

A Simple "Universal" DNA Extraction Procedure Using SDS and Proteinase K Is Compatible with Direct PCR Amplification

Daniel Goldenberger, Inge
Perschil, Michael Ritzler, and
Martin Altwegg

Department of Medical Microbiology,
University of Zürich, 8028 Zürich,
Switzerland

A large number of different protocols for the efficient isolation of highly purified DNA from eukaryotic and prokaryotic cells is extant.⁽¹⁻⁴⁾ These procedures usually include treatment with proteinase K in the presence of SDS, which efficiently lyses the cells and nuclei and liberates the DNA tightly bound in chromatin.⁽⁵⁾ Proteins are then extracted with phenol and chloroform, and the nucleic acids are precipitated with ethanol. This procedure is tedious and time-consuming, and significant amounts of DNA may be lost, especially when working with small specimens (e.g., joint biopsies). Therefore, this approach is not appropriate for diagnostic tests. Direct amplification of digested samples without phenol/chloroform extraction and precipitation is not possible because SDS is inhibitory to *Taq* polymerase at concentrations as low as 0.01%.⁽⁶⁾ Alternative simple DNA extraction procedures have been used but have often resulted in incomplete lysis of the cells. These procedures typically have included detergents (e.g., Triton X-100), chaotropes (e.g., guanidium isothiocyanate or sodium iodide), proteases (e.g., proteinase K), substances that lyse erythrocytes and leukocytes (e.g., saponin), or heat denaturation.⁽⁷⁾ Often nonionic detergents such as Tween 20 or Laureth 12 in combination with proteinase K are used, followed by heat inactivation of the enzyme prior to PCR amplification.⁽⁷⁻¹⁰⁾

In our laboratory we have aimed at establishing an efficient but simple DNA extraction procedure applicable to various types of clinical specimens, including tissue, sputum, liquid specimens, and bacterial cultures. The procedure would allow direct PCR amplification without purification or precipitation of the DNA. Here, we describe the application of the widely used SDS/proteinase K method made compatible with direct PCR amplification, and including the use of uracil *N* glycosylase (UNG) to prevent false positives caused by amplicon carryover.⁽¹¹⁾

MATERIALS AND METHODS

PCR Amplification and Detection

A eubacterial PCR amplification system, which included dUTP instead of dTTP, was used as described previously⁽¹²⁾ except for performing 40 cycles instead of 30. Also, pyrogen-free water was used

that had been shown to be free of contaminating bacterial DNA.⁽¹³⁾

Various amounts of SDS (Fluka, Buchs, Switzerland) and Tween 20 (Sigma Chemical, St. Louis, MO) were added to amplification reactions alone and in combination. This was done to (semi-)quantitate the neutralization effect of Tween 20 on SDS. PCR products were analyzed by agarose gel electrophoresis, Southern blotting, and hybridization as described.⁽¹²⁾

Bacteria and CFU Determination

Clinical isolates of *Enterococcus faecalis*, *Corynebacterium diphtheriae* (nontoxicogenic), and *Escherichia coli* as well as a reference strain of *Staphylococcus aureus* (ATCC 25913) were grown overnight on sheep blood agar at 37°C. One colony was then suspended in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) and incubated at 37°C to a density corresponding to a 0.5 McFarland standard. Aliquots (0.5 ml) of 10-fold serial dilutions (using physiological saline) were stored at -20°C while the number of bacteria (CFU/ml) was estimated by culturing 100 µl of each dilution step for 48 hr on sheep blood agar at 37°C.

DNA Extraction

Aliquots (0.5 ml) of serial dilutions of the bacterial suspensions were thawed and centrifuged for 10 min at 12,000g and the pellets treated according to one of the following procedures.

Procedure A

Bacterial pellets were resuspended in 0.2 ml of digestion buffer [50 mM Tris-HCl (pH 8.5), 1 mM EDTA, 0.5% SDS, 200 µg/ml proteinase K] and incubated for 3 hr at 55°C with agitation (Thermomixer, Eppendorf). After heat inactivation of the proteinase K for 10 min at 95°C, the tubes were cooled to 4°C and centrifuged for 10 min at 12,000g. Ten microliters of the supernatant was used directly for PCR amplification while adding 2% Tween 20 (final concentration) to the amplification mix.

