HEMAgene™•BUFFY COAT

Automated extraction of gDNA from buffy coat samples preserved with HEMAgene™•BUFFY COAT DNA stabilizing reagent using the QIAGEN® QIAsymphony® SP

HEMAgene•BUFFY COAT is a DNA stabilizing reagent designed for ambient temperature transport and room temperature archival storage of buffy coat samples. While HEMAgene•BUFFY COAT allows biobanks and researchers to maximize the use of fresh buffy coat samples for downstream applications, it can also provide additional protection to archived frozen buffy samples. In order to assist in the processing of large numbers of samples, HEMAgene•BUFFY COAT has been optimized for ease of integration into automated extraction systems, such as the QIAsymphony®SP using standard blood protocols.

Introduction

It is common to ship, store and process a large number of buffy coat samples for DNA extraction for genetic research and large population studies. The extraction of DNA from samples using manual methods is time-consuming and labour-intensive, therefore many laboratories have invested in automated equipment to increase throughput and reduce costs by enabling batch processing of samples. The QIAsymphony SP is a system that enables the preparation of genomic DNA from a wide variety of biological samples using paramagnetic silica particle-based technology.

The purpose of this study is to evaluate the performance of the standard QIAsymphony DSP DNA Midi Kit and the DNA Blood 1000 DSP protocol on the QIAsymphony from HEMAgene•BUFFY COAT samples.

Materials and methods

Sample collection

Approximately 7–8 mL of whole blood was collected into a 10 mL EDTA-K Vacutainer tube from each of eight donors. Samples were rocked at room temperature and then centrifuged at 1,200 × *g* for 10 minutes to separate the plasma, buffy coat and red blood cell fractions. Plasma was removed with a Pasteur pipette, leaving approximately 1 mL of plasma above the buffy coat layer. The buffy coat fraction was transferred to a 15 mL conical centrifuge tube using a P200 micropipette. HEMAgene•BUFFY COAT DNA stabilizing reagent (4.5 mL) was added to each buffy coat fraction. Samples were then vortexed to mix. Samples were stored at room temperature until DNA extraction.

DNA extraction

DNA was extracted from a 1 mL aliquot of each 5 mL HEMAgene•BUFFY COAT sample on the QIAsymphony SP using the QIAsymphony DSP DNA Midi Kit (QIAGEN catalogue number 937255) and the DNA Blood 1000 DSP protocol. The elution volume was set to 400 µL. The setup of the QIAsymphony was conducted according to the procedures contained in the QIAsymphony handbook.

DNA analysis

The DNA yield and concentration for each eluted sample was determined using Quant-iT™ PicoGreen® reagent (Life Technologies). Samples were diluted 1/50 prior to quantification. To evaluate DNA purity the absorbance (260, 280 and 340 nm) of each sample was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific). The 260 nm and 280 nm readings were corrected by subtracting the 340 nm reading from each before calculating the A_{260}/A_{280} ratio. DNA integrity was evaluated by running approximately 100 ng of DNA from each sample on a 0.8% agarose gel (90 V, 50 minutes) and staining with ethidium bromide. A Lambda HindIII digest ladder was used to determine the approximate size of the eluted DNA.

For Research Use Only
Not for use in diagnostic procedures

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Results

DNA yield and concentration

From eight HEMAgene•BUFFY COAT DNA samples extracted in triplicate on the QIAsymphony SP, the median DNA yield from a 1 mL aliquot was 19.5 µg and the average yield was 17.6 µg (Figure 1, right axis).

The median concentration of the extracted DNA was 48.6 ng/µL and the mean was 44.0 ng/µL (Figure 1, left axis) with a 95% confidence interval of 34.8 ng/µL to 53.3 ng/µL. The concentration range was 14.2 ng/µL to 74.8 ng/µL.

The average A_{260}/A_{280} ratio of the extracted DNA samples was 1.9.

