Separate Isolation of Genomic DNA and Total RNA from Single Samples Using the SV Total RNA Isolation System

By Paul Otto, Dan Kephart and Rex Bitner, Promega Corporation; Suzanne Huber and Karl Volkerding, Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison

Corresponding author: e-mail to rbitner@promega.com

Promega's SV Total RNA Isolation System[a] *provides a fast and simple technique for the purification of intact total RNA from tissues, cultured cells and whole blood samples (1). We describe here a modification of the protocol that provides a rapid and safe method for the sequential purification of DNA and RNA from the same sample of tissue, cultured cells or whole blood, using Promega's SV Total RNA Isolation System. A simple DNase step minimizes genomic DNA carryover from the purified RNA when only RNA is desired. The method also allows the purification of only genomic DNA, when this is desired. The nucleic acids isolated using this method are of high purity and are ideally suited for applications such as cDNA cloning, RT-PCR, PCR, Northern and Southern blots and RNase protection assays. With a minor modification, this protocol may also be used for the isolation of hepatitis C virus (HCV) RNA suitable for RT-PCR.*

Introduction

Analysis of RNA is important for many studies including cellular development, responses to environmental stimuli and disease states. Promega's SV Total RNA Isolation System provides a rapid and safe method for the purification of high quality total RNA. The SV RNA System is based on a silica membrane that can be used in either a centrifugation ("spin") or vacuum format. Nucleic acid isolation is achieved without the use of organic extractions, and employs a simple DNase extraction step to minimize genomic DNA carryover. When the RNA isolated with this system is analyzed by RT-PCR, genomic DNA contamination generally is not seen. Because the SV Total RNA Isolation System uses guanidine thiocyanate (GTC) to inactivate pathogenic organisms and viruses (2), an additional degree of safety is assured when isolating genomic DNA samples from human blood or tissues.

For certain applications, it is important to be able to isolate genomic DNA and total RNA separately from a single sample of tissue, culture cells or a single sample of whole blood. One example is the detection of translocation-terminating mutations with the protein truncation test (PTT) using the TNT® T7 Quick Coupled Transcription/Translation System[c-f] (Cat.# L1170; 3). PTT has been used in the analysis of the APC tumor suppressor gene, which plays a key role in familial adenomatous polyposis (FAP). Different segments of the APC gene are tested using either genomic DNA via PCR or mRNA via RT-PCR, thus requiring the isolation of both nucleic acids from a single sample.

For other applications where only genomic DNA isolation is desired, this protocol allows the purification of high quality, high molecular weight DNA. Purifications of equivalent quality and yield were achieved from whole blood samples treated with EDTA, citrate or heparin as anti-coagulants. With minor modifications, genomic DNA can be isolated from primate and rodent cell lines, mouse blood and a variety of mouse tissues (e.g., liver, kidney or spleen).

The isolation of hepatitis C virus (HCV) from human blood plasma is of growing clinical importance. With a minor modification to the protocol, HCV RNA can be purified from human blood plasma using the SV Total RNA Isolation System. The RNA isolated with this method is suitable for RT-PCR analyses.

Sequential Isolation of Genomic DNA and RNA Including Viral RNA

Figure 1 outlines the procedure and the various modifications used for the separate isolation of DNA and RNA from a single sample of blood, tissue or tissue culture cells. Protocol modifications for the isolation of HCV RNA are also included.

