Interpreting mRNA electropherograms

Application

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Introduction

An important step in gene expression workflow is the routine quality assessment of mRNA samples to ensure the success of gene expression experiments. In contrast to gel electrophoresis which consumes a significant amount of sample and reveals only very limited information about sample quality, the Agilent 2100 bioanalyzer, using Caliper's LabChip® technology, in combination with the RNA 6000 LabChip® kit, provides detailed information about the condition of mRNA samples in the form of highly sensitive electropherograms. This application is a tool to aid mRNA data interpretation and identify features from mRNA electropherograms that reveal information about mRNA sample quality.

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caliper LabChip®

High quality mRNA

High quality messenger RNA run on the Agilent 2100 bioanalyzer typically has the shape of a broad peak. In figure 1, the bovine lung mRNA transcripts range between 500 and 9000 bases long, with the majority of transcripts in the size range of 1000–4000 bases long. The electropherograms of high quality samples are generally smooth and free of multiple large peaks. However, the Agilent 2100 bioanalyzer is very sensitive and some transcripts that are highly expressed may stand out against the smooth slopes of the rest of the sample. These occasional transcript enrichments will be relatively small compared to the rest of the sample.

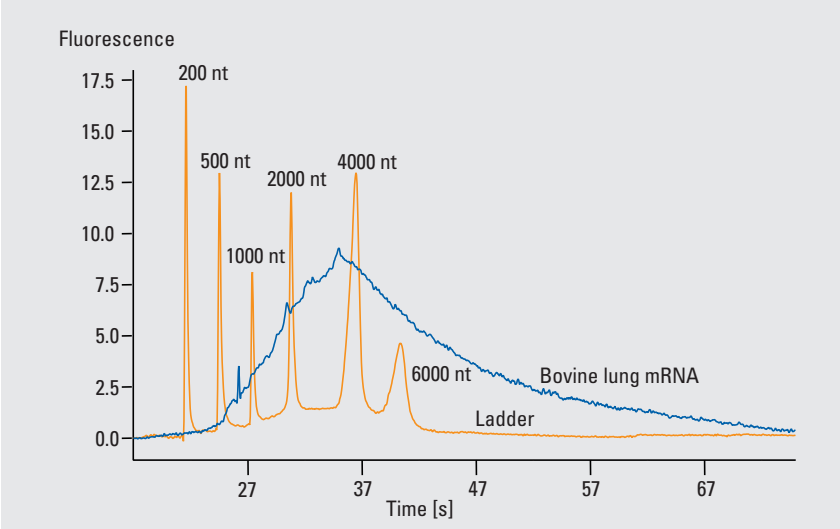

Figure 1
The relative amounts of transcripts falling in a certain size range can be estimated by overlaying the RNA 6000 ladder onto the electropherogram by holding down the control key while using the mouse to click on the ladder lane in the small gel-like image. The figure shows overlaid electropherograms of bovine lung mRNA, 250 ng/ µl, and the RNA 6000 ladder. The ladder contains six fragments of sizes 200, 400, 1000, 2000, 4000 and 6000 nucleotides. The relatively smooth character of the broad bovine lung mRNA peak is typical of a high quality mRNA sample free of ribosomal RNA contamination.

Ribosomal contamination

It is common for high quality mRNA samples to contain low levels of ribosomal RNA contamination which are characterized by the presence of one to two large, well-defined ribosomal RNA peaks. As in figure 2, the ribosomal RNA peaks can appear on either or both sides of the mRNA apex. Low levels of ribosomal RNA contamination do not compromise the quality of mRNA samples, however large amounts of contamination may interfere with future reactions. The Agilent 2100 bioanalyzer software is able to identify and quantitate ribosomal peaks that are five percent of the total mRNA concentration or greater. The peak is clearly identified on the electropherogram, and the percent contamination is recorded in the data table associated with the sample.

