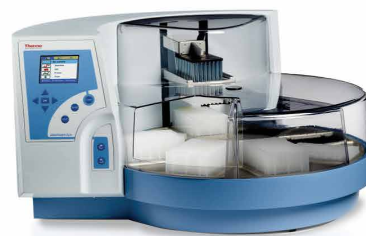
Genomic DNA extraction from bone marrow aspirates and peripheral blood mononuclear cells

Introduction and background

Bone marrow aspirates (BMAs) and peripheral blood mononuclear cells (PBMCs) are commonly used sample types that require reliable sample preparation technology for the research of various diseases. The nucleic acid extracted from BMAs and PBMCs can be used for multiple applications, such as next-generation sequencing (NGS) using the Ion Torrent™ Oncomine™ Myeloid Research Assay, microarray analysis using Applied Biosystems™ CytoScan™ and CytoScan™ HD arrays, real-time PCR using SNP genotyping assays, and many more.

The Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit uses magnetic beads that are compatible with semiautomated or automated genomic DNA (gDNA) extractions from whole blood, saliva, buffy coat, and buccal swabs. Here we evaluate a workflow using the MagMAX DNA Multi-Sample Ultra 2.0 Kit to extract gDNA from BMAs and PBMCs on the Thermo Scientific™ KingFisher™ Duo Prime Purification System. Applied Biosystems™ MagMAX™ Cell and Tissue DNA Extraction Buffer is used for initial processing of samples. High-quality gDNA is produced using this workflow, demonstrated by DNA integrity analysis and real-time PCR performance.



