Ramaciotti Centre for Genomics

UNSW AUSTRALIA

Nucleic Acid QC – Technical Note

Nucleic Acid Quality Control

The ability to measure nucleic acid concentration, purity, and integrity is critical, as it is the starting point of every molecular biology experiment. This document reviews the most commonly used quality control techniques for assessing total RNA and genomic DNA.

Quantification & Assessment of Purity

Spectrophotometric Analysis

Spectrophotometry is commonly used to determine both the concentration and purity of nucleic acids. Most spectrophotometers produce absorbance spectra from 220 nm to 350 nm. All nucleic acids (RNA, DNA, ssDNA and dNTPs) absorb ultraviolet light at 260 nm. Proteins absorb at 280 nm. Some spectrophotometers perform a spectral decomposition for selective quantification of DNA, RNA or protein. This can be helpful in assessing the level of contamination with other nucleic acids. The Ramaciotti Centre uses both the NanoDrop™ and the Trinean Xpose as part of our QC pipeline, allowing us to assess both the purity and composition of nucleic acids submitted for processing.

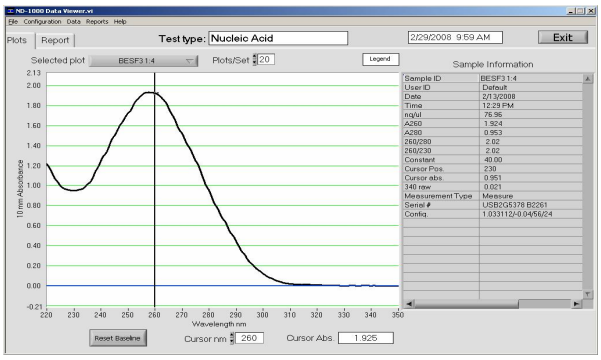
260/280 Ratios

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of nucleic acid preparations. Generally, a ratio of ~1.8 indicates pure DNA, and a ratio of ~2.0 indicates pure RNA. If this ratio is lower it indicates the presence of contaminants that absorb near 280 nm, such as proteins. Protein contamination is problematic, as the presence of RNase and/or DNase will cause degradation.

260/230 Ratios

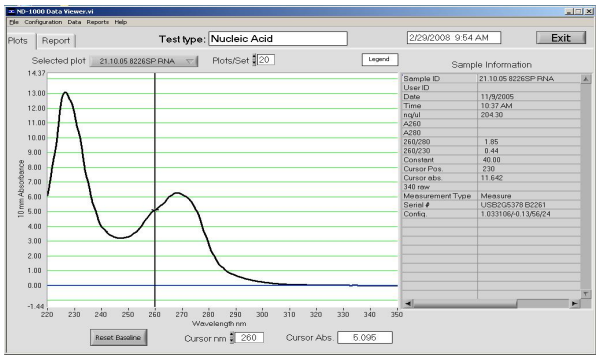
This ratio is also used as a measure of nucleic acid purity. A ratio in the range of 1.8 – 2.2 is acceptable for pure nucleic acid. If this ratio is lower than 1.8, it can indicate the presence of contaminants that absorb at 230 nm. Reagents such as TRIzol® contain phenol which absorbs at both 230 nm and ~270 nm. If this reagent, or similar, is used in the extraction procedure a low 260/230 ratio can indicate phenol, guanidine or isothiocyanate contamination. Phenol contamination causes the overestimation of nucleic acid concentration and also inhibits downstream enzymatic reactions. An additional precipitation or column clean up step will remove this contamination (refer to our precipitation protocol).

Spectra of Pure Nucleic Acid



(NanoDrop™ 1000 Spectrophotomer trace)

Spectra of Sample with Phenol Contamination



(NanoDrop™ 1000 Spectrophotomer trace)

Limitations of Spectrophotometry

- Samples with concentrations less than ~25 ng/ul cannot be reliably qualified using this method.
- Poorly resuspended samples will give inconsistent readings.
- Contaminants that absorb near 260 nm will cause an overestimation of concentration.
- All nucleic acids (RNA, dsDNA, and ssDNA) absorb at 260 nm, and hence contribute to the total absorbance of the sample.
- Spectrophotometry **will not** provide data on the integrity of your sample i.e. is it degraded.

Quantification by Fluorescence Assay

The Ramaciotti Centre routinely uses fluorescence-based assays for quantitation of nucleic acids. We use Quant-iT™ assays from Life Technologies™, and measure concentration on the QuBit. The Quant-iT™ assays have the following advantages:

- Quant-iT™ assays are specific for the nucleic acid of interest, and provide accurate quantification of the molecule of interest.
- They are not affected by the presence of proteins or contaminants carried over from the extraction process.

Limitations of Fluorescence Assays

- Fluorescence assays **will not** provide information on the purity of your sample. It is advised that a spectrophotometric reading is also taken.
- Fluorescence assays **will not** provide data on the integrity of your sample i.e. is it degraded.
- Some fluorescence assays are not nucleic acid specific and will bind both RNA and DNA.

