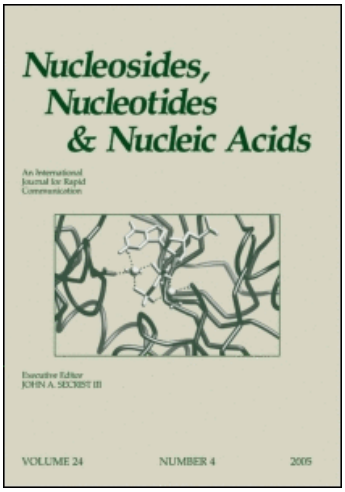

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Svetlana N. Tamkovich[a]; Pavel P. Laktionov[a]; Elena Yu. Rykova[a]; Valentin V. Vlassov[a]
[a] Institute of Bioorganic Chemistry of Siberian Division of Russian Academy of Sciences, Novosibirsk-90, Russia

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Simple and Rapid Procedure Suitable for Quantitative Isolation of Low and High Molecular Weight Extracellular Nucleic Acids

Svetlana N. Tamkovich, Pavel P. Laktionov,* Elena Yu. Rykova, and Valentin V. Vlassov

Institute of Bioorganic Chemistry of Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia

ABSTRACT

The procedure based on binding of nucleic acids with glass surface in presence of chaotropic salts was adapted for efficient isolation of 100–10000 b.p. DNA fragments and 50–10,000 b. RNA fragments. The method provide 90% and 85% efficacy of isolation of 100 b.p. DNA and 100 b. RNA fragments respectively. High molecular weight nucleic acids are isolated with 98% efficacy. Isolated nucleic acids are free from contaminations, influencing nucleic acids modifying enzymes and fluorochromes. The method is rapid, simple and cost-effective.

Key Words: Isolation of DNA; Isolation of RNA; Circulating nucleic acids.

Methodological approaches for nucleic acids (NA) isolation are well known and well investigated. Such methods as phenol extraction with subsequent precipitation of NA with ethanol,[1,2] phenol-guanidinium procedure for nucleic acids extraction,[3] isolation of NA with glass-milk[4] have been generally used to date. Nevertheless rapid

*Correspondence: Pavel P. Laktionov, Institute of Bioorganic Chemistry of Siberian Division of Russian Academy of Sciences, 8, Lavrentier Ave., Novosibirsk-90, 630090, Russia; E-mail: lakt@niboch.nsc.ru.

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Table 1. Effectivity of binding of ^{32}P-labeled NA with GM and TGM.

	% of DNA binding			% of RNA binding	
DNA added, ng	GM	TGM	RNA added, ng	GM	TGM
50	47 ± 5	92 ± 3	100	65 ± 4	87 ± 1
200	55 ± 3	93 ± 3	200	75 ± 3	89 ± 3

expansion of DNA and RNA analysis in medical, biotechnological and basic research has created specific requirements for NA isolation methods. Development of noninvasive method of early cancer diagnostics[5] and posttherapeutic monitoring,[6] which are based on circulating DNA and RNA detection, demand productive, rapid and reproducible methods, which allows isolation of both short and long fragments of NA free from contaminations preventing PCR analysis and quantitative fluorescent assay.

Isolation of NA based on NA absorbtion by the treated glass-milk and following NA elution was shown to be suitable for isolation of circulating NA from human plasma.

Glass-milk (GM) from Sigma (Sigma, S-5631) was size fractionated and treated with hydrofluoric acid as described.[7] Elution Buffer for DNA (EBD) and Elution Buffer for RNA (EBR) described in Refs. [7,8] were tested for their capability to elute NA from GM and size fractionated treated GM (TGM). DNA fragments were obtained by calf thymus DNA hydrolysis with DNase 1 treatment for 1 h at 37°C, enzyme:substrate ratio 1:50 in 0.1 M NaOAc pH 5.2, 5 mM $MnSO_4$. About 100 b.p and 200–500 b.p. DNA fragments were eluted from 1% agarose gel. 100 b. RNA transcript of mdr gene was used for investigation of RNA isolation. DNA and RNA fragments were ^{32}P labeled using T4 polynucleotide kinase. 1V of the sample, containing DNA was mixed with 2V of Binding Buffer 1 (BB 1) containing 6.75 M guanidinium thiocyanate (GuSCN), 60 mM EDTA, 15 mM tris-acetate, pH 6.4 and 3 mg GM or TGM. After incubation for 5–10 minutes at room temperature GM or TGM was washed twice by centrifugation with 0.5 ml of Washing Buffer 1 (WB 1) containing 4.5 M GuSCN, 40 mM EDTA, 10 mM tris-acetate, pH 6.4, than twice with 0.5 ml of Washing Buffer 2 (WB 2) containing 25% propanol-2, 100 mM NaCl, 10 mM tris-HCl, pH 8.0. DNA was eluted by incubation of GM with TE-buffer as described[4] or by incubation of TGM with EBD[7] 3–5 minutes. Supernatants with eluted DNA were collected after centrifugation (1 min at ~12 000 rpm of centifuge ''Eppendorf''). 1V of the sample, containing RNA was mixed with 12.5 V of Binding Buffer 2 (BB 2) containing 20%

Table 2. Recovery of ^{32}P-labeled NA from GM and TGM.

