Interpretation of Bioanalyzer Traces

Genomics Research Center

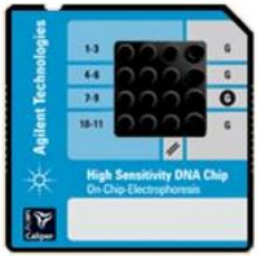
Introduction to Bioanalyzer Assays

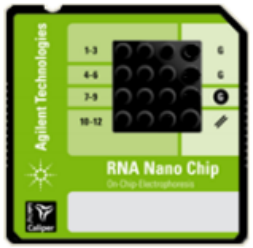
High-Resolution Automated Electrophoresis of DNA, RNA, and Protein Samples

The Agilent 2100 Bioanalyzer system is an established automated electrophoresis solution for the sample quality control of biomolecules. The system integrates an instrument, data processing software, reagents, and a microfluidic chip specific for DNA, RNA, or protein analysis. It is suitable for next-generation sequencing (NGS), gene expression, biopharmaceutical, and genome editing workflows, delivering highly precise analytical evaluation of various sample types. Advantages of microfluidics-based automated electrophoresis over traditional gel electrophoresis include dramatically reduced sample (1 µL for nucleic acids, 4 µL for proteins) and reagent consumption, significantly faster analysis time, and less hands-on activities during sample preparation and data analysis. The Agilent 2100 Bioanalyzer system is ideal for the scientist requiring a versatile instrument for nucleic acids and protein electrophoresis analysis with moderately low throughput (10-12 samples) needs.

Bioanalyzer Automated Electrophoresis for Sample Quality Control | Agilent

Available Bioanalyzer Assays

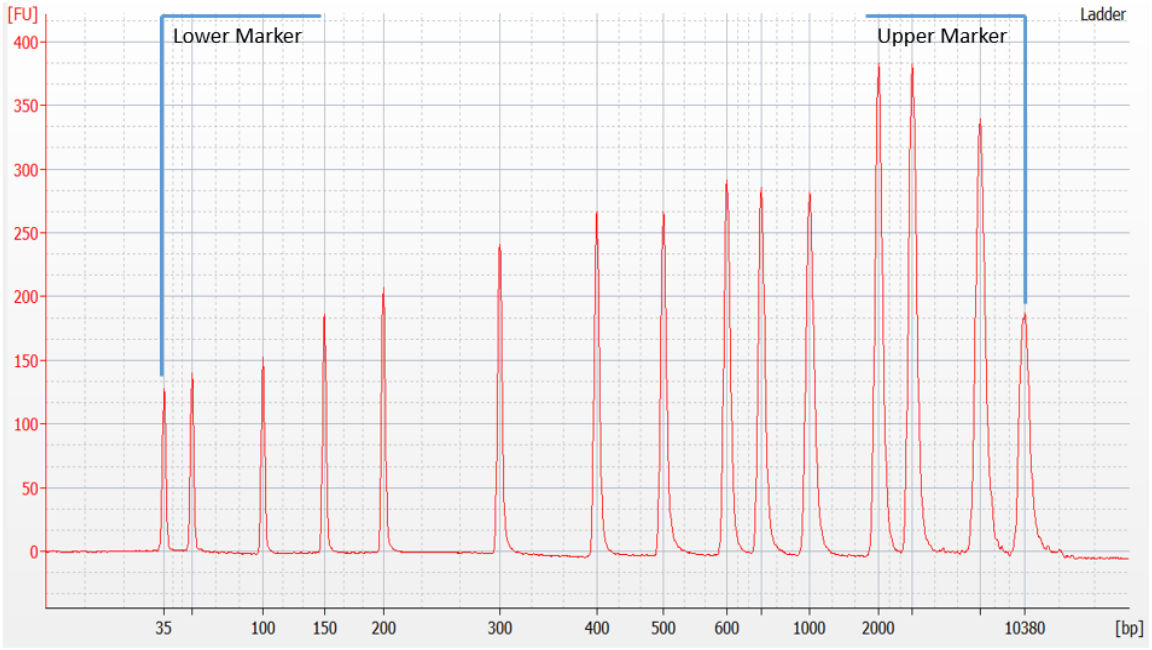




	High Sensitivity DNA	RNA Nano	RNA Pico	Small RNA
Volume	1 uL	1 uL	1 uL	1uL
Size	50 - 7,000 bp	N/A	N/A	6 - 150 nt
Concentration	5 - 1000 pg	25 - 500 ng	0.05 - 10 ng	Total RNA: 1 - 100 ng
				Small RNA: 1 - 20 ng

