

Isolation of Fractionated RNA from HeLa Cells Using Norgen's Cytoplasmic & Nuclear RNA Purification Kit

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INTRODUCTION

High quality total RNA is often isolated from a number of sources as a first step towards various types of gene expression analysis including RT-PCR, Northern blotting, primer extension and RNA protection assays. However when total RNA is isolated, often times the samples are comprised of a very large proportion of a few particular types of RNA species, depending on the source of RNA that is being used. In these cases, expression studies may be difficult to perform due to the overabundance of some RNA species, and the possible masking effects of the signals generated by this RNA. Thus, in certain circumstances it is desirable to be able to isolate fractionated RNA as opposed to total RNA. For example, it may be desirable to isolate only mature, cytoplasmic RNA for some studies on expression profiling. In other cases, it may be preferable to isolate nuclear RNA in order to investigate and study pre-processed (non-spliced) RNA.

Towards this goal of isolating fractionated RNA samples, Norgen Biotek has developed the Cytoplasmic & Nuclear RNA Purification Kit. This kit provides a rapid method for the isolation and purification of both cytoplasmic and nuclear RNA from cultured animal cells and small tissue samples. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The cytoplasmic RNA is preferentially purified from the nuclear RNA and the other cellular components such as genomic DNA and proteins. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including RT-PCR, Northern blotting, RNAase protection assays, primer extension and expression array analysis.

In this application note, Norgen's Cytoplasmic & Nuclear RNA Purification Kit is used to isolate fractionated RNA from HeLa cells growing in a monolayer. The RNA is evaluated for its integrity, as well as for the presence of genomic DNA in the RNA preparations. The isolated cytoplasmic RNA is then used as the template in a RT-PCR reaction to detect the S14 ribosomal gene.

MATERIALS AND METHODS

Mammalian RNA Isolation (Cytoplasmic and Nuclear)

RNA was isolated from 5×10^5 HeLa cells growing in a monolayer cells using Norgen's Cytoplasmic & Nuclear RNA Purification Kit. Briefly, a plate of HeLa cells was washed with PBS, and ice-cold Lysis Solution was added directly to the plate. Once the cells were lysed, the lysate was transferred to a microcentrifuge tube and spun at maximum speed for three minutes. The supernatant containing the cytoplasmic RNA was transferred to a new tube, while the pellet containing the nuclear RNA was left in the same tube. Binding Solution was then added to the supernatant and the pellet, and the solutions were mixed and resuspended well. The RNA samples were then bound to separate spin-columns using centrifugation. Next, the columns were washed twice with 400 μ L of the provided Wash Solution, and the fractionated RNA was then eluted from the columns using 50 μ L of the provided Elution Buffer. The procedure was performed in triplicate.

Gel Electrophoresis

The purified RNA was run on 1.6% formaldehyde-agarose gels for visual inspection. Generally, 5 μ L of each 50 μ L elution were run on the gel. The purified RNA samples were also run on 0.9% agarose gels, again with 5 μ L of each 50 μ L elution being loaded onto the gel.

RT-PCR Assay

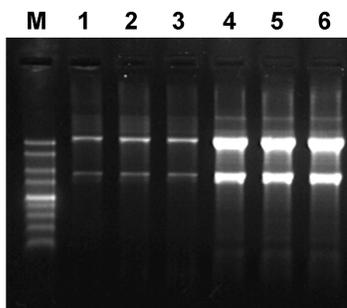
An RT-PCR was performed on 2 of the cytoplasmic RNA samples isolated from the HeLa cells. The purified RNA was used directly as the template, with no DNase treatment to remove possible contaminating DNA. The RT-PCR assay was based on the S14 ribosomal gene, which is a common housekeeping gene. Invitrogen's Superscript II (Carlsbad, California) was used to generate cDNA from 5 μ L of each of the cytoplasmic RNA elutions. A fraction of this was then used at the template for the RT-PCR reaction. The primers generated (based on the sequences published in Takanami *et al.*, 2002) allowed for the detection of the S14 ribosomal gene, and the generation of a 143 bp product. Five microlitres of the final PCR was then run on a 1.5% agarose gel for visual inspection.

RESULTS AND DISCUSSION

The isolation of RNA from various sources is often the first step towards downstream applications involving gene expression and analysis. However, depending on the cell type being used, there are often certain RNA species that may be very abundant upon the isolation of total RNA. These abundant RNA species may then interfere with the downstream gene analysis by masking signals and information from the less abundant RNA species. Thus, it is desirable in some cases to be able to isolate fractionated RNA, as this may help to improve sample utility for expression profiling and other applications by eliminating some of the more abundant RNA species that may be present in different fractions. Norgen has developed the Cytoplasmic & Nuclear RNA Isolation Kit to separately isolate the cytoplasmic RNA and nuclear RNA from mammalian cells or small tissue samples. The cytoplasmic RNA fraction may be used for some particular studies on gene expression and mRNA analysis, while the nuclear RNA fraction may be found to be extremely useful for studying siRNA or pre-processed (non-spliced) RNA.

Norgen's Cytoplasmic & Nuclear RNA Isolation Kit was used to isolate both cytoplasmic and nuclear RNA from 5×10^5 HeLa cells growing in a monolayer. The provided protocols were followed, and each was performed in triplicate. Once the RNA was isolated, it was run on a formaldehyde-agarose gel for visual inspection. In each case, 5 μ L of the total 50 μ L elution was loaded onto the gel. As can be seen in Figure 1, both the 28S and 18S RNA is present in all the nuclear and cytoplasmic RNA fractions, indicating that the kit allows for the isolation of the main RNA species. In all cases the isolated RNA is intact with no signs of degradation, indicating that the isolated RNA is of the highest integrity and quality.

