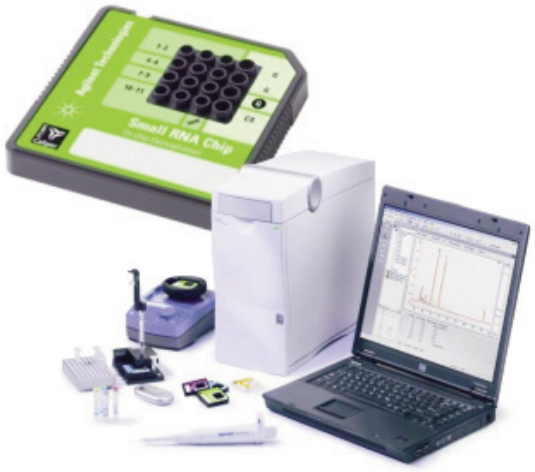
Monitoring extraction efficiency of small RNAs with the Agilent 2100 Bioanalyzer and the Small RNA Kit

Application Note

Genomics



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Abstract

High quality small RNA extraction is a critical aspect in miRNA expression profiling. Various RNA isolation methods using commercially available kits can extract total RNA enriched for small RNA. However, each of these protocols yields small RNA preparations that can differ in total quantity and quality, which may affect down-stream experiments. This Application Note demonstrates the use of the Agilent 2100 Bioanalyzer with the RNA 6000 Nano and Small RNA kits for monitoring RNA integrity and small RNA extraction efficiency. RNAs extracted from three different commercially available kits were tested on the Agilent 2100 Bioanalyzer to determine the quality of the extracted RNA and uncover differences in total and small RNA yields.

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Introduction

RNA quality is a key factor for the success of downstream applications, such as microarray-based gene expression analysis or RT-qPCR[1]. Various commercially available RNA isolation kits have been optimized for miRNA analysis to recover small RNA or offer protocols specific for the enrichment of small RNAs. However, the quality and quantity of the samples may be highly variable due to the methods used and the performance of the kits. RNA quality control is strongly advised prior to proceeding with costly experiments.

The Agilent RNA 6000 Nano and Pico kits provide rapid user-independent measurements and have become the gold standards for assessing RNA integrity. In addition, the Agilent Small RNA kit provides an analytical solution for monitoring small RNA molecules. It measures absolute miRNA content [pg/µl] and its percentage relative to small RNA (miRNA/small RNA ratio %). This allows for the identification and monitoring of miRNA fractions in total RNA extracts, and optimization of RNA isolation and purification protocols.

This Application Note demonstrates the use of Agilent RNA kits for monitoring the performance and extraction efficiency of total and small RNA extraction experiments, with a focus on miRNA content. Three commercially available RNA isolation kits were tested for total RNA and small RNA integrity and quantity analysis. Differences in extraction efficiency and quality of total and small RNA fractions are easily visualized by the Agilent 2100 Bioanalyzer.

Experimental

Cell culture

HEK293 cells were obtained from ATCC and cultured in DMEM (MEM) medium containing 10% FBS, and 1% Pen/Strep. Cells were maintained at 37 °C in a 5% CO_2 atmosphere. Once the cells were confluent, they were harvested and frozen at –80 °C until use.

RNA extraction

Three commercial RNA extraction kits (Kit A, Kit B and Kit C) were used to isolate total RNA according to the manufacturer instructions. These selected kits claim to collect enriched small RNA content in total RNA fraction. Equal cell numbers were used for each extraction (1.75 million cells), within the specifications of the kits.

Total RNA 6000 Nano and Small RNA Assays

RNA samples extracted by the three kits were analyzed on the Agilent 2100 Bioanalyzer using the Agilent 2100 Expert Software (Rev. B.02.05). The RNA 6000 Nano kit was used for quantification of total RNA and integrity assessment (RIN). The Small RNA kit was used for separation of small RNA species and quantification of the miRNA/small RNA ratio.

