The RNA Integrity Number (RIN)

The RNA integrity number (RIN) is a tool designed to help scientists estimate the integrity of total RNA samples. The RIN extension automatically assigns an integrity number to a eukaryote total RNA sample analyzed on the Agilent 2100 bioanalyzer. Using this tool, sample integrity is no longer determined by the ratio of the ribosomal bands alone, but by the entire electrophoretic trace of the RNA sample, including the presence or absence of degradation products. In this way, interpretation of an electropherogram is facilitated, comparison of samples is enabled and repeatability of experiments is ensured.

Scope

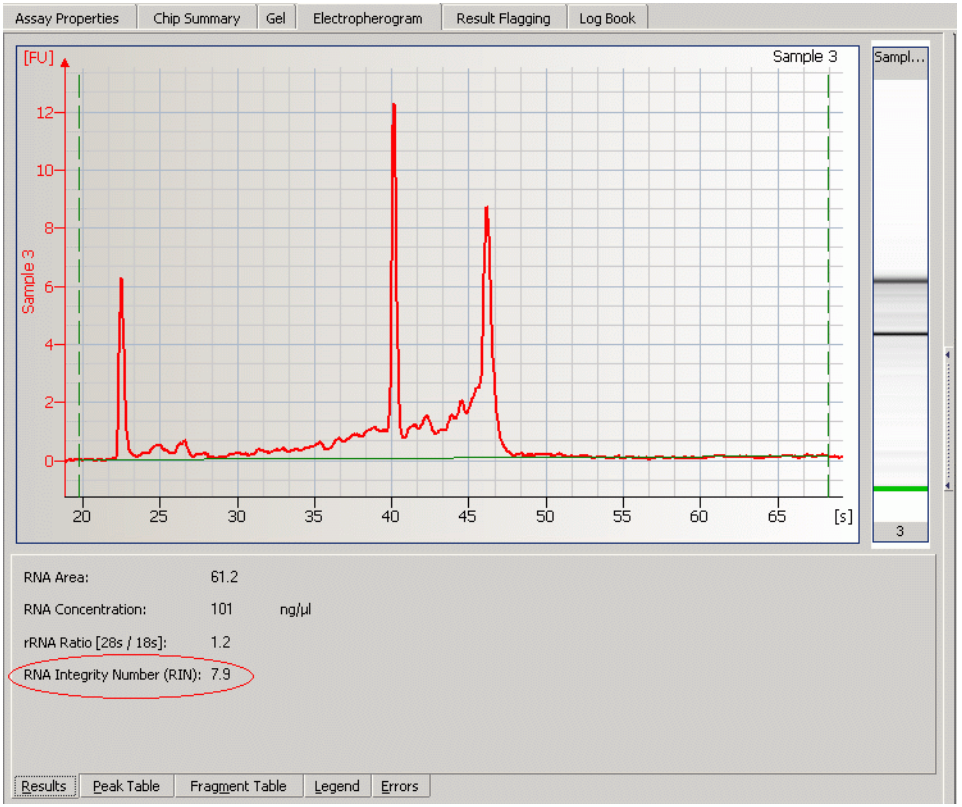
What the RIN can do:

- Obtain an assessment of the integrity of RNA.
- Directly compare RNA samples (e.g. before and after shipment, compare integrity of same tissue across different labs, etc.).
- Ensure repeatability of experiments (e.g. if RIN shows a given value and is suitable for microarray experiments, then the RIN of the same value can *always* be used for microarray experiments given that the same organism/tissue/extraction method was used).

What it CANNOT do:

- Tell a scientist ahead of time whether an experiment will work or not if no prior validation was done (e.g. RIN of 5 might not work for microarray experiments, but might work well for an appropriate RT-PCR experiment. Also, an RIN that might be good for a 3' amplification might not work for a 5' amplification).

The computation of the RIN is part of data analysis for total RNA samples. The computed RNA integrity number is shown on the *Results* sub-tab of the *Gel* or *Electropherogram* tab of the *Data and Assay* context. It is also included in XML export files and in printed reports.



