Comparison of DNA purification kits for whole blood

S Prevost, A Weirich, NA Arbour, R Liu, E Vaillancourt, CJ Harder
Spartan Bioscience Inc.

The Spartan DX™ real-time PCR instrument is compatible with DNA purified from blood using commercial kits from GE Life Sciences, QIAGEN, Invitrogen, and Promega.

Introduction

When DNA is purified from blood, the major inhibitor of PCR is the heme component of hemoglobin. The concentration of hemoglobin in blood is approximately 160 mg/ml and the inhibitory concentration is ≥1 mg/ml (Ref 1). DNA may be purified from blood using homebrew methods such as phenol-chloroform extraction, or with commercial kits based on technologies such as silica membranes.

The purpose of this study was to compare the performance of four DNA purification kits for on the Spartan DX real-time PCR instrument.

Materials and Methods

DNA extraction

Whole blood was collected in BD Vacutainer® SST™ Tubes from a single individual. Using aliquots of blood from the same sample, DNA was purified using four kits according to the manufacturers' instructions. Kits included the illustra™ blood genomicPrep Mini Spin Kit (GE Life Sciences, Cat. No. 28-9042-64), QIAamp DNA Blood Mini Kit (QIAGEN, Cat. No. 51104), PureLink™ Genomic DNA Mini Kit (Invitrogen, Cat. No. K1820-00), and the Wizard® Genomic DNA Purification Kit (Promega, Cat. No. A1123). Purifications were performed in duplicate for each kit. After purification, samples were stored at 4°C.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the human muscarinic acetylcholine receptor subtype M3 gene (*CHRM3*) gene using online software from Biosearch Technologies (www.qpcrdesign.com). The forward primer sequence was 5'-ttg ggt cat ctc ctt tg-3' and the reverse primer was 5'-gca cag ttc tct ttc ca-3'. The expected amplicon size was 74 base pairs. A TaqMan® probe was obtained from Biosearch and consisted of a FAM fluorophore at the 5' end and a Black Hole Quencher® (BHQ-1) at the 3' end. The probe sequence was 5'-tcc ttt ggg ctc ctg cca tct-3'.

TaqMan reactions were performed on the Spartan DX using 0.2 ml flat-cap PCR tubes (VWR Cat. No. 53550-106). Reactions were topped with 15 µl of mineral oil (Sigma, Cat. No. M8662) to prevent evaporation. Components of the real-time PCR amplification mixture are listed in Table 1. Table 2 shows the cycling parameters for a two-temperature program which combines the primer annealing and extension steps.

DNA analysis

Fluorescence values were downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. PCR results were confirmed by agarose gel electrophoresis using 5 µl of the amplification products.

Results

Agarose gel electrophoresis showed that all reactions yielded a single amplicon of the correct size. Real-time PCR graphs showed that all DNA kits gave positive results and the threshold cycle (Ct) values ranged from 23 to 26 (Table 3). Figure 1 shows a representative real-time PCR result.

Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	5.75 mM
dNTP mix (Invitrogen)	0.2 mM
Platinum TAQ Polymerase (Invitrogen)	1 U
TaqMan probe (Biosearch)	0.1 µM
PCR primers (Biosearch)	0.5 µM each
Template DNA	2 µl
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of real-time PCR mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	150 s	1
Denaturation	95°C	30 s	35
Annealing/extension	58°C	55 s	35

Table 2. Cycling parameters.

DNA Isolation Kit	DNA yield/200 µl Blood (µg)	260/280 Ratio	Purification Time (min)	Threshold Cycle
illustra™ (GE)	0.95	1.69	22	23
QIAamp (QIAGEN)	0.45	1.60	23	25
PureLink™ (Invitrogen)	1.45	1.71	28	25
Wizard® (Promega)	0.4	1.55	35	26

Table 3. DNA yields and real-time PCR results.

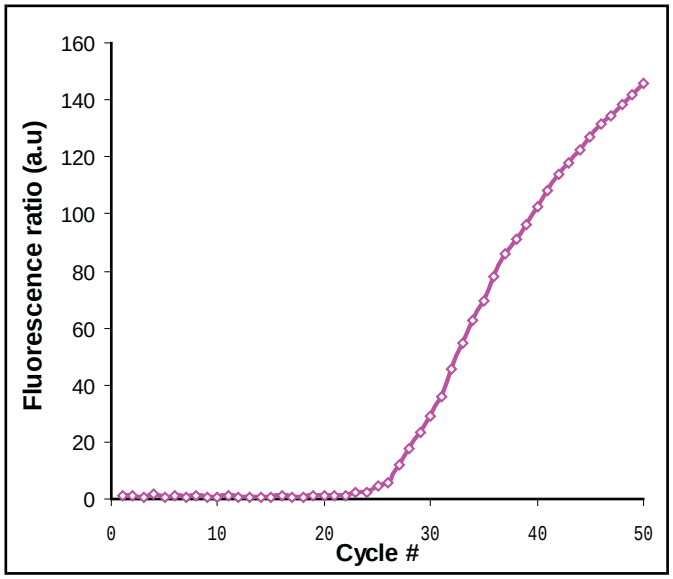

Figure1. Real-time PCR result obtained with the Wizard kit from Promega.

Discussion and Conclusions

All four DNA purification kits generated the predicted real-time PCR results with the Spartan DX, indicating they are effective at removing PCR inhibitors from blood. In terms of speed and ease of use, we found the illustra™ kit from GE Life Sciences to be the most user-friendly. Overall, DNA purified from blood with commercial kits is compatible with the Spartan DX for real-time PCR.

References

1. QIAGEN Inc. (2006). *Critical factors for successful PCR.* Lit. #1022653.