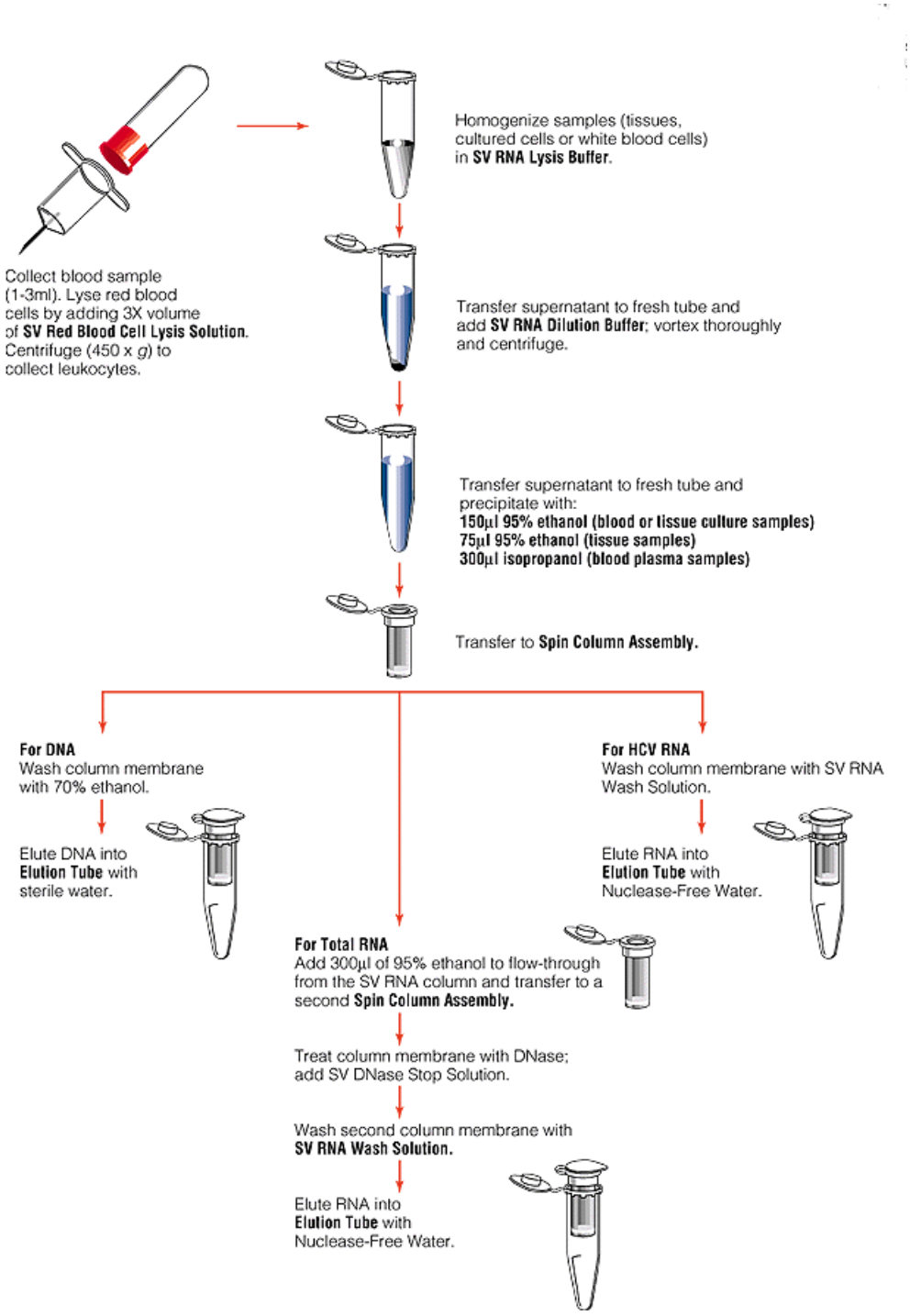

Figure 1. Protocol for sequential DNA and RNA isolation using the SV Total RNA Isolation System. Isolation of DNA followed by RNA from whole blood, tissue or tissue culture cells. The isolation of viral RNA from human blood plasma is also described.

Two important changes in the protocol that allow the separate isolation of DNA and RNA are the vortexing of the sample during the lysis steps and the addition of ethanol in two separate steps (Figure 1). During the isolation of RNA, it is desirable to trap the genomic DNA in the cell debris during lysis, so that it can be effectively removed by centrifugation. However, when genomic DNA isolation is desired, two vortexing steps facilitate the liberation of genomic DNA from the cell debris so that it can be precipitated by the addition of ethanol. The addition of ethanol in two steps allows for the isolation of DNA after the first ethanol addition, while RNA passes through the SV column. The addition of ethanol to the flowthrough allows the isolation of RNA on a second SV System membrane.

Results of DNA and RNA Isolations Using the Modified SV Procedure

The data in Table 1 represent average DNA yields for four samples each of fresh human whole blood, using the modified SV protocol.