High Sensitivity DNA

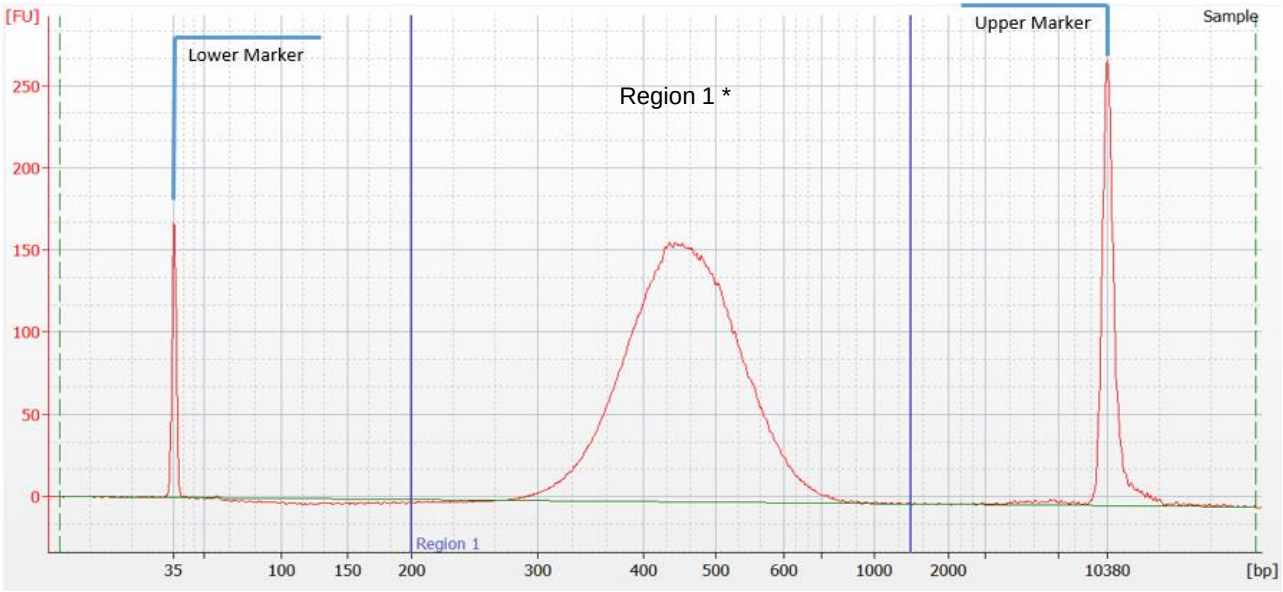
Ladder – A ladder is included with each run. The ladder contains two internal standards (Lower and Upper Markers) to align the ladder data with samples to determine sizing. The concentration of the upper marker is known and used to help determine sample concentration.



[FU] = Fluorescent Units

High Sensitivity DNA

Example Sample Trace – Lower and upper markers are included with each sample and are used to align the sample with the ladder. A region (Region 1) has been set and is indicated by the vertical blue lines on either side of the library trace. This is manually applied to each sample based on the library profile. This calculates the average size of the library within this region and is used for normalization prior to sequencing.



[FU] = Fluorescent Units

*Region 1 is often placed up to 1500 bp even if the library trace extends beyond this limit. In our experience, library fragments larger than 1500 bp do not cluster well and are not considered when determining library size for sequencing normalization.

High Sensitivity DNA

Examples of sample-related Issues that may impact Quality: Presence of peaks prior to the expected library range could be due to primer dimers (Image 1) or adapter dimers (Image 2). Further purification should be performed to remove these peaks prior to next generation sequencing. Large peaks beyond the library trace can indicate Ampure bead carryover (Image 3). Library should be placed on magnet to remove residual beads.

