DNA from buffy coat stored in a novel biostabilizer at room temperature performs equivalently to DNA from frozen buffy coat in Illumina® Infinium genotyping

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Introduction

Compared to whole blood, buffy coat preparations are more suitable for transport and long-term storage for genetic and biobanking applications. Buffy coat provides a concentrated source of nucleated cells from which DNA can be extracted. However, buffy coat samples are not stable at room temperature so they must be kept frozen until use. Transport of buffy coat samples is expensive due to the requirement for special packaging, dry ice and dangerous goods handling fees. Cold chain transport also poses a risk to valuable samples, where shipping delays can cause total sublimation of dry ice and exposure to freeze-thaw damage. Long-term storage requires significant capital investment in ultra-low temperature freezers and monitoring systems resulting in high recurring operational costs. In the event of a power failure, untreated buffy coat samples are at significant risk of DNA loss due to freeze-thaw. Mitigation of these risks requires expensive backup systems and complicated single-use aliquot schemes.

HEMAgene™•BUFFY COAT DNA stabilizing reagent (HG-BCD) is a novel biostabilizer that enables total protection of buffy coat samples both at ambient temperature or when frozen. HG-BCD can stabilize buffy coat samples at ambient temperature for optimal workflow flexibility, or it can confer added protection to frozen samples in the event of a freezer failure or a shipping delay.

This study demonstrates the ability of HG-BCD to protect DNA in buffy coat samples through multiple freeze-thaw cycles and at extended room temperature storage. It also demonstrates that samples treated with HG-BCD exhibit optimal genotyping performance, regardless of storage conditions.

Materials and methods

Sample collection and buffy coat preparation

- Whole blood (approximately 32 mL, 4 tubes) was collected from each donor using EDTA-K tubes
- Blood was fractionated by centrifugation (1200 × *g*, 10 minutes)
- Buffy coat recovered from replicate samples was pooled

Untreated buffy coat vs. HG-BCD treated buffy coat

- One buffy coat aliquot (0.5 mL) from each donor was treated with 4.5 mL of HG-BCD and stored at room temperature until extraction
- A control aliquot was left untreated and frozen at -80°C until extraction
- DNA was extracted from all samples with a QIAGEN® QIAsymphony®, using the Blood 400 DSP protocol for HG-BCD samples and the Buffy Coat 400 DSP samples for untreated buffy coat
- Extracted DNA was quantified by PicoGreen®; DNA integrity was assessed by agarose gel
- Extracted DNA was diluted to concentrations between 25 and 100 ng/µL and run on the Illumina Infinium HumanCore 250K BeadChip genotyping array

Accelerated aging samples

- One buffy coat aliquot (0.5 mL) from each donor was treated with 4.5 mL of HG-BCD
- Treated samples were split into 0.5 mL aliquots
- DNA was extracted immediately from one aliquot to provide baseline data
- Remaining aliquots were stored at -80°C, room temperature and 50°C for 12 weeks (12 weeks storage at 50°C correlates to 48 weeks of storage at room temperature based upon the Arrhenius equation)
- DNA was extracted using the Promega ReliaPrep™ Blood gDNA Miniprep System
- Extracted DNA was quantified by PicoGreen; DNA integrity was assessed by agarose gel
- Extracted DNA was genotyped as mentioned previously

Freeze-thaw samples

- A 0.5 mL aliquot of buffy coat from each donor was treated with 4.5 mL of HG-BCD
- Untreated buffy coat controls were prepared by adding 4.5 mL of 0.9% saline to 0.5 mL aliquots of buffy coat
- Samples and controls were exposed to 20 freeze-thaw cycles consisting of freezing at -80°C for 1 hour followed by thawing at 50°C for 15 minutes
- 200 µL aliquots of the samples and controls were collected before freezing and after 2, 4, 10, 15 and 20 cycles
- DNA was extracted using the Promega ReliaPrep Blood gDNA Miniprep System
- Extracted DNA was quantified by PicoGreen; DNA integrity was assessed by agarose gel
- Extracted DNA was genotyped as mentioned previously

