High Throughput RNA Purification with Ambion® RNAqueous™ Kits on the Robbins® Tango™ Liquid Handling System

Jean Shieh*, Jeannine Carramao*, Joanna Wu*, Erica Samuel*, Jennifer Ho†, Marianna Goldrick†, and Arezou Azarani*

***Robbins Scientific® Corporation, Sunnyvale, CA; †Ambion, Inc., Austin, Texas**

Introduction

Numerous molecular biology applications such as Northern blotting, reverse-transcription, cDNA library construction, *in situ* hybridization, and microarray gene expression analysis require RNA as starting material. The need for RNA therefore generates a crucial requirement for high throughput methods of RNA purification.

Ambion's high throughput RNA isolation kits were specifically designed for the purification of 96 or 384 RNA samples. However, because manual RNA purification approaches have been proven to be inaccurate (due to errors in manual dispensing), laborious, and time-consuming, automation methods need to be developed and incorporated into RNA-isolation protocols. Automation will increase the efficiency, accuracy, and speed of RNA purification.

The Tango Liquid Handling System (Robbins Scientific, California) combines superior capacity, precision, speed, and flexibility to facilitate automated high throughput RNA purification. This system is designed to allow the repetitive and simultaneous aspiration or dispensing of 96 (or 384) samples of submicroliter and microliter volumes. The Tango system can, therefore, be used to automate all RNA purification procedures involving cell distribution, washing, lysis, and RNA elution, as well as subsequent downstream RNA applications such as quantification and RNA-based amplification procedures.

This application note describes a high throughput method of RNA purification using Ambion's RNAqueous™-96 and -384 kits on the Tango Liquid Handling System. Various parameters such as the reproducibility and consistency of RNA quantity and quality, as well as the speed of the purification process, are analyzed in depth.

Figure 1. The Ambion RNAqueous RNA isolation kit.

Figure 2. The Tango Liquid Handling System.

Materials and Methods

RNAqueous-96 (cat. # 1920) and RNAqueous-384 (available as a custom kit) RNA isolation kits (Figure 1), RNaseZap® (cat. #s 9780-82-84), and HeLa S3 cells were provided by Ambion, Texas. For each of the experiments, a Tango Liquid Handling System (Figure 2) equipped with either 96 1mL syringes or 384 100µL syringes, with DuraFlex™ needles, was used for liquid handling. Ethyl alcohol (cat. # A407-500) was purchased from Fisher, Illinois. Filtration was performed with either a Sorvall® RT6000B centrifuge set at 1900x*g*, 4°C, for 2 minutes or a Robbins vacuum manifold (cat. # 1029-42-0). The human β-actin primers (cat. #5402-1) and the Titan™ One-Tube RT-PCR* System (cat. #1855476) were purchased from Clontech, California, and Roche, Mannheim, Germany, respectively. RNA quantification was performed with the RiboGreen® RNA Quantitation kit (cat. # R-11490) from

*PCR patents are owned by Hoffman-LaRoche, Inc., and F. Hoffman-LaRoche Ltd., Nutley, NJ.

Molecular Probes, Eugene, Oregon. Fluorescence analyses were done with a TECAN™ SPECTRAFluor® Plus (TECAN, North Carolina) fluorescence plate reader set at 485nm excitation and 535nm emission wavelength.

The RNA Purification Kit

Ambion's RNAqueous technology is available in multiple formats including a high-throughput, 96 well plate format. A 384 well plate format is also available as a custom kit. The kits are designed for total RNA isolation from 100 to 10^6 cultured cells. The RNAqueous-96 kit is ideal for the isolation of total RNA from multiple samples and very small samples. The procedure utilizes an RNA-binding glass-fiber filter and either vacuum filtration or centrifugation methods to provide high yields of intact RNA. An optional on-the-filter DNase treatment can be performed to ensure removal of genomic DNA for RT-PCR applications.

