

Interpreting Agilent Bioanalyzer Results

The biggest key to successful GeneChip microarray experiments is starting with high quality RNA. Various methods, spectrophotometers and gel electrophoresis, have traditionally been used to analyze RNA quality. Agilent has introduced an instrument, the Bioanalyzer that provides more sensitive qualitative analysis from less RNA than the traditional methods. The AMC has adopted the use of the 2100 Bioanalyzer as a method for checking the quality of incoming RNA and for predicting the probability of successful biotin labeling.

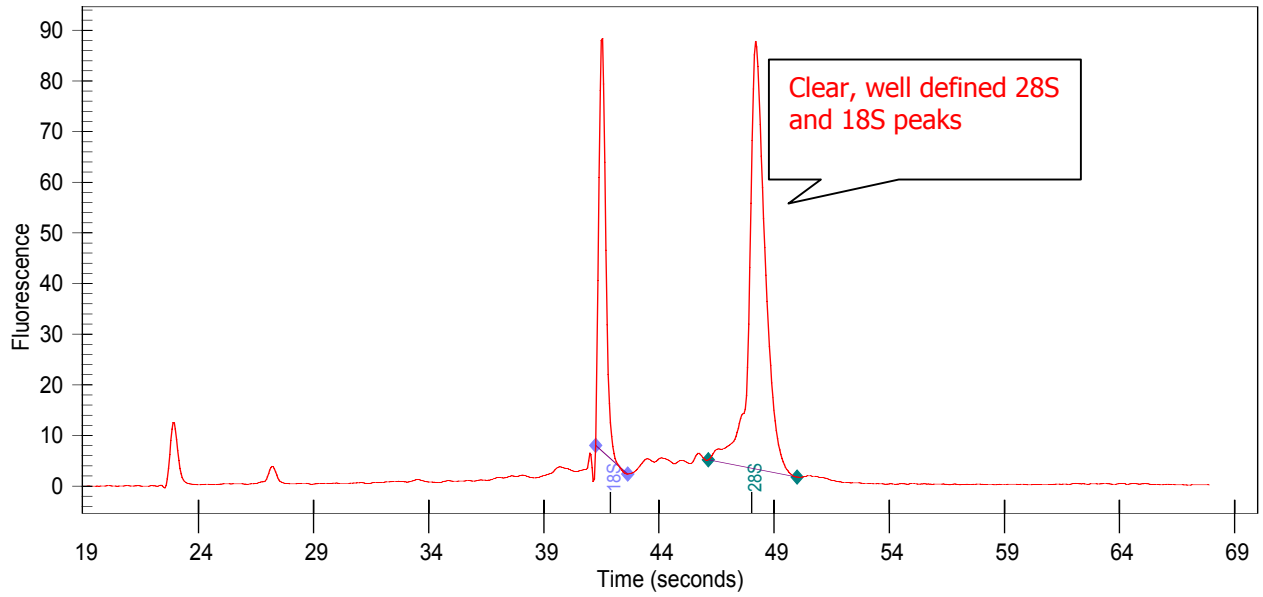
The Agilent 2100 Bioanalyzer provides a platform that uses a fluorescent assay involving electrophoretic separation to evaluate RNA samples qualitatively. The Bioanalyzer measures the amount of fluorescence as the RNA sample is pulsed through a microchannel over time. The Agilent Bioanalyzer software creates a graph called an electropherogram, which diagrams fluorescence over time. Smaller molecules are pulsed through the separation channel quicker than larger ones and will therefore appear on the left side of the electropherogram. For each sample the software creates a gel image to accompany the graph.

Below are diagrams displaying High Quality RNA, Marginally degraded RNA, and Highly degraded RNA. Accompanying the electropherogram are descriptions of the above three categories to help you decipher your bioanalysis results.

