MASSEY UNIVERSITY
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MASSEY GENOME SERVICE

RNA Quality Analysis
using Agilent 2100 Bioanalyzer
Operational since 2011

BIOANALYZER
TECHNICAL INFORMATION BULLETIN
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BULLETIN INCLUDES

Checking the quality of RNA Assay Results
Details of Bioanalyzer Report Summary
Details of Bioanalyzer Service QC Report

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MASSEY GENOME SERVICE (MGS)

RNA Quality Analysis using Agilent 2100 Bioanalyzer and the RNA 6000 Nano Assay

TECHNICAL BULLETIN

Background

This document provides you with information on how to check the quality of your RNA 6000 Nano assay data, and includes information on:

- Checking the quality of the RNA Ladder used for product sizing
- Checking the quality of the total RNA profiles and mRNA profiles

Massey Genome Service (MGS) provides the following two reports to the customer:

- **Bioanalyzer Report Summary:** This report is generated by the Agilent Bioanlyzer 2100 Expert software used for running the instrument. The report is a summary of the quality of your RNA samples and includes the RNA ladder, which is run with each LabChip assay and is used for product sizing.
- **Bioanalyzer Service QC Report:** This report is generated by the MGS and is a summary of our services analysis of the data quality.

Included with these two reports are the **Data Files**, which can be opened using the **Agilent 2100 expert software**. This is the same software used by the MGS for running and analyzing the results. This software can be downloaded from the MGS website at http://genome.massey.ac.nz , by going to the 'Bioanalyzer Service' link.

Checking the Quality of RNA Assay Results

The concentration and integrity of RNA samples can be rapidly characterized using the Agilent 2100 bioanalyzer and RNA 6000 LabChip kit. Degraded or contaminated RNA preparations can be identified before time-consuming protocols such as cDNA synthesis are initiated [3].

RNA 6000 Ladder Well Results

MGS provides a **Bioanalyzer Report Summary** as mentioned above. Included in this summary is the **RNA 6000 Ladder Results**. The RNA 6000 Ladder is run on each RNA 6000 Nano Labchip assay and is used to size call the samples run on the same labchip. The RNA 6000 Ladder consists of 6 RNA products of sizes 200nt, 500nt, 1000nt, 2000nt, 4000nt and 6000nt, which are each labelled with the same fluorescent dye, and a 25nt lower marker. The ladder is run in a separate well on the labchip [figure 1].

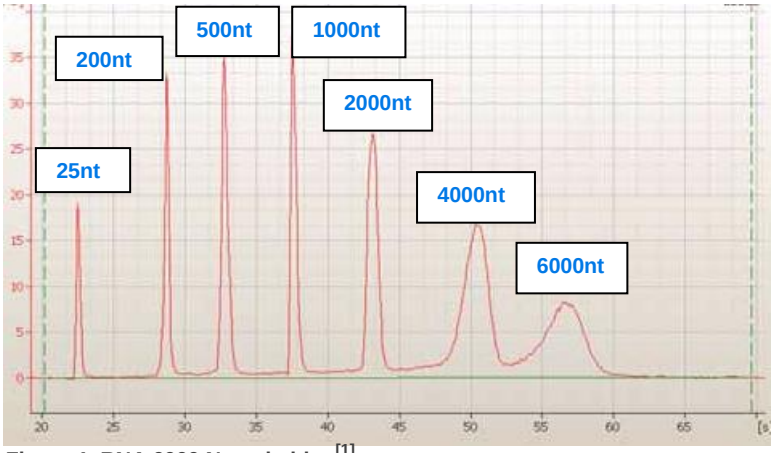

Figure 1. RNA 6000 Nano ladder [1]

Major features of a successful ladder run are:
• 1 marker peak at 25nt
• 6 RNA peaks (2100 expert software calls for 5 first ladder peaks only)
• All 7 peaks are well resolved
• Correct peak size assignment in the electropherogram

RNA 6000 Sample Well Results

Each RNA sample is run in a separate well on the RNA 6000 Nano LabChip. The following assays are run on both these labchips:

- Eukaryotic total RNA
- Prokaryotic total RNA
- Plant total RNA
- mRNA

Total RNA profiles

Major features of a successful total RNA run are [1]:
• 1 marker peak at 25nt
• 2 ribosomal peaks (with successful sample preparation) [figure 2]

The ribosomal ratio and the RNA Integrity Number (RIN) are displayed in the **Bioanalyzer Report Summary**. Details regarding these measurements are in the **'Details of Bioanalyzer Report Summary'** section of this bulletin.

