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# A simple guanidinium isothiocyanate method for bacterial genomic DNA isolation

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**Abstract:** A high-quality and low-cost genomic DNA isolation method is needed for use in microbiology laboratories. In this study, we developed a new modified guanidinium isothiocyanate method to isolate DNA from *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serotype Typhimurium, and *Staphylococcus aureus* and compared it with 4 other DNA isolation methods. The results show that the modified guanidinium isothiocyanate method developed in our laboratory is simple, fast, and inexpensive and yields DNA whose quality and quantity are similar to that of 2 commercial extraction kits but far better than 2 conventional DNA extraction methods used for comparison.

**Key words:** Bacteria, genomic DNA isolation, guanidinium isothiocyanate

## 1. Introduction

In environmental (Bürgmann et al., 2001), clinical (Kocagöz et al., 1993), and food (Elizaquível and Aznar, 2008) microbiology laboratories, high-quality DNA is required for identification of microorganisms in samples. For this purpose, several methods have been developed and are in use. These methods can be classified into 2 major groups: in-house extraction methods and methods using commercial kits. Commercial kits provide ready-to-use solutions like buffers and enzymes and offer convenience. In addition, many commercial kits include special supporting matrices that increase the recovery of DNA and allow more efficient removal of contaminating molecules. Thus, it is possible to obtain higher quality and quantity of DNA when one uses commercial kits. However, commercial kits are more expensive than in-house methods. DNA isolation with in-house methods requires preparation of buffers and thus takes longer. Moreover, lack of supporting matrices results in obtaining less DNA. However, satisfactory DNA yields can be obtained if proper in-house methods are used. Additionally, money can be saved by employing in-house methods, which usually require basic chemicals that can be found in almost every laboratory.

Several in-house methods have been developed to extract DNA from microorganisms. One of them is the guanidinium isothiocyanate method (Pitcher et al., 1989). This method has been modified many times up to now. Some researchers have used it to extract hepatitis B virus DNA from human serum (Manzin et al., 1991), *Yersinia ruckeri* and *Lactococcus garvieae* from culture media (Wilson and Carson, 2001), total DNA from soils and sediments (La Montagne et al., 2002), and DNA from human fecal samples (McOrist et al., 2002).

In the present study, a new modified guanidinium isothiocyanate method was developed to isolate DNA from *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serotype Typhimurium, and *Staphylococcus aureus*, and its efficiency in terms of the purity and quantity of DNA obtained was compared with 4 different extraction methods.

## 2. Materials and methods

### 2.1. Microorganisms

*Escherichia coli* (ATCC 25922) and *Salmonella enterica* subsp. *enterica* serotype Typhimurium (ATCC 14028) were obtained from the Turkish Public Health Agency, Ankara, Turkey, and *Staphylococcus aureus* (ATCC 25923) bacterium was purchased from the German Collection of Microorganisms and Cell Cultures.

*S. aureus*, *E. coli*, and *Salmonella* ser. Typhimurium were grown in tryptic soy broth (Merck, Germany) at 37 °C for 18 h. Cultures were then centrifuged at 16,000 × *g*

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for 5 min. The supernatant was removed and the pellet was resuspended with phosphate-buffered saline buffer (137 mmol $L^{-1}$ NaCl, 2.7 mmol $L^{-1}$ KCl, 10 mmol $L^{-1}$ $Na_2HPO_4$, 2 mmol $L^{-1}$ $KH_2PO_4$, pH 7.4).

The number of microorganisms used for DNA isolation was determined by inoculating serial dilutions of microorganism suspensions on culture plates. Homogeneous microbial suspensions (1000 µL each) were equally distributed into sterile microcentrifuge tubes and centrifuged at 16,000 × *g* for 5 min, and then the supernatant was removed and the tubes were stored at 4 °C until used. The amount of starting material was approximately $1.4 \times 10^8$ CFU/mL, $1.0 \times 10^8$ CFU/mL, and $1.9 \times 10^8$ CFU/mL for *S. aureus*, *E. coli*, and *Salmonella* ser. Typhimurium, respectively.

### 2.2. Methods of DNA extraction

#### 2.2.1. Freeze and thaw method

This freeze and thaw (F&T) method mainly uses freezing and thawing cycles according to a protocol published by Hasde et al. (2002). Briefly, bacterial pellet was resuspended in 100 µL of TE buffer (10 mmol $L^{-1}$ Tris, 1 mmol $L^{-1}$ EDTA, pH 8.0), and the tubes were kept at –80 °C for 5 min and incubated at 60 °C for 10 min. These 2 steps (F&T) were repeated 5 times. The tubes were then centrifuged at 5000 × *g* at 4 °C for 10 min, and the supernatant was transferred to a clean tube and stored at –20 °C until used.

