

Reverse transcription polymerase chain Reaction (RT-PCR) for determination of  
human Pol  $\beta$  mRNA level in human cells

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## **Abstract**

The main goal of this experiment is to determine the concentration of mRNA in human normal and cancer cells through reverse transcription PCR as well as to prepare the PCR product for future gel electrophoresis analysis. The expected results should show the band around 361 bp.

## **Introduction**

PCR, or Polymerase chain reaction, is a common technique used in the lab for DNA studying. The procedures include denaturing dsDNA, annealing of primers, and synthesizing new DNA. During denaturing, the high temperature allows the hydrogen bonds between the DNA strand to break, thus, separating them. Next, annealing occurs at a lower temperature, which causes the DNA primers to attach to the template DNA. Finally, during the synthesis of DNA, an enzyme Taq polymerase, which is used as a starting point for DNA synthesis, makes 2 new strands of DNA using the original strands as templates. Taq DNA polymerase is a thermally stable primer and can undergo high temperatures. This primer is used in PCR because the entire reaction is operated in a thermal cycler. If another type of enzyme (for example, an enzyme that cannot function under high temperatures) would be used, the elongation process wouldn't occur, and the DNA would not be synthesized properly, preventing the completion of a reaction.

The PCR reaction usually runs for about 25 to 40 cycles, allowing for making millions to billions of copies of a fragment, and to amplify a small amount of DNA to a large amount, which makes it easier to study the DNA. The results are analyzed under gel electrophoresis, where the DNA is separated by its molecular weight. PCR is used in the medical field to identify a certain virus or bacteria. For

example, the presence of a viral COVID – 19 mRNA can be determined through the PCR test, as well as the traces of the HIV virus.

## Procedures

First, the template and the OligodT22 primer were assembled. The whole mixture contained 1 microliter of RNA template sample (total 5 ug), 4 ul of 100 uM oligodT22 primer, 5 ul M-MLV 5x Reaction Buffer, 1.25 uL 10 mM dNTPs (final: 0.625 uM), 1 ul (25 units) Recombinant RNasin Ribonuclease Inhibitor (total: 25 units), 1 ul (200 units) M-MLV RT, and 1.1 ul of DEPC – treated water. Next, the mixture was incubated for 30 minutes at 25 degrees Celsius and then incubated again in a heating block for 50 minutes at 42 degrees Celsius. After that, 50 ul of PCR reaction was set up. The reaction contained 5 ul 10 x PCR buffer (Mg<sup>2+</sup>, final: 1.5 mM), 3 ul of 25 mM MgCl<sub>2</sub> (final: 1.5 mM), 2 ul cDNA template, 1 ul of 10 mM dNTPs (final: 0.2 mM), 2 ul of 10 pmol/ul forward primer, 2 ul of 10 pmol/ul reverse primer, 1 ul of Taq DNA polymerase, and 34 ul of water. Next, the PCR reaction was ready, and the sample was prepared for the future 1% agarose gel analysis.

## **Discussion**

The expected results of the gel electrophoresis are the band at 361 base pairs. This result might be slightly different because of the possible source of error, such as improper calculations of the initial amount of RNA template sample and the amount of DEPC – treated water. Another source of error could be the inaccurate measurement of the components.

The reverse transcription can be used to measure the expression of a gene (for example, Pol Beta) in cells. The change in gene expression can be determined by making cDNA copies of mRNA and then measuring the abundance of the gene-specific transcript, which will tell how much of a certain mRNA is present in a sample. RT – PCR is one of the most effective methods to measure the Pol beta gene expression because it has a wide dynamic range, is very sensitive and, thus, elective, has little or no post-amplification processing, and can increase sample capacity. Such a method is capable to amplify specific regions of the DNA it targets.

## Reference

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## Notebook

## Lab 7

260-280 - above 1.9 (level of protein contamination)

PCR  $\rightarrow$  No ethanol contamination

## Types of PCR - 3

RT-PCR, qPCR

Covid-look for mRNA

↓  
cDNA  
RNA starting material

↓  
Lab 8  
1) Assay  
2) bel

↘ cDNA is a starting material

1) RT  $\rightarrow$  cDNA

## 2) PCA

3) bel

## Product - Ampli cant

361 bp band

use:

20 mL is total

$4.643 \mu\text{g} - 1 \mu\text{g}$

$$f = \frac{5}{4.643} = 1.1$$