Automated Poly(A)+ mRNA Isolation

PolyATtract® Automated System for Isolation of Poly(A)+ mRNA

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Abstract

The PolyATtract® Automated System is a high-throughput poly(A)+ mRNA purification system that isolates poly(A)+ mRNA directly from total RNA, tissue culture cells or tissue lysates in a plate-arrayed format. The flexible PolyATtract® isolation can be performed on a liquid-handling workstation such as the Beckman Biomek® 2000 or FX for completely automated, walkaway isolation of poly(A)+ mRNA.

Introduction

The PolyATtract® Automated System isolates poly(A)+ mRNA directly from total RNA, tissue culture cells or tissue lysate. The system is formatted to allow high throughput and maximum flexibility on a variety of automated platforms. The system forgoes ethanol precipitations, phenol extractions, cesium chloride gradients and oligo(dT) cellulose columns by using MagneSphere® Paramagnetic Particle technology for direct purification of poly(A)+ RNA from samples.

The PolyATtract® Automated System has been optimized for isolation of 96 samples in a plate format. No modifications of the standard protocol are necessary when using less than 5mg of tissue, 1×10^6 tissue culture cells or 20µg total RNA.

This approach combines the speed and efficiency of solution hybridization with the convenience and speed (<1 minute) of magnetic separation.

Procedure

The PolyATtract® Automated System combines the disruptive and protective properties of guanidine thiocyanate and β-mercaptoethanol to inactivate the ribonucleases present in cell and tissue extracts. Guanidine thiocyanate (GTC), in association with SDS, acts to disrupt nucleoprotein complexes, allowing RNA to be released into solution and be isolated free of protein. The final GTC concentration allows for hybridization of the poly(A) sequence of most mature eukaryotic mRNA species to a synthetic biotinylated oligo(dT) probe yet maintains complete inhibition of the cellular RNases. Once hybridized, the biotinylated oligo(dT):mRNA hybrids are captured with Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs). The particles are washed at high stringency, and purified mRNA is eluted by the addition of Nuclease-Free Water.

This procedure yields a highly purified fraction of mature mRNA without organic extractions or precipitations. A summary of the procedure is provided in Figure 1.

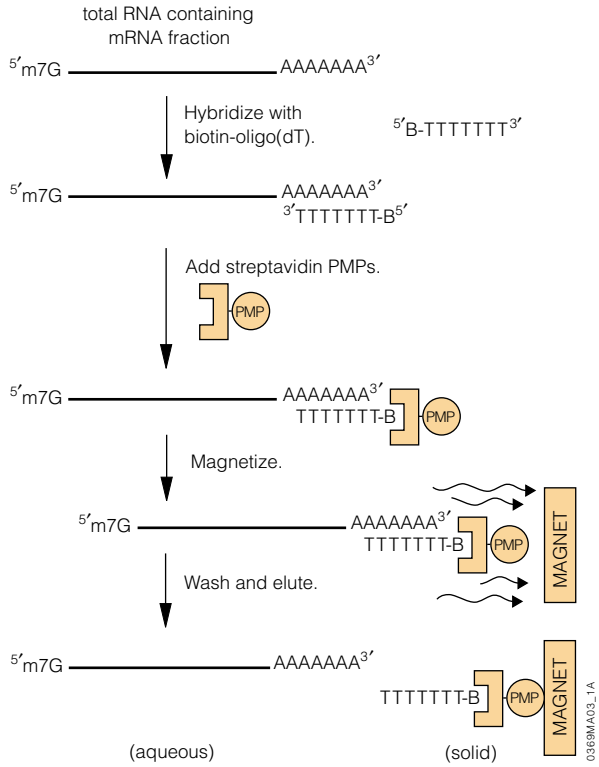

Figure 1. Schematic of the PolyATtract® Automated System protocol.

Paramagnetic particles incorporate iron oxide into submicron-sized particles that have no magnetic field themselves but form a magnetic dipole when exposed to a magnetic field. The use of paramagnetic particles eliminates the need for traditional column chromatography, centrifugation or any other special procedures.

Unlike procedures that use direct coupling of probes to paramagnetic particles, the PolyATtract® Automated System uses a biotinylated oligonucleotide probe to hybridize to the targeted nucleic acid in solution. The hybrids are then captured using coupled Streptavidin MagneSphere® Paramagnetic Particles. This approach combines the speed and efficiency of solution hybridization with the convenience and speed (<1 minute) of magnetic separation.

