How to check your sample quality

Quality of sample is an important factor for fine microarray data.
We recommend to perform the quality check for your samples by yourself before shipping.
For your information, please see the below QC method and its criteria.

If it is difficult to perform the QC by yourself, please ship the sample without prior QC.
In that case, if it does not pass our QC, re-shipment or purification may be required for those samples.
Please note that **sample QC is performed free of charge only twice. More QCs and purification service will be charged additionally.**

Absorbance Determination

To confirm the concentration and the amount of RNA

The concentration and the amount of RNA are confirmed by absorbance determination. Please see the **Table 1** to check your sample.

If your sample is difficult to be obtained, the amount of starting materials can be reduced as shown in the **Table 2**. The total RNA amount required for analysis would be depend on microarray format to be used.

Regardless of concentration, approx. 5uL sample is required for QC, so please prepare 8uL or more of the fluid measure. After using 5uL, please prepare the sample so that the amount of RNA in the **Table 2** will remain.

The amount listed in the **Table 2** is the minimum amount of starting materials required for microarray analysis.
For the same starting materials with the different amount, the more probes can be detected for the material with the larger amount.
So it is recommended to use the same amount of starting materials between the samples to be compared.
If any re-analysis is required due to the defective reagents or slides (the reasons other than samples), it will be made at free of charge. However, re-analysis may not be performed if the amount of the remaining sample is less than the standards specified in the **Table 1**.
If the amount of RNA is enough, please prepare for the sample according to the standards in the **Table 1**.

Table 1 Please check the below items for absorbance determination (HSS recommend standards)

Sample amount	1ug or more
Concentration	100ng/uL or more
A260/A280	1.8 ~ 2.1
A260/A230	2.0 or more

Table 2 The minimum sample amount required for analysis

QC (volume)		5uL
DNA microarray analysis (RNA amount)	**8 x**	10ng
	4 x	25ng
	2 x , 1x	50ng

To confirm RNA purity

The purity of RNA is confirmed by absorbance determination. Please see if your samples clear the requirements listed in the **Table 1**.
Some samples may be available for analysis even though they have the value less than the required standards. For details, please see the below **Appendix 1**.

Please see the **Table 3** for what the absorbance of wavelength is. Even though there is no problem for the absorbance ratio, the absorbance of 260nm wavelength may be affected by polysaccharide. So please prevent Total RNA being contaminated with polysaccharide at the extraction step.

High purity RNA sample shows the absorbance spectrum as **Figure 1**. Such a graph shows the absorbance curve with the minimum value at approx. 230nm and the maximum value at approx. 260nm, and it shows stable absorbance as "0" at the side of long wavelength.

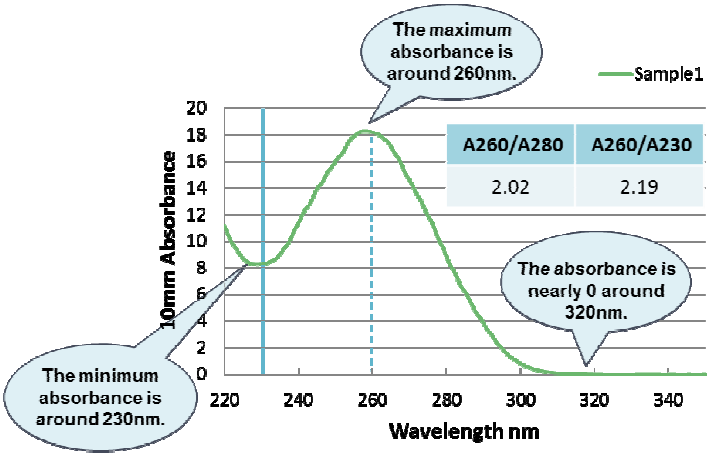

Figure 1 Spectrum of a high purity sample

Table 3 Absorbance at each wavelength

A230	Maximum absorption of salt, sugar, guanidine isothiocyanate and other organic solvent
A260	Maximum absorption of nucleic acids * This value may be affected by polysaccharide.
A280	Maximum absorption of proteins and phenol
A320	A measurement of the turbidity of the sample. The value should be close to 0.0.

Figure 2 shows an example of a sample with A260/A230 rate less than the standard value. In this case, it is highly possible that buffers such as organic solvent, salt or sugar is contaminated. Such buffers have absorbance at the wave length around 230nm, and the enzyme reaction may be inhibited at labelling.

Figure 3 shows an example of a sample with A260/A280 rate less than the standard value. In this case, it is highly possible that proteins or phenol are contaminated. Such substances have absorbance at the wave length around 280nm. The enzyme reaction may be inhibited at labelling, and nucleic acid can not be quantified correctly as the 280nm absorbance affects 260nm absorbance.