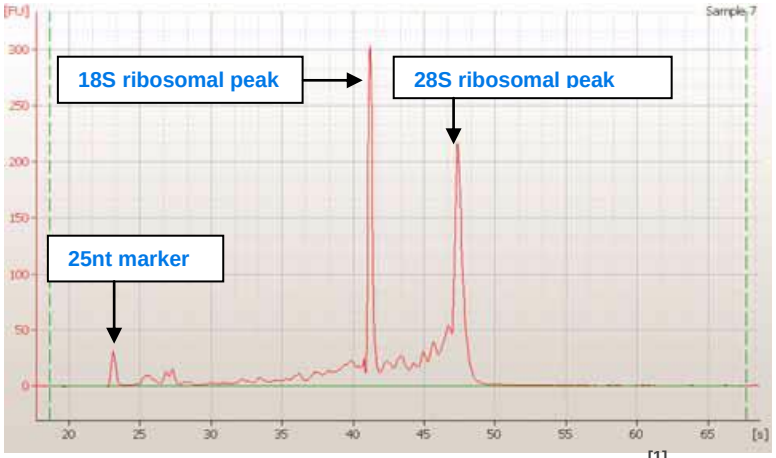

Figure 2. RNA peaks of a successful assay for Eukaryotic RNA [1]

Major features of a degraded total RNA run:

RNase degradation of total RNA samples produces a shift in the RNA size distribution toward smaller fragments and a decrease smaller fragments and a decrease in fluorescence signal [figure 3A] [3]. The 18S and 28S peak can no longer be identified with certainty. With more severe degradation (figure 3B), the spectrum shifts entirely toward early migration times. The overall signal becomes weak as dye intercalation sites are destroyed [3].

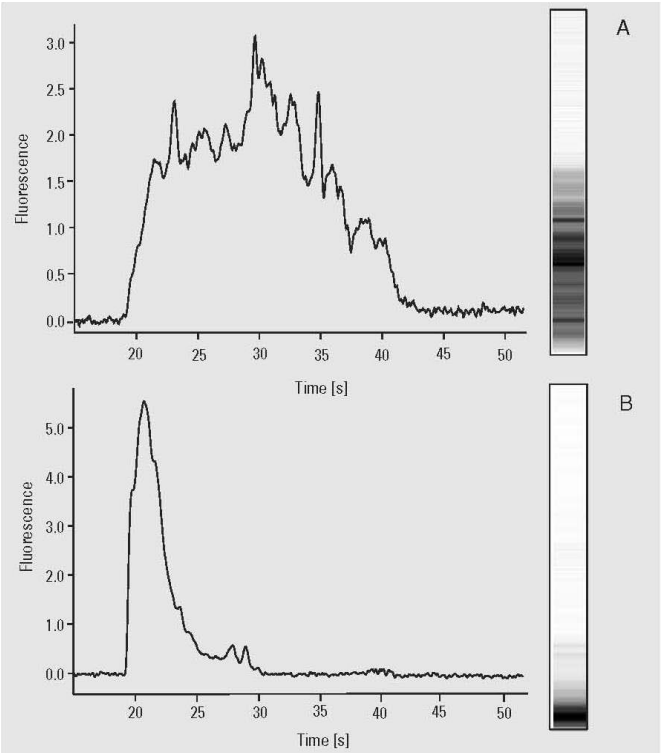

Figure 3A & 3B. Levels of degraded Total RNA

mRNA profiles

Major features of a successful mRNA run are [1]:

- 1 marker peak (25nt)
- Broad peak (with successful sample preparation) [figure 4]
- Contamination with ribosomal RNA shown as 2 overlaid peaks; if present [figure 5] [2]

Figure 4. RNA peak of a successful assay for mRNA [1]

