Interpretation of Fragment Analyzer Traces

Genomics Research Center

Introduction To Fragment Analyzer Assays

Accurate, efficient, and reliable QC analysis of nucleic acids

The Fragment Analyzer systems utilize automated parallel capillary electrophoresis to provide reliable quality control (QC) for nucleic acids. With its unique design and intuitive features, common QC bottlenecks are resolved by the automation of key steps such as gel loading and sample injection increasing lab efficiency. A broad range of kits are available allowing you to easily qualify and quantify DNA and RNA samples. Choose from three models with varying throughputs to match your labs needs.

Agilent Fragment Analyzer Instrumentation

Fragment Analyzer Systems

5300 Fragment Analyzer System

The 5300 Fragment Analyzer system is a capillary electrophoresis instrument that can separate up to 48 or 96 samples in parallel. This system is designed to improve lab efficiency and workflow allowing you to automate the analysis of large numbers of samples and focus on results.

The 5300 Fragment Analyzer system performs DNA QC and RNA QC for a broad range of samples including, gDNA, small RNA, cfDNA, large DNA fragments, and total RNA. The diversity of sample types these systems can separate make these instruments ideal for individualized workflows, including NGS library QC and CRISPR workflows.

Fragment Analyzer Assays

	RNA	High Sensitivity Genomic DNA	Standard Sensitivity Genomic DNA	Next Gen Sequencing Library
Volume	2 uL	2 uL	1 uL	2 uL
Size Range	200 - 6,000 nt	75 - 60,000 bp	75 - 60,000 bp	100 - 6,000 bp
Concentration	5 - 500 ng/uL	0.3 - 12 ng/uL	25 - 250 ng/uL	Fragment: 0.1 - 10 ng/uL Smear: 5 - 100 ng/uL

Fragment Analyzer Application Note

NGS Library Kit

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.

