10 Annexes

10.1.1 Bioanalyzer

It is the standard method utilized for qualitative and quantitative analysis of RNA samples prior to analysis on microarray system. Data is translated into gel-like images (bands) and electropherograms (peaks) (Fig. Annex I and II). With the help of a ladder that contains components of known sizes, a standard curve of migration time versus fragments size is plotted. From the migration times measured for each fragment in the sample, the size is calculated. For RNA only one marker is run with each of the samples bracketing the overall sizing range. The marker is internal standards used to align the ladder data with data from the sample wells. This is necessary to compensate for drift effects that may occur during the course of a chip run.

For the microarray assay, high quality of RNA is especially important. Thus, twelve samples (six of control and six of RTX group) were analysed by a bioanalyzer.
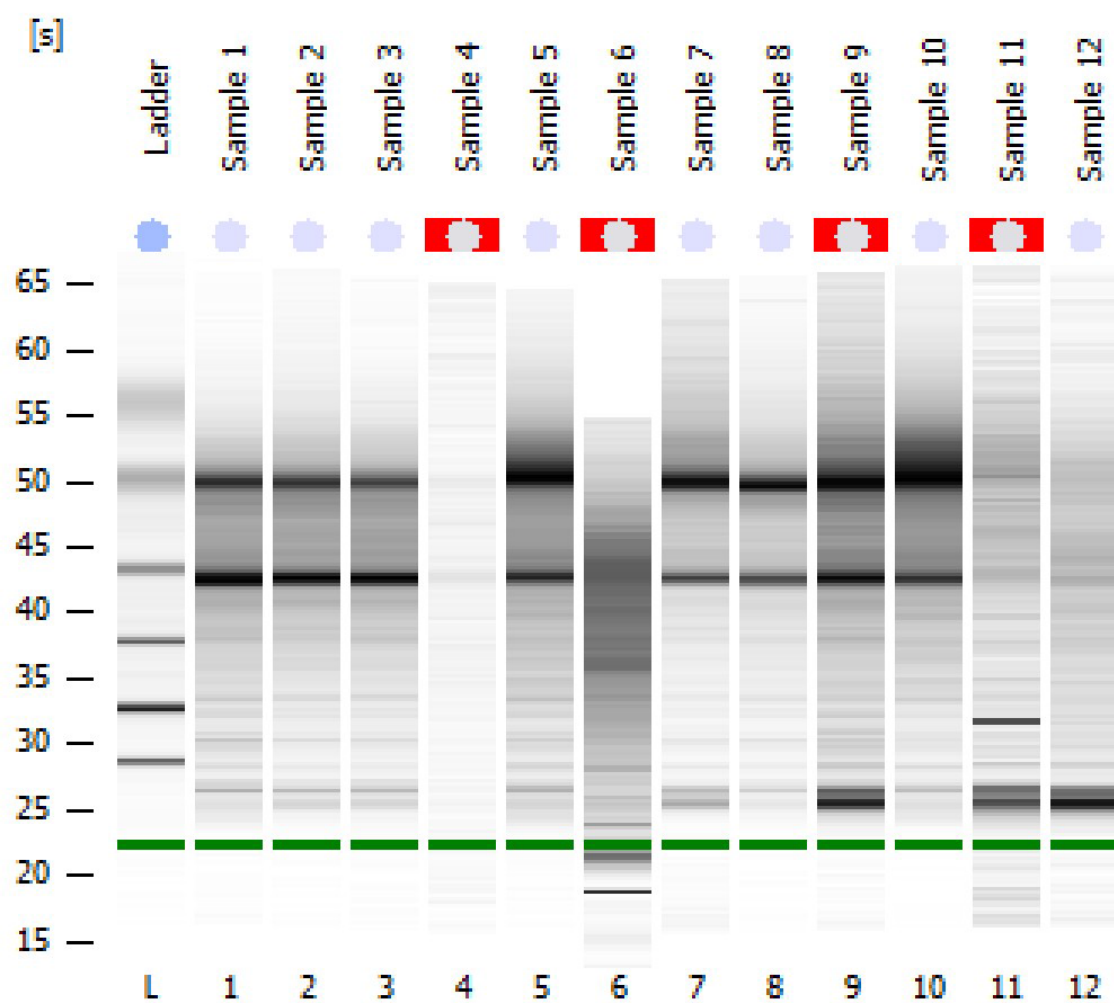
Fig. Annex I. Gel-like images. *Comparison of the samples from the ladder. It is obvious that four of them were degraded (samples 4, 6, 9 and 11).*
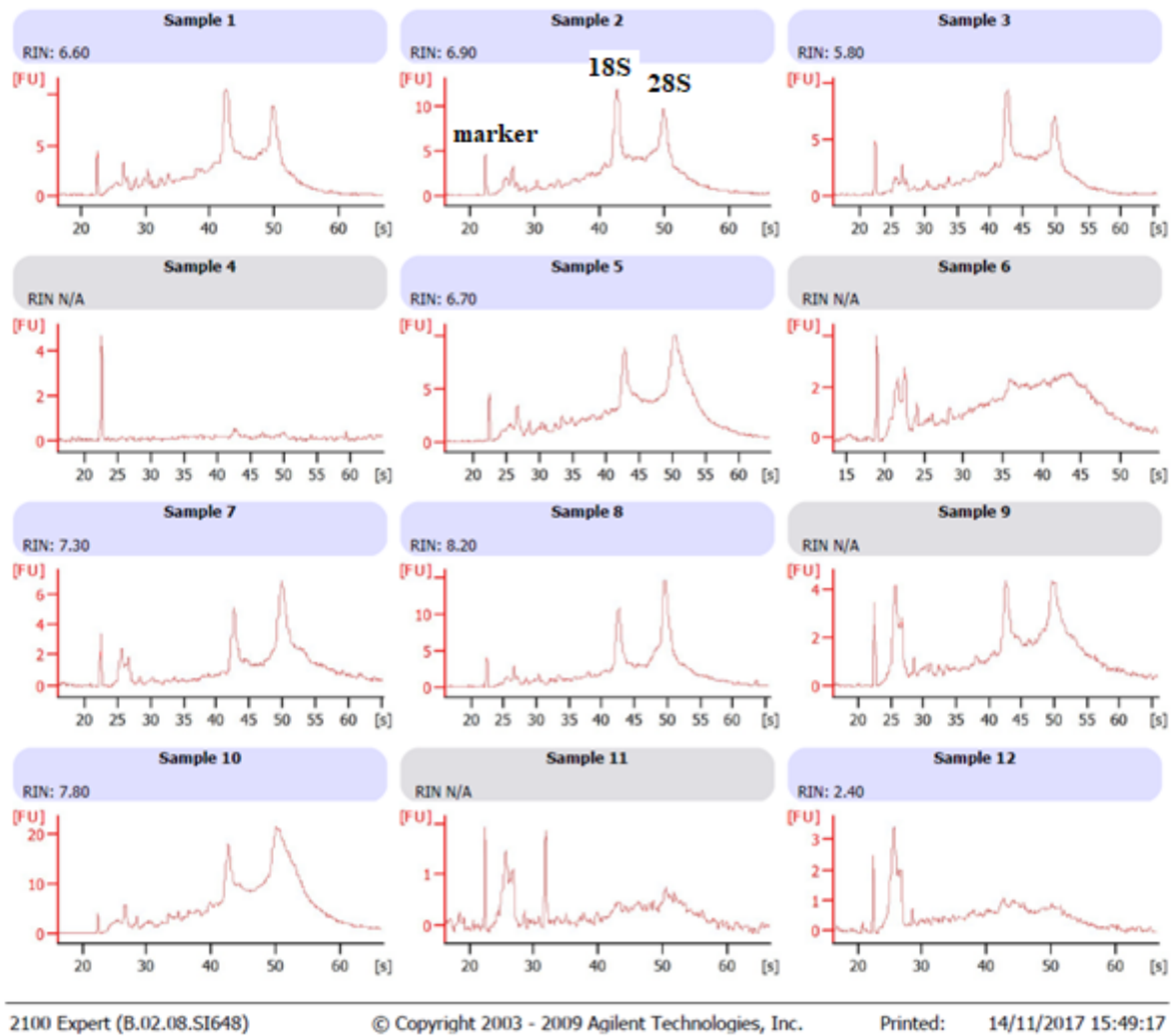
Fig. Annex II. Electropherogram. *The 2100 expert software plots fluorescence intensity versus size/migration time and produces an electropherogram for each sample. RIN is the ratio of the 28S to 18S ribosomal RNA peaks. 28S RNA degrades faster than 18S, thus if 28S RNA is degraded, RIN decreases. It is assumed that ribosomal RNA are less fragile than messenger RNA, and therefore the RIN is representative of total RNA.*