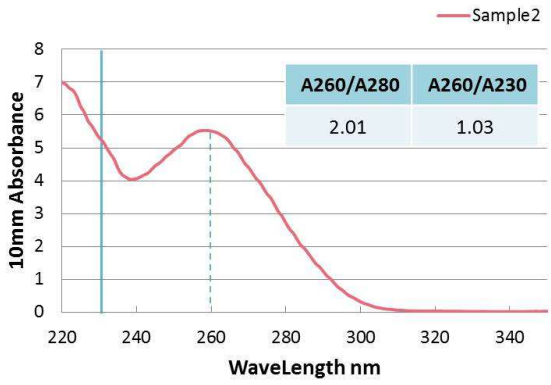

Figure 2 Spectrum with low A260/A230

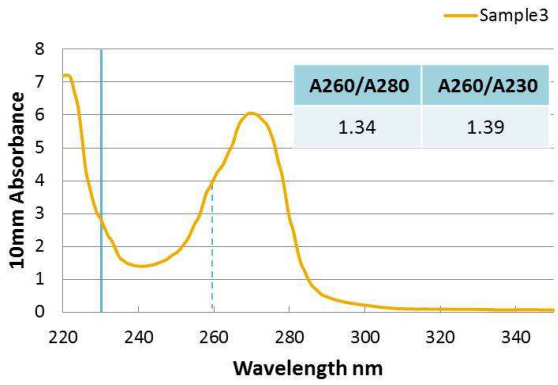

Figure 3 Spectrum with low A260/A280

If the values of A260/A230 and A260/A280 are less than the standard value, please see the **Appendix 1** and perform RNA purification as necessary.

Appendix 1: Samples with absorbance ratio lower than the standard

Even though the absorbance ratio is lower than the standard value, samples as below can be used for analysis as long as their absorption spectrum is not much different.

1. RNA samples purified by spin-column based method

Some samples may obtain low value for A260/A280 and A260/A230 due to the reason derived from samples.

As the reason for the low purity can not be judged if it is caused by any contamination or by the sample itself, we ask our customers if they used spin column for purifying their sample.

For samples purified by spin-column based method, other materials such as buffers may less remain as the solvent was replaced.

2. RNA samples with low concentration

For samples with low concentration, as the measured values are close to the detection limits of the apparatus, the peak of 260nm and the slope of 280nm is nearly same as the background. So it is considered that the accurate absorbance is not measured. (Figure 4)

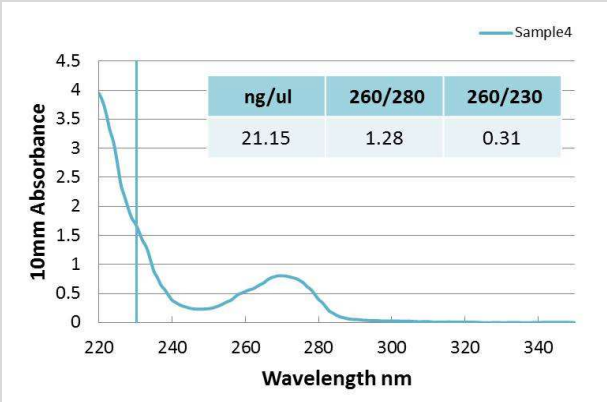

Figure 4 Spectrum of a sample with low concentration

Electropherogram

To confirm RNA integrity

RNA sample integrity can be confirmed by electropherogram (or Bioanalyzer by Agilent Technologies).
"RIN" is an index which provides a quantitative value for RNA integrity measured by a Bioanalyzer, which is indicated from 1 to 10 according to the integrity. The value of 10 indicates that the RNA is intact.

For example, please see the **Fig. 5** for intact samples. Two peaks (bands) of rRNA are clear, and no RNA is hardly detected in the other region.

For less intact samples, degradation products of nucleic acid are detected between two peaks or in the low molecular region, and the peak of rRNA becomes smaller.
Please see the **Fig. 6** for your reference.

For completely degraded samples, no rRNA peak is detected as shown in the **Fig. 7**.

For gene expression microarray analysis, it is revealed that the more a sample degrades, the more false-positive results are produced.
Additionally, as the reverse transcription and the amplification are performed using an oligo dT primer in the labelling step, largely degraded samples may not be labelled well.

HSS recommends **RIN value of 7 or more**.
If Bioanalyzer is available, please prepare for your sample with the RIN value to be more than 7.

If RNA is degraded, the quality of the sample can not be recovered by purification. So degraded samples are required to be replaced with other newly prepared samples.

