



Automated Preparation of Agilent 2100 Bioanalyzer LabChips for RNA Analysis

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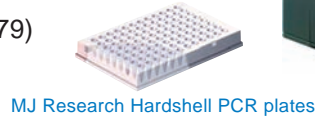
Abstract

Total RNA is the starting point for several applications. These include cDNA synthesis for gene expression, RNase protection assays, probes for microarrays, RNAi transfection experiments, and RT-PCR. Extraction methods involving filter plates, magnetic particles, and precipitation are typically used to isolate the RNA out of the tissue samples. Once the RNA is separated from the extraneous material, its quality and quantity must be determined. Experiments which utilize this RNA can be expensive and/or time-consuming. Knowing that the starting RNA is of sufficient quality is vital for success of these applications.

One quality assurance technique is to use Agilent's 2100 Bioanalyzer to determine RNA integrity¹. The 2100 Bioanalyzer utilizes planar microfluidic labchips. Each chip holds 12 samples and is manually loaded with reagents and samples. This makes analyzing large numbers of samples tedious. The Caliper Sciclone ALH 3000 equipped with the Z-8 eight channel pipettor can load reagents and/or samples to these chips.

Materials

- ❖ Caliper Sciclone ALH 3000 equipped with the Z-8 Eight Channel Pipette
- ❖ Agilent 2100 Bioanalyzer
- ❖ Agilent nanoRNA LabChip Kit (P/N 5067-1511)
- ❖ Agilent DNA 7500 LabChip Kit (P/N 5067-1506)
- ❖ HeLa RNA (Ambion)
- ❖ Planar LabChip Holder (Caliper custom part, call to request)
- ❖ Eppendorf Tube Rack (Caliper P/N 79379)
- ❖ MJ Research Hardshell PCR Plates
- ❖ 100 μ L Tips (Caliper P/N 109079)



Methods

HeLa control RNA was diluted by a factor of 10 with RNase free water to a final volume of 150 μ L. The tube of diluted RNA and RNA ladder (6 μ L) was denatured as described in nanoRNA kit manual. The control RNA was manually distributed to column 1 and rows A-D of column 2 of a PCR plate. Denatured ladder was placed in well A3 of the same plate. The Maestro application was executed to prepare the microfluidic chip, loading all reagents and denatured samples with the Z-8 pipetter. The chip was vortexed as described in the kit manual and analyzed on the Bioanalyzer. While the chip was running, the next chip was prepared on the Sciclone ALH 3000. A total of 3 chips were prepared from the same PCR plate. A single DNA LabChip was prepared on the Sciclone ALH 3000 and analyzed following the DNA 7500 kit instructions.

Results and Discussion

A set of macros were written to aid the user in defining the assay, sample locations, and reagent options. These macros were written in Visual Basic for Applications (VBA) embedded in Maestro software. The main application in Maestro initialized the Sciclone and presented the user with an interface (Figure 1).

On the window were options for preparing the chip(s), loading samples, assigning where the samples are located on the deck and within the sample plate, where to put the samples on the chip, and sample analysis information for the Agilent software. The user could auto-fill a chip with a single row or column or could randomly place samples in the chip. If the user requested all reagents be loaded, a pause was sent to the system for priming the chip. The HeLa control RNA and ladder were denatured offline in an Eppendorf tube. The user interface allowed the denaturing aliquot transfer to be skipped.