Decontamination of the Tango System from RNase

Prior to beginning the RNA purification experiments, the Tango dispensing head (with fixed syringes), wash module, and stage were decontaminated from RNase. The stage and the Tango wash module were wiped with RNaseZap, RNase-free water, and ethanol. The Tango syringes were washed in the wash module for 2 cycles** with a 2% bleach solution and then for 3 cycles with RNase-free water. A 3-cycle wash with 50% RNaseZap was performed next, followed by a 3-cycle wash with RNase-free water. Finally, the head was washed with 100% ethanol for 2 cycles, and the syringes were emptied and then left to dry overnight.

Results and Discussion

The automation performance of Ambion's high throughput 96 and 384 RNA isolation kits on the Tango Liquid Handling System was evaluated by determining the purification period and the quantity and quality of the purified RNA samples. Sample-to-sample consistency in dispensing and reliability in yield and quality were calculated for the isolated RNA samples.

Measuring the Dispensing Precision of the Tango Syringes

To determine the uniformity and consistency of the volumes dispensed across the Tango syringe array, the coefficient of variance (CV) for specific dispensing volumes was calculated. Different volumes of a 10µg/mL fluorescein solution were dispensed into each well of a 96 or 384 well plate containing 0.1M Tris buffer. The final volume in each well after the fluorescein solution was dispensed was 100µL. Each plate was then read in a TECAN SPECTRAFluor fluorescence plate reader, and the CVs were determined across each plate for each of the dispensed volumes. The results, shown in Table 1A and B, indicate high uniformity and consistency of dispense across the array (CVs of less than 10%).

**"One cycle" was defined as an aspiration and a dispense at full syringe volume.

Table 1A: Dispensing Precision Using 1mL Syringe

Volume of Fluorescein Dispensed (µL)	Relative Fluorescence Units	% CV
1	7107	8.45
5	13025	7.03
10	25070	6.35
20	48278	2.79

Table 1B: Dispensing Precision Using 100µL Syringe

Volume of Fluorescein Dispensed (µL)	Relative Fluorescence Units	% CV
0.5	2636	9.44
1	6167	4.38
5	24332	4.30
10	44259	3.07

RNA Purification

HeLa cells in 1X PBS were dispensed at a density of 100,000 cells per well into the RNA filter plates using the Tango system. Figure 3 shows the Tango worktable setup used for cell transfers in these experiments. The protocol can be found on the Robbins website at www.robsci.com/techinfo.html.

Figure 3. Tango Worktable for Cell Transfer.

RNA purification was performed according to the Ambion instruction manual for the kits. Total RNA samples can be purified with a 96 or 384 kit in less than 40 minutes (including a 20-minute DNase treatment period). As the Tango stage is composed of twelve nests, with one nest assigned to the wash module and 7 nests assigned to each of the reaction buffers, four separate 96 or 384 RNA filter plates can be assigned to the remaining 4 nests (Figure 4). This setup allows the simultaneous isolation of a total of 384 (96×4) or 1536 (384×4) RNA samples in less than one hour. Figure 4 shows the Tango worktable setup and protocol used for RNA isolation. The protocol can be found on the Robbins website at www.robsci.com/techinfo.html. Purification speeds for the 96 and 384 kits are recorded in Table 2.

Figure 4. Tango Worktable for RNA Isolation.

RNA Quantity and Quality

To determine reproducibility in the quantity of RNA purified using the Tango system for all automated dispensing (including dispensing of cells into filter plates, dispensing of all reagents for the purification step, and dispensing of the RiboGreen samples and purified RNA samples for quantification), purified RNA samples in 96 and 384 plates were quantified using the RiboGreen assay. The expected RNA yield from 100,000 cells was approximately 2µg[1]. In agreement with the expected results, the total RNA yields purified from the 96 kit were approximately 2346.3ng per well with a CV of less than 12%. With the 384 kit, an average of 1474.2ng of total RNA sample per well were purified (from 80,000 cells) with a CV of less than 15%. Results are shown in Figure 5. Uniformity and consistency in RNA yields among different wells and experiments indicate that the Tango system enables a very reproducible method of automated RNA isolation.

