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A study on total RNA including microRNA isolation from cell lines by different cost effective methods

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Abstract: Small non-coding RNAs have been recognized as potential controllers of various biological functions, including gene expression, molecular growth, and physiological functions. The diverse ways considerably aid the prognosis and diagnosis of many diseases which microRNAs (miRNAs) manifest themselves. It is crucial to successfully isolate miRNAs from various tissues and bodily fluids since they have a wide range of uses as clinical biomarkers for the precise detection of many diseases, cancer risk assessment, and investigation of different metabolic processes and gene activity. The present study compares the available RNA extraction protocols and renders an improvised protocol for isolating RNA and subsequent extraction of miRNA molecules. Different RNA isolation protocols were analyzed: Direct-zol™ RNA MiniPrep Plus kit, TRIzol™ reagent, and non-kit organic RNA extraction methods. The quality of RNA was assessed using spectrophotometry and bleach gel electrophoresis. Here, we have measured the expression of miRNA10b, a specific biomarker candidate for the prognosis of colorectal cancer that plays a major role in metastasis. The amplified expression of miRNA was demonstrated using Real-time PCR. The qualitative and quantitative analysis of the extracted RNA was evaluated. The modified extraction protocol was found to yield good-qualitymiRN. The modified RNA extraction protocol is a simple, cost-effective, efficient method for miRNA isolation from cell lines.

Introduction

Tumor MicroRNAs (miRNA) constitute a novel class of non-coding molecules, approximately 21–25 nucleotides in length, which endogenously regulate major biological and developmental processes (Wahid et al., 2010; Felekkis et al., 2010). Numerous study reports have stated the use of miRNAs in detecting and diagnosing various pathological conditions and tumorigenesis. The altered expression of microRNA in various tumors is the driving factor that has the tumorigenic process (Calin et al., 2004; Bryzgunova et al., 2021). It has been noted that miRNA-133b, miRNA-126, and miRNA-142-3p play an important role as tumor suppressors by focusing on particular genes in colon cancer cells (Weng et al., 2015; Pathak et al., 2015). Additionally, it has been established that colon cancer patients' stool samples overexpress miRNA-221 and miRNA-18a, which are implicated in

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the initiation and growth of tumors (Yau et al., 2014; Evert et al., 2018). Additionally, recent studies have stated the link between the upregulated expression of miR-130a, miR-331, miR-19a, miR-22 and the development of primary tumors in gastric cancer patients compared to non-cancer individuals (Jiang et al., 2015; Zhu et al., 2018).

Interestingly, both primary neoplastic ductal cells and pancreatic cancer cell lines showed elevated expression of miRNA-205, miRNA-18a, miRNA-93, miRNA-221, and miRNA-224 (Zhang et al., 2009). Research has recently revealed the dangerous connection between miR10b and colon cancer growth and metastasis (Jothimani et al., 2018; Das et al., 2021). The target gene of miR10b and homeobox D10 (HOXD10) have closely interacted with promoting molecular mechanisms of colorectal cancer (Wang et al., 2016). Typically, an abnormal expression of miR-10b regulates the clinicopathological features of carcinoma cells in the colorectal tissues. It is crucial to successfully isolate RNA from various tissues, cell lines, and bodily fluids to study the expression of short RNAs like miRNA. Cloning, microarrays, northern blots, Real time-PCR, and nanoscale technologies have all been used to evaluate miRNAs from various cells and tissue samples (Qavi et al., 2010; Ban and Song, 2022). There is ample availability of nucleic acid purification products and extraction kits for the extraction of total RNA for obtaining miRNA from different sample sources. The isolation kits claim high recovery of RNA by the utilization of commonly referred kit methods including MagMAX nucleic acid isolation kit, Ambion's RNAqueous®-96 technology, mirVana™ miRNA isolation kit, RNeasy Mini kit-Qiagen, Magne Sil Total RNA mini-isolation System-Promega, PureLink™ miRNA isolation Kit which either utilize highly efficient spin columns, filter cartridges, silica–magnetic particles or glass-fiber filter techniques. While these kits claim efficient isolation of RNA depending on sample type and quantity, one must take caution to avoid genomic DNA or protein contamination and the possibility of residual RNase activity. However, not all such kits are scalable and come at a high cost, thereby resulting in significant expenses to clinical laboratory budgets.

It is essential to have a nucleic acid extraction methodology that is both successful and rigorous in the field of molecular biology because this stage serves as a prerequisite for many applications that come later in the research process. The recovery efficiency, which can be highly impacted by the various purification processes that are utilized, is one of the most important factors that determines the amount of microRNA that is present in an RNA sample as a whole. Protocols for miRNA extraction that are straightforward, efficient in terms of time, and consistent are required for further growth in research laboratories.

Methods that use guanidine thiocyanate and guanidinium chloride take advantage of the strong chaotropic nature of these salts, which denatures macromolecules and inactivates nucleases in a short amount of time. As a result of the widespread availability of RNase enzyme in the environment, it is common knowledge that this enzyme can compromise the quality and integrity of the RNA that has been isolated. A wide variety of ionic detergents, such as sodium dodecyl sulphate (SDS), potassium ethyl xanthogenate (PEX), Triton-X, Tween-80, and cetyl trimethyl ammonium bromide (CTAB), have been incorporated for the purpose of denaturation of protein, inhibition of enzymes that compromise the nature of RNA, and strong interaction with lipids. It is likely that the action of detergents directly disrupts the protein-nucleic acid connections, which, in turn, ultimately leads to the disintegration of the cytoplasmic membrane, which causes the full lysis of the cell.

The use of a variety of chemicals, such as phenol, chloroform, and isoamyl alcohol, which are all readily available in the proportion of 25:24:1, pH>7.4, has been enormously preferred for the efficient isolation of miRNA, although this method of extraction requires a number of steps of purification to produce samples that are free of phenol. The incorporation of alcohol precipitation is a stage that is both of the utmost importance and absolute necessity. For the most part, 70% to 100% ethanol and isopropanol or 2-propanol have been used for the production of tiny RNA samples in a stable environment. In order to precipitate with isopropanol rather than ethanol, the volume may be increased by up to fifty percent (50%).

