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An Improved Protocol for High Quantity and Quality of Genomic DNA Isolation from Human Peripheral Blood

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Abstract

DNA isolation is the most essential step in molecular studies. The quantity and quality of the isolated DNA may subsequently influence the reliability and reproducibility of experimental data especially those involving downstream analysis such as polymerase chain reaction (PCR). In this study, we report an improved protocol for isolating high quantity and quality of genomic DNA from human peripheral blood that is as competitive to commercial kits. The concentration of the genomic DNA isolated using the improved protocol was >100 ng/µl and the A_{260}/A_{280} absorbance ratio was ranged within 1.604-1.861. When the DNA integrity was measured using Fragment Analyzer™, the isolated genomic DNA was highly intact with a genomic quality number of ≥7.0. The isolated genomic DNA was adequate for further molecular analyses including standard PCR and real-time PCR. More importantly, the improved protocol is able to isolate the genomic DNA of *Plasmodium* parasites that infected human red blood cells, thus enabling them to be correctly identified up to the species level using multiplex PCR.

Keywords: DNA isolation; human blood; polymerase chain reaction; *Plasmodium* parasites
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1. Introduction

Molecular study often involves the isolation of nucleic acids such as DNA. Hence, the quantity and the quality of the isolated DNA are extremely important for reproducible and reliable of experiment data in subsequent molecular analyses including standard polymerase chain reaction (PCR) and real-time PCR. For instance, contaminants such as polysaccharides, organic solvents, proteins, and detergents that are co-isolated with the DNA are reported to inhibit a PCR amplification [1, 2]. In addition, DNA with co-isolated contaminants may also lead to a higher cycle threshold value of the targeted gene in a real-time PCR application, which may then result in an incorrectly interpreted real-time PCR data [3].

There are multiple choices of commercial DNA isolation kits currently available in the market that can isolate a high quality of DNA. However, the commercial kits commonly have a limited number of reactions and are relatively expensive which may not be affordable by some laboratories, especially those in low-income countries. Therefore, an efficient and economic

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alternative DNA isolation method is crucial. We addressed this particular issue with an improved protocol that can effectively and economically isolate high quantity and quality of DNA from human peripheral blood, and empirically validated the protocol using molecular analyses such as standard PCR and real-time PCR.

2. Materials and Methods

2.1 Human blood sample collection

Three ml of peripheral blood sample was collected from volunteers into a BD Vactutainer® blood collection tube with spray-coated K_2EDTA by an experienced phlebotomist. A total of 100 volunteers were recruited in this study with informed consent. The ethical approval for this study was obtained from the Universiti Malaysia Sabah Medical Research Ethics Committee with a reference number: JKEtika 1/15 (7).

2.2 Genomic DNA isolation

Three ml of the collected blood was mixed with 7 ml of chilled 1X Red Cell Lysis Buffer (containing 1.6M of sucrose, 5% of Triton X-100, 25mM of magnesium chloride hexahydrate, and 60mM of Tris hydrochloride) in a Falcon tube and centrifuged at 2500 xg for 5 min at 4°C. The supernatant containing the lysed red blood cells was discarded and the color of the precipitated pellet was observed. The red blood cell lysis step was repeated until the pellet became light pink to white color. Subsequently, the pellet was re-suspended by adding 20 µl of 1 mg/ml Proteinase K, 80 µl of 5X Proteinase K buffer, 20 µl of 20% sodium dodecyl sulfate solution, and 150 µl of sterile distilled water containing 1 µg/ml of RNase, and incubated overnight at 37°C. The next day, the whole mixture content was treated with 100 µl of 5M sodium chloride solution and 250 µl of phenol: chloroform: isopropanol (1:1:1 v/v/v, pH 8.05-8.35) solution. After vortexing and centrifuging at 13,000 rpm for 10 min, the aqueous phase of the mixture was transferred into a microcentrifuge tube containing 500 µl of absolute ethanol. The tube was vigorously shaken until a white clump was formed and was centrifuged at 13,000 rpm for 3 min. The excess absolute ethanol was discarded and the pellet was washed with 500 µl of 70% ethanol. After centrifuged at 13,000 rpm for 2 min, the excess 70% ethanol was discarded and the pellet was air-dried for 5 min at room temperature. Finally, the pellet was re-suspended with 100 µl of 1X TE Buffer (containing 10mM of Tris and 1mM of ethylenediaminetetraacetic acid).

