Maxwell® 16 Buffy Coat Genomic DNA Purification Application

ABSTRACT The Maxwell® 16 Integrated System combines compact instrumentation, prefilled reagent cartridges and optimized automated methods to maximize performance and flexibility while minimizing hands-on time required for DNA extraction. The Maxwell® 16 Instrument can process up to 16 samples in about 30 minutes. The purified DNA is ready for downstream analysis without sample preprocessing, precipitation or rehydration of a DNA pellet. In this article, we describe extraction of DNA from buffy coat samples, the white blood cell fraction of whole blood, using the Maxwell® 16 Blood DNA Purification Kit.

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DNA purification from buffy coat samples requires a new automated method for the Maxwell® 16 Instrument but uses the existing Maxwell® 16 Blood DNA Purification Kit.

INTRODUCTION

Genomic DNA (gDNA) extraction is a routine procedure in clinical research and clinical molecular diagnostic laboratories. Extracted DNA is commonly used in mapping or gene structure analysis as well as for specialized applications such as HLA genotyping to support organ transplant procedures. Research or diagnostic applications in HLA genotyping most commonly use whole blood or white blood cell fraction (buffy coat) as a sample. To improve DNA yield and purity, the anticoagulated whole blood is typically centrifuged to separate the buffy coat from the red blood cells and serum.

Laboratories performing HLA clinical research or HLA molecular diagnostics face increasingly complex assays, pressure to deliver consistent results, the need for ongoing training of lab personnel and limited space for equipment. Manual DNA extraction methods are most commonly used, and low- to moderate-throughput manual purification users can spend more than 30% of their time performing DNA extraction. Until now, there have been limited options for automated DNA extraction that did not require large, complicated instrumentation, considerable capital investment, training and routine maintenance.

We have developed an application of the Maxwell® 16 Blood DNA Purification Kit[a] (Cat.# AS1010) for DNA extraction from buffy coat samples that provides hands-on labor savings, improves consistency of results and is easy to use. This application requires a new automated method for the Maxwell® 16 Instrument but uses the existing Maxwell® 16 Blood DNA Purification Kit. The new method can be downloaded free of charge from

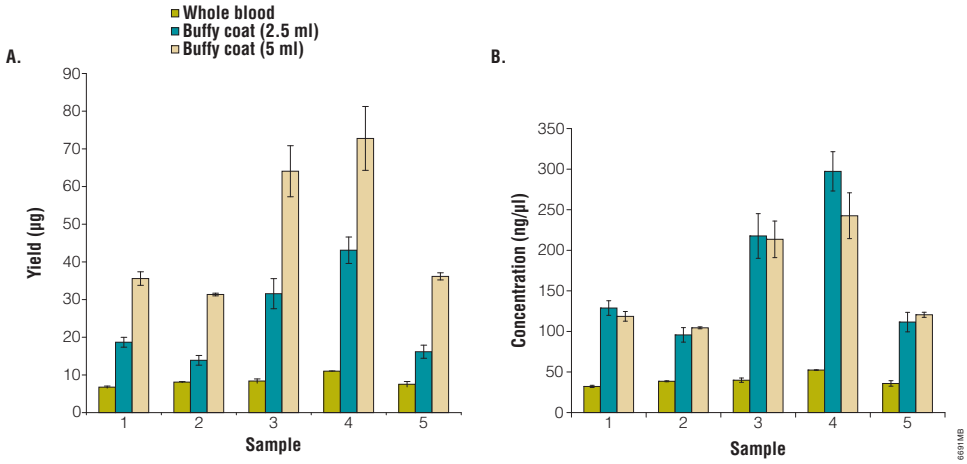


Figure 1. The Maxwell® 16 gDNA method for buffy coat samples delivers high yields of concentrated genomic DNA. Whole blood was collected from five individual donors into 10 ml EDTA-treated Vacutainer® tubes. Buffy coat fractions were prepared by spinning the collection tubes for 10 minutes at 2,500 × *g* and collecting cells at the interface between the lower red blood cell layer and the upper plasma layer. An approximately tenfold concentration of cells was obtained using this technique (i.e., 250 µl or 500 µl of buffy coat represents material from approximately 2.5 ml or 5 ml of blood, respectively). All samples were processed using the Maxwell® 16 Blood DNA Purification Kit. For whole blood processing, 400 µl of sample was added to well #1 of the Maxwell® 16 reagent cartridge, and the method for blood was initiated. For buffy coat processing, either 250 µl or 500 µl of buffy coat fraction was added to well #1 of the Maxwell® 16 reagent cartridge, and the method developed for buffy coat samples was initiated. DNA was eluted in either 300 µl (for whole blood and 250 µl of buffy coat fraction) or 500 µl (for 500 µl buffy coat fraction) of elution buffer provided with the system. Recovered samples were analyzed using a NanoDrop® ND-1000 spectrophotometer. Total gDNA yields were calculated using the concentration of each sample and average volumes recovered from whole blood (average = 210 µl), 250 µl buffy coat samples (average = 145 µl) or 500 µl buffy coat samples (average = 300 µl). Total yields or concentration from whole blood, 250 µl of buffy coat or 500 µl of buffy coat samples were plotted.

www.promega.com/maxwell16/firmware. The buffy coat DNA extraction application provides superior yield and concentration compared to other methods. The Maxwell® 16 Instrument is compliant as General Purpose Laboratory Equipment and General Purpose Reagents in the USA.