Table 1. Genomic DNA Isolated from Human Whole Blood.				
Blood (µl)	**A_{260}**	**A_{280}**	**A_{260}/A_{280}**	**Yield (µg)**
200	0.03	0.02	1.81	2.9
400	0.08	0.05	1.75	5.0
600	0.13	0.07	1.76	10.4
800	0.10	0.06	1.77	9.9
1,000	0.09	0.05	1.74	8.5
Average yields of DNA for four samples of each blood volume using procedure in Figure 1.				

Figure 2, Panels A and B, show typical molecular weight distributions for genomic DNA and RNA purified using the modified SV Total RNA Isolation System protocol. The DNA isolated is of high molecular weight, and the RNA shows banding patterns characteristic of RNA isolated using the standard SV Total RNA Isolation System protocol.

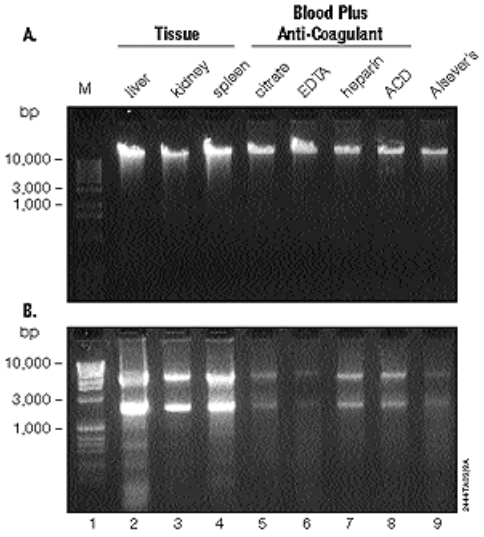

Figure 2. Genomic DNA and RNA isolated from mouse tissue and blood. These gel photos show typical molecular weight distributions for genomic DNA (Panel A) and RNA (Panel B) purified using the SV Total RNA Isolation System modified protocol. **Panel A:** DNA isolated using the modified procedure is of high molecular weight. **Panel B:** RNA isolated using the modified procedure shows banding patterns characteristic of RNA isolated using the standard protocol. Tissue sources and blood anti-coagulants are indicated in the figure. Lane M contains 1kb DNA Ladder (Cat.# G5711).

Table 2 shows purity and yield data for nucleic acid isolated from mouse blood and tissue, using the protocol described in Figure 1. When working with whole blood, red blood cells must be lysed prior to processing samples. Tissue samples must be homogenized before isolating nucleic acids. To isolate intact RNA, it is necessary to inactivate endogenous RNases immediately during cell lysis. The SV Total RNA Isolation System utilizes guanidine thiocyanate (GTC) and beta-mercaptoethanol for their disruptive and protective capabilities (4). The combined effects of GTC, beta-mercaptoethanol and SDS allow the nucleic acids to be isolated essentially free of proteins such as RNases.

Table 2. Genomic DNA and RNA Isolated from Mouse Blood and Tissues.						
Sample/Amount	**A_{260}/A_{280}**	**DNA µg/ml**	**Yield DNA (µg)**	**A_{260}/A_{280}**	**RNA µg/ml**	**Yield (µg)**
Liver/30mg	1.8	88.7	17.7	1.9	257	51.5
Kidney/20mg	1.8	58.0	11.6	2.0	280	56.0
Spleen/15mg	1.8	82.6	16.5	1.9	698	140.0
Blood-citrate	1.8	67.5	13.5	2.0	76.3	15.3
Blood-EDTA	1.8	65.8	13.2	2.0	127	25.4
Blood-Heparin	1.8	73.0	14.6	2.0	79	15.8

DNA and RNA yields as isolated sequentially from the same tissue or blood samples (1ml).

The presence of genomic DNA in RNA preparations can interfere with amplification-based techniques such as RT-PCR. To improve removal of contaminating genomic DNA from the purified RNA, RNase-Free DNase I is applied directly to the SV Total RNA Isolation System membrane. To ensure that the RNA is not contaminated with DNase I, the enzyme is inactivated by the SV DNase Stop Solution. Contaminating salts and cellular impurities are removed by simple washing steps, and the RNA is eluted in Nuclease-Free Water (Cat.# P1193).