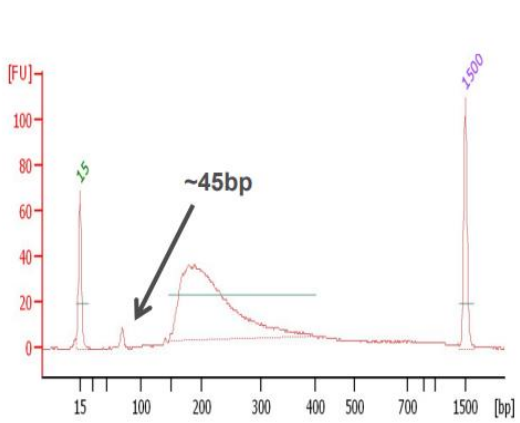


Image 1: Primer Dimer Presence

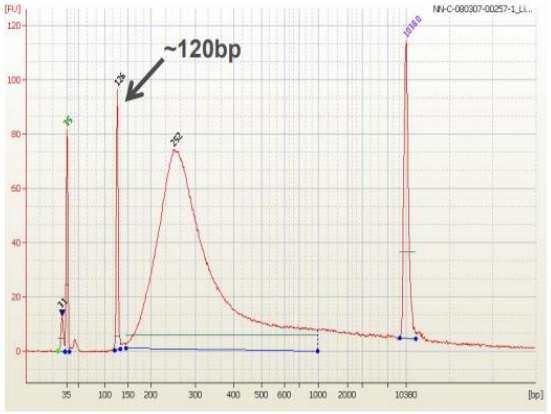


Image 2: Adapter Dimer Presence

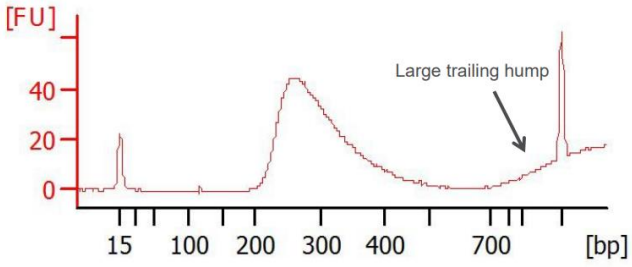


Image 3: Ampure Bead Presence

High Sensitivity DNA

Examples of Mechanical issues: The presence of an electrical spike (Image 4) or artifact peaks (Image 5) may be caused by the instrument and/or assay buffers, and are not necessarily cause for repeating the QC assay. We address these on a case by case basis to determine if the issue is influencing the instruments ability to call QC parameters.

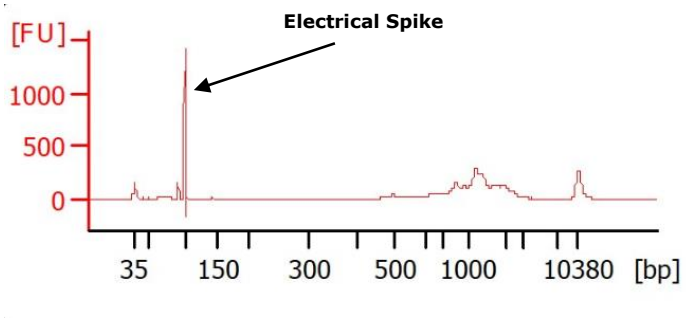


Image 4: Electrical Spike

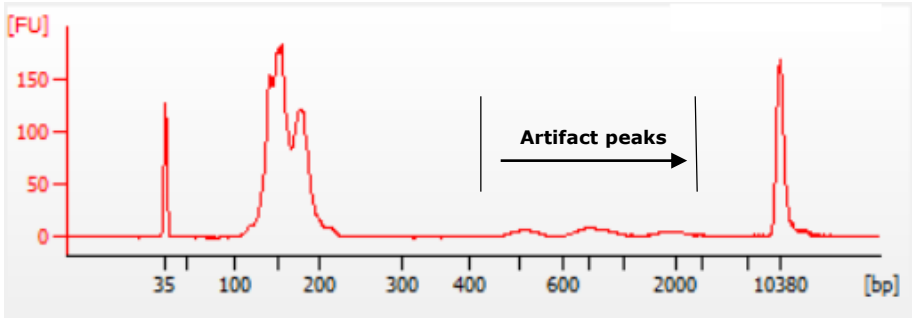
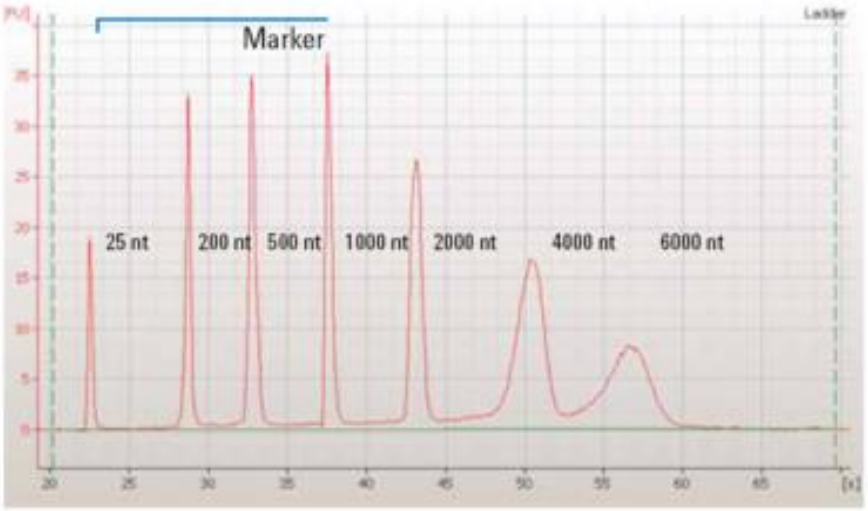


