Sequential Isolation and Purification of RNA, DNA and Protein from a Single Sample of Animal Cells using Norgen's RNA/DNA/Protein Purification Kit

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INTRODUCTION

The advancement in the human genome sequencing project, together with the emergence of high throughput molecular technologies including mass spectrometry for proteins and gene expression microarrays, has led to increased interests in molecular biomarker discovery. In fact, the use of "Systems Biology" – "the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions" (1), has become an important part of both medicine and basic biological sciences. Disease diagnosis and/or monitoring, as well as various areas of research require the simultaneous study of (a) changes in DNA (by mutation or by methlyation), (b) altered gene expression revealed by RNA abundance, and (c) protein level and activity. Traditionally, samples are fractionated prior to purification of each of the aforementioned biomolecules. This results in the purification of each biomolecule independently, and therefore may lead to variability due to inconsistent handling. Moreover, in many instances, samples such as biopsy materials, specific tissues or samples from cell cultures could be precious and difficult to obtain, making pre-isolation fractionation impractical. Therefore, there is a great need for a simple and rapid method for the isolation and purification of total RNA, genomic DNA (gDNA) and proteins from a single sample.

Norgen Biotek's RNA/DNA/Protein Purification Kit provides an innovative and rapid method for the sequential isolation and purification of total RNA, gDNA and proteins sequentially from a single sample of cultured animal cells, tissues, blood, bacteria, yeast, fungi or plants. The procedure is based on spin column chromatography, using Norgen Biotek's proprietary resin as the separation matrix. The purified RNA is of the highest quality and can be used in a number of downstream applications including real-time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array analysis. The gDNA can be used in various applications including PCR reactions, sequencing, bisulfite modification, Southern blotting and SNP analysis. The purified proteins can also be used in a number of different downstream applications, such as SDS-PAGE analysis and Western blots, and mass spectrometry.

In this application note, Norgen's RNA/DNA/Protein Purification Kit is used to isolate RNA, DNA and proteins from a single sample of HeLa cells. Furthermore, downstream applications of RT-PCR for RNA, PCR for DNA and Western blot for protein are performed, indicating the purity and quality of each of the purified biomolecule.

METHODS AND MATERIALS

Sequential RNA-DNA-Protein Isolation

RNA, DNA and proteins were isolated from 5 x 10^5 HeLa cells using Norgen's RNA/DNA/Protein Purification Kit as per the provided protocol (Figure 1). Briefly, the cells were pelleted by centrifugation, the media removed and the pellet washed once with PBS. Lysis solution was then added to the cell pellet, followed by the addition of the ethanol. The lysate was then loaded onto a provided column, and the flowthrough that passed through the column was collected for subsequent protein purification. The column was then washed twice with 400 µL of the RNA Wash Solution, and the purified RNA was then eluted with 50 µL of RNA Elution Solution. The column was then washed once more with the gDNA Wash Solution. This was followed by the elution of purified gDNA using the gDNA Elution Buffer. Next, the spin column was regenerated and activated for protein purification using 500 µL of Protein Column Regeneration Buffer and 500 µL of Protein Column Activation and Wash Buffer respectively. The flowthrough from the first column loading contained the proteins and was then pH adjusted using Protein pH Binding Buffer. The pH-adjusted lysate was re-loaded onto the column in order to bind the proteins. The column was washed once with 500µL of Protein Column Activation and Wash Buffer, and finally the purified proteins were eluted with 100 µL of

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Protein Elution Buffer and neutralized with 9.3 µL of Protein Neutralizer.

RNA Gel Electrophoresis

The purified RNA was resolved on 1.5% formaldehyde-agarose gels for visual inspection. Generally, 3 µL of each 50 µL elution was run on the gel.

DNA Gel Electrophoresis

The purified genomic DNA was resolved on 1% agarose gels for visual inspection. Generally, 10 µL of each 100 µL elution was run on the gel.

Protein Gel Electrophoresis

The purified proteins were resolved on 12% SDS-PAGE gels, followed by Coomassie Blue Staining for visual inspection. Typically, 10 µL of each 100 µL elution was run on the gel. Please note that alternatively, the proteins from the initial lysate may also be directly loaded onto an SDS-PAGE gel (without column purification) using the provided Protein Loading Dye (data not shown).

PCR and RT-PCR Assay

RNA and DNA purified from HeLa cells were used as the template in an RT-PCR and a PCR reaction respectively, using primers specific for the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (2). The same set of primers was used for both RT-PCR and PCR, however the expected molecular weight of each PCR product differed as a non-transcribed intron was flanked by the primer pair used. The expected size of the PCR product for the RT-PCR was 638 bp, and the expected size for the PCR was 902 bp. Ten µL of the final PCR products were then run on a 1.5% agarose gel for visual inspection.