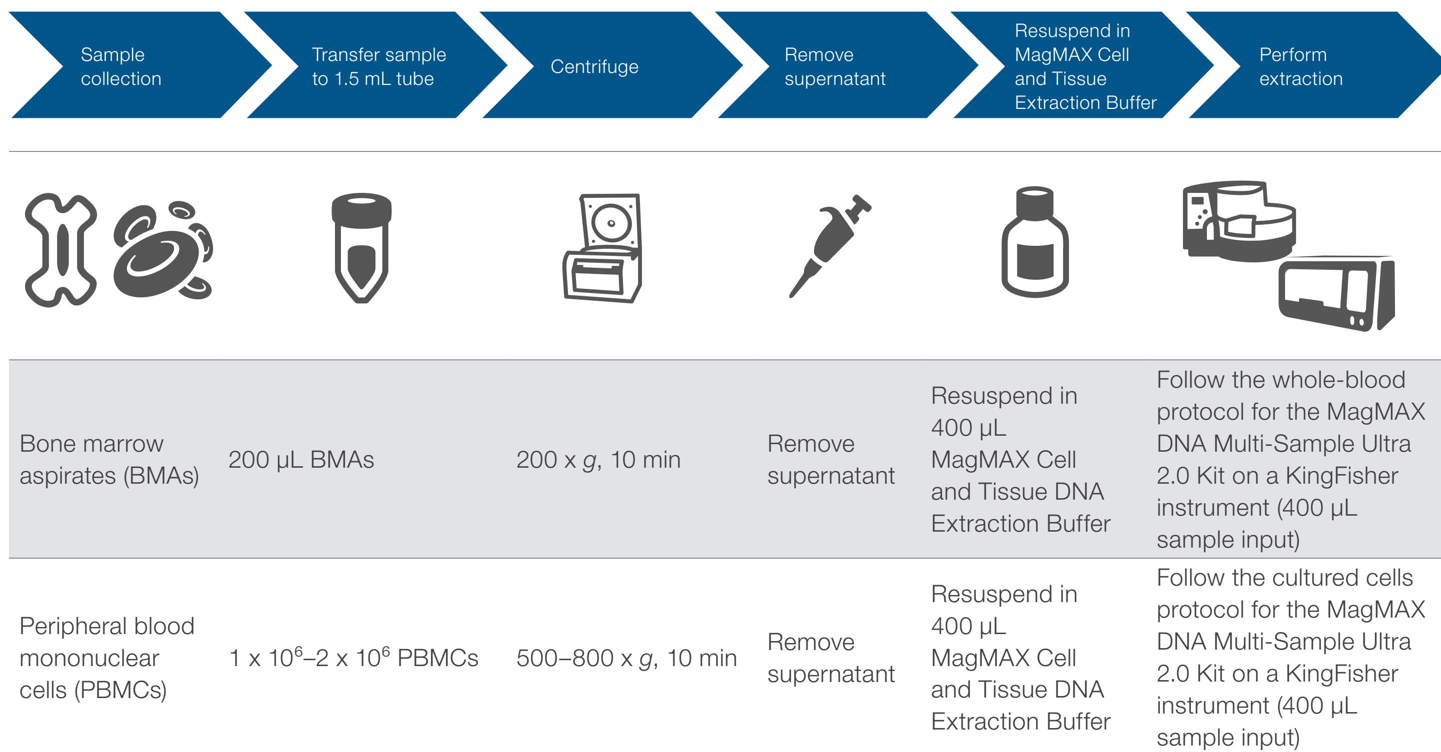
Experimental procedures

Two studies were performed using the MagMAX DNA Multi-Sample Ultra 2.0 Kit to evaluate gDNA extracted from BMAs and PBMCs.

In the first study, three BMA samples from different donors were processed in duplicate extractions using the sample preparation workflow shown in Figure 1. Sample volume of 200 µL was centrifuged at 200 x *g* for 10 minutes in a 1.5 mL Eppendorf™ tube to pellet cells from the BMA sample. The supernatant was removed while being careful not to disturb the pellet formed at the bottom of the tube. The pellet was then resuspended in 400 µL of MagMAX Cell and Tissue DNA Extraction Buffer (included with the MagMAX DNA Multi-Sample Ultra 2.0 Kit in Cat. No. A45721). From this point, the protocol for isolation of DNA from 200–400 µL of whole blood on the KingFisher Duo Prime instrument (**Pub. No. MAN0017325**) was used with the resuspended cell pellet, instead of a whole-blood sample.

In the second study, three PBMC samples from different donors were processed in duplicate extractions using the workflow in Figure 1. Sample input volume of 1 x 10^6–2 x 10^6 cells was transferred to a 1.5 mL Eppendorf tube and centrifuged at 500–800 x *g* for 10 minutes to pellet the cells. The supernatant was removed while being careful not to disturb the pellet formed at the bottom of the tube. The pellet was then resuspended in 400 µL of MagMAX Cell and Tissue DNA Extraction Buffer. From this point, the protocol for isolation of DNA from cultured cells on the KingFisher Duo Prime instrument was followed (**Pub. No. MAN0018808**).

For both studies, extracted double-stranded DNA (dsDNA) was quantified with the Invitrogen™ Qubit™ 1X dsDNA Broad Range (BR) Assay Kit. DNA integrity and size in base pairs (bp) were analyzed on the Agilent™ 4200 TapeStation™ System with a Genomic DNA ScreenTape™ device. To assess DNA quality using qPCR, Applied Biosystems™ TaqMan™ Assays (assay IDs: Hs02758991 and Hs03023880) with TaqMan™ Universal Master Mix II, no UNG, were used to detect *GAPDH* and *ACTB* gDNA targets on the Applied Biosystems™ ViiA™ 7 Real-Time PCR System, 384-well format.



Sample collection	Transfer sample to 1.5 mL tube	Centrifuge	Remove supernatant	Resuspend in MagMAX Cell and Tissue Extraction Buffer	Perform extraction
Bone marrow aspirates (BMAs)	200 µL BMAs	200 x *g*, 10 min	Remove supernatant	Resuspend in 400 µL MagMAX Cell and Tissue DNA Extraction Buffer	Follow the whole-blood protocol for the MagMAX DNA Multi-Sample Ultra 2.0 Kit on a KingFisher instrument (400 µL sample input)
Peripheral blood mononuclear cells (PBMCs)	1 x 10^6–2 x 10^6 PBMCs	500–800 x *g*, 10 min	Remove supernatant	Resuspend in 400 µL MagMAX Cell and Tissue DNA Extraction Buffer	Follow the cultured cells protocol for the MagMAX DNA Multi-Sample Ultra 2.0 Kit on a KingFisher instrument (400 µL sample input)

Figure 1. Workflows for processing BMAs and PBMCs for gDNA extraction using the MagMAX DNA Multi-Sample Ultra 2.0 Kit and KingFisher instrument.

Results and discussion

Yields measured with the Qubit 1X dsDNA BR Assay Kit indicated sample-to-sample variability from the DNA recovered from both BMAs and PBMCs (Figures 2A and 2B). Extractions from BMAs resulted in yields as low as 4.54 ng/µL (sample 2) and as high as 52.40 ng/µL (sample 3). Yields from PBMCs were consistently high across all three samples at greater than 80 ng/µL.

