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Full Length Research Paper

Optimizing factors influencing DNA extraction from fresh whole avian blood

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A study was conducted to optimize the efficient combination of lysis buffer, proteinase K, incubation time, phenol-chloroform-isoamyl alcohol (PCI) volume, spinning rate (rpm), and precipitation agent on quantity and quality of DNA extracted from various volumes of avian blood. Blood samples were collected in EDTA and swiftly transferred to a laboratory for DNA extraction. The lysis buffer used had composition 5 M NaCl, 1 M Tris (pH=8.0), 0.5 M EDTA and20% SDS. The effect of various levels for each factor concerned was examined using General Linear Models or t-test procedures of SAS® software. The volume removed from the top aqueous part following the first and the second PCI washings was included in the models as a continuous variable; the variables of interest were OD_{280}, OD_{260}, OD_{260}/OD_{280} (as quality criterion), total extracted DNA, extraction efficiency (µg DNA/µl blood), assay scores for easiness of removing the top aqueous phase after the first (assay 1) and the second (assay 2) spinning. The optimum level of factors significant for DNA extraction from fresh avian blood was found to be lysis buffer : blood sample ratio of 31:36 (µl : µl), incubation time of 60-70 min at 58°C, two washings with PCI at 1.2:1.3 PCI : top aqueous phase (µl : µl) for the first and 1.4 for the second washing, centrifuge of homogenised sample at 2000 - 2500 rpm for 20 min, precipitation of DNA with 1.5 – 2.0 volume of absolute ethanol.

Key words: DNA extraction, phenol-chloroform, avian, whole blood.

INTRODUCTION

Availability of adequate high quality genomic DNA is essential to succeed in various molecular biological techniques such as sequencing, cDNA synthesis and cloning, RNA transcription, nucleic acid labeling (random primer labeling) etc. Hence, extraction of high quality DNA with minimum time and cost is always of interest in molecular genetic studies. To meet these criteria many DNA isolation procedures have been developed. Literally hundreds of procedures for DNA preparation from various sources of tissue have been published over the last few decades. The Internet is an excellent source (http://www.nwfsc.noaa.gov/protocols.html/, http://bric.postech.ac.kr/resources/rprotocol/ and http://www.protocol-online.org/). Few "classical" procedures such as traditional phenol / chloroform extraction are "tried and true" and nearly always work.

Many modified versions of the conventional phenol / chloroform extraction methods are still in use as they produce reliable high quality DNA (Maniatis et al., 1982; Sambrook et al., 1989; Hillis et al., 1990; Hillis et al., 1996; Palumbi, 1996). The procedure available differ widely concerning the initial volume of blood, time of isolation, reagents required and of most important, precision of the method with reference to quantity and quality of isolated DNA. Manuals of molecular biology procedures often state that nucleic acid preparations are free of protein contaminations when the ratio of absorbance at 260 nm to that at 280 nm is 1.8 - 2.0 (in absence of phenol contamination).

This study was conducted to investigate the efficient combination of lysis buffer volume, proteinase K, incuba-

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Table 1. Effect (mean ± SE) of starting blood sample (µl) on variables studied.

	Blood sample volume (µl)				
Variables	20	22	24	26	28
OD_{280}	0.05±0.00[b]	0.06±0.00[b]	0.05±0.0[b]	0.05±0.0[b]	0.1±0.01[a]
OD_{260}	0.09±0.00[b]	0.11±0.01[b]	0.09±0.0[b]	0.1±0.01[b]	0.2±0.01[a]
$OD_{260/280}$	1.73±0.01[c]	1.80±3.60[a]	1.8±0.02[ab]	1.8±0.01[a]	1.74 ±06[cb]
T. Ex.-DNA[1]	70.0±1.90[c]	81.0±5.70[bc]	92.0±3.8[b]	92.0±4.3[b]	132.0±6.7[a]
Ext. Effi.[2]	3.50±0.09[b]	3.60±0.20[b]	3.7±0.15[b]	3.7±0.17[b]	4.3±0.19[a]
Assay 1	3.30±0.09[a]	2.40±0.11[c]	2.6±0.05[c]	2.9±0.07[b]	2.0±0.00[d]
Assay 2	3.25±0.06[a]	3.06±0.13[a]	3.1±0.07[a]	2.7±0.06[b]	3.0±0.00[a]

[1] Total extracted DNA from the sample (µg).
[2] Extraction efficiency (µg DNA / µl blood).
[a-c] Means with different superscript in each raw differ significantly (P<0.05).

tion time, phenol-chloroform-isoamyl alcohol (PCI) volume, centrifuge speed and and precipitation agent on quantity and quality of DNA extracted from whole fresh avian blood.