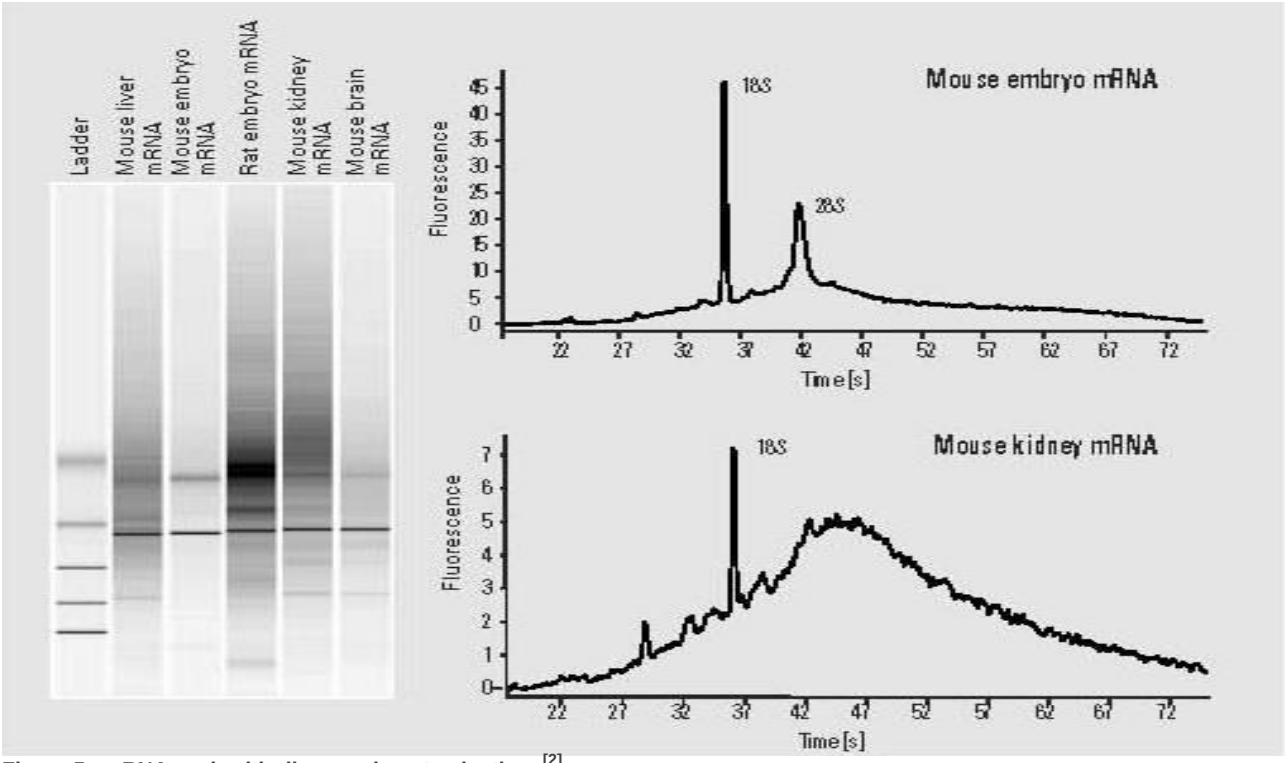

Figure 5. mRNA peak with ribosomal contamination [2]

Intact poly (A)+ RNA samples display broad size distributions [figure 6] and the size range can be estimated by overlaying the RNA 6000 ladder containing RNA fragments of known size. Large transcripts are detected late in the analysis window [3].

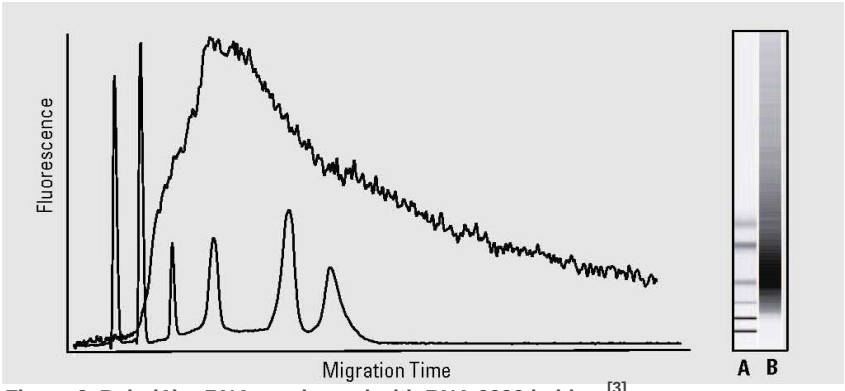

Figure 6. Poly (A) + RNA overlapped with RNA 6000 ladder [3]

As with total RNA, RNase degradation of mRNA shifts the RNA size distribution toward smaller fragments [figure 7]. When cDNA or cRNA pools are produced from partially degraded RNA, these pools will exhibit similarly short size distributions [3].