MAXWELL® 16 BUFFY COAT GENOMIC DNA EXTRACTION PRINCIPLE

The Maxwell® 16 Instrument has a compact design to minimize the amount of laboratory space that it occupies. Optimized methods are preloaded on the instrument. The Maxwell® 16 Instrument can be unpacked and ready to purify 1–16 samples in about 15 minutes (1).

Successful DNA extraction from buffy coat requires the rapid disruption of a large number of white blood cells. In the Maxwell® 16 method, 250 or 500 µl of buffy coat sample is added directly to the cartridge. The Maxwell® 16 Blood DNA Purification Kit buffy coat application provides DNA yield of up to 70 µg and concentration greater than 200 ng/µl, greatly exceeding the DNA yield and concentration obtained from equal volumes of whole blood (Figure 1).

The unique plunger design allows buffy coat samples to be purified without the need for preprocessing steps, such as proteinase K or other enzyme treatment, organic solvents or mechanical grinding. After adding the sample, the reagent cartridges are placed into the Maxwell® 16 Instrument, and simple on-screen prompts guide the selection of the appropriate optimized method. When the Maxwell® 16 Instrument run is completed, the purified DNA is ready for downstream applications such as PCR.

The functionality of the Maxwell® 16 Instrument is based on the sequential capture and release of MagneSil® Paramagnetic Particles (PMPs) into the wells of the reagent cartridges, which are supplied preloaded with reagents (1). The instrument uses a powerful magnet and unique plunger design to lyse and capture target material (e.g., genomic DNA) while washing away impurities. The ability of the instrument to selectively capture and move the PMPs during processing eliminates the need for complex liquid handling and reagent bottles, which can be affected by clogging and contamination issues. The reagent cartridge and plunger come into contact with a single sample; they are removed and discarded after each run. Single-use reagent cartridges also ensure that new reagents are delivered untainted to maximize performance and run reproducibility.

ELUTION VOLUME AND PURITY

Generally, DNA extraction methods must find a balance between maximum yield and maximum concentration while not affecting purity. We examined the Maxwell® 16 buffy coat DNA extraction method by measuring DNA yield and concentration from 250 µl of buffy coat (~1×10^7 white blood cells) using a range of elution volumes (Table 1). As expected, the eluted DNA concentration drops by almost 50% as the elution volume increases from the recommended 300 µl to 600 µl. However, DNA yield also increased with added elution buffer. This flexibility allows users to choose the appropriate amount of elution buffer for their experimental needs.

Table 1. The effect of elution volume on yield and concentration of genomic DNA isolated from 250 µl of buffy coat (1×10^7 white blood cells).

Elution Volume	Average Concentration (ng/µl)	Average Yield (µg)
300 µl	118	17.6
400 µl	98	24.4
500 µl	77	27.1
600 µl	67	30.1

Performance of extracted DNA in downstream applications such as PCR is dependent not only on yield and concentration but also on purity. We initially measured DNA purity by measuring A_{260}, A_{280} and A_{230} absorbance using the NanoDrop® ND-1000 spectrophotometer. The ratio of A_{260}/A_{280} is a useful estimate of purity with respect to protein contamination, while the ratio of A_{260}/A_{230} is a useful estimate of guanidine isothiocyanate contamination. Extracted DNA from either 250 or 500 µl of buffy coat from four blood donors is of high quality for both A_{260}/A_{280} (averaging 1.91 and 1.91, respectively) and A_{260}/A_{230} (averaging 1.82 and 1.71, respectively). Another measure of purity is visualization of ethidium bromide- stained DNA separated by native gel electrophoresis. DNA extracted with the Maxwell® 16 buffy coat application is of high molecular weight with low ethanol carryover (Figure 2).

Using a powerful magnet to transfer the MagneSil® Paramagnetic Particles eliminates the need for complex liquid handling and reagent bottles.