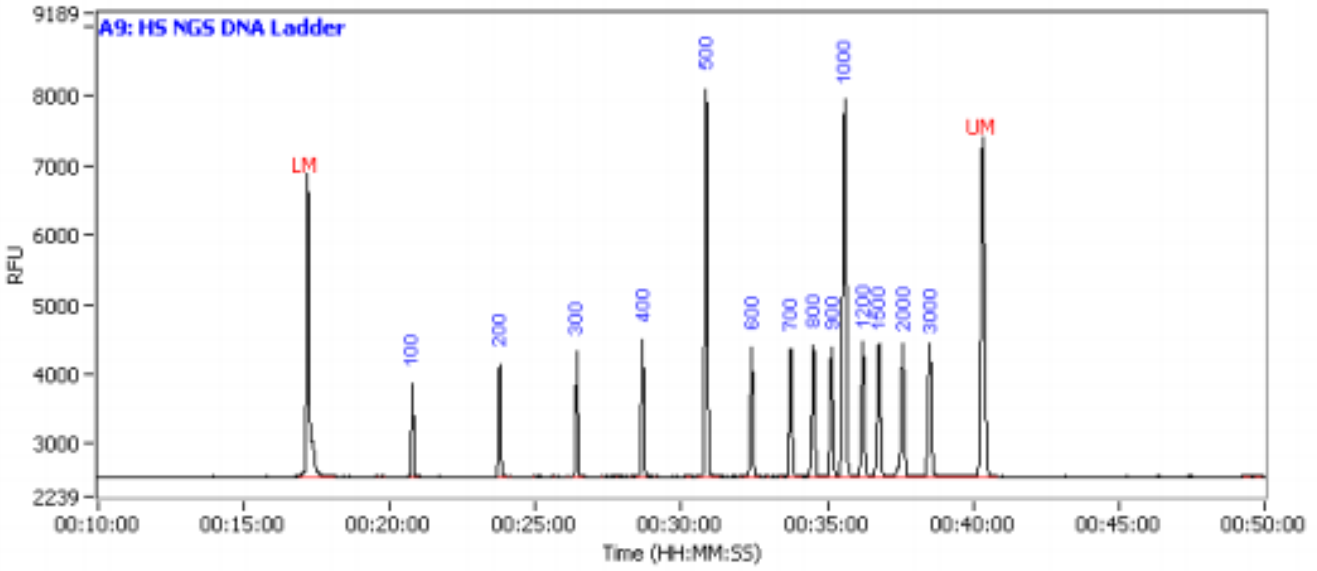
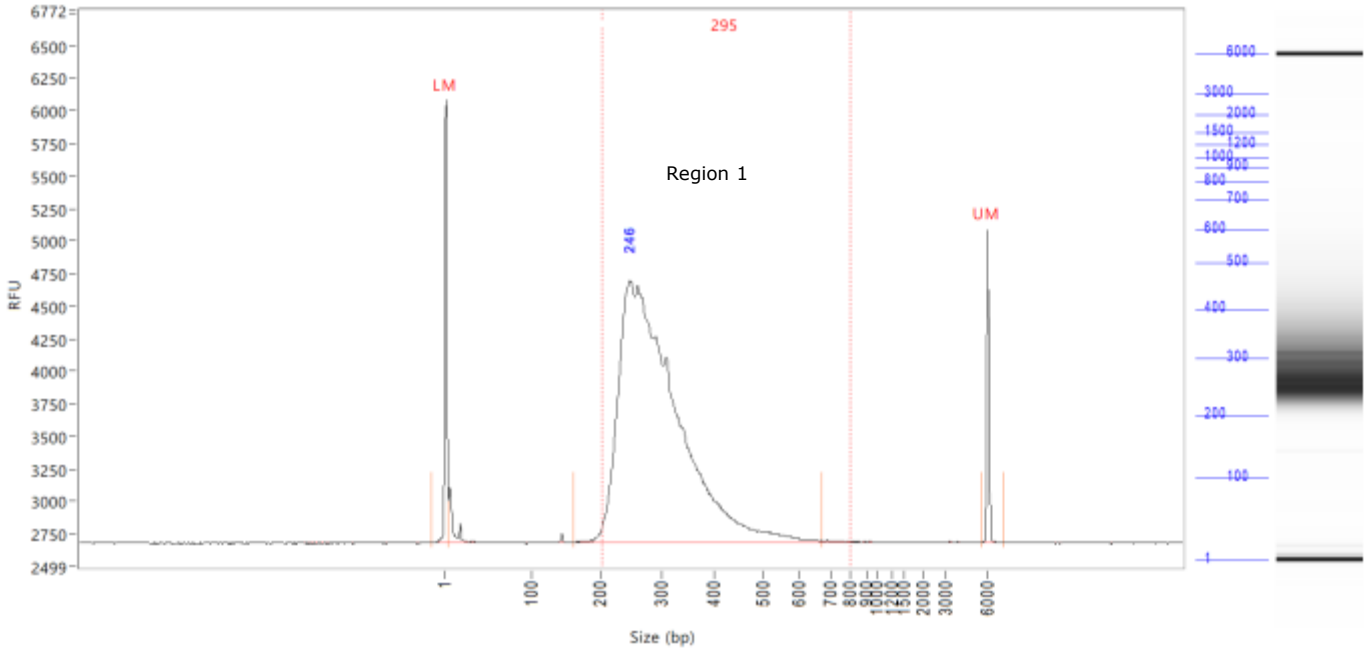


Figure 9. Representative NGS DNA Ladder result using the Fragment Analyzer system with the DNF-474 HS NGS Fragment kit (1bp – 6,000bp). Method: **DNF-474-33** (short array). Peaks annotated by size (bp).

