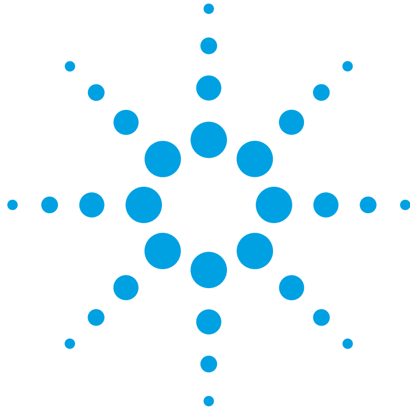


The Total RNA Story

Deborah Vitale

Introduction

Assessing RNA sample quality as a routine part of the gene expression workflow is an important step in successful gene expression experiments. The Agilent 2100 bioanalyzer is the first commercially available microfluidics instrument to provide detailed information about the condition of RNA samples. However, with the introduction of new technology, and a greater amount of available information, the data interpretation can initially seem foreign. This application is a tool to aid RNA data interpretation and identify features from total RNA electropherograms that reveal information about RNA sample quality.

Data from a high quality total RNA preparation

Although a wide variety of ribosomal peak heights and ratios exist across a broad range of RNA sample types, most high quality RNA samples share the same common features:

- The most distinct and intense features are the 18S and 28S ribosomal peaks (16S & 23S prokaryotic).
- There is an absence of smaller well-defined peaks between the two ribosomes. Any mRNA migrating between the ribosomes will be smooth and lack distinct peaks.
- The baseline between 29 seconds and the 18S ribosome is relatively flat and free of small rounded peaks corresponding to smaller RNA molecules.

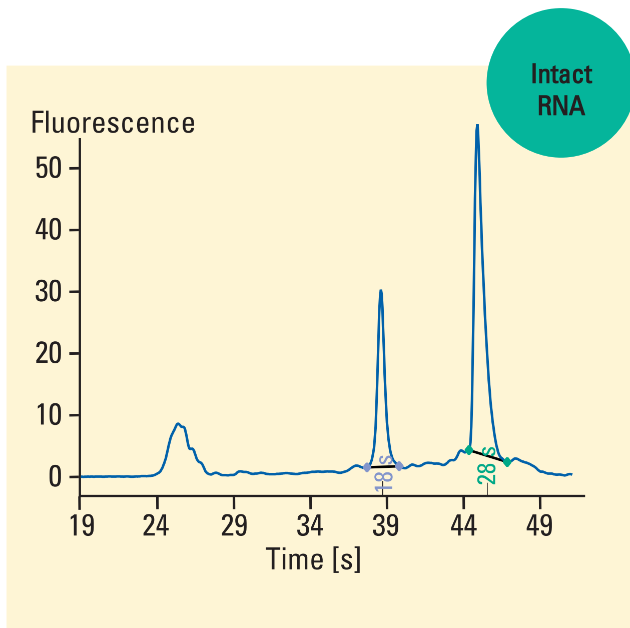
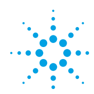


Figure 1
Electropherogram of a high quality, eukaryotic, total RNA sample. The sample was isolated using a Trizol preparation that retains the 5S ribosome and tRNA seen at 25 seconds.

Depending on the RNA extraction method, the small 5S, 5.8S and tRNA may be present in the electropherogram from 24–27 seconds. In figure 1, the ML1 total RNA was prepared with a Trizol extraction, which usually yields sample that includes the smaller RNA molecules. In contrast, Qiagen column purification removes small RNAs, leaving only the mRNA and the two large ribosomal peaks.



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As RNA degrades:

- The peaks begin to shift towards the left of the electropherogram,.
- The baseline between and to the left of the ribosomes becomes jagged. Defined looking peaks appear.
- The intensities of the ribosomal peaks decrease relative to the peaks resulting from their degradation.
- The intensity of smaller degraded RNA increases.The most highly degraded products have a migration time between 22 and 24 seconds.

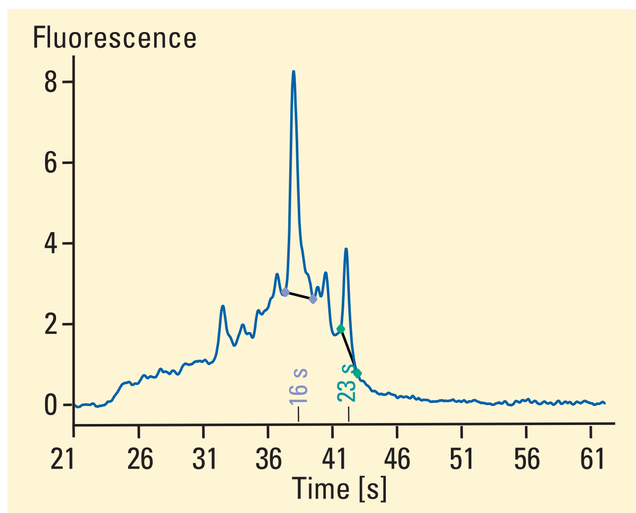

Figure 2
Electropherogram of a partially degraded,prokaryotic, total RNA sample. The 23S ribosome is degraded more quickly than the 16S ribosome.

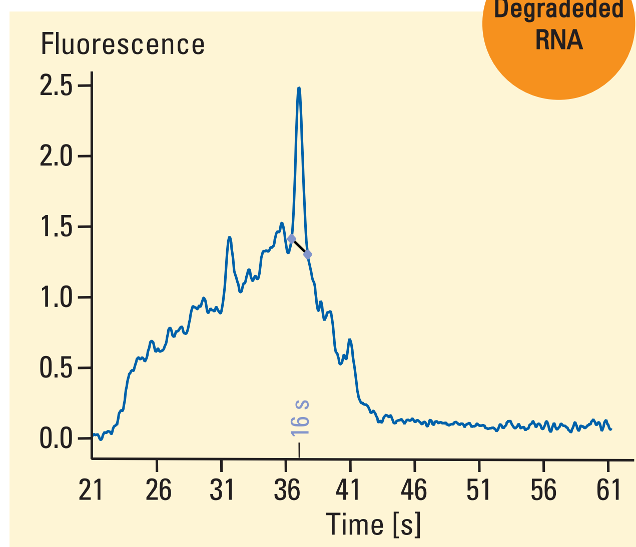

Figure 3
Electropherogram of a degraded prokaryotic total RNA sample. The 23S ribosome is no longer present, while the amount of shorter RNA fragments has increased.

Data from a sample in the process of degradation

Figure 2 shows an E. coli total RNA sample that is partially degraded. Notice that the 23S ribosomal peak is almost completely digested. Not all RNA follows the same pattern during degradation, however, the larger ribosome is typically digested first, resulting in a shortening and broadening of the peak. In figure 3, the 23S ribosome is almost completely digested, and the sample is more highly degraded.

Conclusion

RNA quality can easily be determined through visual inspection of the electropherogram. This simple assessment can also screen for degraded samples that will cause further experiments to perform poorly. Using the Agilent 2100 bioanalyzer to asses the integrity of your RNA provides a faster method of increasing your confidence of sample quality, thereby saving time, money and unnecessary frustration.

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