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Research Note

NON-PHENOLIC METHOD OF DNA PURIFICATION FROM BACTERIA, BLOOD SAMPLES AND OTHER BIOLOGICAL SOURCES FOR RESTRICTION ENZYME ASSAYS AND THE POLYMERASE CHAIN REACTION

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Many methods of DNA purification from eukaryotic and prokaryotic cells have been described. The most common involves the use of phenol and chloroform to remove proteins and lipids from nucleic acids (T. Maniats et al., 1982. In *Molecular Cloning. A laboratory manual,* Cold Spring Harbor, NY, p. 280-281). The major drawback of the method is, however, the caustic and toxic properties of the organic reagents used. Alternative procedures using buffered SDS with proteinase K and extensive dialysis or sodium perchlorate and chloroform or guanidium thiocyanate have been described (J. L. Longmir et al., 1987, *Nucleic Acids Res., 15:* 859; B. J. Malcon & E. P. Jeanne, 1989, *Anal. Biochem., 180:* 276-278; D. G. Pitcher et al., 1989, *Lett. Appl. Microbiol., 8:* 151-156). We adapted a procedure for lambda DNA purification (L. S. Ozaki & Y. M. T. Cseko, 1984. In *Genes and Antigens of Parasites: a laboratory manual.* 2. ed. Fundação Oswaldo Cruz, Rio de Janeiro, p. 172-173) to prepare genomic DNA from prokaryotic and eukaryotic cells that is rapid, requires little manipulation, and used materials and reagents readily available in any laboratory working with molecular biological techniques.

DNA purification. I) STE-ammonium acetate method – various types of cells were prepared and their DNA purified by the STE-ammonium acetate method as follows:

A) *Bradyrhizobium japonicum* – Cells of *B. japonicum* saturated culture (1.5 ml; 1.5 x 10^9 cells) were pelletted in an Eppendorf tube, washed twice with 1 ml of PBS (150 mM NaCl/ 2.6 mM NaH2PO4/ 7.4 mM Na2HPO4, pH 7.2) and resuspended in 500 µl of the same buffer. 50 µl of fresh lysozyme (5 mg/ml in water) and 1 µl of pancreatic RNase (10 mg/ml in water) were added and the mixture kept in ice for 10 min. 1 µl of proteinase K (20 mg/ml in water) or 50 µl of autodigested pronase (10 mg/ml in water) and 100 µl of STE (10 mM Tris-HCl/ 250 mM EDTA/ 2.5% SDS, pH 8.0) were added and the mixture incubated at 37 °C for 15 min. 100 µl of 8,0 M ammonium acetate (pH not adjusted) were added and incubated at room temperature for 15 min. The white proteinaceous precipitate was pelletted by centrifugation in a microcentrifuge for 2 min at room temperature. The supernatant was transferred to another Eppendorf tube, 0,6 volumes of isopropanol were added, mixed at room temperature for 1 min and the fibrous DNA precipitate was harvested by centrifugation. The pellet was washed twice with 1 ml 80% ethanol and then dissolved in 50 µl TE (10 mM Tris-HCl/ 1 mM EDTA, pH 7.5).

B) Human and bovine white blood cells – human and bovine blood (1 ml) was diluted in 5 volumes of PBS. Erythrocytes were lysed with saponine (Sigma Co.) at a concentration of 0.01-0.02% and leucocytes harvested by centrifugation at 3,000 g. The cells were transferred to an Eppendorf tube and washed twice 1 ml PBS, resuspended in 500 µl of the same buffer and processed from this step on as in A) except that lysozyme was omitted.