DNA integrity numbers (DINs) for BMAs were greater than 7.0 with strong gDNA peaks observed in the electropherogram traces across all three samples (Figure 3). For PBMCs, DINs were greater than 9.0 with strong gDNA peaks observed across all samples. qPCR analysis on Applied Biosystems™ ViiA™ 7 Real-Time PCR Software indicated *ACTB* and *GAPDH* gDNA targets had strong amplification across all three samples for both sample types (Figure 4).

Note: During preparation of sample plates, a white precipitate formed when the samples resuspended in MagMAX Cell and Tissue DNA Extraction Buffer were added to the enhancer solution within a 96 deep-well sample plate. The precipitate dissolved during the proteinase K digestion at high temperature and did not impact DNA recovery.

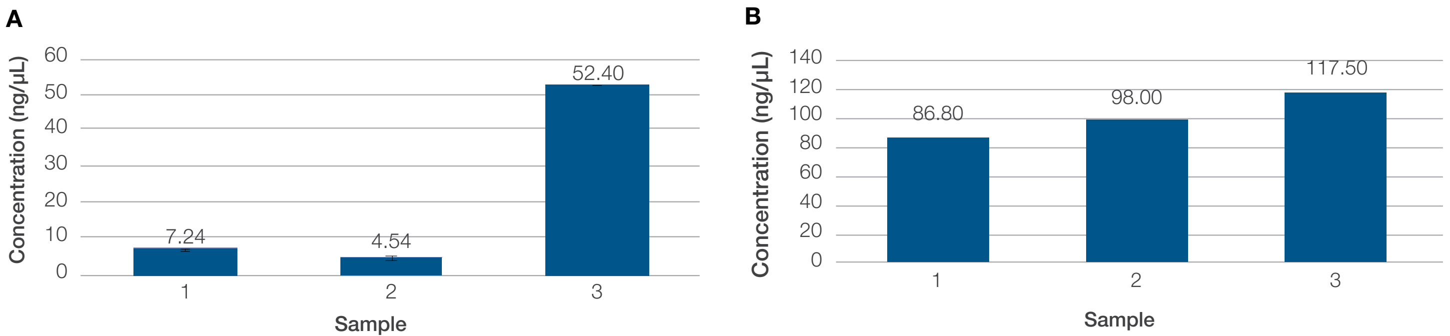


Figure 2. Yields of gDNA for three samples. (A) BMA and **(B)** PBMC samples were processed with the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime instrument. Samples were prepared for extraction with the MagMAX Cell and Tissue DNA Extraction Buffer. Both sample extraction methods were evaluated for dsDNA concentration with the Qubit 1X dsDNA BR Assay Kit.

