HEMAgene™•BUFFY COAT

Compatibility of the QIAGEN® QIAamp® DNA blood mini kit with fresh buffy coat samples in HEMAgene™•BUFFY COAT DNA stabilizing reagent

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0.5 mL buffy coat samples in HEMAgene™•BUFFY COAT DNA stabilizing reagent (HG-BCD) at room temperature and extracted with the QIAGEN® QIAamp® DNA blood mini kit have an average DNA concentration of 13.6 µg/mL by fluorescence quantification. The isolated genomic DNA is of high molecular weight and is suitable for PCR amplification.

Introduction

Buffy coat fractions are often prepared from whole blood because they provide a clean, concentrated source of nucleated cells from which to extract DNA. Unlike whole blood, buffy coat preparations are more conducive to long-term storage and transport as long as they are kept frozen. However, the use of freezers for long-term storage and the use of dry ice for transport are costly and are not a reliable method for stabilizing samples. HEMAgene•BUFFY COAT DNA stabilizing reagent for buffy coat samples offers reliable, ambient temperature preservation of DNA in buffy coat samples for the recovery of high molecular weight DNA. The purpose of this study was to assess the yield and quality of DNA extracted from buffy coat samples, preserved in the HEMAgene•BUFFY COAT DNA stabilizing reagent, using the QIAGEN QIAamp DNA blood mini kit.

Materials and methods

Sample collection, buffy coat preparation and DNA extraction

Nine donors were recruited for this study and two blood draws per donor were made. Approximately 7 mL of whole blood was collected from each donor into 2 × 10 mL EDTA-K Vacutainer tubes (#366643; 16 × 100 mm, 10.0 mL BD Vacutainer® plastic EDTA tube; Lavender BD Hemogard™ closure; Becton, Dickinson & Company). Samples were gently rocked at room temperature and then centrifuged at 1,200 × *g* for 10 minutes at room temperature to fractionate samples into plasma, buffy coat (5–10× concentrated leukocytes) and packed red blood cell (erythrocytes) fractions. Plasma was gently removed from fractionated samples with a Pasteur pipette, leaving ~1 mL of plasma above the buffy coat layer. Using a P200 micropipette (set at 100 µL) and "wide-bore" pipette tips, a 0.5 mL aliquot of the buffy coat fraction was transferred to a 15 mL conical tube and prepared for room temperature storage by the addition of 4.5 mL of HEMAgene•BUFFY COAT DNA stabilizing reagent.

QIAGEN QIAamp DNA blood mini kit protocol for fresh buffy coat samples in HEMAgene•BUFFY COAT DNA stabilizing reagent

1. Fresh buffy coat samples in HEMAgene•BUFFY COAT DNA stabilizing reagent (buffy coat: HEMAgene•BUFFY COAT DNA stabilizing reagent 1:9) were stored at room temperature until required for genomic DNA isolation.
2. QIAamp DNA Mini and blood mini handbook, Third Edition, June 2012; Protocol: DNA purification from blood or body fluids: Page 26.
3. At "Step 2" of the QIAGEN QIAamp DNA blood mini kit protocol (QIAamp DNA mini and blood mini handbook 06/2012, Protocol: DNA purification from blood or body fluids), add 200 µL of the HEMAgene•BUFFY COAT sample (buffy coat: HEMAgene•BUFFY COAT DNA stabilizing reagent 1:9) instead of 200 µL of blood.

Absorbance determination of DNA concentration

DNA yields from the HEMAgene•BUFFY COAT samples were determined using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc.). A 2 µL volume of each DNA sample was placed on the pedestal and scanned from 220 nm to 350 nm with absorbencies measured at 230 nm, 260 nm and 280 nm. Sample DNA concentration (ng/µL), A_{260}/A_{280} ratio, A_{260}/A_{230} ratio were reported by the NanoDrop

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2000c software. The total nucleic acid yield per sample was calculated by multiplying the sample concentration by the respective DNA elution volume.

Fluorometric determination of DNA concentration

DNA yields from the samples were determined using Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen p7581) and a Lambda DNA Standard (Invitrogen, 25250010). PicoGreen is a fluorescent double-stranded DNA-binding dye (480 nm Excitation/520 nm Emission) that enables sensitive quantification of small amounts of double-stranded DNA. An aliquot of each sample was diluted 10-fold with TE buffer. Triplicate 5 µL aliquots of each sample and a standard curve of Lambda DNA (in triplicate; 0–10 ng/µL) were mixed with PicoGreen reagent in a black flat-bottomed 96-well microplate (Greiner Bio-One, 655209). Fluorescence was measured using an Infinite® M200 microplate reader (TECAN®).

Genomic DNA integrity

To assess DNA integrity, 100 ng from each HEMAgene•BUFFY COAT sample was separated on a 0.8% agarose gel by electrophoresis for 1 hour at 80 volts. The gel was stained in 1 µg/mL ethidium bromide in distilled water for 15 minutes at room temperature, rinsed and photographed on a UV transilluminator using a DigiDoc-IT™ imaging system (UVP LLC). The UltraRanger 1 kb DNA Ladder (300 bp–24,000 bp; Norgen Biotek) was used as a size reference for the genomic DNA samples.