Phenol that has been dissolved plays an important part in the process of denaturing proteins while they are still in the aqueous solution. It also plays a function in separating proteins from genomic DNA and disassociating them from it. Denaturation of proteins, peptide bonds, and lipids can also be aided by chloroform or trichloromethane, both of which reduce the degree to which DNA is soluble in organic phases. In order to successfully isolate RNA using organic extraction methods, certain troubleshooting criteria are incorporated into the process. The current work detailed a straightforward process for the isolation of cytoplasmic and nuclear fractions of RNA and short RNA from

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cultured cells for a variety of applications in the future. This protocol was optimized and adapted from other methods that were already in existence (Atherton and Darby, 1974).

Materials and Methods

COLO 320HSR (ATCC® CCL220.1™), 6% sodium hypochlorite, TrizolTM reagent, chloroform, isopropanol, ethanol, phenol, proteinase-K, sodium acetate, guanidine thiocyanate, ammonium thiocyanate, DNase I enzyme, microRNA-10b RT primer (Applied Biosystem), reverse transcriptase enzyme (Takara), TaqMan® MicroRNA Reverse Transcription kit, TaqMan® Small RNA Assay kit, Direct-zol™ RNA MiniPrep Plus kit.

Sample preparation

Aspiration was performed with great care on the medium contained within the COLO 320 cell culture flasks. After thoroughly washing with Dulbecco's Phosphate Buffered Saline, the cells were examined (D-PBS). After detaching the cells from the surface of the plate, they were placed in a new tube, and the cell suspension was moved. After that, the cells were pelleted by centrifuging them for approximately three to five minutes at a speed of 2300 revolutions per minute. The cell pellet was further utilized for the various protocols of RNA extraction while the supernatant was properly discarded.

Cold phenol extraction protocol of cytoplasmic and nuclear miRNA

The cell pellet was added to one volume of lysis solution (0.14 M NaCl, 0.01 M Tris, 1.5 mM $MgCl_2$, -mercaptoethanol), pH 8.5, and thoroughly mixed. After 5 minutes, the mixture was extracted at 0°C with an equivalent volume of cold phenol. Cold phenol was used to extract the aqueous phase once more, and an equal volume of chloroform was added and vortexed. This process was carried out twice. For around 30 minutes, 1 ml of ice-cold with 100% ethanol is added. After 15 minutes of centrifugation, the supernatant was discarded. Two times the ethanol phase was carried out.

Newly manufactured 0.5% SDS and freshly prepared lysis buffer were completely combined with the sample cell pellet to get the nuclear RNA fraction. The mixture was heated at 55^0C for 3 minutes after phenol was added. After adding an equivalent volume of chloroform, the mixture was heated for another two minutes while shaken. After around 10 minutes on ice, the sample was centrifuged. Cold phenol was used to re-extract the aqueous phase. 100% ethanol was used to precipitate the RNA, which was then incubated for an hour. The buffer containing 50g of DNase was used to re-dissolve the precipitate. After vigorous mixing, cold phenol was used to extract the solution. Centrifuging was done after adding an equal volume of chloroform to the isoamyl alcohol supernatant. A volume of 3M potassium acetate was added, and the top aqueous phase was blended by inversion. Ethanol was increased by two times and then incubated on ice. 30 minutes were spent centrifuging at 13,000 rpm and 4^0C. The pellet was eluted in nuclease-free water to prevent degradation until the experimental analysis and frozen at -80^0C (Atherton and Darby, 1974).

Hot phenol extraction of RNA

The cells were suspended in 5 ml of ice-cold sodium acetate buffer before 0.05 volume of 10% SDS and 1 ml of sodium acetate-saturated phenol were added right away. The tube was vortexed for 10 seconds, dropped into a 60°C water bath, and then quickly cooled on ice for 5 minutes. The aqueous phase was eliminated via centrifugation at 800x g. After adding an equivalent volume of chloroform, it was centrifuged. The aqueous phase was eliminated by performing the chloroform extraction procedure one again. The sample precipitated in 2.5 liters of 100% ethanol, which also maintained it at -20^0C. The pellet was collected after 15 minutes at 7500 rpm of centrifugation. The pellet was then cleaned once more using wash buffer (Tris-Hcl-10 Mm, NaCl-0.1 M, and 75% ethanol), and then it was kept in water without nucleases (Ribaudo et al., 1992).

RNA isolation by acid guanidium thiocyanate-phenol-chloroform

The homogenate was given a small amount of denaturing solution and aggressively vortexed. The following reagents were then included: 1 ml of saturated phenol, 0.1 ml of 2M sodium acetate (pH 4), and 0.2 ml of chloroform-isoamyl aggressively vortexing for 10 seconds, then 15 minutes of cooling on ice. 10,000 g centrifuged at 40°C for 20 min. 1ml of isopropanol was added to the collected aqueous phase, which was then stored at -20^0C for an hour. The resulting RNA pellet was then dissolved in 300 µl of denaturing solution and precipitated with 1 volume of isopropanol at -20^0C for an hour after being centrifuged at 10,000g for 20 minutes. Once more After another round of centrifugation for 10 minutes at 4^0C, the RNA pellet was suspended in 75% ethanol and dried by air. The resulting RNA pellet was dissolved in DEPC-treated water (pH- 8) or water devoid of nucleases (Chomczynski et al., 1987).

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Guanidium Method for RNA preparation

The guanidinium solution was diluted with the cell pellet before being vortexed. A step gradient was made by layering the cell lysate on top of 1.5 ml of caesium chloride (5.7 M) in the tube. The sample was centrifuged at 35,000 rpm for approximately 6 to 12 hours before collecting the supernatant. The bottom level's answer was thoroughly considered. In 360 µl of wash solution, the pellet was re-suspended. 40 µl of 3M sodium acetate with a pH of 5.2 and 1 ml of 100% ethanol were also included. The tube was allowed to spin for 15 minutes following an incubation period of roughly 30 minutes. After removing the supernatant, the wash solution procedure was repeated. The pellet was then drained and dissolved in 100 µl of water free of nucleases (Glisin et al., 1974).

