HEMAgene™•BUFFY COAT DNA stabilizing reagent protects DNA in buffy coat samples through multiple freeze-thaw cycles

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HEMAgene™•BUFFY COAT DNA stabilizing reagent (HG-BCD) for buffy coat samples offers reliable, room temperature preservation of DNA in buffy coat samples for the recovery of high molecular weight DNA. A 0.5 mL buffy coat sample stored in HG-BCD can withstand multiple freeze-thaw cycles with minimal DNA loss and no degradation compared to the substantial DNA loss incurred after multiple freeze-thaw cycles of an unprotected buffy coat sample.

	% DNA loss after 2 freeze-thaw cycles	% DNA loss after 20 freeze-thaw cycles
HEMAgene•BUFFY COAT	1%	7%
Unprotected buffy coat	75%	91%

Introduction

Buffy coat fractions provide a clean, concentrated source of nucleated cells from which to extract DNA. Compared to whole blood, buffy coat preparations are more conducive to long-term storage and transport as long as they are kept frozen. Unprotected buffy coat samples are at risk of significant DNA loss after more than one freeze-thaw cycle. According to the University of McGill Green Biobanking survey, 25% of respondents have experienced specimen loss as a result of power failures in the past 5 years[1]. In order to reduce the risks associated with multiple freeze-thaw cycles, the following are implemented: complex and costly cold chain infrastructure, monitoring systems, backup infrastructure and single use aliquot schemes.

A method of protecting buffy coat samples from damage even during multiple freeze-thaw cycles would be beneficial to reduce costs and simplify large population studies and long-term banking of samples. HG-BCD stabilizing reagent for buffy coat samples offers ambient temperature transport and room temperature archival storage of high molecular weight DNA. This study demonstrates the protection of DNA in buffy coat samples through multiple freeze-thaw cycles when stored in HG-BCD as determined by fluorescence quantification and gel electrophoresis.

Materials and methods

Sample collection and buffy coat preparation

Three donors were recruited for this study and 1 blood draw per donor was made. Approximately 7 mL of whole blood was collected from each donor per draw into a 10 mL EDTA-K Vacutainer tube. Samples were gently rocked at room temperature and centrifuged at 1,200 × *g* for 10 minutes at room temperature to fractionate samples into plasma, buffy coat and packed red blood cell 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 buffy coat fraction was transferred to a 15 mL conical tube and diluted with an equal volume of 0.9% (150mM) NaCl . This 1 mL buffy coat suspension was prepared as follows for each donor sample:

PD-WP-00033 Issue 2/2013-09

Buffy coat in HG-BCD: A 0.5 mL aliquot of this buffy coat suspension was transferred to a 15 mL conical tube and processed immediately by the addition of 4.5 mL of HG-BCD, and vortexed. An aliquot was removed at "t=0" for analysis. The remaining sample was stored at -80°C.

Unprotected buffy coat: 4.5 mL 0.9% (150 mM) NaCl was added to the remaining 0.5 mL buffy coat suspension and vortexed. An aliquot was removed at "t=0" for analysis. The remaining sample was stored at -80°C.

Freeze-thaw cycles

These samples were exposed to 20 freeze-thaw cycles consisting of (i) freezing at -80°C for 1 hour followed by (ii) thawing at +50°C for 15 minutes in a waterbath. 200 µL aliquots of the HG-BCD samples and unprotected buffy coat samples were collected before freezing at t=0 and after freezing at 2, 4, 6, 8, 10, 15 and 20 cycles.

DNA extraction

200 µL samples were processed for gDNA using the Promega ReliaPrep™ Blood gDNA Miniprep System as per the following protocol:

1. Promega ReliaPrep Blood gDNA Miniprep System technical manual; Instructions for use of products A5081, A5082; Literature # TM330, Revised 12/12.
2. At "Step 3" of the Promega ReliaPrep Blood gDNA Miniprep system protocol, 200 µL of the HEMAgene•BUFFY COAT sample (buffy coat: HEMAgene•BUFFY COAT DNA stabilizing reagent 1:9) or the unprotected buffy coat: 0.9% NaCl sample was added instead of 200 µL of blood.
3. At "Step 14: of the Promega ReliaPrep Blood gDNA Miniprep system protocol, the samples were eluted in 100 µL of nuclease-free water.

Absorbance determination of DNA concentration

Total nucleic acid yields from the 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 absorbances were measured at 260 nm and 280 nm. Readings were background corrected by subtracting the absorbance at 340 nm. Sample DNA concentration (ng/µL) and A_{260}/A_{280} ratio, were reported by the NanoDrop 2000c software. The total DNA 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). 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. Fluorescence was measured using an Infinite® M200 microplate reader (TECAN®).

Genomic DNA integrity

To assess DNA integrity, 100 ng from each 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 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.