MATERIALS AND METHODS

Blood samples were collected in EDTA (7 µg/µl blood), kept in ice and shifted to the lab for examination. On average, fourteen replicates of blood samples underwent DNA extraction for each combination of lysis buffer volume, proteinase K extent, incubation time, PCI volume, spinning speed, and precipitation agent. The starting blood samples were taken at 20, 22, 24, 26 and 28 µl for DNA isolation. The ratio of lysis buffer (LB): starting blood volume (SBV) (µl : µl) used ranged from 27 - 30 to 51 - 61(µl : µl). The lysis buffer had the composition 5 M NaCl, 1 M Tris (pH 8.0), 0.5 M EDTA and 20% SDS. Proteinase K was used at 0.0 to 5.0 (µg/µl blood). The mixture of lysis buffer and sample was incubated at 58°C for 60, 70 or 90 min. The PCI: removed top layer (RTL) ratio in washing I and washing II ranged from 0.9 - 1.3 and 1.1 - 1.6 (µl: µl), respectively. Spinning was set at 2500 and 10000 rpm in the first round and 3000 and 10000 in the second round. The ease of removing the top aqueous phase following by the first and the second spinning was appraised as assays 1 and 2 and it was scored as 1, 2, 3 or 4 equating to easy, medium difficulty, relatively hard and very hard. The impact of precipitation agent was examined using three agents: ethanol, propanol and isoamyl alcohol.

The collected data on each factor analyzed using General Linear Models or t-test procedures (only for spin rpm) of SAS® software. The volume removed from top aqueous phase after the first and the second PCI washings included in the models as a continuous independent variable.

RESULTS

Genomic DNA is rather fragile. High molecular weight DNA is easily sheared by mechanical forces. The physical as well as chemical treatments involved in DNA extraction can affect both the quantity and quality of the DNA obtained. The starting blood volume ranging from 20 to 28 µl had a significant effect on all parameters considered (Table 1). The blood samples of 28 µl produced the most efficient extraction of DNA. The quality criterion was acceptable for all starting blood volumes with more reliable values for samples ranging from 22 to 26 µl. Despite of limited incremental differences of 2 µl for initial blood sample, it has observed that a blood sample of 22 – 26 µl leads DNA template of higher quality. In the contrary, the greater starting sample resulted in a higher quality sample and also higher efficiency of extraction.

Adding proteinase K from 1 to 5 µg/µl blood resulted in a significant difference in $OD_{260/280}$ ratio compared to extraction of DNA without proteinase K. However, the criterion of quality was located within the accepted range (1.8 - 2) for samples without proteinase K. Increasing levels of proteinase K resulted in more total DNA extracted as well as greater efficiency of DNA isolation (Table 2).

Lysis buffer (LB): starting blood volume (SBV) ratio (µl : µl) was examined in a wide range from 28 to 61 with incremental differences of 2 µl:µl (Table 3). All the variants of interest were significantly influenced by LB: SBV ratio with no clear-cut association between increasing levels of buffer. It seems that the quality and quality of extracted DNA could be accepted even with LB: SBV ratio of less than 28. In contrast to the quality criterion, total extracted DNA and extraction efficiency were significantly affected by incubation time of blood and lysis buffer mixture (P<0.05, Table 4). Both the variables were demonstrated negative association with increasing levels of incubation time. The results clearly reveal that incubation of the mixture of blood and lysis buffer at 58°C must not exceed 70 min.

Total extracted DNA and extraction efficiency were significantly influenced by phenol chloroform isoamyl alcohol (PCI): Removed top layer (RTL) ratio (µl : µl), in the first and the second washings in favour of higher ratios (Tables 5 and 6). The $OD_{260/280}$ was not affected by various ratios of PCI: RTL in the first washings but an inconsistent alteration in $OD_{260/280}$ was observed for different ratios in the second washing. The low spinning rate of 2500 and 3000 rpm for 20 min in the first and second spinning, respectively, resulted in significant differences in all variables of interest (except for DNA extraction efficiency) compared to the high rate of 10000 rpm for 5

Table 2. Effect (mean± SE) of Proteinase K levels (µg : µl blood) on variables studied.