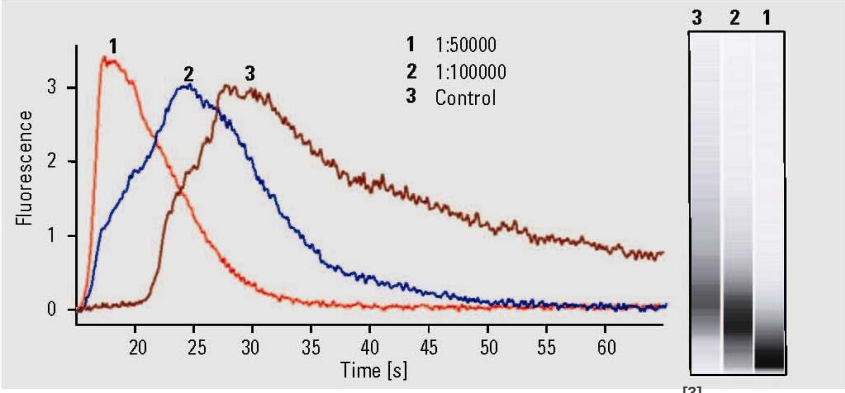

Figure 7. Illustration of increasing degradation of poly (A) + mRNA [3]

Details of Bioanalyzer Report Summary

MGS generates a **Bioanalyzer Report Summary** of the data generated from the Agilent Bioanalyzer LabChip run. This is saved as a PDF document and is sent to the customer by e-mail.

The important information reported in the **Bioanalyzer Report Summary** is:

- The type of assay performed. The assays offered at MGS are:
  - Eukaryote Total RNA Nano
  - Prokaryote Total RNA Nano
  - Plant Total RNA Nano
  - mRNA Nano
- Electropherogram File Run Summary:
  - Equivalent to a gel image
  - Contains the ladder lane, the sample lanes and the internal lower marker used for sizing the products.
- Chip Comments:
  - Identifies the online customer request, so the customer knows which request the data relates to.
- Summary Electropherogram images:
  - Small image of each samples electropherogram profile.
  - Includes RIN numbers for total RNA assays.
- Results Flagging Colour for total RNA assays:
  - **Green colour flag –** Samples with RIN value equal to or greater than the primary threshold of 8.0.
  - **Yellow colour flag –** Samples with RIN value equal to or greater than the secondary threshold of 5.0 and less than the primary threshold of 8.0.
  - **Red colour flag –** Samples with RIN value less than the secondary threshold of 5.0.

  NOTE: MGS sets a primer threshold of 8.0 for the RIN number. This means high quality RNA should have an RIN of ≥8.0. RNA which falls between the primary and secondary thresholds (RIN <8.0, ≥5.0) can be of acceptable quality depending on the down-stream application.

- Electropherogram profiles for each sample:
  - The ribosomal RNA (rRNA) peaks are identified for total RNA assays, and for mRNA assays there should be a single broad peak.
  - The lower 25nt marker is identified.
  - A gel profile is also provided.

Specific Results Details for Total RNA Assays

The specific details reported in the **Bioanalyzer Report Summary** for the **Total RNA assays** are:

- RNA Area
- RNA Concentration (ng/µl)
- rRNA ratio – 28S/18S for eukaryote and plant assays, and 23S/16S for prokaryote assays
- RNA Integrity Number (RIN)
- Result Flagging Colour
- Result Flagging Label

rRNA ratio: MGS requires high quality RNA to have an rRNA ratio of 1.5-2.5.

RNA Integrity Number (RIN): MGS requires high quality RNA to have an RNA Integrity Number (RIN) of ≥8.0, which is equal to or greater than the primary threshold, to be of high quality.

Results Flagging Colour:

- **Green colour flag –** Samples with RIN value equal to or greater than the primary threshold of 8.0.
- **Yellow colour flag –** Samples with RIN value equal to or greater than the secondary threshold of 5.0 and less than the primary threshold of 8.0.
- **Red colour flag –** Samples with RIN value less than the secondary threshold of 5.0.