Trizol method of Isolation

The cell pellet received around 1 ml of Trizol reagent, which was then incubated on ice for 10 minutes. 200 µl of chloroform was added after incubation and vortexed for roughly 20 seconds. The tube was then incubated for 15 to 30 minutes on ice. The aqueous layer was collected in a new tube, and 0.5 ml of isopropanol was added after all the tubes had been centrifuged at 12,000 rpm for approximately 15 minutes at 4^0C. The tube was repeatedly mixed, then incubated for 10 minutes on ice. The samples were centrifuged for roughly 10 minutes at 4^0C using a 12,000 rpm. After the supernatant was removed, a small amount of 70% ethanol was added to the bottom gel-like substance. It was centrifuged once again for 10 minutes at 4^0C and 7500 rpm. The pellet was gathered and dried by air. The pellet received a DEPC water addition of 50–100 µl.

Direct-zol™ RNA MiniPrep Plus

The cell pellets were properly mixed while suspended in the proper amount of TRI Reagent®. The lysed samples received 95–100% ethanol addition. The mixture was then put into the provided Zymo-Spin TM IIICG Column Tube and centrifuged for two minutes at 12,000 rpm. The flow-through was removed, and a fresh collection tube was connected. The column was filled with 400 µl of RNA Wash Buffer before being centrifuged. Then, 75 µl of DNA Digestion Buffer and 5 µl of DNase-I was added. It was then transferred right to the column matrix after being thoroughly mixed. 15 minutes of incubation at ambient temperature (20–30^0C). Centrifuged after adding 400 µl of Direct-zol TM RNA Prewash to the column. The flow-through was disregarded, and the previous action was carried out once more. the column with 700 µl of RNA Wash Buffer, then centrifuged for two minutes at 12,000 rpm carefully place the column into an RNase-free tube. The RNA was eluted by directly adding 100 µl of RNase-Free Water to the column matrix and centrifuging.

Double detergent extraction

Cells were suspended in lysis buffer and extracted mixture for five minutes at 0 degrees Celsius with an equivalent volume of phenol. The nuclear RNA was extracted from the first phenol phase by heating it at 550 degrees Celsius for three minutes while adding an equal volume of lysis buffer. 1% tween-40 and 0.5% sodium deoxycholate were added. Chloroform was added in equal amounts to the tube and heated for 2 minutes with shaking. The aqueous phase was extracted with cold phenol and precipitated with ethanol.The ethanol precipitate was further re-dissolved in buffer and DNase (Atherton and Darby, 1974).

Purification of RNA from animal cells using Diethyl-pyrocarbonate

The cell pellet was diluted with lysis buffer at a volume range of 15-100 volumes. After being heated to 37^0C for five minutes, the cell suspension was allowed to cool at room temperature for the following 15 minutes. One volume of saturated NaCl solution was added and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and a volume of 95% ethanol was added to the pellet and stored at -20^0C.

Quantitative Analysis

UV Spectroscopy

The conventional method for evaluating RNA concentration and purity is UV spectroscopy. Accordingly, diluted RNA samples' absorbance is measured within 260nm and 280 nm. Accurate estimation of nucleic acid concentration is based on the principle of Beer-Lambert law, which predicts a linear change in absorbance with concentration.

As per the guidelines, using this equation, an A 260 reading of 1.0 is equivalent to ~40 µl/ml single-stranded RNA. RNA purity is assessed by calculating the A260/A280 ratio. A value of 1.8- 2.0 of the A 260/A280 ratio indicates highly purified RNA.

Realtime-PCR

TaqMan® MicroRNA Reverse Transcription protocol was utilized with minimal modifications to conduct the reverse transcription process. The procured RNA samples were reverse transcribed using gene-specific microRNA-10b RT primer (Applied Biosystem) and reverse transcriptase enzyme (Takara). The reversely transcribed cDNA was then stored at -20^0C for further studies.

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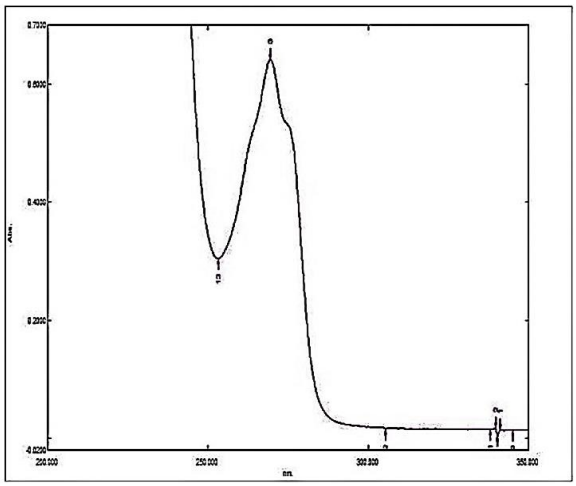
TaqMan® Small RNA Assay kit was used for checking the expression of microRNA-10b in the obtained cDNA samples with the specific microRNA-10b TM primer (Applied biosystem).

Results

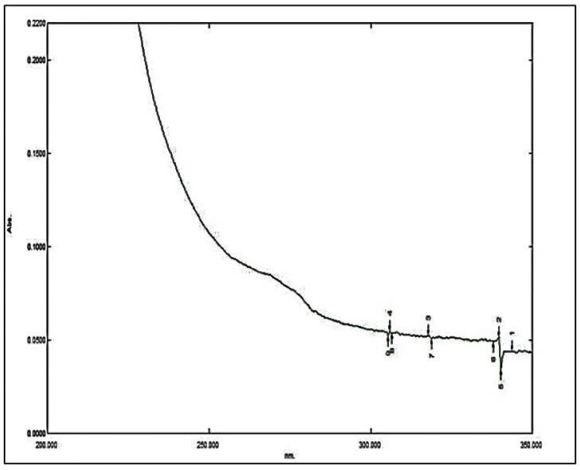
The purpose of this research is to compare the effectiveness of the many different procedures for isolating RNA that have been developed since the 1970s up until the late 1990s, as well as the traditional TRIzol method and the kit approach.