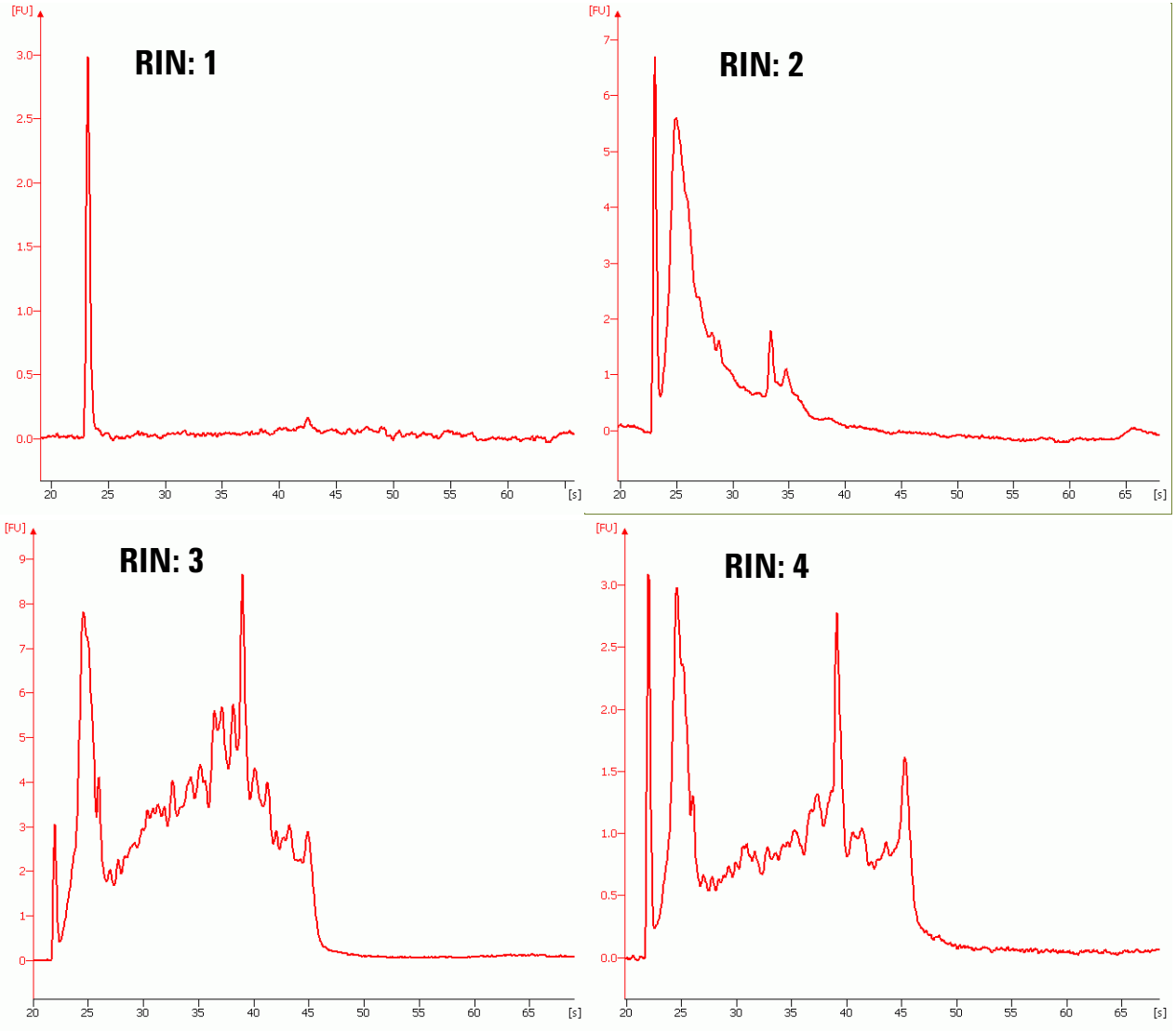
NOTE

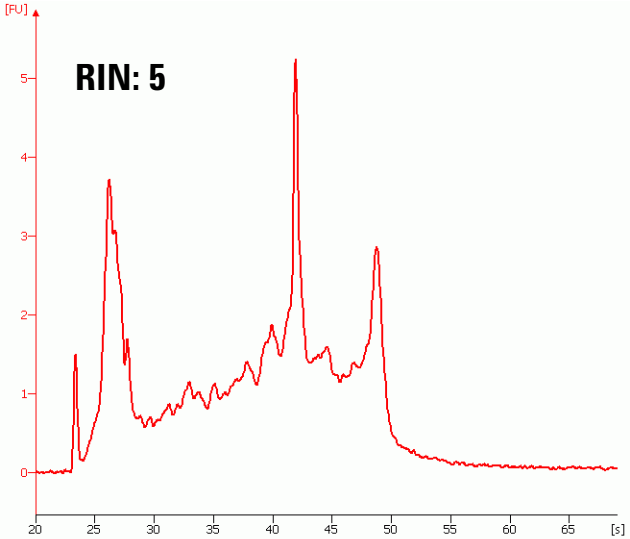
Until now, the computation of the RIN has only been validated for eukaryote total RNA Nano samples. The 2100 expert software also calculates the RIN for prokaryote total RNA samples and for the RNA 6000 Pico assay. Be aware that for these samples, the RIN has not been validated in extensive downstream experiments.

