Chapter 3

Comparison of DNA isolation kits to extract DNA from whole blood samples

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Abstract

Background and aim
Genomic deoxyribonucleic acid (DNA) from white blood cells (WBCs) is widely used for polymerase chain reaction (PCR). Although kits for DNA isolation are in common use, there is scarce information about their performance and the PCR quality of the genomic DNA obtained. Hence, three different kits, QIAamp blood mini kit, High Pure PCR Template Preparation Kit and the Puregene DNA isolation kit were evaluated on these aspects.

Materials and methods
Genomic DNA was isolated from whole blood samples with WBC counts ranging from 0.5 to 20 x 10^9 WBC/l. The WBC count was used to calculate the amount of genomic DNA. The actual amount of genomic DNA isolated, was determined spectrophotometrically. The DNA extraction efficiency was obtained by dividing the actual amount of DNA by the calculated amount yielded. PCR quality was analysed by measuring Cycle threshold (C_t) values with a quantitative real-time PCR β-globin assay.

Results
The extraction efficiency of the three DNA isolation kits was 20% to 40%. Spectrophotometrically determined DNA concentrations correlated inversely with C_t values, regardless of the DNA isolation kit applied, whereas the strongest correlation was obtained for the Puregene DNA isolation kit. WBC counts also correlated inversely with C_t values and here the strongest correlation was found for the QIAamp blood mini kit.

Conclusion
The overall performance of the DNA isolation kits was quite comparable (DNA recoveries of 20 - 40%), albeit the QIAamp blood mini kit yielded the most reproducible extraction efficiencies and lowest C_t values within every WBC count category.

Introduction

White blood cells (WBCs) are easily obtained and therefore a common source of genomic deoxyribonucleic acid (DNA), which is the starting material for a variety of molecular biological techniques. One of the most important applications is the use of genomic DNA in polymerase chain reaction (PCR), for either polymorphism analysis or quantitative analysis with fluorescent real-time PCR. In the latter, the number of genome copies present at the start of the PCR is related to the Cycle threshold (C_t) values[1,2]. DNA isolation kits are commonly used for the isolation of genomic DNA from WBCs. However, it is not known whether the performance of DNA isolation kits is influenced by the number of WBCs present. Neither, has been investigated whether the PCR quality of genomic DNA depends on the DNA isolation kit used. To answer these questions three different DNA isolation kits are tested. The QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) and the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) rely on the preparation of a chaotropic mixture, in which DNA binds to a column and is subsequently eluted. The principle of DNA isolation with the Puregene DNA isolation kit (Puregene, Landgraaf, the Netherlands) is based on a salting out method. The WBC count was used as the gold standard to calculate the exact amount of genomic DNA in a given volume. The diploid genome of one WBC equals the amount of 6.6 pg DNA[2]. The calculated amount of DNA is set as the 100% score. The actual amount of genomic DNA isolated was measured spectrophotometrically. Dividing the actual amount of genomic DNA isolated, by the calculated amount of genomic DNA, yields the extraction efficiency. The C_t value was used as a rough indicator for the PCR quality of genomic DNA, provided that an equal number of genome copies is present at the start of the PCR[1,2]. Genomic DNA with the best PCR quality should result in the lowest C_t value. A quantitative real-time PCR β-globin assay was used in this study to compare the PCR quality of genomic DNA, obtained with the different DNA isolation kits[2,3].

Materials and methods

Sample collection

Thirty ethylene diamine tetra-acetic acid (EDTA) anticoagulated blood samples with known WBC counts were collected from anonymized patients referred to the haematology department for routine investigations. These samples were categorized in six different WBC categories each category consisting of five samples. The range of WBC counts was set at 0.5 x 10^9 WBC/l, being the lowest number of WBC counts that could be counted reliably. The highest level was set at 20 x 10^9 WBC/l, being twice the number of the upper limit of a normal WBC count. Four intermediate categories of 2.0, 5.0, 10 and 15 x 10^9 WBC/l were formed. Each sample was divided into three equal

aliquots of 300 μl whole blood. Until DNA isolation was performed all blood samples were stored at –20°C. WBC counts were performed on a Beckman Coulter GenS (Beckman Coulter Inc., Fullerton, CA, USA).