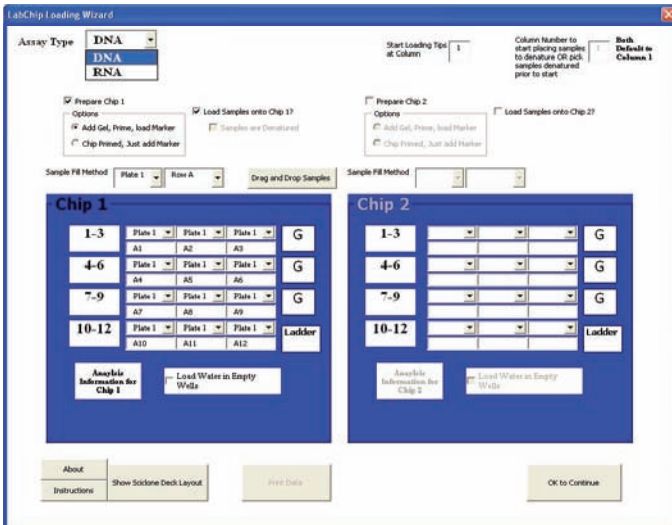


Figure 1. Graphical User Interface for preparing and/or loading LabChips

This reduced the amount time the RNA was at room temperature. If a random selection of samples need to be aliquoted for denaturing, the software could be set to do this.

Quantitation of the total RNA yielded results close to the value stated on the label. The precision for the RNA assay was 10% for all 36 wells (12 wells on 3 chips) (Table 1). The standard technique for quantitation of RNA and DNA is absorbance at 260 nanometers. This assay is for total nucleic acid. It cannot discriminate DNA from RNA nor can it tell you the integrity of the RNA.

	Chip 1 Wells 1-12	Chip 2 Wells 1-12	Chip 3 Wells 1-12	All wells
	Average ng/ μ L	Average ng/ μ L	Average ng/ μ L	Average ng/ μ L
RNA Concentration	107	98	103	103
%RSD	13	11	4	4

Table 1. Results of preparing 3 separate RNA LabChips using the Sciclone ALH 3000 (TV=100 ng/ μ L).

Results and Discussion (cont)



Figure 2. Sciclone ALH 3000 accessing a well on a LabChip

Average Wells 1-12 of a single chip					
Peak	Size(bp)	%RSD		Conc.(ng/ μ l)	%RSD
1	50.00	0.0		8.3	0.0
2	89.75	1.1		10.1	3.5
3	294.58	0.5		9.1	3.9
4	498.08	0.5		10.7	4.4
5	709.00	0.6		10.8	3.7
6	1207.25	2.2		11.5	3.4
7	1758.92	3.7		11.4	3.4
8	2853.58	1.4		11.8	3.4
9	4672.58	2.6		11.9	3.6
10	10380.00	0.0		4.2	0.0

Table 2. Results of loading DNA ladder on LabChip using the Sciclone ALH 3000 (TV= 8 ng/ μ L).

Measuring absorbance at 260 nm gave a result of 1.24 mg/mL compared to 1.03 mg/mL on the LabChip after dilution correction. The slightly higher determination from the optical density measurement was expected because the technique cannot distinguish the different nucleic acids present in the sample.

A single DNA chip was prepared using LabChip 90 ladder as the sample (diluted 5x) (Table 2). The quantitation was an average of 15% high for the sample. While this is within the specified +/-30% quantitation error, the Z-8's accuracy was confirmed to be +/- 7% at 1 μ L using tartrazine dye.

