Application Note

Sequential automation of RNA and DNA preps on the same QIAcube® instrument

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The QIAcube enables fully automated sample prep for all QIAGEN® spin-column purification kits, using the same purification chemistry as the manual kits. Up to 12 samples can be processed per run. Both RNA and DNA preps can be performed on the same instrument without the risk of cross-contamination.

Introduction

Automation of QIAGEN spin-column kits on the QIAcube saves valuable time and ensures standardized results. Since the same QIAcube may be used by multiple researchers for different applications, cross-contamination between samples and preparation technologies must be avoided (e.g., when nucleases are used). The unique instrument design and features minimize contamination between sequential preps, allowing both RNA and DNA preps to be performed on the same instrument. To show the process safety and robustness, we performed alternating automated RNA preps (requiring a DNase step) and DNA plasmid preps (requiring an RNase step). The preps were sequentially performed on the same QIAcube instrument using the RNeasy® Mini Kit and the QIAprep® Spin Miniprep Kit, respectively.

Independently, we performed a series of manually processed preps to compare with the automated preps. RNA and DNA quality and yields were similar between the two methods, showing the absence of carryover of nucleases.

Materials and methods

The RNeasy Mini Kit and the QIAprep Spin Miniprep Kit were sequentially automated on the QIAcube. Twelve samples were processed (RNeasy or QIAprep) per run (Table 1). As a control, manual preps (4 each) were performed using the same kits (Table 1). ▷

Table 1. Sequence of preps

Step	Procedure	Number of preps
QIAcube run 1	RNeasy	12
QIAcube run 2	QIAprep	12
QIAcube run 3	RNeasy	12
QIAcube run 4	QIAprep	12
QIAcube run 5	RNeasy	12
QIAcube run 6	QIAprep	12
QIAcube run 7	RNeasy	12
Manual procedure	QIAprep	4
Manual procedure	RNeasy	4

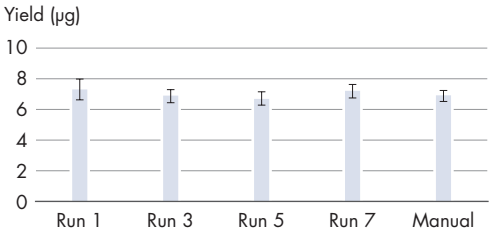

Figure 1. High yields of RNA. RNA was isolated from Jurkat cells using the RNeasy Mini Kit. Preps were automated on the QIAcube (runs 1, 3, 5, and 7) and also performed manually. RNA was quantified by measuring the optical density. High yields were obtained for both automated and manual procedures.

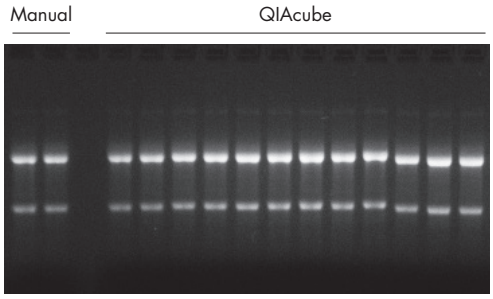

Figure 2. Analysis of RNA by gel electrophoresis. RNA preps were performed using the RNeasy Mini Kit, either manually or automated on the QIAcube. RNA was run on a 1.2% agarose gel in Buffer FA (120 V for 90 minutes). The same amount of each sample was used for agarose gel analysis. The integrity of the RNA was preserved after both manual and automated processing, as can be seen from the agarose gel.

RNA preparation

To isolate RNA, 1 x 10^8 Jurkat cells were lysed in 35 ml Buffer RLT using the TissueRuptor®. The amount of lysate – which was equivalent to 1 x 10^6 cells per sample – was aliquoted and frozen overnight at –20°C. Cells were thawed on ice just before processing the samples manually or on the QIAcube. RNA was purified using the RNeasy Mini Kit on the QIAcube with DNase digestion. Manual preparation of RNA was also performed with the RNeasy Mini Kit using the protocol provided in the *RNeasy Mini Handbook* (see "Protocol: Purification of Total RNA from Animal Cells Using Spin Technology"). As with automated processing, the protocol for manual processing also included DNase treatment and a total of 4 preps were performed (Table 1). RNA was analyzed by optical density measurement, gel electrophoresis, RT-PCR (data not shown) and using the Agilent® 2100 Bioanalyzer.

DNA preparation

pCMVβ plasmid DNA was purified from frozen, pelleted bacteria (1.3 ml aliquoted from one batch of an *E. coli* culture) using the QIAprep Spin Miniprep Kit with the standard protocol on the QIAcube. In this protocol, RNase A treatment was included during the lysis step to remove RNA. The protocol for manual preparation of plasmid DNA was also performed using the QIAprep Spin Miniprep Kit and was comparable to the QIAcube procedure. Both manual and automated procedures were performed from the same batch of *E. coli* culture and included RNA digestion. A total of 4 manual preps were performed (Table 1). After purification, plasmid DNA was analyzed by optical density measurement and gel electrophoresis.

Results

High yields of highly pure RNA

Similarly high yields of highly pure RNA were obtained from both automated and manual procedures (Figure 1). The high quality of the RNA was demonstrated by analyzing samples on the Agilent 2100 Bioanalyzer. Very high RIN values were obtained (Figure 3). The maximum RIN value of 10 was achieved for both manually prepared RNA as well as for RNA preps automated on the QIAcube. No degradation or fragmentation was observed and the integrity of the RNA was preserved, as shown by gel electrophoresis (Figure 2). The 28S/18S rRNA ratio for all samples was between 1.9 and 2.1 (data not shown), which is the optimal range.