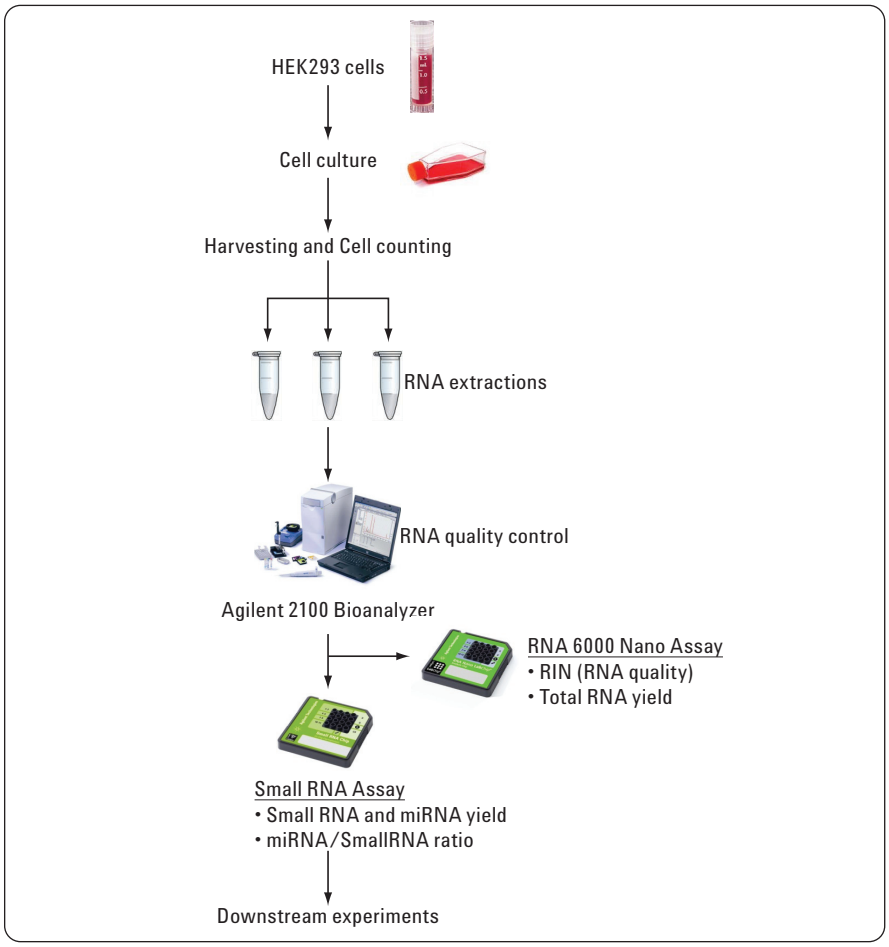

Figure 1
Total and small RNA experimental workflow analysis.

Results and discussion

A typical small RNA extraction workflow is shown in Figure 1. The quality control step assesses critical parameters such as purity, yield and integrity of the RNA. These vital parameters should be reproducible irrespective of RNA extraction protocols.

Total RNA quality and yield

Degradation of total RNA leads to the accumulation of small RNA fragments resulting in an overestimation of the miRNA and small RNA content in samples. Therefore, it is critical that total RNA samples are initially evaluated for integrity. Total RNA samples enriched for small RNA were extracted from HEK293 cells using three commercially available kits, and analyzed on the Agilent 2100 Bioanalyzer. RNA samples were measured in triplicate with the RNA 6000 Nano kit. The average RIN scores (RIN ± SD) were 8.1 ± 0.2, 9.4 ± 0.05 and 9.4 ± 0.5 indicating that overall, high quality RNA was extracted by the Kit A, B and C protocols, respectively. Furthermore, the Agilent 2100 Bioanalyzer results showed major differences in total RNA concentrations between replicate extractions performed by individual kits (data not shown). Although the same number of cells were used, the total yield varied significantly between the protocols (Table 1). The differences in RIN observed by comparing Kit A with Kits B and C, as well as the dissimilarity in RNA yield of replicate extractions may be due to variations in sample handling or kit performance. On average, Kit B showed higher yields of total RNA compared to Kit A and Kit C (Table 1). Variability in total RNA integrity and quantity can influence downstream experiments and are important parameters to consider in experimental design.

RNA 6000 Nano Assay*	Kit A	Kit B	Kit C	Kit Cs
Total RNA concentration [ng/µL]	12	161	34.6	–
Total RNA yield [ng]	600	8050	3460	–
Small RNA Assay*				
Small RNA concentration [pg/µL]	191.1	2729.6	1449.1	473.7
miRNA concentration [pg/µL]	15.9	275.6	113.1	89.3
miRNA/small RNA ratio [%]	8	10	8.3	17
Total small RNA yield [ng]	28.6	409.4	434.7	142.1
Total miRNA yield [ng]	2.4	41.3	33.9	26.7

Table 1
Agilent 2100 Bioanalyzer RNA 6000 Nano Assay and Small RNA Assay results from different commercial RNA extractions kits.
*Average of three Bioanalyzer measurements

Small RNA and miRNA analysis

Extracted RNAs were analyzed with the Small RNA kit (Figures 2 a–c) to monitor the composition of the small RNA fractions. The replicate measurements of each preparation showed reproducible peak profiles in all three protocols with well separated small RNA populations. The major content is comprised of transfer RNA (tRNA) and other small RNAs of larger size (represented by multiple peaks between 40–150 nt). Significant differences in small RNA yields observed between different extraction kits are in accordance with total RNA yield (Table 1).

An alternate protocol for Kit C was performed for the specific enrichment of small RNA (referred to as Kit Cs, Figure 2d). Kit Cs showed a lower number of small RNA fragments of larger size (> 100 nt) resulting in the highest miRNA/small RNA ratio in comparison to the other kits. The data suggest that this protocol enriches the miRNA content in small RNA extractions. This information may be vital when optimizing miRNA extractions for specific downstream experiments where larger small RNA fragments can negatively influence RNA applications.

