Genomic DNA isolation from dried blood using gelatin-coated magnetic particles

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Gelatin-coated magnetic particles were implemented for isolation of human genomic DNA from dried blood spotted onto glass microfibre filters. The DNA quality and quantity were compared to a silica-based membrane purification kit. Conditions for dried blood storage were varied for DNA stability determination. Approximately three times higher DNA yield was recovered by coated magnetic particles than the silica-based kit. In addition, the quality of extracted DNA was determined using β-actin gene amplification by polymerase chain reaction (PCR). It was implied that genomic DNA was stable for at least 28 days in a dried condition. Gelatin-coated magnetic particles are rapid, simple and useful for DNA extraction in field applications.

Keywords: Dried blood, DNA extraction, gelatin, magnetic particles.

GENETIC disorders caused by gene or chromosome abnormalities have created health problems in all ethnic groups worldwide. Thalassaemia and haemoglobinopathies are the most common genetic diseases found in Thailand. Carrier screening and prenatal diagnosis of these genetic defects among the pregnant women are important for antenatal counselling and preventive intervention[1,2]. Nowadays, several gene defects can be studied by molecular typing methods and PCR is the most commonly applied method for detection of the diseases. Thus, nucleic acid separation is an indispensable step for this application. According to the high quality of DNA yield, whole blood (white blood cells) is considered to be the optimal source for human genomic DNA isolation[3,4]. Therefore, this specimen might be limited in terms of long-term storage and sample transportation. Dried blood spots (DBS) represent an attractive alternative to overcome these limitations[5,6].

Various commercially available techniques are employed for nucleic acid isolation from DBS. However, high-speed centrifugation and chemical waste generation are necessary for these methods. Due to the rapid and simple procedures, magnetic particles are widely used for the isolation of nucleic acid from cellular sources, including blood and tissues[7–9]. Several kinds of polymer-coated magnetic particles such as silica[10], aminosilane[11] and polyethylenimine[12] enable improving the separation ability and recovery yield of DNA. Recently, gelatin-coated magnetic particles were formulated for isolation of DNA from bacterial cells[13].

In the present study, gelatin-coated magnetic particles were subsequently applied for isolation of human genomic DNA from DBS. The quantity and quality of extracted DNA were compared to a silica-based commercial genomic DNA purification kit. Additionally, the DNA stability in dried blood spots over time and temperature was studied using housekeeping β-actin gene amplification by PCR.

Artificial gelatin-coated magnetic particles were prepared as previously described[13]. Briefly, the microwave melting gelatin (15% w/v) was added into the divalent and trivalent iron ions and then co-precipitated in alkaline solution (2.5% NH_4OH). The coated magnetite was washed repeatedly with deionized water and dried overnight in a lyophilizer. The dried magnetic particles were kept at 4°C until use. The size of the dried magnetic particles was analysed using the particle size analyser (Zetasizer Nano ZS, Malvern Instruments, UK) and was found to be approximately 600 nm (data not shown).

The procedure for genomic DNA isolation from DBS using gelatin-coated magnetic particles was initially optimized. Approximately 1 ml of fresh whole blood collected in a ethylene diamine tetra-acetic acid (EDTA) tube was obtained with ethical approval from the fourth-year Medical Technology students, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. Seventy-five microlitres of whole blood was individually spotted onto Whatman GF/C glass microfibre filters, circles 21 mm in diameter[14]. To determine the DNA stability, several periods of time and temperature for dried blood storage were varied from 1, 7, 14, 21, 28 and 56 days in either 4°C or ambient temperature (27–35°C) respectively. Prior to extraction of DNA, DBS were cut out into small pieces with sterile scissors and placed into the 1.5 ml microcentrifuge tube. DNA extraction using gelatin-coated magnetic particles was performed by adding 600 µl of alkaline lysis solution (0.2 M NaOH and 1% (w/v) sodium dodecyl sulphate). The solution was gently mixed by aspiration and incubated at room temperature for 5 min. Next, approximately 400 µl of red-cleared supernatant was transferred into a new microcentrifuge tube. Fifty microlitres of gelatin-coated magnetic particles (10 mg/ml) in binding buffer (4 M NaI and 20% (w/v) polyethylene glycol) and 200 µl of binding buffer were added into the solution, mixed by inversion and allowed to stand at room temperature for 5 min. The magnetic particles were immobilized by an external magnet and the supernatant was completely removed. The magnetic pellet was washed three times with 1 ml cold

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70% (v/v) ethanol and dried at room temperature for 5–10 min. Finally, the magnetic particles were resuspended in 50 µl of TE buffer (50 mM Tris-HCl and 1 mM EDTA, pH 8.0) and the bound DNA was eluted at 65°C for 5 min with gentle agitation. The DNA solution was transferred into a new microcentrifuge tube and kept at –20°C until further use. The steps of DNA extraction using gelatin-coated magnetite are illustrated in Figure 1. The commercial genomic DNA isolation method (NucleoSpin Tissue, Macherey-Nagel, Germany) was performed according to the manufacturer's instructions.

