Isolation of total RNA in human cells using TRIzol reagent

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Abstract

The main goal of this experiment is to isolate total RNA from the human HeLa cancer cell using TRIzol reagent, to determine its concentration using a Nanodrop spectrophotometer, and to analyze the purity of results using gel electrophoresis. Once the results were obtained, nothing appeared on the gel, and the concentration of the isolated sample was too low compared to the rest.

Introduction

RNA, or ribonucleic acid, is a nucleic acid present in all living cells. Compared to DNA, RNA is single-stranded, contains sugar ribose, and has a shorter chain of nucleotides. RNA is an important cell component as it helps to regulate gene expression, and viral infection, proceed RNA-guided DNA repair and make proteins. In this experiment, total RNA was isolated from a human cell. Total RNA is contributed by messenger RNA (mRNA, about 2% of total RNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and non-coding RNAs (microRNAs). Messenger RNA is responsible for transferring genetic information from genes to ribosomes for protein synthesis, transfer RNA reads mRNA sequence and transfers amino acids to the ribosome, and ribosomal RNA binds with a corresponding amino acid to a growing peptide chain. Non-coding RNAs are also important as they help to regulate gene expression at mRNA levels.

After the RNA isolation workflow, 3 phases should be seen in a column: aqueous phase (RNA), Interphase (DNA), and organic phase (protein, lipids). To extract the RNA, TRIzol reagent can be used. The name TRIzol comes from the idea that by using this reagent, 3 things listed above can be obtained.

After isolation, it is important to determine RNA concentration as it helps to commit the analysis of specific RNA levels with certain gene expressions in cells. To do so, an apparatus, called a nanodrop spectrophotometer can be used. The

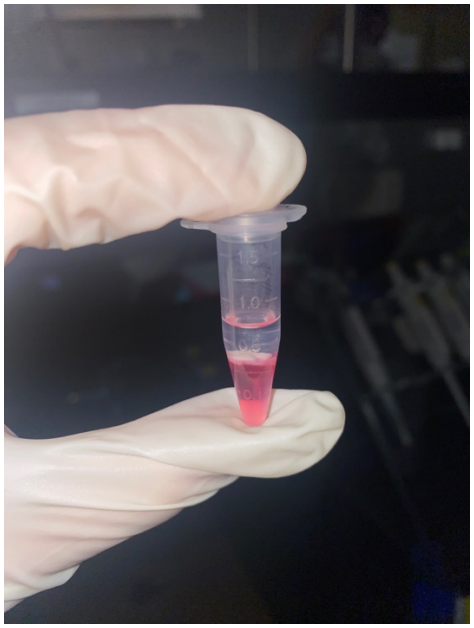
expected concentration of RNA nucleic acid is about 40 ug/ml. In addition to the concentration, it is important to check the quality of isolated RNA, which can be done using gel electrophoresis. Ideal gel results should contain 2 bands: 28S ribosomal RNA and 18S ribosomal RNA. Otherwise, the RNA is contaminated.

Procedures

First, to lyse the cell and to separate the phases, 500 microliters of TRIzol were added to the pellet of 1x10^7 cultured human normal HeLa cancer cells, and the cell lysate was pipetted up and down 6 times. Next, the mixture was incubated for 5 minutes at room temperature. Later, 100 microliters of chloroform were added, and the sample was inverted 3 times. Then, the mixture was incubated for 3 minutes at room temperature. After that, the sample was centrifuged at 12,000 rpm at room temperature for 15 minutes. After the centrifuging was done, the aqueous phase containing RNA was transferred to a new tube by pipetting out the aqueous phase. The next step included the precipitation of total RNA, for which first, 250 microliters of isopropanol were added to the aqueous phase, per 500 microliters of TRIzol reagent from the previous step. Next, the sample was incubated at -20 degrees Celsius for 10 minutes. Later, it was centrifuged at room temperature for another 10 minutes at 12,000 rpm. The supernatant was discarded using a micro-pipettor. The RNA precipitation was done at this point, and the washing of the RNA pellet has been started. For the wash, 500 microliters of ethanol per 500 microliters of TRIzol reagent were added, and the mixture was pipetted up and down several times. Next, the RNA sample was centrifuged at room temperature for 15 minutes at 12,000 rpm. After that, the supernatant was removed using a