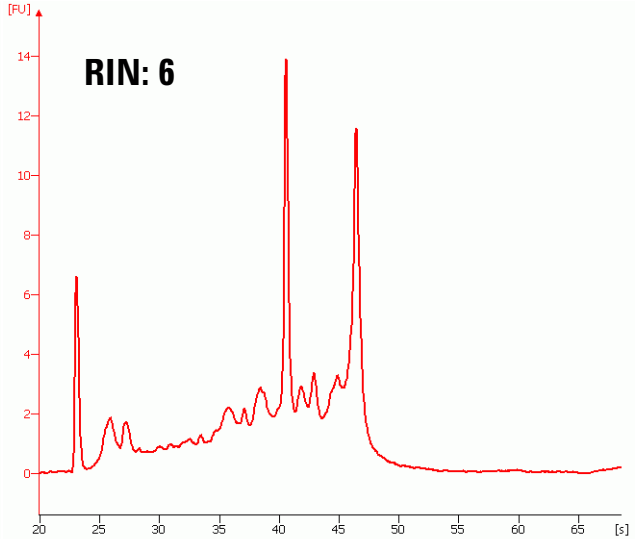
Although the lower quantitative limit of the RNA 6000 Nano assay is specified as 25 ng/µl it is recommended to use at least 50 ng/µl for a meaningful RNA integrity number. When using lower concentrations, higher sample to sample variances of the RIN may be observed.

