Simple salting – out method for genomic DNA extraction from whole blood

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Abstract:
The study conducted in the genetic engineering laboratory ,Biology department,College of Science,University of Tikrit,to modify and optimize DNA extraction protocol passed on salting out method, it carried out at the department of biology, college of science, Tikrit university from November 2009 to April 2010. Ten blood samples were collected in EDTA tube and transferred to a laboratory for DNA extraction. The DNA was extracted by use of modified salting out method . The quality and quantity of extracted DNA was measured spectrophotometricaly at wave length 260nm and 280 nm. The result showed that this method produces good quality high molecular weight DNA, also it was simple, rapid, minimum cost laboratory method.

Keywords: salting-out, DNA Extraction, blood.

Introduction:
centrifuged at 4500 rpm for 15 minutes and the supernatant was discarded. To the remaining white pellet resuspended in the residual supernatant, 1.5 ml of the Protienase K buffer (*20 mmol Tris-Hcl, 4mmol Na2EDTA, 100 mmol NaCl,adjust PH to 7.8*) was added, also 100 µl of 10% SDS was added. The solution mixed until the pellet was re-suspended and well dissolved. Then 20 µl of fresh, refrigerated Proteinase K solution (20mg/ml) was added. The tubes were placed in a water bath at 55C° for 90 minutes. The tubes were placed on ice to cool for 2-3 minutes, 1 ml of 5.3 M Nacl was added and vortexed for 15 second. They were then centrifuged at 4500 rpm for 20 minutes. The supernatant was transferred to a new set of tubes containing equal volume of cold isopropanol. The tubes were inverted 5-6 times gently to precipitated DNA, then centrifuged at 4000 rpm for 5 minutes. The supernatant was discarded and ethanol (70%) was added, again tubes centrifuged. The supernatant discarded and 200-300 µL of distil water was added to re-suspended DNA.

The Optical Densities of the DNA samples were obtained by adding 50 µL of the DNA prepared solution into tubes containing 950 µL of diluent. Each of the new solutions were added to quartz cuvettes that were placed in a spectrophotometer and the Optical Densities were tabulated using distilled water as the blank control. The 260 nm / 280 nm ratio was obtained to give an analysis of the purity of the sample and the concentration of the extracted DNA was also found. This will provide a quantitative analysis of the results.

Agarose gel electrophoresis:
Agarose gel (1gm /100ml) prepared in pH 8.0 buffer containing 89 mmol of Tris-borate, 89 mmol of boric acid and 2 mmol of EDTA (per liter)and were run in a horizontal apparatus. The DNA samples were mixed with loading buffer and electrophoresised at 50 volts for 1 hours. After the run, the agarose gel was stained with ethedium bromide (0.5 µg/ml) for 30 minutes, then photographed on U.V light with digital camera.

Availability of adequate high quality genomic DNA is essential to succeed in various molecular biological techniques. Hence, extraction of high quality DNA with minimum time and cost is always of interest in molecular genetic studies. To met these criteria many DNA extraction procedures have been developed. These procedures which vary in subtle ways, they all have three main stages: lyses of cells, selective release of genomic DNA from cell matrices, and removed of contaminants to recover genomic DNA(2,4). The procedure available differ widely concerning the initial volume of blood, time of isolation, reagents required and most important, precision of the method with reference to quantity and quality of isolated DNA(2,3).

The white blood cells **(*WBC*)** of peripheral blood are usually the most convenient source of human genomic DNA for DNA analysis. It is estimated that 10 ml of whole blood yield approximately 250 µg of DNA, more than sufficient for complete analysis of any gene(1,4).

Some of the problems faced when extracting DNA by standard methods such as requirement of the deproteinizing cell digest with hazardous organic solvent like phenol, chloroform and isoamyl alcohol and the long incubation period for deproteinization ,it also minimize the incubation time which consecuently minimize protocol duration time.The method described in this report avoids the use of any hazardous organic solvent. This is *achieved* by salting out the cellular proteins by dehydration and precipitation with a saturated salt solution(6,8,12).

Materials and methods:
A total of ten blood samples were collected and kept in 4C° and subjected to DNA extraction using modified salting-out method. In this method 500 µl blood was used and the following procedure was adopted: 1.5 ml of R.B.Cs lyses buffer (*155 mmol Ammonium Chloride, 10 mmol Potassium Hydrogen Carbonate , 1mmol EDTA ,adjust PH to 7.6*) was added to the blood samples. Tubes were incubated on ice 15 minutes inverting occasionally. Then

which is maximum at 1.94 and minimum at 1.61 (Table-1).

