Automation of Stratagene Absolutely RNA 96 Microprep Kit with the Bravo Automated Liquid Handling Platform

Application Note

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Abstract

A new method was developed to automate RNA isolation in a 96-well format using the Bravo Automated Liquid Handling Platform and Absolutely RNA 96 Microprep Kit from Agilent Technologies (Automation Solutions and Stratagene divisions, respectively). Current RNA isolation protocols require extensive manual processing including repetitive pipetting and vacuum filtration steps. Using Agilent's VWorks Automation Control software, a script was written for the Bravo Platform to aspirate, dispense, mix, and transfer sample from a source 96-well tissue culture plate to the binding and collection plates of the Absolutely RNA 96 kit as well as to automate sample wash steps using an accessory vacuum filtration unit on the Bravo Platform. RNA quality was assessed on the Agilent BioAnalyzer 2100.

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Introduction

The Stratagene Absolutely RNA 96 Microprep Kit allows high throughput isolation of total RNA from small samples of cultured cells. This simple method eliminates toxic phenol-chloroform extractions and time-consuming ethanol precipitations by selectively binding RNA from cell lysate on 96-well silica fiber matrix plates. However, there are repeated washes, filtration, and centrifugation steps that are amenable to automation. The Bravo Platform has nine plate decks or stations that can fully accommodate the Absolutely RNA 96 protocol. Described below are a set of three protocols, written for the Bravo Platform to automatically handle binding, DNase treating, washing, and isolated RNA elution.

Materials

- Agilent Bravo Automated Liquid Handling Platform (G5409A)
- 96 channel LT Disposable Tip Head (04730-202)
- Agilent Vacuum Filtration Station (G5432A)
- Agilent 96LT 200 µL sterile, filtered pipette tips (08585-102)
- Agilent VWorks Automation Control software
- Stratagene Absolutely RNA 96 Microprep Kit (#400793 or #400794)
- 96-well Tissue Culture dishes (e.g. Costar Flat Polystyrene, #3598)
- Buffer reservoirs and/or 96-well plates for reagent preparation and waste disposal
- Agilent Microplate Centrifuge or other tabletop plate centrifuge (G5405A)
- Sulfolane (Sigma # T22209)
- Ethanol

Protocol Workflow

The Absolutely RNA 96 protocol can be divided into three major steps:

1. Preparing the cell lysate and binding to the silica matrix plate
2. DNase treating on column (optional)
3. Wash and Elution

A summary of the steps for each are the following:

Lysis and Binding

1. Aspirate media from tissue culture dish
2. Dispense lysis buffer to dish
3. Dispense 80% sulfolane to dish and mix
4. Transfer lysate to Absolutely 96 Binding plate
5. Vacuum filter

DNase Treatment (optional)

1. Dispense low salt wash to Binding plate
2. Vacuum filter
3. Centrifuge to dry filter
4. Dispense DNase to Binding plate and incubate

Wash and Elution

1. Dispense high salt wash to Binding plate
2. Vacuum filter
3. Dispense low salt wash to Binding plate
4. Vacuum filter
5. Repeat 3 and 4
6. Centrifuge
7. Dispense elution buffer to Binding plate and incubate
8. Centrifuge

Using VWorks Automation Control software, three scripts were written for each of the three major steps outlined. Unique labware was first defined in the software to allow the Bravo Platform to properly position the tip head relative to the deck platepad. Accessories such as the vacuum filtration unit and tip boxes are predefined in the software. All three scripts are run independently to accommodate the optional DNase treatment.

Absolutely 96 Buffer Preparation

Reagents and buffers for the Absolutely 96 Microprep kit were prepared according to the user manual. Specifically, (1) lyophilized RNase-Free DNase I was reconstituted with supplied buffer and a 100 µL aliquot diluted in 5 mL DNase dilution buffer per 96-well plate, (2) wash buffers diluted to 1X concentration with 100% ethanol, (3) BME added to lysis buffer, (4) 100% sulfolane diluted to 80% with RNase-free water.

For use with the Bravo Platform, it is possible to use the following setup:

80% Sulfolane may be prepared in excess for use in a buffer reservoir (20 mL is sufficient to process 2 runs in 96-well plate format but more is required when using a reservoir). For DNase I, elution buffer, lysis buffer, and high salt buffer required the following volumes per well: 50 µL, 30 µL, 100 µL, and 500 µL, respectively. This necessitates aliquotting to a 96-well plate for dispensing by the Bravo platform. The Bravo Platform can be used to prepare these plates as well if desired. For low salt wash, a reservoir filled with 200 mL for each plate is sufficient.