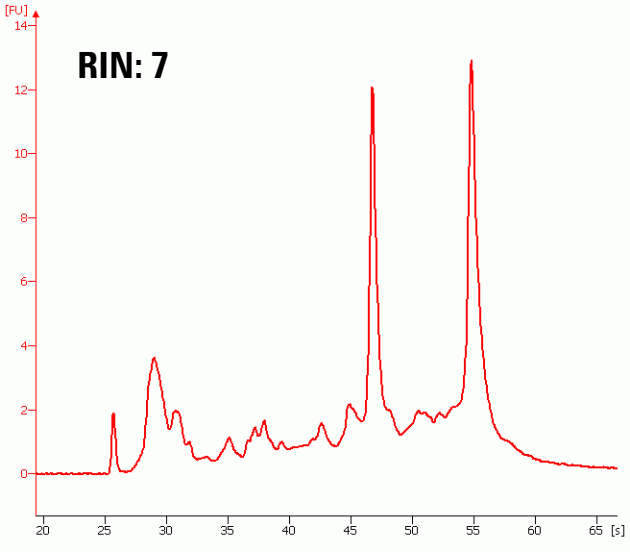
Examples for RNA Integrity Numbers

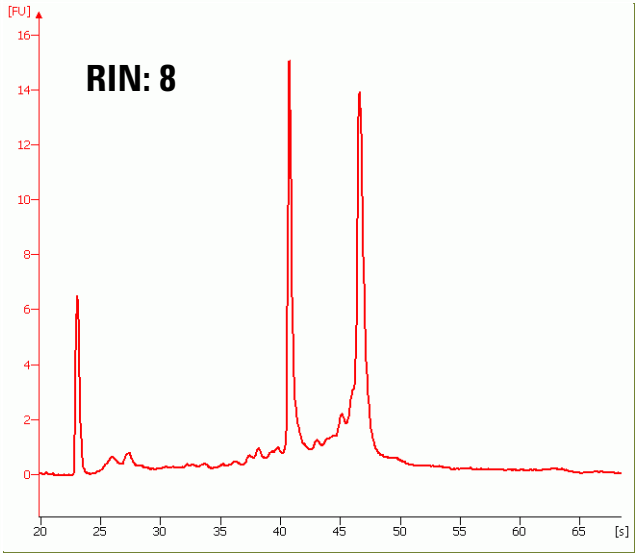
A database of about 1300 mammalian total RNA samples was created using the RNA 6000 Nano assay. The samples came from different species (mainly human, rat and mouse), tissues, preparation methods, concentrations and degradation states. All samples were classified according to their degradation state. Numbers from ‘1’ to ‘10’ were used as labels. ‘10’ stands for a perfect RNA sample without any degradation products, whereas ‘1’ marks a completely degraded sample. The labels in-between are used to indicate progressing degradation states of the RNA sample. The following figure shows typical representatives for each of the 10 RNA integrity classes.

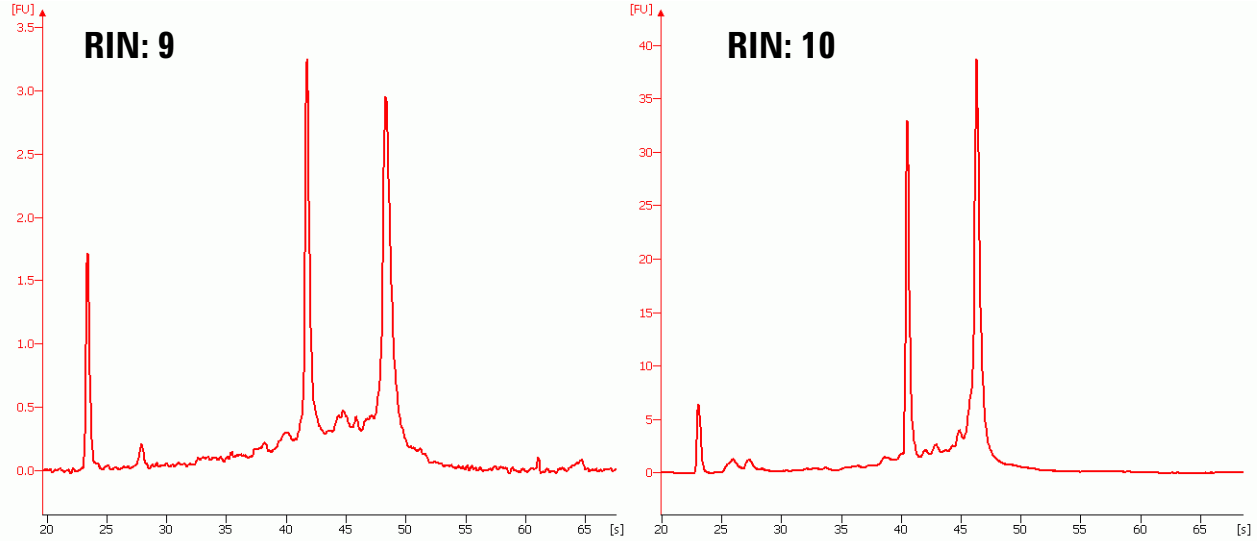

RIN: 1
RIN: 2
RIN: 3
RIN: 4
[FU]
[s]