#### 2.2.2. Modified guanidinium isothiocyanate method

Briefly, for the modified guanidinium isothiocyanate (GuSCN) method, the pellets were resuspended in 600 µL of lysis buffer, which was previously used as an elution buffer for aptamer studies by Qin (2009). This buffer contains 20 mmol $L^{-1}$ Tris HCl, 4 mol $L^{-1}$ guanidinium isothiocyanate, and 1 mmol $L^{-1}$ dithiothreitol (pH 7.7). The suspension was incubated at 60 °C for 5 min. The tubes were then removed and kept at room temperature for 5 min, and 2 µL of RNAse A (100 g $L^{-1}$) was added. The tubes were inverted 3 times (end-over-end inversion) and incubated at 37 °C for 20 min. After the tubes were brought to room temperature, 200 µL of ammonium acetate (2 mol $L^{-1}$) was added, and the tubes were vortexed for 20 s and incubated on ice for 5 min. Each tube was then centrifuged at 14,000 × *g* at 4 °C for 3 min. The supernatant was transferred to a clean tube containing 600 µL of isopropanol, and the tube was gently inverted 10 times and centrifuged at 14,000 × *g* for 2 min. The supernatant was removed, and the pellet was washed by adding 600 µL of 70% ethanol and centrifuging at 14,000 × *g* for 2 min. The ethanol was removed and the tube was dried at room temperature. The final pellet was dissolved in 100 µL of TE buffer (10 mmol $L^{-1}$ Tris, 1 mmol $L^{-1}$ EDTA, pH 8.0) at 65 °C for 30 min. Tubes were stored at –20 °C until used.

#### 2.2.3. Modified guanidinium isothiocyanate-column method

After the lysis step of the modified GuSCN method, a postcolumn purification step was added (High Pure PCR Product Purification Kit, Roche, Germany) for the modified GuSCN-column (GuSCN-C) method. For this, the manufacturer's protocol was used, except that the binding buffer addition step was omitted. DNA was eluted with 100 µL of TE buffer (10 mmol $L^{-1}$ Tris, 1 mmol $L^{-1}$ EDTA, pH 8.0) and stored at –20 °C until used.

#### 2.2.4. GeneSpin method

The GeneSpin (GS) method was used according to the instruction booklet included in the commercial GeneSpin kit (DNA extraction and purification kit, Cat. No. 5224400605, Eurofins, Germany). Although RNAse A was recommended by the instruction booklet of the kit, it was not used in the protocol since RNAse A was not supplied with the kit.

#### 2.2.5. Wizard method

The Wizard (W) method was done according to the instruction booklet of the Wizard Genomic DNA Purification Kit (Cat. No. A1120, Promega Corporation, USA). Following the recommendations of the manufacturer, 60 µL of 10 g $L^{-1}$ lysozyme (from hen egg white; Fluka, Germany) was used to extract DNA from *S. aureus*.

#### 2.2.6. Boiling method

The boiling (B) method mainly uses a boiling step. The bacterial pellet was resuspended in 100 µL of TE buffer (10 mmol $L^{-1}$ Tris, 1 mmol $L^{-1}$ EDTA, pH 7.2), as suggested by Aldous et al. (2005). The tubes were incubated at 95 °C for 20 min and centrifuged at 14,000 × *g* at 4 °C for 3 min. The supernatant was transferred to a clean tube and stored at –20 °C until used.

#### 2.2.7. Quantification of purified DNA samples

Amplification by real-time PCR was performed with the Light Cycler 480 Real-Time PCR System (Roche). Light Cycler 480 SYBR Green I Master Mix (Cat. No. 04707516001, Roche) was used for PCR. The primers were synthesized and purified using HPLC (Metabion International AG, Germany). After an initial denaturation at 95 °C for 5 min, 50 cycles were performed by denaturing at 95 °C for 15 s, annealing at 55 °C for 15 s, and extending at 72 °C for 30 s.