2.3 Quantity and quality of the isolated genomic DNA

The concentration and purity of the isolated genomic DNA were measured using the NanoPhotometer® P-Class (Implen, Germany). Unpaired t-test (https://www.graphpad.com/quickcalcs/ttest1.cfm) was utilized to compare the means of DNA purity between current protocol and other commercial kits as reported by Lee *et al*. [4], including the QIAamp® Blood Mini Kit (Qiagen, Germany), MagNA Pure LC Nucleic Acid Isolation Kit I (Roche Diagnostics, Germany) and Magtration-Magnazorb DNA common kit-200N (Precision System Science Co. Ltd., Japan). In addition, the Standard Sensitivity Genomic DNA Analysis Kit (Advanced Analytical Technologies Inc., USA) was used to measure the integrity of the isolated genomic DNA using the Fragment Analyzer™ Automated Capillary Electrophoresis System (Advanced Analytical Technologies Inc., USA) which represented with a genomic quality number (GQN). GQN of 1 indicates that the genomic DNA is highly degraded and GQN of 10 indicates that the genomic DNA is highly intact.

2.4 Standard PCR

A standard PCR amplifying the cytochrome P450 family 2 subfamily E member 1 (*CYP2E1*) gene sequence that containing the T7678A variant was performed by using the isolated genomic DNA as a template (~100 ng/reaction). The detailed procedure is as previously described by Chong *et al*. [5].

2.5 Real-time PCR

Genotyping targeting the rs3751723 single nucleotide polymorphism (SNP) of the iroquois homeobox 3 (*IRX3*) gene was performed using a real-time PCR and the isolated genomic DNA served as a template (~100 ng/reaction). The detailed procedure is as previously described by Chong *et al*. [6].

3. Results and Discussion

A total of 100 DNA samples isolated using the improved protocol were subjected to quantity and quality measurements (Table 1). The concentration of the isolated DNA ranged between 102 ng/µl and 305 ng/µl. When the purity of the isolated DNA was measured, the A_{260}/A_{280} absorbance ratio ranged from 1.604 to 1.861. The absorbance values did not deviate far from the ideal ratio of 1.800 for genomic DNA [7]. Therefore, the isolated genomic DNA using the improved protocol is able to provide sufficient quantity and quality for reliable data in the subsequent molecular analyses.

Table 1. Results of genome DNA isolated using the current improved protocol in this study

Sample	DNA conc. (ng/µl)	A_{260}/A_{280}	GQN
1	179	1.646	8.2
2	173	1.769	8.1
3	177	1.703	9.7
4	176	1.645	8.5
5	113	1.633	9.1
6	193	1.618	8.7
7	188	1.604	8.1
8	127	1.749	8.1
9	138	1.660	8.6
10	119	1.703	9.2
11	305	1.755	7.3
12	255	1.827	8.7
13	214	1.723	7.2
14	187	1.765	7.9

Table 1. Results of genome DNA isolated using the current improved protocol in this study (cont.)

Sample	DNA conc. (ng/µl)	A_{260}/A_{280}	GQN
15	172	1.776	8.1
16	152	1.701	9.5
17	133	1.675	7.3
18	134	1.661	9.3
19	112	1.695	8.9
20	140	1.826	9.9
21	188	1.671	7.0
22	136	1.751	8.3
23	134	1.759	8.1
24	198	1.754	9.1
25	115	1.715	9.3
26	110	1.861	7.0
27	102	1.659	8.5
28	199	1.663	7.5
29	109	1.618	8.5
30	169	1.797	7.4
31	208	1.717	8.5
32	152	1.801	9.0
33	218	1.845	8.4
34	154	1.642	9.5
35	239	1.755	8.1
36	172	1.646	8.9
37	144	1.718	9.2
38	147	1.648	8.6
39	168	1.689	7.2
40	165	1.636	9.0
41	180	1.762	8.5
42	214	1.755	7.4
43	171	1.670	8.5
44	260	1.638	8.0

Table 1. Results of genome DNA isolated using the current improved protocol in this study (cont.)