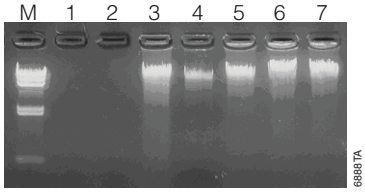


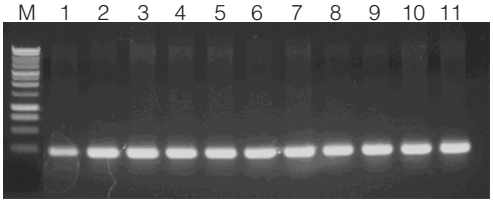
Figure 2. Genomic DNA purified from human blood using Maxwell® 16 buffy coat method. Five microliters of purified gDNA samples were mixed with 1 µl of 6X loading dye; then 3 µl of this mixture was loaded onto a 1.2% agarose gel and visualized by ethidium bromide staining. M, Markers; lane 1, Elution Buffer (–) control; lane 2, Nuclease-Free Water (–) control.

PERFORMANCE IN DOWNSTREAM APPLICATIONS

Increasingly, DNA-based testing is being used in HLA clinical research and HLA molecular testing. In general, HLA DNA can be typed either by hybridizing labeled, sequence-specific oligonucleotide probes to PCR-amplified HLA loci or by amplifying the HLA loci using sequence-specific probes followed by gel electrophoresis or DNA-sequencing. A single-locus target (Factor V) showed strong amplification using DNA isolated from different blood donors and across different extractions and visualized by gel electrophoresis (Figure 3).

Extracted DNA was subjected to low-resolution Sequence-Specific Primer (SSP) testing with 100% concordance (Figure 4). In addition, other laboratories have performed studies with the Pel-Freeze SSP UniTray kit and other HLA genotyping methods with similar concordance (data not shown).

Extracted DNA from either 250 or 500 µl of buffy coat from four blood donors is of high quality for both A_{260}/A_{280} (averaging 1.91 and 1.91, respectively) and A_{260}/A_{230} (averaging 1.82 and 1.71, respectively).



Figures 3. Amplified genomic DNA purified from human blood using the Maxwell® 16 buffy coat method. PCR products were amplified from 1 µl purified sample for Factor V. Ten microliters of PCR product was run on a 1.2% agarose gel and visualized by ethidium-bromide staining. The size of the expected PCR product for Factor V is 267 bp. Lane M, DNA Ladder; lane 1, Human gDNA (+) control.

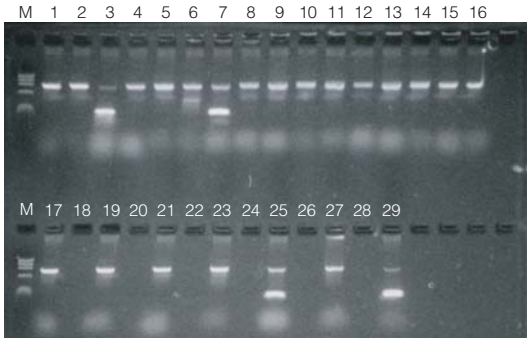


Figure 4. Low resolution DRB1 typing using DNA purified from buffy coat. Genomic DNA was purified from buffy coat derived from human whole blood using the Maxwell® 16 Buffy Coat gDNA purification application and subjected to polymerase chain reaction using sequence-specific primers (PCR-SSP). Amplification products were separated by gel electrophoresis and visualized with ethidium-bromide staining. The appearance and size of amplification products corresponds to assignment of a genotype. Figure courtesy of Dr. Deborah O. Crowe, Dialysis Clinic, Inc. (DCI), Nashville, TN.

CONCLUSION

The Maxwell® 16 System is easy to use and designed to provide labor savings for low- to moderate-throughput extraction of DNA. The system uses a compact and simple instrument that requires minimal training and maintenance combined with prefilled reagent cartridges to improve productivity by freeing up time.

The Maxwell® 16 System can extract pure, high-quality gDNA from whole blood and the white blood cell fraction of whole blood (buffy coat). DNA extracted from buffy coat samples using the Maxwell® 16 Blood DNA Purification Kit shows consistently high performance in common HLA research and clinical testing applications.

ACKNOWLEDGMENTS

We would like to thank Dr. Deborah O. Crowe and colleagues from Dialysis Clinic, Inc. (DCI). DCI is a nonprofit organization founded in 1971 and dedicated to providing rehabilitation of end-stage renal disease (ESRD) patients and constant improvement of patient care. www.DCiiNC.org

REFERENCE

1. Kephart, D. *et al.* (2006) *Promega Notes* **92**, 20–3.

PROTOCOL

- *Maxwell® 16 DNA Purification Kits Technical Manual*, #TM284, Promega Corporation
  www.promega.com/tbs/tm284/tm284.html

ADDITIONAL RESOURCES

For additional details visit:
www.promega.com/PN97Maxwell16

ORDERING INFORMATION

Product	Size	Cat.#
Maxwell® 16 Instrument	1 each	AS2000
Maxwell® 16 Blood DNA Purification Kit	48 preps	AS1010

For Laboratory Use.

[a]This product is licensed for use under U.S. Pat. No. 6,174,704.