DNA isolation procedures

DNA isolation was performed with the QIAamp blood mini kit (Qiagen, Leusden, the Netherlands), the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH Mannheim, Germany) and the Puregene DNA isolation Kit (Biozyme, Landgraaf, the Netherlands), all according to the manufacturers' protocols. Isolated genomic DNA was quantified spectrophotometrically by measuring absorptions at 260, 280 and 320 nm[4-7] with the Ultrospec 2000 (Pharmacia Biotech, Roosendaal, the Netherlands). Absorption at 320 nm (A^{320}) gives information about the presence of e.g. phenolic substances, whereas the ratio of the absorptions at 260 nm *versus* 280 nm ($A^{260/280}$) provides information about impurities due to proteins or RNA.

Quantitative real-time PCR β-globine TaqMan® assay

A quantitative real-time PCR β-globin TaqMan® assay, described by Lo *et al.* for the ABI Prism 7700, was used to obtain C_t values[2]. This quantitative real-time PCR TaqMan® assay was adapted for use on the LightCycler® (Roche Diagnostics by Almere, the Netherlands). The fluorescence signal from the liberated FAM reporter group was measured at the end of the extension step (72°C) in channel 1. The 2nd derivative method was used to calculate C_t values from the sigmoid real-time PCR curves. The PCR mixture contained 7.0 μl sterile water, 4.0 mM $MgCl_2$, 2.0 μl LC Faststart DNA Master Hybridization Probes (Roche Diagnostics, GmbH, Mannheim, Germany), 0.2 μM of the dual labelled FAM/TAMRA TaqMan® probe BGFT13 (Eurogentec, Seraing, Belgium) and 0.70 μM of both primers BGLOBF13 and BGLOBR13 (Eurogentec, Seraing, Belgium). After adding 1.0 μl genomic DNA to the PCR mixture, the total volume was 20 μl. The PCR protocol included the following steps a) denaturation for 10 minutes at 95°C b) 45 cycles at 95°C for 10 seconds, 60°C for 30 seconds and 72°C for 5 seconds. The temperature transition rates were programmed at 20°C/second for all three temperatures. C_t values of thirty DNA samples, isolated with the same DNA isolation kit, were determined within one PCR run.

Statistical methods

Statistical analysis was performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, USA). As initial distributions of the variables extraction efficiencies and C_t values were positively skewed. When appropriate, parametric tests were performed, otherwise non parametric statistics were used. To explore the influence of the WBC count (6 different levels from 0.5 to 20 x 10^9 WBC/l) on the extraction efficiency, the analysis was performed on the dataset where the outliers with extraction efficiency < 1.3% and

> 104% were omitted from the analysis (three for the Puregene method, two for the Roche method). As the data were thereafter readily normalized, univariate ANOVA was performed. The correlation between C_t values and DNA concentrations based on either WBC counts or UV-spectrophotometry was determined with the non parametric Spearman rank correlation. The Spearman correlation coefficient (r_s) indicates the degree of association between either DNA concentrations and the C_t values ($r_{s(UV)}$) or WBC counts and C_t values ($r_{s(WBC)}$).

Results

Following the manufacturers' protocols, the end volume for the genomic DNA solution was 200 µl for the Roche, 100 µl for the Qiagen and the Puregene DNA isolation kit. DNA extraction efficiencies appeared to be similar for all three DNA isolation kits in all WBC count categories, except in the lowest category (Figure 3.1). In this category the chaotropic isolation methods (Qiagen and Roche) seemed to exceed the salting out based method (Puregene). Background A^{320} readings ± standard deviation (SD) showed different optical density (OD) values over the complete range for each DNA isolation kit, but were higher for the chaotropic isolation methods than for the salting out isolation method, with respectively 0.20 ± 0.12 and 0.08 ± 0.06 for the Roche and Qiagen, and 0.02 ± 0.02 for the Puregene DNA isolation kit. The background corrected $A^{260/280}$ ratio of genomic DNA of the WBC count categories of 5.0 x 10^9 WBC/l and higher, varied from 1.8 to 1.9. Only the two lowest WBC count categories had a different background corrected $A^{260/280}$ ratio. Genomic DNA, isolated with the chaotropic isolation methods, showed in this area lower ratios than genomic DNA, isolated with the salting out method, 1.7 and 1.8 for the Qiagen and Roche DNA isolation kits, respectively, and 2.0 for the Puregene DNA isolation kit (Table 3.1). To explore the influence of the WBC counts on the extraction efficiency, the univariate ANOVA analysis was performed including all WBC count categories for each DNA isolation kit. The results were respectively F = 13.4, $P < 0.0001$ for Qiagen, F = 20.8, $P < 0.0001$ for Roche and F = 1.67, $P = 0.186$ for Puregene. This indicates that the mean value of at least one WBC level was significantly different as compared to other WBC levels for the Qiagen and Roche DNA isolation kit. The correlations of different DNA isolation methods (taking all WBC count levels into account) with the WBC count were as follows: r = -0.596, $P = 0.001$ for Qiagen, r = -0.782, $P < 0.0001$ for Roche and r = 0.153, $P = 0.445$ for Puregene. We speculated that two factors might be attributable to these findings. The first one was large variation in the extraction efficiency results in the group with WBC count of 0.5 x 10^9 WBC/l. The second one might be that extraction efficiency is dependent on the WBC count. Therefore, the analysis was performed once more, thereby omitting WBC count group of 0.5 x 10^9 WBC/l. The results of univariate ANOVA were respectively F = 1.33, $P = 0.293$ for Qiagen, F = 22.1, $P < 0.0001$ for Roche and F = 1.87, $P = 0.162$ for Puregene. Only for the Roche DNA isolation kit at least one