The quality and integrity of the RNA samples purified were determined by performing RT/PCR analysis. Human β-actin primers designed to amplify an 838 bp β-actin fragment were used to carry out the RT/PCR experiments. All dispensing was performed by the Tango system. Figure 6 shows the Tango worktable setup used for these experiments. The protocol can be found on the Robbins website at www.robsci.com/techinfo.html. Results, shown in Figure 7, indicate that amplification is reproducible among the RNA samples purified from the different wells of the 96 and 384 kits. These results indicated that the integrity of the RNA samples

Table 2: Purification Period for RNA Isolation Using the Tango System. Times indicated show purification of one 96 or one 384 plate per experiment. ND stands for not determined.

	Purification Time (Min) with DNase Treatment		Purification Time (Min) with No DNase Treatment	
Kit format	Using Vacuum	Using Centrifuge	Using Vacuum	Using Centrifuge
96	28	40	8	20
384	ND	35	ND	15

Experiment 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2379.1	2302.8	2673.7	2588.8	2623.4	2807.4	2783.1	2489.6	2585.6	2730.6	2896.3	2730.6
B	2429.5	2130.5	2245.3	2328.4	1775.2	2899.5	2556.8	2837.2	2837.2	2766.1	2642.2	2766.1
C	2736.2	2362.8	2794.3	2300.2	2387.0	2659.2	2770.2	2606.3	2606.3	2857.3	2773.4	2857.3
D	2254.7	2430.8	2403.6	2302.9	2302.4	2603.2	2727.7	2442.8	2442.8	2707.2	2475.2	2707.2
E	2358.9	2487.7	2744.8	1969.1	2569.7	2854.7	2943.7	2341.1	2341.1	2572.8	2745.5	2572.8
F	2222.4	2421.0	2778.4	1282.5	2801.7	2925.8	2833.4	2617.2	2617.2	2192.1	2651.3	2192.1
G	2479.9	1957.9	2286.5	2799.3	2455.8	1879.1	0	2472.5	2472.5	2594.3	2675.9	2594.3
H	1863.4	2206.1	2203.8	2389.6	2731.0	2466.0	2446.2	2476.2	2476.2	2597.8	2816.5	2597.8

Experiment 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1521.3	1519.4	1763.8	1467.1	1845.9	1870.03	1555.8	1956.2	1815.6	1670.1	1647.7	2095.3
B	1617.2	1369.8	1765.0	1358.7	1568.5	1765.68	1467.6	1506.0	1486.0	1668.4	1570.9	1732.3
C	1687.3	1463.2	1465.8	1414.2	1504.1	1342.66	1537.7	1471.1	1601.2	1528.8	1531.3	1302.8
D	1413.7	2390.4	1446.9	1365.6	1788.2	1436.25	1683.7	1411.1	1594.3	1314.4	1540.4	1340.6
E	1553.5	1561.6	1440.8	1451.6	1559.5	1625.69	1443.0	1277.7	1480.4	1841.8	1265.9	1335.4
F	1599.8	1762.7	1465.7	1617.8	1511.1	1541.58	1303.2	1223.3	1085.2	1397.0	1595.9	1344.8
G	1807.2	1484.1	1452.2	1360.5	1114.7	1396.04	1309.2	1397.8	1416.7	1312.6	1343.0	1216.8
H	1701.7	1788.6	1615.9	1331.9	1498.2	1261.84	1255.3	1163.1	1314.1	1160.5	1513.6	1182.7
I	1743.0	1452.9	1398.2	1414.8	1443.9	1468.6	1187.2	1240.2	1215.8	1285.0	1114.4	1244.3
J	1562.4	1758.2	1282.8	1311.6	1553.9	1409.4	1386.5	1125.9	1341.0	1089.2	1199.4	1325.9
K	1497.3	1474.9	1377.0	1481.3	1359.7	1264.9	1183.7	1438.1	1280.5	1376.4	1231.9	1329.2
I	1246.8	1625.9	1188.0	1522.0	1411.7	1293.8	1216.5	1437.7	1409.5	1354.7	1240.3	1477.1
M	1349.6	1281.9	1271.4	1306.1	1383.0	1196.8	1357.7	1358.1	1277.0	1653.9	1253.4	1456.0
N	1164.8	1591.3	1324.2	1224.4	1455.7	1256.1	1620.5	1329.5	1279.1	1401.5	1754.8	1611.2
O	1612.4	1323.3	1342.8	1581.4	1639.4	1669.8	1850.2	1763.2	1564.4	1560.5	1924.3	1718.7
P	1536.0	2004.4	1706.1	1543.7	1723.1	1754.1	1893.2	1681.0	1525.7	1778.6	1621.4	1599.4

