Nucleic acid isolation

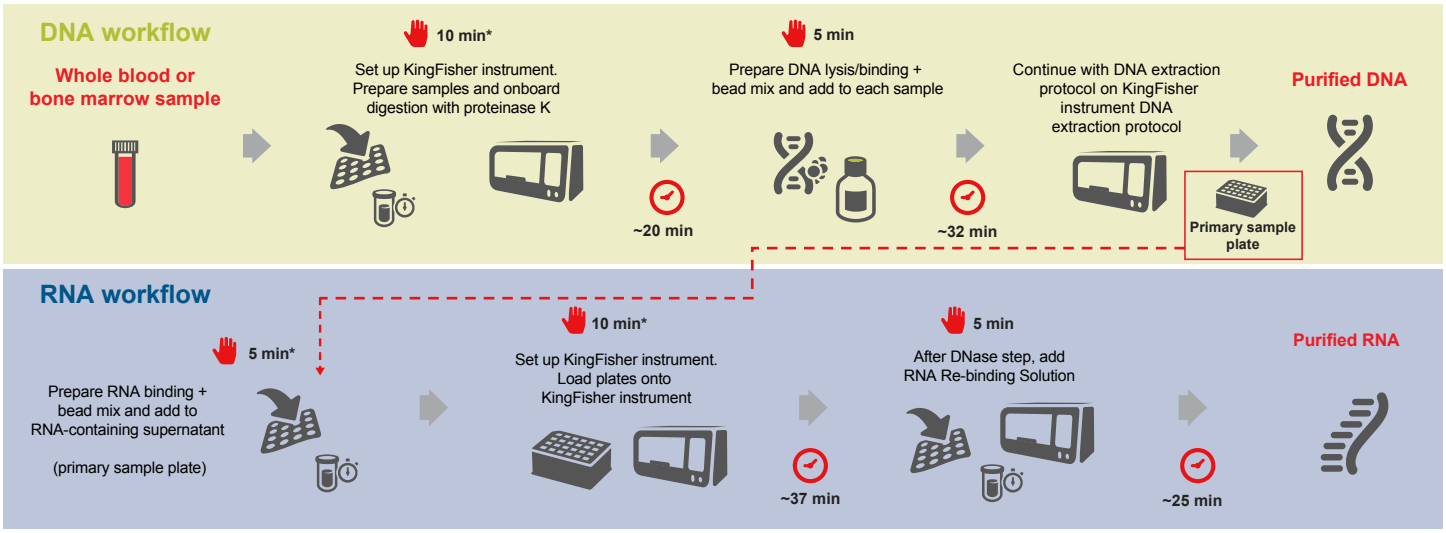
Advancing hematological research with the MagMAX Sequential DNA/RNA Kit

Introduction

High-quality DNA and RNA from precious hematological samples is pivotal for a myriad of downstream molecular applications. Hematological samples like whole blood and bone marrow are rich sources of genetic material that can provide insights into the molecular mechanisms and pathogenesis of various diseases [1].

The Applied Biosystems™ MagMAX™ Sequential DNA/RNA Kit (Figure 1) can help maximize DNA and RNA yields from precious hematological samples, providing separate, ready-to-use eluates compatible with a broad range of molecular applications, including qPCR and sequencing. This kit was developed for processing on Thermo Scientific™ KingFisher™ sample purification systems, providing a scalable automation-enabled workflow for fast results. With the MagMAX Sequential DNA/RNA Kit and KingFisher purification systems, sequential isolation of DNA and RNA can be performed from one sample source in about 2.5 hours from start to finish. Figure 2 shows the workflow for sequential isolation, its expected touch points, and total time. More information can be found in the kit's user guide (MAN0030308), and protocols for the KingFisher system can be found here.

Figure 1. The MagMAX Sequential DNA/RNA Kit comes with 11 individual components in a space-saving design for convenient storage. All components of the kit can be stored at ambient temperatures. The bottles are conveniently labeled by application for easy identification—green, blue, and white bottle cap labels indicate components for DNA isolation, RNA isolation, and both, respectively.