C) *Cultured Plasmodium falciparum* – Saponine was added to 2 ml of an *in vitro* culture of *P. falciparum* (5 x 10^7 parasite cells; 5%

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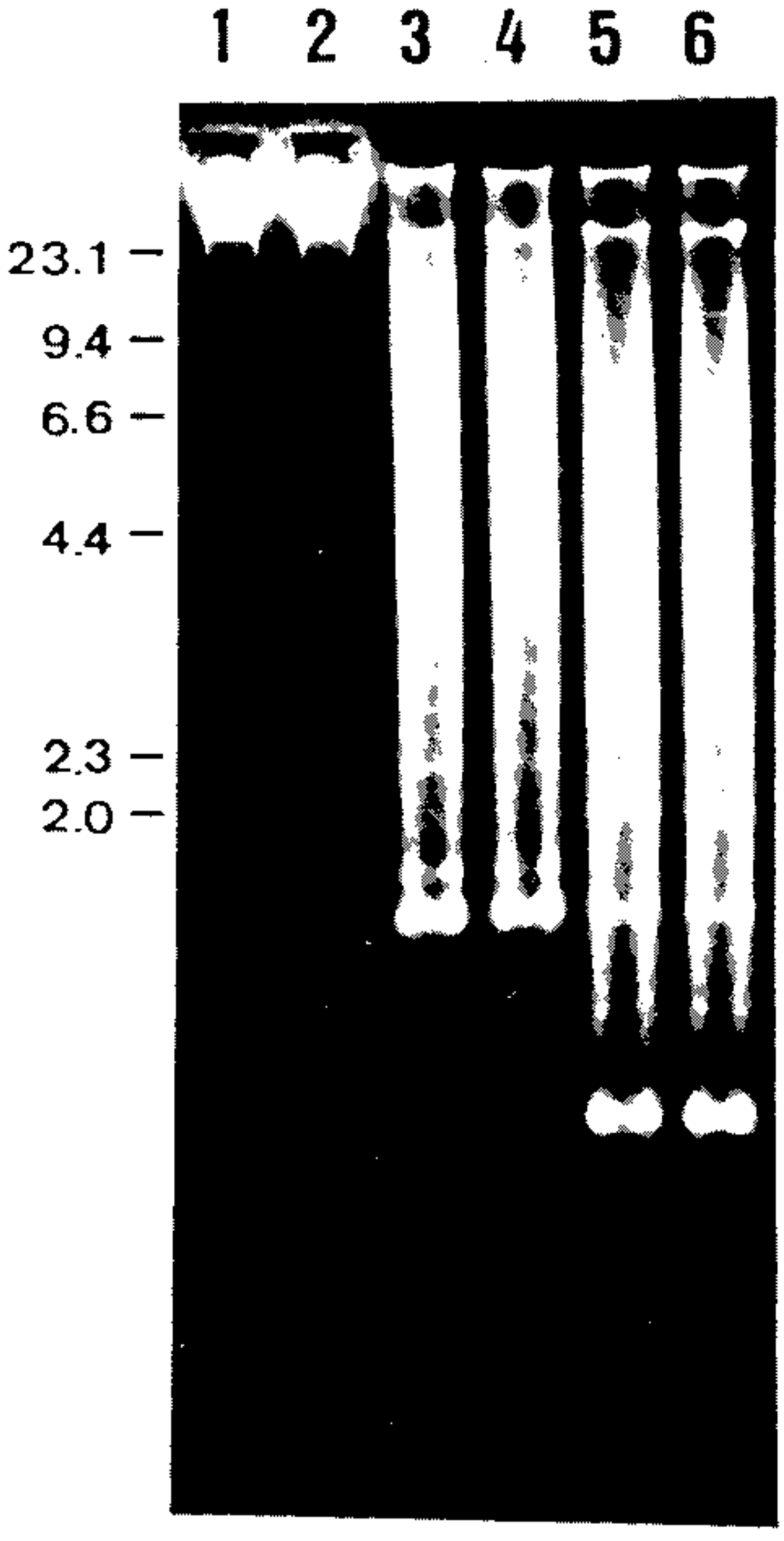

Fig. 1: agarose gel electrophoresis of restriction endonucleases digest of bovine DNA prepared by the phenol-chlorophorm (lanes 1, 3 and 5) and the STE-ammonium acetate methods (lanes 2, 4 and 6). The restriction endonucleases used were EcoRI (lanes 3 and 4) and PstI (lanes 5 and 6). Lanes 1 and 2 uncut DNA controls. Lambda phage DNA digested with Hind III was used as molecular weight markers.

hematocrit and 20% parasitemia) to a final concentration of 0.01-0.02%. Parasite cells were harvested and transferred to an Eppendorf tube. The cells were washed twice with 1 ml of PBS, resuspended in 500 µl of the same buffer, and processed as in B).