Variables	Proteinase K (µg : µl blood)				
	0.0	**1-2**	**2.1-3**	**3.1-4**	**4.1-5**
OD$_{280}$	0.06±0.00^{a}	0.05±0.0^{a}	0.06±0.0^{a}	0.06±0.00^{a}	0.06±0.01^{a}
OD$_{260}$	0.11±0.01^{a}	0.09±0.0^{a}	0.1±0.0^{a}	0.11±0.00^{a}	0.10±0.01^{a}
OD$_{260/280}$	1.70±0.03^{b}	1.8±0.01^{a}	1.8±0.01^{a}	1.80±0.01^{a}	1.80±0.01^{a}
T. Ex.-DNA[1]	66.7±3.55^{b}	94.6±3.8^{a}	82.0±3.1^{ab}	84.0±3.06^{ab}	77.3±8.40^{ab}
Ext. Effi.[2]	3.12±0.21^{c}	3.8±0.15^{abc}	3.5±0.08^{bc}	4.20±0.17^{a}	3.86±0.40^{ab}
Assay 1	2.20±0.11^{c}	3.0±0.00^{b}	2.9±0.08^{b}	3.35±0.15^{a}	3.50±0.00^{a}
Assay 2	2.90±0.12^{cd}	2.8±0.05^{d}	3.2±0.05^{b}	3.4±0.05^{ab}	3.50±0.00^{a}

$^{a-c}$Means with different superscript in each raw differ significantly (P<0.05).
[1]Total extracted DNA from the sample (µg).
[2]Extraction efficiency (µg DNA / µl blood).

Table 3. Effect (mean ± SE) of various ratios of Lysis Buffer: blood volume (µl: µl) on variables studied.

Variables	Lysis Buffer: blood volume (µl: µl)								
	28-30	**31-33**	**34-36**	**37-39**	**40-42**	**43-45**	**46-48**	**49-51**	**51-61**
OD$_{280}$	0.05±0.00^{b}	0.07±0.01^{a}	0.06±0.0^{b}	0.06±0.00^{b}	0.05±0.0^{b}	0.05±0.0^{b}	0.05±0.00^{b}	0.06±0.00^{b}	0.06±0.00^{b}
OD$_{260}$	0.08±0.01^{dc}	0.13±0.01^{a}	0.1±0.0^{bc}	0.1±0.01^{bcd}	0.08±0.0^{d}	0.09±0.0^{bcd}	0.09±0.01^{bcd}	0.11±0.01^{b}	0.10±0.00^{bcd}
OD$_{260/280}$	1.70±0.01^{c}	1.8±0.03^{ab}	1.8±0.01^{a}	1.80±0.01^{a}	1.8±0.03^{ab}	1.8±0.02^{ab}	1.80±0.01^{a}	1.80±0.01^{a}	1.80±0.01^{a}
T. Ex.-DNA[1]	71.1±7.4^{de}	110±7.50^{a}	92.6±2.5^{a}	77.0±5.30^{cd}	60.0±2.74^{e}	89.0±4.3^{bc}	71.9±5.3^{de}	79.2±7.14^{bcd}	74.1±2.30^{de}
Ext. Effi.[2]	3.20±0.26^{bc}	4.02±0.21^{a}	4.04±0.1^{a}	3.60±0.16^{ab}	2.8±0.16^{c}	3.6±0.16^{ab}	3.30±0.18^{bc}	3.60±0.18^{ab}	3.70±0.12^{ab}
Assay 1	3.30±0.08^{ab}	2.7±0.13^{c}	3.2 ±.06^{b}	0.39±0.16^{ab}	3.4±0.24^{a}	2.5±0.03^{cd}	2.40±0.18^{d}	2.50±0.11^{cd}	2.40±0.08^{d}
Assay 2	3.30±0.08^{a}	3.0±0.00^{bc}	2.89±0.1^{c}	3.30±0.10^{a}	3.4±0.07^{a}	3.2±0.07^{ab}	2.90±0.16^{c}	2.80±0.15^{c}	2.75±0.16^{c}
Assay 3	1.50±0.0^{def}	1.35±0.07^{ef}	1.19±.06^{f}	1.72±0.14^{def}	2.2±0.19^{ab}	2.0±0.07^{abc}	2.00±0.23^{abc}	2.30±0.26^{a}	1.75±.16^{bcd}
Assay 4	3.50±0.0^{a}	3.6±0.07^{a}	3.5±0.00^{a}	3.58±0.05^{a}	0.4±0.07^{ab}	3.5±0.06^{a}	3.25±0.16^{bc}	3.07±0.20^{c}	3.50±0.00^{a}

[1] Total extracted DNA from the sample (µg). [2] Extraction efficiency (µg DNA / µl blood). $^{a-e}$ Means with different superscript in each raw differ significantly (P<0.05).