DNA amplification

Purified DNA was evaluated in qPCR for amplification performance using primers targeting the single copy thymidylate synthase gene (TYMS locus; NM001071.2). For each reaction, 50 ng of purified genomic DNA was amplified in a 25 µL volume containing: 1× PCR buffer (20 mM Tris, 50 mM KCl), 2 mM $MgCl_2$, 200 µM dNTPs (Invitrogen™), 50 µg/mL BSA (Sigma Aldrich®), 1 µM SYTO9 dye (Invitrogen), 0.4 µM each of Primer hTSm143F and hTSm143R (Invitrogen), 1U Taq polymerase (Invitrogen). The amplification conditions for the TS143 target were: 1 cycle: 95°C for 5 minutes; 35 cycles: 95°C for 20 seconds, 55°C for 20 seconds, 72°C for 30 seconds and 1 cycle 72°C for 10 minutes. A melt curve program was included and consisted of: 1 cycle 95°C for 30 seconds at a ramp rate of 4.4°C/second (no acquisition), 72°C for 10 minutes at a ramp rate of 2.2°C/second (no acquisition), 95°C at a ramp rate of 0.11°C/second (continuous acquisition). DNA samples were run in triplicate in a Corbett Rotorgene RG-6000 and C_t values for each sample calculated using the Rotorgene 6000 series software 1.7.

Results

As shown in Table 1, DNA yields for the individual HEMAgene•BUFFY COAT samples differ but are within the range expected for donor-to-donor variation. As expected, DNA yields by absorbance (NanoDrop) are slightly higher than those observed by fluorescence (PicoGreen). Quantification of DNA by absorbance is less time-consuming but subject to interference by non-DNA moieties (proteins, RNA, carbohydrates, etc.). Quantification of DNA by fluorescence with DNA binding dyes (PicoGreen) is more accurate. The Nanodrop absorbance (220 nm–350 nm) scans shown in Figure 1 indicate that the genomic DNA isolated from the HEMAgene•BUFFY COAT samples is free of contaminants and inhibitors (organics, chaotropic salts, denaturants, etc. that absorb at 230 nm) that may inhibit or compromise molecular biology applications. The A_{260}/A_{280} values are higher than expected for genomic DNA alone. In this instance, the A_{260}/A_{280} values reflect the co-purification of DNA and RNA by the QIAamp mini spin columns since the optional RNAse A digestion step in the protocol was not performed for these samples.

Sample ID [200 µL input] [200 µL elution]	Fluorescence		Absorbance			
	ng/µL	Total ng	ng/µL	Total ng	A_{260}/A_{280}	A_{260}/A_{230}
Fresh buffy coat 1	15.50	3101	17.40	3480	1.93	1.82
Fresh buffy coat 2	16.82	3363	18.50	3700	1.92	1.72
Fresh buffy coat 3	15.01	3003	17.50	3500	1.94	1.87
Fresh buffy coat 4	13.20	2640	15.20	3040	2.02	1.76
Fresh buffy coat 5	13.45	2690	14.60	2920	1.95	2.05
Fresh buffy coat 6	14.05	2810	15.20	3040	2.00	1.72
Fresh buffy coat 7	11.52	2304	12.50	2500	2.02	1.71
Fresh buffy coat 8	10.99	2198	11.30	2260	1.99	2.02
Fresh buffy coat 9	11.72	2345	13.40	2680	1.89	1.52
Average	**13.58**	**2717**	**15.07**	**3013**	**1.96**	**1.80**

***Table 1**: 200 uL aliquots of HEMAgene•BUFFY COAT samples were used for genomic DNA isolation using the QIAGEN QIAamp DNA blood mini kit. Samples were processed with the supplied binding buffer, wash buffer and elution buffer according to kit protocol.*

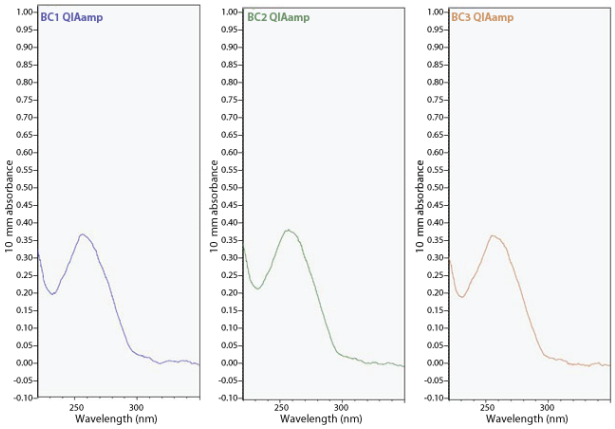

***Figure 1**: 2 µL aliquots of each HEMAgene•BUFFY COAT sample were analyzed and quantified by absorbance on a NanoDrop 2000c spectrophotometer as described in the materials and methods. Triplicate 1 µL aliquots of each HEMAgene•BUFFY COAT sample were also quantified by fluorescence using the QuantiFluor dsDNA system as described in the materials and methods.*

Analysis of the genomic DNA isolated from the HEMAgene•BUFFY COAT samples by agarose gel electrophoresis (Figure 2) shows high molecular weight genomic DNA in each sample and no evidence of degradation.