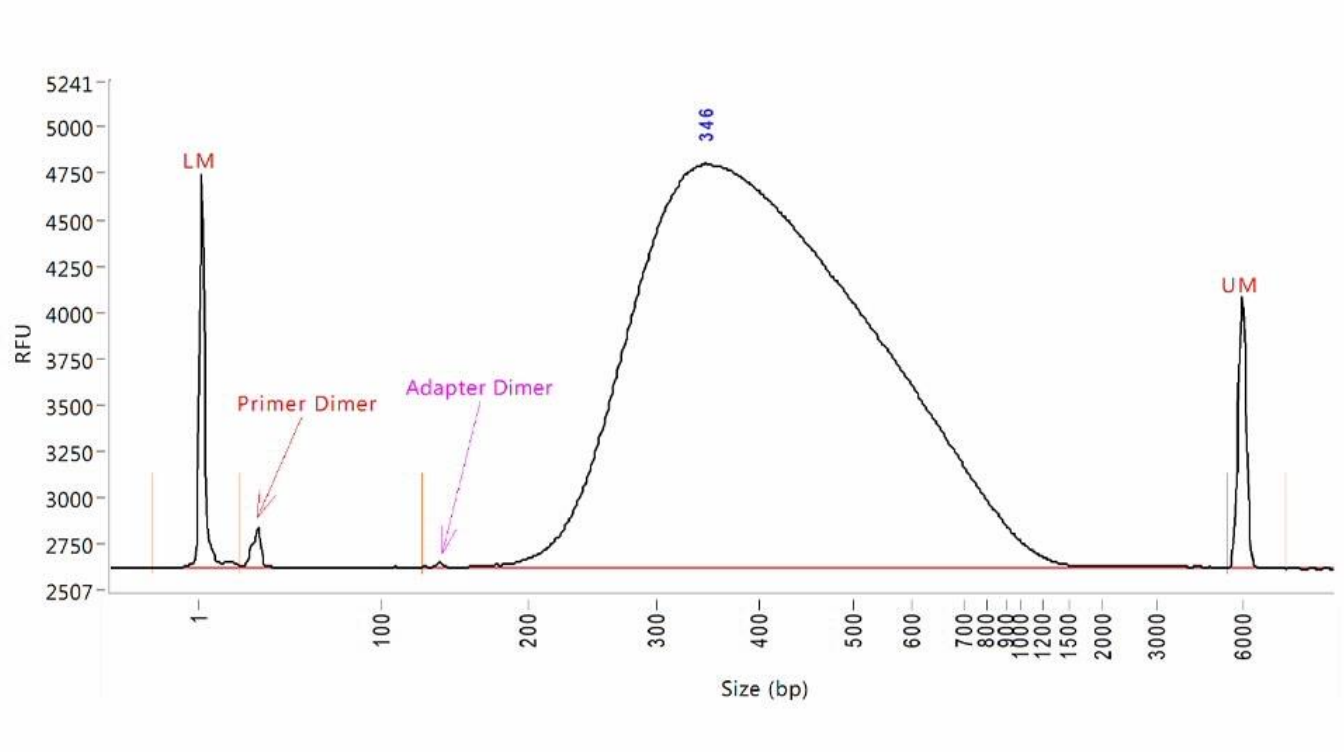
NGS Library Kit

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 red dotted 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.



