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Analysis of small RNAs with the Agilent 2100 Bioanalyzer

The Agilent 2100 Bioanalyzer offers advantages of sensitivity and accuracy for performing RNA separation, detection and quantitation, coupled with a rapid, automated system. Here we demonstrate the performance of the Agilent 2100 Bioanalyzer compared with standard techniques for RNA separation, detection and quantitation.

Isolation of pure and intact RNA is essential, but the concentration and integrity of total RNA preparations is affected by the purification process used. Applications for which RNA will ultimately be used may include reverse transcriptase (RT)-PCR, preparations of targets for microarrays, ribonuclease protection assays, preparations of cDNA libraries and northern blotting. Recently, the importance of low-molecular-weight (LMW) RNAs, such as small interfering RNAs (siRNAs) and small nuclear RNAs (snRNAs), has been outlined in different studies[1]. In particular, the importance of a new class of small RNA, microRNAs (miRNAs), has been highlighted. Each miRNA is thought to regulate multiple genes, and as hundreds of miRNA genes are predicted to be present in higher eukaryotes, the potential regulatory circuitry afforded by them is enormous.

Standard protocols for the isolation of total RNA and mRNA are not optimized for recovering small RNA molecules and may lead to major loss of miRNAs and other small RNAs. Therefore, an efficient, accurate method is needed to determine the integrity and the concentration of total and small RNAs from various sources. The Agilent 2100 Bioanalyzer offers such advantages, coupled with a rapid, automated system (Vitale, D. Comparing performance of the Agilent 2100 Bioanalyzer to traditional RNA analysis techniques. Agilent publication 5980-2206E; 2002; Lightfoot, S. Quantitation comparison of total RNA using the Agilent 2100 Bioanalyzer, ribogreen analysis and UV spectrometry. Agilent publication 5988-7650EN; 2003; Kuschel, M. Analysis of total RNA with the Agilent 2100 Bioanalyzer. Agilent publication 5968-7493E; 2001).

Using the Agilent 2100 Bioanalyzer for small RNA analysis

We compared the performance of the Agilent 2100 Bioanalyzer against the most commonly used techniques to separate, detect and quantitate small RNAs. We evaluated samples extracted using different protocols and from various cell lines.

We purified RNA from two different human cell lines, lymphoblastoid and human brain tumor (U118), using the MirVana™ miRNA Isolation Kit (Ambion) according to manufacturer's instructions. Two different RNA extraction procedures were used. The first is designed to recover total RNA (sample A) and the second is specific for small-RNA enrichment (samples 2 and 4). The resulting RNA preparations are highly enriched for LMW RNAs (5.8*S*, 5*S*, tRNA, miRNAs, siRNAs

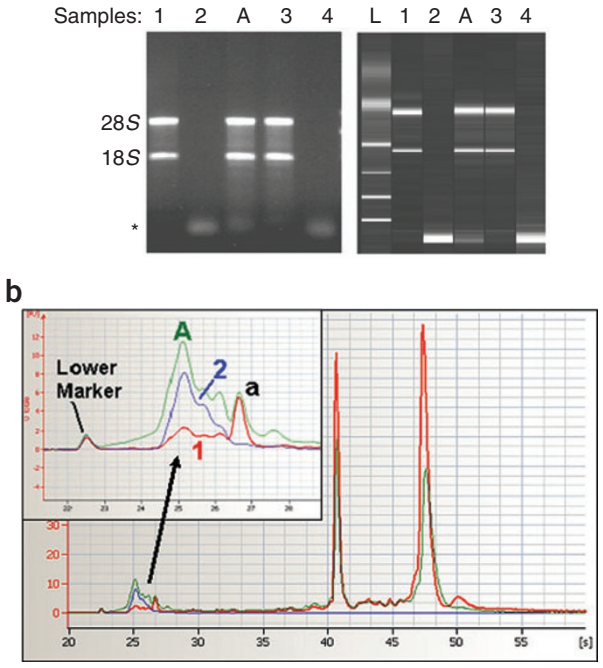

Figure 1 | Comparison of RNA separation by agarose gel electrophoresis against the Agilent 2100 Bioanalyzer analysis. (**a**) Agarose gel (1%, left) of total RNA (sample A), LMW RNAs (samples 2 and 4) and HMW RNAs (samples 1 and 3) compared to the digital gel (right) obtained with the Agilent 2100 Bioanalyzer (L, ladder; 500 ng per sample for all RNAs). (**b**) Screen capture of Agilent 2100 Bioanalyzer electropherograms of sample A (total RNA), sample 1 (HMW RNA) and sample 2 (LMW RNA). Peak labeled "a" (inset) was detected only in total and HMW RNA samples (A and 1) but not in LMW RNA–enriched sample (2).

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and/or snRNAs; both single- and double-stranded species). Samples 1 and 3 consist of high-molecular-weight (HMW) RNAs.

