Notes

DNA Extraction with TRIzol Reagent Using a Silica Column

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TRIzol is a monophasic solution of phenol and guanidine isothiocyanate used for the extraction of RNA, DNA and proteins from tissues or cells. However, few studies have described its application to DNA extraction due to its time-consuming procedure. We present a TRIzol-modified method of extracting DNA from tissues using the TRIzol reagent and a silica column, which requires only one-third of the time required for the classic extraction procedure. Spectrophotometric analysis showed that the 260/280 and 260/230 nm optical density ratio of the DNA extracted using the TRIzol-modified method is ideal and equal to that obtained by the classic method and commercial DNAiso methods. The DNA extracted by the TRIzol-modified method had the same performance in a restriction enzyme digestion and quantitative PCR as that extracted using the classic method. Using the TRIzol-modified method saves time, simplifies the DNA extraction procedure, and facilitates various molecular biology assays.

Keywords DNA, extraction, trizol, silica column, simplify

(Received September 29, 2020; Accepted November 21, 2020; Advance Publication Released Online by J-STAGE November 27, 2020)

Introduction

TRIzol is a monophasic solution of phenol and guanidine isothiocyanate used for extracting RNA, DNA and proteins from tissues or cells. The extracted DNA, RNA or proteins from biological samples can be used in genotype, transcription, or protein expression analysis. Numerous uses of TRIzol for the extraction of RNA and proteins have been reported.[1,2] In contrast, few reports have described its application in the extraction of DNA[3,4] because of its time-consuming procedure and the undesirable purity of the extracted DNA.[5]

Recently, some materials and techniques such as proteinase K,[6] detergent,[7,8] silica column,[9] precipitation,[6] and magnetic particles[10] have been used in DNA extraction with features of rapidity, simplicity, high-purity and integrity. However, these methods usually only extract one nucleic acid, DNA or RNA. Hence, in studies that require simultaneous DNA, RNA and protein assays for meaningful data interpretation, the more common procedure is to divide the sample and treat each portion for DNA, RNA or protein extraction. However, the procedure will induce unforeseeable errors due to the differences in cell mass.[11] Moreover, for scarce and/or irreplaceable samples, simultaneous isolation of DNA, RNA and proteins from a single biological sample is essential.[3,12]

In this case, DNA extraction using the TRIzol reagent is a highly attractive method because of its flexibility in extracting DNA, RNA and proteins. Another advantage of the TRIzol extraction is that the sample dissolved in the TRIzol reagent is very stable and can be maintained for a long time at low temperature without affecting the extraction of nucleic acid and protein, which is incomparable with enzymatic nucleic acid extraction. Moreover, the TRIzol extraction method can be performed at room temperature without the need for a warm bath, which reduces equipment requirements.

The method of extracting RNA and protein using the TRIzol reagent has been optimized and the effect has been relatively good.[11,12] However, the DNA extraction procedure needs to be improved. Using a silica gel column to extract DNA has several advantages in that it is simple, rapid, reproducible, and offers high throughput, despite its minor shortcomings of containing endotoxin and difficulty in retrieving DNA from a TBE solution (http://www.docin.com/p-691188997.html). It can make up for the shortcomings of the TRIzol DNA extraction procedure. Therefore, in this study, we developed a modified TRIzol-based method using silica to efficiently and easily extract DNA from tissue samples without interference on the extraction procedure of RNA and proteins. This method generates a significant quantity of excellent quality DNA in one-third of the extraction time required for the classic TRIzol-base extraction procedure.

Experimental

Ethics statement

The experiment was approved by the Institutional Animal Care and Use Committee of Shenyang Agricultural University (IACUC Issue No. 2019101007). All tests on the animal followed the operation rules for laboratory animals.

DNA extraction

The blood sample was collected from a cock raised in an experimental flock and used to evaluate the DNA extraction methods described below. Blood samples were collected in 9 mL heparinized vacutainer blood collection tubes by wing