If samples can not be re-prepared for some reasons like no more samples are available, it is possible to proceed the analysis using the degraded samples, but please accept the possibility of affecting to the analysis result.

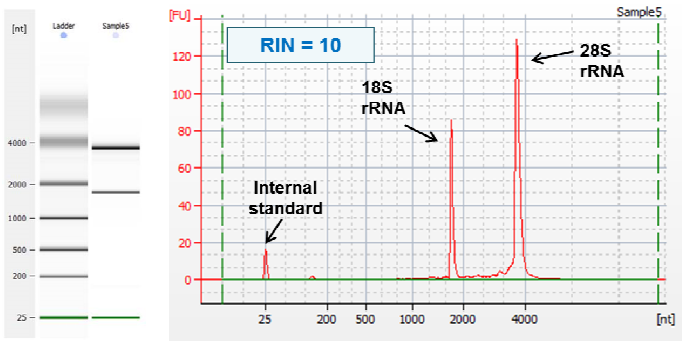

Fig. 5 Electropherogram for a sample with no degradation

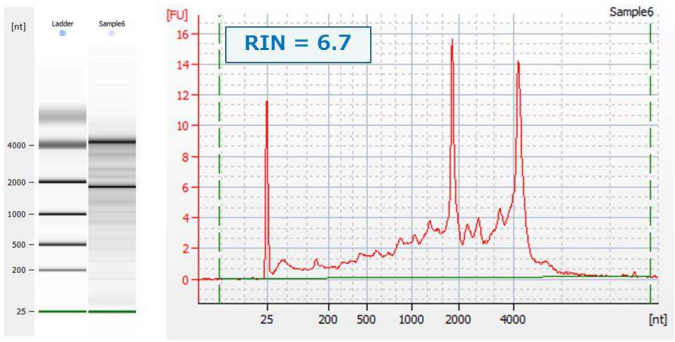

Fig. 6 Electropherogram for a largely degraded sample

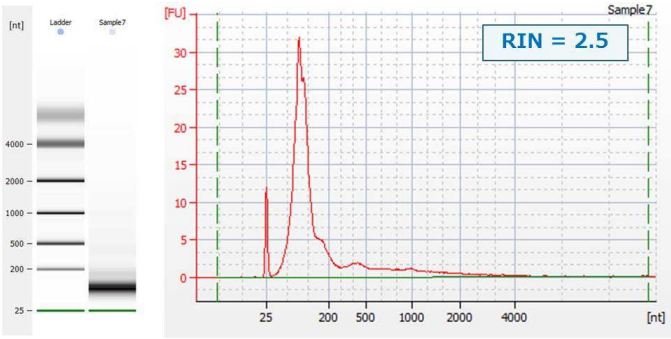

Fig. 7 Electropherogram for a completely degraded sample

Appendix2: Samples other than mammalian

Organisms other than mammal have different rRNA size or Electropherogram pattern. (**Fig. 8, 9**)
In that case, RIN value of Bioanalyzer mostly does not work as an index, so please make sure that the peak of rRNA is clear and nucleic acids are less detected in the other region.

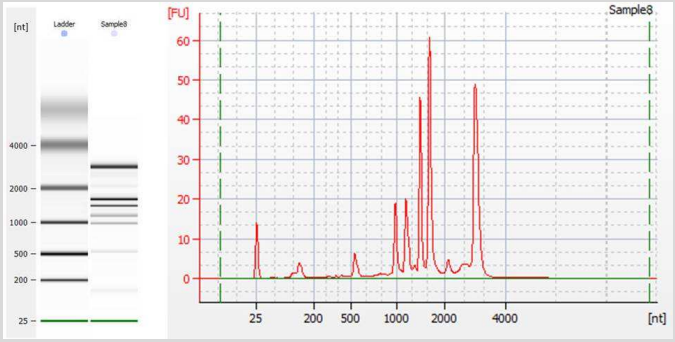

Fig. 8 Electropherogram of an intact plant sample
Peaks other than 28S/18S are detected in photosynthetic tissue where rRNA of organelle are abundantly contained.

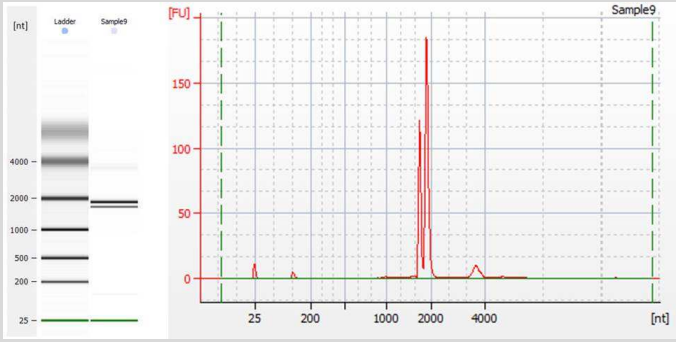

Fig. 9 Electropherogram of an intact insect sample
A hidden break occurred at the maturation step for 28S rRNA of an insect to be cleaved at the size around 18S.

