Optimum Concentration Of DNA Extracted From Human Peripheral Blood

Sarab F. I. Al-Ani , Alaa A. B. Al-khafaji , Omar Abdulkareem Ali
Microbiology Dept., College of Medicine, Al-Anbar University

Abstract:

***Objective*:** our study was to establish a simple, fast and inexpensive method for DNA extraction from human peripheral blood using commercial Laundry detergent (Bonux) from local markets.

Patients & Methods: Four concentrations of laundry detergent (*Bonux*) (10,20,30 and 40% w/v) were evaluated for obtaining the optimum concentration of DNA extraction from human peripheral blood.

Results: The results revealed that DNA purity of blood sample represented by $OD_{260/280}$ ratio for 10% concentration of laundry detergent was 1.428±0.048, while the best concentration of laundry detergent was 30% with DNA purity measured in human blood by OD 260/280 ratio (1.843±0.064), while in proteinase k was 1.769 **.**

***Conclusion*:** we concluded that the commercial laundry detergent powder contain a mixture of detergents, enzymes, chelating complexes, these materials assist in isolation of high molecular weight DNA from human blood. The detergent can be used in the modified procedure instead of proteinase K with 30% concentration of the laundry detergent (*Bonux*) which is not harmful, available and economical in use.

Keyword: DNA extraction, Laundry detergent.

Introduction:

Different approaches have already been described to extract genomic DNA from whole blood [1,2]. Many Protocols exist for the extraction of human DNA from peripheral blood frequently involve detergents (SDS) or enzyme treatment (e.g. proteinases) in specialized buffer systems, whose preparation is often time consuming in its procedure and expensive [3]. However, in our belief none of the published methods in Iraq assembles the composite criteria for yield, purity, reliability, non-toxicity, speed, and price to be used routinely in a laboratory. So the aim of our study was to establish a simple, fast and inexpensive method for DNA extraction from human peripheral blood using commercial Laundry detergent (*Bonux*).

Materials and Methods:

Bonux (Laundry detergent) was obtained from the super market; other chemicals were from Promega, USA. Four concentrations of laundry detergent (*Bonux*, powder contains a mixture of detergents, enzymes, chelating complexes) (10,20,30 and 40% w/v) were evaluated for obtaining the optimum concentration of DNA extraction from human peripheral blood. human peripheral blood was drawn from one volunteer from Ramadi City, Anbar province. The procedure of DNA extraction was performed according to Miller *et al*. [1] with simple modifications , it repeated three times for each concentration of laundry detergent and proteinase k , and was comprised the following steps:

Lysis buffer {0.3 M Sucrose, 0.01 M TrisHCl pH 7.5, 5 mM MgCl2 6HO, 1% Triton X100} was added to 5ml of EDTA-anticoagulated blood into 50 ml centrifuge tube to reach a final volume of 45 ml. Tubes were centrifuged immediately for 5 min. at 2700 xg. 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

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poured quickly into a (2ml) tube. The sediment was resuspended by vigorous mixing and centrifuged for 1 min. at 675 xg. The supernatant was discarded and leukocytes resuspended in 1340 µl of 10 mM TrisCl pH 8. Leukocytes were divided into two (2 ml) tubes, 660 µl each. 660 µl of detergent (*Bonux* Power Henkel) using four concentrations (10, 20,30, & 40% w/v; previously prepared) and a clean glass bead (2 mm diameter) was added to each tube. Tubes with beads were mixed vigorously to homogenize the contents (1 min.). 500 µl of 5 M NaCl was added; tubes were mixed vigorously for 10 sec. and centrifuged for 5 min. at 14000 xg. The supernatant was poured into two new (2ml) tubes and centrifuged for 3 min. at 14000 xg. The supernatants were combined into one tube (10ml) and DNA precipitated by adding 3 ml of 96% ethanol. DNA precipitate was retrieved using a glass pipette with heat-sealed thin end and washed in two (1.5ml) 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 0.5 ml of 10 mM TrisCl pH 8. 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. For the longer term, DNA was stored at -20 °C.

