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ORIGINAL ARTICLE

Optimization of conditions to extract high quality DNA for PCR analysis from whole blood using SDS-proteinase K method

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Abstract In case of studies associated with human genetics, genomics, and pharmacogenetics the genomic DNA is extracted from the buccal cells, whole blood etc. Several methods are exploited by the researchers to extract DNA from the whole blood. One of these methods, which utilizes cell lysis and proteolytic properties of sodium dodecyl sulfate (SDS) and proteinase K respectively, might also be called SDS-PK method. It does not include any hazardous chemicals such as phenol or chloroform and is inexpensive. However, several researchers report the same method with different formulas and conditions. During our experiments with whole blood DNA extraction we experienced problems such as protein contamination, DNA purity and yield when followed some SDS-PK protocols reported elsewhere. A260/A280 and A260/A230 ratios along with PCR amplification give a clear idea about the procedure that was followed to extract the DNA. In an effort to increase the DNA purity from human whole blood, we pointed out some steps of the protocol that play a crucial role in determining the extraction of high quality DNA.
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1. Introduction

Genomic DNA isolation is a key step in various clinical related studies including genetics, genomics, gene polymorphism, DNA fingerprinting and gene sequencing. These studies utilize a number of techniques that include, but are not limited to, agarose gel electrophoresis, restriction fragment length polymorphism (RFLP), real time/polymerase chain reaction (RT/PCR), Sanger-sequencing and microarrays. Whole blood is

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the primary source of genomic DNA in most of the clinical investigations associated with genetics, genomics, pharmacogenomics, genetic diseases and epidemiology (Gao et al., 2015; Lu et al., 2016; Rzehak et al., 2016; Visvikis et al., 1998). Buccal swab or saliva is also used to extract DNA for a number of PCR based studies (Küchler et al., 2012; Ng et al., 2004).

Whole blood yields ample amount of DNA, which is extracted by several methods (Chacon-Cortes et al., 2012; Ghaheri et al., 2016). A very common method includes phenol–chloroform extraction (Di Pietro et al., 2011). The same method may be applied to extract the DNA from tissues as well. However, due to the hazardous nature of phenol and chloroform it remains a major concern when it is used for DNA extraction. A different method utilizes sodium dodecyl sulfate (SDS) and proteinase K. It is often utilized for extraction of genomic DNA from different types of biological samples including whole blood (Goldenberger et al., 1995; Hassani and Khan, 2015; Murray et al., 2016).

Extraction procedure of DNA from whole blood may be divided into three main steps (i) Red blood cell (RBC) lysis and removal, (ii) White blood cell (WBC) lysis and protein removal, (iii) DNA extraction and washing. Based on previously reported formulas and protocol of SDS-PK method we extracted DNA from human whole blood. During the course of the whole procedure of DNA extraction it was noticed that the experimental procedures in all the above three steps, along with reagents and their concentration, play a crucial role in determining the yield's quantity, quality, and integrity. Several modifications were done to fine tune the extraction of a high quality DNA from human whole blood. The present manuscript highlights several crucial points which were identified in the SDS-PK method of DNA extraction from human whole blood. Identification of these points ensured the optimization of experimental protocol and conditions which directly affect the quality and quantity of DNA yield.

2. Materials and methods

2.1. Chemicals and reagents

Sodium dodecyl sulfate, ethylenediaminetetraacetic acid, disodium (disodium EDTA), tris–HCl and proteinase-K were purchased from Bio Basic Inc. Canada. SYBR green PCR master mix was purchased from Applied Biosystems, UK. Ammonium chloride and absolute ethanol were purchased from Avonchem, UK; ammonium acetate was purchased from Winlab UK; sodium bicarbonate from Fluka, Switzerland; sodium chloride from BDH, UK and RNAse A was purchased from Sigma–Aldrich, USA. All other chemicals were of highest purity grade.

2.2. Blood samples

All the procedures were done according to ethical guidelines. Fresh blood samples were obtained from King Khalid University Hospital. Scientific and ethical approval was granted for these experiments by the Institutional Review Board, College of Medicine and King Khalid University Hospital, with approval number 15/004/IRB (Research Project No. E-14-1193). Blood samples were collected from healthy individuals in heparinized vacutainers and stored at 2–8 °C. Collected samples were processed for DNA extraction within 72 h of collection.

2.3. DNA extraction method

Genomic DNA from the whole blood was extracted in the following three steps.

2.3.1. RBC lysis and removal

In a 15 ml centrifuge tube 2 ml blood was taken and 8 ml RBC lysis solution was added which contained NH_4Cl (ammonium chloride) (150 mM), $NaHCO_3$ (sodium bicarbonate) (10 mM) and disodium EDTA (0.1 mM). Tubes were placed in tube rotator for 5 min. All tubes were centrifuged for 10 min at 300× gravitational force. Supernatant was discarded and the white cell pellet was resuspended in 500 µl phosphate buffered saline (PBS) containing NaCl (sodium chloride) (137 mM), KCl (potassium chloride) (2.7 mM), Na_2HPO_4 (sodium phosphate dibasic) (10 mM) and KH_2PO_4 (potassium phosphate monobasic) (1.8 mM), and pH adjusted to 7.4. This RBC lysis and removal step was repeated for three times and at the end, a RBC-free clean white pellet was obtained and resuspended in 500 µl phosphate buffered saline (PBS).

2.3.1.1. Technical point. Initially RBC lysis was done in two steps instead of three, as mentioned above, and it was noticed that extracted DNA analysis in NanoDrop 8000 (Thermo Scientific, Wilmington, USA), A260/A280 ratio was found to be less than what it is expected (1.8).

2.3.2. WBC lysis

In WBC suspension 1.5 ml lysis buffer (pH 8.0) containing tris–HCl (20 mM), disodium EDTA (0.1 mM) and NaCl (25 mM) was added along with 500 µl sodium dodecyl sulfate (SDS, 10%) and 50 µl freshly made proteinase K (10 mg/ml PBS). This mixture was incubated at 50 °C in a water bath for two hours. This incubation is a minimum period to obtain a clear lysed cell solution, if the cell pellet is visible after two hours of incubation, the time may be extended until a clear solution is obtained.

2.3.2.1. Technical point. 10 µl of freshly made proteinase K (10 mg/ml PBS) along with incubation at 55 °C in water bath for two hours. and RBC lysis with two steps of washing could not yield highly pure DNA as observed by NanoDrop. However, increasing the concentration of proteinase K, incubation at 50 °C, and three washing steps in RBC lysis were found to have a positive effect on purity of the DNA.

2.3.3. DNA extraction and collection

After incubation the samples were removed from the water bath and cooled down at room temperature. 500 µl of 7.5 M ammonium acetate was added in each sample and gently vortexed until the solution was homogenous. In this mixture, which is approximately 3 ml, 7 ml of chilled absolute ethanol was added and sample tubes were inverted until a condensed DNA pellet was visible. This DNA pellet was collected with the help of a wide bore pipette tip attached to a100 µl micropipette.

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