Effects of the diluent on OD value

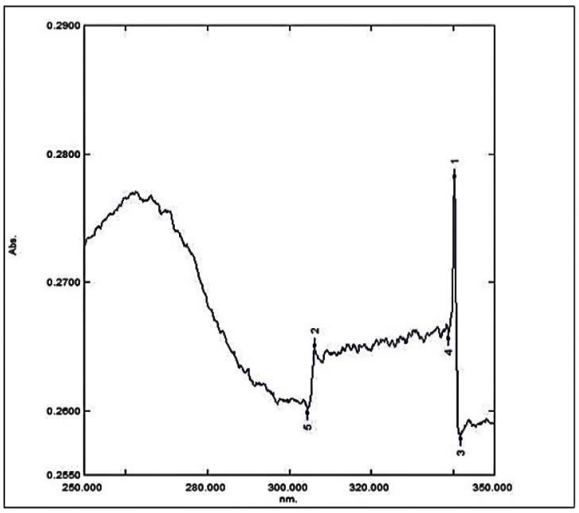
In the course of practical research, it was found that the ratio of A 260/280 is reduced when DEPC water is used as the diluent. In comparison, nuclease-free water produces a higher A 260/280 ratio of 1.85, but TE buffer has a greater propensity to produce a higher value in the range of 2.14. figure 1.



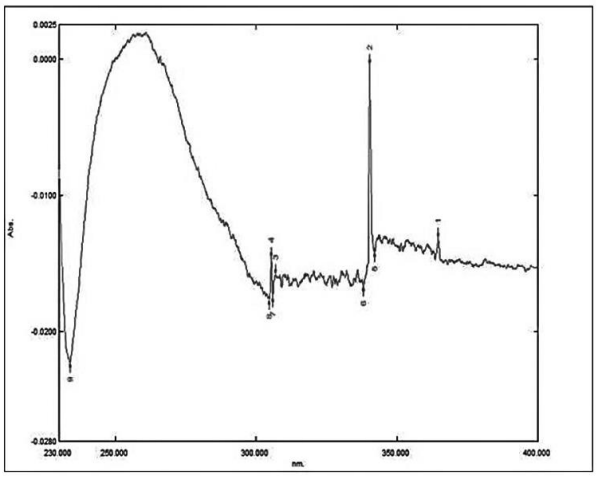
1(h) RNA isolation from leucocytic cell



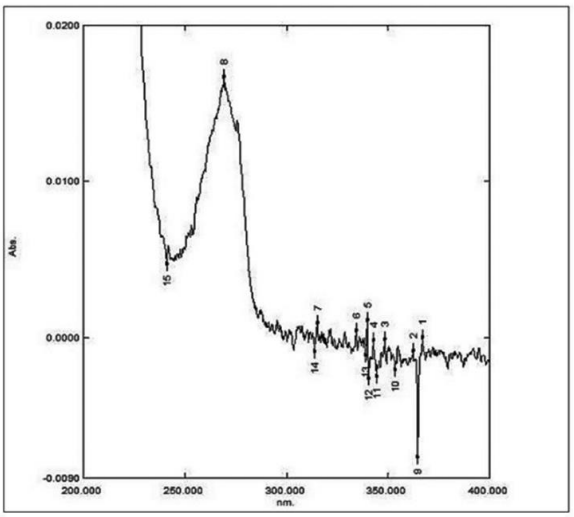
1(i) Isolation of RNA using CTAB



1(j) Double detergent



1(k) DEPC method



1(a) Trizol Method

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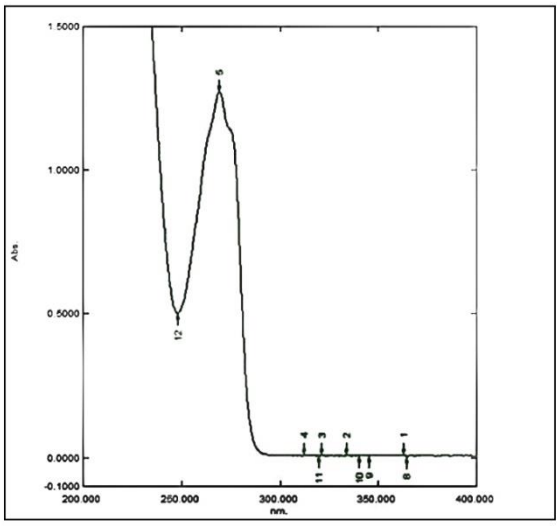
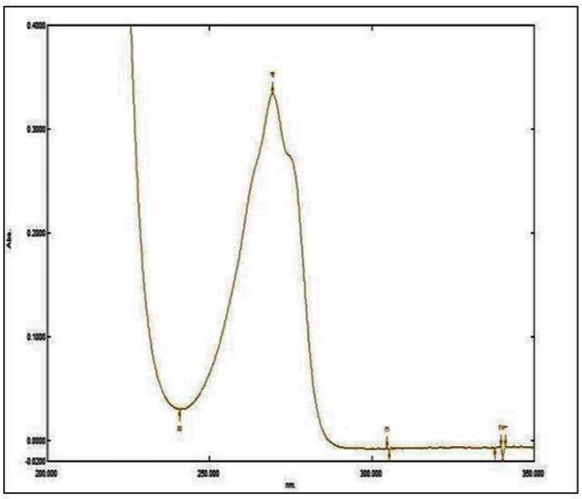
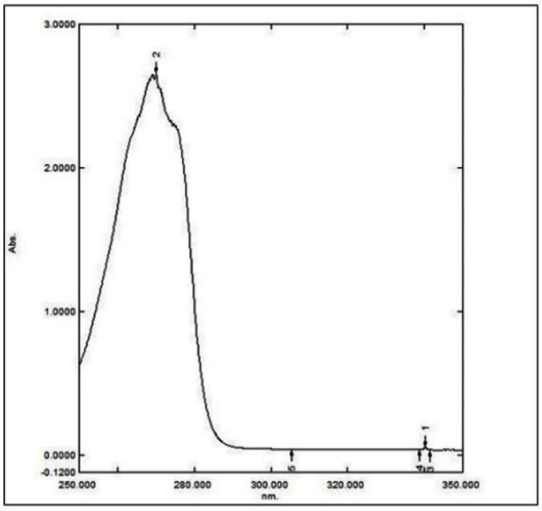


