Simultaneous Extraction of DNA and RNA from Animal Cells by a Modified Laemmli Buffer

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Method Article

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Abstract

Simultaneous investigation of DNA and RNA is often a necessity in genetic manipulation and biological researches. Besides, most of the traditional procedures devised for RNA isolation have some difficulties associated with RNase activity. Therefore, this protocol presents a safer process to extract high purity RNA in shorter time.

Introduction

The protocol describes a simple and less time-consuming method for nucleic acid extraction from animal cell culture with high purity. This process could prove useful for simultaneous study of DNA and RNA extracted from a unique sample. After adding isopropanol to cell lysate, RNA precipitation occurs and simultaneously, DNA containing suspension is isolated, in the following step, RNA and DNA are separately purified by absolute ethanol. Using the present protocol, the yield of total extraction is proportional to the amount of the starting sample; however, the minimum number of the cells with a favorable qualification is 2×106. Moreover, all solutions used in this protocol could be prepared with autoclaved ddH2O diethyl pyrocarbonate \(DEPC) free.

Reagents

• Extraction buffer: 4.4 ml 0.5 M Tris \(pH 6.8), 4.4 ml Glycerol, 2.2 ml 20% SDS, 0.5 ml 2-Mercaptoethanol • Isopropanol • Absolute ethanol • Autoclaved ddH2O diethyl pyrocarbonate \(DEPC) free • TE buffer \(Tris-EDTA buffer): 10 mM Tris-HCl \(pH 8.0), 1 mM EDTA

Equipment

• High speed centrifuge \(Eppendorf 5804 Bench top Centrifuge) • Thermo Scientific NanoDrop 2000 spectrophotometer • Vortex mixer • Spatula • Micropipette with sterile tips \(20-200µl) • 15×10 mm Petri dishes \(Nunc™ Dishes)

Procedure

For mammalian cells grown in monolayer: \(i) Perform general cell culture procedures in 15×10 mm Petri dishes \(Nunc™ Dishes). \(ii) Remove the medium and wash the cells with 1-2 ml of sterile ice-cold PBS. \(iii) Discard PBS and cell lysis perfectly done by adding 100µl extraction buffer to the Petri dish. Collect cell lysis with scraper and transfer it into a 1.5 ml microtube. \(iv) Add 1 ml isopropanol to the lysate, mix and leave it for 5 min. In this level, we can observe either a two-phase mixture or a white coil DNA both with precipitation. \(v) Using a spatula transfer coil DNA to a 1.5 ml microtube. This is as the extracted DNA sample. Otherwise, centrifuge the two-phase mixture at 4 °C for 5 min at 10,000g, then transfer the organic phase to a 1.5 ml microtube as the extracted DNA, and keep the aqueous phase with the precipitant as the extracted RNA sample. \(vi) Add 1 ml of absolute ethanol to the both DNA and RNA

extracts for final purification and complete the procedure by washing the pellet twice with ethanol and centrifuging at 4 °C for 5 min at 10,000g. \(vii) Eliminate the ethanol perfectly by heating at 65ºC for 1 hr \(critical step). \(viii) Dissolve DNA in 70 µl TE buffer and RNA in autoclaved ddH2O diethyl pyrocarbonate \(DEPC) free and leave them for 1 hr at 65ºC or 24 hrs at 37 ºC. \(ix) Evaluate quality of the extracted DNA and RNA by NanoDrop spectrophotometer. Keep the rest of the RNA and DNA at -80°C for long period.

Timing

Steps ii to vi: 30 min Step vii: 60 min Step viii: 60 min

Troubleshooting

Step problem possible reason solution iii Turbidity in mixture Cell debris After transferring lysate into a 1.5 ml microtube, if you see any turbidity, centrifuge mixture at 10,000g at 4 °C for 5 min and remove cell debris.

