Technical Information

Comparing yield and quality of genomic DNA and total RNA from cancer cell lines using AllPrep Kits or dedicated kits for a single nucleic acid type

Introduction

Human cancer-derived cell lines are the most widely used models for studying cancer biology and for testing hypotheses to improve cancer treatment. Using an appropriate in vitro model in cancer research is crucial for investigating genetic, epigenetic and cellular pathways to study proliferation, apoptosis and cancer progression, enabling potential molecular markers to be defined and cancer therapeutics to be screened and characterized. Best cell culture practice is essential for ensuring consistent and reproducible results from the same cancer cell lines. Simultaneous purification of high-quality genomic DNA and RNA from a single biological sample maximizes the use of precious sample material, especially if only a limited number of cells are available. The AllPrep Kits have optimized nucleic acid purification protocols that allow simultaneous purification of DNA and RNA.

We compared the yield and quality of genomic DNA and RNA purified from three commonly used cancer cell lines using our specialized AllPrep Kits and dedicated DNA or RNA prep kits such as DNeasy® and RNeasy®.

Cell culture procedure

Cell lines were cultured in 75 cm^2 tissue culture flasks at 37°C and 5% CO_2 until 80% confluence was reached. The cell culture media used are listed consecutively in the table below. To harvest the cells, the media was removed and the cells were washed with PBS. The cells were removed from the plate after a short treatment (10–30 s) with 1 ml trypsin using a cell-scraper. Cells were pelleted by centrifugation (2 min, 300 *x g* at room temperature) and washed with PBS. The cells were centrifuged again and diluted to 1 x 10^6 cells/ml in PBS.

Cell line	Background	Medium
A431	Human epidermoid carcinoma cells	500 ml DMEM complete (Gibco® 31885-023), + 50 ml FCS (Merck®, S 0415), + 5 ml MEM (Gibco 11140-035), + 5 ml L-Glutamin (Gibco 25030-024), + 5 ml Penicillin/Streptomycin (Sigma P-0781)
HCT116	Human colon cancer cells	500 ml DMEM complete (Gibco 31885-023), + 50 ml FCS (Merck, S0415), + 5 ml MEM (Gibco 11140-035), + 5 ml L-Glutamin (Gibco 25030-024), + 5 ml Penicillin/Streptomycin (Sigma P-0781), + 17.5 ml Glucose (Sigma G8644)
MCF7	Human breast cancer cells	500 ml RPMI (Gibco 31870-025), + 50 ml FCS (Merck, S 0415), + 5 ml MEM (Gibco 11140-035), + 5 ml L-Glutamin (Gibco 25030-024), + 5 ml Penicillin/Streptomycin (Sigma P-0781), + 5 ml Pyruvate (Gibco 11360-039, + 1.25 ml Insulin (Gibco 12585-014)

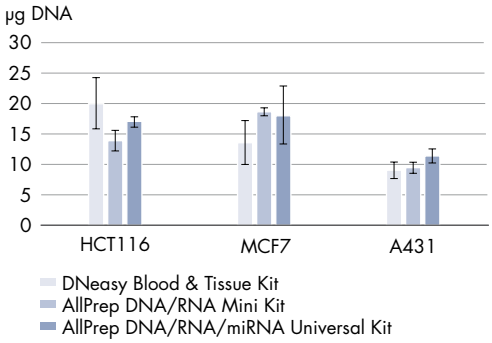


Figure 1. Yields of genomic DNA from various human cancer cell lines. Mean yield of genomic DNA from 4 replicate purifications from 1 x 10^6 cells of HCT116, MCF7 and A431 cell lines using the DNeasy Blood & Tissue Kit, AllPrep DNA/RNA Mini Kit and AllPrep DNA/RNA/miRNA Universal Kit. Yields were determined using the QIAxpert.

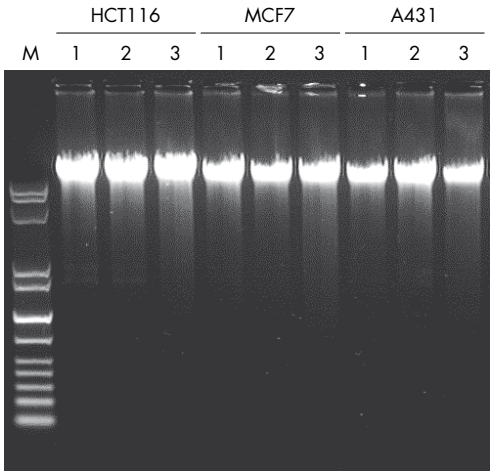


Figure 2. Quality of genomic DNA extracted from various human cancer cell lines. Comparison of genomic DNA quality from 1 x 10^6 HCT116, MCF7 and A431 cells purified with the DNeasy Blood & Tissue Kit (1), AllPrep DNA/RNA Mini Kit (2) and AllPrep DNA/RNA/miRNA Universal Kit (3). Samples analyzed on a 0.8% agarose gel.

