EXPERIMENT 4

RNA ISOLATION WITH TRIZOL REAGENT AND RNA VISUALIZATION & QUANTIFICATION

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Introduction

The genetic material in DNA consists of genetic codes which are in a double-stranded form bound by hydrogen bonds. RNA is a single-stranded form of genetic material which has many purposes which include the transmission of genetic code as well as a variety of purposes. mRNA has the function of transcribing the DNA sequence to be transcribed and messaging it to the ribosome. rRNA acts as a ribosomal subunit. tRNA has the purpose of carrying the amino acids corresponding to each codon to the ribosome to bind and form the protein which was the desired output of this system. miRNA and siRNA act in the regulation of genes by binding to the gene sites in DNA and preventing their transcription, which downregulates or inhibits the transcription of that gene in the cell. The RNA undergoes several alterations such as poly-A tail addition, 5' cap addition which has the purpose of preventing RNA catalysis by the RNAases in the cytoplasm. The RNA also undergoes intron splicing which eliminates the segments in DNA that will not be transcribed. The size of RNA is referred to as S units which is a sedimentation unit named the Svedberg Unit.

DNA and RNA are both genetic materials with significant differences, however, they are similar in the way that they are chains of sugars that are linked by phosphate groups. RNA's sugar is the ribose sugar and DNA's sugar is deoxyribose which lacks one oxygen molecule. RNA's extra oxygen molecule results in making RNA more unstable than DNA is. Additionally, the bases of RNA are A, U, G, C while the bases of the DNA are A, T, G, C.

The purpose of this experiment was to extract RNA from the liver tissue. After the purification of RNA, RNA must be transferred to the membrane using techniques such as Northern Blotting and fixed to the membrane using techniques such as baking or UV cross-linking which fix the backbone of RNA to the membrane [1]. To further observe the effect of the protein coded by purified RNA, cDNA from the purified RNA is injected into organisms and the effect of transcribed proteins is observed. Additionally, the purity and quality are measured quantitatively by the NanoDrop technology which is a machine that utilizes spectrometric measurement and gives feedback on whether the experiment did a good job on RNA purification.

Materials

The liver tissue obtained from the previous animal experiment was used as a tissue. Trisol and Chloroform were used for isolating RNA from liver tissue. The Trisol has Guanidium thiocyanate that lyses the cell and inhibits the RNAase and DNAase such that they cannot lyse the RNA to be purified. Chloroform is used for getting RNA rid of Trisure and distinguishing the phases of DNA, RNA, and proteins. Trisol and Chloroform are worked with under the hood to prevent interaction with the experimenter. Isopropyl alcohol and Ethanol were used for the precipitation of RNA and DNA. Isopropanol also deactivates RNAase. DEPC treated water is used for the purification of RNA from any chemicals. Eppendorf was used to preserve the liver tissue and other samples. The centrifuge was also used for suspension. 2X loading dye was used to be able to view the bands in the gel electrophoresis [2]. A Nanodrop machine was used to quantitate the purity of the purified RNA [3]. Micropipettes are used throughout the experiment to transport solutions in a measured approach and to help the suspension of the solutions.

Methods

For the purification of RNA obtained from the liver tissue of mice, the liver tissue was smashed in the Eppendorf for increasing the surface area to enhance the chemical reactions. The following steps were done under the füme hood to prevent interaction of chemicals with the experimenter through the air and also decrease the contamination. 1ml trisol was put into the Eppendorf that contains the tissue and incubated for 5 minutes for dissociating the tissue. After 0.2 ml of Chloroform is added to the same Eppendorf and the Eppendorf is shaken for 15 seconds and left 2 minutes for incubation for isolating RNA from liver tissue. Then the solution is taken from the hood and the solution is centrifuged for 10 minutes with 13000 rpm speed in 40C condition for achieving different phases in the Eppendorf. After the centrifugation, 3 phases are observed which are RNA, DNA, and proteins from the top of the Eppendorf to the bottom. The clear top part which has the RNA is taken via a micropipette and the rest of the Eppendorf is discarded.

Later, 0.5 ml Isopropanol was added to the taken solution and shaken for 5 seconds, and incubated for 5 minutes at room temperature. The solution is centrifuged at 13000 rpm speed for 10 minutes in 40C condition. After the centrifugation, the RNA is seen as a pellet at the bottom of the Eppendorf. The supernatant is discarded using a micropipette and the Eppendorf was containing only the pellet. The pellet was left to be dried under the füme hood until It dried.

Then, the RNA was to be resuspended. For this purpose 1 ml ethanol is added to the RNA pellet and resuspended using up and down with micropipette as well as using a vortex. The solution is centrifuged with a 13000 rpm speed for 3 minutes. After, the supernatant is discarded via a micropipette, and 50 ml DEPC treated water is added to the solution. Later, 6 ul loading dye and 6 ul of the sample from the Eppendorf are mixed in a separate Eppendorf. The ½ ratio was obtained such that the loading dye will enable the researcher to view bands in the electrophoresis step.

The solution is added to the well in the electrophoresis gel to run. Also, the sample solution which did not contain loading dye is used for NanoDrop machine measurement for quantifying the pureness of the isolated RNA throughout this experiment [3].

Results

NanoDrop Results

In NanoDrop machine results, the nucleic acid concentration was measured as 3649 ng/ul of RNA for our group SB1 where group IA2's nucleic acid concentration was measured as 253 ng/ul of RNA. Meaning our groups' RNA concentration is higher than group IA2s resulting that our purified RNA is more concentrated than theirs which is a positive aspect for our experiment results [4].