The results of this study showed that this procedure eliminates completely the use of any toxic reagents such as phenol, chloroform and isoamyl alcohol, also shows that our procedure is rapid , it was completed in about two hour and half, it eliminates the step of prolonged digestion of samples with Protinase K (overnight), thus saved the time of operation.

(Table -1):Yield and Purity of 10 DNA samples extracted by salting-out method.

Sample no.	Concentration µg/100µl	Purity 260/280 Ratio
1	11.1	1.81
2	10.9	1.78
3	11.2	1.83
4	11.4	1.87
5	12.3	1.94
6	9.8	1.66
7	10.9	1.80
8	10.9	1.78
9	9.4	1.61
10	11.0	1.81
Mean	10.89	1.78

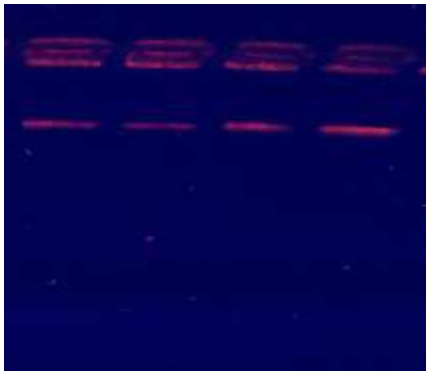

Figure-1:Agarose gel electrophoresis (1%) with DNA extracted from blood samples

Results and Discussion:

The physical as well as chemical treatments involved in DNA extraction can affect both the quantity and quality of the DNA obtained (2).Our study was conducted to optimize the efficient combination of some physical and chemical treatments used in our salting-out method like lysis buffer, protienase k, incubation time on quantity and quality of DNA extracted from (10) samples of blood from different persons.

Lysis buffer (LB) : starting blood volume (SBV) ratio was examined in a wide range.Khosravinia *et.al.*(2006) found that DNA yield and purity were significantly influenced by LB:SBV ratio and by incubation time of blood and lysis buffer mixture.The results of this study showed that the quality and quantity of extracted DNA could be accepted with 3:1 LB:SBV ratio. Total extracted DNA and extraction efficiency were significantly affected by incubation time of blood lysis and buffer mixture(2,4), in this study it was found that 15 minutes incubation time is sufficient for lysis. In contrast to the quality and quantity criterion, total extracted DNA were significantly affected by incubation of proteinease k(2,5).Khosravinia *et al.*(2006) indicated that increasing levels of proteinease k resulted in more total DNA extracted as well as greater efficiency of DNA isolation. Bajorath(1988) and Hilz,(1975) found that proteinease k is typically used with 0.5% SDS at 50-200 µg/ml in nucleic acid preparation at PH 7.5-8.0 and 37 C in 30 minutes-18 hour incubation time.The results of this study showed that using 20 µl of proteinease k (20mg/ml) in 1.5% SDS at PH 7.8 in 90 minutes incubation time at 50 C will increase the extraction efficiency.

This procedure yields good amount (generally in the range 11 µg from 500µl of blood) and high quality DNA which is suitable.for various molecular biological techniques. We have also found less time to extract DNA from blood by this method. The results were tabulated and the mean concentration were calculated (Table -1). The mean 260/280 nm ratio was calculated to assess the purity of the DNA

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استخلاص الدنا الجينومي من الدم الكلي بطريقة التمليح الخارجي المبسطة

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الملخص

أجريت هذه الدراسة في مختبر الهندسة الوراثية /قسم علوم الحياة/كلية العلوم / جامعة تكريت للفترة من تشرين الثاني ٢٠٠٩ ولغاية آذار ٢٠١٠ وأستهدفت أجراء تحوير لعدة طرق والوصول الى طريقة سريعة ومبسطة لاستخلاص الحمض النووي DNA من الدم . تم الحصول على نماذج الدم من عشرة اشخاص بالحقن الوريدي وجمعت النماذج بانابيب حاوية على مانع تخثر EDTA .تم أستخلاص الحمض النووي DNA من نماذج الدم بطريقة Salting-out method .رحلت عينات الحمض النووي المستخلص كهربائيا على هلام الاكاروز للتأكد من وجوده . تم قياس تركيز ونقاوة الحمض النووي بأستخدام جهاز المطياف على الطول الموجي ٢٦٠ و ٢٨٠ نانوميتر. أظهرت النتائج ان الطريقة المستخدمة في هذا البحث هي بسيطة وسريعة وتستلزم مواد كيميائية متوفرة وقليلة الكلفة وتعطي محصول جيد من الحمض النووي DNA.