	13	14	15	16	17	18	19	20	21	22	23	24
A	2347.0	1598.7	1471.5	2347.0	1598.7	1471.5	2347.0	1598.7	1471.5	2347.0	1598.7	1471.5
B	1491.2	1282.3	1726.4	1491.2	1282.3	1726.4	1491.2	1282.3	1726.4	1491.2	1282.3	1726.4
C	1472.5	1126.6	1596.6	1472.5	1126.6	1596.6	1472.5	1126.6	1596.6	1472.5	1126.6	1596.6
D	1385.8	1320.7	1463.1	1385.8	1320.7	1463.1	1385.8	1320.7	1463.1	1385.8	1320.7	1463.1
E	1170.5	1327.8	1183.2	1170.5	1327.8	1183.2	1170.5	1327.8	1183.2	1170.5	1327.8	1183.2
F	1317.2	1079.4	1160.8	1317.2	1079.4	1160.8	1317.2	1079.4	1160.8	1317.2	1079.4	1160.8
G	1292.3	1073.6	1282.9	1292.3	1073.6	1282.9	1292.3	1073.6	1282.9	1292.3	1073.6	1282.9
H	1166.8	1162.1	1106.5	1166.8	1162.1	1106.5	1166.8	1162.1	1106.5	1166.8	1162.1	1106.5
I	1365.8	1394.8	1314.1	1118.5	1460.3	1457.4	1183.1	1527.4	1510.4	1580.8	1456.1	1526.6
J	1300.8	1248.7	1297.3	1130.7	1185.9	1564.9	2058.9	1366.1	1540.9	1375.3	1463.8	1746.4
K	1321.3	1232.3	1228.4	1215.5	1251.9	1362.2	1273.1	1731.4	1686.7	1539.0	1561.3	1573.7
L	1324.2	1058.9	1593.5	1344.4	1305.2	1191.5	1648.2	1717.8	1335.3	1700.0	1665.7	1614.0
M	1489.1	1608.8	1995.8	1383.7	1341.5	1360.1	1729.4	1551.6	1527.1	1545.3	1534.6	1481.9
N	1576.3	1219.3	1561.3	1299.6	1606.4	1483.9	1518.6	1502.9	1673.5	1577.3	1603.7	1668.0
O	1435.8	1356.9	1805.8	1660.3	1469.0	1527.1	1510.2	1398.7	1683.0	1924.3	1709.0	1490.4
P	1365.8	1394.8	1314.1	1118.5	1460.3	1457.4	1183.1	1527.4	1510.4	1580.8	1456.1	1526.6

Figure 5. Yields and CVs of RNA Samples.
The Tango Liquid Handling System was used for the dispensing of the purified RNA samples and the RiboGreen reagent. Yields are recorded in ng.

Experiment 1: Yields and CVs of RNA Samples Purified with Ambion's RNAqueous-96 Kit and Robbins' Tango Liquid Handling System. Total RNA samples were isolated from 100,000 HeLa cells (eluted in 100µL total volume) and quantified (10µL out of 100µL total volume) by the RiboGreen RNA Quantitation kit. The yield in well G7 was discarded from the calculation. Standard deviation (SD) and CVs were calculated for an average of three plates.
Average: 2525.1; SD: 284.6; CV%: 11.27.