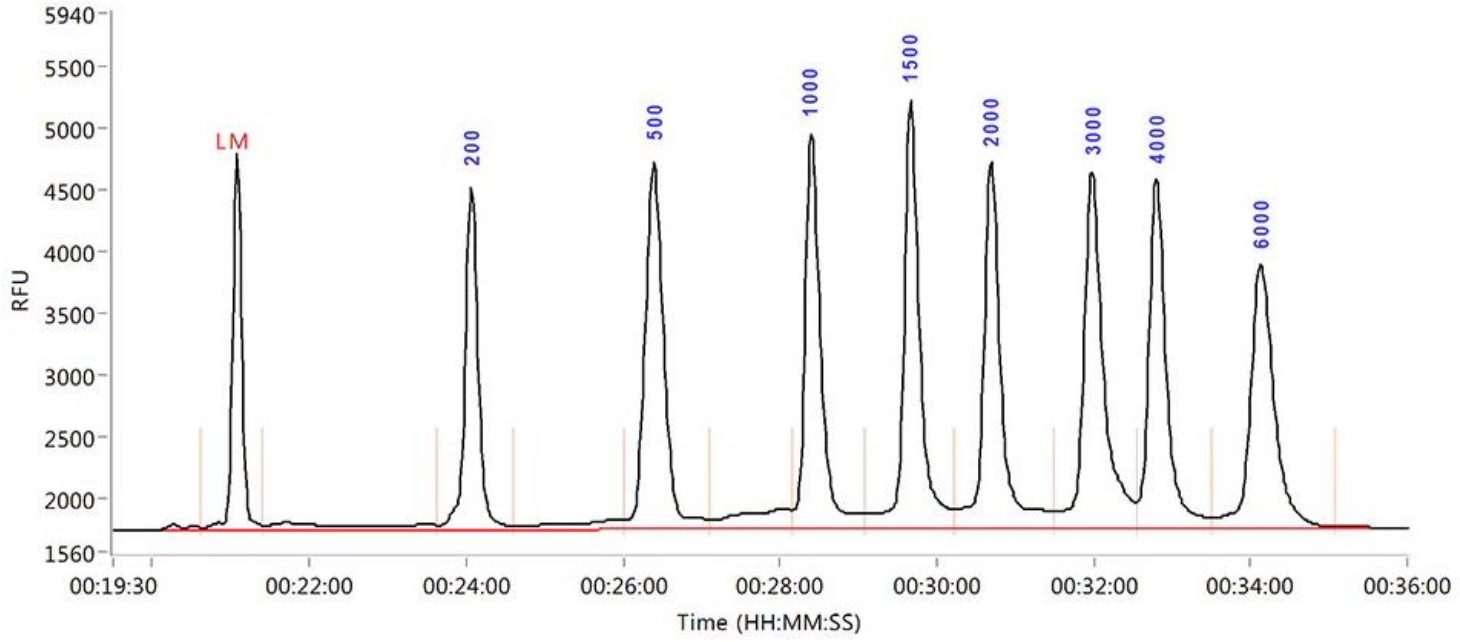
NGS Library Kit

Presence of peaks outside the expected library range could be due to primer dimers or adapter dimers. Further purification should be performed to remove these peaks prior to next generation sequencing.