Centrifugation

As required, plates were removed from the vacuum station on the Agilent Bravo Platform and spun in a tabletop centrifuge at 1100 g. Alternatively, the plate may be moved from the Bravo Platform automatically and spun in the associated Agilent Microplate Centrifuge.

RNA Analysis

Total RNA from 12 wells (4 corners and middle row) were run on the Agilent BioAnalyzer 2100 using an Agilent RNA 6000 Nanochip, and assessed for sample quality using BioAnalyzer 2100 Expert software version B.02.06.

Table 1: Bravo Setup Example

Deck Position	Binding	DNase Treat	Wash/Elution
1	Vacuum station with Absolutely 96 Binding Plate	Vacuum station with Absolutely 96 Binding Plate	Vacuum station with Absolutely 96 Binding Plate
2	Sample Tissue Culture Dish	DNase solution plate*	—
3	Sulfolane reservoir	—	—
4	Low salt wash reservoir	Low salt wash reservoir	Low salt wash reservoir
5	Waste plate	—	—
6	Lysis buffer		Elution buffer*
7	—	—	High salt wash reservoir
8	Filter Tip Box	—	Filter Tip Box*
9	Filter Tip Box	Filter Tip Box*	Filter Tip Box*

* Plates need to be swapped out before the start of the protocol. Note that the Bravo Platform tip head may be equipped with a plate gripper to move plates to a free platepad as required.