RIN: 5
[FU]
[s]


RIN: 6
[FU]
[s]

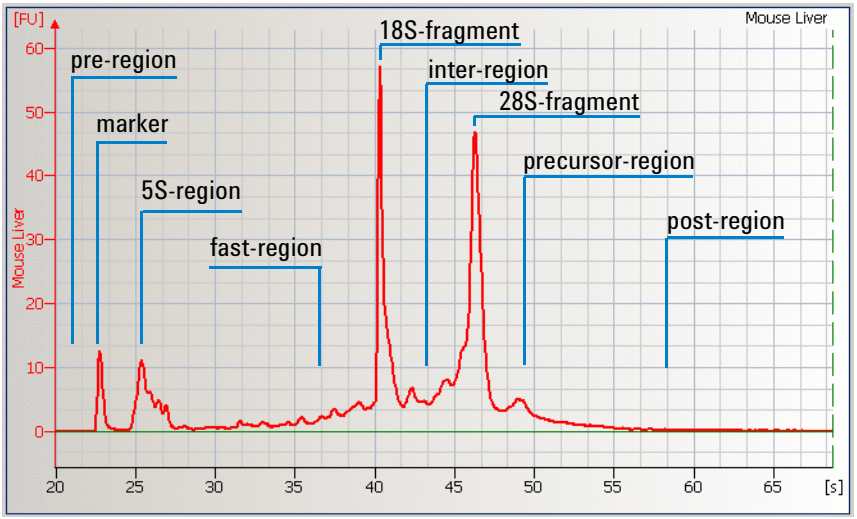

RIN: 7
[FU]
[s]


RIN: 8
[FU]
[s]


RIN: 9
[FU]
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
25
30
35
40
45
50
55
60
65
[s]
RIN: 10
[FU]
40
35
30
25
20
15
10
5
0
20
25
30
35
40
45
50
55
60
65
[s]

Computation of the RNA Integrity Number and Signal Anomalies

For the computation or the RNA integrity number, the electropherogram is partitioned into regions as shown in the figure below. The lower marker and the 18S and 28S fragments divide the electropherogram into nine regions:



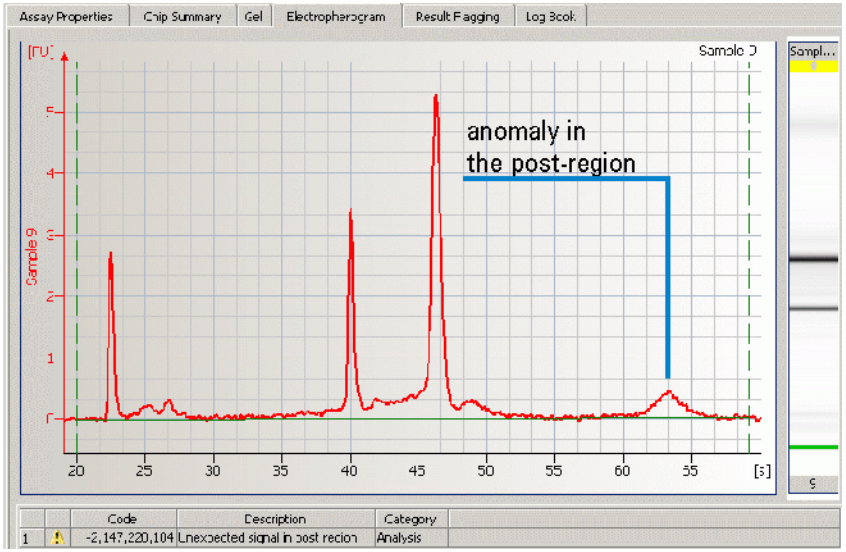
Signal Anomalies

In addition to the computation of the RIN, the data analysis detects various unexpected signals, disturbing the computation of the RIN. Such disturbances are called anomalies. Region anomaly detectors recognize unexpected signals in each region. If detected, the anomaly is displayed in the *Error* sub-tab of the *Electropherogram* and *Gel* tab.

