CPGR | CENTRE FOR PROTEOMIC & GENOMIC RESEARCH

RNA Quality Check List

Quantity

A minimum of 1 µg of total RNA is required for most downstream applications.
Poly(A) mRNA makes up approximately 1-5% of the total RNA. Therefore, up to 50 ng of Poly(A) mRNA can be obtained from 1 µg of total RNA.

NanoDrop and other spectrophotometric readings can overestimate the presence of RNA in the presence of contaminants, such as DNA. Fluorometric quantitation is more accurate, and therefore preferable.

Purity

RNA Purity can be inferred from the UV absorbance ratios of 260 to 280 nm ($A_{260/280}$) and 260 to 230 nm ($A_{260/230}$), usually obtained by NanoDrop analysis.
An **$A_{260/280}$ ratio of between 1.8 and 2.1** and an **$A_{260/230}$ ratio of between 1.8 – 2.2** is generally accepted as pure for RNA. An $A_{260/230}$ absorbance ratio lower than 1.8 indicates the presence of contaminants such as chaotropic salts, often guanidine thiocyanate, as well as polysaccharides and polyphenols.

Purity ratios can often be improved by isopropanol precipitation at room temperature followed by washes with 80% ethanol. All ethanol should be removed/evaporated from samples before resuspension.

RNA samples should also be free of DNA contamination.

Integrity

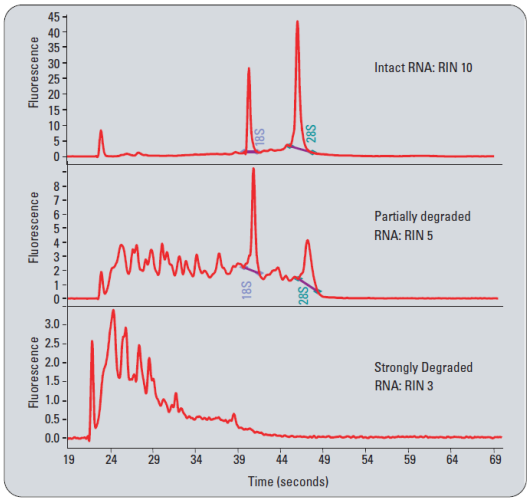
RNA integrity is of utmost importance when preparing RNA sequencing libraries and/or investigating transcript abundance. Lower quality RNA can result in higher read duplication rates, fewer useable reads and an increase in 3' bias of transcripts; it can also result in false positives in differential expression analysis.

High quality total RNA should have an **RNA Integrity Number (RIN) ≥ 8** when analysed on an Agilent Bioanalyzer (Fig. 1A). Plant RNA can be lower (preferably RIN ≥ 6.5)[1] due to the presence of chloroplast ribosomal RNA.

[1] https://www.agilent.com/cs/library/applications/5990-8850EN.pdf

If access to a Bioanalyzer is not possible, integrity can be examined by running total RNA on a denaturing agarose gel and staining with ethidium bromide. RNA should be heated to 70°C for 2 minutes and placed immediately on ice before electrophoresis to eliminate RNA secondary structure (only heat denature RNA to be loaded on gel; RNA can degrade at elevated temperatures in the presence of divalent cations and at high pH). High quality RNA should have a **28S band that is twice as intense as the 18S band of ribosomal RNA** (Fig 1B).

A



B

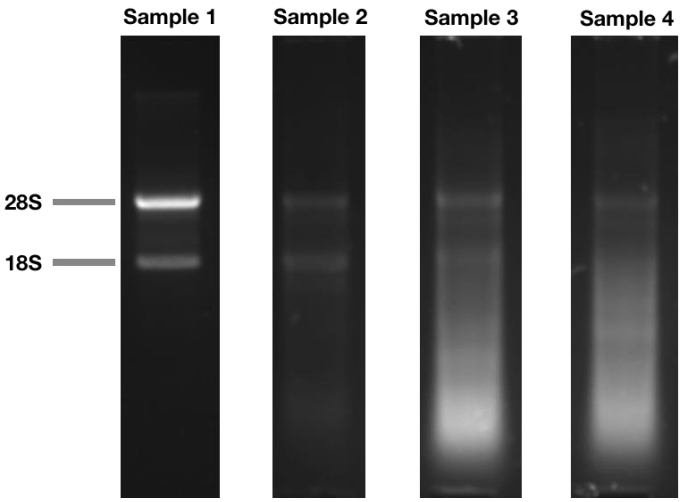


Figure 1: RNA integrity analysis on the Agilent Bioanalyzer (A) showing increasing RNA degradation[2] with associated RINs, and by agarose gel (B) showing increasing RNA degradation[3]. Sample 1 shows intact RNA and Sample 4 shows degraded RNA.

[2] https://www.agilent.com/cs/library/applications/5989-1165EN.pdf
[3] https://www.digitalproteomics.com/from-rna-to-sequencing-qc-matters/