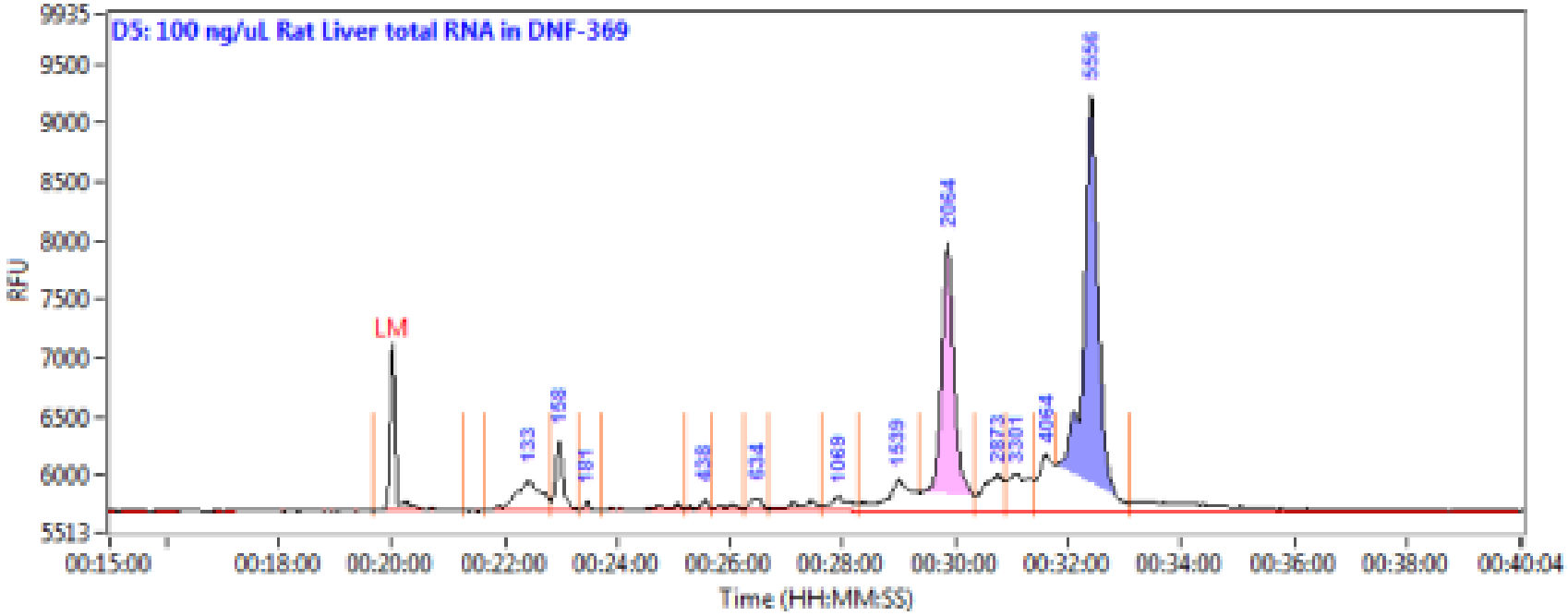
RNA

Ladder – A ladder is included with each run. The ladder also includes a lower marker used to align the sample with the ladder to determine size and concentration.



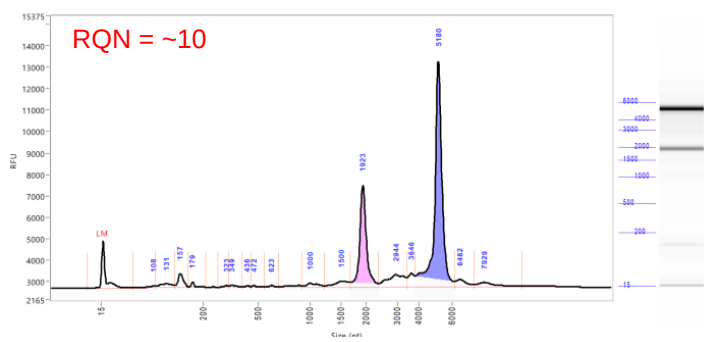
RNA

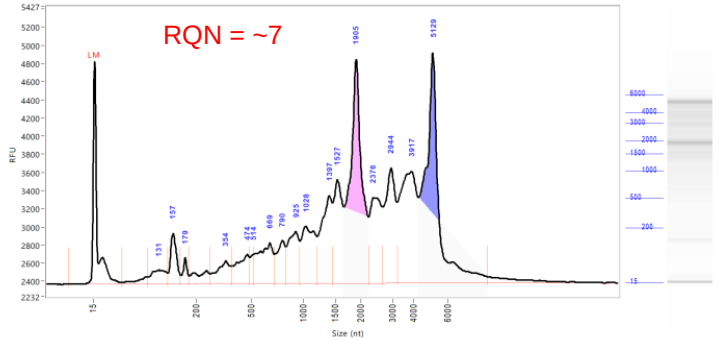
Example Sample Trace - A lower marker (LM) is included with each sample and is used to align the sample with the ladder. Quantification and sizing are determined based on the ladder that is included in each run.

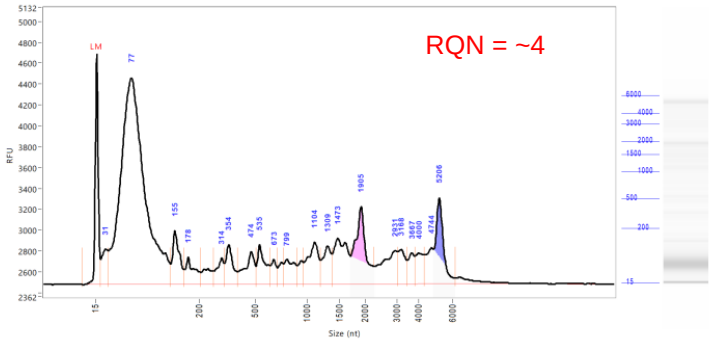


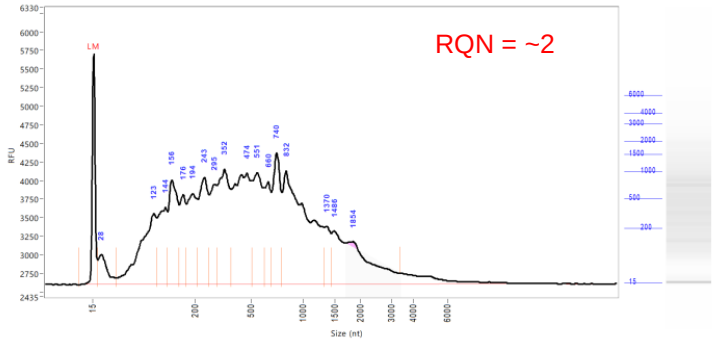
RNA

Fragment Analyzer RNA includes RQN or RNA Quality Number. This is comparable to the RIN or RNA Integrity Number given by the Bioanalyzer. The RQN number is calculated by analyzing the entire electropherogram in addition to the ratio of the ribosomal peaks. It is based on a scale of 1 to 10, with 1 representing degraded RNA and 10 representing intact RNA. Below are examples of different RQN values and their respective traces.