WBC level was significantly different as compared to other WBC levels. The correlations of different DNA isolation methods (omitting WBC count level of 0.5 x 10^9 WBC/l) with the WBC count were as follows: r = -0.398, P = 0.05 for Qiagen, r = -0.844, P < 0.0001 for Roche and r = 0.326, P = 0.138 for Puregene. In the tenfold range of WBC counts, the extraction efficiency varies roughly from 20% to 40%. In the lowest WBC count category, the extraction efficiency is higher for the chaotropic isolation methods than for the salting out isolation method (Figure 3.1). The real-time PCR curves are shown in Figure 3.2.

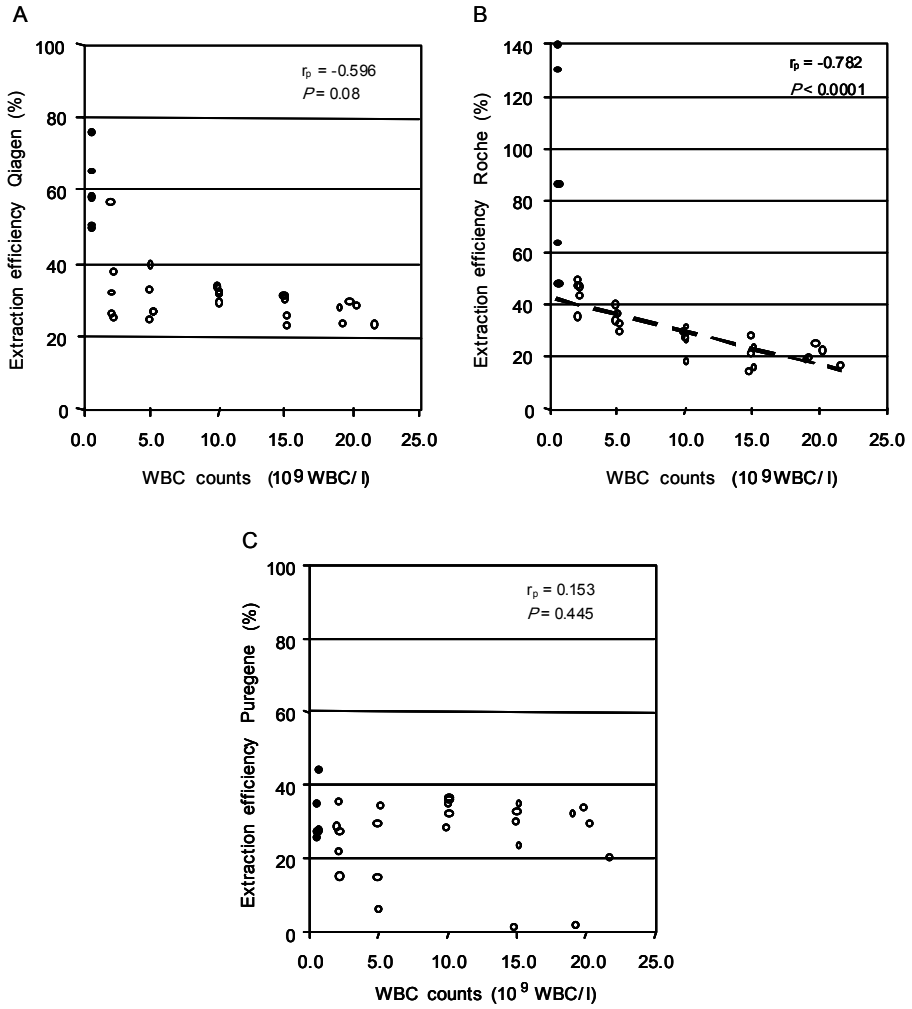

Figure 3.1 White blood cell (WBC) counts *versus* extraction efficiency of (A) Qiagen, (B) Roche and (C) Puregene with a WBC range of ± 0.5 to ± 20 x 10^9 WBC/l. The WBC count category of 0.5 x 10^9 WBC/l is excluded before calculating the r_p and p values of all DNA isolation kits. The closed circles in Figure 3.1B are not included in the trend line.

Table 3.1

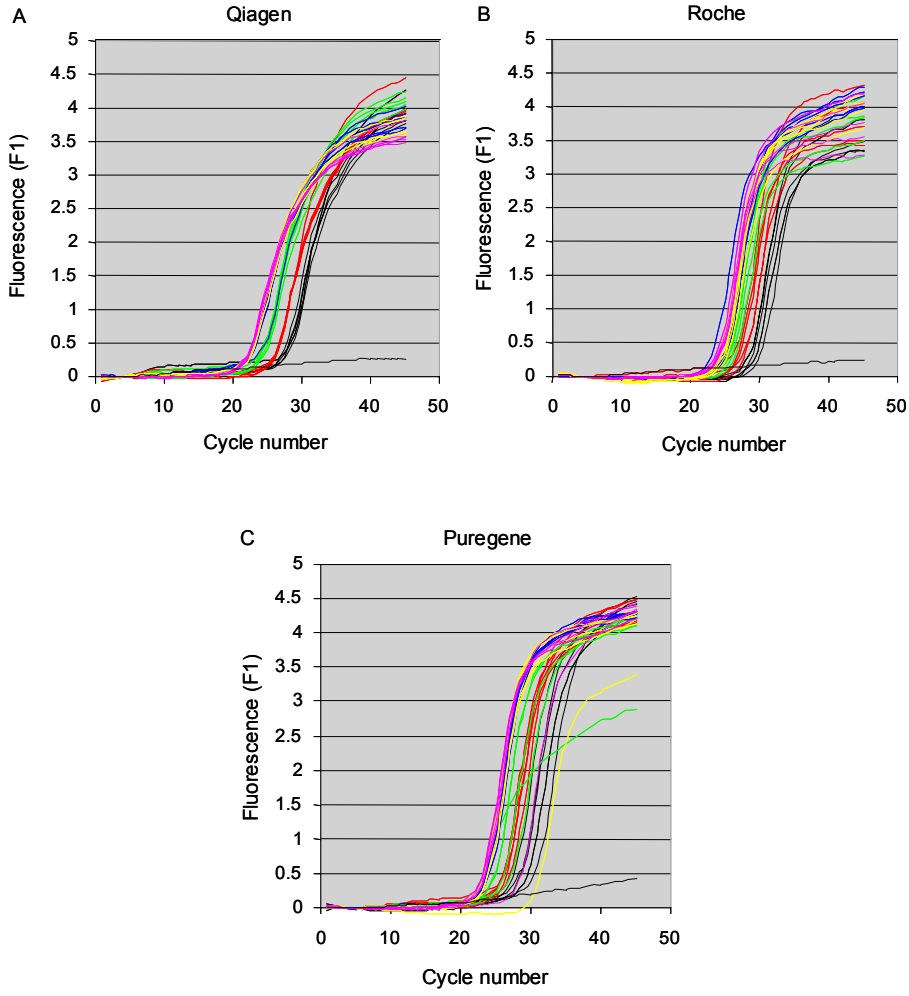

Figure 3.2 Real-time PCR amplification curves of three different DNA isolation kits (A) Qiagen, (B) Roche and (C) Puregene.

