Journal of Medical Genetics and Genomics Vol. 3 (4) pp. 74 - 76, April 2011
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2011 Academic Journals

Short Communication

An alternative method for high throughput RNA isolation from whole blood

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Accepted 09 February, 2011

We have developed an efficient, cost effective, and high-throughput RNA isolation method for whole blood. By optimizing the blood processing steps and using the MagMAX™-96 platform from Applied Biosystems®for high-throughput RNA isolation, we were able to isolate high quality and quantity RNA from whole blood using a simple and cost effective protocol that may be useful on a variety of blood samples.

Key words: RNA, blood, MagMAX, RIN.

INTRODUCTION

A large number of pre-clinical and clinical research study samples are whole blood specimens. RNA isolated from whole blood has been used extensively in biomarker and other genomic studies (Pahl, 2005; Siest et al., 2009). Blood RNA, like the RNA from most tissues, is vulnerable to nuclease degradation and therefore blood samples are prone to low RNA yields, depending on the white cell counts (Vlassov et al., 1998). Although the quality and quantity of isolated RNA from blood have increased over the years, the protocols remain relatively costly and time consuming (Debey-Pascher et al., 2009). Newer high throughput RNA isolation systems have allowed for more efficient RNA extraction, but the cost of numerous (tissue specific) kits and specialized reagents continues to make blood RNA isolation an expensive procedure. Due to increased sample workload in the laboratories, a high throughput method at lower cost is needed. Such a method producing high quality RNA was obtained by deviating from a dedicated blood RNA isolation procedure using the PreAnalytix (Feldbachstrasse, Switzerland) kit to a modified solid tissue RNA isolation method, using the MagMAX-96 robotics instrument. For this protocol, the whole blood was collected in PAXGene blood RNA tubes to maintain the integrity of the RNA prior to isolation.

Currently, one of the fastest ways to isolate RNA from tissues is by using the MagMax 96-well robotic system utilizing magnetic binding beads, for isolation of RNA. We have modified the MagMAX protocol for RNA isolation from tissues to isolate the RNA from whole blood collected in PAXGene blood RNA tubes, using a kit designed for RNA isolation from solid tissues and mammalian cells (AM1839; Ambion, Carlsbad, CA, USA). An evaluation of the time requirement and costs of the traditional PreAnalytix and the modified MagMAX blood RNA isolation procedures demonstrated a significant savings in technician hours and materials.

MATERIALS AND METHODS

Collection of blood samples

All animal studies were conducted using procedures approved by our institutional animal care and use committee. Female Sprague-Dawley rats (150 to 250 g) were purchased from Charles River Laboratories (Wilmington, MA, USA). Blood was collected in PAXGene Blood RNA tubes (Qiagen Inc., Valencia, CA, USA) for downstream RNA isolation, quantification and RT-PCR analysis (Carrol, 2007). Tubes containing whole blood were held at room temperature for at least 2 h, stored frozen at approximately -20°C overnight, and then transferred to a freezer set to maintain a temperature of approximately -70°C until processed.