Isolation of nucleic acids from clinically relevant sources and subsequent analysis using amplification-based methodologies is an extremely powerful approach used in many research and diagnostic applications. DNA and RNA were sequentially isolated using the protocol outlined in Figure 1, and aliquots were tested by PTT analysis (3, 5-7). In PTT, mRNA or exons from genomic DNA corresponding to the gene of interest are amplified using a primer pair designed to introduce a T7 promoter and start codon upstream (5′) of the coding region. The resulting amplified DNA is added directly to *in vitro* coupled transcription/translation reactions such as the TNT® T7 Coupled Wheat Germ Extract System(c,d,f) (Cat.# L4140); the products are analyzed by SDS-polyacrylamide gel electrophoresis for the appearance of truncated proteins.

An aliquot of total RNA isolated using this procedure was amplified using Promega's Access RT-PCR System(b) (Cat.# A1250) with primers corresponding to a region of the human APC gene (8). The expected 937bp product was obtained from amplification reactions using RNA equivalent to 50µl of starting blood (Figure 3, lane 1). The purified genomic DNA was amplified by omitting the AMV reverse transcription step of the Access RT-PCR System protocol. Two primer pairs (designated segment 2 and segment 4) were used to amplify overlapping regions of the APC gene exon 15 (Figure 3, lanes 2 and 3). When aliquots of each unpurified segment 2 or segment 4 PCR amplification product were used as template in Promega's TNT® T7 Coupled Wheat Germ Extract System (Cat.# L4140), the expected protein products were produced from the 1.8kb and 2.2kb templates, respectively (Figure 4).

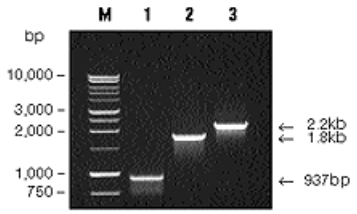

Figure 3. Amplification of RNA and DNA isolated using the SV isolation protocol. Aliquots of total RNA (lane 1) or genomic DNA (lanes 2-3) isolated from 1ml human blood using the SV Total RNA Isolation System were amplified using Promega's Access RT-PCR System(b) (Cat.# A1250). Peripheral human blood was collected in standard EDTA-coated tubes. A 1ml aliquot was placed in a nuclease-free, 1.5ml microcentifuge tube. DNA and total RNA were extracted using the protocol outlined in Figure 1. For RT-PCR, primers corresponding to nucleotides 58-80 (upstream primer) and 935-955 (downstream primer) of the human APC gene were used to generate the expected 937bp product (segment 1) from 15ng of RNA. One hundred nanograms of genomic DNA was used with primer pairs designed to amplify bases 1,978-3,808 (1.8kb product; segment 2) or bases 4,801-7,028 (2.2kb product; segment 4) of the human APC gene (1). Twenty microliters of the segment 1 amplification and 10µl of the segments 2 and 4 amplifications were resolved on a 1.5% agarose gel. Lane M, Promega's 1kb DNA Ladder (Cat.# G5711).

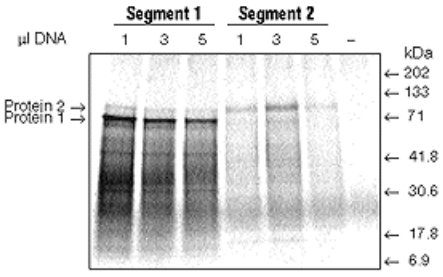

Figure 4. Translation *in vitro*. Increasing amounts of unpurified segment 2- or segment 4-amplified products were used as templates in Promega's TNT® T7 Coupled Wheat Germ Extract System (Cat.# L4140). Translation products corresponding to the two segments were resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. The positions of the expected protein products are indicated.

Figure 5 shows the amplification of HCV RNA after isolation from blood plasma, using the modified procedure detailed in Figure 1. In this modification, the ethanol addition is replaced by addition of 300µl of isopropanol, which allows for a more efficient precipitation of HCV RNA onto the SV RNA membrane, in a single application. Because of the reduced amount of genomic DNA found in human blood plasma, there is generally no need to use DNase.