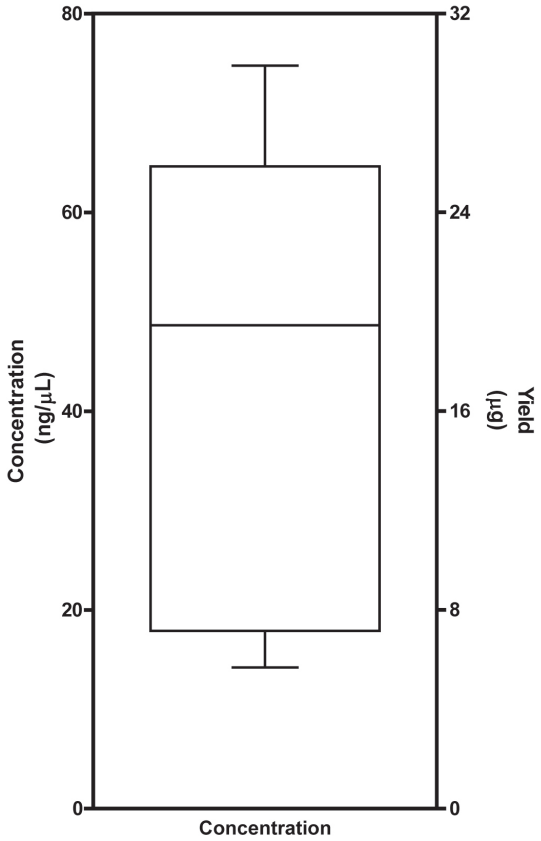

***Figure 1**: DNA concentration and yield obtained from extracting a 1 mL aliquot of HEMAgene•BUFFY COAT DNA sample on the QIAsymphony SP, eluting in a 400 uL volume of elution buffer. The box plot, from top to bottom, represents the maximum, upper quartile, median, lower quartile and minimum values.*

Molecular weight of extracted DNA

As assessed by agarose gel, the DNA extracted from each sample had a molecular weight equal to or greater than 23 kb (Figure 2).

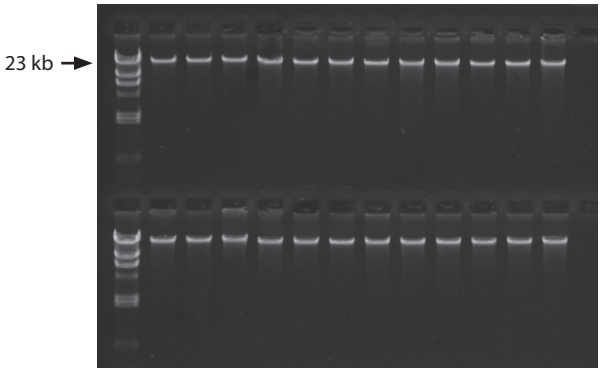

***Figure 2**: Agarose gel of DNA extracted by the QIAsymphony SP from 24 HEMAgene•BUFFY COAT DNA samples.*

Discussion

Using the QIAsymphony SP with the QIAsymphony DSP DNA Midi Kit and a standard protocol (QIAsymphony Blood 1000 DSP) we were able to obtain high quality DNA from HEMAgene•BUFFY COAT DNA samples. DNA concentrations were suitable for a variety of downstream applications and could be increased or decreased as required by selecting different elution volumes on the instrument. In addition to achieving suitable DNA yields and concentration, an additional 4 mL of the HEMAgene•BUFFY COAT sample from each of the donors remained that could be subsequently stored at room or frozen temperature and retrieved for further extraction and analysis upon request.

The QIAsymphony SP, when used with the QIAsymphony DSP DNA Midi Kit and the Blood 1000 DSP protocol was able to extract DNA from 24 HEMAgene•BUFFY COAT DNA samples in approximately 2 hours with no user interaction beyond initial instrument setup. The HEMAgene•BUFFY COAT samples were fully compatible with the QIAsymphony methodology and substituting the starting material with HEMAgene•BUFFY COAT did not require any protocol changes.