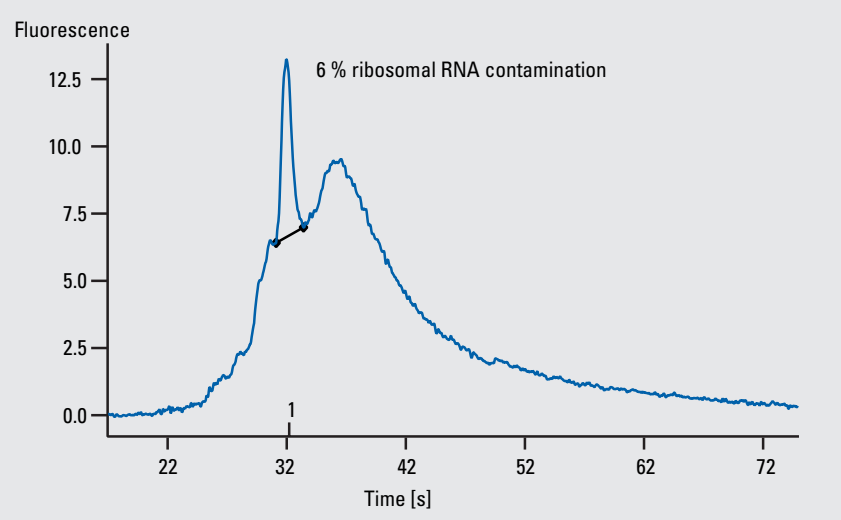

Figure 2
Electropherogram of rabbit liver mRNA, 250 ng/µl. The intense peak identified by the software at 32 seconds is a ribosomal contamination peak, which comprises 6 % of the sample.

Electropherogram profile variability

Some tissues produce electrophoretic traces that are different from the most common mRNA profiles shown in figure 1. The chicken brain mRNA electropherogram in figure 3 contains a majority of RNA transcripts in the 1000 to 2000 nucleotide region but is also enriched with RNA molecules longer than 6000 nucleotides, which appear as a second broad peak. DNase and RNase digestion confirm that this second peak is RNA and not genomic DNA contamination. The enrichment of long RNA molecules is not easy to distinguish when mRNA samples are run on gels, however, the high sensitivity of the Agilent 2100 bioanalyzer enables the clear detection of a second peak in the mRNA electropherograms from a variety of species and tissue samples. It is reproducible in multiple mRNA isolations of the same sample type, and is consistent in successive LabChip experiments. These large peaks are characteristic features of certain sample and tissue types, which add to the variability of mRNA electropherograms. When questionable peaks appear in the electropherograms of mRNA samples, a quick RNase digestion will reveal whether or not they are RNA.

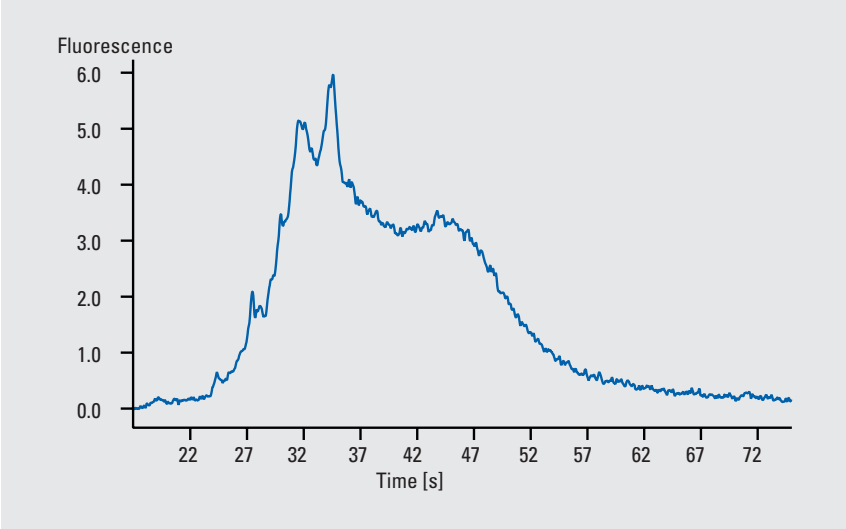

Figure 3
Electropherogram of chicken brain mRNA, 200ng/µl. The electropherogram contains a second broad peak from 40-55 seconds made up of very long RNA transcripts, which is a reproducible feature of this sample.

Conclusions

Using the Agilent 2100 bioanalyzer and the RNA 6000 LabChip kit, the quality and integrity of messenger RNA can easily be determined through visual inspection of the electropherogram. Additionally, the percentage of ribosomal RNA contamination and an estimate of sample concentration is listed in the sample data table. This simple assessment can screen for samples with too much ribosomal contamination that will cause further experiments to perform poorly, and can also be used to identify samples with tissue profiles enriched with longer RNA transcripts. Using the Agilent 2100 bioanalyzer to assess the integrity of your mRNA increases your confidence in sample quality to a degree that was not previously possible using gel electrophoresis.

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