Procedure B

Bacterial pellets were resuspended in 0.2 ml of Triton X-100 buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% Triton

TABLE 1 Neutralization of SDS with Tween 20

Tween 20 (%)	SDS (%)										
	0	0.005	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5
0	+	+	-	-	-	-	-	-	-	-	-
2	+	+	+	+	+	-	-	-	-	-	-
5	+	+	+	+	+	+	+	-	-	-	-
10	+	+	+	+	+	+	+	-	-	-	-

Purified bacterial DNA was amplified in the presence of different amounts of SDS and Tween 20, followed by agarose gel electrophoresis and staining with ethidium bromide. (+) Visible band of the expected DNA length; (-) absence of expected band and primer-dimer fragment indicating inhibition.

X-100] and incubated for 30 min at 95°C with agitation. The tubes were then cooled to 4°C and centrifuged for 10 min at 12,000g and 10 µl of the supernatant was used directly for amplification.

Influence of SDS and Tween 20 on UNG Activity

Approximately 10^7 molecules of the 798-bp PCR product containing uridine instead of thymidine was incubated in PCR buffers with different amounts of SDS, Tween 20, and combinations of the two

with or without UNG for 15 min at room temperature. To prevent inhibition of the subsequent PCR amplification caused by SDS, each sample was diluted 1000-fold and reamplified in the absence of UNG.

RESULTS AND DISCUSSION

High concentrations of Tween 20 ($\leq 10\%$ final concentration) have no influence on amplification efficiency, whereas minute quantities (0.01%) of SDS are sufficient to inhibit *Taq* polymerase signif-

icantly (Table 1). This result agrees with the findings of Gelfand,⁽⁶⁾ who described relative *Taq* polymerase activities of 105%, 10%, and $<0.1\%$ in the presence of 0.001%, 0.01%, and 0.1% SDS, respectively. The fact that 0.5% nonionic detergents such as Tween 20 or NP-40 instantaneously neutralize 0.01% SDS⁽⁶⁾ prompted us to quantitate this effect more thoroughly. Table 1 shows that $\leq 0.2\%$ SDS (final concentration) can be neutralized by Tween 20. We assumed that a commonly used procedure to extract DNA from bacterial cells as well as from eukaryotic tissue—a combination of 0.5% SDS and proteinase K which is expected to be more efficient than using a nonionic detergent as described by Relman⁽⁸⁾—might be compatible with direct amplification, omitting the tedious and not very efficient extraction with organic solvents followed by ethanol precipitation. Therefore, we compared a SDS/proteinase K-based extraction (procedure A) with one including Triton X-100 as detergent and heat (procedure B) for their efficiency in lysing various bacterial species and their compatibility with direct amplification by analyzing