Anticipated Results

The critical step in this protocol is formulation of the extraction buffer. The composition of this buffer is Laemmli buffer Bromophenol Blue free, that can completely break the links between bio-macromolecules \(DNA, RNA and proteins), decrease the possibility for creation of new connections between them and specially stabilize the unfolded proteins by 2-ME and SDS. In addition, SDS and Glycerol aid lipids dissolve in the buffer and alcohols. The precipitation of nucleic acids, particularly RNA, occurs in the presence of isopropanol. Finally, ethanol plays an effective role in obtaining a high-quality nucleic acid extraction \[1]. In fact, in all the steps of this procedure RNase is inactive, thus RNA can be intact in the presence of extraction buffer, isopropanol and ethanol. We examined this protocol for 52 samples of six cell lines \(PLC/PRF/5, MDA-MB231, MCF7, NIH-3T3, SKNMC and HepG2). Data analysis was performed using one sample t-test, SPSS software version 17 \(95% CI). The results showed that, for DNA concentration, the mean ± SE for ratio of OD260/OD280 and OD260/230 were 1464.80±185.63 ng/µl, 1.80±0.007 and 2.08±0.025, respectively. While for RNA concentration, the ratio of OD260/OD280 and OD260/230 were 627.35±56.86 ng/µl, 1.92±0.03 and 1.70±0.04, respectively \(Fig. 1a). It was previously reported that the accepted range for OD260/OD280 and OD260/230 ratios are more than 1.8 and greater than 1.5, respectively \[2-3]. Furthermore, this protocol was applied to extract DNA in order to do DNA fragmentation assay \(Fig. 1b). Also, the quality of the extracted RNA was examined by evaluating the expression of a variety of genes, including p50, HIF-1α, Lgr5 and β-catenin using RT-PCR \(Fig. 2).

References

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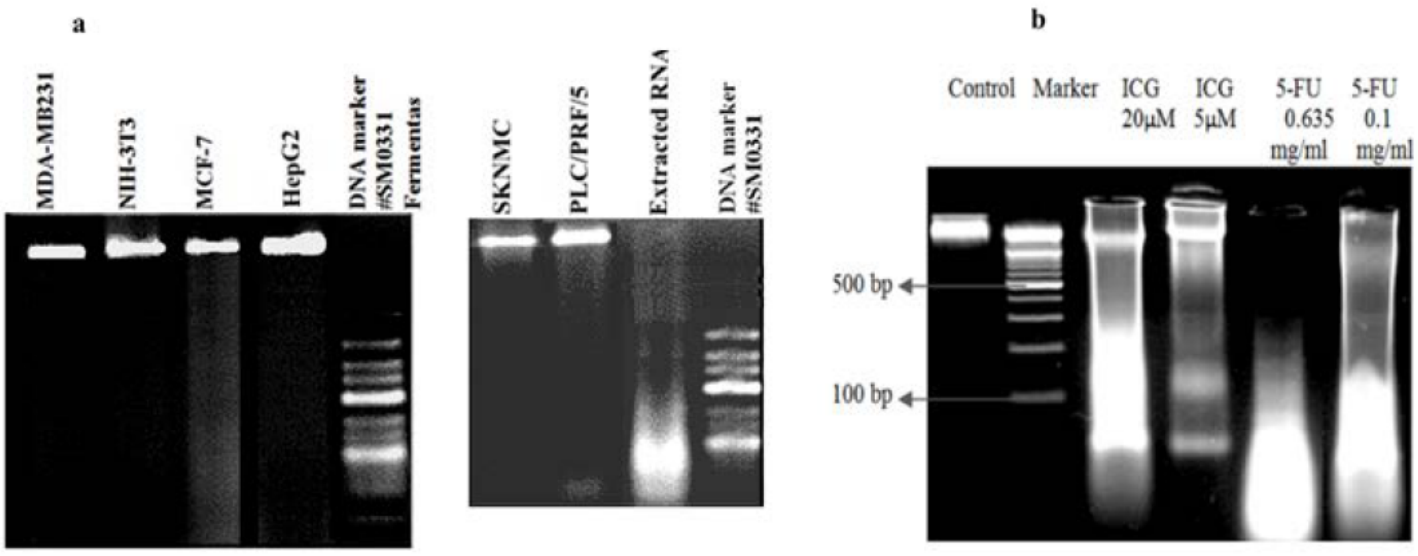
Figures

Fig. 1- Analysis of the isolated DNA and RNA samples using the new protocol.