Sample	DNA conc. (ng/µl)	A_{260}/A_{280}	GQN
45	170	1.678	8.5
46	184	1.642	8.8
47	182	1.657	7.8
48	193	1.681	8.5
49	174	1.640	7.8
50	183	1.616	8.8
51	104	1.658	8.8
52	108	1.666	9.6
53	216	1.812	8.3
54	179	1.791	8.1
55	195	1.776	9.0
56	201	1.719	8.2
57	186	1.729	8.3
58	120	1.788	8.7
59	129	1.669	7.8
60	134	1.639	9.3
61	226	1.795	8.4
62	253	1.768	8.5
63	130	1.772	7.4
64	136	1.771	7.3
65	125	1.728	7.1
66	128	1.753	7.9
67	258	1.756	7.6
68	134	1.789	8.9
69	132	1.632	8.3
70	287	1.783	8.1
71	141	1.778	8.6
72	126	1.684	9.0
73	131	1.785	7.9

Table 1. Results of genome DNA isolated using the current improved protocol in this study (cont.)

Sample	DNA conc. (ng/µl)	A_{260}/A_{280}	GQN
74	245	1.729	8.5
75	299	1.743	9.3
76	108	1.667	8.4
77	181	1.684	8.3
78	173	1.629	8.2
79	199	1.646	8.3
80	174	1.778	7.8
81	178	1.775	7.8
82	192	1.756	8.4
83	169	1.829	7.8
84	118	1.621	7.1
85	199	1.710	8.2
86	177	1.660	9.5
87	178	1.691	9.2
88	195	1.683	7.7
89	147	1.664	9.3
90	182	1.691	8.3
91	156	1.702	7.3
92	165	1.669	8.8
93	184	1.678	9.2
94	175	1.690	7.8
95	176	1.626	7.3
96	166	1.665	8.8
97	150	1.716	10.0
98	175	1.628	8.7
99	194	1.640	7.7
100	185	1.678	7.9
Mean ± S.D.	171.47 ± 42.93	1.71 ± 0.06	8.37 ± 0.70
Range (min-max)	102-305	1.604-1.861	7-10

When compared with other commercial kits that use different isolation principles, the mean of DNA purity using the current protocol was similar to those isolated using the Magtration-Magnazorb DNA common kit-200N (p = 0.724), but statistically different to those isolated using the QIAamp® Blood Mini Kit and MagNA Pure LC Nucleic Acid Isolation Kit I (both p-values <0.001) (Table 2). The mean differences were mainly because of the different technologies utilized in isolating the DNA, the number of DNA samples isolated, and variations in sample handling during the extraction process. However, when a scatter plot of the DNA purity values was mapped (Figure 1), most of the purity values overlapped despite the different protocols utilized in isolating the DNA from human blood, suggesting that DNA isolation using the current improved protocol is as competitive to other commercial kits, yet is much more cost-effective and economical when compared to the commercial kits. However, given the advantage of cost-effective, the limitation of the current protocol is time consuming (~10 h) as compared to commercial kits which have shorter processing times (< 2 h).

Figure 2 shows a representative of 11 genomic DNA analyzed using the Fragment Analyzer™ in this study. The isolated genomic DNA using the improved protocol was highly intact with a genomic quality number (GQN) ranging from 7.0 to 10.0 (Table 1). Although the influence of GQN in subsequent molecular analyses is rarely reported, a previous study reported that nucleic acid with an integrity number <5.0 is unreliable for subsequent analysis such as real-time PCR [8].