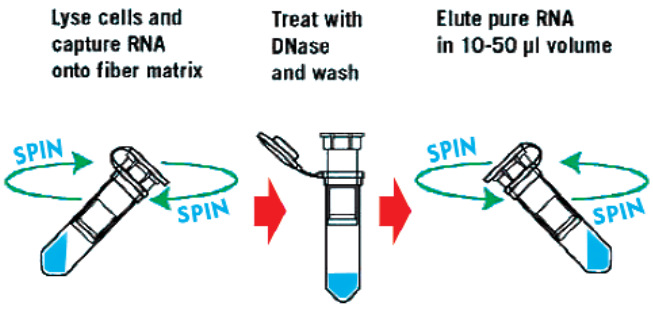

Figure 1. Absolutely RNA Kit Method

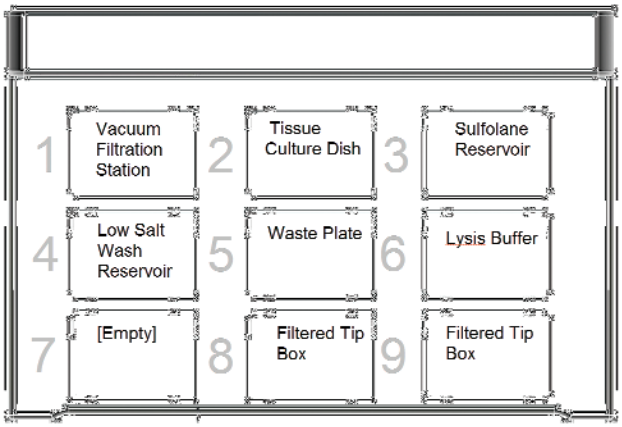

Figure 2. Bravo layout for lysis and binding steps

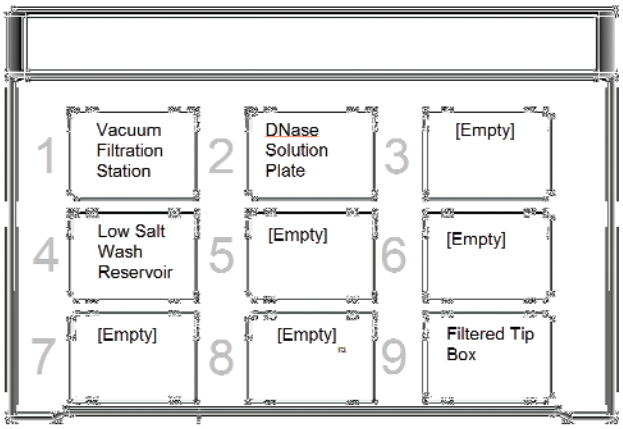

Figure 3. Bravo layout for DNase treatment step

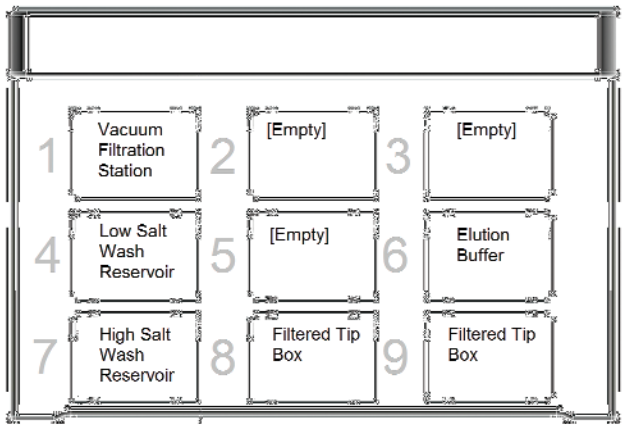

Figure 4. Bravo layout for wash and elution steps

Results and Conclusion

After completion of the RNA isolation, samples from the four corners and middle row of the 96-well tissue culture dish were analyzed using the Agilent BioAnalyzer 2100 to assess both the quality of the isolated RNA, as well as the absence of genomic DNA contamination. See Figure 5 for representative electropherograms produced using BioAnalyzer 2100 Expert software. In this case, samples are overlaid, with the four corners displayed in the top graph, and the middle row in the bottom graph. Note the RNA is fully intact with sharp peaks for 18S and 28S ribosomal RNA, the presence of 'small RNA' peak, and lack of genomic DNA contamination. RNA integrity numbers were also calculated by the 2100 Expert software and ranged from 9.2 to 9.6.

In conclusion, these results demonstrate that the Agilent Bravo Automated Liquid Handling Platform can be used successfully to automate total RNA isolation using the Absolutely RNA 96 Microprep kit.

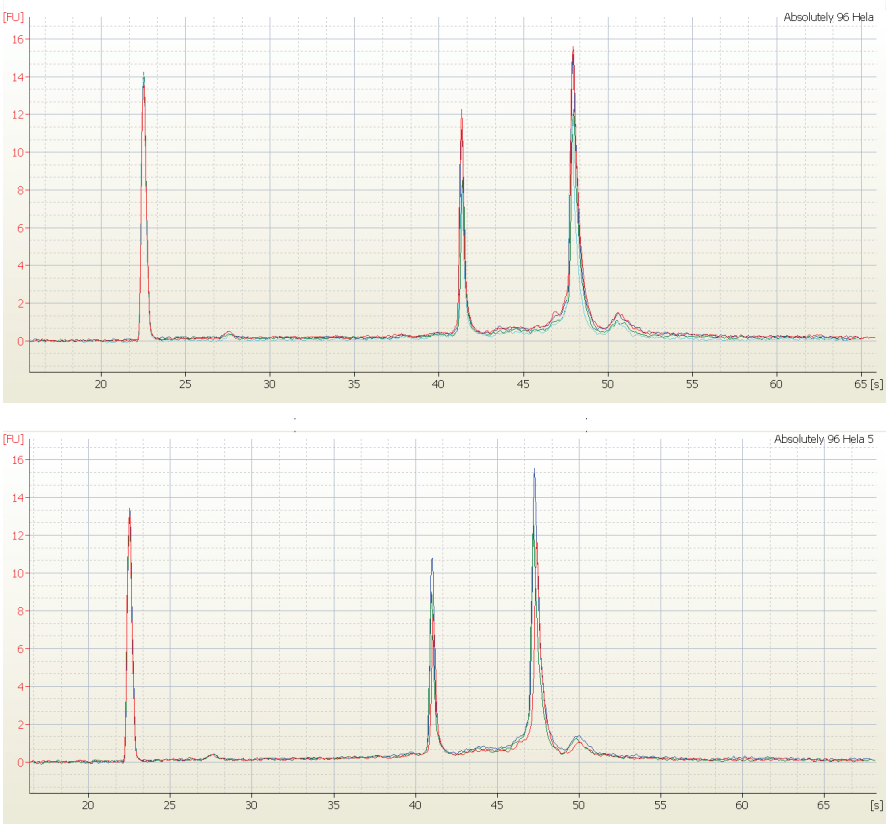

Figure 5: Electropherograms recorded using the Agilent BioAnalyzer 2100. Total RNA was isolated from HeLa cell culture in a 96-well plate using the Agilent Bravo Automated Liquid Handling Platform and Absolutely RNA 96 Microprep kit. Representative samples from the four corners (top) and middle row of the plate (bottom) are overlaid. Samples were analyzed using Agilent 2100 Expert software and assessed for absence of genomic DNA contamination and RNA quality. RNA integrity number (RIN) was determined by the software and ranged from 9.2-9.6.

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