micro-pipettor. The RNA pellet was then air-dried at 37 degrees Celsius for 3 minutes. The RNA washing was done. Next, the RNA was dissolved in DEPC-treated water. For this step, the RNA was resuspended with 30 microliters of DEPC-treated water, and the mixture was pipetted up and down until the pellet was completely dissolved. Next, the RNA solution was incubated for 5 minutes at 37 degrees Celsius. Once the RNA sample was prepared, it was run in a 1% agarose gel, for which 2 microliters of RNA were mixed with 8 microliters of DEPC water and 2 microliters of 6x loading buffer. Then, all 12 microliters of the sample were loaded into the agarose gel. Lastly, the RNA was stained in agarose gel with the use of 100 microliters of 10 mg/ml of ethidium bromide in 30 ml of water in a small container for 10 minutes. The relative amount of the RNA sample was estimated.

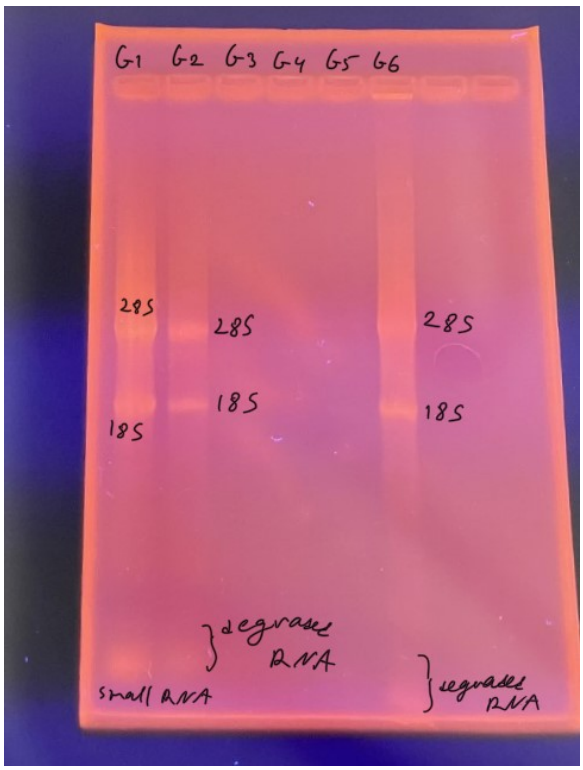
Results



Sample after centrifugation. 3 separate phases can be clearly seen:
Bottom layer – organic layer: protein and lipids
White phase – interphase, DNA
Top phase – aqueous phase, RNA

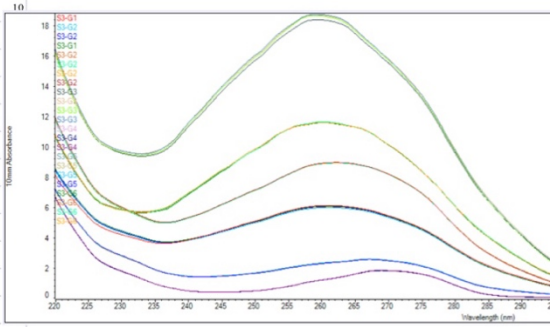
During the next step, extraction/precipitation with isopropanol, the RNA pellet was

formed. The size of the pellet was small but still visible.



For group 5, nothing appeared on the gel, resulting from improper purification.