Besides that, the isolated genomic DNA was molecularly validated using a standard PCR, and our study showed that the *CYP2E1* gene sequence containing the T7678A variant was successfully amplified (Figure 3). In addition, the isolated genomic DNA in this study could also be

Table 2. Comparison of DNA purity isolated from different protocols

Genomic DNA isolation protocol	Isolation principle	Time required	No. of samples tested	Purity range (mean ± SD)	*p*-value	Reference
Current protocol	Salting-out	10 h	100	1.60-1.86 (1.71 ± 0.06)	-	This study
QIAamp® Blood Mini Kit (Qiagen, Germany)	Spin-column	20-40 min	22	1.59-2.04 (1.84 ± 0.09)	<0.001*	Lee *et al.* [4]
MagNA Pure LC Nucleic Acid Isolation Kit I (Roche Diagnostics, Germany)	Magnetic-bead	< 2 h	22	1.60-1.97 (1.88 ± 0.08)	<0.001*	Lee *et al.* [4]
Magtration-Magnazorb DNA common kit-200N (Precision System Science Co. Ltd., Japan)	Magnetic-based	25-40 min	22	1.56-1.90 (1.70 ± 0.08)	0.724	Lee *et al.* [4]

*Statistically significant when compared to the current protocol (p <0.05).

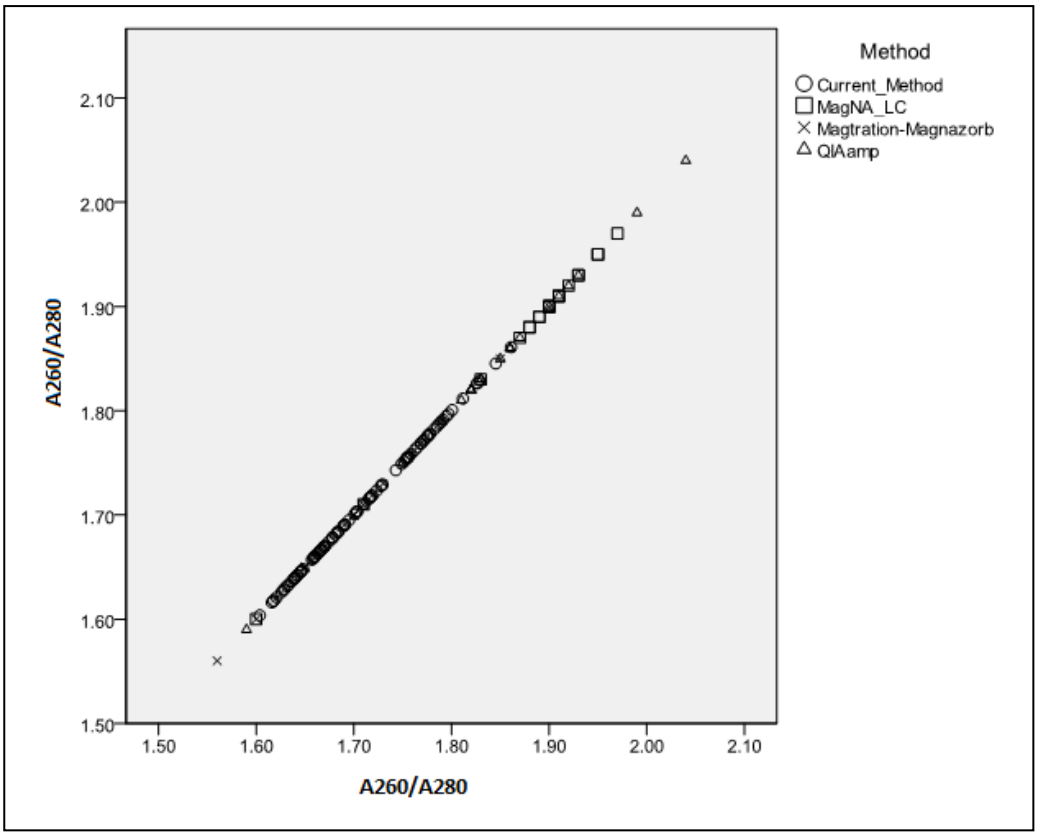

Figure 1. Scattered plot showing the overlapping of purity values between DNA isolated using the current protocol and those reported using other commercial kits

