Characterization of RNA quality using the Agilent 2100 Bioanalyzer

Application Note

Life Sciences

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Introduction

RNA quality is critically important for many applications. These include Northern hybridization, cDNA library construction, S1 nuclease mapping, and differential gene expression measurements using microarray technology. Agarose gel electrophoresis is sometimes used to monitor the quality of RNA preparations, but many researchers forego RNA characterization because of the lengthy protocols involved and the large amount of sample required.

The Agilent 2100 bioanalyzer and the RNA 6000 LabChip® kit provide a simple, rapid alternative for the characterization of RNA samples. The Agilent 2100 bioanalyzer is a small benchtop system that uses LabChip technology from Caliper Technologies Corp. to integrate sample separation, detection, quantification, and data analysis. Each disposable RNA chip is used to determine the concentration and purity/integrity of 12 RNA samples with a total analysis time of about 25 minutes. The system permits rapid screening of RNA preparations for sample degradation and ribosomal RNA contamination.

In this application note, we present guidelines for assessing the quality of RNA samples using the Agilent 2100 bioanalyzer. Examples of three common RNA problems are illustrated:

1. Degradation of total RNA preparations
2. Degradation of mRNA preparations
3. Ribosomal RNA contamination in mRNA preparations

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Results and Discussion

1. Degradation of Total RNA

The analysis of an intact total RNA sample from Jurkat cells is shown in figure 1. The 18S and 28S ribosomal RNA peaks are identified by the Agilent 2100 bioanalyzer software and dominate the electropherogram. The amount of small RNA (peak 1), which can include 5.8S and 5S ribosomal peaks and transfer RNA, highly depends on the preparation protocol. The sample in figure 1 contains little material in the small RNA region, whereas other preparations may contain significant levels of small RNA.

RNase degradation of total RNA samples produces a shift in the RNA size distribution toward smaller fragments and a decrease in fluorescence signal (figure 2A). The 18S and 28S peak can no longer be identified with certainty. With more severe degradation (figure 2B), the spectrum shifts entirely toward early migration times. The overall signal becomes weak as dye intercalation sites are destroyed.

Figure 1
Total RNA from cultured Jurkat cells was isolated using the RNeasy Midi Kit from Qiagen (Hilden, Germany). The RNA was diluted to a concentration of 100 ng/µL and 1.0-uL aliquots were analyzed using the RNA 6000 LabChip kit.