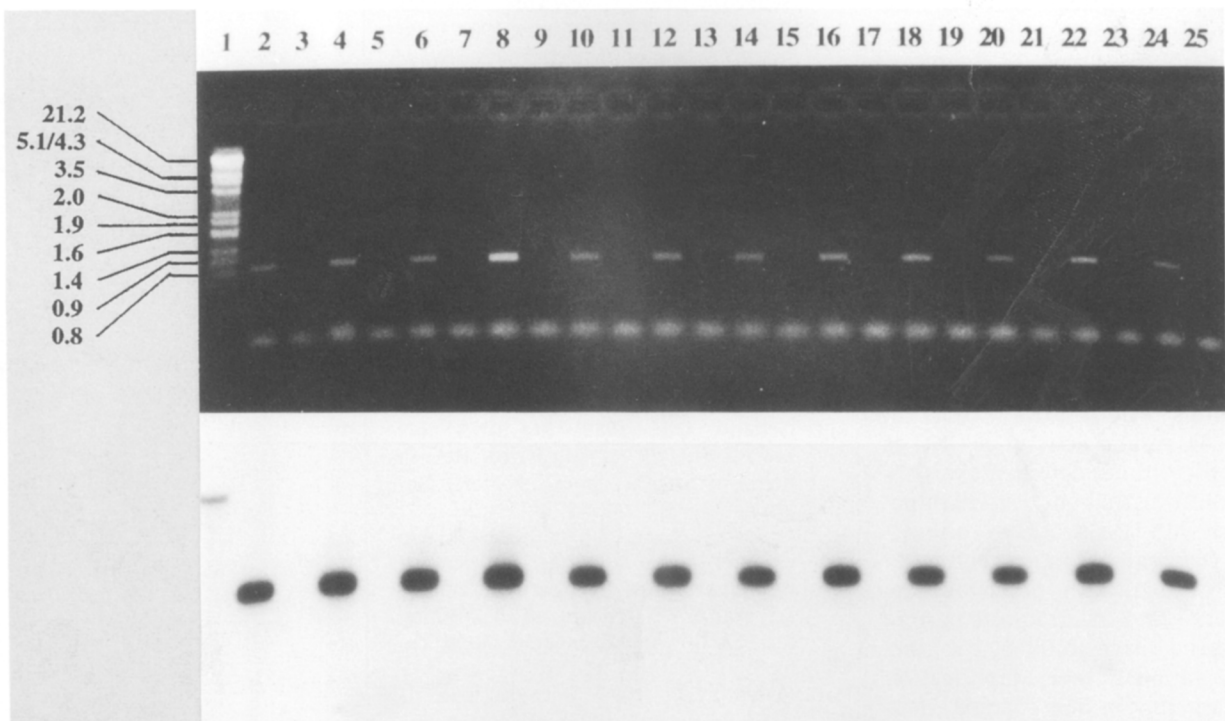


FIGURE 1 Inactivation of dUTP-containing DNA (798 bp) by the activity of UNG in presence of different amounts of SDS, Tween 20, and combinations of the two. DNA molecules ($\sim 10^7$) were incubated without (lanes 2,4,6,8,10,12,14,16,18,20,22,24) and with UNG (0.5 unit) (lanes 3,5,7,9,11,13,15,17,19,21,23,25) for 15 min at room temperature, diluted 1:1000, and 10-µl aliquots were reamplified. SDS/Tween 20 concentrations (%) were 0:0 (lanes 2,3), 0.025:0 (lanes 4,5), 0.05:0 (lanes 6,7), 0.075:0 (lanes 8,9), 0.1:0 (lanes 10,11), 0.15:0 (lanes 12,13), 0.2:0 (lanes 14,15), 0.5:0 (lanes 16,17), 0:2 (lanes 18,19), 0:5 (lanes 20,21); 0.05:2 (lanes 22,23), and 0.1:2 (lanes 24,25). (Lane 1) Molecular size marker.

serial dilutions of bacteria. As expected, Gram-negative bacteria are lysed more easily than Gram-positive bacteria; however, the two procedures were almost equally sensitive. Between seven cells (*E. coli*, both procedures) and 600 cells (*S. aureus*, procedure A) per amplification resulted in a visible band on stained agarose gels (data not shown). Considering the fact that the sensitivity of the procedure can be improved by a factor of at least 10 and up to 100 by Southern blotting and hybridization with a digoxigenin-labeled probe, the sensitivity achieved is sufficient for diagnostic purposes. Procedure A was slightly less efficient than procedure B only for *S. aureus*, but procedure B cannot be used in conjunction with tissue samples. Therefore, procedure A, based on digestion buffer with SDS and proteinase K, is now being used for all of our diagnostic applications.

In our eubacterial PCR system, contaminating DNA is inactivated by exposure to UV light.⁽¹²⁾ The incorporation of 2% Tween 20 into the amplification mix prior to exposure to UV light did not affect DNA inactivation nor amplification efficiency (data not shown).

In all of our amplifications, UNG enzyme and dUTP, instead of dTTP, are used to prevent product carryover from previous amplifications.⁽¹¹⁾ Therefore, we examined the compatibility of our DNA extraction procedure with the UNG system by analyzing the inhibitory effect of SDS, Tween 20, and combination of the two on the UNG enzyme. As can be seen in Figure 1, up to 0.5% SDS does not affect the activity of UNG. This also has been reported for proteinase K, which is just as active in the presence of SDS.⁽⁴⁾ With the combination of 0.05% SDS and 2% Tween 20 (final concentrations for amplifications when using extraction procedure A), similar activity of UNG as in the sample without detergents was observed, indicating that our decontamination system with UNG works in conjunction with DNA extraction procedure A.

The DNA extraction procedure described, based on SDS and proteinase K, has been used successfully in conjunction with various other amplification systems targeting other bacteria (*Bartonella henselae*, *Bartonella quintana*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Borrelia burgdorferi*) and using a variety of clinical samples such as joint and other

body fluids, tracheal secretions, bronchoalveolar lavages (all after centrifugation), sputum (liquefied and centrifuged), and with slight modifications of the procedure (overnight incubation in digestion buffer and sonication), tissue specimens. We conclude that this extraction procedure is easy to use and almost universally applicable. It allows direct amplification without further purification when combined with Tween 20 in the amplification mix and also is compatible with the UNG procedure for the prevention of amplicon carryover.

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