The primers used were Coag2 (5'-ACCACAAGGTACTGAATCAACG) and Coag3 (5'-TGCTTTCGATTGTTCGATGC-3') for *S. aureus* (Aarestrup et al., 1995); SalvInvA-F (5'-TCGTCATT-CCATTACCTACC-3') and SalvInvA-R (5'-AAACG-TTGAAAAACTGAGGA-3') for *Salmonella* ser. Typhimurium (Hoorfar et al., 2000); and uidA-F (5'-AAAACGCCAAGAAAAAGCAG-3') and uidA-R (5'- ACGCGTGGTTACAGTCTTGCG-3') for *E. coli* (Jefferson et al., 1986; Bej et al., 1991).

Real-time PCR was performed with the Light Cycler 480 Real-Time PCR System (Roche). For each amplification mixture, 5 µL of each DNA sample was used as the template and 3 replicates were run.

## 3. Results

### 3.1. Determination of DNA integrity

Extracted DNAs (7 µL) were examined by electrophoresis (Figure 1). As seen in agarose gel images (Figure 1), it was observed that extracted genomic DNA degraded using the F&T method and B method, except for *S. aureus* bacteria. A single genomic DNA band was observed in the GuSCN, GuSCN-C, and GS methods. Although RNA or degraded RNA/DNA bands were not visible with the GuSCN and GuSCN-C methods, ribosomal RNA bands were visible with the GS method. In the W method, intact genomic DNA was isolated at high yields with *E. coli* and *S. aureus*.

### 3.2. Determination of DNA quantity

DNA quantities were determined according to both Ct (threshold cycle) values obtained from real-time PCR quantification (Figure 2A) and absorbance values at 260 nm from a NanoDrop-1000 Spectrophotometer (Figure 2B). Ct values of each isolate were compared with each other, and it was found that the isolation efficiency of the modified GuSCN method was much higher than those of the other conventional methods (F&T and B). The yield of genomic DNA from the GuSCN-C method was lower than that of the commercial kits.

### 3.3. Determination of DNA purity

The purity of the DNA obtained from the samples in this study was calculated according to A260/A280 and A260/A230 values. The A260/A280 ratio was between 1.92 and 2.21 (Figure 2C) for all the methods studied here. A260/A230 ratios were measured between 0.96 and 1.77 for conventional methods (F&T and B); much higher ratios were observed with commercial kits (GS and W). A260/A280 and A260/A230 ratios increased with the GuSCN-C method (Figures 2C and 2D).

## 4. Discussion

The guanidinium isothiocyanate method for DNA isolation was originally developed by Pitcher et al. (1989). Their method includes the following steps: lysis using GuSCN (guanidinium isothiocyanate, EDTA, sarkosyl), extraction with ammonium acetate and chloroform-2-pentanol mixture, DNA precipitation with isopropanol, and, finally, washing with ethanol. In addition to these steps, it uses lysozyme for lysis of gram-positive bacteria. Since its publication, the method has been modified many times by other researchers (Wilson and Carson, 2001; La Montagne et al., 2002; McOrist et al., 2002). The modifications require several enzymes, detergents like SDS, PEG, diatomaceous earth, or phenol–chloroform extractions.

In this study, we used GuSCN buffer (guanidinium isothiocyanate, Tris-HCl, and DTT) as an aptamer elution buffer from proteins (Qin et al., 2009) to lyse the bacteria. A heating step was added to the protocol to increase the yield of genomic DNA isolation. We did not use any enzymes (except RNAse A) like lysozyme to lyse gram-positive bacteria, nor any extraction step using any harmful chemicals like chloroform. However, we were able to obtain genomic DNA in high yield using a few simple steps. In addition, we also tried to use a purification column after the lysis step, which we called the GuSCN-C method. Finally, we compared the efficiency and purity of the genomic DNA isolated by the modified method to

