

GENOMIC DNA ISOLATION FROM HUMAN WHOLE BLOOD SAMPLES BY NON ENZYMATIC SALTING OUT METHOD

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ABSTRACT

Introduction: Human DNA can be extracted from all the nucleated cells such as hair, tissue, blood etc. certain sources contain high levels of proteins & many types of secondary metabolites that effects DNA purification, highly purified DNA is essential for molecular studies. Here we followed salting out method to extract large quantities of human DNA from whole blood.

Methods: Blood sample was used for extraction of DNA by salting out method. Lysis Buffer contains detergent and salts which create a hypertonic condition resulting in lysis of cells. RBC lysis: by using TKM1 buffer, WBC lysis: by using TKM2 buffer, extraction of DNA & storage: by using SDS, NaCl&Tris EDTA buffer.

Results: 1% Agarose gels were used to check the DNA by Gel dock. DNA quantified by using Spectrophotometer, quantity of DNA obtained from 300µl blood is 6 to 10ug/300ul.

Conclusion: Using the Non enzymatic (Salting out) method, good quality DNA samples from a human whole blood can be extract that is enough to perform Polymerase chain reaction to study gene polymorphisms in human population.

Keywords: Nucleated cells, BUFFER, TKM1, TKM2, WBC, RBC, SDS.

INTRODUCTION

DNA was first isolated by Friedrich Miescher who discovered a substance called "nuclein" in 1869. Blood is the main source of DNA for genotype-related studies in humans. A rapid, efficient, and cost-effective method for the isolation of genomic DNA from whole blood is needed for screening a large number of samples. There are many published protocols [1-3].

DNA was isolated from the 201 blood samples by a rapid non-enzymatic method by salting out cellular proteins with saturated solution and precipitation by dehydration [4].

Salting out method is one of the simplest of all the published methods. Following this procedure, it takes anywhere from 3 to 4 hrs to isolate DNA for large number of samples with the yield ranging (6 to 10ug) good quality of DNA from 300uL whole blood. From our experience we suggest salting out method is less time consuming and not cost effective as well as gives better concentration of DNA which required for the genotype studies with large sample size.

MATERIALS AND METHODS

Standard chemicals: This method uses standard chemicals that can be obtained from major suppliers; we used chemicals supplied by Sigma & Himedia.

Materials

Tris-HCl, Potassium Chloride, Magnesium Chloride, EDTA, Sodium Chloride, Sodium dodecyl sulphate (SDS), Isopropanol, Ethanol, Triton-X, 1.5ml eppendorf tubes and micro centrifuge.

Blood collection

Blood collected in EDTA-containing vacutainer tubes. As will all body fluids, blood represents a potential biohazard, thus care should be taken in all steps requiring handling of blood. If the subject is from a known high-risk category, additional precautions may be required. Blood samples stored at room temperature for DNA extraction within the same working day & also at refrigerator for later uses.

Preparation of Reagents

The reagents were prepared as described below:

a. **TKM 1 Buffer / Low salt buffer (500 ml):** 0.605 g of TrisHCl (10mM) pH 7.6, 0.372 g of KCl (10 mM), 1.016 g of MgCl₂ (10 mM), 0.372g of EDTA (2mM) was dissolved in 500ml of distilled water

b. **Triton-X (10ml):** Added 0.1 ml of 100 % Triton-X to 9.9ml of distilled water.

b. **TKM 2 Buffer / High salt buffer(100 ml):** 0.121 g of TrisHCl (10mM) pH 7.6, 0.074 g of KCl (10 mM), 1.203 g of MgCl₂ (10 mM), 0.074 g EDTA (2mM), 0.467 g of NaCl (0.4 M) was dissolved in 100ml of distilled water

d. **SDS:** One gram of sodium dodecyl sulphate was dissolved in 10ml distilled water.

e. **6M NaCl :** 8.765 g of NaCl was dissolved in 25 ml of distilled water.

f. **TE Buffer:** 0.030 g of TrisHCl (10mM) pH 8.0, 0.009 g of EDTA (1mM) was dissolved in 100ml of distilled water

DNA extraction Protocol

RBC Lysis

1. 900 µl of TKM 1 and 50 µl of 1x Triton-X were added to 300 µl of heparinised blood in an autoclaved 1.5 ml eppendorf.