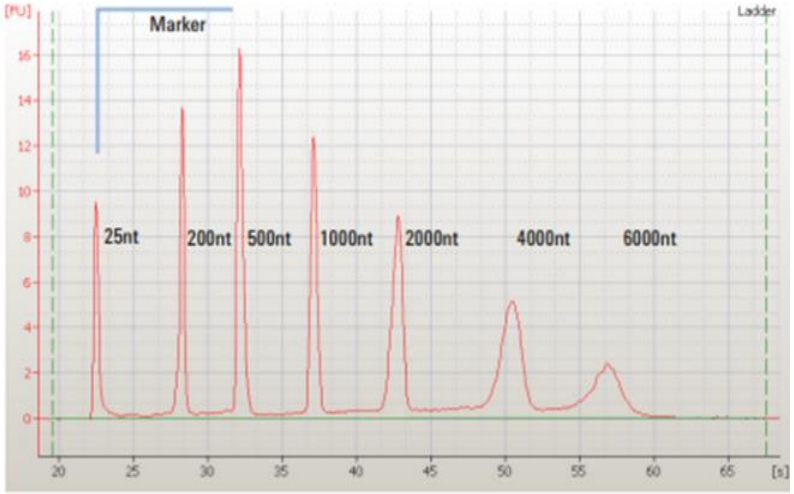
Image 5: Artifact Peaks

RNA

Ladder – A ladder is included with each run. Below are images of an RNA Nano and RNA Pico Ladder which are a mixture of RNA of known concentrations. The ladder also includes a lower marker used to align the sample with the ladder to determine size and concentration.



RNA Nano Ladder (Concentration: 150 ng/uL)

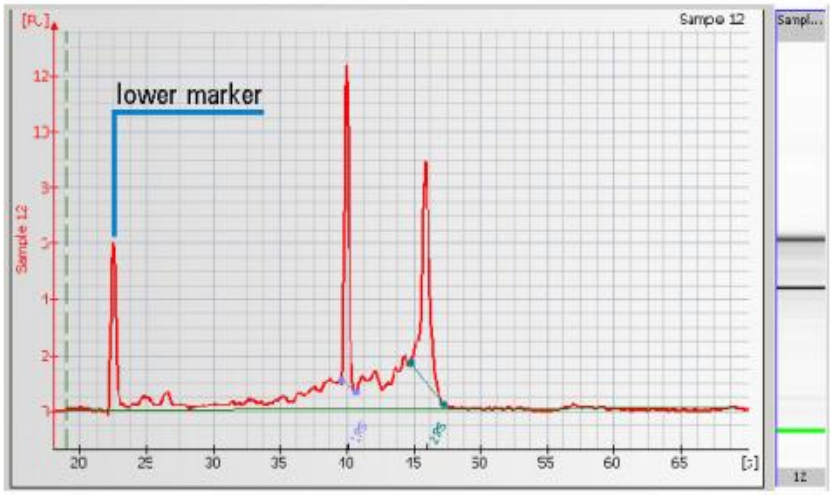


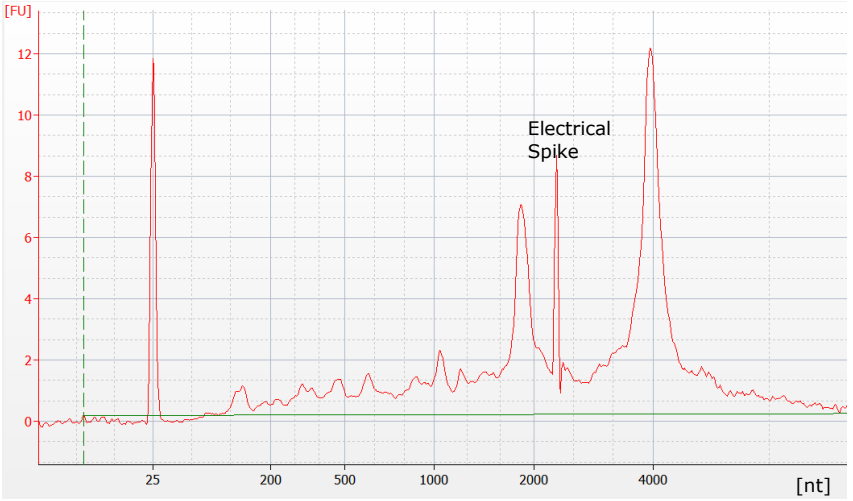
RNA Pico Ladder (Concentration: 1000 pg/uL)

RNA

Example sample trace from either a Nano or Pico bioanalyzer chip. Both assays incorporate a lower marker with each sample. Quantification and sizing are determined based on the ladder that is included in each run.

There are other factors that may impact the baseline and therefore impact concentration such as contaminants. Baseline impacts such as electrical spikes do not impact quantification/quality metrics.