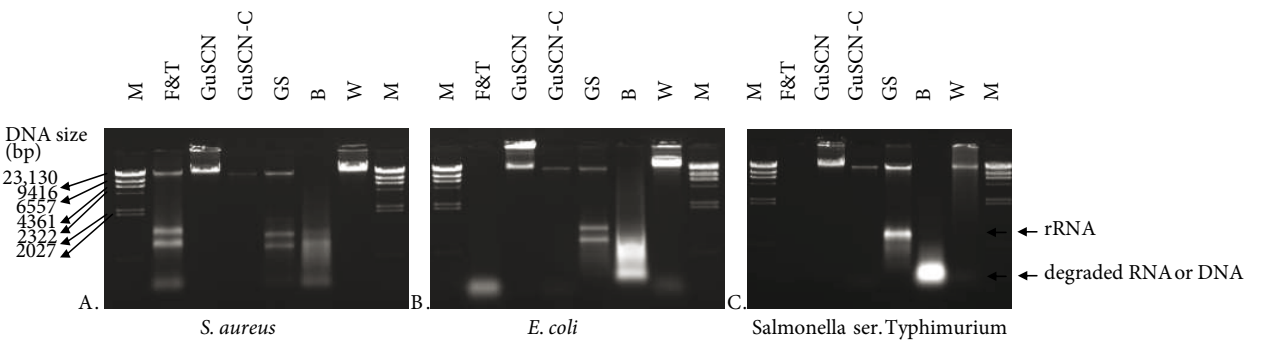


**Figure 1.** Comparison of different genomic DNA isolation methods with respect to quantity, integrity, and nucleic acid purity. Purified genomic DNA was analyzed by DNA gel (0.8% agarose) electrophoresis and visualized under UV light. Genomic DNA from gram-positive bacteria *S. aureus* (A) and gram-negative bacteria *E. coli* (B) *and Salmonella* ser. Typhimurium (C) was purified. Lanes: M, Lambda DNA HindIII digest molecular size standard, marker; F&T, freeze and thaw method; GuSCN, modified guanidinium isothiocyanate method; GuSCN-C, modified guanidinium isothiocyanate method followed by a column purification step; B, boiling method; GS, GeneSpin method; W, Wizard method. Note that the GuSCN method yields intact genomic DNA bands; its efficiency is much higher than other conventional methods and is comparable to that of commercial kits.