The recovery yield of extracted DNA was determined using UV spectrophotometer. It was implied that the yield of genomic DNA extracted using gelatin-coated magnetic particles was approximately three times greater than the commercial kit at either 4°C or room temperature. The mean in duplicate of DNA extracted from DBS stored at 4°C and ambient temperature using gelatin-coated magnetic particles was 104.4 (88.2–123.3) and 115.2 (93.3–131.0) µg/ml, whereas mean of DNA yield utilized from the commercial kit was 33.1 (27.5–45.5) and 38.3 (32.0–57.6) µg/ml respectively. The quality of extracted DNA was subsequently analysed on 1.0% agarose gel electrophoresis. The DNA band over 10 kb in size was obvious, indicating the excellent quality of DNA extracted using gelatin-coated magnetic particles (Figure 2). In addition, the average OD_{260}/OD_{280} ratio was measured for DNA purity analysis. The range of OD ratio was approximately 1.6–1.8 and insignificantly different in both methods, indicating that the DNA quality was acceptable with negligible protein contamination. Likewise, the high quality of extracted DNA using magnetic particles was due to the greater ability to adsorb DNA than proteins and single-stranded RNA in the presence of high salt concentration has previously been reported[15,16].

The quality of extracted DNA was also assessed using beta-actin gene amplification by PCR. The primers pairs used in this study were designed from the sequence available in GenBank database (accession no. M10277). The PCR was performed in 25 µl containing 1 × PCR buffer (75 mM Tris-HCl; pH 9.0, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 75 mM $MgCl_2$, 200 µM dNTPs, 10 pmol of forward (5′-GACATCCGCAAAGACCTGTAC-3′) and reverse (5′-TGAGGACCCTGGATGTGACA-3′) primers, 1.25 U *Taq* DNA polymerase (iNtRON Biotechnology, Inc., Korea) and approximately 5 µl of DNA template. The PCR conditions were as follows: pre-heating at 94°C

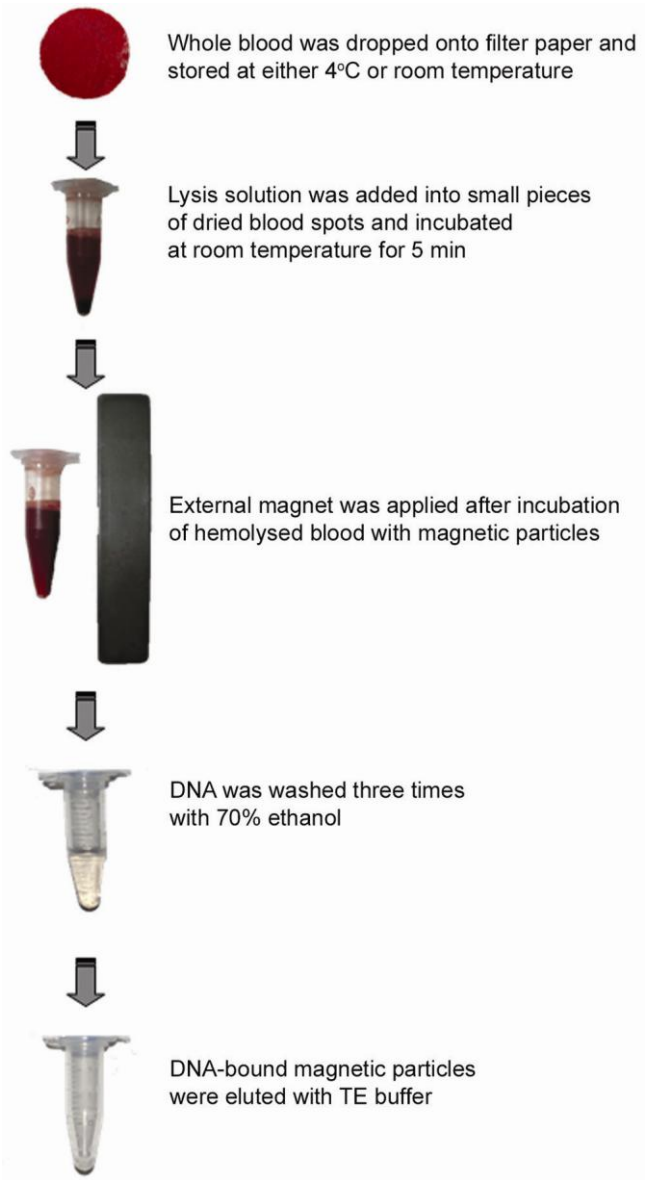

Figure 1. Steps of DNA extraction from dried blood spotted onto glass microfibre filter using gelatin-coated magnetic particles.