* Sample addition time may vary due to number of samples and method of sample addition to the sample plate.

Figure 2. Schematic for nucleic acid isolation workflow using the MagMAX Sequential DNA/RNA Kit on KingFisher purification systems. Complete both DNA and RNA extractions with a total turnaround time of approximately 2.5 hours, including hands-on time for plate and reagent preparation.*

applied biosystems

Here we show the evaluation of the quality and molecular performance of nucleic acid extracted from whole blood and bone marrow samples using the MagMAX Sequential DNA/RNA Kit with KingFisher purification systems.

Materials and methods

Nucleic acid isolation

Whole blood samples from three healthy donors were collected in K_2 EDTA collection tubes and shipped cold overnight for next-day use. Bone marrow from three healthy donor samples was obtained from a commercial vendor and shipped on ice overnight. Nucleic acids from the obtained whole blood and bone marrow samples were extracted on the KingFisher™ Flex, Apex, and Duo Prime purification systems following the procedures outlined in the user guide for low and high sample input volumes, including: 100 µL, 150 µL, 200 µL, and 500 µL for whole blood samples and 50 µL, 150 µL, 200 µL, and 500 µL for bone marrow samples. Extractions from low-volume sample inputs (up to 150 µL for whole blood and bone marrow) were performed in 96 deep-well plastics. Extractions from high-volume sample inputs (200–500 µL) of whole blood and bone marrow were performed in 24 deep-well plastics on KingFisher purification systems. Sequentially eluted DNA and RNA were analyzed for quality and performance. In addition, the eluates obtained from the extractions on the KingFisher automation platforms were compared between runs and between wells to evaluate the reproducibility of the MagMAX Sequential DNA/RNA Kit.

Yield and quality

Extracted DNA and RNA yields and concentrations were analyzed utilizing the Invitrogen™ Qubit™ 1X dsDNA Broad Range Assay Kit and Qubit™ RNA High Sensitivity Assay Kit, respectively, on the Qubit™ Flex Fluorometer. Extracted RNA quality was assessed using the Agilent™ RNA 6000 Pico Kit and reagents on an Agilent™ 2100 Bioanalyzer System.

Functional molecular performance

Assessment of extraction performance and RNA and DNA quality in molecular analysis was conducted by qPCR with respective protocols on an Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. DNA was detected using Applied Biosystems™ TaqMan™ Assays targeting *GAPDH_g1* at 20X with Applied Biosystems™ TaqMan™ Universal Master Mix II (no UNG) with 1 µL of DNA eluate in the reaction. cDNA synthesis was performed on RNA eluates using the Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit following the parameters in the **product information sheet** with 4 µL of RNA eluate in each reaction, on an Applied Biosystems™ Veriti™ Thermal Cycler. Following cDNA synthesis on the extracted RNA, qPCR was conducted with TaqMan Assays targeting *GAPDH_m1* and TaqMan Universal Master Mix II (no UNG) with 1 µL of cDNA material, on a QuantStudio 7 Flex Real-Time PCR System.

For further analysis, sequentially isolated DNA and RNA was functionally evaluated by sequencing with the Ion Torrent™ Oncomine™ Myeloid Assay GX v2 on the Ion Torrent™ Genexus™ Integrated Sequencer following the procedural guidelines from the **user guide** that begin in Chapter 8.

Results and discussion

Yield and quality

Total DNA yields across the extracted eluates from whole blood and bone marrow samples were greater than 200 ng at all sample volume inputs (Figure 3). Similarly, RNA yields were observed to be at least 100 ng from both whole blood and bone marrow at all sample volume inputs (Figure 4). Theoretically, as more sample input is used, higher yield is expected. However, some results showed variation in yield by sample input and sample type. As samples were not normalized by cell count prior to extraction, yields were expected to vary from sample to sample, as observed by the range in yields from Figures 3 and 4. White blood cell (WBC) count can significantly impact sample yields for both DNA and RNA. It is recommended to obtain a WBC count prior to extraction, as the kit recommends a WBC maximum of 15,000 WBC/µL.