Anomaly Description	Critical?
Unexpected baseline signal	Yes
Unexpected signal in pre-region	No
Unexpected signal in 5S-region	Yes
Unexpected signal in fast-region	Yes
Unexpected signal in inter-region	Yes
Unexpected signal in precursor-region	No
Unexpected signal in post-region	No
Unexpected ribosomal ratio	Yes
Unexpected sample type	Yes
Unexpected lower marker (compared to previous well)	No

Two categories of anomalies were introduced, critical and non-critical. Anomalies in regions interfering with the customer sample RNA are considered critical. They are displayed as errors and the corresponding gel lane is flagged red. If a non-critical anomaly is detected, the RIN can still be computed accurately. Therefore non-critical anomalies are displayed as warnings and the corresponding gel lane is flagged yellow. Non-critical region anomalies are pre-region anomaly, precursor-region anomaly and post-region anomaly. The baseline anomaly is detected for signals with fluctuating or

steep baseline. The ribosomal ratio anomaly detects unexpected ratios of the 28S fragment area and the 18S fragment area. The unexpected sample type anomaly is detected for samples which do not fit the standard total RNA profile. The electropherogram below gives an example for a non-critical anomaly in the post-region.

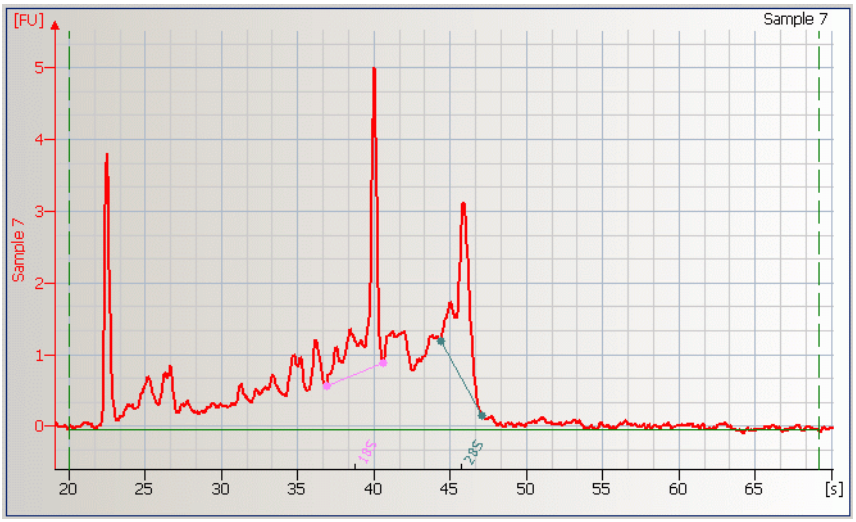


Troubleshooting the RIN

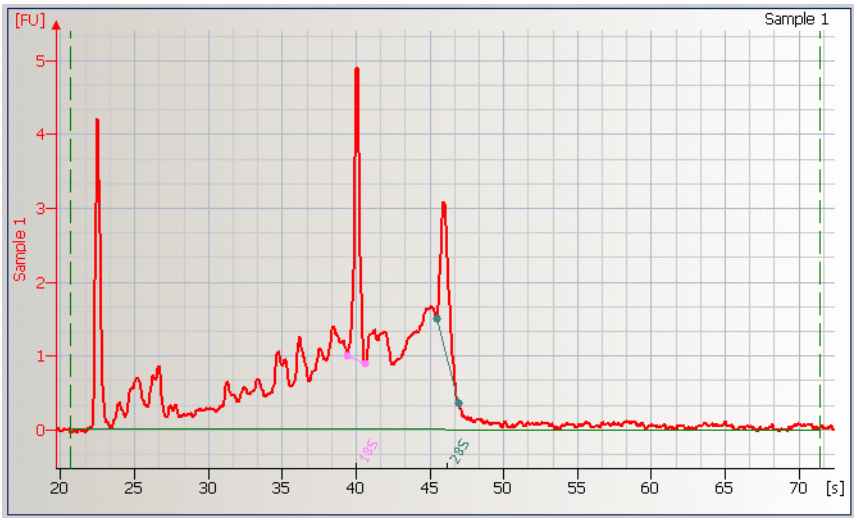
To obtain meaninful and reproducible results, the lower marker and ribosomal bands must be identified correctly. Although the ribosomal fragment identification has been improved, in rare cases (i.e. when analyzing degraded RNA samples) the fragment baseline is not properly set. In this case the user should adjust the baseline settings manually.