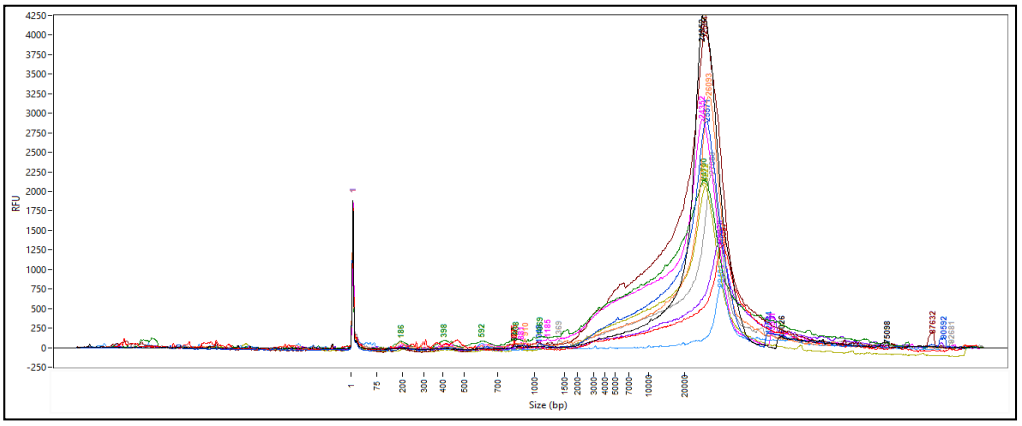

Figure 2. Overlay electropherogram of 11 isolated genomic DNA samples analyzed using the Fragment Analyzer™

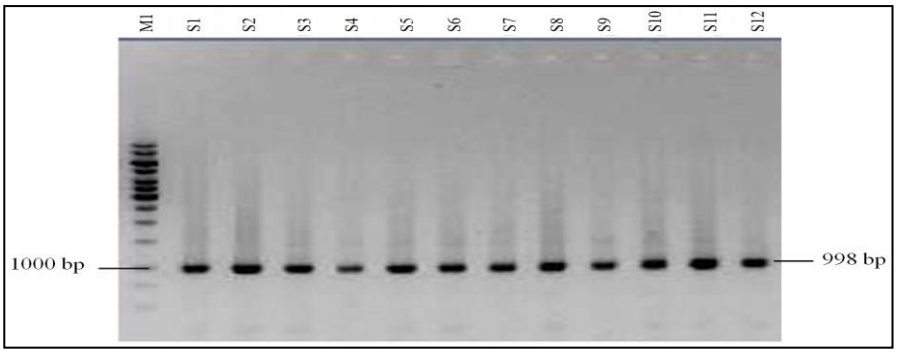

Figure 3. The *CYP2E1* gene sequence containing the T7678A variant was successfully amplified (998 bp). M1: Thermo Scientific GeneRuler^{TM} 1 kb DNA Ladder. S1-S12: DNA samples isolated using the improved protocol

used to generate reliable data for genotyping analysis in real-time PCR. For example, the rs3751723 SNP of the *IRX3* was effectively genotyped using the isolated genomic DNA in this study and could be precisely distributed into the homozygous wild-type, heterozygous, and homozygous variant groups (Figure 4). Both findings indicated that the improved method can maintain the intactness of the genomic DNA throughout the isolation process with a very minimal amount of protein contamination.