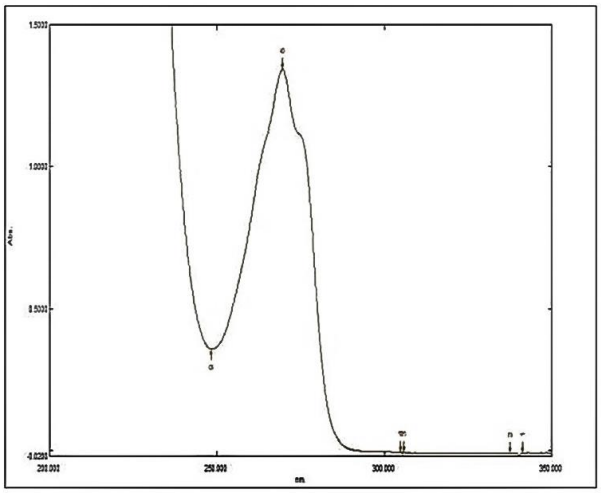
1(b) Kit Method



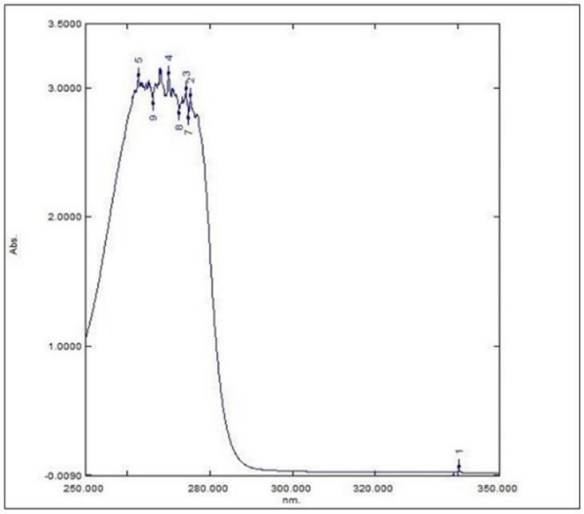
1(e) Hot phenol extraction of RNA



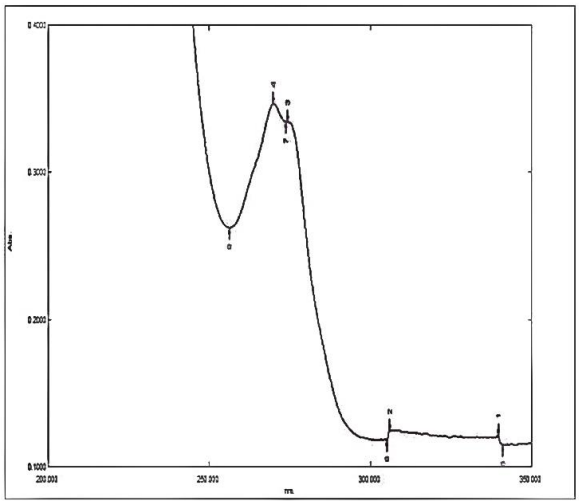
1(c) Cold phenol method- Nuclear RNA



1(f) Guanidinium method for RNA



1(d) Cold phenol method-Cytoplasmic RNA



1(g) Single step Method of RNA isolation

Figure 1. UV spectrophotometry (a) Trizol Method (b) Kit Method (c) Cold phenol method- Nuclear RNA (d) Cold phenol method-Cytoplasmic RNA (e) Hot phenol extraction of RNA (f) Guanidinium method for RNA (g) Single Step method for RNA isolation (h) RNAisolation from leucocytic cells (i) Isolation of RNA using CTAB (j) Double detergent method (k) DEPC method.Thefollowing protocols double detergent and DEPC method showed negative results, no peak was obtained.

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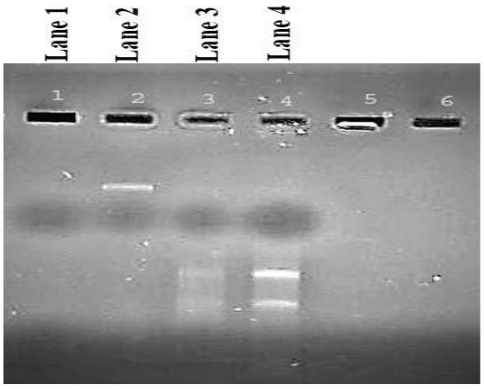
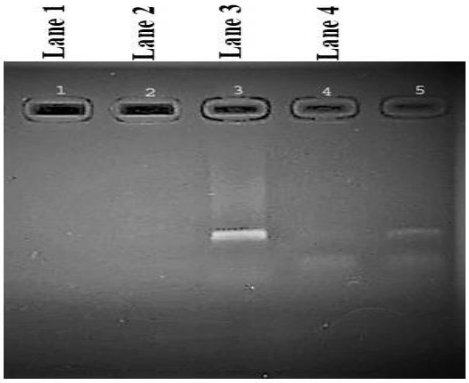
Qualitative Analysis

Bleach gel electrophoresis 1%

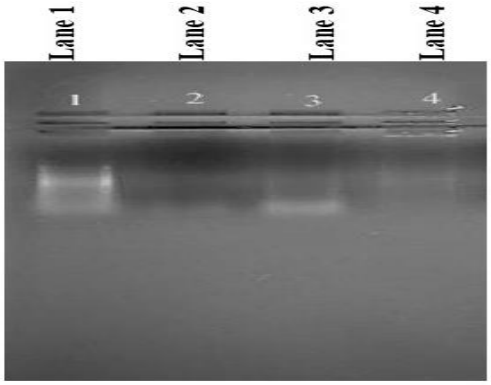
Formaldehyde gel, which has a long history and is considered the industry standard, has been discovered to take more time and is said to be labor-intensive. The quality of the isolated samples can be quickly assessed by including negligible amounts of 6% sodium hypochlorite (commercial bleach) at different concentrations, for instance, 0.5% or 1% to the agarose gels containing TAE buffer prior to electrophoresis. The secondary structure of the respective endoribonucleases is found to be denatured and the potentially contaminating RNases are destroyed. The bleach gel' is used as an inexpensive and safe way to evaluate RNA integrity figure 2.