Results Flagging Label:

- **RIN ≥8.0:** The sample's RIN value is equal to or greater than the primary threshold of 8.0.
- **RIN ≥5.0, <8.0:** The sample's RIN value is equal to or greater than the secondary threshold of 5.0 and less than the primary threshold of 8.0.
- **RIN <5.0:** The sample's RIN value is less than the secondary threshold of 5.0.

Specific Results Details for mRNA Assays

The specific details reported in the **Bioanalyzer Report Summary** for the **mRNA assays** are:

- RNA Area
- RNA Concentration (ng/µl)
- rRNA Concentration %

rRNA Concentration: MGS requires the concentration of rRNA in a high quality purified mRNA sample to be ≤5%.

Details of Bioanalyzer Service QC Report

MGS generates a **Bioanalyzer Service QC Report** and is a summary of our services analysis of the data quality. This is saved as an excel document and is sent to the customer by e-mail.

Bioanalyzer QC report for Total RNA Assays

The report is filled in as follows:

- Customer Name
- Username/request ID
- Assay Performed: Either 'Eukaryotic total RNA', 'Prokaryotic total RNA', 'Plant total RNA', or 'mRNA'.
- LabChip: 'RNA 6000 Nano LabChip'.
- Table contains:
- Sample Name: From the customer online submission.
- Sample Concentration (ng/µl): From the **'Bioanalyzer Report Summary'.**
- RNA Integrity Number (RIN): From the **'Bioanalyzer Report Summary'.**
- 28S/18S Ratio or 23S/16S Ratio**:** From the **'Bioanalyzer Report Summary'.**
- Samples meet/do not meet Quality Standards: The sample is marked with (*) if the sample meets MGS Quality Standards, and the sample is marked with (!) if the sample does NOT meet MGS Quality Standards.
  NOTE: Please refer to the section **'Bioanalyzer Quality Standards'.**
- If any of the samples do not meet MGS quality standards, the section called **'Reasons the sample(s) do not meet MGS quality standards',** with be completed explaining the reasons.
- Name and Authorising Signature: The person running the assay fills in their name and signature.

Bioanalyzer QC report for mRNA Assays

The report is filled in as follows:

- Customer Name.
- Username/request ID
- Assay Performed: Either 'Eukaryotic mRNA', 'Prokaryotic mRNA', or 'Plant mRNA'.
- LabChip: 'RNA 6000 Nano LabChip'.
- Table contains:
- Sample Name: From the customer online submission.
- Concentration (ng/µl): From the **'Bioanalyzer Report Summary'.**
- rRNA Concentration %: From the **'Bioanalyzer Report Summary'.**

- Samples meet/do not meet Quality Standards: Mark the sample with (*) if the sample meets MGS Quality Standards, and mark the sample with (!) if the sample does NOT meet MGS Quality Standards.
  NOTE: Please refer to the section **'Bioanalyzer Quality Standards'.**
- If any of the samples do not meet MGS quality standards, the section called **'Reasons the sample(s) do not meet MGS quality standards',** will be completed explaining the reasons.
- Name and Authorising Signature: The person running the assay fills in their name and signature.

Bioanalyzer Quality Standards

Quality Standards for Total RNA Assays

- MGS requires the RNA Integrity Number (RIN) to be equal to or greater than 8.0 for high quality RNA.
- MGS requires the 28S/18S or 23S/16S ratio to be 1.5-2.5 for high quality RNA.

Quality Standards for mRNA Assays

- MGS requires the rRNA concentration to be less than or equal to 5% for high quality mRNA.

References

1. Agilent Technologies Inc., Checking Your Agilent RNA 6000 Nano Assay Results, Agilent Technologies 2006, Technical notes, pp24-27.

2. Kuschel, M. 2000. Analysis of messenger RNA using the Agilent 2100 Bioanalyzer and the RNA 6000 LabChip® kit, Agilent Technologies Inc., Application notes, p5.

3. Ausserer, W., Kuschel, M. 2000. Characterization of RNA quality using the Agilent 2100 Bioanalyzer, Agilent Technologies Inc., Application notes, pp1-4.