The median C_t values calculated from these curves seemed to be equal for the three DNA isolation kits in the lower three WBC count categories. Albeit that the median C_t value was somewhat lower for the Qiagen DNA isolation kit with the smallest C_t value interval ranges (Table 3.1). The median C_t values in the three higher WBC count categories seemed to be the lowest for the Qiagen DNA isolation kit, with also the narrowest C_t value interval ranges (Table 3.1). It was one and two C_t units lower than the median C_t values of the Puregene and the Roche DNA isolation kits, respectively. The variability of the C_t values within one WBC count category is lower for the

chaotropic isolation methods than for the salting out method (Table 3.1). For all the DNA isolation kits it was found that increased concentrations of genomic DNA was paralleled by decreased C_t values. Likewise, increased WBC counts were related with a decrease in C_t values. However, there seem to be subtle differences in the strength of these associations between the DNA isolation kits. This is apparent from the r_s values obtained with the non parametric Spearman rank correlation (Figure 3.3).

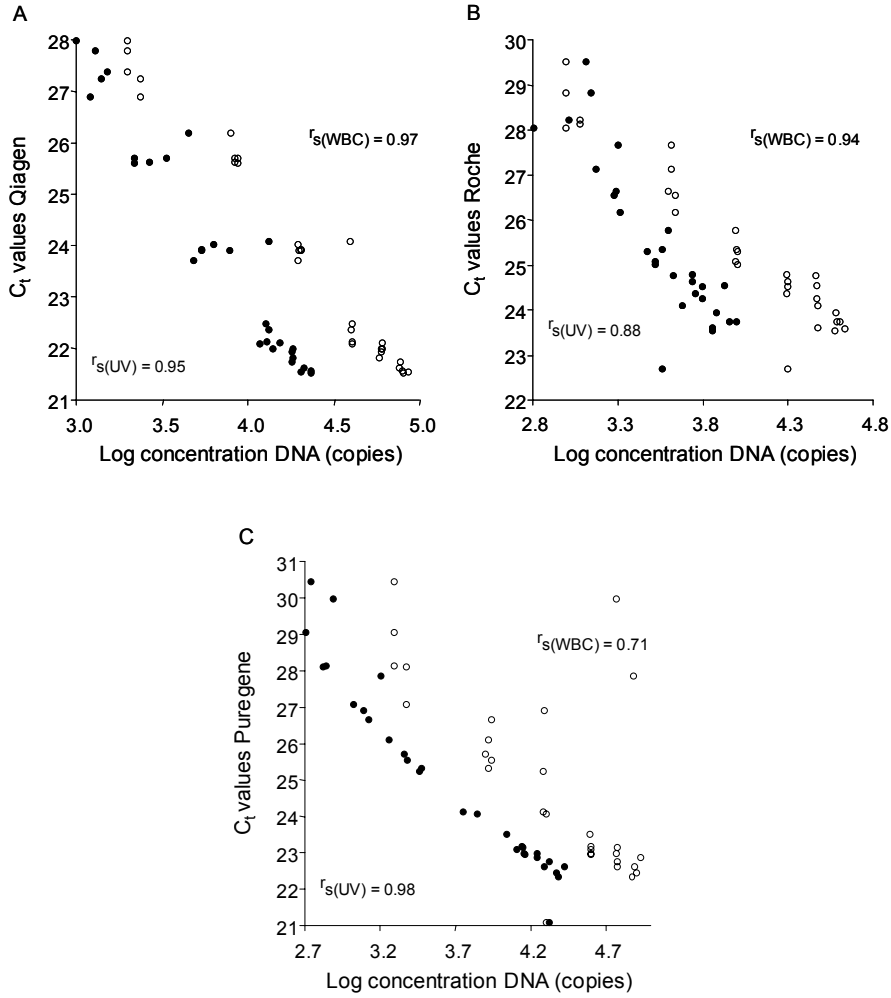

Figure 3.3 Log concentration DNA *versus* C_t values. The Spearman correlation coefficients (r_s) indicate the correlation between the log concentrations DNA in copies *versus* the C_t values of respectively (A) Qiagen, (B) Roche and (C) Puregene. The closed circles indicate the samples of which the DNA concentration is determined UV-spectrophotometrically ($r_{s(UV)}$). The open circles indicate the samples of which the DNA concentration is determined by counting white blood cells ($r_{s(WBC)}$).