Assessment of Nucleic Acid Integrity

The Ramaciotti Centre employs two instruments to assess the integrity or intactness of nucleic acids. The Agilent 2100 Bioanlayzer for RNA and the Perkin Elmer LabChip® GX for both RNA and genomic DNA. The information provided below on assessment of RNA integrity relates to the Agilent Bioanalyzer as this is currently the most widely used instrument. Further information on integrity assessment can be found on our website.

Agilent 2100 Bioanalyzer – Total RNA Integrity

The Agilent 2100 Bioanalyzer is a micro-fluidics platform that enables the quality analysis of RNA using only 1 ul of sample. The Bioanlayzer software generates an RNA Integrity Score (RIN score) for each sample on a scale of 1 – 10 (1 = lowest, 10 = highest). This score is based on the entire electrophoretic trace and not just the ratio of rRNAs. As degradation of RNA occurs the rRNA peak heights decrease and smaller, degraded RNA peaks appear.

Advantages of the Agilent 2100 Bioanalyzer

- RNA integrity number - provides a numerical quality value for RNA.
- Sensitive - small amounts of material required (detection down to 200 pg/ul of total RNA).
- Allows visualisation of genomic DNA contamination of total RNA (some other instruments utilise RNA specific dye).

Limitations of Bioanalyzer

We do not find the concentration estimate generated by the Bioanlayzer to be as accurate as either spectrophotometric or fluorescence based assays.

Agilent Bioanalyzer – Examples of Traces

To help with the interpretation of traces, some examples are provided below, please refer to the Agilent website for further information.

Electropherograms of RNA samples with varying levels of intactness

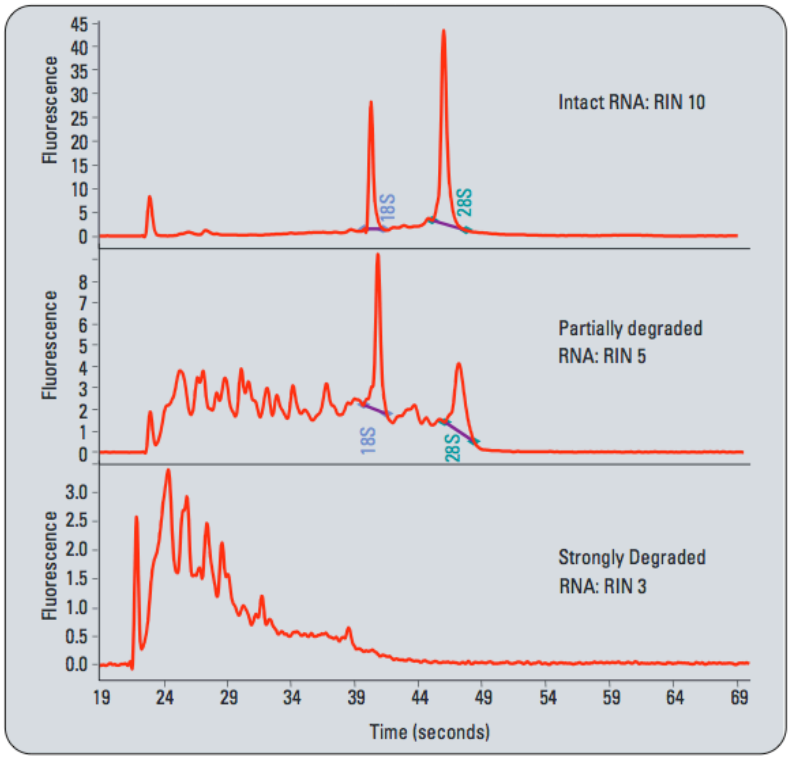


Image from Agilent Technologies Publication Number 5989-1165EN.

Genomic DNA contamination in RNA samples

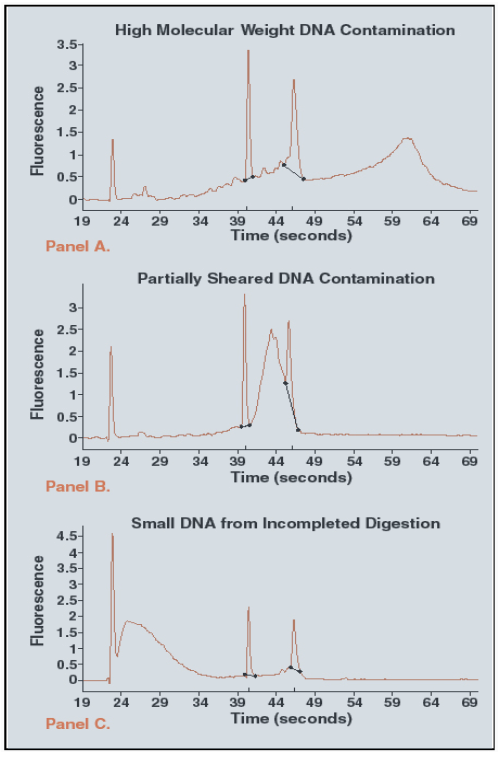


Image from S. Chacko, BioPharm International, 2005.