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A SUGAR, LAUNDRY DETERGENT, AND SALT METHOD FOR EXTRACTION OF DEOXYRIBONUCLEIC ACID FROM BLOOD

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We present a method for DNA extraction from peripheral blood using sugar, commercial detergent, and sodium salt. Our method is simple, fast, and inexpensive; its qualitative parameters do not significantly differ from the standard salting-out procedure.

INTRODUCTION

Many commercial and in-house methods exist for the extraction of mammalian DNA from peripheral blood. However, in our opinion none of the published methods meets the composite criteria for yield, purity, reliability, non-toxicity, speed, and price to be used routinely in a small laboratory. A simple method for DNA extraction from human peripheral blood using sugar, commercial detergent (*Persil*), and sodium salt is presented that fulfils such criteria.

MATERIALS AND METHODS

Persil from Henkel was purchased at the local store; other chemicals were from Sigma. Statistical comparisons were made on DNA extracted from peripheral blood drawn from one pig at time of slaughter. Optimized DNA extractions were done on peripheral blood of cadaveric donors.

The procedure comprised the following steps:

(i) Lysis buffer (0.3 M sucrose, 0.01 M TrisCl pH 7.5, 5 mM $MgCl_2$*$6H_2O$, 1% Triton X100) was added to 5–10 ml of EDTA-anticoagulated blood into 50 ml tube to reach a final volume of 45 ml.

(ii) Tubes were centrifuged immediately for 5 min at 2700 g. Supernatant was discarded and 1 ml of 10 mM Tris pH 8 added to the sediment. The sediment was released from the bottom of the tube by swirling and poured quickly into a 2 ml tube.

(iii) The sediment was resuspended by vigorous mixing and centrifuged for 1 min at 675 g. The supernatant was discarded and leukocytes resuspended in 1 340 ml of 10 mM TrisCl pH8. Leukocytes were divided into two 2 ml tubes, 660 ml each.

(iv) 660 ml of *Persil Green Power* (Henkel; 20 mg/ml; stored in frozen aliquots) and a clean glass bead (4 mm diameter) was added to each tube.

(v) Tubes with beads were mixed vigorously to homogenize the contents (1 min maximally). 500 ml of 5 M NaCl was added; tubes were mixed vigorously for 10 s and centrifuged for 5 min at 17 500 g.

(vi) The supernatant was poured into two new 2 ml tubes and centrifuged for 3 min at 17 500 g. The supernatants were combined into one tube and DNA precipitated by adding 3 ml of 96% ethanol.

(vii) DNA precipitate was retrieved using a glass pipette with heat-sealed thin end and washed in two 1.5 ml tubes with 0.5 ml of 70% ethanol each. Ethanol was removed by squeezing onto the walls of the second tube and DNA dissolved in 1 ml of 10 mM TrisCl pH 8.

(viii) DNA was incubated for 5 min at 70 °C. When DNA remained in bulk, it was resuspended by up- and down- pipetting with a filter-tipped 1 ml pipette.

(ix) For the longer term, DNA was stored at –20 °C.

RESULTS AND DISCUSSION

The standard salting-out procedure of Miller *et al*.[1] consists of buffy coat separation, overnight cell lysis by serine protease from *Tritirachium album* (proteinase K), salting out by NaCl, DNA precipitation by ethanol, and resuspension. In order to accelerate and economize the standard method, we used red cell lysis[2,3] instead of buffy coat separation, and replaced proteinase K with the detergent *Persil*.[4] There were no significant differences between the standard method and ours in terms of DNA yield and purity: we measured yield per ml of porcine blood (standard, 47.0±7.3 μg; our method, 40.9±4.3 μg) and purity by $OD_{260/280}$ ratio (1.844±0.016; 1.841±0.012). DNA was not degraded and it did not inhibit PCR with sequence specific primers [5,6] or digestions by restrictases. During five years of use for urgent medical samples, our method has had less than 2% failure rate and cost ~ 0.5 $ per 10 ml blood sample.