PD-WP-00033 Issue 2/2013-09

Results

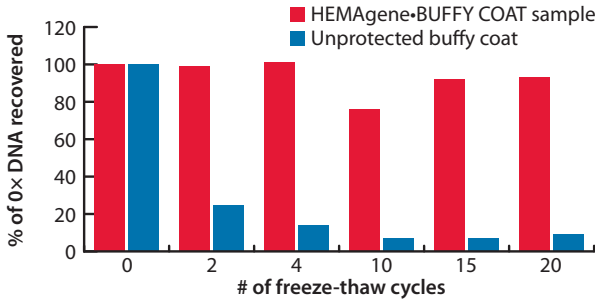
As shown in Table 1, the HEMAgene•BUFFY COAT samples only experienced a 1% loss of DNA after 2 freeze-thaw cycles, compared to a 75% loss of DNA in an unprotected buffy coat sample after 2 freeze-thaw cycles as measured by fluorescence.

The A_{260}/A_{280} values for all samples are within the expected range and are greater than 1.9.

Sample treatment	# of freeze-thaw cycles	Fluorescence		% loss from 0×	A_{260}/A_{280}
		ng/µL	ng per aliquot		
HG-BCD	0×	27.8	2782	n/a	1.94
	2×	27.5	2745	1.3%	1.98
	4×	28.1	2814	-1.1%	1.96
	10×	21.1	2112	24.1%	1.94
	15×	25.6	2562	7.9%	1.94
	20×	25.9	2589	6.9%	1.97
Unprotected buffy coat	0×	23.7	2375	n/a	1.94
	2×	5.9	588	75.2%	2.06
	4×	3.2	322	86.5%	2.24
	10×	1.6	156	93.4%	1.93
	15×	1.7	171	92.8%	2.66
	20×	2.2	220	90.8%	2.47

***Table 1**: 200 µL aliquots were used for genomic DNA isolation and eluted in 100 µL. The average DNA concentrations, yields and absorbance values from both the protected HEMAgene•BUFFY COAT samples (HG-BCD) and the unprotected buffy coat samples (0.9% NaCl) after multiple freeze-thaw cycles (0× to 20×) were determined as described in the materials and methods.*

A comparison of the percent DNA yield after each freeze-thaw cycle compared to the original amount prior to freezing for the protected HEMAgene•BUFFY COAT samples versus the unprotected buffy coat (0.9%NaCl) samples is depicted in Figure 1.



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Analysis of the genomic DNA isolated from the samples by agarose gel electrophoresis (Figure 2) shows high molecular weight genomic DNA, no evidence of degradation and minimal loss in the HEMAgene•BUFFY COAT samples after 20 freeze-thaw cycles. Significant loss is seen in the unprotected buffy coat samples after as few as 2 freeze-thaw cycles.

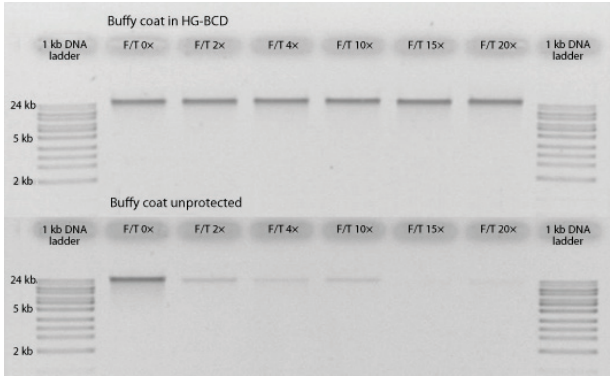

***Figure 2**: After multiple freeze-thaw cycles (0× to 20×), genomic DNA was purified from the unprotected buffy coat sample (0.9% NaCl) and the protected HEMAgene•BUFFY COAT sample (HG-BCD) as described in the materials and methods. Equivalent volumes from each freeze-thaw cycle sample were analyzed by agarose gel electrophoresis as described in the materials and methods.*

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PD-WP-00033 Issue 2/2013-09

Conclusions

DNA stability and integrity are maintained through multiple freeze-thaw cycles in buffy coat samples stored in HEMAgene•BUFFY COAT DNA stabilizing reagent (HG-BCD). The concentration determined by fluorescence demonstrated only a 1% loss of DNA after 2 freeze-thaw cycles and a 7% loss of DNA after 20 freeze-thaw cycles for the HEMAgene•BUFFY COAT samples. In comparison, the unprotected buffy coat samples experienced a 75% loss of DNA after 2 freeze-thaw cycles, and a 91% loss after 20 freeze-thaw cycles. This is a significant finding, since (i) buffy coat samples can be subjected to multiple freeze-thaw cycles due to unexpected temperature changes during shipping, repeated sampling and unexpected power failures, and (ii) the capability to allow freeze-thaw cycles without loss enables maximum flexibility and value from buffy coat samples.

Agarose gel electrophoresis demonstrated that there was no degradation and minimal loss of the HEMAgene•BUFFY COAT samples even after 20 freeze-thaw cycles, however there was significant DNA loss in the unprotected buffy coat samples after as little as 2 freeze-thaw cycles. The high yields in the HEMAgene•BUFFY COAT samples were due to increased solubilization of DNA in HG-BCD, leading to an increase in DNA available for recovery.

HEMAgene•BUFFY COAT DNA stabilizing reagent protects samples from the sample degradation and sample loss associated with multiple freeze-thaw cycles, allowing simplified logistics for both transport and storage.

Reference

1 McCarthy, Connor. A survey on long-term storage of biological specimens at McGill University.

PD-WP-00033 Issue 2/2013-09