ORAgene®•RNA

Interpreting bioanalyzer results for RNA collected using Oragene®•RNA

The Agilent Bioanalyzer is an instrument for assessing the "quality" of RNA used for microarray analysis. The sharpness of the ribosomal RNA peak and the low baseline between peaks on the electropherogram generated by the instrument are used as measures of the integrity of the ribosomal RNA and, by implication, the integrity of messenger RNA. The bioanalyzer uses an algorithm to generate an "RNA integrity number" (RIN) that helps researchers decide if the RNA is of sufficiently high integrity to proceed with microarray analysis.

Most high quality RNA samples share the same common features:

- distinct 18S and 28S ribosomal peaks (or 16S and 23S for prokaryotic samples)
- the absence of smaller peaks between the two ribosomal peaks
- a flat baseline prior to the 18S ribosomal peak

In an intact RNA sample, the 28S and 18S RNA peaks should have a ratio of approximately 2:1. If the RNA has begun to degrade, the peaks will be less sharp as smaller and faster migrating fragments are generated. Degradation will also generate a higher background and the ratio of the two peaks will decrease as the larger 28S RNA tends to degrade quicker than the 18S RNA. Furthermore, the presence of DNA can contribute to an elevated baseline. All of these factors will result in a decreased RIN.

The bioanalyzer is of particular value for assessing the quality of RNA from sterile sources containing a high RNA to DNA ratio such as cultured cells, primary tumour fragments and organ tissue. When dealing with RNA from blood, the RNA to DNA ratio is much lower than from other sources and therefore much more attention must be paid to effectively removing contaminating DNA in order to obtain a high RIN value.

High quality messenger RNA can be obtained from saliva using DNA Genotek's Oragene®•RNA product, however, for several reasons, it is more difficult to obtain a high RIN value. As with blood, saliva has a lower RNA to DNA ratio than that of cultured cells or tissue therefore similar care must be taken to remove contaminating DNA. Additionally, saliva is not a sterile source of nucleic acid; some of the RNA contained in saliva originates from oral bacteria. The ratio between the 16S and 23S bacterial ribosomal RNAs is less than the human 18S:28S ratio. The algorithm used by the bioanalyzer was not designed for use with samples containing both human and bacterial RNA so the instrument generates a lower RIN that is not indicative of the integrity of the RNA.

Instead of relying on the RIN, a visual assessment of the electropherogram can provide valuable insight into the quality of the RNA. The presence of sharp 23S and 16S peaks with a low background is itself a very good indication of the high integrity of the human messenger RNA.

For the reasons indicated above, quantification of total RNA in Oragene•RNA by absorbance or by the bioanalyzer is not a useful measure of the amount or integrity of messenger RNA. Instead, we recommend that the quantity of human-specific RNA be determined by quantitative PCR (qPCR) using human-specific 18S primers. For detailed information about the stability and yield of RNA from saliva collected using Oragene•RNA, see our white paper[1].

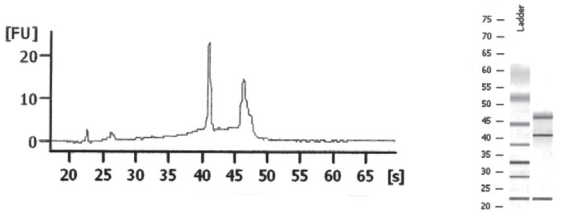

***Figure 1**: A representative bioanalyzer electropherogram and gel image for RNA purified from an Oragene•RNA/saliva sample using the procedure described in our protocol[2]. Distinct 16S and 23S rRNA peaks preceded by an area of low background indicate the presence of intact RNA. Sharp corresponding bands are visible on the gel image. RIN = 7.4.*

References

1 Stability and yield of RNA collected from saliva using Oragene®•RNA for expression analysis. DNA Genotek. PD-WP-008.

2 Oragene•RNA purification protocol using the Qiagen RNeasy Micro Kit for volumes up to 1,000 µL. DNA Genotek. PD-PR-021.

All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.

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