Volume 17 Number 20 1989 Nucleic Acids Research

Efficient DNA isolation within a single gel barrier tube

Ruben F.Moreno, Frank R.Booth[1], Irene H.Hung and Lowell L.Tilzer

Kansas University Medical Center, Department of Pathology, 39th and Rainbow, Kansas City, KA 66103 and [1]Regional Crime Laboratory, Kansas City Police Department, 1525 Holmes, Kansas City, MO 64108, USA
Submitted August 25, 1989

We previously described (1),the method in which organic solvents can be separated from aqueous containing DNA by using silica gel tubes (SST tubes). The role of this tube in DNA isolation can be extended further to include performing the SDS-Proteinase K digestion and alcohol precipitation steps, in addition to the phenol:chloroform extraction. The system is ideal for isolating DNA from large numbers of samples quickly, safely, and easily.

Approximately one half ml of buffy coat from five ml of EDTA anticoagulated blood was obtained by centrifugation at 1500g for 5 minutes. Contamination of buffy coat with plasma and red cells was kept to a minimum. The buffy coat was placed in a 9.5 ml SST tube containing 1.0 ml of 0.4 M Tris-HCl, pH 8.0, 0.1 M EDTA, 1% SDS, and 200 micrograms of Proteinase K. Besides blood, solid tissue homogenates and cultured cells could be used. The mixture was incubated 1 hr. at 50 °C with occasional mixing. Two ml of phenol and chloroform (1:1) were added, shaken five minutes, then the tube was centrifuged 5 minutes at 1500g. The organic layer and digested proteins were trapped beneath the silica gel. One half volume of 7.5 M ammonium acetate and one volume of isopropanol were added to the DNA containing layer above the gel barrier. The tube was inverted several times until the DNA precipitated. The DNA was spooled with a plastic loop and redissolved in 0.01 M Tris, pH 8.0, and 0.0001 M EDTA.

The yield and quality of DNA as determined by spectrophotometric measurement and electrophoresis are excellent. The purified DNA cuts easily with restriction endonucleases. The entire method: protease digestion, DNA extraction, and alcohol precipitation, occurs in a single tube. Due to this streamlining of the technique, the process becomes semiautomated. More phenol:chloroform extractions of a single sample could be performed in the same tube by using the larger 13.5 ml capacity SST tube. After the second centrifugation, both organic phases become trapped beneath the gel barrier. However, for most purposes this second (or third extraction with chloroform alone) is not necessary.

Thus, for DNA isolation from just a few samples, the procedure takes less than 90 minutes. The procedure can be used to extract large numbers of blood samples per day. Performing all the work in a single tube avoids transposition errors when handling multiple specimens. Finally, an instrument could be designed that automates the entire procedure permitting hundreds of samples to be processed with minimal labor and time.

REFERENCES

1. Thomas, S.M., Moreno, R.F., and Tilzer, L.L. (1989) Nucl. Acids Res. 17, 5411.