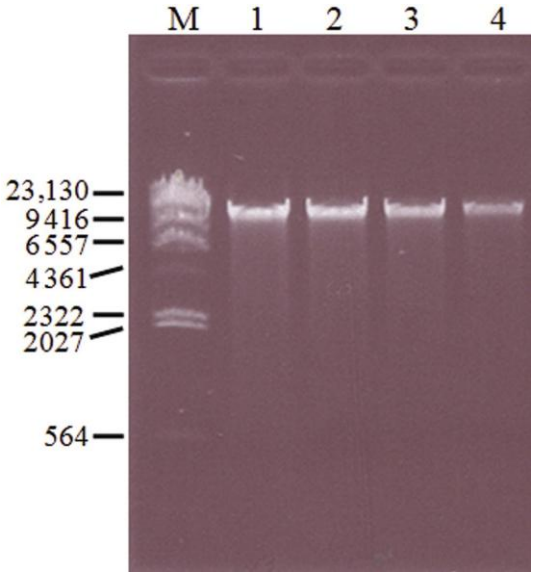

Figure 2. The 1.0% agarose gel electrophoretic pattern of genomic DNA extracted using gelatin-coated magnetic particles. Lane M, Lambda-*Hin*dIII DNA marker; lanes 1, 2 and 3, 4, 15 µl of DNA extracted from dried blood stored at 4°C and room temperature for one day using gelatin-coated magnetite respectively.