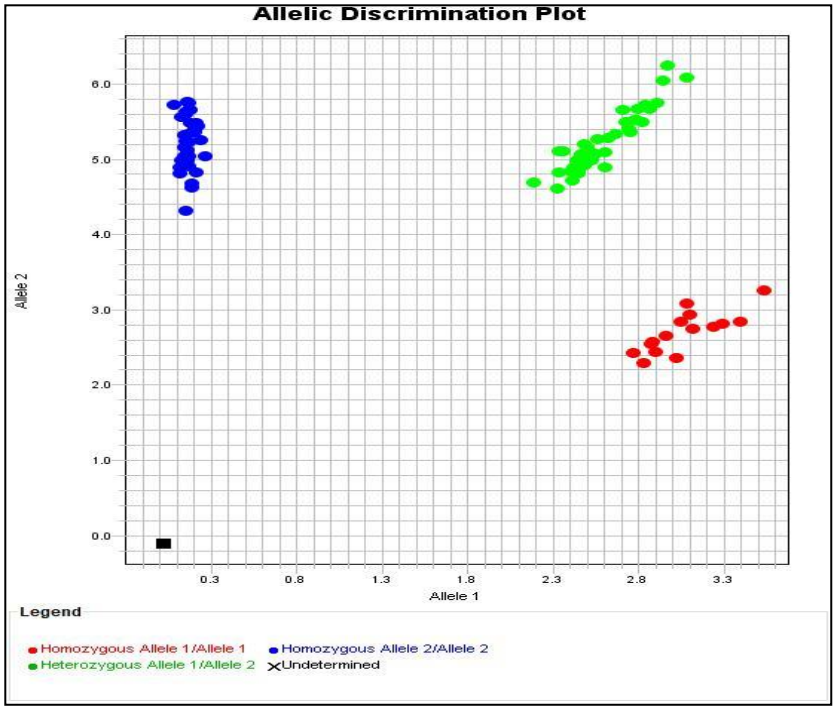

Figure 4. The rs3751723 SNP of the *IRX3* gene was effectively genotyped from DNA samples isolated using the improved protocol

More interestingly, our improved protocol is able to isolate the genomic DNA of *Plasmodium* species that infected the red blood cells, and being correctly identified up to the species level when applied with the PlasmoNex™ system [9], even for a mix-species infection (Figure 5). This finding is obviously important as the malaria incidences, especially those infected with the fatal *Plasmodium knowlesi* species, are evidently increasing in the Southeast Asia countries [10-12]. Therefore, the improved DNA isolation protocol in this study could provide consistent data in *Plasmodium* species detection for effective and rapid malaria treatment.

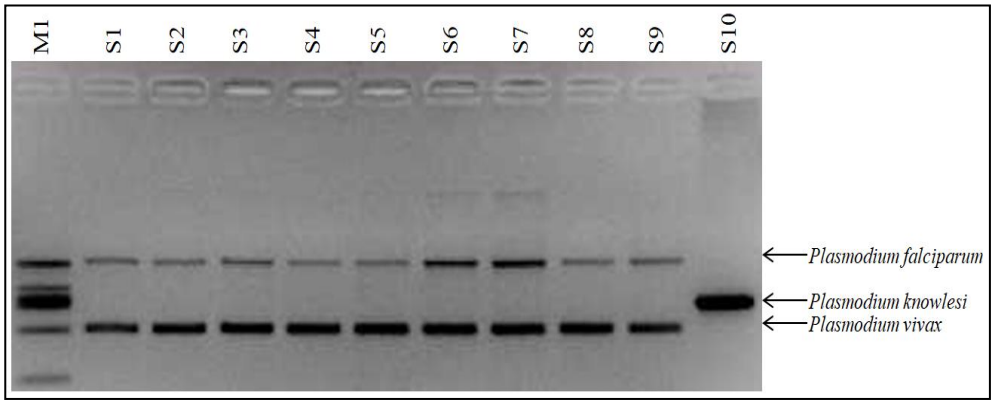

Figure 5. The *Plasmodium* species was correctly identified using the PlasmoNex™ system. M1: PlasmoNex™ DNA Marker. S1-S10: *Plasmodium* DNA samples isolated using the improved method

4. Conclusions

In conclusion, we report here an improved protocol to effectively and economically isolate high quantity and quality of genomic DNA from human peripheral blood, and the present protocol is as competitive to other commercial kits. The isolated genomic DNA is competent for subsequent molecular analyses including standard PCR and real-time PCR. More essentially, this improved protocol is capable to isolate the genomic DNA of *Plasmodium* species and being accurately diagnosed up to the species level in a multiplex PCR.

5. Acknowledgements

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