Analysis of the PolyATtract® Automated System

We tested the performance of poly(A)+ RNA purified with the PolyATtract® Automated System in both yield and recovery, as well as in endpoint and real-time RT-PCR[a]. Figure 2 shows the analysis of yield and recovery of isolated poly(A)+ mRNA using the PolyATtract® Automated System. We observed an average ≥1% recovery of isolated poly(A)+ mRNA relative to total RNA input. To evaluate the ability of the PolyATtract® Automated System to prepare poly(A)+ RNA from low numbers of cultured mammalian cells, small amounts of tissue or total RNA, we used RT-PCR to examine expression of the β-actin RNA (Figure 3). The β-actin message was detected in the poly(A)+ RNA purified from less than 39µg of mouse liver, 5×10^3 HeLa cells or 126ng of total RNA. Results may vary depending on the starting material and amplification primers used.

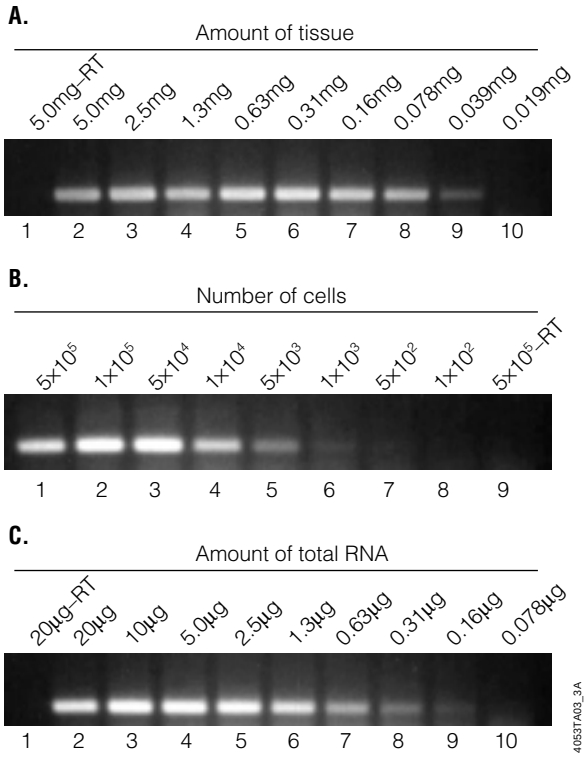

Figure 3. RT-PCR analysis of poly(A)+ RNA isolated with the PolyATtract® Automated System from tissue lysates, tissue culture cells and total RNA. Poly(A)+ RNA was isolated from the indicated amounts of mouse liver tissue lysate or mouse liver total RNA or the indicated number of HeLa cells. Purified RNA was then analyzed by RT-PCR using a β-actin primer pair. **Panel A.** Poly(A)+ RNA isolated from mouse liver tissue lysate and amplified. Lane 1, no reverse transcriptase control. **Panel B.** Poly(A)+ RNA isolated from HeLa cells and amplified. Lane 9, no reverse transcriptase control. **Panel C.** Poly(A)+ RNA isolated from mouse liver total RNA and amplified. Lane 1, no reverse transcriptase control.

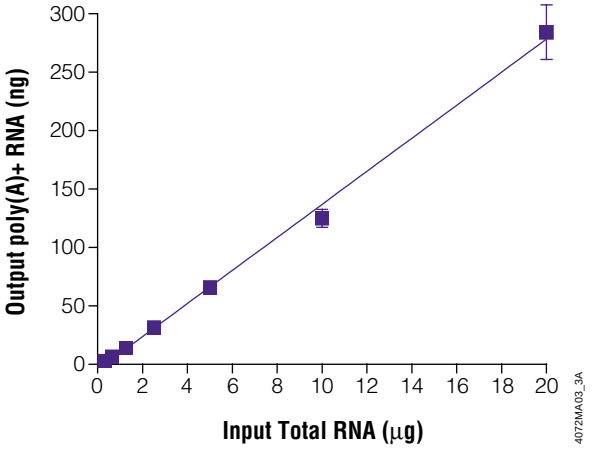

Figure 2. Yield and recovery using the PolyATtract® Automated System. Decreasing amounts of mouse liver total RNA were purified using the Biomek® 2000 method. Twofold serial dilution between 20µg and 0.31µg of total RNA was used. Yields were determined using the RiboGreen® reagent, and percent recoveries were calculated relative to input total RNA. All numbers are an average of 3 isolations, and standard deviations are indicated by the error bars. An average yield of 65ng was obtained from 5µg of total RNA with an average percent recovery of 1.3%.