Example:

'uncorrect' software identification of the ribosomal fragments: RIN=7.2



RNA integrity number after the manual adjustment: RIN=5.7

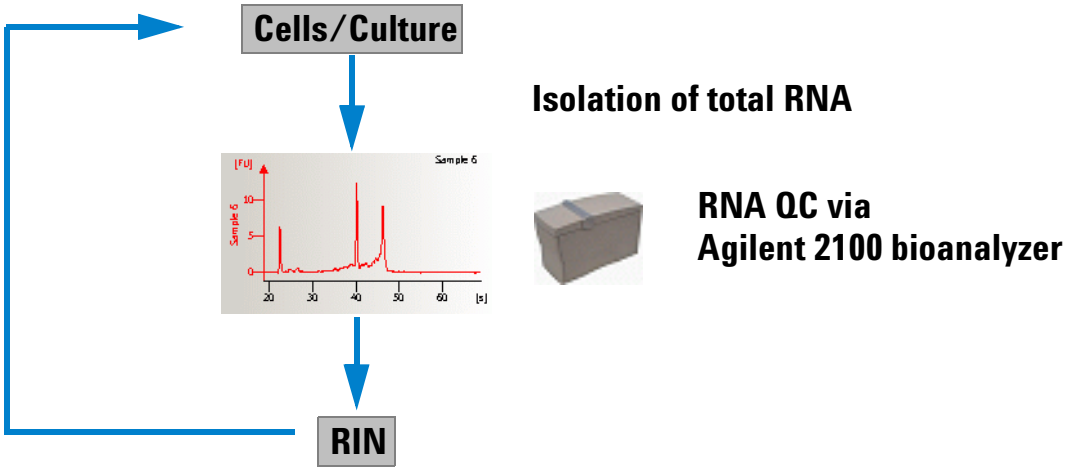


On details on how to adjust the lower marker and ribosomal bands, please refer to "Changing the Data Analysis" on page 125.