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vein venepuncture.[13] These samples were inverted to mix and prevent clotting and immediately placed in isothermic boxes and transferred to the laboratory. Then, 300 μL of anticoagulation blood was mixed with 3 mL TRIzol reagent (ThermoFisher, USA). Next, the solution was divided into six centrifugal tubes after the red cell was dissolved. DNA was extracted from three of these tubes following the manufacturer's protocol as the classic procedure, and the DNA served as the samples of the classic group. DNA was also extracted from the remaining three tubes using the TRIzol-modified method. In this method, after adding ethanol and inverting the tube as in the classic procedure, the mixer was transferred into the silica column (B515115, Sangon BiotechCo., Ltd., Shanghai, China) encased in a 2 mL centrifuge tube. After centrifuging at 12000*g* for 1 min, the filtrate was discarded. Then, 500 μL 0.1 M sodium citrate in 10% (w/w) ethanol was added into the silica column and centrifuged at 12000*g* for 1 min to wash the DNA. After repeated washing, 500 μL 75% (w/w) ethanol was added and centrifuged at 12000*g* for 2 min to wash the DNA. Then, 0.15 mL of 8 mM NaOH was added into the silica column to dissolve the DNA for 10 min. Lastly, the extracted DNA was obtained by centrifugation of the silica column encased in a new centrifuge tube, which served as the samples of the silica column group. The pH of the DNA extract was adjusted to 8.0 with 1 M HEPES. The extraction procedures for both methods are listed in Table 1.

At the same time, the DNA of the corresponding amount of anticoagulation blood was extracted using the commercial DNAiso reagent (Takara Bio, Dalian, China) according to the manufacture's instructions and used as a positive control.

Spectrophotometric evaluation of DNA extraction

The concentration and purity of the extracted DNA were evaluated using a Cytation 5 Multi-Mode Reader (BioTek, VT, USA) with a Take3 Micro-Volume Plate. The DNA concentration (expressed as ng/μL) was calculated directly using the Gen5 Microplate Reader and Image Software of the instrument, and DNA purity was assessed by the presence of protein and guanidine contaminants based on the A260/280 and A260/230 absorbance ratios.[14]

Electrophoretic evaluation of DNA extraction

DNA integrity was assessed by electrophoresis (5 μL of DNA was separated on a standard 0.8% (w/v) agarose gel) to assess whether there was a degraded DNA smear.

Restriction enzyme digestion

DNA digestion with restriction enzymes was performed to ensure the absence of inhibitors of restriction endonucleases that might be present in the extracted DNA. All DNA samples were digested with two different restriction enzymes (*Bam*H I, *Xho* I) for 12 h following the manufacturer's instructions (Takara, Dalian, China). Then, the digested DNA was separated on a 0.8% agarose gel by electrophoresis and photographed using the OmegaLumG Gel imaging system (Aplegen Inc, CA, USA). The grey value of the digested DNA on the photograph was analyzed by the ImageJ 1.52p software (NIH, USA).

Quantitative polymerase chain reaction (qPCR)

DNA amplification was performed using the Applied Biosystems QuantStudio 3 Real-Time PCR Systems (ThermoFisher, MA, USA) to assess the efficiency of the DNA amplification. The primers of chicken PRL gene[13] were synthesized by Sangon Biotech company. The qPCR reaction was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). Each 20 μL qPCR premix contained 10 μL Master Mix, 10 pmol of each primer and 1 μL of extracted DNA. The amplification was performed with an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 15 s and annealing and extension at 60°C for 15 s (Fluorescence collection). The qPCR products were verified on a 1.5% agarose gel by electrophoresis to ensure the specificity of the chicken PRL gene amplification.

Due to the fact that only gross inhibition could be observed at high DNA concentrations, the qPCR was performed with a 100-fold gradient dilution DNA template to observe the trace inhibitors. Each sample in a group was mixed in equal quantities and used as a template for RT PCR amplification (three replicates).

Evaluation of the application of the improved method

DNA from pig kidney tissue purchased in the local supermarket and *E. coli* strain DH5α stored in the authors' lab were extracted using the improved classic and DNAiso methods. The amount of kidney tissue and *E. coli* precipitate was 20 and 4 mg, respectively. At the beginning of the DNA extraction, they were