***DNA Quantitation*:**

The DNA quantitation was performed according to Sambrook *et al.* [4]. The DNA microcentrifuge tubes were quantified by UV spectrophotometric. Readings were taken at wavelengths of 260 nm (OD_{260}) for DNA sample and 280 nm (OD_{280}) for protein concentration of sample. All readings of four concentrations were recorded and the DNA purity of samples should be within the range of (1.6-1.9).

Results and Discussion:

The procedure used by Miller *et al.* [1] which represented the standard salting out method 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 get fast and economic method the standard procedure was modified by using red cell lysis [4,5] instead of buffy coat separation, and replaced proteinase K with four concentrations of the laundry detergent (*Bonux*) [6]. The results revealed that DNA purity of blood sample represented by $OD_{260/280}$ ratio for 10% concentration of laundry detergent was 1.428±0.048 . The optimum concentration of laundry detergent for DNA extraction of human blood was 30% with DNA purity measured by OD 260/280 ratio (1.843±0.064) (Table1); while the mean of DNA purity extracted by with 50 mg/ml proteinase K was 1.769. DNA extracted by laundry detergent was not degraded and it did not inhibit PCR with sequence specific primers or digestions by restrictases [2,7]. The modified procedure may be useful to researchers in Iraq, Because of the high price of proteinase K and time consumption of procedure. The cheap and abundant laundry detergent in local super markets was evaluated to extract DNA from human blood.

According to the company information, detergent contains many components: protease, detergent, builder, silicates, soda ash, polymers, perborate, tetra-acetyl ethylene diamine, optical brightener, dye, sulphate, and perfume. These materials have an important role in lysis of the cells and extraction of DNA, It can be assumed that the makers of *Bonux* engineered their protease and optimalized the whole mixture for release of (in) organic substances (DNA included) from clothes. The procedure need in future to perform a equalization study between proteinase K and different laundry detergents. In our opinion, other studies need to carry out this procedure for extraction the DNA from gram positive and gram negative bacteria. These findings were in agreement with that reported by Bahl & Pfenninger [7] who found that the mean and standard deviation of DNA purity represented by OD $_{260/280}$ ratio was

1.841±0.012. The results study were similar in the mean and standard deviation when compared with that reported by miller *et al* [1] who used salting out procedure in extraction the DNA from human blood, they measured the DNA purity by OD $_{260/280}$ ratio and found to be 1.844±0.016. It can be concluded that the commercial laundry detergent powder contains a mixture of detergents, enzymes, chelating complexes, these materials assist in isolation of high molecular weight DNA from human blood. the detergent used in the modified procedure {replaced proteinase K with four concentrations of the laundry detergent (*Bonux*)} is not harmful, available and economical to use in the laboratories. **Table 1. DNA purity & concentrations extracted from human peripheral blood by Laundry detergent (*Bonux*).**

DNA Extracted by Laundry detergent (*Bonux*)					
Blood Samples		**O.D. 260**	**O.D. 280**	**O.D. $_{260/280}$**	**Concen. of DNA ng/µl**
laundry detergent 10%	**1**	0.042	0.030	1.400	21
	2	0.043	0.029	1.483	21.5
	3	0.042	0.030	1.400	21
Mean ± S.D.		0.042±0.001	0.030±0.001	1.428±0.048	21.167±0.289
laundry detergent 20%	**1**	0.044	0.028	1.607	22
	2	0.044	0.028	1.630	22
	3	0.045	0.027	1.667	22.5
Mean±S.D.		0.044±0.001	0.028±0.001	1.635±0.030	22.167±0.289
laundry detergent 30%	**1**	0.047	0.025	1.880	23.5
	2	0.046	0.026	1.769	23
	3	0.047	0.025	1.880	23.5
Mean±S.D.		0.047±0.001	0.025±0.001	1.843±0.064	23.333±0.289
laundry detergent 40%	**1**	0.045	0.027	1.667	22.5
	2	0.045	0.027	1.667	22.5
	3	0.044	0.028	1.630	22
Mean±S.D.		0.045±0.001	0.027±0.001	1.655±0.021	22.333±0.289

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