Use Models for the RNA Integrity Number

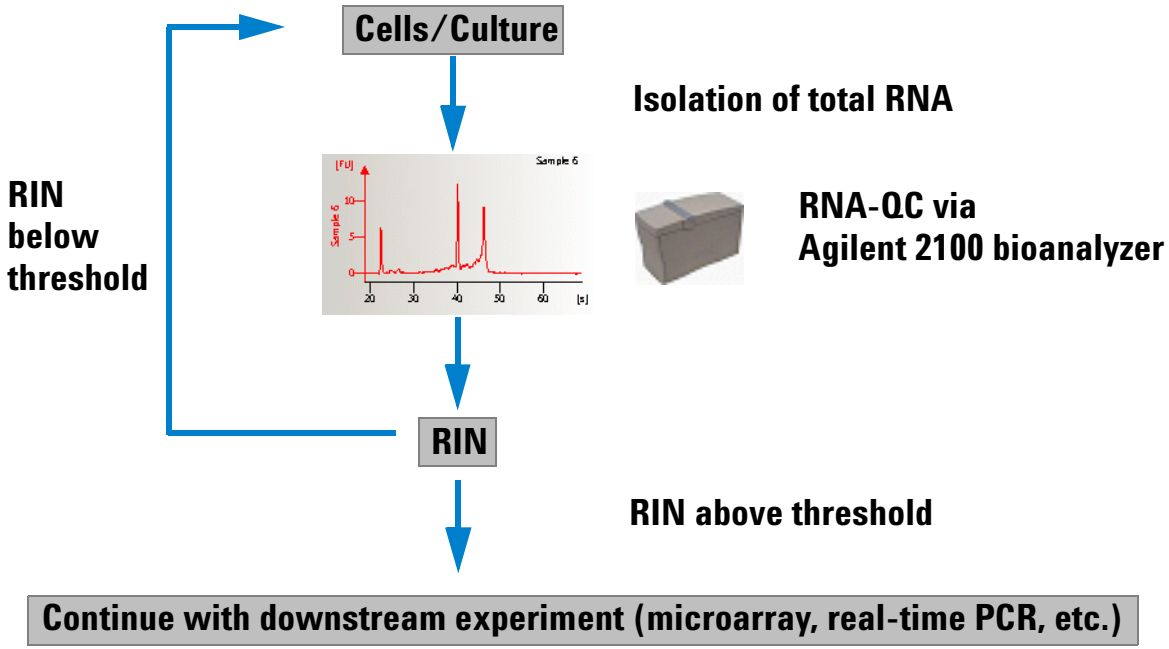
To take full advantage of the RIN feature, a 2 step use-model is suggested:

1 Determine the threshold value for the RIN that results in meaningful downstream experiments:



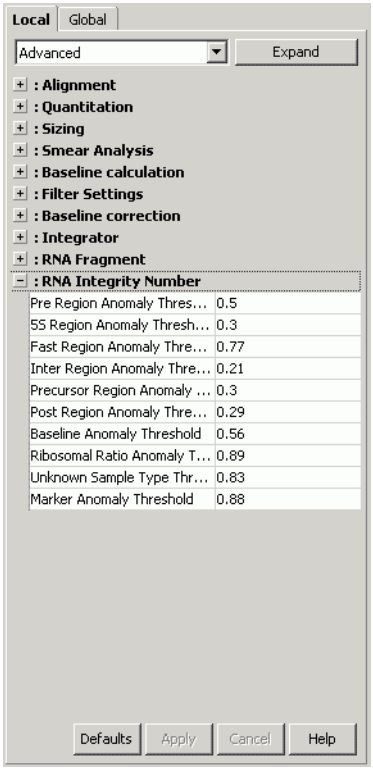
Correlate RIN with downstream experiment and determine threshold RIN for meaningful results (iterative process)

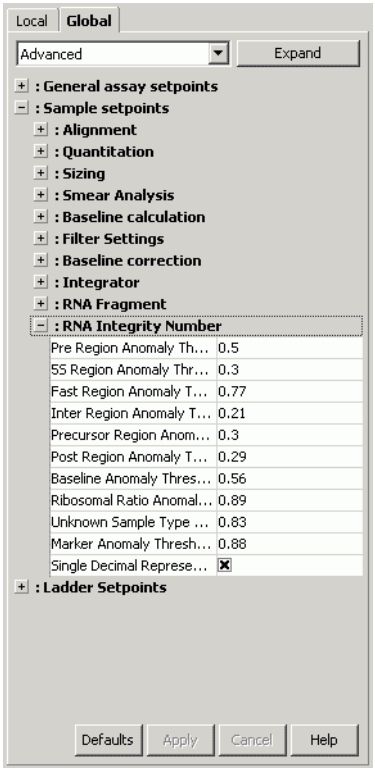
2 Run standard experiment and use RIN to determine if sample integrity id sufficient:



RNA Integrity Number Setpoints

Various setpoints are available in advanced user mode of the setpoint explorer to customize the computation of the RIN.





The user can modify predefined thresholds for anomaly detection. The higher the theshold the less anomalies are detected. Valid threshold values range from 0 to 1. In addition on the global setpoint tab, the user can switch between integer and decimal representation of the RIN.