COMPARISON BETWEEN MANUAL SALTING-OUT AND COMMERCIALIZED DNA EXTRACTION FROM SMALL VOLUME OF PERIPHERAL BLOOD

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ABSTRACT

The techniques of isolation deoxyribonucleic acid (DNA), a crucial step in the process of DNA analysis has progressed rapidly. The manual techniques for DNA purification have been gradually replaced by automation and purification kits. Although expensive, these commercialized techniques are preferred as they are superior in extracting DNA from small volumes of samples. These methods are also safer and rapid. The purpose of this study was to adopt and optimize the manual salting-out procedure for the purification of DNA from small volumes of blood, and to compare the cost of performing the technique to that of the Mini QIAmp DNA extraction kit (Qiagen) and fully automated technique (Magtration 12C, Precision). Apart from being relatively safe and cheaper, we found that the optimized salting-out procedure was able to extract DNA that is comparable in quantity and quality from small volumes of blood.

INTRODUCTION

Research in human genetics has progressed rapidly in the last decade. Many discoveries in genetics has been identified through the study of the human deoxyribonucleic acid (DNA). The isolation of DNA is a crucial step in the process of DNA analysis (Schneider, 1998). Portugal and Cohen (1977) reported that the first attempt to purify DNA from human was done by Friedrich Miescher in the late 19th century. Since then, the method of DNA extraction has progressed by leaps and bounds. Due to increasing demands and need for DNA extraction, the laborious and time consuming manual extraction procedure is now being replaced by automated systems. Manual techniques with commercialized kits are also popular choice for DNA extraction. These techniques are considered less laborious, rapid and cause less technical error (Lien et al., 2008). Although, such kits are expensive and may introduce aerosol cross contamination of the DNA products (Ortuno et al., 2008), they are still popular choices due to their superiority in extracting DNA from small volumes of whole blood. They are also relatively safer procedure.

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The original salting-out method (Miller et al., 1988) used large volume of blood, and the entire procedure took more than 24 hours for completion. Since then several modified procedures have shortened the purification process and made it adaptable to different types of tissue (Aljanabi and Martinez, 1997; Howe et al., 1997; Noguera et al., 2000; Aidar and Line, 2007). The manual salting-out procedure may yield high-molecular weight DNA and avoid the use of toxic phenol and chloroform as compared to the other manual procedures.

The purpose of this study was to adapt and optimize the salting-out procedure for the purification of DNA by using small volumes of whole blood (200 μL) in as short time as possible. In this study, we also compared the cost of the manual salting-out technique with the Mini QIAamp Extraction kits (Qiagen, Germany) and automation technique using the magnetic beads (Magtration 12 C, Precision Japan).

MATERIALS AND METHODS

Sample
The study protocol has been approved by the ethical committee of the International Islamic University Malaysia. Three millilitres (mL) of whole blood was collected via EDTA vacutainer from 12 volunteers. The total white cell count was determined by the blood cell analyzer (ADVIA, USA). Only samples with normal white cell counts were used in the purification process.

Isolation of DNA
Several trials were conducted to optimize the method, until the best protocol that yielded reasonable quantity of DNA from 200 microlitre (μL) of whole blood was determined. The ratio of red blood cell (RBC) lysis solution and nuclei lysis buffer used were kept similar to the amount as used in large quantity of blood samples. The amount of proteinase K (specific activity: >30 units/mg) was increased from 40 mg to 120μg to speed-up the proteinase K by tapping the microtube to breakup the pellet. After digestion for 1 hour at 65ºC, 140μL of 5.3 M NaCl was added to the tube. The mixture was then shaken vigorously for 15 seconds, followed by centrifugation at 16 000 g for 15 minutes. The pellet was washed with 200μL of cold absolute ethanol, inverted several times and centrifuged at 16 000 g for 10 minutes. The supernatant was carefully removed and the washing step was repeated once. The second wash with 200μL of 75% v/v ethanol was centrifuged for 2 minutes at 16 000 g and repeated once. The DNA was allowed to dissolve in 100μL of TE buffer before quantitation. The whole processes took about 2 hours.