Isolating gDNA and RNA

Nucleic acids were isolated from 4 aliquots of 1 x 10^6 cells. Genomic DNA was purified using the AllPrep DNA/RNA Mini Kit, AllPrep DNA/RNA miRNA Universal Kit or DNeasy Blood & Tissue Kit. Total RNA was purified using the AllPrep DNA/RNA Mini Kit, AllPrep DNA/RNA/miRNA Universal Kit or RNeasy Plus Mini Kit. The standard protocol for each kit was used and cell disruption and homogenization were performed using the TissueLyser for 5 minutes at 40 Hz. The QIAxpert® was used to determine gDNA and RNA yields, concentrations and $A_{260}/_{280}$ ratios. The QIAxpert is an innovative high-speed microfluidic spectrophotometer that enables DNA, RNA and protein quantification with additional quality control. gDNA integrity was analyzed on a 0.8% agarose gel and RNA integrity was determined using the Agilent® RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer.

Equivalent gDNA yield and quality

The yields, concentration and A_{260}/A_{280} ratio of gDNA from three different cancer cell lines (HCT116, MCF7 and A431) were comparable between the DNeasy Blood & Tissue Kit, AllPrep DNA/RNA Mini Kit and AllPrep DNA/RNA/miRNA Universal Kit (Figure 1, Table 1). The integrity and overall quality of gDNA eluates were analyzed on a 0.8% agarose gel. All tested cell lines showed similar integrity and overall quality of gDNA independent of the kit used (Figure 2).

Table 1. Quality assessment of gDNA purified from 1 x 10^6 cancer cells

Cell line	DNeasy Blood & Tissue Kit	AllPrep DNA/RNA Mini Kit	AllPrep DNA/RNA/ miRNA Universal Kit
Mean gDNA concentration (ng/µl)/1 x 10^6 cells			
HCT116	181.3	139.7	151.1
MCF7	136.3	186.5	194.8
A431	91.3	95.2	114.0
Mean A_{260}/A_{280} ratio			
HCT116	1.86	1.86	1.85
MCF7	1.86	1.85	1.84
A431	1.86	1.84	1.86

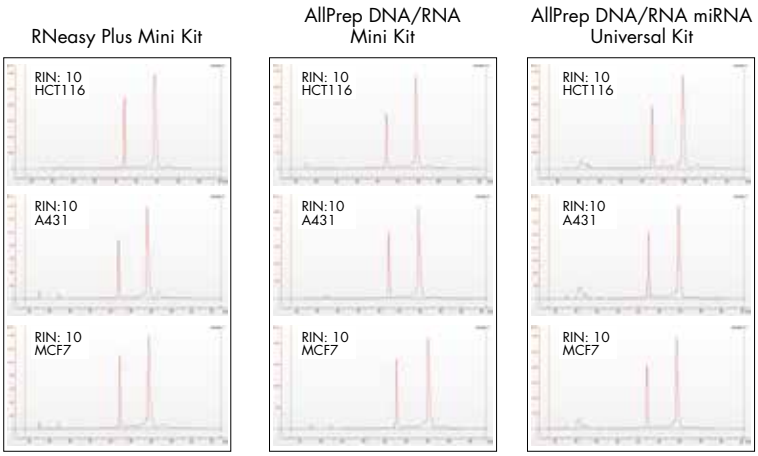
Comparable RNA yield and quality

The average RNA yield, concentration and A_{260}/A_{280} ratio from the cancer cell lines tested were comparable between the RNeasy Plus Mini Kit and the AllPrep DNA/RNA Mini Kit. The average RNA yields and concentrations were slightly higher using the AllPrep DNA/RNA/miRNA Universal Kit (Figure 3, Table 2) as this kit purifies total RNA including miRNA. To compare RNA integrity and overall quality of the RNA, samples were assessed using an Agilent 2100 Bioanalyzer. The electropherograms and gel images exhibited sharp peaks and bands for 18S and 28S ribosomal RNA (Figure 4). The RNA integrity number (RIN) values for all of the samples were comparable regardless of the kit used.