D) *P. falciparum* in blood of infected patients – blood from *P. falciparum* infected patients (E. Kimura et al., 1990, *Gene, 91:* 57-62) (5 ml, 0,06-1% parasitemia) were collected and diluted in 5 volumes of PBS. Saponine was added to a concentration of 0.01-0.02% and cells were harvested by centrifugation at 5,000 rpm, transferred to an Eppendorf tube and washed twice with 1 ml PBS. The cells were resuspended in 500 µl of PBS and processed as in B).

DNA purification. II) phenol chloroform method – DNA from the cells described above was purified in parallel by the standard phenol-chloroform method (T. Maniats et al., *loc. cit.*).

Restriction enzyme assays – DNA samples were digested with 2U/µg of restriction endonucleases. Assay conditions: 1) Eco RI (Enzibiot) – 100 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM MgCl2, 100 µgm/1 BSA, at 37 °C for 1h. 2) Pst I (Biolabs) – 50 mM Tris-HCl pH 7.9, 100 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, at 37 °C for 1h.

PCR amplification – Amplification with the polymerase chain reaction method (R. K. Saiki et al., 1988, *Science, 239:* 487-491) was performed with oligonucleotides specific for the highly conserved regions of 5' end of p190 *P. falciparum* gene using DNA prepared from blood of malarial patients (E. Kimura et al., *loc. cit.*), with 40 cicles (120 s at 95 °C; 90 s at 55 °C and 150 s at 72 °C).

The efficiency of DNA extraction using the STE and the phenol-chloroform methods were compared. There is no significant differences in DNA yields extracted with either method. The amount of nucleic acid purified from various organisms were approximately 2 µg per 10^8 *B. japonicum* cells, 270 µg per 10^8 human or bovine leucocytes and 10 µg per 10^8 *P. falciparum* cells (*in vitro* cultured parasites).

DNAs prepared by either method were digested with EcoRI or PstI and fractionated by agarose gel electrophoresis (Fig. 1). The pattern of digested DNA showed to be equivalent for both type of DNAs (compare Fig. 1, lanes 3-6), suggesting a complete digestion of the DNAs and absence of enzyme inhibitors in the preparations.

We determined the ratio of absorbancy readings (Beckman DU-9 spectometer) at 260 and 280 nanometers (OD260/OD280) of STE prepared DNAs from *B. japonicum,* bovine and human leucocytes. The ratio values obtained were around 2.0 which are comparable to that of phenol-chloroform prepared DNA (T. Maniats et al., *loc. cit.*).