Table 4. Effect (mean ± SE) of incubation time of blood sample and lysis buffer on variables studied.

Variables	Incubation time (min)		
	60	70	90
OD_{280}	0.07±0.01[a]	0.06±0.00[b]	0.05±0.00[b]
OD_{260}	0.12±0.01[a]	0.10±0.01[b]	0.10±0.00[b]
$OD_{260/280}$	1.79±0.02[a]	1.8±0.010[a]	1.84±0.01[a]
T. Ex.-DNA[1]	116±13.0[a]	101±2.33[a]	77.1±1.90[b]
Ext. Effi.[2]	4.20±0.21[a]	4.05±0.09[a]	3.36±0.07[b]

[1]Total extracted DNA from the sample (µg).
[2]Extraction efficiency (µg DNA/µl blood).
[a-e]Means with different superscript in each raw differ significantly (P<0.05).

Table 5. Effect (mean ± SE) of phenol chloroform isoamyl alcohol (PCI): Removed top layer (RTL) ratio (µl: µl), in the first washing on variables studied.

Variables	Phenol: RTL (µl: µl), washing I				
	0.95	1.0	1.1	1.2	1.3
OD_{280}	0.05±0.0[a]	0.06±0.0[a]	0.05±0.0[a]	0.05±0.0[a]	0.06±0.0[a]
OD_{260}	0.09±0.0[a]	0.10±0.0[a]	0.09±0.0[a]	0.08±0.0[a]	0.10±0.0[a]
$OD_{260/280}$	1.9±0.05[a]	1.8±.01b[a]	1.80±0.01[a]	1.7±0.01[a]	1.8±0.01[a]
T. Ex.-DNA[1]	67.8±2.65[b]	85.6±3.2[ab]	94.8±3.3[a]	82.4± 3.5[a]	88.6±3.78[a]
Ext. Effi.[2]	3.40±13b[c]	3.5±0.08[bc]	3.97±0.1[ab]	3.91±0.17[a]	3.97±0.16[a]
Assay 1	1.56±0.19[d]	2.53±0.1[c]	3.00±0.0[b]	3.50±0.0[a]	3.25±0.05[ab]
Assay 2	1.56±0.19[e]	2.91±.09[c]	2.50±0.0[d]	3.50±0.0[a]	3.25±0.05[b]
Assay 3	1.19±0.06[d]	1.88±.08[a]	1.00±0.0[e]	1.50±0.0[c]	1.50±0.0[b]
Assay 4	2.9±0.13[e]	3.35±.05[d]	3.50±0.0[b]	3.50±0.0[a]	3.50±0.0[c]

[1]Total extracted DNA from the sample (µg).
[2]Extraction efficiency (µg DNA / µl blood).
[a-e] Means with different superscript in each raw differ significantly (P<0.05).

Table 6. Effect (mean ± SE) of Phenol Chloroform Isoamyl alcohol (PCI): Removed Top Layer (RTL) ratio (µl: µl), in the second washing on variables

Variables	Phenol: RTL (µl: µl), washing II					
	1.1	1.2	1.3	1.4	1.5	1.6
OD_{280}	0.05±0.00[c]	0.07±0.01[a]	0.06±0.0[ab]	0.07±0.0[a]	0.05±0.00[c]	0.05±0.00[c]
OD_{260}	0.09±0.00[b]	0.12±0.01[a]	0.11±0.01[a]	0.11±0.01[a]	0.09±0.00[b]	0.10±0.01[b]
$OD_{260/280}$	1.84±0.02[a]	1.75±0.02[b]	1.8±0.03[ab]	1.74±0.01[b]	1.80±0.01[b]	1.80±0.02[ab]
T. Ex.-DNA[1]	77.5±3.06[c]	88.9±6.50[c]	80.2±6.2[c]	110.6±7.7[a]	98.6±3.10[b]	105.8±6.5[a]
Ext. Effi.[2]	3.39±0.1[ab]	3.80±0.14[a]	3.6±0.19[ab]	3.32±0.16[b]	3.60±0.12[ab]	3.80±0.26[ab]
Assay 1	2.28±0.12[d]	2.68±0.09[c]	2.1±0.07[d]	1.17±0.17[e]	3.50±0.07[a]	3.00±0.00[b]
Assay 2	2.69±0.15[b]	3.0±0.09[ab]	2.9±0.15[ab]	1.17±0.17[c]	3.20±0.08[a]	2.96±0.04[ab]
Assay 3	1.70±0.07[b]	1.50±0.13[b]	2.3±0.26[a]	1.06±0.06[c]	1.64±0.11[b]	1.46±0.04[b]
Assay 4	3.23±0.07[b]	3.60±0.08[a]	3.2±0.21[b]	2.61±0.11[c]	3.50±0.00a	3.50±0.00[a]