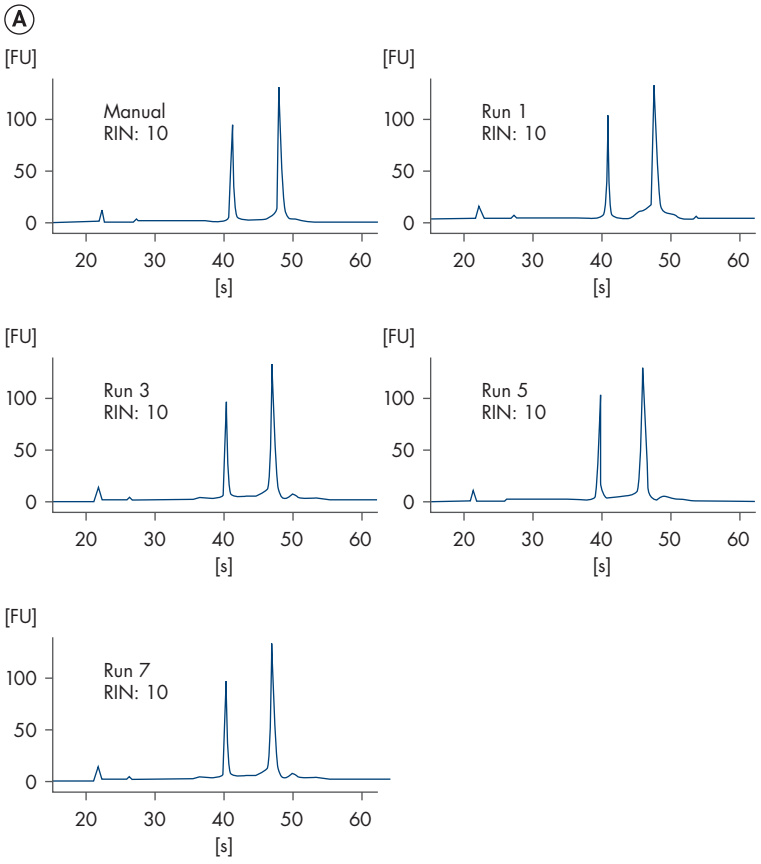

Figure 3. High-quality RNA preps. Preps were automated on the QIAcube (runs 1, 3, 5, and 7) and also performed manually. RNA was analyzed on the Agilent 2100 Bioanalyzer. **A** RIN numbers demonstrate the high quality of the purified RNA. **B** No fragmentation or degradation was observed.

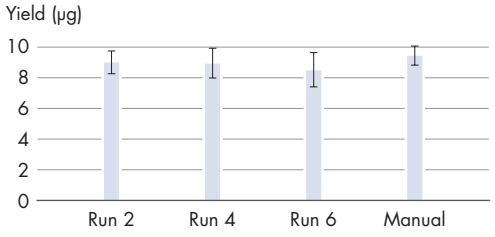

Figure 4. High yields of plasmid DNA. pCMVβ plasmid DNA was purified manually and on the QIAcube. Optical density measurements showed consistently high yields for both methods.

High yields of highly pure plasmid DNA

As shown in Figure 4, a yield of 9 µg of pure plasmid DNA was obtained with the manual procedure as well as with the QIAcube. The high quality of the plasmid DNA was demonstrated by A_{260}/A_{280} ratios, which were between 1.8 and 1.9, and wavelength spectra measurement, which revealed peak absorbance at 260 nm (data not shown). These results were confirmed by agarose gel analysis. No plasmid DNA degradation was evident (Figure 5). The ratio between open circular and supercoiled (covalently closed circular) forms of plasmid DNA was comparable for all samples. Fully intact supercoiled plasmid DNA was obtained for each sample, indicating that there was no degradation by DNase.

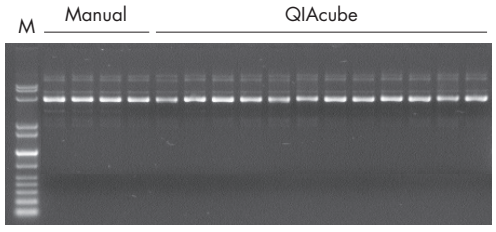

Figure 5. Analysis of plasmid DNA by gel electrophoresis. pCMVβ plasmid DNA was run on an 0.8% agarose gel in Buffer TAE. High yields were obtained for both manual and automated preps and no degradation was evident. **M**: GelPilot® 1 kb Plus Ladder.

Conclusions

- No DNase or RNase contamination was detected when performing sequential RNA and DNA preps on the QIAcube. Highly pure DNA and RNA were obtained every time.
- Equally high yields of DNA and RNA were achieved with both manual and automated processing on the QIAcube. The quality and integrity of the nucleic acids was also comparable for both manual and automated procedures.
- Automation of spin-column kits on the QIAcube provided a convenient and reliable alternative to manual processing. The unique instrument design and its advanced processing technology streamlined nucleic acid purification procedures and minimized contamination.

Ordering Information

Product	Contents	Cat. no.
QIAprep Spin Miniprep Kit (250)*	For 250 high-purity plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106
RNeasy Mini Kit (250)*	250 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-Free Reagents and Buffers	74106
QIAcube	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	Varies†

* Other kit sizes and/or formats available; see **www.qiagen.com**.

† See **www.qiagen.com/qiacube**.

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