Experiment 2: Yields and CVs of RNA Samples Purified with Ambion's RNAqueous-384 Kit and Robbins' Tango Liquid Handling System. Total RNA samples were isolated from 80,000 HeLa cells (eluted in 50µL total volume) and quantified (2.5µL out of 50µL total volume) by the RiboGreen RNA Quantitation kit Standard deviation (SD) and CVs were calculated for one plate.
Average: 1474.2; SD: 211.4; CV%: 14.3.

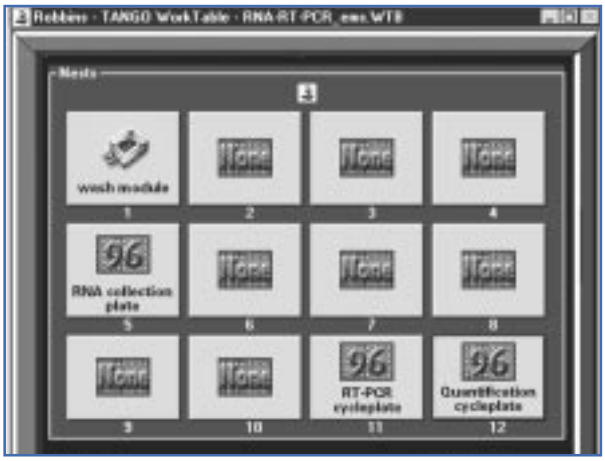

Figure 6. Tango Worktable for RT/PCR Experiment

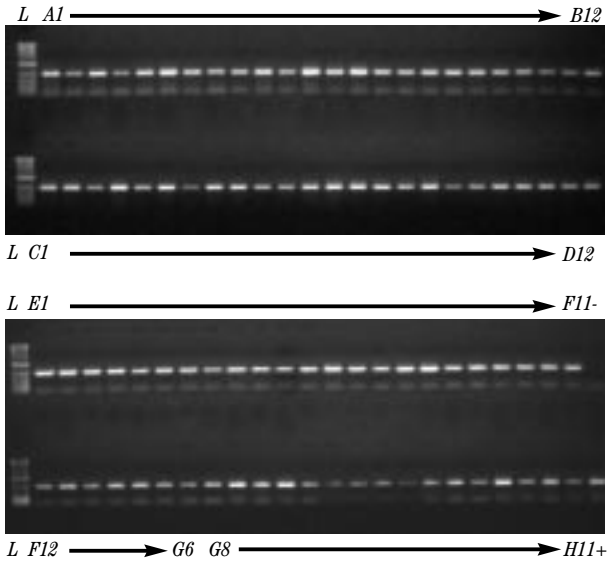

Figure 7. Results of RT/PCR Experiments Performed on the Purified RNA Samples. The experiments were performed according to the Titan RT/PCR kit instruction manual. A total of 30.5µL (out of 100µL total elution volume) of the purified total RNA samples were used in each reaction. Human β-actin primers were designed to amplify an 838 bp fragment. After the reaction samples were thermocycled, aliquots of the RT/PCR reactions were analyzed on a 1% agarose gel.
L: 1-kb DNA ladder, -: negative control with no RNA, +: positive control with total RNA

was preserved (RNA did not undergo degradation) regardless of the fact that fixed syringes instead of disposable sterile tips are used in the Tango head for dispensing (fixed syringes are used in the Tango system to provide a more convenient [less cumbersome] and cost-efficient [less waste produced] method of dispensing)[2].

Conclusion

The Tango Liquid Handling System can be successfully used to automate high throughput purification of RNA. The data obtained in this study clearly indicates that the Tango liquid dispensing system reduces the purification period while maximizing accuracy and precision of RNA purification.

References

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Robbins®
Robbins Scientific Corporation
1250 Elko Drive, Sunnyvale, CA 94089-2213
Phone: 408-734-8500 or 800-752-8585, Fax: 408-734-0300
e-mail: custserv@robsci.com, Internet: http://www.robsci.com

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