Application Note

Quality control of total RNA and cRNA for microarray analysis

Dik van Leenen, Microarray Facility, University Medical Center Utrecht, The Netherlands

In this application note, the suitability of the QIAxcel® system was assessed for quality control of RNA for microarray analysis. The values obtained for the tested quality control parameters indicate that the QIAxcel system is highly suited for analyzing the quality of total RNA and fragmented or intact cRNA.

Introduction

Microarray technology is a powerful tool used to determine the expression levels of thousands of genes in a single experiment and, thus, has become increasingly important in biomedical research and life science applications. Preparation of cRNA, which is then hybridized to microarrays, is a multistep procedure (Figure 1) that is prone to random and systematic errors, especially when large numbers of samples are handled. Therefore, it is critical to minimize experimental noise, standardize processing procedures, and include appropriate experimental controls and replicates.

Monitoring the quality of the initial total RNA sample as well as products generated throughout the entire procedure is crucial, since their quality strongly influences the predictive power obtained from microarray data.

Quality control parameters applied to total RNA samples typically include:

- Determination of the A_{260}/A_{280} absorbance ratio to indicate protein contamination
- Determination of the A_{260}/A_{230} absorbance ratio to detect potential chemical contaminants such as guanidinium thiocyanante
- Determination of the 28S/18S rRNA ratio to check the integrity of the total RNA

While double-stranded cDNA is usually not subjected to quality control due to the limited amount of material produced, the size distribution of unfragmented cRNA is usually analyzed. In addition, efficiency of fluorescent labeling of cRNA is also determined. We evaluated the QIAxcel system for quality control of total RNA and cRNA prior to Cy®3/Cy5 labeling.

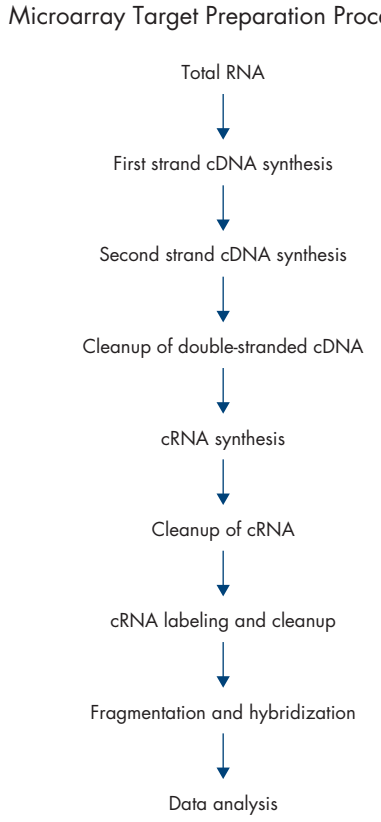

Figure 1. Typical workflow for microarray analysis.

Materials and methods

Total RNA isolation from *Schizosaccharomyces pombe* (fission yeast) was performed according to standard methods. The protocols described below apply to RNA isolated by different methods and from different organisms.

RNA samples were prepared for capillary electrophoresis according to the protocol in the *QIAxcel RNA Handbook*: A 1 µl aliquot of the RNA eluate was mixed with 1 µl RNA denaturing buffer, heated at 70°C for 2 minutes, and cooled in ice-cold water. Sample volume was adjusted to 10 µl with QX RNA Dilution Buffer, and samples were subsequently analyzed on the QIAxcel system using the QIAxcel RNA QC Kit v2.0 and the CM-RNA method.

cRNA generation and labeling

Synthesis of double-stranded cDNA, amplification to cRNA, and labeling were performed as described (1). After amplification, cRNA was normalized to 600 ng/µl and analyzed using the QIAxcel system. After quality control, the cRNA was labeled with Cy3 or Cy5 dyes and then hybridized to self-spotted or commercial microarrays.

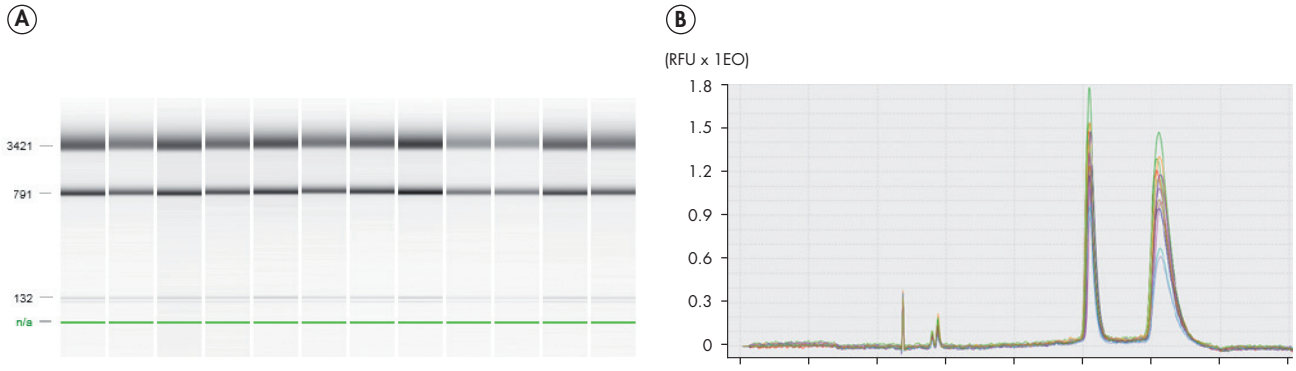

Figure 2. Streamlined RNA analysis using the QIAxcel system. Total RNA purified from *Schizosaccharomyces pombe*. Results presented as **A** a gel image and **B** a superimposed electropherogram view.