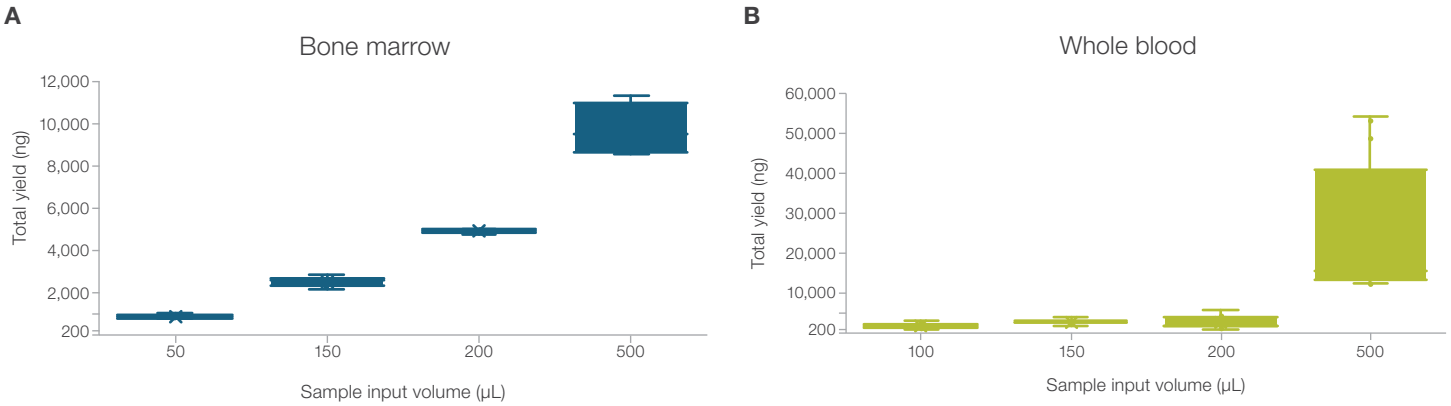


Figure 3. Average total DNA yields. DNA from **(A)** bone marrow and **(B)** whole blood samples from 3 different donors was extracted using KingFisher purification systems at various sample input volumes.