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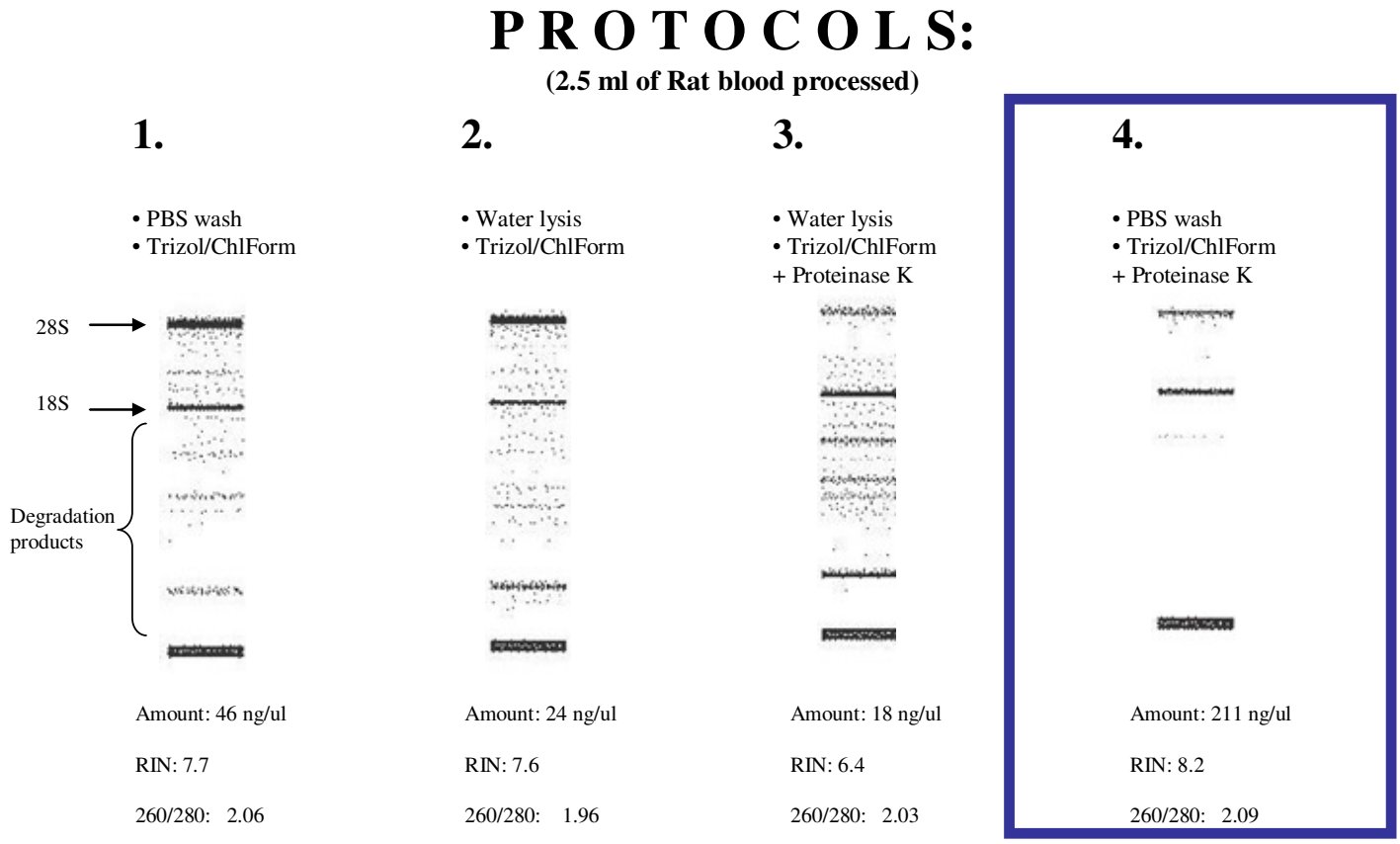

Figure 1. A comparison of capillary electrophoresis gels. Total RNA concentrations, RNA Integrity Numbers (RIN) and absorbance ratios (260/280) represents the 4 methods of whole rat blood RNA isolation tested. Data were generated with an Agilent Bioanalyzer using the Eukaryote RNA assay and the Nanodrop ND-1000 Spectrophotometer.

Table 1. RNA quality comparison. Total RNA concentrations, RNA Integrity Numbers (RINs) and absorbance ratios (260/280) representing the RNA quality and quantity resulting from four protocols tested. Data were generated with an Agilent Bioanalyzer using the Eukaryote RNA assay and the Nanodrop ND-1000 Spectrophotometer.

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
RNA concentration (ng/ul)	46	24	18	211
RNA Integrity Number (RIN)	7.7	7.6	6.4	8.2
260/280	2.06	1.96	2.03	2.09

RNA isolation

For extraction of RNA, various whole blood pellet treatments were finally combined with the tissue RNA isolation method, as outlined in the manufacturers protocol (Ambion, AM1839) (Figure 1 and Table 1). Protocol 1 included a sterile PBS wash of the blood serum pellet followed by a chloroform extraction. Protocol 2 utilized a water lysis step with chloroform extraction. Protocol 3 differed from protocol 2 in that, it also included an RNA nucleic acid purification and digestion step using Proteinase K followed by the chloroform addition. Protocol 4 combined the PBS blood pellet wash with chloroform and proteinase K steps (de Paula et al., 2003; Chomczynski and Sacchi, 2006).