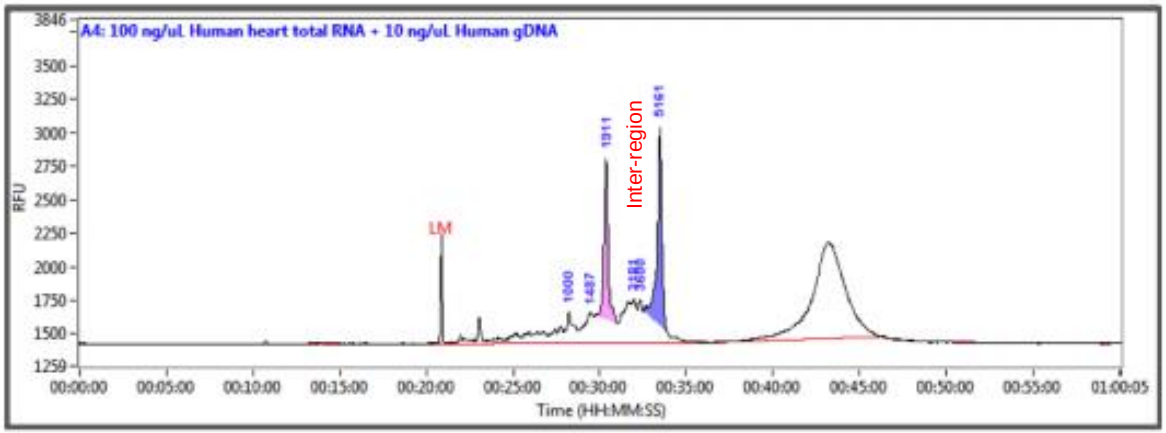


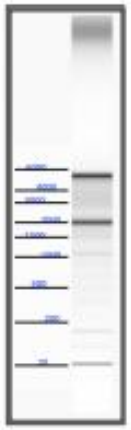




RNA

Genomic DNA Contamination – If there are unexpected large peaks or an increase in the region between the two ribosomal units (inter-region), it is possible your samples are contaminated with genomic DNA*. DNase treatment is required before further processing.