[a-e] Means with different superscript in each raw differ significantly (P<0.05).
[1] Total extracted DNA from the sample (µg). [2] Extraction efficiency (µg DNA / µl blood).

min in both rounds (P<0.01; Table 7).

Precipitation agent had a significant impact on $OD_{260/280}$ and total extraction DNA (P<0.05). Isoamyl alcohol resulted in a greater amount of DNA extracted with higher quality. However, there was no significant difference between results obtained using isoamyl alcohol and ethanol. Considering cost and availability, ethanol is the preferred DNA precipitation agent over isoamyl alcohol.

Table 7. Effect (mean ± SE) of spinning rate on variables studied.

Variables	Spin (rpm)					
	First wash			Second wash		
	2500	10000	t-value	3000	10000	t-value
OD_{280}	0.05±0.00	0.06± 0.0	-2.56[*]	0.05±0.00	0.06±0.00	-3.41[**]
OD_{260}	0.09±0.00	0.1± 0.0	-0.02[*]	0.09±0.00	0.10±0.00	-2.72[**]
$OD_{260/280}$	1.82±0.01	1.75±.01	3.73[**]	1.83±0.02	1.75±0.01	4.17[**]
T. Ex.-DNA[1]	91.1±2.60	75.9±2.8	4.05[**]	88.3±0.01	75.9±2.85	3.22[**]
Ext. Effi.[2]	3.6± 0.08	3.5± 0.9	0.65[ns]	3.64±0.01	3.52±0.09	0.95[ns]
Assay 1	2.27±0.10	3.0±0.09	-5.33[**]	2.44±0.09	3.00±0.09	-4.21[**]
Assay 2	2.40±0.11	3.15±.05	-5.37[**]	2.65± 0.11	3.14±0.05	-4.02[**]
Assay 3	1.45±0.05	1.8±0.08	-3.65[**]	1.51±0.06	1.80±0.08	-2.92[**]
Assay 4	3.20±0.05	3.45±.04	-3.15[**]	3.32± 0.05	3.45±0.07	-2.9[**]

[1] Total extracted DNA from the sample (µg). [2] Extraction efficiency (µg DNA / µl blood).

Table 8. Effect (mean ± SE) of precipitation agent on variables studied.

Variables	Precipitation alcohol		
	Ethanol	Propanol	Iso amil
OD_{280}	0.06±0.00[a]	0.04±0.00[a]	0.05±0.01[ab]
OD_{260}	0.10±0.00[a]	0.08±0.00[a]	0.09±0.01[a]
$OD_{260/280}$	1.77±0.01[b]	2.08±0.02[a]	1.80±0.03[b]
T. Ex.-DNA[1]	83.3±2.15[a]	62.9±3.06[b]	87.4±9.94[a]
Ext. Effi.[2]	3.63±0.07[a]	3.14±0.15[a]	3.50±0.40[a]

[1] Total extracted DNA from the sample (µg). [2] Extraction efficiency (µg DNA / µl blood).
[a-e] Means with different superscript in each raw differ significantly ($P<0.05$).

DISCUSSION

Although only a small sample of DNA is required for molecular biological techniques the quantity of DNA extracted from blood or any other tissue is very important, especially if only traces of a tissue are available. Procedures are compared on the basis of DNA extraction efficiency and total DNA extracted from a µl or µg of tissue (Miller et al., 1988; Glasel, 1995; Laws and Adams, 1996; Philips and Simon, 1995). Isolation of DNA from small samples has many advantages; less chemical and time required, less risk of damage to the material, easy drying off and rehydration. Cell clumps may occur in large blood sam-ples when cells are not completely re-suspended prior to addition of the cell lysis solution. It is clear that greater blood sample compared to smaller eases the handling of samples when transferring to various tubes as well as at removing the top aqueous phase following the first and the second spinning.