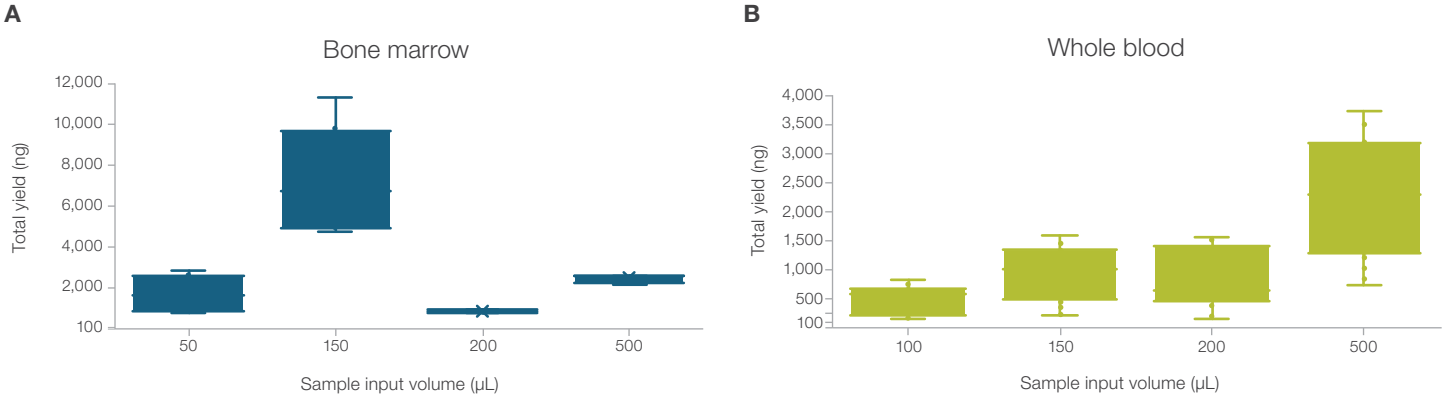


Figure 4. Average total RNA yields. RNA from **(A)** bone marrow and **(B)** whole blood samples from 3 different donors was extracted using KingFisher purification systems at various sample input volumes.

Instrument platform consistency

Analyses of extractions from low input amounts of bone marrow samples (50–150 µL) and whole blood samples (100–150 µL) from three donors each were compared between the KingFisher Duo Prime and KingFisher Flex systems to evaluate run-to-run and system-to-system variation. Table 1 indicates comparable performance across hematological sample types for concentration and total yield, with both KingFisher systems exhibiting minor differences in values.

Table 1. Comparison of results from KingFisher Duo Prime and KingFisher Flex systems to assess reproducibility of the MagMAX Sequential DNA/RNA Kit.

Average concentration (ng/µL) across donors			
Instrument		KingFisher Duo Prime system	KingFisher Flex system
Bone marrow	DNA	17.0	16.4
	RNA	3.4	5.0
Whole blood	DNA	23.8	23.7
	RNA	7.9	9.5
Average yield (ng) across donors			
Instrument		KingFisher Duo Prime system	KingFisher Flex system
Bone marrow	DNA	1,696	1,632
	RNA	272	386
Whole blood	DNA	2,362	2,367
	RNA	636	763

Intactness

The percentage of RNA fragments longer than 200 nucleotides relative to the total RNA content (DV200) was observed from the RNA run on an Agilent Bioanalyzer. DV200 provides valuable information about the degradation status of RNA samples. A higher DV200 fragment size index indicates a higher proportion of intact RNA, while a lower DV200 value indicates increased RNA degradation and fragmentation. Average DV200 values across all RNA samples sequentially isolated after DNA isolation from a single sample source were >65%, indicating that the RNA obtained after DNA isolation is intact within the workflow. Figure 5 details DV200 results obtained and averaged across whole blood and bone marrow samples across all sample volume inputs and both KingFisher Flex and KingFisher Duo Prime systems.