Figure 2
Total RNA (100 ng/µL) from cultured Jurkat cells was incubated for 15 minutes at room temperature with diluted RNase A (2 x 10-6 and 1 x 10-5 mg/mL, resp.). Samples (1.0-µL aliquots) were analyzed using the RNA 6000 LabChip kit. The Agilent 2100 bioanalyzer provides two visual representations of each sample, an electropherogram (left) and a gel-like image (right).
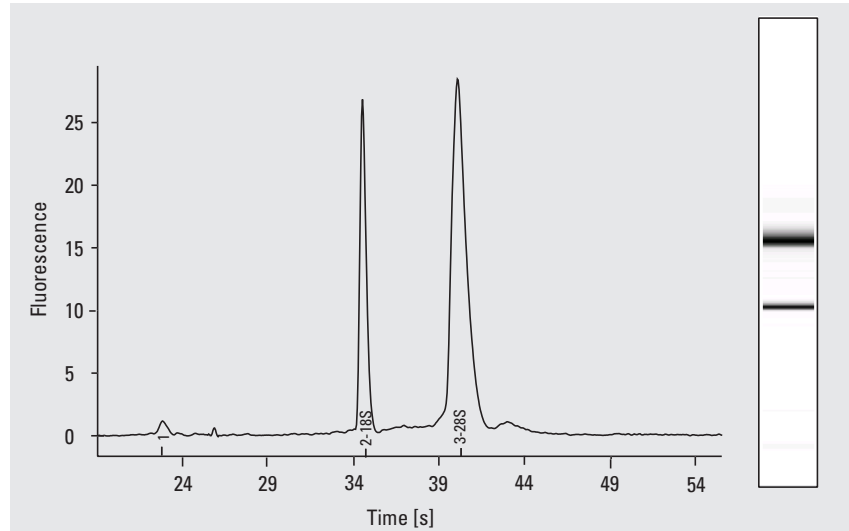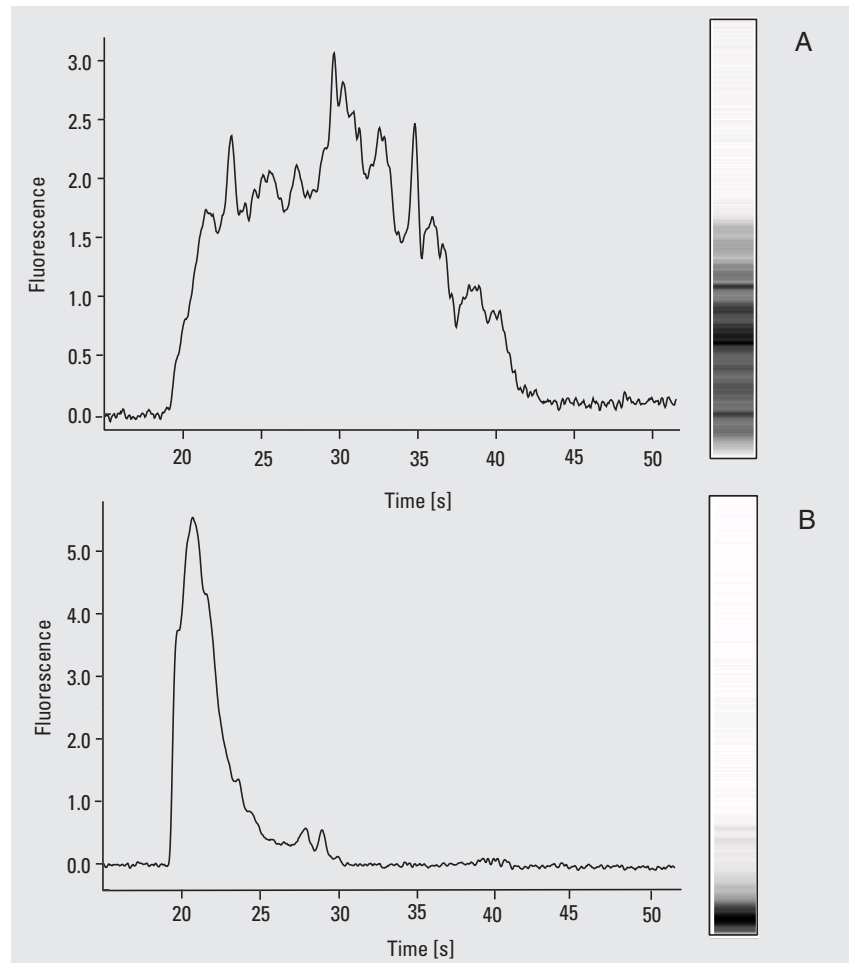
2. Degradation of mRNA

Oligo (dT)-cellulose is commonly used to enrich RNA samples in poly (A)+ RNA. Intact poly (A)+ RNA samples display broad size distributions (figure 3) and the size range can be estimated by overlaying the RNA 6000 ladder containing RNA fragments of known size. Large transcripts are detected late in the analysis window. If multiple rounds of enrichment are not used, ribosomal RNA peaks will appear superimposed upon the poly (A)+ RNA distribution, as described below.

As with total RNA, RNase degradation of mRNA shifts the RNA size distribution toward smaller fragments (figure 4). When cDNA or cRNA pools are produced from partially degraded RNA, these pools will exhibit similarly short size distributions.

Figure 3
Total RNA from cultured Jurkat cells was enriched in poly (A)+ sequences by three rounds of oligo (dT) selection using the Oligotex mRNA Mini kit from Qiagen. The RNA was diluted to a concentration of 60 ng/µL and 1.0-µL aliquots were analyzed using the RNA 6000 LabChip kit. For size comparison the RNA 6000 ladder is overlaid (lane A fragment sizes: 200, 500, 1000, 2000, 4000, 6000 bases).

Figure 4
Poly (A)+ RNA (60 ng/µL) from cultured Jurkat cells was incubated for 15 minutes at room temperature with very dilute RNase A (1 x 10-6 and 2 x 10-6 mg/mL, resp.). Samples (1.0-µL aliquots) were analyzed using the RNA 6000 LabChip kit.