Table 1 The extraction steps of both methods with TRIzol reagent

Step No.	Procedure	Time/min	
		Classic method	Silica column method
1	Add TRIzol	0.5	0.5
2	Incubate	5	5
3	Centrifuge at 12000 × *g*	1	1
4	Transfer the supernatant into a new centrifuge tube	0.5	0.5
5	Add chloroform	0.5	0.5
6	Incubate	3	2
7	Centrifugal at 12000 × *g*	15	15
8	Remove the water phase	1	1
9	Add ethanol	0.5	0.5
10	Incubate	3	3
11	Transfer into silica column	—	0.5
12	Centrifugal	5 (at 2000 × *g*)	1 (at 12000 × *g*)
13	Discard the supernatant (filtrate)	0.5	0.5
14	Add 0.1 M sodium citrate in 10% ethanol	0.5	0.5
15	Incubate	30	—
16	Centrifugal	5 (at 2000 × *g*)	1 (at 12000 × *g*)
17	Discard the supernatant (filtrate)	0.5	0.5
18	Add 0.1 M sodium citrate in 10% ethanol	0.5	0.5
19	Incubate	30	—
20	Discard the supernatant (filtrate)	0.5	0.5
21	75% ethanol	20	—
22	Centrifugal	5 (at 2000 × *g*)	2 (at 12000 × *g*)
23	Discard the supernatant (filtrate)	0.5	0.5
24	Vacuum or air dry	10	—
25	Add eight mM NaOH and incubate	10	10
26	Centrifuge at 12000 × *g*	1	1
27	Transfer the supernatant into a new centrifuge tube	0.5	
28	Adjust pH with 1 M HEPES	0.5	0.5
	Total time	150	49

Table 2 Measured value of the extracted DNA of chicken blood using the three methods

Method	DNA concentration/ ng μL^{-1}	DNA purity		The grey value of digested DNA lane	CT value of qPCR
		260/280	260/230		
DNAiso	50.83 ± 35.56	1.58 ± 0.30	2.30 ± 0.04	2537.25 ± 1308.71	18.94 ± 0.81^{Aa}
Classic	160.57 ± 73.81	1.47 ± 0.25	2.28 ± 0.22	1608.63 ± 122.76	17.36 ± 0.51^{b}
Column	172.93 ± 20.43	1.87 ± 0.05	2.25 ± 0.26	980.65 ± 56	16.6 ± 0.05^{B}

For superscript notations, uppercase letters mean significant difference ($p < 0.01$), and lowercase letters mean significant difference ($p < 0.05$).

Table 3 DNA concentration in different dissolution time in the modified method

Dissolution time/min	6	8	10	12
DNA concentration/ng μL^{-1}	58.62 ± 10.31^{a}	110.84 ± 30.23^{b}	168.57 ± 17.35^{c}	174.18 ± 16.46^{c}

Lowercase letters mean significant difference ($p < 0.05$).

ground in the TRIzol reagent using the grinders to disperse the cells. The purity and concentration of the extract were compared.

Statistical analysis

The statistical analysis was done using the GraphPad Prism 6.0 software (GraphPad Software, CA, USA). The data for the concentration, purity, grey value of the restriction enzyme digestion and qPCR CT value were analyed with the t-test. All the tests were repeated three times.

Results and Discussion

Duration of both DNA extraction procedures

In 1987, researchers began using the TRIzol reagent to extract RNA from plants and animal tissue, and the reagent was later developed to extract DNA and proteins. Recently, the reagent has been widely used for the extraction of RNA, and sometimes proteins. However, it is infrequently used to extract DNA from tissues. This is most likely due to the associated time-consuming procedure and undesirable purity. Although a variety of DNA extraction methods have emerged, the features of extraction with the TRIzol reagent, such as wide adaptability to materials, protease-free and bath-free, make it very attractive.

Silica gel is a kind of material that can specifically bind nucleic acid and has been used for nucleic acid extraction since 1980.[15] Currently, many DNA extraction kits also use a silica column for nucleic acid purification.

The advantages of using a silica gel column to extract DNA are that it is simple, rapid, and reproducible, and it can process multiple samples simultaneously. However, the disadvantage is that the purified products often contain endotoxinsendotoxin, which is not suitable for the transfection test.

However, the use of the silica column in nucleic acid extraction with the TRIzol reagent has never been reported.

In this study, we attempted to use a silica column to extract DNA through the TRIzol-base method. The time required for the two DNA extraction methods are significantly different (Table 1). The classic precipitation method takes 150 min and 27 steps, while the modified silica column method takes 49 min and 23 steps. In the TRIzol-modified method, the time to wash the DNA with 0.1 M sodium citrate in 10% ethanol was reduced from 35 to 1 min and the time to wash the DNA with 75% ethanol was reduced from 15 to 2 min. Therefore, the whole extraction process took only 49 min, which is one-third of the original extraction time. At the same time, in the TRIzol-modified method, the step of discarding the supernatant after the washing steps was eliminated, which reduced the possibility of mistakenly discarding the DNA precipitate and theoretically ensured better uniformity.