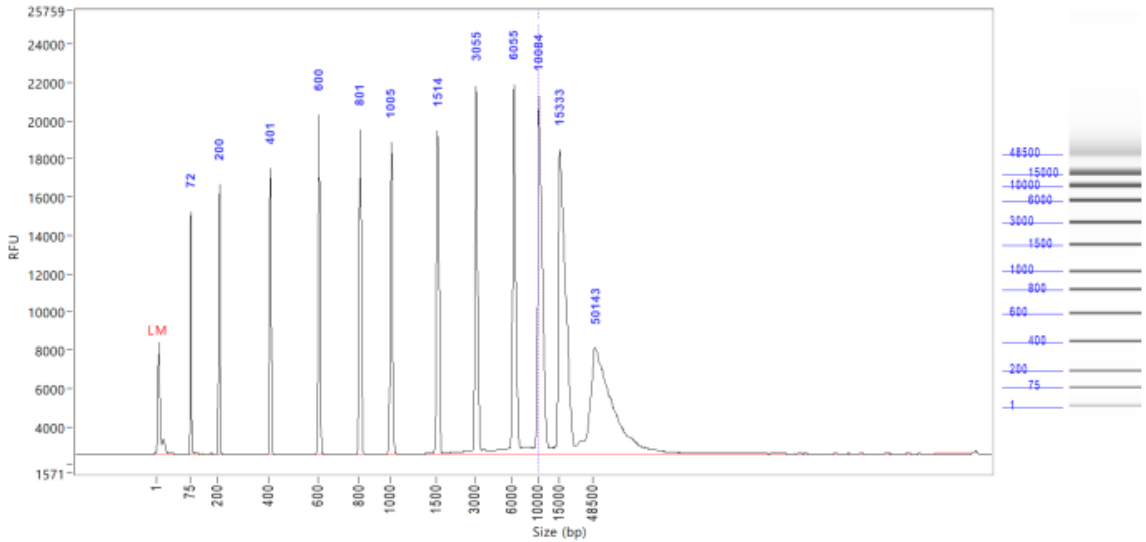


Human heart total RNA electropherograms measured by the Fragment Analyzer™. Top panel shows RNA sample without gDNA; bottom panel shows gDNA contamination in the peak to far right. Blue peak corresponds to the 28S region; red peak corresponds to 18S region.

* Nanodrop is necessary to obtain concentration, 260/280, and 260/230 ratios to help confirm presence of gDNA.

Genomic DNA

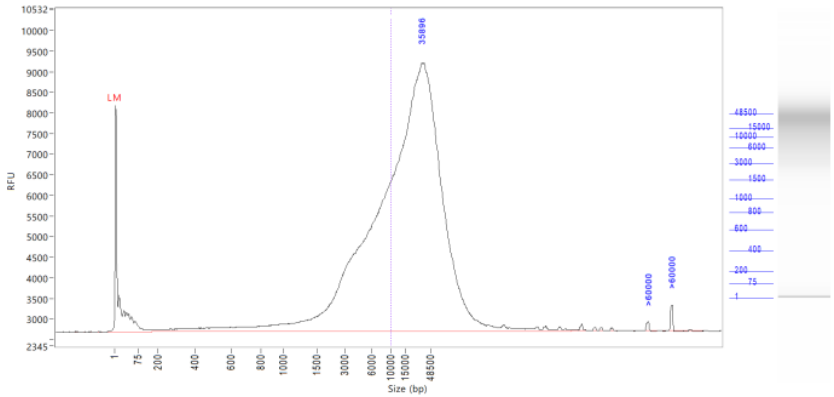
Ladder – A ladder is included with each run. The ladder contains a lower marker (LM) to align the ladder data with samples to determine sizing, concentration, and GQN.



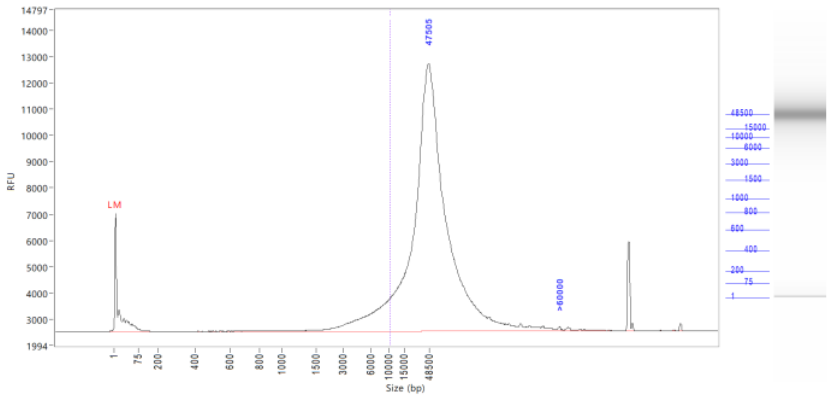
Genomic DNA

Sample Trace – Below is an example of a genomic DNA sample. A Lower Marker (LM) is included with each sample to align to the ladder data.

GQN: Genomic Quality Number is calculated as a percentage of total sample that lies above a set size threshold (the Fragment Analyzer software automatically places a threshold at 10,000). The appropriate threshold can vary depending on organism. GQN values range from 0 to 10 with 10 being 100% of the trace is above the specified threshold.