Table 2. Quality assessment of total RNA purified from 1 x 10^6 cancer cells

Cell line	DNeasy Blood & Tissue Kit	AllPrep DNA/RNA Mini Kit	AllPrep DNA/RNA/ miRNA Universal Kit
	Mean gDNA concentration (ng/µl)/1 x 10^6 cells		
HCT116	559.5	578.4	694.4
MCF7	421.4	438.7	573.0
A431	355.1	370.1	423.4
	Mean RNA Integrity Number (RIN)		
HCT116	10.0	10.0	10.0
MCF7	10.0	10.0	9.9
A431	10.0	10.0	9.9
	Mean A_{260}/A_{280} ratio		
HCT116	2.08	2.08	2.08
MCF7	2.07	2.08	2.06
A431	2.08	2.08	2.05

RNA yields in general depend not only on the cell line or type, but also on the cells' growth rate.



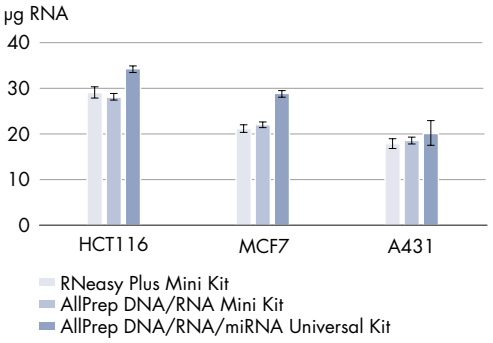


Figure 3. Yields of total RNA from various human cancer cell lines. Mean yield of RNA from 4 replicate purifications from 1 x 10^6 cells of HCT116, MCF7 and A431 cell lines using the RNeasy Plus Mini Kit, AllPrep DNA/RNA Mini Kit and AllPrep DNA/RNA/miRNA Universal Kit. Yields were determined using the QIAxpert.

Figure 4. Analysis of RNA eluates. One microliter of each RNA eluate was applied to an RNA Nano chip and analyzed on an Agilent 2100 Bioanalyzer. Electropherograms from all of the RNA eluates show sharp peaks for the 18S and 28S ribosomal RNA.

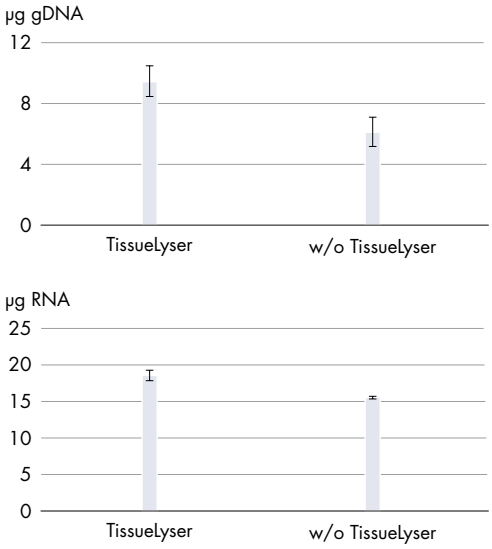


Figure 5. Influence of mechanical disruption and homogenization on nucleic acid yield using the TissueLyser. Mean yield of gDNA and RNA from 4 replicate purifications from 1 x 10^6 A431 cells using the AllPrep DNA/RNA Mini Kit. Yields were determined using the QIAxpert.

Optimal disruption and homogenization

The optimal gDNA and RNA yields for cancer cell lines using the AllPrep Kits can be achieved using the TissueLyser for disruption and homogenization. Average yields of gDNA and RNA from 1 x 10^6 A431 cells disrupted and homogenized with or without using the TissueLyser are shown in Figure 5.

High-quality gDNA and RNA from a single sample in less time

We compared the genomic DNA and total RNA purification efficiencies from common cancer cell lines using sample preparation kits for sequential purification from the same cell lysate compared to the single stand-alone purification kits. The results revealed that the gDNA and RNA yields and quality from different cancer cell lines were comparable between nucleic acids extracted simultaneously from one sample (using the AllPrep DNA/RNA Mini Kit or AllPrep DNA/RNA/miRNA Universal Kit) and nucleic acids extracted with individual procedures (using the DNeasy Blood & Tissue Kit or RNeasy Plus Mini Kit). To gain high gDNA and RNA yields from cancer cell lines when using the AllPrep Kits, we recommend using the TissueLyser for optimal disruption and homogenization. The AllPrep DNA/RNA Mini Kit and AllPrep DNA/RNA/miRNA Universal Kit provide a straightforward and convenient workflow for the purification of high-quality gDNA and RNA from a single sample in less time and with the same quality as with separate purification procedures.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.