High Quality RNA

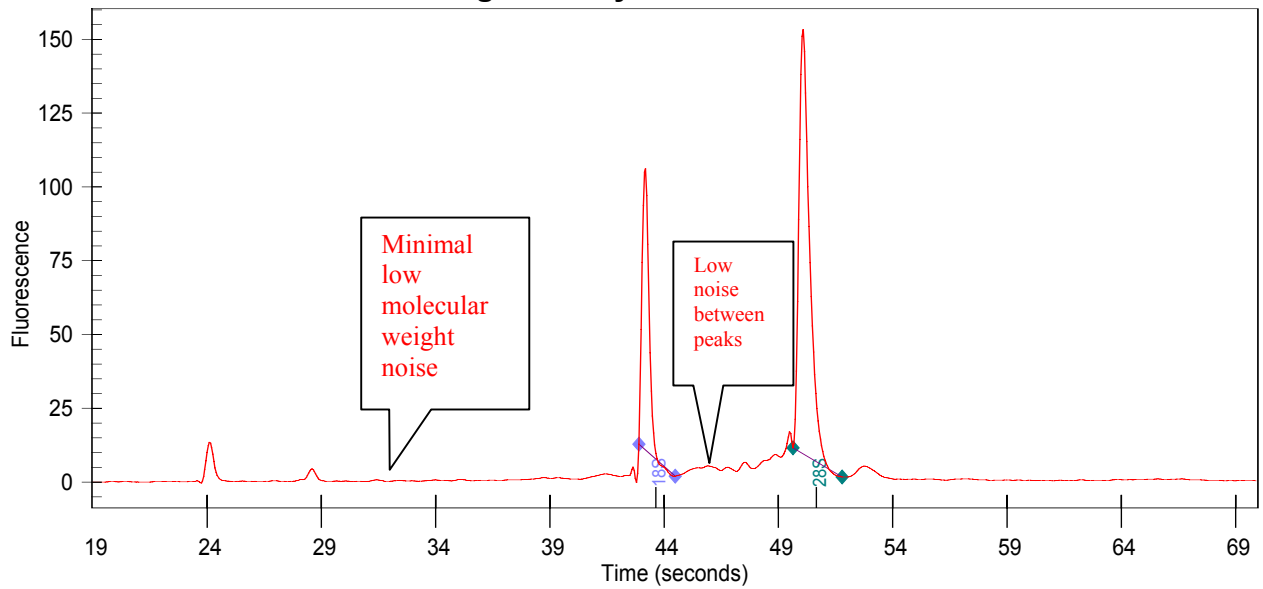
Below are examples of electropherograms from high quality RNA samples. High quality RNA electropherograms show several characteristics. First, there are clear 28S and 18S peaks. Secondly, there should be low noise between the peaks and minimal low molecular weight contamination.