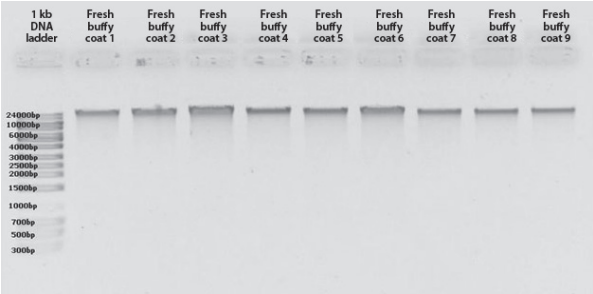

***Figure 2**: 200 µL aliquots of HEMAgene•BUFFY COAT samples were used for genomic DNA isolation using the QIAGEN QIAamp DNA blood mini kit. Samples were processed with the supplied binding buffer, wash buffer and elution buffer according to kit protocol. Aliquots of the eluted DNA samples were analyzed by agarose gel electrophoresis as described in the materials and methods.*

The quantitative real time PCR (qPCR) results shown in Figure 3 indicate that the genomic DNA in each HEMAgene•BUFFY COAT sample is equivalent to a purified human genomic DNA reference/control sample (C_t values). In addition, these results demonstrate that the genomic DNA in each HEMAgene•BUFFY COAT sample is free of contaminants or inhibitors and is suitable for downstream molecular biology applications, including qPCR.

Sample ID [200 µL input] [200 µL elution]	TS143 qPCR C_t value
Fresh buffy coat 1	19.81
Fresh buffy coat 2	19.94
Fresh buffy coat 3	19.81
Fresh buffy coat 4	19.63
Fresh buffy coat 5	19.86
Fresh buffy coat 6	19.58
Fresh buffy coat 7	19.73
Fresh buffy coat 8	19.80
Fresh buffy coat 9	19.84
50 ng human gDNA	19.97
Average	19.80

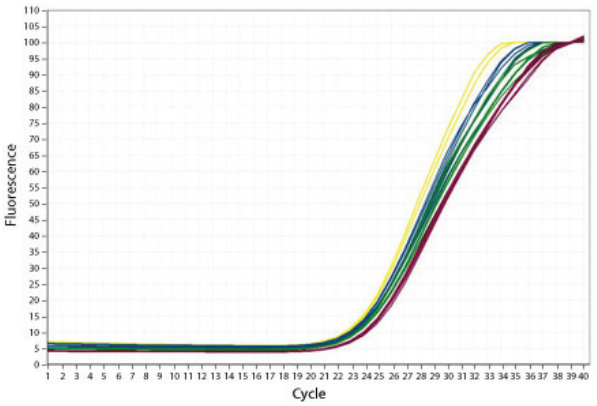

***Figure 3**: 200 µL aliquots of HEMAgene•BUFFY COAT samples were used for genomic DNA isolation using the QIAGEN QIAamp DNA blood mini kit. Samples were processed with the supplied binding buffer, wash buffer and elution buffer according to kit protocol. Aliquots (~50 ng) of the eluted DNA samples were analyzed by qPCR on a Corbett Rotorgene RG-6000 as described in the materials and methods.*

Discussion and conclusion

High yields of high quality DNA were purified from HEMAgene•BUFFY COAT samples using the QIAGEN QIAamp DNA blood mini kit. Agarose gel electrophoresis analysis of the purified samples showed that the integrity and quality of the DNA was excellent.

200 µL aliquots of HEMAgene•BUFFY COAT samples, extracted with the QIAGEN QIAamp DNA blood mini kit, have an average DNA concentration of 13.6 µg/mL by fluorescence quantification (15.1 µg/mL by absorbance). If more concentrated DNA samples are required, elution in smaller volumes (50 µL–100 µL) will increase the DNA concentration but will also reduce the overall DNA yield in these samples.

As shown in the *QIAamp DNA mini and blood mini handbook (06/2012; page 25, Table 4. Total nucleic acid yields with QIAamp kits using successive elutions)*, 200 µL of whole blood yields 3-8 µg of genomic DNA (15–40 µg/mL) after the first elution. Since the buffy coat fraction represents ~50% of the white blood cells (leukocytes) present in whole blood, a 200 µL aliquot of a buffy coat fraction reconstituted back to the original blood draw volume (5–10 mL) would be expected to yield 1–4 µg of genomic DNA (5–20 µg/mL). The yield of genomic DNA purified from a 200 µL aliquot of HEMAgene•BUFFY COAT sample (buffy coat: HEMAgene•BUFFY COAT DNA stabilizing reagent 1:9) using the QIAGEN QIAamp DNA blood mini kit, and only one elution step, has an average yield of 2.72 µg (range 2.20 to 3.36 µg) which is within the expected range for a reconstituted buffy coat fraction (diluted 1:9) and donor-to-donor variation.

In addition, the TS143 qPCR product was amplified successfully from the purified DNA samples, indicating the DNA is free of contaminants or inhibitors and suitable for PCR analysis.