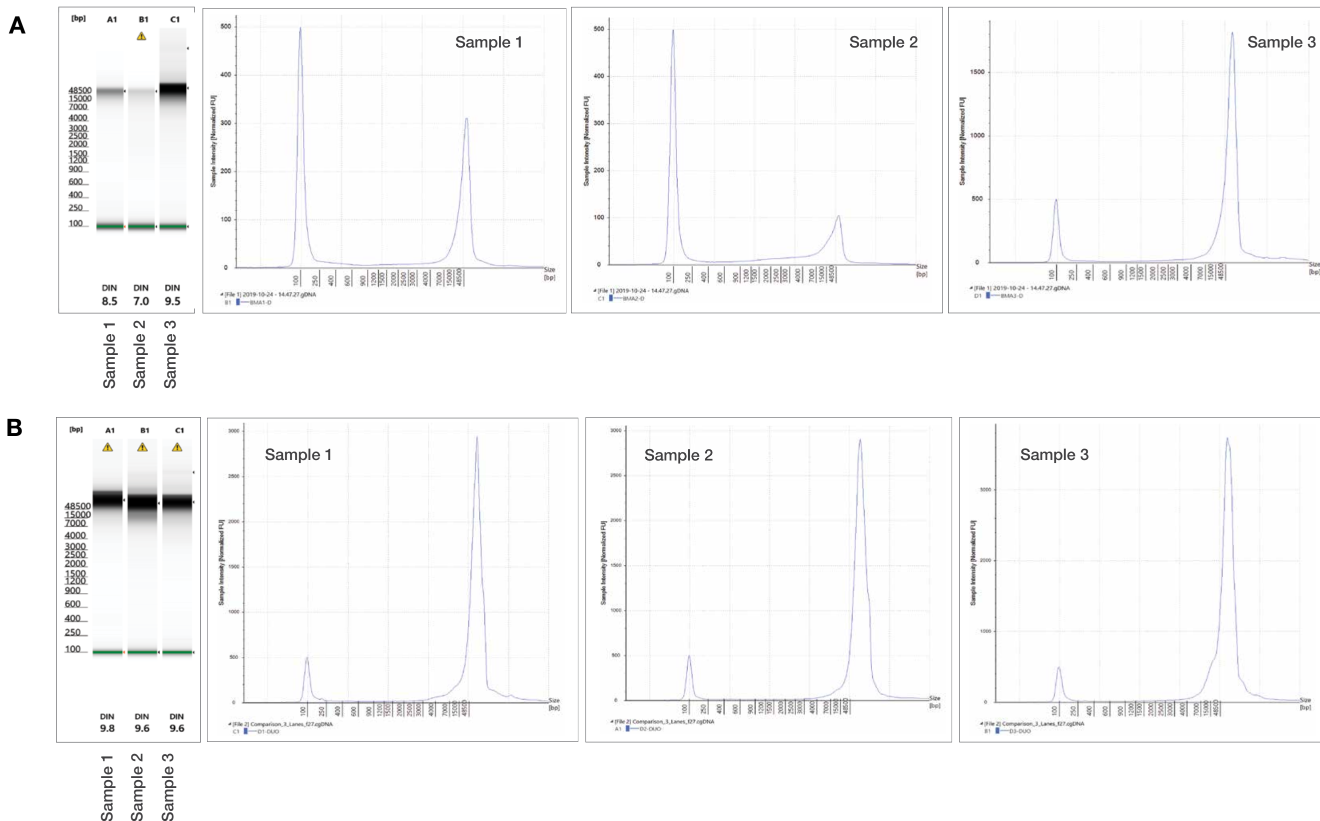


Figure 3. DNA integrity and size data. (A) BMA and **(B)** PBMC samples were processed using the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime instrument. Gel representations (left) from the 4200 TapeStation System display DIN results. Electropherogram traces (right) show the integrity and size in bp of recovered DNA.

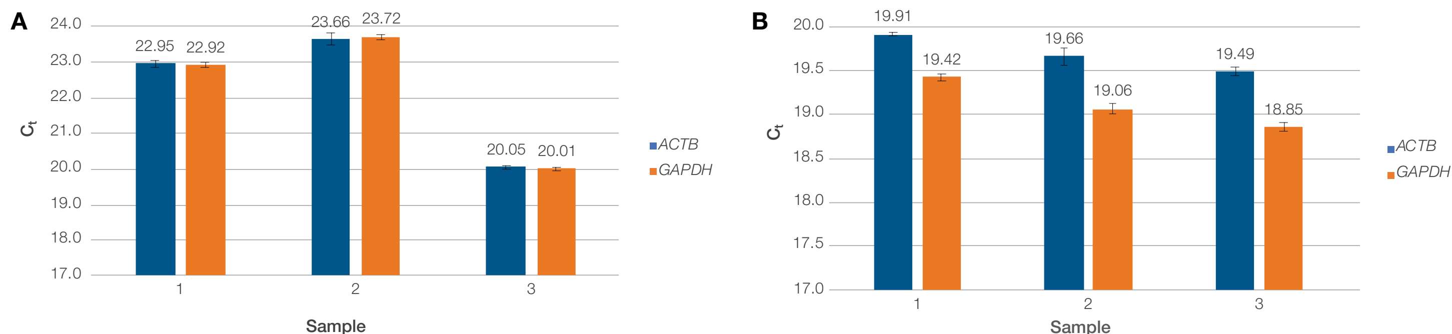


Figure 4. qPCR data from processed samples. (A) BMA and **(B)** PBMC samples were processed with the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime instrument. *ACTB* and *GAPDH* were amplified from gDNA using TaqMan Assays on the ViiA 7 Real-Time PCR System. Data are from ViiA 7 Real-Time PCR Software.

Conclusions

Use of the MagMAX DNA Multi-Sample Ultra 2.0 Kit on a KingFisher instrument can provide a streamlined approach for purification of gDNA from BMA and PBMC samples. This approach expands the type of samples that can be used with the kit and KingFisher platform. The yield and quality of gDNA extracted from BMA and PBMC samples show that the suggested purification workflow is suitable for obtaining high-quality gDNA for various research applications.

Authors

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Ordering information

Product	Cat. No.
MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
MagMAX DNA Ultra 2.0 with Cell and Tissue Extraction Buffer	A45721
KingFisher Duo Prime Purification System	5400110
ViiA 7 Real-Time PCR System with 384-Well Block	4453536
Qubit 1X dsDNA High Sensitivity (HS) and Broad Range (BR) Assay Kits	Q33265

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