Results

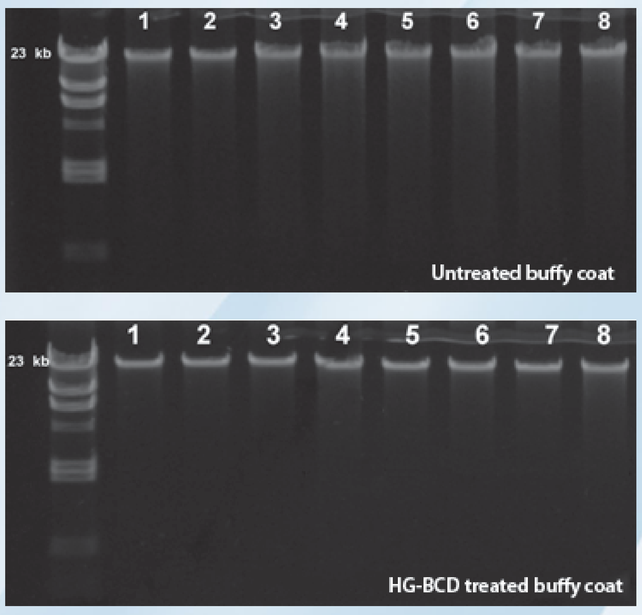

Figure 1: Agarose gels of gDNA extracted from untreated, frozen buffy coat and buffy coat treated with HG-BCD and stored at room temperature.

- Agarose gel electrophoresis reveals that HG-BCD protects DNA in buffy coat from degradation when samples are stored at room temperature.
- DNA molecular weight is similar to that of DNA extracted from untreated, frozen buffy coat.

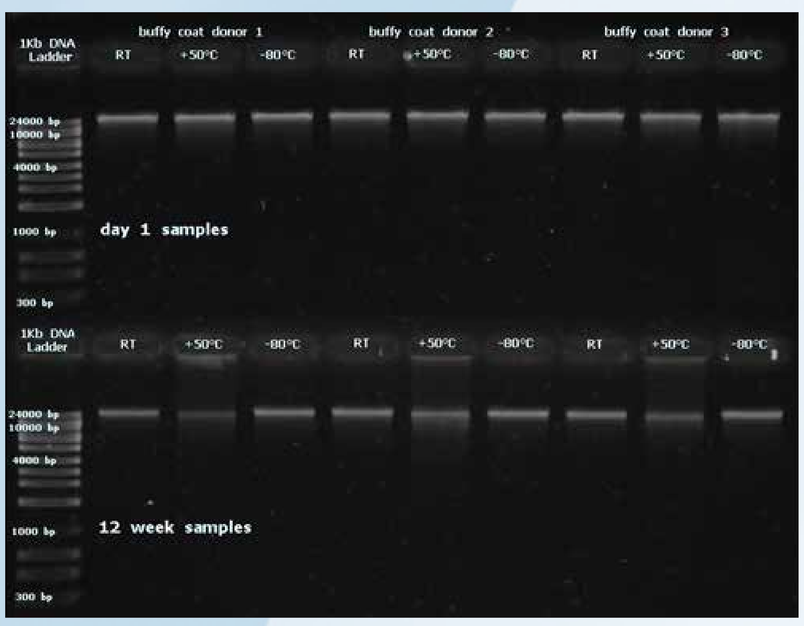

Figure 2: Agarose gel of gDNA extracted from buffy coat treated with HG-BCD after storage at either room temperature, -80°C or 50°C for 1 day and 12 weeks.

- Agarose gel electrophoresis of the DNA extracted from samples stored at -80°C, room temperature and 50°C shows high molecular weight genomic DNA in both the "Day 1" and "12 week" samples.
- There is no evidence of DNA degradation resulting from storage time or storage temperature indicating that HG-BCD is able to protect DNA in buffy coat after extended storage at both room temperature and elevated temperatures, up to 50°C.

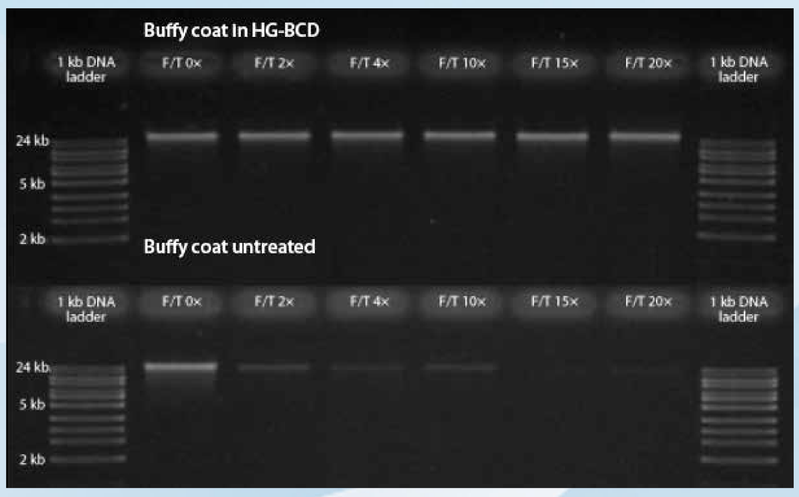

Figure 3a: Agarose gel of gDNA extracted from treated (HG-BCD) and untreated buffy coat samples before freezing and after exposure to 2, 4, 10, 15 and 20 freeze-thaw cycles.