We also evaluated the performance of purified poly(A)+ RNA in real-time RT-PCR (Figure 4, Panel A). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) message was amplified directly from total RNA, poly(A)+ mRNA purified from total RNA or poly(A)+ mRNA purified directly from cells. We found no significant differences in amplification between sources indicating little loss of purified message during the poly(A)+ isolation procedure. Additionally, we analyzed a dilution series using real-time RT-PCR to quantitate the removal of 18S ribosomal RNA during purification. 18S RNA was amplified from a serial dilution of total RNA and from purified poly(A)+ RNA. Comparison of the amplification profiles indicates that >99.5% of ribosomal RNA is removed (Figure 4, Panel B).

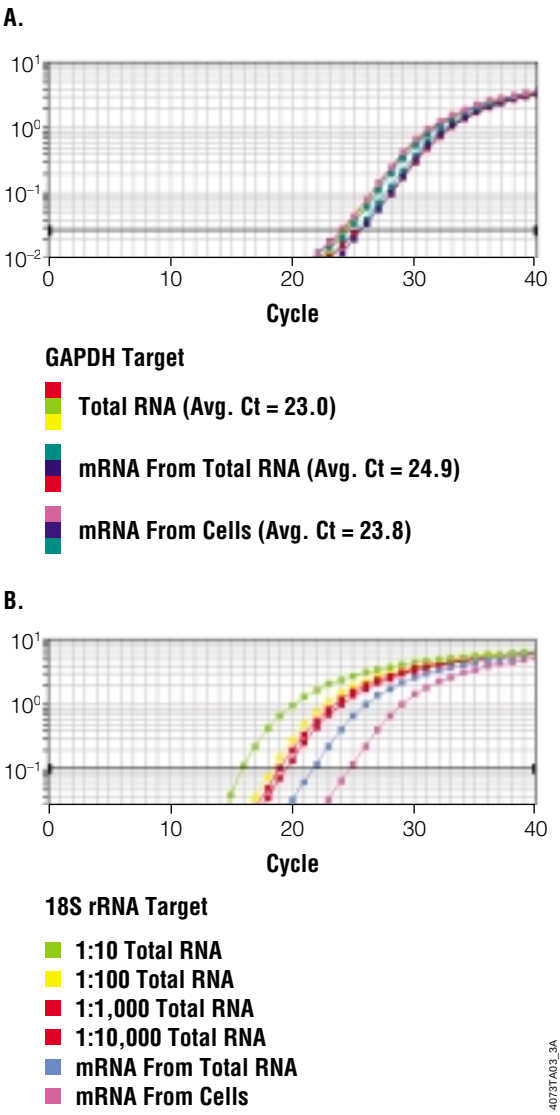

Figure 4. Real-time RT-PCR analysis of purified mRNA. Aliquots of total RNA, mRNA isolated from total RNA, or mRNA isolated directly from 1×10^5 HeLa cells were reverse transcribed. **Panel A.** Five microliter aliquots of the reverse transcription reactions were used for amplification of GAPDH target. **Panel B.** A dilution series of the total RNA reverse transcription reaction was compared to undiluted poly(A)+ RNA by amplification of an 18S ribosomal RNA target. While amplification of the GAPDH mRNA target is equivalent in the total and mRNA samples, dilution series analysis indicates that >99.5% of 18S rRNA is removed during purification of poly(A)+ RNA. All reactions were performed using TaqMan® reagents from Applied Biosystems, Inc.