Western Immunoblotting

The purified proteins were resolved by SDS-PAGE as described above and transferred to a PVDF membrane. Western blotting was performed using an anti-GAPDH antibody.

RESULTS AND DISCUSSION

RNA, DNA and proteins were sequentially isolated from 5 x 10^5 HeLa cells using Norgen's RNA/DNA/Protein Purification Kit as per the provided protocol. The protocol was performed in duplicate, and the entire protocol was completed in 20 minutes. Once the nucleic acids were isolated, they were run on agarose gels for visual inspection while the purified proteins were resolved and visualized on an SDS-PAGE gel.

As can be seen in Figure 2, each of the biomolecules purified was of a high quality. The RNA isolated was of high purity, with no signs of degradation and gDNA contamination (Figure 2, Panel A). The purified RNA contained the entire spectrum of species, including the intact 28S and 18S rRNAs, mRNAs, and even small RNAs (< 200 nt). Similarly, the gDNA isolated was of high integrity and purity, with no signs of RNA contamination (Figure 2, Panel B). The SDS-PAGE analysis indicated that the purified proteins were of a good diversity and a high quality (Figure 2, Panel C). More importantly, the proteins were column-purified without the use of any alcohol precipitations. Thus, Norgen's kit offers a rapid method to isolate high quality RNA, DNA and proteins from a single sample with no need for fractionation.

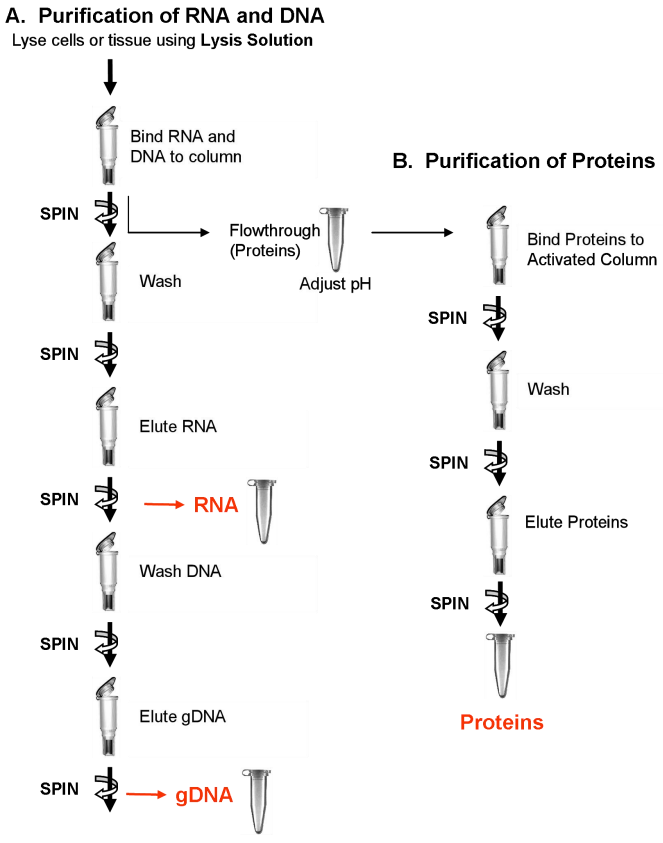

Figure 1. Procedure flowchart for the simultaneous purification of RNA, DNA and proteins from a single sample using Norgen's RNA/DNA/Protein Purification Kit.

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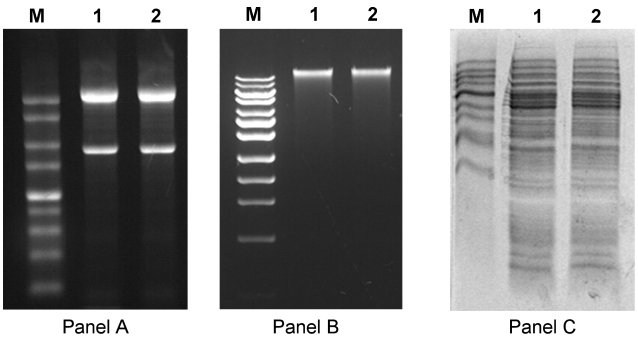

Figure 2. Total RNA, Genomic DNA and Proteins Isolated from 5 x 10^5 HeLa Cells. Panel A is a 1X MOPS 1% formaldehyde-agarose gel showing the RNA that was isolated from 2 different samples of HeLa cells. Lane M is Norgen's 1Kb RNA Ladder, and Lanes 1 and 2 contain 3 µL out of the 50 µL elutions. Panel B is a 1% agarose gel showing the gDNA isolated from the same 2 HeLa cell samples. Lane M is Norgen's UltraRanger DNA Ladder and Lanes 1 and 2 contain 10 µL of each of the 100µL elutions. Panel C is a 12% SDS-PAGE gel that contains the proteins that were isolated from the 2 HeLa cell samples. Lane M is a protein ladder and Lanes 1 and 2 contain 10 µL of the 100 µL elution of proteins that had been column purified.