Purification of DNA with other methods
DNA was prepared from equal volumes from the same blood samples by spin column Mini QIAamp Extraction kits (Qiagen) and Automated magnetic beads purification (Magtration 12C, Precision, Japan) according to the protocol recommended by the manufacturer.

Determination of DNA yield and purity
In all procedures, spectrophotometer (BioRad, USA) was used to measure the optical density of the samples at 260nm and 280nm to quantitate and to determine the purity of samples respectively. The DNA quality was also checked on agarose gel stained with ethidium bromide and electrophoresed in TAE buffer.

Polymerase chain reaction (PCR) amplification of the DNA product
Ten μL of DNA purified by the optimized salting-out procedure was added to 1 X PCR Buffer II (GoTaq Flexi, Fermentas), 3mM of MgCl2, 200μM of dNTPs, forward and reverse primer (30 picoMoles each), 1 unit of DNA Polymerase (Go Taq, Fermentas) and sterile distilled water to make a total reaction volume of 50 μL. The PCR conditions were as follows: initial denaturation for 1 minute (min) at 94ºC followed by 35 cycles consisting of 94ºC for 1 min, 61ºC for 1.5 min and 72ºC for 1 min. Finally, the PCR was completed with elongation process at 72ºC for 5 min. The primers used in this PCR were 5'-GAAGAGTGATGTA TAGCCCCAG-3’ and 5’-TTTAATTCCAGAGC TATGAAAGCCC-3’ for forward and reverse primer respectively (Sanghera et al., 1998), to amplify codon 55 of Paroxonase-1 (PON1) gene. The PCR protocol was chosen as we had used this protocol successfully earlier in our research either with DNA extracted using the manual commercialized kit or automated procedure (Magtration 12C, Precision). The expected product was 140 base pairs (bp).

Time taken to complete each procedure from preparation of sample until the completion of the isolation procedure was noted. The cost per extraction was calculated based on the actual cost.
incurred for the procurement of all reagents, chemicals and kits used. As it was not possible to determine precisely the cost per extraction in salting-out method as only minimal amount of chemicals were used to prepare for the purification solutions, the cost was approximated by calculating the highest possible expenditure.

Data was analysed by SPSS version 12.0.1. The DNA yield were presented as median interquartile range (IQR). Wilcoxon Signed Ranks test was used to compare the yield between the various purification methods. $p$ value at $<0.05$ were taken as statistically significant.

RESULTS AND DISCUSSION

The optimized salting-out procedure gave comparable yields of DNA with automated magnetic beads separation technique (Magtration 12C, Precision) as there was no significant difference statistically between the two procedures. The yields for both optimized salting-out and automated procedure were however significantly lower ($p < 0.05$) when compared to that of spin column DNA isolation kit (Mini QIAamp, Qiagen) (Table 1 and Figure 1). All methods showed good purity with the ratio of optical density at 260nm to 280nm, was between 1.8 and 2.0. The spin column however showed consistent purity of around 1.9 in all samples. The DNA isolated from optimized salting-out procedure was also of high molecular weight and did not degrade similar to the other two methods (Figure 2) and suitable for PCR (Figure 3). Isolation of DNA through the optimized salting-out procedure was about twice as slow compared to the automated technique and kits. However the cost per-extraction in salting-out was more than five times cheaper than the other two methods (Table 2).