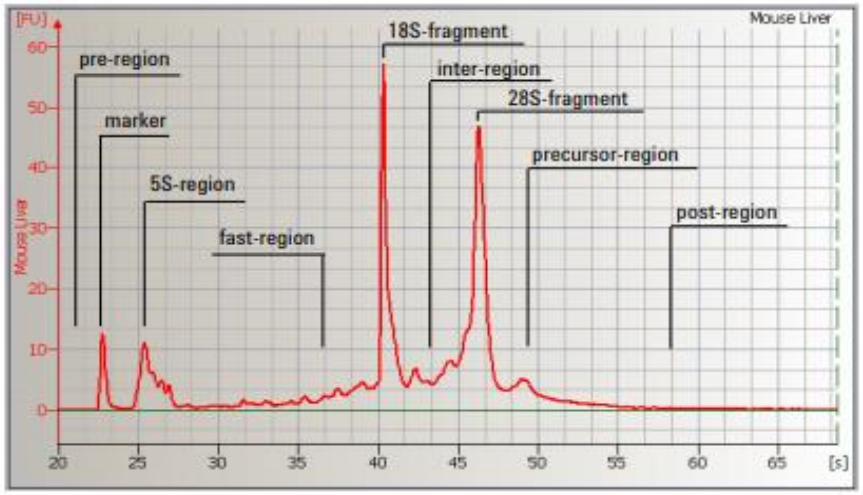




[FU] = Fluorescent Units

RNA

Both RNA Nano and RNA Pico assays provide a RIN or RNA Integrity Number. The RIN indicates the integrity of the total RNA and is based on a scale from 1 to 10 with 1 being degraded RNA and 10 being intact RNA. To calculate RIN, the entire electropherogram is analyzed with an algorithm that assesses the following regions:



Manually Adapted RIN Values - if there are anomalies that impact the accurate calling of RIN values (such as unexpected signals in 5S region, among other factors), adjustments to the anomaly thresholds allow us to manually adapt RIN values for some samples. Agilent does not guarantee the accuracy of this RIN and recommends performing a visual inspection of the data.

RNA

Lower RIN values can indicate degradation within the sample. Below are examples of varying RIN values and the corresponding traces.

Factors that can impact RNA quality:

- Improper handling during extraction
- Improper RNA storage temperature
- Contaminants

Nanodrop used to help determine contaminant

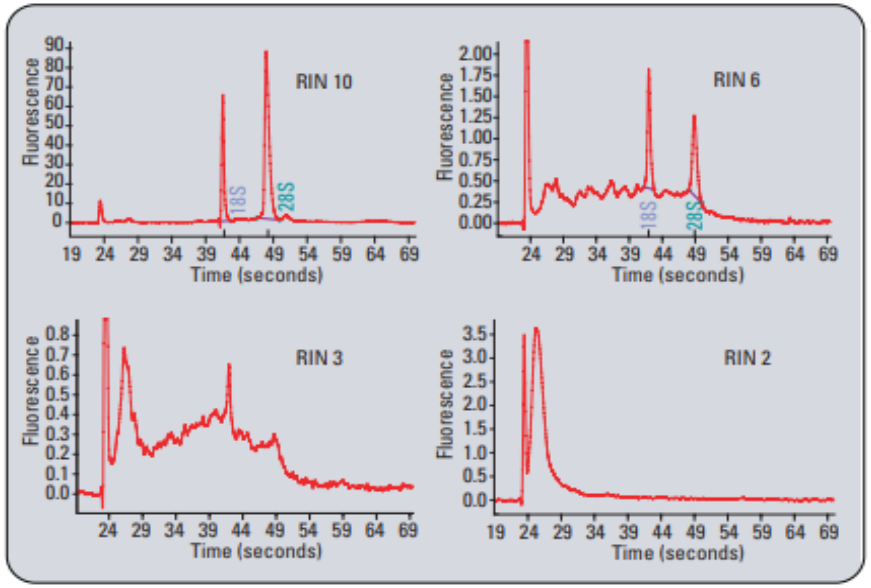
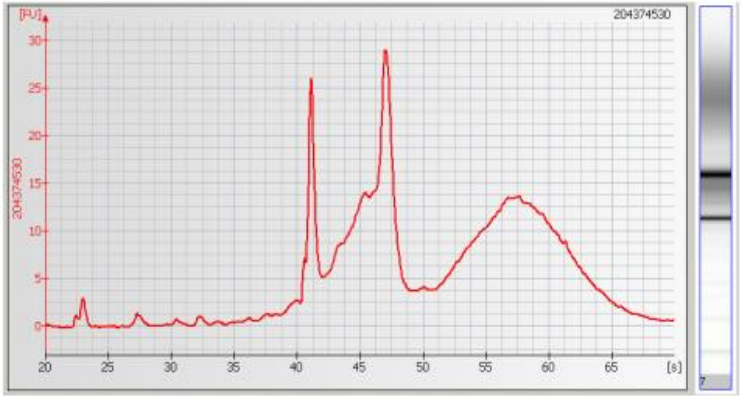


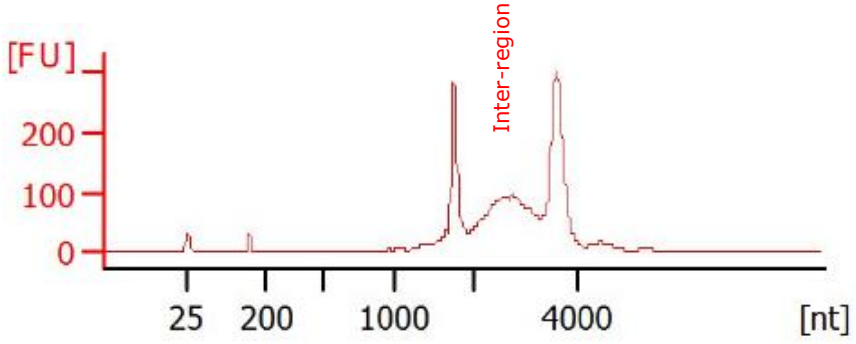
Figure 2
Sample electropherograms used to train the RNA Integrity Number (RIN) software. Samples range from intact (RIN 10), to degraded (RIN 2).