	DNA eluted, %			RNA eluted, %	
DNA added, ng	EBD	TE-buffer	RNA added, ng	EBR	TE-buffer
10	97	–	10	84	–
50	98	45	50	85	45
100	98	51	100	86	51
400	98	53	400	85	53

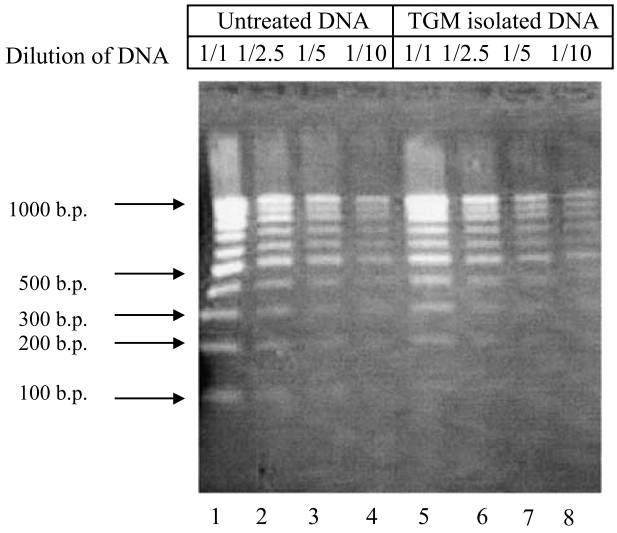

Figure 1. Isolation of DNA marker M15 (100-1000 b.p.). 2.5 μg of M15 (Sibenzyme) DNA in 50 μl of TE buffer was isolated by means of TGM/EB and eluted into 50 μl. Dilutions of starting DNA solution (2.5 μg in 50 μl) and DNA after TGM/EB isolation were applied on 2% agarose gel. Line 1—500 ng, line 2—200 ng, line 3—100 ng, line 4—50 ng of starting DNA solution, line 5—8 of similarity dilution of isolated DNA.

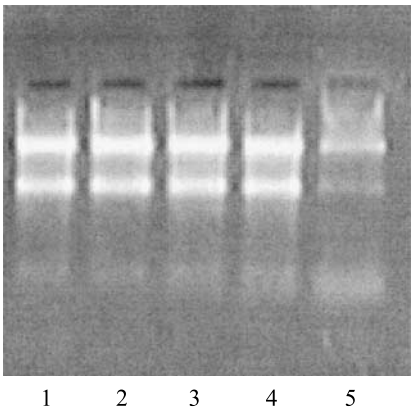

Figure 2. Isolation of RNA from membrane-cytosolic fraction of A9 cells. Membrane–cytosolic fraction from 5×10^6 cell was prepared after addition of lysis buffer (0.6% Nonidet P40, 50 mM Tris-HCl, 0.15 M NaCl, 5 mM NaF, 1 mM PMSF, 2 mM TA, 1 mM Na_3MoO_4). Nuclei were pelleted by configuration and supernatant was collected for RNA isolation from equal aliquots by different protocols: isolation by TGM/EB with or without propanol-2 and isolation by phenol extraction, then isolated RNA was electrophoresed through a 2% agarose gel (containing ethidium bromide). Line 1—not used propanol-2, line 2—with 5% propanol-2, line 3—with 10% propanol-2, line 4—with 20% 2-propanol, line 5—phenol extraction protocol.

Table 3. Isolation of fragmented ^{32}P-labeled DNA from human plasma.

DNA recovery, (%)/Hoechst 32258 fluorescence, arbitrary units			DNA isolation, hours	Method of DNA isolation
Concentration of added DNA, μg/ml				
1	5	200		
76 ± 4/78	74 ± 5/81	73 ± 5/83	4.5	5% SDS, 5 M CH_3COOK, 4 M $KClO_4$, ethanol precipitation.[10]
75 ± 5/75	77 ± 4/385	76 ± 5/15,200	0.7	GM binding[4]-EBD[7]
98 ± 2/100	98 ± 2/500	98 ± 2/20,000	0.2	TGM binding[7]-EBD[7]

propanol-2, 1 M GuSCN, 40 mM EDTA, 10 mM tris-acetate, pH 4.5 and 3 mg GM or TGM. After incubation for 5–10 minutes at 4°C GM or TGM was washed twice by centrifugation with 0.5 ml of WB 1, twice with 0.5 ml of WB 2. RNA was eluted by incubation of GM with TE-buffer as described[4] or by incubation of TGM with EBR[8] 3–5 minutes. Supernatants with eluted RNA were collected after centrifugation (1 min at ~12 000 rpm of centifuge ''Eppendorf''). The quantity of isolated ^{32}P labeled DNA and RNA was estimated by β-counting of the samples.

Scanning electron microscopy demonstrates unification of GM after hydrofluoric acid treatment (data not shown), which leads to increased NA-binding capacity of TGM. 1 mg of TGM bind as much as 2.5 μg of DNA (400–1500 b.p.) and 2 μg of total cellular RNA whereas GM bind 1 μg of DNA and 0.8 μg of RNA per milligram.

The data presented in Table 1 show more effective binding of 100 b.p. DNA fragments and 100 b. RNA transcript with TGM compared with GM. EBD and EBR provide almost 50% increase of the yield of eluted NA compared with TE-buffer (Table 2). To confirm efficacy of NA isolation DNA fragments and total cellular RNA were isolated using TGM/EB procedure. Results presented in Fig. 1 confirms high yield of isolation for 100–1000 b.p. DNA fragments. Results presented in Fig. 2 demonstrates TGM/EB procedure for RNA isolation is more effective than phenol-chloroform extraction method.[1] Addition of propanol-2 to binding mixture increase the yield of 5S RNA up to 20%.

In order to estimate efficacy of NA isolation from human plasma ^{32}P labeled DNA fragments 200–500 b.p. were added to human plasma and isolated with GM and TGM with elution of DNA by EBD. It was shown that isolated DNA is free from contaminations interfering with fluorescence with recovery yield about 98% (Table 3).

The data obtained demonstrate high efficacy of TGM/EB procedure for isolation of DNA and RNA, isolated DNA can be used for concentration estimation with fluorescence based methods and can be successfully used in PCR.[9]

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