Adding proteinase K (final concentration of 100 µl/ml) eases the cell lysis and prevents cell clumping (Herrman and Frischauf, 1987). It seems that a small blood sample (25 µl) is not associated with cell clumping and lyses of the cells involved proceeded even without proteinase K. An initial large blood sample necessitates application of proteinase K to prevent cell clumping and to enhance lyses of the cells.

It was anticipated that higher levels of lysis buffer would result in further lyses of cell walls and exposure of cell contents including genomic DNA for purification in the subsequent steps (Hillis et al., 1990, 1996). The results from this study did not confirm that (Table 3). However, the LB : SBV ratio of 31 to 36 resulted in more reliable DNA both in quantity and quality, for which finding the data in the present study does not offer any explanation.

The results clearly reveal that incubation of blood and lysis buffer mixture at 58°C must not exceed 70 min. It seems that the stability of DNA molecule at a constant temperature is a function of time. The total extracted DNA declined at incubation time of 90 minutes due to partial degradation of DNA. However, the quality of the extracted DNA from all the treatments was pretty constant.

The volume of chloroform-isoamyl alcohol added to the top aqueous layer in the tube was not found to impact significantly on assay scores in either 1 or 2 washings. It was expected that increasing levels of PCI would result in $OD_{260/280}$ near to or even beyond 2 through enhancing the chance of chloroform contamination in the sample extracted.

The DNA extracted using low spinning rate was of better quality and also greater in quantity. However, the ease score for removing the top opaque aqueous phase following spinning was significantly greater for high centrifuge rates (Table 8). These results reveal that sedimentation of various components in the three aqueous phases which formed during the spinning process is a slow process. The association between garish-black phase in the middle and the top opaque phase (containing DNA) lessens as the PCI phase separates.

Quality of extracted DNA is crucial to succeed in implementing molecular techniques considered. Spectro-

photometery is a method of choice to measure the purity of DNA samples by assessing the amount of ultraviolet irradiation that is absorbed by the bases in DNA make up. The $OD_{260/280}$ ratio of 1.8-2.0 indicates that the absorption is probably due to nucleic acid. The ratio of less than 1.7 indicates protein contamination (Glasel, 1995; Rafalski, 1997). The $OD_{260/280}$ ratio higher than 2.0 indicates RNA contamination. Protein contamination (ratio<1.7) is usually caused by exceeding the recommended amount of starting sample materials (Sambork et al., 1989; Stulnig and Amberger, 1994). DNA may be contaminated with protein, phenol, Tris or EDTA. However, other contaminants such as ethanol, sodium acetate and ammonium acetate will lead to incorrect determination of a sample concentration or no identification of sample contamination.

Detailed procedures described in the literature or internet are straightforward instructions on how to extract DNA and no reference is made to any difference in outcome resulting from modifying the procedures. However, the findings from few studies with respect to the quantity of the extracted DNA from fresh avian blood are in fair agreement with the results of this study (Seutin et al., 1991). Elkin et al. (2003) extracted ~60 µg of DNA per 5 µL of blood erythrocytes using commercial blood DNA isolation kit. Pirany (2005) reported average yield of 4.99±0.01 µg DNA/µl of whole blood. A lower than expected yield may be obtained if the cells are not completely lysed. It is very important to use the amount of starting materials specified in the procedure. Too few cells may create an imbalance in the DNA isolation chemistry and inhibit DNA precipitation. Too many cells may overload the system inhibiting complete cell lysis. In either situation, the result is a low yield of DNA.

In conclusion, this study revealed that an approved procedure for DNA extraction from whole fresh avian blood could consist of lysis buffer: initial blood ratio (µl : µl) of 31-36, incubation time of 60 - 70 min at 58°C, twice washing with PCI at 1.2-1.3 times PCI . / top aqueous phase (µl/µl) for first and 1.4 times for the second washing, to cetrifuge hemogenised sample at 2000-2500 rpm for 20 min, precipitation of DNA with 1.5-2 times (v/v) absolute cold ethanol, washing with 70% cold alcohol, draining and finally dissolving in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

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