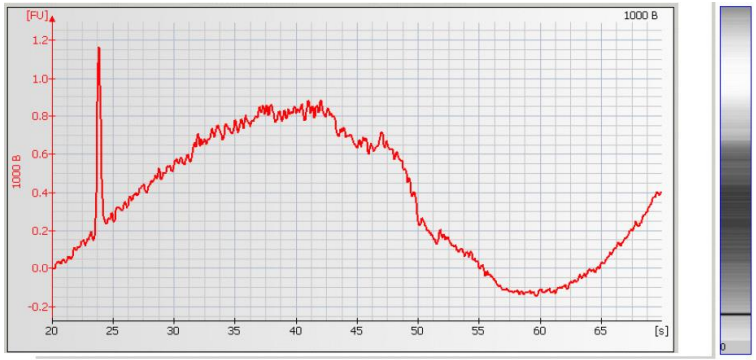
RIN Bioanalyzer Technical Note

RNA

Genomic DNA Contamination – If there are unexpected peaks past the ribosomal peaks or an increase in the inter-region, it is possible your RNA samples are contaminated with genomic DNA. DNase treatment is required before further processing.





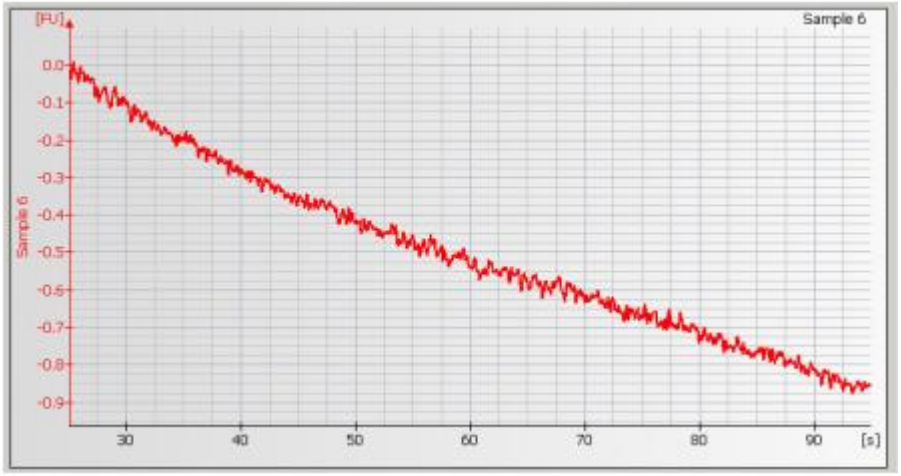


Secondary RNA Structures: Nanodrop is necessary to obtain concentration, 260/280, and 260/230 ratios to help confirm presence of gDNA instead of secondary RNA structures.

RNA

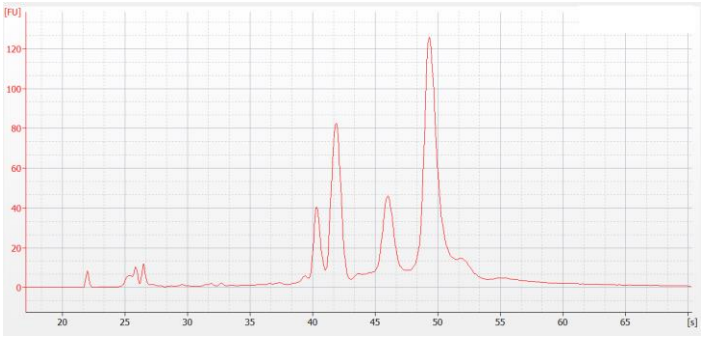
High Salt concentration: The table below shows maximum recommended buffer concentrations, and the image shows an example of the effect high salt concentrations can have on a Bioanalyzer trace.

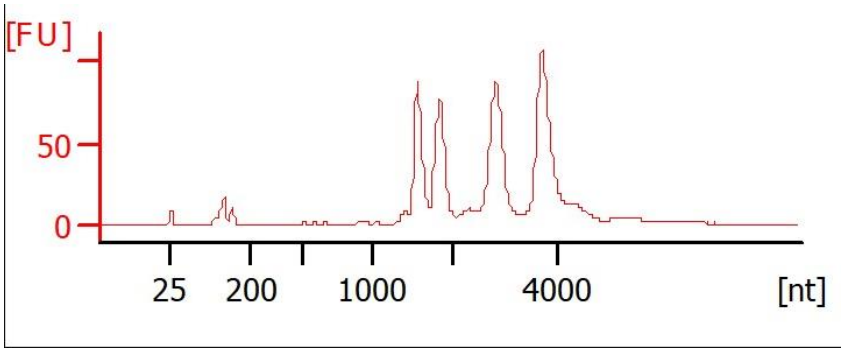
RNA Pico	RNA Nano	Small RNA
50 mM Tris	100 mM Tris	10 mM Tris
15 mM MgCl2	15 mM MgCl2	
0.1 mM EDTA	1.0 mM EDTA	0.1 mM EDTA
50 mM NaCl	125 mM NaCl	