The $r_{s(UV)}$ value for the association of the concentration of genomic DNA with the C_t value was the highest for the Puregene and the lowest for the Roche DNA isolation kit, 0.98 and 0.88, respectively. The $r_{s(WBC)}$ value for the association of the WBC counts with the C_t value was the highest for the Qiagen and the lowest for the Puregene DNA isolation kit, 0.97 and 0.71, respectively. The C_t values of the Qiagen DNA isolation kit correlated very well with the concentration of genomic DNA as well as with the WBC counts. Also, the C_t values of the Roche DNA isolation kit correlated well with the concentration of genomic DNA as well as with the WBC counts. In contrast, the C_t values of the Puregene DNA isolation kit correlated very well with the concentration of genomic DNA but relatively poorly with the WBC counts.

Discussion

WBC counts generate unambiguous data about the amount of genomic DNA present in a given volume of anticoagulated whole blood samples. Hence, the efficiency of a genomic DNA isolation with a DNA isolation kit can be determined unequivocally from the number of WBCs. In this study three different DNA isolation kits were compared, using samples containing low as well as high WBC counts, divided into six different WBC count categories. We showed that the different DNA isolation kits were comparable with regard to the total amount of genomic DNA that can be isolated. This is determined spectrophotometrically[4-7]. However, the background A^{320} readings are substantially higher in the chaotropic isolation methods than in the salting out method, this is especially prominent in the lowest WBC count category. OD readings for samples with low concentrations of genomic DNA, obtained with the chaotropic DNA isolation kits, might therefore overestimate the actual genomic DNA present. Only in the lowest WBC count category there is a marked difference in the calculated extraction efficiencies between the salting out and the chaotropic DNA isolation methods. The salting out method showed an extraction efficiency of about 40%, whereas the extraction efficiency of the chaotropic method was much higher. However, the high background in genomic DNA isolated with the chaotropic method might lead to an overestimation of the actual amount of genomic DNA present, and hence, to an exaggerated extraction efficiency. Turning to 2.0×10^9 WBC/l and higher, there is a remarkable constancy in the extraction efficiency. In a tenfold increase of WBC counts, there is a decrease from 40% to 20% in the extraction efficiency. This is observed for all the DNA isolation kits examined. It seems therefore that extraction efficiency depends on neither the method of DNA isolation nor the WBC count (Qiagen and Puregene), given that the WBC count is 2.0×10^9 WBC/l or higher. The PCR quality of the genomic DNA is evaluated by comparison of C_t values, obtained with a quantitative real-time PCR TaqMan® assay for the β-globin gene. Provided that the same copy number is present at the start of the PCR, genomic DNA with consistently the lowest C_t value, offers the best PCR quality. Although the C_t values are almost identical within a WBC

count category, the median C_t value of genomic DNA isolated with the Qiagen kit seems to be consistently lower than the C_t values obtained with genomic DNA isolated with the other DNA isolation kits. The Qiagen kit also yields genomic DNA with the narrowest C_t value interval ranges (Table 3.1). The largest C_t value interval ranges are observed with genomic DNA isolated with the salting out method. Most likely, this is related to the fact that three samples in three different WBC count categories yielded a very low extraction efficiency. In general, C_t values are inversely related to DNA concentrations. Because the DNA concentration in a whole blood sample is largely determined by the number of WBCs, one should also find an inverse relation between C_t values and WBC counts. Actually, the association found between C_t values and WBC counts for the chaotropic isolation methods is better than the association between C_t values and genomic DNA concentration, as measured with UV-spectrophotometry. For the salting out method this association is the other way around and most likely, due to the very low extraction efficiency of three samples in three highest WBC count categories, which impairs the association of the C_t values with WBC counts, but not the association of C_t values with genomic DNA concentration, as measured with UV-spectrophotometry. It implies that WBC counts could be used to predict the yield of genomic DNA if a chaotropic DNA isolation is used in addition of an UV-spectrophotometric measurement. This would be worthwhile considering if samples with a low WBC count are used.

In conclusion, the use of WBC counts to calculate the amount of genomic DNA in a given volume allowed us to establish that the extraction efficiency ranges roughly from 20% to 40%, independent of the DNA isolation kit used and the number of WBCs present (Qiagen and Puregene). Using C_t values as a measure for the PCR quality of genomic DNA, it was found that the Qiagen blood mini kit seemed to yield the best quality of genomic DNA for PCR in the three highest WBC count categories.

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