High Quality RNA



RNA Area 360.81
rRNA Ratio [28S / 18S] 1.92

High Quality RNA

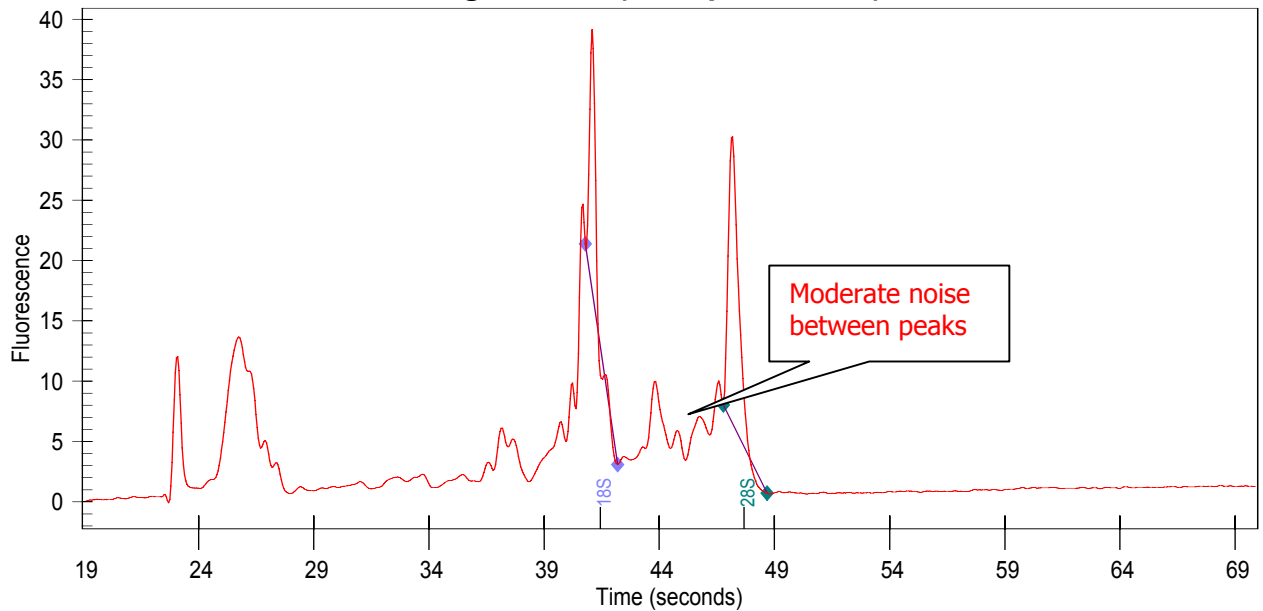


RNA Area 398.29
rRNA Ratio [28S / 18S] 1.71

Marginally Degraded RNA

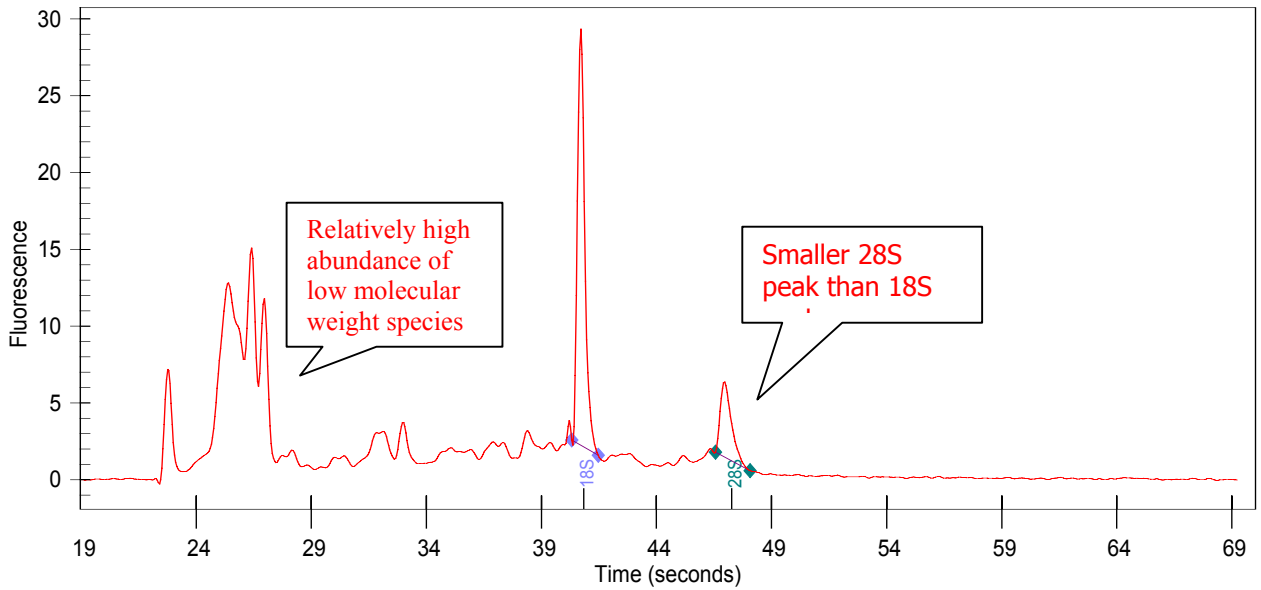
Below are examples of electropherograms from marginally degraded RNA samples. Characteristics include the presence of low molecular weight species, noise between 28S and 18S peaks, and a smaller 28S than 18S peak. These characteristics tend to interfere with the labeling and amplification resulting in reduction of yield and possibly poor array performance.

Moderate Degradation (Inter-peak noise)