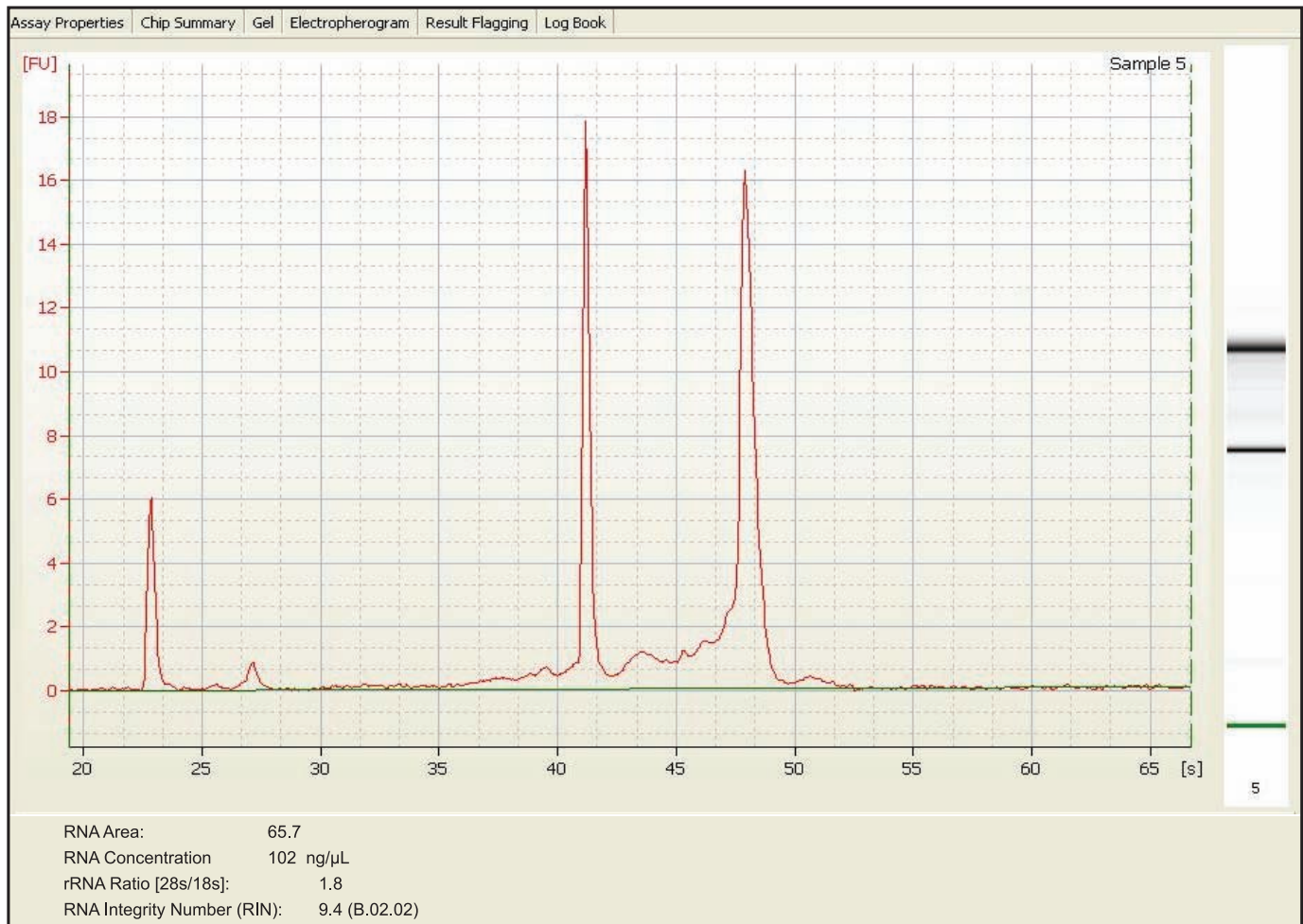


Figure 3. Electropherogram of HeLa control RNA from Agilent Expert Software

Conclusions

The Agilent 2100 bioanalyzer is the recommended instrument to determine RNA integrity. It is also capable of determining the amount and type of contamination as well as the quantity of RNA. There are a number of other applications on the bioanalyzer which could be easily automated. All of these currently require manual chip preparation and sample loading. The Sciclone ALH 3000 equipped with Z-8 is capable of preparing and loading the chips for the Agilent 2100. The Z-8's fixed 9 mm spacing and gantry's excellent motor control results in excellent tip placement in the small loading wells of the chip. This allows for the use of all 8 cannula for chip preparation. The result is faster prep times compared to a single channel device. The automation of chip preparation and loading improves reproducibility and allows the user to work on other tasks in the lab. This application can be scaled up to meet the demands of a lab with more than one bioanalyzer.

¹Andreas Schroeder, Odilo Mueller, Susanne Stocker, Ruediger Salowsky, Michael Leiber, Marcus Gassman, Samar Lightfoot, Wolfram Menzel, Martin Granzow, and Thomas Ragg, *BMC Molecular Biology* 2006, 7:3.