1.0 ul</u>
Total volume =	20.0 ul

Mix all added reagents by micropipetting, vortex it and quick spin to collect entire volume at the end of the bottom of PCR tube. Incubate at 37 °C for 1 hour and followed by incubation at 95 °C for 2 minutes. Immediately after incubation at 95 °C for 2 minutes keep reaction tube on ice, i.e. snap chill. After 5 minutes spin down and proceed for PCR. Store remaining cDNA at -20 °C. *This is actual RT step, where you get single stranded complementary DNA (cDNA) copied from RNA by the action of Reverse Transcriptase (RT) enzyme.*