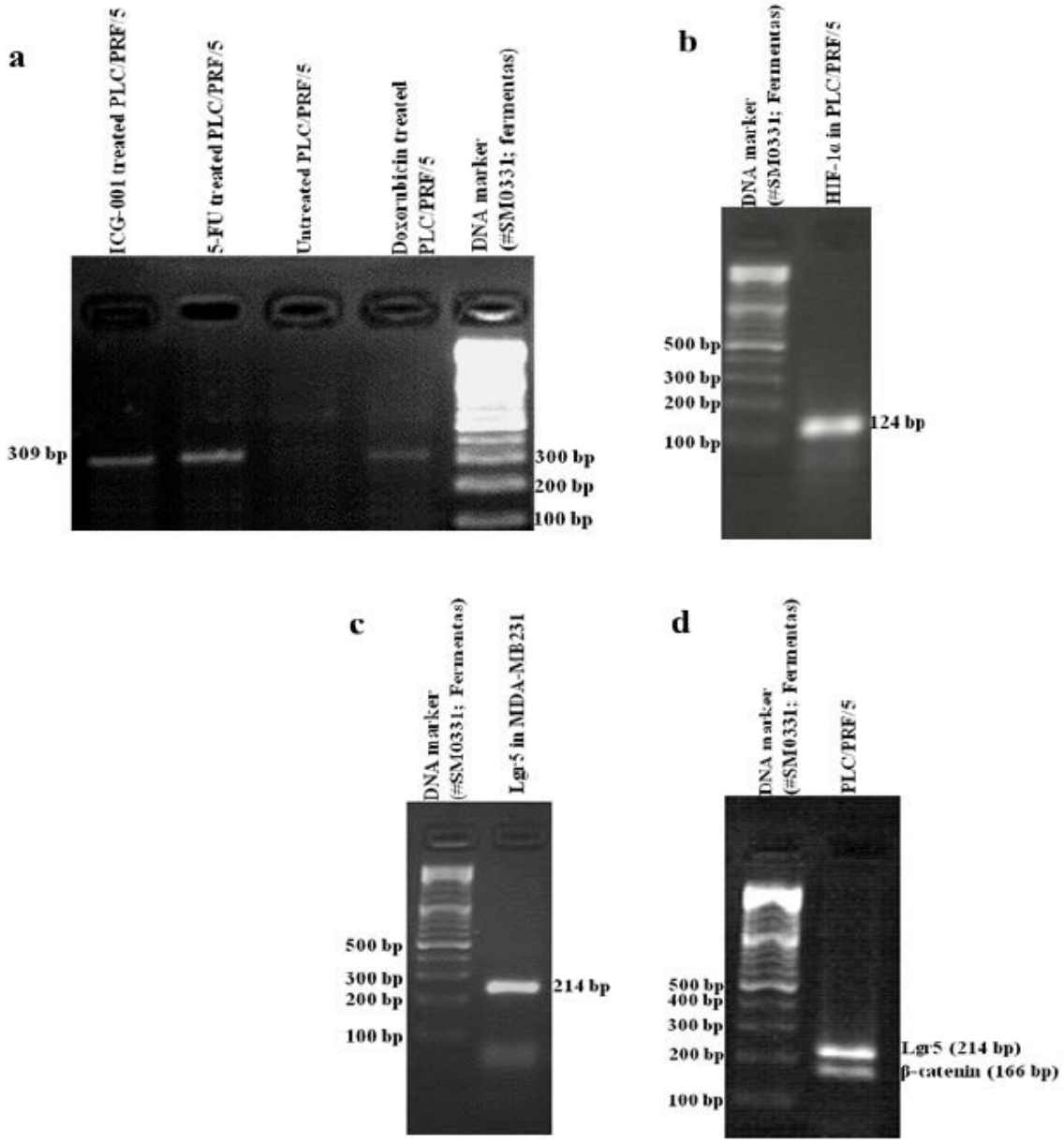


DNA samples isolated from different animal cell lines (normal and cancerous) along with the extracted RNA sample were detected on agarose gel 1.5% with EtBr (a). DNA fragmentation assay was performed for DNA fragments isolated from ICG-001 and 5-FU treated and non-treated PLC/PRF/5 cells and then bands were detected on agarose gel 2% by EtBr (b).