2. Incubated at 37°C for 5 minutes to lyse the RBCs.

3. Cells were centrifuged at 8000 rpm for 3 minutes and the supernatant was discarded.

4. This step was repeated 2-3 times with decreasing amount of 1x Triton-X till

RBC lysis was complete and a white pellet of WBCs was obtained.

Cell Lysis

To the cell pellet, 300 µl of TKM 2 and 40 µl of 10% SDS were added,

Mixed thoroughly and incubated at 37°C for 5 minutes.

At the end of incubation, 100 µl of 6M NaCl was added and vortexed to precipitate the proteins.

Cells were centrifuged at 8000 rpm for 5 minutes.

Precipitation of DNA

The supernatant was transferred into a new eppendorf tube containing 300 µl of isopropanol.

DNA was precipitated by inverting the eppendorf slowly.

Further, the eppendorfs were centrifuged at 8000 rpm for 10 minutes to pellet down the DNA.

Supernatant was discarded, 70% ethanol was added and mixed slowly to remove any excess salts.

Finally the tubes were centrifuged at 8000 rpm for 5 minutes to pellet down the DNA.

Supernatant was discarded and DNA air-dried.

After thorough drying, 50 µl of TE buffer was added to dissolve the DNA.

RESULTS

Agarose gel electrophoresis used to check and Remaining DNA was stored at 4-8 C for downstream applications.

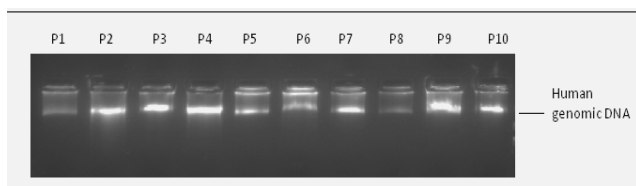


Fig. 1: it shows 1% agarose gel electrophoresis of genomic DNA isolated from human blood samples

We routinely use about 1-2µl per PCR reaction without adverse affects. DNA quantified by Spectrophotometric method and diluted to a working concentration at this point or simply use 1-2 µl per PCR reaction.

Table 1: Amount of genomic dna isolated from human blood samples by this procedure

Sample code	DNA quantity in ug/300ul blood	^A 260/ ^A 280 Purity ratio
P1	6.00	1.79-0.03
P2	8.12	1.81-0.04
P3	9.11	1.82-0.06
P4	7.19	1.77-0.02
P5	7.12	1.72-0.01
P6	6.16	1.69-0.03
P7	10.14	1.77-0.07
P8	6.18	1.56-0.03
P9	9.00	1.67-0.06
P10	10.00	1.78 -0.01

DISCUSSION

Extracted genomic DNA from human whole blood samples is to be use for diagnostic & for genotype studies which will help in personalized medicine. The physical as well as chemical treatments involved in DNA extraction can affect both the quantity and quality of the DNA obtained [5].

In our method, genomic DNA extraction consumes less time and with high quality and quantity by using simple materials and equipments.

Not only was high quality DNA extracted from blood that was stored at 4°C, -20°C In a workday, This study results one person can isolate DNA from more than 50 blood samples using this method. This method has been routinely used to extract DNA from whole blood of human for PCR based applications in our laboratory. It has several advantages such as; economical spending, no need to the specialized and expensive equipments, spending little time, no need to the experimented and experienced staff and more important, DNA extraction from whole blood stored at usual fridges for long time. In this method, genomic DNA with high quality and quantity can be acquired from whole blood . Time of extraction of genomic DNA in our method is 3-4 hrs for 20 samples so within one working day 50 isolations can be done.

Salting out method is a simple rapid isolation method, concentration of DNA in between 1.8 to 2.0 it demonstrate good deproteinization [6]. The results were tabulated and the mean concentration were calculated (Table -1).

The mean 260/280 nmratio was calculated to assess the purity of the DNA.

RESULTS

We expect that the yield of DNA ranged from 6to10 ug from 300µl blood. The purity 260/280 ratios were ranged consistently 1.7 to 1.8 demonstrating good deproteinization.

CONCLUSION

The resulted quantity of DNA is enough to conduct further PCR reactions. Using the above method, good quality DNA samples from a human whole blood were extracted to study gene polymorphisms in human population.

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