We loaded each sample on an agarose gel and stained with ethidium bromide to estimate the quality and quantity of each RNA sample. We also analyzed the RNA samples with the RNA 6000 Nano Kit, which is specifically optimized for the analysis of total RNA with the Agilent 2100 Bioanalyzer. The Agilent 2100 Expert software includes data-collection, presentation and interpretation functions. Data can be displayed as a gel-like image and/or as electropherogram(s). The RNA profile of each sample is automatically displayed as individual electropherograms. We compared the results from the gel electrophoresis to the Agilent 2100 Bioanalyzer digital gel (**Fig. 1a**) and to the corresponding electropherograms (**Fig. 1b**).

We efficiently detected 18*S* and 28*S* ribosomal RNA (rRNA) bands on the gel, but LMW RNA were visualized as faint, smeared bands. This suggests that agarose gel electrophoresis is not effective for detecting the quality and quantity of small RNAs or for verifying enrichment protocol efficiency. The Agilent 2100 Bioanalyzer can easily detect an intense peak at 100–175 base pairs resulting from small RNA fragments located under the 0.2-kilobase band of the ladder. Considering the electropherograms of samples A, 1 and 2, and by examining the early electropherogram region (from 25 to 200 base pairs) in more detail, we could identify different profiles (**Fig. 1b**). The LMW region of sample A shows peaks of small RNAs. Sample 1 shows a profile similar to Sample A in the later part (18*S* and 28*S* RNAs) of the electropherogram but not in the earlier region (small RNAs) where only one peak is retained with comparable concentrations (peak "a" in the inset of **Fig. 1b**). Notably, the two extraction protocols seem to similarly retain the HMW RNAs but not the smaller ones with the same efficiency.

All samples were run on a 15% polyacrylamide gel and stained with ethidium bromide (**Fig. 2**). From the comparison of sample electrophoresis we could efficiently detect 5.85*S*, 5*S* rRNA and tRNA bands in total RNA (sample A) and in LMW-enriched preparations (samples 2 and 4). Although 15% acrylamide gel electrophoresis provides highly resolved profiles of LMW RNAs (5.8*S*, 5*S* and tRNA) it is not feasible for obtaining information about the quality and quantity of HMW and LMW RNAs in the same run.

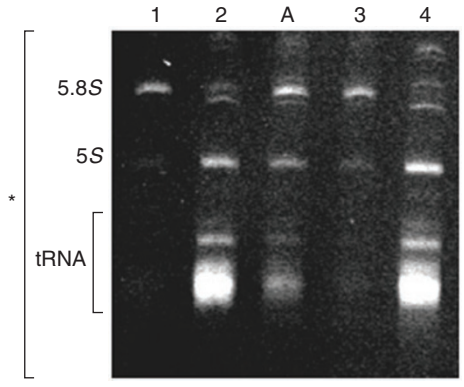

Figure 2 | Polyacrylamide gel analysis of RNAs. Total RNA (sample A), small RNAs (LMW; samples 2 and 4) and HMW RNAs (samples 1 and 3) were run on a polyacrylamide gel (500 ng per sample) and stained with ethidium bromide.

Table 1 | Total RNA dilutions and relative concentrations of small RNAs obtained from the Bioanalyzer software result table

Total RNA (Sample A)	1 µg	0.5 µg	0.25 µg	0.1 µg
Small RNAs	117 ng	70 ng	29 ng	5 ng

We also evaluated the Agilent 2100 Bioanalyzer using six dilutions of sample A (with the total RNA concentration ranging from 1 µg/µl to 100 ng/µl and the corresponding small RNA fractions ranging from 117 to 5 ng), and comparing the electropherograms to 1% agarose gel runs (**Table 1**).

The Agilent 2100 Bioanalyzer clearly detects small RNA bands that cannot be visualized on an agarose gel (data not shown). The Bioanalyzer consistently detected as little as 5 ng of LMW RNA out of 100 ng of total RNA, whereas an agarose gel stained with ethidium bromide required at least 500 ng of total RNA. With limited sample quantities (for example, from biopsies), most of the RNA is consumed in running the gel. The high sensitivity of the Bioanalyzer requires only small quantities of sample, and the new Series II RNA 6000 Pico kit is 100–200× more sensitive than agarose gel electrophoresis.

Conclusion

The Agilent 2100 Bioanalyzer integrates and automates sample handling, separation and analysis. Chip-based analysis also reduces sample consumption while increasing analysis speed and data precision. The Agilent 2100 Bioanalyzer shows excellent performance for quantitative and qualitative analysis of LMW RNA samples. RNA samples (HMW and LMW) are detected and analyzed in real time, and the digital output facilitates data exchange. A valuable advantage of the Agilent 2100 Bioanalyzer is the ability to accurately determine sample concentration while checking integrity and purity of the sample. Automation of both the separation and data analysis makes the Agilent 2100 Bioanalyzer easy to use. The data analysis and comparison functions also offer considerable advantages. Time-consuming steps, such as scanning or densitometric analysis, are eliminated.

The Agilent 2100 Bioanalyzer can be used to measure the quantity, integrity and purity of small RNAs and HMW total ribosomal RNA, and the integration of these two measurements provides many advantages over traditional agarose gel electrophoresis.

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