RNA

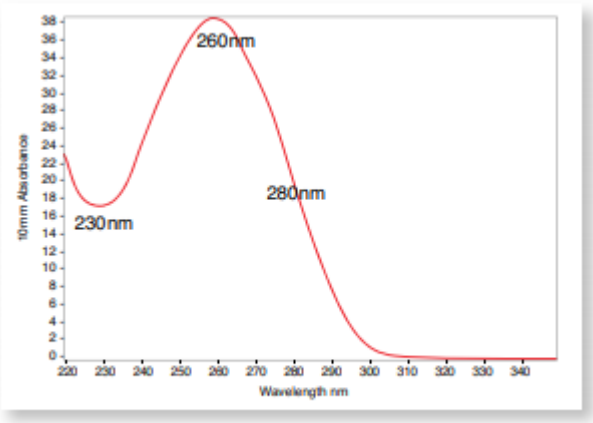
Prokaryotic Contamination: The presence of 16S and 23S peaks within an RNA profile is an indicator of prokaryotic contamination. We recommend assessing the workflow to identify potential sources of contamination.





Nanodrop can be used to assess purity

When an absorption spectrum is measured, nucleic acids absorb light with a characteristic peak at 260 nm. Additional measurements at 230 nm (detection of organic substances) and 280 nm (detection of proteins and phenols) will yield a more accurate estimation with respect to sample quality. The ratios of the absorbance values at the wavelengths 260 nm / 280 nm and 260 nm / 230 nm, respectively, provide a clear picture of the purity of a nucleic acid sample



Typical Nucleic Acid Spectrum

- A 260/280 ratio of ~ 1.8 is generally accepted as "pure" for DNA.
- A 260/280 ratio of ~ 2.0 is generally accepted as "pure" for RNA.
- The 260/230 values for a "pure" nucleic acid are often higher than the respective 260/280 values and are commonly in the range of 1.8 – 2.2.

Nanodrop Technical Note

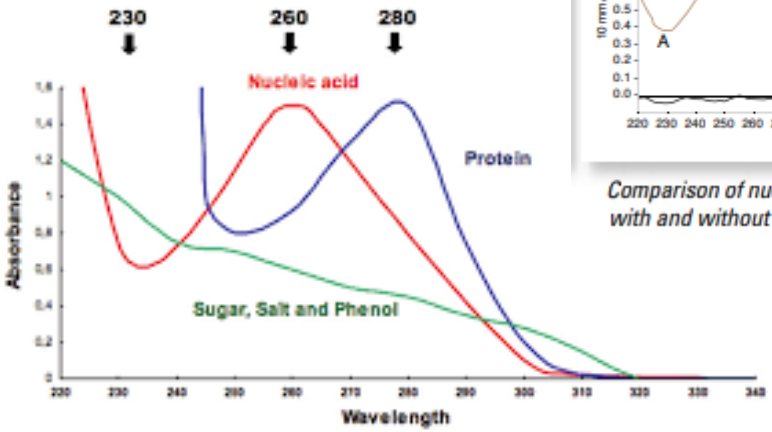
Nanodrop can be used to assess purity

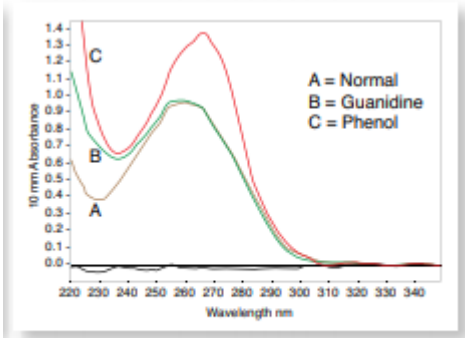
A low A260/A230 ratio may be the result of:

- Carbohydrate carryover (often a problem with plants).
- Residual phenol from nucleic acid extraction.
- Residual guanidine (often used in column based kits).
- Glycogen used for precipitation

A low A260/A280 ratio may be caused by:

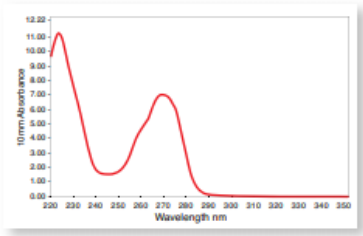
- Residual phenol or other reagent associated with the extraction protocol
- A very low concentration (>10 ng/µL) of nucleic acid