S/N	Sample	Username	Date &Time	Nucleic Acid	Unit	Abs 260	Abs 280	260/280	260/230	Sample time	Factor	Dilution Factor
47	S3-G1	AHC3-510	10/17/22 20:24	243.6	ng/µl	6.089	3.281	1.86	1.46	RNA	40	10
48	S3-G1	AHC3-510	10/17/22 20:25	238.1	ng/µl	5.954	3.175	1.88	1.38	RNA	40	10
49	S3-G1	AHC3-510	10/17/22 20:25	240.6	ng/µl	6.016	3.206	1.88	1.38	RNA	40	
50	S3-G1	AHC3-510	10/17/22 20:26	241.9	ng/µl	6.046	3.22	1.88	1.36	RNA	40	
51	S3-G2	AHC3-510	10/17/22 20:26	354.8	ng/µl	8.869	4.764	1.86	1.5	RNA	40	
52	S3-G2	AHC3-510	10/17/22 20:27	354.3	ng/µl	8.859	4.74	1.87	1.49	RNA	40	
53	S3-G2	AHC3-510	10/17/22 20:27	355.2	ng/µl	8.88	4.757	1.87	1.49	RNA	40	
54	S3-G2	AHC3-510	10/17/22 20:27	355.9	ng/µl	8.897	4.744	1.88	1.48	RNA	40	
55	S3-G3	AHC3-510	10/17/22 20:28	735.6	ng/µl	18.389	9.606	1.91	1.92	RNA	40	
56	S3-G3	AHC3-510	10/17/22 20:28	748	ng/µl	18.701	9.844	1.9	1.93	RNA	40	
57	S3-G3	AHC3-510	10/17/22 20:28	745.8	ng/µl	18.645	9.702	1.92	1.94	RNA	40	
58	S3-G3	AHC3-510	10/17/22 20:28	750.1	ng/µl	18.753	9.775	1.92	1.93	RNA	40	
59	S3-G4	AHC3-510	10/17/22 20:29	46.3	ng/µl	1.159	0.827	1.4	0.67	RNA	40	
60	S3-G4	AHC3-510	10/17/22 20:29	46.3	ng/µl	1.158	0.828	1.4	0.66	RNA	40	10
61	S3-G4	AHC3-510	10/17/22 20:29	46.6	ng/µl	1.166	0.842	1.39	0.66	RNA	40	10
62	S3-G5	AHC3-510	10/17/22 20:31	91.7	ng/µl	2.291	1.357	1.69	0.83	RNA	40	10
63	S3-G5	AHC3-510	10/17/22 20:31	90.7	ng/µl	2.266	1.323	1.71	0.83	RNA	40	10
64	S3-G5	AHC3-510	10/17/22 20:32	91	ng/µl	2.275	1.343	1.69	0.82	RNA	40	10
65	S3-G5	AHC3-510	10/17/22 20:32	90	ng/µl	2.251	1.325	1.7	0.81	RNA	40	10
66	S3-G6	AHC3-510	10/17/22 20:33	463.4	ng/µl	11.586	6.05	1.91	2	RNA	40	10
67	S3-G6	AHC3-510	10/17/22 20:33	463.8	ng/µl	11.594	6.054	1.91	1.98	RNA	40	10
68	S3-G6	AHC3-510	10/17/22 20:33	465.8	ng/µl	11.644	6.087	1.91	2	RNA	40	10
69	S3-G6	AHC3-510	10/17/22 20:33	464.1	ng/µl	11.602	6.062	1.91	2	RNA	40	10



The average value of the nucleic acid concentration for group 5 is low compared to the rest of the values

Discussion

After phase separation using TriZol, 3 separate phases could be clearly seen. This is because of its composition, TriZol Reagent can maintain RNA integrity while lysing the cell and dissolving cell components. Later, the RNA pellet was precipitated with the help of isopropanol because, in the isopropanol, DNA is less soluble than RNA.

However, even though 3 separate phases were seen in the column, based on the final results, the RNA sample of group 5 was not purified well enough.

First, nothing appeared on the gel. Looking at ladder 5, no bands can be seen. This is usually caused by the high ethanol concentration or improper drying of a sample.

Next, the theoretical and actual concentration values did not match. Compare with the highest average concentration value of 744.88 ug/ml, the average value of the obtained concentration of the nucleic acid for group 5 was 90.85 ug/ml, which is lower than it's supposed to be. Thus, based on the proof above, it can be concluded that the sample was not purified properly.

A possible source of error is hair contamination in the test tube, because of which, the RNA sample had to be transferred into a separate tube. During transferring process, some of the samples could be lost.

The best gel results were obtained by groups 1,2, and 6 as both bands of ribosomal RNA (28S and 18S) could be seen, as well as some small RNAs. However, the best concentration results were obtained by group 3 as, compared to the other samples, the average actual value for concentration was the highest one.

In general, despite the fact that the RNA was not purified properly, a basic technique of RNA purification and quality analysis has been learned.

Reference

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Notebook pages

Lab 6

TRIzol - can get 3 things: RNA, DNA, proteins

Proteins -> down layer (organic), lipids

Top - RNA aqueous phase (needed)

Interphase - DNA (white)

Careful when phase separation, only need aqueous phase

Don't submerge pipette to not get DNA and to not get contaminations

Heat block - at 37°C

Contaminations can come very easily

No mRNA on gel (b/w 28S and 18S)

No 28S and 18S -> contaminated

1 bands (28S/18S) -> contaminated

Do not loosen the pellet (must be white)

No more than 3 min for air dry