Automated Poly(A)+ RNA Isolation on the Beckman Biomek® 2000 and FX Robotic Workstations

The PolyATtract® Automated System provides a fast, simple technique that is compatible with automation on liquid-handling workstations. We demonstrate the use of this system on both the Beckman Biomek® 2000 and the Biomek® FX robots. The initial deck configurations for both the Biomek® 2000 and FX liquid-handling workstations are shown in Figure 5. The automated PolyATtract® poly(A)+ purification method purifies intact, high-quality mRNA. Additionally, there is no detectable cross-contamination between samples (Figure 6). The open systems approach of the PolyATtract® chemistry allows implementation on a variety of liquid-handling platforms, in addition to the Biomek® 2000 and FX workstations.

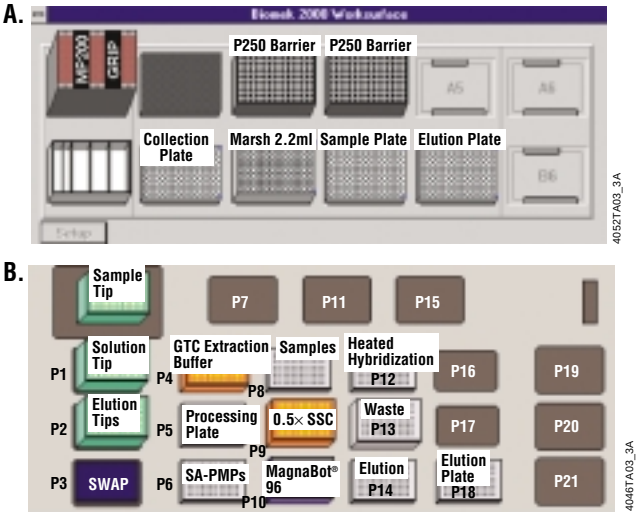

Figure 5. Deck layouts for the Beckman Biomek® 2000 (Panel A) and Biomek® FX (Panel B). More information for methods and a complete listing of method requirements are available from Promega Technical Services and in the *PolyATtract® Automated System Technical Bulletin*, #TB321.

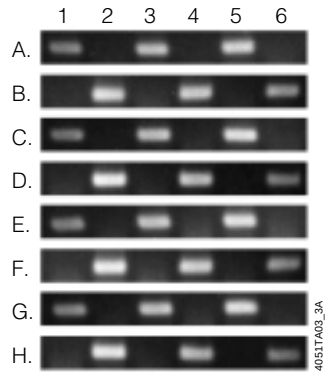

Figure 6. Undetectable cross-contamination. Poly(A)+ RNA was purified in a checkerboard pattern on the Biomek® 2000 workstation from mouse liver lysate made from 5mg of tissue and 100µl of GTC Extraction Buffer. Amplified product is observed only in wells that contained lysate. RT-PCR (40 cycles) was used to assay for cross-contamination.

Conclusions

The PolyATtract® Automated System provides conveniently formatted reagents for automated poly(A)+ RNA purification. We demonstrate isolation of poly(A)+ mRNA directly from total RNA, tissue culture cells and tissue lysates using a single methodology. Average percent recoveries relative to input total RNA are ≥1% with efficient removal of ribosomal RNA. Isolated poly(A)+ mRNA performs well in endpoint and real-time RT-PCR, and there is no detectable cross-contamination observed by RT-PCR.

Protocols

- *PolyATtract® Automated System Technical Bulletin* #TB321, Promega Corporation. (www.promega.com/tbs/tb321/tb321.html)

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Ordering Information

Product	Size	Cat.#
PolyATtract® Automated System*	4 × 96	Z5671
MagnaBot® 96 Magnetic Separation Device[1]		V8151
MagnaBot® Spacer 1/8 inch[3]		V8581
1/4 inch Foam Spacer[2]		Z3301
MagnaBot® Spacer[3]		V8381
MagneSil™ Magnetic Separation Unit[2(b)]		A2231
PolyATtract® GTC Extraction Buffer (with 2% β-mercaptoethanol)*	120ml	Z5531
Biotinylated Oligo(dT) Probe*	35µl	Z5261
Streptavidin MagneSphere® Paramagnetic Particles*	15 × 0.6ml	Z5481
	25ml	Z5482
Nuclease-Free Water*	2 × 25ml	P1193

* For Laboratory Use.

[1] Required by both the Biomek® 2000 and Fx protocols.

[2] Required by the Biomek® 2000 protocol only.

[3] Required by the Biomek® FX protocol only.

[(a)] The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

[(b)] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.