3. Identification of Ribosomal RNA Contamination in mRNA Preparations

Ribosomal RNA contamination in mRNA preparations is easily identified by the presence of ribosomal RNA peaks. The Agilent 2100 bioanalyzer software permits semi-quantitative estimation of ribosomal RNA content. For example, figure 5 shows the overlay of the electrophoretic traces
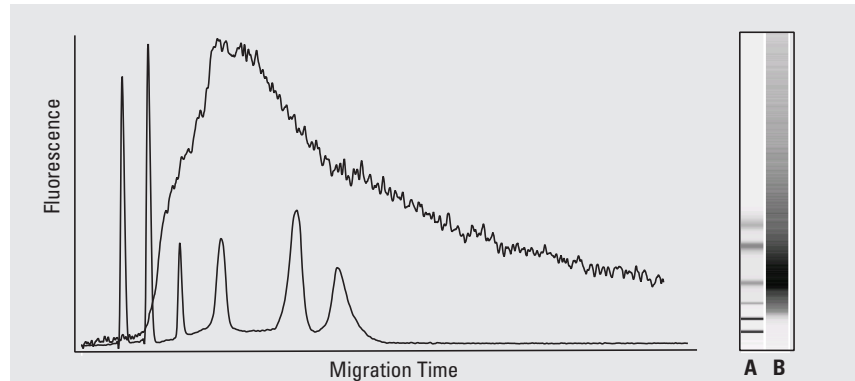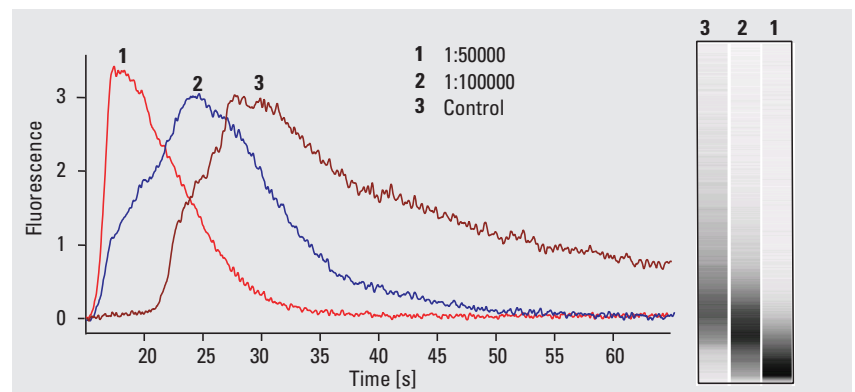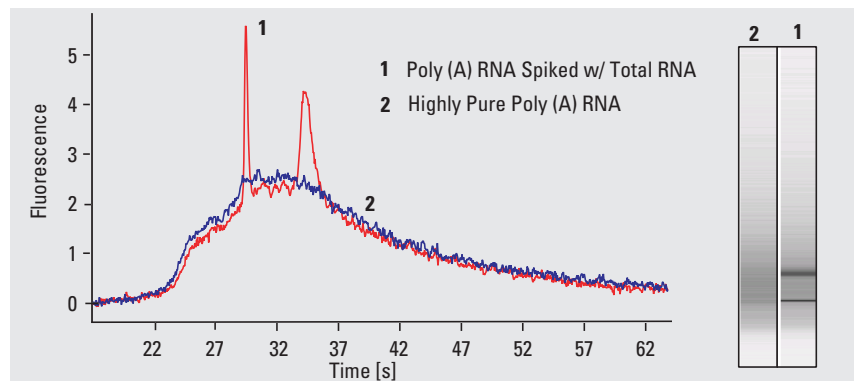

Figure 5
Highly purified poly (A)+ RNA and poly (A)+ RNA (42 ng/µL) spiked with total RNA (10 ng/µL) were analyzed using the RNA 6000 kit. The software integrates the messenger RNA background and calculates the percentage of ribosomal RNA contamination.

of a poly (A)+ RNA sample with no detectable ribosomal RNA contamination and of the same poly (A)+ RNA sample which has been spiked with intact total RNA. The Agilent 21000 bioanalyzer software permits integration of ribosomal RNA peaks that rise above the mRNA distribution to estimate the level of ribosomal RNA contamination in mRNA samples.

Note: In some cases the level of ribosomal RNA contamination is higher than the value estimated by this approach. It is recommended to use this software feature to screen samples for unacceptably high ribosomal RNA contamination. In spiking studies, contamination levels down to 5 % are easily detected.

Conclusion

The concentration and integrity of RNA samples can be rapidly characterized using the Agilent 2100 bioanalyzer and RNA 6000 LabChip kit. Degraded or contaminated RNA preparations can be identified before time-consuming protocols such as cDNA synthesis are initiated.

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