In order to assess the quality of the RNA and DNA isolated, RT-PCR and PCR were performed respectively. A primer set against the human GAPDH gene was used for the amplification. Figure 3 showed that in the RT-PCR, only the presence of reverse transcriptase in the reactions yielded the desired 636 bp PCR product (Lanes 1 and 3). In contrary, no PCR product was generated when no reverse transcriptase was used in the reactions (Lanes 2 and 4), suggesting that the RNA isolated had minimal amount of gDNA cross-contamination.

The same GAPDH primer set was used in a typical PCR reaction using the purified gDNA as a template. Figure 3 (Lanes 5 and 6) showed that the PCR from gDNA yielded a product with molecular weight larger than that of the RT-PCR (902 bp). The increase in molecular weight was due to the presence of an extra non-transcribed intron in the HeLa gDNA. This successful PCR result suggested that the gDNA isolated was of high purity and had retained its biological activity.

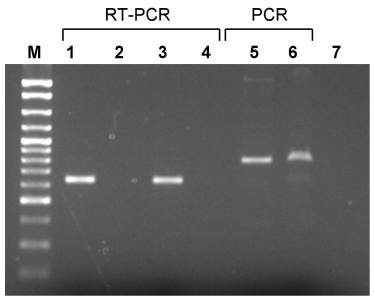

Figure 3. RT-PCR and PCR Results using the Purified HeLa RNA and DNA. RNA isolated from HeLa cells was used as the template in an RT reaction with and without reverse transcriptase in the absence of DNase treatment. The RT reaction was then used in an RT-PCR to detect the GAPDH gene. Lanes 1 and 3 contain the successful RT-PCR results when reverse transcriptase was used, and Lanes 2 and 4 show no amplification in the absence of reverse transcriptase. Genomic DNA was also successfully used in a PCR reaction using the same set of primers to detect the GAPDH gene (Lanes 5 and 6). Lane M is Norgen's CloneSizer 100bp Ladder and Lane 7 is a control with no template. The larger PCR product in lanes 5 and 6 is due to the presence of an intron in the genomic DNA that was used as a template.

The quality of the proteins purified was evaluated by Western immunoblotting (Figure 4). The proteins were first resolved by SDS-PAGE and subsequently transferred to a PVDF membrane. The presence of the GAPDH proteins was detected using an anti-GAPDH antibody. Figure 4 showed that strong signals were detected in Lanes 1 and 2 where purified HeLa total proteins were loaded. On the other hand, no signal was detected when *E coli* protein lysate was loaded (Figure 4, Lane 3). This suggested that the proteins isolated were of high purity and had retained its biological activity.

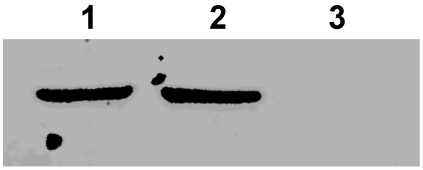

Figure 4. Western Immunoblotting of Total Proteins Isolated from HeLa cells. Total proteins were isolated and column purified from HeLa cells using Norgen's RNA/DNA/Protein Purification Kit, and Western blot analysis of duplicate samples with antibodies against GAPDH was performed. Lanes 1 and 2 contain the expected signal from the GAPDH, while Lane 3 was a negative control using *E. coli* lysate.

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CONCLUSIONS

Through the analysis of the performance of Norgen Biotek's RNA/DNA/Protein Purification Kit in sequentially isolating RNA, DNA and proteins from HeLa cells, a number of conclusions regarding Norgen's kit can be made:

1. **Norgen Bioteks's kit allows for the sequential isolation of RNA, DNA and proteins in less than 20 minutes from a single sample using the same column.** The procedure for the sequential isolation can be demonstrated in the scheme in Figure 1. The protocol uses a minimal number of steps to isolate the 3 different biomolecules. All RNA, DNA and proteins are isolated and purified from the same sample with no splitting of the lysate, thus reducing inconsistency and variability. All RNA, DNA and proteins are column-purified using the same column, and without any alcohol precipitation.

2. **The RNA, DNA and proteins isolated using Norgen's kit are of the highest purity and integrity**. The high purity and integrity of the biomolecules is demonstrated in Figures 2 to 4. Figure 2 indicates that all three molecules isolated are of high purity and show no signs of degradation. Figure 3 shows that the RNA and DNA isolated have maintained their biological activity with minimal amount of cross-contamination. Figure 4 shows that the proteins purified are of a good quality and are suitable for downstream applications like Western immunoblotting.

Thus Norgen Biotek's RNA/DNA/Protein Purification Kit is an excellent tool for isolating high quality RNA, DNA and proteins rapidly from a single sample.

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