Spectrophotometric evaluation of the DNA extraction

Based on the measured data, it was apparent that the TRIzol-modified method produced an equivalent yield and purity of DNA from the chicken blood when compared with the other methods (Table 2). The mean concentration of DNA isolated with the silica column was 172.93 ng/μL, against 160.57 ng/μL in the classic isolation method and 50.83 ng/μL in the DNAiso method. However, the difference was not statistically significant ($p > 0.05$). When comparing the purity (260/280 and 260/230) of the DNA extracted through the three isolation methods, it was found that the purity of the extracted DNA was equal for all the three methods ($p > 0.05$). One of the most important factors is that the dissolution time with 8 mM NaOH solution should not be less than 10 min. Compared with the DNA concentration obtained after 10 min of dissolution, the DNA concentration obtained after 6 and 8 min of dissolution was significantly lower ($p < 0.05$), while the DNA concentration at 12 min of dissolution was not significantly different ($p < 0.05$) (Table 3). Variations in DNA concentration is likely to result from the incomplete dissolution.

Electrophoretic evaluation of DNA extraction

The electrophoresis result demonstrated that two lanes of the DNA isolated by the classic and DNAiso methods, respectively, had a smear or residual particles. Contrarily, there was no obvious DNA smear in all the three lanes of the silica column (Fig. 1A). These data indicated that the DNA extracted by the silica column method has less nuclease or potential prohibiting impurities than that found in the classic method.

Restriction enzyme digestion

The smeared migration pattern on the gel indicated that the DNA extracted by the three methods was digested successfully (Fig. 1A). Meanwhile the digested smears of the DNA extracted

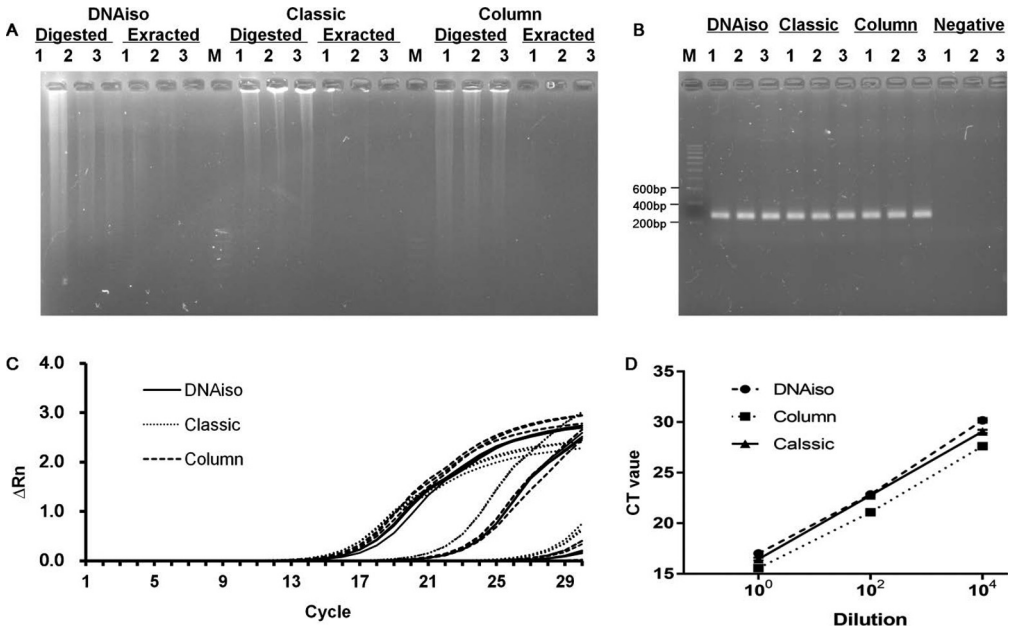

Fig. 1 The electrophoresis and amplification plot of the extracted DNA using the three methods. A, The electrophoresis result of the extracted DNA and digestion DNA. B, The electrophoresis results of qPCR. C, The amplification plot of gradient dilution DNA. D, The CT value variation of the gradient dilution DNA.

