Optimizing a Method to Quantify Cellular RNA Levels

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Abstract

There is evidence that improperly processed or unprocessed RNA can result in dysfunctional RNA that could play a role in the development of diseases, such as cancer. This project will optimize a method that allows us to measure the quantity of processed and unprocessed RNA. RNA is isolated from yeast cells and then reverse transcribed to produce cDNA of varying sizes. The cDNA levels are then analyzed through a Polymerase Chain Reaction (PCR) to quantify the rations of processed vs unprocessed RNA. While this project is only a small part of a much bigger picture, it is important to optimize this technique as it is an important measurement in our research

Background

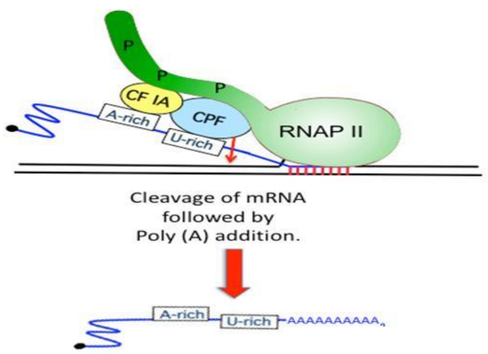


Figure 1. Maturation of mRNA involves placing a cap on the 5' end of the DNA strand. Introns are spliced and the 3' end is processed to leave a newly transcribed strand of mRNA. This newly transcribed strand of mRNA is cleaved and a chain of nucleotides, referred to as a poly (A) tail, is attached to the end.

Materials and Methods

A culture of yeast cells were grown on an agar plate.

Cells were washed and ground up with acid wash glass beads and a series of buffers with a spin column to extract RNA from the cells.

RNA concentration is calculated and Sample 1 out of 2 is then split into two more samples, RT and no RT.

Random primers are added to both samples.

Reverse Transcriptase (RT) is added to one sample (RT sample) and the other will not get any RT (no RT sample).

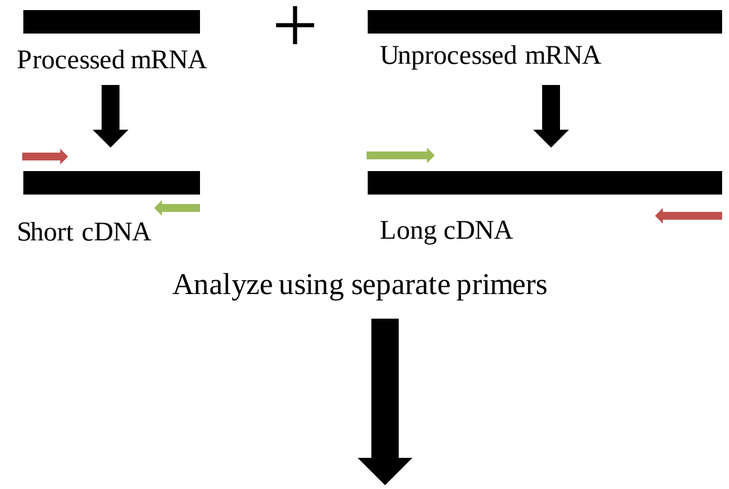
Both samples are incubated and then split once more to make a total of 4 samples. All 4 samples will be diluted differently. There is 1 in 2, 1in 5 RT, 1 in 2 no RT, and 1 in 5 no RT.

Forward and reverse primers and a template are added to the 4 samples. 2 control samples are made, one with a known genome template and another with no template at all.

Samples are put in thermocycler for Polymerase Chain Reaction (PCR) to occur.

Electrophoresis is used to analyze the ratios of processed and unprocessed DNA.

Experimental Model



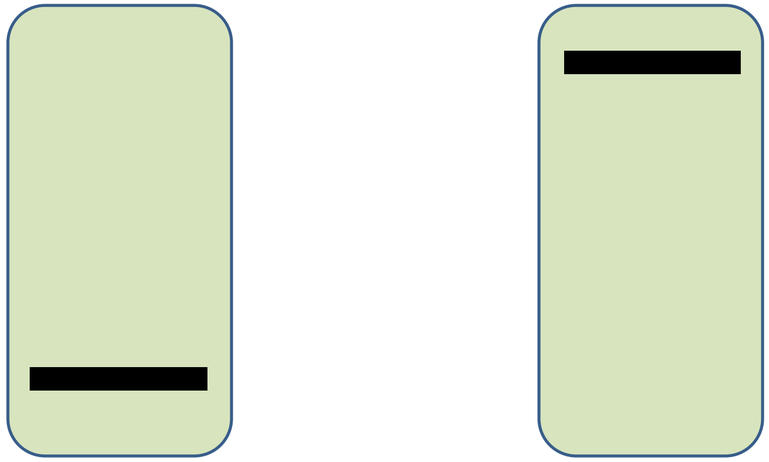


Figure 2. The mRNA has been turned into cDNA and is now being primed as shown by the red arrows. In an agarose gel, the shorter cDNA is towards the bottom while the longer cDNA is towards the top of the gel.

$\frac{\text{Short}}{\text{Long}} = 1$ All RNA is unprocessed

$\frac{\text{Short}}{\text{Long}} > 1$ More processed than unprocessed

$\frac{\text{Short}}{\text{Long}} < 1$ More unprocessed than processed

Within a Eukaryotic cell, mRNA is transcribed from DNA in the nucleus. This mRNA is then translated into proteins within the ribosomes attached to the endoplasmic reticulum (ER). Before translation occurs, mRNA goes through a series of changes. First, the RNA polymerase transcribes the DNA so pre-mRNA is produced. The pre-mRNA is cleaved and polyadenylated, this is when the end of the pre-mRNA has been cut off and a series of adenine nucleotides is added to the cleaved pre-mRNA to form a tail-like structure, producing mature mRNA. Once the mature mRNA has been made, it is ready to be translated into proteins

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