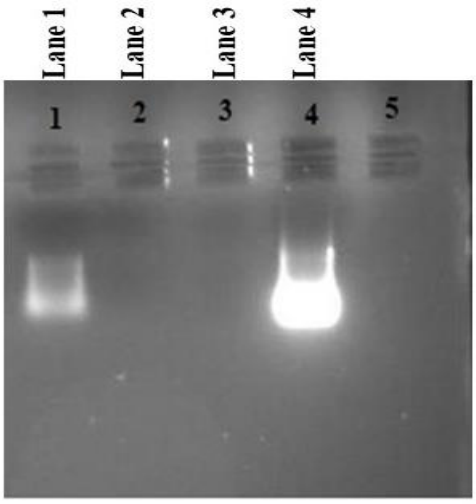
2 (a)



2 (b)



2 (c)



2 (d)

Figure 2. Bleach gel Electrophoresis (a) First gel image (b) Second gel image (c) Third gel image (d) fourth gel image.In the first gel image lane 1 showed the DEPC method, lane 2-Cytoplasmic RNA, lane 3-Nuclear RNA, lane 4- Direct-zol™ RNA Mini Prep Plus. In the second gel image lane 1 showed Double detergent method, lane 2- CTAB method, lane 3 Cytoplasmic RNA, lane 4- Nuclear RNA lane 5-Trizol method. In the third gel image lane 1 showed Nuclear RNA, lane 2-CTAB method, lane 3-Simple method from leucocytic cell line. In the fourth gel image, lane 1 showed the DEPC method and Hot phenol method, lane 2- Double detergent method, lane 4-Cytoplasmic RNA

Standardizing an easy, affordable process for isolating Standardizing an easy, affordable process for isolating miRNA from diverse samples is required. The various isolation techniques used in the study result in a wide range of acquired results. It is essential to choose a procedure that is precise and dependable. The discrepancies in the extraction techniques, which consider several elements such as the chemicals used, temperature, and pH, are explained by the mismatch between the isolated samples and miRNA expression.

In this study, we isolated RNA using various established techniques and identified miRNA from the total RNA content. The outcomes have been compared using the following techniques.

Trizol Method of Isolation

The Trizol method for RNA isolation was found to be effective. The RNA concentration was observed to be 2.46 µg/µl, and its purity was 1.72 in the spectroscopic analysis, which is close enough to the range of the ideal purity value. Hence, the Trizol method successfully produces the extraction of miRNA from the total RNA content.

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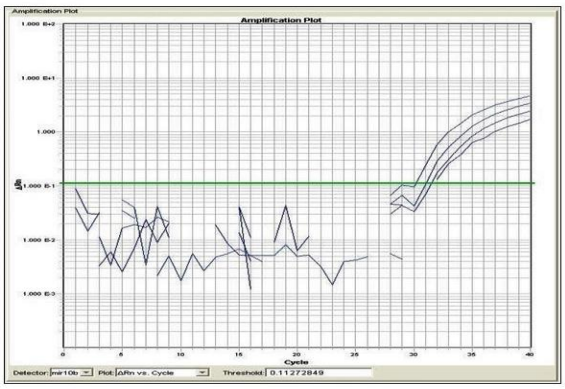
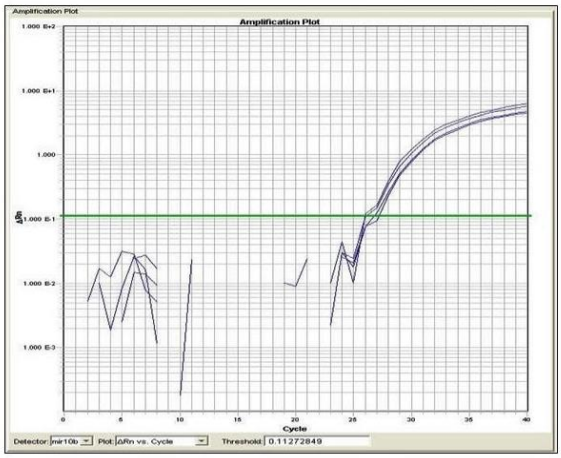
Cold phenol extraction of cytoplasmic and nuclear RNA method

The Cold phenol extraction protocol (1973) for nuclear and cytoplasmic miRNA with necessary modifications successfully isolated miRNA, which was confirmed by the spectroscopic analysis. The nuclear and cytoplasmic RNA concentrations were observed to be 2.12 µg/µl and 2.87 µg/µl, and the purity was1.74 and 1.75 of the A 260/A280 ratio which is close enough to the ideal purity value. Hence, this method tends to be more effective in comparison to the trizol method and equally good as the kit method in terms of yield and cost.

3 (a) Kit method



3 (b) Trizol method



3 (c) Cold Phenol method Nuclear RNA

3 (d) Cold Phenol method Cytoplasmic RNA

Figure 3. Real-time PCR analysis microRNA-10b Expression (a) Kit method (b) Trizol method (c) Cold Phenol-Nuclear RNA method (d) Cold Phenol-Cytoplasmic RNA. The obtained qRT-PCR results indicated positive miRNA-10b expression

Simple method of RNA isolation from human leucocytic cell lines, single-step method of RNA isolation by Acid guanidium thiocyanate-phenol-chloroform extraction and Guanidium Method for RNA preparation

Only a few protocols, including the Guanidinium method for total RNA preparation (1992), the Simple method of RNA isolation from human leucocytic cell lines, and the single-step method of RNA isolation by Acid guanidium thiocyanate-phenol-chloroform extraction (1986), were successful in extracting miRNA from total RNA content. However, the purity of the RNA was observed to have values close to the ideal purity value, which is probably due to the low quality of RNA or loss of small RNA content or may be due to the lack of adequate phase separation and purification.

Hot phenol extraction of RNA (modified), Double detergent extraction, Purification of RNA from animal cells using Diethyl-pyrocarbonate, and Isolation of low molecular weight RNA using CTAB

Methods such as Double detergent extraction (1974), Purification of RNA from animal cells using Diethyl-pyrocarbonate (1975), and Isolation of low molecular weight RNA using CTAB were unable to isolate the RNA from the given cell line appropriately, and no peak was observed in the spectroscopic analysis. This was because the RNA had degraded as a result of degradation

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caused by handling errors or insufficient lysis of cells, as well as improper detergents.