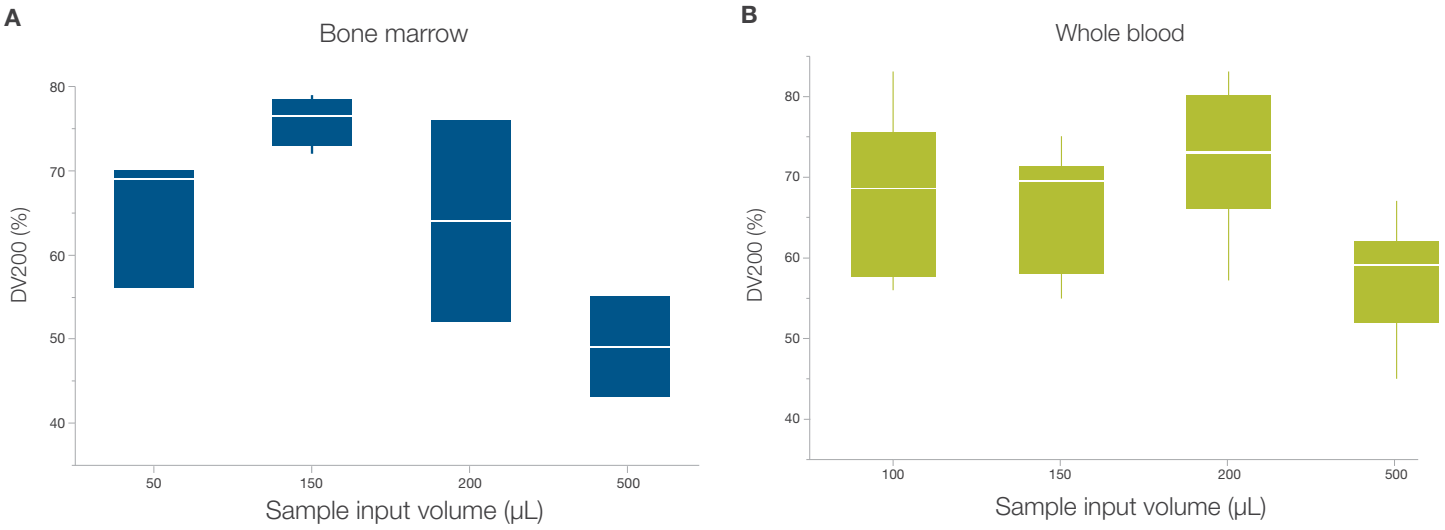


Figure 5. Average DV200 values obtained from RNA eluates from the MagMAX Sequential DNA/RNA Kit from varied sample input volumes. Eluates were obtained from **(A)** bone marrow and **(B)** whole blood samples, following the sequential isolation workflows on different KingFisher systems.

Functional molecular performance

The presence of carryover DNA in RNA eluates and its effect in downstream applications was determined and analyzed in reactions with reverse transcription (+RT) and without reverse transcription (–RT). This allows differentiation of RNA and DNA and enables identification of RNA-specific eluates with minimal DNA contamination to assess the purity of eluates. Minor amounts of DNA carryover were observed within the RNA eluates. The shift in threshold cycle (dC_q) from RNA-specific amplification (occurring only in +RT reaction) and DNA contamination (occurring in both +RT and –RT reactions) was greater than 7 cycles across all sample extractions, indicating distinct selective isolation of DNA and RNA within the eluates, respectively (Table 2).

Molecular performance of the isolated DNA, analyzed by PCR targeting the housekeeping gene *GADPH*, indicated amplification across all samples extracted. Table 2 shows 100% amplification across all samples.

Table 2. Molecular performance of PCR. Percent amplification of housekeeping gene *GAPDH_g*1 by qPCR and ±RT results indicating percentage of samples with $dC_q > 7$ from RNA-specific amplification across different sample types and volumes extracted on KingFisher purification systems with the MagMAX Sequential DNA/RNA Kit.

Instrument	Sample type	Input volume (µL)	*GAPDH_g1* amplification	±RT reactions with $dC_q > 7$
KingFisher Duo Prime	Whole blood	100	100%	100%
		150	100%	100%
		200	100%	100%
		500	100%	100%
	Bone marrow	50	100%	100%
		150	100%	100%
		200	100%	100%
		500	100%	100%
KingFisher Flex	Whole blood	100	100%	100%
		150	100%	100%
		200	100%	100%
		500	100%	100%
	Bone marrow	50	100%	100%
		150	100%	100%
		200	100%	100%
		500	100%	100%

Isolated RNA and DNA were run with the Oncomine Myeloid Assay GX v2 on the Genexus Integrated Sequencer. Typical sequencing metrics were assessed to understand nucleic acid quality for sensitive downstream sequencing applications (Table 3). DNA amplicon uniformity, base uniformity, and hotspot coverage exceeded expectations of 97% coverage with mean read length (MRL) achieving ≥200 bp in size and over 2 million mapped reads. Similarly, NGS metrics generated from RNA exceeded expectations, with mapped reads greater than 400,000 (74%) and an MRL of 103 bp.

Table 3. DNA and RNA quality metrics obtained from the Oncomine Myeloid Research Assay using the Genexus Integrated Sequencer. DNA and RNA were sequentially isolated from samples of various volumes (100 µL, 150 µL, 200 µL, and 500 µL of whole blood and 50 µL, 150 µL, 200 µL, and 500 µL of bone marrow) using the MagMAX Sequential DNA/RNA Kit on KingFisher Flex and KingFisher Duo Prime purification instruments.