Figure 1. 1.6% formaldehyde-agarose gel containing RNA



isolated from 5×10^5 HeLa cells using Norgen's Cytoplasmic & Nuclear RNA Purification Kit. Lane M is Norgen's 1kb RNA marker, Lanes 1 – 3 contain three different samples of nuclear RNA and Lanes 4 – 6 contain three different samples of cytoplasmic RNA.

After the formaldehyde agarose gel was run, the purified RNA samples were also run on a 0.9% agarose gel in order to examine the RNA for the presence of DNA. As can be seen in Figure 2, genomic DNA can indeed be detected in the three nuclear RNA fractions (Lanes 1 – 3). This is to be expected, as the genomic DNA is located in the nucleus of the cell and thus it would be assumed to be present as a contaminating factor in RNA that is isolated from the nucleus. However, no genomic DNA can be detected at all in RNA that is isolated from the three cytoplasmic fractions (Lanes 4 – 6). This is extremely important, as it verifies the fact that the fractions are indeed being collected separately, and furthermore that there is no contamination of the cytoplasmic fraction with the nuclear fraction containing the genomic DNA.

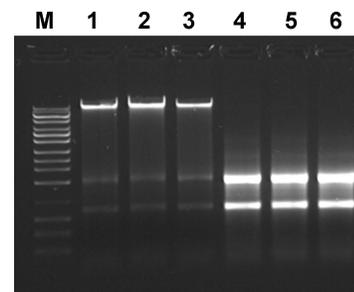
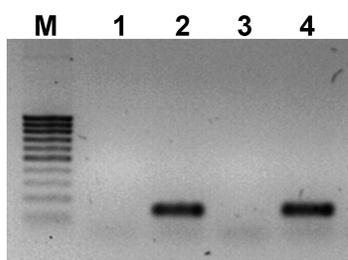


Figure 2. 0.9% agarose gel containing RNA isolated from 5×10^5 HeLa cells using Norgen's Cytoplasmic & Nuclear RNA Purification Kit. Lane M is the Norgen UltraRanger DNA marker, Lanes 1 – 3 contain three different samples of nuclear RNA, and Lanes 4 – 6 contain three different samples of cytoplasmic RNA.

Once the various gels were run to visually analyze the fractionated and purified RNA, the cytoplasmic RNA was used directly as the template in an RT-PCR reaction. The RT-PCR reaction was used to detect ribosomal S14, and the size of the expected product based on the use of RNA as the template is 143 bp. Through the use of NCBI's website and the BLAST application, it was determined that if the primers annealed to genomic DNA that was present in the preparation, the PCR amplification would result in the generation of a product with the expected size of 1200 bp. No pre-treatment of the isolated cytoplasmic RNA samples with DNase was performed. As a control, the RT-PCR was performed without the addition of reverse transcriptase in both cases.

From Figure 3 it can be seen that the RT-PCR was successful for two different samples of cytoplasmic RNA. In both Lanes 2 and 4 the expected 143 bp product can be observed, indicating that the purified RNA is intact and of a high integrity. Furthermore, in all 4 lanes the 1200 bp product is not observed. This indicates that there is no contaminating traces of genomic DNA in the RNA preparations, as this genomic DNA would be detected and amplified in the PCR step of the RT-PCR. This is particularly evident in the control lanes 1 and 3, in which no reverse transcriptase was added to the cDNA reaction. Therefore, in these cases there would be no cDNA present to compete with the genomic DNA as a template. Thus, the cytoplasmic RNA fractions isolated with Norgen's Cytoplasmic & Nuclear RNA Purification Kit do not contain any traces of genomic RNA, and can be used directly in RT-PCR reactions without the need to pre-treat the samples with DNase. Furthermore, the isolated RNA is of a high quality and contains no inhibitors, as indicated by its use a



template in RT-PCR reactions.

Figure 3. Agarose gel electrophoresis of RT-PCR results amplified from cytoplasmic RNA that was isolated from HeLa cells using Norgen's Cytoplasmic & Nuclear RNA Purification Kit. The RT-PCR was based on the S14 ribosomal gene, and the expected size of the product was 143 bp. Lane M is the Norgen PCRSizer DNA Marker, Lanes 1 and 3 are controls that contained no reverse transcriptase, and Lanes 2 and 4 are the RT-PCR with reverse transcriptase, and contain the expected 143 bp product.

CONCLUSIONS

Norgen's Cytoplasmic & Nuclear RNA Purification Kit was used to isolate fractionated RNA species from 5×10^5 HeLa cells. From gel analysis, it was found that the 28S and 18S RNA can be seen in both the nuclear and cytoplasmic RNA fractions, indicating that the kit allows for the isolation of the main RNA species. Also, the isolated RNA is intact and shows no signs of degradation, demonstrating that the

isolated RNA is of the highest integrity and quality. Furthermore, the isolated cytoplasmic RNA contains no traces of genomic DNA, indicating that kit is indeed isolating the separate fractions of RNA. RT-PCR was performed successfully on the cytoplasmic HeLa RNA, again indicating the purity and integrity of the isolated RNA. Lastly, no DNase treatment was performed prior to the RT-PCR, and no PCR products from genomic DNA were detected. This further verifies the fact that the cytoplasmic RNA isolated using Norgen's kit is free of any contaminating nuclear DNA. Thus, Norgen's Cytoplasmic & Nuclear RNA Purification Kit provides an excellent method for isolating fractionated cytoplasmic and nuclear RNA from various sources including mammalian cells and tissues.

REFERENCES

1. Takanami I, Takeuchi K, Watanabe H, Yanagawa T, Takagishi K. Autocrine motility factor receptor gene expression and cell motility in lung cancer cell lines. *Oncology Reports* 2002; 9:125-128.