To confirm DNA contamination

DNA contamination is confirmed by electropherogram (or Agilent Technologies' Bio Analyzer).
In case of DNA contaminated, a signal is detected in the different region from that of rRNA, just shown in the upper of the **Fig. 10** and **Fig. 11**.

For such samples, the concentration calculated by absorbance determination includes DNA concentration, so it is highly possible that the Total RNA volume can not be measured precisely.

The measurement by target specific fluorometer such as Qubit (Invitrogen) may be effective to investigate DNA contamination.
If the concentrations are different between UV measurement and fluorometer, DNA contamination may occur.

If any DNA contamination is suspicious, HSS recommends DNase treatment.

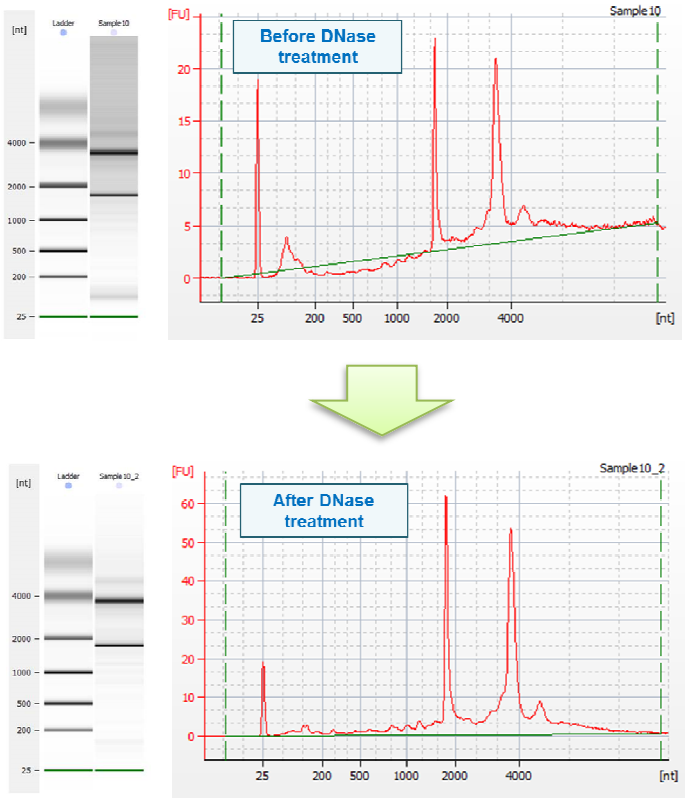

Fig. 10 Difference between the electropherogram pattern of a DNA contaminated sample (above) and a DNase treated sample (below)

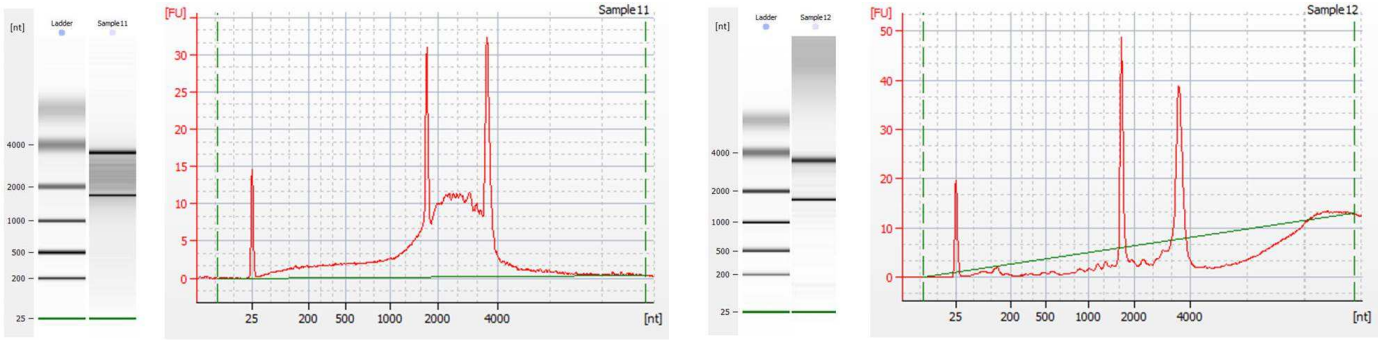

Figure 11 Various electropherogram patterns of a DNA contaminated sample