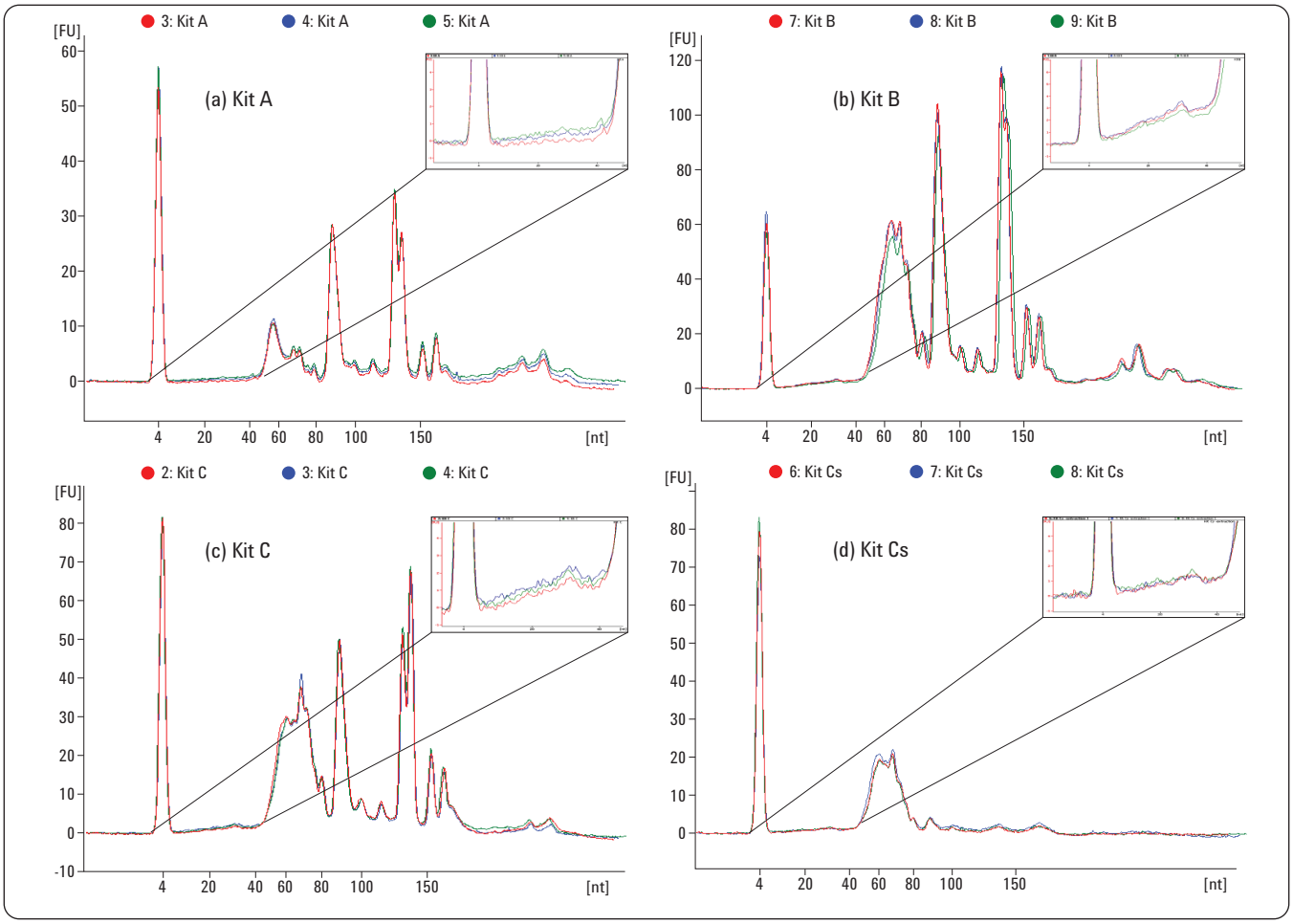

Figure 2

Agilent Bioanalyzer Small RNA assay. Small RNA samples isolated with three different kits (A, B, and C) were subjected to Bioanalyzer Small RNA analysis. Kit Cs refers to results obtained with Kit C using an alternative protocol. Overlays of three bioanalyzer replicate measurements are shown. Insert shows the expanded miRNA region (4–40 nt).

Conclusions

Critical parameters in the small RNA analysis workflow are reproducible miRNA yields and determination of relative concentrations of miRNA in the small RNA population. Therefore, it is important to examine total RNA and small RNA extraction efficiency for successful miRNA expression analysis.

Agilent 2100 Bioanalyzer analysis with the RNA 6000 Nano and Small RNA kits can evaluate the integrity and quantity of total RNA, small RNA and miRNA. Although equal numbers of cells were used for RNA extractions, significant differences in total RNA yield and integrity, as well as miRNA ratio can be observed between different extraction kits.

In summary, the Agilent 2100 Bioanalyzer RNA assays allowed the comparison of various RNA isolation methods for RNA quality and yield consistency. These factors will help optimize small RNA preparation protocols.

References

1.
S. Fleige, M. W. Pfaffl, RNA integrity and the effect on the real-time qRT-PCR performance. *Mol. Aspects Med.* 27, 126–139 (2006).

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