Broadly, for protocol number 4, approximately 2.5 ml of rat blood was collected, inverted several times, and then stored at ambient temperature for approximately 2 h. After ambient temperature incubations, samples were maintained overnight at -20°C and then placed at -70°C for storage until processed. For processing, the blood samples were returned to ambient temperature prior to centrifugation (10 min at 3000×g in a swinging bucket). The supernatant was removed, discarded and 4 ml of sterile PBS was added to the pellet, which was then vortexed until it was visibly suspended. The suspension was centrifuged at 3000×g and the subsequent supernatant was discarded. The resulting pellet was re-suspended again with 400 µl TRI reagent (Ambion. AM9738, Carlsbad, CA, USA) and 30 µl of Proteinase K, transferred to a micro centrifuge tube, vortexed for 5 s, and incubated at 55°C for 10 min on a shaker (500 rpm). Approximately 43 µl of chloroform was then added and samples vortexed for a short period of time.

After a 5 min incubation at ambient temperature, the sample tube

was centrifuged at 12,000×g for 10 min at 4°C. The upper aqueous layer (100 to 200 µl) was transferred to a new micro centrifuge tube and stored at -70°C or transferred to each well of the first 'automation reaction' 96-well MagMAX Express (MME) plate. The Ambion kit AM1839 was then prepared and utilized by following the manufacturer's instructions.

Each protocol resulted in 50 µl elution samples, using the previously outlined MagMAX automated RNA extraction (AM1839, Ambion, USA). RNA integrity numbers (RIN)s (Schroeder et al., 2006) and concentrations were determined using the Agilent 2100 Bioanalyzer and by spectral photometry using ND-1000 Spectrophotometer. 260/280 Absorbance ratio of the elution samples from protocols 1 to 4 averaged 2.0.

RT-PCR

The quality and quantity of the isolated RNA was confirmed using an Agilent 2100 Bioanalyser and ND-1000 'NanoDrop' (NanoDrop Technologies, USA). Reverse transcribed to cDNA using QScript cDNA Supermix (Quanta BioSciences, Gaithersburg, MD, USA) according to the manufacturer's protocol. The 18S gene expression was quantified using an Applied Biosystems dual labeled prime probe set (AM1839 kit), Perfecta qPCR FastMix (Quanta BioSciences), and employing an ABI Prism 7900 HT Sequence Detection platform (Applied Biosystems, USA). The PCR protocol was as follows: 45°C incubation for 2 min (UNG incubation) followed by 95°C for 30 s (initial denaturation) and 40 cycles, each comprising of 95°C for 2 s and 60°C for 20 s respectively.

RESULTS AND DISCUSSION

Protocol number 4 recovered the highest amount of RNA and with superior quality compared to all other methods. The result was reproduced with repeated RNA isolations from rat blood samples wherein 200 to 400 ng/µl of RNA could be recovered with an RNA Integrity Numbers average value was 8.0, and absorbance ratio average of 2.0. To assess the performance of the method with blood from other species, repeated RNA isolations were also conducted from dog blood. The RNA yield and quality from these blood samples were similar to those achieved with rat blood. The quality of the RNA from both rat and dog blood were further confirmed by evaluation of RT-PCR efficiency through amplification of a housekeeping gene (ribosomal 18S subunit). As expected, this method (protocol number 4) produced acceptable results with both rat and dog blood confirming that, it may be useful for extraction of RNA from other species also.

In conclusion, this method for RNA isolation from whole rat blood collected in PAXgene RNA tubes is a more efficient and cost effective method as compared to the traditional total blood RNA isolation methods. Utilizing the tissue isolation kit to also isolate blood RNA has also lead to significant savings. Finally, this procedure resulted in high yield and high quality RNA, acceptable for downstream applications such as microarrays and quantitative Real Time Polymerase Chain Reaction (qRT-PCR) analyses.

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