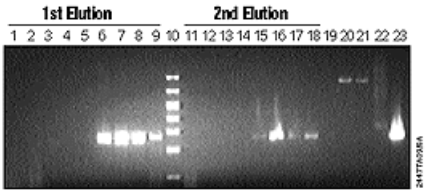

Figure 5. HCV viral RNA isolated and amplified from plasma using a modification of the SV Total RNA Isolation System. RNA was isolated from the blood plasma of an HCV-positive individual, using the modified protocol for viral RNA isolation (Figure 1). Two 100µl elutions of the SV membrane were performed and samples of the first (lanes 1-9) or second elution (lanes 11-18) were collected and run on a 2.5% NuSieve® agarose gel. A high guanidine protocol (R. Bitner) was used to isolate viral RNA in lanes 20 and 21. Lanes 1-5, negative control samples; lanes 6-9, samples from HCV+ controls; lane 10, DNA markers; lanes 11-14, negative SV RNA controls; lanes 15-18, HCV+ controls; lane 19, negative (no RNA) control; lane 20, HCV-negative sample, high guanidine protocol; lane 21, HCV+ sample, high guanidine protocol; lane 22, HCV-TriZOL®; lane 23, HCV+TriZOL®.

SUMMARY

The SV Total RNA Isolation System uses a safe and rapid procedure to purify high quality total RNA. Using simple modifications to the basic SV System protocol, the separate purification of both DNA and RNA from the same sample can be easily processed. In situations where it is desirable to purify only genomic DNA, the RNA purification can be omitted and high quality genomic DNA can be isolated. These procedures do not involve the use of organic extractions or precipitations. The procedures are easy to perform, and can be used to process multiple samples simultaneously.

The SV Total RNA Isolation System protocol can also be modified to isolate viral RNA from sources such as Hepatitis C Virus, providing a safe and rapid procedure. This simple procedure can be used for the rapid processing of multiple samples. For further information on downstream applications such as cDNA cloning, Northern and Southern blotting analyses, and RNA protection assays, please see Promega's *RNA Applications Guide* (9) and *Protocols and Applications Guide* (10).

REFERENCES

1. Brisco, P. (1997) *Promega Notes* **64**, 7.
2. Manuelidis, L. (1997) *J. Neurovirol.* **3,** 62.
3. Cayouette, M. (1997) *Promega Notes* **60**, 8.
4. Chirgwin, J.M. *et al.* (1979) *Biochemistry* **18**, 5294.
5. Roest, P.A. *et al.* (1993) *Hum. Mol. Gen.* **2**, 1719.
6. *TNT® T7 Quick Coupled Transcription/Translation Systems Technical Manual* #TM045, Promega Corporation.
7. Kephart, D. (1998) *Promega Notes* **68**, 20.
8. Kinzler, K.W. *et al.* (1991) *Science* **253**, 661.
9. *RNA Applications Guide* (1997), Promega Corporation.
10. *Protocols and Applications Guide,* Third Edition (1996), Promega Corporation.

Ordering Information

Product	Size	Cat.#
SV Total RNA Isolation System	50 preps	Z3100
SV Total RNA Isolation System, Trial Size	10 preps	Z3101

Related Products

Product	Size	Cat.#
TNT® T7 Coupled Wheat Germ Extract System		L4140
Miniprep Vacuum Adapters[a]	20ea	A1331
SV Red Blood Cell Lysis Solution	200ml	Z3141
SV RNA Lysis Buffer	50ml	Z3051
Wizard® *Plus* SV Minipreps DNA Purification System[a] (with Vacuum Adapters)	50 preps	A1340
	250 preps	A1470

Wizard® Genomic DNA Purification Kit	100 isolations	A1120
	500 isolations	A1125

[a]Patent Pending.

[b]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 or RT-PCR process. Use of this system is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

[c]U.S. Pat. Nos. 5,283,179, 5,641,641 and 5,650,289 and Australian Pat. No. 649289, have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay.

[d]U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, and Australian Pat. No. 660329, have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerase and eukaryotic lysates.

[e]U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217 and Australian Pat. No. 646803 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor (PRI). *Inhibitors of Angiogenin*, which comprises a segment of human PRI, is the subject of U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687 assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

[f]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.