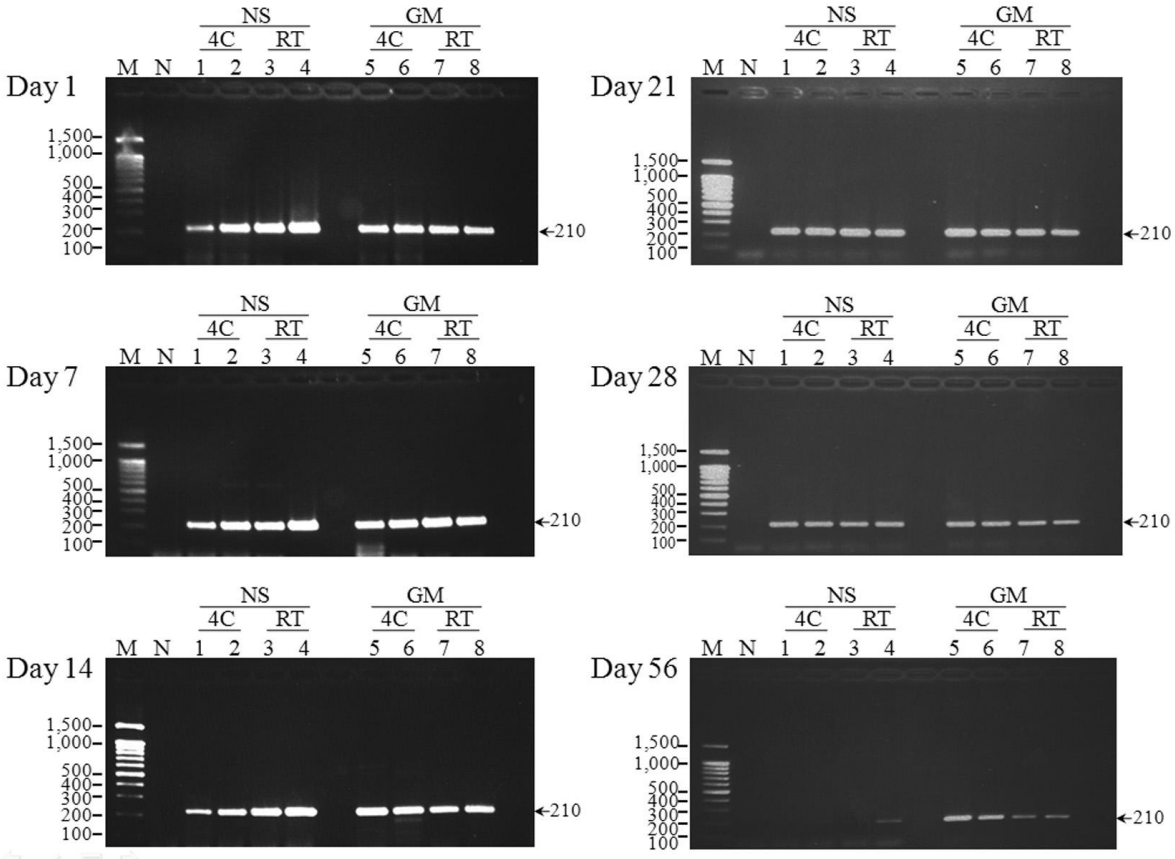

Figure 3. Agarose gel electrophoresis of approximately 210 bp amplified product of human beta-actin DNA extracted from dried blood spots (DBS) using either gelatin-coated magnetic particle (GM) or a commercial DNA purification kit (NS). DBS were stored at 4°C (4C) or room temperature (RT) for 1, 7, 14, 21, 28 and 56 days. Lane M, Standard DNA marker; lane N, Negative amplification control (distilled water); lanes 1, 2 and 3, 4, Amplified products in duplicate obtained from DBS stored at 4°C and room temperature respectively and these were extracted using a commercial kit; lanes 5, 6 and 7, 8, Amplified products in duplicate derived from DBS stored at 4°C and room temperature respectively, and these were purified using gelatin-coated magnetic particles.

for 1 min followed by 35 cycles of 94°C, 30 sec; 62°C, 30 sec; 72°C, 30 sec with a final extension at 72°C for 5 min. A 210 bp amplified product (10 µl) was electrophoresed through a 2.0% agarose gel, stained with ethidium bromide and analysed under a UV transilluminator (Fotodyne Incorporated, USA). The result revealed that genomic DNA was stable for up to 28 days in dried condition, as it could be detected in all blood spotted samples stored at both temperatures (Figure 3). However, the amplification products could still be obtained from 4°C stored DBS using gelatin-coated magnetic particles after 56 days of storage, indicating that the magnetic extraction led to a higher recovery yield than the commercial kit. Additionally, the time for the whole extraction process was also compared. The validated technique, gelatin-coated magnetic particle, was accomplished in an average of 30 min, whereas at least 1.5 h was required for the commercial kit.

In conclusion, DBS have been used for years to screen genetic disorders and infectious diseases[6,17–19]. Therefore, the protocols for nucleic acid extraction from DBS were rarely published. From the results, gelatin-coated magnetic particles are useful for human genomic DNA isolation from DBS. This technique is rapid, simple and cost-effective, attributes that are desired for DNA extraction in resource-limited areas. Moreover, DBS are suitable for large-scale field studies because they are easy to collect and transport, can be stored at room temperature, and can be mailed to laboratories for analysis. Therefore, the study involving larger number of samples should be performed for further assessment of field applications.

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DNA barcodes of some threatened freshwater indigenous fishes in India

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This study was directed to identify early life-history stages of eight culturable species currently under threat through DNA barcodes. A total of 22 sequences of cytochrome *c* oxidase I gene were generated from eight species using multiple specimens producing 655 nucleotide base pairs per taxon. The neighbour-joining tree showed three major clusters for three different orders. Similar species were clustered under the same nodes and dissimilar species were clustered under separate nodes signifying species specificity. This can be utilized for accurate identification of threatened fish species and in turn assist in conservation of fish stock either inside freshwater protected areas or in the river systems.

Keywords: Barcoding, freshwater fish, life-history stages, neighbour-joining tree.

INDIA is endowed with a rich fish genetic biodiversity (2200 fish species) and ranks ninth in terms of freshwater mega biodiversity[1,2]. The small, shallow rivers provide refuge to a large number of varied fish population and are also undergoing major habitat alterations due to anthropogenic stresses. The Mahananda–Tangon–Punarbhaba river system (MTPRS) is one such shallow interconnected river system in Malda district, West Bengal, India, harbouring a rich fish biodiversity throughout the year. Out of this rich fish biota, eight most economically important, culturable species were selected for the present study. They were *Chitala chitala* Hamilton, 1822; *Notopterus notopterus* Pallas, 1769; *Ompok bimaculatus* Hamilton, 1822; *Eutrophichthys vacha* Hamilton, 1822; *Ailia coila* Hamilton, 1822; *Neotropius atherinoides* Bloch, 1794; *Pangasius pangasius* Hamilton, 1822 and *Gudusia chapra* Hamilton, 1822. The populations of these fish fauna are presently in decline[3] and require efforts towards conservation through culture or introduction in fish sanctuary. Propagation of stock in controlled environment requires pure seed collected from nature and hence, precise identification of early life-history stages of these fishes is important.

Appropriate identification tool for early life-history stages is essential to confirm purity of seed for introduction

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