core facility genomics
Medical Faculty of the Universität Münster

Quality Control Workflow for RNA Sequencing

Successful library generation is strongly influenced by the quality of the input RNA. The Quality Control (QC) is performed using the Agilent 4200 TapeStation System. A DNase I digestion is highly recommended.

Procedure QC

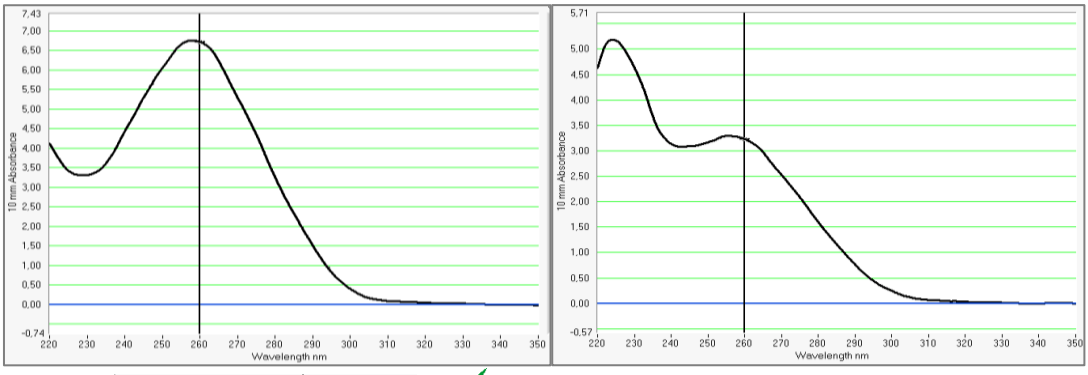
1. Preparation of 4 µl aliquots in PCR stripes
   - ➔ We prefer to protect the original RNA sample (several times of thawing can affect the quality. So we try to work always with directly prepared aliquots.)
2. RNA measurement with the NanoDrop 8000 Spectrophotometer
   - ➔ To gain an indication of the concentration and quality
   - ➔ *If you perform the NanoDrop measurement by yourself, please fill in the data (Concentration and the 260/280 and 260/230 ratios) in the „NGS – sample table". Then we only need 2.5 µl Aliquots (instead of 4 µl as decriped in point 1.)*
3. Depending on the NanoDrop results, the QC is performed by using a RNA ScreenTape or High Sensitivity RNA ScreenTape.
   - ➔ Quantitative Range of the RNA ScreenTape 25-500 ng/µL
   - ➔ Quantitative Range of the High Sensitivity RNA ScreenTape 0.5-10 ng/µl
   - ➔ To decide which Tape is the best for your sample, we need the NanoDrop measurement. If the samples need to be diluted, we will perform the dilution.
4. Interpretation of the TapeStation results
   - ➔ RNA Integrity Number (RIN) as objective evaluation of RNA degradation and quality
   - ➔ RIN values above 6.5 for mRNA Preparation and from 3.5 or higher for rRNA depletion
5. Submission of the original samples

Additional recommendations and information

- ➢ The RNA should not be degraded and should be free of contaminations such as TRIzol reagent, proteins or genomic DNA
  - ➔ The 260/280 ratio indicates potential contamination with genomic DNA
  - ➔ The 260/230 ratio indicates potential phenolic contamination
  - ➔ Both values should be ideally ~2

Examples of the Plots after the NanoDrop measurement

Pure RNA	Phenolic Contamination



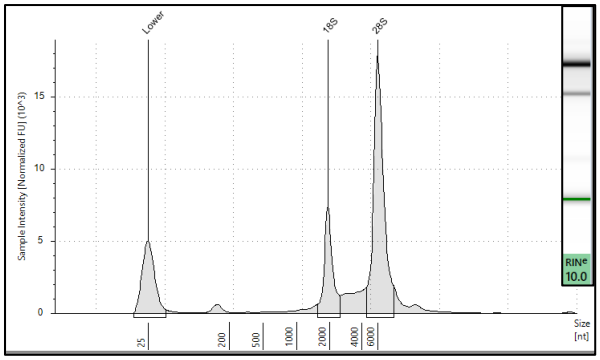
260/280	2,07
260/230	2,03

260/280	2,03
260/230	0,69

➢ The TapeStation can more precisely detect the concentration and quality

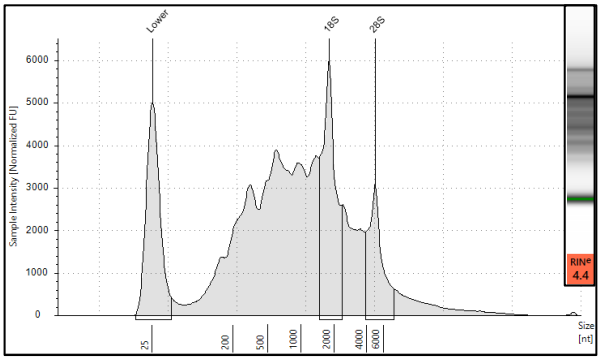
Exemplary TapeStation results

A: RNA with a very good quality



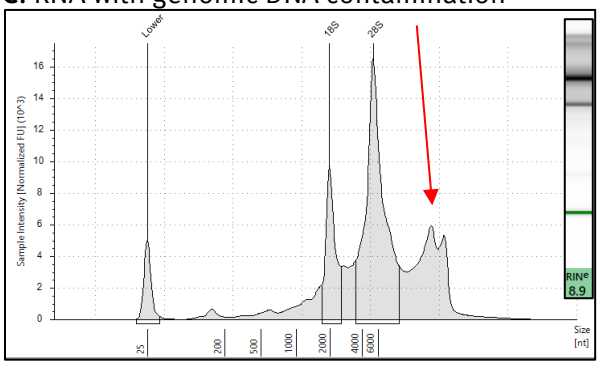
RIN^e	Concentration [ng/µl]	Sample Description
10.0	107	A

B: Degraded RNA (28S ‹ 18S)



RIN^e	Concentration [ng/µl]	Sample Description
4.4	156	B

C: RNA with genomic DNA contamination



RIN^e	Concentration [ng/µl]	Sample Description
8.9	426	C

DNase I digestion is highly recommended!

Examples of Kits:
„RNA Clean & Concentrator (Dnase I included)“ (ZYMO Research)
„On column DNase I digestion“ in RNeasy® Mini Kit (QIAGEN)