Table 4 The CT value of qPCR of the DNA with different dilutions and their different value

Method	Dilution			The difference value between the diluents	
	10^0	10^2	10^4	$10^2 - 10^0$	$10^4 - 10^2$
DNAiso	17.03 ± 0.28	22.86 ± 0.03	30.18 ± 0.22	5.83 ± 0.31^{a}	7.31 ± 0.21^{Aa}
Classic	16.46 ± 0.14	22.76 ± 0.18	29.07 ± 0.16	6.30 ± 0.31^{a}	6.31 ± 0.27^{B}
Column	15.57 ± 0.03	21.08 ± 0.03	27.62 ± 0.16	5.51 ± 0.05^{b}	6.54 ± 0.18^{b}

Superscript uppercase letters mean significant difference ($p < 0.01$), and lowercase letters mean significant difference ($p < 0.05$).

by the silica column method had a more perfect flame shape, compared to that found in the classic and DNAiso methods. The grey value of digested DNA on the photograph analyzed by the ImageJ 1.52p software (NIH, USA) indicated similar restriction enzyme digestion efficiency for the DNA extracted by the three methods (Table 2). The digestion experiment demonstrated the absence of any significant inhibitor of the enzymes used in the DNA digestion.

qPCR result

Results from the quantitative PCR showed that the modified and classic methods had similar Cycle Threshold (CT) values (17.36 and 16.6) (Table 2) and amplification plots for the amplification of the chicken PRL genes (Fig. 1D), and the values were not statistically different ($p > 0.05$). In contrast, they all have a lower CT value than that (18.94) of the DNAiso method ($p < 0.05$ and $p < 0.01$, respectively). The electrophoresis results of PRL qPCR products showed that the PRL target DNA fragment of 259 bp appeared in all lanes in which the extracted DNA served as the qPCR template, whereas no lanes appeared in the negative control (Fig. 1B).

A qPCR with template of 100-fold gradient dilution of mixed DNA was performed to validate the integrity of the DNA and absence of inhibitors. The CT value (Table 4), amplification plot (Fig. 1C) and variation trend of the CT value (Fig. 1D) are relatively consistent in the three extraction methods. However, the DNA extracted with the TRIzol-modified method had a lesser difference of the CT values of the different diluents than that extracted with the classic and TRIzol-modified methods ($p < 0.05$ or $p < 0.01$) (Table 2). The results indicated that DNA extracted with the TRIzol modified method had lesser inhibitor than other methods. It might be the method of choice for other biological experiments that require large quantities of DNA, such as Southern blotting, DNA banking,[14] high throughput next-generation sequencing or microarray techniques.[13]

Evaluation of the application of the improved method

DNA samples from 20 mg pig kidney tissue and 4 mg *E. coli* were extracted using the developed silica column method and were compared with those extracted by the classical and DNAiso methods. The results showed that the DNA extracted by DNAiso was significantly higher concentration than that obtained by the classical and silica column methods, when the kidney tissue was used as the material ($p < 0.01$) (Table 5). The 260/280 value of the DNA was higher than that of DNA extracted by the silica column method ($p < 0.05$), but there was no significant difference in the concentration and purity of DNA

Table 5 The concentration and purity of DNA extracted from different materials

Sample	Method	DNA concentration/ ng μL^{-1}	DNA purity	
			260/280	260/230
Pig kidney	DNAiso	296.43 ± 29.61	1.83 ± 0.15	2.09 ± 0.02
	Classic	302.43 ± 98.45	1.81 ± 0.08	2.13 ± 0.24
	Column	208.43 ± 72.7	1.86 ± 0.01	2.28 ± 0.06
E.coli	DNAiso	80.98 ± 54.77	1.6 ± 0.1	1.99 ± 0.06
	Classic	138.58 ± 27.71	1.54 ± 0.08	2.01 ± 0.12
	Column	116.52 ± 13.91	1.7 ± 0.05	2.17 ± 0.06

extracted by the classical and silica column methods. When *E. coli* was used as the material, the 260/280 values of DNA extracted by DNAiso were significantly higher than that of the classical and improved methods ($p < 0.05$). However, there was no significant difference between the DNA concentration and value of 260/230. It was shown that the silica column and classical methods provide similar levels of purity and concentration, indicating good consistency for both methods.

Conclusions

In summary, this method is suitable for DNA extraction in a laboratory with only one standard microcentrifuge. The relatively expensive proteinase K or pronase,[16] incubators, and coated magnetic bead matrices are not required. This very simple and rapid DNA extraction method provides further opportunities for researchers to reduce costs or raise efficiency when performing their experiments and when offering genetic services.

Acknowledgements

This work was supported by the National Key Research and Development Program Projects (2017YFD0500901, 2017YFD-0500305) and Basic Scientific Research Projects of Liaoning Higher Education Institutions (LSNFW201710). We would like to thank Editage (www.editage.cn) for English language editing.

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