However, in the spectroscopic analysis of the sample from the hot phenol extraction protocol, the concentration were not good to show the amplification of miRNA at early cycles. However, the Trizol method and the cold phenol extraction method were effective in the extraction of miRNA, and the amplification plot was obtained at

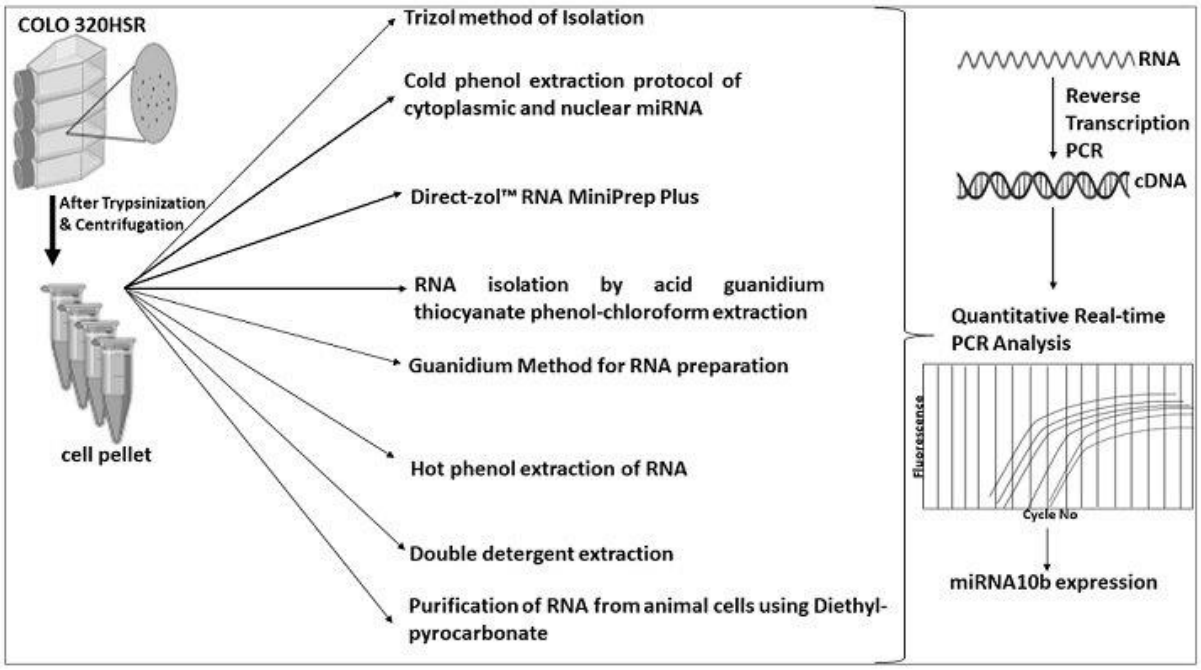

Figure 4. Flow chart depicting various miRNA isolation protocol and its quantification.

of RNA was observed to be 1.24 µg/ml and the purity was 1.72 of the A 260/A280 ratio and miRNA extraction was not undertaken.

Using this comparative analysis as a tool, we were able to determine the method that would be the most accurate and productive for the particular sample. To obtain a relatively effective result according to the standard kit methods sheds light on the possibility of developing a simple protocol for isolating miRNA by implementing the necessary modifications following an in-depth analysis of several protocols. This possibility has the potential to be explored further.

Real-time analysis of micrRNA-10b expression

We could positively demonstrate miRNA expression from the isolated small RNA samples with the assistance of qRT-PCR. This was made possible by the utilization of materials and reagents that were on the less expensive side. As a result, we examined some different procedures for extracting miRNA to achieve substantial yields, lower costs, and shorter extraction times. In addition, the same level of microRNA expression was produced using a method that required the purchase of a costly kit. Real-time PCR can be used to determine miRNA's expression, which significantly adds to the overall findings.

The obtained qRT-PCR results indicated positive miRNA-10b expression in different RNA isolation protocols as depicted in, however, majority of protocols early cycles which can be compared with the amplification plots obtained from the commercially available kit method figure 3. Figure 4 presents a flow chart that illustrates several techniques for isolating miRNA and quantifying the results of using these protocols.

Discussion

Conventional solution-based procedures and solid-phase systems, which may frequently be found in commercial kits, are two of the many specialized methods currently available. There is no shortage of different extraction procedures being made available to choose from. However, there is an immediate need to optimize and improve the existing extraction processes. This must be done as soon as possible. Because RNA degradation products have the potential to obscure or dilute the presence of genuine miRNAs, it is essential to select an RNA extraction process that is both effective and efficient (Ibbersonet al., 2009). The recovery efficiency, which can be highly impacted by the various purification processes utilized, is one of the most important factors that determines the amount of microRNA present in an RNA sample as a whole. As shown in figure 4, the most desirable techniques for RNA extraction do not require the use of any significant pieces of specialized apparatus and dramatically cut down on the amount of expensive and

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potentially harmful solvents used. The development of miRNA-based prognostics and diagnostics, as well as the incorporation of these methods into routine clinical laboratory practice, are both hampered by the absence of straightforward protocols for the extraction and analysis of miRNA that are also less time-intensive and more consistent. A standardized approach to the various procedures that can be used to isolate miRNAs.Most miRNA isolation kits are based on purifying and enriching miRNAs from the larger RNAs with the easy implementation of column-based separation. Although the phase of sample preparation is different for each type of sample (tissues, adherent cultured cells, and cultured suspension cells), these kits provide the greatest results when used on a wide variety of samples.

Isolating small amounts of cell-free RNA presents a number of challenges due to the fact that the majority of the kits currently on the market are quite pricey, and the various extraction procedures require a significant amount of manual labor and are overly dependent on the application of potentially hazardous chemicals. In molecular biology, uncountable DNA and RNA isolation strategies are among the most crucially important marketplaces. This is something that should be brought to your attention.