The quality and quantity of nucleic acid yielded from the optimized salting-out procedure was comparable to that of magnetic bead separation technique as determined by the absorbance measurement. We noted however that the yield with the spin column technique was better. The advantage of spin column technique was that the DNA product was normally free of protein contaminants (Dixon et al., 1998). This explained the consistent purity of around 1.9 derived from all samples. However, despite being the cheapest the salting-out procedure gave good purity of DNA products. As shown in this study, 200 μL of whole blood was able to yield around 4μg/mL of DNA which is sufficient for direct polymerase chain reaction analyses. Typical yield of DNA from 5 mL of whole blood with salting-out procedure is around 100-200 μg/mL DNA. By optimizing the technique using a small volume of blood sample, this technique could be a great advantage for neonates and children blood samples. Another known advantage of manual salting-out procedure compared to the other two methods is that the procedure is adaptable and can easily accommodate greater volume of blood sample. This advantage however is not only seen in the salting-out protocol, as some commercialized kits can also be scaled up, though with significant extra additional costs.

This study has shown that even though the time taken to complete DNA extraction from 12 blood samples using salting-out procedure was twice as longer compared to the manual commercialized kit (MiniQIAamp, Qiagen), the cost incurred was five times cheaper. The cost of labour and additional time taken to complete the manual salting-out method could be an issue for laboratories that conducts large scale studies. Here, full automation technique would be the method of choice. Nevertheless, one still has to consider the cost of a complete automation system, which is often very expensive.

In conclusion, the main advantage of the present DNA preparation was its minimum cost since

| Table 1. The total amount of DNA yield(µg/mL) from various purification methods |
|---|---|---|
| **Sample number** | **Optimized Salting-Out Procedure** | **Mini QIAamp Extraction kit (Qiagen)** | **Automation techniques Whole blood Genomic kit- (Magtration 12C)** |
| 1 | 3.45 | 6.51 | 4.00 |
| 2 | 4.01 | 6.32 | 4.20 |
| 3 | 3.45 | 6.79 | 4.10 |
| 4 | 4.45 | 6.57 | 4.20 |
| 5 | 4.30 | 5.55 | 4.30 |
| 6 | 4.52 | 5.80 | 4.29 |
| 7 | 3.90 | 6.79 | 3.92 |
| 8 | 3.88 | 6.03 | 4.24 |
| 9 | 3.32 | 6.66 | 4.30 |
| 10 | 4.43 | 6.80 | 4.11 |
| 11 | 4.41 | 5.90 | 4.02 |
| 12 | 3.90 | 6.12 | 4.28 |
Fig. 1. Comparison of DNA yield between various purification methods. Data presented as median IQR. * Wilcoxon signed rank test shows significant difference ($p < 0.05$) in the DNA yield between salting-out and spin column and automated magnetic bead and spin column.

Fig. 2. Isolated DNA on 1% (v/v) agarose gel electrophoresis showed high molecular weight product. Lane 1: Lambda DNA/HindIII marker; Lane 2-5: DNA extracted using optimized salting out procedure; Lane 6 & 11: empty (No DNA); Lane 7-10: DNA extracted using miniQIAmp Genomic Kit (Qiagen); Lane 12-14: DNA extracted using automated technique (Magtration 12c, Precision).
Table 2. Comparison of time and cost per extraction of the purification methods

<table>
<thead>
<tr>
<th></th>
<th>Optimized salting-out Procedure</th>
<th>Mini QIAamp Extraction kit (Qiagen)</th>
<th>Automation Technique Whole blood Genomic kit (Magtration 12C, Precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time for DNA extraction for 12 samples</td>
<td>120 minutes</td>
<td>50 minutes</td>
<td>45 minutes</td>
</tr>
<tr>
<td>Cost per extraction</td>
<td>Less than RM 5.00</td>
<td>RM30.90</td>
<td>RM28.75</td>
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Fig. 3. Polymerase chain reaction products on 3% (v/v) agarose gel electrophoresis. Lane 1: Low molecular weight DNA ladder. Lane 2-7: PCR products using DNA isolated by salt-extraction; Lane 8: control negative.

expensive kits were not used. This makes it suitable for use in laboratories with good human resource, but with limited financial support. Furthermore, the optimized procedure enables the purification of DNA from small volumes of blood specimens comparable to the two common commercial kits.

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References