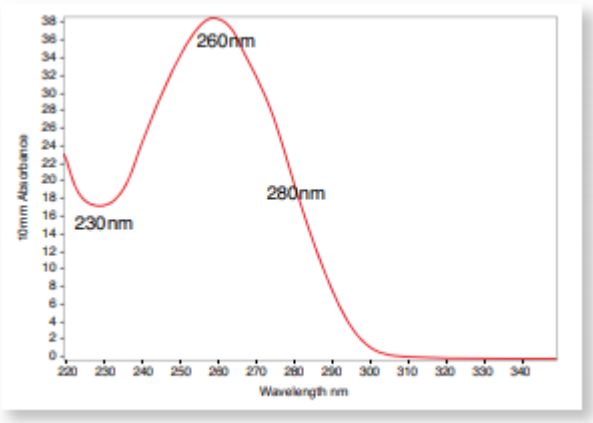
GQN: 6.1



GQN: 8.8

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

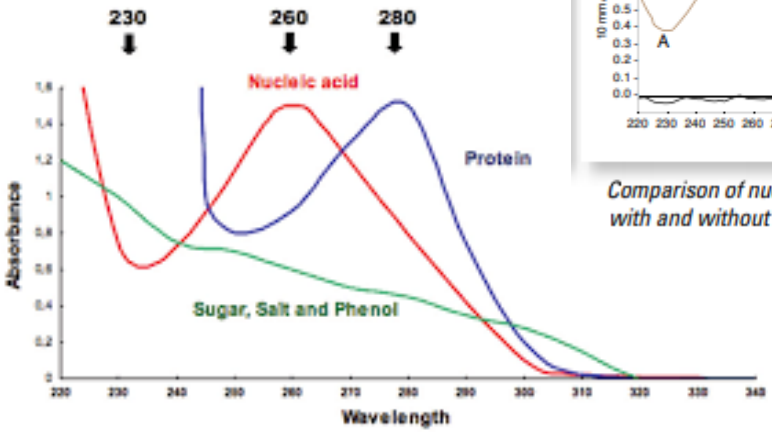
Nanodrop can be used to assess purity

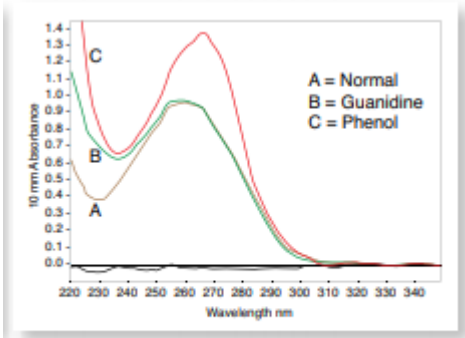
A low A260/A230 ratio may be the result of:

- 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:

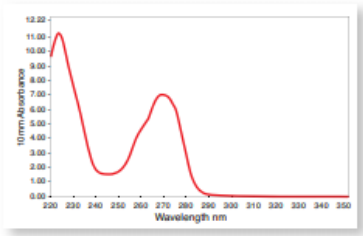
- Residual phenol or other reagent associated with the extraction protocol
- A very low concentration (>10 ng/µL) of nucleic acid



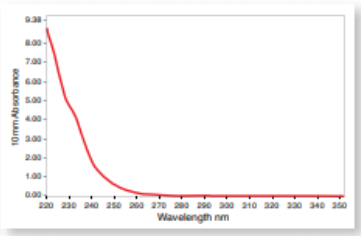


Comparison of nucleic acid sample spectra with and without 2 common contaminants

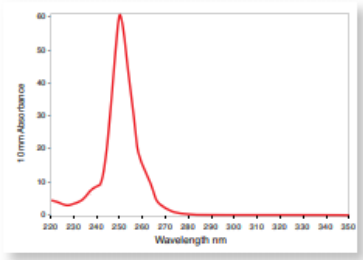
Below are several examples of reagents commonly used with nucleic acids that have absorbance in the 220 – 240 nm range. Note: Phenol also exhibits significant absorbance between 260 – 270 nm which may shift the peak and result in an overestimation of the nucleic acid concentration.



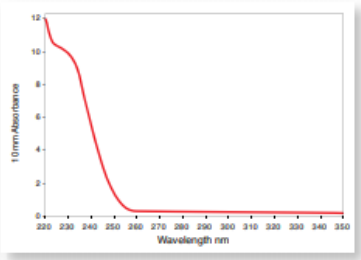
Phenol



EDTA



Guanidine Isothiocyanate



Guanidine HCl

Nanodrop Technical Note

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