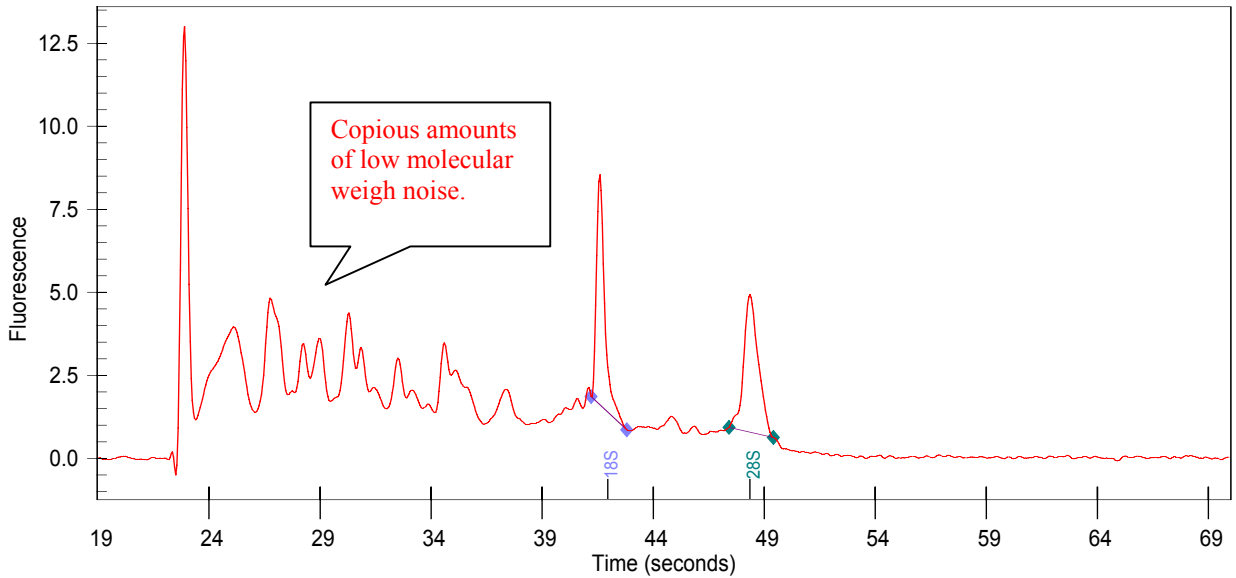
RNA Area 309.05
rRNA Ratio [28S / 18S] 1.70

Moderate Degradation (disproportionate 28S/18S ratio)



RNA Area 227.06
rRNA Ratio [28S / 18S] 0.24

Moderate Degradation (Low Weight Molecular Species)

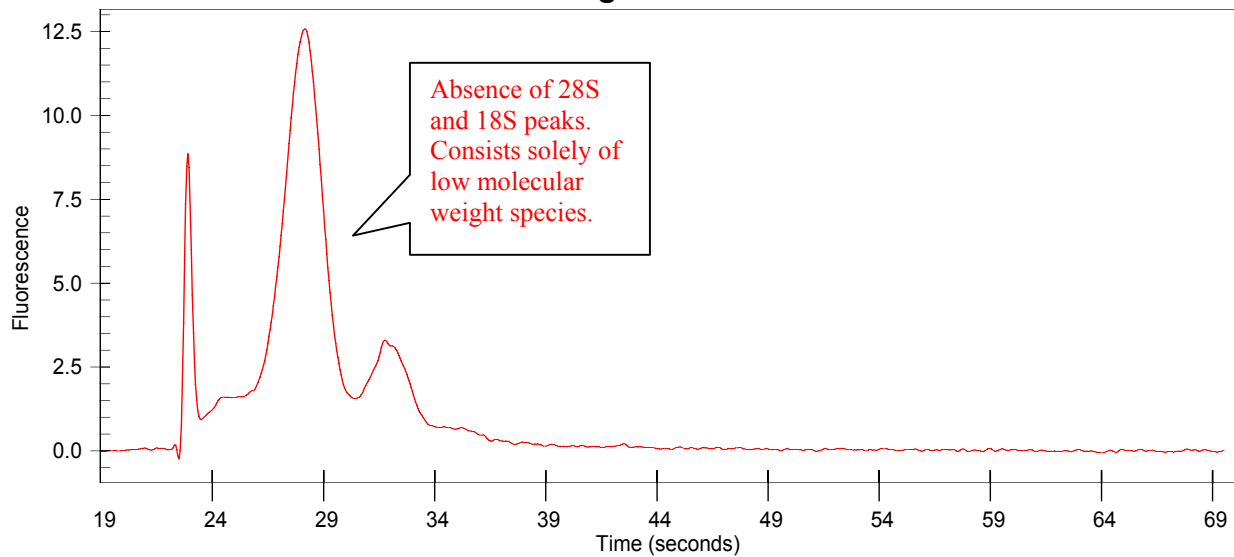


RNA Area 161.17
rRNA Ratio [28S / 18S] 0.91

Highly Degraded RNA

Below is an example of extreme degradation. The example lacks 28S and 18S peaks altogether and consists solely of low molecular weight species. This example would perform poorly in the labeling procedure. If your samples perform in a similar manner on the Bioanalyzer it would be advisable to examine your RNA extraction technique and try submitting new samples.

Extreme Degradation



RNA Area 147.22
rRNA Ratio [28S / 18S] 0.00