Pos.	Plate Id	M	18S	28S	Size 18S	Size 28S	Total RNA Conc.	Ratio
A - 1	100817-TotalRNA	Yes	Yes	Yes	1805.1	3439.9	268.0	1.61
A - 2	100817-TotalRNA	Yes	Yes	Yes	1825.1	3506.2	197.3	1.62
A - 3	100817-TotalRNA	Yes	Yes	Yes	1805.8	3440.6	262.1	1.62
A - 4	100817-TotalRNA	Yes	Yes	Yes	1830.7	3503.7	221.3	1.58
A - 5	100817-TotalRNA	Yes	Yes	Yes	1835.8	3509.0	269.1	1.68
A - 6	100817-TotalRNA	Yes	Yes	Yes	1860.4	3556.8	202.8	1.56
A - 7	100817-TotalRNA	Yes	Yes	Yes	1851.6	3536.3	244.5	1.54
A - 8	100817-TotalRNA	Yes	Yes	Yes	1829.0	3493.8	291.1	1.56
A - 9	100817-TotalRNA	Yes	Yes	Yes	1833.1	3522.1	146.2	1.36
A - 10	100817-TotalRNA	Yes	Yes	Yes	1831.2	3534.1	138.5	1.40
A - 11	100817-TotalRNA	Yes	Yes	Yes	1814.8	3466.0	248.2	1.60
A - 12	100817-TotalRNA	Yes	Yes	Yes	1826.0	3501.6	233.9	1.62

Figure 3. Screenshot showing 28S/18S rRNA peak ratios calculated from samples shown in Figure 2A.

Results

The QIAxcel system is a capillary electrophoresis system that processes samples in batches of 12, for analysis of up to 96 samples without manual intervention. Data can be viewed in both gel image and electropherogram format. Intact RNA is a prerequisite for successful microarray analyses. QIAxcel ScreenGel™ software provides reliable analysis of the size, quantity, and quality of total RNA prior to microarray analysis (Figure 2). The two distinct rRNA peaks and 28S/18Speak ratios of 1.48–1.67 indicate the high quality of the total RNA (Figures 2 and 3).

Analysis of unlabeled cRNA shows expected profiles with a smear of products concentrated in size from 500 bp to 2000 bp. The size distribution should be equal between samples: less intact RNA yields smaller cRNA fragments (Figure 4).

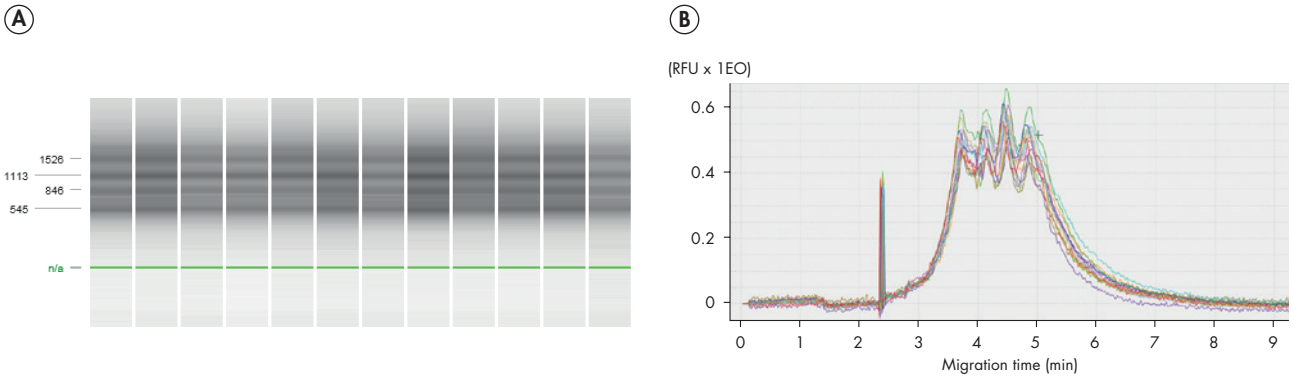

Figure 4. Reliable quality control of unlabeled cRNA generated from yeast total RNA. Results presented as **A** a gel view and **B** a superimposed electropherogram view.

Conclusions

These results demonstrate the high suitability of the QIAxcel system for fast, sensitive analysis of the quality and quantity of total RNA and fragmented or intact cRNA. The 28S/18S rRNA ratio, a measure of RNA integrity, was successfully determined — a crucial factor for meaningful gene expression data. The ease of use, fast processing times, and the ability to analyze up to 96 samples without manual intervention make the QIAxcel system particularly well suited for research laboratories with high-throughput needs.

Reference

1. Lenstra, T.L. et al. (2011) The specificity and topology of chromatin interaction pathways in yeast. Mol. Cell **42** 536.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9001941
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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