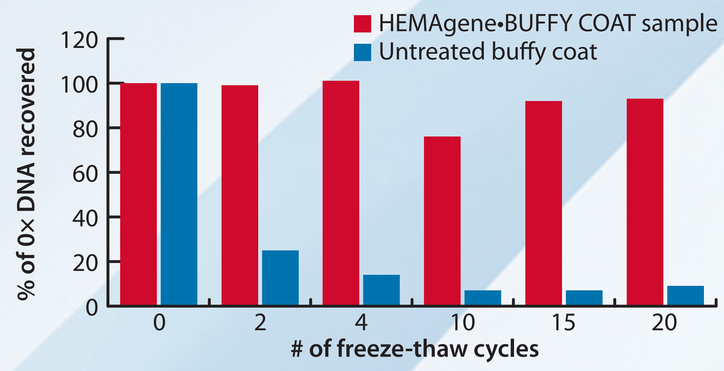

Figure 3b: DNA Recovery from untreated buffy coat and HG-BCD samples after a varying number of freeze-thaw cycles (-80°C/50°C). Each bar represents the average recovery from three independent samples.

- Buffy coat samples without HG-BCD exhibited a 75% drop in DNA recovered after only 2 free-thaw cycles.
- Buffy coat samples treated with HG-BCD exhibited only a 7% loss in DNA recovered after 20 freeze-thaw cycles, whereas untreated buffy coat samples exhibited a 91% drop in DNA recovered after the same number of freeze-thaw cycles (Figure 3b).
- HG-BCD is highly effective at preventing loss of DNA due to sample freeze-thawing.
- Genotyping call rates were 99.5% or higher for all samples tested.

Table 1: Genotyping call rates and concordance for frozen, untreated buffy coat samples and frozen, HG-BCD treated samples.

Donor	Genotyping call rates (%)		Genotyping concordance (%)
	Frozen, untreated buffy coat	Frozen, buffy coat in HG-BCD	
1	99.97	99.97	99.99
2	99.97	99.97	100
3	99.98	99.97	100
4	99.52	99.51	100
5	99.51	99.52	100
6	99.97	99.96	100
7	99.52	99.51	100
8	99.97	99.96	100

- HG-BCD samples perform as well as frozen, untreated buffy coat samples (Table 1).
- Genotyping concordance between untreated buffy coat and HG-BCD samples is 99.99% or higher (Table 1).
- The use of HG-BCD reagent confers added protection to frozen buffy coat samples without interfering with genotyping performance.

Table 2: Genotyping call rates and concordance for samples stored in HG-BCD for 12 weeks at room temperature and at -80°C.

Donor #	Storage temperature (°C)	Timepoint (wks)	Genotyping call rate (%)	Genotyping concordance (%)
1	-80	0	99.96	100
		12	99.96	
	RT	0	99.97	100
		12	99.97	
2	-80	0	99.96	100
		12	99.96	
	RT	0	99.96	100
		12	99.96	
3	-80	0	99.96	100
		12	99.96	
	RT	0	99.96	100
		12	99.97	

- Extended room temperature storage of HG-BCD treated buffy coats does not impact genotyping performance.
- Performance between baseline samples and 12 week samples is identical.
- Genotyping concordance between the baseline and 12 weeks samples is 100%.

Table 3: Genotyping call rates and concordance for HG-BCD treated samples subjected to multiple freeze-thaw cycles.

Donor #	# Freeze-thaw cycles	Call rate (%)	Genotyping concordance (%)	
			0 cycles vs. 2 cycles	0 cycles vs. 20 cycles
1	0	99.97	100	100
	2	99.97		
	20	99.96		
2	0	99.53	100	100
	2	99.52		
	20	99.52		
3	0	99.96	100	100
	2	99.96		
	20	99.86		

- HG-BCD samples continue to perform optimally in genotyping, even after 20 freeze-thaw cycles (Table 3).
- Concordance with baseline remains at 100% after extensive freeze-thawing.
- HG-BCD is a robust biostabilizer that effectively protects DNA in buffy coat samples at both room temperature and throughout multiple freeze-thaw cycles.

Conclusions

- HG-BCD enables room temperature transport and storage of buffy coat samples by preventing DNA degradation, even after many weeks at ambient temperature. In contrast, untreated buffy coat requires cold transport and storage.
- HG-BCD can be added to frozen samples to confer added protection in the event of a freezer failure or a shipping delay.
- HG-BCD effectively prevents the loss of DNA due to freeze-thaw that occurs with untreated buffy coat, even after extensive freeze-thaw exposure.
- DNA from buffy coat treated with HG-BCD is of high quality and is suitable for genotyping on the Illumina Infinium HumanCore 250K genotyping array. Genotyping performance of the HG-BCD samples is unaffected by freeze-thaw cycles and extended room temperature storage.

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