Conclusion

A plethora of RNA and miRNA extraction kits produce results that are almost exactly correct and of high quality. Although numerous reliable procedures for isolation have been established, the preparation stages are extremely time-consuming and inconvenient. However, there is a problem in that these kits are very pricey, and you need very specialized equipment and tools to analyse them. It is impossible to follow a standard clinical practice such as RNA isolation for large-scale sample studies. The majority of the procedures for isolating substances involve phenol-based methods, which have the disadvantage of leaving a remnant of the organic solvents in the sample isolates. These kinds of contaminants are known to lower the quality of the sample. By adding a sequence of processes involving the precipitation of alcohol, it is possible to overcome this challenge. The modification of the protocols that are already in place can assist in creating a method that is uncomplicated, economical, less time-consuming, and productive. Most of the procedures depend on the potency of the chemicals used to lyse and denature all of the other cellular components. Many technical obstacles need to be conquered to make it possible for microRNAs to be used in clinical settings. The scientific and medical sectors have shown a significant interest in a comprehensive investigation of the biomarkers based on miRNA. Standardizing a methodology would make it easier to achieve trustworthy results and cut down on the cost of isolation.

Conflicts of interest

None

References

Atherton, K.T., & Darby, G. (1974). Extraction of RNA from mammalian tissue culture cells. *Analytical Biochemistry*, *57*(2), 403-412. https://doi.org/10.1016/0003-2697(74)90095-5

Ban, E., & Song, E.J. (2022). Considerations and Suggestions for the Reliable Analysis of miRNA in Plasma Using qRT-PCR. *Genes*, *13*(2), 328.https://doi.org/10.3390/genes13020328

Bryzgunova, O., Konoshenko, M., Zaporozhchenko, I., Yakovlev, A., & Laktionov, P. (2021). Isolation of Cell-Free miRNA from Biological Fluids: Influencing Factors and Methods. *Diagnostics*, *11*(5), 865. https://doi.org/10.3390/diagnostics11050865

Calin, G.A., Sevignani, C., & Dumitru, C.D. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences*, *101*(9), 2999-04. https://doi.org/10.1073/pnas.0307323101

Chomczynski, P., & Sacchi, N. (1987). Reagent for the single-step simultaneous isolation of RNA. *Anal Biochem.*, *162*, 536-7. https://doi.org/10.1016/0003-2697(87)90021-2

Das, J., Das, M., Doke, M., Wnuk, S., Stiffin, R., Ruiz, M., & Celli, J. (2021). A small molecule inhibits pancreatic cancer stem cells. *International Journal of Experimental Research and Review*, *26*, 1-15. https://doi.org/10.52756/ijerr.2021.v26.001

Evert, J., Pathak, S., Sun, X.F., & Zhang, H. (2018). A study on effect of oxaliplatin in MicroRNA expression in human colon cancer. *Journal of Cancer*, *9*(11), 2046. https://doi.org/10.7150/jca.24474

Glisin, V., Crkvenjakov, R., & Byus, C. (1974). Ribonucleic acid isolated by cesium chloride

centrifugation. *Biochemistry*, *13*(12), 2633-7. https://doi.org/10.1021/bi00709a025

Ibberson, D., Benes, V., Muckenthaler, M.U., & Castoldi, M. (2009). RNA degradation compromises the reliability of microRNA expression profiling. *BMC Biotechnology*, *9*(1), 102. https://doi.org/10.1186/1472-6750-9-102

Felekkis, K., Touvana, E., Stefanou, C.H., & Deltas, C. (2010). microRNAs: a newly described class of encoded molecules that play a role in health and disease. *Hippokratia*, *14*(4), 236.

Jiang, H., Yu, W.W., Wang, L.L., & Peng, Y. (2015). miR-130a acts as a potential diagnostic biomarker and promotes gastric cancer migration, invasion and proliferation by targeting RUNX3. *Oncology Reports*, *34*(3), 1153-61. https://doi.org/10.3892/or.2015.4099

Jothimani, G., Sriramulu, S., Chabria, Y., Sun, X.F., Banerjee, A., & Pathak, S. (2018). A review on theragnostic applications of microRNAs and long non-coding RNAs in colorectal cancer. *Current Topics in Medicinal Chemistry*. *18*(30), 2614-29. https://doi.org/10.2174/1568026619666181221165344

Pathak, S., Meng, W.J., Nandy, S.K. (2015). Radiation and SN38 treatments modulate the expression of microRNAs, cytokines and chemokines in colon cancer cells in a p53-directed manner. *Oncotarget*, *6*(42), 44758. https://doi.org/10.18632/oncotarget.5815

Ribaudo, R., Gilman, M., Kingston, R.E., Choczynski, P., & Sacchi, N. (1992). Preparation of RNA from tissues and cells. *Current Protocols in Immunology*, *3*(1), 10-1. https://doi.org/10.1002/0471142735.im1011s04

Qavi, A.J., Kindt, J.T., & Bailey, R.C. (2010). Sizing up the future of microRNA analysis. *Analytical and Bioanalytical Chemistry*, *398*(6), 2535-49. https://doi.org/10.1007/s00216-010-4018-8

Wahid, F., Shehzad, A., Khan, T., & Kim, Y.Y. (2010). MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochimica et Biophysica Acta (BBA)-*Molecular Cell Research*, *1803*(11), 1231-43. https://doi.org/10.1016/j.bbamcr.2010.06.013

Wang, Y., Li, Z., Zhao, X., Zuo, X., & Peng, Z. (2016). miR-10b promotes invasion by targeting HOXD10 in colorectal cancer. *Oncology Letters*, *12*(1), 488-94. https://doi.org/10.3892/ol.2016.4628

Weng, W., Feng, J., Qin, H., Ma, Y., & Goel, A. (2015). An update on miRNAs as biological and clinical determinants in colorectal cancer: a bench-to-bedside approach. *Future Oncology*, *11*(12), 1791-08. https://doi.org/10.2217/fon.15.83

Yau, T.O., Wu, C.W., & Dong, Y. (2014). microRNA-221 and microRNA-18a identification in stool as potential biomarkers for the non-invasive diagnosis of colorectal carcinoma. *British Journal of Cancer*, *111*(9), 1765-71. https://doi.org/10.1038/bjc.2014.484

Zhang, Y., Li, M., & Wang, H. (2009). Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. *World Journal of Surgery*, *33*(4), 698. https://doi.org/10.1007/s00268-008-9833-0

Zhu, F., Wu, Q., Ni, Z., Lei, C., Li, T., & Shi, Y. (2018). miR-19a/b and MeCP2 repress reciprocally to regulate multidrug resistance in gastric cancer cells. *International Journal of Molecular Medicine*, *42*(1), 228-236. https://doi.org/10.3892/ijmm.2018.3581

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