DNA quality metric	Target	Result
Myeloid DNA amplicon uniformity	≥97%	100%
Amplicon composition bias	<0.5	0.16
DNA base uniformity	≥97%	99%
Myeloid DNA mapped read	≥800K	2,360,122
Myeloid DNA mean read length (MRL)	≥200 bp	210 bp
Myeloid DNA 350x hotspot coverage	≥97%	100%
Myeloid DNA 350x *CEBPA* gene coverage (high-GC regions)	≥90%	100%
RNA quality metric	**Target**	**Result**
Mapped (mappable fusion) reads	≥50K	403,984
Average mapped reads	≥60%	74%
MRL	≥70 bp	103 bp

Conclusions

The MagMAX Sequential DNA/RNA Kit is an efficient and versatile kit that can be used with automated KingFisher purification systems to maximize productivity in the lab. This is particularly important for expanding hematologic workflows. The MagMAX Sequential DNA/RNA Kit offers the advantage of sequential isolation, allowing for the efficient extraction of DNA and RNA from a single sample source. By eliminating the need for upfront red blood cell (RBC) lysis, the kit saves time and resources, streamlining hematological workflows for genomic biomarker and cancer research with DNA and RNA of high quality, yield, and purity.

In summary, the combination of sequential isolation with automation capabilities makes the MagMAX Sequential DNA/RNA Kit an excellent solution that enhances productivity, expands hematologic workflows, and provides high-quality DNA and RNA for genomic biomarker and cancer research.

Authors

Karessa Garza, Anthony Pedroza, Stacie Lupo, Lillie Manley,
Thermo Fisher Scientific

Reference

1. Cui YY. Sequential extraction of RNA, DNA and protein from cultured cells of the same group. *World J Methodol.* 2023;13(5):484–491. Published 2023 Dec 20. doi:10.5662/wjm.v13.i5.484

Ordering information

Description	Cat. No.
Nucleic acid isolation kits	
MagMAX Sequential DNA/RNA Kit	A65309
MagMAX Sequential DNA Lysis/Binding Solution	A66591
MagMAX Sequential RNA Binding Solution	A66592
MagMAX Sequential RNA Wash Solution I Concentrate	A66593
MagMAX Sequential DNA Wash Solution I	A66594
MagMAX Sequential DNA Elution Buffer	A66595
MagMAX Sequential RNA Elution Buffer	A66596
MagMAX Sequential Proteinase K	A66597
MagMAX Sequential Binding Beads	A66598
MagMAX Sequential Proteinase Digestion Buffer	A66599
MagMAX Sequential DNase I	A66600
MagMAX Sequential DNase Buffer	A66601
KingFisher purification systems and accessories	
KingFisher Duo Prime Purification System	5400110
KingFisher Flex Purification System with 96 Deep-well Head	5400630
KingFisher Flex Purification System with 24 Deep Well Head	5400640
KingFisher Apex Purification System with 96 Deep-Well Head	5400930
KingFisher Apex Purification System with 24 Combi Head	5400940
Quality and molecular analysis instruments	
Qubit Flex Fluorometer	Q33327
QuantStudio 7 Flex Real-Time PCR System	4485701
Veriti Thermal Cycler	4375305
Genexus Integrated Sequencer	A45727
Ion Chef Instrument	4484177
Ion GeneStudio S5 System	A38194
Quality and molecular analysis reagents	
Qubit 1X dsDNA Broad Range Assay Kit	Q33265
Qubit RNA High Sensitivity Assay Kit	Q32852
TaqMan Universal Master Mix II, no UNG	4440043
SuperScript VILO cDNA Synthesis Kit	11754050
SuperScript IV VILO Master Mix	11756050
Oncomine Myeloid Assay GX v2	A50694
Oncomine Myeloid Research Assay	A51770

Learn more at **thermofisher.com/magmaxsequential**