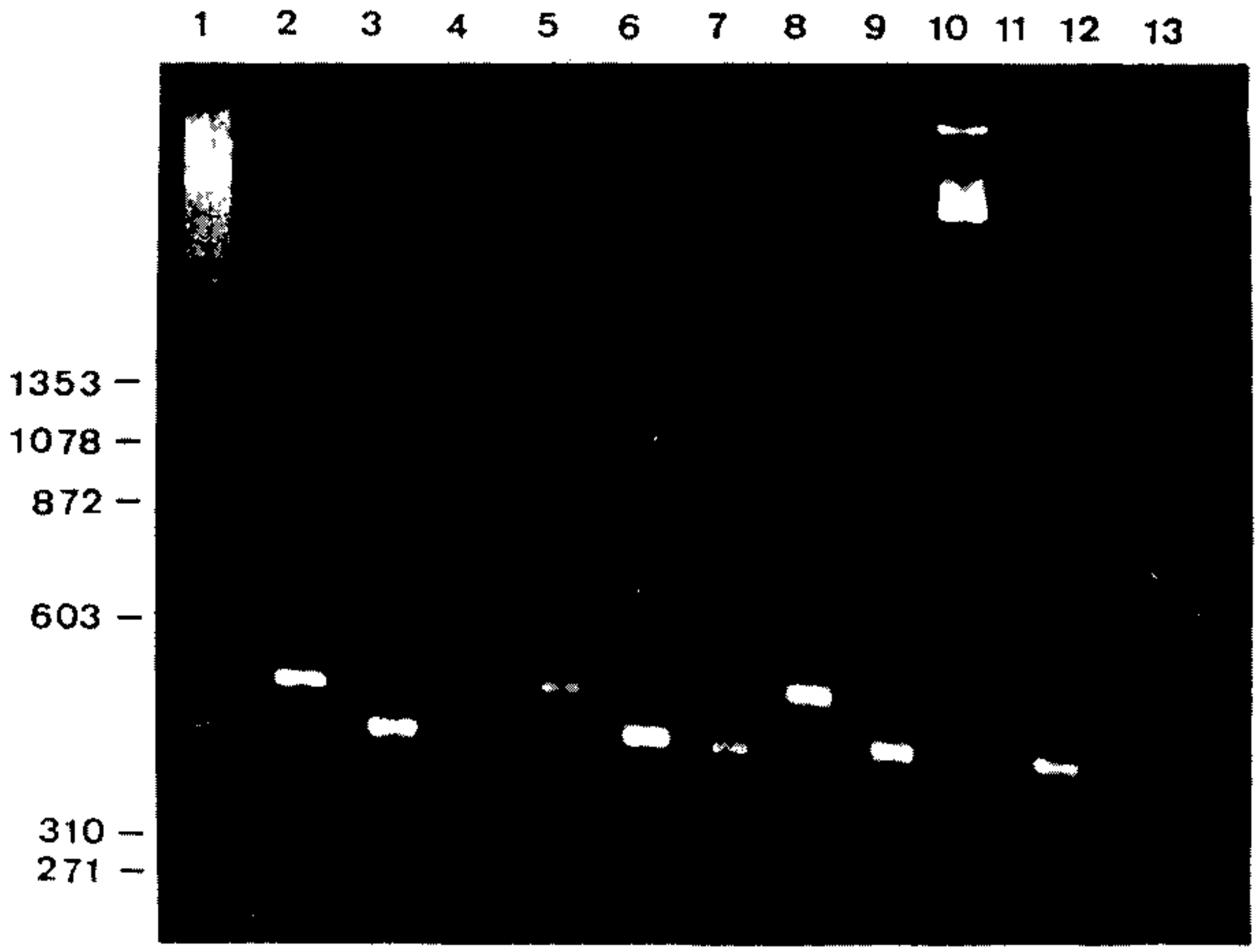

Fig. 2: PCR of a *Plasmodium falciparum* sequence in DNA prepared from blood of malarial patients by the STE-ammonium acetate method. Lanes 1-12, infected blood samples, lane 13, negative control. ϕ 174 RF DNA digested with Hae III was used as standard for molecular weight determination.

DNA amplification by polymerase chain reaction (PCR) were performed with total DNA from blood of *P. falciparum* infected human patients purified by the STE-ammonium acetate method and parasite specific oligonucleotides (E. Kimura et al., *loc. cit.*). Fig. 2 shows the ethidium bromide stained bands of amplified sequences run in agarose gel. Specific parasite sequences are readly amplifiable by Taq polymerase with the oligonucleotides based on the low copy number p190 gene of *P. falciparum* (K. Tanabe et al., 1987, *J. Mol. Biol., 195:* 273-287). The fragments size variation is due to a polymorphic region of gene p190 that is specific for each *P. falciparum* strain (K. Tanabe et al., *loc. cit.*).

We adapted a non-phenolic method for extraction of nucleic acid of lambda phage for the purification of DNA from various biological sources. The method proved to be useful for preparing DNA from *B. japonicum,* human and bovine leucocyte, and *P. falciparum.* It is particularly convenient for processing a large number of samples as is the case when screening blood from patients for malarial DNA sequence with PCR (E. Kimura et al., 1990, *loc. cit.*). No more than 500 ng of total DNA from blood of patients with 0,06 to 1% parasitemia were required.

The STE-ammonium acetate procedure has also been used for the purification of DNA from other organisms such as *Rhizobium* spp, *Bradyrhizobium spp, Escherichia coli, Yersinia pestis, Saccharomyces cerevisiae, Trypanosoma evansi, Babesia spp* and the bovine tick *Boophilus microplus* (results not shown). Enzymatic assays with DNaseI, and various restriction enzymes were performed on the purified DNAs and no inhibitors for these enzymes were observed (results not shown).