Our DNA extraction may be useful in small laboratories with limited budget or as inspiration for teaching science in grammar schools. According to the company information, *Persil* contains many components: protease, detergent, builder, silicates, soda ash, polymers, perborate, tetra-acetyl ethylene diamine, optical brightener, dye, sulphate, and perfume. We have not tested what is the exact cause of the equslization proteinase K and *Persil*. It can be hypothesised that the makers of *Persil* engineered their protease and optimalized the whole mixture for release of (in)organic substances (DNA included) from clothes and the high quality of released DNA is incidental. Further, other experiments[7,8] imply that other laundry detergents than *Persil* may be used as well.

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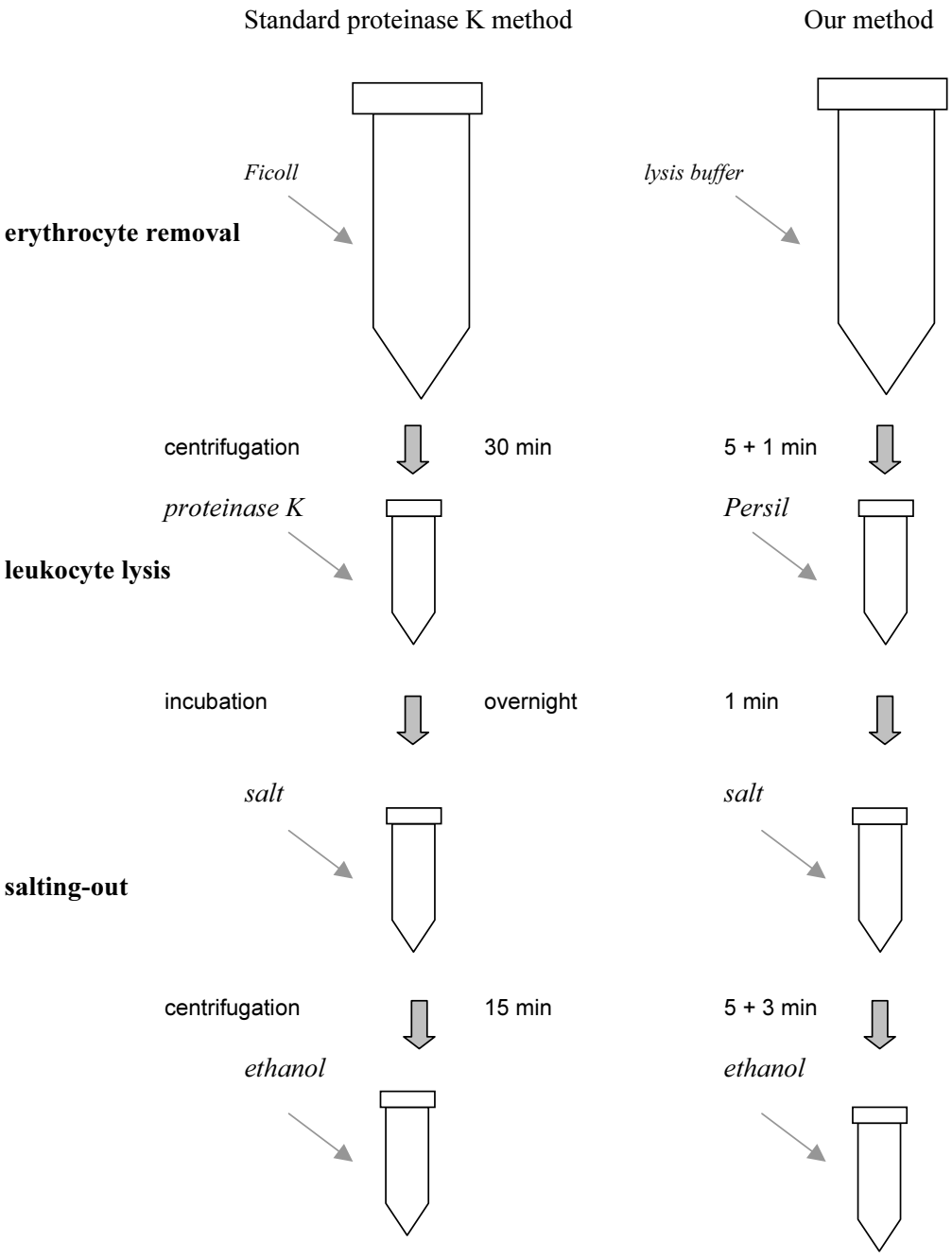

Schema 1. Comparison of standard proteinase K salting-out method with our method