Figure 1

Analysis of the isolated DNA and RNA samples using the new protocol. DNA samples isolated from different animal cell lines (normal and cancerous) along with the extracted RNA sample were detected on agarose gel 1.5% with EtBr (a). DNA fragmentation assay was performed for DNA fragments isolated from ICG-001 and 5-FU treated and non-treated PLC/PRF/5 cells and then bands were detected on agarose gel 2% by EtBr (b).

Figure 2: RT-PCR analysis of some gene expressions by the extracted RNA of the new protocol.



Expression profile of p50 in the presence of ICG-001 and 5-FU in PLC/PRF/5 cells (a) HIF-1α expression in Methotrexate treated PLC/PRF/5 (b) Multiplex PCR of β-catenin and Lgr5 in MDA-MB231 (c) Multiplex PCR of β-catenin and Lgr5 in PLC/PRF/5 (d).

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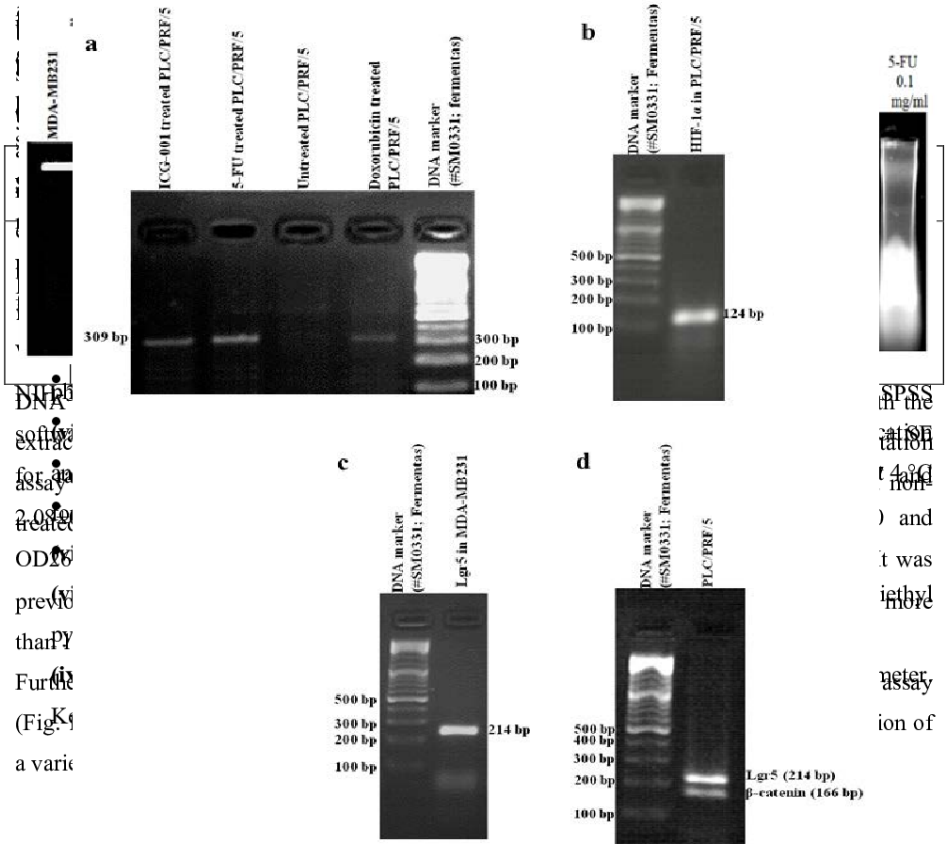

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Figure 3

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