The A260/A280 value reflects the purity ratio regarding DNA and RNA [4]. Our group's value for that is 1.99 and IA2's concentration is 1.68. As our group's ratio is greater than the ratio of IA2's and closer to 2 which reflects pure RNA, our RNA is purer than group IA2's.

The A260/230 value reflects the ratio regarding the purity of nucleic acid and It should be expected to be between 2.0 to 2.2 for ideal results [3]. Our value is 1.94 and IA2's value is 0.35. Hence, our group's nucleic acid purity is higher than that of group IA2's.

Gel Image Results

Our group worked on the lane labeled as SB1. In gel electrophoresis, we observe 3 bands which can be of rRNAs of length 28S, 18S, and 5S. Hence, the gel image enables the researcher to measure the sizes of the purified RNA.

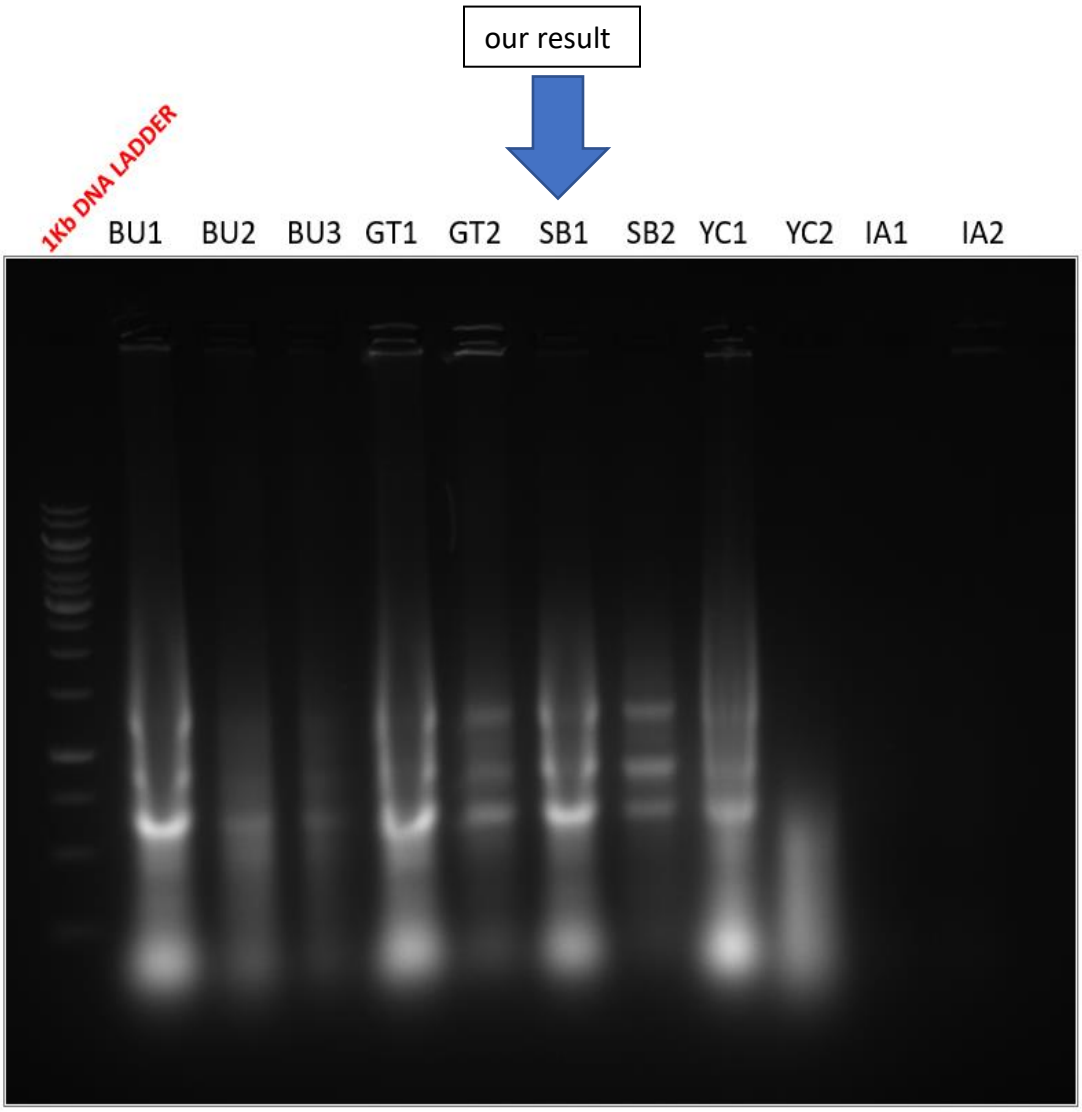

Figure I: Gel Electrophoresis of each group.

Discussion

Regarding the results of our experiment of RNA purification. Our purity ratios and RNA concentrations were mainly higher than that of IA2's results. Our A260/280 and A260/230 results were precisely close to 2.0 which was the ideal purity quantity. Hence, our RNA purification was a success and the purified RNA can be used in cDNA experiments without a doubt.

In the gel image resulted from the gel electrophoresis, 3 bands can be seen without giving the researcher trouble where the researcher can distinguish the lengths of the rRNA as 28S, 18S, and 5S for instance. However, the dye is overflowed as we can see an excess amount of dye at the sides of the lane. The reason for this can be a human error that occurred during loading the solution into the well with a micropipette.

All in all, the RNA isolation from the liver tissue was a success and It can proceed with further cDNA experiments.

References

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