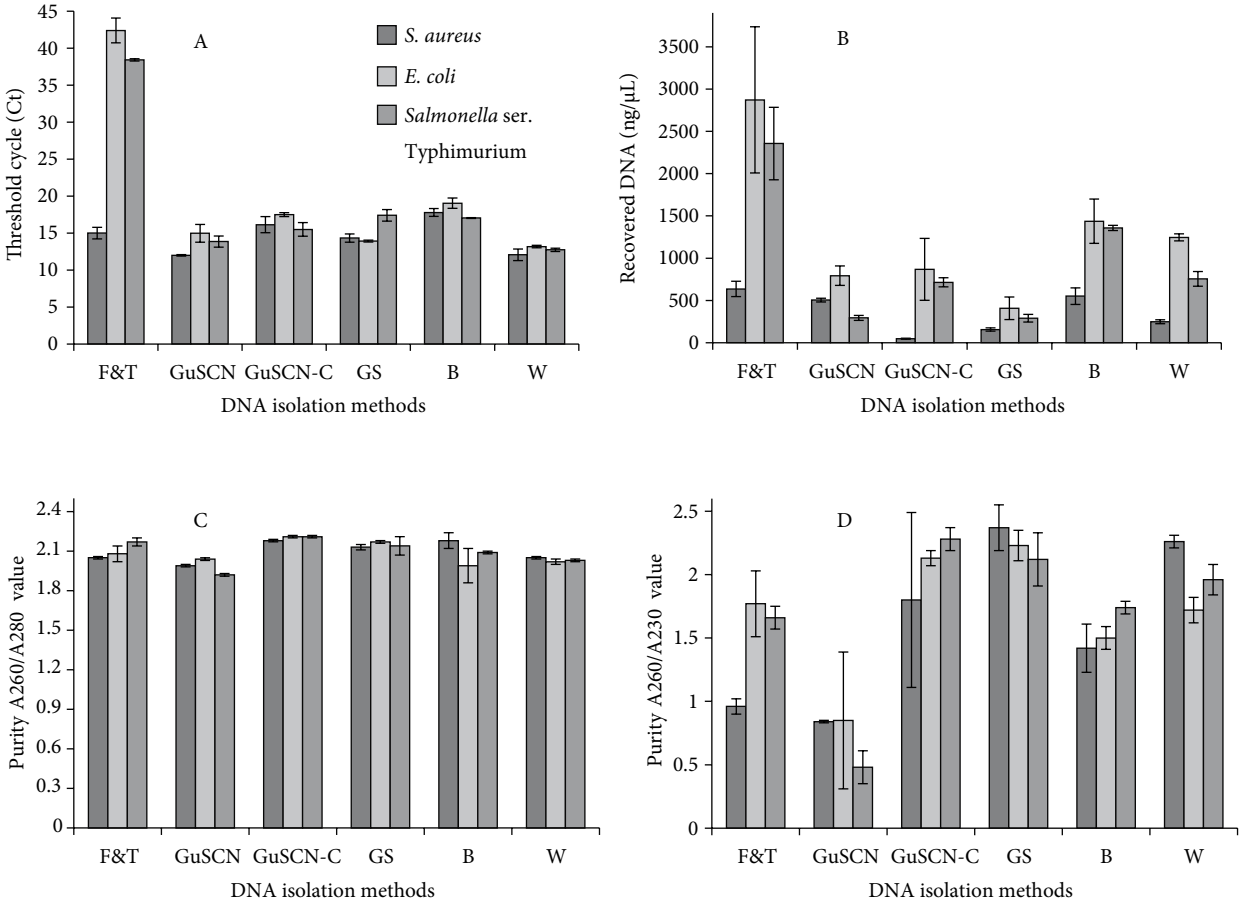


**Figure 2.** Comparison of different genomic DNA isolation methods with respect to quantity and nucleic acid purity. Threshold cycle (Ct) after RT-PCR (A); amount of DNA recovered by each DNA purification method (ng/µL) (B); efficiency of elimination of proteins: purity (A260/A280 value) (C); elimination of carbohydrates: purity (A260/A230 value) (D). Final elution volumes of all purification methods were 100 µL.

other conventional DNA isolation methods [the F&T of Hasde et al. (2002) and the B of Aldous et al. (2005)] and commercial kits [GeneSpin kit (GS) and Wizard kit (W)] by isolating DNA from the gram-positive bacteria *S. aureus* and the gram-negative bacteria *E. coli* and *Salmonella* ser. Typhimurium.

Since spectrophotometric methods alone may not be reliable in measuring the amount of genomic DNA accurately, in this study, purified genomic DNAs were examined by gel electrophoresis and the quantity of amplifiable genomic DNA was determined using real-time PCR. As shown in Figure 1, the F&T method failed to extract genomic DNA from gram-negative bacteria (*E. coli*, *Salmonella* ser. Typhimurium) but was partially successful for gram-positive bacteria (*S. aureus*), as confirmed by real-time PCR analysis (Figure 2A). Although the boiling method did not reveal any intact genomic DNA bands (Figure 1), even the degraded DNA, seen in the gel, could be amplified by PCR. Both the GeneSpin (GS) kit and the Wizard (W) kit extracted intact genomic DNA that was visualized as bands in gels (Figure 1), which also corresponded to lower Ct (threshold cycle) values (Figure 2A). Our modified GuSCN method revealed intact genomic DNA bands in gels with no sign of degradation (Figure 1). The isolation efficiency of the modified GuSCN method was much higher than that of the other conventional methods (Figure 2A) and extracted DNA whose yield and quality were comparable to those of kit methods (GS method and W method; Figures 2B–2D).

The amount of protein contamination in isolated DNA was assessed by calculating the ratio of absorption at 260 nm to that at 280 nm. All of the methods studied here yielded 260/280 nm absorption ratios between 1.92 and 2.21 (Figure 2C), which indicates effective removal of protein. Carbohydrate contamination, measured by the ratio of absorption at 260 nm to 230 nm, was also evaluated, and it was found that while conventional methods (F&T and B) produced genomic DNA with 260/230 absorbance

ratios between 0.96 and 1.77, commercial kits (GS and W) exhibited much higher absorbance values (Figure 2D). Since some impurities may lead to PCR inhibition, purified DNA preparations were diluted and their Ct (threshold cycle) values were determined and compared (data not shown). It was found that the change in Ct corresponds to the amount of starting template DNA, which suggested that PCR was not affected by impurities. Adding a purification column step to the GuSCN method increased both A260/A280 and A260/A230 ratios, indicating the removal of residual contaminants. However, addition of this purification column step decreased the amount of DNA obtained; we do not recommend its use.

Our modified GuSCN method is inexpensive, simple, rapid, and efficient for purification of genomic DNA from gram-positive bacteria such as *S. aureus* and gram-negative bacteria such as *Salmonella* ser. Typhimurium and *E. coli*. Moreover, the method does not require any enzymes such as lysozyme, detergents, SDS, PEG, or diatomaceous earth. The modified GuSCN method was also used for genomic DNA extraction from *C. albicans* and the yield was comparable to other conventional methods (data not shown).

In conclusion, the results of this study provide researchers with a new isolation method to obtain genomic DNA from bacteria in high yields that is suitable for PCR amplification.

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