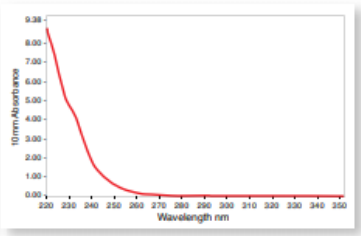


Comparison of nucleic acid sample spectra with and without 2 common contaminants

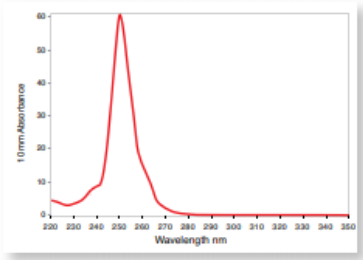
Below are several examples of reagents commonly used with nucleic acids that have absorbance in the 220 – 240 nm range. Note: Phenol also exhibits significant absorbance between 260 – 270 nm which may shift the peak and result in an overestimation of the nucleic acid concentration.



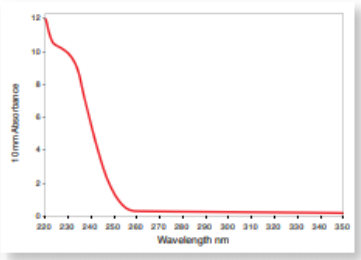
Phenol



EDTA



Guanidine Isothiocyanate



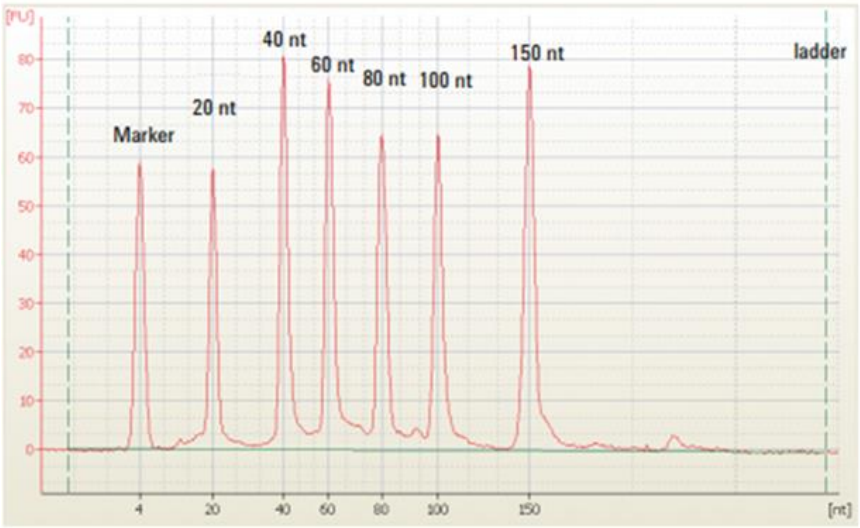
Guanidine HCl

Nanodrop Technical Note

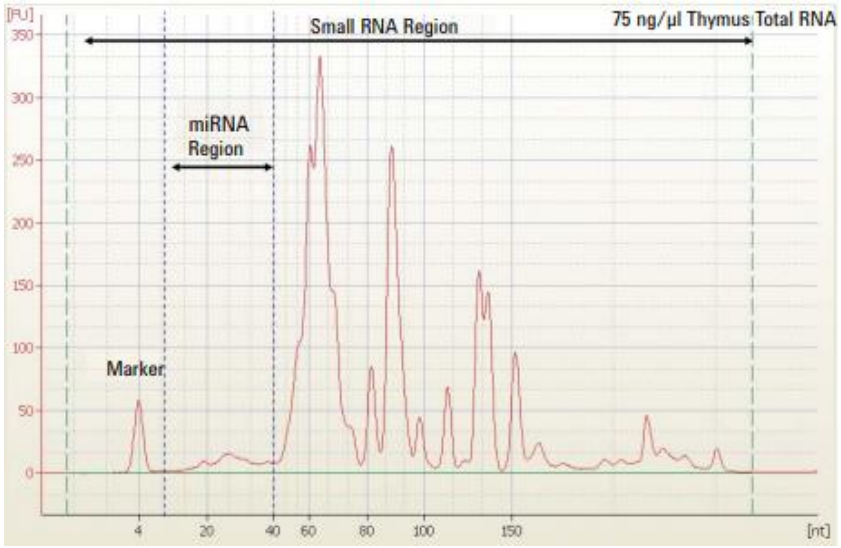
Small RNA

Small RNA – A ladder is included with each run. Below is an image of the Small RNA ladder. A lower marker is included to align the ladder with sample data.

Example Sample Trace - A lower marker is included to align the sample with the ladder data. The Small RNA Region extends from 0 to 150 nt while the micro RNA (miRNA) Region is from 10-40 nt.



Small RNA Ladder



Contact Us

Genomics Research Center
Wilmot Cancer Center
601 Elmwood Ave., Box 704
Rochester, NY 14642

Phone: (585) 275-7250

Website

Email: URGenomics@urmc.Rochester.edu

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